Chapter 10

Biology of Larvae and Spat

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At present, observers are divided into two schools. One class believes that spawn "strikes" very near where it is emitted. A second class believes that it may be carried miles away. Perhaps under certain conditions either result might be reached. Is it worth while to solve a problem of this sort? How long will it require to get perfect evidence bearing on these questions? Let those who think that a few scientific experiments can revolutionize oyster culture in a short time, reflect.

Julius Nelson (1892)

INTRODUCTION

The study of oyster larvae is entering its fourth century. Stafford (1913) reported that the eggs and larvae of the European flat oyster, *Ostrea edulis L.*, were first examined under the microscope by Brach in 1690, and that Leeuwenhoek in 1695 described sperm of *O. edulis* and the larval swimming organ he called the velum. In North America, intensive study of the eastern oyster, *Crassostrea virginica* (Gmelin), began with experiments by Brooks (1879) and Ryder (1881) on development and culture of oysters from Chesapeake Bay. Much has since been learned but much remains to be accomplished. However, the future of larvae-centered research appears bright because of renewed interest in recruitment of marine and estuarine organisms and because technological advances promise to provide new ways to answer unsolved questions.

By way of introduction to this chapter, the early life history of the eastern oyster, which involves a number of developmental stages and associated agents of mortality (Fig. 1), can be summarized briefly as follows. Adult eastern oysters are most common in estuaries, where they reproduce by releasing gametes into the water column at salinities above 5 to 10 ppt. External fertilization occurs, and a non-feeding trochophore larva develops. Subsequent larval stages are planktotrophic, grazing on phytoplankton, detritus, and bacteria and growing over a period of 2 to 3 weeks. Initially the larva (D-stage) has a straight hinge, which becomes modified after a few days as an umbo develops. During its pelagic existence, the larva is distributed by water movements. Larvae are subject to mortality from predation and disease, and possibly as a result of reduced viability due to poor food supply (quantity or "quality") or poor condition of spawning females that leads to reduced lipid content in eggs. Temperature and perhaps food supply affect the length of the larval period, with any increased time spent in the water column undoubtedly leading to decreased survival because of increased exposure to predators and disease (Underwood and Fairweather 1989).

Larvae that survive in the plankton eventually attain the benthic settling stage (late-stage, eyed-stage) during which they spend time crawling on hard surfaces as pediveligers, apparently sensing cues associat-

ed with the substrate, called "cultch."[1] If the appropriate stimuli occur, a larva cements its left valve to the cultch and metamorphoses, whereupon it is called a "spat."[2] Over time, and in the absence of physical disturbance and extensive harvest by humans, large reefs (also called "rocks," beds, bars) comprised of shells of oysters are built up into the surrounding water column where currents carry food over the reef and transport waste and sediment away. These shells form the primary settlement material for subsequent generations of eastern oysters in sediment-dominated estuaries.

Numerous factors influence the ultimate recruitment of oysters to the benthos (Fig. 1). Production of gametes depends on appropriate environmental cues to stimulate spawning. Until they attach to cultch, the microscopic larvae are subject to the vagaries of the pelagic environment, including suitability of physical conditions (e.g., temperature, salinity, dissolved oxygen, suspended particles), appropriateness of available food, and presence of disease organisms and predators. Movement of larvae occurs in

[1,2] "Cultch" and "spat" are terms that were in use in England at least by 1669 (Stafford 1913).

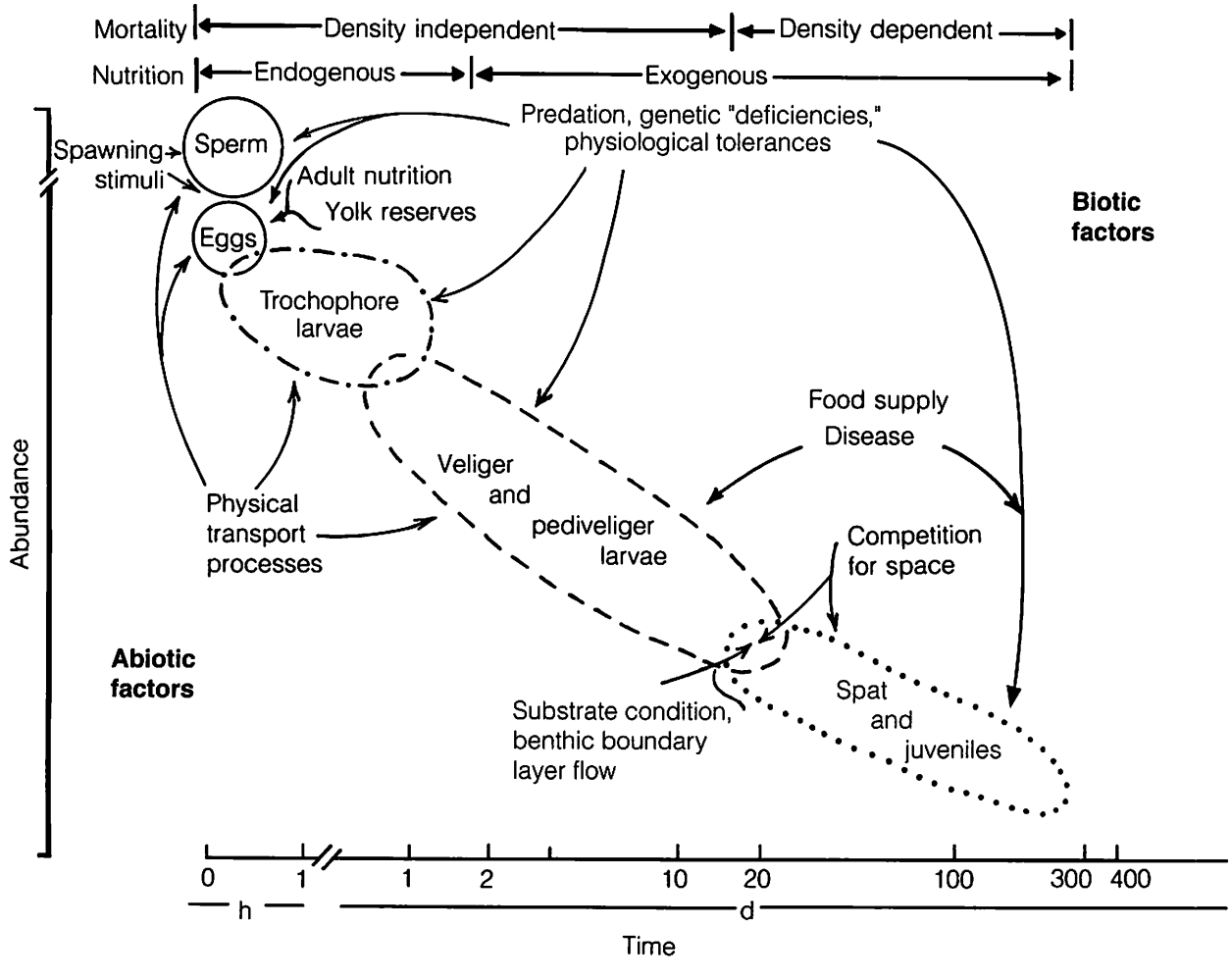

Figure 1. Conceptual diagram of recruitment to the benthos and first-year survival in the eastern oyster, highlighting biotic and abiotic factors that influence mortality of early life history stages. Log_{10} scale is used on the time axis. Adapted from a concept in Houde (1987) and insights in Rumrill (1990).

what is to them a relatively viscous medium, and one that is often moving at speeds beyond the fastest swimming speeds that they can generate. Settlement and metamorphosis are triggered by physical and chemical cues, with survival of young oysters affected by the presence and quality of cultch and by associated benthic predators and competitors for space.

Clearly, comprehension of the early life history of the eastern oyster requires a broad understanding of the aquatic milieu in which it lives as a pelagic larva and as a benthic juvenile, and of the biological interactions that affect its survival and recruitment to the benthos. However, our knowledge of these matters is still limited in many ways, in spite of the time and effort devoted by numerous scientists over the years.

In this chapter, I will focus on the biology, behavior, and ecology of eastern oyster larvae and spat. I begin with an overview of the estuarine habitat, emphasizing those macroscale and microscale physical factors that influence distributional patterns of larvae. Then I describe our knowledge of larval swimming behavior and larval dispersal in nature. This is followed by information on settlement behavior and factors influencing survival of larvae and spat. Supplementary information can be found in other chapters in this book that consider aspects of eastern oyster anatomy (Eble, Chapter 2), shell (Carriker, Chapter 3), feeding (Newell and Langdon, Chapter 5), digestion (Langdon and Newell, Chapter 6), reproduction and larval development (Thompson et al., Chapter 9), responses to natural environmental factors (Shumway, Chapter 13), predation (White and Wilson, Chapter 16), and commercial culture (Castagna, Chapter 19).

THE ESTUARINE HABITAT

Oyster larvae inhabit a dynamic and complex environment in which they are subject to macroscale and microscale physical forces. On the macroscale level, they are relatively weak swimmers in a water column within which water may be moving in different horizontal and vertical directions. Thus, maximum distribution of larvae and their survival to settlement will be governed by the length of their pelagic existence and the rate and direction of transporting currents (Scheltema 1986). On the microscale level, because of their small size, larvae exist in a viscous environment that influences their ability to swim and capture food.

An understanding of the pelagic existence and distribution of oyster larvae thus requires a familiarity with the physics of estuarine water circulation and mixing, as well as an understanding of the behavior of objects in viscous fluids. What follows then is a brief description of these important factors, set in the context of estuarine structure. The description can be supplemented by reference to the fuller accounts of Purcell (1977), Vogel (1981), Scheltema (1986), Okubo (1994), and relevant chapters in Mann and Lazier (1991).

Classes of Estuarine Structure

Pritchard (1967) defined an estuary as a semi-enclosed coastal body of water with a free connection to the sea and within which seawater is measurably diluted by freshwater from land drainage. Geomorphologically, estuaries can be classified into four categories (Pritchard 1967), two of which (drowned river valleys, bar-built estuaries) are prime eastern oyster habitat. Drowned river valleys are the predominant type in the mid-Atlantic coastal plain of the U.S. coast. Examples include the Delaware and Chesapeake Bays. Bar-built estuaries are separated from the ocean by barrier islands and sand spits that have one or more inlets that are small relative to the estuary behind the barrier; as a consequence, tidal action is much reduced. Examples include coastal New Jersey estuaries, Albemarle and Pamlico Sounds in North Carolina, and many estuaries in the Gulf of Mexico.

Circulation within an estuary represents the integrated hydrodynamic response to a variety of near-field and farfield forcings, including freshwater runoff, tidal forces, wind stress, and long wave propagation from regions distant from the estuary. Consequently, in addition to a classification based on geomorphological criteria, estuaries can be classified broadly according to hydrodynamic criteria that are relevant to an understanding of transport or retention of oyster larvae (Pritchard 1967, 1989; Biggs and Cronin 1981).

Continuing research is revealing the inherent complexity of estuarine circulation patterns, but for our purposes we can consider a simplified hydrodynamic scheme (see Biggs and Cronin [1981] and Mann and Lazier [1991] for more detail): (1) strongly stratified, salt-wedge, or Type A estuaries in which freshwater inflow predominates and lighter fresh water flows out over denser salt water. There is a steep density gradient at the interface of the two water layers, flow is dominated by density currents, and limited mixing occurs as a result of breaking internal waves (e.g., Southwest Pass in the lower Mississippi River). (2) Partially stratified, Type B estuaries in which the vertical density gradient remains pronounced, but in which moderate tidal action prevents the river flow from dominating the circulation by providing energy for turbulent mixing of fresh and salt waters (e.g., mainstem Chesapeake Bay and some of its tributaries, such as the James River; Pamlico Sound; Long Island Sound). These estuaries usually serve as excellent eastern oyster habitat. (3) Well mixed or vertically homogeneous estuaries (Type C) within which tidal action dominates the mixing process and provides enough energy to reduce any vertical salinity gradient (i.e., salinity is usually homogeneous from surface to bottom). Such estuaries (e.g., lower Delaware Bay; North Inlet, South Carolina) can provide good to excellent eastern oyster habitat.

The classification scheme just described can be useful for a general understanding of types of oyster habitat, but not all estuaries fall neatly into broad categories (Pritchard 1989). Indeed, tributary estuaries or subestuaries often exhibit marked departures from the proposed sequences of categories, at least partly because the geomorphology of an estuary interacts with its hydrodynamics to produce a circulation unique to that water body. In Maryland's portion of Chesapeake Bay, for example, there are patterns such as the three-layered circulation of Baltimore Harbor, which is a deep tributary with a small freshwater drainage system (Pritchard 1989), or the upstream two-layered and downstream three-layered flow patterns of the Patuxent River (Carter and Pritchard 1988; Pritchard 1989) and the Tred Avon River (Boicourt 1982). Even within tributaries of major estuarine systems, circulation patterns can vary greatly over time. For example, Elliott (1976) recorded as many as six circulation modes in the partially mixed Potomac River estuary of Chesapeake Bay over the course of a year (Fig. 2). These modes were recorded for vertical-longitudinal components of the Potomac River's circulation. Lateral variability adds to the system's complexity.

As a consequence of this variability in circulation patterns, estuaries that have been studied by investigators interested in distributional patterns of oyster

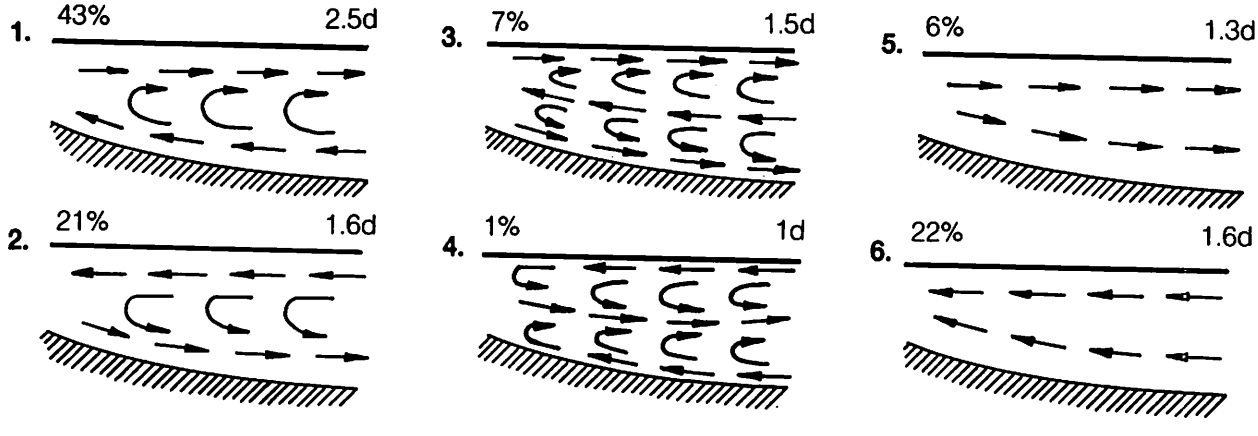

Figure 2. Schematic depictions of six circulation modes recorded in the partially mixed Potomac River estuary over the course of a year (Elliott 1976). Percentage occurrence and average duration (d = days) are shown for each mode. Modified after Day et al. (1989).

larvae have often differed widely in physical characteristics and hydrographic regime (Andrews 1979). They include the shallow, still, clear lake-like estuaries of Bras d'Or, Nova Scotia; deep, turbulent, open waters of Long Island Sound; shallow, stratified bar-built lagoons on New Jersey's coast; muddy, tidal river estuaries such as Delaware Bay and the James River in Chesapeake Bay; and trap-type low-flushing tributaries such as the St. Mary's, Great Wicomico, and Piankatank rivers of Chesapeake Bay. The different flushing, tidal, stratification, and wind regimes of these regions, in concert with basin geometry, may strongly influence larval abundance and distribution and thus make comparisons among field studies difficult.

Physical Factors Influencing Larvae

Water movement occurs on a variety of scales. At the large end of the scale are geophysical flows that influence horizontal (and perhaps vertical) larval transport. At the small end of the scale, viscous forces affect larval swimming, feeding, and settlement. I now provide a brief summary of the salient macroscale physical factors (circulation, mixing, fronts) that influence estuarine water movement, and therefore transport of larvae, and follow with discussions of microscale physical factors that affect swimming by larvae and of the benthic boundary layer that larvae encounter as they settle on hard surfaces.

Macroscale Physical Factors

Circulation. Estuarine water circulation is a dynamic physical process that is influenced by four main driving forces (Fischer et al. 1979): gravitational circulation (a result of freshwater input); tidal circulation; wind-driven, locally forced circulation; and meteorologically-influenced, remotely forced circulation (the latter two types being perhaps the most variable of the four circulations). A given estuary may be dominated by one or more of these circulation types at any given time, and the combination of circulations that predominates may change as time passes (e.g., when a wet season is followed by a dry season, or when spring snowmelt produces high runoff in a watershed). Gales or hurricanes may pass over an estuary and cause destratification or, if offshore, cause coastal sea water to be forced into an adjacent estuary. Neap tide-spring tide variations in tidal height can weaken or destroy vertical stratification, such as in some subestuaries of the western shore of Virginia's Chesapeake Bay (Haas 1977).

Gravitational circulation, the first circulation type, is driven by pressure gradients induced by differences in density and elevation between inflowing salt water and freshwater runoff in the estuarine mixing bowl (Mann and Lazier 1991). Less dense fresh water overrides denser salt water, pressure surfaces tilt seaward in the fresh surface layer, and there is a net outflow of water to the sea. Contrarily, the pressure surfaces of the saltier bottom water (the "salt wedge") tilt up-estuary and salt water flows toward the land. At some point in the water column the pressure surfaces become horizontal, gravitational forces are constant, and the net flow or circulation ceases. This region of the estuarine water column is the "level of no net motion," usually found above mid-depth. Obviously, the distribution of particles will be influenced by their position in the water column.

Estuaries in which less dense water rides over denser water exhibit the two-layer "classical" circulation. The friction between the salt wedge and the overlying fresher water causes upward transport, or advection, of salt water from the breaking of interfacial waves in the water column. This transport is enhanced by the additional energy of tidal action. Gravitational circulation may also be driven by temperature, but this is uncommon in temperate estuaries, except perhaps in shallow tributaries with limited input of low salinity water.

Tidal circulation (or tidal "pumping"), the second circulation type, operates independently of density gradients and wind stress and is most notable in shallow estuaries with a large tidal range. This circulation type coexists with gravitational circulation in many estuaries, especially those with moderate or high freshwater input and a tidal range of about 2 m or higher. The interaction of tidal currents and bathymetry results in complexity in strength and duration of ebb and flood. Turbulent mixing occurs as friction is generated by tidal flow over the bottom or by tidally enhanced shear at the fresh-saltwater interface.

The third circulation type — wind-driven or locally forced circulation — can dominate in regions with extensive open water and shallows, and with limited freshwater input and tidal range. The high variability (hours to weeks) of wind velocity and direction over the estuary surface obviously influences this type of circulation. It is clear that gravitational circulation can be enhanced by unchanging wind blowing downstream along the course of an estuary, with the result that particles in the surface or bottom layers may be moved greater distances than if there were no wind. On the other hand, steady upstream winds may reverse the net surface current so that it flows upstream, with a resultant diminished net bottom-flow upstream and perhaps a consequent midlayer of ebbing water moving down-stream between the surface and bottom layers. Boicourt et al. (1996) have shown how wind events can reverse water flow in a shallow, broad subestuary (Broad Creek, Maryland) in Chesapeake Bay while having little or no effect on a closely adjacent, narrower, sinuous subestuary (Tred Avon River). Under constant wind conditions, the transport direction and speed of particles in these subestuaries would therefore depend on their position in the water column and the shape of the estuary.

Remotely forced circulation, the fourth type, is similar to the third type, except that local water motion is influenced by meteorological forces acting on water bodies outside the local region. At the remote location, atmospheric pressure gradients or wind stress cause water level changes that propagate as waves to drive circulations some distance removed from that location. For example, Chesapeake Bay tributaries are influenced by a meteorologically based, quarter-wave seiche that operates in the mainstem of the Bay (Chuang and Boicourt 1989). Remote wind forcing may also act to drive additional salt water into the mouth of an estuary (Cannon et al. 1990; Sanford and Boicourt 1990).

Mixing. Because estuaries are regions where salt and fresh waters mix, suspended particles may also be mixed throughout the water column. Thus, an understanding of mixing processes is needed in any consideration of larval distributions. Mixing, redistribution, or dispersal of larvae within water masses may result from advection (transport by a current, with resultant movement of the center of the mass of larvae) or dispersion (spatial separation of larvae from each other) (Fig. 3A). Dispersion of larvae is accomplished by various means including small-scale eddies and rapid changes in the velocity field (shear). Eddy diffusion or random turbulence, which occurs over periods shorter than a few minutes, also causes particle dispersion (or "diffusive transport").

Estuarine mixing is effected by interactions between tidal currents and physical boundaries (tidal forcing) and by winds, non-tidal waves (usually caused by wind events), and river input. The varying influences of these energy sources affect advection and dispersion of suspended material, including larvae, providing a challenge to those wishing to predict larval dispersal patterns in estuaries. In addition, the fact that residual circulation moves water in a certain direction is no guarantee that particulate materials will also move in that direction (Day et al. 1989). Comprehension of natural patterns of larval distribution will depend on deeper insight into transport and mixing processes in estuaries, as well as into larval behavior.

Fronts. Fronts delineate the convergence of dissimilar masses of water and are sites of laterally convergent flow and strong vertical water movement (Mann and Lazier 1991; Franks 1992). They often

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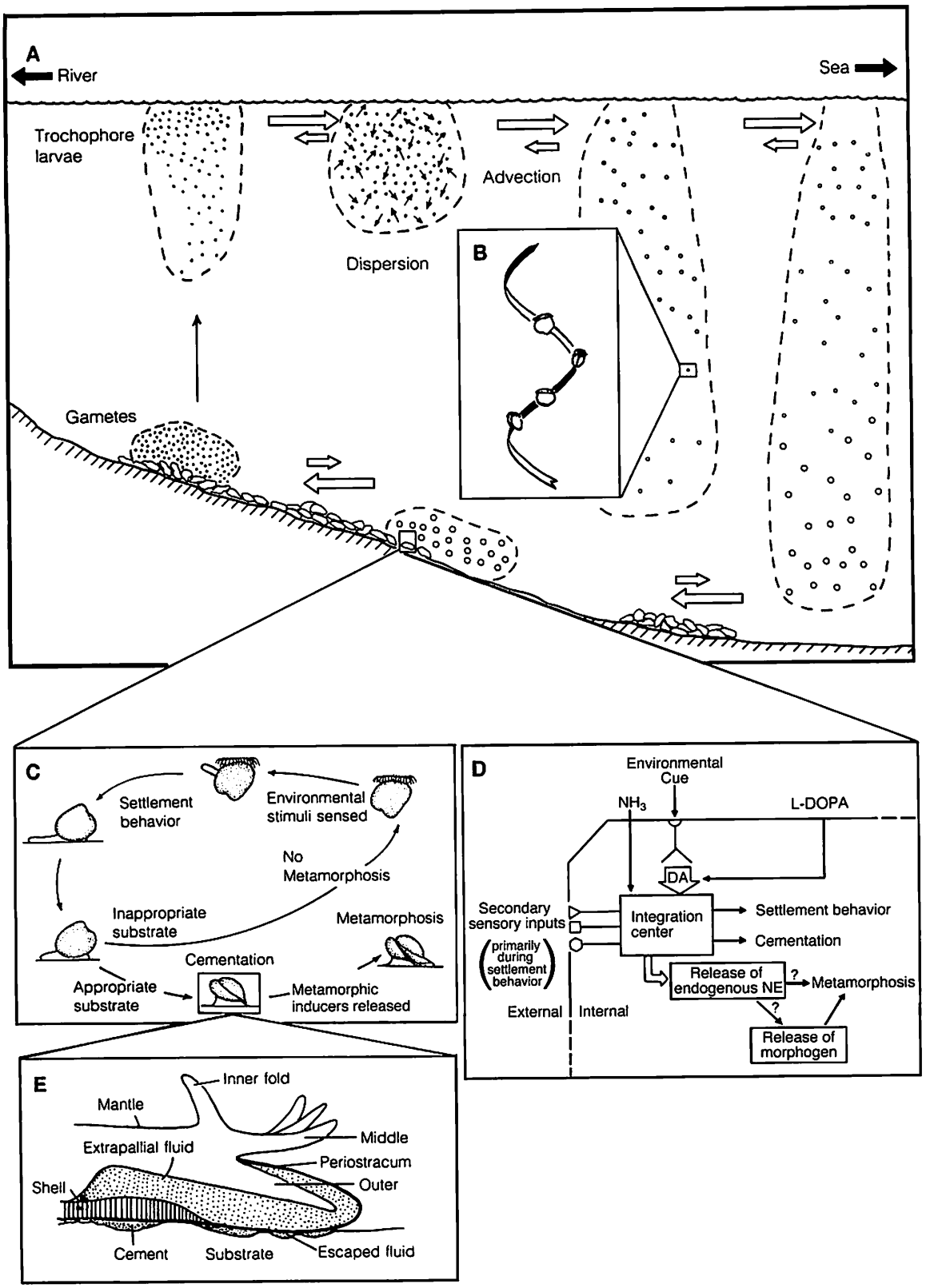
Figure 3 (opposite page). (A) Diagram of hypothetical distribution of gametes and larvae by dispersion (small closed arrows) and tidal advection (large, open arrows: upper, ebb tide; lower, flood tide) in a Type B estuary. The various locations of the larval "clouds" illustrate the hypothesis of estuarine transport and retention, i.e., down-estuary initially, then up-estuary as larvae grow and spend more time near the bottom. (Note that there is no evidence to date that larvae from one spawning event remain together in "clouds" or that larvae settle on or near their natal bed.) In the diagram, moderate mortality is represented and size of circles codes roughly for larval size. (B) Diagram of helical swimming path of an ascending veliger. Modified from Cragg (1980). (C) Diagrammatic model for natural settlement and metamorphosis of Pacific oys-



ter, *C. gigas*, larvae. Modified from Coon et al. (1985). (D) Schematic representation of the parallel dopaminergic and adrenergic pathways hypothesized to function during settlement and metamorphosis in *C. gigas* larvae. Modified from Coon et al. (1990a). DA [dopamine]; NE [norepinephrine]; L-DOPA [L-3,4-dihydroxyphenylalanine]. (E) Diagram of the mechanism of cementation in ostreid bivalves. Modified from Harper (1991).

contain accumulated biomass (Franks 1992), a fact long known to observers. For example, Nelson (1913) noted that tidal slicks (quiet ripple-free strips of surface water) in New Jersey and Connecticut waters often contained higher concentrations of bivalve larvae than were found a short distance away.

In estuaries, such fronts develop from contact between water masses of two different salinities, the resulting pycnocline curving to intersect the estuarine surface or bottom. For example, frontal systems develop on flood tide in the James River, Virginia, where Mann (1988) found evidence that bivalve larvae, including oyster larvae, were entrained in upstream-flowing high salinity water that moved through the frontal system before plunging toward the estuary bottom below the downstream-flowing fresher water.

In conclusion, it is clear that a change from one circulation mode to another can result from a variety of environmental factors, which also influence mixing and formation of fronts. In addition, currents in surface waters may not be synchronized with those in bottom waters. Given these complexities, the prediction of larval transport in estuaries is complicated, with more data needed on circulation in conjunction with collections of oyster larvae in the field. Clearly, interpretation of the results of any larval sampling program will depend on adequate knowledge of the estuary under study.

Microscale Physical Factors

Before one considers the behavior of larvae, one must understand the microscale features of the water column they inhabit. A complete discussion can be found in Vogel (1994), with additional insights in Purcell (1977) and Mann and Lazier (1991).

Briefly, oyster larvae inhabit a world of very low Reynolds number — a world of "creeping motion" — in which viscous forces (dynamic viscosity and kinematic viscosity) dominate. The dynamic viscosity of a fluid can be envisioned as the interlaminar stickiness or friction between very thin sheets of the fluid sliding over each other. It is strongly affected by temperature, with salinity having a much smaller effect. The character of a fluid flow depends more directly on the kinematic viscosity, which is the ratio of the dynamic viscosity to the fluid's density. Kinematic viscosity reflects the ease with which the fluid flows or forms vortices, and is also strongly affected by temperature. Dynamic and kinematic viscosity are also called molecular viscosity (the internal resistance of the fluid), and should not be confused with the larger-scale eddy viscosity familiar to oceanographers.

In addition to the viscous forces discussed above, inertial forces within a fluid affect an object's motion. Because a solid object in motion possesses the characteristics of dimensionality and velocity, it is subject to inertial forces, i.e., the object will continue to move in a given direction until drag overcomes the inertia and slows the object to a stop. Thus, viscous forces act to retard motion whereas inertial forces tend to maintain motion. For example, compare the almost instantaneous cessation of motion when stirring (the imparting of inertial forces) stops in a beaker of chilled molasses (viscosity >> inertia) with the more gradual slowing of flow when stirring stops in a beaker of hot water (inertia >> viscosity).

Different flow regimes can be characterized by the relative importance of inertial and viscous forces. The ratio of inertial forces to viscous forces is the (dimensionless) Reynolds number, which is lower in (say) chilled molasses than in (say) hot water. Reynolds number (Re) is given by the equation:

$$\mathrm{Re} = \frac{\text{fluid density} \times \text{velocity} \times \text{object's linear dimension}}{\text{fluid viscosity}}$$

As the equation indicates, Reynolds number is influenced by an object's size. Vogel (1994) estimated Reynolds numbers to be about 3×10^8 for a large whale swimming at 10 m s^{-1}, 3×10^2 for a copepod "hopping" at 20 cm s^{-1}, and 3×10^{-1} for a 0.3 mm-long invertebrate larva travelling at 1 mm s^{-1}. Consequently, although the whale and the larva may occur in the same water body, the whale's world is dominated by inertial forces and viscosity is of no consequence whereas the larva's world is dominated by viscous forces and inertia is of no consequence. At the speed at which larvae swim, cessation of swimming results in an almost instantaneous stoppage of forward progress because such progress depends entirely on the swimming forces at that moment and not on forces that acted in the past.

At the low Reynolds numbers of the oyster larval world, the presence of a solid surface nearby — the "wall effect" — can greatly affect flow patterns and forces experienced by larvae. To avoid this effect, the ratio between the distance of the larva from the surface and the diameter of the larva must increase as Reynolds number decreases. Such wall effects should be taken into account in laboratory studies of larval swimming. Vogel (1994) offers the rule of thumb that wall effects will be negligible if y/l >20/Re, where (y) is the distance to the nearest wall and (l) is an object's "characteristic" length, i.e., the greatest length in the direction of flow. Assume an oyster veliger of 250 μm shell length is moving at 1 mm s^{-1} (Re = 0.3) and that its greatest length in the direction of flow is its shell height of 150 μm. Thus, a chamber wall should be about 10 mm away to minimize wall effects. Faster moving larvae would be subject to higher Reynolds numbers, and thus would be less subject to wall effects.

Boundary Layers

One final attribute of the physical environment of oyster larvae remains to be explored here, namely the boundary layer and its flow dynamics (Mann and Lazier 1991). The boundary layer is a region near a surface (e.g., the estuary bottom) where viscous forces result in diminished flow, with speed of flow decreasing to zero at the surface. The lower the Reynolds number, the thicker the boundary layer, with thickness defined as the distance from the surface to a point at which local velocity equals 99% of the "free-stream" or non-affected velocity (Vogel 1994).

In laminar flow at low Reynolds number, fluid particles move roughly parallel to each other in smooth paths as if "layers" of fluid were slipping easily across one another. In general, laminar flow is experienced by tiny, slow-moving organisms. Larger fast-moving organisms experience turbulent flow, in which fluid particles move erratically even if the fluid as a whole is travelling in one direction. Increased fluid speed, increased fluid density, or decreased fluid viscosity will abruptly shift flow from laminar to turbulent.

Laminar boundary layers apply only to flows at very low Reynolds numbers such as are found around microorganisms. Almost all other boundary layers in nature are turbulent. There are two types of turbulent flow. Smooth turbulent flow occurs where there is fully-developed, uniform, steady flow over hydraulically smooth surfaces. A sublayer develops in which viscous flow dominates, with the occasional disruptions from the intrusion of overlying turbulent fluid from the "logarithmic" or inertial layer. Rough turbulent boundary layers occur as increased roughness of the bottom material or increased flow velocity leads to the eventual loss of the viscous sublayer and establishment of hydraulically rough flow. Complicating the pattern, the presence of large obstacles (e.g., oyster shells) produces accelerated and decelerated flow over and around these obstacles. These aspects of benthic flow environments are described more fully by Nowell and Jumars (1984) and Butman (1987).

Flow environments can influence three aspects of oyster life history — delivery of larvae to the attachment surface, maintenance of position during and after settlement, and subsequent survival and growth (Nowell and Jumars 1984). Delivery of larvae to a potential attachment surface is undoubtedly regulated or strongly influenced by boundary-layer flow dynamics, but the responses of oyster larvae to such boundary layers have not been delineated. Once attached, the oyster is subject to local flow forces of varying magnitude and direction. Such forces may dislodge newly attached spat or carry suspended sediment particles that might cause damage by abrasion (Shelbourne 1957). Vertical and horizontal delivery of food particles to all ages of attached bivalves is also dependent on near-bottom flux but this subject remains to be explored for eastern oysters (see Newell and Langdon, Chapter 5).

LARVAL MOVEMENT

Larval Swimming

Ciliary Propulsion. Because movement at low Reynolds numbers is hampered by viscous forces, there are few mechanisms available to microscopic swimming organisms to produce movement. A "flexible oar" is required because a solid oar would just move back and forth while imparting no net motion

(Purcell 1977). Flagella and cilia serve as such flexible oars. Organisms like oyster larvae that use cilia for propulsion tend to be larger than those that use flagella (Sleigh and Blake 1977).

The ciliary beat is two-phase, being effected through alternate power and recovery strokes — the beat cycle (Sleigh and Blake 1977; Vogel 1994). The power stroke starts with the relatively straight cilium extended vertically, then bending basally so that it moves stiffly and normal to its long axis in an arc towards the body surface (Fig. 4). The recovery stroke has the cilium flexed, with the region of bending now being propagated from the base to the tip, causing the cilium to move parallel to its long axis. Its tip remains near the body surface as it approaches the cilium's base and then rises to the vertical position, whereupon the power stroke resumes. The force exerted on the surrounding water in the power stroke is twice as great as in the recovery stroke (Sleigh 1989). The extent of the "water zone" carried by the power stroke exceeds that carried by the recovery stroke because of the variation of the cilium's height above the cell surface during the beat cycle. Consequently, the cilium scoops much more water to one side during the power stroke than is returned to the other side by the recovery stroke. There is no continual flow because viscous forces dominate over inertial forces (Sleigh 1989). The beating of cilia is coordinated in a regular pattern of metachronal waves that sweep across the body surface. The pattern of metachronal waves on the velum of molluscan larvae is described as laeoplectic, with the recovery stroke moving clockwise (seen from above) and the power stroke occurring to the left of the line of metachronal wave propagation (Knight-Jones 1954).

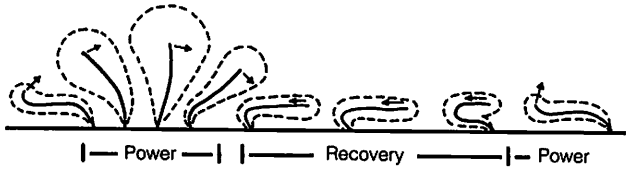


Figure 4. Diagrammatic representation of the beat cycle (power stroke and recovery stroke) of a typical cilium, and the associated "capture zone" of water (- - -). Note the difference in amount of water carried to the right in the power stroke compared to that returned to the left in the recovery stroke. Redrawn from Sleigh (1989).

Sleigh and Blake (1977) note that the propulsive efficiency of ciliated organisms varies with the ratio of body length to the multiple of number of cilia times cilia length. The most suitable length for ciliated organisms ranges from 20 to 350 μm (Sleigh and Blake 1977), a range that includes oyster larvae. The actual propulsive efficiencies for oyster larvae have not been determined.

Swimming of Bivalve Larvae. Development of the prototroch, or ciliated crown, on the ventral side of the newly formed trochophore larva allows for sporadic and disorganized movement (Galtsoff 1964). About 15 to 20 min after the prototroch forms, rotation around the larva's dorsoventral axis begins, with the prototroch directed forward and toward the water surface. The oval, ciliated velum or swimming organ develops from the prototroch within a day or two. The role of this specialized and rapidly maneuvered structure in swimming and feeding is described by Galtsoff (1964) and Elston (1980) for *C. virginica* (see also Newell and Langdon, Chapter 5), with additional details provided for other bivalves by Strathmann and Leise (1979), Cragg and Gruffydd (1975), Cragg (1980, 1989), and Waller (1981).

After the formation of the larval shell and the ciliated velum (Cragg 1980), the negatively buoyant larvae swim vertically up or down along a cylindrically helical path (Fig. 3B) assisted by the beating of their large marginal (preoral) cirri, or aggregated cilia. (The aggregation of cilia into a cirrus allows for an increase in swimming velocity, a cirrus being a more effective propulsion structure than a cilium; Cragg 1989.) The pitch and diameter of the helix are variable in the blue mussel, *Mytilus edulis,* (Bayne 1963), *O. edulis* (Cragg and Gruffydd 1975), the scallop, *Pecten maximus* (Cragg 1980), and also in *C. virginica* (pers. obs.). Hidu and Haskin (1978) observed that eastern oyster larvae usually swim up or down in a "loose" clockwise spiral, with maintenance of position being associated with a slow spiral movement. Measurements of the pitch and helix diameter have yet to be reported for *C. virginica.*

Larval *O. edulis* (Cragg and Gruffydd 1975) and *P. maximus* (Cragg 1980) rotate about their dorsoventral axis such that one valve continuously faces the center of the helix as the larva spirals upward, usually in a clockwise direction when seen from above. Jonsson et al. (1991) report a similar helical swimming behavior for larval cockles, *Cerastoderma edule*, but do not state if the larvae continuously face inward. Downward movement in bivalve larvae may result from retraction of the velum and shell closure with consequent rapid sinking, or may occur more slowly with the velum extended and the cilia beating or still (*O. edulis*, Cragg and Gruffydd 1975; *C. virginica*, Hidu and Haskin 1978; *P. maximus*, Cragg 1980; *C. edule*, Jonsson et al. 1991). Larvae may also swim downward actively. Horizontal movement is less common and is accomplished in the eastern oyster by vertical helical swimming in an oblique direction that carries the larvae sideways as well as upward or downward (pers. obs.).

In laboratory beakers, eastern oyster larvae will swim up or down at different speeds. Nelson (1903) noted a common phenomenon in such containers, where streams or columns of larvae, often occurring in tumbling clumps, can be seen descending from the surface to the bottom (perhaps similar to swimming-driven convection patterns of algal cells; Kessler 1986). Nelson and Perkins (1931) reported that clumped groups of up to a dozen eyed larvae were often observed near the water surface of a container, with the larvae striking the tips of their velar cilia against those of their neighbors for varying periods of time. The explanations for these behaviors are not clear, nor is it known if they occur in nature.

Behavior changes as bivalve larvae grow. Laboratory observations reveal that most trochophores congregate near the top of their container, with greater dispersal throughout the water column with age (e.g., *P. maximus*, Cragg 1980; *C. gigas*, Wang and Xu 1989). The oldest larvae remain near the container bottom, either swimming about 1 mm above it or crawling on it when they develop into pediveligers. Nelson and Perkins (1931) reported that eyed larvae of eastern oysters displayed limited swimming activity in still water in the laboratory; most rested on the bottom with their umbo downward and with their velar cilia beating gently. If a current was established or if a larva was pulled through the water while attached to a mucous thread, the larva's velum became extended, the velar cilia beat faster, and the larva rose from the bottom. When the current stopped, some larvae dropped to the bottom immediately, with most of the rest returning to the bottom within 10 to 20 min. Introduction of higher salinity water in conjunction with a current caused velar activity to continue for a longer period and more larvae to continue swimming than occurred in the absence of higher salinity. Introduction of lower salinity in conjunction with a current slowed activity and minimized swimming.

Swimming Speeds. Vertical swimming speeds have been measured for some bivalve species (Table 1). The speeds represent the net rate of vertical movement, or vertical velocity (influenced as it is by the variability of the diameter and pitch of the helix), rather than the true or instantaneous linear velocity at which a larva swims along its spiral path (Cragg 1980). Of all the species listed in Table 1, eastern oyster larvae are, with few exceptions, the fastest swimmers at their usual environmental temperature. Their swimming speeds are comparable to the range of 0.7 to 2 mm s^{-1} reported by Mileikovsky (1973) for marine invertebrate larvae. These swimming speeds are sufficient to overcome most vertical water movement in the estuarine water column, but are about 10 to 100 times slower than the usual speeds of horizontal water movement.

Swimming speeds of eastern oyster larvae are influenced by larval size. With a three-fold increase in larval length (from about 90 μm to 275 μm) during development, vertical swimming speed up or down can increase about three times (Hidu and Haskin 1978). Swimming speeds may be affected by various environmental variables, although data are limited. Hidu and Haskin (1978) observed that higher temperatures resulted in higher swimming speeds. Mann and Rainer (1990) found that larvae continued to swim under conditions of lowered oxygen concentration (20 to 25 min experiments), with significantly slower swimming rates (compared with 100% oxygen saturation) occurring only for 118 μm larvae at

Table 1. Estimates of vertical velocity (upward swimming) of bivalve larvae measured in the laboratory at normal atmospheric pressure and habitat salinity. Estimates are usually for a range of larval sizes.

Species	Velocity (mm s^{-1})	Temperature (°C)	Reference
Crassostrea virginica	0.8 to 2.3 (means)[a]	25	Hidu and Haskin 1978
Crassostrea virginica	1.0 to 3.1 (means)[a]	22	Mann and Rainer 1990
Arctica islandica	0.3 to 0.4	12	Mann and Wolf 1983
Cerastoderma edule	0.7 (mean)	15?	Jonsson et al. 1991
Lyrodus pedicellatus	7.7 (mean)	20-28?	Isham and Tierney 1953
Mercenaria mercenaria	1.2 to 1.3	?	Turner and George 1955
Mulinia lateralis	0.3 to 0.5	23	Mann et al. 1991
Mytilus edulis	1.1 (mean)	?	Konstantinova 1966
Ostrea edulis	1.2 (mean)	?	Cragg and Gruffydd 1975
Pecten maximus	0.3, 1.4 (means)[b]	14	Cragg 1980
Rangia cuneata	0.4 to 0.5	25	Mann et al. 1991
Spisula solidissima	0.3 to 0.4	23	Mann et al. 1991

[a] Means are for four (Mann and Rainer 1990) or five (Hidu and Haskin 1978) different size classes.
[b] Means are for 30-h and 33-d old larvae.

10% saturation and 290 μm pediveligers at 21% saturation. The effects of environmental variables and ontogenetic changes on swimming of eastern oyster larvae needs further attention. In addition, the hydrodynamic disturbances produced by swimming may be significant in feeding and predator avoidance (Gallager 1993); this topic has not been explored for oyster larvae.

LARVAL DISPERSAL

Dispersal of invertebrate larvae has attracted renewed interest (Levin 1990). Oysters, being prolific spawners that live in semi-enclosed habitats, would make excellent objects of study if their larvae (especially the younger stages) could be easily and rapidly identified in nature or in samples. Mass spawning is probably the norm over most of the spawning season, with eggs and sperm discharged in streams that may be produced for a few seconds or up to an hour or more, depending on the readiness or responsiveness of the spawning individuals (when the end of the spawning season approaches, males may spawn for several hours until spent; Carriker 1986). The water over a bar of spawning oysters may turn milky with expelled gametes (Galtsoff 1964; Quayle 1988). As the tide ebbs and flows, the larval aggregates form into lanes along the tidal axis and the milkiness disappears. The planktonic character of oyster larvae, their residence in the water column for up to three weeks depending on temperature and food conditions, and their small size and relatively weak swimming ability mean that the larvae will probably be distributed away from the parental bar to a presently unknown extent. Greater understanding of their dispersal, whether active or passive, has been difficult to obtain, mainly because of size- and sampling-related problems.

Tracking Methods

It has not yet been possible to follow a brood of larval oysters from fertilization to settlement, for three reasons. First, most zooplanktonic studies tend to focus on organisms (e.g., copepods, fish larvae) that are much larger than bivalve larvae. Thus, mesh sizes in these plankton samplers are often too large to retain bivalve larvae so data on their abundances are limited. Second, early straight-hinge larvae of many bivalves, including oysters, are externally indistinguishable, hindering their ready recognition in plankton samples until development of features such as a characteristic

umbo. Third, older oyster larvae may be relatively scarce, making quantitative sampling difficult and unreliable (e.g., Nelson 1910; Galtsoff 1964). For example, Pritchard (1953) calculated that the large commercial sets of oysters (the equivalent of 40 spat per shellface per week on suspended cultch) in the James River, Virginia, required a density of only about one late-stage larva per 100 L of river water. Thus, large quantities of water must be filtered to provide reliable samples.

Use of the shell hinge (provinculum) with its species- or genera-specific arrangement of teeth has been shown to be a suitable aid for the identification of bivalve larvae (Lutz et al. 1982). The arrangement of the eastern oyster's hinge teeth has been described by Carriker and Palmer (1979), and Carriker (see Chapter 3) has expanded upon these matters in greater detail. Hu et al. (1993) display a comprehensive sequence of scanning electron micrographs delineating ontogenetic changes in larvae and post-larvae of four ostreid species, including *C. virginia.*

Although examination of hinge teeth can result in reliable identification of species, the sorting of larvae from many samples and their subsequent preparation for inspection of hinge teeth is tedious and time consuming. New technologies are needed to facilitate these tasks. In addition, in studies such as that of Wood and Hargis (1971), larvae were not actively tracked, but were sampled as they moved past static platforms (ships). Clearly, there is no guarantee that a single larval cohort can be followed in this fashion unless some sort of natural or artificial tag is available.

To that end, Levin (1990) reviewed methods used to mark marine invertebrate larvae and monitor their dispersal. She noted that attempts to track bivalve larvae stained with neutral red (Loosanoff and Davis 1947; Manzi and Donnelly 1971), alizarin sodium monosulfonate or alizarin red (Hidu and Hanks 1968; Manzi and Donnelly 1971), or other stains (Manzi and Donnelly 1971) have not succeeded. Some stains did not persist for long, were not intense enough, or were toxic. Only Millar (1961) has succeeded in tracking tagged larvae (European flat oysters stained with neutral red), and then only for 12 to 18 h.

Levin (1990) noted that the ability to follow dispersal of known assemblages of larvae in the field may be enhanced in the future by advances in methods of labelling larvae and in instrumentation and technology. Earlier, Carriker (1988) had recommended use of radioisotopes as tags in limited situations, but popular sentiment against release into the environment of radioactive material, no matter how diluted, may prevent this. Levin (1990) suggested that use of fluorescent dyes detectable by flow cytometry or use of rare-earth elements detectable by neutron activation analysis might prove feasible. Ikegama et al. (1991) developed a monoclonal antibody that recognizes antigens uniquely present in developmental stages of a species of starfish and used the antibody to detect embryos and larvae of that species in plankton collections; that technique could be applied to the identification of oysters. Finally, Hu et al. (1992) used a modified electrophoretic technique to distinguish among three species of oyster larvae in the laboratory; they felt this technique would work with field samples. Given the formidable task of sorting and identifying bivalve larvae, even stained larvae, from plankton samples, some sort of tag that can be identified by an efficient technique or automated instrument is highly desirable.

Studies of Oyster Larval Dispersal

Recently, with scientists recognizing that hydrodynamics are an important driving force of aquatic ecosystems, attention has turned to the need to couple the study of biological and hydrodynamic processes ("dynamic biological oceanography"; Legendre and Demers 1984). Modern studies have revealed how complex the interaction between physical, chemical, and biological factors can be as hydrodynamic variability is transmitted through these factors to the biota (Legendre and Demers 1984). Oyster biologists at the turn of the century had an intuitive idea of the importance of such interactions. From 1889 to the mid-1900s, research on biology of larval oysters in New Jersey waters and Delaware Bay was performed, first by Julius and then by Thurlow C. Nelson. Their students and others have continued this effort to the present time, with additional studies stimulated by the Nelson's work being performed in Chesapeake

Bay. I will discuss this work in some detail because of its early and extensive emphasis on environmental influences on larval distributions (see Carriker 1947 and Nelson 1953, 1955 for additional summaries).

Field Studies: Larvae as Active Agents

Initially, J. Nelson related oyster spawning to temperature in an effort to predict spat settlement at coastal sites like Barnegat Bay, New Jersey (Nelson 1891). The bay supported extensive spat settlement in a sheltered, shallow (< 2.5 m) system with extensive beds of seagrasses, a tidal range of about 15 cm, and a tendency to salinity stratification in calm weather (Nelson 1923). Most of the spawning oysters lay near the Bay's inlet, with numbers declining in the upper half of the Bay because of unsuitably soft bottoms (Nelson 1955). Contrarily, larval numbers increased away from the inlet and up-Bay. For example, over a 12-d period of daily sampling in 1916, a cumulative total of 2,900 larvae were collected near the adult beds, about 6,700 at the upper-Bay station, and around 4,000 at each of three intermediate stations (Nelson 1955). Concurrent samples in the inlet found small numbers of larvae in the sea-going ebb tides and similar numbers in in-coming flood tides.

Continued work in Barnegat Bay and elsewhere revealed correlations between the tidal cycle and abundance of larvae. In 1911 in Barnegat Bay, Nelson (1912) collected 212 plankton samples spread over eight time periods from one low tide to the next. Because larval numbers fell with the approach of low water and rose on flooding tides to a maximum value as the tide began to ebb (Table 2), he attributed these fluctuations to changes in larval swimming behavior with tidal phase. This relationship was not as strong in Barnegat Bay in 1913, but it held at two other sites that year (Table 2) where fewer larvae were collected on ebb tide than on flood tide (Nelson 1914; these two sites had a greater tidal range [ca. 1.5 m] than Barnegat Bay). Nelson (1917) also collected more larvae on the flood tide than on ebb tide in Prince Edward Island, Canada.

Plankton tows by T.C. Nelson in late June-early July, 1922 (15 stations) and 1923 (13 stations) showed that straight-hinge larvae appeared first and abundantly in the lower, more saline part of Barnegat Bay, with larger larvae subsequently becoming more numerous up the Bay, especially over flats and in creek mouths (Nelson 1923, 1924a). Nelson (1926) explained such distributions, and his father's earlier observations that larvae swam upward on flood tides and sank on ebb tides, by implicating density (salinity) changes associated with tidal change.

In Barnegat Bay, strong vertical stratification that occurred in quiet weather had important effects on larval distributions. Larvae of eastern oysters, other bivalves, and gastropods were often found concentrated above the halocline. For example, 54 oyster larvae 100 L^{-1} were captured at one station in pumped samples at 0.2 m above the bottom (23.3°C; 18.9 ppt) compared with 12,550 oyster larvae (plus "swarms" of gastropod larvae) at 0.4 m above the

Table 2. Data of Nelson (1912) and Nelson (1914) on distribution of oyster larvae (all sizes combined) in water samples from three New Jersey locations at four tidal stages, collected from mid-June through late August (1911) and June to September (1913). N = number of samples. $\overline{X}$ = average number of larvae per sample.

	1911		1913					
	Barnegat [a]		Barnegat		Edge Cove		Scullville	
Tidal Stage	N	$\overline{X}$	N	$\overline{X}$	N	$\overline{X}$	N	$\overline{X}$
Flood	25	21	55	26.6	55	19	103	20+
High	80	49	48	25.5	80	32	176	24+
Ebb	45	71	69	26.7	78	15+	131	15
Low	62	11	39	21.3	47	15.5	145	10+

[a] Nelson (1912) used 7.5 L buckets to collect these samples (reported in Nelson [1913]). These data have been adjusted to 1.5 L to match the sample size subsequently used by Nelson (1914).

bottom (23.7 °C; 16.5 ppt) (Nelson 1927). Similarly, Nelson and Perkins (1931) reported an instance at high tide of 66,110 eastern oyster larvae above a halocline (ca. 7 ppt; 1 m depth) and 102 larvae near the bottom (ca. 12 ppt; 2 m).

The relation of oyster larvae and the halocline was further demonstrated for pumped samples collected in 1931 in conjunction with use of a current meter (Nelson and Perkins 1932). As before, larvae were concentrated in the vicinity of the halocline when it was present (Fig. 5; left panels). When the halocline was weak or absent, pumped samples and current meter records revealed that larvae were often (but not always; see Korringa 1941 and Carriker 1947) concentrated at the level(s) of greatest current velocity (Fig. 5; right panels). Nelson and Perkins (1932) reported that when currents were negligible and in the absence of a halocline, larvae tended to be concentrated near the bottom (note however that their figure in support of this claim shows that currents in the surface waters were not negligible — Fig. 5; lower right panel). In commenting on these data, Korringa (1941) did not believe that the currents were sufficient to affect larval distributions, a point supported by Carriker (1947). The question that still needs answering is: although currents can stimulate larvae to swim, is salinity more of a stimulatory factor?

In Barnegat Bay, larval distributions in relation to haloclines such as are reported above were consistent over seven years of observations (Nelson and Perkins 1932). Thus, the authors concluded that the more intense setting over the Bay's shallow flats compared with setting in deeper water (>2 m) occurred because persistence of the halocline allowed larvae to be carried over the flats where salinity stratification was absent. They proposed that the less common strong sets in deep water were associated with southerly winds that mixed the water column, destroyed the halocline, and allowed passage of larvae to the bottom.

Nelson and Perkins (1931) explained the concentrations of larvae above the halocline by suggesting that larvae moving toward the bottom would encounter higher salinity water and be stimulated to swim upward. Thereafter, upon encountering lower salinity water the larvae would become less active, sinking again into higher salinity water and being stimulated once more to rise. In the laboratory, eastern oyster larvae that I have introduced into the bottom of a plastic column in which a layer of lower salinity sits upon one of higher salinity (ca. 2 to 5 ppt higher) have swum up, hesitated at the salinity interface, penetrated it, and congregated at the interface after a few minutes (pers. obs.). Similarly, hard clam, *Mercenaria mercenaria*, trochophores swam up through layers of decreasing salinity before swimming in circles just above the 15 to 20 ppt interface (Turner and George 1955). Mann et al. (1991) found that the larvae of three coastal mactrid bivalves also concentrated in salinity discontinuity layers established in the laboratory.

In nature, Tremblay and Sinclair (1990a) found sea scallop, *Placopecten magellanicus*, larvae over Georges Bank in the western Atlantic Ocean to be evenly distributed in the water column when waters were mixed, and to be concentrated above the pycnocline when it was well developed. Tremblay and Sinclair (1990b) suggested that the more even distribution might be caused by the overwhelming of larval swimming speeds by increased turbulence associated with decreased stratification. Jonsson (1989) speculated that density asymmetry of marine planktonic ciliates that concentrate in the vicinity of natural pycnoclines may result in passive geotaxis (a type of behavior modified by morphology). This behavior, influenced by turbulence, may affect the ciliates' distribution near a pycnocline. Jonsson (1989) proposed that molluscan veliger larvae might be affected similarly. This topic requires additional exploration.

Carriker (1947) summarized the conclusions that had been reached up until that time by the Nelsons and their colleagues on oyster larval distributions and responded to objections (see p. 390) raised by Korringa (1941). He wrote that tidal currents were thought to distribute larvae into horizontal assemblages, unevenly dispersed in "lanes" (fronts) upstream and downstream from beds of spawning oysters, with heaviest sets occurring on cultch under these lanes. There was little evidence of lateral distribution of larvae. In areas with strong tidal currents, e.g., Little Egg Harbor, New Jersey, most larvae were found on flood tide. In regions with weak tidal currents, e.g., Barnegat Bay, New Jersey, there were about equal numbers of larvae on flood and ebb tides. Ear-

ly-stage larvae tended to be homogeneously distributed throughout the water column, whereas late-stage larvae remained near the bottom. Younger larvae occurred further downstream, whereas late-stage older larvae were more numerous upstream. Transport of older larvae upstream was thought to be the result of larval sensitivity to increases in salinity and (perhaps) current velocity caused by the flooding tide as mentioned above.

Carriker (1951) performed an extensive series of planktonic studies in Barnegat Bay and Great Bay, New Jersey for four summers to examine oyster larval

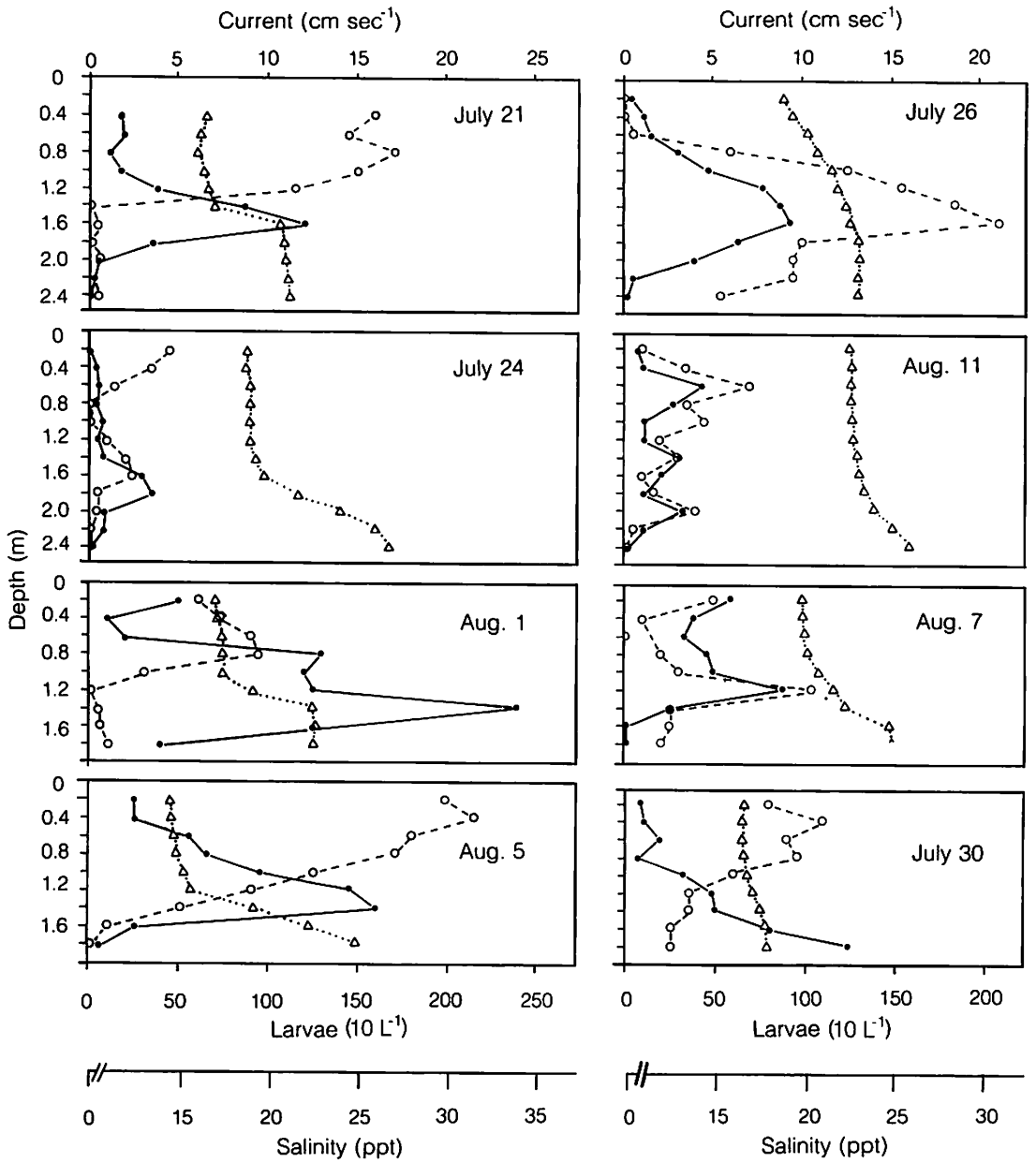


Figure 5. Vertical distribution of eastern oyster larvae (solid circles) in Barnegat Bay, New Jersey, in relation to salinity (triangles) and the presence of a halocline (left panels), and to current velocity (open circles) in the absence of a halocline (right panels). Note that oyster abundances are numbers 10 L^{-1}, except for August 1 (left panel) and July 30 (right panel) when they are numbers 1 L^{-1}. Sizes of larvae were not stated. Redrawn from Nelson and Perkins (1932).

distribution in relation to geographic location, depth (1.5 to 3.5 m), and tidal cycle. Samples were pumped from discrete depths at hourly intervals over a tidal cycle. On a number of occasions, the greatest number of larvae occurred just above haloclines. At three of four sites, more larvae of all stages were collected during flood than during ebb tides (Table 3). Although the reverse was true for Landing Creek, plankton samples were also collected at that site directly on bottom, and eyed larvae, although scarce, were more numerous on the bottom during ebb than flood tide (Table 3).

Using data on tidal current velocity in Delaware Bay, Carriker (1951) calculated that inert particles would be carried about 10 km upstream from a point of release on an average spring flood tide, yet larvae set on cultch located over 30 km upstream from the beds of spawning adults. Further, larvae would be expected to be carried about 1 km downstream in each succeeding ebb (a total of about 30 km in 2 weeks) if they were transported as inert particles because the ebb tide runs longer than flood in Delaware Bay. Thus, Carriker (1951) concluded that larvae must swim for longer periods on a flood tide than on an ebb tide in the Bay.

To determine the effect of hydrography on oyster settlement in Chesapeake Bay, Pritchard (1953) studied the James River, Virginia, in an area with consistently dependable oyster spat settlement and production of juvenile or "seed" oysters. The study revealed the two-layered circulation system mentioned on p. 374. The slope of the level of no net motion separating fresher surface flow moving seaward from the denser, deeper layer moving upriver was tilted from the left side of the river down to the right side, looking downstream. This tilt placed the interface between the two layers of different-density water over the oyster beds on the shallow northeast side of the river. Coincidentally, this was where the river's productive seed beds were located. Thus, Pritchard (1953, 1989) proposed that the denser inflow could serve to replenish upriver beds with larvae from downstream spawning beds, and attributed the high seed-oyster production on these shallow upriver bars to slow upwelling over these grounds of the deeper waters with their associated larvae.

In Chesapeake Bay, Manning and Whaley (1954) studied St. Mary's River, a meandering tributary at the mouth of the Potomac River, Maryland that, at the time, experienced dense oyster sets. The tributary has numerous headlands and creeks, is about 15 km long, varies in depth from about 10 m at the mouth to about 2 m near the head, and at the time of the study had 88% of its oyster beds near the mouth, decreasing to 3% near the head. Manning and Whaley (1954) found the abundance of newly settled spat to increase from 0.7 cm^{-2} of shell cultch surface at the river mouth to 14 cm^{-2} in the middle reach and 33 cm^{-2} near the head, with older oyster larvae being more common lower in the water column. They proposed that the river acted as a larval trap because of its sluggish circulation and the prevailing southerly winds; larval concentrations could thus be the result of passive transport and retention of larvae spawned within that system.

Table 3. Tidal stage and numbers of eastern oyster larvae collected in pumped samples over a tidal cycle at each of four locations in Barnegat Bay (BB) and Great Bay (GB), New Jersey. At Landing Creek, larvae were sampled on and off the bottom. Modified from Carriker (1951).

Tidal stage	Lanoka Lagoon, BB (71)[a]	Winter Creek, GB (27)[a]	Turtle Island, GB (75)[a]	Landing Creek, GB (48)[a]	
				On bottom	Off bottom
Flood	2,582	305	7,380	8 (75)[b]	1,394 (9)[b]
Ebb	1,224	143	2,948	482 (40)[b]	1,828 (6)[b]

[a] Number of samples collected during the tidal cycle.

[b] Percentage of total numbers of larvae that were mature or eyed.

In Delaware Bay, which is weakly stratified due to tidal and wind mixing, Kunkle (1957) examined over 600 plankton samples over a 3-year period and found that younger stages (straight hinge, early umbo, and late umbo) of oyster larvae were almost uniformly distributed vertically during all phases of the tidal cycle. Late-stage (mature, eyed) larvae tended to congregate on or near the bottom during low slack and high slack, as well as during ebb tide. During early flood and maximal flood, late-stage larvae were generally homogeneously distributed vertically. In late flood they tended to concentrate near the bottom. Haskin (1964) provided additional data collected, but not reported, by Kunkle (1957), demonstrating how eyed larvae disappeared from surface waters more rapidly than from bottom waters as the tide ebbed, with lowest counts occurring at slack low water (Fig. 6, top left). On the flood, larval counts increased, with surface numbers being more than double just off-bottom numbers at full flood.

Wood and Hargis (1971) sampled field distributions of oyster and other larvae over many tidal cycles in James River, Virginia and reported on the results of one 24-h period. In their sampling area, coal particles of a size (44 to 210 μm) and density similar to those of oyster larvae were common, apparently because of the proximity of a coal-loading facility located a few kilometers downstream. The distributions of these passively transported particles and of bivalve larvae differed in time and space (Fig. 6, top right; see also Fig. 3 in Shumway, Chapter 13). The highest numbers of coal particles captured by sampling gear usually coincided with current-speed maxima, regardless of current direction, whereas maximal numbers of larvae coincided in most cases with salinity increases that accompanied flood tide (Plate 1 in Wood and Hargis 1971). When ebb tide increased to its peak after high slack tide, larval numbers decreased in both instances, with mixed results (note arrows in Fig. 6, top right) as the ebb tide slowed to low slack tide. As flood tide increased to its peak thereafter, larval numbers increased, with mixed results again (Fig. 6, top right) as the flood tide slowed to high slack tide.

Wood and Hargis (1971) concluded from their results that larvae were responding actively to salinity cues, with larvae in the deeper channel and northeast shoal waters being transported upstream, in agreement with the conclusions of Pritchard (1953, 1989). However, the coal particles were also transported upstream from the downstream loading facility, so passive transport was also occurring.

In Prince Edward Island, Canada, Drinnan and Stallworthy (1979b) pumped samples of eastern oyster larvae from various depths and found that mature larvae were more densely aggregated near the bottom. They too reported higher concentrations of all larval stages on rising tides.

Seliger et al. (1982) attempted to explain the long-term differences in spat settlement between two adjacent tributaries of the Choptank River in Maryland's Chesapeake Bay. Based on two, day-long samples collected in one summer and a single day-long sample in the following summer, they speculated that larvae were transported from a common spawning area at the mouth of the river along an upstream transport region to seed bed regions in each tributary. They proposed that the differences in spat settlement between the tributaries were the result of the tributary that experienced greater settlement success being located closer to the proposed common spawning area than is the tributary with less successful settlement. However, the lower Choptank River, the two tributaries themselves, and the Choptank River further upriver beyond the two tributaries have numerous oyster beds that contain spawning adults (Kennedy and Krantz 1982; Boicourt et al. 1996), so there is no reason to propose that a common spawning area at the downstream end of the system is the sole source of larvae. Boicourt et al. (1996) demonstrated that the differences between the two tributaries were probably the result of different circulation patterns that exposed the less productive tributary to fewer larvae, no matter what their source.

Pritchard (1989) re-examined his earlier data (Pritchard 1953) and related his findings to results of weekly larval tows and spatfall measurements collected by scientists in the region, who had shown that spatfall in the James River estuary (and in the other tributary estuaries on Virginia's western shore of Chesapeake Bay) was heaviest both on north shore oyster bars in the river and later in the summer. Pritchard (1989) concluded that most larvae in the channel were in the upper half of the upstream-flowing layer;

he stated that the similarities in salt concentration and water density between this area of larval concentration and the seed beds means that minimal energy is required to transport the larvae from the channel up the slope of the river bed and onto the north shore seed beds. He also concluded that differences in larval and spat distributions in the James River over a spawning season clearly reflected flow patterns

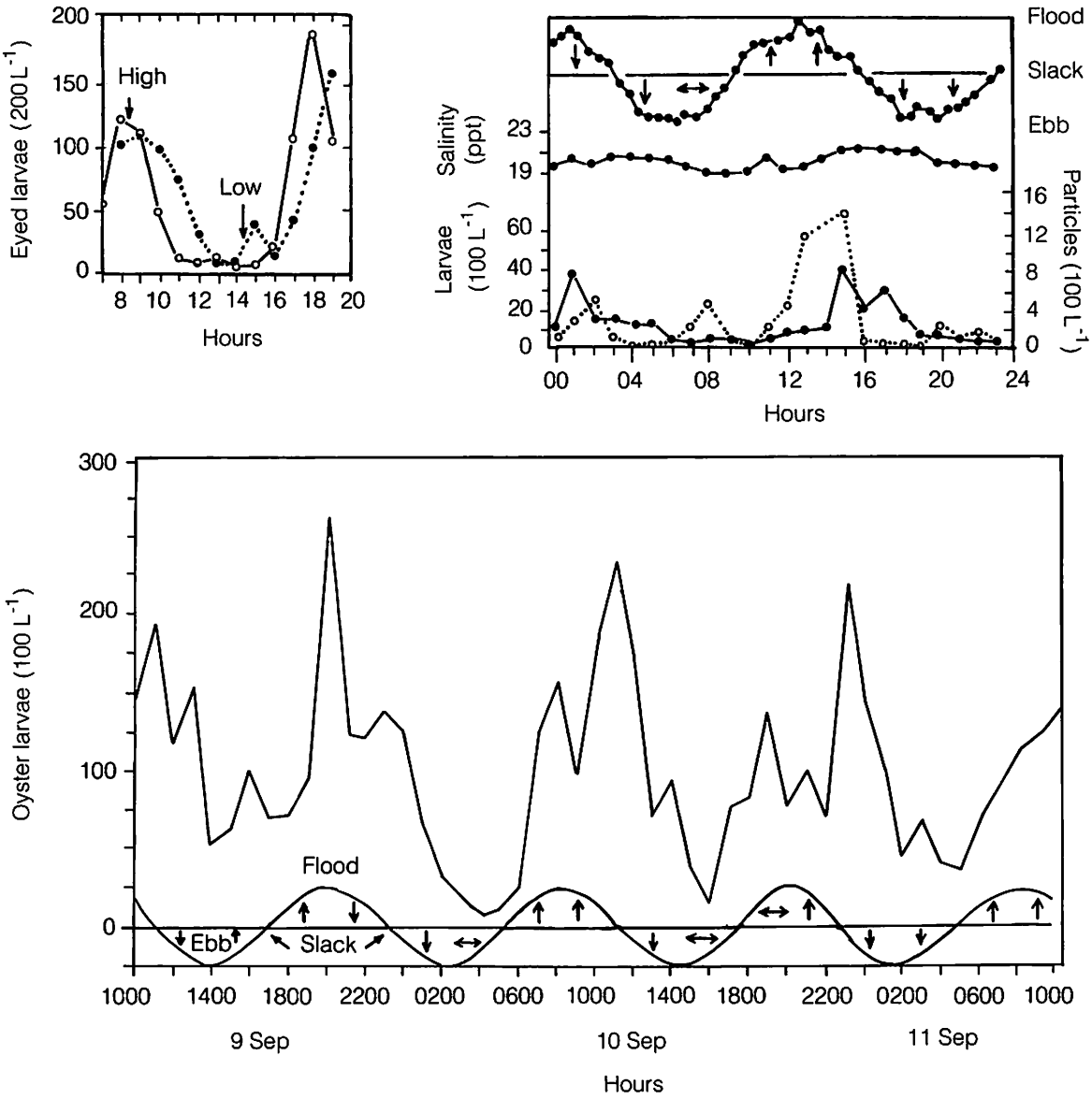


Figure 6. Top left: distribution of eyed larvae in the water column (○: surface; ● : bottom) over a tidal cycle at a Delaware Bay station, August 20, 1956. Arrows indicate occurrence of high and low water (redrawn from Haskin 1964). Top right: distribution of oyster larvae (•——•) and coal particles (◦·····◦) in James River,Virginia (7 to 10 m at Station CU, September 1, 1965) in relation to salinity and tidal stage. Redrawn from Wood and Hargis (1971). Bottom: changes in abundance of oyster larvae in relation to tidal stage in the James River, September 1965. Redrawn from Andrews (1983). In the top right and bottom panels, vertical or horizontal arrows indicate an increase (↑), decrease (↓), or no change (↔) in larval numbers as ebb or flood increases or decreases.

that change as the river varies from a strong to a weak, partially-mixed estuary over the summer period of larval settlement.

Changes in the vertical distribution of bivalve larvae associated with changes in the tidal cycle are not limited to eastern oyster larvae. Yasuda (1952) collected molluscan larvae (species not named) hourly for 24 h at Maeshiba, Japan. Numbers of bivalve larvae increased as the tide rose, reached a maximum around high tide, and decreased suddenly as ebb tide began, reaching a minimum near low tide.

The fact that most studies of the distribution of eastern oyster larvae over time were performed in regions with widespread beds of adult oysters hinders the tracking of larval cohorts over time (see Carriker 1961 for such a study of hard clam larvae). Perhaps the best demonstration of distribution of oyster larvae from a single source is for Pacific oysters that spawned in Ladysmith Harbour, British Columbia, in 1932 and 1936 (Quayle 1988). During that time, adults of this introduced species were present only at the head of Ladysmith Harbour, where the most intense spat settlement occurred (Fig. 7). Numbers of spat per unit area decreased with increasing distance away from the spawners; however, in 1936, some spat were found on rocks 32 km north and 56 km south of Ladysmith Harbour.

Field Studies: Larvae as Passive Agents

Results of the studies reviewed above have led to the hypothesis that eastern oyster larvae, while basically transported passively by horizontal water movement, can modify that transport by moving vertically in response to some stimuli. One such stimulus is thought to be a change in salinity; another is current speed. Other researchers have questioned the idea that oyster larvae can actively influence their distribution within the water column in a way that would allow estuarine circulation patterns to retain the larvae within an estuary or move them to upstream settlement sites. Korringa (1941) found patterns of distribution of larvae of the European flat oyster in the Oosterschelde oyster grounds of the Netherlands to be different from patterns reported for eastern oyster larvae in the U.S. mid-Atlantic region. However, the Oosterschelde differs from Barnegat Bay in that it is a region of strong tidal currents, with much water movement into and out of the system and a tidal range of 3 to 4 m, and *O. edulis* releases older larvae because it is a brooding species. Nevertheless, Korringa (1952) continued to be skeptical about interpretations of data collected on the eastern oyster, including Carriker's (1951) work, and saw no evidence that larvae performed rhythmic vertical migrations that enabled them to travel up-estuary. deWolf (1973, 1974) performed an extensive study of barnacle larval dispersal in the Dutch Wadden Sea and concluded that retention of larvae (barnacle or bivalve) can be explained by mechanical processes alone, with no need to appeal to patterns of swimming behavior as an additional mechanism.

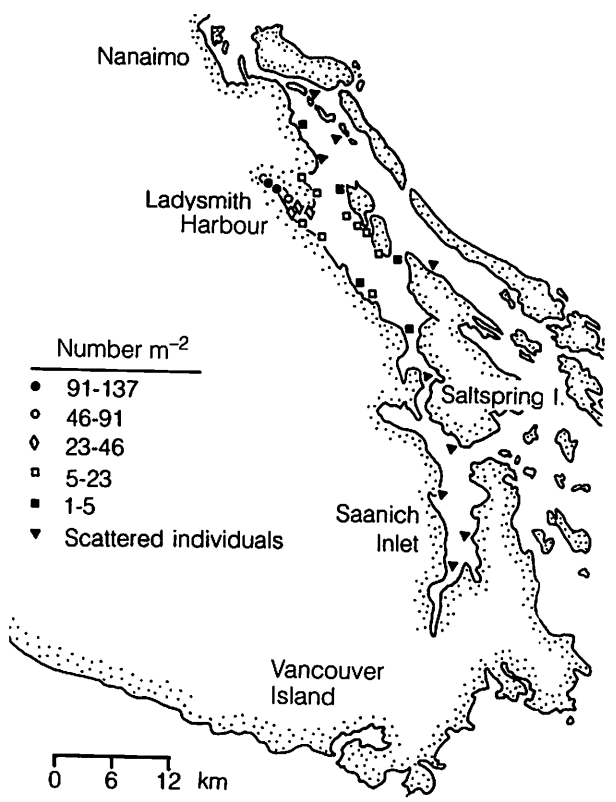


Figure 7. Distribution of Pacific oyster spat (number m^{-2}) produced by a spawning population in Ladysmith Harbour, British Columbia in 1936. Redrawn from Quayle (1988).

In Milford Harbor, a Connecticut tributary of Long Island Sound, Prytherch (1929) examined 140 plankton samples and found oyster larvae to be most abundant during the "low-water period" (last 2 h of ebb tide, slack low water, first 2 h of flood) and minimally abundant during the "high-water period" (last 2 h of flood, slack high water, first 2 h of ebb). When numbers of umbo larvae were examined in relation to tidal stage over three tidal cycles, larvae were most abundant in bottom and mid-depth samples at the start of the ebb stage, distributed throughout the water column during low slack, most abundant in surface samples during first flood, and mostly near the bottom or at mid-depth during high water. This pattern is different from that observed by Wood and Hargis (1971) and Andrews (1983), but no explanation for this discrepancy has been advanced.

Andrews (1979, 1983) argued that oyster larvae are predominantly distributed passively, and discussed a different set of data (Fig. 6, bottom) collected during the same survey from which Wood and Hargis (1971) obtained their data. Larvae of a number of bivalve species were collected and showed similar fluctuations in abundance in relation to tidal cycle (Andrews 1983). Captured oyster larvae, the major component of the larval populations, did not increase in size but remained predominantly straight-hinge during the 12-day sampling period. This would indicate a steady recruitment over the period, rather than the presence of a single brood of siblings. Weekly placement of cultch on a grid of 19 stations revealed that spat settlement increased or decreased synchronously throughout the river from week to week. Andrews (1983) concluded that groups of larvae became widely distributed before setting, an indication of constant dispersal.

Andrews' (1983) data were similar to those of Wood and Hargis (1971) in that there were regular rhythms of larval abundance with tidal stage. When ebb tide increased to its peak after high slack, larval numbers decreased in all four instances and as flood tide increased to its peak, larval numbers increased three out of four times (note arrows in Fig. 6, bottom). There were mixed results (i.e., increased or decreased numbers or no change) as the ebb slowed to low slack or as the flood slowed to high slack. Andrews (Virginia Institute of Marine Science, pers. comm.) found that the James River usually displayed little variation in salinity (ca. 1 ppt) or temperature (ca. 1°C) between surface and bottom in August when setting was most intensive. He therefore considered that there was an insufficient environmental signal for the larvae to be using as a cue for dispersal.

Andrews (1983) postulated that downstream broodstock had once provided the larvae that eventually recruited upstream in the James River. With the onset of disease that decimated the broodstock, most larvae were now derived from adults spawning on the upstream seed beds, with gradual downstream transport and dispersal of younger larvae on the southern side of the river and an upstream, passive return of the older larvae in channel waters that welled up over the seed oyster beds in the upriver shallows. If larvae survived predation pressures and were not flushed from the estuary before they developed into the umbo stage, they would then remain in the channel waters and perhaps be entrained upriver. However, Andrews (1983) believed that upstream entrainment was less successful in estuaries with high flushing rates, such as the James River, than in less strongly flushed habitats (e.g., lower Delaware Bay). In summary, Andrews believes that although there is evidence that eastern oyster larvae are transported up-estuary, there is no need to postulate that the larvae participate actively in this transport.

Experimental Studies. One of the problems raised by critics of the concept that oyster larvae play an active role in their estuarine dispersal concerns what kinds of cues would stimulate larval behavior that could take advantage of possible upstream transport mechanisms. If larvae are capable of swimming actions that would result in their entrainment in appropriate water masses for retention in estuaries, they must have some means of sensing environmental cues. Our knowledge of oyster larval response to possible environmental cues is extremely limited. However, some research has been conducted on larval response to salinity, because salinity varies with the tidal cycle. Nelson and Perkins (1931) showed that oyster

larvae respond to increases in salinity by swimming more actively. To demonstrate the effects of salinity change on behavior of mature and eyed larvae, Haskin (1964) used small observation chambers (2 × 2 × 2 cm) notched into paraffin blocks, into which waters of varied salinity flowed at 1 ml min^{-1} in the presence of about 30 larvae per experiment. Filters maintained light at a transmission maximum of 575 nm. Observing larvae that were actively swimming off-bottom, Haskin (1964) found that rapid salinity changes of as much as 13 ppt over a 2-h period and slower changes of about 8 ppt over a 2-h period modified larval activity, which rose or fell directly with salinity change (see Fig. 2 in Shumway, Chapter 13). He concluded that larvae were thus able to play an active role in their estuarine transport.

In similar paraffin chambers as used by Haskin (1964), Hidu and Haskin (1978) found that eastern oyster larvae swam up or down at speeds up to 14 cm min^{-1} (2.3 mm sec^{-1}). At these speeds, larvae could move 7 to 8 m vertically in an hour, which would allow them to exploit tidal transport systems. As noted earlier, larval speed was also a function of size. Hidu and Haskin (1978) used the ontogenetic difference in swimming speeds to explain Kunkle's (1957) observations of differential distributions of larvae by size in the water column by postulating that the smaller larvae were poor swimmers and thus subject to relatively passive distribution through the water column, whereas the larger larvae were better able to affect their position by swimming in response to salinity changes.

One criticism of Haskin's (1964) and Hidu and Haskin's (1978) experiments is that they were performed in such small experimental chambers (2 × 2 × 2 cm) that larval swimming behavior could have been affected by wall effects (see discussion of Reynolds number on p. 378), especially for those larvae not swimming in or near the center of the chamber. Nevertheless, the pattern of responses of the larvae to salinity that Hidu and Haskin (1978) observed is clear and in agreement with the observations of others.

Modeling Studies

The potential retention of eastern oyster larvae in the lower James River, Virginia was studied with a physical model by Ruzecki and Hargis (1989). They manipulated a hydraulic model of the river by injecting fluorescent dyes at six release points to simulate potential locations of brood stock concentrations. The model was cycled and dye dispersion monitored for up to 40 tidal cycles after dye release. This technique allowed them to use dye to simulate density distributions of oyster larvae at maximal spatfall after late summer spawning in this once-productive region of the James River. Dye concentrations revealed a cyclonic circulation pattern, with water moving upriver on the northern side of the estuary and eventually crossing over to the southern side near the upper extent of the traditional seed beds. Downstream flow on the southern shore eventually became shunted over to the opposite shore in the vicinity of the traditional broodstock beds. This circulation pattern would retain suspended particles for varying periods of time, although there would be some degree of loss to upriver regions of lower salinity and to downriver regions of higher salinity. Ruzecki and Hargis (1989) noted that this pattern is a combination of Andrews' (1983) proposed trap-type and flushing circulations.

The role of physical processes in the retention of oyster larvae in Delaware Bay was examined by Jacobsen et al. (1990) who used two- and three-dimensional numerical models to calculate particle trajectories over time. There was an indication that larvae could be retained along the New Jersey shore if winds were light or southwesterly. Strong northeast winds might move larvae into the channel, where currents flow downstream away from the seed beds. However, the two models differed in many of their predictions, and need to be refined.

Overview of Dispersion Studies

The study by Wood and Hargis (1971) demonstrates the role of passive transport up-estuary of inert coal particles. The principle of "Occam's Razor" or parsimony of explanation supports the arguments by some authors that such passive transport is sufficient to maintain oyster populations within estuaries without the need for an active role on the part of the larvae. However, as reviewed above, field observations have clearly found the vertical position of *C. virginica* larvae in the water column to be correlated with changes in tidal salinity and (perhaps) changes in current speed.

Also, laboratory observations have shown that eastern oyster larvae respond to salinity change and water movement. In relation to Carriker's (1951) report that older oyster larvae apparently remained on the bottom during ebb tide, note that Jonsson et al. (1991) observed cockle, *Cerastoderma edule,* larvae entrained in 10 cm s^{-1} benthic water flow to rest on the bottom of the experimental flume for up to 10 s.

There is evidence that larvae of the European flat oyster can also regulate their position in the water column. In a Norwegian fjord stratified into a surface freshwater layer, a thin intermediate saline layer, and a de-oxygenated bottom layer, Gaarder and Bjerkan (1934) found the larvae to remain in the saline layer. And in the Helford River, England, larval abundances were usually similar at the surface and at 3 m, except during sunny days when they were greater at depth (Cole and Knight-Jones 1949).

Based on the field and laboratory data cited above, it seems reasonable to conclude that reactions by oyster larvae to environmental cues may supplement the effects of passive transport and enhance retention in estuaries. However, as noted above, one objection concerns how larvae might sense cues, such as a change in tides. After all, larvae are thought to be entrained in a body of water that is moved about by the tides. How then do they sense changes in salinity? Or are other cues involved?

The question of sensory abilities is being investigated for other estuarine organisms such as ciliates (Jonsson 1989; Crawford and Purdie 1992) that avoid being flushed from estuaries or that aggregate around pycnoclines. Crawford and Purdie (1992) speculated that organismic responses to turbulence caused by shearing of currents, or gravitational instability of surface waters, might help retain ciliates in estuaries. Such a mechanism may or may not apply to oyster larvae. However, the point is that organisms much smaller than oyster larvae appear to sense environmental cues on a microscale that is poorly understood but apparently real. There is no reason to think that oyster larvae are any less attuned to microscale influences.

Recent advances in the use of flumes for the study of behavior of invertebrate larvae promise to open fruitful new avenues of research. Jonsson et al. (1991) observed the swimming behavior of larval cockles in boundary-layer flow. Settling larvae in moderate to fast water flows (5 to 10 cm s^{-1}) seemed to be trapped in the viscous sublayer, moving with the flow with their shells tilted and the velum pointing downstream. Stoppage of flow led to the larvae re-orienting with the velum upward and the usual vertical, helical swimming pattern resuming. Flows greater than 15 cm s^{-1} resulted in the larvae tumbling and being resuspended in bed-load transport. Jonsson et al. (1991) postulated that cockle larvae were confined in boundary-layer flows at moderate current speeds because the velocity gradient imposed a near-bottom shear on the larval body, viscous torque dominated, and the larvae became tilted and unable to swim vertically. As eastern oyster larvae are likely to be affected similarly, the resultant near-bottom drift would allow them to sense chemical cues such as ammonia (Fitt and Coon 1992) or to stop swimming and crawl on cultch. Indeed, recent work by Turner et al. (1994) demonstrates that eastern oyster larvae can respond to dissolved chemical cues under conditions of water flow such as are found in nature.

Patchiness of Larval Distributions

The question of patchiness of distributions of oyster larvae in nature remains to be explored. During his study, Pritchard (1953) noted that samples of larvae over time at one station showed concentration peaks that were more pronounced than one would expect if larvae were just passively suspended in the water. He proposed that the larvae may have been "swarming," thus retaining a more compact configuration for the population. In another study, late-stage oyster larvae (captured with 153 μm mesh plankton nets) in a Louisiana estuary exhibited pronounced patchiness, smaller than the study's sampling scale (1 min tows; Vecchione 1987). Jacobsen et al. (1990) found distributions of late-stage larvae to be patchy in Delaware Bay, but they attributed that patchiness to physical processes and not to larval behavior.

Patchiness has also been reported for larvae of other oyster species. Talbot (1974) examined published data on the distributional patterns of larvae of European flat oysters in the Blackwater and Roach-Crouch

estuaries in England in light of physical theories of how diffusion (i.e., passive turbulent mixing) works in estuaries. He concluded that the larvae did not appear to diffuse as rapidly as inactive particles would be expected to if affected simply by physical factors. That is, the larval distributions did not seem to track the water movements completely, leading Talbot (1974) to propose that the larvae might move to the bottom and not be subject to mixing and movement. Long term studies in Pendrell Sound, British Columbia have shown that Pacific oyster larvae spread horizontally throughout the Sound within a week after spawning begins and that their distribution is aggregated rather than random (Quayle 1988).

It is not clear how aggregation of larvae of these oyster species would be accomplished in nature, given the turbulence of the water column and the relative dilution capacity of the water in relation to larval abundances. As Legendre and Demers (1984) indicate, investigations of horizontal and vertical structure of planktonic populations (i.e., patchiness) and of planktonic dispersal need to take into account the tight coupling between biological dynamics and hydrodynamics. The authors noted that such an investigation would have four components — physical and biological variables, and temporal and spatial scales — that are interdependent, and that there is no single temporal or spatial scale on which different hydrodynamic processes or biological responses occur. Thus, for oysters, short-term biological processes such as gamete release and fertilization or substrate exploration and settlement by pediveligers would be influenced predominantly by turbulent mixing. Longer-term biological processes such as the necessary development and growth period to metamorphic competence should allow turbulent diffusion and advection to operate on larval distribution and patch structure. Further research into these matters would be challenging, but enlightening.

EXPERIMENTAL LABORATORY STUDIES ON SETTLEMENT

Settlement

The switch to a settled benthic existence by a meroplanktonic larva involves changed morphology and behavior, apparently in response to environmental and internal cues. The general topic of settlement and metamorphosis in marine invertebrate larvae has been reviewed by Burke (1983) and by Rodríguez et al. (1993). Burke (1983) described a neurological and behavioral model for the induction of metamorphosis. The model proposes that larval receptors (sensory organs) are activated by an environmental factor(s) associated with a preferred habitat. The receptors communicate with effectors, or larval tissues, which respond in turn with metamorphic change. Rodríguez et al. (1993) took more recent literature into account in their review of ecological, physiological, and biotechnological aspects of settlement in benthic marine invertebrates.

For oysters, Burke (1983) defined settlement to include larval exploratory activity that occurs before permanent attachment and during which attachment is reversible. Thus, settlement, the ending of planktonic existence, is a behavioral activity that can be repeated or reversed by the settling larvae (Scheltema 1974). Settlement is followed by metamorphosis, an irreversible developmental process that begins with cementation of the shell to substrate and continues through the processes of tissue modification during which morphological (e.g., velar loss, foot resorption, gill development) and physiological and metabolic changes occur.[3] These changes modify planktonic-related attributes in favor of attributes that enhance benthic survival.

There is a characteristic progression of larval behavior during the processes of settlement and metamorphosis of oyster larvae (Nelson 1924b; Cranfield 1973b; Coon et al. 1990a; Tamburri et al. 1992). Older umbo larvae develop a pair of "eye spots" and a foot containing a byssal or pedal gland. When ready to set, larvae exhibit a stereotypical "swim-crawl" behavior, including swimming with the foot extended

[3] Baker and Mann (1994a) have characterized four phases of metamorphosis in *C. virginica*. Attached larvae that have retained larval characteristics are called settlers. Subsequently, prodissoconch postlarvae are those that have experienced degeneration of the velum. Dissoconch postlarvae display shell growth but the foot persists. Juveniles have lost all larval organs and have completed metamorphosis.

beyond the shell (Fig. 3C). This is the point during larval development when larvae have become "behaviorally competent" to respond to stimulation and to exhibit settlement behavior (Coon et al. 1990a). Larvae are usually located near the estuarine bottom at this stage, but they can settle on hard surfaces suspended throughout the water column. When contact is made with a surface, the larvae (now called pediveligers) crawl thereon, usually in circular paths that become more sharply angled if the surface is stimulatory enough to induce more localized exploration. Unattractive surfaces do not stimulate the larvae sufficiently and they resume swimming. However, if appropriate cues are available, crawling ceases and the larvae cement their left valve to the surface in the first step of metamorphosis into spat.

Tamburri et al. (1992) used motion analysis of video-taped behavior of eastern oyster larvae to measure changed behaviors in the presence of waterborne settlement inducers (described below). Within the first 3 min of exposure to an inducer, larvae rapidly swam downward. Stimulated larvae then swam horizontally at swimming speeds (grand mean ± 1 SD: 0.51 ± 0.04 mm s^{-1}; N = 4 sets of multiple measurements) that were only about half as fast as control speeds in the absence of inducers (0.98 ± 0.07 mm s^{-1}; N = 5). Larvae also modified their path trajectories by increasing their horizontal turning near the bottom, subsequently attaching to the bottom with their foot.

As noted, metamorphosis usually follows the onset of settlement behavior. However, larvae can be "morphogenetically competent" to metamorphose whether or not settlement behavior has been induced (details below). Morphogenetic competence is signaled by the ability to respond to endogenous cues by metamorphosing physiologically and morphologically (Coon et al. 1990a). The sum of behavioral and morphogenetic competence is "metamorphic competence," usually referred to simply as "competence." Coon et al. (1990a) found that cultured larvae of the Pacific oyster could remain competent while delaying metamorphosis for at least 30 d. Because eastern oyster larvae responded to settlement and metamorphic inducers in a fashion similar to the responses of Pacific oyster larvae (Coon et al. 1986), they are probably also able to delay metamorphosis.

Colonization of surfaces by marine invertebrate larvae has attracted much attention over a number of decades, and the topic has often been reviewed (Meadows and Campbell 1972; Crisp 1974; Scheltema 1974; Chia and Rice 1978). I now examine those studies that pertain to oysters.

Physical Factors

Eastern oyster larvae may be stimulated to settle by various physical factors in the environment. Nelson (1909) noted that peaks of spat settlement in Barnegat Bay were correlated with warmer temperatures, and Hidu and Haskin (1971) and Lutz et al. (1970) showed that larvae settled in response to increased temperatures such as might be experienced over a sun-warmed tidal flat by larvae carried inshore on an incoming tide. Hidu and Haskin (1971) found no settlement response to increased salinity. Eastern oyster larvae respond to the proteinaceous component of the surface of oyster shells (Crisp 1967), and also exhibit rugotropism, settling in small pits and irregularities on surfaces (Nelson 1953; Galtsoff 1964). Settlement behavior in relation to light intensity, surface angle, and current speed has been studied for a number of oyster species with contradictory results that may be due to experimental conditions (e.g., Cranfield 1970).

The eastern oyster exhibits a variety of settlement responses to the environment throughout its range (Table 4). The broadest generalization seems to be that pediveligers tend to settle on undersurfaces, presumably to avoid light and silt. Eastern oyster larvae were found to settle more readily in shade than in light in the laboratory (Ritchie and Menzel 1969). Nelson (1953) reported that oyster larvae consistently set on the undersurfaces of experimental shells placed in the field. However, in 1952, most of the larvae settled on the upper surfaces of the shells at his study site in Delaware Bay. Nelson (1953) attributed this change to the increased turbidity (and lower light intensity) caused by high densities of phytoplankton during the setting period. In tributaries of the Choptank River, Kennedy (1980) found increased settlement on upper surfaces of spat-recruitment plates, a change from the situation during similar studies a decade earlier. Like Nelson (1953), he too attributed

the change to increased turbidity and consequent light attenuation.

Conspecifics

As do many sessile invertebrates, oysters have larvae that settle gregariously, producing aggregations of conspecifics. Such gregarious behavior was first described for European flat oysters by Cole and Knight-Jones (1949). Nothing was known of the gregarious response of eastern oyster larvae until Crisp (1967) demonstrated that immersion of cultch in tissue extracts of eastern oyster bodies enhanced settlement on the cultch by larvae, although Nelson (1926) attempted unsuccessfully to test the hypothesis that adult oysters might, through their secretions, attract competent larvae to settle nearby. Hidu (1969) showed that cultch

Table 4. Settlement surface preferences (upper, under) of *Crassostrea virginica* spat on different kinds of material and in different light conditions (where known). Abbreviations refer to states in the USA.

Surface		Settlement substrate	Remarks	Authority
Upper	Under			
			Field Data	
	+	Shell	More and larger spat on under surface in shell bags (SC).	Smith 1949
	+	Shell	Shell bags, held at 1.3 to 1.7 m for 7 d. Upper side not silted (MD).	Sieling 1951
+		Cement board	Held at 0.3 to 2.7 m in 0.3 m intervals. Setting seemed higher by day than by night (FL).	Butler 1955 (4-yr study)
	+	Cement board	Held at 3 m, just 2.5 cm above bottom. 50% of the set occurred on this bottom plate.	Butler (same study as above)
	+	Concrete-coated cardboard	At various depths in 2.3 m of clear calm water. Spatfall increased with depth and was heavier by day than by night. No fouling or silting occurred (Canada).	Medcof 1955
Not stated		Not stated	Setting heaviest 0400-0830, then 1630 to 1940, 0830 to 1630, and 1940 to 0400. Under constant light, larvae preferred darkened areas (NC).	Chestnut 1968
+		Cement board panels	Suspended throughout water column; collected weekly. Delaware Bay (NJ).	Hidu 1978
+		Asbestos plate	Three sampling levels in water column (ca. 3 m) in Mobile Bay (AL). Peak Secchi disk visibility: 1.5 to 2.8 m.	Lee 1979
+		Asbestos cement	Held 20-30 cm above bottom in 1 to 3 m depth. Collected weekly in Choptank River (MD).	Kennedy 1980
Varied with site and tidal level		Asbestos cement	Larvae responded differently at different tidal levels to light, desiccation, sediment.	Kenny et al. 1990
	+	Shell	Over 50% of spat set on under surface, and at 3 m versus 1 m (NC).	Ortega and Sutherland 1992
			Laboratory Data	
	+	Shell	In open Pyrex dishes	Crisp 1967
	+	Shell	If undersurfaces were illuminated, setting decreased greatly (FL).	Ritchie & Menzel 1969
Not stated		Scallop shell or fiberglass plate	Setting higher in darkness; partly inhibited by light (Canada).	Shaw et al. 1970

with attached 1-day or 2-month old spat attracted significantly more spat than spat-free cultch. Also, larvae settled in larger numbers on clean shells held outside a very fine mesh plankton bag containing 2-month old spat than on control shells outside a similar bag with no spat; this suggested that a water-borne pheromone produced by the spat was involved. Keck et al. (1971), Veitch and Hidu (1971), and Hidu et al. (1978) subsequently investigated and characterized the waterborne chemicals that acted as "gregarious factors" to stimulate settlement near adults or newly-settled spat. Most recently, Zimmer-Faust and Tamburri (1994) have shown the cues to be peptides of low molecular weight and with arginine occupying the C-terminal.

Surface Films

Bacterial surface films are among those factors that stimulate exploration of and settlement on surfaces by invertebrate larvae. Oyster biologists knew decades ago that cultch should be placed on the estuarine bottom a few days before larval settlement is expected so that a suitable surface film can develop (e.g., Nelson 1908). Recent studies have advanced our understanding of the effects of such films. Weiner et al. (1985) studied a bacterium first isolated from an oyster hatchery's tanks that held eastern oyster spat. When attached to a surface such as an oyster shell, the bacterium, *Shewanella colwelliana*, produces L-3,4-dihydroxyphenylalanine (L-DOPA), other melanin precursors, and melanin, and enhances settlement of the eastern oyster (Weiner et al. 1985, 1989) and the Pacific and European flat oysters (Fitt et al. 1990; Tritar et al. 1992).

Specific Chemicals

Attention has recently turned to the behavioral and biochemical influences of settlement stimulants. Coon et al. (1985) discovered that L-DOPA induced both settlement and metamorphosis in the Pacific oyster, whereas the neuroactive catecholamines, epinephrine and norepinephrine, induced metamorphosis without settlement. Exposures of 5 to 10 min duration to optimal concentrations of L-DOPA led to characteristic settlement behavior, with some larvae cementing and metamorphosing and others resuming normal swimming behavior with their foot retracted. Maximal inductive activity of L-DOPA occurred at 2.5×10^{-5} M, with prolonged exposure (hours) to concentrations above 5×10^{-6} M being toxic and concentrations above 10^{-4} M being lethal. When exposed to epinephrine and norepinephrine, Pacific oyster larvae were induced to metamorphose immediately without exhibiting settlement behavior (specifically, crawling and cementing) and cultchless spat were the end result of exposure of the larvae to the two inducers (Coon et al. 1985). The optimal concentration of these catecholamines was 10^{-4} M, and near-maximal induction of metamorphosis resulted from exposures as short as 1 h. The inducers were non-toxic, and epinephrine was somewhat faster and more potent than norepinephrine (Coon et al. 1986). There seemed to be no negative effects associated with exposure to epinephrine, because spat appeared to be normal after 12 months (norepinephrine-treated spat were not tested). Coon et al. (1986) found that eastern oyster larvae responded to the catecholamines much as did Pacific oyster larvae, but their responses were less consistent and less intense (lower percentage responses) than were those of the Pacific oyster.

Coon et al. (1985) developed a model for the response of the Pacific oyster to L-DOPA and the two catecholamines (Fig. 3C). The response was not dependent on contact with a surface, in that behavioral responses were elicited by the soluble products.

After a larva begins to crawl on and explore a surface, subsequent settlement may involve encounters with environmental cues that initiate cementation and metamorphosis. Such cues may include light, chemical signals, and surface composition, texture, or rugosity. Because the catecholamines studied by Coon et al. (1990a) induced only metamorphosis in the laboratory, these chemicals or their mimics are more likely to be endogenous in nature, not to be released until after cementation, whereupon they induce metamorphosis by interacting with $alpha_1$-adrenergic receptors (Coon and Bonar 1987).

Coon et al. (1990a) developed a mechanistic model of two serial pathways controlling settlement and metamorphosis to supplement the model of Coon et al. (1985) (Fig. 3D). They proposed that a dopa-

minergic pathway exists that responds during settlement to sensory cues, releasing dopamine and stimulating the hypothetical integration center or neural network that initiates and sustains settlement behavior. As settlement behavior leads to cementation, an adrenergic pathway controls metamorphosis as the integration center stimulates the release of norepinephrine or epinephrine. In turn, these components induce metamorphosis via $alpha_1$-adrenergic receptors, either by acting directly on the appropriate tissues or by stimulating the release of one or more morphogenic agents.

Upon review of the settlement-related literature cited above, it is possible to categorize larval responses to waterborne substances as (1) those associated with conspecifics and (2) those associated with bacterial films on surfaces. Tamburri et al. (1992) have managed to separate experimentally the effects of conspecifics from the effects of bacterial films to show that eastern oyster larvae respond to both, with larvae from populations in Chesapeake Bay, Virginia, and Galveston Bay, Texas, responding similarly.

Undoubtedly, the topic of chemical cues has not been exhausted. Coon et al. (1990b) and Fitt and Coon (1992) found that ammonia (NH_3) induced settlement behavior (foot extension, crawling) in eastern and Pacific oysters in the laboratory, with 7.1 µM being the lowest ammonia concentration that induced such behavior in eastern oysters. Newly competent larvae of the eastern oyster were less responsive to ammonia than were older larvae. Water in which scrubbed adult eastern oysters had been held for 24 to 48 h contained sufficient ammonia to induce settlement behavior in larvae of both species (Zimmer-Faust and Tamburri [1994] caution that the concentration of ammonia necessary to stimulate settlement behavior in these experiments may actually be poisonous, with the behavior thus not being natural settlement behavior). Fitt and Coon (1992) measured ammonia concentrations in water overlying Georgia oyster beds during a tidal cycle and in water collected at low tide from shell surfaces and crevices between shells. Concentrations of ammonia in the overlying waters were never high enough to stimulate settlement behavior in larval eastern oysters, but concentrations in crevices were indeed high enough. Such a response by oyster larvae to natural concentrations on an oyster bed could stimulate larvae to contact shell surfaces for further exploration.

Further research should help us understand the variety of settlement and metamorphosis cues available, their interactions, and the behavioral physiology involved in larval responses (see Havenhand [1991] for a review that is relevant, although concerned with gastropod larvae). Such information should be useful to aquaculturists as oyster farming becomes more common.

Cementation

Cranfield (1973a, b, c, 1974, 1975) thoroughly studied settlement and attachment during the first 6 h of benthic existence of spat of European flat oysters. The larval foot's pedal gland produced and deposited a tanned mucopolysaccharide, a pedal cement, when settlement occurred. Thereafter, as shell growth continued and the larval foot was resorbed, a mantle fold secreted a similar cement. Tomaszewski (1981) studied cementation in eastern oyster spat up to a week old and reported that the mantle and the periostracum were involved in attachment and adhesion. Harper (1991) expanded this work by examining post-larval cementation in four oestrid species, including the Pacific oyster, for up to 6 weeks after settlement. During this time, a thin (<0.5 µm) periostracal sheet was produced from the periostracal groove between the middle and outer mantle folds (Fig. 3E). An extra-periostracal, crystalline cement accumulated between the shell and the experimental cultch. This external cement had a similar elemental (calcium carbonate) composition to that of the spat shell, and was not produced by a mantle fold. Harper (1991) proposed that the cement was derived from extrapallial fluid that leaked through the continuously forming periostracum to fill the spaces between the cultch and the new spat shell. The crystallizing fluid served as a smoothing agent that enabled the cultch and shell surfaces to come close enough to ensure adhesion as a result of electromagnetic interactions.

Metamorphic Changes

Once cemented in place, the spat resorbs larval structures (some if not all of the velum, as well as the velar muscles, foot, eyespot, and anterior adductor

muscle) and rearranges permanent structures (Galtsoff 1964; Baker and Mann 1994a). The labial palps develop, the gills proliferate, and the posterior adductor muscle, mouth, and anus move counterclockwise (as seen from the spat's right side). Details of the functional morphology and development of larvae of eastern and European flat oysters can be found in Elston (1980) and Waller (1981). Feeding activities during settlement and metamorphosis have been studied by Baker and Mann (1994b).

SETTLEMENT AND RECRUITMENT IN NATURE

Numerous field studies of settlement and recruitment[4] by the eastern oyster have been performed. Nelson (1906) photographed spat on upper and lower surfaces of tile, pottery, crockery, glass, and bivalve shell and postulated that spat settled on under surfaces because they are free of sediments. He remarked on the importance of the development of a surface film or "slime" to make cultch surfaces attractive to settling larvae (Nelson 1908). Truitt (1929, 1931) found oyster shell to be more suitable as cultch in Chesapeake Bay, attracting more larvae than did glass, gravel, slag, or wood.

Korringa (1941, 1952) believed that two important factors governed settlement success of the European flat oyster in Holland. One was the number of mature larvae available to settle per unit volume of water and the second was the current velocity at the time of settlement. Of lesser importance, but still of significance, were the suitability of the bottom and the presence of predators. In a similar vein, Osman et al. (1992) stated that three factors can influence the success or failure of recruitment of benthic invertebrates. These factors are the availability or supply of settling larvae, the density-dependent mortality that occurs after settlement, and species-specific mortality of the earliest life-history stages.

[4] Often confused with "settlement," recruitment refers to the survival of settled spat at some arbitrary length of time after settlement (Keough and Downes 1982). The number of recruits is a function of the number of settlers and some probability of survival.

Settlement of oysters in nature has attracted attention from scientists and managers concerned about future recruitment to commercial oyster populations. Although we are learning more in the laboratory about factors involved in stimulating settlement and metamorphosis, it is still unclear as to how oyster larvae encounter and respond to a suitable habitat in nature. Here I review a variety of field studies to examine what we know and to outline areas meriting further study.

Settlement and Recruitment Variability

Site-specific Settlement

Settlement and recruitment over the natural range of the eastern oyster is variable (Table 5), periods being limited in length in northern waters and more extensive in southern waters (see Thompson et al., Chapter 9 for data on spawning periods). Kenny et al. (1990) conclude that the start and extent of the period of larval recruitment are relatively consistent among years within a given location along the geographic range of the species. On a scale smaller than the eastern oyster's natural range, biologists have known for some time that some regions of an estuary may have consistently higher recruitment than others over the long term (e.g., Kennedy 1980). The James River, Virginia, consistently has had later sets than elsewhere in Chesapeake Bay, perhaps because a decline in freshwater discharge allows net upriver flow of saline, larvae-bearing water (Andrews 1983).

It is common, however, for seasonal and local variability in recruitment to be high. Nelson (1903) encountered high seasonal variability in spat recruitment even on the most productive oyster beds in New Jersey and implicated poor condition of the gametes resulting from negative prior influences on the females (Nelson 1905). (Subsequently, Helm et al. [1973] demonstrated that nutritive stress imposed on adult females of European flat oysters reduced larval viability.) In addition, Nelson (1903) noted that oyster planters in New Jersey were familiar with areas where settlement on cultch was limited but where growth of planted oysters was excellent (Rochford [1951, 1952] made similar observations about Australian waters for the "Australian oyster" [species not named, but presumably *Saccostrea commercialis*]). Loosanoff and Nomejko (1956) found that, although the

Table 5. Timing of recruitment of *Crassostrea virginica* larvae throughout the species' natural range. Abbreviations refer to states in USA.

Location	Study period	Timing of recruitment	Peak of recruitment	Authority
Bideford River, Prince Edward Is.	1936 to 37	Jul to Aug	Mid Aug 1936; Late Jul 1937	Medcof 1939
Nova Scotia	1939 to 40	Jul to Aug	Varied	Medcof 1955
Milford Harbor, CT	1925 to 26	Jul 20 to Sep 1	Mid Aug	Prytherch 1929
Long Island Sound, CT	1937 to 61	Start: Jul 9 to Aug 11 End: Aug 26 to Oct 17	Usually 2; timing variable	Loosanoff 1966
Delaware Bay: Cape May, NJ	1965 to 66	Late Jun to early Sep	Late Aug 1965; early Jul 1966	Hidu 1978
Chesapeake Bay: Maryland	1920 to 28 (5 years)	Early Jun to late Oct	2 peaks: mid Jul; late Aug-early Sep	Truitt 1928
	1942 to 53	Late May to Oct	Varied: late Jun to Sep, but usually Jul; Sometimes 2 peaks	Beaven 1955
	1962 to 66	Jun to late Aug	Usually 2 peaks of varying intensity	Shaw 1969
	1977 to 79	Late May to late Sep	Varied with year and location (see Table 6)	Kennedy 1980
Virginia	1946 to 54	Early Jul to early Oct	Usually 2 peaks: mid Jul; mid Aug to mid Sep	Andrews 1955
North Carolina	1988 to 90	Not stated	Concentrated in Sep to Oct	Ortega and Sutherland 1992
South Carolina	Many years	Early May to early Oct	Usually 2 peaks: early Jun to early Jul; Aug to early Sep	McNulty 1953
	1982 to 86	Start: late Apr to early May. End: late Oct to mid Nov	Usually 2 peaks: early Jun; late Jul to early Aug	Kenny et al. 1990
Gulf of Mexico:				
Apalachicola Bay, FL	1949 to 50	Mid Apr to mid Nov	Varied with location	Ingle 1951
Santa Rosa Sound, FL	1951 to 61	Start: early Mar to mid May End: mid Sep to late Nov	Varied	Butler 1965
Mobile Bay, AL	1977	22 Jul to 7 Oct	Two peaks; Sep peak larger	Lee 1979
Galveston Bay, TX	1929	Early May to Sep?	Usually 2 peaks: varied with location	Hopkins 1931
	1969	May to Oct	Jul and Sep to Oct	Moore and Trent 1971
Texas	1951 to 52	Mid May to mid Oct	Not stated	Menzel 1955

Brockport area of Long Island Sound tended to produce more oysters than other areas from 1944 to 1955, the rank order of 10 sampling stations in the Brockport area varied irregularly in recruitment intensity from year to year. In Canada, Drinnan and Stallworthy (1979b) found differences in eastern oyster recruitment over time in different tributaries of the Bideford River. Differences have also been observed for decades in two tributaries of the Choptank River in Maryland (Table 6), although Broad Creek has consistently experienced higher recruitment than has the adjacent Tred Avon River (Kennedy 1980).

Andrews (1983) summarized years of observations in Virginia and elsewhere by noting that high-flushing estuaries such as the James River would usually have consistent annual recruitment of low to moderate intensity, with a decline in recruitment intensity as one moved upriver from the mouth. In contrast, trap-type estuaries with their sluggish circulation and small drainage areas would experience higher but irregular recruitment (sometimes complete failure), with the intensity of recruitment increasing upstream. Andrews (pers. comm.) notes that Chesapeake Bay's small coastal plain subestuaries with low freshwater flow, sinuous morphology, shallow flats, grassy marsh margins, and deep channels allow extensive trapping of larvae.

Oyster larvae have been found to recruit differentially on an even smaller scale than estuary-wide. For example, Nelson (1903) quoted an experienced New Jersey oyster planter that "It sometimes happens that a

Table 6. Variability over time and space of peak recruitment periods (measured weekly as numbers of spat per 100 cm^2) of oyster larvae in two tributaries of the Choptank River, Maryland (1962 to 1966, Shaw 1969; 1977 to 1979, Kennedy 1980; 1983 to 1991, Newell and Kennedy, pers. obs.). Where two or more peaks occurred in a tributary in any one year, the major peak is italicised. After Kennedy (1986).

Year	Tred Avon River	Broad Creek
1962	Early August	Early and late July
1963	Early July; early August	Early July; *early August*
1964	*Early July*; mid August	*Early July*; early August
1965	Mid June; *early July*; late August	Early June; *late June-early July*; late August
1966	Late June; early July	Mid-late July
1977	Late September	*Late July*; Late August
1978	Almost no set	Minor peak in late July
1979	Late May; mid June; early July	Mid June; early July
1983	Mid July (very little set)	Early July; early September (limited set)
1984	No spatfall	No spatfall
1985	Early July; early August	Same weeks as Tred Avon River
1986	Mid July; early August	Late July
1987	Late July	Same week as Tred Avon River
1988	Almost no set	Late August (minor peak)
1989	No spatfall	No spatfall
1990	Minimal set	Minimal set
1991	Early July: *late July*; mid August	Late July: *mid August*

fair set will adhere to shells on one part of an oyster lot [bed] and none on other parts." In recent studies, Bushek (1988) found eastern oysters in Galveston Bay, Texas, to recruit more abundantly to piers within 10 m of shore and in quieter water than was available beyond 10 m in a seaward direction. He proposed that this horizontal difference in recruitment was the result of a preferential response by larvae to some combination of factors not revealed by the study. Kenny et al. (1990) found that recruitment at three locations within North Inlet Estuary, South Carolina, was similar in timing and abundance from 1982 to 1986, but that location of cultch with depth was a dominant source (36%) of recruitment variability.

Differences in settlement patterns may also occur at the microhabitat level. Nelson (1909) reported that shells exposed in nature side-by-side often differed greatly in numbers of spat. Of two wire bags of shell lying ca. 1 m apart on a tidal flat, one was covered with oyster spat while the other had almost none (Nelson 1953). Drinnan and Stallworthy (1979b) examined eastern oyster recruitment on scallop shells that they had attached to the same point on a stake driven into the bottom but that were deployed at right angles to each other at the four points of the compass. There were recruitment differences (to take just one example) such as a high of 394 spat per shell on shells oriented to the east and a low of 59 per shell on those on the same stake but oriented to the south. Microscale differences in settlement intensity also occur under experimental conditions. For example, Hidu (1969) reported that it was common for one or two of many exposed cultch shells to receive most of the settled spat in laboratory experiments.

Explanations for the intermediate and microhabitat differences just described are lacking, but probably involve variations in current movement and transport patterns, larval patchiness, physiological tolerances, and the presence of competitors and predators. Further exploration of the influence of scale on settlement and recruitment of the eastern oyster throughout its range is warranted. Michener and Kenny (1991) have done so for sites in South Carolina and found biological and physical factors to operate at different scales and intensities. Their paper provides a useful review of the relevant literature.

Effects of Environmental Factors

Peaks of settlement and recruitment intensity do not seem to occur with predictable regularity, even within a single location (Table 5). Indeed, they can vary from year to year and between closely adjacent tributaries (Table 6). However, there is evidence that some environmental factors may operate over a large area and affect recruitment success. For example, Drinnan and Stallworthy (1979c) found peak recruitment periods in Bideford River, Prince Edward Island, often to be synchronous throughout the estuary and its tributaries, an indication that (unknown) environmental factors were exerting a simultaneous influence on settlement and recruitment in the face of patchy distribution of such settlement. Similarly, in 1948, recruitment was reduced from 1947 values over most of Maryland's part of the Chesapeake Bay (Sieling 1950) as well as in the James River, Virginia (Andrews 1951). Salinity (and rainfall) is certainly implicated in widespread patterns of spat production, as demonstrated by a multivariate analysis of data for central Chesapeake Bay (Ulanowicz et al. 1980), but other factors undoubtedly have an influence and need to be examined.

Little is known about the influence of hydrography on settlement and recruitment patterns. Prytherch (1929) noted that larvae in Milford Harbor attached from low slack water through the first two hours of flood tide and claimed that a velocity of 10 cm s^{-1} inhibited setting. In areas without current, spat evenly covered collectors on all sides, but above 10 cm s^{-1}, spat were found mostly on the lee or protected side. Galtsoff et al. (1930) also found the zone of heaviest setting to coincide with the level of low slack water. Orton (1937) briefly reviewed this topic in relation to some bivalve species, noting that European flat oyster larvae settled more commonly where eddies occur, or in relatively still water. Korringa (1941) disputed these ideas, finding it difficult to understand how dispersed larvae could be reaggregated.

Seliger et al. (1982) proposed that success of spat recruitment in two tributaries of the Choptank river was negatively correlated with the persistence of strong local winds. For one tributary (Tred Avon River), spat recruitment appeared to be correlated nega-

tively with increased wind velocities. However, examination of his published data shows that this correlation does not hold for the adjacent, equally shallow tributary (Broad Creek) a few kilometers away.

Oxygen concentration may also affect settlement and recruitment. The presence of hypoxic conditions reduces larval settlement and anoxia almost completely prevents settlement (Baker and Mann 1992).

Nelson (1923) reported a correlation between the abundance of oyster larvae (no sizes given) in the water over a given site and the numbers of larvae recruiting at that site. Truitt (1931) also noted that recruitment was high in areas of Chesapeake Bay with abundant oyster larvae. However, Pollard (1973) found no correlation between total larval abundance and recruitment in Louisiana. And, after seven summers of study of oyster larvae in Prince Edward Island, Drinnan and Stallworthy (1979d) concluded that recruitment and the concentration of mature larvae in a region were not always correlated, and that other, unknown factors were involved. This topic merits further exploration.

In conclusion, more attention to settlement behavior in nature as well as in the laboratory is needed to reach clear understanding of the factors attractive to larvae and responsible for stimulating settlement. In addition, there has been much recent interest in the potential role of larval recruitment in influencing population ecology of benthic marine invertebrates (e.g., Roughgarden et al. 1985, 1988) and subsequent community ecology (e.g., Fairweather 1988; Underwood and Fairweather 1989). Attention has focused mainly on rocky intertidal systems, but oysters would be suitable as study material to enhance our understanding of these matters in estuaries. Finally, "supply-side" ecology has management implications (Fairweather 1991) that remain to be explored for oyster populations.

LARVAL AND SPAT SURVIVAL AND GROWTH

Estimates of mortality of larvae and spat are necessary for adequate management of oyster stocks, yet such estimates are difficult to procure. Ideally, obtaining such estimates requires the ability either to monitor larval cohorts in the plankton or to contrast the production of larvae with the subsequent production of recruits (i.e., spat). Nelson and Perkins (1931) reported that the season's first brood of larvae yielded about 79,400 larvae per 100 L of pumped Barnegat Bay water on day 4 after spawning, 4,275 by day 9, and 84 (eyed or mature) by day 13. These values yield an instantaneous daily mortality coefficient (Rumrill 1990) of about -0.76 (this value seems high and it should be noted that Nelson and Perkins [1931] reported that larvae collected in the summer of 1930 showed evidence of disease and that spat settlement was poor that year).

Quayle (1988) summarized years of data from Pendrell Sound, Canada, on the relationship between initial numbers of straight-hinge larvae of the Pacific oyster and numbers of competent larvae. He reported that mortality generally varied between 91 and 99.5%, with total mortality occurring in some years. A 7-year study of production of eastern oyster larvae in Bideford River, Canada, found (for example) a range of daily mortality of 5% (July) to 30% (August) in 1958 (Drinnan and Stallworthy 1979a) and an average daily mortality of 20% in 1959 (Drinnan and Stallworthy 1979b). These values are generally similar to the daily mortality rates of 13 to 28% presented by Rumrill (1990) for a number of species of marine invertebrates with meroplanktonic larvae (note that Jorgensen [1981] reported the daily mortality in the plankton for a single cohort of blue mussel larvae to be 13%).

Mortality rates are affected by environmental variation, and a relatively small change in larval mortality can have a major influence on recruitment (Houde 1987; Underwood and Fairweather 1989). These effects can be illustrated for the eastern oyster (Table 7) by using the average daily mortality coefficient of 20% developed by Drinnan and Stallworthy (1979b) for 1959 and their estimate of a 20-d period of larval existence (Drinnan and Stallworthy 1979a). In this example, the presence of "favorable" conditions would result in the settlement and metamorphosis of 183,200 spat from the single spawning of one female (estimated at 1×10^7 eggs). A 25% increase in mortality or 25% delay in metamorphosis

would each produce a loss of 63% of potential spat. If the increased mortality accompanied the delayed metamorphosis, there would be a decline of 89% in spat compared with "favorable" conditions.

Variations from "favorable" conditions may be very common in nature. For example, Quayle (1988) reported that for Pacific oysters in Pendrell Sound, British Columbia, from 1950 to 1955, the length of the larval period nearly doubled from about 18 d at ca. 22°C to about 30 d at ca. 18°C. Such delayed growth would have a substantial effect on survival (in my example above of 20% daily mortality, 273,000 spat would be produced at 22°C but only 25,000 at 18°C, a decline of 91%). As Houde (1987) indicated, field surveys of larval survival are probably not capable of distinguishing differences of 25% in instantaneous natural mortality coefficients, yet my example shows that such a difference strongly influences variability in spat recruitment (Table 7). Houde (1987) proposed that such coarse controls in the larval stage for fish may have more significance for recruitment than finer controls in the juvenile stage when changes in mortality and growth rate are smaller. This relationship may be true for oysters also.

Only a limited amount of quantitative information is available on the extent of mortality suffered by oyster spat. Korringa (1941) estimated that only about 250 flat oyster larvae out of each one million produced in a Dutch estuary survived to metamorphose, and of the resultant spat, 95% died before winter. This estimate compares well with Waugh's (1972) estimate of 93% mortality for European flat oyster spat after 1 year in English waters. Estimates for young eastern oysters are also high. For Long Island Sound in 1937, Loosanoff and Engle (1940) found spat mortality over a 51 to 52 d period in summer to range between 86 and 100% (the result they thought, not of chemical or physical factors, but of predation by starfish and oyster drills). Nelson and Chestnut (1945) noted that only about 1 of 630 eastern oyster spat per 6 cm^2 survived at the end of a year under crowded conditions. In South Carolina, spat younger than 6 weeks and up to 15 mm long that were transplanted in July from the intertidal region to a depth of about 0.7 m below low water suffered 100% mortality within a month, with many of the emptied shells bearing drill holes (Smith 1949). Recently, Osman and Abbe (1995) implicated periodic intrusions of hypoxic and anoxic water into Chesapeake Bay shallows in the low survivorship and decreased growth of post-settlement eastern oysters.

Field studies have shown that settlement and recruitment of oyster larvae often has high inter-regional and interannual variability. Whereas Nelson (1957) reported that natural sets failed completely in only 2 of 30 summers on the Cape May flats of Delaware Bay, Loosanoff (1974) wrote that between 1904 and 1961 in Long Island Sound, excellent recruitment of oyster larvae occurred in only 8 years, with the remaining sets being "marginal" or nonexistent. Because the Sound contained large numbers of spawning oysters over this period, he attributed the lack of

Table 7. Hypothetical production of eastern oyster spat resulting from spawning of 1×10^7 eggs by a single female under conditions that are favorable and unfavorable (25 % increase in mortality; 25 % delay in metamorphosis). M = instantaneous mortality coefficient (d^{-1}), from Drinnan and Stallworthy (1979b). N = number of spat, rounded to nearest 100.

Condition	M	Age at metamorphosis (d)	N	Decline (%)
Favorable	0.20	20	183,200	—
Increased mortality	0.25	20	67,400	63
Delayed metamorphosis	0.20	25	67,400	63
Increased mortality and delayed metamorphosis	0.25	25	19,300	89

recruitment to larval mortality, and went on to examine possible causes of mass mortalities in nature. He did not believe that food was limiting in the Sound, and found no relationship between setting intensity and water temperature, salinity, or pH. He indicated that even a hurricane in 1955 did not hinder setting immediately after its passage, in spite of the associated prolonged and intensive turbulence and turbidity. He was not persuaded that predation was the cause of mass mortalities, pointing out that larval populations of one species would suddenly disappear from regularly-collected plankton samples while the larvae of other species maintained their numbers. He concluded that disease and the presence of external metabolites from dinoflagellate blooms were the major potential causes of mass mortalities of eastern oyster larvae (supplemented in later years by anthropogenic toxins).

There may indeed be a limited number of causes of mass mortalities of larvae in nature. Nevertheless, there are a variety of factors that have been implicated in mortality in general, and I consider them briefly.

Food and Feeding

Relatively little is known about the food of oyster larvae and spat and its effects in nature, with most studies concentrated on aquaculture-related topics (feeding and digestion by larval eastern oysters are described in more detail by Newell and Langdon in Chapter 5 and Langdon and Newell in Chapter 6). Baldwin and Newell (1991) have shown that eastern oyster larvae are omnivores, ingesting bacteria, phagotrophic protozoans, and phototrophs, organisms common in estuaries in summer. Baldwin (1995) and Baldwin and Newell (1995a, b) also demonstrated that eastern oyster larvae adjusted their feeding rates depending on food particle size and plankton cell type or chemical composition.

His and Seaman (1992) have shown that the availability of food within about 3 d after spawning is important to the survival of larval Pacific oysters in the laboratory, apparently because lack of food impairs subsequent digestive ability. Food availability has implications for larval survival in nature where food supplies may be more dilute than in the laboratory. However, larval eastern oysters may not be as sensitive as Pacific oysters to limitations in food supply; Loosanoff (1974) noted that his studies and those of others showed that eastern oyster larvae tolerated days or weeks of starvation, resuming growth when food was supplied again (R. Mann, Virginia Institute of Marine Science [pers. comm.] believes that nutriment may have inadvertently been a contaminant in the "starvation" protocols, thus maintaining the eastern oyster larvae). Millar and Scott (1967) also found *O. edulis* larvae released from the female to be resistant to starvation.

It is not clear if oyster larvae are food limited in nature. Jorgensen (1981) did not find this to be true for the cohort of blue mussel larvae he studied. Olson and Olson (1989) reviewed the topic of food limitation for marine invertebrate larvae and concluded that the evidence was equivocal as to whether or not larval starvation was important to the success of subsequent recruitment in molluscs, including oysters. They postulated that nearshore or estuarine species of invertebrates were less likely to be food limited. Fritz et al. (1984) found no evidence that eastern oysters in Delaware Bay would be subject to critically low feeding rates (see also Loosanoff [1974] for Long Island Sound and Abbe [1986] for Chesapeake Bay). As noted earlier, eastern oyster larvae are omnivores (Baldwin and Newell 1991) and apparently resistant to starvation stress (Loosanoff 1974). Although more evidence is needed, it seems reasonable to hypothesize that recruitment success of eastern oysters is not usually affected by starvation of larvae. However, the presence of blooms of nutritionally inadequate or inappropriate algae may indeed play a role in larval mortality, as demonstrated for bay scallop, *Argopecten irradians*, larvae (Nelson and Siddall 1988). In addition, hypoxia and anoxia may hinder feeding and energy metabolism (Widdows et al. 1989; Baker and Mann 1994b).

Strathmann et al. (1993) demonstrate that developing veligers (> 200 μm) of *C. gigas* reared in low concentrations of algal food (3,000 cells ml^{-1}) had significantly larger velar lobes and longer prototrochal cilia than veligers fed high concentrations of algae (30,000 cells ml^{-1}). This observation suggests that developmental plasticity is an adaptive advantage for

producing larger organs for food capture under conditions of food scarcity. It may be possible to use some aspects of relative size of velar structures of oysters as an index of food availability in nature.

Depletion of Biochemical Reserves

Even if food quantity and quality are satisfactory for development of the feeding veliger stages, the amount of food reserves available to the pre-feeding larva during embryogenesis and to the metamorphosing spat may be limiting (see Thompson et al., Chapter 9). As the fertilized egg develops to the initial veliger stage, it depends on endogenous energy supplies placed in the egg by the female (Holland 1978). Such supplies may be negatively influenced by the presence of stressors that affect the adult, with a resultant reduction in larval vigor (Bayne 1976).

Lannan (1980a, b) demonstrated in a hatchery system that non-genetic (artificial rearing conditions) and genetic (optimally conditioned gonads) factors affected the variance in survival of larval Pacific oysters. The genetic variance was non-additive, depending largely on the gonadal development of parents. That is, for maximum larval survival, gametes should be shed by the parents when gonadal development is "optimal." Following from this finding, Gallager and Mann (1986) related the state of gonadal development in *C. virginica* to fluctuations in egg lipid concentrations and examined the potential for depressed concentrations to have a negative effect on the pre-feeding larval stages. They found the content of egg lipids (predominantly triglycerides and phospholipids) to be correlated with the yield of straight-hinge larvae derived from a single spawning, but not with subsequent larval growth. This finding suggests that such reserves significantly affect embryogenesis but not subsequent larval development after exogenous feeding begins.

Compositional changes in biochemical reserves mobilized by eastern oysters during embryogenesis and metamorphosis have received less study (see Chu and Webb 1984) than such changes in European flat oysters (e.g., Holland and Spencer 1973; Rodriguez et al. 1990) and other bivalves (e.g., Marty et al. 1992; Whyte et al. 1992). Similarly, the investigation of biochemical factors that influence growth in larvae, such as the work of Delaunay et al. (1992) on differential growth among hatchery-reared batches of scallop larvae, remains to be performed on eastern oyster larvae.

Predation, Competition, and Recruitment

Predation on oyster larvae in nature is undoubtedly extremely high and deserves greater study (see White and Wilson, Chapter 16 for more details). Briefly, a variety of filter-feeding zooplankters such as ctenophores prey on larvae in the water column (e.g., Purcell et al. 1991). Nelson (1925a, b) found 126 early eastern oyster larvae in one 3-cm ctenophore, *Mnemiopsis leidyi*, and reported good recruitment of oysters where ctenophores were relatively rare in 1921 and 1922. However, Nelson and Perkins (1931) noted that significant declines in larval abundances in Barnegat Bay in 1930 occurred in the absence of the ctenophore. Loosanoff (1966) disputed Nelson's (1925a) hypothesis that ctenophores can prey so heavily on larval oysters that settlement is compromised, citing an instance of excellent setting intensity in Long Island Sound in 1944, also a year of abundance for ctenophores. As Purcell et al. (1991) suggested, although the ctenophore is a predator of oyster larvae, the proportion lost to this predator in nature may be slight.

Losses to benthic organisms may also affect abundances of oyster larvae. Sea anemones and barnacles feed on or capture larvae (MacKenzie 1977; Steinberg and Kennedy 1979), as do solitary ascidians (Osman et al. 1989), the benthic scyphistoma stage of the cnidarian, *Chrysaora quinquecirrha* (Purcell et al. 1991), and perhaps benthic ciliates (Loosanoff 1959). Nelson (1921a) reported that molluscan larvae could be found in stomachs of adult eastern oysters, and presented a photograph of some of the 63 oyster larvae at the umbo stage that he removed from one oyster's stomach. He also reported finding 71 oyster larvae in one oyster's feces, with most capable of swimming when placed in fresh sea water. Quayle (1988) also stated that Pacific oyster larvae (live and dead) could be found in the feces and pseudofeces of adults. (André et al. [1993] examined predation by adult cockles on settling cockle larvae and estimated that one-third of the larvae were inhaled by the adults

within a 5 cm^2 region around the adults' siphons, and that benthic suspension feeders could affect recruitment to the benthos negatively.) Finally, Nelson (1903) noted that an unnamed species of *Gammarus* could capture oyster "spawn" but gave no further details.

Spat on shell and spat produced in the absence of cultch ("cultchless oysters") can suffer almost total mortality from predation by blue crabs, *Callinectes sapidus* (Lunz 1947; Krantz and Chamberlin 1978), and young oyster drills, *Urosalpinx cinerea* (Carriker 1951). Mud crabs are also important predators of spat (McDermott 1960; Abbe and Breitburg 1992) in central Chesapeake Bay. The predatory flatworm, *Stylochus ellipticus*, is able to open and eat spat as large as 61 mm long (Landers and Rhodes 1970). Microscopic juvenile *S. ellipticus* prey heavily on newly-settled eastern oysters in Maryland's Chesapeake Bay (Newell and Kennedy, pers. obs.). The potential role of micropredators in determining recruitment (Osman et al. 1992) onto oyster beds invites further study.

Competition is probably more important in the early benthic phase of the eastern oyster's existence than in the pelagic phase. Osman et al. (1989) found that limited growth and early mortality of newly settled spat were associated with the presence of a variety of sessile invertebrates that also occupied the cultch. Of the potential factors that may have contributed to reduced growth and survival of spat (physical factors in the environment, predation, competition for space, competition for food) it appeared that competition for food was of major significance (Zajac et al. 1989). However, as Osman et al. (1990) demonstrated with additional experiments, ontogenetic changes in trophic relationships can complicate interactions among eastern oyster larvae and spat and associated predators and competitors. Roegner (1991) has found evidence that mortality of newly-settled oysters in nature is initially density independent, but that does not allow for dependable estimation of settlers from data on recruits because of subsequent high mortality. Intraspecific competition for space also occurred among Roegner's (1991) study animals. Continued research is needed to tease apart the roles of predation, competition, and abiotic factors in settlement and recruitment of eastern oysters over a variety of time and space scales.

Disease

Infectious diseases (those caused by parasites or pathogens) are little understood in natural populations of oyster larvae, much of our knowledge being based on studies of cultured larvae (see Elston 1979 and Sindermann 1990 for reviews). Bacterial infections seem to be more common than viral or fungal infections in larvae; Ford and Tripp review information on larval vibriosis in Chapter 17. Non-infectious diseases (abnormalities in structure and function related to genetic and environmental factors) are also not well understood, especially for natural populations of larvae (Sindermann 1990). Elston (1984) reports on ways to prevent or control infectious disease in culture systems. However, the importance of disease in survival, growth, and settlement of oyster larvae is an area ripe for exploration.

Physiological Tolerances

The tolerances of larvae and spat of eastern oysters to natural environmental stressors are reviewed by Shumway in Chapter 13. Carriker (1986) has provided an extensive review of the literature on the effects of suspended particles on eastern oyster larvae. Both papers provide a broad entry into the relevant literature.

Larval and Spat Growth

Larvae

There is much variability in the maximum shell lengths of larvae recorded over the eastern oyster's range. In Canada, Stafford (1912) reported a height (umbo to opposite shell margin) of 359 μm and a length (longest anterior to posterior distance, roughly parallel to shell margin) of 386 μm for the largest free-swimming larva he collected.[5] Medcof (1939)

[5] Loosanoff and Davis (1963) drew attention to the disparity between the length:height relationship reported by Stafford (1912) and those reported by other investigators such as Medcof (1939). The drawings of larvae in Stafford (1913) are consistent with his reported lengths and heights, with lengths remaining greater than height in older larvae. No one else has found oyster larvae to maintain such a relationship after the umbo begins to grow.

gave a modal height of 365 μm and a length of 335 μm for the prodissoconchs of 194 freshly-settled spat in Prince Edward Island. For Milford Harbor, Connecticut, Prytherch (1934) estimated an average size of 330 × 220 μm for wild metamorphosing larvae, and Loosanoff and Davis (1963) found most cultured larvae to metamorphose between 275 and 315 μm in length in the laboratory (rarely, free-swimming larvae measured 355 μm). Nelson (1921b) reported that setting larvae in New Jersey were about 300 μm long, and Carriker (1951) collected a 306 μm long larva in Great Bay, New Jersey. In Florida, Forbes (1967) reported that the apparent longitudinal axis (the length when viewed from directly above) of newly settled spat averaged 317 ± 16.1 μm (N = 150), with the mean length of the left valves of disarticulated prodissoconchs being 301 ± 18.2 μm (N = 50).

Clearly, the size at settlement of eastern oyster larvae decreases between Canada and more southerly locations. This point was first made by Nelson (1916), who reported his personal observations that pre-metamorphic larvae in Canada were 25% larger at setting than larvae in New Jersey, and who wondered if this indicated the existence of a boreal variety of the species. It is not clear that temperature is responsible for this latitudinal difference. The length of larval life has been estimated for a number of locations and temperatures (Table 8). As expected, the period of time spent as a larva decreases as temperature increases. However, when the data are graphed (Fig. 8), they show that larvae in Prince Edward Island remain longer in the plankton before settling than southern larvae at the same temperature, perhaps accounting for their greater size (see below).

Data on growth of eastern oyster larvae in nature are limited (Fig. 9). The daily growth exhibited by the first brood of larvae to appear in Barnegat Bay in 1922 over a temperature range of 20 to 24°C (Nelson 1923) was faster than that reported for Canadian locations at temperatures within the lower temperature range for Barnegat Bay (Medcof 1939; Stallworthy 1979). This observation may be even more significant in that the Bideford River data are for shell heights, whereas the Barnegat Bay data are for shell lengths, a lesser dimension. Within Bideford River, however, there were differences in daily growth rates measured by different observers (Fig. 9). Growth in the Bras d'Or Lakes to the east of Prince Edward Island was slightly slower than in Bideford River. The available data do not allow for statistical testing of the data for significant differences. The subject of growth of oyster larvae in nature, both within and among regions, requires additional research.

In contrast to the field data above, comparative data for two laboratory experiments show that larvae from Canadian oysters grew as rapidly as Virginia oysters at the same general temperatures for at least the first 8 d (Fig. 9; the results shown are for the fastest brood out of eight reared in the Canadian experiments). If Canadian larvae can indeed grow about as rapidly as their southern counterparts while remaining longer in the plankton, their greater size at settlement can thus be explained.

Salinity has a less limiting effect on larval growth than does temperature. In the laboratory, eastern oyster larvae grew optimally between 30.0 and 32.5°C in salinities of 10 to 27.5 ppt (Davis and Calabrese 1964). However, there was no optimal salinity for growth, with maximum growth occurring over a wide salinity range (about 15 or 20 ppt to 27 ppt) when temperatures ranged between 17.5 and 32.5°C. These data are for larvae from one location, Long Island Sound. Comparative data for other regions and for various salinity regimes are limited. Newkirk (1978) found that larvae produced by oysters from four populations in eastern Canada and reared at four salinities exhibited as much difference in survival between groups from the same estuary as between estuaries.

Widdows et al. (1989) determined relationships among shell length (SL, μm), dry mass (DM, μg), and ash-free dry mass (AFDM, μg) of larvae reared in Virginia. These relationships are: $DM = (2.48 \times 10^{-5}) SL^{2.073}$; $AFDM = (9 \times 10^{-6}) SL^{2.066}$.

Spat

The influence of environmental factors on growth of spat is a topic that has received minimal study. As part of a larger study on oxygen and eastern oysters, Baker and Mann (1992) found hypoxia (20% of air saturation) to inhibit postlarval growth up to 144 h post settlement. Osman and Abbe (1993) associated

low oxygen concentrations in deeper water with a significant decrease in growth rate of post-settlement eastern oysters with increasing depth. The influence of oxygen and other abiotic factors on spat is an area ripe for study.

There is also a need for comparative studies of spat growth. Beaven (1950) described growth of oysters set on shell and held in rafts at different sites in central Chesapeake Bay, noting differences in growth on different oyster beds. Paynter and DiMichele (1990) compared growth in two experimental groups of cultchless oysters held in trays in mesohaline Chesapeake Bay. The parents of one group were from a wild population, whereas the other parents were from a population that had been selectively bred for rapid growth over 18 generations. Newly-settled spat from the selected population exhibited fast growth (48 mm from July through October, 1987). First and second year growth rates were about 15 mm and 12 mm month^{-1}, respectively, for the "selected" spat, significantly higher rates than those of about 10 mm and 8 mm month^{-1}, respectively, for the "wild" spat. Growth ceased in both groups in late November at 10°C. Growth rates achieved by oysters from both populations were about 5 to 10 times higher than for spat of New Brunswick oysters reared in Prince Edward Island

Table 8. Estimated length of larval life (days to settlement) of the eastern oyster from Canada to Florida. Abbreviations refer to states in the USA.

Location	Water temperature (°C)	Larval life (d)	Reference
Bideford River, Prince Edward Island	19	30	Medcof 1939
	20	26	Medcof 1939
	21	24	Medcof 1939
	18	30	Needler 1940
	20	26	Needler 1940
	21	24	Needler 1940
	19 to 20	24.3	Stallworthy 1979
	21 to 22	19.8	Stallworthy 1979
Great South Bay, NY	21	12 to 14	Churchill and Gutsell 1921
Milford Harbor, CT	20 to 23.2	13 to 18	Galtsoff et al. 1930
Barnegat Bay, NJ	21 to 24	14 to 16	Nelson 1923
	20	17	Nelson 1928
	23 to 25	13	Nelson 1928
	? to 29	12	Nelson and Perkins 1932
Milford,CT (laboratory studies)	23	18	Loosanoff and Davis 1963
	30	10	Loosanoff and Davis 1963
Gulf Breeze, FL (laboratory studies)	24	19	Forbes 1967

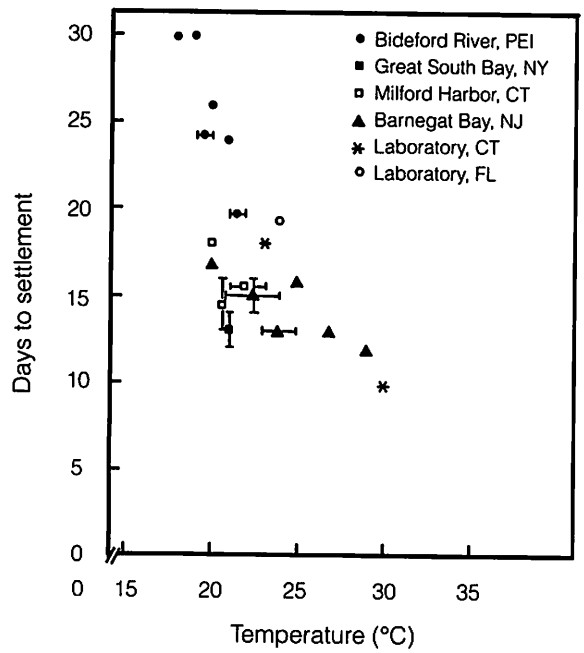


Figure 8. Days to settlement of eastern oyster larvae in relation to temperature at Bideford River, Prince Edward Island; Great South Bay, NY; Milford Harbor, CT; Barnegat Bay, NJ; Milford Laboratory, CT; and Gulf Breeze Laboratory, FL (see Table 8 for references). Lines associated with symbols encompass the range(s) of days or temperatures, with the symbol at the mid-point of the range(s).

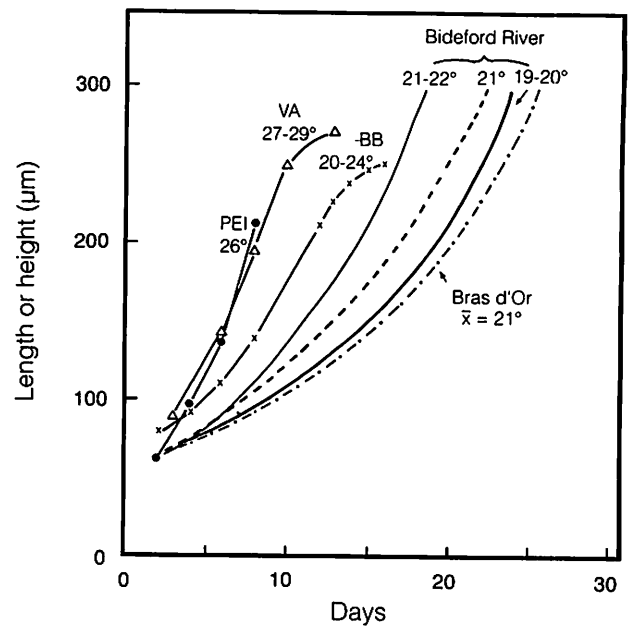


Figure 9. Daily growth of eastern oyster larvae in Barnegat Bay, NJ (BB: Nelson 1923), Bideford River, Prince Edward Island (dashed line: Medcof 1939; solid lines: Stallworthy 1979), and Bras d'Or Lakes, Nova Scotia (Stallworthy 1979), and in laboratories in Virginia (VA: Chu et al. 1987) and Prince Edward Island (PEI: Mallet and Haley 1983; the data are for the Bouctouche oysters). The BB, PEI, and VA data are for shell lengths; the remaining data are for shell heights.

waters (Mallet and Haley 1983). Finally, Crosby et al. (1991) found different growth rates for recently settled spat within and between intertidal and subtidal habitat in South Carolina tidal creeks. Initially, growth rates subtidally outpaced intertidal rates, but the latter surpassed the subtidal rates about 36 d post-settlement.

Little research has been performed on the relation between growth of larvae and of spat. Newkirk et al. (1977) found larval and spat growth rates in the eastern oyster to be highly correlated and capable of being selected. Heritability of larval growth rate was 0.25 to 0.50, with much of the genetic variation being additive. Losee (1979) discovered that spat derived from faster-growing larvae (i.e., from larvae that set during the first 3 d of a setting period) were significantly larger 29 weeks later than spat that settled later in the setting period. The topic is important and merits further attention.

FUTURE RESEARCH DIRECTIONS

Despite the extensive research reviewed here, there are still numerous gaps in our knowledge of the biology, behavior, and ecology of oyster larvae and spat. However, because the eastern oyster is a commercially and ecologically important species facing population declines due to disease, overfishing, habitat destruction, and pollution, there is much interest in protecting the resource. Successful efforts in these directions will require continued emphasis on research into how larvae are dispersed in nature, and how they survive, encounter and test cultch, and settle. Oyster farming practices (aquaculture) may also require such insights, but more likely will continue to focus on ways to produce viable larvae in hatcheries, rear them successfully

to settlement in the least time possible and with the least mortality, and protect the young spat as much as possible in "growout" facilities.

Beyond the application of commercially-relevant knowledge, there are major questions to be answered about the basic biology of eastern oyster larvae and spat. Many of the questions posed by ecologists interested in mechanisms of recruitment to and competition within benthic assemblages and communities can be answered through use of the eastern oyster as a model. The species can be spawned and cultured readily and the sessile spat stage is easily manipulated experimentally. In addition, the roles of the young stages in energy flow and trophic interactions are only sketchily understood, but new techniques of encapsulating food and tracing its passage are being developed. Finally, the development of sensitive technology should soon allow larvae to be tracked and perhaps even manipulated in field experiments, thus adding to our understanding of dynamic biological oceanography.

ACKNOWLEDGMENTS

I am indebted to J. Andrews, S. Baker, P. Baker, W. Boicourt, M. Carriker, S. Coon, W. Hargis, H. Haskin, E. Houde, J. Kraeuter, R. Mann, D. Meritt, R.I.E. Newell and L. Sanford for comments on the manuscript, and to Deborah Coffin Kennedy for the figures. UMCEES Contribution No. 2471 of the Center for Environmental and Estuarine Studies.

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Chapter 11

Biochemical and Population Genetics

PATRICK M. GAFFNEY

INTRODUCTION

In Galtsoff's time, little was known about the genetics of the eastern oyster, *Crassostrea virginica*; indeed, the word genetics is not indexed in his treatise (Galtsoff 1964). Since then, our knowledge of oyster genetics has grown considerably, owing to (1) the development of hatchery methods for routine spawning and rearing of oysters, allowing controlled breeding experiments, and (2) the advent of techniques (e.g., gel electrophoresis) for the examination of genetic variation at relatively large numbers of gene loci. The past two decades have witnessed a variety of electrophoretic studies of the population genetics of species of *Crassostrea*, particularly the eastern oyster. In recent years molecular methods, e.g., analysis of restriction fragment length polymorphisms (RFLPs) in nuclear and mitochondrial DNA, have begun to deepen our understanding of population structure in the eastern oyster.

BIOCHEMICAL GENETICS

Early studies of biochemical variation that might be genetic in origin (as opposed to clearly non-genetic variation, e.g., seasonal variation in carbohydrate content) were typically undertaken in order to delineate patterns of geographical variation. For example, Hillman (1964) reported a consistent difference in chromatograms of hemolymph taken from oysters from Long Island Sound and James River, Virginia, despite a long period of acclimation by the oysters to a common laboratory environment. Li et al. (1967) reported that oysters from a population resistant to Malpeque disease (Malpeque Bay, Prince Edward Island) possessed a serum antigen lacking in a disease-susceptible population (Bras d'Or Lake, Nova Scotia) and suggested this difference might be genetic in origin. A major drawback to studies of this kind was the unknown nature of the peptides or antigens that differed among populations, making it difficult to ascertain that these differences were indeed of genetic origin. The introduction of electrophoretic methods to population biologists in the 1960s was a signal event because it allowed known gene products (enzymes) to be routinely screened in large numbers of individuals (see Lewontin 1974 for review).

Enzyme-coding Loci

Several electrophoretic surveys of eastern oyster populations have documented considerable genetic variation in enzyme-coding loci. Electrophoretic mobility variants (electromorphs) of an enzyme coded by different alleles at a single locus are termed allozymes (in contrast to isozymes, electromorphs produced by different gene loci). Thirty-one of 42 loci (74%) detected by electrophoretic methods in *C. virginica* are found to be polymorphic, where a polymorphic locus is defined as one for which the common allele has a frequency of less than 0.95 in at least one population sample (Table 1). This level of polymorphism is comparable to or higher than that found in other invertebrate species (Selander 1976; Nevo 1978).

Table 1. Enzyme systems that have been examined electrophoretically in the eastern oyster *C. virginica*. Numerical suffixes attached to locus designations indicate electrophoretic mobility, in decreasing order from the most rapidly migrating isozyme locus. For some enzymes, the number of loci indicated is questionable, as homologies among various studies are not always apparent. E.C. = Enzyme Code (- = terminal number not assigned); N = maximum number of alleles recorded in a single study; m = monomorphic (frequency of most common allele > 95%).

Enzyme	E.C.	Locus	N	References[a]
acid phosphatase	3.1.3.2	AcP-3	6	1, 3, 7, 8
		AcP-2	m	
adenylate kinase	2.7.4.3	Adk-1	6	1, 3, 7, 8, 10
		Adk-2	m	
alanopine dehydrogenase	1.5.1.17	AlpDH	3	14
aldehyde oxidase	1.2.3.1	Ao	4	4
aminopeptidase	3.4.13.-	Ap-1	6	1, 3, 5, 7, 8,
		Ap-2	m	9, 11, 13
aspartate aminotransferase	2.6.1.1	Aat-1	4	1, 2, 3, 4, 5,
		Aat-2	4	7, 8, 10, 13
carboxylesterase	3.1.1.1	Est-1	7	1-9, 11
		Est-2	m	
		Est-3	5	
catalase	1.11.1.6	Cat	2	1
creatine kinase	2.7.3.2	Ck	m	1
fructose bisphosphate aldolase	4.1.2.13	Ald	2	3, 8
glucosephosphate isomerase	5.3.1.9	Gpi	8	1-13
glucose-6-phosphate dehydrogenase	1.1.1.49	G6pd	2	1
glutamate dehydrogenase	1.4.1.2	Gdh	3	1, 10
glyceraldehyde-phosphate dehydrogenase	1.2.1.12	Gly3pd	3	3, 8, 10
glycerol-3-phosphate dehydrogenase	1.1.1.8	αGlypd	m	3
hexokinase	2.7.1.1	Hk	m	1,3,8,10
L-iditol dehydrogenase	1.1.1.14	Sdh	4	1,3,7,8
isocitrate dehydrogenase (NADP+)	1.1.1.42	Idh-1	4	1,3,7,8
		Idh-2	3	
leucine aminopeptidase	3.4.11.-	Lap-1	7	1-4,6-13
		Lap-2	7	
malate dehydrogenase	1.1.1.37	Mdh-1	4	1,3,7,8,10
		Mdh-2	3	
malate dehydrogenase (decarboxylating) (NAPD+)	1.1.1.40	Me	m	1,3,8
mannosephosphate isomerase	5.3.1.8	Mpi-2	7	1, 7, 8, 13
phosphogluconate dehydrogenase (decarboxylating)	1.1.1.44	6Pgd	6	1, 3, 7, 8, 10, 13
octanol dehydrogenase	1.1.1.73	Odh	3	1
phosphoglucomutase	2.7.5.1	Pgm-1	7	1-13
		Pgm-2	4	
purine nucleoside phosphorylase	2.4.2.1	Nsp	m	1
pyruvate kinase	2.7.1.40	Pk	m	1
superoxide dismutase	1.15.1.1	To-1	m	1,3,8
		To-2	2	
strombine dehydrogenase	1.5.1.-	StrDH	4	14
triosephosphate isomerase	5.3.1.1	Tpi	4	10
xanthine dehydrogenase	1.2.1.37	Xdh	2	1,3,4,8,10,13

[a]References:

(1) Schaal and Anderson 1974
(2) Singh and Zouros 1978
(3) Buroker et al. 1979
(4) Zouros et al. 1980; Foltz et al. 1983
(5) Groue and Lester 1982
(6) Koehn and Shumway 1982
(7) Buroker 1983a
(8) Buroker 1983b
(9) Gaffney and Scott 1984
(10) Hedgecock and Okazaki 1984
(11) Rodhouse and Gaffney 1984
(12) Rose 1984
(13) Foltz and Chatry 1986
(14) Gaffney, unpublished data

Despite the large number of geographical surveys of allozyme frequencies in the eastern oyster, there have been few detailed studies characterizing allelic variation at individual loci. Martin (1979) described kinetic and biochemical properties of three common glucosephosphate isomerase (*Gpi*, also abbreviated *Pgi*) genotypes in *C. virginica*. He found few differences among the genotypes, with the exception of specific activity, in which the M/M genotype exceeded the other two genotypes (M/S and S/S) by 50%.

Pogson (1989) purified and characterized the four most common allozymes at the *Pgm-2* locus in the Pacific oyster, *Crassostrea gigas*. He observed significant differences among genotypes in kinetic parameters, attributable largely to the divergent properties of the $Pgm\text{-}2^{92}$ allozyme. Inferred fitness differences could not explain allelic frequencies in cultured and natural populations, however, nor could they account for the association reported between heterozygosity at this locus and size (see p. 426).

Non-enzyme Proteins

Relatively little attention has been paid to genetic variation in non-enzyme proteins in *C. virginica*, probably due to the relative abundance of enzyme variants. Hedgecock and Okazaki (1984) described three electrophoretically invariant muscle proteins; these are probably the same as those described by Buroker (1983b), who reported that one muscle protein locus, *Mp-1*, was polymorphic in the western Gulf of Mexico but monomorphic elsewhere. Populations from Nova Scotia and western Florida were described as fixed for different alleles at this locus by Buroker et al. (1979).

Effects of Biochemical Genetic Variation on Physiology

The question of whether the genetic variation frequently observed at enzyme loci is of adaptive importance or is merely neutral molecular "noise" has occupied the attention of population geneticists ever since the introduction of gel electrophoresis to population biology (Lewontin 1974; Crow 1985). As it became apparent that survey data on allelic frequencies in natural populations were insufficient to resolve this question definitively, investigators turned to scrutinizing allelic variants at individual polymorphic loci in order to assess their effects, if any, on fitness (Dykhuizen and Hartl 1983; Hartl and Dykhuizen 1985). This approach has been exemplified by the studies of Koehn and colleagues on the leucine aminopeptidase (*Lap*) polymorphism in the blue mussel, *Mytilus edulis* (Koehn 1978; Koehn and Hilbish 1987). According to this school, the following criteria must be satisfied in order to demonstrate that an allozyme polymorphism is of adaptive significance: (1) the enzyme variants (allozymes) must differ in one or more catalytic properties; (2) the catalytic differences must cause physiological differences *in vivo*; (3) these physiological differences must result in phenotypic differences; and (4) the differences in phenotype must lead to differences in fitness among individuals. These criteria have been met in studies of *Lap* in *M. edulis* (Koehn and Hilbish 1987).

Most studies of genetic variation in *C. virginica* have been simple allozyme surveys; no other enzyme polymorphism in bivalve molluscs has been the subject of such rigorous scrutiny as that devoted to *Lap* in *Mytilus edulis*. However, several studies have pointed to physiological differences among individual *C. virginica* associated with allozyme genotype.

Singh and Zouros (1978) observed significant differences in growth rate among genotypes at the *Got-1* (glutamate oxaloacetate transaminase, synonymous with aspartate aminotransferase, *Aat*) locus in a year-old cohort of *C. virginica* from Nova Scotia. Homozygotes of the 4/4 genotype were significantly larger than the 2/4 heterozygote and the 2/2 homozygote. (Numerical designations of alleles refer to their mobilities on electrophoretic gels. Thus, a 4/4 individual is a homozygote, carrying two copies of the 4 allele; a 2/4 individual is a heterozygote, with one copy of the 2 allele and one copy of the 4 allele.) Two years later, another cohort from the same population showed the 2/4 and 4/4 genotypes to be similar in size and larger than the 2/2 genotype (Zouros et al. 1980). An association between size and aspartate aminotransferase genotype has also been found in cultured populations of *Crassostrea gigas*; Sugita and Fujio (1982) reported that individuals of the B/B genotype at the *Aat-1* locus were 50% larger than A/B individuals, which were in turn twice as large as A/A oysters.

There is a tendency in these studies to conclude that the physiological effects associated with allozyme genotype are in fact due to functional differences in the protein products of the allozyme loci. This inference is rarely justified, however, unless it can be shown that the individuals being compared do not differ in genetic background or environmental history. This degree of rigor can seldom be attained with laboratory animals, much less with natural populations of marine invertebrates. For example, Martin (1979) considered size differences among genotypes at the *Gpi* locus in several North Carolina eastern oyster populations to be the physiological manifestation of molecular differences among *Gpi* allozymes. In the absence of demographic information on the populations, it is impossible to exclude an alternative hypothesis, namely that the allozyme genotypes simply distinguished age classes which differed coincidentally in genotype frequencies.

In a careful study of *Pgm-2* allozymes in *C. gigas* from a seminatural population in British Columbia, Pogson (1991) found that the three most common heterozygous genotypes exhibited significantly greater enzyme activity levels than either homozygotes or the less common heterozygotes. The cause of these differences in specific activity, which appear to reflect differences in steady-state enzyme concentrations rather than kinetic properties, is unknown. One explanation is the presence of a tightly linked overdominant regulatory locus, in strong gametic phase disequilibrium (nonrandom association among alleles at different loci) with the *Pgm-2* locus (Pogson 1991).

Ironically, studies that attempt to discern fitness differences among allozyme genotypes are usually only capable of detecting effects so large that they cannot reasonably be attributed to allozyme genotype per se. As Eanes (1987) noted, studies of null enzyme alleles show that even a 50% reduction in enzyme activity is accompanied by a negligible (and in practice, immeasurable) loss in fitness; among functional (wild-type) allelic variants, fitness differences would likely be even smaller. Therefore, in the absence of detailed biochemical, physiological, and population data — in other words, for virtually all enzyme loci in all bivalves, with the exception of *Lap* in *Mytilus edulis* and possibly *Pgm-2* in *C. gigas* — the interpretation of phenotypic differences associated with allozyme genotypes remains largely speculative.

Correlates of Heterozygosity

The seminal work of Zouros and colleagues (Singh and Zouros 1978; Zouros et al. 1980) on the relationship of allozyme heterozygosity to fitness-related traits in *C. virginica* has been of considerable interest to evolutionary and population geneticists (Mitton and Grant 1984; Zouros and Foltz 1987). These studies demonstrated in *C. virginica* a positive relationship between multilocus heterozygosity (i.e., the number of allozyme loci at which an individual is heterozygous) and traits generally considered to be positively related to fitness, such as growth rate and viability.

Growth Rate and Viability

Singh and Zouros (1978) reported that in a year-old cohort of naturally set eastern oysters, faster growth was associated with heterozygosity at four of the five allozyme loci scored. (The remaining locus, *Got-1*, is discussed above). A subsequent study (Zouros et al. 1980) with a larger sample size and two additional allozyme loci confirmed this result. The effects of heterozygosity at individual loci were independent and additive (Foltz et al. 1983), resulting in a positive correlation between overall (multilocus) heterozygosity and growth rate (Fig. 1).

These reports inspired a series of studies on natural populations of other bivalve species. Some of these obtained similar results (e.g., Koehn and Gaffney 1984; Gentili and Beaumont 1988; Koehn et al. 1988; Zouros et al. 1988; Alvarez et al. 1989) whereas others found no relationship between allozyme heterozygosity and growth rate (Beaumont et al. 1985; Gaffney 1986, 1990). The relationship is typically not found in the progeny of pair matings or small mass spawnings (Beaumont et al. 1983; Gaffney and Scott 1984; Foltz and Chatry 1986). In such cases allozymes act as markers for entire chromosomes or large segments thereof, masking the relatively minor effects of the allozyme loci themselves (Gaffney and Scott 1984).

Juvenile viability also appears to be positively correlated with multilocus heterozygosity. Zouros et al. (1983) found higher survival of more heterozygous eastern oysters from two weeks post-settlement to three years of age (Fig. 2). In *Mytilus edulis*, a significant change in the heterozygosity distribution of a 0-year class that occurred within less than two months was ascribed to differential mortality (Diehl and Koehn 1985). However, a subsequent experiment in which mortality and migration were directly monitored in another 0-year class of the same population failed to show any mortality-induced changes in the heterozygosity distribution (Gaffney 1990). Foltz and Zouros (1984) found that heterozygosity was positively correlated with age in the giant scallop, *Placopecten magellanicus*, although there was no association of size and heterozygosity within year classes. Alvarez et al. (1989) found a strong negative relationship between juvenile survival and heterozygosity in the European flat oyster, *Ostrea edulis*, and hypothesized that larger (more heterozygous) oysters were more likely to die from infestations of the haplosporidian parasite, *Bonamia ostreae*.

A hypothesis is required to account for the sporadic appearance of a positive correlation between heterozygosity and fitness-related traits such as growth and viability. Unfortunately, we suffer from a surfeit of such hypotheses and the inability to distinguish critically among them. This issue has been addressed in an extensive study of growth in relation to heterozygosity in the coot clam (dwarf surf clam), *Mulinia lateralis* (Koehn et al. 1988; Gaffney et al. 1990). Briefly, the major hypotheses involve overdominance, inbreeding, null alleles, and chromosomal anomalies.

Overdominance, or heterozygote superiority, at allozyme loci is the most obvious interpretation of the enhanced growth rate and viability of more heterozygous individuals. It is arguable, though, whether genotype at the allozyme locus itself is responsible for these effects, for two reasons: (1) allozyme-as-

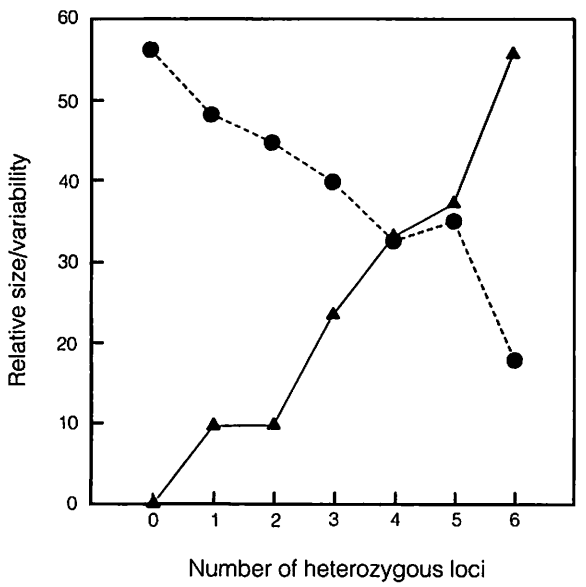

Figure 1. Size (▲) and variability in size (●) of year-old eastern oysters in relation to degree of heterozygosity at seven allozyme loci. Size is expressed as the percentage by which the mean log-transformed total body weight of a heterozygosity class exceeds the mean log-transformed weight of the 0-heterozygote class. Variability in size is expressed as the coefficient of variation of log-transformed total body weight. Data from Zouros et al. (1980).

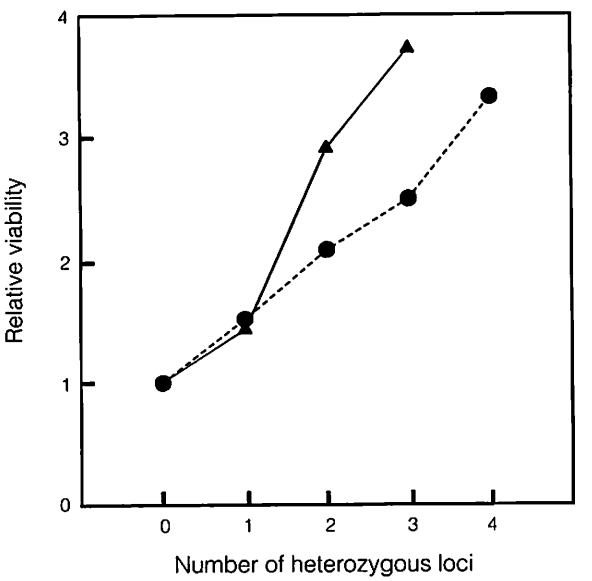

Figure 2. Estimates of relative viability (probability of surviving relative to the 0-heterozygote class) in eastern oysters in relation to heterozygosity at four enzyme loci. Viability (▲) from three weeks to one year in a population from Prince Edward Island. Viability (●) from one year to three years in a population from Cape Breton. Viabilities calculated by Alvarez et al. (1989) from data of Zouros et al. (1983).

sociated phenotypic differences are often simply too large, as discussed above, and (2) there is little biochemical evidence for heterozygote superiority at the enzyme level (Kacser and Burns 1981; Zouros and Foltz 1987). An alternative explanation, associative overdominance, attributes the superior fitness of allozyme heterozygotes to the effects of alleles (e.g., deleterious recessives) at other loci that are nonrandomly associated with alleles at the allozyme locus. Unfortunately, because it relies on the presence of unseen and in practice essentially unknowable loci, this hypothesis has proven resistant to rigorous testing (Zouros and Foltz 1987). A promising model system for the study of associative overdominance is *Pgm-2* in *C. gigas*, in which the common heterozygotes show substantially greater enzymatic specific activity and higher growth rates than homozygotes (Pogson 1991).

The inbreeding hypothesis states that some degree of inbreeding occurs in oyster populations as the result of matings among relatives, and that inbred individuals, which are on average less heterozygous, show reduced growth and viability due to inbreeding depression. This hypothesis is generally discounted in view of the reproductive biology of the typical bivalve species; external fertilization and the planktonic larval stage are assumed to ensure random mating. A more compelling reason to reject the inbreeding hypothesis is the observation that heterozygote deficiencies vary significantly among loci, in contrast to the uniform heterozygote deficiencies expected under inbreeding (Zouros et al. 1980; Gaffney et al. 1990).

Another argument against the inbreeding hypothesis would be a gametic incompatibility system that reduced the incidence of consanguineous matings in *C. virginica* (Longwell and Stiles 1973). However, the poor fertilization and survival of progeny these authors observed in full-sib (brother-sister) crosses do not necessarily indicate the existence of a true gametic incompatibility system (Mulcahy and Mulcahy 1983), which in marine invertebrates may be limited to clonal organisms with self versus nonself recognition systems. Mallet and Haley (1983) observed no gamete incompatibility in full-sib matings of *C. virginica*. They also found no inbreeding depression in larval or juvenile growth or survival, and suggested that the results of Longwell and Stiles (1973) may have been due to differences in gamete maturity between full-sib families and control groups. As Lannan (1980) pointed out, full-sib matings may often produce poor progeny simply because sibling parents rarely have mature eggs and sperm at the same time, in contrast to unrelated individuals. In a series of crosses within and among several hatchery lines and populations of wild oysters, Gaffney et al. (1993) found evidence of gametic interactions affecting fertilization, but no systematic bias in favor of within-line or among-line crosses. Reports of experimental self-fertilization in *C. virginica* (Mallet and Haley 1983) and *C. gigas* (Lannan 1971) further militate against the existence of a gametic incompatibility system in oysters.

Null alleles (allelic variants that code for a nonfunctional protein product) could account for a heterozygosity-fitness relationship, if null heterozygotes (which would be scored on a gel as homozygotes, because they carry only one active allele) are less fit than wild-type individuals. As Zouros et al. (1980) showed, the maintenance of null alleles at frequencies high enough to cause an appreciable effect requires unusually high mutation rates, for which there is no evidence; in the fruit fly, *Drosophila melanogaster*, the average mutation rate to null alleles at allozyme loci is estimated at 3.86×10^{-6} per gene per generation (Voelker et al. 1980b). In species in which null allelic frequencies have been directly measured, such as *D. melanogaster* (Voelker et al. 1980a) and pine trees (Allendorf et al. 1982), null alleles typically occur at frequencies of 0.2% in natural populations, too low to cause a detectable heterozygosity-fitness relationship. Furthermore, if the average reduction in fitness experienced by a null heterozygote is so small that it can only be detected with great difficulty (Eanes 1987), clearly null alleles cannot account for the relatively large phenotypic effects associated with allozyme heterozygosity.

Foltz (1986a) interpreted aberrant genotypic proportions in progeny of pair matings in *C. virginica* as evidence that frequencies of null alleles in natural populations of oysters are orders of magnitude higher than the 0.2% observed in other organisms. This unusual finding may be more readily accommo-

dated by an alternative hypothesis that involves aneuploidy, the occurrence of individuals lacking chromosomes or fragments thereof.

Dixon (1982) and Thiriot-Quiévreux (1986) reported high frequencies of aneuploidy occurring in natural populations of bivalves. Loss of a single chromosome (or chromosome segment) will cause the afflicted individual to be hemizygous, i.e., possessing a single copy rather than the usual two copies of genes located on the missing chromosome. For such genes, hemizygotes will be scored as homozygotes on electrophoretic gels. Because aneuploid individuals generally suffer seriously reduced fitness from the loss of genetic material, a spurious correlation between apparent enzyme heterozygosity and fitness will result. Furthermore, because the frequency of aneuploidy appears to be related to environmental stress (Dixon 1982), the strength of a heterozygosity-fitness correlation due to aneuploidy might vary among populations in relation to their environmental history. This variation could account for the irregular occurrence of a heterozygosity-fitness relationship within the same species. Variation among loci in the phenotypic effects of heterozygosity could reflect variation among chromosomes or chromosome segments in the frequency of meiotic errors.

Direct evidence in support of this hypothesis is lacking. Thiriot-Quiévreux (1986) and Thiriot-Quiévreux et al. (1988) reported that the frequency of aneuploid cells observed in mitotic spreads from gill tissue of juveniles was inversely correlated with growth rate in *C. gigas*, and argued that this finding provided support for the hypothesis outlined above. However, the aneuploidy described by these authors was not uniform among cells within an individual, but rather was in the form of somatic mosaicism, which would not likely lead to the mis-scoring of allozyme heterozygotes.

In a subsequent study, Thiriot-Quiévreux et al. (1992) scored 83 full sibs of *C. gigas* at nine segregating allozyme loci and counted chromosomes from 30 metaphase preparations of gill tissue from each oyster. There was a significant relationship between the number of missing chromosomes and body size, but no relationship between allozyme heterozygosity and either size or degree of aneuploidy. The authors concluded that "within families a much larger component of variation in growth rate is due to aneuploidy than to allozyme genotype, but this conclusion cannot, at present, be extended to natural populations."

Energetics

In an attempt to account for the higher growth rates of more heterozygous oysters, Koehn and Shumway (1982) measured standard metabolic rate (oxygen consumption of oysters starved for two weeks) in relation to genotype at five polymorphic allozyme loci. They observed a significant negative correlation between the degree of multilocus heterozygosity and metabolic rate, with five-locus heterozygotes using only half the amount of oxygen consumed by a five-locus homozygote. At each locus, homozygotes consumed 10 to 20% more oxygen than heterozygotes. The contributions of each locus were highly significant, and appeared to be additive; overall, multilocus heterozygosity explained 66% and 57% of the variation in weight-standardized oxygen uptake under ambient and stressed conditions, respectively. Koehn and Shumway (1982) concluded that this genetic difference in metabolic rate provided an energetic explanation for the higher growth rate of more heterozygous oysters.

The interpretation that these effects are due to the allozyme loci themselves must be treated with the same skepticism afforded studies that purport to demonstrate large selection differentials among allozyme genotypes. It is very unlikely that variation at five gene loci, out of many thousands in the oyster genome, could explain more than half of the observed variation in metabolic rate. One alternative interpretation includes aneuploidy (i.e., aneuploid oysters showed higher metabolic rates because of impaired physiological integrity, or because their condition was poorer, resulting in a higher proportion of metabolically active tissue and consequently a higher weight-specific oxygen consumption). Another hypothesis involves inbreeding depression. The oysters used by Koehn and Shumway (1982) came from a hatchery mass spawning involving an unknown number of parents of unknown origin. In these oysters, allozyme heterozygosity may have served as an

index to overall genomic heterozygosity, which influenced physiological performance.

Subsequent attempts to explain the heterozygosity-growth correlation in terms of energy budget components have proved inconclusive. Rodhouse and Gaffney (1984) found no genotypic differences in rate of oxygen consumption of *C. virginica* from a natural population from New York, but noted that the discrepancy between their results and those of Koehn and Shumway (1982) may have arisen from differences in technique (Winkler method versus oxygen electrode). In a sample of juvenile *M. edulis*, a multiple regression of weight-standardized oxygen consumption rate on dry weight and heterozygosity at five loci showed a significant negative relationship between oxygen consumption and heterozygosity (Diehl et al. 1985). However, a simpler analysis of these data, using a multiple regression of log oxygen consumption on log dry weight and heterozygosity, showed no significant effect of heterozygosity (Gaffney 1986). This discrepancy suggests that if there was a correlation between heterozygosity and metabolic rate in this sample, it was marginal.

A subsequent study of *M. edulis* (Diehl et al. 1986) showed a positive relationship between individual heterozygosity and standard rate of oxygen consumption, counter to the Koehn-Shumway (1982) hypothesis. Finally, a study on the energetics of the coot clam in relation to heterozygosity (Garton et al. 1984) found a significant negative correlation between weight-standardized oxygen consumption and heterozygosity at six allozyme loci. Other energy budget components (ammonia excretion, clearance rate) were uncorrelated with heterozygosity. In their analysis, data from seven of the 112 experimental clams were discarded without statistical justification; a re-analysis of the original data set shows a suggestive ($P < 0.10$) but not significant negative relationship between oxygen consumption and heterozygosity (Gaffney 1986). Holley and Foltz (1987) found a slight but significant positive association between heterozygosity and clearance rate in the brackish water clam *Rangia cuneata*.

On balance, these early tests of the Koehn-Shumway hypothesis have provided ambiguous results. Because the association between heterozygosity and growth rate is usually a modest one ($r^2 = 0.05$), it is not surprising that attempts to sort out individual physiological causes have often been inconclusive. Recently, more sophisticated studies of energy metabolism in *M. edulis* have shown that the growth advantage associated with allozyme heterozygosity is attributable to lower rates of both protein synthesis and protein breakdown; the energy saved as a result of reduced protein turnover is used to increase feeding activity (Hawkins et al. 1989). The mechanism by which protein metabolism and allozyme heterozygosity are associated remains fertile ground for further investigation.

Heterozygote Deficiencies

Despite the evidence discussed above for the general occurrence of heterozygote superiority, frequencies of heterozygotes in natural populations of marine bivalves are typically below the values expected in large, randomly breeding units (Hardy-Weinberg expectations). Hypotheses advanced to account for this paradox are generally the same as those invoked to explain the heterozygosity-fitness correlation. Three of these (i.e., those involving null alleles, inbreeding, and aneuploidy) have been discussed above.

Null alleles are probably too uncommon at most loci to serve as a general explanation of large heterozygote deficiencies, whereas inbreeding has been rejected on the basis of both reproductive biology and the observed heterogeneity of heterozygote deficiencies among loci. Aneuploidy, on the other hand, may occur with sufficient frequency in natural populations as to generate substantial heterozygote deficiencies. In a large-scale study of 15 allozyme loci in a natural population of *M. lateralis*, Gaffney et al. (1990) noted a striking correlation between the magnitude of heterozygote deficiency and the effect of heterozygosity on size; loci that showed the largest heterozygote deficits were those for which heterozygosity had the greatest (positive) effect on size. This relationship could reflect the propensity of particular chromosomes or chromosome segments to experience meiotic transmission failures; loci in regions most prone to aneuploidy would show the largest heterozygote deficiencies and the greatest reductions in fitness of the apparently homozygous genotypes.

Heterozygote deficiencies may also originate from another factor: sampling a population that in fact comprises two or more genetically differentiated subpopulations (the Wahlund effect). The long-lived planktonic dispersal stage of marine bivalves such as *C. virginica* makes it possible in theory for larvae of different geographical origins to settle at the same site, generating potential Wahlund effects. However, the heterozygote deficiencies observed are often much larger than expected from mixing larvae of different geographical origins (Koehn et al. 1976; Zouros and Foltz 1984). In the case of the eastern oyster, mixing of differentiated populations may contribute to heterozygote deficiencies in some instances — particularly at the boundaries of the putative major populations — but is unlikely to be the sole explanation of large and widespread heterozygote deficiencies.

Finally, it has been suggested that both heterozygote deficiencies and allozyme-associated heterosis may result from strong selection that favors homozygotes during the larval phase and heterozygotes afterwards (Zouros et al. 1983; Mallet et al. 1985). This hypothesis would not appear to be generally applicable, in that it requires large selection coefficients, as discussed above; moreover, the effects of linked deleterious recessive alleles cannot be invoked to explain selection *against* heterozygotes. In any case, it has yet to be tested directly (Gaffney et al. 1990). In the only study to date of allozymes in oyster larvae, both natural and cultured populations showed genotypic frequencies in Hardy-Weinberg equilibrium (Hu et al. 1992). However, this study focused on a locus (*Gpi*) that typically does not show large heterozygote deficiencies.

Direct analysis of allozymes in larval stages may prove to be of limited use, because only some loci can be resolved in individual larvae. In principle, this obstacle could be overcome by examining the DNA sequences of the enzyme-coding genes, using modern molecular methods (e.g., amplification of targeted DNA sequences from individual larvae by the polymerase chain reaction [PCR], coupled with DNA sequence analysis by digestion with restriction enzymes or direct sequencing).

POPULATION GENETICS

Most studies on the population genetics of *C. virginica* have focused on geographical variation in allozyme frequencies; to date, only one study (Foltz 1986b) has reported linkage relationships (the location of individual genes on the same or different chromosomes) for allozyme loci. More recently, attention has been directed to characterizing mitochondrial DNA sequence variation in natural and cultured stocks of oysters.

Geographic Variation

Allozymes

In general, relatively little differentiation at the allozyme level has been observed in oyster populations throughout much of their range, i.e., along the Atlantic and Gulf coasts of North America. Estimates of genetic similarity (Nei 1972) based on 32 enzyme loci among populations from Cape Cod to Corpus Christi ranged from 0.962 to 0.997 (Buroker 1983b). Similar observations have been made by Weir et al. (unpub. data, cited in Reeb and Avise 1990). This degree of similarity is characteristic of local populations within a species (Ayala 1975), and is consistent with (but not proof of) the existence of considerable gene flow among populations along the Atlantic coast. It is possible that to some extent this homogeneity can be attributed to human intervention (relaying and transplantation of oysters among locations along the Atlantic and Gulf coasts).

In contrast to the relative homogeneity of oyster populations from Cape Cod to Corpus Christi, populations from sites to the north (Canada) and south (Laguna Madre, Texas, and Bay of Campeche, Mexico) differ markedly in terms of allelic frequencies. These geographically extreme populations show reduced levels of genetic similarity characteristic of populations at the subspecies level of evolutionary divergence (Table 2).

Biological interpretations of the coefficients of genetic similarity presented in Table 2 must be made with caution. Combining studies from different laboratories invariably introduces some error, because electrophoretic methods and allelic designations vary.

For example, where multiple loci encode the same enzyme, Buroker et al. (1983b) designate the most rapidly migrating isozyme as "*-1*," whereas Hedgecock and Okazaki (1984) use this suffix for the most slowly migrating isozyme. In addition, data used for comparing populations are taken from only those loci common to both studies. Nevertheless, the picture that emerges is one of a species showing fairly homogeneous allele frequencies across most of its range, with distinct populations at the northern and southern extremes (Fig. 3).

At some loci, the differences among populations are striking. The Nova Scotia and western Florida populations are fixed for different alleles at the *Ald*, *Mdh-1* and *Mp-1* loci, and have substantial allele frequency differences at the *AcP-3*, *Ap-1*, *Aat-2*, *Est-3*, *Lap-2*, and *Pgm-2* loci (Buroker et al. 1979). Similarly, western Florida and Bay of Campeche populations have no alleles in common at the *Aat-2* locus and have significantly different allelic frequencies at the *GPI*, *Lap-1*, *Lap-2*, *Mdh-2*, *Tpi*, and *Xdh* loci (Hedgecock and Okazaki 1984; locus designations converted to match Buroker et al. 1979). The Laguna Madre (Brownsville, Texas) population differs from other Gulf coast populations at the *Lap-2*, *Mdh-1*, and *Pgi* loci (Buroker 1983b) and an esterase locus (Groue and Lester 1982).

Table 2. Measures of genetic similarity (Nei 1972), based on data from 10 loci common to Buroker et al. (1979), Buroker (1983b), and Hedgecock and Okazaki (1984): *Aat-1*, *Aat-2*, *Glypd*, *Lap-1*, *Lap-2*, *Mdh-1*, *Mdh-2*, *Mp-1*, *6Pgd*, and *Pgi*. The Atlantic and Gulf category comprises 20 population samples from localities ranging from Cape Cod to Corpus Christi; for comparisons involving these populations, the range of similarity values obtained is shown. CA = Bay of Campeche.

Location	NS	A&G	LM	CA
Atlantic & Gulf coasts (A & G)	0.645 - 0.694	0.984 - 0.998	0.781- 0.829	0.814 - 0.842
Nova Scotia (NS)			0.617	0.466
Laguna Madre (LM)				0.651

Buroker (1983a) described macrogeographic clines in allelic frequencies at the *Lap* and *Gpi* loci in Atlantic and Gulf coast eastern oyster populations. These may be statistical artifacts, in that spatial patterns in allelic frequencies will occur on occasion simply by chance when many loci are examined. If the critical significance level is adjusted to take into account the multiple tests made (Buroker's study involved more than 40 alleles), the spatial variation at the *Lap* and *Gpi* loci is no longer significant. Weir et al. (unpub. data, cited in Reeb and Avise 1990) likewise found little allozyme differentiation among eastern oyster populations but did note shifts in allelic frequencies at the *Got-2* and *Lap-1* loci along the mid-coast of Florida and at the mouth of the Mississippi River, respectively.

In the Gulf of Mexico, Buroker (1983b) observed a correlation between allelic frequencies at the *Lap-2* locus and the salinity gradient created by the Mississippi River, reminiscent of the salinity-related clines at the *Lap* locus in *M. edulis* (Koehn and Hilbish 1987). Salinity-correlated allele frequency varia-

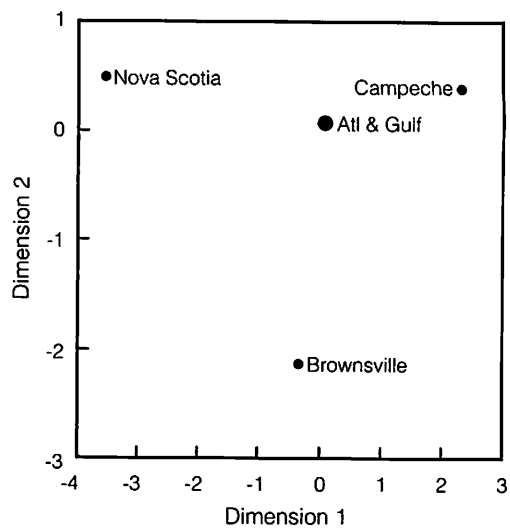

Figure 3. Nonmetric multimensional scaling (monotonic regression by the Kruskal method; Wilkinson 1990) of the genetic distance matrix (Nei 1972) for 23 populations of the eastern oyster, using data for 10 loci common to Buroker et al. (1979), Buroker (1983b), and Hedgecock and Okazaki (1984). The distance matrix is well depicted in two dimensions (stress = 0.0028, R^2 = 0.9999). The Atlantic (Atl) and Gulf category comprises 20 populations.

tion has also been observed at the other *Lap* locus (*Lap-1*) in Chesapeake Bay samples of *C. virginica* (Rose 1984). In view of the presumed homology of the *Lap* loci among bivalve species (Koehn et al. 1980) and response of oyster hemolymph leucine aminopeptidase activity to salinity changes (Yoshino and Cheng 1976), associations between *Lap* genotype and salinity comparable to those observed in *M. edulis* are not surprising.

On a smaller scale, spatial variation in allelic frequencies has been described and attributed to local population structure. In a survey of ten eastern oyster bars in Chesapeake Bay, Buroker (1983a) reported that although all populations were very similar (Nei's genetic similarity values of 0.985 to 0.998), there was significant heterogeneity in allelic frequencies at 12 of 18 polymorphic loci. Buroker (1983a) interpreted the results of a principal component analysis of the frequencies of the 28 most common alleles to indicate the existence of four geographical subpopulations within Chesapeake Bay. This interpretation is debatable; the putative subpopulations (Figure 1 of Buroker 1983a) comprise oyster bars that are not geographically clustered. An alternative analysis using nonmetric multidimensional scaling of the genetic distance matrix derived from Buroker's data likewise provides little evidence of geographical clustering (Fig. 4). In view of the extensive relaying of oysters within Chesapeake Bay by commercial operators and management agencies for more than a century, as well as the potentially widespread dispersal of oyster larvae, it would be surprising if significant spatial structure were found.

In a survey of ten eastern oyster populations from the northern Gulf of Mexico (Galveston Bay to Mississippi Sound), Grady et al. (1989) noted significant heterogeneity in allelic frequencies for six of thirteen polymorphic loci examined. Again, all populations were genetically very similar (0.94 to 0.98), and there was no evidence of any geographical structure.

The pattern observed in these data — spatial variation in allelic frequencies which evinces no geographical pattern — has been termed "chaotic genetic patchiness" by Johnson and Black (1982), who argued that accidents of recruitment and localized selection accounted for irregular spatial and temporal patterns of allozyme frequencies in an intertidal gastropod. Patterns of fine-scale spatial variation observed at a single point in time do not constitute evidence of permanent population structuring; rather, in the eastern oyster, the general picture is one of large-scale geographic homogeneity, upon which localized and ephemeral heterogeneity may be superimposed.

From a biological point of view, it is important to distinguish between immigration and gene flow among populations. Substantial migration among bivalve populations may occur as the result of larval dispersal, yet immigrants may, as a result of physiological and genetic adaptation to their source environment, be unable to survive and reproduce in their new environment (Hedgecock 1986). Failure to reproduce successfully may also result from gametogenic asynchrony with the native population; in either case, the immigrants do not contribute genes to

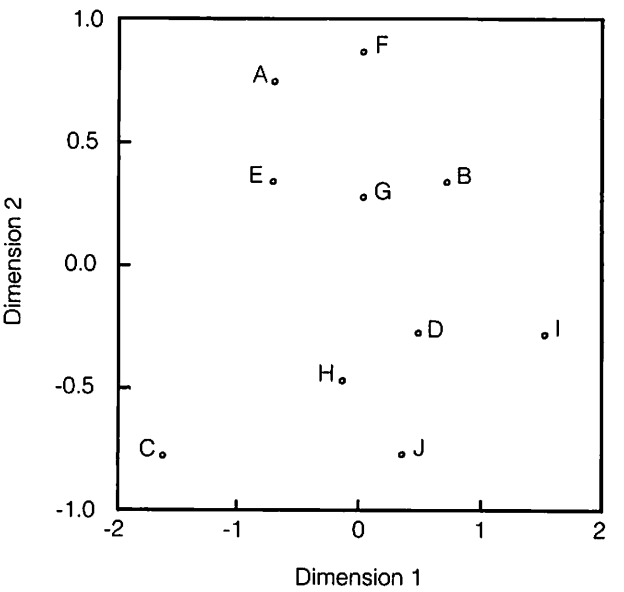

Figure 4. Nonmetric multimensional scaling (monotonic regression by the Kruskal method; Wilkinson 1990) of the genetic distance matrix (Nei 1972) for 10 Chesapeake Bay populations of the eastern oyster. The populations are labelled A to J in order from northernmost (Swan Point, Maryland) to southernmost (James River, Virginia). Allozyme frequencies for 12 loci used by Buroker (1983a) in a principal component analysis of interpopulation variation were used to derive genetic similarities. The distance matrix is well represented in two dimensions (stress = 0.084, $R^2 = 0.959$).

the next generation. Many coastal species possess a high capacity for dispersal yet appear to display limited gene flow among populations (Burton and Feldman 1982; Hedgecock 1986).

Admixture should be detectable by the presence of gametic phase disequilibrium at loci that differ markedly among populations in allelic frequencies. The evidence on this point is limited. Foltz et al. (1983) observed few significant allelic associations in an analysis of seven loci in an eastern oyster population from Prince Edward Island; however, two of the three significant associations involved loci (*Lap-2* and *Est-3*) at which Buroker et al. (1979) found Nova Scotia and United States populations to differ substantially in allelic frequencies. Similarly, if substantial exchange occurred between the Laguna Madre and Corpus Christi populations (hydrographic data suggest the potential for unidirectional migration of larvae from north to south), one might expect significant disequilibrium among those loci that differ greatly between the two localities (*Lap-2*, *Mdh-1*, and *Gpi*) if the immigrants do not successfully reproduce in their new habitat. For example, if the Laguna Madre population represented a mix of 90% "natives" (fixed for the $Mdh\text{-}1^{96}$ allele, frequency of 0.02 for the Gpi^{106} allele) and 10% immigrants from Corpus Christi (fixed for the $Mdh\text{-}1^{100}$ allele, frequency of 0.50 for the Gpi^{106} allele), a gametic disequilibrium of 0.043 between the *Mdh-1* and the *Gpi* loci would result. This is not far from the average disequilibrium value of 0.035 reported by Buroker (1983b).

Migration among differentiated populations would also produce heterozygote deficiencies by the Wahlund effect. Therefore, we might expect loci with marked geographic differentiation to show large heterozygote deficits in populations containing immigrants. The Laguna Madre population showed no heterozygote deficits at two of three strongly differentiated loci (*Mdh-1*, *Gpi*), whereas at the third locus, *Lap-2*, heterozygote deficits were no larger than in other Gulf populations (Buroker 1983b). However, deviations from Hardy-Weinberg equilibrium frequencies may be influenced by a number of factors other than migration (see p. 430-431), making it difficult to infer the presence or absence of migration from heterozygote frequencies alone.

From these data, it is difficult to determine what proportion, if any, of the oysters present in the lower Laguna Madre are immigrants from northern populations. A detailed electrophoretic study that employed larger sample sizes and examined higher-order multilocus associations as well as two-locus disequilibria would likely be able to resolve this question. The population genetics of these marginal northern and southern populations clearly deserves further study.

Mitochondrial DNA

Restriction enzyme analysis of mitochondrial DNA (mtDNA) sequence variation has provided a powerful new tool for the analysis of intraspecific genetic variation, adding a phylogenetic dimension to the interpretation of population structure. Mitochondrial DNA possesses several attractive features: it is generally maternally inherited, is not subject to recombination, evolves rapidly, and often exhibits extensive intraspecific polymorphism (Avise et al. 1987).

Biparental inheritance of mitochondria appears to be common in *Mytilus* (Hoeh et al. 1991; Zouros et al. 1992) and possibly in freshwater mussels (Hoeh et al. 1991). However, neither Reeb and Avise (1990) nor Brown and Paynter (1991) found evidence of significant paternal leakage in *C. virginica*. This discrepancy is intriguing, as paternal sperm-borne mitochondria appear to be incorporated into the egg during fertilization in both *M. edulis* (Meves 1915; Longo and Anderson 1969) and *C. virginica* (Wale and Gould 1977).

In an analysis of restriction site variation in populations of *C. virginica* from Prince Edward Island to Brownsville, Texas, Reeb and Avise (1990) observed that virtually all individuals from the St. Lawrence River to Cape Canaveral, Florida, clustered in one phenetic group (the Atlantic assemblage) whereas all individuals from Miami, Florida, to Brownsville, Texas, clustered into a Gulf assemblage (Fig. 5). Representatives of both mtDNA arrays were present in one geographically intermediate locale (West Palm Beach, Florida). This geographic pattern of divergence has been observed in several coastal marine or salt marsh organisms, and is consistent with the observation that

the mid-Atlantic coast of Florida represents a zoogeographic boundary maintained by water current patterns (Reeb and Avise 1990).

Reeb and Avise (1990) noted that these results — a sharp genetic discontinuity in mtDNA patterns that is not accompanied by parallel differentiation at the allozyme level — show a remarkable concordance with earlier studies of allozyme and mtDNA variation in the horseshoe crab, *Limulus polyphemus* (Selander at al. 1970; Saunders et al. 1986). Taken together, these data suggest that gene flow is severely limited between Atlantic and Gulf assemblages, and that homogeneous allozyme gene frequencies are the result of natural selection (Karl and Avise 1992).

Another approach to the analysis of mtDNA variation is direct sequencing of fragments of mitochondrial genes amplified by PCR. Ó Foighil et al. (in press) sequenced a 450 bp portion of the 16S ribosomal gene from 20 *C. virginica* and detected variation at two nucleotide sites, resulting in five haplotypes. Haplotype A was found in two Long Island Sound oysters; haplotype B was found in seven Delaware Bay oysters; haplotype C was found in five South Carolina oysters and one Delaware Bay oyster; haplotypes C and D were found in five Texas oysters. Despite the small sample sizes, the geographical heterogeneity is statistically significant ($p < 0.0001$), suggested the existence of oyster "races" within the western Atlantic region. Additional studies are needed to confirm these results, and to delineate the geographic distributions and performance characteristics of the identified subpopulations.

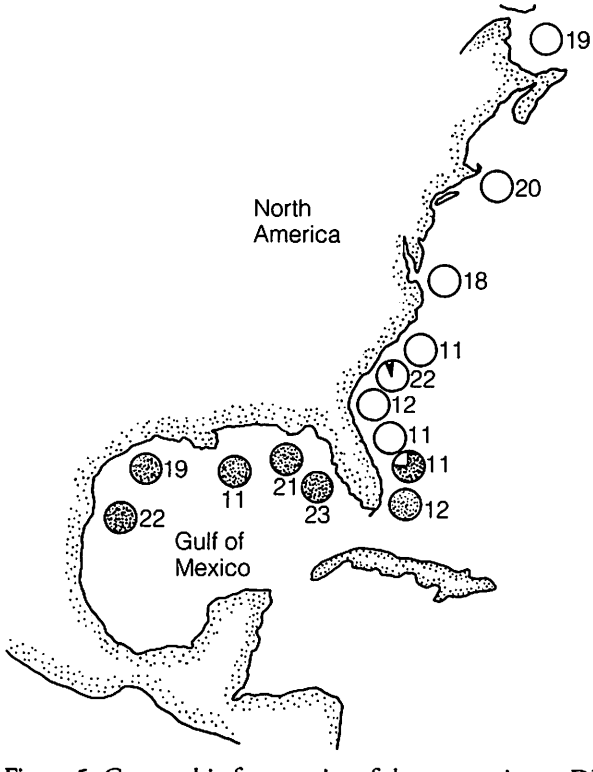

Figure 5. Geographic frequencies of the two major mtDNA clonal assemblages (Atlantic and Gulf coasts) observed in the eastern oyster. Number of individuals analyzed is listed for each site. From Reeb and Avise (1990).

On a smaller scale, Brown and Paynter (1991) found no significant heterogeneity in the frequencies of mtDNA haplotypes among three oyster populations from Chesapeake Bay. This apparent lack of geographical structure parallels the results of Buroker's (1983a) allozyme survey, and presumably reflects considerable genetic mixing from larval dispersal and perhaps the effects of repeated transplants by oyster culturists.

Nuclear DNA

A recent analysis of geographic variation in allelic frequencies at four "anonymous" nuclear loci resulted in a picture of a Gulf-Atlantic discontinuity similar to that observed for mtDNA (Karl and Avise 1992). This approach involves the amplification of polymorphic single-copy nuclear DNA (scnDNA) sequences, followed by restriction enzyme digestion of PCR products to detect allelic variants. The function of the DNA sequences examined is unknown, hence the term anonymous loci. Karl and Avise (1992) concluded that because these loci showed a pattern of geographic discontinuity similar to that observed for mtDNA, the geographical uniformity of allozyme frequencies is likely maintained by balancing selection, and that "Such balancing selection on allozyme polymorphisms could counter the influence of genetic drift, even in the face of population subdivision due to historical demographic events or contemporary restrictions on gene flow that are reflected in geographically divergent frequencies of neutral genetic markers." However, the scnDNA data are limited by comparison with the allozyme data set — only four loci were scored, and sample sizes were small — and the possible functions of the anonymous loci are unknown. Additional data on other anonymous nuclear loci, together with some understanding of their func-

tion, are needed to confirm or disprove the hypothesis proposed by Karl and Avise (1992).

The interpretation of data gathered from the use of ever more sophisticated methods in molecular genetics is not always straightforward. As Avise (1989) noted, it may soon be possible to detect genetic differences that separate local populations, family units, and even individuals. Although such information may prove invaluable to population biologists, it will not necessarily provide clear guidelines to the resource manager seeking a genetic basis for the management of unit stocks.

Physiological Races

Well before the advent of electrophoretic methods, it was suggested on the basis of differences in spawning physiology that the eastern oyster comprised a series of latitudinally separated "physiological races" (Stauber 1950; Loosanoff and Nomejko 1951). Stauber (1950) argued that oyster populations inhabiting major estuaries (e.g., Chesapeake Bay, Delaware Bay, Long Island Sound) might represent genetically distinct populations that had been effectively isolated by hydrographic barriers until human activities intervened.

Latitudinal gradients in physiological traits are well known in various marine species. In some cases these have been shown to have a genetic component, judging by the persistence of differences among populations through several generations of laboratory rearing in a common environment (Levinton and Monahan 1983; Lonsdale and Levinton 1986). After several generations of culture in New Jersey, oysters from Long Island Sound and Virginia continue to display different reproductive timing from native Delaware Bay lines (Barber et al. 1991), suggesting the possibility of important genetic differences among populations that are effectively identical in terms of allozyme frequencies and mtDNA restriction site variation.

On the other hand, the existence of pronounced differences in reproductive timing often observed among spatially proximate bivalve populations (e.g., intertidal versus subtidal), which may well have derived from the same larval pool, calls into question the notion that all differences in reproductive physiology imply genetic distinctness (Newell et al. 1982; Borrero 1987; Hilbish and Zimmerman 1988; MacDonald and Thompson 1988). Transplant experiments (e.g., Widdows et al. 1984; Mallet et al. 1987; Kautsky et al. 1990; Tedengren et al. 1990) typically show most of the physiological differences among populations to be environmental in origin, with a minor component attributable to either genetic differences or to irreversible phenotypic adaptation (permanent physiological entrainment to a particular environmental regime). In view of how little is known about the genetic basis of variation in reproductive physiology, it would be premature to conclude that physiological races, if they do exist, correspond to discrete genetic entities. A quantitative genetic analysis of physiological variation within and among putative physiological races of *C. virginica* is clearly required to settle this question.

Conclusions Regarding Population Structure

What sort of population structure is suggested by the apparent homogeneity of allozyme frequencies in oysters from Cape Cod to eastern Texas, the sharp separation in mtDNA and scnDNA RFLP patterns between Atlantic and Gulf assemblages, and at the same time, the possibility of distinct physiological races within these assemblages? On the basis of the available allozyme data, one would conclude that the eastern oyster comprises four genetically distinct populations, among which limited gene flow occurs. These are: eastern Canada, Cape Cod to Corpus Christi, Laguna Madre, and southern Gulf of Mexico. This view is supported by the presence of fixed allelic differences at several loci. Unless allelic variants at these loci are subject to strong selective pressures — an unwarranted assumption for the majority of allozyme loci, the case of *Lap* in *M. edulis* being the exception to the rule — the presence of even a miniscule amount of gene flow (on the order of one immigrant per generation) would suffice to homogenize gene frequencies among populations, according to a variety of theoretical models (reviewed by Felsenstein 1976).

On the other hand, the distribution of mtDNA haplotypes and scnDNA RFLP patterns indicates the

existence of two discrete assemblages, between which little gene flow occurs. In contrast to the allozyme data, the southern Texas (Brownsville) and Canadian populations are not distinct from the Gulf Coast and Atlantic assemblages, respectively, in their mtDNA patterns.

Overall, these studies indicate the presence of discrete Atlantic and Gulf eastern oyster populations separated by a zoogeographic barrier. Genetic drift has led to the divergence of neutral or weakly selected genes (i.e., mtDNA, scnDNA), while selection acts both to maintain homogeneous allozyme frequencies across wide areas and to create distinct regional differences in physiological traits (e.g., spawning time). This view can only be validated through additional studies on "neutral" molecular markers, more information on demographic factors in oyster populations, and a better understanding of the genetic control of physiological traits.

Thus it appears that a seemingly straightforward question — to what extent and in what ways are oyster populations genetically different? — does not have a simple answer. To the evolutionary biologist, an acceptable model of oyster population structure must account for the discrepancy between allozyme, mtDNA, and scnDNA patterns. For a resource manager, the high level of genetic similarity within broad geographic regions suggests that transplants within regions may be allowable, at least from a genetic point of view. To the aquaculturist, genetic differentiation evidenced by allozymes or DNA sequence variation may serve primarily as a guide to geographic differences in quantitative traits such as growth and disease resistance, about which woefully little is known.

SUMMARY

Several aspects of the population genetics of the eastern oyster warrant further attention. Additional allozyme and DNA studies are needed to delineate micro- and macrogeographic population structure; these should emphasize the geographic border zones separating the major coastal assemblages and the relative roles of migration and selection in maintaining their distinctness. Secondly, it is not clear whether distinct physiological races exist in the eastern oyster. A quantitative genetic analysis of variation in physiological traits within and among the putative major populations would settle this question. Finally, the causes of heterozygote deficiencies and the correlation between heterozygosity and fitness traits remain to be determined. Two possible sources of heterozygote deficiencies in oysters are aneuploidy and the Wahlund effect. The association between heterozygosity and fitness may be attributed to either aneuploidy or the presence of gametic disequilibrium between allozymes and linked deleterious recessive alleles.

Although it has proven extremely difficult to demonstrate rigorously the Wahlund effect or associative overdominance in natural populations of marine invertebrates, hypotheses involving chromosomal aberrations are more amenable to testing. By expanding the range of genes that can be examined, and by allowing analysis of all developmental stages, new molecular techniques will provide more powerful tests of many of these questions. Their resolution will be a significant step forward for evolutionary biologists and oyster culturists alike.

ACKNOWLEDGMENTS

I thank Dennis Hedgecock, Stan Allen, and Roger Newell for helpful comments. This research was supported by the Northeastern Regional Aquaculture Center through grants 87-CRSR-2-3217 and 88-38500-4070 from the Cooperative State Research Service, U.S. Department of Agriculture. Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Chapter 12

Chromosomes, Biology, and Breeding

A. CROSBY LONGWELL AND S.S. STILES

INTRODUCTION

This chapter provides an account of eastern oyster, *Crassostrea virginica*, chromosome division as it occurs during oocyte maturation and fertilization (meiosis). We describe the basic features of *C. virginica* chromosomes, compare them to the chromosomes of other oysters, note their frequently abnormal numbers in zygote mitoses, and present photomicrographs of them during oogenesis, fertilization, and embryo cleavage. As an introduction to this treatment of the eastern oyster, mitosis and the common form of meiosis are reviewed and compared. The hereditary and evolutionary consequences of alterations or elimination of meiosis are pointed out. We discuss the possibility of structural polymorphisms of *C. virginica* chromosomes in natural and artificially bred populations as they can now be revealed by molecular techniques, and the general biological and population significance of such chromosomal change. We review artificially induced triploidy of the eastern oyster from the cytogenetic perspective, and consider other alterations of its breeding system through experimental manipulation, spontaneous change or elimination of the two meiotic chromosome divisions. Cytogenetic characteristics shared by most individuals or groups with more than two chromosome sets (polyploids) are given as a background for understanding the biology and partial sterility of artificially induced oyster triploids (polyploids with three chromosome sets).

Throughout, we point out the utility of cytogenetics as an auxiliary to other genetic and non-genetic studies of the oyster and its breeding and hybridization. This is in addition to the use of cytogenetics as a primary approach to particular problems of eastern oyster biology, taxonomy, culture, and management.

MEIOSIS

Introductory Review

The halving of the somatic chromosome number that occurs in gametogenesis of most plants and animals is accomplished by meiosis. Meiosis consists of two specialized mitotic chromosome divisions in the course of which there is only one replication of DNA. At fertilization, the two gametes, each with one (haploid) chromosome set, fuse to form a zygote with two (diploid) chromosome sets. Offspring usually differ genetically from their parents. By contrast, in mitotic division of somatic nuclei in all animals (with exception of the Protozoa), each chromosome simply divides longitudinally. The two resulting nuclei are genetically identical.

In the beginning phase of the first meiotic division (prophase), the matching (homologous) chromosomes of the maternally and paternally derived set move together and pair. This synapsis allows recombination of genes by exchange of chromosome segments between the two members of each chromosome pair. Such pairing is an essential prelude to normal segregation of the chromosomes in the first meiotic division, as discussed in Bernardi (1989) and Gillies (1989). Chromosome misdivision resulting from failed synapsis of structurally dissimilar chromosome

pairs is one basis for the sterility commonly associated with interspecies hybridization in both animals and plants.

At metaphase of the division cycle, paired chromosomes orient randomly on the spindle (cell division apparatus). The distribution of maternally and paternally inherited chromosomes to the two resulting nuclei at the next division phase (anaphase) is consequently also random.

Departures from the standard form of meiosis, or its elimination entirely, have profound hereditary consequences and change the evolutionary mechanisms of the species. They alter the very nature of the breeding system, from true sexual reproduction with contribution of genetic material from the male, to development of the egg with only physiological stimulation by the male gamete (gynogenesis), to the egg's spontaneous development (both being forms of parthenogenesis). Such deviations have occurred in diverse plant and animal groups, including Bivalvia, through mutations that inhibit meiosis or change the behavior and segregation of the meiotic chromosomes, often in association with either or both polyploidy and hybridization of closely or remotely related species or even genera (White 1973; Baker et al. 1976; Yamaguchi and Lucas 1984; Okamoto and Arimoto 1986; Ó Foighil and Thiriot-Quiévreux 1991). Establishing the nature of meiosis is consequently basic to a full understanding of the genetics and breeding of any species group as approached from all perspectives, and essential to its successful manipulation in the production of polyploids.

More detailed explanation of technical terms used throughout this chapter can be found in several dictionaries of genetics and cytogenetics, e.g., King and Stansfield (1985).

Normal Meiosis

The meiotic chromosome behavior of the eastern and some other oysters was described by Longwell et al. (1967) and by Longwell and Stiles (1968a). Except where otherwise designated, the following account is based on this work, or on related observations of these authors. The same authors also described a procedure for improving the quality of chromosome preparations of very yolky oyster eggs so as to facilitate examination of their meioses (Longwell and Stiles 1968b).

The paired mid-prophase (pachytene) chromosomes of the yet unspawned, maturing oocyte (taken from the eastern oyster gonad) are seen as small, separate, nucleus-like bodies. Amplification of ribosomal RNA genes at the nucleolus-organizing loci of the chromosomes occurs during this period of meiosis (Raven 1958, 1961; Costantini et al. 1978; Guraya 1986), accounting for the atypical appearance of these prophase chromosomes.

Fully mature, pre-spawned eggs of the eastern oyster, and also of the Pacific oyster, *Crassostrea gigas*, and European flat oyster, *Ostrea edulis*, have been observed arrested at the post-pachytene (diplotene-diakinesis) stage of the first meiotic prophase. When sampled directly after spawning, eastern oyster eggs are at the succeeding metaphase stage. This is true whether they are allowed to spawn naturally, or thermally stimulated to spawn, and whether or not sperm are present in the water. Occasionally a spawned egg is observed at prometaphase of the first meiotic division (Fig. 1).

Unripe, overripe, or otherwise poor quality eggs probably account for the abnormally clumped first meiotic metaphase chromosomes observed in some experimental spawnings of eastern oyster eggs. This basic chromosomal phenomenon was clearly shown in amphibians and mammals to be associated with gametic aging (Blandau 1975). The fact that full normal maturation of molluscan eggs is associated with meiotic as well as physiological maturation was recognized by Longo (1983).

The early stage of meiotic maturation at which molluscan eggs are spawned may further contribute to the frequent irregularity of their meiosis. Metaphase is many times more sensitive than telophase (when the chromosomes near the spindle poles) to chemical and physical perturbations, and appears to be so in all animal groups (von Borstel and St. Amand 1963). It was noted in the earliest studies of chromosome division conducted on a variety of marine invertebrates that externally fertilized eggs completing meiosis after fertilization are particularly susceptible

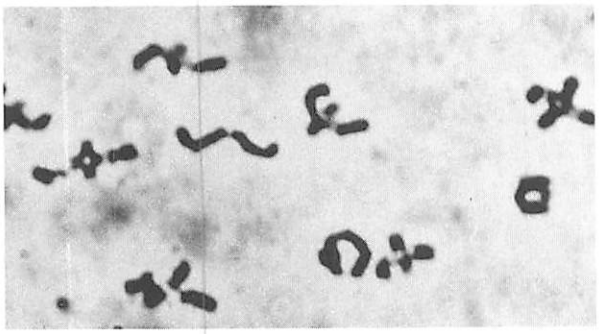

Figure 1. The 10 chromosome pairs of a spawned eastern oyster egg at prometaphase of their first meiotic division (100 × phase-contrast objective). From Longwell et al. (1967).

to abnormalities of one or both meiotic divisions (Wilson 1953). This sensitivity has been used as a measure of the effect of contaminant toxicity to the process of meiotic maturation of eastern oyster and hard clam, *Mercenaria mercenaria*, eggs (Stiles 1990; Stiles-Jewell 1994; Stiles et al. 1991).

Observations of prometaphase or diakinesis chromosome configurations in spawned eastern oyster eggs indicate that recombination of genes occurs during oogenesis of this species. This evidence comes from chiasmata, i.e., knot-like contortions of the chromosome pairs that occur as a consequence of the exchange of homologous segments between paired parental chromosomes. Frequency and distribution of chiasmata can vary in response to environmental change (Riley 1948; Rees and Jones 1977), thereby affecting genetic variability. Chiasmata variability has not been examined in any oyster species.

The dense nucleus of the sperm head is recognizable in the ooplasm of the eastern oyster egg shortly after fertilization (Fig. 2). The oocyte chromosomes, arrested at first meiotic metaphase until fertilization, divide shortly after entry of the sperm. They are clearly observable during this process.

One of the two nuclei resulting from the first meiotic division passes directly onto the division apparatus of the second meiotic metaphase. The other becomes the first polar body nucleus. Eastern oyster chromosomes appear at this time as short, contracted bodies. The second division, which rapidly ensues, yields the second polar body nucleus and the fully mature female gamete.

By the end of the first meiotic chromosome division, the dense sperm nucleus has developed into the diffuse male pronucleus. Chromosomes of the male parent emerge from this nucleus during the second meiotic division of the oocyte. The chromosomes of the male and female gamete can be distinguished from one another, in that those of the female tend to be more elongate (Fig. 3). Fertilization allows better discrimination of the male set of chromosomes than is possible of spermatocytes in maturing male gonads.

Meiosis in the eastern oyster egg is of the common type found in most vertebrates and higher plants. Recently Guo (1991) described the same type of meiosis for *C. gigas*. This species is the only oyster aside from *C. virginica* in which meiosis has been thoroughly studied (Lu 1986). Nonetheless, more limited observations on the meiosis of other species of *Crassostrea* and *Ostrea* reported in conjunction with determination of their chromosome numbers (below) indicate that meiosis during oogenesis of all these oysters is probably of this general type.

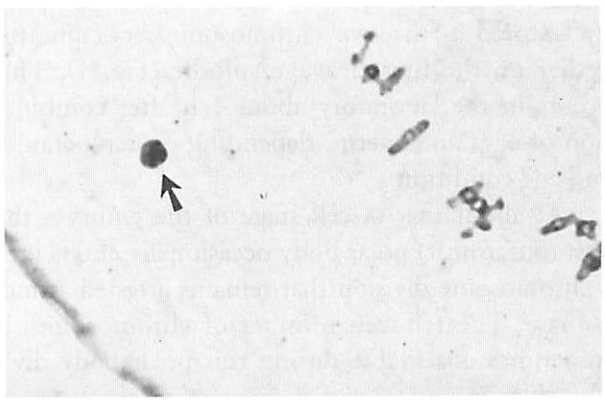

Figure 2. The 10 eastern oyster chromosome pairs in a fertilized egg. These bivalent chromosomes are aligned on the spindle and ready for separation in their first meiotic division. The dense, round nucleus developed from the sperm [arrow] appears somewhat apart from the bivalents (100 × phase-contrast objective). From Longwell and Stiles (1968).

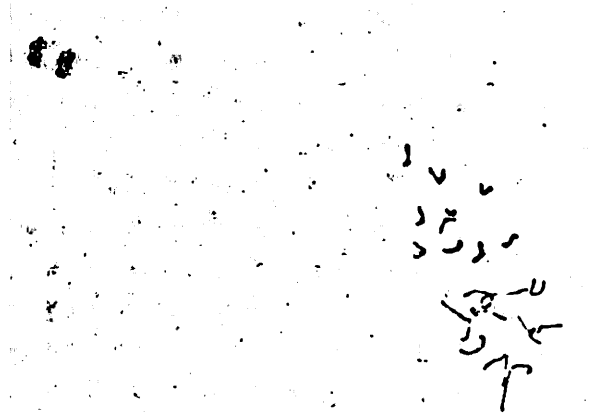

Figure 3. The reduced chromosome number of eastern oyster male and female gametes (10 each) before they come together on the first cleavage spindle of the egg. The more elongate chromosome group is that of the female. The two small dense bodies at top left are polar body nuclei (100× phase-contrast objective). From Longwell and Stiles (1968).

Observations on both *C. virginica* and *C. gigas* further indicate that meiosis in oyster spermatogenesis is also of the common type. Genetic crossing-over occurs in the chromosomes of oyster spermatocytes just as it does in the oocytes (Ahmed 1973).

The reduced chromosome number of the eastern oyster egg and sperm are combined and diploidy restored as the two chromosome sets come together on the first cleavage spindle (Fig. 4). This occurs in the laboratory about 1 h after combination of eggs and sperm, depending on temperature and egg condition.

At about the 64-cell stage of the embryo, the first (outermost) polar body occasionally enters into a chromosome division that remains arrested at metaphase. The reduced number of chromosomes is sometimes observable during this polar body division (Fig. 5).

A study conducted on *C. gigas* showed temperature to be the best predictor of the duration of meiosis, although it does have significant interaction with salinity (Lu 1986). Temperature does not affect the proportion of time taken by the various division phases. The duration of meioses varies more for some increment changes in temperature than it does for others.

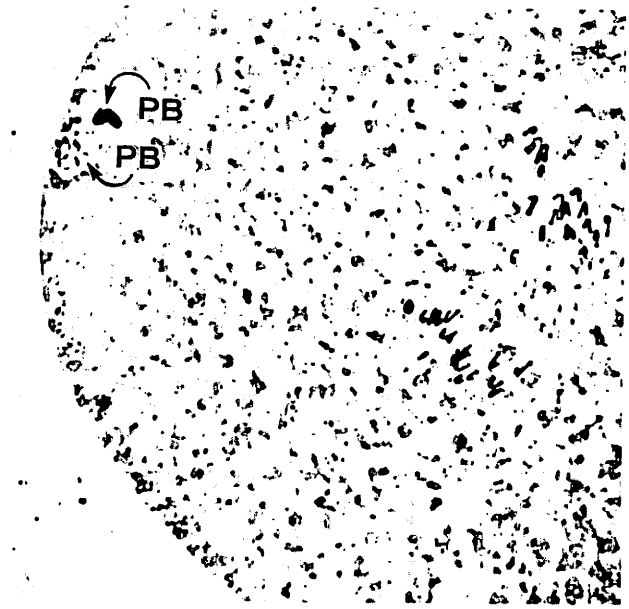

Figure 4. The 20 chromosomes of an eastern oyster egg shown here at mitotic anaphase of the first cleavage division of the egg. The dense body and reticulate body at the edge of the egg are the two polar body [PB] nuclei (100× phase-contrast objective). From Longwell and Stiles (1968).

Fertilization, Abnormal Meiosis, and Cleavage

Despite the constancy in number of oocyte chromosome pairs, about 10% of the cleavage embryos from mass spawnings of about 850 wild eastern oysters were either haploid, polyploid, hyperdiploid (more than two but less than three full chromosome sets), hypodiploid (less than two but more than one set), or mosaics of cells with different chromosome numbers (Stiles and Longwell 1973). Frequencies of numerical chromosome abnormalities in oyster zygotes, many at least resulting from irregular meiotic divisions of the oocyte, differed from spawning to spawning. Abnormalities of eastern oyster oocyte meioses were also briefly remarked on by Longwell et al. (1967) and Longwell and Stiles (1968a). These occur as well in the Pacific oyster (Ahmed and Sparks 1967). The exact magnitude of numerical chromosome abnormalities in zygotes is not known for many species, but they can make a large contribution to gametic wastage, devel-

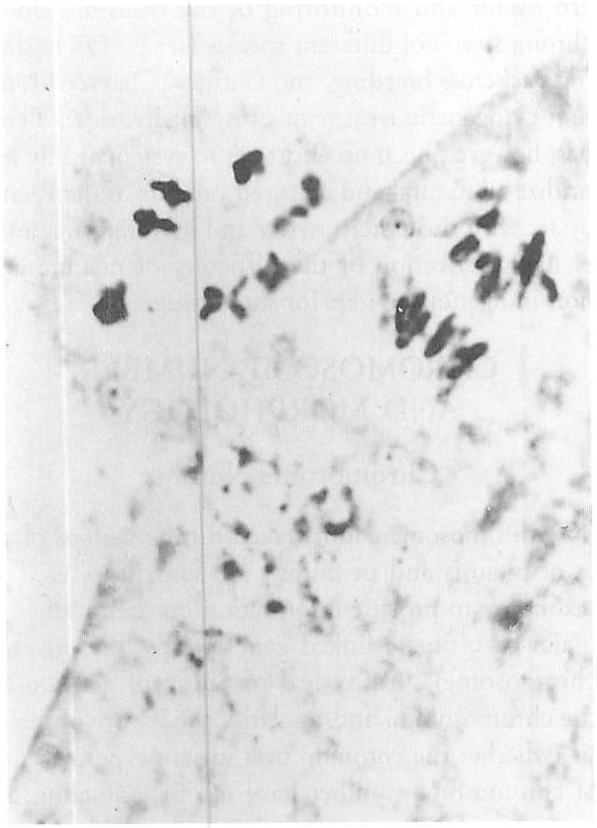

Figure 5. An arrested chromosome division in the polar body of a cleaving eastern oyster egg. The polar body is the bleb-like projection at the upper left edge of the egg (100× phase-contrast objective). From Longwell and Stiles (1968).

opmental abnormality, and zygote mortality (Khush 1973; Gillies 1989).

Fertilization, meiosis, and ensuing embryo cleavage mitoses often have higher frequencies of abnormality in interspecies than intraspecies crosses. Sometimes fertilization and meiosis fail altogether (Riley 1948; Swanson 1981). A measure of meiotic abnormality in interspecies crosses is, therefore, a potentially useful gauge of species relatedness.

Considerable normal fertilization, meiosis, and cleavage occur in interspecies fertilizations of the eastern with the mangrove oyster, *C. rhizophorae*, and also in crosses of *C. virginica* with *C. gigas* (Menzel 1969, 1972, 1973; Stiles 1973, 1978, 1981; Stiles et al. 1985; Scarpa and Allen 1992). There is no evidence that successfully developing zygotes of these crosses are parthenogenetic. Similar results are obtained in cross fertilizations of the Pacific with the Portuguese oyster *C. angulata*, a possible synonym or subspecies of *C. gigas* (see Carriker and Gaffney, Chapter 1; Menzel 1969, 1972, 1973). Somewhat similar results were obtained in crosses of the Pacific with *C. rivularis*, identified as the "Suminoe oyster from Oregon" (Scarpa and Allen 1992) but most likely *C. ariakensis* (see Carriker and Gaffney, Chapter 1).

The incidence of effective, normal fertilization, meiosis, and cleavage is, nonetheless, lower in interthan in intraspecies fertilization of the eastern, Pacific, and mangrove oysters (Stiles 1973, 1978, 1981; Stiles et al. 1985). Scarpa and Allen (1992) also found depressed fertilization in crosses of the eastern with the Pacific oyster but not with the Suminoe oyster. Fertilizability of Pacific oyster females with *C. rivularis* males was depressed as judged by polar body elevation (Allen and Gaffney 1993).

Although Menzel (1969, 1972, 1973) reported generally normal fertilization, meiosis, and cleavage mitosis in crosses of the Pacific with the Portuguese oyster, Stiles (1973) noted lower than usual fertilizability of *C. angulata* with *C. virginica*. Unfortunately, only a single female was among the small group of *C. angulata* available for the fertilization trial, pointing up the difficulty of obtaining and maintaining non-indigenous oysters in good spawning condition for studies such as these. The comparative cross-fertilizability of the Pacific and Portuguese oysters with the eastern oyster merits further study.

In contrast to the somewhat subtle differences in crossability of the eastern with the Pacific and mangrove oysters, and of the Pacific with the Suminoe oyster, *C. ariakensis*, fertilization, meiosis, and cleavage were mostly abnormal in crosses of the eastern, Pacific, and Portuguese oysters with the slipper-shaped oyster *C.* (formerly *Saccostrea*) *iredalei* (Menzel 1969, 1973). Irregular ploidy levels, described in cleavage division of these crosses, suggest that fertilization may not have been genetically effective, and that whatever development ensued was mostly parthenogenetic. Crosses of the oysters listed above with the Sydney rock oyster, *Saccostrea commercialis,* did not result in any embryonic cleavage (Menzel 1969).

The ability of some oysters to cross, as discussed above, is probably influenced by genes of the type previously documented to regulate the crossing ability of several higher and lower plants, bacteria, protozoa, and insects (Brewbaker 1964; Williams 1964). Such systems probably exist as well in the hermaphroditic tunicates (Morgan 1942), and in two hermaphroditic clams (Okamoto and Arimoto 1986; Ó Foighil and Thiriot-Quiévreux 1991).

Although normal fertilization and meiosis are an essential prelude to successful species hybrids, these alone do not assure that the hybrid will be viable. The eastern and Pacific oyster hybrid has consistently failed to survive to metamorphosis[1] despite considerable normal early cytological development (Davis 1950; Imai 1977; Imai and Sakai 1961; Stiles 1978, 1981; Stiles and Choromanski 1990; Gaffney 1990; Downing 1991; Scarpa and Allen 1992; Allen et al. 1993). The hybrid of the eastern oyster and *C. ariakensis* similarly fails to metamorphose, as do triploid hybrids of the eastern oyster and either *C. ariakensis* or the Pacific oyster (Allen et al. 1993). This hybrid inviability may be attributed to species incompatibilities at the nuclear or cytoplasmic gene level not involving behavior or structure of the chromosomes. However, cryptic structural differences in chromosomes of the eastern and Pacific oysters influencing gene expression remain a possible contributing factor.

Use of an intermediate species hybrid, the third parent serving as "bridge," may lead to the successful combination of *C. virginica* and *C. gigas* genes, particularly in combination with backcrosses to the eastern oyster and monitoring of the transmission of chromosomes of different species (see p. 457 regarding backcross breeding, and Gaffney, Chapter 11, for non-cytogenetic treatment of oyster hybrids). There has, however, been no effort yet to systematically hybridize all natural and cultured variants of the Pacific oyster with the eastern oyster and its variants, another likely reflection of the difficulty of maintaining non-indigenous oysters for such studies.

[1] R.W. Menzel stated in a public discussion at the first Aquaculture Genetics Conference, Galway, Ireland, that he had been in error about having obtained a successful hybridization of *C. virginica* and *C. gigas* (Menzel 1969). His statement, however, fails to be included in G.T. Newkirk, 1983, Applied breeding of commercially important mollusks: a summary of discussion, pages 415-422 in N.P. Wilkins and E.M. Gosling, editors. Genetics in Aquaculture. Elsevier Science Publishers, Amsterdam.

CHROMOSOME NUMBER AND MORPHOLOGY

Chromosome Number

Chromosome counts taken during various phases of meiosis and in mitosis are sometimes used in taxonomy to provide information on the number of major or ultimate linked gene groups (one to each chromosome), in physical mapping of genes onto the chromosomes, and to clarify the nature of meiosis (whether the common or a variant type). Counts of chromosome number have use in evaluating the outcome of interspecies hybridizations or experimental manipulations of meiosis or mitoses for the purpose of inducing polyploidy or parthenogenesis.

The number and morphology of eastern oyster chromosomes (karyotype) were determined by Longwell et al. (1967) and Longwell and Stiles (1968a) in relation to their study of meiosis, fertilization, and cleavage. Except where otherwise cited, the description of *C. virginica* chromosomes given here is based on these two reports.

Oocytes of the eastern oyster in prometaphase and metaphase of the first meiotic division clearly have 10 pairs of chromosomes (Figs. 1 and 2). Ten chromosomes can again be counted at the first meiotic anaphase, and at the second meiotic metaphase and telophase. The group of chromosomes developed from fertilizing sperm also has 10 chromosomes (Fig. 3), as does the first polar body (Fig. 5). Counts of chromosomes in 2 to 32-cell embryos give a diploid number of 20 (Fig. 4). Twenty chromosomes are observable in premeiotic mitoses of the gonads, and in mitoses of *C. virginica* gill cells.

Fifteen species of *Crassostrea* and six species of *Ostrea* examined have a haploid number of 10, as do three species in the genus *Saccostrea. Ostrea lapèrousii* (in Japan, the Hiroshima race of *C. gigas*) and *O.* (= *Crassostrea*) *ariakensis* may have a haploid number of 12 and slightly different karyotype (Kobayashi 1952, 1954). Information on chromosome numbers of these diverse oysters comes from the following citations: Ahmed and Sparks 1967; Longwell et al. 1967; Menzel 1968, 1969; Ahmed 1973, 1975; Ieyama and Inaba 1974; Ieyama 1975; Rodríguez-Romero et al. 1979a; Thiriot-Quiévreux and Ayraud 1982; Nakamura 1985; Stiles et al. 1985; Vitturi et al. 1985.

The bronze oyster, *Dendostrea folium,* of the subfamily Lophinae (Harry 1985) has one chromosome pair less than the usual 10 reported for Crassostreinae and Ostreinae oysters (Ieyama 1990). One of three members of the family Pycnodonteinae (deepsea oyster, *Neopycnodonte cochlear* [Poli, 1795]) likewise has only nine chromosome pairs (Vitturi et al. 1985). The other two Pycnodonteinae examined (*Parahyotissa imbricata* and *Hyotissa hyotis*), however, have 10 pairs (Ieyama 1990; Stiles, unpub. obs.).

It is interesting to note that oyster species have the lowest chromosome number for bivalves; numbers in bivalves can range up to 23 (Murray 1975). Gastropod molluscs have a broader general range, with haploid chromosome numbers of 5 to 44 in non-polyploid species. The generally lower chromosome number of bivalves is, however, not reflected in the triploid clam, *Corbicula leana* (Corbiculidae) (Okamoto and Arimoto 1986), nor in polyploid congeners of the minute hermaphroditic clam, *Lasaea australis* (Leptonidae), with its widely ranging number of regular chromosomes as well as its supernumerary chromosomes numbering up to 340 (ÓFoighil and Thiriot-Quiévreaux 1991). (Supernumerary chromosomes are small chromosomes present in some species in varying numbers with no apparent harmful effect.)

Improvement in techniques for displaying oyster chromosomes now allows a better view of them in oyster larvae, making it possible to trace the fate of eastern oyster embryos with irregular chromosome numbers, and the fate of chromosomally abnormal cells in otherwise diploid embryos (Stiles and Choromanski 1983, 1987; Komaru and Wada 1985). *In situ* DNA hybridization described below, in addition, has a special utility for detecting aneuploidy and polyploidy as it occurs in particular cell types within tissues.

It was recently determined that mosaic embryos composed of diploid cells and cells with more or less than the diploid number of chromosomes (aneuploid number) can survive to the juvenile stage in C. *gigas* and in *Ostrea denselamellosa* (an Asiatic species belonging to the subgenus *Ostrea* [*sensu stricto*] (Harry 1985, Thiriot-Quiévreux et al. 1988, 1992; Insua and Thiriot-Quiévreux 1991) (some non-mosaic *C. gigas* aneuploids also seem to survive [Guo and Allen 1994]). At least in *C. gigas,* these chromosomal chimeras (mixoploids) exhibit reduced growth (Thiriot-Quiévreux et al. 1988, 1992). The slowest growing juveniles have the highest percentage of cells with less than the normal complement of 20 chromosomes, and aneuploidy is not correlated with allozyme variability. As Thiriot-Quiévreux et al. (1992) point out, these observations raise important questions about the basis of the large variation in growth rate observed in young Pacific oysters, something seen also in young eastern oysters.

Chromosome Morphology

Each member of each first meiotic chromosome pair of eastern oyster chromosomes is of similar morphology and staining characteristics (Fig. 1). There is no evidence of a heteromorphic sex chromosome pair as occurs in mammals and insects.

Chromosomes of embryo cleavage cells are in general somewhat longer than those of the fertilizing sperm, and longer than the second meiotic metaphase chromosomes. Combined total length of all 10 second metaphase chromosomes of the eastern oyster is only about 13 μm (Longwell et al. 1967). At metaphase of cleavage mitoses, individual lengths of these chromosomes range from about 1 to 7 μm if one considers both untreated embryos and those

conventionally treated with the drug colchicine to disrupt the spindle, and shorten and straighten the chromosomes for karyotyping (Figs. 6, 7). Measurement of individual chromosomes of treated embryos showed the longest to be about 1.5 to 3 times the length of the shortest. Mean length is from 2.0 to 3.3 µm. Taking chromosome length as roughly equivalent to DNA, each chromosome pair carries from about 7.5 to 13% of the total genetic material of *C. virginica*.

Position of the centromere (the spindle attachment region of the chromosomes) on the eastern oyster chromosomes is median or submedian, dividing each chromosome into two equal or near equal arms. The most equal arm ratio is 1.0; the most unequal is 2.0.

One or two of the longest median chromosomes of the eastern oyster sometimes show a constriction about two-thirds the distance from the centromere to one end of the chromosome. Such constrictions correspond to the primary, transcriptionally active nucleolar or ribosomal gene locus (Swanson 1957; Longwell 1987a). A diagrammatic representation of the eastern oyster karyotype is given in Fig. 8.

Most species of Ostreidae examined appear to have the same general chromosome morphology and length just as they have the same number of chromosomes, but few thorough karyotypic comparisons have been made. Those that have been conducted reveal some interesting variations.

Rodríguez-Romero et al. (1978, 1979a, b) reported a difference in number and position of metacentric and submetacentric chromosomes among karyotypes of *C. virginica*, the Columbian oyster, *C. columbiensis*, and *C. rhizophorae*. This variation in the karyotypes of the eastern oyster and *C. rhizophoae*, along with protein and sequence differences in sub-unit ribosomal DNA (see Carriker and Gaffney, Chapter 1), is consistent with their status as separate species. The number of metacentric and submetacentric chromosomes in *C. sikamea* (the Kumamoto oyster) may also differ from closely related *C. gigas* (Ahmed 1973).

Thiriot-Quiévreux and Ayraud (1982) reported two pairs of telocentric chromosomes (single-armed with the centromere at one end) for the European

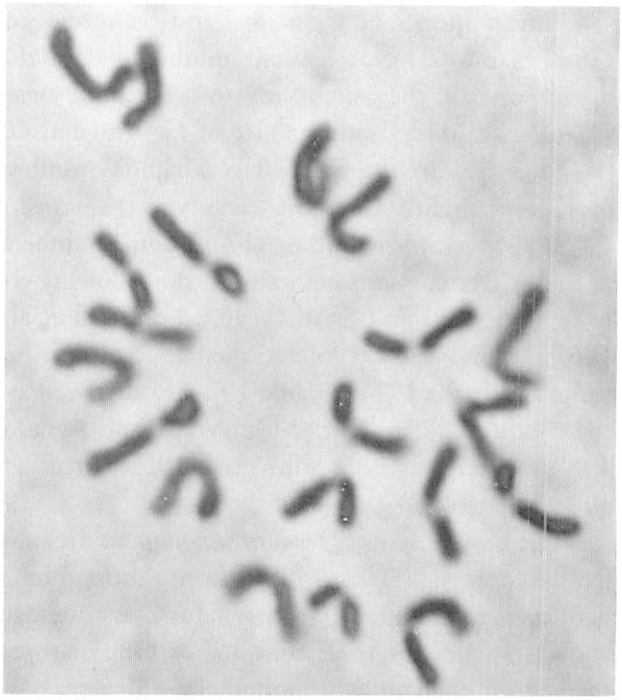

Figure 6. The 20 chromosomes of an eastern oyster egg at prometaphase of a cleavage division (100× phase-contrast objective).

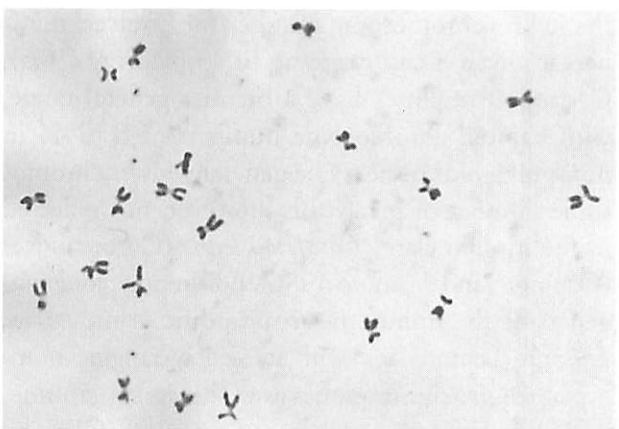

Figure 7. The 20 chromosomes of an eastern oyster cleavage division after treatment for karyotyping. The chemical colchicine was used to disrupt the spindle and contract the chromosomes as they began their division process (100× phase-contrast objective). From Longwell et al. (1967).

flat oyster, and Ieyama (1990) reported the same for the nine chromosome pairs in *D. folium* (Lophinae). The basic karyotype of *O. denselamellosa* is similar to that of the Olympia oyster, *Ostreola conchaphila* (Ahmed and Sparks 1967), and differs from that of all *O. edulis* populations examined (Thiriot-Quiévreux 1984).

The karyotype of the nine chromosome pairs in *Neopycnodonte cochlear* (Pycnodonteinae) differs from that of the Ostreidae (Vitturi et al. 1985). However, *Parahyotissa imbricata* of the same family has a karyotype similar to that of species in the genus *Crassostrea*, as well as the same number of chromosomes (Ieyama 1990).

Dissimilarity of species chromosome structure is measured more definitively by examining gametogenesis in F_1 species hybrids than by chromosome karyotype alone (Riley 1948). Lapses in chromosome pairing caused by structural differences in the chromosomes of two parental species and their related failure to form chiasmata (a reason for hybrid sterility) are sometimes directly observed at meiotic prophase (Gillies 1989). The irregular chromosome pairing that occurs in hybrid gametogenesis is, nonetheless, more often measured less precisely as the consequent misdivision of the chromosomes at their first meiotic anaphase. It is worth noting here that Menzel (1972) reported observing normal meiosis in both *C. gigas* × *C. angulata* and *C. virginica* × *C. rhizophorae* hybrids. The rather small variation in number and centromere position of the chromosomes in *C. virginica* and *C. rhizophorae* must not be sufficient to interfere greatly with their pairing in prophase of meiosis.

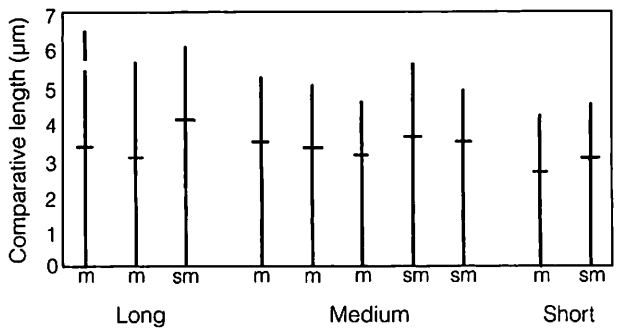

Figure 8. Diagrammatic representation of the eastern oyster chromosomes of three relative lengths. The centromere of each is designated by the crossline, and a secondary constriction on the longest chromosome by the break in the line near one chromosome end. The "m" stands for metacentric chromosome, and "sm" for submetacentric chromosome. Adapted from Longwell et al. (1967).

None of the studies of oyster chromosome morphology have had the advantage of modern automated image analysis which can greatly improve the precision with which small differences in karyotype are measured.

DNA CONTENT

Low total DNA content of the eastern oyster (Hinegardner 1974) is in agreement with the size and small number of its chromosomes. Each chromosome of all eucaryotes (organisms with true nuclei that undergo mitosis and meiosis) is believed to contain a single gigantic molecule of DNA (Friefelder 1987). Eastern oyster DNA consists of about 4.7×10^8 nucleotide pairs, near the extreme low for bivalve molluscs. This is only about half the DNA content of the surf clam, *Spisula solidissima*, and one-eighth that of the nut clam, *Acila castrensis*, which has the largest bivalve genome measured biochemically (Hinegardner 1974). It is interesting to note that the combined length of the nine chromosome pairs of *D. folium* (Ostreidae, Lophinae) is only about half that of the 10 chromosome pairs of *P. imbricata* (Pycnodonteinae) with its *Crassostrea*-like karyotype (Ieyama 1990).

At least 60% of the DNA in *C. virginica* consists of unique nucleotide sequences coding for cell structural or enzymatic proteins. These unique sequences are interspersed with nucleotide sequences that occur in tandem repeats along the length of the chromosomes (Goldberg et al. 1975). General function of repetitive DNA is uncertain, but it is important in discerning fine structural change in chromosomes.

INTRASPECIFIC CHROMOSOME POLYMORPHISM

Structural chromosome change has been associated with adaptation and evolution of other animal and plant groups (Wright 1968; Merrell 1981). Such alterations of chromosomes promote gross morphological modifications by their effects on chromosome pairing and segregation (Wilson et al. 1974; Bush et al. 1977; Syvanen 1984; Krimbas and Powell 1992). They simultaneously act as a major isolating mechanism because half the gametes of an animal having even one such change in its complement of chromosomes can be genetically unbalanced (Wilson et al. 1974; Bush et al. 1977; Syvanen 1984). The often profound biological effect of structural chromosome changes (translocation of a segment of one chromosome to another, and inversion of a segment within a chromosome such as would alter the centromere position of the oysters' chromosomes described above) make their identification useful in hybrid breeding programs and in tumor research, as well as in ecological and biodiversity studies.

The small size of the chromosomes of the eastern and other oysters does not allow detection of much polymorphism when only basic procedures are used to differentiate and compare members of chromosome pairs within and between individuals, or even to compare the complete chromosome complements of different populations or species. Nonetheless, a variation in basic chromosome number and structure was found in some populations of *O. edulis* (Thiriot-Quiévreux and Ayraud 1982).

Without further study, it is impossible to know whether the seeming lack of gross karyotypic differences in the eastern oyster is matched by an equivalent lack of the finer structural variability revealed by technologies for staining discrete chromosome sectors (bands), or for hybridizing DNA sequences onto the chromosomes *in situ* (Longwell 1987a). There is evidence for a link between gross structural change of the chromosomes and variation in gene function of value in both research and breeding (Bennett et al. 1981; Brandham and Bennett 1983; Committee on Biotechnology 1984). It is not likely that eastern and other oysters are exempt from regulatory mechanisms having a basis in chromosome structure. Systems of balanced chromosome polymorphisms could help explain the presence of considerable meiotic and zygotic abnormalities in the eastern oyster, and be a basis for enzyme heterozygote deficiency and perplexing aspects of bivalve population genetics for which other tentative explanations have been provided (Zouros 1988; Thiriot-Quiévreux et al. 1992).

The only study on eastern oyster chromosomes employing other than a basic technology concerned chromosome G-bands (Rodríguez-Romero et al. 1979c; Sumner 1990). The G-bands are characterized by a richness of particular DNA bases, and their staining depends largely on variation in DNA base composition (Comings 1978). It is of interest that the eastern oyster was found to have a rather large number of G-bands because there is generally little compartmentalization of DNA base composition in cold-blooded vertebrates (Medrano et al. 1988; Hillmer et al. 1991).

Consistent C-bands (late replicating, repetitive, transcriptionally inactive DNA, of the class to which transposable genetic elements belong) occur about the centromeres of four of the 10 chromosome pairs of *O. denselamellosa* (Insua and Thiriot-Quiévreux 1991). Two other chromosomes of *O. denselamellosa* occasionally have bands about their centromeres. Terminal C-bands were occasionally observed on the ends of six pairs of chromosomes. Polymorphisms in constitutive heterochromatin, as these late replicating chromosome regions are referred to, are generally known to distinguish species, populations, and individuals (Brutlag 1980; Longwell 1987a). They sometimes account for significant species differences in DNA content in the absence of comparable change in chromosomal length and number.

There are NOR-bands (nucleolus organizer regions) on the four *O. denselamellosa* chromosome pairs with C-bands about their centromeres, and also on another pair without C-bands (Insua and

Thiriot-Quiévreux 1991). The NOR-bands represent ribosomal gene clusters that were transcriptionally active during the prior interphase, and are known to differ widely between individual vertebrates, including fish (Foresti et al. 1981). There is a heteromorphism in the number of active NOR sites both within and between individuals of the *O. denselamellosa* broodstock studied. There are heteromorphisms of NOR-band number within *O. edulis* (Thiriot-Quiévreux and Insua 1992). The location of NOR bands differs within the karyotypes of *O. denselamellosa*, *O. edulis* and *C. gigas* (Insua and Thiriot-Quiévreux 1991). The size of the NORs varies intraspecifically in *C. gigas* (Thiriot-Quiévreux and Insua 1992). One eastern oyster NOR site was identified by Longwell et al. (1967) as being on the longest chromosome pair of this species from an observed secondary constriction of the type normally associated with the major nucleolus. No NOR-band studies, however, have been conducted on *C. virginica.*

The more recently developed procedures for nucleic acid hybridization onto chromosomes *in situ* (Hamkalo and Narayanswami 1985; Sharma 1985; Lichter et al. 1991) may be applicable to the study of oysters, their taxonomy, and their breeding. Even the fine structural organization of the entire chromosome complement can be revealed by DNA hybridization once a full array of DNA probes becomes available for hybridization. These procedures can be used on chromosomes prepared as those shown for the eastern oyster in Figs. 1 to 7, as well as on small chromosomes that have been electrophoretically separated (Schwarz and Cantor 1984; Chu et al. 1986). Importantly, *in situ* techniques are applicable to non-dividing, and even to terminally differentiated nuclei of cells and tissues. Aneuploidy and polyploidy are detectable *in situ* in both dividing and non-dividing cells.

In the eastern oyster, repetitive sequence DNA, the type most often used for *in situ* hybridization, is of two classes and similar to that of other molluscs (Collier 1971; Kamalay et al. 1976; Kidder 1976a, b; Collier and Tucci 1980). The shorter class with about 20 repeats comprises about 28% of all *C. virginica* DNA (Kamalay et al. 1976). The larger class with about 3,000 repeats comprises about 10% of eastern oyster DNA. Longer tandem arrays of DNA comprise the ribosomal genes. Some of this DNA should be detectable with C- and NOR-banding procedures as well as by *in situ* techniques.

POLYPLOIDY AND CHROMOSOME ENGINEERING

Polyploidy in Nature

In nature, polyploid individuals seem to arise most commonly through the occasional failure of meiosis (Beatty 1957; Swarup 1959; Stebbins 1970; White 1973; Lewis 1980; Gillies 1989). An egg with the unreduced diploid number of chromosomes is fertilized and results in a triploid individual. Oocytes from these triploids in turn often fail to undergo meiosis. When fertilized, they yield more regularly breeding tetraploids (polyploids with four chromosomes sets; see below).

Two general characteristics of all polyploids are their increased cell and nuclear size and reduced reproductive potential (Beatty 1957; Swarup 1959; Stebbins 1970; Lewis 1980). Polyploid species often occupy a different habitat than their diploid relatives, grow under a wider range of conditions, and occasionally exhibit a gigantism or change in shape or tissue proportions. Polyploid congeners of the clam, *L. australis,* have a far wider distribution than diploid forms, and have direct developing instead of planktonic larvae (Ó Foighil and Thiriot-Quiévreux 1991). Even polyploid tumor cells of mammals are more invasive than non-polyploid tumor cells, grow more rapidly, and possess greater adaptability (Brodsky and Uryvaeva 1985).

There is a selective polyploidization that accompanies development, differentiation, and growth of most diploid species, including gastropod molluscs. Thus it is unlikely that there is a completely diploid organism when both somatic and germinal tissue are considered (Nagl 1978, 1985; Brodsky and Uryvaeva

1985). Such polyploidization occurs either by programmed arrest of mitosis leading to multinucleation of cells, or by chromosome reduplication without mitosis (endoreduplication).

Absence of Natural Polyploidy

The several species of *Crassostrea* and *Ostrea* examined for chromosome number provide no evidence of evolutionary polyploidy, despite the labile nature of meiosis in oysters which should facilitate chance occurrence of polyploids. Polyploidy, however, occurs in the freshwater clam, *C. leana* (Okamoto and Arimoto 1986), and in the highly successful congeners of *L. australis* (Ó Foighil and Thiriot-Quiévreux 1991).

Animals with low DNA content, such as oysters, generally have more somatic endoreduplication and less polyploidy of the germ line. They have more rapid growth and development than those that depend more heavily on mitosis for these processes (Nagl 1978, 1985). These factors may explain the absence of natural polyploidy in oysters and the paucity of mitoses even in rapidly growing oysters relative to the much larger incidences of mitoses occurring in slower growing mammals and in plants. (The low mitotic index in oyster somatic tissue is obvious to any experienced cytogeneticist studying their chromosomes.) In their early analysis of eastern oyster chromosomes, Longwell et al. (1967) made observations suggestive of somatic chromosome pairing and somatic reduction of chromosome number in the eastern oyster, phenomena often associated with endoreduplication (Rieger et al. 1976). Now, new technology for chromosome studies discussed above allows definitive analysis of such cellular phenomena in oyster species with small chromosomes and few somatic mitoses. Nonetheless, too little work has yet been done on oysters to further address the matter of their growth by endoreduplication.

Induced Triploids

Oyster triploids are of interest and importance in aquaculture because their lesser expenditure of energy in gonad development leads to better condition and growth during seasonal gametogenesis (Allen 1987; Allen and Downing 1991). There may be other benefits as well (see Gaffney, Chapter 11).

Oyster eggs can be artificially polyploidized by deliberately inhibiting the meiotic division of their chromosomes. Spawned eggs are treated with either chemicals (usually cytochalasin B), heat or cold shock, or high pressure (Longo 1972; Chourrout 1984; Quillet and Panelay 1986). The ploidy level of the zygote depends on how the chromosomes behave when the division for which they have replicated is arrested (Stiles et al. 1983; Stiles and Choromanski 1986). It will depend as well on the behavior of the centrioles or spindle organizing centers (Mazia 1961) which, like the chromosomes, will have replicated in preparation for the aborted division.

Triploidy has been induced in the eastern and also in the Pacific, European flat, and Sydney rock oysters, and triploid individuals reared (Stanley et al. 1981, 1984; Downing and Allen 1984; Quillet and Panelay 1986; Gendreau and Grizel 1990; Nell et al. 1994). It has been induced as well in several other molluscs (Beaumont and Fairbrother 1991). See the following for information on optimization of triploid induction, detection of triploids, and evaluation of the treatment: Allen 1983, 1987; Downing and Allen 1984; Stanley et al. 1984; Chaiton and Allen 1985; Allen et al. 1989; Uchimura et al. 1989; Beaumont and Fairbrother 1991; Barber et al. 1992; Scarpa et al. 1994.

Because oyster eggs are spawned at the first meiotic division, they are potentially polyploidized by arresting either the first or the second phase of their meiosis. The genetic composition of individuals resulting from blockage of the first meiotic division should differ from those resulting from blockage of the second division. Stanley et al. (1984) provided some evidence from allozyme analyses that the first meiotic division of the eastern oyster can be successfully blocked to produce triploid individuals.

It should be noted that until recently neither experimental nor hatchery production of triploid

oysters has been directly followed by cytogenetic observations of meiosis and early embryo mitosis. Stiles and Choromanski (1986, 1990), however, conducted cytogenetic studies on the disruption of the first meiotic division of the eastern oyster and found the outcome irregular. Some embryo cleavages were tetraploid and some triploid, and some embryos were mosaics of polyploid and aneuploid constitution. These findings were recently corroborated on the Pacific oyster by Stephens and Downing (1988), Guo (1989, 1991), and Guo et al. (1991, 1993). *In situ* DNA hybridization technology now makes possible more exact analysis of chromosome segregation, even as to individual members of the full chromosome complement.

Triploidizing the eastern and Pacific oysters, the pearl oyster, *Pinctada fucata martensii*, and the blue mussel, *Mytilus edulis*, by blocking meiosis has been reported as variously successful. Results have ranged from high incidences of triploid induction and good survival of triploid individuals to near complete loss of triploidy (Downing and Allen 1984; Chaiton and Allen 1985; Stiles and Choromanski 1986; Yamamoto and Sugawara 1988; Uchimura et al. 1989). Some individuals have both tetraploid and aneuploid cells.

It is not surprising that oyster larvae and juveniles reared from eggs treated to induce development as triploids should sometimes consist of cells or tissues of varying ploidy levels and aneuploid chromosome numbers, or that triploidy may seemingly be lost over development and growth. Mosaics of diploid, polyploid, and aneuploid cells have been observed even in embryos from untreated eastern oyster eggs, and in juvenile Pacific oysters reared from untreated eggs (see above). Cells with deviant chromosome numbers are unlikely to contribute equally to development and growth, so their measured frequencies in a larval culture are apt to change over time. Some cell types can be lost entirely.

Efficacy of the triploidizing treatment will also depend on the time of meiosis at which it is administered (Allen et al. 1989). Inefficient treatment may have no effect, or lead to abnormal chromosome divisions. Because the first and second meiotic divisions vary cytogenetically, the arrest of one will be associated with different subsequent division failures than arrest of the other, as described above (Stiles and Choromanski 1986; Guo 1989, 1991). Unless the meiotic stage is very synchronized among the oocytes, cultures of oyster eggs treated to induce triploidy should contain higher incidences of chromosomal chimeras than those of untreated eggs. Synchronization of meiosis in the oyster depends on the condition and maturity of the eggs that are artificially spawned or stripped and varies considerably in the eastern oyster (Longwell and Stiles 1968a). The longer duration of meiosis in the European flat oyster may allow better synchronization of its two meiotic divisions (Gendreau and Grizel 1990).

Extra centrioles from arrested meioses or mitoses have the potential for organizing multipolar instead of normal bipolar spindles during ensuing chromosome divisions of diverse cell types (Mazia 1961; Brodsky and Uryvaeva 1985; Alieva and Vorobjec 1991). Multipolar spindles cause either a reduction in ploidy level of the daughter cells, or such irregular chromosome division that these cells die. Some reports documenting the diploidization of somatic sectors, the germ line, or premeiotic cells of different allo- and autopolyploid plants are Wettstein (1932), Brown (1947), Gottschalk (1960), Stebbins (1970), Lewis (1980), and Finch (1983). Similar phenomena occur in tissues of hybrid and non-hybrid invertebrates and vertebrates (including mammals) (Beatty 1957; Chu et al. 1964; Lentati 1966; Bickham et al. 1985).

The exceedingly large number of germ-line mitoses associated with the enormous fecundity of oysters allows ample opportunity for depolyploidization of triploid cells to occur. Any reversal of polyploidy in the germ line can be detected by counting the number of first meiotic metaphase chromosomes in mature eggs of sexually ripe triploid oysters. This has not been done for triploids of oysters or any other bivalve.

Finally, even a slight change in the usual diploid chromosome constitution of an organism can increase the occurrence of cells with unstable chromosome numbers (Khush 1973). Polyploid individuals with their extra gene copies tend to tolerate chro-

mosome loss better than diploids in which less than a full chromosome set is more likely to lead to cell death.

Triploid Sterility

The unequal number of chromosome sets in oyster triploids precludes the regular pairing required for normal chromosome segregation during the first meiotic division. The effect of triploidy on chromosome pairing is so elemental a phenomenon it can be generally extended to triploids of any normally diploid organism. Most gametes will be genetically unbalanced with extra numbers of different chromosomes. In evolution, successful triploid organisms circumvent the sterility imposed by their unequal number of chromosome sets by suppressing meiosis and adopting a non-sexual form of development (see below), or they eliminate an entire chromosome set before or during meiosis (Ohtani 1993). Although polyploidy was initially supposed to be an irreversible process, even the diploidization of polyploids has since been considered an evolutionary mechanism in some plants (White 1978).

Without any modification of meiosis, abnormal chromosome pairing alone does not assure absolute sterility of newly induced triploids as may be desired to prevent propagation of a non-indigenous species in nature. Despite pairing irregularities, triploids can produce an occasional normal or a diploid gamete (theoretically between 0.1 and 0.2% in species such as the eastern oyster that have 10 chromosome pairs, assuming general conditions of chromosome behavior) (Dawson 1962; Khush 1973; Brodsky and Uryvaeva 1985; Gottschalk 1985; Gillies 1989; Tyagi and Dubey 1989). Also, even fertilization of two chromosomally unbalanced gametes sometimes yields a balanced diploid zygote. Crosses of triploids with diploids could yield diploid offspring if rare chromosomally balanced zygotes meet with ecological conditions favoring their survival. The offspring of triploid Japanese pearl oysters, *Pinctada fucata martensii*, fertilized with spermatozoa from diploids has recently been shown to include a low number of both diploid and triploid juveniles (Komaru and Wada 1994).

Triploid oysters with diploid gonad sectors (as above) will have the potential of undergoing normal meiosis in the diploid portions of their gonads, thereby yielding proportional numbers of normal gametes. This phenomenon will occur if the triploid state is not accompanied by a physiological imbalance causing total reproductive failure for reasons not associated with chromosome pairing (see Gaffney, Chapter 11).

Tetraploidy

Production of triploid oysters increases the possibility of chance occurrence of viable tetraploid lines through failure of meiosis in fertilized triploid eggs, particularly if triploid females are crossed with diploid males. As noted above, this is the mechanism whereby tetraploidy seems to have arisen in nature, although usually in conjunction with interspecies hybridization.

Tetraploids can be induced from diploid oysters in several ways, three of which are through blockage of meiosis and blockage of the first cleavage division of the zygote (Stiles et al. 1983; Stiles and Choromanski 1986; Guo 1991; Guo et al. 1991, 1993), and blockage of somatic mitoses in developing germ tissue (Longwell 1987a). When all four chromosome sets derive from a single species, the tetraploid is referred to as an autotetraploid to distinguish it from tetraploids with parents of two different species, i.e., allotetraploids. Autotetraploid embryos have been variously induced in the eastern oyster, the Pacific oyster, and European flat oyster, the hard clam, the dwarf surf clam, *Mulinia lateralis*, and the Manila clam, *Ruditapes philippinarum* (Stiles and Choromanski 1986; Diter and Dufy 1990; Gendreau and Grizel 1990; Beaumont and Fairbrother 1991; Guo 1991). None of these tetraploids reached metamorphosis or set. A few tetraploid spat of the blue mussel and of the Manila clam have been obtained (Scarpa et al. 1993; Allen et al. 1994). Some tetraploid *C. gigas* produced from triploids fertilized by the sperm of diploids followed by blockage of the first polar body division have survived to the juvenile stage, and are being evaluated (Guo and Allen 1994a).

Though usually less vigorous than triploids, tetraploids offer some advantages to the breeder that triploids do not, aside from potential increase in size. Chromosome pairing in tetraploids tends to be more regular so more genetically balanced gametes are produced. Fertility is sometimes restored to sterile species hybrids through the doubled number of chromosomes. When sufficiently vigorous, tetraploids can be crossed with diploids to produce triploids, thereby eliminating the need for repeated experimental treatment of eggs to induce triploidy. An alternate possibility is the artificial tetraploidization of developing gonad tissue of diploid male or female oysters to produce diploid gametes which would yield triploid embryos upon fertilization with regular haploid gametes (Longwell 1987a).

Allotetraploids have a use in the selective introgression of chromosomes or genes from one species into another by backcrossing progeny to the overall best species. Backcrossing is a traditional means whereby disease resistance genes in plants are transferred from resistant to susceptible species (Longwell 1987b). During the backcross generations, diploidy is restored and most of the chromosomes of the less desirable species parent are lost (Longwell 1987b; Gillies 1989). There should be strong natural selection against the most severely unbalanced gametes of the triploid parent, and of the zygotes resulting from crosses of these with the diploid native species. This facilitates directed selection back to diploidy, the intensity of which is determined by the breeder and size of the culture. The number of generations required to reestablish diploidy, as reconstructed with one or more chromosomes of the two initial species parents, will depend on pairing propensities of the different species' chromosomes and how well the oyster tolerates aneuploidy, as well as on the intensity of the artificial selection. A small number of diploids (and triploids) would probably occur in the second backcross generation based on probable pairing and segregation of oyster triploid chromosomes. Introgressive breeding programs can now be expedited by recognition of otherwise indistinguishable chromosomes of the two species parents via *in situ* DNA hybridization technology.

Parthenogenesis

Most species in the animal kingdom that have replaced sexual reproduction with various types of asexual reproduction (parthenogenesis) have been polyploids of hybrid origin (Cuellar 1974; Cherfas 1981; Swanson 1981; Gillies 1989). Change occurs through the alteration or suppression of meiosis. Interspecies hybrids of enough animals and plants develop by spontaneous parthenogenesis to warrant examination of the meiosis of any new species hybrid intended for further breeding. Polyploidy and parthenogenesis may co-occur in evolution because both originate through some variation or suppression of meiosis.

Just as they are a potential source of natural tetraploids, induced triploid hybrid oysters and any interspecies or intergeneric hybrids may be looked to as a potential source of naturally occurring parthenogenetic offspring. The hermaphroditic clam, *C. leana* — of probable polyploid origin — is suspected of reproducing parthenogenetically (Ikematsu and Yamane 1977; Okamoto and Arimoto 1986), as do non-planktonic forms of the hermaphroditic clam, *L. australis* (Ó Foighil and Thiriot-Quiévreux 1991). Polyploid *L. australis* congeners are suspected of having arisen from rare hybridizations of divergent forms of the species (Ó Foighil and Thiriot-Quiévreux 1991).

There is a low incidence of spontaneous parthenogenesis in the normal diploid eastern oyster, but no evidence that such eggs survive (Longwell and Stiles 1968a; Stiles and Longwell 1973). Spontaneous parthenogenesis may occur more frequently in inbred lines (Longwell and Stiles 1973), or alternately in crosses of oysters with different genetically determined reproductive times (Lannan 1980).

No attempt has been made to deliberately provoke gynogenetic development of oyster eggs through their fertilization with sperm of incompatible species or genera. Some attempts were made to induce parthenogenesis in the eastern oyster by fertilizing eggs with radiation-inactivated sperm (Stiles 1978; Stiles et al. 1983). As with *M. lateralis* and *M. edulis* (Scarpa 1985; Beaumont and Fairbrother 1991), no known

parthenogenetic progeny were produced. More recently, however, diploid gynogenetic eggs of the Pacific oyster were successfully reared to metamorphosis (Guo et al. 1993). Gynogenetic diploid individuals of dwarf surf clams have been reared to sexual maturity (Guo and Allen 1994b), as have blue mussels to the juvenile stage (Fairbrother 1994). (Further work is being done on the Mediterranean mussel, *M. galloprovincialis* [Scarpa et al. 1994].) Possibly gynogenetic embryos of eggs from fertile interspecies hybrids or from hybrid polyploid oysters would be more viable than those of non-hybrid eastern oyster eggs. Interestingly, sperm inactivation occurs naturally in the hermaphroditic self-fertilizing congeners of *L. australis* (Ó Foighil and Thiriot-Quiévreux 1991).

The genetic outcome of parthenogenesis depends on how the process of meiosis is altered and if meiosis occurs at all (Beatty 1957; Gillespie and Armstrong 1980; Cherfas 1981; Streisinger et al. 1981; Purdom 1983). Population genetic variation, nonetheless, is reduced. This condition could be favorable in stable aquaculture environments, possibly in combination with polyploidy.

Haploid parthenogenetic embryos of diploid female oysters are unlikely to be viable. Parthenogenetic embryos made diploid by arresting the second meiotic division will be highly inbred, the degree of homozygosity depending on the amount of genetic recombination in the preceding meiotic prophase. When eggs of individuals developing from these embryos are in turn induced to develop gynogenetically, each produces a separate, more genetically homozygous line than would otherwise be obtainable in just two generations of breeding.

Any surviving tetraploid, parthenogenetic offspring resulting from arrest of the first meiotic division in oysters will be heterozygous and carry the full set of maternal genes, though differently arranged because of genetic recombination (Stiles et al. 1983; Stiles and Choromanski 1986). Any surviving diploid, parthenogenetic offspring will be heterozygous partial copies or partial clones of the maternal genome. How close these come to being exact maternal copies depends on the behavior of the arrested first division chromosomes, and on the amount of genetic crossing-over that occurred in the preceding meiotic prophase.

Induced parthenogenetic development of regularly bred fertile tetraploid oysters could yield genetically heterozygous diploid clones of the maternal genome (Longwell 1985, 1987a, b). This would occur when there is selective pairing of the exactly identical homologous chromosomes as opposed to random pairing of identical and non-identical maternal and paternal homologues (see Longwell 1985; 1987a, b). Selective pairing of perfectly and partly identical homologous chromosomes is best known in allotetraploids of other organisms, but can also occur in autotetraploids.

CONCLUSIONS

Crassostrea virginica undergoes the typical form of meiosis exhibited by most organisms during both oogenesis and spermatogenesis. There is exchange of chromosome segments between maternal and paternal chromosomes during prophase of its meiosis. Maternal and paternal chromosomes randomly assort to the four products of every meiosis, each of which has half the basic chromosome number of this oyster.

Meiosis and early cleavage mitosis in the eastern oyster, however, are often abnormal. About 10% of the cultured embryos of laboratory or hatchery spawnings can have irregular chromosome numbers. Abnormalities of meiosis and cleavage mitosis must account for much of the zygote mortality of the eastern oyster. As is the situation for *C. gigas*, these abnormalities may provide an explanation for the large size differences between *C. virginica* juveniles reared under uniform conditions in the same cultures.

The chromosomes of *C. virginica* are small compared to those of most animal species studied cytogenetically. The eastern oyster has only 10 rather short chromosome pairs, and the DNA content of its genome is equivalently small. The basic features of *C. virginica* chromosomes have been described, but no known genes have yet been mapped onto them. The primary ribosomal gene cluster is proba-

bly located near the end of one arm of the longest eastern oyster chromosome.

There is evidence that the number and structure of oyster chromosomes and DNA content are less fixed among individuals, populations, species, and higher taxonomic groups than initially supposed. Technology for hybridizing DNA and RNA onto chromosomes and nuclei *in situ* provides a basis for determining how *C. virginica* chromosomes may vary in this regard and will be of help in the difficult problems of oyster taxonomy.

Incipient crossing barriers occur within and between oyster species, including the eastern oyster. A marked crossing barrier was observed in intergeneric crosses of *C. virginica* with *Saccostrea commercialis.* Embryos of the cross *C. virginica* × *C.* (formerly *Saccostrea*) *iredalei* had irregular ploidy levels and development may have been parthenogenetic.

Although no natural oyster polyploids have been found, artificial manipulation of *C. virginica* meiosis is presently used to produce triploid oysters for aquaculture and for research. There is, as well, interest in other manipulative alterations of eastern oyster meiosis to produce tetraploid and parthenogenetic oysters, and some promise that these manipulations will eventually be successful. Recently, some tetraploid Pacific oysters were induced from triploids in a manner similar to the spontaneous occurrence of tetraploids from natural triploids of other plant and animal groups.

Polyploids are of use in breeding programs designed to introgress chromosomes or genes of one species into another. In combination with induced parthenogenesis, tetraploids may possibly be used to produce genetically heterozygous maternal clones.

The spontaneous evolutionary suppression or variation of meiosis associated in plants and other animals with natural polyploidy and asexual development — often in conjunction with wide hybridization — is now known to occur in Bivalvia in one and possibly two clams. Artificially induced polyploid oysters, in particular polyploid hybrids, are a potential source of naturally occurring parthenogenetic offspring of utility in oyster breeding programs.

In the future, *in situ* markers identifying most or all the *C. virginica* chromosomes will allow chromosome transmission in hybrid backcrosses to be followed, and these markers will be used to ascertain the exact chromosome makeup of chromosomally engineered oysters. Because gene function is so dependent on chromosome structure, molecular cytogenetics will play an increasingly important role in the breeding, management, and basic study of the eastern oyster.

ACKNOWLEDGMENTS

A. Longwell dedicates this paper to the memory of Dr. Ernest R. Sears for his life-long studies of polyploidy and chromosome engineering of crop plants that serve as a model and inspiration for work on other species. Thanks go to E. Willis for typing many drafts of this review.

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Chapter 13

Natural Environmental Factors

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INTRODUCTION

Gunter (1957) wrote, "temperature is the most important single factor governing the occurrence and behavior of life," a point reiterated by Kinne (1970) who stated that, "with regard to life on earth, temperature is, next to light, the most potent environmental component." It can act directly on the organism as a factor affecting physiological performance, and it can also be a factor in evolution of the species, acting as a selective force in speciation. Prytherch (1928) stated that, "in the environment of the oyster, temperature is the most important factor as it controls, either directly or indirectly, the growth and reproduction of the organism." In the eastern oyster, *Crassostrea virginica*, as in many other organisms, the processes of reproduction, development, and growth are intimately linked seasonally to climatic conditions and to the availability of energy resources. In addition, thermal effluent may, in many instances, induce detrimental changes in reproduction, gametogenesis, fecundity, and larval development. Conversely, heated effluents from power plants have been of benefit to oyster growers in their efforts to extend the growing season.

For estuarine species, salinity is also an important and influential factor, limiting distribution of many aquatic organisms (Gunter 1961; Wells 1961). Butler (1949c) suggested that the single most important factor affecting oyster populations is salinity. Salinity variations in estuaries may be diurnal, seasonal, or spatial and changes may be gradual or abrupt.

Of all the abiotic factors that can affect the biology of an estuarine organism such as *C. virginica*, the synergistic effects of temperature and salinity probably have the most profound effects. As pointed out by a number of researchers (e.g., Alderdice 1972; Vernberg and Vernberg 1972), two or more environmental variables working in concert can have more profound biological consequences than any one of those factors acting independently.

Temperature or salinity affect virtually every aspect of oyster biology including feeding, respiration, utilization of food reserves, gonadal development and time of spawning, parasite-disease interactions, predation rates, growth, and distribution. Temperature and salinity can affect rate functions, can be the trigger that initiates a process, or can be a threshold factor such that a particular temperature-salinity combination is necessary for continuation of specific processes. Further, effects can vary with specific stages of the oyster's life cycle.

In addition to temperature and salinity variations, oysters experience other environmental factors such as variations in seston concentrations, light, and pH. There is an extensive literature available on the effects of all of these factors on marine invertebrates, and only those studies that specifically pertain to *C. virginica* are discussed here. This review will elucidate the physiological and behavioral mechanisms that allow the eastern oyster to survive and flourish under often harsh estuarine conditions.

DISTRIBUTION, TOLERANCE, AND SURVIVAL

Adult oysters are highly tolerant of extremes in ambient temperatures and are commonly found in waters where the annual range is from –2° to 36°C (Butler 1954; Gunter 1954; Galtsoff 1964). Loosanoff and Engle (1940) reported an elevated body temperature of 35.7°C for oysters immersed in shallow (25 cm) tidal pools; only 12 h later on the following tide, the water temperature was 22°C lower. In some shallow-water habitats, emersed oysters can be frozen solid in the winter and, if not disturbed, will thaw out and survive when covered by water (Loosanoff 1965). If frozen oysters are shaken or dropped, however, death occurs. Henderson (1929) reported a thermal death point for *C. virginica* of 48.5°C and Fingerman and Fairbanks (1956, 1957) noted appreciable death and weight loss in oysters exposed to temperatures above 41°C. Galtsoff (1964), however, reported that oysters can survive intertidal temperatures of 46° to 49°C when emersed at low tide. This finding was supported by Ingle et al. (1971) who reported survival of intertidal Gulf oysters at 49.5°C even when temperatures between 44° and 49.5°C were sustained for 3 h. Further, Vernberg et al. (1963) demonstrated that excised gills survived for 100 min at 44°C.

Rate of change of temperature seems to have a greater effect than temperature level per se, i.e., the slower the rate of temperature increase, the lower the upper lethal temperature. Oysters can also be killed by short exposure to high temperatures or longer exposure to lower temperatures. Fingerman and Fairbanks (1957) demonstrated experimentally that the rate of thermal increase can greatly influence the final median tolerance levels. Oysters acclimated to 24°C and exposed to increases of 0.74°C h^{-1} suffered 50% mortality at 41°C. In contrast, oysters exposed to an increase of 13.2°C h^{-1} experienced 50% mortality at 47.5°C. Quick (1971) reported 54% mortality after 5-d exposure to 35°C in oysters acclimated to 16°C. A direct relationship between the survival time of buried oysters and temperature was reported by Dunnington (1968). He found that under these anaerobic conditions oysters survived for 2 d in summer (25°C) and for over 5 weeks in winter (< 5°C) and attributed the differential survival rates to a reduced metabolic rate at low temperature.

As with temperature, oysters have a wide tolerance of salinity. Commercial production occurs in areas with annual salinity variations from 0 to 42.5 ppt (Ingle and Dawson 1950a, b, 1953). The species normally occurs from about 5 to 40 ppt (Galtsoff 1964; Wallace 1966) and Menzel et al. (1966) gave a range of 1.2 to 36.6 ppt. Amemiya (1926) reported the lower and upper salinity limits for this species to be 1.5 and 39 ppt. Loosanoff (1953a) and Wells (1961) gave minimum values for normal survival of 7.5 ppt and 7 ppt, respectively. Several authors set the minimum salinity for indefinite survival at 4 to 5 ppt (Arnold 1868; Ryder 1885; Belding 1912; Loosanoff 1932). The optimum salinity range is generally considered to be about 14 to 28 ppt (Moore 1900; Butler 1949c; Chanley 1958; Galtsoff 1964) although this optimum range can vary geographically. R. Newell (University of Maryland, pers. comm.) suggested that intermediate salinities of about 15 to 18 ppt represent a physiologically optimum range.

Gunter (1950, 1953) noted that although *C. virginica* is uncommon below 5 ppt, it can survive salinities as low as 2 ppt for a month, or even fresh water for several days when water temperatures are low. Self-sustaining populations occurred where salinities were as low as 0.2 to 3.5 ppt for five consecutive months annually (Butler 1952), and permanent oyster communities flourished in 10 to 30 ppt (Butler 1954). Distinct types of oyster reefs occur at different salinity regions in the Gulf of Mexico (Butler 1954). Reefs near the head of an estuary experience salinity ranges of 0 to 15 ppt (average 10 ppt) annually, and because of high annual mortality rates, the populations are sparse. Oysters from low-salinity areas tend to be small and rounded with smooth whitish shells, and spatfall is low and growth slow. Where salinity fluctuates from 10 to 20 ppt (average 15 ppt), populations tend to be dense as a result of high reproductive ability, availability of oyster shell upon which larvae can metamorphose, and low concentration of predators. Near the mouth of a typical Gulf Coast estuary with a salinity of about 25 ppt, growth rates are usually high and reproductive potential is at its maximum; however, competition and predation are also

maximal. Where the estuary opens into high-salinity Gulf waters, oysters are sparse, growth is slow, and mortality is high. Suitable cultch is lacking and the high concentration of predators leads to low spat survival. The commercial importance and reproductive capacity of oyster beds in these high salinity regions are negligible.

Long-term exposure to high salinities can also be detrimental. Eastern oysters can survive in open ocean waters for some time; however, they usually do not reproduce or grow well, although there are exceptions. W. Menzel (late of Florida State University, pers. comm.) observed rapid growth, profuse spawning, and spatfall in salinities of 35 ppt and higher at Port Aransas, Texas. Breuer (1962) described a commercial population of *C. virginica* at Port Isabel in the lower Laguna Madre, Texas, that spawned and grew rapidly in a range of 32 to 42 ppt. Moreover, some spat survived salinities of 1.4 to 4.2 ppt in this same location as evidenced by successful propagation during periods of flood and reduced salinities. Breuer (1962) suggested that this Texas population might constitute a new physiological race because its salinity tolerance is lower than for *C. virginica* from northern populations. The possibility that populations of oysters have different salinity optima warrants further investigation.

Few data exist on effects of salinity on juvenile oysters. Loosanoff (1953a) found that juvenile oysters (spat) could resist reduced salinities as efficiently as do adult oysters. In a later study, Chanley (1958) showed that juvenile oysters less than 1 year old (0.3 to 2.2 mm) from Chesapeake Bay survived waters of 5 ppt and that the optimum salinity for growth of recently set oysters was 15 to 22.5 ppt. Juveniles differed somewhat from larvae in their salinity requirements and responded to reduced salinities as did adults, i.e., with no growth below 5 ppt, slow growth below 12 ppt, and normal growth from 12 to 27 ppt.

The effect of salinity on mortality rate in eastern oysters is highly dependent on ambient temperature as shown by variable survival during spring floods and heavy rains, mostly in the southern portions of the range. Oyster mortality from excessive freshwater runoff is fairly common, as has been reported by numerous authors (see Baughman 1948; Galtsoff 1972; Joyce 1972). Specific areas most frequently affected include the mouth of the Mississippi River (Gunter 1950, 1953; Butler 1952), the upper reaches of the Chesapeake Bay (Beaven 1946; Engle 1946; Andrews et al. 1959), the Santee River in South Carolina (Lunz 1938; Burrell 1977), and areas of Louisiana (Owen 1953; Andrews et al. 1959; Dugas and Perret 1975). The situation is exacerbated because these periods of runoff usually coincide with periods of high temperature and increased metabolic energy demands. Major mortalities in Chesapeake Bay also occurred after Tropical Storm Agnes in 1972 (Cory and Redding 1976; Haven et al. 1976). In some areas, it is the combination of high temperature and high salinity that causes mass mortalities of oysters, as in southeast Louisiana (Owen 1953) and Texas (Copeland and Hoese 1966).

In Long Island Sound, oysters survived in freshwater or at 3 ppt for 70 and 115 d when water temperatures ranged from 8° to 12°C; at the same salinities, all oysters died within 15 d when the temperature ranged between 23° and 27°C (Loosanoff 1948). Andrews et al. (1959) reported that oysters conditioned to low salinity and low temperature combinations were able to withstand low salinities in a state of "narcosis" for as long as closure was continuously enforced by fresh water or other factors.

The tolerance or susceptibility of oysters to pollutants can be exacerbated by temperature and salinity stresses, especially in the early stages of development. Mandelli (1975) exposed juvenile and adult oysters to diluted desalination brines and found that both groups were adversely affected by copper concentrations in the water (19 to 43 μg Cu L^{-1}) and that spring and summer mortalities (elevated temperatures) were higher than those in autumn and winter. MacInnes and Calabrese (1979) reported an influence of temperature in the toxic effects of heavy metals on oyster embryos, with the highest susceptibility to metal toxicity at either 20° or 30°C and the lowest toxicity at 25°C. They also demonstrated that, while interaction of temperature and salinity on survival of embryos and larvae was only significant at high concentrations of copper, low concentrations of copper produced intolerable stress during periods of persistently low salinities and low or high temperatures. They also showed that veliger larvae were more tolerant to temperature and salinity changes than were de-

veloping embryos. A synergistic effect of temperature on the toxicity of free chlorine and chloramine to oyster larvae (7-d old) was also demonstrated by Capuzzo (1979); also see Capuzzo, Chapter 15.

Larval Distribution

Distribution of oyster larvae and their behavior during development is governed by a number of factors, not the least of which is salinity; their response to these factors, coupled with tidal currents, plays a most significant role in their distributional patterns. For years, scientists have debated whether or not horizontal or vertical distribution of oyster larvae and their retention in estuaries at spawning are controlled by active swimming in response to environmental factors, or whether purely mechanical forces of strong currents and turbulences at mid-tides keep larvae in suspension. Some authors believe that oyster larvae are carried at random by currents and exhibit no differential vertical position with tidal stage, whereas others believe that there is a response by larvae to increased salinity and propose this increased swimming as a mechanism in estuarine movement.

Julius Nelson (1908, 1909, 1911 to 1917) found more larvae in the water column during flood than ebb currents. He postulated that larvae were more active during increasing salinities and therefore would tend to rise more during flood currents and be carried up the estuary, i.e., he postulated that the oyster larvae overcome strong, non-tidal seaward drift by rising and swimming on the flood tides and settling to the bottom during ebb tides. His ideas were later confirmed by Nelson (1931) and other authors (Carriker 1951; Kunkle 1957; Haskin 1964; Wood and Hargis 1971).

Nelson (1931) summarized a long-term study of factors that affect vertical distribution of oyster larvae. Oyster larvae tended to be concentrated at the top of the halocline. The sharper the transition zone between the overlying brackish water and the underlying saline water, the more marked the concentration of larvae. The concentration was deemed to be a passive, physical effect with no evidence for any active selection of an optimum salinity by larvae. Nelson and Perkins (1931) were the first to demonstrate increased activity of oyster larvae exposed to increased salinities. They concluded that oyster larvae are usually found at a point midway between the surface and bottom in the water column and that this area of greatest concentration could not be correlated with fluctuation in temperature, pH, or salinity, but rather with areas of greatest current velocity. They believed that, in the absence of a halocline, oyster larvae are most abundant in numbers where current is strongest; where salinity gradients are present, the greatest numbers of oyster larvae were found just above the halocline. Thus, Nelson and Perkins (1931) demonstrated that it is a combination of larval swimming activity, salinity, current, and halocline presence that determines vertical distribution of larvae in the estuary. In the presence of a halocline, larval swimming is stimulated as larvae sink into the more saline water, which causes them to rise into the less saline overlying water. If no halocline is present, or if it moves with the tidal current, larvae are distributed in proportion to speed of the current, being most abundant where current is most rapid (see also J. Nelson 1917). When current is negligible, larvae are found in greatest numbers on the bottom. Contrary to the conclusion of Nelson and his colleagues, Prytherch (1928, 1934) concluded that larvae remain on the bottom during most of the tidal cycle and that they remain within several hundred meters of spawning beds. However, Prytherch's conclusions were not supported by other research.

Loosanoff (1932) found that larvae in the water column were most abundant at and around slack water, i.e., when current velocity was at its minimum, and were practically absent during the peak of flood and ebb. Larvae were most numerous near the bottom during late ebb and least abundant at the beginning of flood tide. These observations led Loosanoff to conclude that oyster larvae swim only during periods of weak tidal currents and remain on the bottom during most of their existence. Later, Loosanoff (1949) did not find any significant correlations between larval number and stage of the tidal cycle; he concluded that larvae do not settle during ebb tides and are thus rapidly dispersed in tidal currents.

Carriker (1947) provided a most insightful survey of the literature extant on evidence for the horizontal movements of oyster larvae. He agreed with previous workers' findings that larvae, especially older stages, rise on the flood and sink on the ebb tide. He concluded by pointing out that much more exhaustive work had to be done both in the laboratory and in the field before this question could be resolved. His detailed field study of larval distribution in New Jersey waters and of larval movements with respect to tidal cycles demonstrated that mature and eyed larvae can be present close to the bottom in relatively large numbers during the ebb tide. Within the larval "swarm," larvae were heterogeneously distributed, with the swarms tending to remain in definite "lanes" or areas (see also Nelson 1952). Carriker concluded that salinity gradients as well as current velocities are influential in larval movements, based on evidence that different larval stages exhibited different vertical distributional patterns, i.e., younger stages tended to sink near or onto the bottom on ebb tide and swim upward on the flood. He also showed that younger stages ebbed and flowed passively with the tide, with older stages tending to sink onto the bottom on the ebb and rise into the water on the flood. Finally, Carriker (1947) determined that relatively large numbers of mature and eyed larvae were present on the bottom during ebb tide. These observations led him to conclude that older larvae tend to migrate into headwaters of estuaries to set beyond the distance made possible by tidal conveyance alone, as a result of an active vertical movement by the larvae. These findings were disputed by Korringa (1952) and Verwey (1966) who believed that swimming movements of the larvae were not important, though Carriker's studies were supported by subsequent research (see below).

Pritchard (1953) provided a detailed study of hydrographic conditions prevailing in the James River, Virginia, and he predicted the distribution of oyster larvae. His theoretical predictions did not agree with observed distributions of oyster larvae, so he suggested that oyster larvae could not be considered simply as passive particles. Rather, they appeared to exhibit some ability to remain closely grouped, independent of the physical character of the circulation and mixing processes.

In contrast to earlier studies, Loosanoff (1949) found no relationship between stratification of larvae and tidal stages in Milford Harbor, Connecticut, and no evidence that larvae in advanced stages of development were more common near the bottom. He believed that oyster larvae do not descend to the bottom during periods of rapid tidal flow but are widely dispersed by tidal currents. These data supported earlier findings of Prytherch (1928) and Galtsoff et al. (1930) who reported the distribution and abundance of oyster larvae in Milford Harbor to be extremely irregular.

Andrews (1954) believed that too much attention had been given to larval activity and too little to the physical system of currents, tides, wind, and turbulence. He believed that larvae are distributed passively with their own active motion essentially limited to vertical migrations. Further support for the passive particle theory came from Manning and Whaley (1954) who concluded that the estuarine circulation system, which provides a means of slow upstream transport and ensured retention of larvae, was apparently the major factor in determining horizontal distribution of oyster larvae and spatfall in St. Mary's River, Maryland. They also suggested that water density can be a controlling factor in larval vertical distribution. This suggestion, though, is in keeping with previous studies in that it is just this vertical migration and activity that ensures distribution via physical means.

Kunkle (1957) found that older larval stages tended to congregate on or near the bottom at both slack and ebb tides, but were in the water column during early and maximum flood. Young larvae (up to 8-d old) were uniformly distributed throughout the water column on both ebb and flood tides. His work thus strongly confirmed that of the Nelsons and Carriker reported above.

Haskin (1964) extended this work with extensive field studies coupled with laboratory experiments. His experiments were designed to determine whether or not salinity is indeed an adequate stimulus to induce activity changes in oyster larvae (Nelson 1931). He

demonstrated that larvae disappeared from surface waters as the tide ebbed and later increased with the flood tide. His data provided strong evidence that there is a different distribution of older stage larvae on flood and ebb tides (Fig. 1). His laboratory data further indicated that large changes in salinity (although greater than those normally experienced in the field [Fig. 2A]) were definitely correlated with increased swimming activity. Under conditions of more subtle salinity changes, there was still a strong, though not as pronounced, pattern of activity change as the salinity varied (Fig. 2B). Although Haskin's (1964) data do not prove conclusively that salinity accounts for vertical distribution of oyster larvae over the tidal cycle, they do demonstrate that salinity can play an important role in regulating larval activity. The accumulated data lend support to the possible role of "ebb tide eddies" as larval traps, evidence for which is found in heavy spatfalls that commonly occur in mouths and lower tributary streams of estuaries (Nelson 1931).

Wood and Hargis (1971) found that coal particles, present in nearly all their samples from the James River, Virginia, had a density similar to that of oyster larvae. Wood and Hargis (1971) took advantage of this built-in "control" and compared distributions of oyster larvae with those of the coal particles. The temporal pattern in concentration maxima was different between larvae and coal particles so Wood and Hargis (1971) concluded that selective swimming by the larvae must be involved. Their data suggest (Fig. 3) that fluctuations in abundance of coal particles coincide with variations in tidal current velocities, whereas those of bivalve larvae coincide with increases in salinity. They claimed that larvae can swim vertically at speeds of 60 cm min^{-1}. With currents of up to 480 cm min^{-1} present in the region, this behavior alone is not sufficient to provide any advantage in distribution. Larvae could, however, rise in the water column through about 10 m of water in 15 min at this rate,

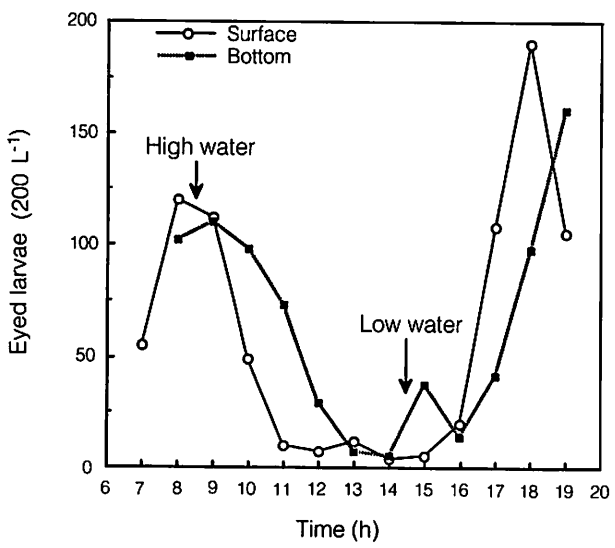


Figure 1. Numbers of eyed larvae collected during tidal cycle observations at the Paris Green Station, Delaware Bay, on August 20, 1956. After Haskin (1964).

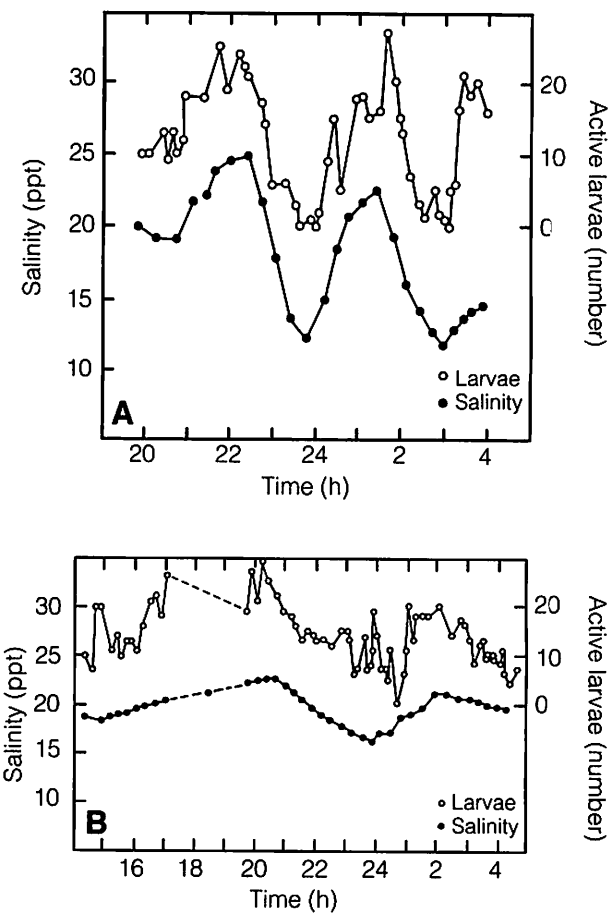


Figure 2. (A) Swimming activity of eyed larvae in response to salinity changes, August 21, 1958 and (B) swimming activity of eyed larvae in response to salinity changes during laboratory experiments, August 30, 1958. After Haskin (1964).

which is sufficient speed to allow them to capitalize on tidal changes in water flow. During tidal ebb (salinity decreases), larvae descend to the bottom where currents are weakest. When the tide floods, larvae stimulated by increased salinities may then swim up into surface waters to be carried upstream. Wood and Hargis (1971) concluded that bivalve larvae are not transported passively, but exhibit an active swimming response that results in movement upriver. These researchers also demonstrated that the swimming behavior was correlated with increases in salinity, not with increasing current speed as had been sug-

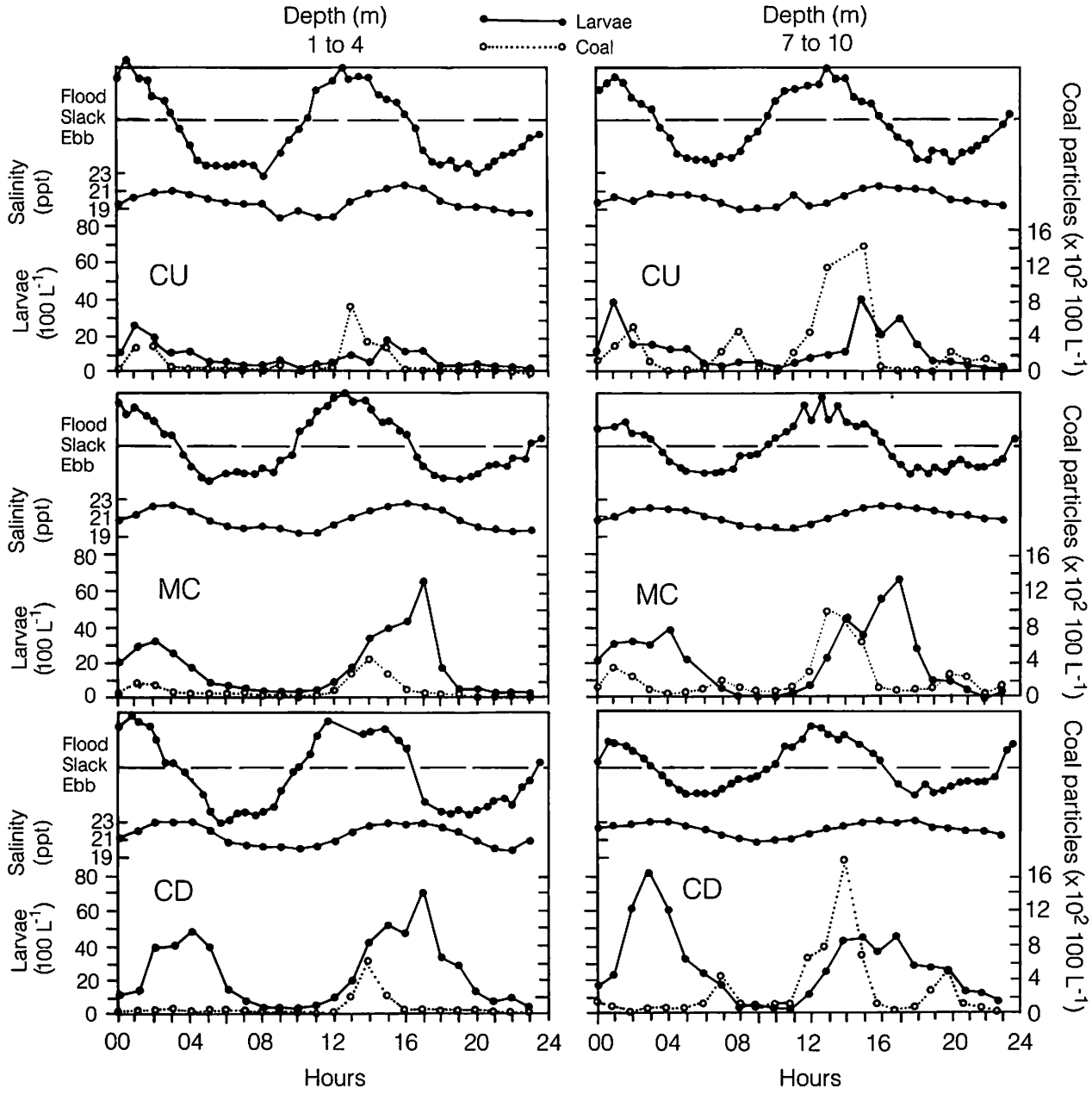


Figure 3. Hourly measurements of relative flow (upper curve), salinity (middle curve), and density of larvae and particles (two lowest curves) averaged for each of three channel stations [CU, MC, CD] in James River, Virginia, over average depths of 1 to 4 m (left) and 7 to 10 m (right). After Wood and Hargis (1971).

gested previously. Menzel (1955) suggested that late umbo larvae of *C. virginica* are really influenced by salinities in relation to the specific gravity of the larvae themselves, so that in high salinities (>35 ppt) they tend to "float" near the surface. This might help to explain intertidal spatfall in high-salinity areas.

Hidu and Haskin (1978) monitored swimming speeds of oyster larvae of various stages in response to differing salinities. Straight-hinge larvae (75 μm shell length) swam vertically between 0.6 and 2 cm min^{-1}, whereas eyed larvae (300 μm) swam at 5 cm min^{-1}. Hidu and Haskins (1978) demonstrated that larvae could move up or down at a rate of up to 14 cm min^{-1}; at these speeds they could move through a linear distance of 7 to 8 m h^{-1}, an ample speed to place larvae well above the bottom in time to "take advantage of" available tidal transport systems. Much higher swimming rates of 67 cm min^{-1} were reported by Wood and Hargis (1971). Different swimming speeds of different larval stages reported by Hidu and Haskin (1978) can partially explain some of the differences of vertical position noted in the work of Kunkle (1957) and others.

Temperature effects on vertical distribution have not been as widely studied as those of salinity. Nelson (1908, 1916) speculated that high water temperatures caused larvae to rise to the surface whereas low temperatures tended to drive them deeper into the water column; however, Nelson and Perkins (1931) found no correlation between temperature and larval distribution. For further discussion of larval behavior see Kennedy, Chapter 10.

REPRODUCTION PATTERNS

Gametogenesis and spawning in oysters are directly correlated with water temperature. In addition, the condition index (the ratio between dry meat weight and shell cavity volume; Grave 1912; Hopkins 1937) of oysters is strongly influenced by season and environmental factors and not solely by temperature. Major changes associated with variation in condition are a function of the gametogenic cycle. For further discussion of the gametogenic cycle in eastern oysters, see Thompson et al., Chapter 9.

Coe (1936) first discussed the role of temperature in determining sex in *C. virginica*. In the northern part of its range, the oyster appeared to be mainly or wholly protandric, but in somewhat warmer waters a higher proportion of young oysters functioned as females during their first breeding season (end of Year 1). Coe (1934) suggested that more favorable conditions for rapid growth of young oysters led to omission or abortion of the initial male phase and that localities and seasons in which 1-year old oysters attained the largest mean size usually had the largest proportion of females in the first breeding season. From North Carolina southward, young eastern oysters of both sexes reached functional maturity within the same reproductive season that they settled. Animals from Connecticut to North Carolina increased not only in mean size of individuals, but also in the proportion of females in yearling populations under favorable conditions (Coe 1936).

A number of authors have proposed various equations to predict maturation of gametes at a given temperature. Loosanoff and Davis (1952) maintained oysters at experimental temperatures ranging from 10° to 30°C in an effort to determine the number of days needed for formation of the first physiologically mature gametes of each sex. They found that almost no gametogenic activity took place at 10°C, whereas a temperature of 15°C proved adequate for ripening and spawning in some animals. Their equation for the prediction of average times needed for development of mature gametes in 50% of the oysters at different temperatures is:

$$D = 4.8 + 4205e^{-0.3554\,T}$$

where D is the average time needed, T is temperature (°C), and e is the base of natural logarithms. Development time ranged from 26.5 d at 15°C to 4.9 d at 30°C (mature gametes); however, calculated data did not always coincide with empirical data. Loosanoff and Davis (1952) suggested that the amount of glycogen in oysters at the beginning of gonadal development could control the quantity of spawn produced.

Price and Maurer (1971) were able to predict the exposure temperature necessary for laboratory maturation of eastern oyster gametes from Delaware Bay. They found that these oysters required 6 to 7 times as long to ripen at temperatures between 12° and

22°C as did oysters from Long Island Sound. The cumulative temperature exposure was more significant in the ripening of Delaware Bay oysters than exposure to "high" temperatures per se, and ripening did not occur at 12°C and below. Their equation is:

$$D = \frac{700}{T\text{-}12}$$

where D is exposure time in days and T is the daily mean exposure temperature within the approximate range of 12° to 22°C. Their equation was specific in that it was only applicable to Delaware Bay oysters removed from the field in winter and spring before ambient water temperatures had risen above 12°C. They estimated that the average number of days for 50% of eastern oysters to produce ripe gametes was 150 d at 15°C, 56 d at 20°C, and only 35 d at 25°C.

Kaufman (1978) proposed a revised version of the formula given by Loosanoff and Davis (1952) such that:

$$D = kT^{(b + a \cdot \log T)}$$

where D and T are the same as in Loosanoff and Davis' equation; k, a, and b are coefficients, the values of which were determined from the experimental data of Loosanoff and Davis (1952):

Event	k	a	b
Time of appearance of mature gametes	10288	−0.29	−1.96
Time of first spawning	348×10^8	3.84	−13
Time of mass spawning	36×10^{18}	7.68	−24

The revised formula allows accurate description of the maturation rate of gametes as a function of ambient temperature, and it can be used to predict both when spawning will be initiated and the time of mass spawning. Kaufman's (1978) calculations for *C. virginica* over temperature changes of 1°C ($15° < T < 30°$) and the experimental data of Loosanoff and Davis (1952) are in agreement.

Kaufman (1978) further concluded, based on calculations using his revised formula, that at lower temperatures, a slight temperature change in the environment causes a more significant change in the rate of gametogenesis than at higher temperatures: at $20° \leq T \leq 30°$ all three processes had a Q_{10} of 2, in agreement with van't Hoff's rule, whereas at $15°C \leq T \leq 21°C$, the figures deviated significantly from the rule. Thus, the time at which the first ova appear increases by a factor of four while the time at which the first spawning occurs is reduced by a factor of five and the time of mass spawning is reduced by a factor of eight.

Salinity is also known to affect gametogenesis, condition index, and spawning in oysters, although to a lesser degree than temperature. Like the effects of temperature, those of salinity also vary among populations. Amemiya (1926) reported normal egg development from 18 to 40.1 ppt, with optimal development in the range of 19.3 to 35.1 ppt. Davis (1958) found egg cleavage from 7.5 to 35 ppt, with normal development from 10 to 22.5 ppt.

Gametogenesis is arrested or depressed at low salinities. Loosanoff (1953a, b) showed that normal gonadal development proceeded in salinities near 7.5 ppt and he placed the lower limit somewhere between 7.5 and 5 ppt. Calabrese and Davis (1970) reported mature gonads and spawning activity at 27.5 ppt, but eggs did not develop at 10 ppt. At 12.5 ppt they rarely developed to the straight hinge larval stage. Larvae reared to the setting size at 27.5 ppt could successfully complete metamorphosis in salinities as low as 9 to 10 ppt. Successful spawning has been reported at salinities ranging from 32 to 42 ppt in the lower Laguna Madre, Texas (Breuer 1962). Butler (1949c) found that gametogenesis was inhibited in oysters held in brackish water for long periods, but that the condition was reversible if oysters were returned to normal conditions. He, too, recorded a lower limit of about 6 ppt for successful gametogenesis and suggested (as did Loosanoff 1953a, b) that the marked variation and suppression of gonadal activity at low salinities could also be caused by variations in food availability. Further, oysters apparently do not "fatten" or increase proportionally in dry weight when

salinity drops below 20 ppt in Canadian waters (Medcof and Needler 1941; Medcof 1946). Ingle and Dawson (1953) suggested that the low glycogen content and poor quality of oysters from Apalachicola Bay, Florida, could be due to great ranges in salinity to which oysters were exposed.

The glycogen content of *C. virginica* is intimately linked to the reproductive cycle which in turn is strongly affected by season. Generally, condition index and glycogen content decline during the breeding season and at elevated temperature. This is a result of more rapid conversion of glycogen to glucose in response to elevated metabolic demands (see Mitchell 1917; Medcof and Needler 1941; Medcof 1946; Galtsoff et al. 1947; Chipman 1948; Menzel and Hopkins 1952; Lee et al. 1960; Haven 1962; Sakuda 1966). In a comprehensive review, Walne (1970) summarized data available on condition index in various oyster species including *C. virginica.* He found that the median condition index increased exponentially with the latitudinal location of the species or population and suggested that populations in cooler waters generally tend to be in better condition than those from warmer waters. Figures 4 and 5 summarize data on seasonal variations in the proportion of glycogen and in condition indices for *C. virginica,* respectively.

Condition index is often high in winter and declines to a lower level in summer once spawning has occured. Condition indices vary among populations. Engle (1958) reported meats in Chesapeake Bay to be thinnest in summer, with an increase in condition index during winter. In contrast, Galtsoff et al. (1947), working in a different section of the Bay, reported higher indices in summer than in winter. Galtsoff et al. (1947) demonstrated that glycogen content of oysters from the York and Piankatank Rivers in Virginia varied seasonally, but that quantitative variations were different between the two areas. They further demonstrated that the glycogen content of York River oysters decreased from the mouth of the river towards its head; the process was reversed in the Piankatank River, where oysters from upper parts of the river were richer in glycogen than those from the lower part of the river. They also showed that transplanting oysters with low glycogen content from an environment where oysters generally grew poorly to an environment where oyster growth and condition was better could increase the glycogen content of the transplanted oysters.

Swift and Ahmed (1983) showed that eastern oysters exhibit some regulatory mechanism for maintaining concentrations of glucose, total Lowry-positive substances (LPS), and triacylglycerols in the hemolymph. Over a 27-d period of starvation, oysters purchased commercially (Washington, D.C.) and held at constant temperatures and salinities maintained concentrations of their hemolymph glucose and LPS; environmental extremes, however, caused variations in these hemolymph constituents (Swift

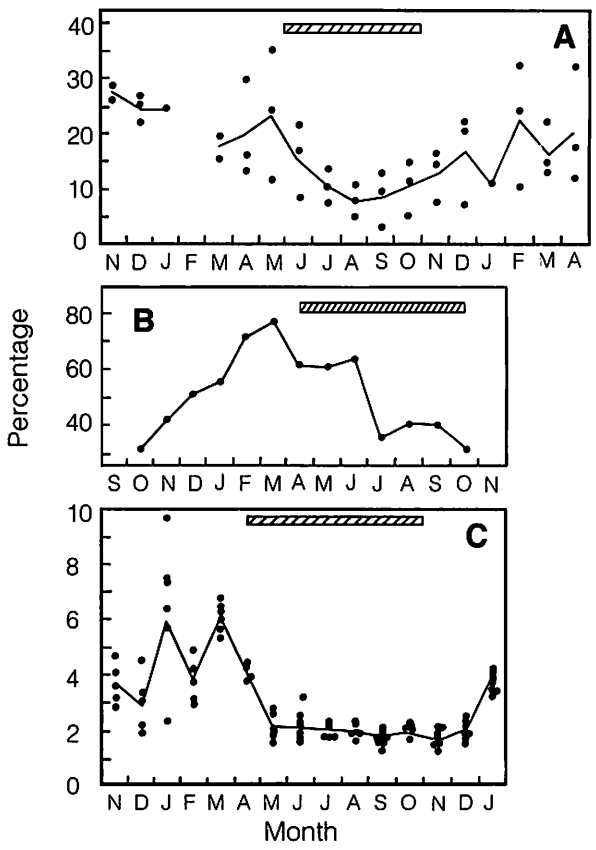


Figure 4. Seasonal variation in the percentage of glycogen (or carbohydrate) in the tissues of *C. virginica.* Data from (A) Galtsoff et al. (1947), (B) Lee et al. (1960), and (C) Menzel and Hopkins (1952). Hatched bar indicates approximate time of spawning. After Walne 1970.

and Ahmed 1983). Oysters maintained at 4°C had significantly higher concentrations of hemolymph glucose and LPS than did oysters held at 20°C. Oysters maintained at low salinity (12 ppt) had significantly lower concentrations of glucose in their hemolymph and concentrations of LPS than animals kept at either 18 or 24 ppt.

Seasonal changes in protein and carbohydrate content of oyster hemolymph have also been reported. Fisher and Newell (1986) showed in laboratory experiments that temperature, salinity, and nutrition did not play a major role in affecting concentrations of these constituents, thus indicating good short-term regulation. They did find, however, that there were large differences in hemolymph constituents between oysters taken from different localities. They attributed these differences to long-term differences in environmental condition or to population differences. They

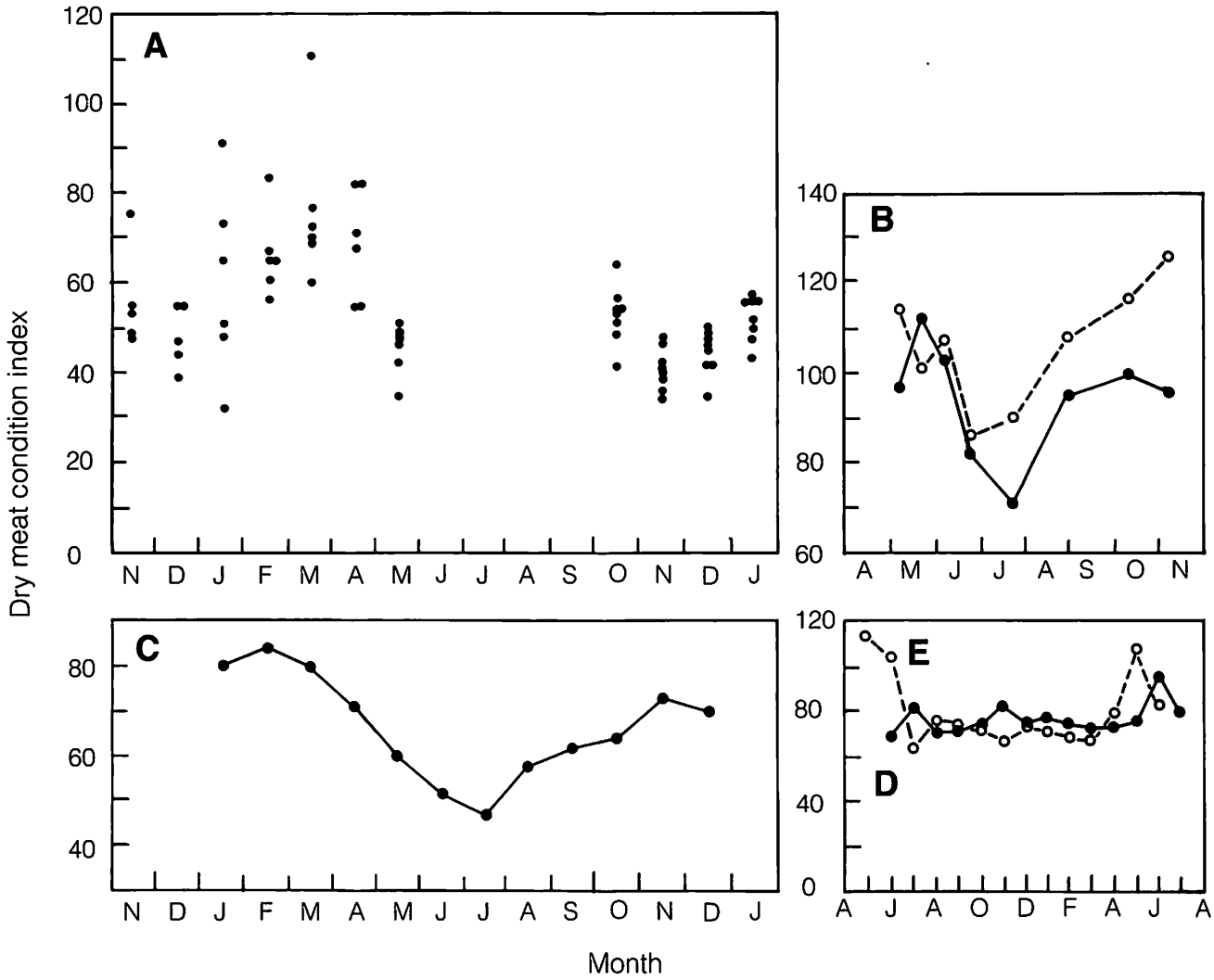


Figure 5. Seasonal variation of the dry meat condition index in *C. virginica.* Data from (A) Menzel and Hopkins (1952) and (B) Medcof (1946), in which solid circles and full line are samples from the upper part of the Bideford River, Prince Edward Island, and open circles and broken line are for samples from the lower part of the river. Additional data are from (C) Sakuda (1966) for oysters in Hawaii, (D) Haven (1962) in the York River (•), and (E) Haven (1962) in the Rappahannock River (o). Data from Haven (1962) are composite curves of four years' observations. After Walne (1970).

also suggested that the relationship between hemolymph protein and carbohydrate concentrations is linked to the reproductive cycle and stressed the importance of establishing good controls before using hemolymph composition to assess effects of other variables such as parasitism or environmental stress.

SPAWNING

Temperature is undoubtedly the single most important factor governing spawning of eastern oysters. For many years, the concept of a "critical temperature" for spawning was believed to apply to oysters in general. Nelson (1928b) wrote that oysters spawn after water temperature reaches 20°C "over all parts of their range, with no adjustment to the extremes of their distribution" (see also Townsend 1893; Stafford 1913; Churchill 1921; Gutsell 1924; Nelson 1924a; Prytherch 1924). This was supposedly confirmed in the laboratory by Galtsoff et al. (1930), but as will be demonstrated later, his results were a direct consequence of geographic location.

The proposition that ambient water temperatures must attain 20°C in order for eastern oysters to spawn has since been refuted by several authors (Loosanoff 1932; Galtsoff 1938; Loosanoff and Davis 1950; Stauber 1950b; Loosanoff and Nomejko 1951; Bardach et al. 1972). Indeed, Nelson (1931) wrote that spawning of ripe gonads is triggered by a rapid rise in temperature but is not determined by a specific critical temperature as others had originally suggested. This belief was reiterated by Medcof (1939) who stated that spawning occurs with rising temperatures "which may or may not reach 20°C." It is now well established that there is a fairly direct relationship between latitude and the minimal temperature required for spawning (with some exceptions), and the existence of physiological races based on these differences. Although minor, irregular spawnings are known to take place in most populations, a required minimal temperature must be reached before a major, mass spawning will occur. In general, oysters in more southern portions of their range exhibit longer spawning periods than their more northern counterparts and mass spawning temperatures are considerably higher, e.g., 26°C in Apalachicola Bay versus only 17°C in Long Island Sound (Fig. 6). The major period of spawning (spring) reported for Mexican oysters by Garcia and Ramirez (1981) is very short; however, minor spawnings also occurred during winter and summer.

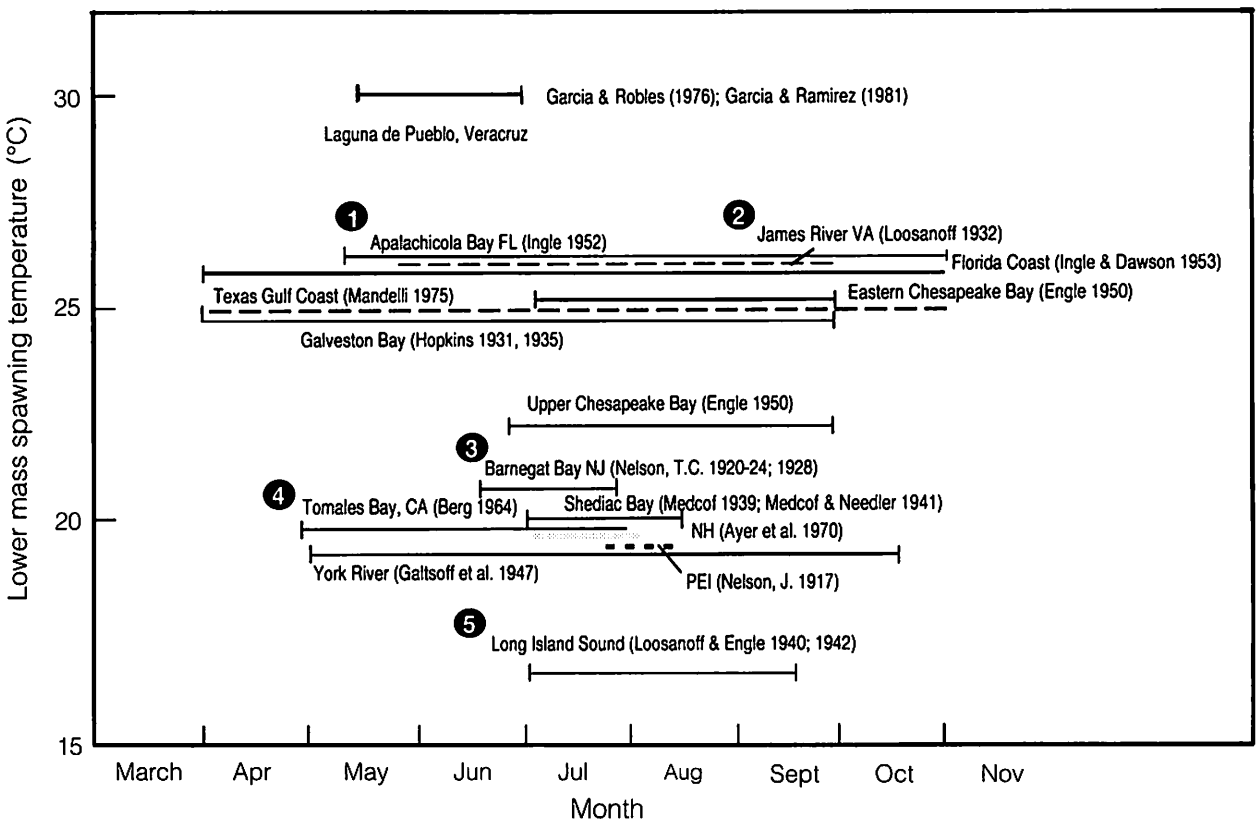
Temperature is not always the only critical factor governing spawning. For example, Matthiessen (1971) reported that although water temperatures in parts of Nova Scotia, New Brunswick, and Prince Edward Island in Canada became sufficiently high during the summer to stimulate spawning, overall the conditions necessary for successful reproduction were marginal and inconsistent from year to year.

Dependence of spawning on temperature has had major ramifications for hatcheries. Loosanoff (1945a) first proposed the "conditioning" of oysters out of season by exposure to increased temperatures. Loosanoff and Davis (1951) were able to delay spawning activity in *C. virginica* from Long Island Sound by transporting nearly ripe oysters to Boothbay Harbor, Maine, where temperatures were adequate to permit continued gametogenesis but too low to stimulate spawning. These eastern oysters could then be used in autumn when ripe oysters from natural populations were unavailable to hatcheries. The ability to control gametogenesis and to induce spawning has assured the supply of oyster larvae on nearly a year-round basis.

The increasing number of electricity-generating power plants built during the 1960s and 1970s prompted a number of studies on effects of heated effluent on various phases of oyster biology. Roosenburg (1969) found significantly higher initial oyster mortalities near a power plant in Maryland when oysters were placed in the effluent between May and September, presumably due to weakened conditions brought about by gametogenic activity or spawning. Similar results were reported by Quick (1971), who found that condition of oysters before exposure to elevated temperatures was critical to their reproduction at the high temperature. Oysters in good condition and not spawning were better able to maintain or improve their condition when exposed to elevated temperatures than those not in good condition. Winter mortalities of oyster spat were significantly reduced in a heated effluent in New Hampshire (Ayer et al. 1970).

Tinsman and Maurer (1974a, b) reported both beneficial and detrimental effects of exposure to thermal effluents. They found that meat weights and condition indices were low during warm months, but more favorable winter conditions in the effluent allowed both body weight and condition of oysters to improve and exceed the weight and condition of oysters from control areas. They also noted increased rates of meat growth and shell heights throughout the study in the heated effluent.

Oysters kept in heated effluents in Connecticut during winter and spring were superior in growth and biochemical composition compared with control oysters, and gonadal development began about 4 months earlier (Ruddy et al. 1975). Protein, carbohydrate, and condition index increased by 56%, 109%, and 22% respectively in winter and spring, and thicker shells were produced. Ruddy et al. (1975) estimated that the growing season could be extended from the normal 6-month period to about 9 months and at the same time allow oysters to take advantage of the spring phytoplankton bloom that normally occurs when water temperatures are still too low for oysters to feed efficiently under normal conditions. Interestingly, even



1 Assuming larval period of approximately 2 weeks; Ingle (1952) reported spat settlement into the second week of November.

2 Temperature was above 20°C for several months; Loosanoff (1932) speculated that other factor(s) influenced spawning

3 Mean average temperature 24 h before spawning ranged from 21.6° to 23.4°C for the years 1924 to1927; temperature continued to increase throughout spawning season.

4 *Crassostrea virginica* introduced; although spawning occurred, successful propogation of larvae was lacking.

5 Spawning in shallow water completed by mid-August; only deep water oysters (lower temperature) spawned through September.

Figure 6. Lower mass spawning temperature for *C. virginica* from various geographic locations.

though the gonadal maturation cycle began substantially earlier (4 months) in oysters in the effluent, the onset of spawning was delayed and once begun, extended over a longer period of time.

Finally, Price et al. (1976) studied effects of heated effluent in Maine and found that, although increased temperatures promoted growth, food became a limiting factor during the late autumn when food concentrations decrease in spite of temperatures remaining warm.

LARVAL SETTLEMENT

Patterns of larval settlement vary from location to location and year to year. A number of factors have been suggested that could affect setting behavior of oyster larvae, including mechanical disturbance, increased oxygen supply, increased food supply, light, type of cultch, and waterborne chemicals from previously set oysters (Lutz et al. 1970). One factor that has received particular attention is temperature. Ryder (1885) reported that the most favorable temperature for settlement was approximately 19° to 24°C. Loosanoff and Engle (1940) reported setting at temperatures as low as 16.9°C. Lutz et al. (1970) studied effects of temperature on setting and reported that a thermal shock (24° to 29°C) administered for 4 h to larvae of settlement size increased the percentage that settle, i.e., a rapid rise of only 5°C had a significant effect on settlement rate. Lutz et al. (1970) added that other factors such as responses to conspecifics can be equally important in settlement. This possibility was studied by Hidu and Haskin (1971) who found that both increased temperatures and a gregarious settlement response play important roles in determining the settlement pattern of oyster larvae. Diaz (1971, 1973) found that settlement was unaffected by a thermal shock of 10°C, whereas shocks of 15° and 20°C significantly decreased settlement rates.

Salinity is also a possible factor in settlement behavior of *C. virginica*. Hopkins (1935) reported that settlement periods appear to be correlated with periods of high salinity. Menzel (1955) suggested that *C. virginica* larvae were unable to set effectively in deep water of high salinity but that it was not an all-or-nothing phenomenon. Even at Port Aransas, Texas, where salinity was about 35 ppt, a few spat were found on the bottom. Menzel (1954) did point out that oysters settled abundantly in waters of high salinity, but only in the intertidal zone. Breuer (1962) found successful settlement in the lower Laguna Madre at 32 to 42 ppt. Haskin (1964) demonstrated that a gradual increase in salinity will stimulate older stage larvae to swim and that with a decrease in salinity, the larvae tend to remain quiescent on the bottom.

Kenny et al. (1988) studied settlement patterns of *C. virginica* in North Inlet Estuary, South Carolina, over a 5-year period. Although the timing and duration of settlement was similar each year, the pattern of spatfall within years varied significantly. There were no consistent relationships between settlement intensity and late stage larval density in the water column, water temperature, or salinity.

The precise effects of temperature and salinity on settlement behavior of oyster larvae are not clearly understood. Although it is evident that both factors play a significant role in settlement behavior, there are undoubtedly a number of factors working in concert that influence larvae, such as tidal current, sedimentation, predation, nutrition, competition, and gregariousness (Kenny et al. 1988). Settlement in the eastern oyster is considered in more detail by Kennedy in Chapter 10.

Development and Larval Growth

Nelson (1891) reported that sperm collected from freshly opened oysters were active for up to 5 h, with eggs losing their ability to be fertilized about 1 h after being stripped. Clark (1935) reported on effects of temperature and salinity on early larval development. The temperature range of his experiments appears to be 5° to 40°C. Sperm survival was higher at low temperatures than at higher temperatures (148 h at 5°C, 60 h at 20°C, and 9 h at 40°C). Eggs remained viable for shorter periods and were less tolerant of increased temperature than sperm and showed no development or fertilization after more than 6 h at 20°C, after 1 h at 35°C, and after less than 5 min at 40°C. Successful fertilization and development to the first swimming stage occurred between 15° to 30°C. The time between fertilization and first swimming stages varied from 25 h at 15°C to 3 h at 30°C.

Sperm were active in seawater from 4.6 to 40.7 ppt, with salinities of about 23 ppt providing greatest survival. Normal development of the first swimming larval stage occurred from 14.5 to 39 ppt; however, below this range, there was moderate egg development but no swimming larvae were produced.

Unfertilized eggs are known to be less tolerant of heat than post-fertilization developmental stages (Davis and Calabrese 1964; Hidu et al. 1974). Fertilized eggs did not tolerate temperatures greater than 32.5°C (Davis and Calabrese 1964) and Roosenburg et al. (1970) reported that normal development of oyster embryos was impaired at temperatures between 30° and 34°C during 1 to 16 h exposures. Hidu et al. (1974) reported that LD_{50} values decreased at 40°, 36°, 34°, and 31°C with increasing exposure times (10 sec, 1 min, 10 min, 1 h) for oyster eggs acclimated to a constant 24°C. Larval stages suffered similar mortalities, but calculated LD_{50} values averaged 4°C and 10°C higher for ciliated gastrula and veliger larvae, respectively, compared with eggs during equal exposure periods.

Larval development takes place over a much narrower range of temperatures and salinities than those commonly experienced by adult oysters. Early studies give values for successful development of oyster larvae ranging from 5.6 to 7.5 ppt minimum salinity (Hopkins 1932; Butler 1949a, b; Loosanoff 1948, 1953a) to 30 to 33 ppt (Amemiya 1926; Prytherch 1934). Nelson (1909) reported finding larvae in plankton samples in New Jersey when salinities were as low as 11.5 ppt. Larvae in New Jersey appeared to develop equally well at both 10 and 28 ppt (Nelson 1931). Ranson (1948) gave a value for optimum larval development in *C. virginica* of 12 to 19 ppt. Korringa (1957) disagreed strongly with the low upper salinity value given by Ranson (1948), citing populations in Long Island Sound (27 to 28 ppt); Beaufort, North Carolina (over 30 ppt); and Port Aransas, Texas (36 ppt) as examples. A similar array of values is given in the early literature for the effect(s) of temperature on larval development and growth. Hopkins (1935) found that larval development was retarded by low salinity, apparently as a result of effects of salinity on food supply. Medcof (1939) demonstrated that eastern oyster larvae had an increased development time of 6 d when larvae were exposed to only a 2°C difference in temperature (Fig. 7). Length of larval life varied from 24 to 30 d at 19° to 21°C. Prytherch (1928) reported an even shorter larval period of 13 to 16 d at 21.3° to 23.2°C.

Many discrepancies regarding optimal salinities for development were resolved by the work of Davis (1958) and Davis and Calabrese (1964) who showed that larval development appears to be governed by the salinity at which the parent eastern oysters undergo gametogenesis. They further showed that degree and rapidity of salinity change is probably more important than actual salinity under field conditions.

In his first series of experiments, Davis (1958) showed that the optimal salinity for development of eggs from eastern oysters that developed gonads at a salinity of 26 to 27 ppt was about 22.5 ppt; oysters that developed gonads at about 8.74 ppt had optimal salinity for egg development between 10 and 15 ppt, although some normal development was noted at salinities as low as 7.5 ppt. Optimal salinity for development of straight-hinge larvae from eggs at 8.7 ppt ranged from 7.5 to 22.5 ppt whereas the range for eggs from oysters conditioned at 26 to 27 ppt was from 12.5 to 35 ppt. Optimal salinity for growth of

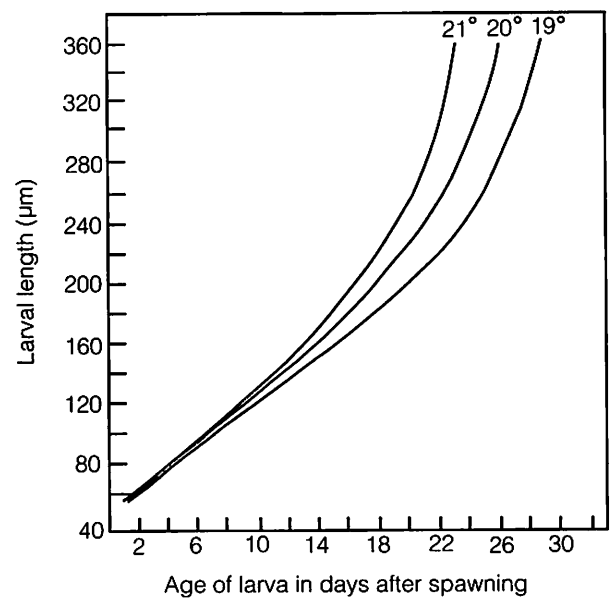


Figure 7. Approximate growth curves for Bideford River, Prince Edward Island, oyster larvae at constant surface temperatures of 19°, 20°, and 21°C. After Medcof (1939).

larvae of oysters from Long Island Sound, conditioned and spawned at 26.0 to 27 ppt was 17.5 ppt (Fig. 8). Optimal salinity for growth of larvae from Hodges Bay, Maryland (8.7 ppt), conditioned and spawned at 26 to 27 ppt, appeared to be about 22.5 ppt. Although not conclusive, it appeared that larvae from Maryland oysters did not tolerate lower salinities than did larvae from Long Island Sound oysters conditioned at the same salinity. Davis (1958) reported a minimal salinity for successful metamorphosis of 10 ppt for larvae of unstated parentage. Similar results were reported by Chanley (1958) who found some growth of recently metamorphosed larvae at 5 ppt, but optimal growth between 12.5 and 25 ppt.

Davis and Calabrese (1964) studied the combined effects of temperature and salinity on development of eastern oyster eggs and larval growth. Rates of growth and development were poor at the extremes of temperature ranges and satisfactory survival rates (70% or better) were limited to temperatures of 27.5° to 32.5°C and salinities of 10 to 27.5 ppt. As salinity decreased, the tolerated range of temperatures narrowed. Optimal temperature for larval growth was between 30° and 32.5°C for all salinities except 7.5 ppt where the optimum was 27.5°C. Conversely, there was no well-defined optimal salinity for larval growth at any temperature as maximal growth occurred in salinities varying from 15 to 27 ppt at some temperatures and from 20 to 27 ppt at 17.5°C. Thus, the effect of reduced salinities on larvae was to reduce the range of temperature tolerance.

Loosanoff (1965) provided further data on larval growth and development at various temperatures and salinities in Long Island Sound. He reported that no eggs developed into normal, straight-hinge larvae at 15.5°C, about 97% developed to fully formed, straight-hinge stage at 17.7°C, most fertilized eggs developed normally at 30°C, and only about 50% of the eggs developed to the straight-hinge stage and many were abnormal at 33.3°C. Optimal salinity for egg development was 22.5 ppt; some normal larvae developed at 15 ppt and at 35 ppt; below 22.5 ppt, the percentage of normally developed larvae decreased. Optimal salinity for larval development from eggs was about 17.5 ppt. Good larval growth was recorded at 15 ppt, with appreciably slower growth at 12.5 ppt and almost no growth at 10 ppt. The older larvae were better able to withstand low salinity. Wright et al. (1983) also studied thermal tolerance of larval stages of *C. virginica* and found that larval mortality generally increased with exposure to higher temperature and with increased exposure time at any one temperature. Nevertheless, at temperatures as high as 40° to 41°C, straight hinge larvae sustained low mortality (11%) when exposed for up to 1 h.

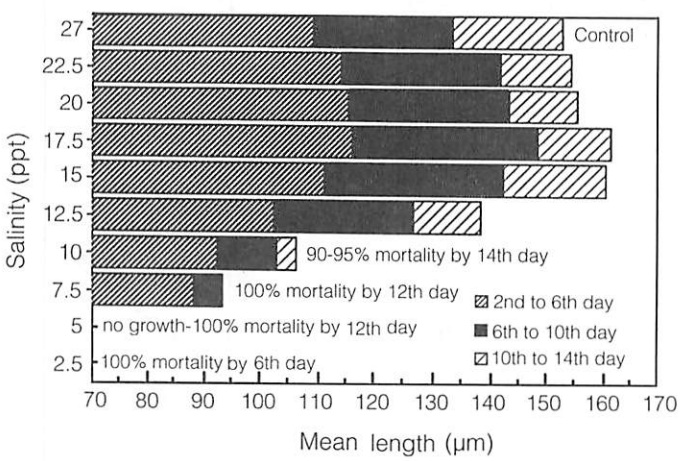


Figure 8. Growth of oyster larvae at different salinities. Samples from each of the duplicate cultures at each salinity were taken on the 6th, 10th, and 14th days. The lengths (μm) of one hundred larvae from each sample were measured. After Davis (1958).

Diaz (1973) found that growth of larval eastern oysters was not affected by brief temperature increases of 10° or 15°C, but a 20°C increase resulted in permanently impaired growth of surviving larvae.

Loosanoff (1965) collected oysters from Chesapeake Bay at 8.7 ppt and spawned them. Some eggs developed into normal larvae at 10 ppt and even at 7.5 ppt, although abnormally small individuals were common at the latter salinity. Optimal salinity for development of eggs in this group of oysters ranged between 12 and 15 ppt, with a salinity of about 22 ppt being the upper limit for normal development.

Hidu et al. (1974) demonstrated that the fertilized egg and ciliated gastrula were considerably more temperature sensitive than later stages of larval development. Time of exposure greatly affected the temperature tolerance of the larvae in that longer exposures led to increased mortality.

Amemiya (1926) studied effects of salinity on early development of *C. virginica* cultured over the salinity range of 12.3 to 52.1 ppt; 24.5 to 29.8 ppt was the optimum salinity for development, and the range of 22 to 33 ppt was favorable. He provided a description of larval development at 24 separate salinities to which the reader is referred for details. He also reported that only a small proportion of oyster larvae develop normally between 31 and 34 ppt. MacInnes and Calabrese (1979) reported 25°C and 26 ppt as the optimum temperature and salinity for normal embryonic development.

Although temperature and salinity have a direct effect on larval growth, availability of suitable food items to support growth is also of considerable importance. Davis and Calabrese (1964) grew larvae on monocultures of the unicellular algae, *Dunaliella euchlora* and *Chlorella* sp., and on a mixture containing *Chlorella* sp., *Dicrateria* sp., *Isochrysus galbana*, and *M. lutheri*. Although there was a distinct effect of temperature on growth rate regardless of food supply, oyster larvae fed the mixture of four algal species showed highest growth rates (Fig. 9).

In a further experiment to determine upper and lower limits for growth of oyster larvae, Davis and Calabrese (1964) found no growth at 15°C, minimal growth at 17.5°C, and maximal growth at 30° and 33°C (Fig. 10). A re-evaluation of these data by Lough (1975) gives a clear expression of the combined effects of thermal and salinity variations on oyster larvae. Lough (1975) used response-surface techniques to show that maximal survival of 2-d old

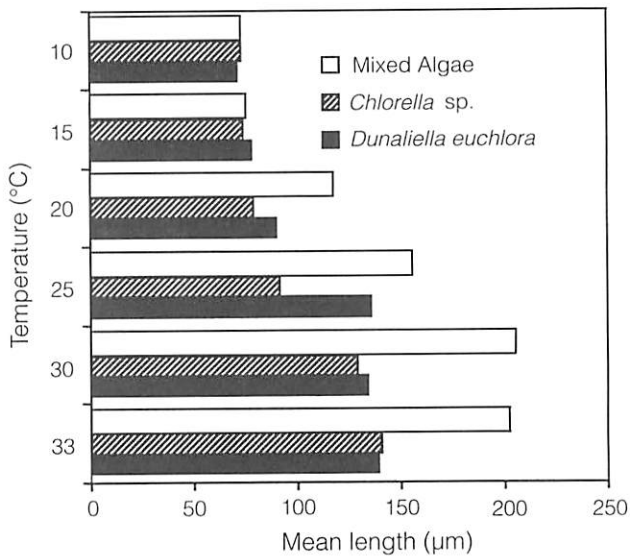


Figure 9. Growth of oyster larvae receiving different foods and reared at different temperatures. Plots are based on mean length of 100 larvae from each temperature at each measuring period. After Davis and Calabrese (1964).

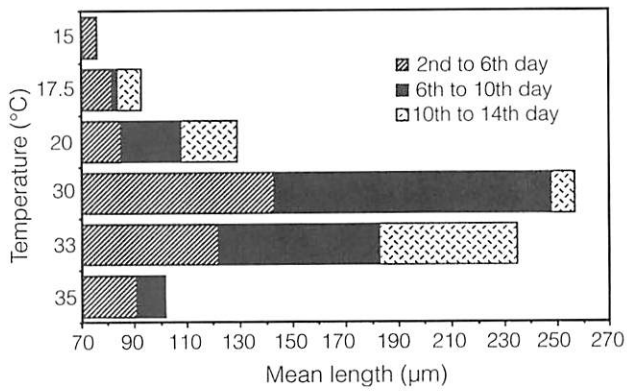


Figure 10. Growth of oyster larvae receiving a mixture of foods and reared at high and low temperatures. Plots based on mean length of 100 larvae from each duplicate culture at each temperature at each measuring period. Many of the larvae kept at 30° and 33°C set between the 10th and 14th days and were not included in the 14-d samples. After Davis and Calabrese (1964).

larvae (80% contour, Fig. 11A) occurred between 19° and 30.5°C and 19 and 30 ppt. Maximal survival after 8 d (60% contour, Fig. 11B) occurred above 21°C and between 8 and 30.5 ppt, with a much higher tolerance to higher temperature and a wider salinity range than the 2-d old larvae demonstrated. Maximum growth (100% response contour) (Fig. 11C) was estimated to occur above 33°C and 19 ppt. Differences between survival and growth at 8 d indicated that a significantly higher salinity range is required for optimal growth than for optimum survival. Lough (1975) estimated that the optimal (80% contour) temperature and salinity conditions for maximizing both larval survival and growth are above 30°C and between 18 and 35 ppt.

Generally speaking, within the zone of tolerance, the higher the temperature the faster the development of eggs and growth of larvae. Because factors apart from temperature can also affect these processes, it is probably best to define optimal conditions for development as those at which mortality is lowest, rather than those at which development is fastest. Development of eastern oyster eggs and larvae is considered further by Eble in Chapter 2 and Thompson et al. in Chapter 9.

Adult Growth

Growth rate of adult eastern oysters is as strongly affected by temperature and latitude (Table 1) as are egg and larval development. Butler (1953) believed that a clear-cut differential in oyster growth exists at different latitudes and that differences in shell growth do not necessarily reflect differences in tissue or meat yield. Measurement of shell volume provides a more critical evaluation of growth than does shell height. As an example, 2-year old oysters from a South Carolina clustered reef yielded about 1.2 kg of meats per bushel (average height ~75 mm, Butler 1953). Oysters grown individually in the same area can require 3 years to attain the same size, but the yield increases to ~3.4 kg per bushel.

As a rule, growth is more rapid in warm waters such as those of the Gulf of Mexico where a marketable oyster (90 mm) can be grown in 2 years. In northern waters, e.g., Long Island Sound, 4 to 5 years are required to attain the same size. Butler (1953) pointed out, however, that oysters growing in the Chesapeake region tend to grow faster and produce more meat per unit time than oysters growing north or south of this region. Loosanoff and Nomejko (1949) in observations of monthly shell growth rates for oys-

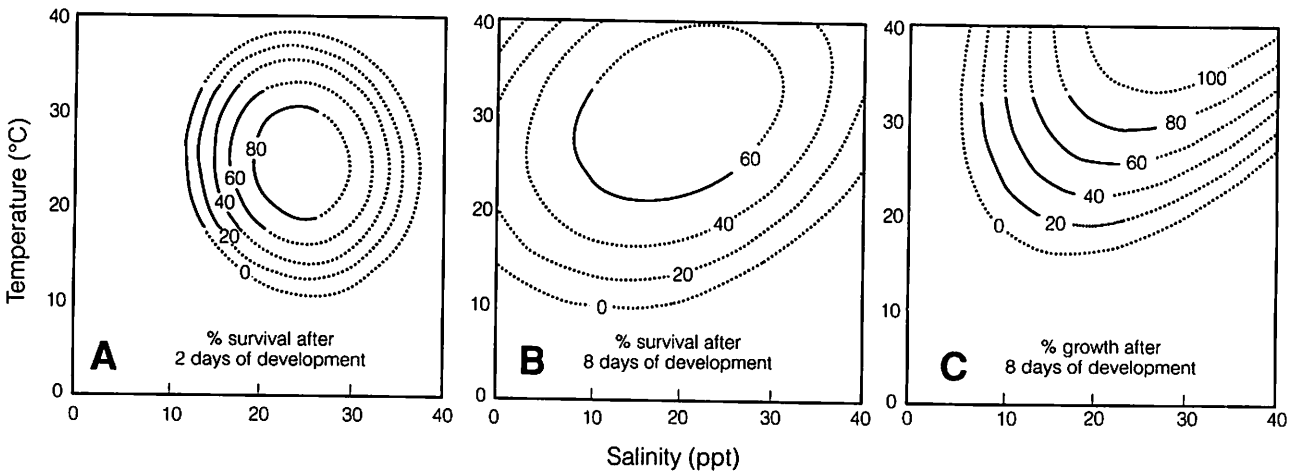


Figure 11. Response surface estimation of percent survival of *C. virginica* larvae (A) after 2 d of development and (B) after 8 d of development at experimental temperature and salinity combinations given in Davis and Calabrese (1964) and (C) response surface estimation of percent growth of veliger larvae after 8 d of development at these temperature-salinity combinations. Contours extrapolated beyond the experimental data are given as dotted lines. After Lough (1975).

Table 1. Summary of growth rate studies on *Crassostrea virginica* (Gmelin) and time required to produce equivalent growth at Apalachicola rate. From Ingle and Dawson (1952).

Place	Linear growth	Elapsed time	Time required in Apalachicola	Reference	
Canada	Setting to 2 in	24 mo	5 mo	Stafford	1913
Long Island	1.4 to 3 in	6 mo*	12 wk*	Moore	1905
Long Island Sound	Setting to 4.5 in	4 yr[1]	1.4 yr[1]	Churchill	1921
New Jersey	3.7 to 4.2 in	12 mo	6 mo	Nelson	1922
Chesapeake Bay	0.8 in	44 d	42 d	Ryder	1885
Chesapeake Bay	1.5 in	12 mo	3.5 mo	Ryder	1885
Chesapeake Bay	3.5 in	23 mo	15 mo	Ryder	1885
Chesapeake Bay	Setting to 0.75 in	3 mo	1.5 mo	Winslow[2]	1913
Beaufort, NC	Setting to 1 in	2 mo	2 mo	Osborn	1883
North Carolina	3.4 to 4.3 in	2 mo	.....	Glaser	1905
North Carolina	5.2 to 5.9 in	2 mo	.....	Glaser	1905
North Carolina	7.2 to 7.6 in	2 mo	.....	Glaser	1905
North Carolina	Seed to market size	2 yr	.....	Higgins	1940
North Carolina	Setting to 3 in	6 mo*	4 mo*	Higgins	1940
South Carolina	Marketable size	One season	.....	Dean	1892
South Carolina	Setting to 1.5 in	2-3 mo*	7 wk*	Moore	1905
South Carolina	Setting to 2.5 in	6-7 mo	6.2 mo	Moore	1903
Tarpon Springs, FL	Setting to good sized	10 mo	.....	Brice	1896
Louisiana	Setting to 1 in	6 wk*	5 wk*	Moore	1899
Louisiana	Setting to 3.5 in	18 mo	15 mo	Moore	1899
Louisiana	Setting to 4 or 6 in	23 mo	17 mo[1]	Moore	1899
Louisiana	Setting to 3.1 in	10.3	9 mo	Gunter	1951
Louisiana	Setting to 4 in	39 wk*	31 wk*	Menzel	1951
Texas	Setting to 3.7 in	12 mo[3]*	28 wk[3]*	Gunter	1951

* Maximum growth rate.

[1] Time refers to 4 inches growth.

[2] See Stafford 1913.

[3] The data of Gunter (1995) are not easily compared with Apalachicola Bay findings inasmuch as the oysters he observed were growing under unusual ecological conditions and his samples were small.

ters in Long Island Sound, found that growth was limited to about 8 months of the year, although most individuals showed growth increases only during 6 or 7 months; no growth occurred after the point of induction of cold coma in the Milford Harbor area. If the water temperatures were maintained above the point of cold coma, growth would continue. There was a strong relationship between changes in rate of increase in shell volume and changes in water temperature.

Ingle and Dawson (1950a, b; 1952) and Copeland and Hoese (1966) reported exceptionally rapid growth of oysters from Apalachicola Bay, Florida, and south Texas. Growth was continuous throughout the year and basic growth curves remained the same despite seasonal environmental changes, i.e., oysters that set in autumn had very nearly the same growth curves as those that set in the spring. Reported growth rates are faster than for any other oyster populations; both sets of authors attributed high growth rates to high

temperature. Ingle and Dawson's (1950a, b; 1952) estimates were based on shell size and may not necessarily reflect tissue growth. The authors summarized growth data over the entire geographic range of *C. virginica* (Table 1); their recorded values for Apalachicola Bay (column 4) remain among the highest known.

Only Loosanoff (1953a, 1965) and Shaw (1966) have provided data on effects of salinity on growth rate. Loosanoff (1953a, 1965) noted that oysters adapt rapidly to salinity change, but that growth was stunted at 7.5 ppt and almost nonexistent at 5 ppt. He suggested that 10 ppt was the minimum salinity at which adult oysters grew at a normal rate. Shaw (1966) transplanted seed oysters from low-salinity waters in Chesapeake Bay to a low-salinity area (Broad Creek in Chesapeake Bay; 8 to 16 ppt, average 12 ppt) and a high-salinity area (Chincoteague Bay; 17 to 35 ppt, average 30 ppt). Over a two-year period, shell growth rate was similar in both areas.

Activity

There have been few studies concerned with effects of temperature and salinity on valve activity or closure in *C. virginica*, with most data collected as a by-product of other studies. Galtsoff (1946) subjected oysters exposed in air for 24 h at 5°C to temperatures of 22°C and found irregular shell movements accompanied by a complete cessation of pumping until the third or fourth day after transfer, after which normal pumping resumed. Loosanoff (1953a) noted that at the lowest salinities tested (0 to 5 ppt), valve movement and water transport were abnormal and growth was inhibited (see also section on growth). Valvular activity also becomes irregular above 30°C, with some eastern oysters closing their valves completely, and with pumping activity frequently inhibited (Loosanoff 1958). There was a reduced rate of pumping even during periods when valves were open. Between 34.1° and 36°C, these symptoms became exaggerated and oysters remained closed about 67% of the time above 36.1°C. When oysters were exposed to a sudden increase in temperature, there was an immediate opening of the valves and inhibition of pumping (Loosanoff 1958). Galtsoff (1964) stated that while temperature has no direct influence on the duration of shell opening, lowered salinity results in partial or complete contraction of the adductor muscle and a slowing or cessation of water current through the gills.

Numerous authors have reported mass mortalities due to fresh water flooding, predominantly in southern United States waters. Flooding is a common phenomenon in these regions, and in some areas low salinity flood conditions can last up to a month. Oysters are thus left inundated in fresh water at high temperatures, and mortalities up to 100% are not uncommon. The magnitude of the effect(s) of environmental perturbations (such as salinity changes) on oysters depends on the range of fluctuations and abruptness of these changes (see Hand and Stickle 1977). The ability of oysters to withstand such changes in salinity is enhanced by their ability to close the shell valves when exposed to extreme conditions such as protection from sudden freshwater input from floods and freshets.

Salt sensitivity at the mantle margin would be an advantage to oysters as an early warning system. Hopkins (1932), developed a method for studying the latent period of reactions of *C. virginica* to chemical stimulation. He monitored the latent period of reaction of tentacles on the oyster mantle to 21 different salts (most of the chlorides, iodides, bromides, nitrates and sulphates of potassium, sodium, ammonium, lithium, and magnesium) and was able to group the different ions according to their stimulating efficiency. He found that effectiveness of an ion as a stimulant depends in a direct manner on its atomic weight and that sensory stimulation of oyster tentacles by the salts is primarily a function of the cations present. He gave the following order of stimulating efficiency: cations, $K > NH_4 > Na > Li$; anions, $I > Br > NO_3 > Cl$. It is not clear what role this sensitivity might play in stimulating valve closure during exposure to various salinities, but it may confer an advantage.

Valve closure can only serve as a temporary means of protection against such adverse environmental conditions as reduced salinities. Even a slight contraction of the valves will result in a reduced rate of water flow that will in turn affect the rate of feeding and gas ex-

change (see below). Long-term valve closure will result in eventual mortality, especially when coincidental with high temperatures.

A distinction must be made between pumping rate (the velocity of water movement through the mantle cavity) and filtration rate (the amount of water completely cleared of particles larger than a specified size per unit time). Obviously, the two are intimately related, but not necessarily synonymous. If animals are not actively pumping, feeding cannot occur; conversely, even though some pumping activity can be underway, feeding still may not occur, especially at very low temperatures.

In an early study of pumping rate in *C. virginica*, Galtsoff (1928 a,b) calculated a maximum pumping rate of 3.9 L h^{-1} for a 76 to 102 mm oyster at 25°C (Fig. 12). Nelson (1938) disagreed with Galtsoff's findings, believing that Galtsoff's method interfered with activity and that the correct rate was actually a much higher value of 26 L h^{-1}. Subsequent studies (Loosanoff 1950a, 1958; Loosanoff and Nomejko 1946) reported values similar to those given by Nelson. While it is possible that Galtsoff's experimental design resulted in low values for pumping rates, it is also possible that other factors were responsible for differences.

Several authors have demonstrated that pumping rate is affected by both temperature and salinity. Most of our knowledge of pumping activity in *C. virginica* is from the work of Loosanoff (1958). His data show that pumping rate increased steadily as temperature rose from 8° to 28°C (Fig. 12). Pumping was reduced or non-existent below 2°C, whereas above 34°C oysters began to show distress that resulted in a marked decrease in pumping rate and abnormal shell movements. Highest flow rates were measured at about 29°C. Even though the absolute values for pumping rate measured by Loosanoff (1958) and Galtsoff (1928a) differ, they are in agreement that the upper temperature range of 35°C is the limit for normal pumping activity. W. Menzel (pers. comm.) observed oysters open and pumping at temperatures of 36° to 37°C. Other temperature values for maximal pumping rate are given by Nelson (1936) and Collier (1959) as 30°C and 20° to 25°C, respectively. It appears that optimal levels are sustained in the region of 25°C regardless of acclimation temperature of oysters.

Loosanoff (1953a) studied effects of salinity on pumping activity. Exposure to an abrupt reduction from 27 ppt to 20, 15, 10, and 5 ppt resulted in a decrease in pumping rate of 24, 89, 91, and 99.6% respectively for about 6 h after transfer. Thereafter, normal pumping activity resumed and there were no long-term effects on pumping rate. Oysters conditioned to live in lower salinities ceased or resumed pumping water and closed or opened their valves at lower salinity concentrations than did oysters from higher salinities.

Pumping rate of *C. virginica* is affected by a number of factors other than salinity and temperature. Nelson (1936) stated that pumping is increased during active shell secretion and by addition of fresh oyster sperm to incurrent water of the male oyster; the increase is typically from 5.9 to 11 L h^{-1}. No response occurs in female oysters unless they are induced to spawn, at which time water flow is markedly reduced. Subsequent research by Nelson and Allison (cited by Galtsoff 1964) suggests that pumping activity in spawning female oysters is actually enhanced although efficiency of particle retention on the gill is reduced. Nelson (1936) suggested that reduced pumping by females reduces the likelihood of retaining eggs on the gills whereas increased pumping by males permits wider and more rapid dissemination of sperm.

Eastern oysters can feed and grow at temperatures much lower and higher than required for spawning (Gunter 1957). Studies that deal specifically with the effects of temperature and salinity on feeding in *C. virginica* are scant. Galtsoff (1928a) reported that no current was produced and no feeding took place at or below 5°C. This statement was later modified by Loosanoff (1958; see below) but the generalization remains true. Prytherch (1928) concluded that gonadal development was dependent on the amount of food consumed by oysters and that, in years when ambient temperatures were above normal, higher rates of spat production due to the increased feeding activity and subsequent gonadal development might be expected.

Loosanoff (1953a, 1958, 1965) provided the most detailed studies of effects of temperature and salinity on feeding activity. He fed 90 oysters on a

culture of *Chlorella* sp. at 2° to 3°C and found that only one produced true feces, whereas 15% produced pseudofeces (Loosanoff 1958); at 3° to 4°C, about 50% of the oysters produced pseudofeces but still only one produced feces. Between 5° and 6°C, 11 of the 90 oysters expelled feces and over 75% produced pseudofeces. Thus, feeding occurs below 5°C only as an exception and the ability to produce pseudofeces may be a function of ciliary activity (as demonstrated by Galtsoff [1928a] who showed that frontal cilia are able to transport particles at a temperature of 3°C, whereas lateral cilia generally only produce a feeding current when the ambient temperature is about 5°C).

In terms of salinity, no feeding was seen in oysters maintained at 3 ppt or lower (Loosanoff 1953a; 1965). Animals exposed to 5 ppt exhibited abnormal activity, and their feces often appeared white or greenish and were composed principally of blood cells. Oysters were, however, producing both feces and pseudofeces, indicating that feeding activity and ingestion were not totally impaired. Loosanoff (1958, 1965) again demonstrated that oysters stopped feeding and "hibernated" [sic] below 5°C. Feeding rate increased rapidly between 13.9° and 27.8°C, with a further, less rapid increase up to 32.2°C. Above 33.8°C, a marked decrease in pumping rate was noted (Fig. 12) and shell movements became abnormal.

Davis and Calabrese (1964) demonstrated that the ability of oyster larvae to digest food is temperature dependent and that some food items are more easily digested at lower temperatures than others. Thus, naked flagellates were more easily digested than were algae with cell walls. No similar studies exist for adults, but it seems reasonable to assume that temperature must affect digestion rate in adults as it does in other species of bivalves (Bayne and Newell 1983 and references therein).

HEART RATE

Heart beat increases with increased temperatures within the range of 5° to 30°C (Fig. 13), depending on geographic location (Federighi 1929; Prytherch, cited in Higgans 1931; Stauber 1940; Stauber, unpublished cited in Stauber 1950a; Menzel 1955, 1956; Feng 1965). Dimock (1967) reported that salinity affects *C. virginica* heart activity by interfering with osmotic balance of heart tissue and with the activity of acetylcholinesterase in the heart (decreased salinity inhibits acetylcholinesterase activity, the extent of the inhibition being influenced by the oyster's ambient salinity).

Feng (1965) found a simple linear relationship between number of leucocytes in suspension in the hemolymph and the temperature of the external medium (Fig. 14). He suggested that leucocytes are nearly 100% settled out at 0°C because the heart is probably quiescent at this temperature. The number of leucocytes in suspension was strongly influenced by the heart rate, which was affected in turn by temperature or other stimuli. Feng and Van Winkle (1975) subsequently used *C. virginica* from two populations (New Jersey and Connecticut) and found that the time of acclimation to low salinities did not significantly affect recovery of heart beat for either stock, although the time of acclimation was very short (4 to 5 h versus 18 h). Above 10°C, heart beat of New Jersey oysters was

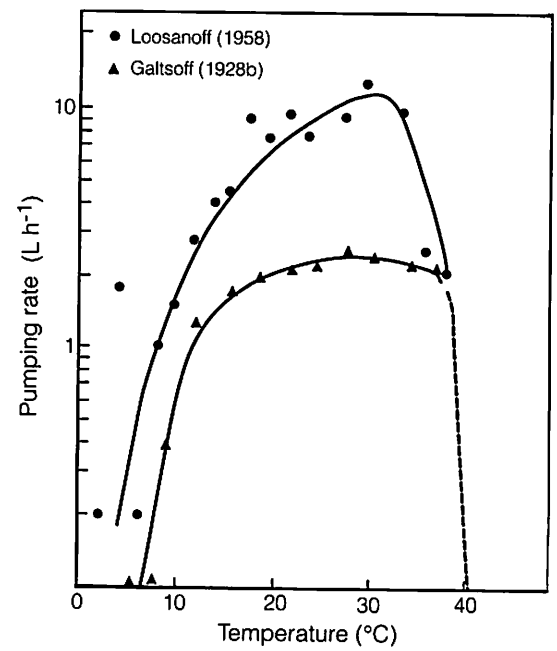


Figure 12. Pumping rate of *C. virginica*. Data taken from Loosanoff (1958) (plotted as upper level of each 2°C temperature interval tested) and Galtsoff (1928b). No size specified in Galstoff (1928b). Loosanoff (1958) gives size range as 100 to 110 mm length; 80 to 85 mm width; 30 to 35 mm depth; 85 to 100 ml volume; he provided no information on sample sizes.

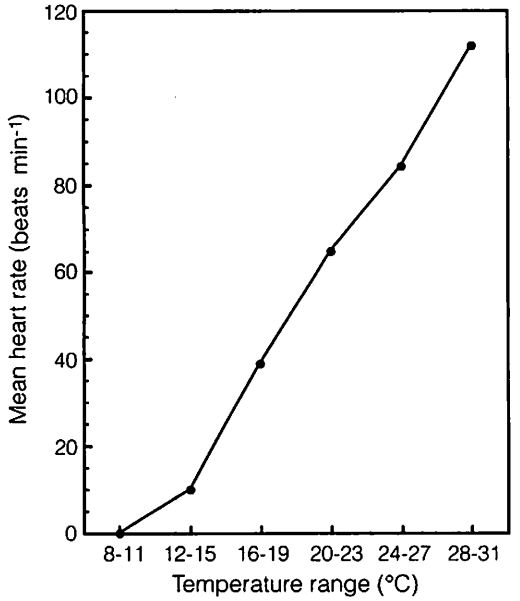


Figure 13. Average rate of heart beat min^{-1} at various temperature ranges for *C. virginica.* After Menzel (1956).

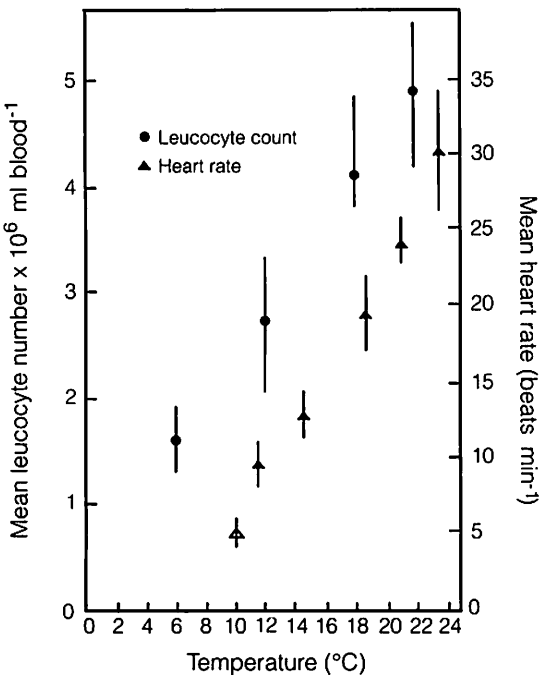


Figure 14. The effect of temperature on leucocyte numbers and heart rate of oysters. Each point represents average heart rate of three determinations on 10 oysters. The mean leucocyte count in heart blood at 6°, 12°, 18°, and 22°C is obtained from a group of 21 oysters. The vertical lines are ranges of the means. After Feng (1965).

less inhibited after a gradual decrease (3 to 4 d) in salinity from 5 to 10 ppt than after an abrupt decrease in salinity. Oysters from Connecticut exhibited a maximal rate of heart beat at intermediate salinities (14 and 19 ppt) at 20° to 30°C with heart rate relatively insensitive to salinity at lower temperatures (5° and 10°C). Feng and Van Winkle (1975) further concluded that the effect of salinity on heart rate is probably not very strong in that it is readily masked at low temperatures. Again, acclimation periods in this study were short and results should be considered as acute responses rather than acclimation responses to reduced salinity.

The only information available on effects of temperature on accessory hearts is given in a personal communication by Stauber (cited in Feng 1965) who stated that the two accessory hearts are as temperature-dependent as the systemic heart.

RESPIRATION

Surprisingly few data are available on effects of temperature or salinity on respiration rate in *C. virginica.* Galtsoff (1964) reported no significant change in respiratory rate after 3 d of acclimation to water of lowered salinity, although he based this conclusion on only nine individuals and a salinity change of 31.6 to 24.1 ppt.

A number of studies followed that of Galtsoff (1964) in which authors reported on the effects of temperature and salinity on excised tissues, with conflicting results. Van Winkle (1968) reported that oxygen consumption ($\dot{V}O_2$; volume respired per unit time) of excised gill tissue was relatively constant over a range of temperatures (10°, 18°, and 26°C) and salinities (5, 10, 15, 20, and 30 ppt) during both summer and winter. Percy et al. (1971) monitored $\dot{V}O_2$ in excised mantle, adductor muscle, and gill tissue of eastern oysters from Trinity Bay, Newfoundland. Respiration rates of gill tissue increased during exposure to dilute salinities, remained constant for mantle tissue, and declined for adductor muscle. All tissues exhibited an increased $\dot{V}O_2$ with increased temperature. Respiratory maxima for both mantle and adductor muscle were about 32°C, whereas gill $\dot{V}O_2$ increased continuously up to 40°C. There were also marked seasonal effects on $\dot{V}O_2$.

Wegener (1971) reported that the $\dot{V}O_2$ of gill tissue from eastern oysters collected at Beaufort, North Carolina, was unaltered by osmotic stress. Bass (1977) also reported on the effects of temperature and salinity on the metabolism of excised gill, mantle, and adductor muscle from Chesapeake Bay oysters. He demonstrated that cold-acclimated gill tissue showed good acclimatory ability, whereas mantle tissue showed little and muscle none. Gill and mantle showed partial acclimation and muscle none when acclimated to warm temperatures. When exposed to dilute seawater, gill and mantle tissues showed elevated respiration rates and muscle tissue did not change. None of the tissues exhibited any alterations in respiration rates when exposed to increased salinities. In contrast, Percy et al. (1971) found an increased respiration rate in both gill and mantle tissue when exposed to dilute salinities and no change in adductor muscle for Newfoundland oysters. Bass (1977) also showed that cold-acclimated tissues were, on average, better able to acclimate to perturbations of temperatures than warm-acclimated tissues.

The data available for $\dot{V}O_2$ in whole oysters are limited, and it is clear that the responses of individual tissues are not always applicable to whole oysters (see Shumway 1982 for review). Shumway and Koehn (1982), in the only comprehensive study to date of the combined effects of temperature and salinity on respiration in *C. virginica*, measured the acclimated and acute rates of oxygen consumption under nine temperature-salinity regimes. They presented a series of multiple regression equations relating acclimation and exposure temperature and salinity to standard $\dot{V}O_2$ measured in starved oysters. As acclimation salinity decreased, the effect of exposure temperature became more pronounced and the effect of exposure salinity decreased. As acclimation temperature increased, the effect of exposure salinity decreased and the effect of exposure temperature increased (Table 2; Fig. 15). The overall multiple regression equation is:

$$R = 0.0015 + 0.0004\ T_a - 0.0019\ S_a + 0.0178\ T_e - 0.0049\ S_e$$

where R = $\dot{V}O_2$ (ml O_2 0.4 $g^{-1}h^{-1}$); T_a and S_a = acclimation temperature and salinity; and T_e and S_e = experimental temperature and salinity. This regression is significant at $p < 0.001$ and explains almost 92% of the total variance in $\dot{V}O_2$. Interaction terms, T_eS_a and T_aS_e, are negligible. Little evidence exists for any temperature regulation, even after three weeks of acclimation.

Values of Q_{10} for warm-acclimated individuals were higher than those for cold-acclimated individuals when exposed to low experimental temperatures, the only exception being oysters acclimated at 7 ppt and exposed to 28 ppt (Shumway and Koehn 1982). At any given experimental salinity, highest Q_{10} values were observed between 20° and 30°C. The multiple regression relating Q_{10} to acclimation and exposure salinity and temperature is:

$$Q_{10} = 4.401 - 0.003S_a + 0.0674T_a - 0.1457T_r - 0.0082S_e$$

where T_r = mean of the temperature range considered; and S_a, S_e, and T_a are as before. Although the regression was significant at $p < 0.001$, it only accounts for 36% of the observed variation in Q_{10}, indicating that other factors also influence Q_{10} values (e.g., acclimation time, starvation, or phase of gametogenic cycle). Finally, Shumway and Koehn (1982) demonstrated that oysters regulated $\dot{V}O_2$ when exposed to declining oxygen tensions at all temperature-salinity combinations tested; however there was no clear pattern of response between exposure conditions and ability to regulate $\dot{V}O_2$. Generally, the degree of regulation decreased with increased temperature or decreased salinity.

Widdows et al. (1989) found that the tolerance of *C. virginica* larvae to anoxia increases with developmental stage and body size. Like juvenile and adult oysters, oyster larvae maintained rates of heat dissipation and oxygen uptake independent of PO_2 down to low critical pressures of oxygen. Further, prodissoconch larvae maintained relatively high rates of heat dissipation under anoxic conditions (34% of normoxic rate), whereas pediveliger and juveniles lowered their anoxic rates of heat dissipation to 3% of the normoxic rate.

Newell (pers. comm.) studied oysters from both a high (30 ppt) and low (10 ppt) salinity environment in Chesapeake Bay and found that those from

the high-salinity population had significantly lower clearance rates coupled with higher metabolic rates than those from the low-salinity population. There was no evidence that oysters held in 6 ppt seawater had acclimated to low salinity after a 14-d acclimation period, supporting the contention of Shumway and Koehn (1982) that the degree of regulation of respiration rate decreases with decreased salinity.

Shumway and Koehn (1982) found no evidence for capacity adaptation or acclimation in oxygen consumption of starved *C. virginica*. It is, however, undoubtedly the possession of this rather "elastic" or "euryplastic" (Alderdice 1972) physiology that allows *C. virginica* to use available oxygen over a wide range of temperature-salinity combinations and thus to sustain an energy gain from a constantly fluctuating environment.

OSMOTIC REGULATION

The effects of salinity on osmotic and ionic regulation in marine and estuarine invertebrates have been the subject of numerous reviews (see Spaargaren 1979 and references therein; Burton 1983). Most marine bivalve molluscs have little, if any, capability for extracellular osmotic regulation and *C. virginica* is no exception. It is poikilosmotic, i.e., an osmotic conformer with no ability for osmotic regulation of the extracellular fluid (hemolymph). When exposed to waters of increased or decreased salinities, the hemolymph becomes concentrated or diluted to remain in osmotic equilibrium with the surrounding seawater. This lack of extracellular regulation puts a burden on cells with regard to maintenance of cell volume compatible with cell function and maintenance of cellular constituents. Newell (pers. comm.) demonstrated that although eastern oysters are tolerant of extended exposure to low salinities, they are physiologically stressed by such conditions.

Loosanoff (1953a) found that the body fluids of *C. virginica* are isosmotic with the external medium as long as shell valves remain open. Fingerman and Fairbanks (1955a, b, 1956) reported that the species

Table 2. Multiple regression equations relating $\dot{V}O_2$ (R; ml O_2 0.4 g^{-1} h^{-1}) of *C. virginica* from Long Island Sound to acclimation (T_1, S_1) and experimental (T_2, S_2) temperatures and salinities (r = correlation coefficient). From Shumway and Koehn (1982).

Acclimation condition			
S_1 (ppt)	T_1 (°C)	Regression equation	r
	10	$R = 0.0160 + 0.0140\,T_2 - 0.0095\,S_2$	0.950
28	20	$R = 0.1050 + 0.0133\,T_2 - 0.0085\,S_2$	0.910
	30	$R = 0.0181 + 0.0202\,T_2 - 0.0107\,S_2$	0.859
	10	$R = -0.0048 + 0.0153\,T_2 - 0.0037\,S_2$	0.965
14	20	$R = -0.0902 + 0.0189\,T_2 - 0.0024\,S_2$	0.984
	30	$R = -0.0286 + 0.0210\,T_2 - 0.0074\,S_2$	0.913
	10	$R = -0.0597 + 0.0192\,T_2 - 0.0022\,S_2$	0.994
7	20	$R = -0.0779 + 0.0175\,T_2 - 0.0006\,S_2$	0.986
	30	$R = -0.1580 + 0.0211\,T_2 + 0.0001\,S_2$	0.977

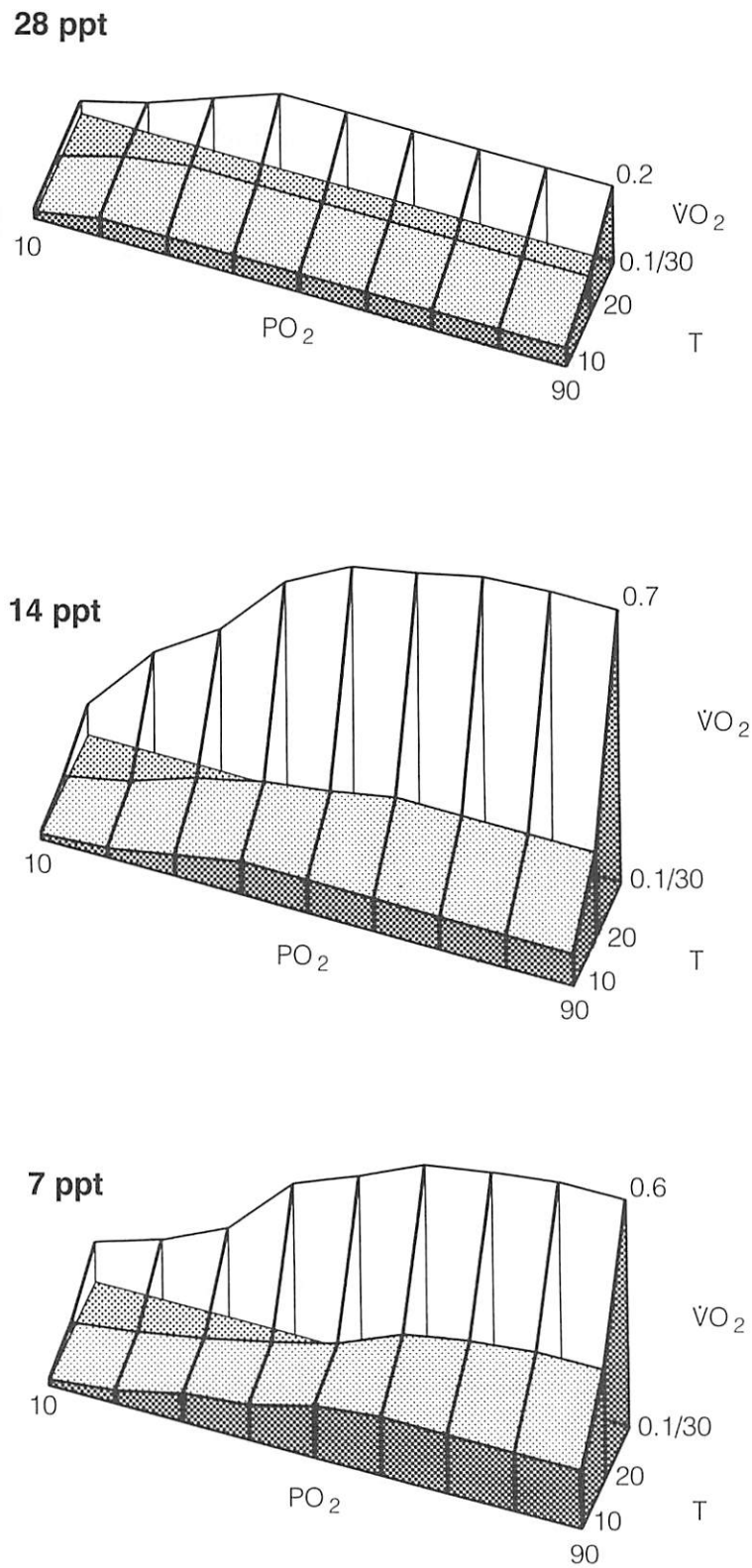


Figure 15. Graphic representation of the combined effects of temperature (T, °C), salinity (28, 14, and 7 ppt) and declining oxygen tension (PO_2) on rate of oxygen consumption $\dot{V}O_2$ (ml O_2 h^{-1}) for a standard oyster of 0.4 g dry weight. After Shumway and Koehn (1982).

has a limited ability to osmoregulate; however, this conclusion was based on hemolymph analyses taken only 4 to 8 h after transferral of oysters adapted to 17 ppt into salinities ranging from 10 to 36 ppt. Galtsoff (1964) stated that, if a salinity change of 10 ppt is maintained for several hours, it reduces the amount of time *C. virginica* remains open and pumping. He also showed that oysters attained osmotic equilibrium in about 120 h when transferred from 31 or 32 ppt to 16.7 or 17.7 ppt. It seems likely that oysters used by Fingerman and Fairbanks (1955a, b, 1956) had closed their valves in response to the salinity change and thus gave the impression of osmotic regulation. These researchers stated in their summary (1955b) that "oysters must be free to open and close their shells for weight and volume regulation. Oysters prevented from completely closing their shells lost weight both in and out of water due to secretion of body fluids." Anderson and Anderson (1974, 1975) demonstrated quite clearly that osmotic and chloride ion concentration of body fluids of the oyster conformed to those of ambient seawater over the non-lethal range of salinities (Fig. 16).

Hand and Stickle (1977) exposed *C. virginica* to simulated tidal fluctuations of salinity of 20 to 10 to 20 ppt and 15 to 10 to 15 ppt and monitored pericardial fluid osmolality and concentrations of Cl^-, Na^+, Mg^{++}, K^{++}, Ca^{++} and ninhydrin-positive substances during both short-and long-term experiments. Their results reconfirmed that oysters are osmotic conformers and that their pericardial fluid remained slightly hyperosmotic to the surrounding seawater (Fig. 17). They also demonstrated that oysters exposed to gradual fluctuations of salinity remain open and pumping for a greater percentage of time than do oysters exposed to sudden and abrupt salinity alterations. Hand and Stickle (1977) suggested that oysters close their valves to partially dampen the osmotic stress imposed when faced with continuous salinity fluctuations for prolonged periods.

In the absence of any extracellular osmotic regulation of the hemolymph, the cells must bear the burden of volume regulation. During the late 1950s and early 1960s, attention became focused on the role of intracellular free amino acids (FAAs), and their function in osmotic regulation by marine invertebrates has been studied extensively (Duchateau et al. 1952; Simpson et al. 1959; Jeuniaux et al. 1961; Bricteaux-Gregoire et al. 1962, 1964a, b). Lynch (1965) and Lynch and Wood (1966) collected eastern oysters from areas of various salinities (i.e., field acclimatized animals) and measured concentrations of 20 free amino acids and ammonia in adductor muscles (Figs. 18, 19). The total concentration of FAAs of the muscle tissue increased proportionally with increased salinity, with taurine, glycine, alanine, and proline accounting for most of the observed increases. Interestingly, concentration changes of individual free amino acids were not proportional to the salinity changes. Histidine was the only FAA that exhibited a decrease in concentration with increased salinity over a portion of the range tested. Lynch and Wood (1966) suggested that alterations in Na:K ratios associated with salinity changes could play a role in regulation of the free amino acid concentration.

Wegener (1971) used isolated mantle tissue as well as whole oysters acclimated in the laboratory to half-strength seawater (17 to 19 ppt) to study by-

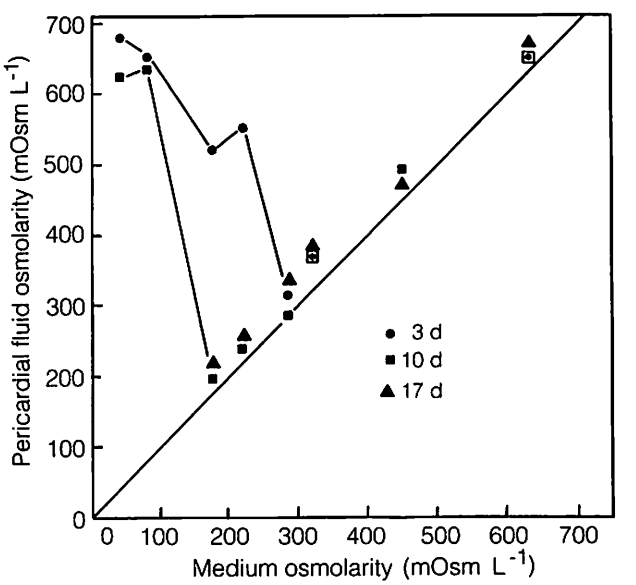


Figure 16. Relationship between osmolarity of the medium and that of oyster pericardial fluid. Each point represents the average osmolarity after 3, 10, and 17 d of three oysters transferred from 620 mOsm L^{-1} to the various media. The isosmotic line is shown. Data points are connected for convenience. After Anderson and Anderson (1975).

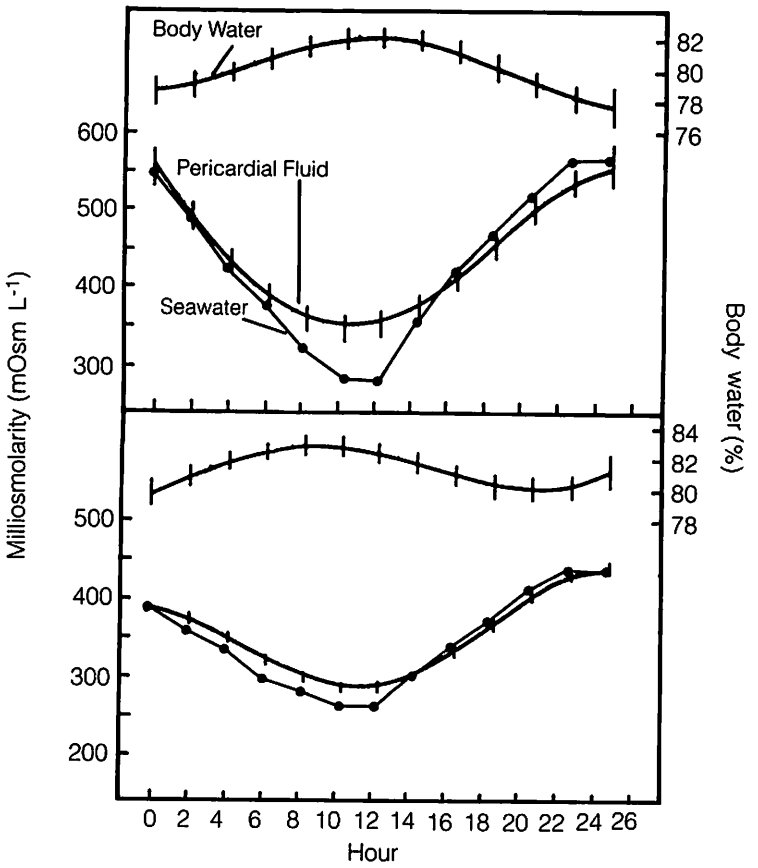


Figure 17. Curves for percent body water and pericardial fluid milliosmolality for 20-10-20 ppt (above) and 15-10-15 ppt (below) diurnal experiments. Actual seawater milliosmolality values are denoted by circles. Vertical lines represent 95% confidence intervals at each sample point along the regression lines. After Hand and Stickle (1977).

products of amino acid metabolism and the fate of both precursors and individual amino acids during salinity stress. Her values for total FAA concentrations are similar to those of Lynch (1965) and taurine was again found to be the most abundant free amino acid. Wegener (1971) found that both whole oysters and isolated gill tissue maintained a constant cell volume when exposed to reduced salinity and that measured changes in ammonia excretion rates of isolated mantle tissue indicated a shift in amino acid metabolism. Adjustments of the FAA pool were shown to be rapid, occurring within less than 3 h of exposure to reduced salinity. Conversely, FAAs were rapidly synthesized by isolated mantle in response to increased external osmotic pressure. When tissues were exposed to 17 ppt seawater, there was an immediate decrease in the incorporation of pyruvate-1-^{14}C in FAAs and a concomitant release of a portion of the FAA pool and some metabolic by-products, including FAAs, NH_3, $^{14}CO_2$, and a non-FAA fraction known to originate from alanine-U-^{14}C. The FAAs released accounted for about 30% of the net reduction in the tissue FAA pool of free amino acids in animals acclimated in 50% seawater. Powell et al. (1982) demonstrated that factors other than salinity changes, including anoxia, turbidity, and drilling effluents, can cause alterations of the FAA pool in *C. virginica*. Their results with regard to salinity induced changes are in agreement with those of the two previous studies mentioned; again, taurine was the most prominent FAA present.

Pathways of synthesis and degradation of FAAs are controlled by ionic concentration of intracellular fluid that in turn is determined by ambient salinity

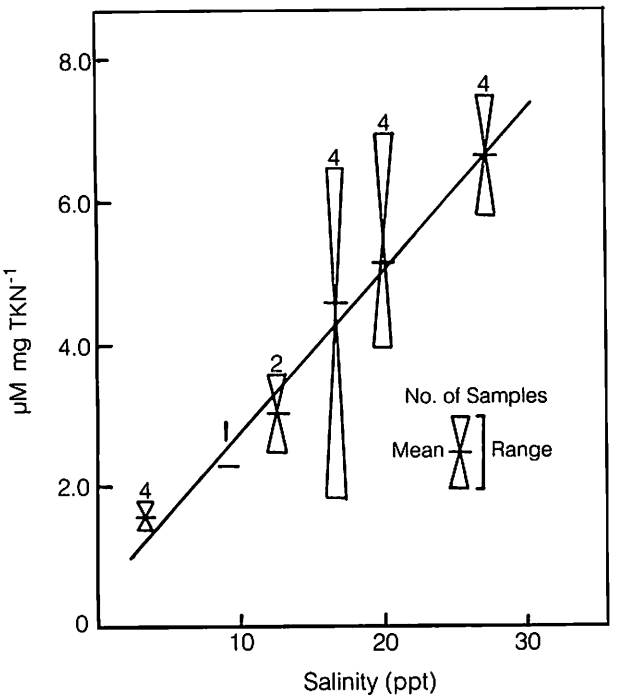


Figure 18. Mean concentration of total free amino acids in adductor muscle of *C. virginica* from various salinities. After Lynch (1965). TKN = Total Kjeldahl nitrogen.

and hemolymph concentration (Gilles 1969). Following the studies discussed above that demonstrated a relationship between salinity and concentration of free amino acids, other studies were undertaken to determine the effect(s) of salinity on enzymes implicated in amino acid metabolism. For example, Sarkissian (1974) studied citrate synthase (an enzyme reversibly inhibited *in vitro* by increasing ionic strength) in *C. virginica* and found that catalytic activity was affected by increased concentration of salt, with the apparent K_m increasing approximately six-fold, i.e., citrate synthase was slightly inhibited by salt. Sarkissian and Gomolinski (1976) demonstrated that malate dehydrogenase from the oyster was not significantly affected by changes in ionic strength of the reaction mixture.

Wickes and Morgan (1976) examined the effects of salinity on glutamate dehydrogenase (GDH), pyruvate kinase (PK), and glutamate-oxaloacetate transaminase (GOT) activity, in adductor muscle and gill tissue of eastern oysters. There was no measurable effect of salinity on PK, suggesting that it does not play a regulatory role in the build-up of FAAs in *C. virginica* during isosmotic intracellular regulation. Activity of GOT, however, increased substantially with increased ionic concentrations. Because they could not demonstrate an increase in aspartic acid with salinity in adductor muscle, Wickes and Morgan (1976) speculated that a direct pathway exists for synthesis of alanine from decarboxylation of aspartic acid, whereby aspartic acid formed from oxaloacetate could immediately undergo decarboxylation to form alanine. In turn, this process would prevent build-up of aspartate as the result of increased GOT activity and would lead to an increased concentration of alanine. This pathway for alanine synthesis remains to be demonstrated. Activity of GDH showed a high positive correlation with salinity in adductor muscle but GDH activity was absent in gill tissue.

In the only other similar study on eastern oysters, Cripps (1977) studied effects of salinity on six enzymes, including GOT, PK, MDH, glutamate dehydrogenase (GDH), lactate dehydrogenase (LDH), and phosphoenolpyruvate carboxykinase (PEPCK) in gill, adductor muscle, and mantle tissue. His results were in general agreement with Wickes and Morgan (1976) and he pointed out that responses of individual tissues were variable for acute salinity stress.

In other related studies, Feng et al. (1970) reported seasonal variations in hemolymph FAAs as well as a shift in the FAA pool in eastern oysters induced by parasitic infestations of *Bucephalus* sp. and *Haplosporidium* (=*Minchinia) nelsoni*. These alterations could conceivably interfere with osmoregulatory capabilities of infected oysters under some conditions. Prusch and Hall (1978) calculated diffusional water permeabilities (P_d) for eight different marine bivalve species and found that *C. virginica* had one of the lowest P_d values of any species studied, $3.01 \pm 0.28 \times 10^{-5}$ cm s^{-1}. This low permeability to water movement undoubtedly aids the ability of *C. virginica* to resist desiccation in high intertidal areas.

The role of amino acids in intracellular osmotic regulation is complicated, and there are obviously numerous mechanisms operating simultaneously. Data reported for *C. virginica* are similar to, although not in total agreement with, data reported for other bivalves with regard to their osmotic regulation and amino acid pools (Lange 1963, 1972; Shumway et

al. 1977; Shumway and Youngson 1979). The ability of eastern oysters to cope with a wide amplitude of salinity variation at the cellular level represents but one more means by which they have successfully invaded estuaries.

GENETIC ADAPTATIONS TO ENVIRONMENTAL STRESSORS

The existence of physiological races that are geographically separated has been demonstrated quite

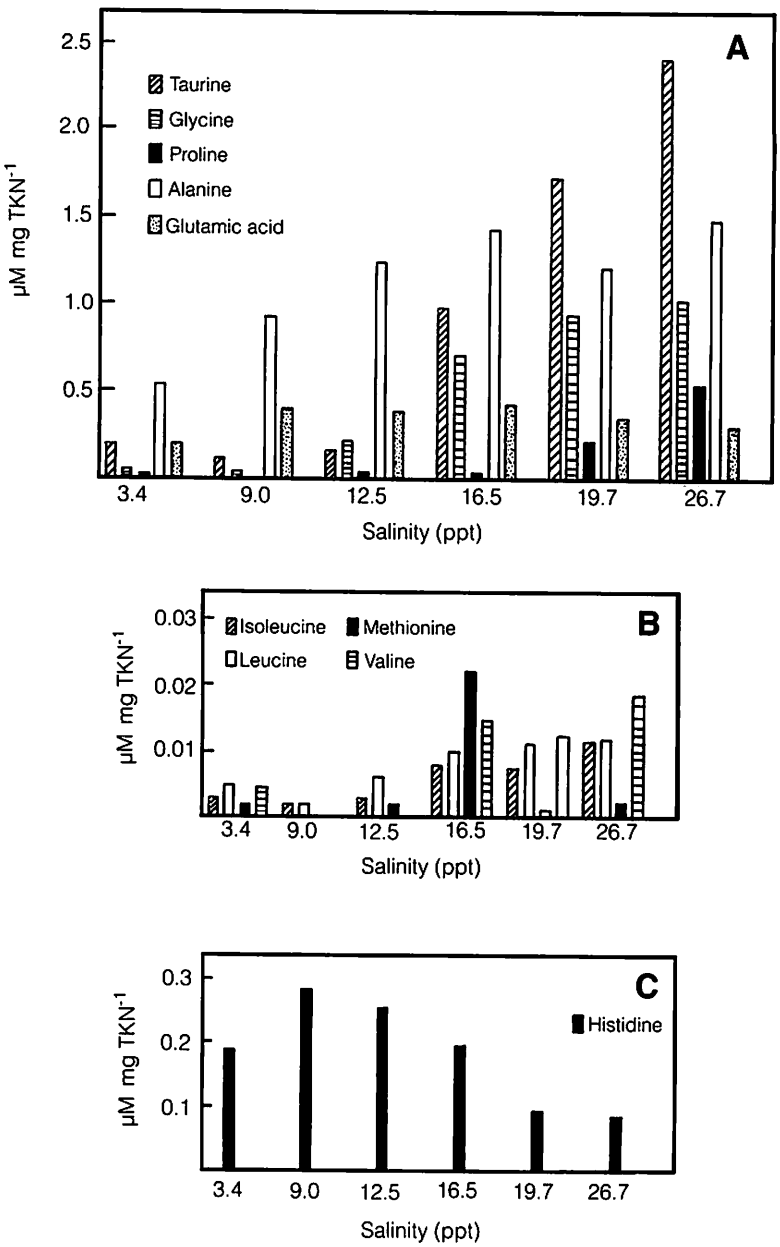


Figure 19. Mean concentrations (µM mg TKN^{-1}) of (A) taurine, glycine, proline, alanine, and glutamic acid; of (B) isoleucine, leucine, methionine, valine; and of (C) histidine in adductor muscle of *C. virginica* from various salinities. TKN [Total Kjeldahl nitrogen]. After Lynch (1965).

clearly, and differences can exist between populations located in close geographic proximity. Longwell (quoted in Loosanoff 1969) stated that "there is sufficient justification for the interesting speculation that genetic differences play a role in the differences in gametogenic activities of South Carolina oysters exposed to the same temperatures that failed to elicit a response from the other southern groups studied." Unfortunately, the prospect is still speculative. Newell and co-workers (pers. comm.) found significant differences in attributes such as scope for growth, feeding rate, and oxygen consumption between oyster populations from high and low-salinity areas; however, they were unable to demonstrate any difference between the two populations through electrophoretic analyses of nine enzyme loci. The question remains as to whether or not separated populations of oysters exhibiting different tolerances to various environmental factors represent merely physiological races or are in fact genetically separate units.

Newkirk et al. (1977) showed that two populations of *C. virginica* exhibited genetic differences in regard to tolerance of reduced salinities and that some of the genetic variation was non-additive. In a more complete study, Newkirk (1978) used oysters from four populations and found evidence of overdominance in survival in one of their hybrids, although there were no significant differences in survival among the populations. Salinity also affected the expression of differences in growth rate demonstrated among populations. Non-additive genetic effects in the hybrid crosses were also seen, but direction and magnitude were dependent upon salinity. Expression of survival differences seen between populations was dependent on the environment in which larvae were reared and whether larvae were from a pure or a hybrid cross. Interestingly, there was as much difference in survival between populations from the same estuary as there was between populations from geographically isolated populations. Even though Newkirk (1978) was able to demonstrate evidence for genotype-environment interaction on growth rate, the presence of non-additive effects interacting with the environment precluded assigning these survival differences either to adaptation to particular environments or isolation of the populations.

Koehn and Shumway (1982) showed that more heterozygous individuals of *C. virginica* were at an energetic advantage compared to their homozygous siblings when exposed to temperature or salinity stress. They found that the metabolic energy demand of high temperature and low-salinity was over twice as great for multiple locus homozygous individuals as for the most heterozygous individuals, and concluded that homeostasis represents a magnification of inherent genetic differences between individuals.

PARASITES, PREDATORS, AND DISEASES

Although salinity per se can affect distribution of oysters, the indirect role of salinity on the incidence of parasites, predators, and diseases can also control the distribution of eastern oysters. The estuarine habitat and associated freshwater influxes can have lethal effects on stenohaline carnivorous gastropods, starfish, and other pestilential taxa. It has been noted that high temperature and salinity combinations generally tend to increase the threat of disease and predation. For further discussion of effects of diseases of and predation on eastern oysters, see Ford and Tripp, Chapter 17 and White and Wilson, Chapter 16, respectively.

One of the most severe diseases of oysters (Dermo) is caused by the protistan parasite *Perkinsus marinus*, and is most prevalent in oysters exposed to conditions of high temperature and salinity (Mackin et al. 1950; Mackin 1951, 1956, 1961; Hewatt and Andrews 1956; Andrews and Hewatt 1957; Quick 1971; Quick and Mackin 1971; Ogle and Flurry 1980). The infectious agent was first erroneously described as a fungal disease by Mackin et al. (1950) and initially named *Dermocystidium marinum* (later changed to *Labyrinthomyxa marina*, Levine 1958; see Mackin and Ray 1966). The relationship between this parasite and high temperature and salinity conditions was quickly established (Mackin 1951), as was the incidence of high mortalities of infected oysters during warm summer months (Mackin 1953; Hewatt and Andrews 1954). Ray and Chandler (1955) established that temperatures above 20°C favored development of the disease in the Gulf of Mexico.

In a later study, Mackin (1956) more clearly defined the effects of salinity on Dermo. He demonstrated conclusively that low temperature-salinity combinations retarded parasite development and concluded that oysters can exist and grow vigorously in salinities slightly lower than the minimum tolerated by Dermo. He hastened to point out, however, that the margin of tolerance is so narrow that, for practical purposes, it does not exist. He summarized his results and others as follows: in the estuary there is generally a positive correlation of high salinity with high incidence and weighted incidence of the disease, although infection by *P. marinus* is reduced at low salinity. The salinity tolerance range of the parasite is wide, in some instances varying from 8 to 50 ppt. From laboratory experiments, he concluded, that, while there can be a retarding effect of low salinity per se, the disease may nevertheless develop in oysters at these low salinities and there is probably no physiological handicap for *P. marinus* produced by low salinities. He suggested that dilution of infective particles by freshwater inflow, coupled with the preponderance of ebb over flood current rates, tended to eliminate infective cells in low-salinity areas and to concentrate them in high-salinity areas.

Hewatt and Andrews (1956) found that oysters infected by *P. marinus* all died within about four weeks at 28°C. When infected oysters were held at 15°C, infection was arrested and mortalities caused by the disease were negligible. Hewatt and Andrews (1956) also suggested that oysters taken from an endemic area were less susceptible to infection by Dermo than were oysters collected from nonendemic waters. Andrews (1965) later showed that temperatures above 25°C were necessary to cause high mortalities, and that *P. marinus* growth is dependent on warm seasons.

Soniat (1985) studied the quantitative relationship between intensity of infection by *P. marinus* and the interaction of water temperature and salinity in Galveston Bay. Although the temperature-salinity interaction explained more variability in weighted incidence of *P. marinus* than either temperature or salinity alone, most variability was still unexplained. Soniat (1985) suggested that this variability was probably the result of individual differences in resistance to infection.

Other diseases known to show chronic infections only at high salinities are *Haplosporidium costalis* (SSO) and *Haplosporidium nelsoni* (MSX), with the latter tolerating a wide range of temperatures and posing a serious and increasing threat to oyster populations (Andrews 1967, 1988; Andrews and Ray 1988). Newell (1985) demonstrated a marked reduction in clearance rate and condition index of *C. virginica* with systemic infections of MSX. He also noted that there were no differences in the rate of oxygen consumption between infected and uninfected oysters. Decreased feeding rates coupled with sustained metabolic rates lead to a decreased condition index and imparts a severe physiological stress on oysters (Newell 1985). Barber et al. (1988a, b) demonstrated that condition index and fecundity in *C. virginica* are a function of the infection intensity of MSX. Uninfected individuals have higher values than epithelially infected individuals, which in turn have higher values than systemically infected individuals. The condition indexes of oysters with gill infections of MSX and systemic infections were 13% and 31% lower than uninfected oysters, respectively. Fecundity was reduced in oysters with gill infections (35% reduction) and systemic infections (81% reduction) and Barber et al. (1988a, b) concluded that reduced fecundity is most likely the result of metabolic stress whereby MSX reduces food intake and competes for energy reserves. These authors also demonstrated that all biochemical components (lipid, glycogen, protein) generally decreased in concentration with increasing MSX infection, intensity, and duration. Thus, even at sublethal levels, meat yield and recruitment potential of *C. virginica* are reduced by the presence of MSX.

Fisher and Newell (1986) provided a possible explanation for the marked seasonal trends in infection by diseases such as Dermo, SSO, and MSX. They suggested that high salinity reduces the oysters' defense capacity, leaving them more susceptible to pathogenic parasites. Fisher and Newell (1986) studied effects of salinity on granular hemocytes in *C. virginica* from Chesapeake Bay. These oyster hemolymph cells are responsible for most phagocytic activity that provides the primary line of defense against foreign particles. Fisher and Newell (1986) demonstrated that increases in acute salinity retarded activity of hemocytes and that decreases in acute salinity

enhanced hemocytic activities. They speculated that differences in hemocyte activities found between high and low-salinity areas could provide oysters with greater disease resistance, i.e., oysters that are able to maintain maximal hemocytic activity can be at an advantage when faced with possible infection. This topic has been treated in greater detail by Cheng in Chapter 8.

In addition to parasites and diseases, many oyster predators are also limited to more saline waters (Grave 1905; Butler 1954; Wells 1961; Maurer and Watling 1973). Indeed, Gunter (1955) proposed that one advantage conferred to eastern oysters living in less saline estuarine water was reduced predation and competition. Probably the most completely studied of these predators are the drills, *Thais haemastoma, Thais lapillus, Urosalpinx cinerea,* and *Eupleura caudata.* These species constitute one of the largest groups of predators on oysters and as such their tolerance and functional capabilities have been studied by numerous authors (see Carriker 1955; Hanks 1957; Manzi 1970; Zachary and Haven 1973; Bayne and Scullard 1978).

Manzi (1970) exposed drills (*Eupleura caudata* and *Urosalpinx cinerea*) to 12 salinity-temperature combinations, and showed that feeding rates on oyster spat increased with each increase in salinity and temperature. Maximum rates were measured at the highest salinity (26.5 ppt) and temperature (25°C) tested; little or no feeding was seen at 12.5 ppt. Manzi (1970) concluded that this salinity is near the lower limit for feeding. He further showed that at all temperature and salinity combinations studied, *U. cinerea* consumed more oyster spat than did *E. caudata.*

Garton and Stickle (1980) found that both predation and ingestion rates of *T. haemastoma* feeding on eastern oysters are sensitive to temperature and salinity, and that the temperature threshold for predation is between 10 and 12.5°C; no feeding occurs below 7.5 ppt. Garton and Stickle (1980) also exposed drills to diurnal tidal fluctuations of salinity, more closely representing the estuarine environment than did previous studies, and demonstrated that drills had predation rates significantly lower than those at the optimal constant salinity of 20 ppt at 30°C; however, there were no significant differences among salinities at 20°C. Predation and ingestion rates in fluctuating salinity cycles were not significantly different from rates for drills at constant acclimation salinities of 10 and 30 ppt. Garton and Stickle (1980) were able to show that *T. haemastoma* can tolerate changes in its physiology that accompany changes in environmental temperature and salinity and are able to function as an efficient oyster predator under these conditions.

Various other oyster predators are also known to be limited in their distribution by salinity. The starfish, *Asterias forbesi,* has a lower salinity threshold of about 16 to 18 ppt (Loosanoff 1945b; Wells 1961) as does the whelk, *Fasciolaria hunteria* (Wells 1961). In Virginia, Hopkins (1962) reported on the distribution of species of the boring sponge, *Cliona* spp., on the eastern shore of Virginia in relation to salinity. He found that *Cliona celata* was most abundant in high-salinity bays and the least abundant in lower salinity, whereas *Cliona truitti* was the most abundant species in low salinity areas and became increasingly prominent as salinity decreased (see also Old 1941). The flatworm, *Stylochus ellipticus,* is also a predator of oysters (Loosanoff 1956) that is tolerant of low salinities.

Various species of crabs also pose a threat to oysters. The commensal pea crab, *Pinnotheres* sp., frequently occurs in oysters in high-salinity water on the Atlantic coast but is uncommon in Gulf waters (Butler 1954). MacKenzie (1970) showed that mud crabs (family Xanthidae) prey on oyster spat and Little and Quick (1976) reported that prolonged periods of high salinity (greater than 25 ppt) foster proliferation of xanthid crabs, among other species. The stone crab *Menippe mercenaria* preys on eastern oysters but is not tolerant of low salinities and is eliminated by freshwater intrusion (Menzel et al. 1966). Menzel et al. (1966) also reported that the blue crab, *Callinectes sapidus,* becomes a more serious predator when large oysters are weakened by high temperatures.

Finally, the sea anemone, *Diadumene leucolena,* is a predator of oyster larvae in the Chesapeake Bay region (MacKenzie 1977; Steinberg and Kennedy 1979). Clearly, the fact that oysters can tolerate lower salinities than those that inhibit predators has aided in the proliferation of oysters in the upper reaches of estuarine systems.

LIGHT, pH, AND TURBIDITY

Orton (1929) first suggested that oysters do not feed during late night or early morning hours. Loosanoff and Nomejko (1946) studied the possible effects of tidal stage and periods of light and darkness on the feeding activity of about 1,400 *C. virginica.* In darkness, the percentage of oysters with full stomachs was comparable to that of the individuals examined in daylight, the oysters fed actively, and the average rate of pumping was comparable to that by day. Shell valves remained open 94% of the time during daylight and darkness and the oysters were feeding all or most of the time when their shells remained open. Thus, no experimental evidence exists to support the early theory that oysters feed only by day, and it is now generally accepted that light has no discernable effect on their feeding activity.

Light has, however, been shown to affect other aspects of oyster biology. Medcof and Kerswill (1965) reported that shading increases linear shell growth of oysters about 150% but reduces the ratio of thickness to length. Further, exposure to light increases plumpness of meats, specific gravity of the body tissue, and shell fluting and pigmentation. These results are in agreement with those reported previously by Medcof (1949). No physiological mechanisms were suggested for these differences.

Medcof (1955) indicated that light favors setting of *C. virginica* larvae. Ritchie and Menzel (1969) and Shaw et al. (1970), however, demonstrated that eyed larvae of *C. virginica* are light sensitive and that larval setting is encouraged by darkness and partially inhibited by light (Fig. 20). Larval behavior is covered in greater detail by Kennedy in Chapter 10.

Early data on the effects of pH on the biology of *C. virginica* are anecdotal. Based on a few field observations, Prytherch (1928) suggested that spawning is inhibited by low pH. Loosanoff and Tommers (1948) demonstrated that pH affects pumping rate such that oysters kept in waters of pH 4.25 pump only 10% as much water as control animals at 7.75 pH, even though the oysters remain open about 75% of the time. Calabrese and Davis (1966, 1969, 1970) demonstrated that the minimum and maximum pH levels at which the oyster will spawn are 6.0 and 10.0, respectively. Moreover, they found that oyster eggs and sperm released outside the range of pH 6.0 to 10.0 lose their viability rapidly within 2 to 4 h, with the lowered viability due to a combination of pH and aging (Fig. 21). The pH range for normal embryonic development is 6.75 to 8.75, with a lower pH limit of 6.00 for larval survival. Normal growth was found over the pH range of 6.75 to 8.75 and growth rate decreased rapidly at pH levels below 6.75. Optimum pH for growth of oyster larvae is 8.25 to 8.5.

The effect of varying quantities of suspended material (seston) on the biology of *C. virginica* has been a matter of discussion for many years, yet seston remains one of the least studied environmental variables. Suspended materials may be natural (floods, storms) or anthropogenic (dredging) in origin. Opinions of researchers have ranged from the claim that oysters feed only in clear waters (Kellogg 1915,

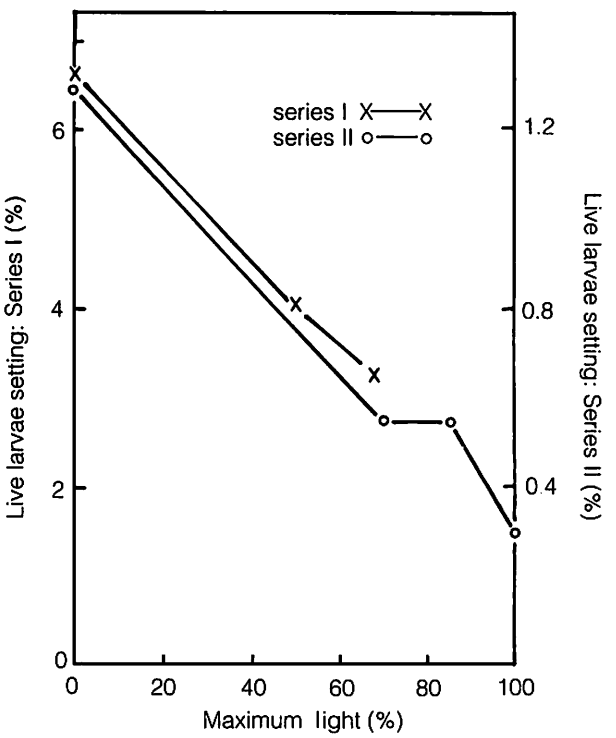


Figure 20. Effects of light on numbers of oyster larvae setting. Total spat setting over 3 to 4 d expressed as percent of living larvae present, plotted against the proportion (%) of each 24 h period during which tanks were illuminated. After Shaw et al. (1970).

1916) to that of oysters being unaffected by highly turbid waters (Grave 1916), and have provided the basis for several debates in the early literature. Nelson (1921c) demonstrated that oysters fed in waters of high levels of seston (up to 0.4 g dry weight L^{-1}). Loosanoff and Engle (1947a) presented evidence indicating that Kellogg (1915, 1916), Grave (1916), and Nelson (1921c, 1951) were all partially correct in their conclusions. They supported Kellogg's (1915, 1916) contentions that oysters feed most efficiently in clear waters; however, they also found that oysters can feed in water containing relatively large numbers of microorganisms, although under such conditions the rate of feeding is decreased. If the concentration of planktonic organisms is too great, feeding ceases. A more detailed account (Loosanoff and Engle 1947b) provided data on the effects of high concentrations of microorganisms on feeding and pumping by *C. virginica*. The changes in feeding activity of oysters in response to increases in seston concentration are discussed by Newell and Langdon in Chapter 5.

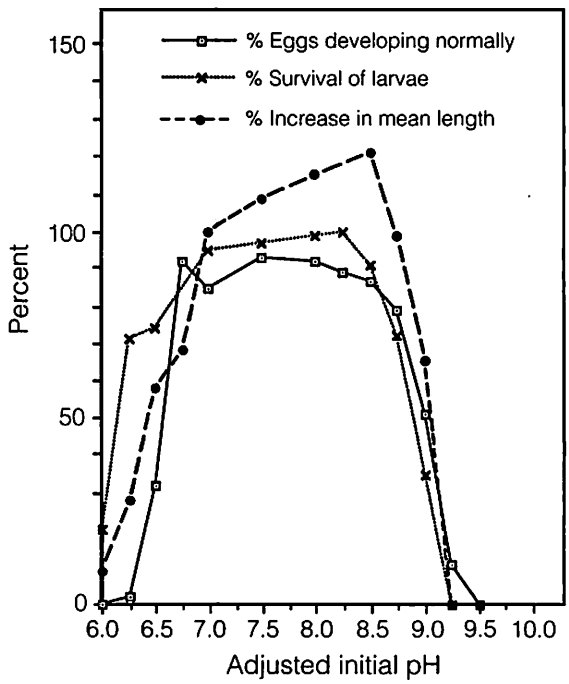


Figure 21. The pH tolerance of oyster embryos and larvae as indicated by percentage of eggs that developed normally, survival of larvae, and increase in mean length. After Calabrese and Davis (1966).

The effects of turbidity (silt or seston concentration) on the activity patterns of oysters has been the focus of several studies, especially in relation to dredging activities. Increased concentrations of suspended materials can induce a reduction in pumping rate, a clogging of the gill apparatus, a subsequent reduction in growth rate, and death. As pointed out by Stern and Stickle (1978), although the effects of turbidity and suspended material may not necessarily be lethal, quite often the associated sedimentation may smother and kill both juvenile and adult oysters.

Lunz (1938) performed a field study during the dredging of the Intracoastal Waterway of South Carolina. Unless adult oysters were completely buried, their mortality near the dredging activity was no higher than in areas remote from the dredging operations and there was no evidence of changes in the physiological condition of the oysters. The intensity of setting of oysters adjacent to the dredging operations did not differ from setting intensity in areas remote from such activities. Lunz (1938) concluded that dredging apparently had no effect on spawning and setting success. Much subsequent work has found deleteriuous effects of siltation on cultch cleanliness. Even a thin layer of silt reduces spat settlement as reviewed by Mackenzie in Chapter 21.

Loosanoff (1948) and Loosanoff and Tommers (1948) demonstrated that concentrations of silt (0.1 g L^{-1}; note that this is over four times the maximum levels commonly observed in estuarine waters) caused a 57% reduction in pumping rate of adult oysters in Long Island Sound. The reduction in the average pumping rate was more than 80% at 1 gL^{-1} and 94% in concentrations of 3 and 4 g L^{-1}. Similar results were shown for kaolin and chalk. Fuller's earth at a concentration of 0.5 g L^{-1} reduced the rate of pumping by 60% (Fig. 22). Although the efficiency of feeding was greatly reduced, the oysters could ingest small quantities of particles in very turbid waters. This finding supports the earlier statement of Nelson (1921b) that oysters could feed in turbid waters.

Loosanoff and Tommers (1948) also demonstrated that the shell movements of oysters in turbid waters were greater in amplitude and of a different type than those under normal conditions. Movements in

turbid waters were usually associated with the expulsion of large amounts of pseudofeces. Loosanoff and Tomers (1948) speculated on the possibility of physiological races of oysters with varying degrees of tolerance to turbidity. These findings were confirmed by the work of Hsiao (1950) who reported that the more turbid the water, the more irregular the respiratory and feeding movements of the shells of *C. virginica*. A reduction in the turbidity was followed immediately by increased shell movement. Hsiao (1950) also showed that in very turbid seawater, where the silt was allowed to settle on the oysters, there was an immediate cessation of shell movement for 16 to 19 h. The animals subsequently attempted to reopen their shells in an effort to remove the silt. If the silt deposits remained for more than 3 d, death resulted.

Engle (1958) found no detrimental effects of suspended silt on eastern oysters hung in baskets adjacent to dredging activities in Chesapeake Bay. He suggested that dredging might provide an increased supply of organic detritus that would in turn increase the condition of the oysters. McKinney and Case (1973) reported similar results for oysters suspended in experimental cages in San Antonio Bay, Texas, although populations on the bottom were killed by the accumulation of dredged particles. Mackin (1956, 1962) and Mackin and Hopkins (1961) found that turbidities up to 0.7 g L^{-1} were not harmful to eastern oysters and that it was apparently impossible to maintain a suspension of high concentration long enough to cause mortality of oysters. They did report an inverse relationship between turbidity and mortality of oysters; however, these differences in mortality rates were probably more a function of salinity than turbidity (see also Butler and Engle 1950; Gunter 1953; Cory and Redding 1976). Mackin (1956) also stated that high turbidities neither enhance nor depress the effect of Dermo disease.

The most detailed studies on the effects of turbidity on pumping and shell valve activity in eastern oysters is that of Loosanoff (1962). He demonstrated that 0.1 g L^{-1} of silt can noticeably affect the behavior of adult oysters. The average reduction in rate of pumping was 57%; however, occasionally the oysters appeared to be stimulated and pumped faster. In silt and kaolin concentrations ranging from 0.1 to 4 g L^{-1}, rate of change in pumping ranged from −100 to +18%, with average reductions ranging from 57 to 94% for silt and from 46 to 85% for kaolin. Over a similar range of concentrations of chalk, the rates of change ranged from −12 to −94%, with average reductions of 38 to 89%. At 0.5 g L^{-1}, Fuller's earth reduced pumping by an average of 60%.

In addition to the reductions in pumping rates, the oysters formed and discharged large quantities of pseudofeces containing silt and increased their shell-valve activity, presumably in association with the rapid expulsion of the pseudofeces. In heavy concentrations of silt, oysters closed their shells entirely for extended periods of time, sometimes remaining closed for a pe-

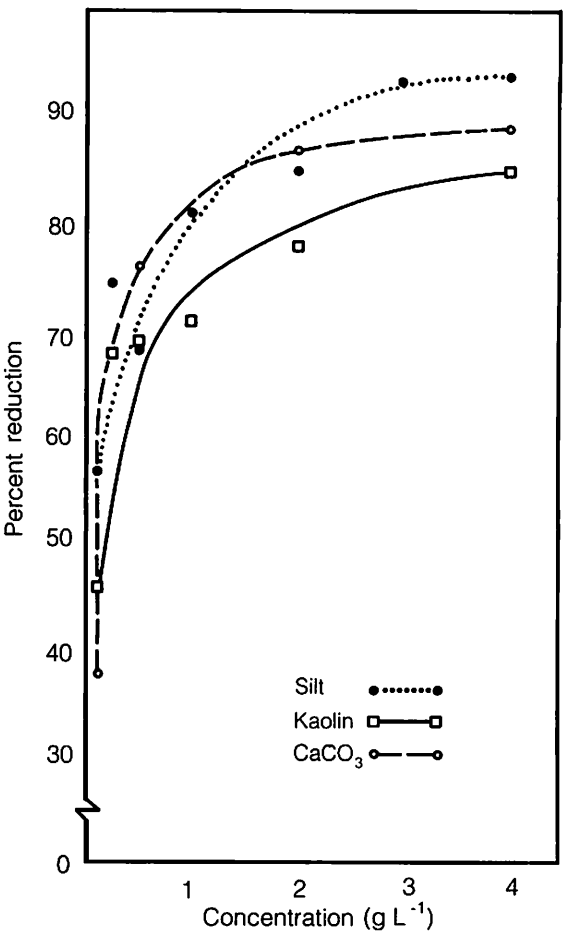


Figure 22. Percent reduction in pumping rate of eastern oysters subjected to different concentrations of turbidity-creating substances. After Loosanoff (1948).

riod of several weeks. Loosanoff (1962) concluded that oysters "feed most efficiently in relatively clear water."

Of equal or greater importance than the effects of seston on adult oysters is its effect(s) on eggs and larval stages. Davis (cited in Loosanoff, 1962) and Davis and Hidu (1969) showed clearly that silt was harmful to oyster eggs. Egg mortality was 27% at concentrations of 0.25 g L^{-1} of silt and 69% at 0.5 g L^{-1}. Silt was more harmful to oyster eggs than either kaolin or Fuller's earth. Oyster larvae could withstand highly turbid water (2 g L^{-1} silt and up to 4 g L^{-1} of either kaolin or Fuller's earth) for up to 14 d.

Carriker (1986) has recently reviewed the available literature concerning the effects of silt on planktonic stages of *C. virginica* ranging from eggs and spermatozoa to pediveligers. His review demonstrates the paucity of information on this topic. He suggests that the free swimming trochophore and veliger stages may be particularly vulnerable to the presence of silt that could clog the highly sensitive feeding apparatus. He also points out that there are undefined, yet beneficial, concentrations of silt for embryos (below 0.25 g L^{-1}) and for veligers (0.75 g L^{-1}), but that the optimum range of particle concentrations for the differing stages of development has not been determined. Clearly, the effects of silt and turbidity on oyster larvae require further research.

SUMMARY

In conclusion, a fundamental requirement of the eastern oyster is the mixture of salt water with fresh water from land drainage (Butler 1954). In light of their sessile nature, oysters are particularly susceptible to environmental perturbations and yet they thrive in the often harsh and constantly fluctuating environs of the estuary. Through an array of physiological and behavioral mechanisms, oysters are highly tolerant of different habitats and environmental variations. It is the possession of a rather plastic physiology, acquired through geologic ages of adaptation, that has allowed the eastern oyster to tolerate estuarine conditions and flourish under such unpredictable conditions and to establish itself as one of the true estuarine species.

ACKNOWLEDGMENTS

I am indebted to Pam Shepard-Lupo and Mary Lou Gilmor, librarians without whom this review would not have been possible. Jan Barter, John Hedley, and Kristin Geib performed many thankless tasks and their help is greatly appreciated. Thanks also to Kim Knowlton and Jim Rollins who prepared the figures and to Martha Hernandez-Davis who typed the manuscript. M. Carriker and the late W. Menzel kindly read an earlier version of the manuscript and made many helpful suggestions.

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