Chapter 14

Environmental Factors: Response to Metals

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INTRODUCTION

Human activities are considered to be major factors that influence the geochemical cycle of metals (Salomons and Förstner 1984). The total annual toxicity of all anthropogenically-mobilized metals (based on dilution to drinking water standards) is reported to exceed that of other classes of compounds such as radioactive or organic wastes (Nriagu and Pacyna 1988). Aquatic environments, both marine and freshwater, are affected by this anthropogenic mobilization of metals.

The ability of marine bivalves to concentrate metals in soft tissues to high concentrations is well-known. Increased concern over anthropogenic contamination of the marine environment by metals and the possibility of human exposure through consumption of shellfish has stimulated interest in understanding the bioaccumulation of metals by bivalves and the ecological implications of such bioaccumulation. The concentrations of metals in bivalve tissues, the potential detrimental effects of metals on the health of exposed organisms and their consumers, and the biological and physical-chemical factors that control related processes have been topics of wide concern.

Many metals are natural constituents of the environment and are essential to cellular function. Marine animals normally accumulate metals from a seawater environment that is extremely low in metal concentrations. Concentrations of metals in the tissues of marine animals are normally four to five orders of magnitude higher than those in surrounding seawater (usually tens of nanograms per liter or lower in seawater compared to milligrams per kilogram and greater in tissues [Simkiss et al. 1982]). In oysters, as in numerous other marine animals, the enhanced bioaccumulation that results in environments subjected to anthropogenic contamination is superimposed on this natural ability to concentrate metals. Processes that control bioaccumulation and intracellular distribution are responsible for the observed concentrations of metals in tissues. These processes include chemical events that result in the presence of bioavailable metal species and biological processes that control metal metabolism.

It is well established that metal concentrations in oyster tissues are highly variable and influenced by natural and anthropogenically-induced changes in environmental conditions. Ranges of concentrations of selected metals in the eastern oyster, *Crassostrea virginica,* are shown in Table 1. These reflect low metal concentrations in oysters in relatively uncontaminated environments, as well as elevated concentrations in oysters from contaminated environments. Seasonal variability would be expected to affect oysters in both contaminated and uncontaminated environments.

Monitoring efforts, such as the National Status and Trends Program of the U.S. National Oceanic and Atmospheric Administration (Lauenstein et al. 1990), have provided information on metal concentrations in *C. virginica* from various habitats. This chapter focuses on our understanding of factors that

Table 1. Range of metal concentrations (μg g dry weight^{-1} tissue) in *Crassostrea virginica* from the Atlantic coast of North America (summarized from NOAA 1987).

Element	Range
Antimony	0.07 to 4
Arsenic	3.17 to 42.67
Cadmium	0.39 to 13
Chromium	0.12 to 4.9
Copper	14.7 to 1603
Mercury	0.01 to 0.48
Nickel	0.55 to 12.57
Selenium	0.93 to 5.67
Silver	0.3 to 7
Tin	0.02 to 0.58
Zinc	300 to 13000

influence variability in metal bioaccumulation, turnover of metals, metal toxicity, and cellular mechanisms of metal sequestration in the eastern oyster. Sections are also included on the effects of organotin on oysters (a special case in which oysters are recognized target organisms) and the public health implications of metal bioaccumulation in oysters. Studies on other bivalve molluscs, particularly species of oysters other than *C. virginica,* are included where appropriate. A comprehensive review of responses of bivalve molluscs to metals in the environment, which includes much of the early work on *C. virginica,* was published previously (Cunningham 1979). Additionally, relatively recent reviews on metal bioaccumulation (Luoma 1983; Rosejadi and Robinson 1993) and the mechanisms of metal toxicity (Viarengo 1989) in aquatic organisms are available.

VARIABILITY IN METAL BIOACCUMULATION AND IMPLICATIONS FOR BIOMONITORING

In reviewing the early literature on metal concentrations in *C. virginica,* Galtsoff (1964) noted relationships between concentrations in oyster tissues and the degree of industrialization near oyster habitats. Notably, concentrations of certain metals such as copper, lead, and iron were higher in areas of greater human activities. The influence of season and geographical location on the variability of metal concentrations in oyster tissues was recognized as well. Numerous studies have now shown that the concentrations of metals in *C. virginica* exhibit considerable individual and seasonal variability (Engel 1988). The range of concentrations (Table 1) can be quite large for some metals and reflects both natural variability and anthropogenic contamination.

Factors that can contribute to variability in oyster metal concentrations are of environmental and biological origin. Seasonal cycles in metal concentrations in oysters are governed by local environmental conditions that determine availability of metals and food supplies and by biological processes, such as reproduction, which influence endogenous cycles (Frazier 1975). Superimposed on these factors would be the individual variability associated with genetic variation and nonsynchronous physiological and biochemical processes.

The geochemistry of the surrounding environment and chemical speciation of metals in the external medium are recognized as major environmental factors that determine local bioavailability and are responsible for the amount and chemical form of metals in the environment. For many metals, the ionic form is regarded as the form that is available for uptake by marine organisms, although uptake of organic complexes can occur (e.g., George and Coombs 1977). Salinity and the presence of organic substances such as humic and fulvic acids (Morel and Hering 1993) are recognized as major environmental factors that affect metal speciation in coastal waters. Thus, in marine environments with different or fluctuating conditions of salinity, organic matter, or suspended particulate matter, the bioavailability of metals may vary considerably and result in considerable variability in tissue metal concentrations in resident organisms.

In the case of cadmium, for example, the ionic species, which is the bioavailable form, is complexed

by chloride ions and rendered unavailable for uptake (Sunda et al. 1978). Thus, cadmium bioaccumulation is expected to decrease as salinity increases. Accordingly, a decrease in cadmium bioaccumulation is correlated with an increase in the $p[Cd^{2+}]$, the negative logarithm of the molar concentration of cadmium ions (Fig. 1). For both cadmium (Phelps et al. 1985) and silver (Sanders et al. 1991), bioaccumulation is inversely correlated with salinity in natural oyster populations. In contrast, the presence of organic rather than inorganic complexes has been found to be most important in controlling bioavailability of copper to oysters (Zamuda and Sunda 1982; Zamuda et al. 1985). This finding is consistent with the general body of information regarding bioavailability of copper in marine environments. Most of the naturally-occurring copper in marine and estuarine waters is considered to be unavailable as a result of binding to dissolved and particulate organic substances. With silver, binding to suspended particulate matter and incorporation into phytoplankton reduces bioavailability in comparison with the dissolved forms of the metal (Abbe and Sanders 1990). Temperature has also been shown to influence the rate of accumulation of available forms of metals in oysters (Zaroogian and Cheer 1976; Hung 1982).

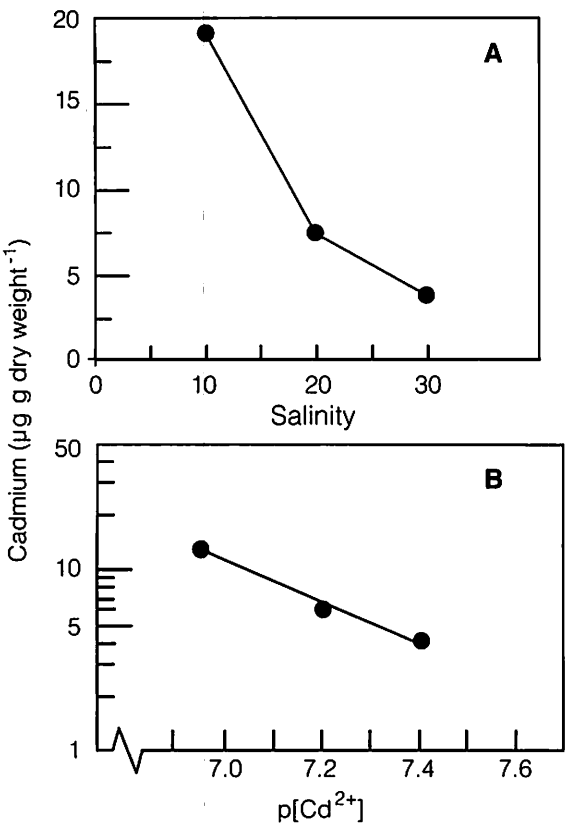

Figure 1. Relationship between cadmium bioaccumulation by *Crassostrea virginica* and either (A) salinity or (B) $p[Cd^{2+}]$. Bioaccumulation is reduced as both salinity and $p[Cd^{2+}]$ increase. Higher $p[Cd^{2+}]$ indicates lower concentrations of the free cadmium ion. There is a linear log-log relationship between accumulated Cd and $p[Cd^{2+}]$. Oysters were exposed to 1000 µg Cd L^{-1} for 7 d. Redrawn with data from Engel et al. (1981).

The reproductive cycle of the oyster, which is characterized by seasonal changes in nutrient reserves and release of gametes (see Thompson et al., Chapter 9), also contributes to the variability in metal content in oysters (Engel 1988). In some cases, the variability is not the result of net movement of metals into or from organisms, but simply reflects changes in biomass associated with use or storage of nutrient reserves and amount of gametes. These changes in biomass can alter metal concentrations by diluting or concentrating the pool of metals contained in the tissues. Such changes can confound assessment of metal bioaccumulation because changes (or lack of changes) in metal concentrations may not accurately reflect the dynamics of metal loss or gain in individuals (Fig. 2). In this example, tissue cadmium concentrations in cadmium-exposed *C. virginica* transferred and held unfed in clean seawater were not greatly altered, suggesting minimal release of cadmium after cessation of exposure. However, cadmium content calculated on a mass-per-individual basis rather than as concentration indicated a net loss of 56% of the accumulated cadmium. This apparent discrepancy was explained by a decrease in tissue mass that accompanied the loss of cadmium after oysters were starved in cadmium-free seawater. In a similar vein, natural populations of the Pacific oyster, *Crassostrea gigas*, exhibited seasonal variability in the concentrations of cadmium, cobalt, copper, iron, lead, nickel, and zinc, which was mainly attributable to fluctuations in the mass of tissues rather than the mass of

the metals in the tissues (Boyden and Phillips 1981). The exception was manganese which was less affected by fluctuations in tissue mass.

Differences in capabilities for accumulating metals can also arise in different populations of *C. virginica*. For example, two separate oyster populations in Chesapeake Bay can be differentiated on the basis of their growth (i.e., one stunted, one not) and large differences in their abilities to accumulate copper and zinc (Phelps and Hetzel 1987). The actual basis for the latter difference has yet to be determined, although genetic variability in metal-metabolizing capabilities was suggested as a possibility. Oysters collected from Chesapeake Bay (Maryland waters) and Beaufort, North Carolina, have also been shown to differ in their abilities to bioaccumulate copper under similar experimental conditions (Engel 1988), the Maryland oysters exhibiting a greater capacity for bioaccumulation.

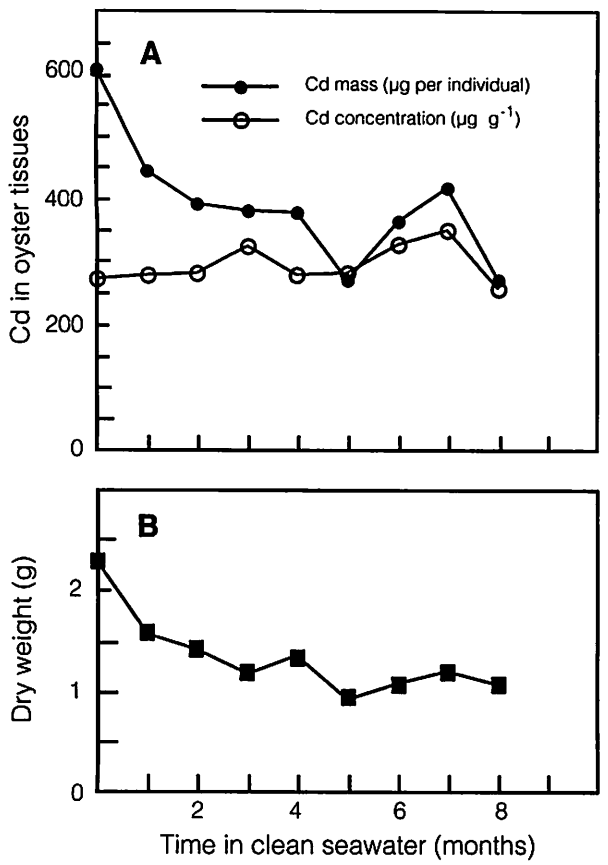

Figure 2. Cadmium content expressed as either (A) mass individual^{-1} or concentration and (B) the dry weight of cadmium-exposed *Crassostrea virginica* after transfer to clean seawater. Both mass of cadmium and oyster dry weight decline over time. As a result, the cadmium concentration does not exhibit any discernable change after the transfer. Oysters were exposed to 14.6 to 15.4 µg Cd L^{-1} for 40 weeks before transfer to clean seawater. Redrawn with data from Zaroogian (1979).

This variability in metal content can serve to confound attempts to use oysters to monitor environmental metal contamination. If the monitoring period is of relatively short duration, contamination of oysters attributable to elevated concentrations of metals in the environment may be obscured by long-term variability. This is illustrated by data on zinc from two studies that examined the response of *C. virginica* transplanted to a Chesapeake Bay tributary (Fig. 3). In one (Frazier 1975), cultivated oysters were transplanted to an uncontaminated reference site in September 1971 and monitored through May 1973. During that period, zinc concentrations in oyster tissues exhibited seasonal variability, ranging from about 2,000 to 5,000 µg g dry weight^{-1} (Fig. 3). Relatively low and stable periods between October and June were interrupted by increases that peaked at about August. In a second study (Frazier 1976), the timing of which coincided with the second year of the one described above (Frazier 1975), oysters acclimatized to conditions at the same reference site were transplanted to a metal-contaminated site in September 1972 (Fig. 3). Oysters were also maintained at the reference site as a control. The effect of transplant to the contaminated site was an increase in the zinc concentration to about 5,000 µg g^{-1}, in comparison with a mean of about 2,000 µg g^{-1} for oysters at the reference site. However, the variability and high values for oysters at the reference site in the first study obscures the biological significance of the observed elevation in the second (Frazier 1975).

Considerable year-to-year variability in seasonal changes was also observed with cadmium in oysters maintained at the same reference site over a four-year period (Frazier 1979). Cadmium concentrations during the four years fluctuated from 2.5 to 17 µg g^{-1}, a seven-fold range. Peak cadmium concentrations dur-

ing two of those years exceeded values attained by oysters transferred to the contaminated site in the transplant study above (Frazier 1976). Although contamination by metals can probably account for the difference in the metal content of the oysters at the contaminated site and its reference group, the biological significance of the elevated bioaccumulation at the contaminated site is ambiguous in light of fluctuations over the longer term in oysters at the reference site (Frazier 1975). These examples emphasize the importance of considering the implications of long-term variability in tissue metal concentrations when interpreting the results of short-term studies. The significance of observed changes attributable to anthropogenic contamination exists in the context of the overall natural variability.

Several approaches have been used in monitoring protocols in dealing with the problem of variability in metal content. Among these are the use of individuals of similar size; coordination of sampling according to season; use of transplant protocols rather than analysis of indigenous individuals; and use of inbred, cultured strains in transplant protocols in order to reduce genetic variability between samples. Preliminary estimations of sample sizes required to show statistically-significant differences for desired levels of probability have facilitated later analysis of data (Boyden and Phillips 1981; Wright et. al. 1985). However, the apparent changes or differences in concentrations of metals that may have been the result of differences in oyster reproductive condition rather than metal content have yet to be adequately addressed. Analysis of specific tissues such as gills, which are considered to be less influenced by seasonal changes in biomass (Lytton et al. 1985), may aid in reducing variability in metal concentrations. Normalization of the metal content to a conservative marker of oyster size that is directly related to cell number, such as DNA content, rather than to weight should also be considered as an alternative method of expressing metal concentration.

Huggett et al. (1973) proposed that ratios among accumulated metals would be relatively constant under "natural" conditions, and that departures from constancy could be interpreted as the influence of "non-natural" or anthropogenic metal inputs and, therefore, could be useful in environmental monitoring. The requisite assumptions were that (1) natural weathering of rocks is the contributor of metals in non-industrialized areas, (2) the ratios of these metals to each other are relatively constant within a drainage basin, and (3) oysters accumulate a constant percentage of each element available to them. Through such analysis, Huggett et. al. (1973) designated several lo-

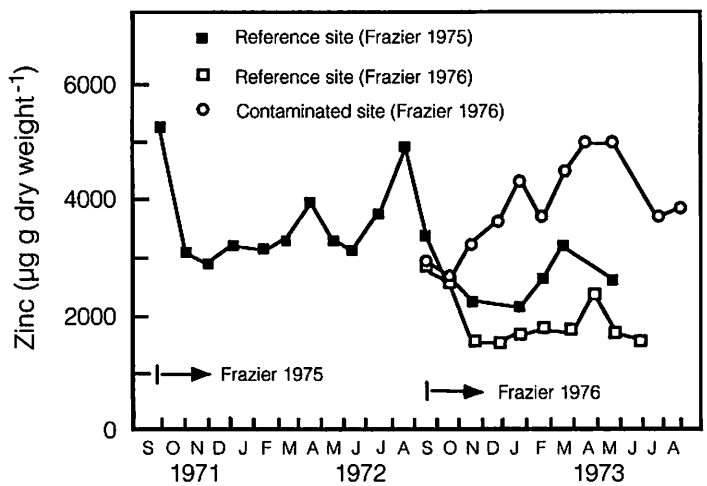

Figure 3. Zinc concentrations in *Crassostrea virginica* transplanted to two sites in the Rhode River, a tributary of the Chesapeake Bay: a reference site considered to be minimally affected by human activities and a contaminated site in the vicinity of a boat manufacturing company and a marina. Redrawn from data derived from the results of two studies (Frazier 1975, 1976).

cations in Chesapeake Bay as sites of copper- and zinc-contamination. Abbe and Sanders (1986) applied this method in demonstrating that elevations in copper in oysters transplanted to a site near a power plant were due to accumulation of copper that was discharged from the plant, an observation reinforced by analysis of copper in the surrounding water (Wright and Zamuda 1991). Although shown to be useful under specific circumstances, this approach has not been widely adopted. Its general applicability is weakened by the third assumption, which requires that, under normal conditions, organisms will reflect the same relative geochemical composition as seen in the environment.

A consensus on the best approaches for using the metal content of oysters to monitor environmental metal-contamination has yet to emerge, although the use of oysters as sentinel organisms is a common activity. There is a need for identifying sources of variability prior to instituting monitoring programs with oysters (Engel 1988). An understanding of the underlying basis for variability in metal concentrations in oyster tissues and incorporation of derived concepts into the interpretation of data will contribute to improving our ability to separate "signal from noise" and to interpret the signal in the context of long-term variations.

METAL TURNOVER IN EASTERN OYSTERS

Information on turnover of metals is central to understanding seasonal cycles in metal content and the toxicokinetics of metals. Additionally, the importance of oysters as a human food resource requires that concentrations of metals in commercial populations are within acceptable levels for consumption. An understanding of the dynamics and mechanisms that underlie metal release is also necessary for evaluating the feasibility of relaying contaminated oysters for depuration of metals, as is currently done for cleansing of microorganisms.

The changes in oyster metal content, whether increases or decreases, are best viewed as the result of continuous processes that are defined physiologically as the outcome of the instantaneous processes of influx (uptake rate) and efflux (release rate). The direction of the netflux (influx-efflux) determines whether substances are bioaccumulated or lost as a function of the relative magnitudes of the unidirectional fluxes. Most studies have described changes in metal concentration or mass, which reflect changes in netflux. Influx and efflux have rarely been measured directly in studies on trace metals.

Relatively large changes in the magnitude and direction of netflux of metals in oysters in the natural environment can be inferred from the reported patterns of metal bioaccumulation as a function of time. For example, over the course of a single year, the amount of cadmium in *C. virginica* (mass Cd individual^{-1}) in a relatively clean environment increased about 7-fold, then dropped to a level 0.4 times that of the maximum (Frazier 1975). Copper, iron, manganese, and zinc in *C. virginica* (Frazier 1975, 1979) and cadmium, cobalt, copper, iron, lead, manganese, nickel, and zinc in *C. gigas* (Boyden and Phillips 1981) also exhibited seasonal fluctuations, although of lesser relative magnitude.

Greig and Wenzloff (1978) reported little or no decline in the concentrations of silver, cadmium, copper, and zinc in *C. virginica* transferred from a metal-contaminated environment in Connecticut to a reportedly clean environment in North Carolina. Zaroogian (1979) also observed no decline in cadmium in laboratory-exposed oysters transferred to uncontaminated seawater when the cadmium content was expressed as concentration, although a decline had actually occurred in the cadmium body burden (expressed as mass Cd individual^{-1}) (Fig. 2). The biological half-life for cadmium estimated from the latter study is about 110 d, based on loss of cadmium mass. Van Dolah et al. (1987) reported that the rate of cadmium loss is at least biphasic, with an overall half-life of about 200 d, which is in the same order of magnitude. The rate of cadmium loss was also shown to be accelerated by increases in salinity, dissolved, organic compounds in the seawater, and temperature (Van Dolah et al. 1987). Cadmium in gills of *C. virginica*, with a half-life of about 35 d (Roesijadi and Klerks 1989), appears to be turning over more rapidly than in the whole animal, suggesting that the gill

cadmium is translocating to compartments within the organism that turn over more slowly

The half-life of inorganic mercury in mercury-exposed oysters transferred to uncontaminated water is 9 to 35 d, depending on temperature (Cunningham and Tripp 1975a). At an exposure of 10 μg Hg L^{-1}, the decline occurs over the first 18 d after transfer, with little change thereafter (Cunningham and Tripp 1975a). Accumulation of inorganic mercury by *C. virginica* was reported as biphasic, with an initially-exponential accumulation phase lasting about 40 h followed by a linear accumulation phase (Mason et al. 1976). In this study, mercury was released during the exponential phase with a half-life of 0.2 to 1.2 d at exposure concentrations ranging from 10 to 100 μg L^{-1}. During the linear phase of accumulation that occurred afterward, mercury uptake was described as irreversible because no release was detected during this period. Thus, the general patterns of mercury turnover described in these two studies are similar, although the actual values are not in agreement. With regard to the inorganic and organic forms of the mercury, different patterns of change occur in specific tissues when mercury-exposed *C. virginica* is transferred to clean seawater (Cunningham and Tripp, 1975b). This occurrence is indicative of differential loss and redistribution of the two forms. Loss of nickel after an experimental exposure was reported to occur with a half-life of about 200 d (Zaroogian and Johnson 1984).

Although of interest for their mechanistic considerations, discrepancies in experimental procedures, particularly in the bioaccumulation phases that precede the measurement of loss, can complicate comparisons of studies such as those described above. Bioaccumulation of cadmium over sustained periods of time, which probably occurred in the field study of Greig and Wenzloff (1978), can result in the formation of stable cadmium-ligand pools that require extremely long times for loss to be observed. Conversely, short-term laboratory exposure may result in the presence of metals in relatively labile pools that are more rapidly lost when the individual is transferred to a clean environment. As seen above with mercury (Cunningham and Tripp 1975a; Mason et al. 1976), differences in experimental procedures can also result in quantitative discrepancies, although general patterns of change are preserved. The methods for expression of metal content in terms of mass or concentration can also influence conclusions. Given such qualifications, the general summary is that accumulated metals decline at relatively slow rates in *C. virginica.* Slow turnover, coupled with continued uptake, contributes to the high levels of metals that have been reported for oysters.

CELLULAR MECHANISMS OF METAL SEQUESTRATION

Mechanisms involved with the cellular sequestration of metals serve as the basis for the ability of organisms to accumulate metals and can also confer protection against metal toxicity. These mechanisms include deposition in granules associated with granular hemocytesand binding by metal-binding proteins such as metallothionein, which are two of the most extensively studied processes associated with metal sequestration in molluscs.

Granular Deposits

Early investigators reported the existence of "green" oysters whose color was due to high concentrations of accumulated copper (see citations in Galtsoff 1964 and Bryan et al. 1987). Consumption of green individuals of the European flat oyster, *Ostrea edulis,* is believed to be related to an outbreak of human poisoning in France in the mid-1800s (described in Bryan et al. 1987). These oysters were collected in industrialized areas where environmental concentrations of copper were extremely high due to past mining activities. A similar example of green specimens of copper-rich *C. virginica* has been observed in a copper-contaminated environment, i.e., in the vicinity of effluent discharges from a power plant using copper-nickel heat exchangers (Roosenburg 1969).

Although copper-laden oysters may have been harmful to human consumers, the oysters themselves are apparently tolerant of high concentrations in tissues with little ill effect (Shuster and Pringle 1969). Concentrations of copper exceeding 3,000 μg g dry $weight^{-1}$ have been reported in *O. edulis* without caus-

ing apparent toxicity to the oyster. Cellular mechanisms for metal detoxification would be expected to be responsible for this tolerance.

It was recognized several decades ago that oysters possess specialized cells, the granular hemocytes, that serve to concentrate copper and zinc (see citations in Bryan et al. 1987). They occur normally and, in copper-exposed individuals, the sequestration of large amounts of copper by these cells is responsible for the characteristic coloration of the "green" oysters. Metal concentrations as high as 13,000 μg Cu g^{-1} and 25,000 μg Zn g dry $weight^{-1}$ have been observed in granular hemocytes of "green" *O. edulis* from an estuary in southwest England that was the site of past mining activities (George et al. 1978). The metals in these cells are typically sequestered in membrane-bound granular structures resembling lysosomes. Three types of metal-sequestering granular hemocytes are reported to occur in *O. edulis* (Pirie et al. 1984) (Fig. 4, 5): (1) copper-sequestering, (2) zinc-sequestering, and (3) mixed copper- and zinc-sequestering. Only the last type has been observed in other oyster species to date (Pirie et al. 1984).

Granular hemocytes can sequester over 90% of the total body burden of copper and zinc in *C. gigas* (Thomson et al. 1985). They naturally infiltrate the interstitial space between the cells of solid tissues, and tissues that are rich in these cells exhibit greater capability for concentrating metals than those that are not (Ruddell and Rains 1975; Lytton et al. 1985). Estimates for mantle tissues of *C. virginica* showed that the granular hemocytes represent about 2 to 8% of total cell numbers (calculated from Ruddell and Rains 1975). In *C. gigas*, the copper and zinc concentrations in different tissues correlate well with the abundance of granular hemocytes, with concentrations in gills > digestive glands > gonads (Lytton et al. 1985). In this species, a high density of granular hemocytes is observed in tissues whose mass is not substantially affected by changes in reproductive and nutritional state (i.e., gills, mantle, and digestive gland). A low density is observed in tissues that are affected to a high degree by such changes, i.e., gonads and glycogen stores (Lytton et al.1985). Results for *O. edulis* (Pirie et al. 1984) are consistent with these findings. The individual variability observed in whole-oyster metal concentration is correlated with fluctuations in the mass of tissues that harbor low populations of these cells. These fluctuations either dilute or amplify the contribution of granular hemocytes to the whole-oyster metal concentration.

Iron and calcium have also been detected in granular hemocytes, although at much lower concentrations than either copper or zinc (Thomson et al. 1985). Cadmium was not detected in the granular hemocytes of gills of cadmium-exposed *O. edulis*, although a considerable amount was accumulated in the whole gill (George et al. 1983). In contrast, in *C. gigas*, cadmium was shown to accumulate in granular hemocytes in the gills and other organs (Martoja and Martin 1985). This difference in cadmium sequestration between the two species has yet to be explained. It may represent interspecific differences in the intracellular distribution of cadmium, a factor that has been noted for these two species (Frazier and George, 1983). However, because the studies of George et al. (1983) and Martoja and Martin (1985) also differed in sample preparation and analytical procedures, it is possible that methodological differences are responsible for the discrepancy.

The general phenomenon of metal sequestration in membrane-bound vesicles has been ascribed a role in metal detoxification (Coombs and George 1979). For copper and zinc, sequestration of metals in the granular hemocytes is a normally-occurring process that results in the accumulation of considerable amounts of these metals by the whole oyster. Thus, exposure to elevated copper or zinc results in an enhanced metal content in structures already involved in the routine metabolism of metals.

Granular hemocytes are best understood for their central role in cellular defense mechanisms. Thus, the natural occurrence of high concentrations of copper and zinc in these cells led to early speculation that these metals may play a role in the inflammatory response (Ruddell 1971a), possibly as anti-metabolites against pathogens (Ruddell and Rains 1975). These cells migrate to areas of tissue damage and extrude a greenish, copper-rich substance with apparent bactericidal action at sites of wound repair in *C. gigas* (Ruddell 1971a). Zinc is also released from the granules of these cells as the latter swell in response to

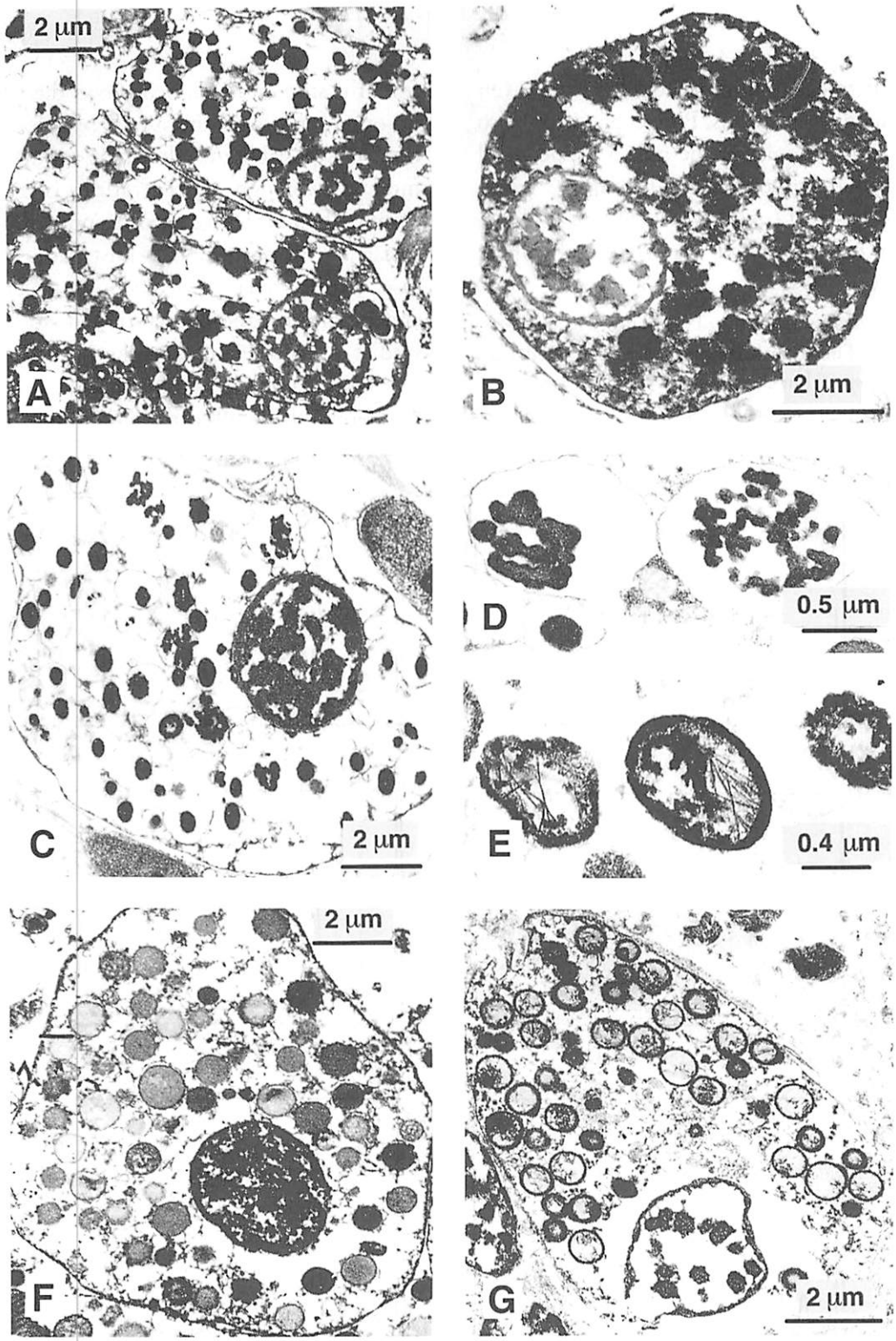

Figure 4. Electron micrographs of hemocytes in gills of three oyster species. *Ostrea edulis:* (A) zinc-specific cell; (B) copper-specific cell; (C) copper/zinc mixed cell; and (D) structural detail of granule contents in mixed cell. *Crassostrea gigas*: (E) structural detail of granule contents in mixed cell. *O. puelchana:* (F) copper/zinc mixed cell. *C. gigas:* (G) copper/zinc mixed cell. From Pirie et al. (1984).

physical or chemical trauma (Ruddell 1971b). On the basis of these observations, Pirie et al. (1984) also proposed a defensive role for the mixed copper- and zinc-rich granular hemocytes in *O. edulis, C. gigas,* and the mud oyster, *O. puelchana*[1], although direct evidence was not presented. Unfortunately, apart from the studies mentioned above, there appear to be no other reports on this aspect of amoebocyte function. It is possible that the ability of oysters to accumulate such high concentrations of metals in the granular hemocytes is related to normal functions associated with protection against pathogens and disease. Further research is obviously needed to clarify and expand on the observations made in the early studies of Ruddell and coworkers.

The localization of copper and zinc in the membrane-bound granules of the granular hemocytes is

[1] Note that the taxonomic status of austral ostreid species is controversial. These authors orginally identified this species as *O. angasi,* but the editors have renamed it here after the convention of Carriker and Gaffney (Chapter 1).

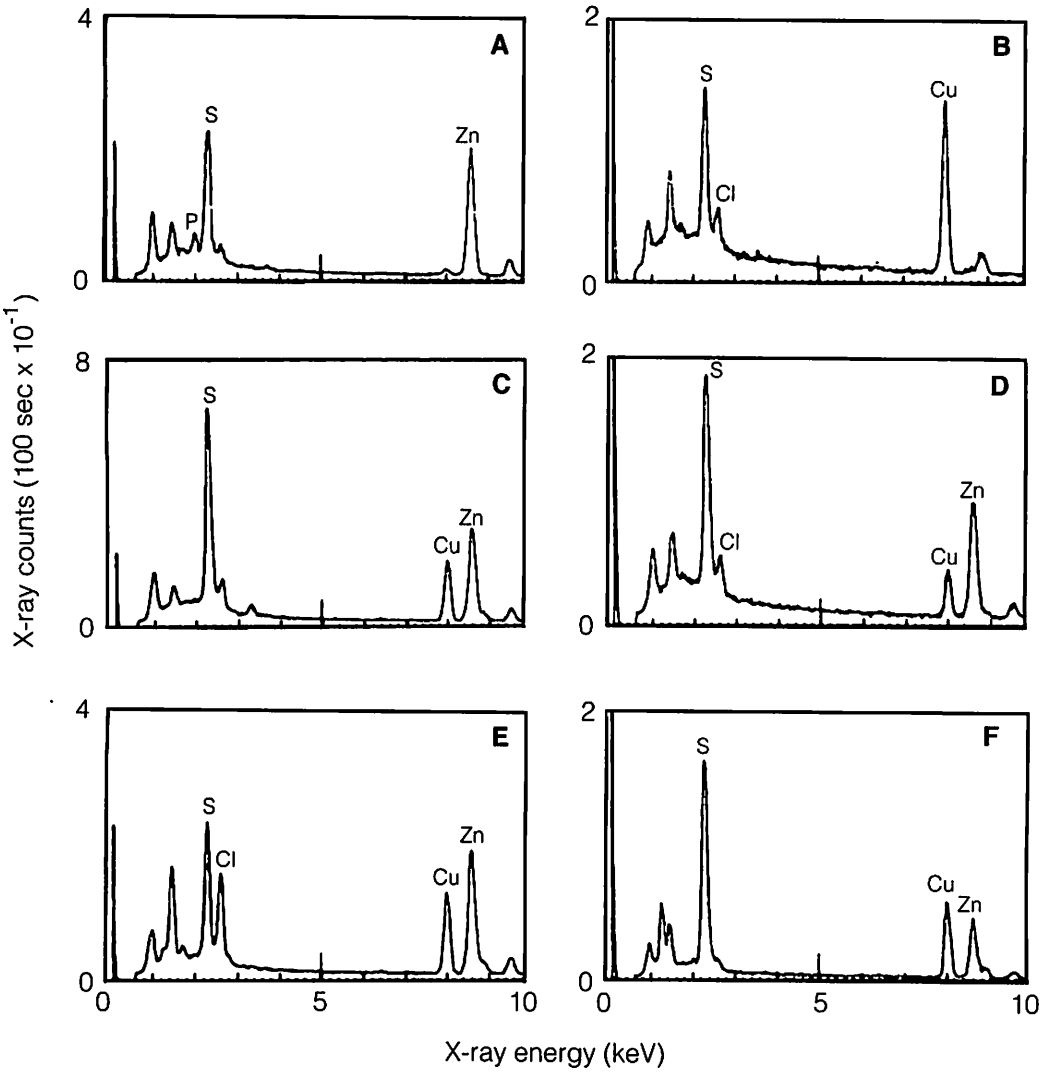

Figure 5. X-ray spectra of hemocytes in gills of three oyster species: (A) *Ostrea edulis,* zinc-specific cell; (B) *O. edulis,* copper-specific cell; (C) *O. edulis,* copper/zinc mixed cell; (D) *O. edulis,* single granule mixed cell; (E) *O. puelchana,* copper/zinc mixed cell; (F) *Crassostrea gigas,* copper/zinc mixed cell. From Pirie et al. (1984).

consistent with the results of early biochemical studies on the distribution of zinc in oyster tissues (both *C. virginica* and *O. edulis*). Relatively large amounts of zinc (about 40% of the total cellular zinc) are associated with a heavy pelleted fraction that sediments at 7,500 to 9,000 g min^{-1} (Wolfe 1970; Coombs 1974) and would include the dense granules of the hemocytes. Most of the balance of zinc was associated with low molecular mass compounds, reportedly taurine, lysine, ATP, and homarine (Coombs 1974). The metabolic significance of this zinc, which is considered to greatly exceed the requirements of zinc-dependent enzymes, has yet to be determined.

Metallothioneins and Other Metal-binding Proteins

Metallothioneins represent a class of low molecular mass, metal-binding polypeptides that are widely distributed in organisms as diverse as prokaryotes, protists, fungi, plants, and animals. These molecules are regarded as central in the metabolism and detoxification of certain metals, notably copper, zinc, cadmium, and mercury (see Hamer 1986 and Kagi and Kojima 1987 for recent reviews on metallothionein biochemistry). The biochemical characteristics for Class I metallothionein (see Fowler et al. 1987 for nomenclature), which are based on proteins isolated from mammals, include (1) molecular mass of 6,000 to 7,000 daltons, (2) high cysteine content (ca. 30% of total residues) and absence of aromatic amino acids, (3) the ability to be induced by metal-exposure and to bind large amounts of the metals, and (4) a unique primary structure based on the locations of the numerous cysteines, which are responsible for the high metal-affinity. Of special note from the ecological perspective is that induction of metallothionein by metal exposure is associated with increased tolerance to metal toxicity (e.g., Roesijadi et al. 1987) and adaptation to metal-contaminated habitats (Klerks and Weis 1986). Induction of metallothionein also has implications for the trophic transfer of metals. The increased cellular capacity for sequestering metals conferred by metallothionein induction and the associated tolerance to metal toxicity may enable exposed individuals to survive and pass on higher amounts of metals to consumers. Many invertebrate species have been shown to possess proteins that resemble metallothionein in some properties: low molecular mass, high amounts of bound metal, and induction by metal exposure. However, few of these proteins have been purified and characterized to the extent that has permitted their classification as me-tallothionein. Thus, progress in understanding structure-function relationships in invertebrate species has occurred slowly.

Crassostrea virginica was the first invertebrate species for which a cadmium-binding protein with some properties similar to metallothionein was reported (Casterline and Yip 1975). Subsequent work on this species has extended the initial observations to include physiological observations and more detailed characterization of metallothionein (e.g., Engel and Fowler 1979; Frazier 1979; Ridlington and Fowler 1979; Engel and Brouwer 1982; Fowler et al. 1986; Roesijadi and Klerks 1989; Roesijadi et al. 1989, 1991) and metallothionein cDNA (Unger et al. 1991).

Two metal-binding proteins that can be classified as Class I metallothionein have been purified and characterized for *C. virginica* from Chesapeake Bay (Table 2). They exist mainly as cadmium-, zinc-binding proteins and are identical in amino acid sequence. They differ from each other only in that one is N-acetylated and the other is not (Roesijadi et al. 1991; Unger et al. 1991). Molecular masses are 7,187 and 7,229 daltons for the non-acetylated and acetylated forms, respectively (Unger et al. 1991). A comparison of the amino acid sequence with metallothioneins from other species, apart from other molluscs (Dallinger et al. 1993, Mackay et al. 1993), indicated that those from the oyster are most similar to metallothioneins from vertebrates rather than to those for other invertebrates reported to date (e.g., sea urchin, brachyuran crabs, nematode [Roesijadi et al. 1989; Unger et al. 1991]).

Crassostrea virginica from the North Carolina coast (e.g., Engel and Brouwer 1982; Fowler et al. 1986) has been reported to possess metal-binding proteins that occur as two molecular mass variants, one similar to that above for oysters from Chesapeake Bay in molecular mass (i.e., about 10,000 daltons from

Table 2. Deduced amino sequence for oyster metallothionein derived from sequencing clones of PCR-amplified cDNA and primer extension reaction off mRNA (Unger et al. 1991). In the mature protein, the N-terminal methionine residue is not present and serine is the N-terminal amino acid residue (Roesijadi et al. 1989, 1991). The mature protein occurs in N-acetylated and non-acetylated forms (Roesijadi et al. 1991). The positions of the cysteines, which are considered to be characteristic of metallothionein, are shown in bold type.

								10									20		
Met	Ser	Asp	Pro	**Cys**	Asn	**Cys**	Ile	Glu	Thr	Gly	Thr	**Cys**	Ala	**Cys**	Ser	Asp	Ser	**Cys**	Pro
Ala	Thr	Gly	**Cys**	Lys															
								30					40						50
Cys	Gly	Pro	Gly	**Cys**	Lys	**Cys**	Gly	Asp	Asp	**Cys**	Lys	**Cys**	Ala	Gly	**Cys**	Lys	Val	Lys	**Cys**
Ser	**Cys**	Thr	Ser	Glu															
										60							70		
Gly	Gly	**Cys**	Lys	**Cys**	Gly	Glu	Lys	**Cys**	Thr	Gly	Pro	Ala	Thr	**Cys**	Lys	**Cys**	Gly	Ser	Gly
Cys	Ser	**Cys**	Lys	Lys															

Sephadex G-75 gel chromatography) and another of twice the mass (i.e., about 20,000 daltons), possibly in dimer conformation (Fig. 6). Although the identity of the 10,000 dalton peak most likely is the metallothionein already characterized for this oyster (Roesijadi et al. 1989, 1991; Unger et al. 1991), the 20,000 protein(s) has yet to be analyzed in detail. Cadmium-binding proteins of similar masses also occur in the blue mussel, *Mytilus edulis*. In this case, amino acid sequencing has verified their identity as metallothioneins (Mackay et al. 1993), the 20,000 MW variants existing as dimers with subunits of roughly 10,000 daltons. The basis for the discrepancy in the two groups of oysters has not been determined, although procedural variations among investigators seem an unlikely explanation because similar procedures have been used in the various studies (in some cases, the same investigators have observed the two variants). According to Engel (1988), Cape Hatteras is the demarcation that divides *C. virginica* into northern populations that possess the 10,000 dalton forms and southern populations that possess both 10,000 and 20,000 dalton forms. An intriguing question on the biochemical diversity of metallothioneins in different populations of *C. virginica*, thus, remains to be further investigated.

The gill of cadmium-exposed oysters is an important organ for the uptake and sequestration of cadmium (George et al. 1983; Roesijadi and Klerks 1989). In *C. virginica*, metallothioneins have been further studied in this organ and have been found to be the main cadmium-binding molecules in the cytosol of gill cells (Roesijadi and Klerks 1989). Non-thionein-bound cadmium is mainly associated with particulate structures, most likely with granular hemocytes. Normally, about half of the gill cadmium is bound in the particulate fraction of gill tissues (Roesijadi and Klerks 1989). The balance is in the cytosol, with about half the cytosolic content bound to metallothioneins. Basal metallothionein synthesis is low and favors synthesis of the N-acetylated form of the protein over its non-acetylated counterpart (Roesijadi et al. 1991). The basal concentrations of the two proteins are also very low, with the N-acetylated protein being the dominant form. These observations contrast with earlier work that reported no such basal levels of metallothionein-bound cadmium in *C. virginica* (Siewicki et al. 1983b). Procedural differences may account for the lack of detection in the latter study.

Experimental induction of gill metallothioneins in *C. virginica* occurs within 1 to 4 d after initiation of exposure to 50 μg $Cd\,L^{-1}$ at 16 to 20°C (Roesijadi and Klerks 1989; Roesijadi et al. 1991). Induction results in increased rates of cadmium-binding to these proteins and reduced rates of cadmium-binding to other soluble proteins in the cytosolic fraction and to

the particulate structures in the pelleted fraction (Fig. 7). As a result, the latter structures accumulate cadmium at a reduced rate (Roesijadi and Klerks 1989). Formation of the acetylated form of metallothionein predominates immediately after induction, although after 7 d both the acetylated and non-acetylated forms are synthesized at similar rates (Roesijadi et al. 1991). Half-lives for turnover of the two forms of metallothionein are about 4 d in the absence of elevated cadmium concentrations in the external medium and 10 to 20 d in its presence (Roesijadi et al. 1991).

In natural environments contaminated by metals, the concentrations of metals, although elevated, are still very low in comparison with exposure concentrations normally used to elicit responses in the laboratory. Analysis of metallothioneins in gills of *C. virginica* from the lower Patuxent River (Maryland), a population that is elevated in cadmium (MDE 1990), indicated that the acetylated form is predominant (Roesijadi, unpub. data). This observation is consistent with our findings that basal and near-basal synthesis favors the acetylated form (Roesijadi et al. 1991).

The work conducted thus far with *C. virginica* suggests that this species may be unique among oysters in displaying a significant role for metallothionein in intracellular cadmium-binding. Studies on *C. gigas* and *O. edulis* indicate a lesser role for metallothionein in such binding, with greater involvement by small molecules whose size is < 3,000 daltons (Howard and Nickless 1977; Frazier and George 1983). Additional research will be required to resolve this question of interspecific variability in the use of metallothionein in metal sequestration.

The coordinated function of the granular hemocytes and metallothioneins has yet to be studied in detail. Cadmium-induced metallothionein is reported to occur in hemocytes of the blue mussel (Steinert

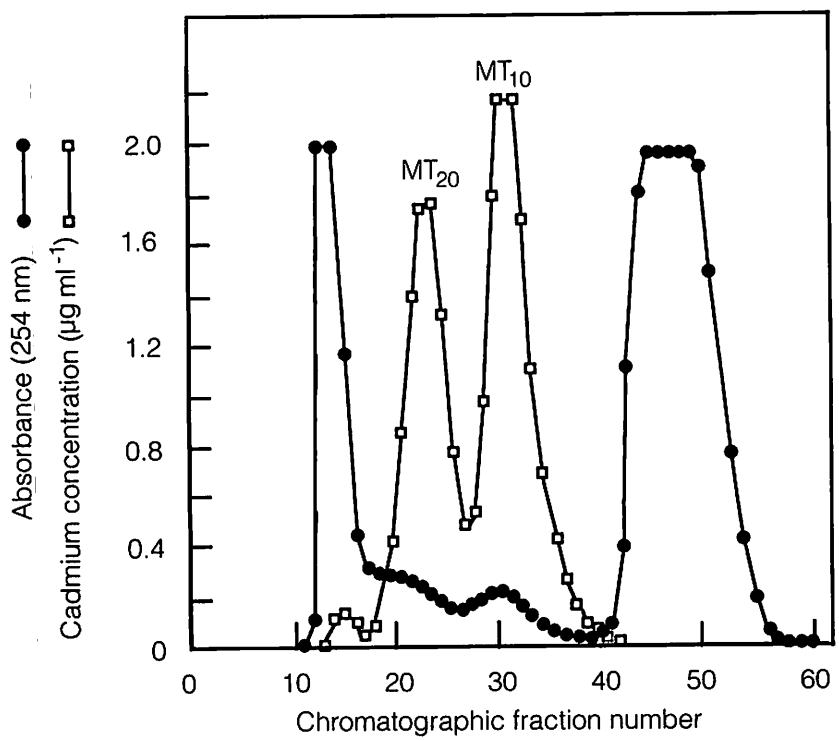

Figure 6. Sephadex G-75 gel chromatogram of cytosol of *Crassostrea virginica* exposed to cadmium in the laboratory. Two variants, designated MT20 and MT10, are evident and correspond to molecular masses of about 20,000 and 10,000 daltons, respectively. Unpublished data from D.W. Engel, NMFS Laboratory, Beaufort, N.C., pers. comm.

and Pickwell 1988). In *C. gigas*, the presence of cadmium-binding proteins in hemocytes of cadmium-exposed oysters was inferred from the co-occurrence of cadmium and sulfhydryl groups in cells examined in histological sections (Martoja and Martin 1985).

Apart from metallothionein, few other metal-binding proteins have been identified in oysters. Webb et al. (1985) described the iron-binding protein ferritin in the tropical rock oyster, *Saccostrea cucullata*. In other bivalves, high molecular mass proteins (>70,000 daltons) are involved in binding metals in cell-free hemolymph (Carpene and George 1981; Robinson and Ryan 1988). These circulating proteins have been proposed as a possible transport form for metals in the hemolymph and are analogous to albumins in the blood of higher animals.

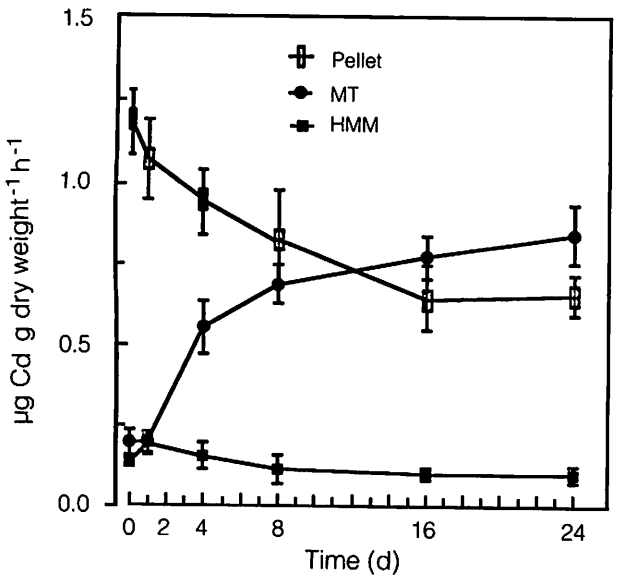

Figure 7. Cadmium-binding rates for metallothionein (MT), the pelleted fraction, and high molecular mass cytosolic proteins (HMM, <70,000 daltons) of gills of *Crassostrea virginica* during continuous exposure to 50 µg Cd L^{-1}. Induction of metallothionein and increased cadmium-binding rates for metallothionein after 1 d are associated with reduced binding rates of cadmium to the other structures. Mean ±1 standard error. Redrawn with data from Roesijadi et al. (1989) using a factor of 5 × for conversion of the cadmium-binding rate in the cytosolic fractions, which were originally normalized to wet weight.

TOXIC EFFECTS OF METALS ON OYSTERS

The toxicity of metals is based, to a large degree, on coordination chemistry and its influence on the binding of metals to intracellular ligands. Metals with greater covalent characteristics will have higher affinity for O, N, and S ligands that are prevalent in biological macromolecules (Niebohr and Richardson 1980). If not intercepted by mechanisms for sequestration and detoxification, metal ions are free to interact and bind to sensitive cellular sites. Inappropriate binding of metals to structures such as enzymes and membranes, followed by subsequent disruption of vital processes, has been considered to be one of the major mechanisms for producing toxicity (Mahler 1961).

Some metals are also capable of stimulating production of free radicals that can undergo peroxidation reactions and result in the destabilization of membranes (Viarengo 1989). For example, copper can destabilize the lysosomal membrane of the mussel, *M. edulis*, with a resultant decrease in the latency of lysosomal enzyme activities (Harrison and Berger 1982). Because these types of effects by metals are considered to be widespread in animal systems, similar responses can probably be expected in oysters under appropriate conditions.

Adult oysters are relatively tolerant of metals that have been accumulated by tissues as noted above in the discussions of bioaccumulation and the cellular disposition of metals. Moreover, our understanding of the toxicity of metals to oysters is mainly derived from the results of laboratory experiments, which typically have used very high exposure concentrations to elicit responses in comparison with concentrations that exist in the natural environment. Of the metals cadmium, chromium, copper, and zinc, only cadmium at 100 and 200 µg L^{-1} caused significant mortalities in *C. virginica* during 20 weeks of exposure, although considerable accumulation of all the metals had occurred (Shuster and Pringle 1969). General emaciation of tissues and inhibition of shell growth were also observed in the cadmium-exposed oysters. Consistent with emaciation, condition index was reduced in oysters exposed to 200 µg Cd L^{-1} for 2 weeks (Roesijadi and Klerks 1989). Cadmium at 100 µg L^{-1} did not af-

fect gill respiration rates and morphology, but did elevate respiration rates at 600 μg L^{-1} (Engel and Fowler 1979). Copper at 50 μg L^{-1}, the lowest exposure concentration of this study, significantly increased respiration and disrupted gill ultrastructure (Engel and Fowler 1979). At 100 μg Cu L^{-1}, the water content of gill tissues was also significantly elevated over those of unexposed individuals.

Elevated concentrations (normalized to dry weight) of cadmium, copper, and zinc in tissues of oysters transplanted to a metal-contaminated site was accompanied by a reduction in shell thickness (Frazier 1976). The manganese concentration of the shell was also greatly reduced in this study, although it is not known whether this is related to processes that result in impairment of shell growth. The reduction in shell thickness was attributed to the effect of cadmium, although direct evidence was lacking. Decreased shell growth has been observed in oysters exposed to cadmium, but not copper, zinc, or chromium (Shuster and Pringle 1969). Cadmium is also known to inhibit enzymes involved in shell formation (e.g., carbonic anhydrase) and can also compete with calcium in calcium transport pathways (Hinkle et al. 1987).

There is also some indication that metal exposure may have adverse effects on the cellular defense mechanisms of oysters (see Cheng, Chapter 8). George et al. (1983) reported that cadmium exposure at 100 μg Cd L^{-1} results in a reduction in the numbers of copper-zinc-bearing granular hemocytes of *O. edulis*. These cells are similar to those proposed to have defensive properties in *C. gigas* (Ruddell 1971a, b) and are likely related to cells that have been characterized as having the most bactericidal and phagocytic activity in *C. virginica* (Cheng et al. 1975; Cheng and Garrabrant 1977). Exposure of intact *C. virginica* to high cadmium concentrations (1,000 μg L^{-1}) results in cadmium-inhibition of phagocytosis by individual granular hemocytes Cheng (1988a, b). However, exposure of isolated hemocytes *in vitro* to high concentrations of various metals, including cadmium, at 1,000 and 5,000 μg L^{-1} either stimulated or had no effects on phagocytosis (Cheng and Sullivan 1984). Thus, exposure of isolated cells *in vitro* did not elicit the same response as that of cells sampled from exposed individuals. Taken together with the results of George et al. (1983), it appears that cadmium exposure of oysters can adversely affect the function of granular hemocytes. With copper, exposure at 1,000 μg L^{-1} decreased the relative numbers of phagocytosing cells, but increased the number of bacterial cells phagocytosed per granulocyte, which was the opposite of that seen with cadmium. It should be noted for these studies that exposure concentrations of 1,000 μg Cd or Cu L^{-1} are extremely high. In the case of cadmium, it is at least five times that which results in overt toxic effects in adult *C. virginica* (Shuster and Pringle 1969) and over four orders of magnitude above natural background concentrations in marine waters (Bruland 1983). Additionally, the normal function of granular hemocytes in sequestering large amounts of metals, particularly copper and zinc, needs to be considered when assessing the toxic effects of metal exposure to these cells.

The early developmental stages of bivalve molluscs are most sensitive to metal toxicity. These stages undergo rapid cellular differentiation and, thus, are highly susceptible to perturbations associated with inappropriate metal-binding. Reports that metals such as mercury, cadmium, and copper are capable of inhibiting RNA-polymerase (Novello and Stirpe 1969; Hidalgo et al. 1976), an enzyme that is central to gene expression, are consistent with the enhanced sensitivity of early life stages that are undergoing rapid cellular differentiation. Metal toxicity in embryos and larvae of *C. virginica* is ranked as mercury > silver > copper > nickel (Calabrese et al. 1973, 1977). Embryos are slightly more sensitive than larvae. When copper toxicity in adult *C. gigas* (Okazaki 1976) is compared with that of larvae, the larvae are much more sensitive (12 d LC_{50} of larvae = 33 μg Cu L^{-1}; 4 d LC_{50} of adults = 560 μg Cu L^{-1}). Synergistic effects of copper, mercury, and zinc on embryonic development are also observed in *C. virginica* (MacInnes 1981). With copper, both salinity and temperature influence toxicity, with low salinity (i.e., 17.5 ppt) and low and high temperature (i.e., 20° and 30° C) being particularly detrimental (MacInnes and Calabrese 1979).

Unsuccessful settlement of spat was reported when high concentrations of copper were present in

the "aufwuchs" (or organic/bacterial film) of oyster shells on which spat normally attach (Phelps and Mihursky 1986).

Exposure of adult *C. virginica* to 15 μg Cd L^{-1} for 37 weeks results in the production of abnormal larvae as offspring (Zaroogian and Morrison 1981). The cause for such abnormalities may be related to toxic effects on gonadal development in adults, which carry over to effects on cellular differentiation in larvae. The effects on early developmental stages may be related to complications arising from metal-induced changes in energy metabolism of developing gametes. Respiration rate in oocytes is low compared with the respiration rate in embryos, and serves to conserve nutrient reserves prior to fertilization (Akberali et al. 1984). This rate may be stimulated by metal exposure. Cadmium, for example, is considered to be a potent uncoupler of oxidative phosphorylation (Jacobs et al. 1956). With copper, stimulation of respiration rates in unfertilized eggs has been attributed to an uncoupling of oxidative phosphorylation in *M. edulis* (Akberali et al. 1984). Metal-induced uncoupling of oxidative phosphorylation in eggs prior to spawning may result in the release of nutrient-depleted, energetically-inferior eggs. If carried over into embryonic development, nutrient reserves, already reduced at the egg stage, may not be sufficient to support the ATP production needed for anabolic processes during embryogenesis.

TRIBUTYLTIN AND OYSTERS: AN ONGOING CASE HISTORY

Unlike most contaminant compounds for which toxicity has generally been extremely difficult to demonstrate *in situ*, organotin compounds, principally tributyltin, from anti-fouling paints have proved the exception. Organotins are compounds in which the tin atom is covalently bonded to organic moieties. In this regard, they are similar to organomercurial and organolead compounds and exhibit a behavior based on lipophilic characteristics. This behavior distinguishes them from inorganic metals whose behavior is governed by stability constants and affinities for charged ligands. Having been formulated to kill organisms that settle on the surfaces of submerged structures, tributyltin-based paints are extremely effective for use as anti-fouling agents. Concern over using them had arisen because their distribution could not be restricted to the structures to be protected and because leaching from painted surfaces resulted in exposures to non-target organisms. Comprehensive treatment of this subject is summarized in the proceedings of several symposia (MTS 1986, 1987; Mee and Fowler 1991; see also Capuzzo, Chapter 15).

The proximity of many oyster populations to areas of heavy organotin use inadvertently targeted them for toxic effects. In France, severe reductions in *C. gigas* abundances, which adversely affected a commercial fishery, were attributed to the effects of tributyltin that had leached from the painted hulls of nearby boats. Concentrations that adversely affect early life-history stages of oysters are very low and within the range of values detected in contaminated environments. Forty-eight-hour LC_{50} values for tributyltin for oyster embryos and larvae, including those of *C. virginica*, range from 0.4 to 4 μg L^{-1} (Thain 1986; Rexrode 1987). Tributyltin concentrations as low as 0.02 μg L^{-1} retard growth of *O. edulis* spat. Such effects are believed to have been responsible for the reduced recruitment of oysters into tributyltin-affected areas in France (Alzieu 1986).

Exposure of adult *C. gigas* and *O. edulis* to tributyltin results in deformed shell growth characterized by abnormal thickening of shells and a phenomenon referred to as chambering (Alzieu 1986), in which spaces filled with a gelatinous protein (Krampitz et al. 1983) are formed within the shell. In *C. gigas*, shell-thickening can become so severe as to cause oysters to grow into a ball-shaped structure, a response considered to be diagnostic of tributyltin exposure. Based on chambering as a criterion of toxicity, reports of tributyltin toxicity on *C. gigas* populations have been reported from areas as far-reaching as France, England, and the Pacific coast of the United States.

Effects of tributyltin on sexual differentiation have also been observed in *O. edulis* (Bryan et al. 1987). Populations exposed to tributyltin tend to favor a predominance of male individuals suggesting possible effects on hormonal mechanisms reminiscent of the imposex, or appearance of male character-

istics in females, observed in certain gastropods exposed to tributyltin (Bryan et al. 1987).

Although data on the effects of tributyltin on *C. virginica* are limited, it appears that this species is more tolerant than either *C. gigas* or *O. edulis*. The 48h LC_{50} of 4 μg L^{-1} for larvae of *C. virginica* (Roberts et al. 1987) is higher than the range of 0.4 to 1.9 μg L^{-1} reported for larvae of *C. gigas* (Rexrode 1987). The value of 2 μg L^{-1} required to inhibit spat growth in *C. virginica* (Thain 1986) is also considerably higher than the range of 0.02 to 0.2 μg L^{-1} reported for *C. gigas* and *O. edulis* (Rexrode 1987). For adult *C. virginica*, the only reported effect of tributyltin has been mortality at 1.1 μg L^{-1} at 4 weeks of exposure (Roberts et al. 1987). No effects were observed on fertilization of gametes spawned from exposed adults or on sexual differentiation in *C. virginica*, in contrast to what has been observed in *O. edulis*.

The symptoms of tributyltin toxicity to oyster populations are reversible, and oysters moved to uncontaminated areas are able to resume normal shell growth. In France, previously-affected areas have seen recoveries in populations of *C. gigas* after institution of a partial ban on the use of organotin-based paints (Alzieu 1986, 1991). Similar bans in the United Kingdom (Abel et al. 1987) and the United States (Anonymous 1988) should benefit the health of resident oyster populations. Environmental monitoring of butyltin and tributyltin residues in the tissues of *C. virginica* in the Gulf of Mexico indicates a reduction in the environmental loading of these substances between 1989 and 1990 (Wade et al. 1991), after limitations in their use in antifouling paints had been instituted in the United States.

PUBLIC HEALTH IMPLICATIONS OF METAL BIOACCUMULATION IN OYSTERS

The potential for human poisoning from ingestion of metal-contaminated shellfish served as the basis for early studies on metal bioaccumulation in oysters (e.g., Shuster and Pringle 1969; Zaroogian and Cheer 1976). In a few instances, occurrences of such poisoning have been attributed to metal-contaminated oysters (cited in Lytton et al. 1985; Bryan et al. 1987). However, few studies have directly addressed this topic by examining the effects of consuming metal-contaminated oysters in higher animals.

Hardy et al. (1984) constructed an experimental food chain linking phytoplankton, *C. virginica*, and a strain of laboratory mouse, *Mus musculus*, in order to determine the efficiency of trophic transfer of cadmium and to estimate potential harm to a mammalian consumer. Eastern oysters exposed for 13 d in a medium that contained a total of 10.4 μg Cd L^{-1} (70% in phytoplankton; 30% dissolved in seawater) accumulated 8.2 μg Cd g dry $weight^{-1}$. When cadmium-laden oysters were incorporated into a mouse diet, only 0.83% of the cadmium in the oyster tissues was retained by mice. Although the transfer efficiency of cadmium from oysters to mice is low, enhanced retention of hepatic and renal cadmium occurs in mice fed diets containing cadmium-contaminated oysters (Sullivan et al. 1984; Siewicki et al. 1987) in comparison with other forms of cadmium-amended diets (Siewicki et al. 1987). Thus, cadmium in *C. virginica* appears to be present in a form that is more easily assimilated by mice. A similar phenomenon has also been observed with the distribution of cadmium when mice are fed cadmium-contaminated *C. gigas*, hard clam, *Mercenaria mercenaria*, and soft-shell clam, *Mya arenaria* (Siewicki et al. 1987). By extrapolating the mouse data to humans, it was estimated that a nonsmoking individual would have to consume 11,071 oysters over a 40 year period (i.e., 5 oysters a week) to increase the cadmium content of the kidneys, a known target of cadmium toxicity, by 5% (Hardy et al. 1984). Cadmium concentrations that would result in renal tubular damage would require an increase in the kidney cadmium of about 400%. It was concluded that only an extremely high rate of consumption of contaminated oysters would be expected to constitute a public health concern (Hardy et al. 1984).

In a related study (Siewicki et al. 1983a), mice were fed a diet containing 1.8 μg Cd g dry $weight^{-1}$ of which 20% was cadmium-contaminated oyster tissue. The oyster tissue component of the diet was 9 μg Cd g dry $weight^{-1}$ (normalized to the mass of the oyster tissue component of the diet). After 28 d, mice on this

diet exhibited reduced hematocrit and hemoglobin levels that are symptomatic of altered iron metabolism (Siewicki et al. 1983a). These latter changes can be related to the disruption of pathways for heme biosynthesis and degradation and the anemia normally associated with cadmium toxicity in mammals. Again, cadmium associated with oyster tissue was more toxic to mice than equivalent amounts of cadmium salts spiked directly into the basal diet. Concentrations of cadmium in *C. virginica* that approach or exceed the 9 μg Cd g dry weight^{-1} in eastern oysters used by Siewicki et al. (1983b) have been reported in oyster tissue samples in various locations (Shuster and Pringle 1969; Frazier 1975; NOAA 1987; MDE 1990). These locations include Copano Bay, Texas; Delaware Bay, Delaware; and Chesapeake Bay, Maryland. Concentrations of cadmium in oysters from a tributary of Chesapeake Bay exceeded 30 μg Cd g dry weight^{-1} (calculated from MDE [1990] using a factor of 5× for conversion of the cadmium concentration originally normalized to wet weight). Lauenstein et al. (1990) have shown that the cadmium content of oysters from some locales may have undergone declines, although sites with contaminated oysters were still observed.

When the data on cadmium residues in oyster populations are considered together with the results from Siewicki et al. (1983b) described above, the possibility exists that cadmium concentrations in some oyster populations may represent a human health concern to heavy consumers of cadmium-contaminated oysters over time-scales shorter than the 40 years estimated by Hardy et al. (1984). At the present time, however, the evidence consists of a limited number of studies and the responses of mice continuously fed a diet containing a high proportion of cadmium-contaminated oyster tissues. The health-related issue of consumption of metal-contaminated shellfish by humans remains a topic of continuing concern (Ahmed, 1991; Fowler, 1991).

SUMMARY

There is now a considerable body of literature on metals in oysters. Our general understanding of metal bioavailability and bioaccumulation has benefited from studies on these organisms. Some of the cellular mechanisms for metal regulation in oysters have been identified, although studies to date on this topic are at an early stage and have largely been descriptive. In view of the central role that structures such as granular hemocytes and metallothionein appear to play in the sequestration of metals, continued study of these structures and their contribution to the regulation of metals at the cellular and organismal levels should provide a better understanding of the biological basis for metal accumulation, turnover, and variability. It appears also that the chemical form of some metals (e.g., cadmium) in oyster tissues may have unique properties with respect to tissue distribution and effects of metals in mammalian consumers.

The relationship between metal toxicity and the viability of *C. virginica* populations is of practical concern due to the current population declines and the continued input of anthropogenically-derived metals to oyster habitats. Oysters in metal-contaminated habitats are known to possess elevated concentrations of metals in tissues. However, the contribution of metal contamination to population declines is currently conjectural and must be considered together with other factors such as fishing pressure, changes in trophic dynamics, the influence of infections by pathogenic organisms, and contamination of the environment by other anthropogenically-derived chemicals. Increased understanding of the regulation of metals at the cell and whole animal levels, the dual role of metals as both facilitative and toxic agents in the function of cellular defense mechanisms, toxicological ramifications of exposures to metals at environmentally-realistic concentrations, and interactions with other physico-chemical and biological factors is needed to better address the question of anthropogenic metal contamination as a potential limiting factor to the viability of oyster populations.

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Chapter 15

The Bioaccumulation and Biological Effects of Lipophilic Organic Contaminants

JUDITH MCDOWELL CAPUZZO

INTRODUCTION

Bivalve molluscs have been used extensively during the past two decades as sentinel monitors of chemical contamination (Butler 1973; NRC 1980; Farrington et al. 1983) and more recently as organisms in the monitoring of biological effects (Bayne et al. 1988). As the relationships between concentrations of chemical contaminants and biological responses in bivalve molluscs continue to be explored, the toxic action of specific compounds and groups of compounds have been elucidated. However, our knowledge of cause and effect relationships among tissue burdens of many contaminants and biological consequences in many species is still incomplete. Lipophilic organic contaminants such as PAHs (polycyclic aromatic hydrocarbons), PCBs (polychlorinated biphenyls), and other synthetic compounds for which PAHs and PCBs serve in part as model compounds, are highly resistant to degradation in the marine environment. Thus, such compounds or their metabolites may accumulate to high concentrations in animal tissues and interfere with normal metabolic processes that affect growth, development, and reproduction (Capuzzo et al. 1988). The bioavailability, bioconcentration, and toxic effects of lipophilic contaminants are related to their pharmacological and toxicological properties (Abernathy et al. 1986; Capuzzo 1987; Widdows et al. 1987; Donkin et al. 1990). The limited capacity of bivalve molluscs to detoxify organic contaminants (Livingstone and Farrar 1984; Stegeman 1985) results in their uptake and accumulation in high concentrations.

Biological effects associated with bioconcentration of lipophilic contaminants have been attributed to the uptake of specific compounds or their metabolites, rather than the total body burden of hydrocarbons or chlorinated hydrocarbons (Anderson et al. 1980; Malins and Hodgins 1981; Widdows et al. 1982, 1987; Capuzzo et al. 1984). Biological effects of organic contaminants have been observed at all levels of biological hierarchy from cellular through community (McIntyre and Pearce 1980; Capuzzo 1987; Moore et al. 1989). For bivalve molluscs, exposure to organic contaminants has resulted in impairment of physiological mechanisms (Widdows 1985); histopathological disorders (Lowe 1988; Moore 1988); and loss of reproductive potential (Neff and Haensly 1982; Berthou et al. 1987). Empirical data suggest that linkages exist among (1) histopathological, bioenergetic, developmental, and reproductive abnormalities; (2) the physiological and molecular processes involved in uptake, retention, and loss of contaminants; and (3) the toxicity or transformation of lipophilic contaminants (Capuzzo et al. 1988). Numerous reviews have examined the biological effects of lipophilic organic contaminants on aquatic organisms (e.g., McIntyre and Pearce 1980; Capuzzo 1987; Moore et al. 1989). This chapter reviews the data available on the bioaccumulation and biological ef-

fects of organic contaminants on the eastern oyster, *Crassostrea virginica*. Such information is important not only in assessing the ecological effects of bioaccumulation but also in assessing the public health risks associated with the consumption of a commercially valuable resource.

BIOACCUMULATION

General Considerations

Uptake and bioconcentration of organic contaminants by marine bivalves are dependent on the bioavailability of specific compounds, the duration of exposure, and the physiological condition of populations. Species differ in their rate of uptake due to variations in both biotic and abiotic factors (Stegeman and Teal 1973; Clark and Finley 1974; Neff et al. 1976; Vandermeulen and Gordon 1976; Augenfeld et al. 1980; Boehm et al. 1982; and Farrington et al. 1982). Variations in filtration rates may influence the volume of water exchanged by an organism, and thus influence the contact between an organism and contaminants in both aqueous and particulate phases. Lipid content of a bivalve mollusc may vary seasonally, influencing both the total body burden of contaminants and the distribution to specific tissues. Finally, habitat differences may result in variations in particle size, particle reactivity with contaminants, and pore water availability. Bioavailability and release of organic contaminants from bivalve molluscs varies as a function of compound solubility, concentration, and partitioning between tissues and water as estimated by the octanol/water partition coefficients (K_{OW}) of individual compounds. Differences in contaminant concentrations among species of bivalves from different habitats may be the result of physico-chemical differences in the availability of sediment-bound contaminants.

Bioconcentration patterns of different organic contaminants are influenced by physico-chemical properties such as molecular configuration or stearic properties of specific compounds that influence biotransformation and membrane transfer kinetics (Shaw and Connell 1984; Farrington et al. 1986), by biological factors such as partitioning between storage lipids and structural lipids (Neff 1979; Chiou 1985), and by differential distribution of contaminants among different tissues (Widdows et al. 1982). Steady-state pharmacokinetic models generally used to describe the kinetics of uptake and release of lipophilic organic contaminants consider that partitioning between the environment and the organism is controlled by steady-state uptake and depuration and the organism is considered to be a single homogeneous unit (Moriarty 1975). In such a model, the log of the bioconcentration factor (K_B) is linear to the log of the octanol-water partition coefficient (K_{OW}) of a specific compound (Fig. 1; Geyer et al. 1982; Mackay 1982; Pruell et al. 1986).

For bivalves, however, empirical data on the relationship of K_B to K_{OW} often deviate from the predicted values derived from single compartment models, suggesting the interaction of several factors in determining bioconcentration, with the potential for major seasonal differences to occur. Such factors may include differential uptake and release rates, dietary input, differential distribution within the organism, seasonal variability in lipid content, and metabolic transformations. Seasonal variation in biological responses to contaminant uptake and bioconcentration may be related to dependency on specific metabolic processes, such as storage and mobilization of lipid reserves during reproductive events (Capuzzo 1987; Capuzzo et al. 1989). Distribution patterns of specific classes or organic contaminants are discussed in the following sections.

Petroleum Hydrocarbons

The uptake of petroleum hydrocarbons by oysters has been documented in numerous studies and is consistent with observations made on other species of bivalve molluscs. While eastern oysters exposed to No. 2 fuel oil accumulated aromatic compounds to a higher degree than aliphatic compounds relative to the composition of No. 2 fuel oil, they also released aromatic compounds more rapidly upon transfer to uncontaminated seawater (Stegeman and Teal 1973). Bieri and Stamoudis (1977) also observed accumulation and release of various hydrocarbons by eastern oysters exposed to No. 2

fuel oil in a semi-natural exposure system. Alkanes, branched alkanes, and olefins were accumulated and released first followed by the alkylnaphthalenes (with up to five alkyl carbons), the biphenyls (with up to two alkyl carbons), and the fluorenes (with up to one methyl group). Highly substituted naphthalenes, biphenyls, and fluorenes, in addition to dibenzothiophenes and phenanthrenes, were accumulated and released last. Polycyclic aromatic hydrocarbons accumulated even after aqueous concentrations had fallen to undetectable levels; ingestion of detrital particles was suggested as a possible mechanism of uptake of hydrocarbons (Bieri and Stamoudis 1977).

Berthou et al. (1987) reviewed hydrocarbon accumulation data for the European flat oyster, *Ostrea edulis,* and the Pacific oyster, *Crassostrea gigas,* after the wreck of the oil tanker *Amoco Cadiz* off the coast of Brittany, France. Uptake and depuration of specific hydrocarbons reflected differences in hydrocarbon availability in the sediments resulting from weathering processes in addition to physico-chemical properties of specific compounds. Rapid accumulation of a wide range of hydrocarbons was followed by an initial rapid loss of alkanes and low molecular weight hydrocarbons, but the persistence of polcyclic aromatic hydrocarbons and dibenzothiophenes was also noted (Laseter et al. 1981; Frioucourt et al. 1982). In transplant experiments where *O. edulis* and *C. gigas* were transferred to heavily oiled areas, Berthou et al. (1987) confirmed the rapid accumulation of hydrocarbons by both species. Depuration of hydrocarbons by both species of oysters growing naturally at the spill site and the transplanted oysters transferred away from the spill site revealed a strong dependency of depuration rates on length of exposure. *Crassostrea gigas* exposed to oil contamination for 18 months in the Aber-Benoit showed no depuration five months after transfer to the uncontaminated site whereas oysters exposed at the same location for a shorter duration (45 d) showed rapid depuration. *Ostrea edulis* also showed more rapid depuration than *C. gigas.* Residual hydrocarbons consisting primarily of PAHs including dibenzothiophenes persisted in both species long after the initial depuration. Seven years after the spill, Pacific oysters kept at the oiled site showed

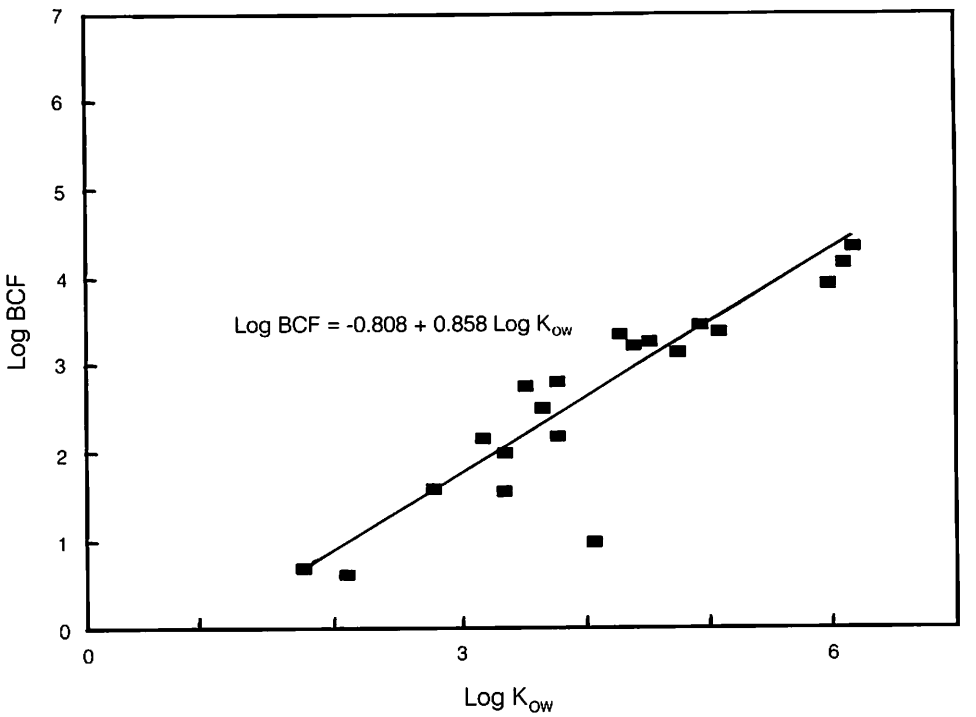

Figure 1. Relationship of bioconcentration factors (BCF) to octanol-water partition coefficients (K_{ow}). Data for *Mytilus edulis* from Geyer et al. (1982).

residual hydrocarbon concentrations two to five times the concentrations at the uncontaminated sites.

A two-phase depuration of hydrocarbons observed in both species of oysters after the *Amoco Cadiz* spill is consistent with observations made on other species of bivalve molluscs. Neff (1979) proposed that a two-phase depuration was consistent with the mobilization of molluscan lipid stores. He suggested that uptake and storage of hydrocarbons in depot lipids, such as energy reserves or gonadal reserves, may be retained until those reserves are mobilized for nutritional or reproductive needs. Hydrocarbons associated with more hydrophobic compartments, such as membrane lipids, may be more rapidly released when ambient concentrations decrease. Farrington et al. (1982) concluded that molecular weight and solubility of hydrocarbons were the main controlling factors in the release of hydrocarbons, although molecular type and configuration were additional factors. The role of lipid compartments in depuration processes in bivalve molluscs may be more complex than that suggested by Neff (1979) because simple hydrocarbon/lipid ratios have not been observed (Berthou et al. 1987; Capuzzo et al. 1989) and multiple lipid compartments may be involved.

Bender et al. (1988) examined the distribution of PAHs in eastern oysters from the Elizabeth River, Virginia, and conducted laboratory studies comparing the uptake and depuration of PAHs by eastern oysters and hard clams, *Mercenaria mercenaria*, with exposure to contaminated sediments from the Elizabeth River. Animals were exposed to contaminated sediments for 28 d, followed by a 28-d depuration phase. Oysters accumulated three to four times more total PAH than clams. Although uptake rates for both species are similar, clams had higher depuration rates. Bioconcentration factors for oysters ranged from 1600 for phenanthrene to 36,000 for methylpyrene (Table 1; Fig. 2).

Wade et al. (1988a) examined the distribution of PAHs in sediments and eastern oysters at 153 stations in the Gulf of Mexico. They observed comparable concentrations of total PAHs in sediments and oysters, but the molecular distribution of PAHs differed. Oyster samples showed a predominance of 2-,

Table 1. Bioconcentration of polycyclic aromatic hydrocarbons by the oyster, *Crassostrea virginica*.[a]

Compound	Uptake k1	Depuration k2	Bioconcentration Factor BCF[b]
		3-ring compounds	
Phenanthrene	330	0.206	1,604
Methylphenanthrene	529	0.103	5,151
		4-ring compounds	
Fluoranthene	821	0.118	6,965
Pyrene	921	0.104	8,857
Methylpyrene	2365	0.066	36,074
Chrysene	1187	0.046	26,019
Benz(a)anthracene	1310	0.045	28,846
Benzo(a)fluorene	508	0.066	8,796
Benzo(b)fluorene	1410	0.072	19,666
		5-ring compounds	
Benzo(a)pyrene	639	0.032	19,673
Benzo(e)pyrene	790	0.023	33,731
Benzofluoranthene	794	0.009	84,217
Perylene	817	0.075	10,861

[a] Data from Bender et al. (1988).
[b] k1 and k2 are rate constants per hour; BCF is determined by k1/k2.

3- and 4-ring PAH compounds, whereas sediment samples had low concentrations of low molecular weight PAHs (2- and 3-ring) and higher concentrations of 5-ring compounds (Fig. 3). Based on ratios of phenanthrene to anthracene in both oyster and sediment samples, the authors suggested that the dominant source of PAHs at most sampling sites was pyrolysis of fossil fuels.

Polychlorinated Biphenyls and Other Chlorinated Compounds

Chlorinated hydrocarbons, including PCBs and chlorinated pesticides such as DDT, are among the most persistent organic contaminants in the marine environment. Distribution of these contaminants in the eastern oyster has been examined several times during the past two decades (Butler 1973; Farrington et al. 1983; Sericano et al. 1990). In the most recent survey as part of the NOAA Status and Trends Program, eastern oysters from 51 sites in the Gulf of Mexico were sampled and analyzed for PCBs and chlorinated pesticides (Sericano et al. 1990). The PCBs were analyzed both as isomer groups (e.g., trichlorobiphenyls, tetrachlorobiphenyls, etc.) and specific chlorobiphenyl congeners. The distribution of PCBs in oysters was significantly different from that observed in sediments sampled from the same sites. Sediment samples showed a high prevalence of penta- and hexachlorobiphenyls, with some tetra- and heptachlorobiphenyls; the four isomer groups comprised >89% of the total sedimentary PCB concentration. In oysters, pentachloropbiphenyls were present in the highest proportion (46.8%) with some tetra- and hexachlorobiphenyls (21.0 and 22.3%, re-

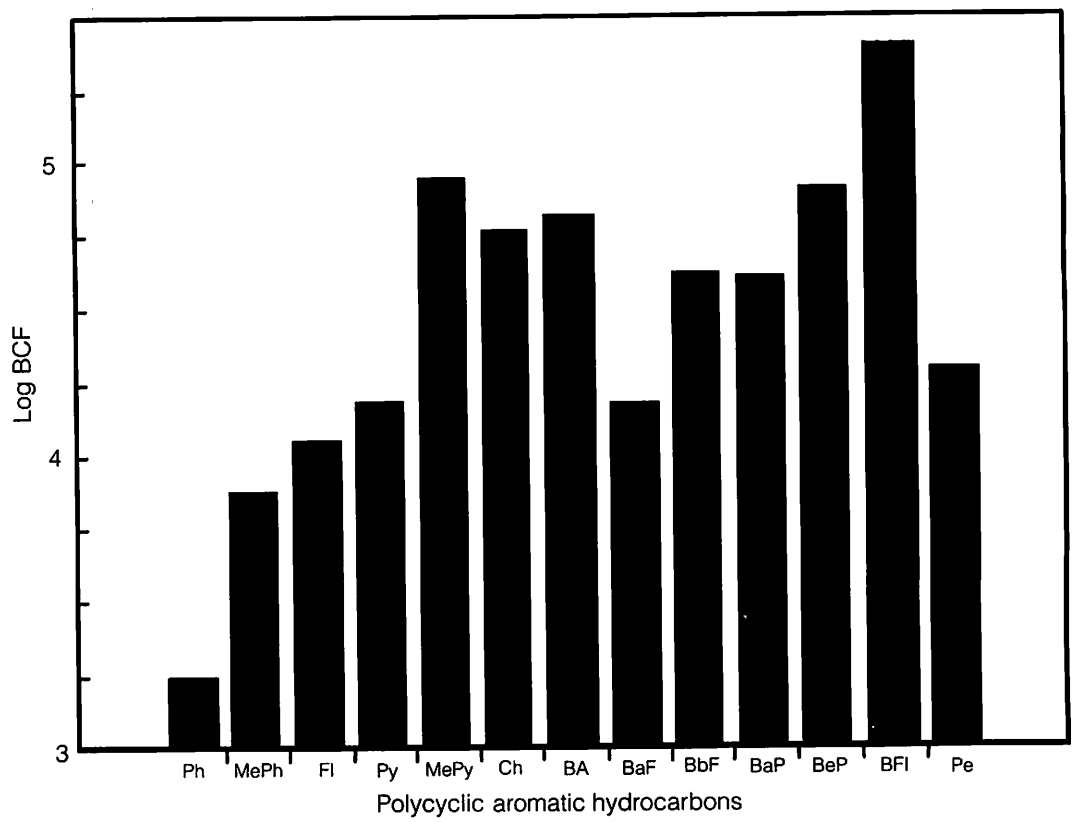

Figure 2. Bioconcentration (BCF) of PAHs in *Crassostrea virginica* from the Elizabeth River, Virginia. Data from Bender et al. (1988); Ph-phenanthrene, MePh-methylphenanthrene, Fl-fluoranthene, Py-pyrene, MePy-methylpyrene, Ch-chrysene, BA-benz(a)anthracene, BaF-benzo(a)fluorene, BbF-benzo(b)fluorene, BaP-benzo(a)pyrene, BeP-benzo(e)pyrene, BFl-benzofluoranthene, and Pe-Perylene.

spectively (Fig. 4). This observation is consistent with observations made by other investigators on the distribution of PCBs in organisms from PCB-contaminated areas (Shaw and Connell 1982; Boon et al. 1985; Farrington et al. 1986; Capuzzo et al. 1989). The lesser chlorinated chlorobiphenyl congeners (di-, tri-, tetra-) are highly water soluble and tend not to accumulate. Higher chlorinated congeners have unfavorable stearic configurations to allow bioaccumulation to occur (Shaw and Connell 1984). Highest concentrations in oyster and sediment samples were found in Matagorda Bay, Texas; Galveston Bay, Texas; Tampa Bay, Florida; and Choctawhatchee Bay, Florida.

Sericano et al. (1990) also evaluated the distribution of hexachlorobenzene, lindane, aldrin, heptachlor, heptachlor epoxide, alpha-chlordane, trans-nonachlor, dieldrin, mirex, and DDT and its metabolites (*o-p'* DDE, *p-p'* DDE, *o-p'* DDD, *p-p' DDD, o-p'* DDT, and *p-p'* DDT) in bivalves and sediment from the Gulf of Mexico. In general, sediment concentrations of chlorinated hydrocarbons were relatively low throughout the range of stations sampled. Exceptions include relatively high concentrations of non-DDT pesticides in sediment samples at sites east of the Mississippi River, such as Choctawhatchee Bay, Naples Bay, Tampa Bay, and St. Andrews Bay, Florida. A large number of samples from the survey contained trans-nonachlor, alpha-chlordane, and dieldrin, whereas relatively few samples contained lindane, aldrin, heptachlor, and heptachlor epoxide.

Concentrations of non-DDT pesticides in eastern oysters were generally 10 to 30 times higher than concentrations found in Gulf of Mexico sediments. The most prevalent compounds were alpha-chlordane, trans-nonachlor, and dieldrin, with concentrations ranging over two orders of magnitude. "Hot spots" for non-DDT pesticides in oysters included Galveston Bay, Mississippi Sound, Tampa Bay and St. Andrews Bay.

Total DDT (sum of the parent compound and each of its metabolites) was the most prevalent chlorinated pesticide in sediment and oyster samples during the survey, with 88 to 95% of the samples having measurable concentrations of DDT over the 2-year sampling period. Highest concentrations of DDT were found in sites in the region between Mobile

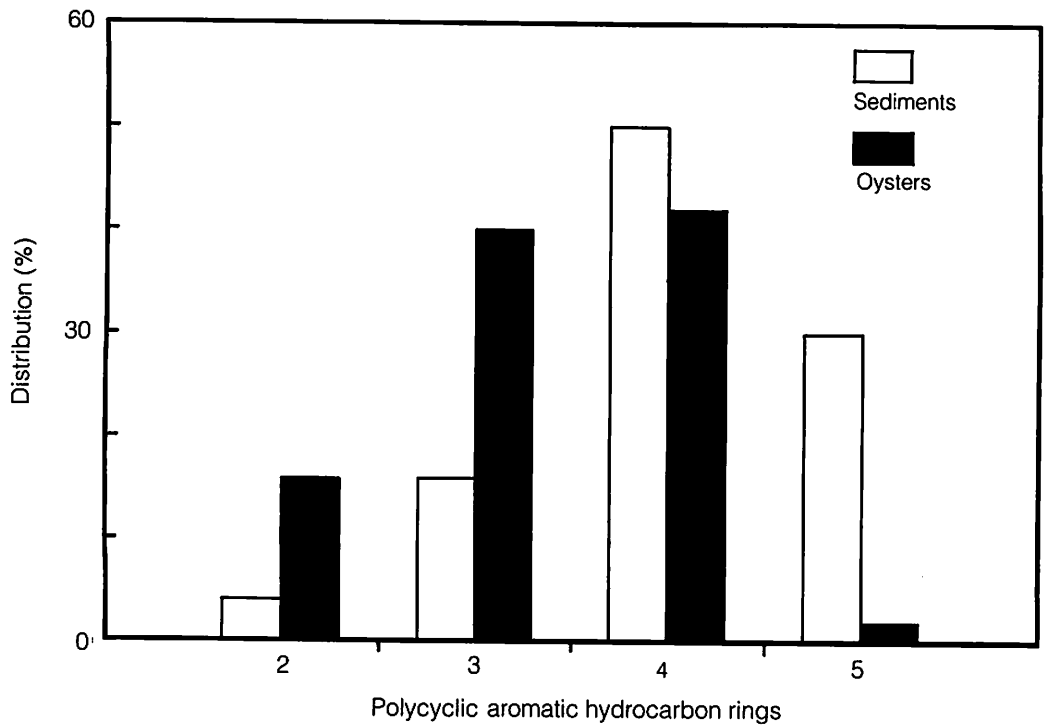

Figure 3. Comparison of PAH distribution in eastern oysters and Gulf of Mexico sediments. Data from Wade et al. (1988a).

Bay, Alabama, and St. Andrews Bay, Florida, and at Matagorda Bay and Galveston Bay, Texas. The relative distribution of DDT and its metabolites differed to some extent between oyster and sediment samples, with a higher proportion of total DDT in oysters being DDE and a significantly lower proportion as DDT. Butler and Schutzmann (1978) also observed a higher ratio of DDE to DDT in marine organisms. Sericano et al. (1990) compared their data on DDT distribution in oysters with data collected by Butler (1973) from 1965 to 1972 and suggested that total DDT concentrations have declined by a factor of 2 to 10-fold over the past 15 to 20 years, which is consistent with the decline in DDT use in the U.S. during the past two decades.

Organotin Compounds

The widespread use of organotin compounds in anti-fouling paints, especially tributyltin (TBT), has led to great concern during the past decade about potential accumulation and effects of TBT on non-target organisms in coastal embayments (see also Roesijadi, Chapter 14). Wade et al. (1988b) examined the distribution of tributyltin and its less toxic breakdown products (dibutyltin and monobutyltin) in bivalves collected from the U.S. coastline. Bivalves, including *Crassostrea virginica* and *Ostrea sandwichensis,* concentrated organotin compounds and TBT accounted for 25 to 100% of the organotin burden (mean = 74%). Concentrations of TBT in bivalves varied by site, with the highest concentrations being detected in those collected from harbor areas. A more detailed study of organotin distribution in *C. virginica* from Galveston Bay was conducted to assess potential differences in spatial and temporal variability (Wade et al. 1988b). Sample sites within Galveston Bay were selected on the basis of relative differences in TBT input and samples were collected from January through December 1986. The results indicated that organotin accumulation in oysters was responsive to the predicted trends in TBT input. Seasonal variation revealed the lowest proportion of TBT (relative

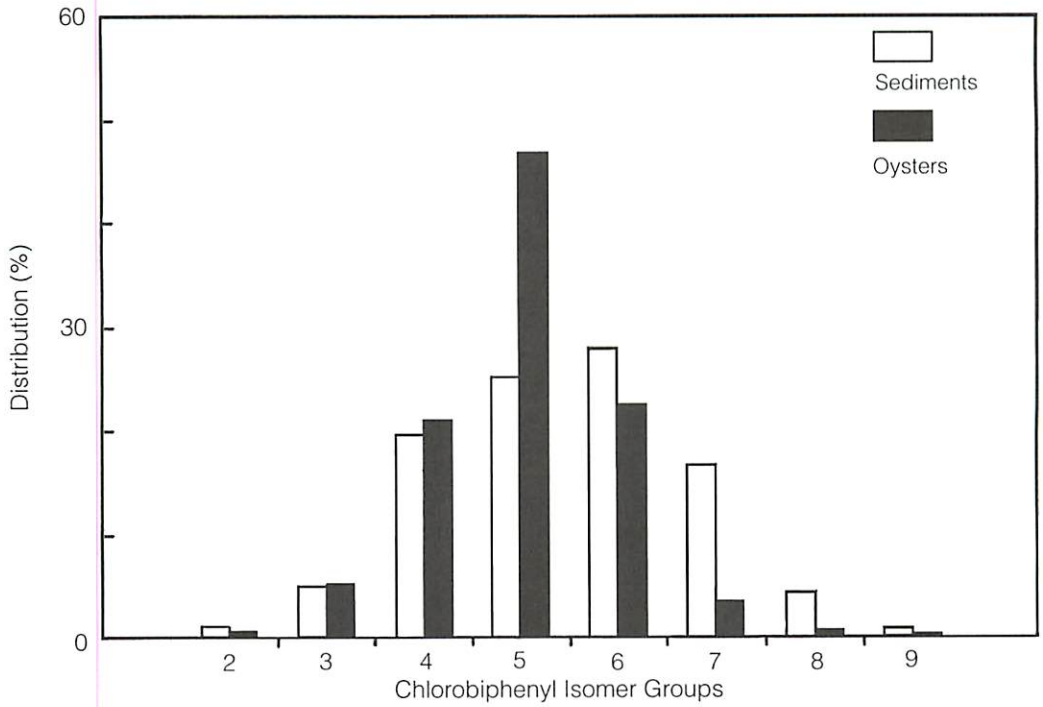

Figure 4. Comparison of PCB distribution in eastern oysters and Gulf of Mexico sediments. Data from Sericano et al. (1990).

to di- and monobutyltin) in summer months, which may be the result of higher biodegradation rates of TBT in the water column or higher biotransformation rates in oyster tissues; ambient water concentrations of TBT were not reported.

Bivalves rapidly accumulate tributyltin, reach an equilibrium plateau, and slowly depurate. Hall and Pinkney (1985) reported bioconcentration factors for TBT of between 2,300 to 11,400. Bioconcentration of TBT may be governed by more than physical-chemical factors because the bioconcentration factors exceed those predicted from octanol-water partition coefficients (K_{OW}; Cardwell and Sheldon 1986; Laughlin et al. 1986). Laughlin (1986) suggested that nonspecific binding of TBT may also be an important process in bioconcentration of TBT in invertebrates.

BIOLOGICAL EFFECTS

General Considerations

The responses of organisms to toxic chemicals can be manifested at four levels of biological organization: (1) biochemical and cellular; (2) organismal, including the integration of physiological, biochemical, and behavioral responses; (3) population, including alterations in population dynamics; and (4) community, resulting in changes in community structure and dynamics (Table 2). Biological effects can be manifested at biochemical, cellular, and organismal levels of organization before disturbances at the population level develop (Capuzzo 1981). All responses are not disruptive in nature and do not necessarily result in degeneration at the next level of organization. Only when the compensatory or adaptive mechanisms at one level begin to fail do deleterious effects become apparent at the next level. An important aspect of comparing responses at various levels of biological organization is ascertaining the degree to which adaptive responses at each of the four levels can persist with increasing concentrations of contaminants. The initial responses in each case are the triggering of mechanisms to reduce or resist the effect of the toxicant; these mechanisms may include the induction of toxicant-metabolizing processes (at the biochemical level) or the selection of toxicant resistant forms (at the population level). Adaptive processes are capable of countering disruptive processes until the system reaches a threshold for the toxicant, at which point the adaptive potential is completely overridden by the degeneration imposed on the system by disruptive effects.

For predictive purposes, it is important to understand the early warning signs of stress at each level of organization before compensatory mechanisms are surpassed. From the biochemical level to the community level, the degree of system complexity, the number of compensatory mechanisms available, and the lag time to measure a response increase dramatically, thereby increasing the predictive difficulties at each level. Chronic exposure to chemical contaminants can alter reproductive and developmental potential of populations of marine organisms, resulting in possible changes in population structure and dynamics. It is difficult to ascertain, however, the relationship between chronic responses of organisms to contaminants and large-scale alterations in the functioning of marine ecosystems or the sustainable yield of commercially valuable species.

Although a wide range of sublethal stress indices have been proposed for evaluation of chronic responses of organisms to contaminants, few have been linked to the survival potential of the individual organism or the reproductive potential of the population. Experimental studies directed at determining effects on energy metabolism or effects that influence growth and reproduction would be most appropriate for linking effects at higher levels of organization.

The effects of lipophilic organic contaminants on marine bivalve molluscs have been examined extensively during the past decade. Most studies have been conducted on the blue mussel, *Mytilus edulis* (e.g., Bayne et al. 1985, 1988), with an effort to integrate responses over several levels of biological hierarchy and to examine responses linked to specific classes of contaminants. Recent work has extended this approach to other species of bivalve molluscs, such as the subtropical turkey wing mussel, *Arca zebra* (Addison and Clarke 1990; Widdows et al. 1990).

Research on the effects of contaminants on the eastern oyster has been more limited in scope; studies

on oysters have focused to a large extent on histopathological responses and there are few studies on physiological or biochemical responses. The link between molluscan disease research and contaminant exposure is poorly understood in many instances, thus leading to a discontinuity between studies of contaminant bioavailability and distribution and studies of biological damage in oyster populations. Cause and effect relationships between a specific contaminant and a biological response have been difficult to interpret. Although such a relationship has been suggested in some instances, data are incomplete to link toxic mechanisms with an observed response.

Biotransformation and Disease Responses

Bivalve molluscs generally have been considered to have a relatively low capacity for detoxifying xenobiotic compounds through cytochrome P-450 mono-

Table 2. Types of responses of marine organisms to chemical contaminants at different levels of biological hierarchy.

Level	Types of Responses	Effects at Next Level
Biochemical-Cellular	Toxication	Toxic metabolites
	Metabolic impairment	Disruption in energetics and cellular processes
	Cellular damage	
	Detoxication	Adaptation
Organismal	Physiological changes	Reduction in population performance
	Behavioral changes	
	Susceptibility to disease	
	Reproductive effort	
	Larval viability	
	Adjustment in rate functions	Regulation and adaptation of populations
	Immune responses	
Population	Age/Size structure	Effects on species productivity and
	Recruitment	coexisting species and community
	Mortality	
	Biomass	
	Adjustment of reproductive output and other demographic characteristics	Adaptation of population
Community	Species abundance	Replacement by more adaptive competitors
	Species distribution	
	Biomass	Reduced secondary production
	Trophic interactions	
	Ecosystem adaptation	No change in community

oxygenase reactions with low rates of *in vitro* PAH metabolism being detected (Anderson 1978; Livingstone and Farrar 1984; Stegeman 1985). Although the dominant metabolites of benzo(a)pyrene detected in molluscs have been primarily quinone derivatives, rather than the diol derivatives observed in fish (Stegeman 1985; Stegeman and Lech 1991), relatively high concentrations of diol derivatives have been detected in some bivalve molluscs (Anderson 1985). Stegeman (1985) suggested that PAH metabolism in bivalve molluscs may proceed through several catalytic mechanisms including peroxidative mechanisms in addition to cytochrome P-450 monooxygenase. The importance of other catalytic mechanisms in the biotransformation of PAH in molluscs has not been examined.

Metabolism of other compounds such as aromatic amines and tributyltin has also been investigated. Biotransformation of aromatic amines appears to proceed rapidly and yield metabolites with mutagenic properties (Anderson and Doos 1983; Kurelec et al. 1985; Kurelec and Krca 1987; Knezovich et al. 1988). Lee (1985) demonstrated limited ability of the eastern oyster to metabolize tributyltin oxide to dibutyltin.

Moore et al. (1980) suggested that because of the low level of enzymatic activity of components of the detoxication system, xenobiotic metabolism did not play a major role in the removal of xenobiotic compounds from bivalve tissues; however, it may be implicated in the formation of toxic metabolites that induce histopathological changes such as neoplasms and that alter lysosomal function. Stegeman and Lech (1991) discussed the evidence that might suggest a relationship between biotransformation of organic contaminants and disease processes in bivalve molluscs. The formation of mutagenic metabolites of aromatic amines (Anderson and Doos 1983) and the formation of DNA adducts by these same compounds (Kurelec et al. 1988) suggest possible toxic mechanisms for at least some compounds to influence histopathological damage. Nacci et al. (1992) reported an increased incidence in DNA strand breaks in blue mussels and eastern oysters from contaminated areas (New Bedford Harbor, Massachusetts, and the Elizabeth River, Virginia, respectively).

The occurrence of neoplastic diseases in bivalve molluscs and the relationship with body burdens of toxic chemicals (especially PAHs) has been reviewed by Mix (1986, 1988). He concluded that most studies published before 1988 indicated no correlation between PAH concentration and the prevalence of disease in bivalve molluscs. He also suggested, however, that our limited understanding of the distribution of toxic chemicals in the environment, their effects on cellular and physiological processes, and mechanisms of biotransformation, hinder our ability to explore the relationship between contaminant exposure and disease progression.

Gardner et al. (1991) have provided strong empirical evidence of a causal relationship between contaminant exposure and the prevalence of neoplasia in the eastern oyster. They examined the induction of neoplasia with exposure to sediments from Black Rock Harbor, Long Island Sound. Contaminants in the sediment mixture that accumulated in oyster tissues included compounds with carcinogenic and tumor promoting potential (Table 3). Neoplastic conditions were observed in eastern oysters within 30 to 60 d of laboratory exposure to contaminated sediments and 30 to 40 d of field exposure. The neoplasia most commonly observed in laboratory studies were of the renal excretory system, gills, gonads, gastrointestinal system, heart, and embryonic neural tissue, whereas in field studies neoplasia of the gill, kidney, and gastrointestinal system were observed.

Chu and Hale (1994) examined the relationship between contaminant exposure and susceptibility to infectious diseases in the eastern oyster. Oysters were exposed to 0%, 15% and 30% dilutions of water soluble fractions from contaminated sediments collected in the Elizabeth River (New Jersey) and then challenged with meronts of the protozoan parasite *Perkinsus marinus* (Dermo). Contaminant exposure enhanced preexisting infections and increased the oysters' susceptibility to experimentally induced infection. Contaminant analysis of the water soluble fractions from contaminated sediments showed a high

prevalence of PAHs and heterocyclic compounds. The authors concluded that contaminant exposure may play a significant role in increasing susceptibility of the eastern oyster to Dermo infections, either through enhanced virulence of *P. marinus* or decreased host resistance.

Effects of Petroleum Hydrocarbons and PAHs

The long term effects of oil exposure were examined for oyster populations (*C. gigas* and *O. edulis*) on the Brittany coast after the spillage of crude oil from

Table 3. Concentration (ng g dry weight^{-1}) of organic contaminants in eastern oysters, *Crassostrea virginica*, and in sediments from Black Rock Harbor, Long Island Sound, to which oysters were exposed (Gardner et al. 1991). "–" = no data.

Compound	Oysters	Sediments	Ratio Oysters/Sediment
	Sufficient evidence as carcinogens		
Benz(a)anthracene	695	3450	0.20
Benzo(a)pyrene	88	3160	0.03
Benzfluoranthene	364	5970	0.06
Indeno(1,2,3-cd)pyrene	22	–	–
Dibenz(a,h)anthracene	9	–	–
Hexachlorobenzene	0.2	–	–
Chlordanes	100	–	–
	Limited evidence as carcinogens		
Chrysene	1260	4450	0.28
	Inadequate evidence as carcinogens		
Benzo(e)pyrene	264	2880	0.09
Fluorene	42	635	0.07
Phenanthrene	553	4020	0.14
Perylene	17	504	0.03
Benz(g,h,i)perylene	37	–	–
Coronene	1.3	–	–
	No evidence as carcinogens		
Anthracene	191	1330	0.14
Fluoranthene	1777	5800	0.31
	Cancer promoters		
DDT and metabolites	1183		
PCBs (Aroclor 1254)	1143	7170	0.16
Pyrene	2950	7250	0.41

the *Amoco Cadiz* (Berthou et al. 1987). Lesions of the digestive tract epithelium, gonads, interstitial tissue, and gills were observed and correlated with hydrocarbon exposure (Neff and Haensly 1982; Balouet et al. 1986). The highest prevalence of lesions in the digestive tract epithelium and gonadal epithelium was observed during the year of the spill (1978), returning to background levels within 3 to 5 years. These findings are consistent with the observations of severe alterations in the histology of the digestive gland and gonad in the eastern oyster with chronic exposure to petroleum hydrocarbons (Barszcz et al. 1978).

Cellular and physiological responses of bivalve molluscs to petroleum hydrocarbons and specific polycyclic aromatic hydrocarbons have been examined in detail during the past decade (reviewed by Moore et al. 1989). Alterations in lysosomal structure and function are consistent with observations of degeneration of digestive gland epithelium, atrophy of digestive tubules, and degeneration (or atresion) of reproductive tissues (Lowe et al. 1981; Moore and Clarke 1982; Couch 1984; Lowe and Pipe 1985, 1986, 1987; Pipe and Moore 1985). Lysosomal disturbances result in autolytic and autophagic activities linked to atrophy of digestive cells (Moore et al. 1989). High levels of lipophilic contaminants in the blue mussel coincided with the formation of pathologically enlarged lysosomes in the digestive epithelium, aberrations in lipid distribution and alterations in digestive cell architecture (Lowe 1988). Effects of lipophilic organic contaminants on the reproductive system of *M. edulis* included a reduction in the volume of storage cells in the mantle, reduction in the volume of ripe gametes, and increased degeneration or atresion of oocytes (Lowe and Pipe 1985, 1986, 1987). Lowe and Pipe (1987) suggested that the reallocation of energy reserves from resorbed oocytes to storage cells might serve as a resistance strategy to counter the deleterious effects of hydrocarbon exposure.

The most important physiological changes associated with exposure to petroleum hydrocarbons or other lipophilic organic contaminants are those responses that may affect an organism's growth and survival and thus its potential to contribute to the population gene pool. Alterations in growth potential may take place as a result of changes in feeding behavior, respiratory metabolism, or digestive efficiencies. Deviations from control values for physiological rate functions (e.g., respiration rates and feeding rates) and reductions in energy balance, measured as scope for growth (Widdows et al. 1982; Bayne et al. 1985) and carbon turnover (Gilfillan et al. 1976; Gilfillan and Vandermeulen 1978), have correlated with reduced growth rates measured for bivalve populations from oil contaminated habitats. Alterations in bioenergetics and growth of bivalve molluscs appear to be related to tissue burdens of aromatic hydrocarbons (Gilfillan et al. 1977; Widdows et al. 1982; 1985, 1987; Donkin et al. 1990). Widdows et al. (1982) demonstrated a negative correlation between cellular and physiological stress indices (lysosomal properties and scope for growth) and tissue concentrations of aromatic hydrocarbons with long-term exposure of *Mytilus edulis* to low concentrations of North Sea crude oil. Recovery of blue mussels after long-term exposure to low concentrations of diesel oil coincided with depuration of aromatic hydrocarbons (Widdows et al. 1987). Donkin et al. (1990) suggested that reductions in scope for growth in *M. edulis* were related to the accumulation of 2- and 3-ring aromatic hydrocarbons, because these compounds induced a narcotizing effect on ciliary feeding mechanisms.

Effects of Organotin Compounds

The effects of tributyltin on bivalve molluscs, especially oysters, have been examined by several investigators, those studies stemming primarily from concerns of the effects of TBT on growth and reproduction of oysters in growing areas in Europe (Alzieu 1986). His and Robert (1983) examined the effects of TBT acetate on embryogenesis and larval development in the Pacific oyster. Responses ranged from slow growth at concentrations greater than 0.1 $\mu g\ L^{-1}$ to inhibition of fecundity at concentrations above 100 $\mu g\ L^{-1}$. Shell malformations or "chambering" (Alzieu et al. 1982; Waldock and Thain 1983), reduced condition index (Henderson 1986; Davies et al. 1988), and reduced growth of transplanted animals (Stephenson et al. 1986) have been observed among field populations of *C. gigas* where TBT was a

suspected contaminant. Thain (1986) examined effects of TBT on reproductive performance in *Ostrea edulis*. Impairment of ovarian development and consequent reduction in larval production were observed at concentrations of 0.25 mg L^{-1} or more; reduction in spat growth was also observed in *C. gigas* but not in *O. edulis*.

Histopathological responses of *Crassostrea gigas* to tributyltin fluoride were examined by Chagot et al. (1990). Aberrations included effects on digestive gland epithelium, gill epithelium, hemocytosis, and shell chambering. The digestive gland was the most susceptible organ to TBT exposure with effects being observed at concentrations as low as 2 ng L^{-1}. Other responses include chromosomal aberrations (Dixon and Prosser 1986), reduced larval survival (Beaumont and Budd 1984; Roberts 1987), and altered defense mechanisms of hemocytes (Fisher et al. 1990). A summary of the responses of oysters to tributyltin is presented in Table 4.

Exposure to Multiple Contaminants

As indicated by the distribution of chemical contaminant data discussed above, many contaminants do not occur in isolation, but organisms are often exposed to complex environmental mixtures of several lipophilic organic contaminants. Examination of responses to multiple classes of contaminants and the interaction of environmental factors, such as seasonal

Table 4. Effects of tributyltin on the Pacific oyster, *Crassostrea gigas*.

Concentration (μg L^{-1})	Effect	Reference
100	Inhibition of fecundity	His and Robert (1983)
10	Absence of the formation of trochophores	His and Robert (1983)
3-5	Absence of veligers Malformation of trochophores	His and Robert (1983)
1	Abnormal veligers Malformation of trochophores	His and Robert (1983)
0.5	Numerous larval anomalies, total mortality – 8 d	His and Robert (1983)
0.2	Perturbation in larval food assimilation	His and Robert (1983)
0.15	Shell chambering	Waldock and Thain (1983)
0.1	Normal D-larvae, slow growth high mortality – 12 d	His and Robert (1983)
0.06	Histopathological changes in digestive gland and gill	Chagot et al. (1990)

variability in response, can add new insight to the additive, synergistic or antagonistic effects of multiple contaminants. Differences in interspecific sensitivity and seasonal variation in intraspecific responses may be related to dependency on specific metabolic processes, such as storage and mobilization of lipid reserves during reproductive events (Capuzzo 1987).

Responses of *Mytilus edulis* collected along a contaminant gradient in Langesundsfjord, Norway, included alterations in lipid distribution, reductions in scope for growth, and increased occurrence of histopathological responses coincident with accumulation of PAHs and PCBs (Capuzzo and Leavitt 1988; Lowe 1988; Moore 1988; Widdows and Johnson 1988). Blue mussels transplanted from an uncontaminated site on Cape Cod, Massachusetts, to a site heavily contaminated with both PCBs and PAHs in New Bedford Harbor, Massachusetts, showed alterations in energetics, reduced allocation of lipids to reproductive processes, and reduced physiological condition, coincident with accumulation of high concentrations of both PCBs and PAHs (Capuzzo et al. 1989; Capuzzo, In press).

Widdows et al. (1990) examined changes in scope for growth in the turkey wing mussel collected along contaminant gradients in the waters surrounding Bermuda. Reduction in scope for growth as a result of both reductions in feeding rates and increases in metabolic expenditures correlated with significant accumulation of lead, tri- and di-butyltin, petroleum hydrocarbons and their polar oxygenated derivatives, and PCBs. Widdows et al. (1990) suggested that the decreases in feeding rates could be attributed to non-specific narcosis associated with the accumulation of low molecular weight hydrocarbons (Donkin et al., 1990), whereas the increases in energy expenditures through increased metabolic rates could be related to the accumulation of TBT through effects on oxidative phosphorylation. Turkey wing mussels collected along the same gradient showed changes in biochemical composition, especially in the ratio of neutral to polar lipids and carbohydrate content (Leavitt et al. 1990).

Capuzzo et al. (1989) observed the greatest differences in condition index and lipid reserves of blue mussels from contaminated and uncontaminated sites during the pre-spawning period, consistent with the accumulation and use of lipid reserves for reproductive development. After spawning, no differences in condition index were evident and lipid reserves were diminished to minimum levels. Roper et al. (1991) examined seasonal fluctuations in several biochemical and condition indices in *Crassostrea gigas* at a polluted and an unpolluted site in Manukau Harbor, New Zealand. Biochemical and condition indices varied seasonally and with site, with oysters from the contaminated site showing consistently lower condition indices and disruption in normal reproductive development. The observed responses could not be attributed to a specific contaminant, although the additional observation of shell chambering suggested that TBT exposure may at least have been partially responsible.

SUMMARY

Bioaccumulation of lipophilic organic contaminants in oysters is consistent with patterns of bioaccumulation predicted from general patterns observed in other species of marine bivalve molluscs. The major factors controlling the distribution of organic contaminants are the relative concentrations of individual contaminants in ambient waters and sediment pore waters of benthic habitats, modified to some extent by differences in partitioning between organisms and water (as indicated by differences in K_{OW}), and seasonal variations in lipid content. For some contaminants, such as tributyltin, both physical-chemical factors and non-specific binding may influence the ultimate fate of TBT. Although the range of studies conducted on biological responses of oysters to organic contaminants is more limited in scope than studies using the blue mussel, patterns of response at various levels of biological hierarchy are consistent and suggest that our understanding of toxic mechanisms may be transferred from one species to another.

Specific questions that remain unanswered are:

1. What are the mechanisms by which neoplastic conditions of bivalve molluscs are induced by lipophilic organic contaminants?

2. To what extent is biotransformation of contaminants involved in the responses of bivalve molluscs at different levels of biological hierarchy?

3. How does exposure to multiple classes of contaminants alter biological responses of bivalve molluscs?

4. Are responses in bivalve molluscs reversible with depuration of organic contaminants?

The eastern oyster may serve as an ideal test species to resolve these questions, particularly in estuarine habitats and the Gulf of Mexico where ambient concentrations of organic contaminants have been well documented.

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Chapter 16

Predators, Pests, and Competitors

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INTRODUCTION

Oyster bed communities, dominated in eastern North American coastal waters by the eastern oyster, *Crassostrea virginica*, furnish diverse habitats for many species of small fish, worms, crabs, and other invertebrates. These organisms may function as commensals, predators, pests, or competitors. Individuals typically classified as predators, pests, or competitors of oysters actually may function commensally under some circumstances, depending on food and space availability and the life stage of the organism. Commensal organisms neither noticeably hurt the structural integrity of the oyster's shell or internal body nor do they compete for food resources. Commensal behavior will not be considered further in this chapter.

Predators can easily locate oysters as prey; however, the oyster's thick carbonate shell must be penetrated before the tissue can be consumed. Some crabs and fish have developed appendages, or oral structures, or both, that are capable of quickly crushing the exterior shell, especially of smaller oysters. Larger eastern oysters, (> ca. 8 cm) — those often termed "market-size" — suffer less predation due to their large size and shell thickness. The wide euryhaline tolerance of oysters also prevents more stenohaline predators from reaching their potential prey. With the exception of the blue crab, *Callinectes sapidus*, most major predators of eastern oysters are restricted to saline waters above 15 ppt (Wells 1961).

Pests may at first appear similar to commensals but detract from the health of the oyster by either weakening the shell or body, or by competing for food. Fortunately for oyster populations, extensive damage from pests rarely occurs because pests are often small or uncommon.

It is often difficult to ascertain if an organism is a competitor because, rather than directly injuring oysters, these organisms out-compete them for space, food, or both. Ultimately, mortality of larval and juvenile oysters is increased. Long-term damage to the oyster community is minimized because most competing organisms are seasonal.

In this chapter, we discuss the important predators, pests, and competitors of eastern oysters in that order. In general, within these broad categories the species or groups that are responsible for the most harm are considered first.

PREDATORS

Predatory Gastropods

Predatory gastropods, common inhabitants of marine environments, can inflict severe damage on oyster populations. Three species, *Urosalpinx cinerea*, *Eupleura caudata*, and *Thais haemastoma*, are particularly destructive (Butler 1954; Galtsoff 1964; Hofstetter 1977). *Urosalpinx cinerea* is the most widely distributed of these species, being found at depths from the intertidal to ca. 30 m along the eastern and western Canadian shores, the entire east coast of the United States, and in Washington and California (Carriker 1953; Galtsoff 1964). *Eupleura caudata* is

found in the same waters as *U. cinerea* but is not as abundant (Galtsoff 1964). *Thais haemastoma* is distributed along the South Atlantic and Gulf coasts; the genus *Thais* is documented on the Atlantic and Pacific coasts (Galtsoff 1964).

Also known as drills and borers, these gastropods are characterized by heavy shells, slow movement, voracious appetites, and the characteristic hole that they bore in the prey's shell (Butler 1954; Carriker 1955b). Although the mechanism of boring by predatory gastropods is not well understood, boring seems to be accomplished through a combination of mechanical and and chemical action. Carriker (1961, 1973) suggests a chemo-mechanical action, with the abrasive action of the radula working on the shell after it has been softened by secretions from an accessory boring organ (ABO).

In *U. cinerea and E. caudata*, the ABO is a separate organ located in the anterior mid-ventral portion of the foot (Carriker 1961). Secretions from the ABO are neutral, not acidic, and may be a chelating agent or an enzyme (Carriker 1961). These secretions soften and loosen the surface crystals of shell calcite and also etch the non-biological inorganically deposited calcite and aragonite crystals in the shell matrix (Carriker 1961). Laboratory observations of these gastropods indicate that they alternate the use of the ABO and the radula in boring. During the early stages of boring, the snail rasps the periostracum from the shell surface. When active boring begins, the snail rasps for a few minutes; then it retracts the proboscis and brings the ABO directly over the hole. The ABO is then extended into the hole and is left there, sealed from outside seawater by the foot, for periods of a few to 55 minutes. The ABO is withdrawn and rasping continues. These processes alternate until the shell is successfully bored (Carriker 1961).

Apparently, *T. haemastoma* employs two methods to kill oysters. In the first, it drills mechanically, using radular movement termed the "band-over-pulley" method (Butler 1954; Gunter 1979), then inserts its long proboscis through the hole and extracts the oyster flesh (Burkenroad 1931; Chapman 1956). About 30% of oysters killed by *T. haemastoma* show no signs of mechanical injury to the shell, indicating that the snail may also use paralytic material to gain entry between the valve margins (McGraw and Gunter 1972; Gunter 1979). McGraw and Gunter (1972) reported the presence of a yellow mucus secreted by the hypobranchial gland that inhibited ciliary movement in the oyster, possibly acting as an anesthetic. Carriker (1961) suggests that *Thais* spp. also have accessory boring organs, so they may also use the chemo-mechanical method described above. It is possible that drilling is a secondary process in larger *T. haemastoma*, but is more important in smaller drills that may not be capable of producing sufficient toxin (McGraw and Gunter 1972).

Urosalpinx cinerea and *E. caudata* are the most common carnivorous gastropods along the Atlantic coast, being found from Canada to Florida (Chestnut 1956; Mackin 1959; Galtsoff 1964). Numerically, these gastropods can be very abundant on oyster beds; Chestnut (1956) reported as many as 106 m^{-2} in North Carolina, and MacKenzie (1970) found abundances ranging from less than 1 to 20 individuals m^{-2}. These drills are responsible for considerable mortality in commercial oyster populations, especially among juveniles (spat), because of their high abundances (Carriker 1955b; Galtsoff 1964). For Long Island Sound, MacKenzie (1981) reports consumption by *U. cinerea* and *E. caudata* to be 0.13 oysters $week^{-1}$ $drill^{-1}$ from early to mid June, increasing to 0.7 oysters $week^{-1}$ $drill^{-1}$ through late July. Total mortality of the oysters on the bed neared 15% by late July and about 50% by late August; about 33% of spat were killed during this period (MacKenzie 1981).

Salinity is one of the main physical factors governing the distribution of drills. *Urosalpinx cinerea* and *E. caudata*, although estuarine species, are limited to salinities above 15 ppt (Zachary and Haven 1973). MacKenzie (1981) reported a salinity requirement of greater than 12 to 17 ppt. Drills are euryhaline organisms and can tolerate exposure to unfavorable environmental conditions for short time periods by tightly closing their operculum and isolating themselves from the surrounding environment (Shearer and MacKenzie 1959). The duration of exposure to unfavorable salinity conditions is important in determining the effect of these fluctuations on drills (Zachary and Haven 1973). Both species tolerate short-term fluctuations in salinity better than

the greater stress of long-term exposure to low salinities (Zachary and Haven 1973).

At low salinities, *U. cinerea* and *E. caudata* decrease or halt drilling activity. As salinity increases above 15 ppt, activity increases in a characteristic pattern (Carriker 1955b; Zachary and Haven 1973). First, drills can attach themselves to substrates, then their locomotion increases, drilling and feeding commences, and, finally, at optimal salinities, they can deposit eggs (Zachary and Haven 1973).

Extremes in environmental temperatures also limit activity of drills (Manzi 1970a, b). Feeding ceases in most species of drills at about 10°C (Carriker 1955b; MacKenzie 1970, 1981; Manzi 1970a). Specifically, Manzi (1970a) demonstrated that the feeding rate of *E. caudata* increased as temperatures rose from 10 to 27°C and then decreased again as temperatures increased to 30°C. Maximum feeding occurred from 25 to 27.5°C. *Urosalpinx cinerea* reached peak feeding rates at 25°C and ceased feeding at 30°C (Hanks 1957). Feeding stopped in *U. cinerea* at low temperatures, and, in the northern part of their range, 75% of these gastropods also buried themselves in the substrate and became totally inactive (Carriker 1955a).

The combined effects of sub-optimal salinities and temperatures are particularly crucial. For example, *U. cinerea* can survive salinities as low as 12 to 17 ppt during warm temperatures but can survive even lower salinities in the winter (Carriker 1955b). Greatest mortality of *U. cinerea* occurred when fluctuating salinities fell below 9 ppt (Zachary and Haven 1973).

Urosalpinx cinerea and *E. caudata* are not limited to feeding on oysters: they also eat barnacles and mussels (Carriker 1955b; MacKenzie 1981). When feeding on oysters, *U. cinerea* preferentially attacks small or thin-shelled individuals (Carriker 1955b), possibly young oysters, that are growing rapidly, have high metabolic rates, and release metabolites that attract the drills (Haskin 1950). Drills can also more easily penetrate these oysters. Wood (1968) introduced the term "ingestive conditioning" to describe the tendency for predators to respond preferentially to effluents of previously ingested prey. *Urosalpinx cinerea* exhibits ingestive conditioning, selecting prey species on which they had previously fed rather than newly introduced prey species (Pratt 1974). In fact, Rittschof et al. (1984) established the existence of prey preference in the embryonic and larval stages of drills by exposing these stages to prey odors. Both *E. caudata* and *U. cinerea* are cannibalistic in the laboratory, even when other prey species are available (Carriker 1955b; Manzi 1970a). Cannibalism in *E. caudata* was positively correlated with temperature and feeding rates (Manzi 1970a).

The southern oyster drill, *Thais haemastoma*, is the most destructive eastern oyster predator from North Carolina to the Gulf of Mexico (Chestnut 1956; Menzel et al. 1958; Hofstetter 1977; MacKenzie 1977a). Two subspecies have been identified: *T. h. floridana* from North Carolina to Florida and the Caribbean and *T. h. haysae* found in Florida, Louisiana, and Texas (Galtsoff 1964). *Thais haemastoma* can be extremely abundant on productive oyster beds, even to the point of being more numerous than oysters. Population densities range from zero on beds in the low salinity Mobile Bay, Alabama, area to over 27,000 individuals hectare^{-1} in some high salinity areas (Hofstetter 1977). Drill larvae in the Gulf of Mexico are present in the water column from May to September, with peak abundances in July or August (Pollard 1973). Excellent reviews of the biology and ecology of *T. haemastoma* may be found in Butler (1954) and Gunter (1979).

The range of *T. haemastoma* is limited by its intolerance of low salinity (Chapman 1959; Hofstetter 1977). In nature, *T. haemastoma* requires salinities greater than 15 ppt (MacKenzie 1977a); at salinities below 10 ppt, the drills become immobile, and exposure to 7 ppt for 1 to 2 weeks is lethal (Galtsoff 1964). In laboratory studies, however, *T. haemastoma* has been shown to be more tolerant, surviving salinities as low as 5 ppt at 20°C for at least 10 d (Stickle and Howey 1975), whereas *T. haemastoma* were never observed in nature at salinities below 7.5 ppt (Stickle and Howey 1975; Garton and Stickle 1980). Therefore, it is likely that drills living in areas with diurnal tidal fluctuations are exposed to salinity regimes that are periodically less than optimal (Garton and Stickle 1980).

Under flood conditions producing sustained salinities below 15 ppt, drill populations are eliminated. Breithaupt and Dugas (1979) reported that high Mis-

sissippi River discharge accompanied by high rainfall in 1973 completely eliminated drills from oyster seed grounds east of the Mississippi in the Gulf of Mexico. They further found that even after two years, reinfestation had not occurred; however, they cautioned that repopulation rates may differ regionally due to physically controlling factors such as bottom type, current patterns, available food, and most importantly, salinity regimes.

Thais haemastoma is also affected by variability in temperature: it becomes torpid below 12°C and inactive at temperatures below 10°C (Gunter 1979; Garton and Stickle 1980). The temperature threshold for initiation of predation by this species is between 10 to 12.5°C, but varies with salinity (Garton and Stickle 1980).

Thais haemastoma is an extremely efficient predator and consumes large numbers of oyster spat (Chapman 1959). For example, in Mobile Bay, Alabama, it killed more than 80% of young oysters in a nine-month period in an area where salinity averaged over 20 ppt (May and Bland 1970; Hofstetter 1977). Chapman (1959) reported that *T. haemastoma* destroyed most of the spat on already depleted oyster beds and about half of the spat and large oysters on more densely populated, productive oyster beds. Although this predation can represent an enormous reduction in oyster populations, *T. haemastoma* prefers oysters as prey only if other prey species are scarce or absent (Butler 1954; Chapman 1959). In laboratory experiments, *T. haemastoma* selected mussels over eastern oyster spat, and chose barnacles, clams, and hydroids over larger oysters (Butler 1954).

Prey selection in *T. haemastoma* can be explained by food value (Garton 1986). This species appears to be an optimal forager, being able to recognize the food value of prey and then to select prey that will result in optimal growth (Palmer 1983; 1984). Foraging efficiency for *T. haemastoma* increases with its size and age because its ability to attack larger oysters increases with size (Brown and Richardson 1987). Small snails lack the individual ability to feed on large oysters but can group together to sucessfully attack oysters (Garton 1986; Brown and Richardson 1987). Group feeding is profitable to the individual only if the prey is large and difficult to handle. Thus, prey selection by different size classes of *T. haemastoma* can be explained based on the energetic considerations of food value versus handling time of prey (Brown and Richardson 1987).

Other species of predatory gastropods, often less abundant and ubiquitous, may have an effect on oyster populations. The crown conch, *Melongena corona*, occurs at densities of about one individual 100 m^{-2} in the Gulf of Mexico (Hathaway 1958) where it lives on soft, protected intertidal bottoms (Gunter and Menzel 1957; Menzel et al. 1958). It is possible that these numbers are an underestimate because individuals are often buried in the substrate (Menzel and Nichy 1958). *Melongena corona* feeds on a variety of molluscs and detritus and is often associated with oyster beds (Hathaway 1958), but probably only kills small numbers of oysters. Research by Hathaway (1958) and Menzel et al. (1958) suggests that *M. corona* may not excrete toxins, but may kill oysters directly or cause their adductor muscle to relax. Observations of oysters clamping the proboscis of crown conchs between tightly closed valves for hours (Hathaway 1958; Menzel et al. 1958) also support the idea that conches do not secrete a toxin that is deleterious to the oyster.

Busycon contrarium, the lightning whelk, is another species of gastropod that feeds on oysters. It occurs on oyster beds, often buried in the substrate, and may number as many as one individual 25 m^{-2} of reef surface (Menzel and Nichy 1958). It attacks oysters by chipping at the outer margin of the shell and then wedging the valves apart with its foot until the proboscis can be inserted (Carriker 1951; Menzel and Nichy 1958). During a summer study of inter- and subtidal oysters on a native oyster bed in Alligator Harbor, Florida, Nichy and Menzel (1960) observed mortality of at least 75 to 80% of oysters within the middle and lower subtidal levels of the reef. They attributed this mortality to *B. contrarium*.

Many methods of controlling carnivorous gastropods have been investigated because these species are so numerous and can cause large losses of commercially valuable oyster populations. These methods may be divided into three categories: physical, chemical, and biological.

Physical methods of controlling drills include trapping and burying. Trapping success depends on using bait as an attractant, replenishing the bait fre-

quently, and maintaining good quality bait in the traps (Andrews 1955b; McHugh 1956, 1957). McHugh (1956) used a wire mesh tray filled with seed oysters to attract and catch the drills. The catch rate of drills declined with the amount of time the trap was left out because the number of drills attracted to the bait reached equilibrium, i.e., the number of drills present on the bait remained constant. The amount and quality of bait are also very important; thus, traps must be checked and rebaited often for the catch rate to remain high (McHugh 1957). Trapping of drills has been used rarely as a method of control. Another method used a modified agricultural plow to bury drills 5 to 6 cm below the sediment surface (Loosanoff and Nomejko 1958). However, this method was ineffective as nearly all the drills crawled back to the surface. Furthermore, this is not a method that could be applied to oyster beds even if it was effective, as the oysters would also be killed by burial.

Chemical methods for controlling drills include the use of substances toxic to drills, such as copper (Glude 1957). Drills, in common with other gastropods, exhibit reluctance to cross barriers of metallic copper, due to a characteristic of the copper and not the physical presence of a barrier (Glude 1957; Huguenin 1977). To be effective, the copper barrier must be as wide as the largest drill to be excluded (Huguenin 1977). Such barriers are not totally effective for thaids because the pelagic larvae can be carried over the barrier, or larger drills can be transported across the barrier by other organisms such as horseshoe crabs (MacKenzie 1962; Huguenin 1977). *Urosalpinx cinerea* and *E. caudata*, which do not have pelagic larvae, are more easily controlled and reinfestation rates are lower (Pollard 1973). This method has never been used on a commercial scale.

Two pesticides, "Sevin" (a methyl carbamate) and "Polystream" (a mixture of chlorinated benzenes), were studied several decades ago for possible use in control of drills (Wood and Roberts 1963; Haven et al. 1966). "Sevin" is highly toxic to crustaceans and does not kill the drills immediately, whereas "Polystream" kills the snails immediately and was thought at that time to inflict little or no damage on bottom communities (Wood and Roberts 1963). Treatments with these pesticides were typically most effective during April and May (MacKenzie 1971). Drills that were not killed immediately did not feed normally for a few months, resulting in substantially decreased oyster mortality during that period (MacKenzie 1971). This method was used in Long Island Sound in the 1960s, when about 100 hectares of oyster bottom were effectively treated. Since then, use of these chemicals on natural oyster bars has not been approved by the U.S. Food and Drug Administration. Today, it is widely recognized that the application of toxic chemicals may have far-reaching adverse effects beyond the use intended. Thus, toxic chemical applications are more closely regulated.

Biological methods for controlling drills include the introduction of natural predators into the local environment. *Parorchis acanthus*, a digenetic trematode parasite of *T. haemastoma* (Cooley 1958, 1962), destroys the drill's liver, and to a lesser extent, its gonad. Although individual infections of *P. acanthus* in *T. haemastoma* can be heavy, natural infection incidence is low, so use of this parasite as an effective method of biological control is doubtful (Cooley 1958, 1962). A second possible but unlikely biological control may be the moon snail, *Polinices duplicata*, that feeds on drills (Flower 1954). Ecological implications must be carefully considered when using any of these methods. Both oysters and benthic community constituents may be injured or destroyed along with the predators if the methods of control are not wisely chosen. In addition, the absence of drills may result in overcrowding of spat because drills can greatly influence spat survival (Andrews 1955a).

Predation by Crabs

Crabs kill many eastern oysters, particularly spat and juveniles (Galtsoff 1964). The stone crab, *Menippe mercenaria*, mud crab, *Panopeus herbstii*, rock crab, *Cancer irroratus*, and blue crab, *Callinectes sapidus*, are all responsible for considerable mortality in natural oyster populations along the Atlantic and Gulf coasts of the United States (Menzel and Hopkins 1956; Krantz and Chamberlin 1978; Bisker and Castagna 1987).

Species of stone crabs occur along the Atlantic coast from North Carolina to the Gulf of Mexico (Menzel and Hopkins 1956). On the Gulf coast, *M. mercenaria* is the most numerous species on oyster

beds and is responsible for considerable oyster mortality (Menzel and Nichy 1958; Mackin 1959; Hofstetter 1977). Stone crabs have large, heavy claws so *M. mercenaria* is capable of crushing even large, market-size oysters (Mackin 1959). It is probable that the damage to and loss of market-size oysters by stone crabs is often underestimated because the destruction of the shell is so complete (Mackin 1959).

Stone crabs are intolerant of low salinity, being limited to regions where the average salinity remains above 12 to 15 ppt throughout the year (Menzel et al. 1958). High salinity episodes during droughts, similar to that which occurred in Apalachicola Bay, Florida, during 1956, probably allow the stone crab to become established in oyster communities that had previously been outside their salinity range (Menzel et al. 1966). Stone crabs occur in lower intertidal and subtidal areas and will feed only when covered by water, remaining in their burrows if exposed by low tides (Nichy and Menzel 1960).

Population density estimates for stone crabs are limited; however, Menzel et al. (1958) estimated one crab (50 to 100 mm carapace width) m^{-2} on Florida oyster beds. In Louisiana, density estimates were as high as 8,649 stone crabs $hectare^{-1}$ (Menzel and Hopkins 1956). Cumulative predation rates on all sizes of oyster by stone crab populations have been estimated to be about 0.6 oysters $crab^{-1}$ d^{-1} or 219 oysters $crab^{-1}$ $year^{-1}$ (Menzel and Hopkins 1956). Stone crabs may prefer barnacles as food when available, but in their absence will typically choose small oysters over large ones (Powell and Gunter 1968).

The mud crab, *Panopeus herbstii,* is the largest xanthid crab in Chesapeake Bay, and the cause of considerable mortality on Chesapeake Bay oyster beds each year, with average predation rates in laboratory experiments as high as 21.5 spat $crab^{-1}$ d^{-1} (Bisker and Castagna 1987). Reported population densities vary from 13 to 103 crabs m^{-2} (Bisker and Castagna 1987). *Panopeus herbstii* remains active at temperatures below 12°C and tolerates salinities from 10 to 34 ppt (Schwartz and Cargo 1960; Bisker and Castagna 1987). It is not migratory and remains a constant threat to oyster populations throughout the year (Bisker and Castagna 1987).

Panopeus herbstii is a very efficient predator with a large moliform tooth on the crushing edge of the dactyl of the major claw. This tooth allows the crab to open oysters twice the size of those opened by other crab species of similar carapace size (Bisker and Castagna 1987). Predation rates for *P. herbstii* are directly proportional to crab size and inversely proportional to oyster size (Bisker and Castagna 1987). In laboratory studies, mud crabs with mean carapace widths of 7.1 and 25.2 mm caused significant mortality to oyster spat that were 8.1 and 24.6 mm shell height, respectively. Daily predation rates for various species of mud crabs have been estimated at 0.4 to 19 oyster spat $crab^{-1}$ d^{-1} (Bisker and Castagna 1987).

The mud crab, *Neopanope texana,* demonstrates different predation patterns on spat depending on whether the spat are attached or unattached to substrates. It will only feed on attached individuals less than 10 mm in size, but will attack spat up to 25 mm if they are unattached. Spat that are attached to substrate are much more difficult for the crab to manipulate and open successfully (Krantz and Chamberlin 1978). Bisker and Castagna (1987) suggest that this difficulty may be true for other species of mud crab as well.

The blue crab, *Callinectes sapidus,* also preys on oysters along the Atlantic and Gulf coasts (Menzel and Hopkins 1956; Menzel et al. 1958; Hofstetter 1977). Nichy and Menzel (1960) observed that blue crabs followed the incoming tide as it covered an oyster bed and tested each oyster, bypassing many until they found a suitable choice. Some researchers have postulated that *C. sapidus* passes over adult oysters because they are too large for the crabs to open the shell (Menzel and Hopkins 1956; Nichy and Menzel 1960); however, *C. sapidus* have been observed to open 7 cm (shell length) clams, *Rangia cuneata,* a species with an exceptionally thick and strong shell (V. S. Kennedy, Horn Point Environmental Laboratory, pers. comm.).

Blue crabs use different methods for opening oysters, depending on the size of the prey. Small spat, less than 15 mm shell height, are typically crushed, whereas the shells of larger oysters are usually chipped around the edge with the chelae. Meat is extracted with mouth parts and chelae tips. The largest crabs are able to crush an adult oyster's umbo (Krantz and Chamberlin 1978). Cultchless oyster spat, initially set on mylar sheets, are particularly easy for *C. sapi-*

dus to open. These spat, which have a weak spot on the lower valve where they were attached to mylar substrate, are easily crushed (Krantz and Chamberlin 1978). Krantz and Chamberlin (1978) reported 79 to 99% mortalities in cultchless oysters, 3 to 40 mm long, kept in uncovered trays in the upper Chesapeake Bay. The ability to open larger spat increases with increasing crab size; blue crabs that were 9.3, 24.5, and 85.5 mm in carapace width caused significant mortality to spat that were 3.4, 13.9, and 24.4 mm in shell height, respectively (Bisker and Castagna 1987). Menzel and Hopkins (1956) estimated predation rates of blue crabs on oysters to be 19 spat crab^{-1} d^{-1} on Florida oyster beds. Bisker and Castagna (1987) found a high efficiency of predation in blue crabs, particularly on spat, with a rate of 16.7 spat (24.4 mm shell height) crab^{-1} d^{-1} in Virginia.

Blue crabs are very mobile, making population densities difficult to estimate (Nichy and Menzel 1960); however, Wells (1961) estimated about four crabs for every 6 m of oyster reef edge. The activity of *C. sapidus* is limited by low temperatures such that the crabs cease feeding when temperatures are below 13°C (Bisker and Castagna 1987).

Elner and Lavoie (1983) compared predation rates for the rock crab, *Cancer irroratus*, mud crab, *Neopanope sayi*, and the American lobster, *Homarus americanus*, on eastern oysters. They found these crustaceans could not attack oysters larger than 30 to 35 mm shell length. The best method for controlling crab predation on oyster populations appears to be isolation of the spat in protected trays, possibly until they reach 30 to 35 mm. This technique is impractical for natural oyster beds, however.

Polyclad Flatworms

Polyclad turbellarian flatworms are common members of many oyster bed communities along all three coasts of North America (Woelke 1957; Mackin 1959; Landers and Toner 1962; Finucane and Campbell 1968; Hofstetter 1977). *Stylochus ellipticus* and *Stylochus frontalis* (=*inimicus*) are the most common species along the Atlantic and Gulf coasts. The northern limit of the range of *S. ellipticus* is Prince Edward Island, but the species is common south of Massachusetts and its range extends to Texas (Hyman 1940). It is the most important species in Chesapeake Bay (Webster and Medford 1961; Christensen 1973). *Stylochus frontalis* is common from Florida to Texas, perhaps reaching as far north as the Carolinas (Hyman 1940). Also known as "oyster leeches" (Pearse 1938; Pearse and Wharton 1938; Galtsoff 1964; Finucane and Campbell 1968) and "oyster wafers" (Menzel et al. 1958), these species have been associated with incidences of high mortality in eastern oyster populations, particularly among spat (Hopkins 1955; Loosanoff 1956; Woelke 1957; Mackin 1959; Provenzano 1961). Nevertheless, their relative importance as oyster predators in natural populations remains undetermined (Gunter 1953).

The turbellarian flatworms are thin, flat, and elliptical, with slightly wrinkled margins (Pearse and Wharton 1938; Webster and Medford 1961). They vary in color within a species from brown or gray to dark pink or orange. Paired tentacles are located near the anterior end and numerous eyespots are found around the anterior margin and also on the tentacles. The mouth with the pharynx, which can be everted a great distance, is about halfway back from the anterior end, on the ventral side (Webster and Medford 1961).

Flatworms are hermaphroditic and extremely fecund: estimates of egg production for *Stylochus frontalis* are 21,970 eggs flatworm^{-1} month^{-1} (Pearse and Wharton 1938; Woelke 1957) and up to 39,000 eggs over 48 h at 21°C for *S. ellipticus* (Chintala and Kennedy 1993). The larvae, which are pelagic and metamorphose after 11 to 21 d (Pearse and Wharton 1938; Woelke 1957), often settle in very high numbers on oysters, as many as one flatworm shell^{-1} d^{-1} (Provenzano 1961). Flatworms grow rapidly (Pearse and Wharton 1938) and become fairly large. *S. frontalis* has been reported as long as 51 mm, and *S. ellipticus* as large as 25 mm (Pearse and Wharton 1938; Webster and Medford 1961). Most species live for one year (Pearse and Wharton 1938), becoming sexually mature early in their life cycle. *Stylochus frontalis* has been reported to become sexually mature after just two months (Pearse and Wharton 1938).

Flatworm species vary in their tolerance to changes in salinity and temperature. *Stylochus frontalis* flourishes at rather high salinities, usually greater than 15 ppt, but can also tolerate salinity as low as 6 ppt (Pearse and Wharton 1938). Pearse and Wharton

(1938) further noted that *S. frontalis* does not lay eggs at salinities below 15 ppt, and does not crawl at temperatures below 12°C and above 34°C.

Stylochus ellipticus also is somewhat limited by temperature and salinity. In laboratory experiments, Landers and Toner (1962) showed that it can survive abrupt changes in salinity from 27 ppt to 7.5 ppt at room temperature. There was 20% mortality at 5 ppt and 100% mortality at salinities from 0 to 2.5 ppt. Individuals acclimated to 5 ppt, however, could survive salinities of 2.5 ppt if the transition was slow. Provenzano (1961) reported that settlement of *S. ellipticus* occurred only when the salinity was below 20 ppt. The rate and extent of locomotion of *S. ellipticus* varies erratically with temperature but normally decreases as temperature decreases (Landers and Toner 1962). Landers and Rhodes (1970) reported that temperatures below 10°C led to a progressive decrease in predation rate and that the lower salinity limit for *S. ellipticus* appeared to be about 5 ppt. The combined effects of changes in both temperature and salinity are probably more important than if only one factor is changed (Landers and Toner 1962).

Flatworms of the genus *Stylochus* enter oysters through their partially gaping valves. *Stylochus ellipticus* preferentially attacks small oysters but large flatworms can kill oysters as large as 6 cm (Landers and Rhodes 1970). In laboratory studies, as few as three *S. frontalis* killed and ate an adult oyster in a week (Finucane and Campbell 1968). *Stylochus* spp. may preferentially attack already weakened or diseased oysters (Hofstetter 1977).

Densities of flatworms vary according to year, location, and season. Densities of 1.4 to 50 *S. frontalis* m^{-2} have been reported from Apalachicola Bay, Florida (Menzel et al. 1958). *Stylochus* spp. are also common on beds in Galveston Bay, Texas, where as many as 500 flatworms per sample have been collected (Hofstetter 1977). In Florida, 1,300 flatworms $bushel^{-1}$ of oysters have been reported on some beds. This infestation resulted in 30 to 90% mortality in the spat population (Hofstetter 1977).

Evidence for the importance of flatworms in oyster predation comes from laboratory feeding experiments and field observations of flatworms in newly dead oysters. For this reason, there is still some disagreement as to the extent of damage caused by these animals to natural oyster populations (Hofstetter 1977). Recent field research by Newell and Kennedy (1992) examined the survival of three sizes of spat in mid Chesapeake Bay. They found that at 7 mm spat are relatively immune to flatworm predation. Based on their field and laboratory studies, they postulated that in early and mid-summer, flatworms may be too small to feed on oysters; thus, in years when spatfall is in the early summer, increased recruitment may occur. Results of this research and work by Newell et al. (1989) indicated that there is a very low survival rate of spat smaller than 2 mm on natural cultch, probably due to predation by *S. ellipticus*. In an early study, Provenzano (1961) reported 100% mortality of spat in isolated salt water ponds used for oyster culture in Martha's Vineyard, Massachusetts. These ponds had limited exchange with the surrounding environment and other natural oyster predators, with the exception of *S. ellipticus*, were absent. As many as 15 *S. ellipticus* were found within the valves of a single oyster shell, thus implicating this flatworm as the culprit in the mass mortality.

Although flatworms will attack barnacles and oysters, prey preference may be more a consequence of environmental factors than of choice (Christensen 1973). In studies comparing the preference of flatworms for barnacles or oysters, flatworms preferred prey items that corresponded to those common in their natural habitat, e.g., flatworms that were taken from areas where barnacles were common and oysters were rare preferred barnacles (Landers and Rhodes 1970). Ultimately, salinity may be the governing factor in prey selection. If barnacles tend to be more common in higher salinity regions and oysters more common in lower salinity regions, prey availability may be controlled by salinity and, thus, prey preference may be limited by the environment (Landers and Rhodes 1970). Another possibility is that temporal events in a location can control prey availability. In some regions, barnacles settle before oysters, providing the basis of the prey population for the flatworms.

Extensive mortality of oysters due to these polyclad flatworms has typically occurred under crowded mariculture conditions (Woelke 1957; Provenzano 1961). Under most conditions, barnacles are probably the preferred prey; however, mariculture condi-

tions of high oyster density may yield circumstances conducive to predation on oysters by turbellarian worms (Christensen 1973). Control of flatworms in trays of hatchery oyster seed may be accomplished by submersing the seed in either fresh or highly saline water (Gunter 1953; Woelke 1957; Provenzano 1961). Such submersion immediately eliminates flatworms but further precautions must be taken to protect oysters from reinfestation.

Starfish

The starfish, *Asterias forbesi,* is a common and highly destructive oyster predator in Long Island Sound and areas northward (Mackin 1959; Galtsoff 1964; MacKenzie 1970). It is responsible for considerable mortality each year, especially of spat, and during years of extreme abundance can cause catastrophic losses in oyster populations (Mackin 1959; Galtsoff 1964; MacKenzie 1970). As with many oyster predators, starfish cannot invade beds where salinity falls below 16 to 18 ppt (Galtsoff 1964).

Mackin (1959) reported that sudden, large increases in starfish populations appeared to occur in 7-year cycles. These "plagues" of starfish do not depend on availability of oysters, but are probably the result of a high percentage of survival of the free-swimming larvae and a successful set of young starfish (Mackin 1959; Galtsoff 1964). In New England waters, starfish reproduce just before oysters spawn, so a year class of oyster spat can be eliminated by an unusually successful starfish set (Galtsoff 1964).

Starfish move slowly and randomly across the bottom (Galtsoff 1964) and during these feeding forays can move a few hundred meters in several weeks (MacKenzie 1970). Galtsoff (1964) stated that starfish can also be carried passively by tidal currents, and in this way are transported to new oyster grounds, but this is probably not correct.

MacKenzie (1970) reported that young starfish on 15 commercial oyster beds in Connecticut waters were responsible for the death of about 1% of the oyster spat during a 6-month period in 1963, whereas adult starfish killed about 25% of the spat. Starfish use two different methods to open oysters: (1) they use force and (2) they secrete an anesthetic substance from their stomachs to numb the oysters and cause them to gape (Galtsoff 1964; MacKenzie 1970). When the valves gape about a millimeter, the starfish extends its thin-walled stomach into the opening and begins to digest the oyster. Although crabs leave chip marks on oyster shells and boring gastropods often leave holes, it is uncertain whether starfish leave any identifying mark on their prey. Galtsoff (1964) contends that they leave none, whereas MacKenzie (1970) reports that the shells of oysters preyed upon by starfish have the distinct appearance of being filed to a blunt edge around at least part of the circumference of the shell where the starfish's tube feet pulled off the thin edge of the oyster's shell.

In oyster-producing areas, control of starfish populations becomes particularly important when the starfish are highly abundant. Starfish can be killed by submersion in highly saline water or by the addition of quicklime spread over the oyster beds (Shearer and MacKenzie 1959; Galtsoff 1964; MacKenzie 1970). Mops dragged over the bottom have also been used to entangle and capture starfish. The formerly common practice of chopping up captured starfish and dumping the bodies overboard only results in increased numbers of starfish, as the chopped-up pieces regenerate new arms.

Fish

Fish are common oyster predators in many estuarine communities, with species composition varying with community, depth, temperature, and local conditions. Fish efficiently locate oysters through visual contact, although this is sometimes supplemented by chemoreception. For instance, the goby, *Gobiosoma bosci,* uses volatile compounds emitted from the oyster to determine the oyster's location (Hoese and Hoese 1967). In Chesapeake Bay, fish predators include toadfish, *Opsanus tau,* and the cow-nosed ray, *Rhinoptera bomasis,* (Krantz and Chamberlin 1978). MacKenzie (1970) reported that the summer flounder, *Paralichthys dentatus,* caused damage to oyster populations in Connecticut waters. Sheepshead, *Archosargus probatocephalus,* and skates, *Raja* spp., can be major oyster predators in the Gulf of Mexico (Menzel and Hopkins 1956; MacKenzie 1977a). Drumfish, *Pogonias cromis,* may be a particularly significant oyster predator, ranging along the eastern

United States and Gulf coasts. In Louisiana, drum fish consume oysters by crushing their shells (Matthiessen 1971). Nearly all oyster predators are limited to consuming smaller oysters but drumfish and cownosed ray can prey upon oysters over ca. 8 cm (Smith and Merriner 1978).

Some fish may also function as a positive influence in oyster communities. Toadfish, for example, although capable of eating oysters, prefer crabs and thus reduce overall predation on the oyster population (McDermott 1964). Skilletfish, *Gobiesox strumosus,* an estuarine and marine species on the Gulf and Atlantic coasts, lays its eggs in empty oyster shells, resulting in shells that are in cleaner condition for subsequent settlement by oyster larvae (Runyan 1961). The author states, however, that *G. strumosus* may act as a vector for oyster parasites. The above complex interactions exemplify why great caution must be exercised when determining the role of an organism in the community because organisms often function in more than one ecological capacity.

Predation on Oyster Larvae

Adults of many benthic invertebrates, especially sessile species, are unable to escape the physical and biological variability of their environment. Thus, many adult organisms, being quite vulnerable, have developed elaborate defense, feeding, and protective mechanisms. Larvae, however, are smaller, less protected and more vulnerable to predation; they are fed upon by pelagic and benthic filter feeders (Mileikovsky 1974). Benthic predators that have been studied as to their effects on oyster larvae include the sea anemone, *Diadumene leucolena,* barnacle, *Balanus improvisus,* and two species of solitary ascidians (MacKenzie 1977b; Steinberg and Kennedy 1979; Osman et al. 1989).

Diadumene leucolena has been thought to limit oyster populations throughout the Chesapeake Bay region (MacKenzie 1977b). Densities of sea anemones on oyster beds have been estimated at 104 individuals m^{-2} for live oyster beds and 176 individuals m^{-2} on shell beds and sea anemones have been estimated to occupy about 15 to 25% of the surface area of live shells (MacKenzie 1977b; Steinberg and Kennedy 1979). It has been thought that on oyster beds where high densities of *D. leucolena* occur, many oyster larvae are consumed by this species. Observations by Steinberg and Kennedy (1979) show that barnacles, under laboratory conditions, are capable of ingesting or damaging oyster larvae in sufficient numbers to cause a large reduction in larval abundance. Also in support of these findings is the presence of semi-digested oyster larvae in the guts of barnacles. Osman et al. (1989) conducted experiments with the solitary ascidians *Ciona intestinalis* and *Styela clava* in the presence of eastern oyster larvae. The ascidians preyed on 29% to 96% of larvae in the experiments.

Controlling sea anemone and barnacle populations on productive oyster beds has been suggested as a means to increase successful setting of oyster larvae and thereby the number of oysters reaching market size (MacKenzie 1977b). However, later work by MacKenzie (1981) in both laboratory and field studies suggest that although potentially important, most benthic invertebrates (including oysters) play at most a minor role in larval mortality.

Purcell et al. (1991) examined predation on planktonic larvae by the scyphozoan, *Chrysaora quinquecirrha,* and ctenophore, *Mnemiopsis leidyi.* Their work showed that medusae of *C. quinquecirrha* do not ingest or digest bivalve veliger larvae; however, scyphistomae of the species do digest veliger larvae. The ctenophore, *M. leidyi,* does prey on bivalve veligers. Although *C. quinquecirrha* medusae may consume some bivalve veligers, ultimately they may reduce predation on these larvae by also feeding on ctenophores.

PESTS

Cliona spp.

Some of the most common intertidal pests of oysters are estuarine sponges belonging to the family Clionidae of which seven species of the genus *Cliona* are found along the Atlantic and Gulf coasts of the United States. Two of the most important species to oyster ecology are *Cliona truitti,* found in lower salinity regimes, and *Cliona celata,* more abundant at higher salinities (Hopkins 1962). These two species are sometimes used as indicator species; abundant *C. celata* signifies that high salinity has prevailed for several months, whereas *C. truitti* indicates prevalence

of salinities of 10 to 15 ppt (Hopkins 1962). These species are easily distinguished: *C. celata* has yellow papillae that form large coarse holes in the oyster shell, whereas *C. truitti*, and all other estuarine clionids, forms small holes.

All *Cliona* spp. can excavate habitations in oyster shells. The oyster's calcareous substratum completely encloses the sponge body except for papillae that extend through the shell to the outside environment. Hatch (1980) describes three major hypotheses about the mode of penetration of Clionidae into their substratum: "an extensive chemical dissolution of the substratum, usually assumed to be the action of some acidic etching agent; an exclusively mechanical removal of the substratum; and a chemomechanical mechanism in which the substratum is chemically softened or loosened and subsequently mechanically removed. The most tenable mechanism is the combination of a localized chemical dissolution coupled with mechanical dislodging of fragments of the substratum and their subsequent transport out of the sponge galleries."

Oyster beds have varying levels of sponge infestations but some geographical regions appear to be more prone than others (Galtsoff 1964). It is not always obvious why oysters become infested. On the eastern shore of Chesapeake Bay, Hopkins (1962) observed that at one study site, *C. celata* was found on most *Urosalpinx cinerea* but rarely on oysters. *Cliona* spp. also may inhabit dead oyster shells. Guida (1976) states that the burrowing alpha stage of *C. celata* is restricted to the shell; however, it can grow out of its galleries and eventually cover the shell (beta stage) and even eventually become free-living (gamma stage). The beta and gamma stages are subject to greater predation pressure due to their exposure outside the shell.

Guida (1976) observed that many species of organisms, including molluscs, crustaceans, polychaetes, and echinoderms eat exposed *C. celata* under laboratory conditions. His field studies corroborated his laboratory observations, showing that predation by these species may be important in controlling sponge populations. Furthermore, Guida (1976) postulated that sponges are potentially important to community structure and energy flow in oyster reef communities because they are consumed by many predators within the community, not just a few rare specialists.

Oyster shells are weakened by the excavations of *Cliona* spp. The extensive matrices or galleries formed by the sponge allow the shell to be broken easily. Heavily perforated shells predispose the oyster to predation by organisms such as fish and crabs. In addition, some oyster competitors, for example the hooked mussel, *Ischadium recurvum*, can insert byssal threads into the holes made by the sponge and become more firmly attached (Turner 1985).

Cliona spp. rarely erode the oyster shell through to the shell cavity, usually doing so only in old, heavy infestations. If the oyster shell is penetrated completely, the oyster quickly lays down conchiolin to prevent contact between tissues and the sponge (Galtsoff 1964). If the oyster is unable to protect its tissues through shell deposition, the sponge becomes pathenogenic, forming adhesions on the oyster's tissue (Overstreet 1978).

The continuous expenditure of energy by the oyster to deposit new shell might have implications on reproduction and sex determination in oysters. Bahr and Hillman (1967) conducted a study examining four groups of oysters: "fed" oysters with normal and filed shells and "starved" oysters with normal and filed shells. "Starved" oysters had marked retardation of the gonads and males appeared to predominate in oysters that were filed. The authors speculated that whether or not food was limited, oogenesis might require more energy than production of sperm. If the shells of oysters are damaged and gametogenesis occurs, the limited energy available may then favor sperm production. Although this is a single study, it brings up interesting questions. For example, when oysters are damaged, do the above processes become important in influencing population structure? More work needs to be conducted to answer this question (see also Thompson et al., Chapter 9).

Polydora spp. and *Boccardia* spp.

Members of the genus *Polydora*, also known as mud worms, are found along the Atlantic and Gulf coasts of the United States. The two main species of Polydora of importance to oyster ecology, *P. websteri* and *P. ligni*, live subtidally and bore into oyster shells,

resulting in mud-filled shell blisters (Larsen 1978). These species, together with *Boccardia hamata*, another mud worm, are frequently found in association with oysters, although associations with hermit crabs, other bivalves, barnacles, and objects such as floating wood, also have been recorded (Galtsoff 1964; Larsen 1978).

The early life histories of *P. websteri* and *P. ligni* are similar: both species lay encapsulated eggs that are attached to the inner surface at the tube in which the mud worm lives (Galtsoff 1964). *Polydora ligni* lives in tubes consisting of mud particles held together by mucus secreted by the antennae and body surface (Galtsoff 1964). The eggs develop in 4 to 8 d, depending on the temperature; larvae have a planktonic stage of an undetermined duration (Galtsoff 1964). The larvae settle on the outer surface of an oyster shell and immediately bore into the shell cavity, subsequently crawling between the mantle and shell (Haigler 1969). *Polydora websteri* uses a chemical agent to penetrate all layers of the oyster shell; *P. ligni* does not penetrate the calcareous substrate or form blisters (Haigler 1969). The boring process for *P. websteri* requires about 24 h to penetrate the chalky deposits, 7 d for calcite-ostracum, and 30 d for conchiolin (Haigler 1969).

Polydora spp. do not directly attack the host's tissues; however, mortality or ill health may result indirectly. For instance, when oysters are infected with high abundances of *P. websteri*, energy is directed towards shell maintenance and repairs rather than growth or reproduction (Owen 1957; Larsen 1978). The ensuing poor health also may make the oyster more susceptible to disease (Korringa 1952). High infestations make the shell brittle (Skeel 1979), rendering the oyster prone to predatory attacks.

Boccardia hamata is closely related to *Polydora* spp. It occurs in the internal galleries of *C. virginica* shells and in tubes on the oyster shells. It is quite possible that this species also actively burrows into the shell, thus making it a potential oyster pest (Larsen 1978). *Boccardia hamata* deposits its eggs in a long string. Development proceeds in the string until the 6-setiger stage when the larvae are released into the plankton (Dean and Blake 1966). The pelagic larvae grow to the 16 or 17-setiger stage and then metamorphose, with the entire pelagic stage lasting about 3 weeks (Dean and Blake 1966).

Two types of chemical control could possibly be used for *Polydora* spp. and *Boccardia* spp. (MacKenzie and Shearer 1959): (1) vermifuges that cause the mud worms to emerge from their tubes and leave the host's shell, or (2) vermicides that kill the mud worms in the tubes, ordinarily not eliciting emergence. Sodium chloride, however, is probably the most practical method for killing these mud worms; 87 to 98% of *P. websteri* were killed after a 10 to 15 minute submersion in saturated salt solution followed by at least 15 minutes of drying (MacKenzie and Shearer 1959). In the past, oysters were immersed in O-dichlorobenzene for not longer than 3 h and then transferred to seawater to facilitate emergence of mud worms (MacKenzie and Shearer 1959). Today chemicals such as these are known to be highly toxic and are no longer used.

Pea Crabs

The pea crab, *Pinnotheres ostreum*, is symbiotic with a variety of hosts including bivalves, polychaetes, and echinoderms (Kruczynski 1974). Pea crabs enter the shell cavity of oysters and remain there for most of their lives. Reported incidences of infection vary among oyster beds and range from 6 to 80% (Sandoz and Hopkins 1947; Haven 1958).

Like many other oyster commensals and pests, *P. ostreum* is restricted to high salinities. Beach (1969) found that embryonic development was not possible at salinities below 15 ppt. As salinity increases, there is an increase in the number of oysters infected with *P. ostreum* (Flower and McDermott 1952). In addition to salinity, submergence also appears to be important, as highest infection rates occur subtidally. Beach (1969) hypothesized that pea crabs have a greater opportunity to find new hosts with increasing periods of submergence.

The life history of pea crabs has been well documented by Silas and Alagarswami (1967). Pea crabs undergo five life stages before reaching maturity. First stages of males and females are adapted to swimming, searching, and invading a host (Christensen and McDermott 1958). Pea crabs grow to maturity

in the mantle cavity of the oyster (Stauber 1945). Copulation occurs within the oyster with the small, free-living, hard-stage male entering an oyster containing a female, copulating with the female, and then returning to its free-living existence (Christensen and McDermott 1958; Barnes 1980). Eggs are carried by the female for 3 to 5 weeks, followed by a larval period of 3 to 4 weeks. Infestation of oysters by new pea crabs most likely occurs in summer or autumn (Stauber 1945; Christensen and McDermott 1958).

Pinnotheres ostreum probably locates new hosts through combined visual and chemosensory techniques. Chemoreceptors have been observed on the antennules of *P. maculatus* and have been indicated as important in several of its life stages (Derby and Atema 1980). The pinnotherid crabs live primarily on the oyster's gills in the mantle cavity, except at very small sizes when they can be found in the water conducting channels in the back of the gills (Haven et al. 1978). Pearce (1964) notes that some life stages of *P. maculatus* are free-living while others are found in the host. Indirectly, pea crabs affect their hosts by removing food particles captured by the oyster gill and thereby depriving the oyster of possible nutrition. Oysters with pea crabs were found to have less meat per shell cavity volume (Haven 1958). Pea crabs also inflict direct injury when attached to the oyster's demibranchs, causing gill erosion and interfering with both the ability and efficiency of food collection (Stauber 1945). Even though gill damage may be extensive, rapid recovery can occur once the pea crabs are removed.

Boonea impressa

The ectoparasitic gastropod, *Boonea impressa,* is commonly found on oyster beds along the Atlantic and Gulf coasts of the United States. Numbers of up to one hundred snails have been reported on a single oyster (Hopkins 1956; Robertson 1978). *Boonea impressa* feeds, as do other pyramidellids, by attaching its proboscis to the oyster's mantle and then piercing the host's body wall with a buccal stylet and using a buccal pump to suck the body fluids (Fretter and Graham 1949; Allen 1958; Maas 1965).

Boonea impressa has a life span of about one year. Larval development lasts from 3 to 5 d, followed by a lecithotrophic period of one week (White et al. 1985). Powell et al. (1987a) observed that on some oyster beds, juvenile *B. impressa* preferred the slipper shells *Crepidula plana* as a host, whereas adults were found almost exclusively on eastern oysters. Powell et al. (1987a) further commented that juvenile snails may feed on hosts different from those of adults for a variety of reasons, including: (1) mechanically, it is easier for small snails to feed on a smaller host, (2) a wider host preference by juveniles avoids a shortage of food supply, and (3) it allows for avoidance of competition between adult and juvenile snails.

Boonea impressa forms patches or aggregations on oyster beds. Patches occur independently of the host and usually remain stable for a period of 5 to 6 d (Wilson et al. 1988b). Powell et al. (1987b) found that snails were contagiously distributed and positively spatially autocorrelated on Texas oyster beds. Compared with wave-exposed beds, patches were larger and better developed on sheltered or enclosed beds where snail movement could occur more freely (Powell et al. 1987b). Snail aggregations become very important in oyster bed ecology because although the effect of an individual snail may be limited, the damage done by aggregations may be considerable. The damage inflicted will depend upon snail patch size, patch duration, snail behavior and size distribution, and oyster size distribution (Wilson et al. 1988b).

Boonea impressa was initially thought to be incapable of significantly damaging oyster health but recent evidence indicates that large numbers can inflict substantial harm. In laboratory and field experiments, oysters parasitized by large numbers of *B. impressa* gained significantly less weight than unparasitized oysters, with mortality occurring in small oysters (White et al. 1984; 1988a; Wilson et al. 1988a). Ward and Langdon (1986) found that *B. impressa* reduced the energy available to oysters for growth and maintenance. White et al. (1988a) developed a model based on energy budgets to estimate the effect of *B. impressa* on oysters; model predictions indicated that *B. impressa* could reduce the energy available for growth and reproduction by 2 to 12% on natural oyster beds.

In addition, *B. impressa* parasitism altered the biochemical composition of the oyster, resulting primarily in reduced carbohydrate concentration (White et al. 1988b). Possibly the most significant effect of this ectoparasite is its ability to both transfer and intensify existing infections of *Perkinsus marinus* (White et al. 1987), a main cause of oyster disease and mortality in some Gulf regions and north to the Chesapeake.

Clams and Mussels

Clams and mussels are not as abundant or ubiquitous as other oyster pests, yet in given localities they may be found in sufficient numbers to influence oyster populations. In the northwest Gulf of Mexico, oysters have been found to be bored by the polyclad clam, *Diplothyra smithi*, and the mussels, *Lithophaga bisulcata* and *Lithophaga aristata* (Ogle 1976). *Ischadium recurvum* may be capable of reducing oyster health depending on abundance. Dense clumps of mussels with one or more oysters in poor condition in the middle of the clump can be found in some years (Engle and Chapman 1952). Although only limited information is available on their effect, mussels and clams appear to be important in oyster communities, but more extensive research is necessary to determine their effects on oyster bed communities.

COMPETITORS

The presence of competitors can reduce the success of oyster populations by competing for food or space. (Organisms that compete with oysters for settlement space are often referred to as fouling organisms.) Ultimately this competition may result in oyster death or injury when competitors are abundant. Although competitors usually influence larvae and juveniles, in severe cases adults also may be affected. Organisms from many marine phyla may function as competitors for all or part of their lives, e.g., algae, arthropods, anemones, bryozoans, sponges, polychaetes, annelids, and molluscs.

Effects of competitors include: (1) consumption of oyster larvae before settlement (see section on Predators), (2) prevention of oyster larval settlement by coverage of oyster setting area, (3) emission of a noxious chemical to repel possible oyster settlement, (4) overgrowth resulting in death from physical trauma, (5) poisoning of oyster spat by adjacent organisms, and (6) degradation of the structural integrity of the oyster shell. Coverage is probably the most common of the above effects on oyster communities, occurring when the oyster shell is overgrown by the competing organisms.

Competitors are common in almost all oyster bed communities, although specific oyster beds may be completely free of competitors. Unlike the community dominants, competitors display a marked seasonal cycle, with high mortality occurring in the fouling communities with the onset of colder temperatures (Galtsoff 1964). As temperatures warm, oyster larval settlement and the settlement of fouling organisms often occur at roughly the same time (Shaw 1967). Coincident setting can be detrimental to oyster larvae because surface area for settlement is limited. In addition, oyster spat mortality may also be high because many competitors have more rapid post-settlement growth (MacKenzie 1970). It is fortunate for oysters, however, that most competitors are seasonal and, therefore, injurious effects on oyster populations are somewhat minimized. If the abundances of fouling organisms were not reduced seasonally, then available settlement space for oyster larvae might be eliminated.

The eastern oyster has been studied as part of the fouling communities of eastern Long Island Sound (Osman et al. 1989; Zajac et al. 1989) and at Woods Hole, Massachusetts (Osman et al. 1990). A variety of sessile invertebrates (ascidians, barnacles, bryozoans) affected settlement success and post-settlement growth and mortality of oysters (Osman et al. 1989). Most species removed settlement space by virtue of their presence. After settlement, oyster spat survival and growth was usually associated negatively with the presence of sessile species. Osman et al. (1989) and Zajac et al. (1989) suggested that competition for food was the most likely cause of reduced survival and growth. Additional experiments by Osman et al. (1990) revealed that ontogenetic changes

in trophic relationships can result in increased complexity of interactions among eastern oyster larvae and spat and sessile competitors and predators.

Morales-Alamo and Mann (1990) examined the effect of differences in time of cultch planting on settlement and survival of oyster spat in Maryland. Their studies showed that recruitment and growth of oyster spat varied depending on the time when the cultch was planted. This was a result of variations in fouling during the study period. Rheinhardt and Mann (1990) studied epibenthic fouling in Virginia waters and found that the initial dominant biofouling species was replaced by other species through time. These authors postulated that this is a result of disturbance: oyster shells were moved around on the bottom, freeing space and allowing new organisms to settle and eventually replace community dominants. Thus, in the oyster bed environment the community structure may change continually over time (Rheinhardt and Mann 1990).

Some of the most common and abundant of the fouling competitors of oysters are the slipper shells, *Crepidula fornicata* and *Crepidula plana.* They are suspension feeders, whose long gill filaments provide increased surface area for food collection (Barnes 1980). Slipper shell larvae and oyster larvae appear to settle at about the same time, though until about two months of age, slipper shells grow more rapidly than oyster spat. Oyster spat mortality resulting from slipper shell overgrowth was as high as 58% in one region of Connecticut (MacKenzie 1970). Slipper shells also may interfere with oyster spat success by competing for food. Although reduced food intake is unlikely to result in mortality, growth may be slowed.

Adult *Crepidula* spp. can have entirely different effects on oyster populations compared with juveniles. Through deposits of feces and pseudofeces, *C. fornicata* can alter hard substrates to muddy ones, making them unsuitable for oyster larval settlement (Barnes et al. 1973). *Crepidula fornicata* also may be capable of ingesting large numbers of young oyster larvae (Kennedy and Breisch 1981). Another very indirect yet potentially important relationship is that between *Crepidula* spp. and juvenile *Boonea impressa,* which may depend on *Crepidula* spp. as its primary food source (Powell et al. 1987a).

Bryozoans, another phylum whose members are common space competitors in marine communities, are found on oyster beds from New England to the Gulf of Mexico. In a study conducted by Marie Lambert, cited by Galtsoff (1964), five species of Bryozoa (=Ectoprocta) were found in a Massachusetts oyster community, the most common species being *Bowerbankia imbridcata* and *Schizoporella unicornis.* Kennedy and Breisch (1981) report the presence of *Membranipora tenuis* and *Conopeum tenuissium* in Chesapeake Bay waters.

Bryozoans compete with oysters most commonly for space by covering oyster shells with their colonies. For example, the calcareous bryozoan, *Schizoporella unicornis,* overgrows spat, with spat mortality either resulting from total coverage or by the valves being forced apart (MacKenzie 1970). Spat mortality from overgrowth occurred when the spat were less than one month old or less than 5 mm long, with oyster mortality reaching as high as 8% in some communities. Some bryozoan species may also be capable of ingesting oyster larvae, further decreasing oyster success. An unusual form of competition may be displayed by the foliose bryozoan, *Bowerbankia gracilis,* that inhibits spat from settling within a 1 cm proximity, possibly by releasing a toxic or noxious chemical (MacKenzie 1981).

Barnacles that are common on oyster beds along the western Atlantic and Gulf coasts are like the other competitors discussed in that they often compete with oysters for available space. Some authors have postulated that if the set of barnacles is very dense, oyster settlement may be affected (Galtsoff 1964; Kennedy 1980). Oyster mortality from overgrowth by barnacles may reach as high as 5% for oysters less than 5 mm long (MacKenzie 1970, 1981). Like bryozoans, barnacles also ingest oyster larvae, sometimes in large numbers (see section on Predation on Larvae).

A variety of less common yet locally or regionally important competitors exist. In North Carolina, the scorched mussel, *Brachiodontes exustus,* can out-com-

pete adult and young oysters for space in areas exposed to wave action (Ortega 1981). Jingle shells, *Anomia simplex*, can be common in Connecticut (MacKenzie 1970). As with slipper shells, jingle shells can cause oyster spat mortality by growing over the spat (MacKenzie 1981). Galtsoff (1964) reported that oysters were often covered with the tunicate, *Molgula manhattensis*. In Chesapeake Bay, tunicates can be so densely packed that anoxic conditions can develop close to the substrate surface and possibly kill many spat (R.I.E. Newell, Horn Point Environmental Laboratory, pers. comm.). Overgrowth by protozoans and sponges can also occur. Galtsoff (1964) reported that the red sponge, *Microciona prolifica*, is a common, non-damaging species found on many oyster bottoms. He also stated that the protozoan, *Folliculina* spp., lived on oyster shells in brackish waters. In different regions of North America, different species of protozoans occur on oyster beds, often abundantly (Andrews 1944).

An unusual form of overgrowth occurs when oysters overgrow themselves. In areas of heavy set, larger spat frequently overgrow smaller ones, resulting in mortality. Mortality occurs mostly in the first four months and can reach 2.5% in some regions (MacKenzie 1970). Although mortality occurs, such overgrowth may add stability as overcrowding of adults would cause food-limitation and thereby reduce the health of the entire population.

Plants also compete with oysters for space. Green algae, common in clear waters, have the potential for causing oyster mortality. *Codium fragile* can cause mortality in oysters when root patches become extensive and collect silt (MacKenzie 1981). Numerous normally harmless algae are associated with oyster communities, e.g., *Gracillaria, Enteromorpha, Ulva, Griffitsia, Ceramium, Chondria, Champia,* and *Scytosipon* (Galtsoff 1964). In great abundance, any of these genera may be competitors with oysters. Like algae, most seaweeds that grow in association with oysters are not injurious; however, when excessive growth occurs, oyster "suffocation" can result directly from the plant holdfast or indirectly from accumulated sediment. For instance, the watermilfoil, *Myriophyllum spicatum*, causes no injury to oyster populations when abundance is low but when overpopulated, the decomposing leaves and stems can cover oyster grounds (Galtsoff 1964) and, depending on conditions, deplete oxygen.

SUMMARY

Oysters are a crucial ecological dominant in many estuarine communities. They are particularly subject to pressure from predators, pests, and competitors because of their sessile nature. Oyster predators, pests, and competitors encompass many diverse groups including gastropods, crabs, flatworms, starfish, sponges, anemones, bryozoans, arthropods, and fish. Mortality may be greatest during the larval and juvenile stages. Although the adult oyster's thick calcareous shell provides protection, mortality of adults does occur. Either predation or competition for space can cause greater mortality but generally mortality results from both factors. In most regions, oyster fecundity and survival is sufficiently high to offset mortality; nevertheless, in some areas, oyster populations are greatly diminished. Generally, mortalities of oysters are much lower in salinities from 5 to 15 ppt than in higher salinities where predators, pests, and competitors are much more abundant. When oyster productivity is important to the ecosystem or local fisheries, chemical, mechanical, or biological measures can be taken to protect oysters. Careful site selection (i.e., salinity, temperature, known predators, competitors, and pests) will be most important in ensuring that restoration of natural oyster bars is successful and that aquaculture enterprises are profitable. Nevertheless, oyster predators, pests, and competitors will certainly remain a component of oyster communities.

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Chapter 17

Diseases and Defense Mechanisms

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INTRODUCTION

Disease has been a recognized problem in the culture of eastern oysters, *Crassostrea virginica*, at least since the early part of the twentieth century when an epizootic mortality occurred in Prince Edward Island, Canada. The etiological agent responsible for what is now referred to as "Malpeque Bay disease" was never identified, but epizootiological aspects of the disease were described by Needler and Logie (1947), who also detailed evidence for the development of resistance in native stocks as a result of natural selection. Since the outbreak of Malpeque Bay disease, a number of reportedly new and highly lethal infectious diseases of *C. virginica* and of other oyster species have been described (see Table 1).

Studies of the defense mechanisms available to oysters as protection against infectious organisms are more recent. Such studies began with the experimental work of Stauber (1950, 1961), were extended by field and histopathological investigations of Mackin and co-workers in the 1950s, and enhanced by the work of many researchers stimulated to enter the field after mass oyster mortalities in the mid-Atlantic states during the late 1950s and early 1960s. A clear understanding of disease requires insight into how affected organisms respond to the etiological agent, as well as a knowledge of the agent itself, for the outcome of the disease process is determined by interactions among the disease organism, the host, and the environment.

Here, we describe several major infectious diseases of *C. virginica* and organismic responses to them. We then pursue the subject of defense mechanisms by describing interactions between cellular and humoral elements in oysters and a variety of biotic and abiotic agents, from both experimental and natural systems. Throughout this review, we will use a number of terms that we now define. The term "infection" refers to the presence of an infectious or foreign organism in tissues of a host. "Disease" is a broad term designating damage to cells sufficient to cause dysfunction of the organism. Disease may be caused by (1) genetic defects reflected in abnormal cellular structure and function, (2) nutritional imbalance that deprives cells of essential nutrients, (3) extrinsic physical or chemical agents that injure cells, or (4) infectious agents that damage cells by their physiological action or physical presence.

An infection may occur without causing disease if the host destroys the invading organism or if genetic adaptations by host or parasite reduce destructive activity toward each other. Recovery from disease also involves the defense system, which functions in the repair of damaged tissues. The term "epizootic" refers to a disease that is rapidly spreading throughout an animal population, whereas "enzootic" refers to a disease that is prevalent in an animal population. We define "resistance" as the relative ability of an organism to avoid infection or to withstand the effects of disease.

Table 1. Summary of reported diseases and parasites of *Crassostrea virginica.*

Disease/Parasite	Description	Reference
Malpeque disease	Caused high mortalities in eastern Canada beginning in 1915. Etiological agent unknown, but candidates currently under investigation are labyrinthulid parasites (Phylum Labyrinthulata) and a hematopoetic sarcoma. Disease symptoms include heavy concentrations of ceroid cells, edema, and focal infiltration of hemocytes in the connective tissues.	M. F. Li 1976 Olive 1982 S. McGladdery, Dept., Fisheries and Oceans, Canada,(pers. comm.), 1989
Herpes-like virus	Found in gaping oysters at elevated temperatures in Maine. Intranuclear inclusions in cells around blood sinuses; hexagonal; 70 to 90 nm, single coat.	Farley et al. 1972
Papova-like virus	Found in oysters from Piscataqua River, Maine and maintained in NMFS Laboratory recirculating system in Oxford, Maryland for 2 weeks. Basophilic intranuclear inclusions in hypertrophied cells of gonad; five to six sides; 53 nm.	Farley 1976a
Rickettsia and mycoplasms	Basophilic inclusions in epithelial cells of digestive tract in oysters from the middle Atlantic states. No evident pathology.	Harshbarger et al. 1977 Meyers 1981
Cardiac edema associated with *Vibrio anguillarum* (bacteria)	Enlargement of pericardial chamber and heart. Found in four of 10,000 oysters sampled from Chesapeake Bay.	Tubiash et al. 1973 Elston et al. 1982
Hinge ligament disease of juvenile oysters associated with *Vibrio* spp. and *Cytophaga* spp. (bacteria)	Affects juveniles from post-setting to about 10 mm in grow-out nurseries. Bacteria destroy protein of hinge ligament, leaving oyster unable to open valves. Treatment with dilute sodium hypochlorite may reduce losses.	Elston 1984 Kraeuter and Castagna 1984 Dungan et al. 1989
Shell disease caused by *Ostracoblabe implexa* (fungus)	Reported by Galtsoff (1964) for *C. virginica* from the southern United States, but probably restricted to *O. edulis* in Europe and eastern North America. Hyphae penetrate and proliferate in shell, causing secretion of conchiolin-rich shell, including wart-like depositions at adductor muscle attachment, giving name "maladie du pied."	Korringa 1951 Galtsoff 1964 Alderman and Jones 1971 M. F. Li et al. 1983
Hexamita sp. (protozoan)	Probably a normal inhabitant of digestive tract, which becomes a secondary invader at low temperatures in stressed oysters.	Scheltema 1962 Feng and Stauber 1968
Dermo disease *Perkinsus marinus* (protozoan)	Prevalent in the Gulf of Mexico and on the U.S. east coast to Massachusetts. Proliferates most rapidly at temperatures between 25° and 30°C and salinities greater than 15 ppt, but survives at much lower temperatures and salinities. Transmitted directly from oyster to oyster. Causes high mortalities.	See this chapter

Table 1. continued

Disease/Parasite	Description	Reference
MSX disease *Haplosporidium nelsoni* (protozoan)	Prevalent along the mid-Atlantic coast of the US but reported from Maine to Florida. Active at temperatures above 10°C and intolerant of salinities below 10 ppt. Mode of transmission unknown. Causes high mortalities.	See this chapter
SSO disease *Haplosporidium costale* (protozoan)	Prevalent in high salinity (>28 ppt) bays in Maryland and Virginia, but reported as far north as Maine. Detected in oyster tissue only from April through June and results in high mortalities in May and June. Mode of transmission unknown. Causes high mortalities.	See this chapter
Bucephalus sp. (trematode)	Prevalent in small, low-salinity, enclosed estuaries. Branching sporocycts in gonad, gill, and digestive gland. Causes castration.	Hopkins 1954, 1957 Cheng and Burton 1965b, 1966
Neoplasia	Rare cases (<1%). Characterized by mitotically active cells of various proposed origins: germ cell, hemocyte, connective tissue. Cells undifferentiated with large nuclei, prominent nucleoli, and high nuclear-to-cytoplasm ratio.	Farley 1969, 1976b Harshbarger et al. 1979
Juvenile Oyster Disease (JOD)	Heavy mortalities occurring in northeastern U.S. Mostly affecting cultured oysters <25 mm shell height. Etiology presently unknown.	See this chapter

PRINCIPAL INFECTIOUS DISEASES

Overview

In the 1940s, about twenty-five years after the original Malpeque Bay epizootic, another serious oyster disease was reported, this time in the Gulf of Mexico. The causative agent is a protozoan parasite (*Perkinsus marinus*). Originally thought to be a fungus, it was initially named *Dermocystidium marinus*, and the disease is still commonly referred to as "Dermo." Two new diseases, MSX and SSO, also caused by protozoans, resulted in epizootic mortalities of oysters in the mid-Atlantic states beginning in the late 1950s (Andrews et al. 1962; Haskin et al. 1966). Like *P. marinus*, the causative organisms, *Haplosporidium nelsoni* (MSX) and *H. costale* (SSO), had never been observed before they were associated with mortalities. In addition to diseases affecting adult oysters in nature, the production of larvae and juveniles in hatcheries and nurseries often results in the proliferation of naturally-occurring microorganisms that may become highly pathogenic for bivalves cultured in high density conditions (Elston 1984). Juvenile Oyster Disease (JOD), which has recently been reported in young, cultured oysters in the northeastern United States, may be an example of this (Bricelj et al. 1992).

Species of oyster other than *C. virginica* also suffer from diseases that cause epizootic mortalities. In western Europe, a virus (gill necrosis virus = GNV) has been associated with mortalities of the Portuguese oyster, *C. angulata* (Comps 1988), and two protozoans, *Marteilia refringens* and *Bonamia ostreae*, have caused high losses of the European flat oyster, *Ostrea edulis* (Balouet et al. 1979; Figueras and Montes 1988; Grizel et al. 1988). *Bonamia ostreae* was first identified in experimental *O. edulis* sampled in 1963 on the east coast of the United States (Farley et al. 1988) and later in commercial plantings on the west coast (Elston et al. 1986; Friedman et al. 1989) that produced seed transplanted to Europe before the outbreak of bon-

amiasis there (Elston et al. 1986). A west coast source was inferred for *B. ostreae* recently discovered in Maine (Friedman and Perkins 1994). Resistance to *B. ostreae* may have developed in some oyster stocks (Elston et al. 1987b; Elston and Holsinger 1988). An organism resembling *B. ostreae* has been implicated in heavy mortalities of the New Zealand dredge oyster, *Ostrea puelchana*[1] (= *Tiostrea lutaria*) (Bucke 1988). Protozoans similar to *Bonamia ostreae*, but belonging to a newly created genus *Mikrocytos*, cause mortalities of the Pacific oyster, *Crassostrea gigas* (*Mikrocytos mackini*), and the Australian rock oyster, *Saccostrea commercialis* (*M. roughleyi*) (Farley et al. 1988). "Summer mortality" affects *C. gigas* on the west coast of the United States and in Japan (Koganezawa 1975; Perdue et al. 1981; Beattie et al. 1988). Deaths occur during the warmest part of the summer and have been linked to glycogen depletion during the reproductive cycle (Perdue et al. 1981). Recently, a bacterium in the genus *Nocardia* has also been implicated in summer mortality (Elston et al. 1987a; Friedman and Hedrick 1991a). Selective breeding at the University of Washington produced strains resistant to the disease (Beattie et al. 1980, 1988).

Reports of oyster diseases are typically considered to represent first-time occurrences. Unfortunately, lack of historical data usually makes such reports difficult to verify. Anecdotal evidence, however, suggests that some diseases existed long before they were first "discovered" by biologists. For instance, mortalities characteristic of those caused by Dermo disease (i.e., occurring during periods of elevated temperature and high salinity) were reported in the Gulf of Mexico in the early 1900s (Mackin and Hopkins 1962) and after *P. marinus* was identified as the etiological agent in the late 1940s, the parasite was discovered in archived oyster tissues fixed around 1930 (Owen, pers. comm. in Ray 1954). Survivors of a heavy mortality in the Rappahannock River, Virginia, in 1949 were the first Chesapeake Bay oysters in which *P. marinus* was identified (Andrews and Hewatt 1957). Andrews and Hewatt (1957) considered it likely that the parasite had existed in Chesapeake Bay before that time, a belief supported by the fact that despite high prevalences of the parasite, the Virginia industry harvested as many oysters annually between 1950 and 1959 as it had during the previous two decades, before *P. marinus* was discovered (Haven et al. 1978). *Haplosporidium costale* was identified serendipitously during a search for *H. nelsoni* along the Atlantic coast of Virginia and Maryland. It caused characteristic heavy mortalities and the fact that oyster growers considered these normal suggests that SSO disease was not new (Andrews et al. 1962).

Whereas *P. marinus* and *H. costale* were most likely present and causing mortalities well before they were first identified, *H. nelsoni* almost certainly did not cause significant mortalities in Delaware and Chesapeake Bay before the late 1950s. Laboratories had been conducting oyster research, including field studies, in both estuaries long before epizootic mortalities were first reported (Waller 1955; Haven et al. 1978). More specifically, Andrews and colleagues had been investigating causes of oyster mortalities in lower Chesapeake Bay for a decade before the first *H. nelsoni*-caused oyster losses occurred there. Consequently, there is an excellent pathological record for the period before the onset of the Virginia MSX epizootic in 1959. Subsequently, Andrews (in Andrews and Wood 1967) examined tissue slides collected in Chesapeake Bay in the mid 1950s and found two oysters infected with protozoans similar to *H. nelsoni*. Organisms resembling *H. nelsoni* have been reported sporadically in oysters in Georgia, Florida, and Maine, without concurrent mortality, also suggesting that the parasite could have existed in the mid-Atlantic states before the 1957 to 1959 epizootics, without causing high mortality.

Reports of apparently new diseases have sometimes followed the importation of native or exotic oysters (Needler 1931; Andrews 1979; Rosenfield and Kern 1979; Elston et al. 1986). Much of the evidence is circumstantial, however, and convincing documentation exists only for parasites known to be transmissible from oyster to oyster such as *P. marinus* and *B. ostreae* (Christensen 1956; Elston et al. 1986).

Many other diseases and parasites of both adult and larval *C. virginica* have been described, but they are rarely associated with significant mortality and the

[1] The authors originally identified this species as *Tiostrea lutaria*. The editors have renamed it following the convention of Carriker and Gaffney (Chapter 1).

information available for them is often no more than a single reported occurrence. A number of publications have summarized the known diseases and parasites of oysters (Lauckner 1983; Andrews 1984a, b; Sparks 1985; Sindermann and Lightner 1988; Fisher 1988a; Elston 1990; Sindermann 1990; Elston 1993; Perkins 1993; Bower et al. 1994). Pertinent information is outlined in Table 1 and host responses are reviewed in the section entitled Cellular Defense Mechanisms. The present section will consider only principal infectious diseases for which a substantial amount of information is available, and will examine in detail what is known about the host-parasite relationship and environmental influences on this association.

Larval Vibriosis

History and Distribution

When research laboratories and early commercial hatcheries began producing bivalve larvae in the 1950s, they were often faced with rapid, epizootic mortalities in their cultures (Tubiash et al. 1965). Microscopic examination showed vibrating particles around or inside moribund and dead larvae (Fig. 1). The particles were subsequently identified as bacteria.

Vibriosis affects larvae of a number of cultured bivalve species, including the hard clam, *Mercenaria mercenaria*, the bay scallop, *Argopecten irradians*, the shipworm, *Teredo navalis*, the European flat oyster, and the eastern oyster (Tubiash et al. 1965). Tubiash et al. (1970) speculated that natural epizootics, stimulated by high temperature and the abundance of larvae in summer, may limit recruitment of bivalve species. Natural densities of larvae, however, are many times lower than those in hatchery culture and no outbreaks have been documented in the field. The disease appears to be largely a phenomenon associated with mass culture conditions, and specifically with poor husbandry and improper sanitary practices in the hatchery.

Etiological Agent

Bacteria isolated from larval cultures experiencing epizootics have been characterized as belonging to the genus *Vibrio* (Tubiash et al. 1970; Brown and Losee 1978). Two species have been identified, *V. anguillarum* and *V. alginolyticus*, and at least one other has been implicated but not named (Tubiash et al. 1970). The bacterial cells are Gram-negative rods, 0.6 to 1.0 μm long, and motile with polar monotric-

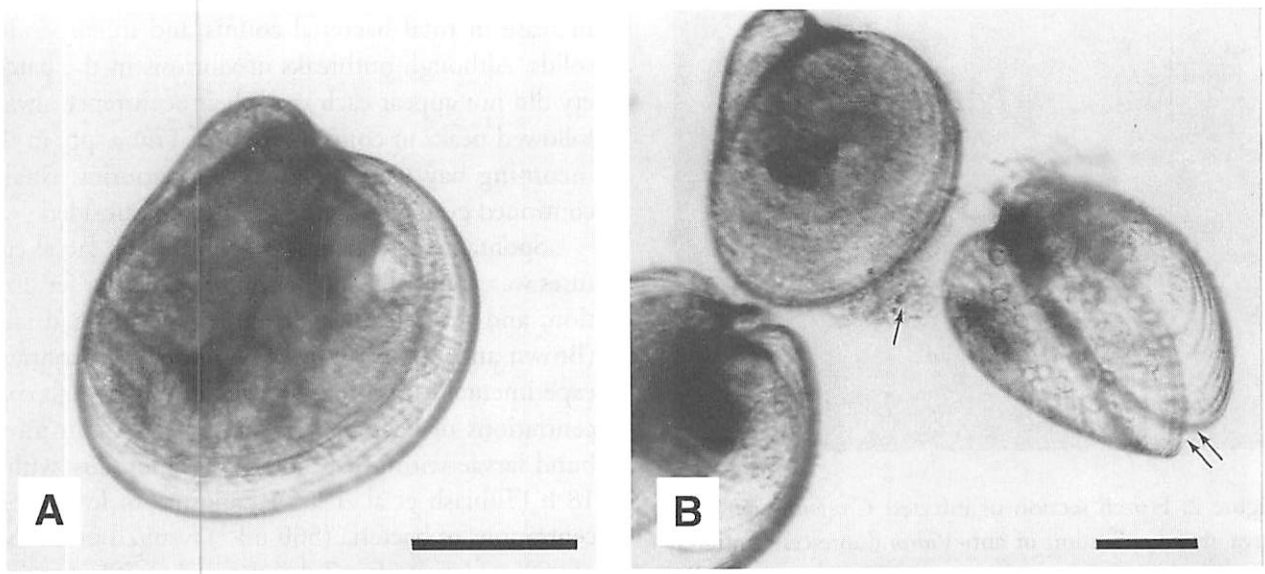

Figure 1. (A) Healthy and (B) *Vibrio*-infected larvae of *Crassostrea virginica.* Note bacteria (single arrow) and gaping valves of infected larva (double arrows). Scale bars = 100 μm .

hous flagella. They form colonies that are 1 to 4 mm in diameter, smooth, off-white to gray, translucent when young, and later becoming opaque. They can be isolated from overtly healthy bivalve molluscs as well as from the water column in highly productive commercial shellfish growing areas. Diagnosis is commonly made by culturing samples on TCBS (Thiosulfate Citrate Bile Salt) bacteriologic medium (Elston 1990).

Infection and Disease Processes

Elston and Leibovitz (1980) induced vibriosis in cultures of larval *C. virginica* and described the associated pathology. They found that disease progression followed one of three patterns. In the first, which affected larvae of all ages, bacteria attached to the external shell periostracum and proliferated inward along the internal shell surface (Fig. 2), invading mantle tissue and then spreading into the visceral cavity. Most larvae ceased feeding and became inactive within 1 d. Some individuals remained active but showed abnormalities of the velum, including loss of the preoral ciliary ring.

The second type of pathogenesis, which affected only Prodissoconch I and early Prodissoconch II stages, resulted in severe deformation of the velum without bacterial invasion of tissues until late in the disease. Larvae remained active but swam abnormally. After 2 or 3 d, pathological symptoms appeared, including erosion of the digestive tract epithelium, rounding up and loss of ciliated velar cells, and destruction of the velar retractor muscles. These lesions were accompanied by reduced feeding and inactivity. Death was probably caused by starvation.

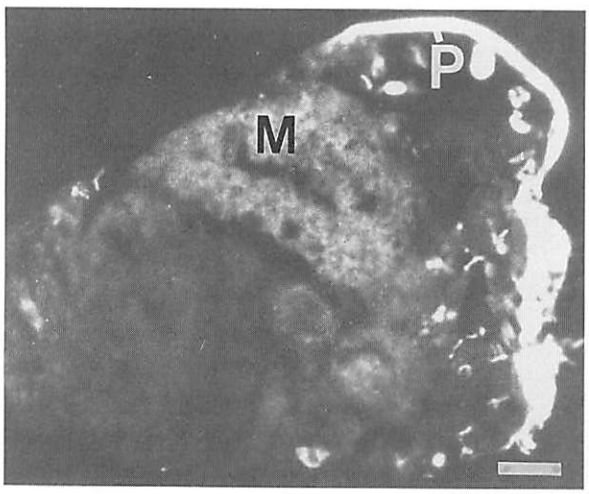

Figure 2. Frozen section of infected *Crassostrea virginica* larva after application of anti-*Vibrio* fluorescent antibody and showing initial attachment of bacteria at the periostracum (P) and progressive growth through mantle (M) tissue. Scale bar = 10 µm. From Elston and Leibovitz (1980).

Late-stage Prodissoconch II larvae showed a third form of the disease. They became inactive and stopped feeding 1 d after cultures were inoculated with bacteria. Visceral atrophy and cellular sloughing were evident 2 d later, after which lesions of the velum appeared. The digestive tract was the most severely affected tissue. Bacteria were seen attached to epithelial surfaces of this organ and, in some specimens, focal necrosis associated with bacteria was observed in absorptive cells of the digestive gland.

Epizootiology

In a study of vibriosis in a Long Island shellfish hatchery, Leibovitz (1978) found that *Vibrio* spp. occurred in very low concentrations in shellfish-growing water in most months, but dominated the bacterial flora for a variable period in the spring and summer of each year. This dominance coincided with an increase in total bacterial counts and in suspended solids. Although outbreaks of vibriosis in the hatchery did not appear each year, their occurrence always followed peaks in concentration of *Vibrio* spp. in the incoming bay water. Hatchery epizootics usually continued even after natural peaks had subsided.

Spontaneous epizootics of vibriosis in larval cultures were generally apparent 6 to 10 d after fertilization, and mortality usually subsided 6 to 8 d later (Brown and Losee 1978; Brown 1981). In contrast, experimental exposure of larval cultures to high concentrations of bacteria (10^6 ml^{-1}) resulted in moribund larvae within 8 h and 100% mortality within 18 h (Tubiash et al. 1965). Exposure to lower concentrations of bacteria (560 ml^{-1}) resulted in survival of 48% of larvae after 7 d compared to 70% for controls (Brown and Losee 1978). Susceptibility to bacterial disease decreased as larvae aged (10-d old < 2-d old < up to 48-h old; Brown 1973). Brown (1973)

found no effect on developing larvae of culture broth filtered to remove bacteria, but later Brown and Losee (1978) reported that fertilized eggs treated with a filtrate of bacterial culture broth had a survival of 68% at the straight hinge stage (Prodissoconch I), compared to 87% for control larvae not exposed to bacteria and 85% for larvae exposed to heat-killed bacteria. Brown and Losee (1978) suggested that a toxin was involved in the disease, and Brown and Roland (1984) confirmed this with experiments showing an LC_{50} value of 47 µg of purified toxin L^{-1} for newly fertilized eggs exposed over a 2-d period. The exotoxin was inactivated by heat, but it also demonstrated a bacteriostatic capacity that was not diminished by high temperature. In fact, heat-treated toxin was associated with improved larval survival, perhaps because of its bacteriostatic property.

Resistance

Elston and Leibovitz (1980) reported finding phagocytes containing ingested bacteria, bacterial antigens, and cellular debris in all three disease conditions described for larvae challenged with *Vibrio* spp. Cells with phagocytosed material were most common in the visceral cavity, indicating migration of hemocytes through the velum or peripheral velar membrane (Fig. 3). In addition, host cells were capable of sequestering bacteria and necrotic tissue. Although most larvae die during vibriosis epizootics, some survive and set (Brown and Losee 1978), indicating that these responses may be effective defense mechanisms. The presence of surviving larvae capable of setting also suggests that selection for resistance to the disease might be possible if survivors could be reared to adulthood and bred.

Control Measures

Although larvae can be treated with antibiotics (e.g., streptomycin [Combistrep®] and chloramphenicol, Tubiash et al. 1965; neomycin, Brown and Losee 1978), routine application is not recommended because of the potential for developing resistant bacterial strains (Brown and Losee 1978; Elston 1984). A suspected problem with vibriosis should be followed with confirmatory diagnosis by bacterial plate cultures. Bacteria may enter larval cultures through incoming sea water, with the algal food supply, or from the broodstock, but their proliferation to disease-causing levels usually indicates poor hygienic procedures in the hatchery (Elston 1984). Once the source has been identified, steps can be taken to clean or sterilize equipment, containers, and water. Contaminated broodstock and larval cultures should be destroyed. Because vibriosis is an opportunistic disease, it can be minimized by reducing stress factors such as overcrowding, high temperatures, insufficient food, or inappropriate oxygen tension, as well as by good sanitary practices (Tubiash 1975; Elston 1984).

Juvenile Oyster Disease

History and Distribution

Since the mid-1980s, high, unexplained mortalities of juvenile hatchery-reared eastern oysters, maintained under intensive culture, have been documented in the northeastern United States. Although sporadic, the mortalities are a major problem for both nascent and established oyster culture operations from New York to Maine. An etiological agent has not yet been identified, but a disease syndrome associated with the mortalities has been described and is known as Juvenile Oyster Disease (JOD). Relatively

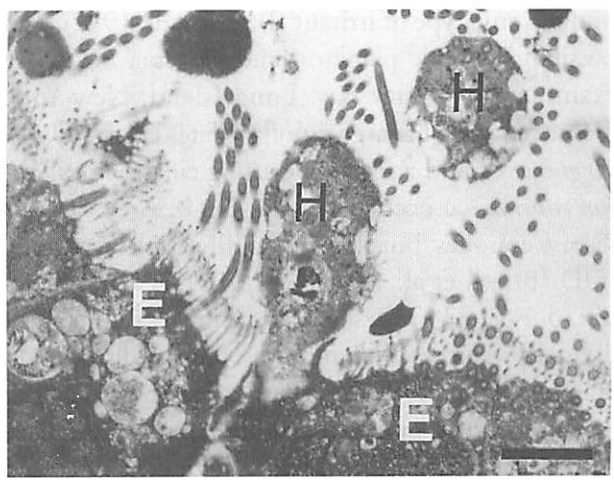

Figure 3. Migration of a *Vibrio*-laden hemocyte (H) through the epithelial surface (E) of an infected *Crassostrea virginica* larva. Scale bar = 10 µm.

little has yet been published about JOD; thus, the following description is largely from abstracts and unpublished reports. Nevertheless, the serious nature of this disease warrants consideration of all information currently available.

Etiological Agent

Several etiologies have been proposed for JOD. Early reports indicated that affected oysters were emaciated and might be suffering from a nutritional deficiency; however, there is little evidence that the disease is linked directly with food availability. In fact, good growth up to the onset of mortality is a characteristic of JOD, although both shell and soft tissue growth are often severely reduced during the mortality episode (Bricelj et al. 1992; Davis and Barber 1994).

Mantle lesions in affected oysters often contain coccoid bodies of varying size up to about 8 μm in diameter (Bricelj et al. 1992; Farley and Lewis 1993). Farley and Lewis (1993) consider these to be a protistan parasite associated with JOD. Others have described the bodies as phagocytosed, degenerate oyster cells and debris, and report no consistent association of protozoans with the disease (Bricelj et al. 1992).

Certain pathological correlates with JOD, including severe mantle retraction followed by rapid secretion of a conchiolin layer around the shrunken tissue and lesions of the mantle epithelium, suggest reaction to some type of irritant (Bricelj et al. 1992). Two possibilities, toxic plankton and bacteria, have been examined in Oyster Bay, Long Island, New York. Dense blooms of a large dinoflagellate, *Gymnodinium sanguineum*, and a red-tide causing ciliate, *Mesodinium rubrum*, co-occurred in the Bay before or during disease episodes, but showed no direct relationship to JOD (Bricelj et al. 1992; Lee 1995). A *Gymnodinium* sp. isolated in Oyster Bay during a JOD episode did not produce JOD symptoms, but did disrupt normal feeding behavior of juveniles for about 2 weeks during which they produced abundant pseudofeces (Wikfors and Smolowitz 1994). High densities of *M. rubrum* and *Gymnodinium* spp. frequently lead to bacterial (especially *Vibrio* spp.) blooms, as the bacteria use nutrients from the decaying plankton (Romalde et al. 1990a, b). Thus, the possibility exists that high plankton densities may make oysters more vulnerable to another disease agent or provide stimulus for bacterial blooms.

In tissue section, bacteria can be found in mantle lesions and between layers of anomalous conchiolin deposits of some oysters (Bricelj et al. 1992). Lewis and Farley (1994) reported that erythromycin reduced mortalities in JOD transmission experiments. Regular sampling of three juvenile oyster cohorts in Oyster Bay, each deployed at a different time in 1993, showed an exponential increase (from 10^3 to 10^4 colony forming units [CFU] g wet weight^{-1} of oyster tissue) in bacteria of the genus *Vibrio* one to two weeks before the onset of mortalities in each group (Lee 1995). Most of the bacteria isolated were phenotypically similar to *V. anguillarum*, *V. alginolyticus*, *V. fluvialis*, and *V. splendidus*. These are common marine species, some of which may become pathogenic to cultured organisms (see p. 585). In fact, the isolates did cause mortalities when 20 to 35-mm oysters were exposed to high doses injected into the shell cavity and applied to incubation water (Lee 1995). The conchiolin deposit characteristic of JOD was not found, however, and it was not determined whether any of these isolates was responsible for mortalities in the field or was a secondary invader.

A bacterial etiology was also suggested because JOD has certain similarities to a disease of cultured Manila clams, *Tapes philippinarum*, in western Europe, which is caused by a *Vibrio* sp. The disease occurs in high density cultures, causes mortalities of up to 80%, and is termed "Brown Ring Disease" (BRD) because of the characteristic organic deposit that rings the edge of the inner shell in affected clams (Paillard et al. 1994). The disease agent, *Vibrio* P1, is phenotypically similar to *V. splendidus* and *V. pelagius* (Castro et al. 1992), has been isolated from disease clams, and causes BRD when injected into nondiseased animals (Paillard et al. 1994).

Transmission. Although the causative agent for JOD has not been identified, the disease can be transmitted in the laboratory. Typical conchiolin deposits, followed by mortality, can be induced in unaffected oysters by proximity to diseased individuals (Lewis 1993).

Infection and Disease Processes

Diagnosis. The most characteristic symptom of JOD is the presence of an anomalous organic deposit on the inside of one or both valves (Fig. 4 A, B). It is formed between the mantle and inner shell, and is usually raised into a ridge around the mantle edge. Because the mantle has typically retracted when the layer is deposited, the ridge is several millimeters from the shell edge. In addition, the left valve of affected oysters is often deeply cupped and gives the appearance of having grown more rapidly than the right valve. Soft tissue pathological changes also occur (see below) but are not unique to JOD. Until a causative agent is clearly identified, the most useful diagnostic feature of JOD is the inner shell deposit.

Portal of Entry. The location of primary lesions on the outer mantle epithelium suggests that the etiological agent may invade the shell cavity, perhaps from the exterior shell surface, in a manner similar to that observed in larval and juvenile oyster vibriosis (Elston and Leibovitz 1980; Dungan and Elston 1988; see also p. 585).

Disease Development and Histopathology. The earliest evidence of JOD is observed in tissue section as disruptions of the apical portions of epithelial cells at the periphery of the mantle and, often, adjacent to the hinge ligament (Bricelj et al. 1992). Lesions progress along the outer mantle epithelium and are characterized by degeneration, necrosis, and sloughing of epithelial cells; hemocyte accumulations in the epithelium and underlying tissue; and layering of anomalous conchiolin along the mantle (Fig. 4C). In some oysters, the attachment of the adductor muscle to the shell degenerates. Coccoid bodies are often present intra- and extracellularly in the lesions. They range in diameter from <1 to 8 μm and when examined by electron microscopy, have the appearance of degenerate oyster cells (Fig. 4D; Bricelj et al. 1992). Bacteria and ciliates are observed in some lesions and are common in moribund oysters.

Sections through the anomalous conchiolin show that it is a layered structure with hemocytes, bacteria, and debris contained in chambers between layers as well against both inner and outer surfaces of the conchiolin sheet itself (Fig. 4E; Bricelj et al. 1992). The conchiolin layer is sometimes deposited between the adductor muscle and the shell, causing the muscle to detach. Deposition of anomalous conchiolin and mortality typically begin within a week or two of the first lesions (Ford1994). Prevalence of mantle lesions in severely affected cohorts was highly correlated with prevalence of anomalous conchiolin and with subsequent mortality at Oyster Bay, New York (Ford 1994).

Effects of Disease on Oyster Metabolism

The onset on JOD is sudden. It typically affects oysters that have been growing rapidly, and there is only minor evidence of distress before mortalities start. Shell and soft tissue growth decrease somewhat just before the first deaths occur, but the most severe growth interruption coincides with mortality (Bricelj et al. 1992; Davis and Barber 1994; Ford 1994). Survivors resume growth after the mortality episode, although they sometimes exhibit calcified conchiolin deposits and externally visible growth checks (Fig. 4 B).

A number of factors, singly or in combination, appear to cause death. Myoepithelial degeneration or deposition of conchiolin between the adductor muscle and the shell result in detachment of the muscle, causing the soft tissue to fall out of the shell. Bacteria and ciliates invade tissues exposed by sloughing of the epithelium, which also permits excessive loss of hemolymph. Although hemocytes infiltrate mantle lesions and are present in and on the conchiolin layer, their role in the disease process is undefined.

Epizootiology

Infection and Mortality Periods. Mortalities associated with JOD may occur from late June through September, with about a 1-month delay between New York and Maine (Bricelj et al. 1992; Davis and Barber 1994; Ford 1994). First evidence of disease, in the form of growth inhibition and mantle lesions, can be detected only about 1 week before the onset of mortality and the mortality episode itself lasts 4 to 6 weeks. In one study at Oyster Bay, New York, 6 co-

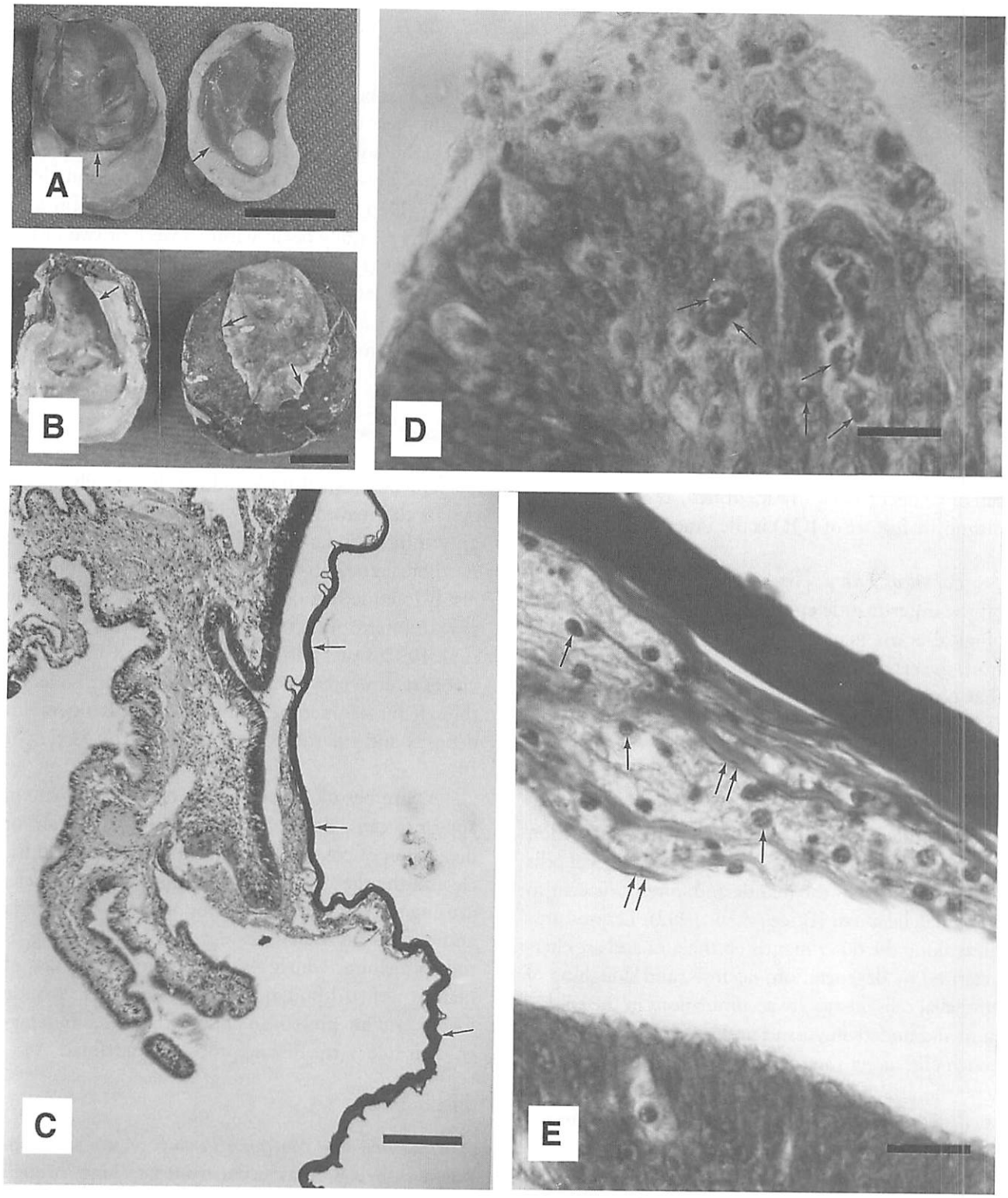

Figure 4. The Juvenile Oyster Disease (JOD) Syndrome. (A) Interior valves of affected oysters showing anomalous conchiolin deposits (arrows); (B) valves of survivors showing internal calcified deposit (left, arrow) and pronounced external shell check (right, arrows); (C) section through mantle and anomalous conchiolin (arrows) of JOD-affected oyster; (D) typical early lesion of mantle epithelium showing "coccoid" bodies of various sizes (arrows); (E) section through mantle epithelium and adjacent anomalous conchiolin, showing lamellar appearance of conchiolin (double arrows) with hemocytes (single arrows) and debris between layers. Scale bars = 10 mm in A and B; 100 µm in C; and 10 µm in D and E.

horts of different ages were deployed in ambient-water nursery trays at approximately 3-week intervals from April 6 to July 6. All began to die within 2 weeks (July 17 to 31) of each other, suggesting that they were all affected by the disease at about the same time (Ford 1994). Cohorts deployed in August and September also died, indicating that the disease agent was present during the remainder of the summer.

Prevalence Levels and Mortality Rates. Prevalence of oysters with anomalous conchiolin deposits ranges from 25 to 100%, with cumulative mortalities of 20 to 90% (Bricelj et al. 1992; Davis and Barber 1994; Ford 1994). Mortalities caused by JOD are highly size/age specific. In the multiple cohort study at Oyster Bay, groups averaging 5 to 20 mm shell height when JOD symptoms were first noted suffered total mortalities of 60 to 90%; those averaging 25 to 42 mm had losses of 0 to 30% (Ford 1994). Davis and Barber (1994) found an identical size refuge (>25 mm), in the Damariscotta River, Maine.

Larger/older oysters experience lower mortalities than smaller/younger individuals, but they may show high disease incidence (Ford 1994). For instance, at Oyster Bay, a cohort with mean shell height of 13 mm at the onset of JOD had a maximum conchiolin prevalence of 95% and a total mortality of 90%. Conversely, a cohort that was 24 mm at the same time also had a peak conchiolin prevalence of 95%, but mortality was only 24%. Thus, increased size/age protects against mortality, but not necessarily the disease itself.

In the Oyster Bay study, JOD developed and caused mortality in cohorts deployed at bottom stations, as well as in surface trays (Ford 1994). Offspring of both inbred and wild stocks were affected equally by JOD and wild-set spat that were held in nursery trays in Oyster Bay developed the disease. Occasional oysters, even large ones, are found with JOD-like conchiolin deposits in Long Island Sound, but it is not known whether the etiology is the same for these individuals as for JOD. The JOD syndrome has not been associated with mortality in wild stocks and the disease appears to be one of densely cultured oysters only.

Environmental Influences

Temperature. Mortalities caused by JOD typically occur at water temperatures between about 21 and 26°C (Bricelj et al. 1992; Davis and Barber 1994). Lewis (1993) reported that laboratory transmission occurred at 18°C, but resulting mortalities were delayed and reduced compared to those at higher temperatures. Maintaining oysters at a constant 25°C inside the hatchery, however, did not stimulate JOD, and there appears to be no consistent relationship between the timing of the spring temperature rise and the onset of mortalities (Ford 1994).

Salinity. Salinity ranges from about 25 to 32 ppt at sites where JOD occurs (Bricelj et al. 1992; Davis and Barber 1994; Ford 1994). The disease process is inhibited at lower salinity as Lewis and Farley (1994) reported that experimental transmission rates were inversely related to salinity. At 10 ppt, some mortality occurred, but without evidence of conchiolin deposits.

Control Measures

Several studies have shown that the incidence of JOD (as measured by conchiolin deposition) and consequent mortality can be reduced by measures that increase the water circulation in containers of juvenile oysters. These include reducing density, increasing mesh size, and increasing flow rate in upwellers (Bricelj et al. 1992; Ford 1994; G. Rivara, Cornell University, pers. comm. 1994). Reducing density is a method also recommended to combat Brown Ring Disease of Manila clams (Flassch et al. 1992). Although antibiotics were shown to lower JOD (Lewis and Farley 1994), their routine use in culture is not recommended, nor would it be a practical treatment for nursery stocks maintained in ambient waters. One of the most effective strategies for avoiding JOD, however, is to spawn and deploy oysters as early in the season as possible so that they have passed the critical size threshold of about 25 mm before JOD appears (Davis and Barber 1994; Ford 1994). Preliminary reports also suggest that resistance can be developed by breeding survivors of JOD epizootics (Davis et al. 1995; Farley et al. 1995).

Dermo Disease

History and Distribution

Dermo disease was identified in the late 1940s as the cause of extensive oyster mortalities in the Gulf of Mexico. The etiologic agent was originally thought to be a fungus and was named *Dermocystidium marinum* by Mackin et al. (1950). Subsequently, it was reclassified as a labyrinthulid, *Labyrinthomyxa marina* (Mackin and Ray 1966). Perkins (1976a, b), however, found that it had ultrastructural similarities to parasitic coccidians rather than to labyrinthulids. Based on Perkins' studies, Levine (1978) placed the parasite in the phylum Apicomplexa and renamed it *Perkinsus marinus*. Currently, *P. marinus* causes documented mortalities from the western Gulf of Mexico to Long Island Sound on the east coast of North America (Fig. 5; see also Ray 1954; Quick and Mackin 1971; Burrell et al. 1984; Craig et al. 1989; Ford, unpubl. data). In addition, organisms resembling *P. marinus* were associated with epizootic losses of *C. virginica* planted in Pearl Harbor, Hawaii, in 1972 (Kern et al. 1973). Eastern oysters grown in lagoons of Tabasco, Mexico, on the Yucatán peninsula, carry high prevalences of *P. marinus*, but associated mortality has yet to be convincingly documented (Burreson et al. 1994b).

Perkinsus marinus is so abundant in oyster-growing areas along the Gulf of Mexico that a survey of 49 sites from Florida to Texas found only one with a prevalence of less than 50% (Craig et al. 1989). The parasite is found consistently along the Atlantic coast of southeastern United States, including Chesapeake Bay (Quick and Mackin 1971; Burrell et al. 1981, 1984; Andrews 1988a; Crosby and Roberts 1990; Lewis et al. 1992). Andrews and Hewatt (1957) remarked on its absence in the high salinity bays of coastal Virginia at the same time that it was widespread in the estuarine water of the lower Chesapeake Bay. In the late 1980s, however, *P. marinus* began to appear in locations on the Atlantic coast of Virginia (E. M. Burreson, Virginia Institute of Marine Science, pers. comm. 1988).

In the mid 1950s, *P. marinus* was found in Delaware Bay after the importation of infected seed oysters from lower Chesapeake Bay, but it effectively disappeared when imports were embargoed (Ford and Haskin 1982). An epizootic recurred in Delaware Bay beginning in 1990, however, associated with abnormally high winter temperatures (Ford 1992). Since

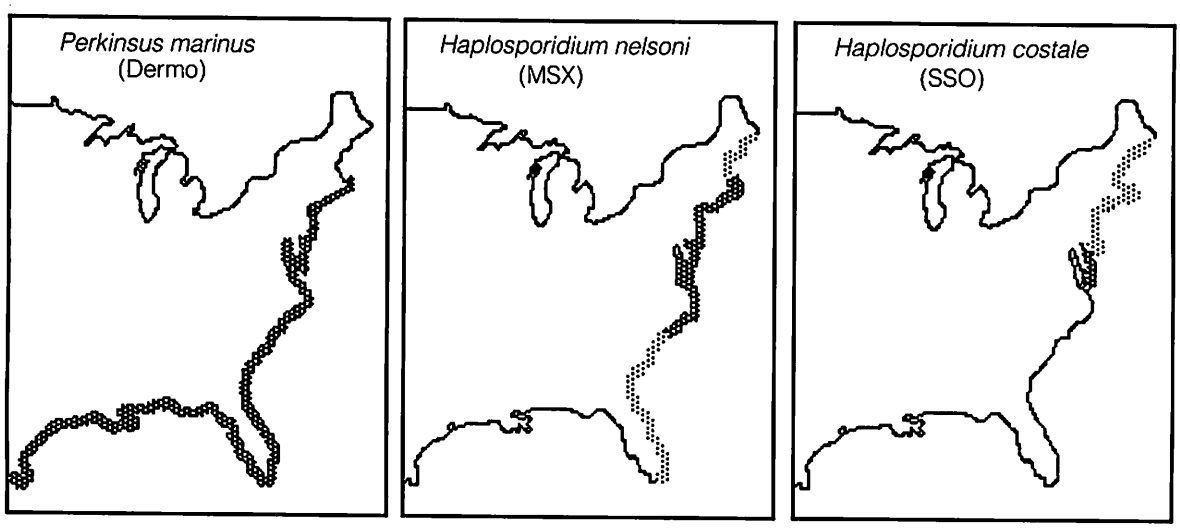

Figure 5. Distribution of diseases caused by *Perkinsus marinus*, *Haplosporidium nelsoni*, and *H. costale* in eastern U.S. Dark shading indicates regions where the diseases are epizootic or enzootic; light shading indicates regions where parasites have been reported, but are not causing recognizable mortalities.

1991, the pathogen has been found in many locations in Connecticut, New York, and Massachusetts (E. J. Lewis, Oxford Biological Laboratory, pers. comm. 1992; Ford, unpubl. data), although several previous surveys north of New Jersey had failed to detect it (Newman 1971; Meyers 1981; Cooper and Durfee 1982; E. J. Lewis, pers. comm. 1992).

Etiological Agent

Life Cycle. Perkinsus marinus is the only organism in the class Perkinsasida, phylum Apicomplexa (Levine 1978). Although there remains some uncertainty about its classification, *P. marinus* is considered an apicomplexan because the anterior end of the zoospore possesses an apical complex, a characteristic apicomplexan structure consisting of a network of microtubules known as the conoid, as well as pedunculate organelles called rhoptries. The rhoptries are thought to contain lytic substances that aid in host cell penetration (Vivier and Desportes 1990). Despite its morphological affinities with the Apicomplexa, recent comparisons of small subunit ribosomal RNA gene sequences suggest that *Perkinsus* spp. may be more closely related to dinoflagellates than to apicomplexans (Goggin and Barker 1993) or that they share a common ancestor with dinoflagellates (Fong et al. 1993b; Siddall et al. 1995). The following description uses terminology for Apicomplexan life stages (Vivier and Desportes 1990), although we acknowledge that these may change as additional information about *Perkinsus* spp. phylogeny becomes available.

In nature, infective stages of *P. marinus* are distributed in the water (T. Li et al. 1994) and are encountered as oysters feed (Mackin 1951a; Perkins 1988). The earliest recognized stages in oyster tissue are unicellular trophonts that enlarge, forming a prominent, eccentric vacuole (Fig. 6A, E). An irregular refringent body, the vacuoloplast, is present in the vacuole and is a diagnostic characteristic in fresh preparations (Perkins 1988). The vacuole forces the nucleus to one edge of the cell, resulting in the "signet ring" stage described by Mackin et al. (1950). Further development occurs by successive nuclear and protoplasm divisions from the trophont into multicellular meronts (Figs. 6A, B, C, D; 7D, F). The meronts rupture releasing individual merozoites, which develop into meronts and repeat the cycle. A similar progression is observed during *in vitro* culture of *P. marinus*, although repeated nuclear division occurs before protoplast division (La Peyre et al. 1993). Under certain conditions (fluid thioglycollate incubation and occasionally in moribund oysters), parasites enlarge to form cells with a very large vacuole. These cells are referred to as "hypnospores," but they are functionally prezoosporangia. When released into water, hypnospores undergo successive bipartitioning to form sporangia containing large numbers of biflagellated zoospores (Figs. 6F, G; 7E, G, H; Perkins and Menzel 1966). Zoosporangia are also formed in culture (Bushek 1994). Zoospores escape through a discharge pore in the sporangium and are capable of infecting live oysters and excised oyster tissue (Perkins and Menzel 1966; Goggin et al. 1989). Recent development of antibodies against *P. marinus* (Dungan and Roberson 1993) and methods for its *in vitro* propagation (Gauthier and Vasta 1993; Kleinschuster and Swink 1993; La Peyre et al. 1993) will greatly promote efforts to understand the biology of this parasite.

Transmission. Zoospores of *Perkinsus* spp. can initiate experimental infections (Goggin et al. 1989) and may cause infections in nature (Andrews 1988a). Perkins (1988), however, reported that experimental infection rates from zoospores were very low compared to those resulting from exposure to vegetative (trophont/meront) stages. A similar conclusion was reached by Volety and Chu (1994) who found that oysters experimentally infected with meronts isolated directly from oysters became more heavily infected than those challenged with prezoosporangial stages (hypnospores) from Ray's fluid thioglycollate medium (see p. 596).

Many infections are initiated by parasites released into the water from the disintegration of dead oysters (Andrews and Hewatt 1957). Cells may also be distributed by scavengers feeding on the tissues of dead oysters (Hoese 1963). Release from dead oysters is unlikely to be the only source of infective particles, however. T. Li et al. (1994) used flow cytometry to enumerate cells found in ambient Chesapeake Bay water that were morphologically similar to *P. marinus* and

reacted with an anti-*P. marinus* antibody. These bodies were most numerous in late May, which is well before the onset of mortality (see p. 603). Although the origin and positive identity of these cells is unknown, live oysters can also be a source of infection because parasites are shed into the gut (Dungan and Roberson 1993) and can be recovered in feces (Bushek et al. 1994a). Additionally, infected oysters may be fed

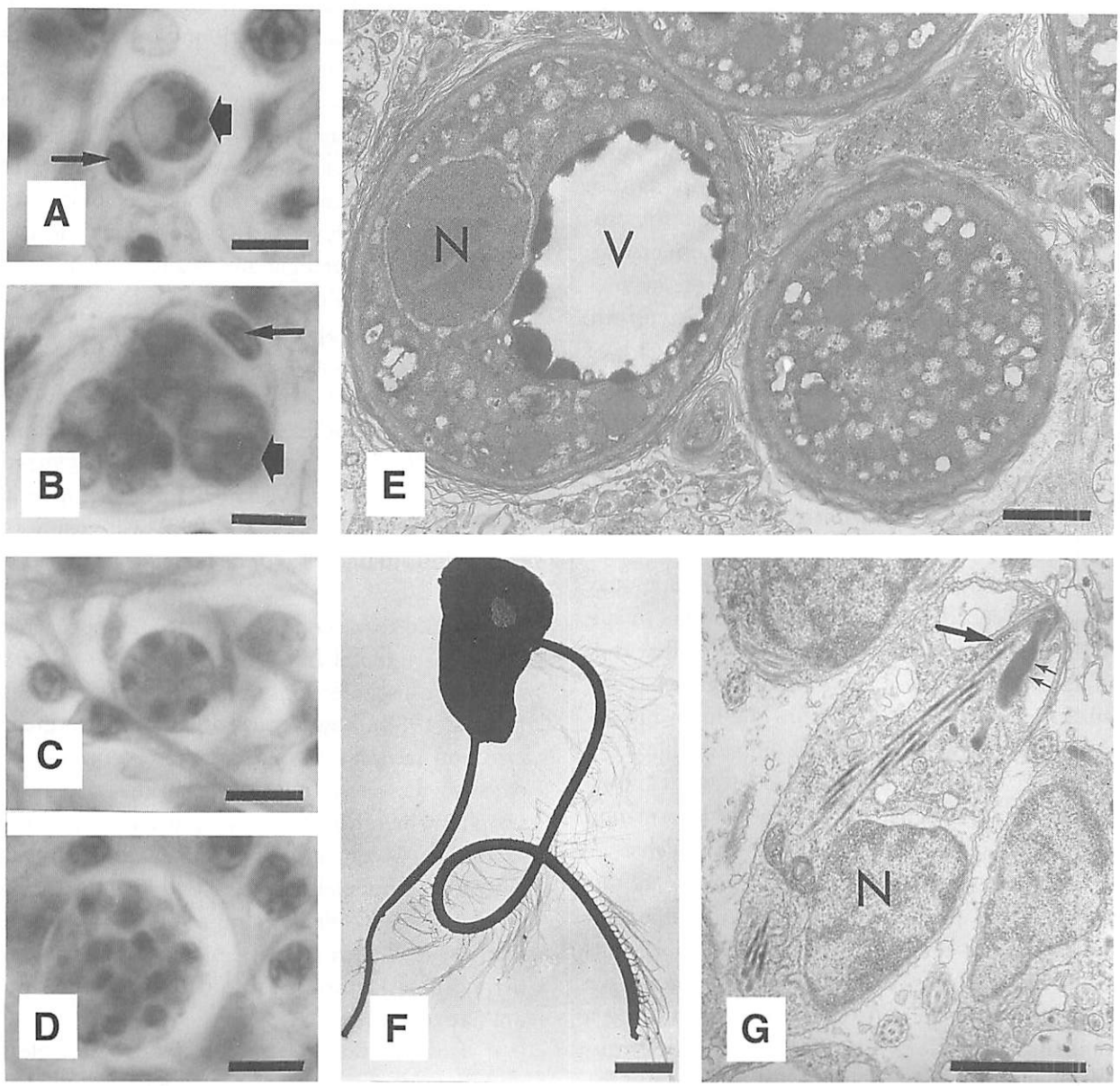

Figure 6. Life stages of *Perkinsus marinus*: (A) unicellular trophont within oyster hemocyte ("signet-ring" stage) — slender arrow points to hemocyte nucleus, thick arrow points to parasite nucleus; (B) several trophonts in oyster hemocyte — slender arrow points to hemocyte nucleus, thick arrow points to parasite nucleus; (C) meront containing wedge-shaped merozoites; (D) meront containing numerous merozoites; (E) unicellular trophonts showing vacuole [V] and eccentric nucleus [N]; (F) biflagellated zoospore showing hair-like mastigonemes along anterior flagellum; (G) longitudinal section through zoospore showing nucleus [N] portion of apical complex containing the conoid (single arrow), and rhoptry (double arrow). Scale bars = 5 μm in A to D and 1 μm in E to G.

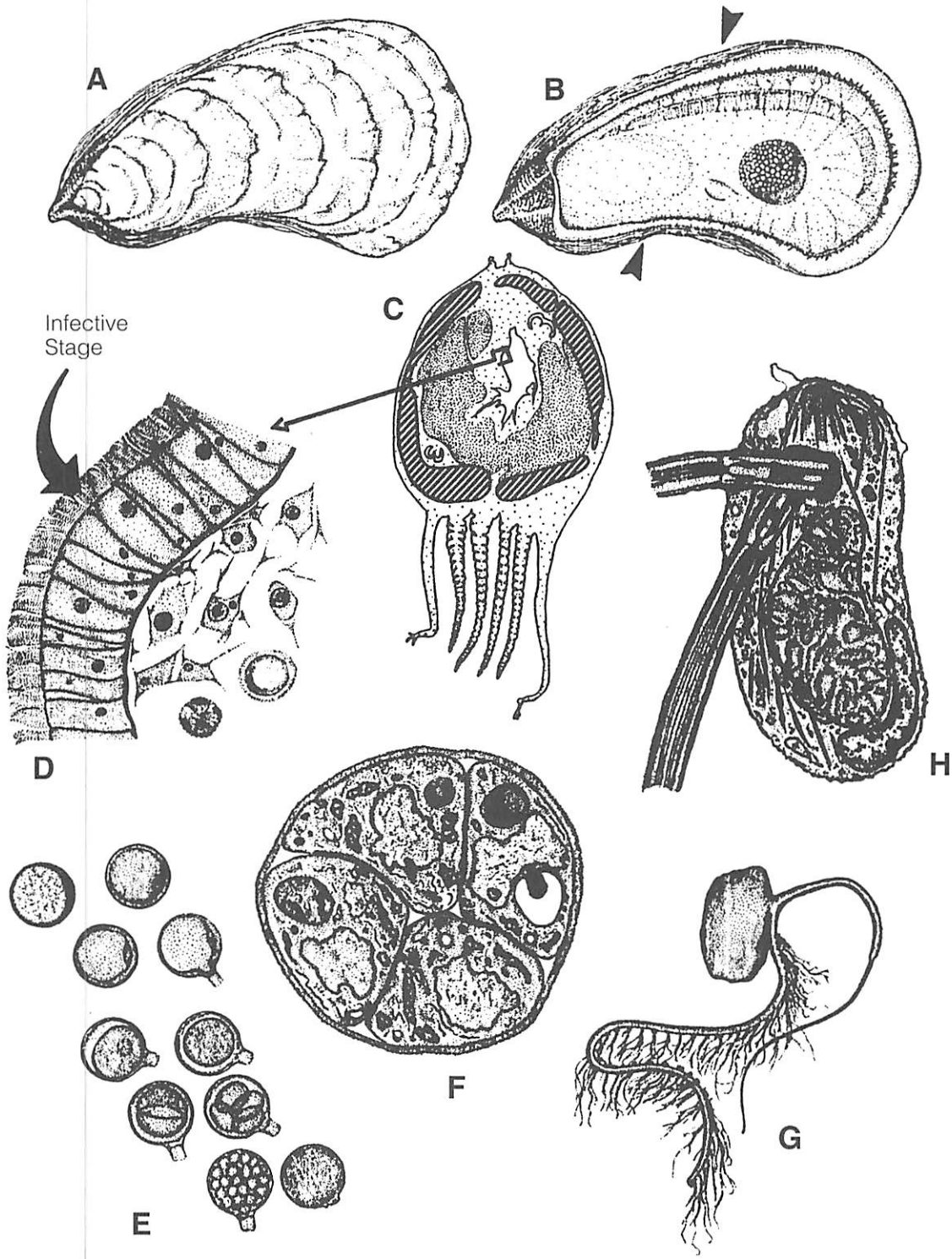

Figure 7. Diagram of stages of *Perkinsus marinus* seen in *Crassostrea virginica*. (A) Intact oyster; (B) oyster with right valve removed showing plane (arrows) of section in (C); (D) stomach epithelium (portal of entry) and underlying connective tissue showing unicellular trophont and multicellular meront; (E) development from early sporangium (top) to mature zoosporangium containing 1 to 2 thousand zoospores (bottom), drawn from Perkins and Menzel (1966); (F) meront containing wedge-shaped merozoites, drawn from Perkins (1988); (G) and (H) biflagellated zoospore, drawn from Fig. 6F, G. See Figure 6 for ultrastructural details.

upon by the hemolymph-sucking snail, *Boonea impressa*, which acts as a vector in the transmission of *P. marinus* to other live oysters (White et al. 1987, 1989). Early studies indicated that dispersion was typically restricted to distances less than 15 m (Andrews 1988a). Epizootics in Chesapeake and Delaware Bays in the late 1980s and early 1990s, however, were characterized by an apparent movement of infective stages over several miles in a single season (J. D. Andrews, Virginia Institute of Marine Science, pers. comm. 1991; Ford, unpubl. data).

Many species of bivalve (e.g., hard clam, *Mercenaria mercenaria*; soft clam, *Mya arenaria*; Baltic macoma, *Macoma* spp.) other than the eastern oyster are infected by *Perkinsus*-like organisms in the United States and elsewhere (Andrews 1954; Ray 1954; Da Ros and Canzonier 1985; Goggin and Lester 1987; Azevedo 1989). Attempts to transmit these organisms by proximity from non-oyster to oyster hosts have consistently failed (Ray 1954; Andrews and Hewatt 1957), and Andrews (1954) reported that non-oyster hosts had lower intensity infections than oysters. Ray (1954) was able to infect some *Mya arenaria* by injection with material from infected oysters, but concluded that overall, species of *Perkinsus* were host-specific. Goggin et al. (1989) reported experimental cross-infections among nine Australian species of molluscs, including both bivalves and gastropods, using *Perkinsus* spp. zoospores. A polyclonal antibody raised against *P. marinus* hypnospores from eastern oysters reacted with *Perkinsus* spp. in *Macoma balthica* and *Mya arenaria* in the United States, and in several molluscan species from Europe and Australia (Dungan and Roberson 1993). It failed to recognize *Perkinsus* spp. in other Australian molluscs and *P. karlssoni* in scallops from Canada. In another comparison among *Perkinsus* species, Goggin (1994) found that the length and sequence of internal transcribed spacers (rapidly evolving noncoding regions) of *P. marinus* ribosomal RNA differed significantly from four other isolates from hosts in Australia and Europe. She suggested that the Australian isolates (*P. olseni* and two unnamed "species") and that from Europe (*P. atlanticus* from Portugal) actually belong to the same species, which is distinct from *P. marinus*.

Infection and Disease Processes

Diagnosis. Initially, infections by *P. marinus* were detected by use of tissue sections (Mackin 1951a, b). Shortly after the parasite was discovered, however, Ray (1952) developed a diagnostic technique using fluid thioglycollate medium in sea water (RFTM) (see also Ray 1966a for modifications). It has become the detection method of choice because it is generally more sensitive than histology as well as being faster and less expensive. Pieces of tissue (typically the rectum and a section of mantle or gill) are incubated in the anaerobic medium for 5 to 7 days at 25° to 30°C (technique referred to as the Ray/Mackin tissue assay). In the medium, parasites enlarge to form thick-walled "hypnospores," but do not replicate. After the tissue is removed from the medium, it is stained with Lugol's iodine, which turns the parasites blue-black. Hemolymph also can be incubated in RFTM to detect circulating parasites (Gauthier and Fisher 1990), a technique that permits repeated, quantitative, and nondestructive sampling of individual hosts. Polyclonal antibodies that recognize all known cell types, including zoospores, have been developed from hypnospores (Dungan and Roberson 1993) and are being used to detect pathogens in ambient water (T. Li et al. 1994). Neither the Ray/Mackin assay nor the polyclonal antibody can differentiate *P. marinus* from other *Perkinsus* species, but gene-probe assays specific for *P. marinus* are being developed (Marsh and Vasta 1995).

Infection intensity in the Ray/Mackin tissue assay is usually rated from 0 (uninfected) to 5 (heavy) (Mackin 1962). Choi et al. (1989) developed a technique for isolating and quantifying hypnospores in infected tissue. They reported that RFTM-incubated oyster tissue could be dissolved in NaOH without harming the hypnospores. Parasites were recovered and counted. The number of hypnospores per gram wet tissue weight (body burden) was found to be exponentially related to the Ray/Mackin rating system (Fig. 8). In a one-year study, Bushek et al. (1994b) compared the hemolymph technique (using 250-µl volumes compatible with repeated sampling), the Ray/Mackin method, and body burden counts. They found that the hemolymph and the Ray/Mackin as-

says were equally sensitive (i.e., able to detect the parasite). Each gave a reasonable estimate of the average infection intensity in the population, but not in the individual oyster. Further, neither reliably detected parasites until their density in the oyster was above 1,000 cells g wet weight $^{-1}$ (Fig. 9A, B; Bushek et al. 1994b). Consequently, both seriously underestimated true prevalence in the spring when intensities were low (Fig. 10 A, B). Ragone Calvo and Burreson (1994) reported that hemolymph diagnosis (300-µl samples) was equivalent to the Ray/Mackin assay in late autumn and early winter, but gave false negatives in the spring. Although both studies employed relatively small volumes of hemolymph, low sensitivity of the hemolymph technique in spring was found even when oysters were completely exsanguinated to provide the sample (Ford, unpubl. data). Bushek et al. (1994b) recommended use of the total body burden assay when highly accurate measures of infection intensity are needed, the Ray/Mackin assay for routine monitoring because of its simplicity and accuracy at the population level, and the hemolymph technique when oysters must be kept alive.

Portal of Entry. Infections are thought to be initiated when a parasite is ingested and crosses the epithelium of the stomach or intestine. The apical complex of zoospores may secrete lytic substances that aid in penetration (Perkins 1976a); however, parasites may also be ingested and carried through the epithelium by phagocytes (Mackin 1951b; Mackin and Boswell 1956; Perkins 1976b; Alvarez et al. 1992). Although infection can also occur through the gill or mantle epithelium (Perkins 1976b), the earliest le-

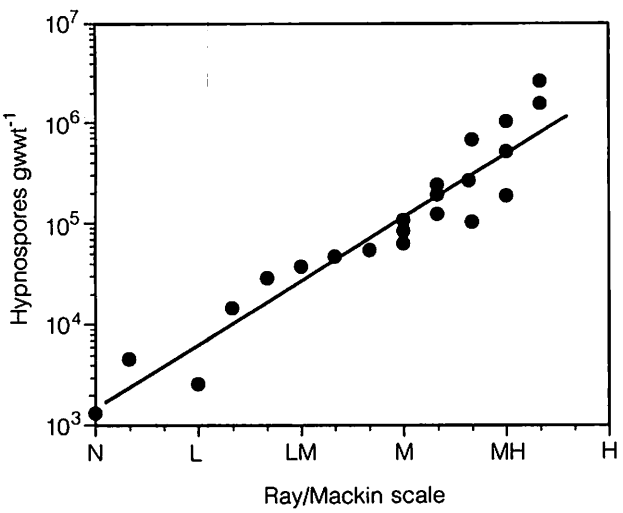

Figure 8. The relationship between the number of hypnospores (prezoosporangia) g wet weight^{-1} (gwwt^{-1}) of oyster tissue and the Ray/Mackin tissue assay for *Perkinsus marinus* infections. The exponential relationship is y (number of hypnospores gwwt^{-1}) = 1409.9 ($10^{0.64296X}$). Ray/Mackin ratings are N = no hypnospores found, L = light, LM = light-moderate, M = moderate, MH = moderate-heavy, H = heavy. From Choi et al. (1989).

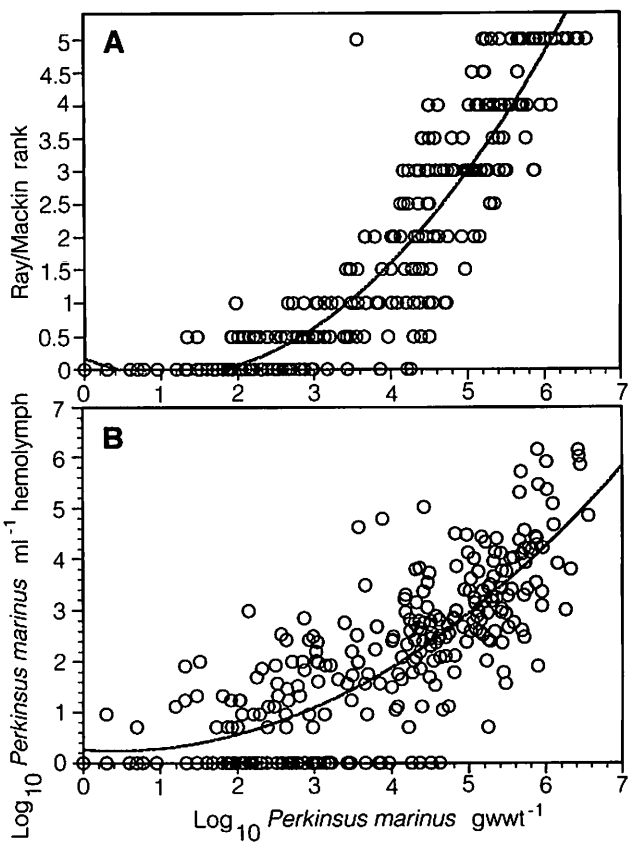

Figure 9. Regressions of (A) the Ray/Mackin tissue assay and (B) the hemolymph assay for diagnosis of *Perkinsus marinus* infections on the body burden assay (number of hypnospores gwwt^{-1}). Values were pooled from all samples collected during a 1-yr study in Delaware Bay. (A) y = 0.176 – 0.463x + 0.205x^2, r^2 = 0.842, $p < 0.001$. (B) y = 0.266 – 0.111x + 0.129x^2, r^2 = 0.642, $p < 0.001$. gwwt = gram wet weight. From Bushek et al. (1994b).

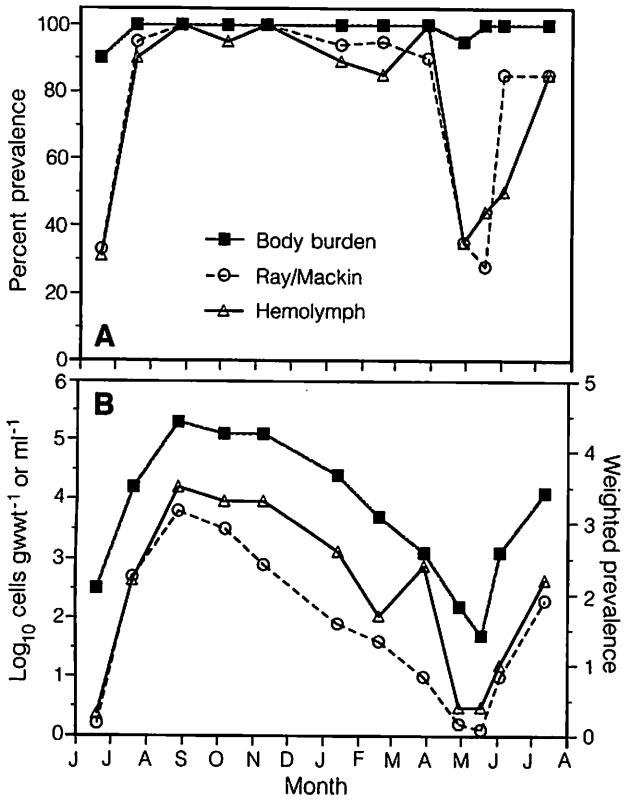

Figure 10. (A) Seasonal percent prevalence and (B) parasite density of *Perkinsus marinus* by three RFTM (Ray's Fluid Thioglycollate Medium) assays: hemolymph, Ray/Mackin, and total body burden. Log_{10}-transformed counts of *P. marinus* for hemolymph and body burden assays are monthly averages; weighted prevalence is the average tissue rank obtained from the Ray/Mackin assay. Key in (A) applies to both figures. gwwt = gram wet weight. From Bushek et al. (1994b).

sions are found in the epithelium of the stomach and intestine (Mackin 1962). The exterior epithelium and that of the digestive tubules are less often parasitized.

Development of Infection and Histopathology. In a series of field experiments employing radiolabelled DNA precursors, Saunders et al. (1993) found that the proliferation rate of *P. marinus* in an oyster was inversely related to the density of parasites in that oyster. Doubling times ranged from a minimum of 1 to 10 h at cell densities of $<10^4$ g dry weight^{-1} to >1 yr at 10^7 parasites g dry weight^{-1}. The slower replication at high densities was attributed to a decrease in available nutrients within the host.

As infections intensify, they are often accompanied by sloughing of hemocytes, parasites, and digestive epithelium into the lumina of the stomach and intestine (Mackin 1951b). The epithelia of digestive tubules atrophy (Gauthier et al. 1990) and tissue lysis is extensive (Fig. 11A, B; see also Mackin 1951b; Ray 1954). Hemolymph vessels of *P. marinus*-infected oysters become occluded by parasites and hemocytes (Fig. 11C). Abscesses containing parasites and hemocytes may break through external epithelia and form ulcers at the mantle surface (Fig. 11D; see also Mackin 1951b). One of the most obvious characteristics of *P. marinus*-infected oysters is an increase in the number of pigmented or brown cells (Fig. 11A; see also Stein and Mackin 1955). Brown cells line the walls of the auricles in all oysters and are scattered systemically among connective tissue cells. They contain lipofuchsin, an indigestible pigmented residue of lysosomal activity that is a product of cell aging, disease, and tissue degeneration (Bloom and Fawcett 1975).

Effects of Disease on Oyster Metabolism

Growth. Menzel and Hopkins (1955) followed growth of a cohort of individually marked oysters over 16 months of exposure to *P. marinus*. At the end of the experiment, survivors were examined histologically for the parasite. Individuals with light infections had deposited new shell at a slower rate than uninfected oysters for the preceding 2 months, whereas those with heavy parasitism had failed to add any new shell for the previous 5 months (Fig. 12). Andrews (1961) found similar results using underwater weighing. Burreson (1991) and Paynter and Burreson (1991) reported an almost complete cessation in growth of several selectively bred (for MSX-disease resistance and for rapid growth) oyster strains when they became infected with *P. marinus*. Native wild stocks also became infected, but they experienced less growth inhibition, suggesting that inbreeding may have heightened sensitivity of these selected oysters to Dermo disease.

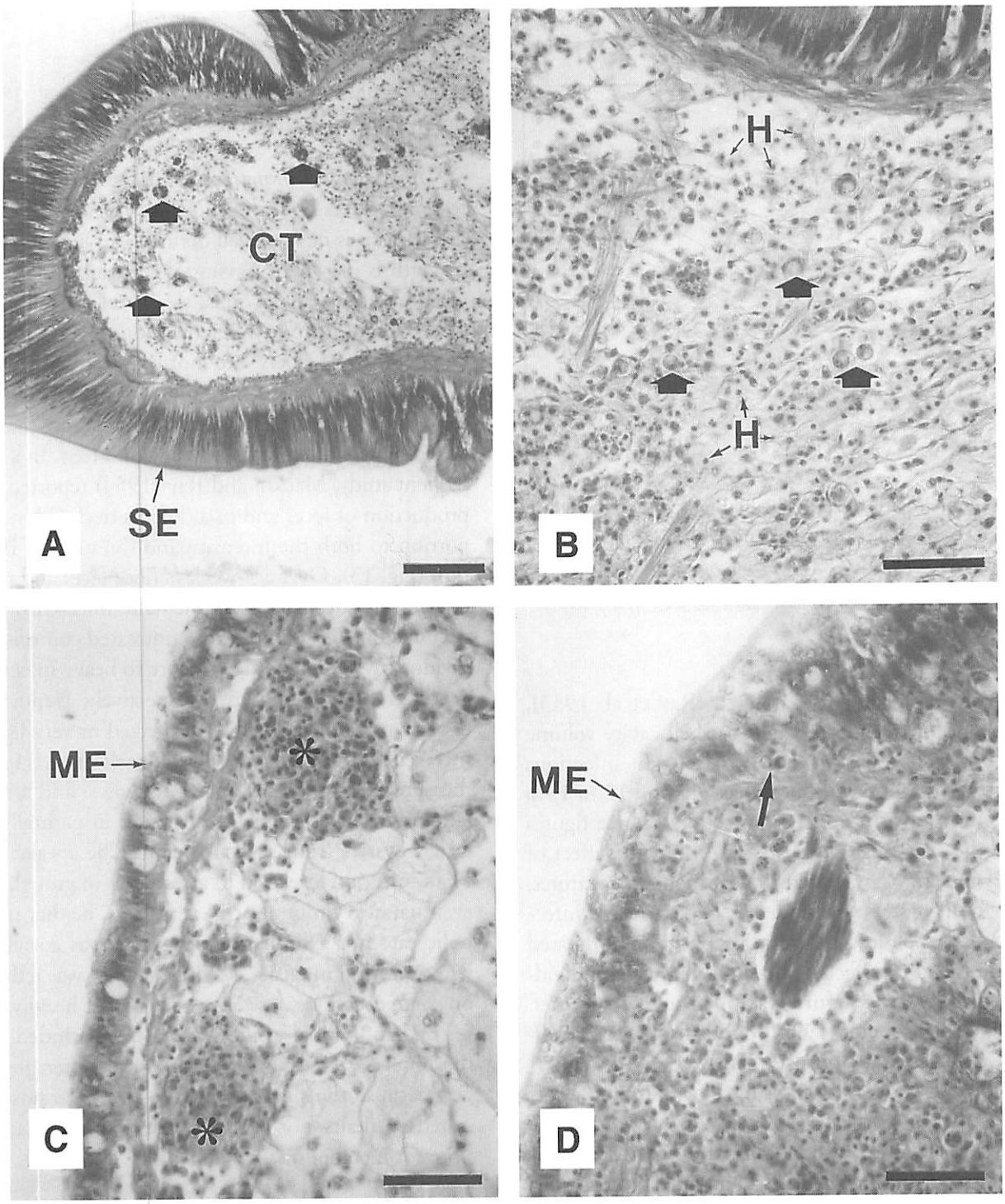

Figure 11. Histopathology caused by *Perkinsus marinus* in tissues of *Crassostrea virginica*: (A) disruption and lysis of storage-connective tissue [CT], brown cells (arrows), and morphologically normal stomach epithelium [SE]; (B) massive hemocyte [H] infiltration associated with presence of trophonts (broad arrows) in digestive gland storage-connective tissue; (C) occlusion of hemolymph vessels (*) beneath outer mantle epithelium [ME]; (D) migration of *P. marinus*-laden hemocytes (larger arrow) through outer mantle epithelium [ME]. Scale bars = 100 μm in A and 50 μm in B to D.

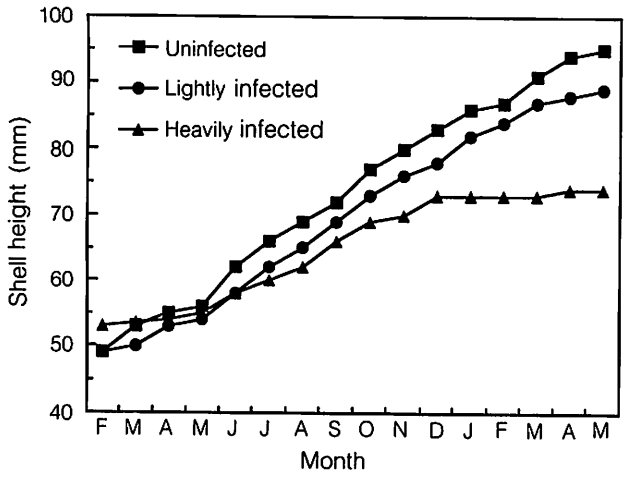

Figure 12. Growth of individually marked oysters held in trays in Terrebonne Bay, Louisiana, over a 16-month period and then diagnosed for *Perkinsus marinus*. Infection ratings were made from tissue sections by J. G. Mackin. From Menzel and Hopkins (1955).

Soft tissues are also affected (Ray et al. 1953). The ratio of wet meat weight to shell cavity volume was 8 to 9% lower in oysters with light infections compared to those with no infections and 12 to 15% less in those with moderate infections. These figures were the same regardless of season, but the effect of heavy infections was intensified at high temperatures. In winter, oysters diagnosed as having heavy infections had 20% lower wet meat condition compared to combined uninfected and lightly infected individuals, whereas this figure was 50% in the summer. Crosby and Roberts (1990) found that oyster condition index (the ratio of soft tissue dry weight to internal shell volume as defined by Lawrence and Scott [1982]) was negatively correlated with *P. marinus* intensity ($p < 0.001$), although only 5% of the variability in condition could be explained by infection intensity. Craig et al. (1989), Gauthier et al. (1990), and Paynter and Burreson (1991) also reported a loss of condition in *P. marinus*-infected oysters, although this is often significant only in advanced infections (Dittman 1993). The reduction of dry-weight condition index, a measure directly correlated with metabolic reserves (Widdows 1985), in parasitized oysters could be caused by disruption of feeding (Mackin and Ray 1954), depletion of glycogen by parasites (Choi et al. 1989), lysis of tissues (Ray et al. 1953), or any combination of these factors.

Physiological Functions. Although *P. marinus* infection reduces both shell and soft tissue growth, the mechanism is not yet well defined. Adductor muscle strength is impaired, leaving oysters, especially those with the heaviest infections, weak and prone to gape when removed from water (Mackin 1962; Gauthier et al. 1990). ("Gapers" are moribund or dead oysters in which the soft tissues are still present.) Working in the Gulf of Mexico, Hewatt (1952) found that infected oysters often failed to open and feed. In a subsequent study, Mackin and Ray (1954) reported that production of feces and pseudofeces declined in proportion to both the intensity and duration of infection (Fig. 13). Over a 3-week period, feces and pseudofeces produced by experimentally infected oysters declined by 40% compared to untreated controls. Individuals with light and moderate to heavy infections produced only 57 and 43%, respectively, as much feces and pseudofeces as did uninfected or very lightly infected oysters. Conversely, Newell et al. (1994) measured clearance and metabolic rates, and assimilation efficiency over a 1-year period in naturally infected oysters from several sites in Chesapeake Bay. High infection levels led to a cessation in growth and eventual heavy mortality. Nevertheless, neither metabolic rate nor assimilation efficiency was correlated with infection intensity. Feeding activity was reduced on some occasions, but only in the most heavily parasitized individuals. These authors concluded that the observed effect of *P. marinus* on oyster growth was because the parasite outcompeted the host for stored nutrients, not because it affected energy acquisition or metabolism.

Biochemical Composition. Histochemical tests revealed that glycogen is depleted in animals with advanced infections as parasites and hemocytes proliferate and replace storage cells (Stein and Mackin 1957). Conversely, both Wilson et al. (1988) and White et al. (1988) found higher tissue glycogen concentrations when infected oysters were analyzed biochemically and speculated that *P. marinus* might stimulate gluconeogenesis. This comparison should be made with

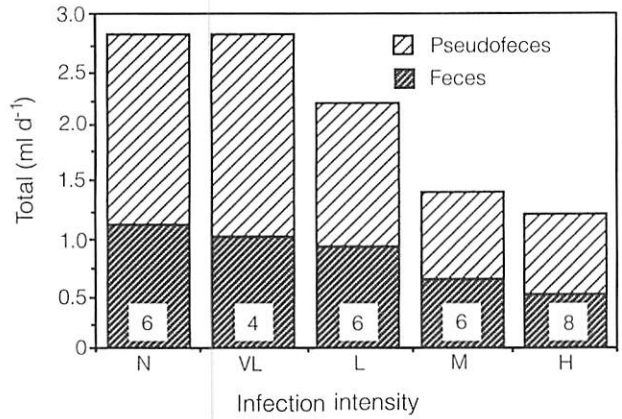

Figure 13. Production of feces and pseudofeces by oysters with different degrees of *Perkinsus marinus* infection. The parasite was injected into the shell cavity of experimental oysters. Oysters were held in experimental chambers through which water from Barataria Bay was pumped for a period of 21 d. Feces and pseudofeces were collected daily and measured wet in a graduated cylinder after settling for 24 h. Oysters were 2.5 to 3.5 cm in shell height and each experimental oyster was matched with an uninjected control oyster of the same size. Infection ratings, obtained with the Ray/Mackin assay, are given in legend to Figure 8; VL = very light. Number of oysters is shown in bars. From Mackin and Ray (1954).

caution, however, because oysters in the biochemical studies were also parasitized by the snail, *Boonea impressa*, techniques differed between studies, and season of collection was not always indicated. Lipid metabolism is clearly altered by infection, as demonstrated by increases in lipid phosphate and fatty acid content (Wilson et al. 1988) and by the large increase in the number of lipofuchsin-containing brown cells characteristic of *P. marinus*-infected animals (Stein and Mackin 1955).

Amino acids and proteins are also altered by *P. marinus* infection. Tissue amino acid concentration decreased with increasing infection intensity due to the reduction of most individual amino acids (Soniat and Koenig 1982; Paynter et al. 1995). Soniat and Koenig (1982) documented an increase in the taurine-to-glycine ratio similar to that reported for molluscs stressed by other infectious and noninfectious diseases (Jeffries 1972; Livingstone 1985). Chu and La Peyre (1989, 1993) and Chu et al. (1993) found considerable variation associated with season, temperature, and salinity, but no consistent significant association between cell-free hemolymph protein or lysozyme concentrations and infection intensity in oysters that had mostly light to moderate infections. Conversely, Chintala et al. (1994) reported a negative correlation of hemolymph protein with (1) *P. marinus* infection intensity and (2) survival time post infection; however, this occurred only after most oysters had moderate to heavy infections.

Reproduction. Like other physiological and metabolic functions, gametogenic development in oysters exposed to *P. marinus* appears to be impaired primarily after infections become advanced. For instance, Dittman (1993) reported that during their first year of exposure when infections were generally light, there was no significant effect of the parasite on percent gonad area of Delaware Bay oysters whereas in the second year, "the percent gonad area of the individuals with advanced infections was significantly lower than that of the individuals with no infections and in most cases was lower than of the individuals with light infections." A similar finding was reported by Choi et al. (1994) in the Gulf of Mexico, where the spawning period is more extended. They measured reproductive effort using an immunological probe for oyster egg protein and *in vivo* incorporation of radiolabelled protein precursors into oysters placed in the field. They found no measurable impact of *P. marinus* in the spring, when most infections were light, but a clear negative association between reproductive development and parasitism in the late-summer/fall, when infections were heavier. Mackin (1962) found that reproductive inhibition was most severe when heavy infections coincided with the earliest stages of gametogenesis. He also found no difference in infection intensities between pre- and post-spawning oysters, and concluded that spawning "stress" was not responsible for the acceleration of disease development and mortality in late summer.

Cause of Death. Many investigators have concluded that extensive tissue lysis and occlusion of major

hemolymph vessels cause death of *P. marinus*-infected oysters (Mackin 1951b; Ray 1954; Perkins 1976b). They have discounted the presence of a toxin because most oysters die only after infections have become extensive. Mackin and Ray (1954) also dismissed starvation as a cause of death because the disease can become lethal within a few weeks of infection, whereas oysters may survive many months without food (Ray 1966b). Evidence for lytic factors has been strengthened by recent findings that an anti-*P. marinus* antibody recognized soluble and noncellular particulate antigen in tissue sections (Fig. 14), where it appeared to be diffusing from infection foci (Dungan and Roberson 1993) and by the fact that cultured *P. marinus* secrete proteases (Faisal et al. 1994). Another factor possibly contributing to pathogenesis is the production of cytotoxic reactive oxygen intermediates (ROIs) by hemocytes of heavily infected oysters (Anderson et al. 1992c), although it is not certain that the relatively small (~2-fold) differences between lightly and heavily infected oysters would be sufficient to cause extensive pathology (Anderson et al. 1995).

On the other hand, Choi et al. (1989) estimated the total energy demand of *P. marinus* parasites at several infection intensities from values for other protozoans and concluded that energy depletion caused by the metabolic demands of the parasite was sufficient to result in the observed reduced growth rate, impaired reproduction, and mortality of infected oysters. The same conclusion was reached by Newell et al. (1994) after a suite of physiological measurements failed to link infections with changes in assimilation efficiency, metabolic rate, or (except in some heavily infected individuals) feeding rates. The pathology and nutrient depletion hypotheses are not mutually exclusive as the parasites surely exert a metabolic drain on the host that increases with infection intensity. As infections become more advanced, the extensive associated cell and tissue pathology must also contribute to death.

Host Response. Infections by *P. marinus* are accompanied by an increase in circulating hemocytes and their infiltration into affected areas of the epithelium and subepithelial tissues, where host cells respond to the parasite by both phagocytosis and encapsulation (Figs. 6A, B; 11B, C, D; see also Mackin 1951b; Perkins 1976b; Anderson et al. 1992c; Chu and La Peyre 1993; Anderson et al. 1995). Zoospores and vegetative stages are phagocytosed by oyster hemocytes *in vitro* (Chu 1988; La Peyre et al. 1995) and infective forms may be brought across the stomach epithelium in phagocytes (Mackin 1951b; Perkins 1976b; Alvarez et al. 1992). In the eastern oyster, as many as half of the parasites may be contained within hemocytes, where they are found in phagosomes (Perkins 1988). Some parasites appear to be destroyed within the phagocytes (La Peyre et al. 1995), but others continue to develop within host cells and eventually destroy them (Mackin 1951b). Hemocytes withdrawn from oysters with advanced *P. marinus* infections have elevated levels of ROIs, which are considered to act in destruction of microbes (Anderson et

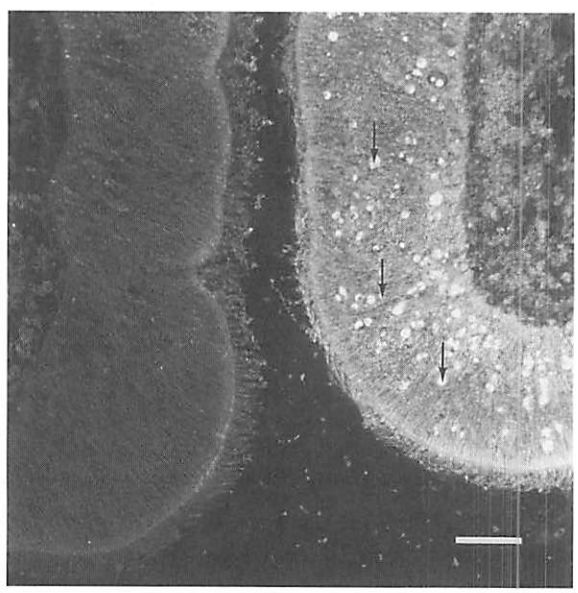

Figure 14. Fluorescence immunostained (Dungan and Roberson 1993) focal *Perkinsus marinus* lesion in *Crassostrea virginica* stomach epithelium. Individual *P. marinus* cells (arrows) infecting the ciliated epithelium on right are intensely stained, whereas no pathogen cells are seen within the epithelium on the left. In addition to cellular immunofluorescence, the infected portion of the epithelium shows diffuse, noncellular immunofluorescence suggesting antibody localization of exported, soluble *P. marinus* antigens in the infected epithelium, its underlying connective tissue, and the adjacent apical surface of cells in the uninfected epithelium. Scale bar = 50 μm.

al. 1992c; Anderson et al. 1995). Their significance in Dermo disease is not clear, however, as evidence from *in vitro* experiments suggests that ingested *P. marinus* are able to suppress intracellular production of cytotoxic oxygen radicals (Anderson et al. 1995; Volety and Chu 1995). Anderson et al. (1995) proposed that many types of particles, including *P. marinus*, might be able to nonspecifically activate hemocytes, but further ROI responses from activated cells would be limited to certain agents, not including *P. marinus*.

Migration of *P. marinus*-laden hemocytes occurs across digestive and external epithelia (Fig. 11D), but does not appear to be effective in ridding the oyster of infection in warm weather because parasites proliferate too rapidly. It may, however, reduce parasite numbers in cooler periods (Mackin 1951b). Perkins (1976b) also reported that attempts by hemocytes to encapsulate small foci of infection in the epithelium and connective tissue failed because the parasites multiplied too rapidly. In fact, Mackin (1951b) concluded that there appeared to be no effective "physiological" response to development of *P. marinus* infections in eastern oysters and selection for *P. marinus* resistant strains has proved more difficult than selection for resistance to MSX disease (see p. 606).

Epizootiology

Infection and Mortality Periods. In Chesapeake and Delaware Bays, the northernmost estuaries where seasonal cycles of *P. marinus* are well-documented, infective stages are present throughout the warm months, probably from May through October (Andrews and Hewatt 1957; Ford unpubl. data). In the lower Chesapeake Bay, newly introduced oysters show initial infections in July with prevalence peaks between September and November and a major mortality period in September and October (Fig. 15). Prevalence in surviving oysters declines dramatically over the late winter and early spring, and parasites frequently become undetectable by the standard Ray/Mackin assay (Fig. 10); however, some parasites remain and proliferate as temperatures rise in the spring (Andrews 1988a; Bushek et al. 1994b; Ragone Calvo and Burreson 1994). Infected and dying oysters release stages that initiate another round of infections. This cycle occurs earlier and more frequently each year that oysters remain exposed and Andrews (1967) estimated that it required from one to three years for a full-fledged epizootic to develop from natural sources in Chesapeake Bay. During the recent Delaware Bay epizootic, *P. marinus* infections were first found in late summer 1990 and widespread mortalities occurred the following summer (Ford, unpubl. data).

In the southeastern United States and the Gulf of Mexico, infection and mortality periods are not as discrete, most likely because temperatures are never low enough to suppress activity of either host or parasite (see p. 608). New infections can probably occur as long as oysters are feeding. Both prevalence and intensity are generally low from January through April and highest from August through November (Ray 1954; Quick and Mackin 1971; Burrell et al., 1984; Soniat 1985; Crosby and Roberts 1990). Mortalities are greatest from July through November (Mackin and Sparks 1962; Quick and Mackin 1971).

Prevalence Levels and Mortality Rates. Prevalence of *P. marinus* varies seasonally and the maximum found in a given year is considered an estimate of infection pressure for that year and location. During fully epizootic conditions, peak prevalences of *P. marinus* infections determined after RFTM incubation are between 80 and 100% (Andrews and Hewatt 1957; Ray 1966a; Quick and Mackin 1971; Burreson and Andrews 1988; Craig et al. 1989). Because some infected oysters undoubtedly die before maximum prevalence is reached in the population, and some light infections go undetected, it is probable that virtually all oysters become infected under these conditions. In North Inlet estuary, South Carolina, Crosby and Roberts (1990) found only 4 months (February-May) between March 1988 and March 1989 in which prevalences were below 100%. Prevalences of less than 30% were typical of enzootic areas in Delaware Bay when *P. marinus* was introduced in the mid-1950s and caused no reported mortalities (Christensen 1956).

Hofstetter (1977) reported *P. marinus*-associated mortality rates averaging 50% per year in oysters in Texas. In Florida, annual mortality rates were estimated to be between 50 and 100% (Quick and Mackin 1971). In experiments with oysters held in

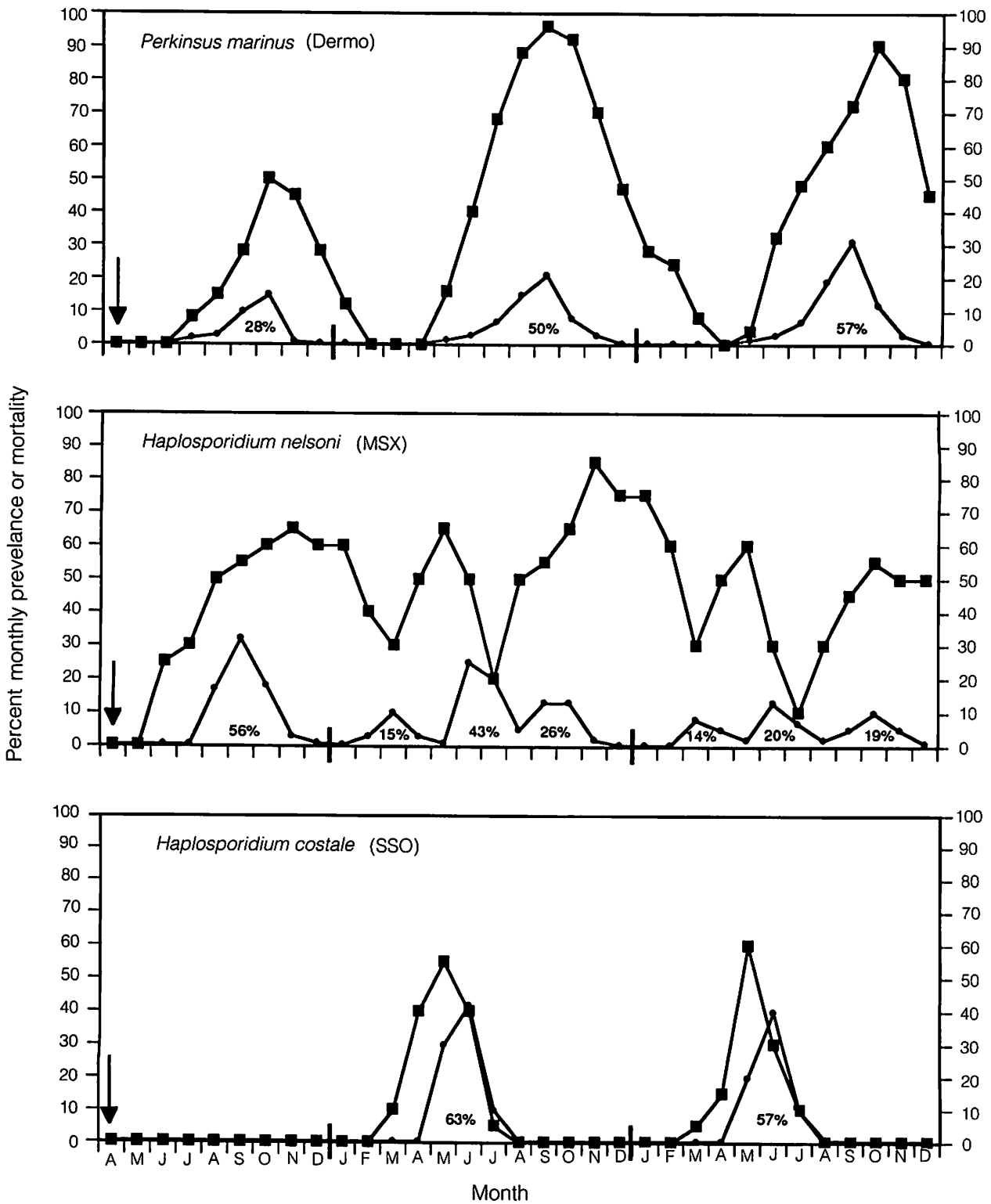

Figure 15. Typical monthly prevalence (■) and mortality (●) patterns over 3 years in susceptible eastern oyster stocks of diseases caused by *Perkinsus marinus*, *Haplosporidium nelsoni*, and *H. costale* on the mid-Atlantic coast of the United States. Percentages annotated under peaks are the cumulative mortalities for the areas under that peak. Arrows mark initial exposure. Data adapted from Andrews and Hewatt (1957); Andrews (1966); Andrews and Castagna (1978); Ford and Haskin (1982).

trays and exposed to *P. marinus* in Louisiana, Mackin and Sparks (1962) recorded cumulative mortalities between March and September of up to 80%. In Chesapeake Bay, it is more difficult to assign causes of mortality because *Haplosporidium nelsoni* (causative agent of MSX disease), as well as *P. marinus*, causes deaths. During the 1950s, before *H. nelsoni* became a factor in the lower Chesapeake Bay, however, mortalities were caused primarily by *P. marinus* and they ranged from 25 to 60% annually (Andrews 1988a). In the Long Island Sound and southern Massachusetts areas, mortalities have been relatively low (<25% annually) or even absent despite infection prevalence and intensities that rival those in more southern regions (Ford, unpubl. data).

Infection and mortality rates depend to some extent on oyster age (Mackin 1951a; Ray 1954; Andrews and Hewatt 1957). Young oysters are generally less likely to be infected and more likely to have lower mortality rates than older oysters (Table 2). When this phenomenon was initially recognized, it was interpreted as "immunity" in young oysters, which was lost as they became older (Ray 1954). Subsequently, Andrews and Hewatt (1957) demonstrated that spat could be easily infected under experimental conditions, and even in nature they can show high prevalences (Ray 1987). The comparatively low prevalences in spat are now thought to result from the relatively small volume of water filtered by young oysters and the reduced chance of encountering infective particles. When dosage is high enough to ensure a high probability that spat will ingest infective stages, they become as heavily infected as older oysters (Ray 1987; Andrews and Ray 1988).

Infectivity and Pathogenicity. The ability to transmit *P. marinus* under laboratory conditions has permitted studies to define its infectivity and pathogenicity as well as the influence of environmental variables on the disease process. In the laboratory, the infectious agent can be transmitted by proximity to infected oysters, by feeding minced infected tissues, or by injection of infected material into the mantle cavity or adductor muscle sinuses of recipient oysters (Ray 1954; Andrews and Hewatt 1957; Mackin 1962; Bushek 1994; Volety and Chu 1994). In general, infections, mortalities, or both, appear earliest (10 to 20 d post-inoculation) when parasites are injected directly into the adductor muscle or mantle cavity. Feeding of minced tissues causes death within 3 weeks, whereas death resulting from proximity to infected individuals begins in 3 to 6 weeks. Under laboratory conditions such as these, cumulative mortality can exceed 90% within 2 months (Ray 1954). Mortality rates and days to first mortality are roughly proportional to the number of *P. marinus* hypnospores (prezoosporangia) injected into the shell cavity; however, fewer than 500 cells injected caused no deaths and more than 10^5 cells did not further increase mortality (Fig. 16; see also Mackin 1962).

Perkinsus marinus cultured in the laboratory can be significantly less virulent than natural cells as measured by infection and mortality rates after challenge (Bushek 1994). In a direct comparison, mortalities

Table 2. Percent annual mortality rates of oysters exposed to *Perkinsus marinus* in lower Chesapeake Bay, showing differences associated with age (years of exposure) and geographical source. From Andrews and Hewatt (1957).

Source	Age (= Years of Exposure)		
	Yearlings	2 Years Old	≥ 3 Years Old
Chesapeake Bay	<10	17 to 26	26 to 67
Seaside of Virginia	16 to 30	37 to 74	46 to 71
South Carolina	7 to 26	10 to 12	22 to 26

Prevalence of *P. marinus* in dead oysters was ≥ 90% except in South Carolina yearling and 2-year old oysters, where it was 25 and 56%, respectively.

were 10 times greater over a 3-month period in oysters exposed to natural cells than in those exposed to cultured cells (Ford and Bushek, unpubl. data). Loss of virulence in cultured parasites is not uncommon and may be due to adaptive changes that permit the organisms to live in culture rather than in a host (Giannini 1974; Moody et al. 1990). Among the factors influencing virulence of *P. marinus* is genetic variation of parasites from different geographic regions. In a factorial experiment using cultured cells, Bushek (1994) demonstrated that *P. marinus* isolated from Atlantic coast oysters caused significantly heavier infections than did parasites from Gulf coast oysters.

During an epizootic caused by *P. marinus*, about 90% of the dead and dying oysters are parasitized and nearly all of them have advanced infections (4 to 5 on the Mackin scale) (Mackin 1951a; Ray 1954; Andrews and Hewatt 1957; Andrews 1965). Andrews (1988a) reported that mortalities are likely to occur when the mean infection intensity for a population exceeds 1 on the Mackin scale; however, it is becoming clear from studies in locations north of Chesapeake Bay that the attainment of high infection intensities does not necessarily predict death if this level is reached late in the season. As water temperatures start to decline in the autumn, parasites can be suppressed before they cause a lethal disease. Oysters

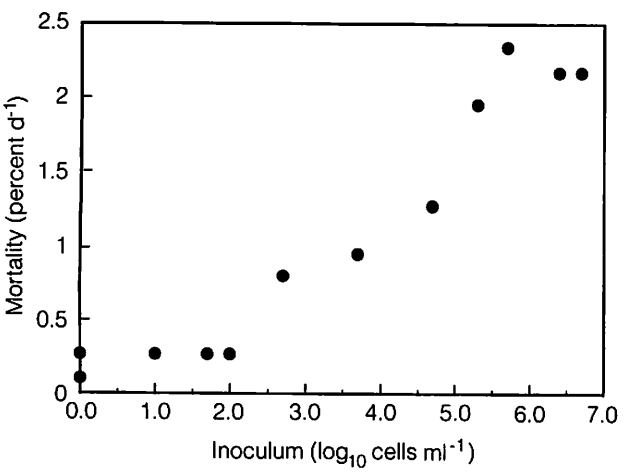

Figure 16. Mortality of eastern oysters versus dose of *Perkinus marinus* cells injected into the shell cavity. Parasites were incubated for 1 d in fluid thioglycollate medium before injection. From Mackin (1962).

with infections rated 4 or 5 on the Mackin scale may appear grossly healthy (exhibit good shell growth and meat quality) and can recover over the winter and spring, although parasites proliferate again in the summer with rising water temperatures (Ford, unpubl. data).

Epizootic/Enzootic Periodicity. Comparison of recent and past data from surveys for *P. marinus* suggest that parasite abundance has been relatively stable over time in the Gulf of Mexico (Craig et al. 1989). Short-term, local changes in this region appear to be the result of fluctuations in fresh-water flow into estuaries, which reduce numbers of both host and parasite. In contrast, there was a steady spread and intensification of the disease north along the Atlantic coast during the late 1980s and early 1990s. This range extension was associated with drought, above-normal temperatures, and unintentional introduction of infected oysters or shucking wastes (Andrews 1988a; Burreson and Andrews 1988; Chu and Green 1989; Ford 1992).

Resistance

The first reported differences among oyster populations in resistance to Dermo disease was by Andrews (1954). He found that oysters from Chesapeake Bay, where *P. marinus* was enzootic, survived better than those from the ocean side of Virginia, where the parasite had (at that time) not been found. Oysters imported from South Carolina, however, survived better than either group of Virginia oysters (Table 2). Similarly, Mackin and Sparks (1962) exposed two groups of oysters, one from an enzootic area and one from a nonenzootic area, to *P. marinus* in Louisiana. They reported that cumulative mortality of the previously unexposed oysters was two-and-a-half times greater than for previously exposed animals. Despite this strong suggestion that selective mortality in nature had produced resistant stocks, clear evidence for heritable resistance was lacking for many years. In fact, Andrews (1954) noted that 6-year-old Chesapeake Bay oysters exposed to *P. marinus* in nature were "still dying at the same rate as three years ago." Further, he was unable to select strains

for resistance to Dermo disease, although he did produce strains resistant to MSX disease (Andrews 1968, 1984a).

To determine whether oysters resistant to MSX disease were also resistant to Dermo disease, Valiulis (1973) inoculated MSX disease-resistant oysters with *P. marinus* isolated directly from infected oysters. He found that MSX disease-resistant strains survived better than unselected strains at doses of up to 10^4 *P. marinus* cells per oyster. At higher doses, this relationship failed (see Ford 1988). Recent exposures of MSX disease-resistant oysters in Chesapeake and Delaware Bays resulted in high mortalities associated with *P. marinus* (Burreson 1991; Chintala and Fisher 1991). Thus, the original suggestion of Valiulis (1973) that a common mechanism may underlie resistance to both MSX and Dermo diseases has not been validated by field tests. The failure of Andrews (1984a) to develop resistant strains through selective breeding in Chesapeake Bay may be because *H. nelsoni* generally kills oysters before *P. marinus* does (Andrews 1965, 1967). Andrews' strains were exposed to both diseases, but the most intense selection may have been for resistance to MSX disease. Andrews' results further suggest that resistance to the two diseases involves separate mechanisms.

In the Gulf of Mexico, where *P. marinus* is prevalent and causes widespread mortality, it is surprising that resistant stocks have not been reported. Several hypotheses have been proposed to explain this apparent anomaly (Ray 1954 and pers. comm. 1988): (1) resistant populations have developed, but have not been discovered; (2) harvesting of large (presumably old and well selected) oysters removes those individuals most likely to produce resistant offspring; (3) selected individuals are most likely to exist in relatively high salinity water and if their offspring settle in the same region, they are apt to be killed by predators that are also most prevalent in high salinity areas; and (4) oysters in the Gulf can reproduce during their first year, before they suffer significant selective mortality. It also seemed possible that no effective survival mechanism existed on which natural selection could act. While it was puzzling that clear evidence of resistance had not been discovered in the Gulf over the nearly half-century since *P. marinus* was first described, controlled comparisons with known unselected oysters, similar to those performed by Haskin and Ford (1979) to demonstrate resistance to MSX disease, had not been conducted until recently to document possible resistance to Dermo disease.

The recent intensification and range extension by *P. marinus* has stimulated renewed efforts to document and develop resistance. In a controlled, factorial experiment, Bushek (1994) demonstrated for the first time that heritable resistance to Dermo disease does exist. He compared four geographically separated oyster stocks that had been spawned and reared in a common environment. Oysters were injected with parasites that had been established in culture. Oysters from New Jersey and Maine, which had undergone little or no selective pressure, developed significantly heavier infections than oysters from Texas and Virginia, where populations have been parasitized for more than 40 years.

The Pacific oyster, *Crassostrea gigas*, appears to be resistant to diseases that affect other oyster species in Europe and has been proposed as an alternative to the eastern oyster for culture on the Atlantic coast of the United States (Mann et al. 1991). Recent studies showed that *C. gigas* experimentally challenged with *P. marinus* became infected, but that infections failed to develop or cause mortality (Meyers et al. 1991; Barber and Mann 1994).

Environmental Influences

Temperature. Perkinsus marinus proliferates within, spreads between, and kills oysters most rapidly at temperatures above 25°C (Andrews 1965; Hewatt and Andrews 1955; Fisher et al. 1992). Infected oysters held at 25° to 32°C had six times the mortality of those held at 18°C, even though prevalence was the same in both groups (Mackin 1951a). Infections were lighter at the lower temperature, suggesting to Mackin that the metabolic rate of the parasite was reduced at the lower temperature. In Chesapeake Bay, Chu and La Peyre (1993) found an apparent temperature threshold between 15° and 20°C. When oysters acclimated to 10°, 15°, 20° and 25°C were inoculated with *P. marinus* and maintained at those temperatures for 46 d, final prevalence in the four groups was

23, 46, 91, and 100%, respectively, with advanced infections appearing at 20° and 25°C only.

Both prevalence and intensity of the parasite decrease over winter and early spring in nearly all areas sampled, but especially at the northern end of its range where localized infections in the gut epithelium (Ragone Calvo and Burreson 1994) persist over winter. They are not usually detected by standard RFTM methods, but proliferate when temperatures rise in the spring (Bushek et al. 1994b; Ragone Calvo and Burreson 1994). Very low temperature itself, however, does not seem to be lethal for *P. marinus*. Holding infected oysters for up to 6 weeks at 1° or 4°C and 10 ppt failed to significantly reduce either prevalence or intensity, but when these oysters were warmed to 12°C at 3, 6, or 15 ppt, infection levels decreased within 2 weeks (Burreson and Ragone Calvo 1993). Also, *P. marinus* apparently can survive freezing, as indicated by enlargement in RFTM (Andrews and Hewatt 1957), and parasites can be detected by RFTM only if they were viable upon incubation (Ray 1954).

In vitro experiments corroborate field observations. Ray (1954) found that the optimum temperature range for enlargement of hypnospores in RFTM was between 25° and 30°C. Enlargement was retarded at 35°C and death occurred at 37°C. Enlargement also occurred in RFTM kept at 10° to 24°C, but to progressively smaller final sizes with decreasing temperature. At 0° to 4°C there was no size change, but when returned to room temperature after 60 h, hypnospores enlarged in a manner similar to controls grown at 25°C. Sporulation of sporangia in sea water occurred in 2 to 4 d at 28°C, 2 to 8 d at 18° to 20°C, and failed to occur at 0°C (Chu and Green 1989). Both prezoosporangia and zoospores tolerated temperatures of less than 4°C for only 1 to 4 d *in vitro*. In culture, proliferation rates are also highly dependent on temperature (Bushek 1994; Dungan and Hamilton In press; Gauthier and Vasta 1995).

Salinity. Reduced prevalence of *P. marinus* in low salinity has been a consistent finding of field distribution studies (Mackin 1951a, 1956; Christensen 1956; Andrews and Hewatt 1957; Soniat 1985; Ray 1987; Craig et al. 1989; Soniat and Gauthier 1989; Gauthier et al. 1990). The parasite appears intolerant of salinities below 8 to 9 ppt in the field (Mackin 1956) and 12 ppt is usually described as the salinity required for a full epizootic. Ragone and Burreson (1993) held naturally infected oysters for 8 weeks in the laboratory at temperatures of 20° to 25°C and at four salinity regimes: 6, 9, 12, and 20 ppt. Prevalence remained between 80 and 100% and advanced infections persisted at all salinities, but the development of lethal infections was retarded at 6 and 9 ppt. Mortalities were half as great (~14%) at 6 and 9 ppt compared to those at 12 and 20 ppt (~31%). Lethal infections and resulting deaths were increasing in the 6 and 9 ppt treatments near the end of the experiment, however, suggesting that low-salinity control of infections was not permanent. Similarly, the onset of infections in oysters fed minced tissues from parasitized oysters was delayed by one month in oysters kept at 10 to 13 ppt compared to those kept at 26 to 28 ppt, but total mortality reached 100% in both groups after 3 months (Ray 1954). Movement of infected oysters into salinity as low as 11 ppt had relatively little effect on the parasite, especially at high temperature (Mackin 1956; Andrews and Hewatt 1957).

A threshold salinity of about 9 to 10 ppt, below which *P. marinus* has reduced pathogenicity, has been supported in another set of *in vivo* experiments in which oysters were inoculated with a known concentration of parasites isolated directly from infected oysters. The oysters were held at 3, 10, and 20 ppt for 5 weeks at 22°C (Chu et al. 1993). Prevalences were 50, 79, and 82%, respectively, at the conclusion of the test. Heavy infections developed only in 10 and 20 ppt groups. Although only light infections developed at 3 ppt, the fact that they developed at all indicates how tolerant *P. marinus* is of low salinity.

Several *in vitro* tests of *P. marinus* viability at various salinities have been conducted. In RFTM, hypnospores enlarged at all salinities between 4 and 50 ppt, but parasite walls were thinner at the lower end of the range and final size was slightly less at the higher end, compared to the middle range (Ray 1954). Chu and Green (1989) found that zoospores could survive up to 28 d at 4 ppt and 20° to 28°C. At 28°C, *in vitro* sporulation occurred in 2 to 4 d at 10

to 34 ppt, in 3 to 7 d at 6 to 8 ppt, and did not occur below 4 ppt. Cultured *P. marinus* are less tolerant of low salinity. Burreson et al. (1994c) exposed cultured cells to *in vitro* salinities of 3, 6, 9, 12, and 22 ppt at temperatures of 1°, 5°, 10°, 15°, and 28°C. After 1 h, parasite mortality was significantly affected by salinity, but not by temperature (Fig. 17A). No parasites survived at 0 and 3 ppt; mortality decreased from 85 to 90% at 6 ppt to about 25% at 12 ppt and to less than 5% at 22 ppt. After 24 h, mortality increased at all salinities except 22 ppt and was inversely related to temperature. At 6, 9, and 12 ppt, mortality was elevated at low temperatures although this was significant only at 9 ppt (Fig. 17B).

At least part of the correlation between low prevalence and low salinity in the field may be produced by dilution of infective stages (Mackin 1956) or the destruction of host oysters during periodic floods and the fact that re-establishment of parasite populations lags behind re-establishment of host populations (Hofstetter 1977; Ray 1987).

Some studies have attempted to determine the relative importance of temperature and salinity on *P. marinus* infections. For instance, in a South Carolina estuary where salinity ranged from 29 to 35 ppt and temperature from 10° to 28°C over the year, Crosby and Roberts (1990) found a positive correlation ($p < 0.001$) between seasonal values for both variables and infection intensity. Seasonal changes in salinity, however, explained only 3.6% of the variance in *P. marinus* intensity whereas temperature accounted for 16.7%. *In vivo* experiments in which infected oysters were held at different temperature-salinity combinations also demonstrated that temperature was more important (Fisher et al. 1992). Conversely, in a survey of Gulf of Mexico oysters, Craig et al. (1989) found that salinity explained 20% of site-to-site variability in disease intensity ($p < 0.001$), but had no relationship to temperature. These apparently contradictory results are probably linked to the type of study (field survey or laboratory test), the location studied (whether salinity and temperature regimes are ever limiting to *P. marinus*), and perhaps race-specific characteristics of both host and parasite (Bushek 1994).

Prevalence and intensity of *P. marinus* infections are related to climate change as well as seasonal temperature fluctuations. Long-term monitoring in the Gulf of Mexico shows that multi-year climate cycles are a significant influence on *P. marinus* infection prevalence and intensity in that region (Powell et al. 1992). Extension of high disease levels into the northeastern U.S. in the early 1990s occurred during an unusually warm period (Ford 1992). It is becoming increasingly apparent, however, that the combination of low temperature and low salinity reduces infection intensity more effectively than either factor alone (Burreson et al. 1994c; Ragone Calvo and Burreson 1994).

Other. Besides temperature and salinity, two other factors are known to influence the course of *P. marinus*-caused disease. Parasitism by the hemolymph sucking snail, *Boonea impressa,* increases the severity of disease in previously infected oysters, even after the snails have been removed (White et al. 1987; Wilson et al. 1988). Like *P. marinus,* the parasitic snail alone poses an energetic drain on oysters by removing metabolites and interfering with feeding (Ward and Langdon 1986; White et al. 1988; Wilson et al. 1988). The magnified effect of two parasites is not surprising, and underscores the importance of multiple stressors affecting oyster health.

Several studies have reported a link between environmental contaminants and *P. marinus* infection development. For instance, experimental exposure to the chemical carcinogen n-nitrosodiethylamine (DENA) caused infections to intensify in oysters parasitized by *P. marinus* (Winstead and Couch 1988). Although DENA by itself is not acutely toxic to oysters, mortalities of parasitized oysters were elevated and gapers had advanced infections. The pathological condition of experimental oysters was abnormal, however. There was no systemic invasion of the connective tissue and parasites were found in the epithelium of the digestive tubules, which are not usually infected. Also, there was a conspicuous absence of hemocyte infiltration. Winstead and Couch (1988) speculated that DENA may have impaired a "non-specific" cellular defense mechanism or may have stimulated parasite growth.

Perkinsus marinus-infected oysters experienced a slightly higher mortality compared to controls (56 vs 46%) after a 60-d exposure to chlorine-produced ox-

idants (1 ppm added chlorine) at 21 to 25 ppt (Scott et al. 1985). A similar pattern was not found at lower salinities nor with lower chlorine concentrations. Recently, Chu and Hale (1994) reported that exposure of oysters to the water soluble fraction (WSF) of highly contaminated sediment from the Elizabeth

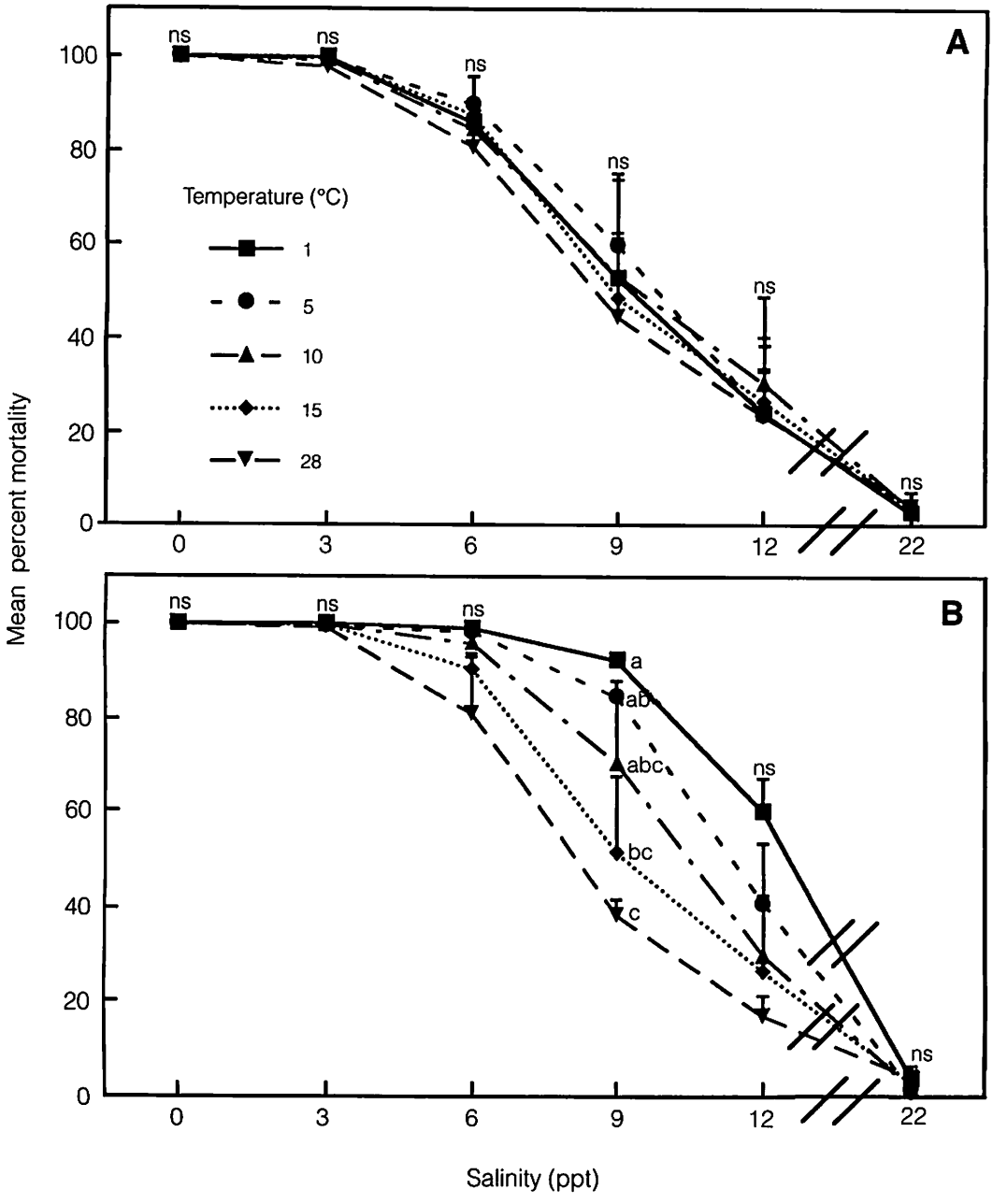

Figure 17. Mortality of *in vitro*-cultured *Perkinsus marinus* cells (A) after 1 h and (B) after 24 h at various temperature and salinity combinations. ns = no significant temperature effect at that salinity. At 9 ppt, values sharing a common superscript are not significantly different at the $\alpha = 0.05$ level. Vertical bars represent standard error of the mean. Key in (A) applies to both figures. From Burreson et al. (1994c).

River, Virginia enhanced the development of *P. marinus* infections. Oysters were maintained at 0, 15, or 30% WSF for 35 d before injection with *P. marinus* (10^3 meront stages oyster^{-1}) and then maintained at the same WSF concentrations for another 35 d. At the conclusion of the experiment, infection prevalences were 15, 45, and 50% at concentrations of 0, 15, and 30%, respectively. Advanced infections were detected only in the contaminant-exposed oysters. Mortalities were low and unrelated to parasitism. The contaminants used by Chu and Hale (1994) were from a grossly polluted site; however, environmentally more relevant levels (30 to 100 parts per trillion) of tributyltin, an additive to antifouling paint, also intensified *P. marinus* infections and resulted in oyster mortality (Anderson et al. 1996; Fisher et al. 1995). Clearly, some environmental contaminants can accelerate *P. marinus* disease development, but there is no correlation between the distribution of the disease and pollution in nature, and it should not be concluded that pollution is responsible for the recent expansion of Dermo disease.

Wilson et al. (1990) found a significant positive correlation between the mean prevalence of *P. marinus* and mean total polyaromatic hydrocarbons (PAHs) in oyster tissue when the units of comparison were bay systems along the Gulf of Mexico. However, they were unable to demonstrate a significant relationship between these variables when the analysis was made at the level of individual samples. They argued that the large-scale correlations are associated with latitudinal variation in spawning, a process through which oysters may rid themselves of contaminants. That is, oysters infected with *P. marinus* do not spawn as abundantly as uninfected individuals and consequently do not rid themselves of PAHs as effectively. Further, the contaminant levels were low throughout the study range, leading the investigators to conclude that the observed correlations originated "either in biological control of contaminant body burden or coincident control of both [contaminant and biological status] directly by climatic cycles rather than the impact of pollution on organism health which might result at higher exposure levels" (Wilson et al. 1992).

Control Measures

Because *P. marinus* is easily transmitted from oyster to oyster, it is imperative to avoid moving infected seed oysters and to refrain from planting disease-free seed near sources of infection such as native populations on natural beds, pilings, bridges, or piers (Andrews and Ray 1988). As a further measure, planters should remove all residual oysters from a ground on which they intend to place seed stock and, if possible, should let grounds lie "fallow" for one or two years after harvest so that infected oysters can die. There is no evidence that the intertidal height of oysters influences infection acquisition or development (Burrell et al. 1984; Gibbons and Chu 1989)

A variety of organic and inorganic compounds, including anticoccidial and antifungal substances, kill *P. marinus in vivo*; however, most are also toxic to oysters (Calvo and Burreson 1994; Krantz 1994). Ray (1966b) found that the fungicide cycloheximide inhibited development of the parasite and stopped mortality for as long as 164 d when applied at concentrations of 1 to 50 μg L^{-1} in aquaria, but that the parasite recovered and began causing deaths as soon as the treatment ceased. Calvo and Burreson (1994) confirmed the short-term therapeutic effect of cycloheximide and that infections relapsed when the treatment ceased. In contrast to low salinity immersion, which merely slowed *P. marinus* development (Ragone and Burreson 1993), cycloheximide caused a true decrease in parasite densities and might be used to curb infections in small lots of oysters, such as broodstock (Calvo and Burreson 1994). In nature, however, elimination of parasitized oysters as a source of infective stages and maintaining normal freshwater input into affected estuaries appear, at present, to be the most reasonable ways of controlling *P. marinus* (Andrews and Ray 1988).

Modeling Perkinsus marinus *Epizootics*

Recently, a physiologically based model that couples eastern oyster and *P. marinus* population dynamics has been developed to investigate biological and environmental factors controlling the prevalence and intensity of Dermo disease and the conditions that produce or terminate epizootics (Powell et al., 1994;

Hofmann et al., 1995). Infections become lethal after a threshold parasite burden is reached; consequently, the model predicts that the balance between oyster and parasite growth rates is crucial to the outcome of the disease. Both temperature and food supply are key elements in this equation because they affect the amount of energy ingested by the oyster, how it is allocated between somatic and reproductive tissue, and how fast the parasite population multiplies. An individual oyster can survive if it adds somatic tissue faster than the parasite mass increases. Model simulations show that in southern locations such as the Gulf of Mexico, high recruitment of (uninfected) juveniles combined with rapid growth rates allows oyster populations to survive even though individual oysters eventually die. Conversely, at higher latitudes such as the mid-Atlantic, where oyster recruitment and growth rates are considerably lower, the parasite will have a much more devastating effect on the host population.

Model simulations for the Gulf of Mexico showed that factors affecting food supply, temperature, and salinity are important in starting and stopping epizootics. For instance, a dry summer followed by a warm winter could start an epizootic, but factors that reduced food supply were more important than either salinity or temperature. Simulations also showed that conditions triggering an epizootic could disappear as much as 18 months before the onset of mortalities. A cool, wet year might stop an epizootic, but more extreme conditions were required to terminate an epizootic than to start one. The model suggests that the most important factor in stopping an epizootic, at least in southern United States, is a large recruitment that dilutes *P. marinus* in the host population. The model predicts that no epizootic will occur following the recruitment unless triggering conditions (e.g., warm winter, high salinity, low food) return.

MSX Disease

History and Distribution

The disease commonly known as MSX was first recognized as the cause of massive oyster mortalities in lower Delaware Bay in 1957 and two years later in the lower Chesapeake Bay (Haskin et al. 1965; Andrews and Wood 1967). The etiological agent, a protozoan, was originally given the acronym "MSX" because it was found as a multinucleated (plasmodial) stage with unknown affinity, thus "multinucleated sphere X" (Haskin et al. 1965). Its spore stage has since been described and the parasite has been named *Haplosporidium* (formerly *Minchinia*) *nelsoni* (Couch et al. 1966; Haskin et al. 1966; Levine et al. 1980).

In the early and mid-1960s, *H. nelsoni* was found in the coastal bays of North Carolina, Virginia, Maryland, Delaware, New Jersey, Connecticut, and New York (Haskin et al. 1965; Sindermann and Rosenfield 1967), but mortalities were not clearly associated with the parasite north of New Jersey and south of Virginia. It was present at least as early as 1967 in Wellfleet Harbor on Cape Cod Bay, but was not found in samples from 12 other sites in Massachusetts (Krantz et al. 1972). Periodic sampling along the entire Atlantic and Gulf Coasts between the mid 1960s and the mid 1980s failed to detect the parasite south of North Carolina (Kern 1988). Beginning in 1984, however, new reports indicated an apparent range extension, from the Damariscotta River, Maine, to Biscayne Bay, Florida (Fig. 5; see also Haskin and Andrews 1988; Hillman et al. 1988; Kern 1988; Ford, pers. obs.). Spore stages have been identified in oysters from Virginia to Cape Cod, whereas reports from other areas are based on plasmodial stages only.

Etiological Agent

Life Cycle. The causative agent of MSX disease, *Haplosporidium nelsoni*, is a spore-forming protozoan that was placed in the Phylum Ascetospora, class Stellatosporea, order Balanosporida, and family Haplosporidiidae by the Committee on Systematics and Evolution of the Society of Protozoologists (Levine et al. 1980). Corliss (1984) suggested a new phylum, Haplosporidia, which excludes organisms such as *Marteilia* spp. because they form cells within cells. Perkins (1990) tentatively agreed and proposed subdivision into class Haplosporea, order Haplosporida, and family Haplosporidiidae. A recent phylogenetic

comparison using 16S (small subunit)-like ribosomal RNA gene sequences suggested that the haplosporidans are more closely related to alveolates (ciliates, dinoflagellates, and apicomplexans) than to other spore-forming protozoans such as microsporidians (Siddall et al. 1995).

Haplosporidans are parasites of marine, and a few freshwater, invertebrates (Sprague 1982). In the eastern oyster, the predominant form of *H. nelsoni* is a multinucleated plasmodium in which nuclei have a peripheral nucleolus (Figs. 18A, B, E; 19D, E). The infective stage has never been recognized, although it is presumed to be a uninucleated form released by a spore (Farley 1967). Multinucleated stages develop by successive nuclear and cytoplasmic divisions (see Perkins 1968). Plasmodial stages have been maintained *in vitro* for several weeks during which time developmental stages including plasmotomy and budding have been observed, but continuously proliferating cultures have yet to be achieved (Kleinschuster et al. 1994).

Sporulation is extremely rare in adult oysters (Farley 1965; Andrews 1979; Ford and Haskin 1982), although prevalences as high as 40% have been found in spat (Andrews 1979; R.D. Barber et al. 1991; Burreson 1994). Plasmodia range in diameter from 5 to more than 100 μm. Spores are operculate, approximately 6 × 8 μm in fixed material, with overhanging lids; filamentous projections are attached to the spore wall (Figs. 18F; 19F). Spores develop exclusively in the epithelium of the digestive tubules (Figs. 18C, D; 19H). Sporulation occurs predominantly within a few weeks in late June and early July and over a more prolonged period in autumn (Farley 1975; Andrews 1979; R.D. Barber et al. 1991; Burreson 1994). All stages from uninucleate cells to spores are found for many other species of Haplosporidiidae; yet, to date, the complete life cycle has not been described for any of them, nor has transmission been rigorously documented.

Transmission. Controlled transmission of *H. nelsoni* has not been achieved despite many attempts at several laboratories (Canzonier 1968, 1974; Sprague et al. 1969; Andrews 1979). Unlike *P. marinus*, *H. nelsoni* infective pressure is independent of oyster density and is fairly uniform over a large area within the high salinity portion of affected estuaries. With favorable salinities, it can spread rapidly over large distances (Andrews and Wood 1967; Andrews 1979; Haskin and Ford 1982). For these reasons, as well as the comparative rarity of sporulating stages, an alternate or intermediate host has been hypothesized (Andrews 1968; Ford and Haskin 1982; Burreson 1988; Haskin and Andrews 1988), but the parasite has not been positively identified in any other species. Spore stages of a haplosporidan parasite of the shipworm, *Teredo* spp. resemble those of *H. nelsoni* (Hillman 1978), but ultrastructural differences and lack of immunological cross reactivity now show that the parasites are not the same (McGovern and Burreson 1989, 1990) and the teredinid parasite has been named *Minchinia teredinis* (Hillman et al. 1990). A haplosporidan also resembling *H. nelsoni* has been found in Pacific oysters, *Crassostrea gigas*, from Korea (Kern 1976), Japan (Friedman et al. 1991), and California (Friedman and Hedrick 1991b). Sporulation of the haplosporidan in *C. gigas* occurs in the digestive tubule epithelium, as is the case with *H. nelsoni*. A possible relationship between the parasites awaits confirmation.

Haplosporidan spores resembling *H. nelsoni* in size and shape have been found ingested by oysters in areas where *H. nelsoni* is enzootic and during the season when oysters become infected by *H. nelsoni*, indicating that they are widely distributed in the water column (R.D. Barber and Ford 1992). Their abundance was not correlated with subsequent *H. nelsoni* infection prevalence, however, suggesting that if they are a stage in the life cycle of *H. nelsoni*, the spores are not directly infective to oysters, but may infect an alternate host. Incomplete knowledge of its life cycle and mode of transmission is a critical gap in understanding the biology of *H. nelsoni*.

Infection and Disease Processes

Diagnosis. Nearly all diagnoses of *H. nelsoni* infection have been performed by histological examination of tissue slides. Screening of hemolymph samples will detect most systemic infections, but it is not reliable for localized parasites (Burreson et al. 1988;

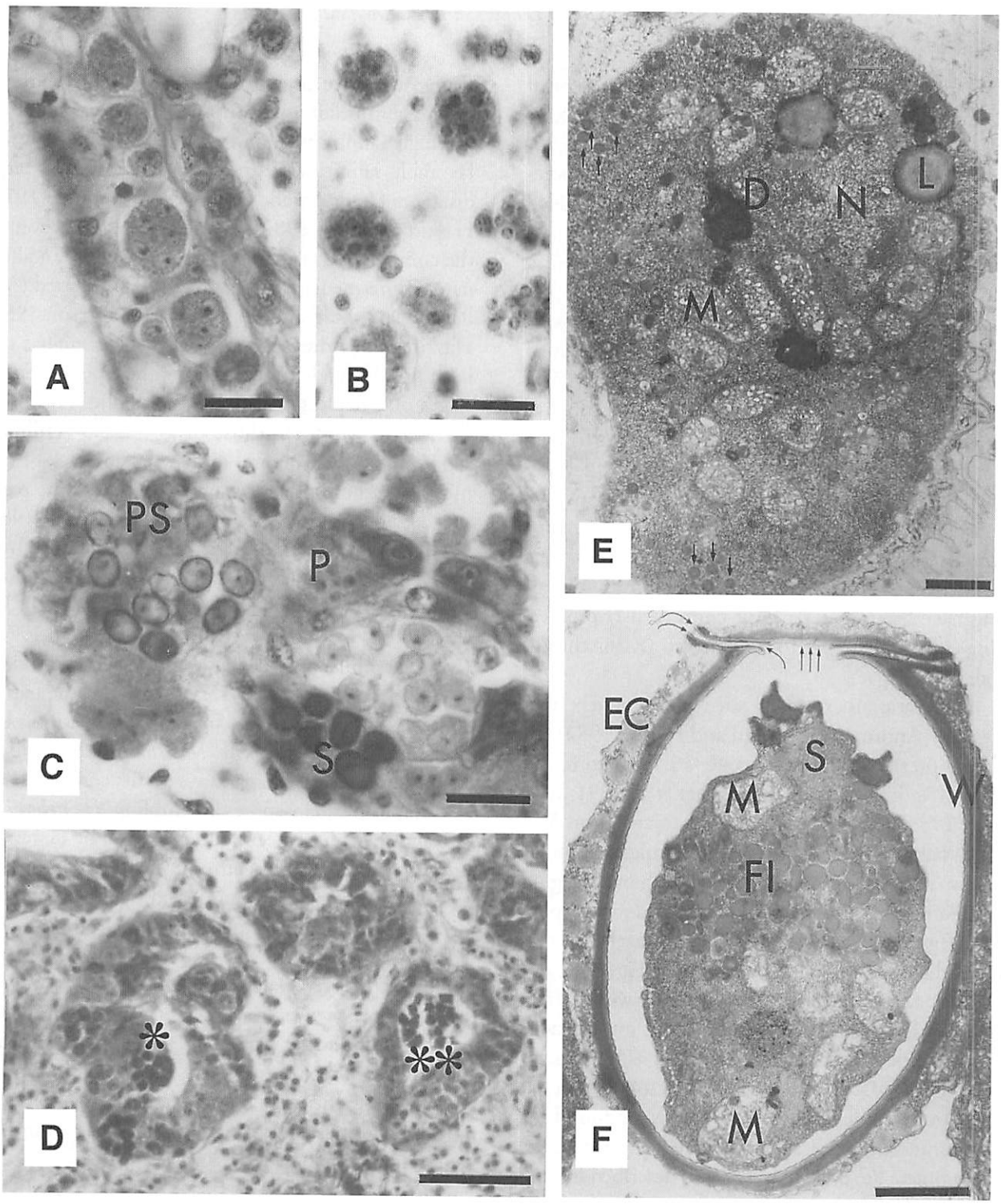

Figure 18. Stages of *Haplosporidium nelsoni* found in *Crassostrea virginica*: (A) plasmodia in gill epithelium (earliest recognized stage); (B) systemic plasmodia in blood vessel; (C) plasmodial [P], prespore [PS], and spore [S] stages; (D) sporulating stages in epithelium (*) and lumen (***) of digestive tubules; (E) plasmodium showing mitochondria [M], nucleus [N], lipid body [L], digestive lamellae [D], and haplosporosomes (arrows), from Scro and Ford (1990); (F) mature spore showing spherule [S], mitochondria [M], spore wall [W], operculum [straight arrows], juncture between operculum and lip of spore wall [curved arrows], formative inclusions [FI] thought to produce haplosporosomes, and epispore cytoplasm [EC], from Rosenfield et al. (1969). Scale bars = 10 μm in A to C; 50 μm in D; 1 μm in E and F.

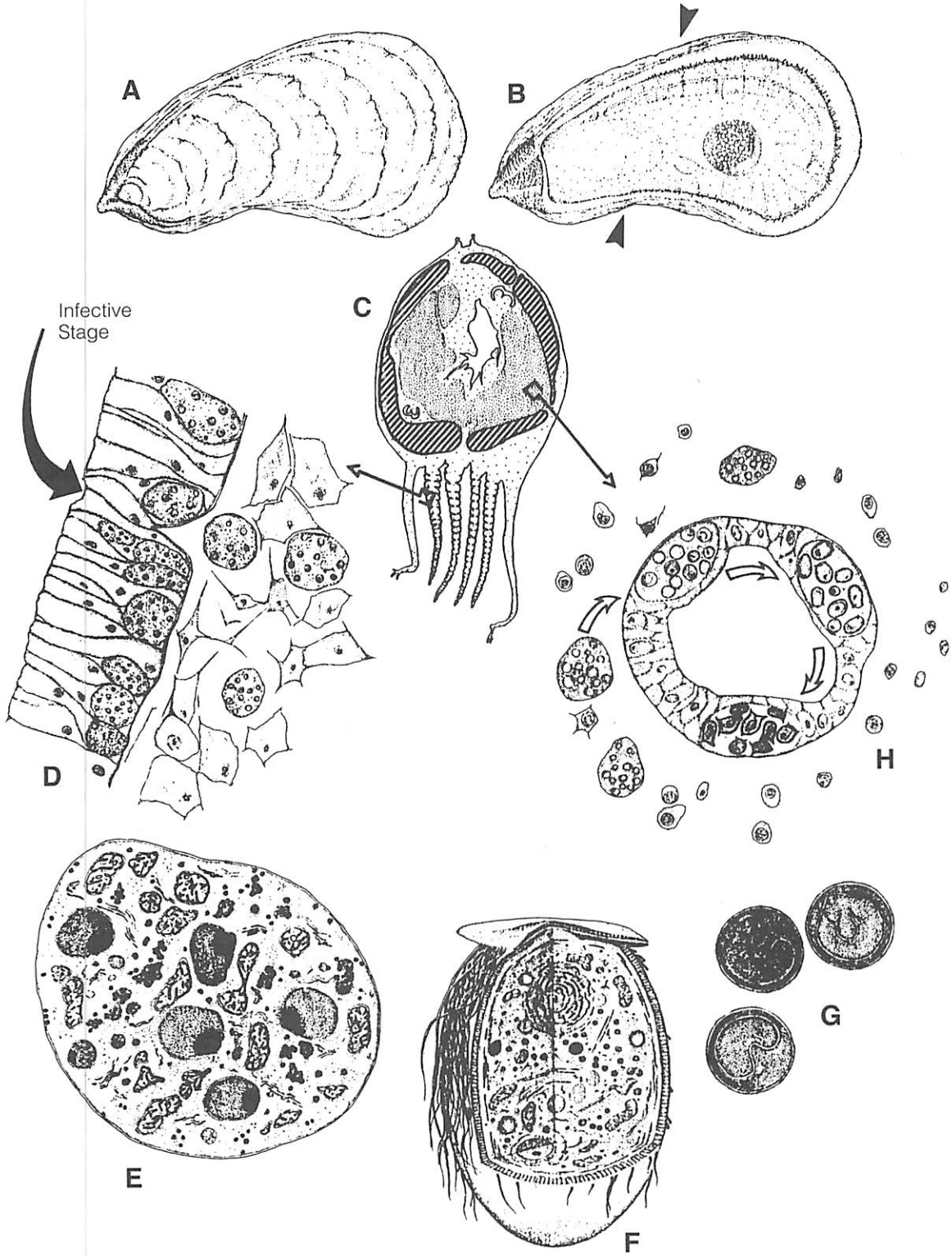

Figure 19. Diagram of stages of *Haplosporidium nelsoni* seen in *Crassostrea virginica*: (A) intact oyster; (B) oyster with right valve removed showing plane [arrows] of section in (C); (D) water tube epithelium (portal of entry) showing plasmodia intercellularly along basal lamina and in blood spaces beneath the epithelium; (E) plasmodium; (F) spore; (G) haplosporosomes; (H) development of plasmodia into sporocysts in epithelium of digestive diverticulum [open arrows]. See Figure 18 for ultrastructural details.

Ford and Kanaley 1988). Nevertheless, because it is rapid and economical, this method is being used in some areas (C. A. Farley, pers. comm. 1991; Morrison et al. 1992). Diagnostic assays involving immunological or nucleic acid probes may become available in the future (Fong et al. 1993a; Stokes and Burreson 1995).

Portal of Entry. The earliest infections of *H. nelsoni* are found in gill and palp epithelia (Fig. 19C, D; see also Haskin et al. 1965; Farley 1968). This contrasts with the apparent portal of entry of *P. marinus* and *H. costale*, the other two major parasites of eastern oysters. Both of these are also waterborne and acquired through feeding, but invade the host through the lining of the digestive system (see pp. 597, 630). Ford and Figueras (1988) found rare instances (<1%) of infections apparently initiated by *H. nelsoni* invading through the gonoducts.

Development of Infection and Histopathology. Parasites multiply along the basal lamina between gill epithelial cells, often causing extensive sloughing of both parasites and host cells (Fig. 20A). Myhre and Haskin (1968) estimated a doubling time for *H. nelsoni* of 24 h in early stage infections. Penetration into underlying tissues occurs almost immediately in susceptible oysters, the infection spreads rapidly to all tissues, and oysters begin to die within a month (Haskin et al. 1965; Andrews 1966; Couch and Rosenfield 1968). The epithelial barrier is an important component of defense against *H. nelsoni.* Most oysters do not die until infections become systemic (Andrews 1966; Ford and Haskin 1982, 1987) and the ability to contain infections at the epithelial level is characteristic of strains selected for resistance to the disease (Myhre 1973; Ford and Haskin 1987).

Gross pathology includes mantle recession, pale "digestive gland", and emaciation (Farley 1968). "Scabby shell blisters" reported by Farley (1968) are rare (Andrews 1988b; Ford, pers. obs.). Hemocytes, often in very high numbers, usually accumulate at sites of infection (Fig. 20A, B, C; see also Farley 1968; Ford 1988). Proliferating parasites cause mechanical disruption and lysis of tissues, an increase in pigment cell numbers, metaplasia of the digestive tubule epithelium, and fibrosis (Fig. 20B; see also Farley 1968). Hemocyte accumulations and tissue damage are associated with both initial and chronic infections, and are roughly proportional to the intensity of infection (Ford 1985a; Ford et al. 1993a).

Effects of Disease on Oyster Metabolism

Growth. Susceptible oysters infected with *H. nelsoni* typically stop growing two to three weeks before they die (Fig. 21; see also Andrews 1966; Matthiessen et al. 1990). Death of highly susceptible oysters is so rapid that it occurs without loss of soft tissue condition (Ford et al. 1988). In contrast, more tolerant oysters, which survive for longer periods, show clear reduction of condition index correlated with infection intensity (Fig. 22; see also Newell 1985; Barber et al. 1988a; Ford et al. 1988).

Physiological Functions. Newell (1985) found that clearance (feeding) rates of infected oysters were less than half those of uninfected oysters, but that oxygen consumption was not related to infection (Fig. 23). This relationship held for oysters collected at three times during the year and measured at ambient temperatures of 10°, 20°, and 24°C. Newell (1985) proposed that a decline in food consumption, perhaps associated with parasite-caused disruption to the gill epithelium (see Fig. 20A, C, D), without compensatory reduction in metabolic rate would cause a negative energy balance in infected oysters and result in the observed depletion of stored metabolic reserves. Moreover, when oysters were subjected to acute temperature elevation, infected individuals responded with 70% greater oxygen uptake than uninfected ones (Littlewood and Ford 1990). This response would further depress the energy balance and suggests that parasitized animals have reduced ability to buffer the effects of rapid environmental change that might occur in an estuarine or intertidal location.

B.J. Barber et al. (1991) compared seasonal clearance and oxygen consumption rates of MSX disease-resistant oysters with those of susceptible oysters.

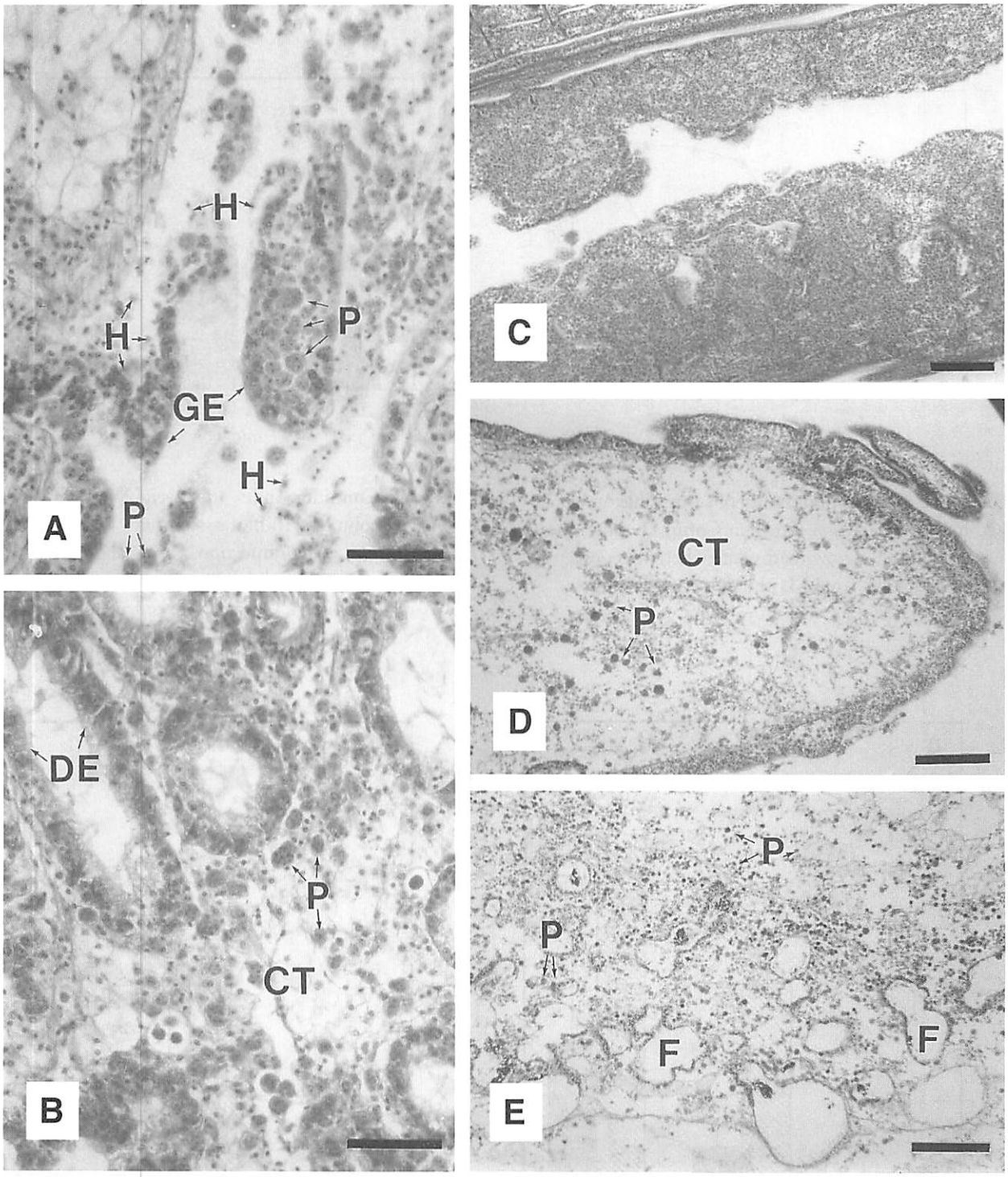

Figure 20. Histopathology caused by *Haplosporidium nelsoni* [P] in tissues of *Crassostrea virginica*: (A) sloughing of gill epithelium [GE], parasites, and hemocytes [H]; (B) heavily infected section of digestive gland showing lysis of storage-connective tissue [CT] and metaplasia of digestive tubule epithelia [DE]; (C) intense hemocyte infiltration in infected gill; (D) disruption and lysis of storage-connective tissue [CT] and loss of normal gill filament architecture at distal demibranch; (E) extreme degradation of the gonad with empty follicles [F]. Scale bars = 50 μm in A and B; 100 μm in C to E.

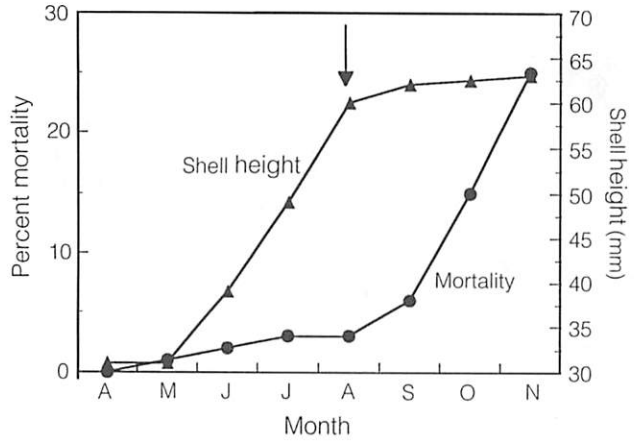

Figure 21. Growth and mortality of susceptible oysters exposed to *Haplosporidium nelsoni* in Cotuit Harbor, Massachusetts, in 1988. Arrow marks date when infections were first detected. Data from Matthiessen et al. (1990).

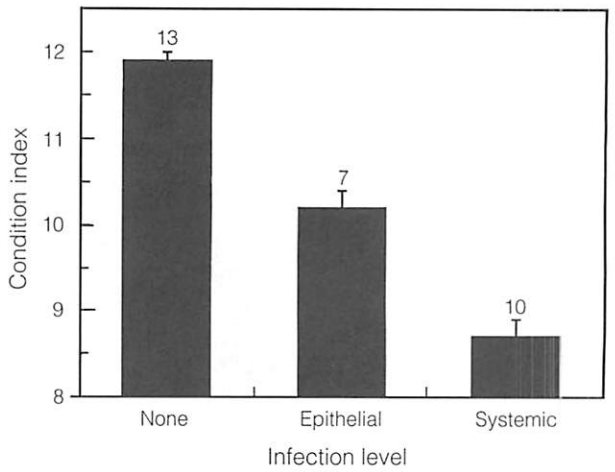

Figure 22. Condition index (dry weight of soft tissue ÷ shell cavity volume × 100) of oysters with various levels of *Haplosporidium nelsoni* infections. Vertical bars represent the standard error of the mean. Number of oysters is shown above columns. Oysters were collected in Delaware Bay in November, 1985. Data from Barber et al. (1988a).

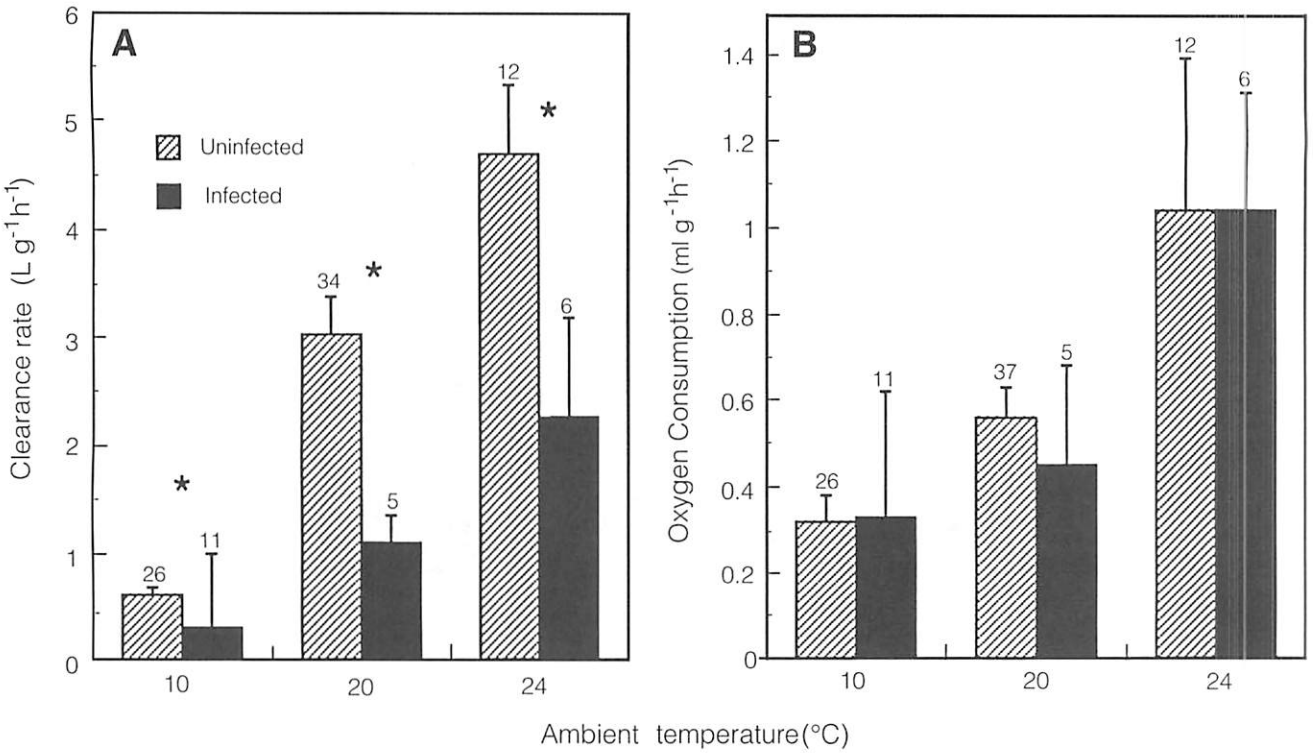

Figure 23. (A) Clearance and (B) oxygen consumption rates of oysters with and without *Haplosporidium nelsoni* infections. Vertical bars represent standard error of the mean. Number of oysters is shown above columns. Asterisks indicate differences significant at the $\alpha = 0.05$ level. From Newell (1985).

They found no differences before challenge by *H. nelsoni.* In July and August, rapidly proliferating infections killed 80% of the susceptible oysters whereas resistant oysters showed few patent infections and only 3% died. At the same time, the difference between energy potentially taken in as food and energy respired was significantly ($p < 0.002$) greater in the resistant group, primarily the result of greater clearance rates. In contrast to Newell (1985), B.J. Barber et al. (1991) found no association of infection with physiological rates (comparison possible in susceptible oysters only). Although they did not discount the possibility that the energetic advantage of the resistant strain was because the oysters were healthier, they also speculated that the greater clearance rates of resistant animals might reflect a physiological state in the gill (the site of initial infections) that inhibited parasite development. They also found that after 80% of the susceptible stock had died, the survivors, which were still heavily infected, had similar clearance and oxygen consumption rates to the resistant oysters, which were largely uninfected. Because the 20% survivors were, by definition, the most "resistant" members of the susceptible group, the authors suggested that metabolic adjustment to parasitism might be an important physiological aspect of MSX disease resistance.

Biochemical Composition. Glycogen, lipid, and protein were all reduced in oysters with *H. nelsoni* infections but glycogen was depleted to a greater extent than either of the other components (Barber et al. 1988b). Oysters with systemic infections showed more severe loss of constituents than those with gill infections. The glycogen fraction of total dry weight in systemically infected oysters ranged from a low of 13% in May to a high of 32% in September compared to 19 and 48%, respectively, in uninfected animals. Values for oysters with gill infections were 20% in May and 43% in September. There were no differences in the percent lipid associated with infection, and because of the loss of glycogen in infected animals, protein formed a relatively higher proportion of tissue mass in these oysters than in uninfected individuals.

Whereas glycogen was depleted by parasitism, hemolymph glucose concentrations were highly variable and showed no consistent relationship to *H. nelsoni* infection intensity (Douglass 1977). Conversely, total hemolymph protein fell in proportion to infection level in MSX-disease susceptible oysters undergoing initial exposure to *H. nelsoni* (Douglass 1977; Ford 1986). Oysters with infections localized in the gill had protein concentrations similar to uninfected animals, but susceptible oysters with heavy systemic infections lost about two-thirds of their hemolymph protein (Ford 1986). Resistant oysters are much less likely to have systemic infections (Ford 1988) so that the opportunity to measure hemolymph constituents in such animals has been limited. Nevertheless, resistant oysters appear less likely than susceptible individuals to experience depletion of hemolymph protein when they are systemically infected (Feng and Canzonier 1970; Douglass 1977), which may be a biochemical reflection of their ability to tolerate infections that would kill more susceptible animals.

Feng et al. (1970) reported that, in summer, infected oyster hemolymph had about half the total free amino acid concentration of uninfected animals. Most individual amino acids decreased, but taurine increased in animals infected by both *H. nelsoni* and the trematode *Bucephalus* sp. Conversely, the total hemolymph amino acid concentration of oysters collected in winter was not associated with *H. nelsoni* infection, although taurine again increased in oysters with both single and double infections. Jeffries (1972) used the amino acid data of Feng et al. (1970) to strengthen his argument that an increase in the ratio of taurine to glycine is an indicator of stress in marine bivalves (see p. 601). He proposed that a ratio of less than 3 was normal; a ratio between 3 and 5 indicated chronic stress, and a ratio greater than 5 signified acute stress. Although the ratios of taurine to glycine did increase in oysters infected with only *H. nelsoni,* they were still below 3 (data of Feng et al. 1970). In oysters with *Bucephalus* sp., or both *H. nelsoni* and *Bucephalus* sp., the ratio increased to 4.5. It is curious that *Bucephalus* sp., a parasite considered well adapted because it does not kill the oyster, was associated with a greater stress index than *H. nelsoni,* a highly pathogenic parasite.

Large increases in the activities of alanine and aspartate amino transferases were reported in the hemolymph of oysters with early gill lesions (Douglass

and Haskin 1976), perhaps indicating an attempt to replace amino acids lost from damaged tissues or, more probably, a leakage of the enzymes themselves. Similarly, Ling (1990) found increased serum acid phosphatase and leucine aminopeptidase activities with increasing parasite burdens during early stages of infection, although there was also considerable seasonal variability. In general however, a reduction in enzyme activities associated with advanced infections has been reported for both alanine and aspartate amino transferases (Douglass and Haskin 1976), for phosphohexose isomerase (Mengebier and Wood 1969), and for alkaline and acid phosphatases and esterase (Eble 1966), and is probably associated with the general metabolic decline that accompanies heavy parasite burdens (Ford 1988).

Reproduction. Gonadal development can be severely impaired by *H. nelsoni* infections (Fig. 20E; see also Farley 1967; Couch and Rosenfield 1968; Ford and Figueras 1988). In a small percentage of cases, this impairment is caused by direct invasion of the gonoducts by parasites and the ensuing hemocyte invasion (Ford and Figueras 1988), but in most instances it appears to be part of the overall metabolic disruption associated with disease (Barber et al. 1988a). Inhibition of gametogenesis by *H. nelsoni* is most severe during the earliest stages of gamete development (Ford and Figueras 1988), as it is in the case of *P. marinus* infection (Mackin 1962). It is also proportional to parasite burden (Fig. 24). On average, the gonads of animals with localized gill lesions occupied 70% of the cross-sectional area occupied by gonads of uninfected individuals (Barber et al. 1988a). In systemically infected oysters, this figure was only about 20% and most oysters with advanced infections showed no evidence of gametogenesis at all (Fig. 20E). When mature ova developed, however, they were the same size regardless of infection intensity (Barber et al. 1988a).

Ford and Figueras (1988) presented evidence that infections in many native Delaware Bay oysters went into remission in early summer. This allowed oysters, which had been clearly retarded in gonad development earlier in the spring, to recover, undergo gametogenesis, and spawn. Given the documented loss of energy reserves that accompany parasitism by *H. nelsoni*, however, it is possible that the gametes produced by recovered oysters are fewer and of lower quality than would be the case for oysters that had never been infected (Barber et al. 1988a).

Cause of Death. The manner in which *H. nelsoni* causes the death of infected oysters is not completely understood. Highly susceptible oysters usually appear in good condition when they die; they are fat and, histologically, the storage cells of the visceral mass are large and intact (Ford, pers. obs.). In contrast, more resistant oysters show clear evidence of tissue damage and loss of metabolic condition at death. They are grossly emaciated and the storage cells appear shrunken and disrupted. Evidence gathered in a number of *H. nelsoni* transmission experiments at the Haskin Shellfish Research Laboratory shows that oysters injected with material from infected oysters have a very rapid high mortality compared to oysters injected with tissue from uninfected oysters, even though the parasite does not become established in recipient oysters (Canzonier 1968; Ford et

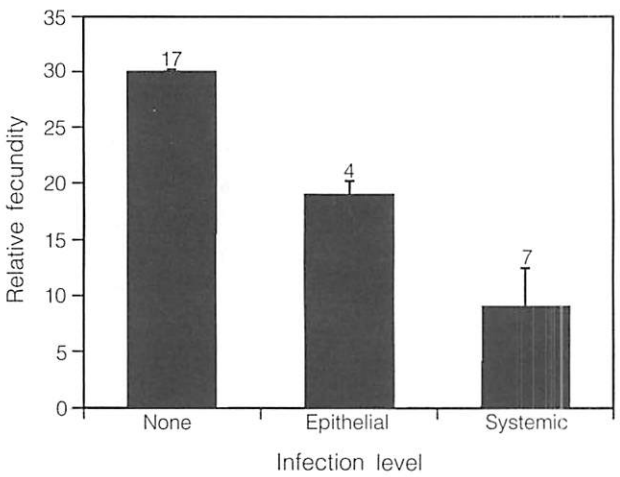

Figure 24. Relative fecundity (area occupied by gonad ÷ total area of visceral mass × 100) estimated from tissue sections of oysters with various levels of *Haplosporidium nelsoni* infections. Vertical bars represent the standard error of the mean. Number of oysters shown above columns. Oysters were collected in Delaware Bay in June, 1985. From Barber et al. (1988a).

al. 1988; H. Haskin, Haskin Shellfish Research Laboratory, pers. comm. 1988). Ford et al. (1988) suggested that mortality of very susceptible oysters, occurring within weeks of infection, may be induced by a toxin that causes rapid death. Death of more resistant individuals, occurring months to years after infection, is increasingly associated with loss of metabolic condition, which may result from depletion of energy reserves through competition with parasites, impaired physiological functioning of the host, or both.

Host Response. Hemocytes congregate at the sites of new gill infections, and the number of cells circulating in hemolymph or infiltrating tissues increases with increasing parasite burden (Ford et al. 1993b). Nevertheless, their role in the disease process is far from clear and seasonal factors have a larger impact on the numbers of circulating hemocytes than do parasite burdens. Phagocytosis of living *H. nelsoni* plasmodia is uncommon both *in vivo* and *in vitro* (Douglass 1977; Scro and Ford 1990; Ford et al. 1993a). It is not yet clear whether failure to ingest and destroy the pathogen results from similarities in surface receptors between host and parasite (i.e., lack of nonself recognition) or production of an inhibitory substance by the parasite (Kanaley and Ford 1990). As infections intensify and become systemic, the proportion of agranular hemocytes increases (Farley 1968; Douglass 1977) until very few granular cells remain. In histological section, the predominant agranular cell at sites of infection is a small, undifferentiated cell with a large nucleus-to-cytoplasm ratio referred to as a hyaline hemocyte (Farley 1968; Feng et al. 1971; Cheng 1981). An increase in circulating hyaline hemocytes, however, is not obvious, although an elevation in the proportion of degranulated or "spent" granulocytes often accompanies infection (Scro and Ford 1990; Ford et al. 1993b).

Despite the fact that strains resistant to MSX disease have been developed (see p. 623), no clear defense mechanism has yet been described. Feng and Canzonier (1970) described a shift in the ratio of two hemolymph protein fractions associated with infection, but Ford (1986) was unable to correlate electrophoretic patterns in hemolymph proteins with either degree of infection or genetic strain. Also, Douglass and Haskin (1975) reported no differences in numbers or types of hemocytes in histological sections of resistant and susceptible stocks exposed to infection. *In vitro* tests showed that hemocytes from resistant oysters were no better at phagocytosing *H. nelsoni* plasmodia than were hemocytes from susceptible oysters and that plasmodia survived equally well (95 to 97%) during short-term incubations (1 to 4 h) in serum from both types of oysters (Ford et al. 1993a). Similarly, hemocytes of the Pacific oyster, *C. gigas*, do not attack plasmodia *in vitro* (Ford et al. 1993a), even though *H. nelsoni* infections fail to develop in this species (Burreson et al. 1994a). Clearly, resistance to MSX disease can exist in the absence of typical antiparasite cellular activities. Ford (1988) hypothesized that selection for improved survival had succeeded by favoring oysters that were insusceptible to *H. nelsoni* because they did not provide proper conditions for parasite development, not by favoring oysters with an effective response to the parasite.

Epizootiology

Infection and Mortality Periods. Sequential importations of disease-free, susceptible oysters into enzootic areas have shown that infection and mortality periods for MSX disease are similar from the lower Chesapeake Bay, Virginia, to Cape Cod, Massachusetts (Haskin et al. 1965; Andrews 1966; Couch and Rosenfield 1968; Leibovitz et al. 1987; Matthiessen et al. 1990), but are slightly delayed towards the north. Oysters usually become infected beginning in mid-May in Chesapeake Bay, early June in Delaware Bay, and late June on Cape Cod. The infective period can last through October, but infection pressure in the late summer and autumn is much more variable than it is in early summer and infections may fail to occur (Andrews 1982; Ford and Haskin 1982).

In susceptible oysters, infections acquired in early summer develop rapidly, causing mortalities from July through October (Fig. 15). Despite the deaths of infected oysters, prevalence and intensity levels remain high over winter, resulting in a second mortality period in late winter and spring. In many instances, infections acquired late in the season remain

subpatent over winter, proliferating only when water temperatures rise the following spring. These infected oysters die in June and July of the summer after infections were acquired, resulting in a third annual mortality period (Haskin et al. 1965; Andrews 1966). After initial infections, some oysters may survive with chronic infections for several years (Ford 1985a). Chronic parasitism displays the same seasonal cycle that occurs in enzootic areas, with infections entering remission during summer and early spring then relapsing in autumn and late spring.

Prevalence Levels and Mortality Rates. Infection levels and consequent mortality rates vary seasonally, annually, regionally, and according to previous selective mortality (Andrews and Frierman 1974; Haskin and Ford 1979; Ford and Haskin 1982; Haskin and Andrews 1988). Andrews (1966) reported that 80% of nonpredation loss in lower Chesapeake Bay was associated with *H. nelsoni*; Ford and Haskin (1982) found this figure to be 70% in Delaware Bay native stock. These proportions are important to the interpretation of total (predation and nonpredation) losses given in the following paragraph.

Andrews and Frierman (1974) and Farley (1975) reported that maximum infection prevalence in lower Chesapeake Bay rarely exceeded 50% in introduced susceptible stocks, with cumulative losses of 70 to 80% during 18 to 24 months exposure. Concurrent with severe drought, however, mortality increased to nearly 80% in only 6 months (Burreson and Andrews 1988). Infection prevalence in stocks introduced into Delaware Bay is commonly 100% just before mortality begins, and losses are 80 to 99% in 2 years. Ford and Haskin (1982) frequently recorded sample prevalences of 70 to 90% in native oysters on Delaware Bay planted grounds and believed that, in many years, all oysters in the lower Bay were infected. Despite this, cumulative losses of Delaware Bay native oysters over two years averaged only 50%, underscoring the fact that the oysters had developed some resistance (Haskin and Ford 1979). In Wellfleet and Cotuit Harbors on Cape Cod, Massachusetts, prevalences in susceptible oysters also approached 90% after one season, with cumulative mortalities exceeding 85% after two seasons' exposure (Matthiessen et al. 1990; Ford, unpubl. data). Samples collected in North Carolina in 1988 and 1989 showed *H. nelsoni* throughout Pamlico Sound and south to Cape Lookout with prevalences as high as 64% (E. M. Burreson, pers. comm. 1989; Morrison et al. 1992). Disease-related mortalities were reported, but the proportion associated with *H. nelsoni* could not be differentiated from that caused by *P. marinus*, which was also present (J. Levine, pers. comm. 1991).

Spat frequently have fewer infections and lower mortality rates than older oysters (Andrews 1984a). This is not due to lessened susceptibility, because when infection pressure is high, spat exhibit high prevalences (Myhre 1973). More likely it is because they filter less water and encounter fewer infective stages than do older, larger animals (see also p. 603).

Infectivity and Pathogenicity. Because *H. nelsoni* has not been transmitted under controlled conditions, the minimum infective dose is not known. Myhre and Haskin (1968) believed that infections might develop from a single infecting organism. A relationship between dosage and subsequent infection levels is suspected (Ford and Haskin 1982; Haskin and Andrews 1988), but has not been experimentally verified.

Despite the fact that extensive damage can occur in the gills of infected oysters, most oysters have advanced, systemic infections when they die (Andrews 1966; Ford and Haskin 1982, 1987), indicating that gill infections are rarely lethal. Also, many symptoms of disease, including pale digestive gland, loss of condition, reproductive impairment, and depletion of circulating protein, become obvious or significant only after infections become systemic.

Epizootic/Enzootic Periodicity. Fluctuations in prevalence levels of *H. nelsoni* from year to year were first noted in Delaware Bay when infection activity was reduced for several years after the original epizootic in the late 1950s (Ford and Haskin 1982). Since then, three more periods of low activity of one to two years duration, interspersed with five to six years of high activity, have been recorded. These were not correlated with salinity, but each low-activity period was preceded by an extremely cold winter (Ford and Haskin 1982). Ford and Haskin (1982) and Haskin and Andrews

(1988) speculated that extreme cold might diminish the supply of infective particles by killing a possible alternate or intermediate host. A fourth, prolonged period of low activity in Delaware Bay began in 1989, at the onset of a *P. marinus* epizootic. During much of this period, winters have been unusually warm, not cold. A different mechanism may be at work, including the possibility that *P. marinus* has in some way diminished *H. nelsoni* abundance.

Most fluctuations in *H. nelsoni* prevalence within Chesapeake Bay have been correlated with river flow and salinity changes (Andrews and Frierman 1974; Farley 1975), probably because the oysters sampled are in zones where salinity becomes low enough to prevent infections (Andrews and Wood 1967). During most of the 1980s, river flows into Chesapeake Bay were well below normal, resulting in higher salinities that permitted both *H. nelsoni* and *P. marinus* to move upbay (Burreson and Andrews 1988). More recently, however, *H. nelsoni* prevalences have not been as closely tied to salinity (E. Burreson, pers. comm. 1988, 1994; G. Krantz, Oxford Cooperative Laboratory, pers. comm. 1988). Clearly, factors in addition to temperature and salinity influence the distribution and abundance of *H. nelsoni.*

Resistance

Resistance to MSX disease developed in native Delaware Bay oysters within a few years of the original epizootic that killed 90 to 95% of all oysters in the lower Bay and 50 to 60% of those in the upper Bay (Haskin and Ford 1979). Under heavy infection pressure, the Delaware Bay stock developed an average survival of 30% at market size (2.5 to 3 years), compared to about 7% for introduced stocks. The latter figure is comparable to the survival of the original Delaware Bay stock between 1957 and 1959 (Haskin et al. 1965), indicating that the abundance and pathogenicity of *H. nelsoni* remained high and that there was about a four-fold improvement in survival of the native stock. Native oysters in Mobjack Bay, Virginia, generally survive quite well compared to introduced James River stocks exposed in lower Chesapeake Bay, but both these and Delaware Bay natives suffered very high losses when challenged by the extremely heavy infection pressure occurring in mid-Atlantic estuaries between 1984 and 1988 (Haskin and Andrews 1988).

Selective breeding programs to develop strains resistant to MSX disease began at Rutgers University (Delaware Bay) and the Virginia Institute of Marine Science (VIMS – Chesapeake Bay) shortly after the initial epizootics. The strains were developed from native or imported eastern oysters that had survived natural epizootics. Succeeding generations were produced by exposing their offspring in nature and spawning individuals that had survived for 3 or more years (Haskin and Ford 1979). Andrews (1968) reported that strains developed at VIMS had mortality rates of 10% per year, or less, and rarely had patent infections. This compared with losses of 65 to 75% over the first year in unselected control oysters newly introduced from the James River. Ford and Haskin (1987), describing results of the Rutgers breeding program, reported that survival increased, on the average, with each selected generation. After a 33-month exposure, the best selected strains (5th to 7th generations) reached market size with only 30% mortality compared to 90 to 95% for offspring of unselected control groups. The difference in infection and mortality levels between VIMS and Rutgers strains is probably because the exposure site at Rutgers (tidal flats on the Delaware Bay shore of the Cape May peninsula, New Jersey) is subjected to considerably higher infection pressure than that at VIMS (lower York River) (see Haskin and Andrews 1988).

In contrast to Andrews' report (1968) that VIMS resistant strains had very low prevalences, studies at Rutgers showed that both selected and unselected strains could have high infection rates, but that selected oysters were able to contain and tolerate infections (Myhre 1973; Douglass 1977; Ford and Haskin 1987; Ford 1988). Oysters survived with chronic infections for as long as 4 years but they were debilitated by the disease (Ford 1985a). Ford and Haskin (1987) showed that whereas initial mortality rates of susceptible oysters were very high and decreased with further exposure, deaths of highly selected strains were low at first but increased with exposure so that after 5 to 6 years, most oysters had died with MSX disease (Fig. 25).

Thus, repeated reinfection, chronic parasitism, or both, finally killed even the most tolerant oysters. Elevated mortality rates in selected stocks, however, generally occurred after oysters had reached market size (75 to 100 mm). Ford and Haskin (1987) cautioned that improved survival was not uniform among strains, with some showing unexpected MSX disease-associated mortalities after the fourth generation. Heterozygosity was preserved during the selection process despite the loss of some rare alleles (Vrijenhoek et al. 1990). Hu et al. (1993) argued that heterozygosity was maintained because lethal homozygous loci became more frequent with continued inbreeding. Deaths of homozygous individuals (inbreeding depression) occurred predominantly in the larval stage, thus resulting in an unexpectedly high proportion of heterozygotes among survivors.

Pacific oysters, *C. gigas*, exposed to *H. nelsoni* infections in the lower Chesapeake Bay did not become patently infected, even though prevalence in control eastern oysters exceeded 80%, with many advanced infections (Burreson et al. 1994a). Whether Pacific oysters are truly resistant to infection by *H. nelsoni*, or become infected at levels too low for detection, is not known. Pacific oysters become infected by *P. marinus*, but infections remain very light and non-lethal (Meyers et al. 1991; Barber and Mann 1994).

Environmental Influences

Temperature. Temperature is a major factor affecting the *H. nelsoni*-oyster relationship and is largely responsible for the annual cycle of infection development and remission (Ford and Haskin 1982; Ford 1985a). It is also a critical element affecting the expression of resistance to the parasite. Myhre (1973) and Douglass (1977) showed that in autumn and winter, prevalences of *H. nelsoni* were approximately equal in all exposed oysters, regardless of whether they had been selected for improved survival. In spring and summer, when temperatures were above 20°C, the selected oysters were able to eliminate or suppress infections and to recover.

To examine experimentally the influence of temperature on infection development, Haskin and Douglass (1971) brought previously infected oysters into the laboratory from ambient water temperature of 5°C and placed them at temperatures of 5°C, 10° to 15°C, and 19° to 22°C. They measured *H. nelsoni* prevalence and

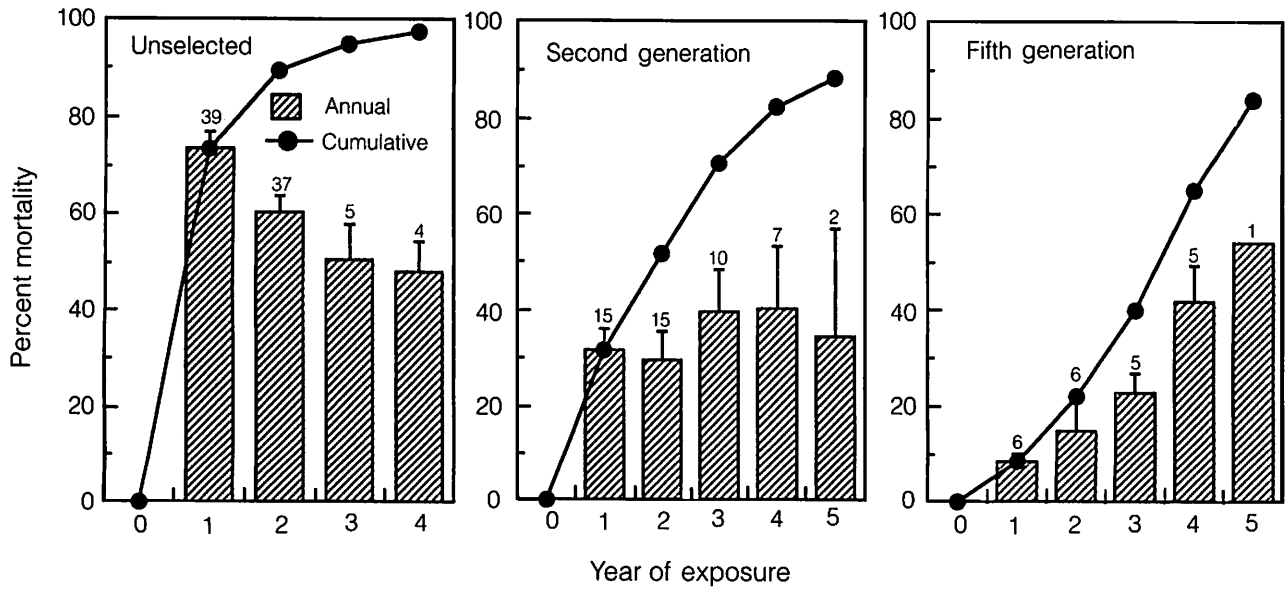

Figure 25. Annual mortality rates and cumulative mortalities associated with *Haplosporidium nelsoni* over a 4 to 5 year period for unselected oysters and strains selected for improved survival for two and five generations. Number of strains is shown above columns. Adapted from Ford and Haskin (1987).

infection intensity weekly. At 5°C, there was no change in infection parameters during the experiment. At 10° to 15°C, parasite burdens increased for the first 2 weeks, then remained static for the following two. At 19° to 22°C, infection levels also increased for 2 weeks but then declined.

Ford and Haskin (1982) combined results of field and laboratory investigations to propose three critical temperature ranges for the eastern oyster-*H. nelsoni* interaction: below 5°C, both organisms are essentially inactive; between 5° and 20°C, the pathogen proliferates more rapidly than the oysters can control it; above 20°C, resistant oysters can overcome the parasite whereas susceptible oysters are killed. The ability of resistant oysters to tolerate infections harbored over the winter may be because metabolic rates of both parasite and host are reduced at low temperatures. If selection increases nonsusceptibility, rather than an active defense response, seasonal fluctuations could be interpreted as temperature-controlled variations in production of substances essential to the parasite (Ford 1988).

Salinity. The absence, or limited presence, of *H. nelsoni* in the upper estuaries and in tributary creeks and rivers of both Delaware and Chesapeake Bays was one of the clearest patterns found in early epizootiological studies (Andrews 1964; Haskin et al. 1965; Andrews and Wood 1967; Farley 1975). The most obvious correlate was low salinity, a finding that was supported during the mid-1960s when a 5-year drought, with consequent elevated salinities, resulted in the movement of *H. nelsoni* up-estuary into regions that had formerly been free of the parasite (Farley 1975; Haskin and Ford 1982). With the return of normal precipitation levels in the late 1960s and 1970s, *H. nelsoni* returned to its previous downbay locations. Most of the 1980s were again drought years and critical spring and early summer periods were especially dry. The pathogen spread even farther up estuaries than in the 1960s, contributing to severe losses of oysters throughout Chesapeake Bay (where deaths were also due to *Perkinsus marinus*) and Delaware Bay.

The correlation of low salinity with reduced *H. nelsoni* prevalence could be due to dilution of infective particles at a distance from a downbay source, as was proposed for *P. marinus* (Mackin 1956); however, low salinity itself is a critical influence. Andrews (1964, 1983) reported that *H. nelsoni* disappeared from oysters on James River seed beds when they were exposed to salinities of 10 ppt or less for about 2 weeks during spring freshets. Haskin and Ford (1982) found the same pattern on the oyster seed beds in Delaware Bay. At temperatures above 20°C, patent infections disappeared within two weeks from oysters moved experimentally from high (>20 ppt) to low (<10 ppt) salinity (Ford 1985b). The disappearance was permanent because infections did not reappear when oysters were returned to a high salinity, non-enzootic area. Further attempts at the Haskin Shellfish Research Laboratory (unpubl. data) to use low salinity immersion to remove *H. nelsoni* from experimental oysters have met with less satisfactory results. These tests showed that at temperatures lower than about 20°C, infections are not completely cleared. They are reduced in intensity, often to the point that they are histologically undetectable, but they frequently become patent again after oysters are returned to higher salinities.

Andrews (1983) noted that high temperature and low salinity were correlated with reduction of infections in the James River seed area. Because this occurred only after oysters became metabolically active in late spring, he proposed that low salinity enhanced the resistance mechanism, permitting oysters to actively rid themselves of the parasite. Ford and Haskin (1988a), on the other hand, provided evidence of direct killing of *H. nelsoni in vitro* by low salinity (Fig. 26) and argued that enhancement of resistance need not be invoked to explain the absence of the parasite in low salinity. It is possible that both factors are involved. Oyster hemocytes do not phagocytose live *H. nelsoni* plasmodia, but they do ingest dead and moribund parasites (Ford et al. 1993b). Parasites killed by low salinity could thus be cleared from tissues by phagocytes, but because the rate of phagocytosis and intracellular degradation is temperature dependent (Feng and Feng 1974; Alvarez et al. 1989), histological sections would show the disappearance of dead parasites only after temperatures reached levels high enough for required cellular activities.

Other. In nature, the distribution of MSX disease is not associated with the presence of pollutants, but controlled experiments to examine possible exacerbation of disease by exposure to contaminants, such as that reported for *Perkinsus marinus* (Scott et al. 1985; Winstead and Couch 1988; Chu and Hale 1994), have not been performed. Concurrent infestation of shells by the mudworm *Polydora websteri* and infections by *H. nelsoni* were correlated with a greater reduction in the condition index of oysters than with either factor alone (Wargo and Ford 1993), indicating that the metabolic effects of MSX disease on oysters may be measurably aggravated by other stressors (see also Littlewood and Ford 1990).

Control Measures

The presence of nearby parasitized oysters is not required to initiate infections in newly exposed oysters. Therefore, control measures useful for *P. marinus*, such as removing old infected oysters from a planting ground, do not reduce the spread of *H. nelsoni*. As with *P. marinus*, placing oysters at different intertidal levels does not affect *H. nelsoni* prevalence or intensity (Littlewood et al. 1992), nor does height in the water column (Ford and Haskin 1988b). Probably the most effective means to combat the disease is to use resistant strains produced in hatcheries and reared using aquacultural techniques, although this is not practical in areas where wild seed is abundant and inexpensive. When wild seed is used, oysters should be kept in disease-free areas (usually low salinity) as long as possible, even at the expense of slower growth. If they must be moved into high salinity areas, where *H. nelsoni* is prevalent, for growth and conditioning before market, the move should be timed to avoid the early summer infection period and oysters should be harvested the same autumn or the following spring (Ford and Haskin 1988b). Low-salinity immersion to rid oysters of *H. nelsoni* infections is potentially valuable for both experimental and perhaps commercial purposes, but considerably more study is required to pinpoint critical time-temperature-salinity thresholds that will completely rid oysters of infections.

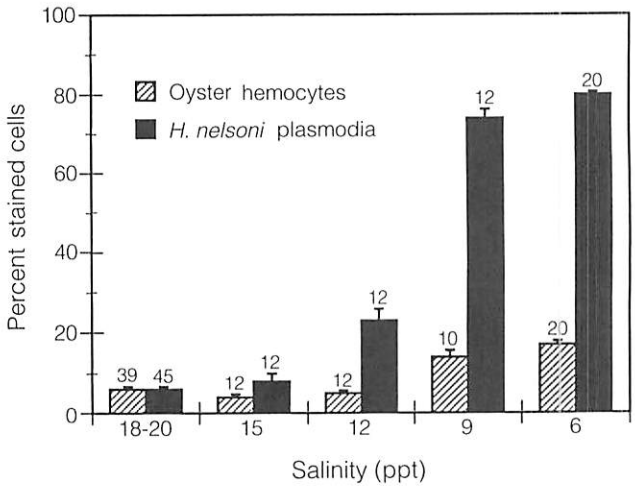

Figure 26. Mortality of *Haplosporidium nelsoni* plasmodia and *Crassostrea virginica* hemocytes, measured by failure to exclude trypan blue dye after incubation for 10 min at various salinities. Vertical bars represent standard error of the mean. Number of tests is shown above columns. From Ford and Haskin (1988a).

SSO Disease

History and Distribution

Shortly after MSX disease had been reported in Delaware Bay, Wood and Andrews (1962) discovered another disease of eastern oysters caused by a protozoan closely related to *H. nelsoni*, which they named *H. costale*. The causative agent is also known by the acronym "SSO," which stands for "Seaside Organism," a reference to the fact that it was discovered in oysters in high salinity bays on the Atlantic coast of Virginia. The pathogen was most likely present well before it was first described because high mortalities were common in oysters held more than 18 months in the seaside bays (Andrews et al. 1962). Since its initial description, *H. costale* has been identified in oysters from coastal Delaware, Maryland, and New Jersey (Andrews 1988c), and organisms with histologically similar plasmodial stages have been found, but not positively identified, along the Atlantic coast as far north as Maine (Fig. 5; see also Newman 1971; Meyers 1981; Andrews 1988c). Also, plasmodial and spore stages of a haplosporidan resembling *H. costale* were found in *C. virginica* that had been held in Tomal-

es Bay, California, for up to 18 months after shipment from the Connecticut shore of Long Island Sound (Katkansky and Warner 1970).

Etiological Agent

Life Cycle. Haplosporidium costale was classified a member of the Phylum Ascetospora, Order Stellatosporea, by the Society of Protozoologists (Levine et al. 1980) (but see p. 613 for discussion of alternative classification in Phylum Haplosporidia). Infective particles are thought to be ingested and the earliest recognized stages in the oyster are multinucleated cells (Figs. 27A, E; 28D, E) found just beneath the digestive tubule epithelium (Andrews and Castagna 1978). Multinucleated plasmodia develop by successive nuclear and plasmodial division, and spread throughout host tissues. Plasmodia eventually develop into sporocysts as cytoplasm condenses around individual nuclei (Figs. 27B, C, D; 28D, F). Spore walls form within the condensed cytoplasm, each enclosing a single nucleus (Perkins 1969). Sporulation occurs regularly and synchronously in adult oysters within a short period during May and June of each year. Mortality occurs at the same time. The prevalence of spore stages in live oysters is only about 5 to 10%, but about half of the dead oysters contain spores (Andrews et al. 1962; Andrews and Castagna 1978). Nevertheless, very few spores reach maturity before the host dies, perhaps because nutrients are insufficient for the huge number of parasites that normally occur in advanced infections (Andrews and Castagna 1978). Andrews (1982) suggested that the regular and abundant sporulation of *H. costale* indicated a better adapted parasite than *H. nelsoni*, in which spores were found only rarely.

Spores and plasmodial stages of *H. costale* resemble those of *H. nelsoni* but are smaller. The spores are operculate, with overhanging lids, and are covered by filamentous wrappings (Figs. 27F, 28F). Spore dimensions average 3×5 μm and plasmodia are generally less than 10 μm in diameter (Wood and Andrews 1962). Nuclei are also small and, in contrast to *H. nelsoni*, have centrally rather than peripherally located nucleoli (cf., Figs. 27E, 28E and Figs. 18E, 19E). Concurrent infections, in which both parasites are sporulating, have been reported (Couch 1967), but it can be difficult to distinguish between the two species when only plasmodial stages are present. Spores of *H. costale* develop in all tissues except the epithelia (Perkins 1969). This contrasts with sporulation of *H. nelsoni*, which occurs only in the epithelia of the digestive tubules. The presence of spores is currently the most reliable way of identifying the organism by light microscopy, particularly when a suspected case appears in a "new" area. In transmission electron microscopy, difference in size and shape of haplosporosomes (Perkins 1979) can be an identifying character (Figs. 19G, 28G).

Transmission. Experimental transmission of *H. costale* by feeding and injection of spores has not been successful (Andrews 1979). Therefore, it is not known if the parasite is transferred directly from oyster to oyster or if another host or vector is involved. The potential for direct transfer is more compelling than is the possibility of direct transfer of *H. nelsoni*, however. Regular production of spores in oysters would provide an abundant source of a resistant stage to move between hosts. Also, oysters become infected only during exposure periods when sporulating *H. costale* are found in previously infected oysters (Andrews and Castagna 1978). The relatively low proportion of mature spores produced in oysters before they die suggests that another host may be involved, but the absolute numbers released by dead oysters may still be sufficient to infect many other oysters.

Infection and Disease Processes

Diagnosis. Diagnosis of *H. costale* is commonly made from tissue sections (Wood and Andrews 1962). Screening of hemolymph may be useful for the diagnosis of systemic infections, but plasmodia are small and their detection may be more difficult than the detection of *H. nelsoni* in hemolymph samples (E. Burreson, pers. comm., 1991).

Portal of Entry. Patent infections of *H. costale* typically appear in the early spring, after exposure in May and June of the previous year. Infections are light, but already systemic (Andrews and Castagna 1978). A uninucleate haplosporidan found "sparingly" in the epithelium of digestive tubules in June and July, immediately after oysters were exposed to epi-

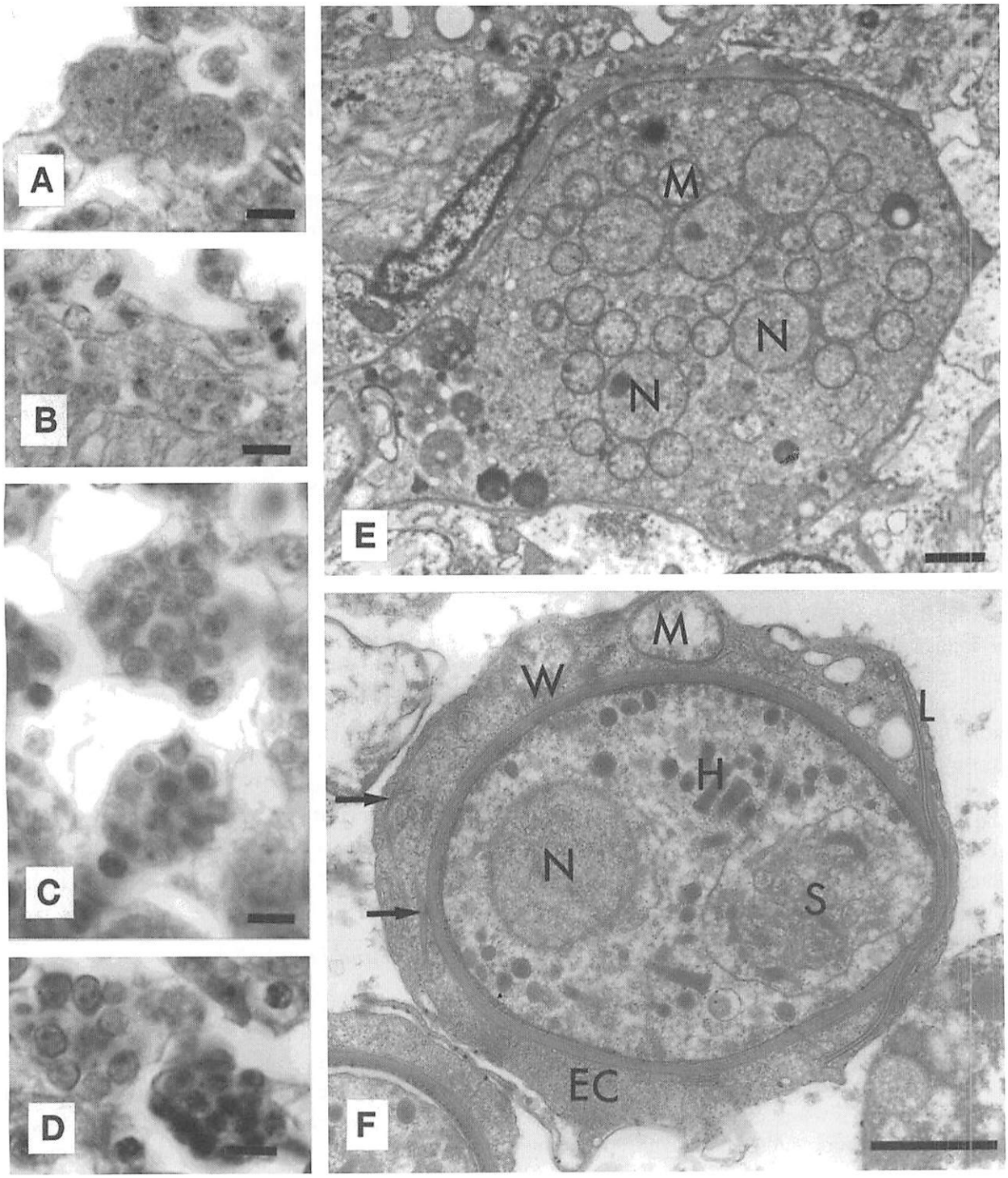

Figure 27. Stages of *Haplosporidium costale* found in *Crassostrea virginica*: (A) plasmodia; (B) early sporonts; (C) sporonts; (D) early (left) and late (right) sporocysts; (E) plasmodium showing nucleus [N] with central nucleolus, and mitochondria [M]; (F) immature spore showing lid [L], wall [W], nucleus [N], spherule [S], epispore cytoplasm [EC], haplosporosomes [H], and spore wrappings [arrows]. Scale bars = 5 µm in A to D; 1 µm in E and F.

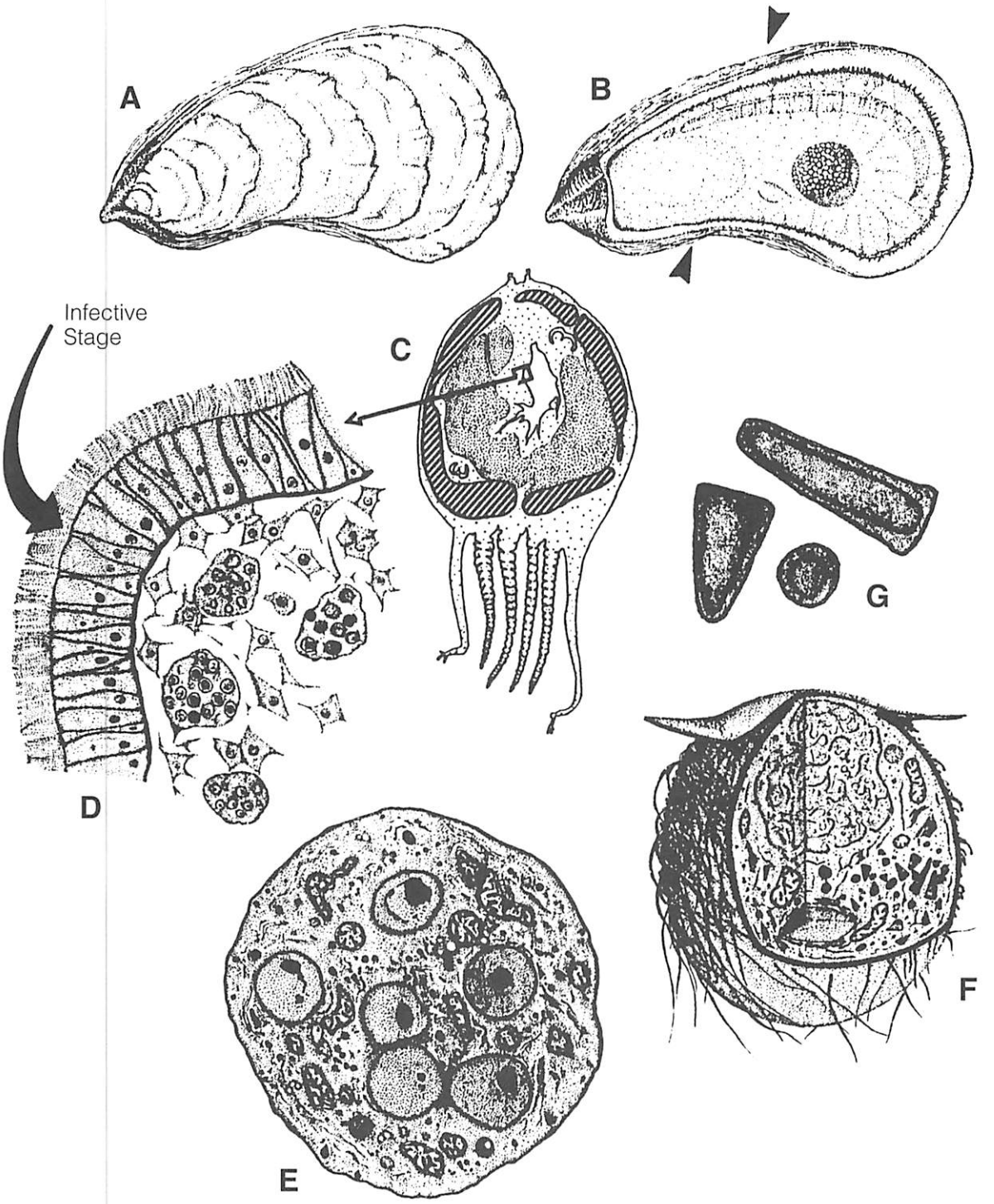

Figure 28. Diagram of stages of *Haplosporidium costale* seen in *Crassostrea virginica*: (A) intact oyster; (B) oyster with right valve removed showing plane (arrows) of section in (C); (D) stomach epithelium (portal of entry) and underlying connective tissue showing plasmodium, sporont, and sporocysts; (E) plasmodium; (F) spore; (G) haplosporosomes. See Figure 27 for ultrastructural details.

zootic mortalities, may be the earliest recognized stage (Andrews and Castagna 1978). Histology suggested that this organism was invading connective tissues and developing into plasmodia, implying that entry into the oyster was through the digestive epithelium.

Development of Infection and Histopathology. It is presumed that infections develop from localized sites, probably the digestive epithelium, in much the same manner as that described for the proliferation of *H. nelsoni* from the gill epithelium. In contrast to *H. nelsoni*, however, localized epithelial lesions containing *H. costale* are rare and nearly all diagnosed infections are systemic (Andrews and Castagna 1978). Consequently, epithelial sloughing at infection sites, a common pathological occurrence in Dermo and MSX diseases, is not reported for SSO (Fig 29). The abundance of parasites disrupts storage or connective tissue cells (Fig. 29), resulting in a "precipitated" or "curdled" appearance of preserved tissue (Andrews and Castagna 1978). Hemocytes infiltrate connective tissues as parasites multiply and can become abundant in heavy infections (Andrews 1988c). That *H. costale* sporulates only in connective tissue and is scarce in epithelia contrasts markedly with *H. nelsoni*. These differences probably reflect distinct life-cycle requirements of the two parasites.

Epizootiology

Infection and Mortality Periods. Sequential importations of uninfected oysters into enzootic areas of Virginia have firmly established that the infection period for *H. costale* occurs in May and June of each year, synchronously with the deaths of previously infected oysters (Couch and Rosenfield 1968; Andrews and Castagna 1978). It is not until the following March and April, however, that patent infections first appear (Fig. 15). Plasmodia proliferate rapidly and sporulation occurs in May and June. Mortality occurs at the same time and by late July, patent infections have disappeared. Mortalities can continue in the same stocks over several years, indicating that reinfection, or perhaps relapse of subpatent infections, occurs each year (Andrews and Castagna 1978) as it does with *H. nelsoni* (Ford 1985a).

Andrews (1983) has pointed out the similarities between the late-summer/autumn infection and mor-

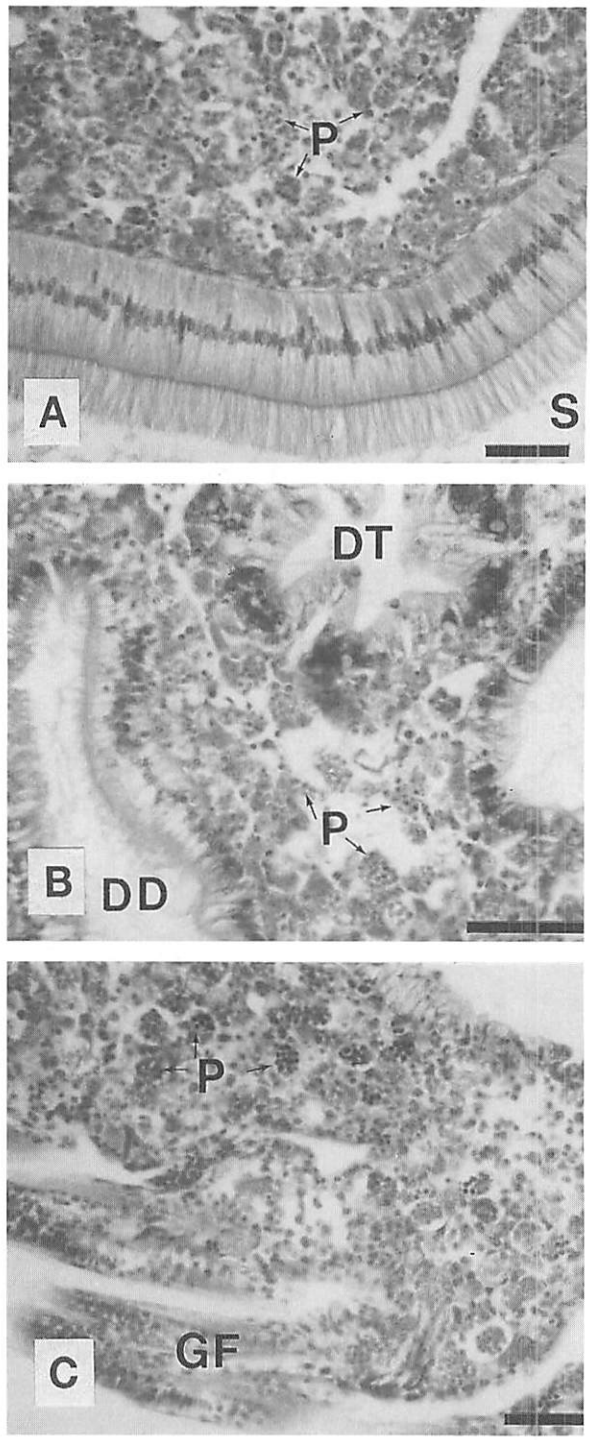

Figure 29. Histopathology caused by *Haplosporidium costale* in tissues of *Crassostrea virginica*: (A) heavy infections with intense hemocyte infiltration near stomach [S]; (B) digestive ducts [DD] and tubule [DT]; (C) gill [gill filament = GF]. Note absence of parasites [P] in, and good condition of, the epithelia. Scale bars = 50 μm.

tality pattern for *H. nelsoni* and that for *H. costale*: they both involve long incubation periods, rapid proliferation of parasites in spring immediately preceding mortality, and a discrete, short mortality period in June and July.

Infection Levels and Mortality Rates. Peak prevalences occur just before mortality and thus the estimate of new cases is not complicated by concurrent acquisition of infections and deaths of diseased oysters, as it is for both *Perkinsus marinus* and *H. nelsoni.* Prevalences of 30 to 40% precede mortalities of 20 to 60% indicating that nearly all infected oysters die (Andrews et al. 1962; Andrews 1984a).

Infectivity and Pathogenicity. As in the case of *H. nelsoni*, the infective dose requirement for *H. costale* is not known because controlled transmission has not been achieved. The high ratio of deaths to cases diagnosed (80 to 90%), as well as the speed with which it develops, indicates that the disease is highly lethal. Sixty to ninety percent of the dead oysters collected during the May to June mortality period along the Atlantic coast of Virginia have been positive for *H. costale* (Andrews et al. 1962; Couch and Rosenfield 1968; Andrews and Castagna 1978).

Resistance

Andrews et al. (1962) noted that oysters native to enzootic areas suffered smaller losses to SSO disease than did imported oysters, but no attempts have been made to determine whether this is a heritable trait. Andrews (1984b) tested MSX disease-resistant stocks for resistance to SSO disease, but reported that results were "inconclusive because of interference [by *H. nelsoni*]." Lightly infected oysters that are not killed during the May to June mortality can recover (Andrews 1984a) and Andrews and Castagna (1978) noted apparent regression of plasmodia, characterized histologically by dense staining and a gradation into brown cells, in early spring. Phagocytosis of apparently moribund plasmodia was observed, but death of the parasite is probably an indication of unfavorable conditions inside the host over winter rather than of resistance. *Haplosporidium nelsoni* plasmodia frequently show an identical condition in early spring concurrent with a reduction in prevalence and infection intensity among parasitized oysters, but the remission is short lived and plasmodia reappear from subpatent infections within a month or two (Ford 1985a). Andrews (1988c) stated that despite the infiltration of hemocytes accompanying *H. costale* infection, hemocytes "seem ineffective in phagocytosing or killing pathogen cells."

Environmental Influences

Relatively little information is available concerning environmental influences on SSO disease. It is usually restricted to high salinity water (above 25 ppt) although occasional cases are found at somewhat lower salinities (20 to 23 ppt) (Andrews et al. 1962). Infections regress when oysters are moved into water below 20 ppt (Andrews 1979). There are no experimental data on the effects of temperature on SSO disease, but it seems likely that seasonal temperature patterns influence disease cycles, as they do with both Dermo and MSX diseases.

Control Measures

The development of lethal infection by *H. costale* requires exposure during a well-defined 2-month period followed by an incubation period of nearly a year. It is therefore possible to place oysters in enzootic water immediately after the May to June infection period and maintain them 21 months before significant losses occur (Couch and Rosenfield 1968; Andrews and Castagna 1978). Thus, planting and harvesting schedules can be designed to avoid mortality while still providing one-and-a-half growing seasons in high salinity water, a schedule that oyster planters in affected bays have learned to use (Andrews et al. 1962).

DEFENSE MECHANISMS

Hemocytes and Their Capabilities

The structure and function of bivalve hemocytes has been reviewed by Cheng (1981), Fisher (1986), and Auffret (1988). For details of *C. virginica* hemocyte structure and function, see Cheng, Chapter 8.

In addition Feng (1988) has reviewed cellular defense mechanisms of oysters and mussels.

The source of bivalve hemocytes has not been demonstrated clearly, but it has been suggested that they arise by differentiation of connective tissue cells (Cheng 1984; Auffret 1988). There have been many descriptions of oyster hemocytes including those of *C. virginica* (Stauber 1950; Galtsoff 1964; Feng et al. 1971; Foley and Cheng 1972; Cheng 1975a; Renwrantz et al. 1979). These studies support the concept that there are two primary lines of cellular development leading to the production of cells lacking stainable cytoplasmic granules (hyalinocytes or agranulocytes) and cells with prominent organelles (granulocytes). Cheng (1981) has postulated that these represent distinct cell lines and that different developmental stages account for the variety of cells seen in oyster hemolymph. Subpopulations of hemocytes vary in their enzyme content (Cheng and Downs 1988) suggesting even greater variability within and between individual oysters. Pigmented or brown cells, often associated with pathological conditions, may represent a third cell line (Cheng 1981).

Determination of hemocyte types and frequencies is often tedious and difficult. Morphological distinctions are sometimes blurred and variability is great. Nevertheless, flow cytometry, which rapidly identifies cells according to light-scattering qualities, consistently shows three distinct subpopulations in eastern oysters from the mid-Atlantic region: agranular cells (hyalinocytes and degranulated granulocytes), large granulocytes, and small granulocytes (Ford et al. 1994; K. A. Alcox, Haskin Shellfish Research Laboratory, pers. comm. 1994). In contrast, eastern oysters from the Gulf of Mexico, also measured by flow cytometry, were found to have only a single hemocyte population, although the type of cell involved was not described (Friedl et al. 1988).

Bivalve hemocytes are involved in wound repair, shell repair, digestion and transport of nutrients, excretion, and internal defense (Cheng 1967, 1981, 1984). Here we will examine response of hemocytes to experimentally-introduced foreign material and their role in tissue repair; responses to infection are summarized in sections concerning specific diseases.

Inflammation

The inflammatory response in oysters has been described by Stauber (1950), Tripp (1960), Pauley and Sparks (1965, 1966, 1967), Feng and Stauber (1968), Feng and Feng (1974), and others. It is clear that the type of tissue affected and type of damage sustained influence the nature of the cellular response but some general observations can be made. Injection of nontoxic particles elicits a hemocytic response that tends to localize the foreign material and to remove phagocytosed particles. For example, Stauber (1950) injected carbon particles intracardially in *C. virginica* and clusters of particles were deposited in blood vessels and sinuses throughout the body. Hemocytes engulfed particles and migrated across epithelial layers to remove carbon from the tissues. A similar response of phagocytosis and migration occurred with injected red blood cells but clearance was supplemented by intracellular digestion (Tripp 1958). Similarly, when talcum powder was injected into connective tissue or the adductor muscle of *C. gigas*, hemocyte infiltration was evident within 16 h and typical granulomatous structures were formed (Sparks and Morado 1988). Phagocytosis of particles proceeded and in time the injected material was cleared from the tissues. Elements of this response are seen also in infection with helminth parasites (Cheng 1967) although certain large foreign bodies elicit other kinds of responses, primarily encapsulation (Cheng and Rifkin 1970).

Oyster hemocytes accumulate around foreign material (or degraded self-material), adhere to or phagocytose it, and degrade it or remove it from the tissues. The details of subcellular mechanisms at work within these cells, including hydrolytic enzymes and reactive oxygen intermediates, are beginning to be revealed (e.g. Anderson et al. 1992a; Cheng 1992a, b). The net effect is neutralization or isolation of potentially damaging substances. The question of whether and how oyster cells may recognize specific foreign substances has been addressed by Cheng (1987) and Chu (1988). The models proposed feature lectins reacting with specific sugars on foreign substances (e.g., red cells, bacteria) and binding them to hemocyte membranes to facilitate phagocytosis.

Response to Mechanical Injury

Sparks and Morado (1988) reviewed the responses of molluscs to wounds and the healing process. Oysters may be damaged mechanically when infested by crabs of the genus *Pinnotheres.* Stauber (1945) established that the walking legs of *P. ostreum* damage gill tissue of *C. virginica* as it moves over the gill surface seeking food embedded in mucous strings. Rapid healing often follows (Stauber 1945), but no histological study of this damage and repair process has been reported.

Des Voigne and Sparks (1968) made sterile wounds in the visceral mass of *C. gigas* and observed the repair process. Initially (24 to 48 h) the wound was filled with round hemocytes and fibroblasts; later (144 h) the hemocytes elongated and collagen was deposited. The scar tissue was gradually replaced by a pseudoepithelium at the surface near digestive tubules. Eventually both hemocytes and collagen were replaced by Leydig cells. Ruddell (1971) performed similar experiments wherein mantle tissue of *C. gigas* was cut and then examined by electron microscopy during repair. He reported that agranulocytes were the primary respondents to tissue damage and that they differentiated into myoblasts, a view not accepted by Cheng (1981), who contended that the "agranulocytes" were actually degranulated granulocytes.

Overall, the oyster's response to mechanical damage of its tissues is a rapid aggregation of hemocytes that sequester cellular debris. They organize into a plug-like structure, and fibrocytes deposit collagen-like material and later displace amoeboid hemocytes. New epithelium is formed as adjacent epithelial cells migrate to replace damaged cells.

Response to Chemical Injury

The injection of soluble toxic material into connective tissue or adductor muscle of *C. gigas* causes cellular necrosis and edema; hemocytic infiltration begins within a few hours (Pauley and Sparks 1965). Necrotic cells and debris are phagocytosed and phagocytes migrate to the exterior across epithelia. The fluid cannot be localized, however, so it diffuses into and damages adjacent tissue as time passes; widespread cytotoxicity may lead to the death of the affected animal (Sparks 1972). Repair of chemically damaged tissues was not reported (Pauley and Sparks 1965).

Bivalves, including oysters, have been studied as bioindicators of environmental pollution (see Roesijadi, Chapter 14 and Capuzzo, Chapter 15). There is abundant evidence of uptake and tissue accumulation of hydrocarbons by eastern oysters (e.g., Stegeman and Teal 1973; Lee et al. 1978) but physiological effects are usually measured without histopathological examination. Effects of xenobiotics on bivalve defense mechanisms have been reviewed by Anderson (1988). Hard clams exposed to known concentrations of hydrocarbons and subjected to extensive histopathological analysis revealed no consistent pattern of cellular damage (Tripp and Fries 1987). Blue mussels (*Mytilus edulis*) exposed to oil showed changes in hemocyte populations and a reduced phagocytic response that may have significance for other species (McCormick-Ray 1987). Bivalves exposed to heavy metals have been subjected to chemical analysis and physiological responses have been measured, again usually without detailed histopathological studies (Cunningham 1979). Farley (1988), however, has reported inflammatory responses in oysters (*C. virginica*) obtained from waters contaminated with copper. Sunila (1988) has reported that blue mussels show inflammatory reactions, including phagocytosis of sperm in follicles, and fusion of gill filaments when exposed to copper in nature or in the laboratory.

Cheng (1988a, b) reported that *C. virginica* exposed to heavy metals exhibit significant changes in hemocyte numbers and activity and Anderson et al. (1992b) found that *in vitro* exposure of hemocytes to high (~77 ppm), but sublethal, cadmium concentrations diminished their production of cytotoxic oxygen products, as measured by chemiluminescence. Tributyltin (TBT) also inhibited chemiluminescence and hemocyte locomotion (Fisher et al. 1990), the fungicide Triforine reduced both hemocyte viability and phagocytic ability (Alvarez et al. 1991), and polycyclic aromatic hydrocarbons altered the relative proportions and sizes of hemocyte subpopulations (Sami et al. 1992). These data, in addition to recent studies showing a positive relationship between laboratory exposure of oysters to extracts of highly contaminated sediments from the Elizabeth River, Virginia, and increased levels of *Perkinsus marinus* (Chu

and Hale 1994), indicate that high concentrations of anthropogenic contaminants can adversely affect oyster hemocytes and defense mechanisms. Nevertheless, the effect of exposure to more typical ambient chemical concentrations in nature is still largely unknown, and there is no correlation between locations where the major oyster pathogens are enzootic and pollution. It should also be noted that natural factors such as temperature and salinity affect *in vitro* aggregation, spreading, locomotion, and foreign particle binding of *C. virginica* hemocytes (Fisher 1988b). Further, subpopulations (i.e., granulocytes and agranulocytes) of *C. virginica* hemocytes vary in relative proportion and mobility according to season, location, and individual (McCormick-Ray and Howard 1991; Ford et al. 1994; Oliver and Fisher 1995).

Response to Foreign Proteins

Feng (1965) performed a series of experiments in which eastern oyster hemocytes were exposed to various foreign proteins either *in vivo* or *in vitro*. Pinocytosis of these materials by hemocytes occurred and the rate of uptake was affected by protein concentration and temperature. Histological examination showed that protein-laden hemocytes migrated through various epithelia and were thus eliminated from the body. It is clear that individual oyster hemocytes recognize soluble foreign proteins, ingest them, and dispose of them.

Response to Transplanted Tissue

Transplantation of tissue from one organism to another is an important experimental tool for studying the ability to recognize and react to foreign or self cells. In molluscs, transplantation experiments are technically difficult and have had only limited success. Canzonier (1974) performed a series of allografts in *C. virginica* to demonstrate that successful ectopic transplantation can be achieved in this animal. Hemocytic infiltration occurred when damaged tissue was implanted, but healthy tissue fused with host epithelia for successful transplantation. The presence of *H. nelsoni* in transplanted tissue did not influence the success of the transplant and the parasite did not spread into the new host's tissue. It is clear that healthy tissue can be transplanted within genetically compatible stocks of *C. virginica* without eliciting rejection. Similar results were previously obtained with *C. gigas* (Des Voigne and Sparks 1969).

Responses to Various Biological Agents

Viruses

A variety of viruses have been associated with oysters, including *C. virginica*, in three different contexts: (1) contamination of oysters with human pathogens, (2) natural infections of viruses that replicate in oyster tissues, and (3) viruses injected into oysters for experimental purposes.

The association of human enteroviruses with oysters and other shellfish is well known and of great public health importance. Adult *C. virginica* have been found to harbor viruses, but it is quite clear they are accumulated passively by feeding in water that is naturally (Gerba and Goyal 1978) or experimentally (Metcalf and Stiles 1965) contaminated with various viruses. There is no published evidence that the presence of these viruses elicits a hemocytic response.

A reo-like virus was isolated from *C. virginica* larvae in a Long Island, New York, hatchery (Meyers 1979) and was presumed to be a contaminant from some vertebrate host (Meyers and Hirai 1980). Virus-like particles have been found in lesions in larval *C. gigas* (Elston 1979) and associated with a fatal disease affecting the velum and other epithelial layers (Elston and Wilkinson 1985).

The information about viruses naturally infecting adult oysters and other marine molluscs has been summarized by Farley (1978), Johnson (1984), and Sindermann (1990). Eastern oysters dying after transplantation to warm water effluent (28° to 30° C) from a power plant contained a herpes-like virus. Because healthy oysters were also infected with the virus, heat stress may have led to the diseased condition (Farley et al. 1972). Diseased oysters had aggregates of hemocytes in affected tissues, but whether that aggregation was a limited response to infection or proliferation of neoplastic cells is not clear (Farley et al. 1972).

A papovavirus has been reported in germinal cells of *C. virginica* from several Atlantic seaboard states as

well as from *C. gigas* and *Ostreola conchaphila*[2] (= *O. lurida)* from Oregon, Washington, Korea, and Japan and *C. rhizophorae* from Puerto Rico (Farley 1985). In all of these species, infected cells enlarge and lyse, causing hemocytes to accumulate around them (Farley 1985). A birnavirus has been isolated in fish-cell cultures from a variety of marine bivalves, including *C. virginica* from Canada (Johnson 1984). None of these strains appears to be pathogenic or to elicit a cellular response in host molluscs. Two other possible infectious viruses have been reported from oysters by Farley (1978): a togavirus budding from the membrane of a hemocyte of *O. edulis* and a possible retrovirus with c-type particles budding from digestive gland epithelium of *C. virginica.* Neither of these infections caused a hemocytic response by the host. Similarly, virus particles seen in larval *C. gigas* did not elicit a cellular reaction (Elston 1979).

J.S. Feng (1966) injected *Staphylococcus aureus* phage 80 into *C. virginica* intracardially and found the rate of clearance was temperature dependent. The virus was stable in normal oyster hemolymph and sea water at 5°, 15°, or 25°C for up to 170 h. Rapid deterioration of the virus occurred after 60 h at 25°C in hemolymph of oysters previously exposed to the virus, whereas no such deterioration was observed at 5° or 15°C. Hemocytes seemed to play only a small role in the *in vivo* clearance of the injected virus (J. S. Feng 1966) and presumably little phagocytosis occurred. In contrast, *C. virginica* hemocytes have been shown to phagocytose the blue-green algal (Cyanobacterium) virus LPP-1 *in vitro* (Fries and Tripp 1970).

Acton (1970) found that T2 coliphage injected intracardially into *C. virginica* were cleared from the circulation rapidly for the first 24 h but a decreased rate of clearance was noted for the next 48 h. After a second injection, the same initial clearance rate continued for 72 h. This secondary response was nonspecific and in the absence of demonstrable neutralizing humoral factors was attributed to "education" of hemocytes (Acton 1970).

[2] The author originally identified this species as *Ostrea lurida.* The editors have renamed it following the convention of Carriker and Gaffney (Chapter 1).

Bacteria

Post-metamorphic oysters contain a complex mixture of bacteria similar to the bacterial flora in the water in which they live. The genera *Achromobacter, Bacillus, Cytophaga, Flavobacterium, Pseudomonas,* and *Vibrio* comprise the normal flora of *C. virginica* (Murchelano and Bishop 1969) and *C. gigas* (Colwell and Liston 1961). Immature oysters either prevent infection or control these bacteria in the tissues so the oysters are not subject to great mortality except occasionally in crowded nursery culture (Elston 1984).

The effects of bacteria on adult oysters are undramatic. Tubiash (1974) exposed adult *C. virginica* to presumed bacterial pathogens and found that only one strain of *Vibrio anguillarum* caused significant mortality: 35% on single short exposure and 80% after chronic exposure (22 weeks). He concluded that bacteria are "of marginal significance as primary pathogens of adult American [eastern] oysters." Colwell and Sparks (1967) isolated a variety of Gram-negative bacteria from healthy *C. gigas*; Murchelano and Brown (1968) found a similar mixture of bacterial genera in *C. virginica. Pseudomonas enalia,* an apparent pathogen, was isolated from dead and dying *C. gigas* and, when injected into adult oysters, hemocytic infiltration and phagocytosis of bacteria followed. Unfortunately, control oysters also exhibited inflammation and often died (Colwell and Sparks 1967) so that the status of *P. enalia* as a pathogen of oysters is uncertain. More recently, a presumptive *Nocardia* spp. has been isolated from *C. gigas* hemolymph and adductor muscle lesions (Friedman and Hedrick 1991a). Azevedo and Villalba (1991) have reported a benign association between bacteria and "giant rickettsia" in the gill filaments of *C. gigas* from Spain.

A condition named "cardiac edema" was found in 4 of 10,000 adult *C. virginica* from Chesapeake Bay (Tubiash et al. 1973). Gram-negative bacteria isolated from pericardial fluid proved to be *Vibrio anguillarum* but attempts to reproduce this condition experimentally were unsuccessful. Affected oysters showed no other histopathology attributable to the bacteria (Tubiash et al. 1973).

Tripp (1960) injected adult *C. virginica* intracardially with either vegetative bacteria (*Staphylococcus aureus, Bacillus mycoides, Escherichia coli, Pseudomonas*

fluorescens, Flavobacterium invisible), bacterial spores (*B. mycoides*), or yeast (*Saccharomyces cerevesiae*) and followed their fates histologically or by culture. Most of the vegetative bacteria were removed from the hemolymph rapidly (i.e., by 2 d) but persisted in tissues for several more days. Yeast cells were cleared from the hemolymph more slowly and bacterial spores were most resistant to clearance. Histological examination revealed extensive phagocytosis of bacteria as early as 1 h post injection. Hemocytes then migrated into tissues and digested phagocytosed microbes or moved across epithelial barriers to the exterior. Vegetative bacteria were most susceptible to intrahemocytic digestion, yeast less susceptible, and spores insusceptible. Bacteria are endocytosed and digested intracellularly by hemocytes of *C. virginica* and the degradation products, presumably simple sugars, are used in glycogen synthesis (Cheng and Cali 1974; Cheng and Rudo 1976). Also, certain bacteria may induce the release of hydrolases from *C. virginica* hemocytes (Cheng 1992a).

S.Y. Feng (1966) injected *C. virginica* intracardially with either *Bacillus thuringiensis, Mycobacterium smegmatis*, or a *Pseudomonas*-like bacillus. All three bacteria species were eliminated but different clearance patterns were noted. The mycobacteria persisted at initial concentrations for 5 d and then rapidly disappeared. Most of the vegetative *B. thuringiensis* was cleared quickly but small numbers persisted for as long as 10 d. A pseudomonad isolated from dead and dying oysters (A-3) established a relapsing course of infection over a three-week period at temperatures of 22° to 27°C but was cleared steadily from the tissues at 9° and 16°C. Histological studies showed that oyster hemocytes phagocytosed injected bacteria and either digested them or carried them across epithelial layers to the exterior (S. Y. Feng 1966). Bacteria (*E. coli, Shigella typhimurium*, and *S. flexneri*) injected intracardially or added to the water and ingested were cleared from the hemolymph within 24 h at 20°C (Hartland and Timoney 1979). At 6°C, intracardially injected bacteria were cleared from the hemolymph after 72 h.

Miscellaneous Protozoa

Reports of an apparently pathogenic haplosporidan described in rock oysters, *Saccostrea* (= *Crassostrea*, see Carriker and Gaffney, Chapter 1) *commercialis*, in Australia (Wolf 1972) and subsequently named *Marteilia sydneyi* (Perkins and Wolf 1976) do not address the details of histopathology or host cell response. *Ostreola conchaphila* in Oregon are infected with an unidentified haplosporidan to which there is a strong hemocytic response in light infections and a moderate response in heavy infections (Mix and Sprague 1974).

Hexamita nelsoni is a cosmopolitan, flagellated protozoan infecting oysters, including *C. virginica, C. gigas, S. commercialis, Ostreola conchaphila*, and *O. edulis*, around the world (Schlicht and Mackin 1968; Sprague 1970). Mackin et al. (1952) described the histopathology of this disease and concluded that it is basically a disease of the hemolymph. Hemocytes occur at certain foci in very large numbers and block blood vessels. Flagellates are found within hemocytes and degeneration of host cells follows, possibly reflected in hemocytopenia in later stages of disease (Mackin et al. 1952). A similar pathological progression is seen in *Ostreola conchaphila* (Stein et al. 1961). It is clear that the flagellate is associated with severe disease, but it has been argued that this protozoan is an opportunistic secondary invader rather than a primary pathogen. Scheltema (1962) attempted to clarify the flagellate-oyster relationship without success, although he concluded that the protozoan does not contribute significantly to oyster death. A study by Feng and Stauber (1968) on experimental hexamitiasis in *C. virginica* showed that the relationship between the two organisms is complex and affected by the oyster's physiology as well as the reproductive capacity of the protozoan; both of these factors are influenced by environmental and experimental conditions (i.e., ambient temperature, size of inoculum, route of entry).

In *C. gigas* females infected with an unknown protistan, Becker and Pauley (1968) found that hemocytes surround but do not encapsulate infected eggs. An unidentified thigmotrichid ciliate infecting *C. gigas* generally elicited no cellular reaction but in one case a general inflammatory response was noted (Pauley et al. 1967). Similarly, infection of *S. commercialis* by *Hartmanella tahitiensis* elicited no apparent cellular reaction to the amoebae (Cheng 1970).

A gregarine-like parasite of *C. virginica* causes a

sloughing of intestinal epithelial cells but no inflammatory reaction (Sawyer et al. 1975). Two species of *Nematopsis*, i. e., *N. ostrearum* and *N. prytherchi*, infect *C. virginica* along the Atlantic and Gulf coasts of the United States (Sprague and Orr 1955). Feng (1958) demonstrated that *N. ostrearum* spores are phagocytosed by hemocytes, distributed in various oyster tissues, and gradually eliminated from the tissues.

Bonamia ostreae has not been found in species of *Crassostrea*, but it has devastated populations of *O. edulis* in Europe as well as *Ostreola conchaphila* in New Zealand (Bucke 1988). This parasite infects granulocytes, is spread by them throughout the tissues, and ultimately lyses the host cell (Balouet et al. 1983). There is some evidence of genetic resistance among previously exposed *O. edulis* populations that have been exposed for several generations, but the basis for this resistance is unknown (Elston and Holsinger 1988). *Bonamia ostreae* cells are engulfed by hemocytes of susceptible *O. edulis* and insusceptible *C. gigas*: they survive in *O. edulis* cells, but may degenerate in *C. gigas* cells during short-term *in vitro* studies (Chagot et al. 1992).

An unidentified haplosporidian was observed in *C. gigas* from Japan where it caused a diffuse hemocytic infiltration in connective tissue among digestive tubules; mature and immature spores replaced normal digestive tubule epithelium (Friedman et al. 1991).

Nematodes

The agnathostomatid nematode *Echinocephalus crassostreai* has been found in *C. gigas* from Hong Kong and Chinese waters (Cheng 1975b). Third stage larvae are found primarily in the lumen of the gonoducts where they elicit formation of a conspicuous tunic of connective tissue fibers, hemocytes (primarily granulocytes), myofibers, and Leydig cells. Histochemical studies of the tunic demonstrated hypersecretion of mucus by cells lining gonoducts near parasites as well as abnormal numbers of brown cells (Cheng 1975b).

Twenty-one days after first-stage larvae of the metastrongylid nematode, *Angiostrongylus cantonensis*, were injected into the soft tissues of *C. virginica*, second- and third-stage larvae were recovered from tissue digests (Cheng and Burton 1965a). Histopathological studies suggest that, in naturally occurring infections, larvae invade oysters via the digestive tract and migrate through tissues by way of blood vessels (Cheng 1966a). Larvae in connective tissue elicited a conspicuous cellular response at 10 and 14 d but not earlier; hemocytes formed a loose, incomplete layer around larvae but did not encapsulate them. Perivascular amebocytic reactions suggested the release of a soluble factor by the nematode that attracts hemocytes (Cheng 1966a). Chesapeake Bay oysters are infected by larval nematodes and respond by forming hemocyte capsules (C. A. Farley, pers. comm. 1991). Eastern oysters held under laboratory conditions may be infected by bacteria and secondarily infected by nematodes to which there is no cellular response (Meyers et al. 1985).

Cestodes

Crassostrea virginica from Hawaii may be infected with metacestodes of *Tylocephalum* sp. (Sparks 1963; Cheng 1966b). The parasite does not seem to damage the host significantly, but a thick fibrous cyst is formed around the metacestode (Sparks 1963). Cheng (1966b) showed that the host does not respond appreciably while the parasite is penetrating the gut wall, but reacts to it when it comes into contact with the underlying connective tissue by enclosing it with a complex capsule of connective tissue fibers, hemocytes, and brown cells (Rifkin et al. 1969). Hemocytes play only a small part in the formation of this capsule, which eventually leads to the death of the parasite, but they are prominent in the resorption of dead metacestodes (Cheng 1966b). Rifkin and Cheng (1968) emphasize the lack of initial participation by hemocytes, but they believe that encapsulating fibers are derived from fibroblasts that differentiate from hemocytes. In the Indian backwater oyster, *Crassostrea madrasensis*, metacestodes are enclosed within a connective tissue capsule in the gill (Stephen 1978).

Trematodes

Trematodes of the genus *Bucephalus* are well known parasites of fresh water and marine bivalves and fish. Adult trematodes live in the intestinal tract of carnivorous fish. Eggs are shed into the water where

miracidia hatch and penetrate the tissues of the first intermediate host, a bivalve mollusc. Sporocysts develop in digestive and reproductive tissues, often causing parasitic castration (Cheng 1967; Sindermann 1970). The sporocysts give rise to cercariae that escape from the mollusc and attach to the second intermediate host, a small fish. When the small fish is eaten by a larger one, the cycle is complete.

The eastern oyster is infected by *B. cuculus*, the "American" species found in waters of low salinity and different from the "European" species, *B. haimeanus*, found in *Ostrea edulis* growing in high salinity waters (Hopkins, 1954). A detailed histopathological study by Cheng and Burton (1965b) showed that the sporocysts of *Bucephalus* sp. affect primarily the digestive gland with physical compression of digestive tubules. They also noted deleterious effects on the gonad as well as penetration of sporocysts into gills, palp, and mantle. There is generally no aggregation of hemocytes around developing sporocysts and no other evidence of host reaction, leading Cheng and Burton (1965b) to conclude that *C. virginica* is a "compatible" host. Douglass (1975), on the other hand, reported finding several cases in which young sporocysts elicited an "intense cellular response," including one case in which phagocytes were found within the sporocyst. He suggested that environmental conditions may have altered the normal (i.e., no host response) host-parasite relationship. There are increased numbers of brown cells in tissues of *Bucephalus* sp.-infected oysters as has been reported in other oyster diseases (Stein and Mackin 1955; Cheng 1967).

Menzel and Hopkins (1955) noted that *Bucephalus cuculus*-infected oysters grew faster than normal oysters and that they were heavier before the terminal stages of disease. Cheng (1965) observed a reduction in neutral fat and fatty acids in Leydig cells of infected oysters with a concurrent increase in total fats in the digestive diverticula. In addition, glycogen stored in Leydig cells between the digestive diverticula is depleted during *Bucephalus* sp. infection (Cheng and Burton 1966).

Little et al. (1966) reported nearly 100% infection of oysters from the southern tip of Texas with metacercariae of *Acanthoparyphium spinulosum*. There was an average of 45 metacercariae per oyster but no histopathology was performed to determine host response to these parasites.

Crassostrea virginica infected experimentally with cercariae of *Himasthla quissestensis* have metacercariae in the heart and blood vessels enclosed in a loose wall of adhering hemocytes (Cheng et al. 1966). Metacercariae were found on gill surfaces of *C. gigas* but not in the tissues, suggesting differential susceptibility between oyster species (Cheng et al. 1966). Font (1980) demonstrated that hemocytes of *C. virginica* are attracted to and attach to dead cercariae, but not live cercaria, *in vitro*.

Crustacea

The parasitic copepod, *Mytilicola* spp., has been reported from oyster species around the world (Sparks 1985), although no records exist for *C. virginica*. *Mytilicola intestinalis* is a common parasite of mussels in Europe, but it will invade European flat oysters if blue mussels are not available (Hepper 1956; Davey and Gee 1988). This species has been recorded in *C. gigas* in France (His 1979). In North America *Mytilicola orientalis* has been found in *C. gigas* from the Pacific coast (Katkansky et al. 1967), and Sparks (1962) described metaplastic changes in the gut of infected oysters. Normal tall columnar epithelium was reduced to squamous or cuboidal epithelium and cilia were lost from cells in contact with the parasite. In some cases the parasite penetrated the gut wall and the mucosa was destroyed (Sparks 1962). This damage did not elicit an increase in hemocytes even when the gut was perforated, but parasites in abnormal sites elicit a marked hemocytic response (Sparks 1985). Fibrosis of tissue underlying the intestinal epithelium was suggestive of a host response (encapsulation) that was an attempt to wall off a relatively massive parasite (Sparks 1962).

Pseudomyicola spinosus infects *C. glomerata* in New Zealand very heavily (78 to 85% prevalence) and causes hemocytosis in the connective tissue beneath the epithelium of the gut wall where parasite appendages are inserted (Dinamani and Gordon 1974).

Response to Tumors

Excellent detailed reviews of tumors in marine bivalves have been recently published by Peters (1988),

Elston et al. (1992), and Peters et al. (1994). Earlier descriptions of oyster tumors are also available (Farley 1969; Pauley 1969; Sparks 1972). There is general agreement that neoplasms do occur in molluscs but there is little agreement about causes, cell origin, and appropriate terminology.

Oyster tumors arise naturally and their frequency varies for unknown reasons, but compared to some other marine bivalves, it is very low (see Table 1 in Peters 1988). Prevalence data are not collected in a standard way so the magnitude of the problem in oysters is unknown. Causative agents and effects on field populations are also largely unknown and the biology of oyster tumors is poorly understood.

In some cases there seems to be a hemocytic reaction associated with oyster tumors, but it is difficult to interpret these reactions. Smith (1934) found a benign nodular tumor in *C. virginica* attached to the outer surface of the pericardium, with an intense inflammatory reaction beneath the surface epithelium. Sparks and co-workers described a heavy hemocytic infiltration among the collagen fibers and in the Leydig cell area of a mesenchymal tumor (1964a), beneath the epithelial surface of another mesenchymal tumor (1969), and around a large tumor-like fecal impaction (1964b) in the rectum of *C. gigas*. A fibrous tumor in *C. gigas* described by Pauley and Sayce (1968) also showed infiltration of hemocytes, although that part of the tumor appeared to be composed of normal Leydig cells and increased collagen. The Leydig cells surrounding the tissue were not infiltrated by hemocytes but did contain unusual numbers of brown cells. In another instance, an invasive epithelial neoplasm of *C. gigas* extended from the mantle through the gonad and into the digestive gland with an intense inflammatory reaction where hemocytes were engulfing necrotic cellular debris among abnormal tissue elements (Pauley and Sayce 1972). A marked inflammatory reaction was reported by Pauley et al. (1968) in association with "a bizarre proliferation of nervous tissue and collagen"; increased numbers of brown cells were also observed. Wolf (1969) reported tumors in *S. commercialis* that showed intense inflammation with phagocytes abundant in cavities formed by proliferating tissue, perhaps in response to necrotic tissue in the center of the lesions.

Farley (1969) reported a proliferative disease of hyaline hemocytes of oysters (five *C. virginica* and one *C. gigas*) with heavy infiltration of connective tissue. Wolf (1971) found no connective tissue response or any significant inflammatory reaction to a very large tumor in a rock oyster, *S. commercialis*. Farley and Sparks (1970) summarized neoplastic conditions of oysters from the east and west coasts of the United States where the hemocytes are abnormal but where no defensive reaction is detectable. An unusual lesion in the mantle of *C. virginica* contained neoplastic cells of possible blastoid or mesenchymal origin, but there was no evidence of a defensive cellular reaction (Couch 1969).

These observations suggest that there is little or no reaction of oysters to neoplastic cells that arise among their tissues. The presence of masses of hemocytes may be due to their scavenging of necrotic cell debris caused by tumor-associated pathologies or, in some cases, by injury or infection that causes unusual oyster cell proliferation and hemocyte invasion. Much more information about basic mechanisms of neoplastic growth in oysters is needed before any meaningful generalization can be made. A variety of neoplastic conditions have been reported in *C. virginica* exposed to contaminated sediments in the laboratory and in the field (Gardner et al. 1988). If similar results can be obtained regularly, the biology of oyster tumors may become clearer in the future.

Humoral Factors

Lectins

The hemolymph of many invertebrates contains constitutive factors that interact with foreign materials (Tyler 1946; see also, Cheng, Chapter 8). These substances are not antibodies, but they may exhibit specific activity that suggests a possible defensive role (see review by Fries 1984). The ability of phagocytic cells to bind and ingest foreign particles and cells is often dependent on the presence of proteins or glycoproteins that bind specifically with sugars; these proteins are termed lectins (Ofek and Sharon 1988).

Particle-cell or cell-cell interactions occur in the following ways:

1. sugars on the surface of phagocytes interact with lectins on the surface of other cells;
2. lectins on the surface of phagocytes interact with sugars on the surface of cells or particles;
3. soluble lectins react with sugars on the surfaces of both phagocytes and particles and thus bridge the two (Ofek and Sharon 1988).

Molluscs have a variety of membrane receptors. For example, the snail, *Biomphalaria glabrata,* has four classes of membrane components: (1) glycoproteins reactive with lectins concanavalin A and *Ricinus communis,* (2) snail hemolymph-like components, (3) fibronectin-like components, and (4) determinants similar to murine Thy-1 antigen (Yoshino 1983). Activities and interactions of snail defense cells and humoral factors have been reviewed by van der Knapp and Loker (1990), and it is clear that these molluscs have complex recognition systems involving a variety of cells and humoral factors. Renwrantz (1986) and Olafsen (1988) summarized the information available for the presence and usefulness of molluscan lectins in hemolymph as well as on hemocytes and tissue cells.

The details of immunorecognition mechanisms in oysters are not well known. A hemagglutinin described from the hemolymph of *C. virginica* has some specificity for red blood cells of different species and an opsonic effect on phagocytosis of rabbit red blood cells (Tripp 1966). Calcium is required for agglutination of human cells but not for those of nonhuman species; in the absence of calcium, the substance is unstable and degrades into protein subunits that lack agglutinating activity (McDade and Tripp 1967a). Column chromatography (Sephadex G75) of *C. virginica* hemolymph yields two hemagglutinating proteins, but the specificity of these substances for red cells is not clear (Li and Flemming 1967). The native hemagglutinin is a 200,000-dalton polymer composed of 20,000-dalton protein subunits (Gaumer 1966; Acton et al. 1969). Its total and terminal amino acid content is significantly different from mammalian immunoglobulin (Acton et al. 1969). McDade and Tripp (1967a) found that oyster lectins react primarily with N-acetyl-D-glucosamine and N-acetyl-D-galactosamine and with D-glactose and D-glucosamine to a lesser extent; however, Kanaley and Ford (1990) reported that sensitivity to the galactosamine residues was considerably less than that for glucosamine. Oyster hemolymph also agglutinates a large number of bacterial species (Fisher and DiNuzzo 1991) and can selectively agglutinate all *Vibrio cholerae* of the 01 serotypes and biotypes, perhaps by binding to N-acetyl-neuraminic acid (Tamplin and Fisher 1989).

Lectins of known specificity (e.g., concanavalin A) have been used to study specific receptors on oyster hemocyte membranes. These studies reveal that oyster hemocytes can be divided into subpopulations by centrifugation and further subdivided on the basis of their ability to bind specific lectins (Yoshino et al. 1979; Cheng et al. 1980). Vasta et al. (1982) have demonstrated two membrane-bound lectins in *C. virginica* hemocytes, one of which is identical to one of the hemolymph lectins. They have also presented evidence that the reactive lectin is an integral part of the hemocyte's membrane and not merely adsorbed onto membranes from the hemolymph (Vasta et al. 1984).

In *C. gigas,* a hemagglutinin for several types of red blood cells has been described and its action attributed to binding of sialic acids (Hardy et al. 1977b). This material also enhances phagocytosis of bacteria (Hardy et al. 1977a). Further investigation of these substances indicates that they contain fractions (Gigalin E and Gigalin H) with abilities to differentially agglutinate red blood cells and certain bacteria (Olafsen et al. 1992). The pearl oyster, *Pinctada fucata martensii,* contains a hemolymph lectin with a molecular weight of 440,000 daltons composed of identical subunits of 20,000 daltons each (Suzuki and Mori 1989). This material is inhibited by D-galactose and N-acetylgalactosamine. The lectin is not associated with hemocyte membrane or cytoplasm (Suzuki and Mori 1990).

Thus the role of lectins in the detection of non-

self by oyster cells is still uncertain and their importance to the internal defense system remains to be clearly demonstrated. Kanaley and Ford (1990) documented a loss of lectin-like molecules from the serum, and a concurrent increase in lectin-binding receptors on hemocytes of *C. virginica* systemically infected with *H. nelsoni*. They concluded that the changes were related to alterations in hemocyte subpopulation ratios and loss of serum glycoproteins in diseased animals, and probably were not involved in defense. Chintala et al. (1994) reported no relationship between pre- or early post-exposure hemolymph agglutinin levels (that might reflect pre-existing or induced protective capacity) and parasite burdens or survival time after eastern oysters were challenged by *P. marinus* and *H. nelsoni*. Cheng (1987) has contributed a thoughtful analysis of possible mechanisms of parasite recognition by oyster hemocytes and how they might be affected by environmental factors resulting in reduced immunocompetence.

Lysozyme

Lysozyme is found in bodily secretions of many vertebrates and is an important nonspecific defense mechanism. It destroys Gram-positive bacterial cells by digesting the peptidoglycan layer that is part of the cell wall. Lysozyme was first found in *C. virginica* hemolymph (McDade and Tripp 1967b) and later demonstrated in high concentration in mantle mucus (McDade and Tripp 1967c). The hemolymph lysozyme was further characterized by Rodrick and Cheng (1974) and found to be similar to egg white lysozyme. Conversely, Feng (1974) found oyster lysozyme to be very different from egg white lysozyme when analyzed by ion exchange chromatography and disc electrophoresis. Feng and Canzonier (1970) showed seasonal variations in oyster hemolymph lysozyme activity and some possible effect of parasitic infections. Chu and La Peyre (1989) also found seasonal variation in oyster hemolymph lysozyme concentration and Chu et al. (1993) demonstrated experimentally that salinity and lysozyme were inversely related. No consistent relationship was evident, however, between lysozyme concentration and infection by *P. marinus* (Chu and La Peyre 1989, 1993; Chu et al. 1993). Cheng and Rodrick (1975) postulated that lysozyme in whole hemolymph of *C. virginica*, along with several other lysosomal enzymes that are released from hemocytes, may be effective against certain invading organisms. It is not clear whether lysozyme is released from phagocytosing *C. virginica* hemocytes as has been described for *M. mercenaria* hemocytes (Cheng et al. 1975).

Miscellaneous Humoral Substances

Cheng et al. (1966) described experiments in which *C. virginica* cell-free hemolymph added to *Himasthla quissestensis* cercariae caused them to encyst (*C. gigas* hemolymph had a similar effect). Heated (60° or 95°C) serum lost this property. Tissue extracts of any of seven marine bivalves (including *C. virginica* and *C. gigas*) killed cercariae. The absence of metacercariae in the tissues of *C. virginica* and *C. gigas* was attributed to the immobilization of cercariae by plasma seeping into the mantle cavity to prevent tissue penetration and normal encystment in tissue (Cheng et al. 1966). In contrast, Font (1980) found that *C. virginica* plasma (cell-free hemolymph) had no effect on five different species of cercariae from the marine snail, *Nassarius obsoletus*. Unfortunately, *H. quissestensis* was not among the species tested. There is a demonstrable correlation between hemolymph protein composition and infection with *H. nelsoni* alone (Ford 1986) or concurrently with *Bucephalus* sp. (Feng and Canzonier 1970).

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