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Effects of sublethal infection by the parasite *Haplosporidium nelsoni* (MSX) on gametogenesis, spawning, and sex ratios of oysters in Delaware Bay, USA

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ABSTRACT: *Haplosporidium nelsoni* (MSX) is an ascetosporan parasite that has caused heavy mortalities of oysters *Crassostrea virginica* (Gmelin) on the mid-Atlantic coast of the United States. Oysters in some areas have developed resistance to mortality, which involves an ability to restrict infections and tolerate parasitism for prolonged periods. Effects of such sublethal infections on gametogenesis and spawning were examined in native oysters in Delaware Bay, an estuary where the parasite is enzootic. A comparison of infection levels with gonad state in histological sections of 2700 oysters demonstrated a clear inhibition of gametogenesis, in proportion to infection intensity, during late spring when parasite levels were high. Subsequently, however, temperature-associated infection remission occurred; many oysters recovered, developed mature gonads, and spawned before new or recurrent infections proliferated in fall. Inhibition of early gametogenesis was more severe in males than in females. There was no evidence that spawning 'stress' accelerated the development of infections. There was no correlation between year-to-year fluctuation in parasite abundance and oyster setting in Delaware Bay.

INTRODUCTION

Epizootic mortalities of marine bivalves have been reported, over the past 40 yr, from many areas in the world (Mackin et al. 1950, Haskin et al. 1965, Andrews 1968, Farley 1975, Alderman 1979, Balouet et al. 1979, Grizel 1983, Elston 1986, Elston et al. 1986). In most cases, the epizootics have been associated with newly recognized pathogens attacking a highly susceptible population. Of great concern have been the large losses to commercial fisheries, in some cases approaching 90 to 95 % of the harvestable population. Far less attention has been paid to sublethal effects of disease, which may also disrupt population structure and diminish the value of the fishery. Particularly important could be effects on reproduction, an obviously critical process in maintaining or replenishing natural stocks.

The potential effects of sublethal disease pressure may increase in relative importance when populations begin to develop resistance to mortality as a result of natural selection. Improved survival, measured against introduced stocks, has been reported in a number of locations for native oysters that have experienced epizootic mortalities (Needler & Logie 1947, Andrews & Hewatt 1957, Andrews 1968, Farley 1975, Andrews & Castagna 1978, Haskin & Ford 1979, Elston et al. 1987). Although detailed information is presently lacking for most of these cases, the available evidence indicates that hosts become resistant to mortality, but not necessarily to infection (Myhre & Haskin 1970, Ford & Haskin 1982, 1987, R. Elston pers. comm., J. D. Andrews pers. comm.).

The development of resistance to mortality has been particularly well documented for oysters *Crassostrea virginica* (Gmelin), infected by the ascetosporan parasite *Haplosporidium nelsoni* (MSX) (Haskin et al. 1966, Levine et al. 1980) in Delaware Bay, USA (Haskin & Ford 1979, Ford & Haskin 1987). While *H. nelsoni* continues to infect most oysters in high salinity areas of the Bay, a significant fraction are able to restrict infections to localized, non-lethal infections and to tolerate

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parasite burdens that would kill susceptible oysters (Ford & Haskin 1987). In fact, parasitized oysters may live for several years, although their condition is generally much poorer than non-parasitized individuals (Ford 1985).

Except for investigations into parasitic castration by trematode parasites (reviewed by Lauckner 1983), there is almost no published information on the effects of parasitism on reproduction of bivalve molluscs. Farley (1968) described destruction of gonad tissue in oysters infected with *Haplosporidium nelsoni,* but also noted that infected individuals in a surviving population often had gonad development. As part of a larger study of sublethal metabolic effects of *H. nelsoni* parasitism on oysters, we have analyzed reproductive development in oysters exposed to heavy infection pressure in lower Delaware Bay. We were particularly interested in oysters parasitized during the months preceeding and during gametogenesis, when nutrients are accumulated, stored, and converted into gametes (Galtsoff 1964, Eble 1969). Our objectives were to determine (1) whether and to what extent *H. nelsoni* affects gametogenesis and spawning of infected oysters; (2) whether *H. nelsoni* influences sex ratios, or affects males and females differentially; (3) whether spawning weakens oysters so as to accelerate the development of infections; and (4) whether there is a correlation between annual variation in *H. nelsoni* infections and spawning/setting cycles of oysters in Delaware Bay.

METHODS

Oysters sampled. Oysters *Crassostrea virginica* were collected in lower Delaware Bay, USA, between 1980 and 1985 as a part of a program to define levels of *Haplosporidium nelsoni* in native stocks (Ford & Haskin 1982). Collections used in the present study were made at 4 periods during the reproductive and post-reproductive season: late May/early June, late July, late August, and late September (Table 1). These collection dates were designed to measure parasite levels at the onset and early development of infections (July through September) and at the culmination of the annual infection cycle (late May) (Andrews 1966, Ford & Haskin 1982). Collection dates were almost the same, and temperatures varied by only a few degrees, from year to year (Table 1).

In Delaware Bay, most oysters infected with *Haplosporidium nelsoni* are found in the high salinity (20 to 26 ppt) regions of the lower estuary (Fig. 1). This is the

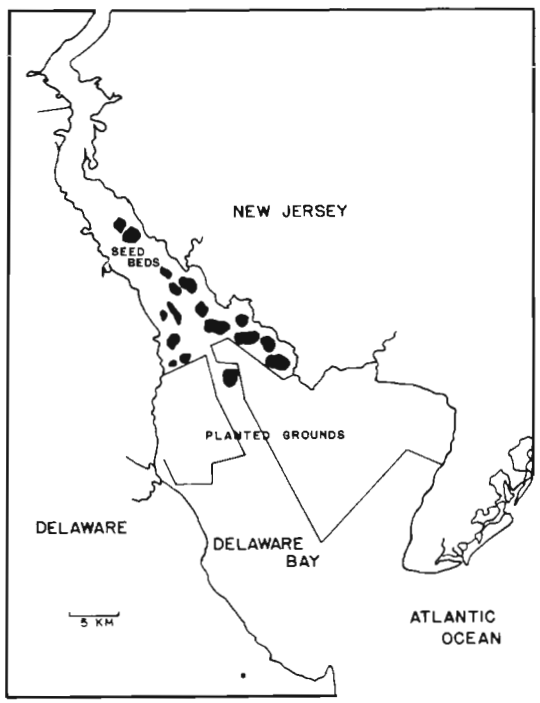


Fig. 1. Oyster seed beds and planting grounds in Delaware Bay, USA

Table 1. Collection dates and bottom water temperature (°C) for 1980–1985 lower Delaware Bay samples

	1980	1981	1982	1983	1984	1985
Late May/Early June						
Dates	23 May	–	31 May–9 Jun	6–13 Jun	6 Jun	28–29 May
Temperature	18–19		20–21	20–22	18–21	19–20
Late July						
Dates	28 Jul	30 Jul	27–29 Jul	25–27 Jul	26 Jul	–
Temperature	26–27	25–26	26–28	25–26	24	
Late August						
Dates	–	27 August	–	26–29 Aug	24 Aug	–
Temperature		22–24		25–26	24	
Late September						
Dates	24 Sep	21–25 Sep	24–29 Sep	28 Sep	24 Sep–4 Oct	–
Temperature	19	19–21	19–20	20	17–21	

location of the 'planting grounds' where seed oysters are placed each year for growth and conditioning before market. Seed oyster beds are located in relatively low salinity (9 to 18 ppt) water where *H. nelsoni* is inhibited (Andrews 1964, Haskin & Ford 1982). Thus, seed oysters have relatively few and light infections when they are transplanted to the lower Bay in May and June at the beginning of the summer infective period (Haskin & Ford 1982). Samples were therefore divided into 2 groups: (1) oysters that had survived 1 to 2 yr of exposure to *H. nelsoni* in the lower bay; and (2) newly transplanted seed oysters undergoing their first exposure to heavy infection pressure.

In addition to the recent collections, an archived series of slides was examined from a group of James River, Virginia, USA, seed oysters planted in lower Delaware Bay in spring 1964. They were sampled monthly for a period of 5 yr, from June 1964 through June 1969. The relatively closely spaced samples in this collection provide a better comparison of temporal infection and reproduction cycles than do the more widely spaced recent samples. Further, they demonstrate the effect of long-term chronic parasitism on reproduction.

Rating of gonad development. Oysters were fixed in Davidson's solution, sectioned through the gill and visceral mass just below the palps, and processed for histological examination according to Ford & Haskin (1982). Each oyster was assigned to one of 5 gonad developmental stages (Loosanoff 1942). To facilitate comparison of gametogenic and infection cycles in the James River series, a 'Gonad Index' (GI) was estimated by assigning numerical values to developmental categories and calculating a mean for each sample.

Undifferentiated: gonad follicles small and widely scattered just beneath the mantle, sex not clear (GI = 1).

Early development: follicles beginning to expand, primary and secondary gametocytes present (GI = 2).

Late development: follicles greatly expanded and coalesced, although substantial connective tissue remaining between them; gametocytes and mature gametes present (GI = 3).

Mature: follicles packed with mature gametes have replaced nearly all connective tissue between mantle and digestive gland (GI = 4 if gonad ≤ 25 % of diameter of visceral mass and 5 if >25 %).

Spawned: includes partially and completely spawned individuals; follicles and gonaducts distended, but number of gametes reduced. Gametocytes and mature gametes may remain indicating potential for further spawning. Hemocyte infiltration of gonad may occur in completely spawned oysters (GI = 0).

Rating of *Haplosporidium nelsoni* levels. Each oyster was rated according to parasite numbers and degree of proliferation (Ford & Haskin 1982). A 'Weighted Incidence' was determined for the James River series by scoring each oyster according to infection intensity and calculating a mean for each sample. Scores ranged from zero for a patently uninfected individual to 15 for one with a heavy, systemic infection.

None: no patent infection (parasites not detected, but a subpatent infection could exist) (Intensity = 0).

Gill: parasites confined to the gill, usually epithelial or localized subepithelial lesions (Intensity = 1 to 4).

Light Systemic: rare to light systemic infections (Intensity = 4 to 9).

Advanced Systemic: moderate to heavy systemic infections (Intensity = 10 to 15).

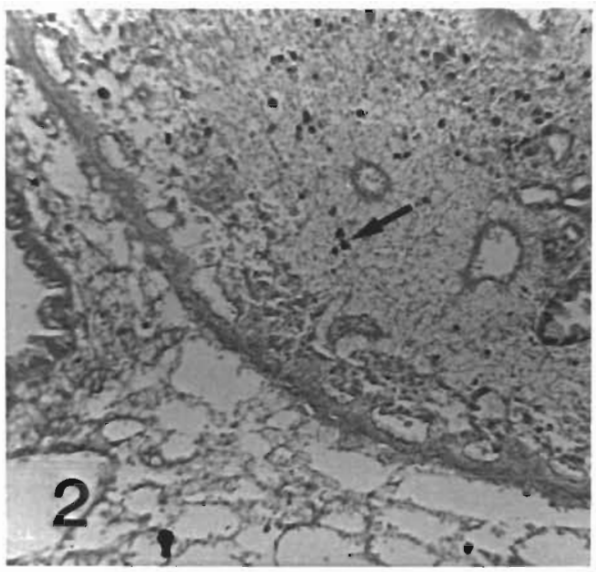
Statistics. Histological sections from 2700 oysters in 136 samples (usually 20 oysters per sample) were examined. Data from the 1980–1985 collections were pooled, according to collection period, into 2 groups: oysters that had been exposed to heavy infection pressure for 1 yr or more and newly transplanted oysters undergoing initial exposure. Differences in gonad development between the 2 groups, between males and females, and between oysters with different infection levels were subjected to '*G*' tests for independence (Sokal & Rohlf 1981).

Comparison of *Haplosporidium nelsoni* levels with oyster setting in Delaware Bay. A mean *H. nelsoni* prevalence was calculated for each year between 1961 and 1984 using data from representative stations in the lower Bay (Ford & Haskin 1982). Prevalences were arcsine-transformed to place them in a normal distribution (Sokal & Rohlf 1981). Spawning and setting success for each year was estimated by computing a mean cumulative spat shell^{-1} season^{-1}, as measured on test shells suspended and changed at 1 to 2 wk intervals between June and October at 30 to 35 stations throughout the Bay (D. Kunkle pers. comm.). Setting on test shells is significantly correlated with both larval abundance and actual set on the bay bottom (Kunkle & Ford unpubl.). Correlation analysis was then performed between spat set and arcsine-transformed *H. nelsoni* prevalences in the same and in the preceeding year.

RESULTS

Histopathology

There were 2 general pathological conditions of the gonad associated with parasitism. The first involved arrested gametogenesis that occurred in early development and was found mostly in late May samples. The most extreme cases occurred in oysters with advanced infections, in which follicles were small or non-existent (Fig. 2). Frequently, the germinal epithelium was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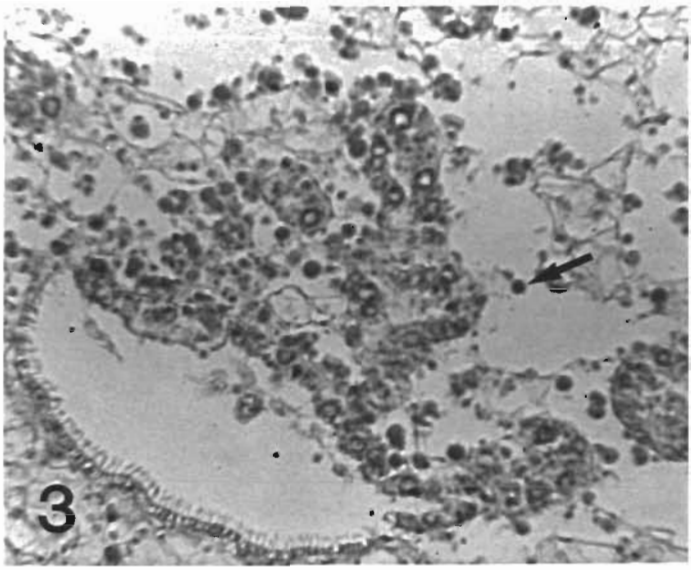
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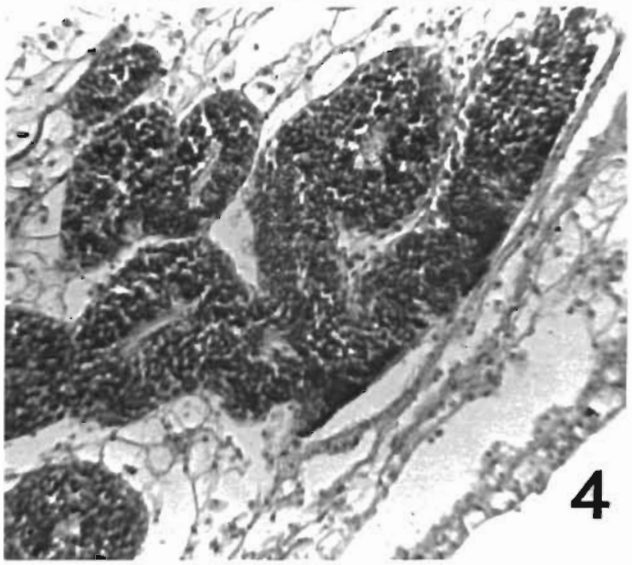
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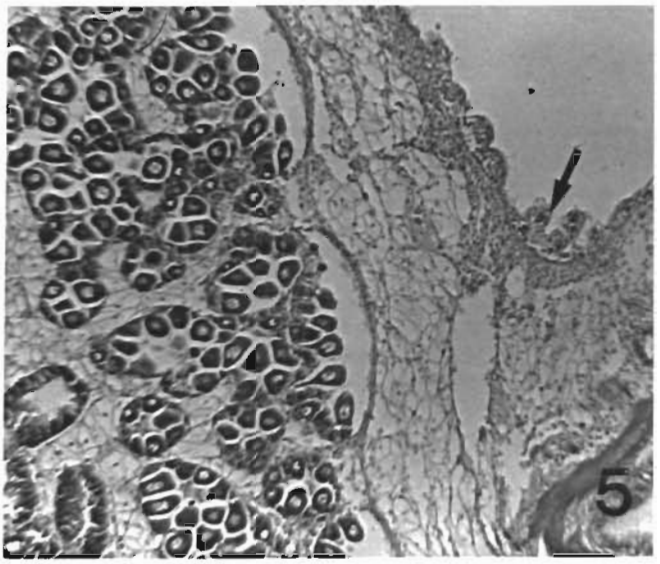
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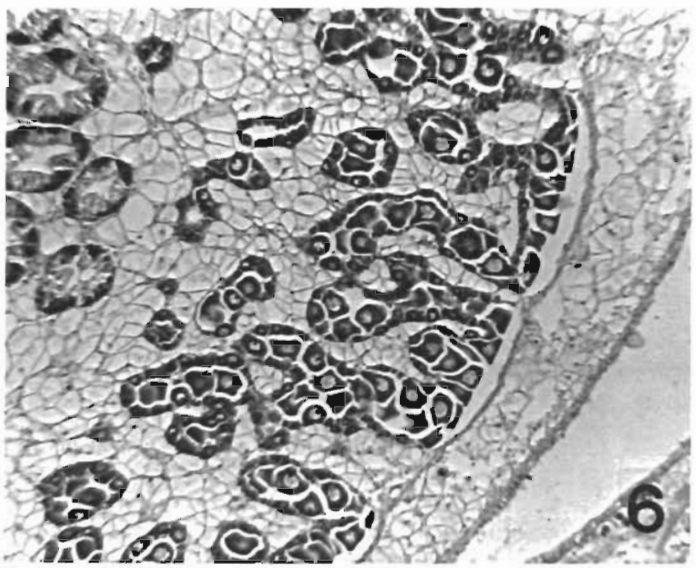
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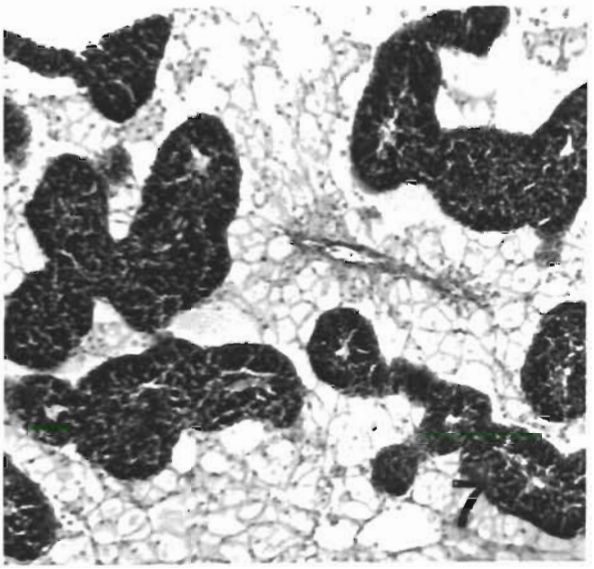
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entirely lacking, and when present, cells were sexually undifferentiated. Parasites and hemocytes were abundant in the gonad (as they were in other tissues) and there was a general destruction of tissues throughout the oyster.

Severe, but not complete, inhibition occurred in other systemically infected oysters. In these, gametocytes lined the follicles, but development was asynchronous and retarded compared to uninfected individuals, and many cells appeared to be undergoing autolysis (Fig. 3). In many systemically infected oysters, and in most individuals with gill infections, there were no obvious pathological conditions in the gonad; gametes appeared normal, but they were somewhat less abundant and well developed (Figs. 4 and 5) than those in uninfected oysters (Figs. 6 and 7).

In late July and late August, infected oysters were found with mature or spawned gonads. In most cases, gonads in individuals with light-systemic or gill infections could not be distinguished from those without infections (Fig. 8). Some oysters with advanced infections also showed relatively little pathological damage to the gonad (Fig. 9); parasites were scattered in all tissues, but gametes appeared normal and evidence of spawning was the same as in uninfected oysters. In other advanced cases, tissue disruption was extensive, yet the oysters appeared to have spawned (Fig. 10).

The second pathological condition occurred in well-developed gonads, and was generally found in July and August collections. These cases involved complete, but localized, destruction of sex products (Figs. 11 and 12). A similar case was reported by Farley (1968). Parasites were present, along with mature gametes in various stages of degradation, in the lumina of follicles and gonoducts. No parasites were evident in connective tissue between follicles or in other parts of the visceral mass, suggesting that invasion of the gonad had been through the gonoducts. Hemocytes were extremely abundant at the infection site and there was sharp gradation between unaffected and totally destroyed gonad. The area involved usually represented a relatively small portion of the entire gonad and the rest of the tissue appeared normal. This was an extremely rare type of lesion with a frequency of less than 1 % among all oysters examined.

Another rare, but interesting, condition involved an apparently normal, well-developed gonad in juxtaposition to extremely heavy parasite concentrations, accompanied by intense hemocyte aggregations and tissue destruction, in the digestive diverticula (Fig. 13). This type of lesion is characteristic of chronic infections, in which recurrent outbreaks of parasites often develop from digestive epithelia (Farley 1968), usually in late summer and fall, after the reproductive season (Ford 1985).

Infection level vs gonad stage, Delaware Bay natives 1980–1985

There were no significant differences ($p > 0.05$) between males and females in frequency distribution among gonad stages for oysters in the same infection category. Therefore, data were combined for both sexes in the same gonad stage and infection category.

Late May

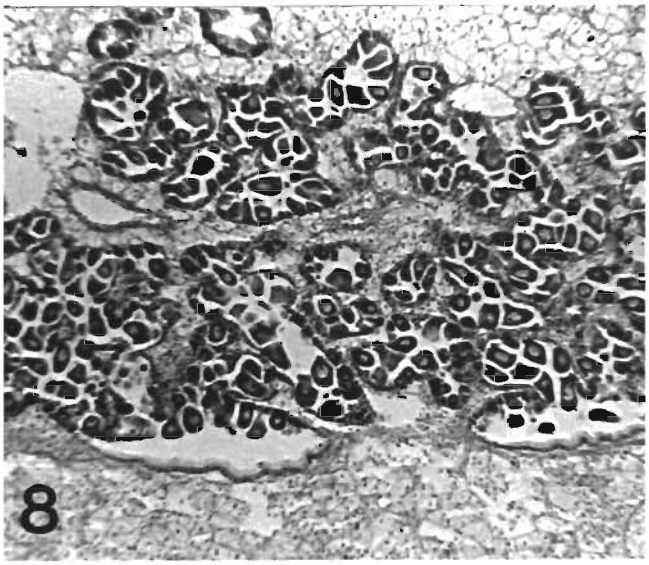
Approximately 60 % of the oysters collected in late May from the planted grounds, where they had been exposed to *Haplosporidium nelsoni* for at least 1 yr, had patent infections. Of these, 10 % had advanced infections and the remaining 50 % was approximately evenly divided between gill and light systemic infections. Parasitism was clearly associated with retarded gonad development, a condition that became more severe with increasing infection intensity (Fig. 14A). Among oysters with no patent infections, 13 % were undifferentiated, 52 % had developing gonads, and 36 % were classified as mature. In contrast, 75 % of the individuals with advanced infections had undifferentiated gonads, 22 % were developing, and only 3 % were mature. Water temperature during this collection period averaged about 20 °C (Table 1).

Although gonad development in oysters with gill infections appeared somewhat retarded compared to those without patent infections, the differences were not statistically significant. Other comparisons between infection levels, however, were significantly different (Table 2).

Late July

Collections made in late July, late August, and late September included groups that had been recently (May and June) transplanted from the seed beds to the

Figs. 2 to 7. *Crassostrea virginica*. Sections of oysters collected in late May. Fig. 2. Section through a heavily parasitized oyster showing complete gonad destruction. Arrow points to a parasite (100×). Fig. 3. Infected female oyster showing severe, but not total, inhibition of gametogenesis. Arrow points to a parasite (250×). Fig. 4. Apparently normal gonad development in a systemically infected male oyster (100×). Fig. 5. Apparently normal gonad development in a female oyster with a gill infection. Arrow marks location of parasites in gill epithelium (100×). Fig. 6. Developing gonad of an uninfected female oyster (100×). Fig. 7. Developing gonad of an uninfected male oyster (100×)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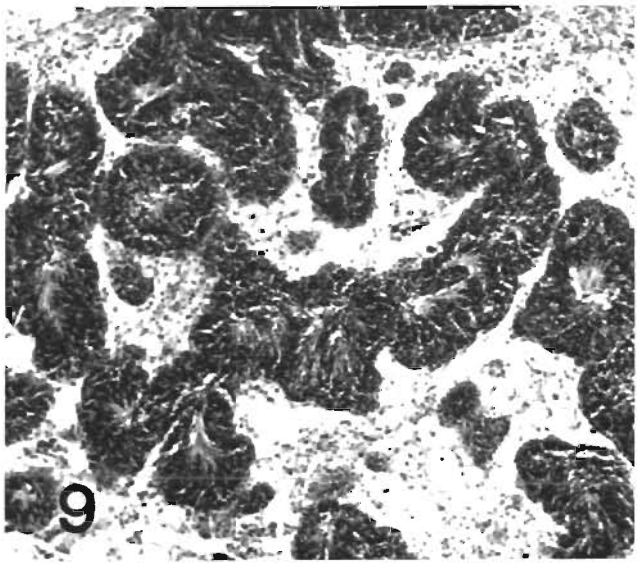
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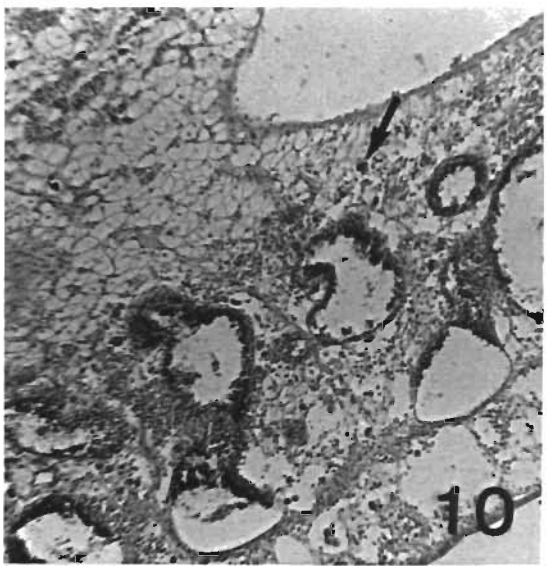
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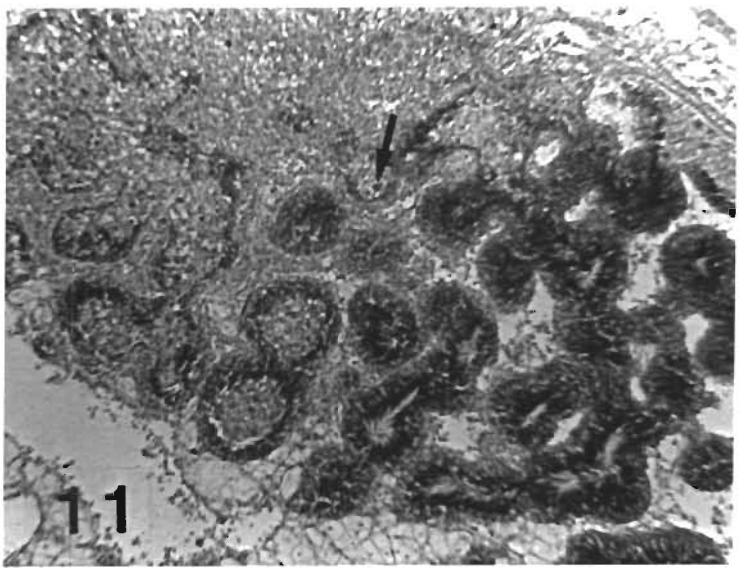
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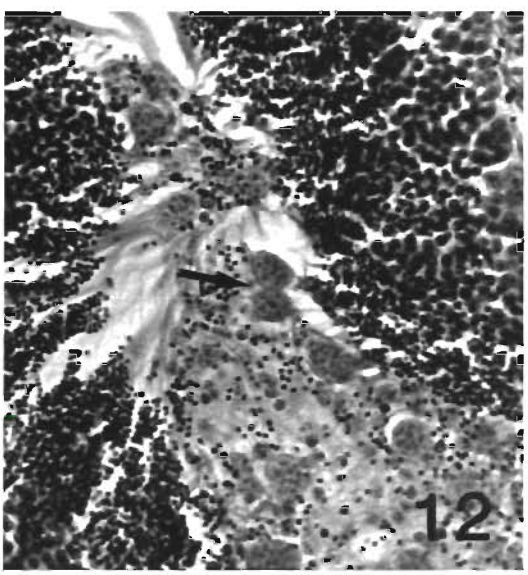
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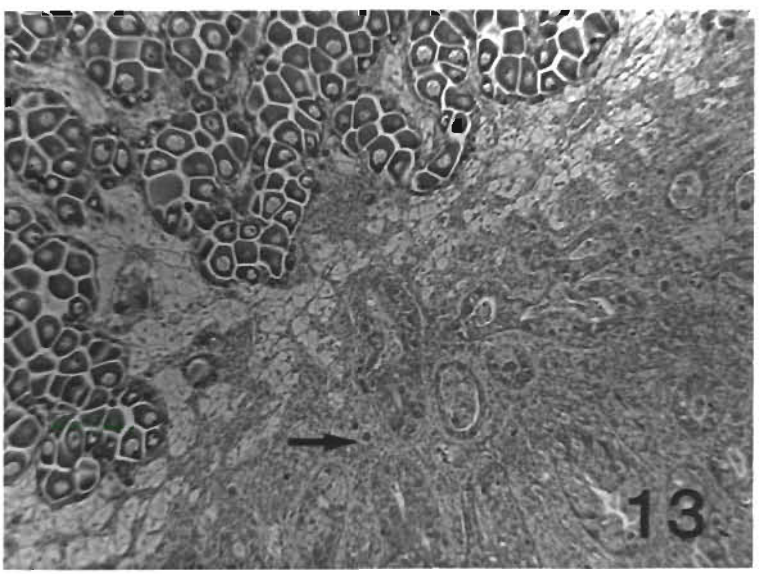
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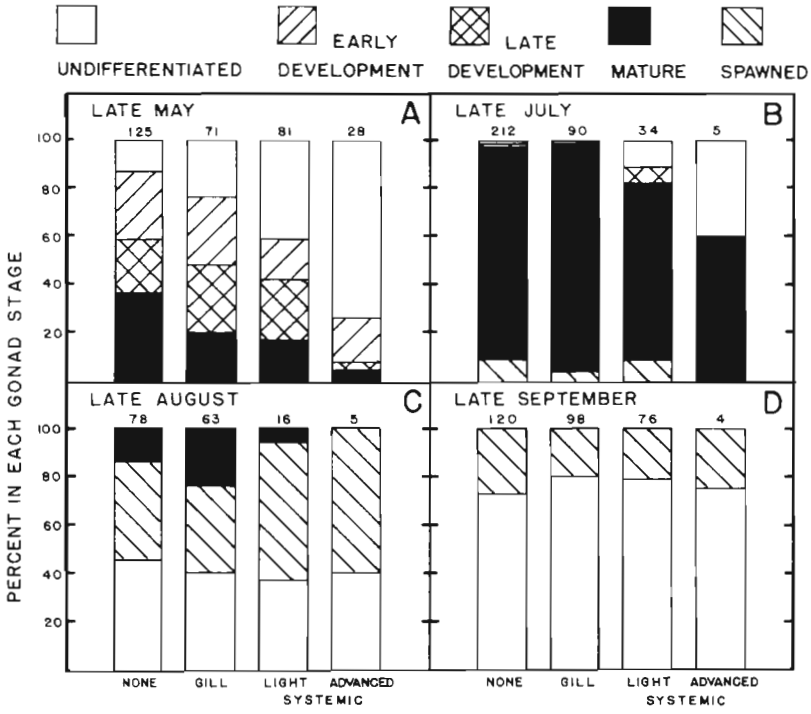

Fig. 14. *Crassostrea virginica*. Distribution of oysters according to gonad stage and *Haplosporidium nelsoni* infection level at 4 dates during the reproductive season. Oysters were collected between 1980 and 1985 from the planting grounds of Delaware Bay. Number of oysters in each infection category is shown above bars

lower bay as well as those that had been in the lower bay for a year or more. Despite different exposure histories, no significant differences ($p > 0.05$) were found in gonad development between the 2 groups at any sampling period. Therefore, data for both were pooled for further analysis.

In late July, Bay temperatures averaged 25 to 27 °C. *Haplosporidium nelsoni* levels were lower than in late May; most oysters in all infection categories had mature gonads and a few had spawned (Fig. 14B). Only 5 of 341 oysters had advanced infections, but gonad development in 3 of these was classified as mature. Twelve % of oysters with light systemic infections and 2 of 5 individuals with advanced infections remained undifferentiated. As in late May, there were no significant differences between patently uninfected oysters and those with gill infections, but both were significantly different from pooled systemically infected oysters (Table 2).

Late August

At the end of August, half the oysters were parasitized, but only 13 % had systemic infections. Water temperatures remained above 25 °C. Most oysters showed evidence of having spawned, including those with systemic infections, and many had returned to the undifferentiated state (Fig. 14C). The distribution of reproductive stages was statistically the same for all infection categories (Table 2).

Late September

Temperatures averaged somewhat less than 20 °C in late September, and parasite levels increased in the typical pattern. Sixty % of the oysters had patent *Haplosporidium nelsoni* and 27 % had systemic infections. Gonads in most of the oysters had returned to the undifferentiated state, although some of those in systemically infected oysters may never have developed (Fig. 14D). No significant differences in gonad stage were associated with infection category (Table 2).

James River imports, 1964–1969

The weighted incidence of *Haplosporidium nelsoni* in James River transplants over 5 yr of sampling

Figs. 8 to 13. *Crassostrea virginica*. Sections of oysters collected in July and August. Fig. 8. Partially spawned female oyster with a light, systemic infection (100×). Fig. 9. Mature gonad in a heavily infected male oyster (100×). Fig. 10. Heavily infected male oyster with a spawned gonad. Arrow points to a parasite (100×). Fig. 11. Localized invasion and destruction of mature male gonad by invading parasites and host hemocytes. Arrow points to a parasite (100×). Fig. 12. Parasites in lumen of male follicle. Same oyster as Fig. 11. Arrow points to a parasite (1000×). Fig. 13. Massive lesion of digestive diverticula involving parasites and hemocytes, in juxtaposition to a well-developed female gonad. Arrow points to a parasite (100×)

Table 2. *Crassostrea virginica*. Contingency table tests for independence (G statistic) between gonad stage and *Haplosporidium nelsoni* infection level

Month	Infection level comparison[a]	Number	G	p
May				
(df = 4)	N vs G	196	7.49	NS[b]
	N vs LS	206	25.98	<0.01
	N vs AS	153	47.41	<0.01
	G vs LS	152	6.67	NS
	G vs AS	99	23.89	<0.01
	LS vs AS	109	12.37	<0.01
July				
(df = 4)	N vs G	302	3.34	NS
	N vs LS & AS	236	15.97	<0.01
	G vs LS & AS	129	13.06	<0.01
August				
(df = 3)	N vs G	119	0.56	NS
	N vs LS & AS	85	2.02	NS
	G vs LS & AS	74	3.17	NS
September				
(df = 3)	N vs G	219	2.36	NS
	N vs LS & AS	200	2.86	NS
	G vs LS & AS	177	2.02	NS

[a] N: no patent infection; G: gill infections; LS: light systemic infections; AS: advanced systemic infections
[b] NS: not significant

demonstrated the characteristic annual infection cycle in Delaware Bay with peaks in winter and late May (Ford & Haskin 1982). In each year, a sharp decrease in parasite levels followed the May peak and coincided with a new infection period (Fig. 15). Each year, the Gonad Index was relatively low (1.5 to 1.7) in late May. In fact, gametogenesis progressed very little compared to native Delaware Bay oysters, between early and late May, despite rising water temperature (Fig. 15). We attribute this to differences in the timing of reproduction associated with physiological races of oysters from different geographical regions (Stauber 1950, Loosanoff & Nomejko 1951, Ford & Figueras unpubl.). Between late May and early July, coincident with reduced infection prevalence, the Gonad Index rose sharply. It was at its highest at about the same time that infection levels were lowest, and spawning was completed before parasite levels peaked again in fall.

The James River oysters were imported from an area that had experienced virtually no selective mortality from *Haplosporidium nelsoni* (Andrews 1964), therefore, they suffered very heavy losses when first placed in Delaware Bay. After initial exposure, nearly all of the oysters had patent infections (Ford & Haskin 1982). By May 1965, cumulative mortality was 65 % and approximately 97 % of the oysters had died by May 1969. To determine whether the consistent decrease in parasitism between late May and early July was the result of recovery or of death of infected oysters, mortalities during this interval were compared with infection level changes (Table 3). The decrease in prevalence of systemically infected oysters ranged between 40 and 75 percentage points. At the same time, mortality was 0 to 18 %. The greatest mortality (18 % in 1965) could account for less than half of the systemic infection decline in that year, and except in 1965, mortality

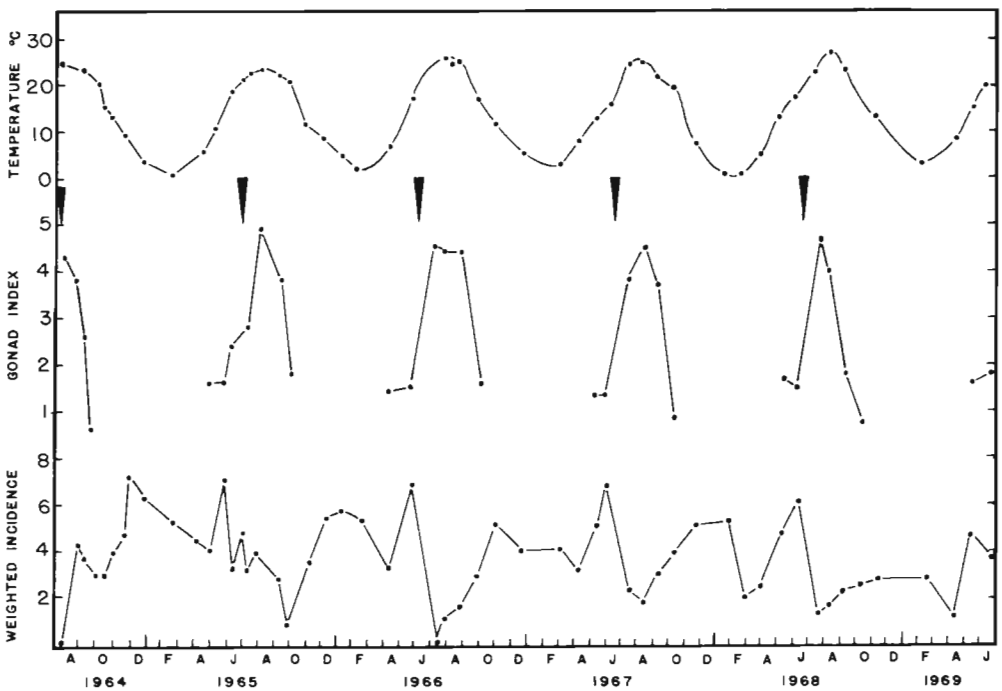

Fig. 15. *Crassostrea virginica*. Weighted incidence of *Haplosporidium nelsoni* and gonad indices in James River seed oysters transplanted to Delaware Bay in spring 1964 and sampled through mid-1969. Bottom water temperature was taken at the time of collection. n = 20 for each sample. Arrows mark major infection periods

Table 3. *Crassostrea virginica*. Decrease in prevalence of *Haplosporidium nelsoni*, and mortality between May and July in James River transplants, 1965–1969

Year	% Prevalence of *Haplosporidium nelsoni* [a]									% Mortality
	Late May			July			May to July decrease			May to July
	TOT	LS	AS	TOT	LS	AS	LS	AS	LS & AS	
1965	70	35	30	40	5	20	30	10	40	17
1966	90	40	35	5	0	0	40	35	75	18
1967	75	30	30	25	5	5	30	25	55	8
1968	70	35	35	25	10	5	15	30	45	0

[a] TOT: total; LS: light systemic infections; AS: advanced systemic infections

accounted for less than half of the loss of advanced systemic infection.

Spawning and infection

To examine the possibility that spawning weakened oysters, so that they were more easily infected and more susceptible to developing disease, we compared infection levels in pre- and post-spawning oysters in late July and late August samples from the 1980–1985 collections. The comparison included oysters undergoing first exposure and those that had been exposed for a year or more. If the above notion were true, we would expect to find more infections among spawned than among pre-spawned oysters. Instead, the distribution of infected individuals was the same, regardless of whether or not they had spawned or how long they had been exposed (Table 4).

Parasitism and sex ratios

Sex ratios were statistically the same, at each sampling, in lower bay and lower seed bed oysters collected between 1980 and 1985 and in James River transplants sampled between 1964 and 1969. Data from all groups were therefore pooled. Also, there were no statistically significant differences in sex ratios among the 3 categories of infected oysters, so these data were pooled for comparison with patently uninfected oysters.

In late May, individuals with undifferentiated gonads were twice as frequent among infected oysters as among uninfected ones (38 vs 19 %) (Table 5). Among the remaining, sexually differentiated oysters, the ratio of females to males was nearly twice as great in the infected group (2.9:1) as in the uninfected group (1.6:1), a difference that was statistically significant (Table 5). By late July, when most oysters had mature gonads, as well as later in the season, female-to-male ratios were similar in both infected and uninfected groups, and averaged approximately 1.5:1.

Females predominated at all times and the sex ratio was significantly different from 1:1 in most cases (Table 5).

Haplosporidium nelsoni fluctuations and oyster setting variability in Delaware Bay

Year-to-year fluctuation in spat set on test shells was not correlated with *Haplosporidium nelsoni* levels in either the same ($n = 28$, $r^2 = 0.10$) or the previous year ($n = 27$, $r^2 = 0.001$).

Table 4. *Crassostrea virginica*. Comparison of the prevalence of *Haplosporidium nelsoni* infections in pre- and post-spawned oysters. Percentages are based on the total in each infection category. There were no statistically significant differences between infected and uninfected oysters in any category

	First exposure		Chronic exposure	
	Pre-spawned	Post-spawned	Pre-spawned	Post-spawned
Late July				
Uninfected	182 (90 %)	20 (10 %)	124 (89 %)	15 (11 %)
Infected	182 (95 %)	7 (5 %)	43 (90 %)	5 (10 %)
Late August				
Uninfected	11 (14 %)	67 (86 %)	10 (15 %)	55 (85 %)
Infected	16 (19 %)	68 (81 %)	12 (16 %)	62 (84 %)

Table 5. *Crassostrea virginica*. Sex ratios of oysters infected and uninfected with *Haplosporidium nelsoni*. Sex ratios of infected and uninfected oysters were compared using contingency table analysis for independence (G statistic). Ratios within each category were tested for Goodness of Fit to a 1:1 ratio: $^{*}p<0.05$; $^{**}p<0.01$; NS: not significant

	Number of oysters[a]			Ratio	Infected vs uninfected
	?	F	M	F:M	
Late May					$G = 8.78$, $p<0.01$
Uninfected	49	127	82	1.55 *	
Infected	130	156	54	2.89 **	
Late July					
Uninfected	7	187	125	1.50 **	$G = 0.68$, NS
Infected	14	49	40	1.23 NS	
August/September					
Uninfected	228	76	54	1.41 NS	$G = 0.55$, NS
Infected	232	80	47	1.70 *	

[a] ?: undifferentiated; F: female; M: male

DISCUSSION

There was a clear and consistent retardation of gonad development associated with *Haplosporidium nelsoni* parasitism at all stages of gametogenesis. The most severe inhibition, however, occurred in the early stages of development and coincided with a late spring prevalence peak at the end of the annual infection cycle. Later gonad development and spawning occurred when parasite burdens were at a relatively low point in the cycle; fewer oysters were infected and there was less inhibition associated with parasitism.

Interpretation of these results depends on a knowledge of the infection cycle for *Haplosporidium nelsoni* (Ford & Haskin 1982). The infection period in Delaware Bay extends from June through October; infections are detectable by late July and intensity increases to a winter peak. Mortalities occur after infections become systemic, but are reduced during the coldest months of the winter. A late May prevalence peak, frequently showing the highest parasite levels of the year, culminates the infection cycle, and is followed by a rapid disappearance of parasites from the hosts. Oysters found to be infected in late spring have thus been parasitized since the previous summer or early fall, although the level of parasitism during the interim may have been lighter or heavier than that found in the May sample.

Haplosporidium nelsoni infections are associated with a number of biochemical and physiological changes that become progressively more severe with increased infection intensity. They include depressed filtration rates (Newell 1985), enzyme levels (Eble 1966, Mengebier & Wood 1969), hemolymph protein concentrations (Ford 1986), and stored glycogen levels (Barber et al. 1988). All of these conditions indicate major disruptions of physiological/metabolic processes that would diminish the substrates needed for all energy-demanding functions, including gametogenesis. These data along with the frequently poor condition of systemically infected oysters suggests that lack of gonad development is part of a general host debilitation rather than a specific effect on the gonad.

The lack of mortality sufficient to explain the loss of systematic infections between late May and early July is true of Delaware Bay native oysters as well as the James River imports (Ford & Haskin 1982). It indicates that many of the oysters with well-developed and spawned gonads late in the summer had recovered from the disease, which presented a clear stress in May. Farley (1968) also reported histological evidence of infection remission in Chesapeake Bay (USA) oysters, although he did not report when it occurred. The recovery is thought to result from a temperature-mediated response or condition in oysters that destroys parasites at or above 20 °C (Myhre & Haskin 1970, Ford & Haskin 1982, Ford 1985). Recovery appeared to be reasonably complete as far as gametogenesis was concerned, since by late July approximately 96 % of the oysters had mature or spawned gonads. Further, there were no significant differences, by late July, between oysters newly transplanted from the seed beds to the lower bay and those that had been under heavy disease pressure for a year or more. Another indication that recovery was an important factor allowing gametogenesis and spawning is that the gonad index of the James River oysters followed essentially the same pattern and reached the same levels during the reproductive season in 4 consecutive years of heavy infection pressure, in which at least 75 % of the oysters (surviving to that time) had patent infections each year.

The establishment and development of new infections during the summer appeared to have little effect on reproduction of newly exposed oysters. During the reproductive period, new infections are for the most part confined to the gill epithelium, where they may cause severe local damage, but where they rarely cause general symptoms of disease (Ford & Haskin 1982, Ford 1986).

Under the proper conditions of temperature and food or stored energy reserves, gametogenesis can be very rapid in *Crassostrea virginica*. Loosanoff & Davis (1952) reported that the time required for undifferentiated Long Island Sound (USA) oysters to produce ripe gametes was 8 d at 20°C and 5.4 d at 25°C. Price & Maurer (1971) calculated temperature requirements for gamete maturation of Delaware Bay oysters using degree days. Using their figure of 130 degree days (above 12°C) for oysters held in the field with access to natural food and an approximate mean temperature of 23°C between late May and mid-July, it would be possible for healthy oysters to go from an undeveloped to a mature state in about 2 wk. This capacity, combined with the relatively rapid decrease of parasite prevalence in June, helps to explain the advanced reproductive condition, in late July, of oysters that only 6 to 8 wk before had evidenced severe inhibition of gametogenesis.

Mackin (1962) investigated the relationship between infection by another oyster pathogen, *Perkinsus (Dermocystidium, Labyrinthomyxa) marinus* (Mackin et al. 1950, Levine 1978) and spawning of oysters in Louisiana, USA. It had been noted that heavy mortalities occurred after spawning and Mackin wished to examine the possibility that spawning so weakened oysters that they became more susceptible to infection and disease. After examining histological sections of oysters before and after spawning, he concluded that there was no relationship between disease and spawned condition or even advanced gonad development. He did find, however, that oysters which were heavily infected during the period of early gametogenesis were 'castrated'. Mackin's results are very similar to our own, including the lack of evidence supporting the hypothesis that spawning enhances the probability of infection and disease development.

Two other studies also reported that parasitism delayed, but did not prevent, breeding of bivalves. Williams (1969) found that infestation of mussels *Mytilus edulis* by the copepod *Mytilicola intestinalis* delayed reproduction, while Ford (1985) reported delayed gametogenesis and spawning associated with chronic infections of *Haplosporidium nelsoni* in oysters. In all cases, the parasites were probably interfering with the apportionment of energy to developing gametes, either through competition or by disrupting normal physiological/metabolic processes, thus lengthening the time required to amass the quantity of nutrients needed for gonad maturation. Another possibility, suggested by Williams (1969), is that parasitism interfered with hormonal control of reproduction by disrupting neurosecretory cycles that are closely associated with reproduction in many bivalves (Sastry 1979).

The disproportionately high number of females among infected oysters compared to uninfected individuals in the late May samples has several possible explanations: (1) females are more prone to infection; (2) infected females are less likely to die; or (3) early gametogenesis in females is less inhibited by infection than it is in males. The large number of undifferentiated oysters at this date and the fact that ratios were no longer related to infection later in the summer, when most individuals were differentiated, favors the last explanation and suggests that male gonads developed primarily after infection pressure was reduced.

Crassostrea virginica is capable of changing sex between breeding seasons (reviewed by Galtsoff 1964). The variables found to influence sex have been thoroughly reviewed and discussed by Kennedy (1983). For the interpretation of our results, the most pertinent information comes from studies in which oysters were experimentally injured. Anemiya (1935) and later Egami (1953) (both cited in Kennedy 1983) removed portions of gill and found that injured groups had lower female-to-male ratios than controls. They proposed that interference with feeding increased the tendency toward maleness. Many other reports also indicate that poor food conditions favor the development of males (Kennedy 1983). In another set of experiments, Bahr & Hillman (1967) and Davis & Hillman (1971) filed the growing shell edges repeatedly during the fall and spring, forcing oysters to continuously repair shell. When sexes were determined in early summer, there were significantly more males in the shell-damaged groups. These investigators hypothesized that diversion of energy to shell repair had limited the amount available for gametogenesis and resulted in the production of males.

Implied in the above reasoning is that energy requirements for females are greater than those for males, thus conditions that interfere with provision of resources to the gonad should push the sex ratio in favor of males. As noted by Kennedy (1983), however, equal amounts of gametes may require equal amounts of energy, regardless of sex. Our data contradict the hypothesis that stress favors the production of males, since *Haplosporidium nelsoni* disease is clearly a severe stress, yet at all times and in all infection categories, females outnumbered males. The evidence that disease retarded male development to a greater

degree than female development is also at variance with this hypothesis. The sex ratios found in Delaware Bay were in the same range as those reported for upper Chesapeake Bay (where *H. nelsoni* is usually absent) by Kennedy (1983) and indicate that the disease is not associated with a disruption in the balance of sexes that might adversely affect recruitment.

Two important parameters not measured by this study were gamete quality and quantity. Larval viability is diminished when parent stocks are stressed by lack of food (Helm et al. 1973, Gabbott & Bayne 1975, Bayne et al. 1975, 1978). Also, oysters exposed to low levels of crude oil for several weeks during gametogenesis could not be induced to spawn, even though histological examination showed moderate gonad development, compared to controls, which spawned readily (Manger 1976). If *Haplosporidium nelsoni* parasitism has disturbed gametogenesis and spawning more than the histological evidence indicates, then some of the year-to-year variability in spawning and setting of oysters in areas enzootic for *H. nelsoni* might be caused by fluctuations in parasite abundance (Ford & Haskin 1982). The lack of correlation between long-term parasite fluctuations and setting cycles in Delaware Bay may indicate that gametes produced by 'recovered' or newly infected oysters are perfectly viable. A more likely explanation, however, is that the majority of broodstock in the Bay is located on the upper Bay seed beds where low salinity protects oysters from significant disease pressure. The effect of sublethal parasitism on the quantity and quality of gametes produced by lower Bay oysters may still be important and is currently being examined at this laboratory.

The initial massive mortalities in lower Chesapeake and Delaware Bays in the late 1950s and early 1960s undoubtedly reduced set potential (Andrews 1979) in both regions and the continued scarcity of oysters in the high salinity areas probably still diminishes recruitment. Nevertheless, both regions have experienced very good sets in some recent years, indicating the potential for recovery from epizootic mortality in a population of organisms such as oysters, which produce huge numbers of gametes.

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