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Enhancement of protozoan pathogen *Perkinsus marinus* infections in American oysters *Crassostrea virginica* exposed to the chemical carcinogen n-nitrosodiethylamine (DENA)*

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ABSTRACT: American oysters *Crassostrea virginica* exposed to high concentrations (600 mg l^{-1}) of n-nitrosodiethylamine (DENA) during winter (February to May) showed significant enhancement of an epizootic apicomplexan parasite, *Perkinsus marinus*. The parasite reproduced and caused atypical lesions in exposed oysters in water temperatures at its lower range (20°C). The reasons for this enhancement are not clear but may reflect damage to the oysters' nonspecific, cellular defense mechanisms by the DENA without concomitant negative effects on the parasite.

INTRODUCTION

Perkinsus marinus, formerly *Dermocystidium marinum*, is epizootic in American oysters *Crassostrea virginica* and a significant cause of mass mortality in Gulf coast and mid-Atlantic estuaries of the USA (Mackin 1951, Ray 1954a,b, Dunnington 1956, Mackin & Hopkins 1962, Lauckner 1983, Sparks 1985). The taxonomy, morphology and pathology of *P. marinus* have been extensively reviewed (Perkins 1976, Levine 1978, Lauckner 1983, Sparks 1985).

Previous investigators indicated that *Perkinsus marinus* infections in oysters are enhanced by temperatures above 20°C (Mackin 1962, Quick & Mackin 1971, Andrews 1976), salinities above 15‰ (Mackin 1956, Scott et al. 1985), close proximity or crowding (Mackin 1962, Andrews 1965, 1979), and spawning state of the oysters (Ray 1954a, Andrews & Hewatt 1957, Mackin 1962, Sinderman 1970). Scavengers (Hoese 1964) and ectoparasitic snails (White et al. 1987) which feed on oysters infected with the parasite may act as vectors in transmitting the disease to other oysters.

This paper reports the first experimentally induced enhancement of *Perkinsus marinus* in *Crassostrea virginica*, with the chemical carcinogen DENA. Histopathological consequence of the infections and possible reasons for the enhancement phenomenon are also discussed.

MATERIALS AND METHODS

American oysters *Crassostrea virginica* were collected from East Bay, Santa Rosa County, Florida, USA, in November 1986 and held in flow-through seawater tanks until experiments were begun in February 1987. A baseline sample (50 oysters) was taken at the collection site and 17 individuals were sampled for histological examination 3 d prior to tests. Exposed and control oysters were held in static 12 l aquaria during each test. No attempt was made to feed exposed or control oysters during tests. Temperature and salinity were maintained at 20°C and 20‰, respectively. Experimental oysters were exposed to 600 mg l^{-1} of n-nitrosodiethylamine (Sigma N0258) for up to 28 d.

Four separate tests were conducted over a 14 wk period (Tables 1 and 2). Test 1 was designed to determine acute effects of high concentrations of the carcinogen on exposed oysters. Samples of 3 exposed and 2 control oysters were taken for histological examination at Days 1, 2, 4, 7, 11 and 14. Additionally, 4 exposed and 3 controls were taken when tests were

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Table 1. *Crassostrea virginica*. Results from tests exposing oysters to DENA (600 mg l^{-1}). In Test 1, 22 oysters were taken for histological examination during test (+); 23 survivors were transferred to clean seawater and held for 3 wk after test termination before histological examination (*)

Test	Date of test	No. exposed	Days exposed	No. left at test end	Percent sampled at test end with *Perkinsus marinus* enhancement
1	Feb 87	45+	27	23*	100
2	Mar 87	20	17	5	100
3	Apr 87	20	28	6	67
4	May 87	20	26	7	86

Table 2. *Crassostrea virginica*. Control oysters from DENA exposure tests. In Test 1, 15 samples were taken during 27 d (+)

Test	Date of test	No. of controls	Days held	No. left at test end	Percent sampled at test end with *Perkinsus marinus* enhancement
1	Feb 87	25+	27	10	0
2	Mar 87	20	17	20	0
3	Apr 87	20	28	20	0
4	May 87	20	26	20	0

terminated (27 d). Surviving exposed oysters (23 total) were transferred to clean seawater and histologically examined 3 wk later.

Tests 2, 3 and 4 were conducted between March and May 1987 to verify the *Perkinsus marinus* enhancement observed in Test 1. In each test, 20 oysters were exposed to the carcinogen and sampled for histological examination after mortalities were greater than 50%. Test 2 was terminated after 17 d with 5 surviving test specimens and Tests 3 and 4 were terminated after 28 and 26 d with 6 and 7 survivors, respectively (see Tables 1 and 2). Control oysters were also sampled at the same time exposed oysters were taken.

Sampled oysters were grossly examined and 1 cm thick transverse cuts were taken across the digestive gland, fixed in Davidsons' fluid (Shaw & Battle 1957) and embedded in Paraplast®. Tissue was sectioned at 7 µm and stained with Harris' hematoxylin and eosin (Luna 1968).

Fig. 1 illustrates the general experimental design used to evaluate *Perkinsus marinus* enhancement in oysters exposed to DENA. Microscopic quantification of *P. marinus* infections in test oysters was done to obtain relative prevalence/intensity values. The number of parasite stages per field (at 400×) infecting the outer gut loop epithelia (Fig. 2) was quantified by observing 6 microscopic fields on each of 10 slides prepared from control oysters and 10 each from exposed and baseline oysters. Values for respective groups of 10 slides were averaged to obtain the infection values. Light infections averaged 3 observable parasites per field, moderate infections averaged 16, and heavy infections averaged more than 170.

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RESULTS

Histological examination of baseline oysters sampled 3 d before tests showed all individuals with healthy digestive gland epithelia and vesicular connective tissue (VCT). Most individuals had light to moderate infections of *Perkinsus marinus* in gut epithelia concomitant with some hemocytic response to the pathogen but no severe histologic damage was observed (Fig. 3).

Results of the 4 tests are shown in Tables 1 and 2. Experimental oysters in Tests 2, 3 and 4 began to have significant mortalities 17 d postexposure to DENA; only 18 individuals (30%) survived when the tests were terminated (Table 1). In contrast, no control oysters died (Table 2). Histological examination of moribund or 'gaper' oysters indicated the probable cause of death to be heavy *Perkinsus marinus* infections. Examination of exposed and control oysters, after tests were terminated, showed very heavy infections of *P. marinus* in

Figure 1. Experimental design used to study the effects of DENA exposures on oysters.

Test animals divided into 3 groups

Control	Baseline	Exposed
Oysters not exposed to DENA, but maintained in identical holding tanks as exposed groups	Histologically examined 3 days before tests to determine prevalence and intensity of pathogen. (100% with light to moderate infections concomitant with some hemocytic response)	Oysters exposed to 500 mg l^{-1} of DENA for 17 to 28 days (4 separate tests)

Results

Control	Exposed
a. No mortality	a. Increased intensity, pathosis and mortality from heavy infections of *P. marinus* in gut epithelia
b. Histology shows light *P. marinus* infections in gut epithelia with light to moderate hemocytic response to the pathogen	b. Atypical light to moderate hemocytic response from oysters to such heavy infections
	c. Atypical invasion and destruction of oyster ciliated digestive tubules by the parasite

Results of Depuration

Histology of surviving DENA-exposed oysters transferred to clean seawater for 3 weeks shows most oysters with light infections of *P. marinus* in gut epithelia concomitant with light to moderate hemocytic response

Fig. 1 Experimental design of methods used to evaluate results of DENA-exposed tests

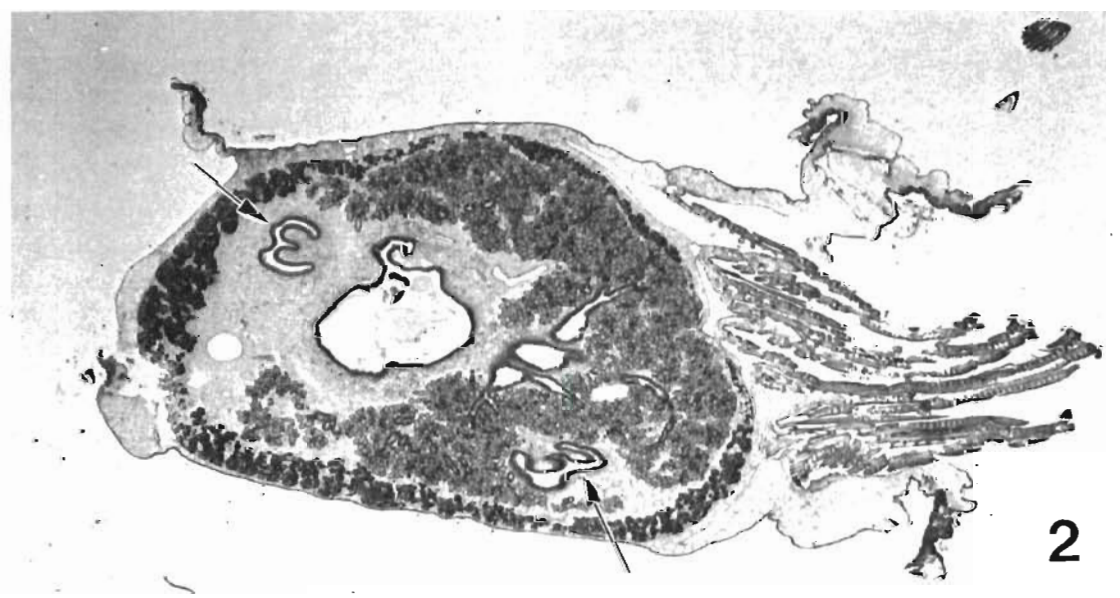
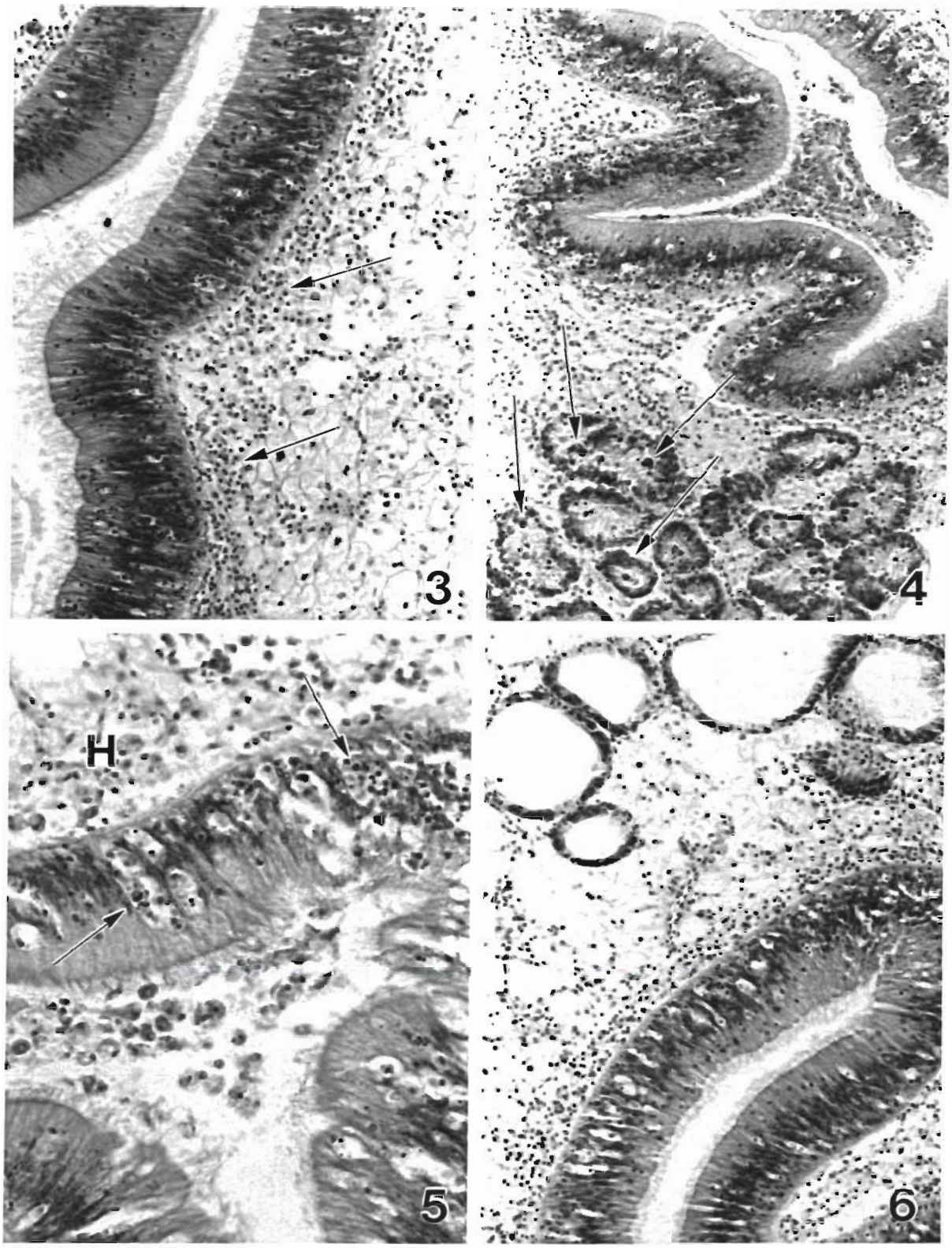


Fig. 2. *Crassostrea virginica*. Cross section of an oyster taken through the visceral mass just below the palps showing the general area examined. Note the 2 outer intestinal loops (arrows) used to determine microscopic quantification of *Perkinsus marinus* prevalence/intensity values (arrows). (×7)

gut epithelia from 15 of 18 (83 %) experimental oysters (Figs. 4 and 5) and only light infections in gut epithelia of controls (Fig. 6).

Test 1 had no mortalities in experimental or control oysters. Of 18 exposed and 14 control oysters histologically examined 14 d postexposure, 83 % of exposed and



100 % of control oysters showed light infections with the parasite. After 27 d, all exposed oysters sampled (4) showed heavy infections of *Perkinsus marinus* in gut epithelia identical to Tests 2, 3 and 4 (Figs. 4 and 5) while all controls had only light infections of the parasite (Fig. 6).

All exposed oysters with heavy *Perkinsus marinus* enhancement showed similar histopathological effects. A massive increase of uninucleate meronts and multinucleate schizonts in gut epithelia concomitant with sloughing of epithelia and pathogen into the digestive lumen was observed (Fig. 4). No significant hemocytic infiltration into gut epithelia or foci of these cells beneath the epithelial basement membrane was seen (Figs. 4 and 5). The lack of a significant hemocytic response to such heavy *P. marinus* infection in gut epithelia is atypical. Fig. 7 illustrates a typical hemocytic response in feral oysters with heavy infections of the pathogen in gut epithelia and should be compared with the hemocytic response in DENA-exposed oysters (Figs. 4 and 5). In addition, a massive invasion and replacement of nonciliated digestive tubule epithelia by meronts with no significant hemocytic response to the parasite were observed (Figs. 8 and 9). Control oysters with *P. marinus* infections in gut epithelia showed typical light to moderate hemocytic responses to the pathogen with localized foci of such cells beneath gut epithelial basement membranes (Fig. 6).

All test oysters showed diminished digestive tubule epithelia, which is normal in bivalves held in static aquaria without food for several weeks. Surviving oysters from Test 1, histologically examined 3 wk after transfer from DENA exposure aquaria to clean water, showed only light infections of *Perkinsus marinus* in gut epithelia and healthy nonciliated digestive tubules free of the parasite (Fig. 10).

A summary of data (Tables 1 and 2) from all tests shows that of 105 exposed oysters, 42 (40 %) died within an average of 24 d after tests began. Of 22 exposed oysters remaining alive and histologically examined at the end of tests, 19 (86 %) showed heavy infections of the pathogen. In contrast, no control oysters died or showed histological evidence of heavy *Perkinsus marinus* infections during or after tests (Table 2).

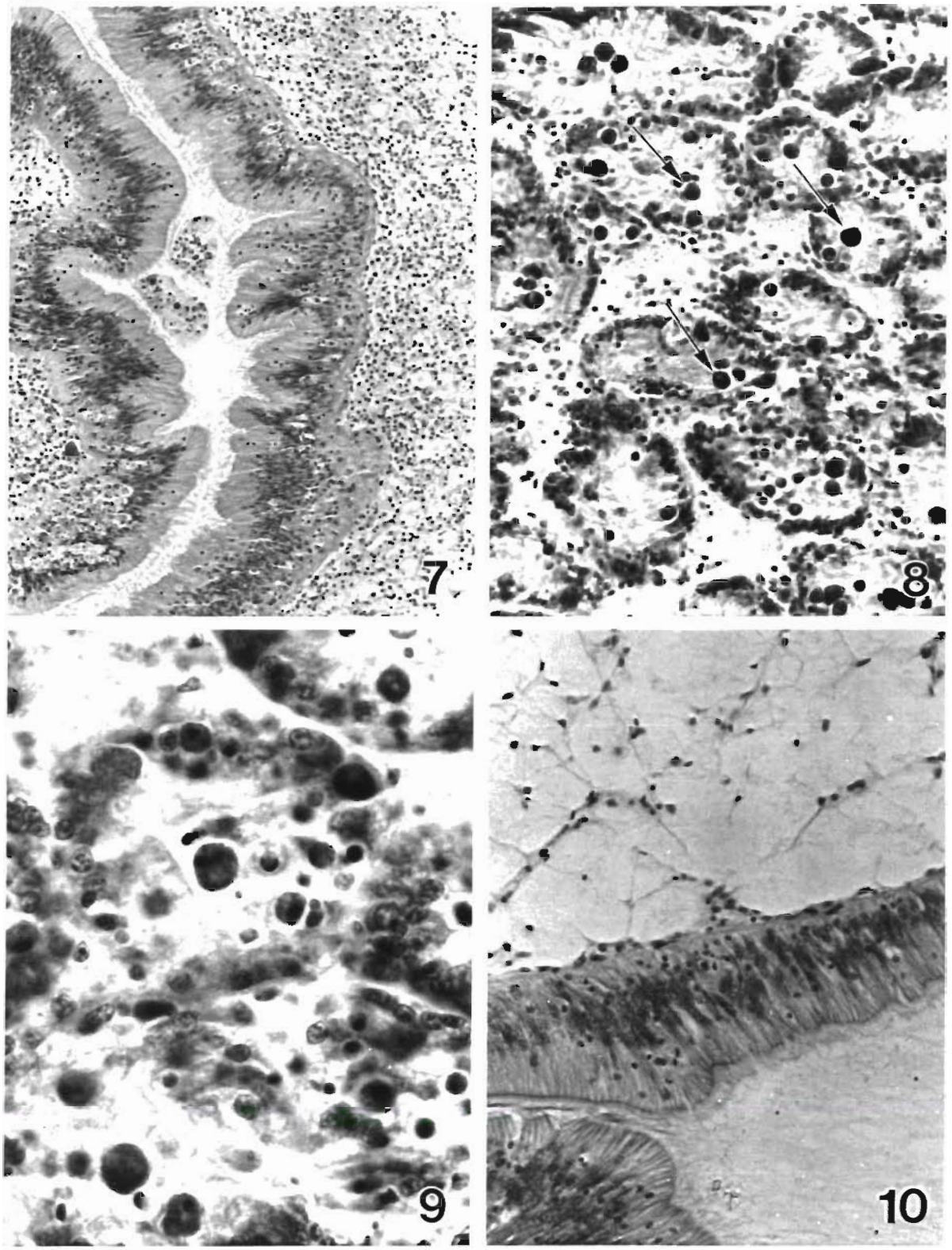
DISCUSSION

These tests were designed to determine acute toxicity of DENA to oysters for later carcinogen assay tests, and the *Perkinsus marinus* enhancement discovery was an ancillary observation. High concentrations of the carcinogen (600 $mg\,l^{-1}$) did not appear to induce the same acute histopathologic effects as reported for other bivalve molluscs exposed to nitrosamines. Examination of DENA-exposed oysters showed no congestion of hemolymph sinuses or branchial blood vessels by blood cells or necrotic lesions in VCT reported in *Mytilus edulis* exposed to 100 $mg\,l^{-1}$ of dimethylnitrosamine (DMN) for 14 d (Rasmussen 1982). Also, no focal hyperplasia of gut epithelia accompanied by nodular proliferations of basophilic cells described in *Unio pictorum* exposed to DMN or DENA (400 $\mu g\,l^{-1}$) for 4 wk were observed (Khudoley & Syrenko 1978). The reason oysters exposed to DENA in our tests did not show similar acute effects may reflect differences in species sensitivity or response potential. It may be that nitrosamines are not as toxic to post-spawn oysters in cool water temperatures as to other spawning bivalve species. Oysters have been shown to be more susceptible to xenobiotics during spawning when water temperatures are above 25 °C (Scott & Vernberg 1979, Scott et al. 1985).

Significant mortalities occurred in Tests 2, 3 and 4 but there were none in Test 1. The reasons are not clear and may reflect a variation in tests.

Although some of the lesions of *Perkinsus marinus* infections or classic 'Dermo disease' described by earlier investigators were observed in exposed oysters, there were significant differences. Classic 'Dermo disease' begins as a chronic disorder and may take months to produce mortality in the host (Menzel & Hopkins 1955). The enhancement phenomenon was acute and caused accelerated mortality in test oysters in less than 3 wk. Histologic examination of oysters with the enhancement phenomenon showed some hemocytic response to *P. marinus* in gut epithelia and vesicular connective tissues of body and mantle, but the response was atypically light for such heavy infections of this parasite (compare Fig. 7 with Figs. 4 and 5). Heavy *P. marinus* infections in oysters are thought to

Figs. 3 to 6. *Crassostrea virginica*. Fig. 3. Baseline oyster 3 d before tests showing moderate *Perkinsus marinus* infection in gut epithelia with light hemocytic response beneath gut epithelia basement membrane (arrows). (×860). Fig. 4. Typical heavy infection of *P. marinus* in gut epithelia of oysters exposed to DENA (600 $mg\,l^{-1}$) for 17 to 28 d. Note sloughing of parasite and epithelia into gut lumen, moderate hemocytic response by the oyster and atypical invasion of the parasite into nonciliated digestive tubules (arrows). (×860). Fig. 5. Higher magnification of *P. marinus* infection in oyster gut epithelia exposed to DENA (600 $mg\,l^{-1}$) for 17 to 28 d. Note numerous parasite stages in gut epithelia (arrows) with a moderate hemocytic response (H) beneath the gut epithelial basement membrane. (×1720). Fig. 6. Control oyster from DENA exposure tests with a light infection of *P. marinus* in gut epithelia. A moderate hemocytic response to the pathogen concomitant with normal diapedesis by blood cells in gut epithelia is occurring due to starvation stress. Note diminished epithelia of nonciliated digestive tubules with no *P. marinus* invasion. (Compare with Figs. 4, 7, 8 and 9). (×860)



Figs. 7 to 10. *Crassostrea virginica*. Fig. 7. Typical heavy *Perkinsus marinus* infection of oyster gut epithelia. Note heavy hemocytic response and sloughing of parasite and gut epithelia into gut lumen. (×860). Fig. 8. Atypical invasion and destruction of non-ciliated digestive tubules by *P. marinus* in oysters exposed to DENA (600 mg l^{-1}) within 17 to 28 d (arrows). (×1720). Fig. 9. Higher magnification of Fig. 8 showing definite parasite meronts in the epithelia and lumen of exposed oysters' nonciliated digestive tubules. (×2400). Fig. 10. Gut epithelia of oysters exposed to DENA (600 µg l^{-1}) for 28 d and depurated in clean flowing seawater for 3 wk. Note no parasites present in gut epithelia or hemocytic response by the oyster (×860)

cause mortality via systemic invasion and damaging blood sinuses (Mackin 1951, Sparks 1985) but this was not observed in DENA-exposed oysters. A striking difference between classic 'Dermo disease' and pathosis in exposed oysters was a massive invasion and destruction of nonciliated digestive tubule epithelia by the pathogen. This is atypical because nonciliated digestive tubules are not generally invaded and destroyed by *P. marinus*, like gut epithelia, but become necrotic due to destruction of supporting connective tissue and blood sinuses by the parasite (Mackin 1951, Perkins 1976, Sparks 1985).

All baseline oysters histologically examined before tests had light to moderate infections of the parasite in gut epithelia. This coincides with reports that the primary portal of entry is gut epithelia (Mackin 1951) and the pathogen can lie dormant in host tissue for months in water temperatures of 20 °C or less (Hewatt & Andrews 1954, Ray 1954a, Mackin 1962, Quick & Mackin 1971). Prevalence and intensity of infections increase linearly at temperatures above 18 °C (Mackin & Sparks 1962, Quick & Mackin 1971), and the parasite does not readily multiply or cause significant pathosis in oysters below this temperature (Andrews & Hewatt 1957, Andrews 1966). Thus, to date, temperature has been established as the most important environmental factor affecting virulence of *Perkinsus marinus* infections in oysters, with the most intense infections occurring above 25 °C (Ray 1954a, Mackin & Sparks 1962, Ray 1966, Lauckner 1983). However, *P. marinus* did not remain dormant in DENA-exposed oysters but reproduced in large numbers causing pathologic damage and significant mortality (86 %) in temperatures of 20 °C. Conversely, the pathogen remained dormant in control oysters and did not multiply or become pathogenic at the same temperature.

It appears the DENA either directly or indirectly enhanced proliferation and lethality of *Perkinsus marinus* in exposed oysters since control oysters showed no signs of pathogen intensity enhancement and surviving oysters transferred to clean water showed no mortality or histological evidence of enhancement 3 wk after transfer.

The reasons why *Perkinsus marinus* was able to multiply in tremendous numbers, infect tissues not generally invaded by the parasite, and become lethal in DENA-exposed oysters at temperatures below its normal pathologic range are not clear. It is possible the DENA 'causes a reduction in the effectiveness of internal defense mechanisms or modifies epithelial barriers and biochemical processes' in exposed oysters (Sinderman 1980). Bivalves do not possess humoral immune factors (antibodies) like vertebrates, but respond to pathogenic agents via nonspecific cellular mechanisms, such as leukocytic infiltration, phagocytosis, encapsulation and diapedesis (Sparks 1972, Cheng 1973). *P. marinus* does not reproduce readily or become intensely pathogenic in oysters below 20 °C in natural environments; however, parasite meronts are capable of enlargement in thioglycollate media at 18 °C (Ray 1954a). Also, bivalves *(Mytilus edulis)* are capable of producing histologically detectable hemocytic responses to chemical injury in water temperatures below 15 °C (Rasmussen 1982). Because control oysters showed no *P. marinus* enhancement and the carcinogen did not appear acutely toxic to test oysters, evidence suggests the DENA may have been toxic to the oysters' nonspecific cellular defense mechanisms. Prior studies indicate carcinogenic pesticides [such as organochlorines (DDT, Dieldrin) and carbamates (Urethan)] reduce phagocytic capacity and viability of macrophages and granular leukocytes in vertebrates (Exon et al. 1987). Also, similar enhancement of a pathogen *(Baculovirus)* in a crustacean *(Penaeus duroarum)* exposed to a polychlorinated biphenyl (Aroclor 1254) has been reported (Couch & Nimmo 1974, Couch & Courtney 1977). An earlier investigation of *Crassostrea virginica* exposed to chronic low levels of DDT, Toxaphene and Parathion (Lowe et al. 1971) may be relevant to the present study. The consulting pathologist, Mr Gilbert Pauley, reported heavy *P. marinus* infections in all exposed oysters examined, with atypically light hemocytic response to the pathogen. In contrast, few control oysters examined from the study were infected with the parasite. However, unlike the present study, the exposed oysters were sampled when salinities were very high (29 ‰) and water warm (25 °C).

If the resistance-compromising hypothesis is correct it would explain why the pathogen was capable of such tremendous proliferation and lethality in exposed oysters. However, it does not explain why the parasite was able to proliferate in such tremendous numbers at a temperature near its lower range. As already discussed, the most important factor which appears to control *Perkinsus marinus* infections in oysters is temperature. *P. marinus* is capable of enlargement in thioglycollate media at 18 °C but has not been reported to be as active as observed in the DENA-exposed oysters in this study. Another possibility relating to the enhancement phenomenon may be that DENA is capable of stimulating the pathogen's growth directly. Few studies address the direct effects of chemicals on disease agents in aquatic animals (Esch et al. 1975, Lauckner 1983) and the present study indicates more research is needed. Prior investigators have suggested that certain diseases in economically important estuarine animals may be caused or enhanced by chemical pollutants in the environment (Sparks 1972, Couch & Nimmo 1974, Fries & Tripp 1976, Overstreet & Howese 1977, Couch

& Courtney 1977, Sinderman 1980, Couch 1985, Couch & Harshbarger 1985) even though there are few studies which directly link specific pollutants to specific diseases. The DENA enhancement of *P. marinus* infections in *Crossostrea virginica* appears to substantiate the thesis that chemical enhancement of certain pathogens can occur in some aquatic animals (Couch & Nimmo 1974, Couch & Courtney 1977, Couch & Harshbarger 1985). This may be relevant in epizootiological areas where *P. marinus* causes the greatest mortalities in oysters since some studies indicate prevalence and intensity of some infections to be heavier in estuaries impacted with more chemical pollution (Couch 1985). We do not mean to imply that every host-pathogen/parasite relationship will be umbalanced in favour of only the pathogen at the expense of the host. In certain cases, it is possible that the parasite may be equally or more vulnerable to chemical influence. Each potential chemical effector must be evaluated on a case-by-case basis (empirically) until more is known about their mechanisms of action.

The exact mechanisms for the *Perkinsus marinus* enhancement phenomenon are not understood, but it is possible that DENA may be toxic to the cells of the oysters nonspecific defense mechanisms, or be capable of stimulating the pathogen's growth directly. Because of the economic importance of *Crassostrea virginica* in epizootiological areas of *P. marinus*, further studies should be conducted to determine: (1) What nonspecific cellular defense mechanisms exist in oysters with the capacity to keep *P. marinus* pathogenicity suppressed in cold temperatures when this study shows the parasite can be very active in these temperatures? (2) Why is *P. marinus* atypically active and pathogenic in oysters in the presence of a stressing agent in water temperatures below its normal pathogenic range? (3) Is DENA affecting the pathogen's growth directly? (4) Are other xenobiotics capable of causing similar *P. marinus* enhancement in oysters in cold or warm water temperatures?

LITERATURE CITED

Andrews, J. D. (1965). Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. Chesapeake Sci. 6: 60–67

Andrews, J. D. (1966). Oyster mortality studies in Virginia. V. Epizootiology of MSX, a protistan pathogen of oysters. Ecology 47: 19–31

Andrews, J. D. (1976). Epizootiology of *Dermocystidium marinum (Labyrinthomyxa marina)* in oysters. Proc. 1st Int. Colloq. Invertebr. Path. (Kingston, Canada), p. 172–174

Andrews, J. D. (1979). Oyster diseases in Chesapeake Bay. Mar. Fish. Rev. 41: 45–53

Andrews, J. D., Hewatt, W. G. (1957). Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. Ecol. Monogr. 27: 1–25

Cheng, T. C. (1973). Immunity to parasites. In: Cheng, T. C. (ed.) General parasitology. Academic Press, New York, p. 89–120

Couch, J. A. (1985). Prospective study of infectious and noninfectious diseases in oysters and fishes in three Gulf of Mexico estuaries. Dis. aquat. Org. 1. 59–82

Couch, J. A., Courtney, L. (1977). Interaction of chemical pollutants and virus in a crustacean: a novel bioassay system. Ann. N. Y. Acad. Sci. 298: 497–504

Couch, J. A., Harshbarger, J. C. (1985). Effects of carcinogenic agents on aquatic animals: an experimental overview. Environ. Carcinog. Revs. 3: 63–105

Couch, J. A., Nimmo, D. (1974). Detection of interactions between natural pathogens and pollutants in aquatic animals. In: Proc. Gulf Coast Regional Symp. Diseases of Aquatic Animals, Louisiana State University, Center for Wetland Resources, LSU-5G-74-05, p. 261–265

Dunnington, E. A. (1956). Oyster parasite distribution studies in Maryland Waters. Maryland Tidewater News 12: 1–3

Esch, G. W., Gibbons, J. W., Bourque, J. E. (1975). An analysis of the relationship between stress and parasitism. Am. Midl. Nat. 93: 339–353

Exon, J. H., Kerkvliet, N. I., Talcott, P. A. (1987). Immunotoxicity of carcinogenic pesticides and related chemicals. Environ. Carcinog. Revs. C-5: 73–120

Fries, C., Tripp, M. R. (1976). Effects of phenol on clams. Mar. Fish. Rev. 38: 10–11

Hewatt, W. G., Andrews, J. D. (1954). Oyster mortality studies in Virginia. I. Mortalities of oysters in trays at Gloucester point, York River. Tex. J. Sci. 6: 121–133

Hoese, H. D. (1964). Studies on oyster scavengers and their relation to the fungus *Dermocystidium marinum*. Proc. natl Shellfish. Ass. 53: 161–174

Khudoley, V. V., Syrenko, O. A. (1978). Tumor induction by N-nitroso compounds in bivalve molluscs *Unio pictorum*. Cancer Letters 4: 349–354

Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In: Kinne, O. (ed.) Diseases of marine animals, Vol. 2. Biologische Anstalt Helgoland, Hamburg, p. 477–961

Levine, N. D. (1978). *Perkinsus* gen. n. and other new taxa in the protozoan phylum Apicomplexa. J. Parasitol. 64: 549

Lowe, J. I., Wilson, P. D., Rick, A. J., Wilson, A. J. (1971). Chronic exposure of oysters to DDT, Toxaphene and Parathion. Proc. natl Shellfish Ass. 61. 71–79

Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology, 3rd edn. McGraw Hill, New York, p. 258

Mackin, J. G. (1951). Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier Bull. mar Sci. Gulf Caribb. 1: 72–87

Mackin, J. G. (1956). *Dermocystidium marinum* and salinity. Proc. natl Shellfish Ass. 46: 116–128

Mackin, J. G. (1962). Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publs Inst. mar. Sci. Univ. Tex. 7: 132–229

Mackin, J. G., Hopkins, S. H. (1962). Studies on oyster mortality in relation to natural environments and to oil fields in Louisiana. Publs Inst. mar. Sci. Univ. Tex. 7: 1–131

Mackin, J. G., Sparks, A. K. (1962). A study of the effect on oysters of crude oil loss from a wild well. Publs Inst. mar. Sci. Univ. Tex. 7: 230–261

Menzel, R. W., Hopkins, S. H. (1955). The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. J. Parasitol. 41: 333–342

Overstreet, R. M., Howse, H. D. (1977). Some parasites and diseases of estuarine fishes in polluted habitats of Mississippi. Ann. N. Y Acad. Sci. 298: 427–462

Perkins, F. O. (1976). *Dermocystidium marinum* infection in oysters. Mar Fish. Rev. 38: 19–31

Quick, J. A., Mackin, J. G. (1971). Oyster parasitism by *Labyrinthomyxa marina* in Florida. Florida Dept. of Natural Resources, Marine Research Lab. Prof. Papers Series No. 131. 1–55

Rasmussen, L. (1982). Light microscopical studies of the acute toxic effects of N-nitrosodiethylamine on the marine mussel, *Mytilus edulis*. J. Invert. Pathol. 39: 66–80

Ray, S. M. (1954a). Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. Rice Inst. Pam. Special Issues, November 1954, p. 1–114

Ray, S. M. (1954b). Experimental studies on the transmission and pathogenicity of *Dermocystidium marinum*, a fungus parasite of oysters. J. Parasitol. 40: 235

Ray, S. M. (1966). Cycloheximide inhibition of *Dermocystidium marinum* in laboratory stocks of oysters. Proc. natl Shellfish. Ass. 56: 31–36

Scott, G. I., Vernberg, W. B. (1979). Seasonal effects of chlorine produced oxidants on the growth, survival and physiology of the American oyster, *Crassostrea virginica* (Gmelin). In: Vernberg, W. B. (ed.) Marine pollution: functional responses. Academic Press, New York, p. 501–516

Scott, G. I., Oswald, E. O., Sammons, T. I., Baughman, D. S., Middaugh, D. P. (1985). Interactions of chlorine-produced oxidants, salinity, and a protistian parasite in affecting lethal and sublethal physiological effects in the Eastern or American Oyster In: Jolley, R. L., et al. (ed.) Water chlorination: chemistry, environmental impact and health effects; Vol. 5. Lewis Publishers, Inc., Chelsea, Michigan, p. 463–480

Shaw, B. L., Battle, H. I. (1957). The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). Can. J. Zool. 35: 325–347

Sinderman, C. J. (1970). Bibliography of diseases and parasites of marine fish and shellfish (with emphasis on commercially important species). Tropical Atlantic Biological Laboratory, Informal Report No. 11, p. 1–440

Sinderman, C. J. (1980). A critical examination of the relationships between pollution and disease. Int. Counc. Explor Sea (ICES), Special Meeting on Diseases of Commercially Important Marine Fish and Shellfish (Copenhagen, 1980) No. 53

Sparks, A. K. (1972). Invertebrate pathology. Noncommunicable diseases. Academic Press, New York, p. 1–382

Sparks, A. K. (1985). Protozoan diseases. In: Synopsis of invertebrate pathology: exclusive of insects. Elsevier Science Publishers B. V., New York, p. 239–311

White, M. E., Powell, E. N., Ray, S. M., Wilson, E. A. (1987). Host-to-host transmission of *Perkinsus marinus* in oyster *(Crassostrea virginica)* populations by the ectoparasitic snail *Boonea impressa* (Pyramidellidae). J. Shellfish Res. 6: 1–5

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