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A COMPARISON OF *CRASSOSTREA GIGAS* AND *CRASSOSTREA VIRGINICA:* EFFECTS OF TEMPERATURE AND SALINITY ON SUSCEPTIBILITY TO THE PROTOZOAN PARASITE, *PERKINSUS MARINUS*

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ABSTRACT The susceptibility of diploid and triploid (2N and 3N) *Crassostrea gigas* to *Perkinsus marinus* was compared, in the laboratory, with that of *Crassostrea virginica* at three test temperatures (10, 15, and 25°C) at 20–22 ppt and at three test salinities (3, 10, and 20 ppt) at a temperature of 19–22°C. Experimental oysters were challenged twice with freshly isolated *P. marinus* meronts, after acclimation to test temperatures and salinities. Although infection prevalence and intensity increased with temperature ($p = 0.0001$) and salinity in *P. marinus*–challenged oysters of both oyster species, they were highest in *C. virginica* groups. Infection intensity was significantly ($p = 0.001$) higher in *P. marinus*–challenged *C. virginica* than *C. gigas* (2N and 3N) at all temperatures; however, infection prevalence was not statistically different at any temperature treatment. In all salinity treatments, prevalence and infection intensity were significantly higher ($p = 0.0001$) in *P. marinus*–challenged *C. virginica* than 2N and 3N *C. gigas*. Because high infection prevalence and intensity were found in non-challenged *C. virginica*, part of the recorded prevalence and intensity in challenged *C. virginica* was probably attributed to latent infection carried over from the field. High mortality occurred in both 2N and 3N *C. gigas* during temperature and salinity adjustment, particularly at 25°C and 3 psu.

KEY WORDS: Pacific oyster, eastern oyster, *Crassostrea gigas*, *Crassostrea virginica*, oyster disease, *Perkinsus marinus*, temperature, salinity

INTRODUCTION

The eastern oyster, *Crassostrea virginica*, has historically supported a major fishery on the East Coast of the United States. Beginning in the late 1950s, severe mortality in oyster populations has been caused by the two endoparasitic pathogens, *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) in the mid-Atlantic region. The introduction of a non-native species, the Pacific oyster (*Crassostrea gigas*) to the waters of this region has been proposed to revitalize the oyster fishery (Mann et al. 1991). The Pacific oyster has been successfully introduced and cultured along the West Coast of the United States and in Europe. This oyster species is rarely infected by the protozoan parasite, *Bonamia ostreae*, which has caused severe losses of the European oyster (*Ostrea edulis*) industry in Europe and on the West Coast of the United States over the last decade (Grizel 1985, Elston et al. 1987, Grizel et al. 1988). Results from recent laboratory studies also indicate that the Pacific oyster is less susceptible than the eastern oyster to *P. marinus* (Meyers et al. 1991, Barber and Mann 1994).

Pacific oysters usually propagate in habitats of salinities >18 ppt and temperatures ≤15°C, although they can tolerate temperature as high as 35°C and salinity as low as 10 ppt (Mann et al. 1991). Information regarding temperature-salinity tolerance in *C. gigas* is, however, limited, and the definitive temperature and salinity tolerances of this species have not been established in the laboratory. Therefore, the competence of the Pacific oyster against *P. marinus* under different salinity and temperature regimes is of particular concern, before its introduction into the mid-Atlantic region. This study evaluates in the laboratory the competence of triploid and diploid Pacific oysters and eastern oysters against *P. marinus* under different temperature and salinity conditions.

MATERIALS AND METHODS

Experiment 1: Temperature Effect

Eastern oysters, *C. virginia* (shell length [SH], 7–8 cm), were collected on January 8, 1992, from Ross Rock in the Rappanhannock River, a tributary of the lower Chesapeake Bay. Oysters from this area typically have a low prevalence of *P. marinus* infection (Burreson 1992, Ragone Calvo and Burreson 1994, Ragone Calvo and Burreson 1995). The ambient temperature and salinity at the time of collection were 8°C and 10 ppt. Triploid (3N, assayed to be 95%) and diploid (2N) Pacific oysters (age, 16 mo; SH, 6–7 cm) were progenies from a spawning conducted by Dr. Standish Allen (Haskin Shellfish Laboratory, Rutger's University) in late July of 1990. The spawning was produced from second-generation parents of 1989 broodstocks from Washington, and juveniles were raised at the Virginia Institute of Marine Science, in quarantined flumes with flowing raw York River water (YRW ambient temperature, 8°C and salinity, = 20 ppt, at the time of experiment). Before the start of the experiment, initial assessment was performed on a subsample of 20 *C. gigas* and 25 *C. virginica* for *P. marinus* infection by use of the tissue thioglycollate assay (Ray 1952, Ray 1966) described below. All groups tested negative. The remaining *C. virginica* and *C. gigas* were held separately in aerated 55-gallon tanks and gradually adjusted to the three test temperatures (10, 15, and 25°C, 2°C per 2 d) at salinities of 20–22 ppt (1 μm filtered YRW). Before temperature adjustment, *C. virginica* was first acclimated (3 ppt per 2 d) from ambient salinity (i.e., 10 ppt) to the experimental salinity (i.e., 20–22 ppt). After adjustment to the desired test temperatures and YRW salinity, oysters were maintained in aerated 1 μm filtered YRW in 40-l

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aquaria (20–22 oysters per aquarium). Oysters were fed with algal paste (a mixture of Tahitian *Isochrysis galbana* and *Thalassiosira pseudonana*, 0.1 g/oyster) daily, and mortality was recorded throughout the course of the experiment. If oysters died at the beginning of temperature adjustment, they were replaced. Thus, the number of oysters among groups was similar (N = 37–41) when *P. marinus* challenge was initiated. All experimental oysters were challenged twice with freshly isolated *P. marinus* meronts. Twenty-nine days after the initiation of temperature acclimation, oysters were inoculated with 0.1 ml of meront/merozoite suspension (2.5×10^5 meronts/oyster) into the shell cavity. Control oysters were inoculated with filtered YRW (0.22-μm-pore-size filter). Forty-one days after the first challenge, challenged oysters were inoculated with a second dose of meronts (7.0×10^3 meronts per oyster). Sixty-eight days after the first challenge (27 d after the second challenge), 10 control and 10 challenged oysters from each temperature treatment were sacrificed and rectal tissues were removed to determine infection by use of the tissue thioglycollate assay (Ray 1952, Ray 1966). Eighty-four days after the first challenge, the remaining oysters were sacrificed and the same parameters mentioned above were measured. Data from the two samplings were pooled to determine the disease prevalence and intensity.

Experiment 2: Salinity Effect

The experimental protocol of this experiment was similar to that of the temperature effect experiment. *C. virginica* (7–8 cm) were collected on May 11, 1992, from Ross Rock, Rappahannock River (Ambient temperature, 19°C; salinity, 6 ppt). *C. gigas* (3N and 2N, 6–8 cm) was from the same stock used for the temperature effect experiment. Initial assessment of *P. marinus* infection on 20 *C. gigas* and 25 *C. virginica* showed that, with the exception of a single *P. marinus cell* detected in one of the diploid *C. gigas*, no oysters were infected with *P. marinus*. The ambient temperature and salinity of YRW at the time of the experiment were 19–22°C and 20 ppt respectively. Both *C. virginica* and *C. gigas* were placed in aerated 200-l tanks, and salinities were gradually adjusted (3 ppt per 2 d) to salinities of 3, 10, and 20 ppt, at 19–22°C. After salinity adjustment was completed, oysters were maintained in aerated 40-l aquaria. During the salinity adjustment period, heavy mortality occurred in both diploid and triploid *C. gigas* at 3 ppt. Consequently, the susceptibility of *C. gigas* and *C. virginica* to *P. marinus* was compared only at 10 and 20 ppt. As in experiment 1, test oysters were challenged twice by freshly isolated meronts/merozoites (2.0×10^5 cells/oyster, 21 d after the initiation of salinity adjustment and 5.0×10^3 cells per oyster 12 d after the first challenge). Again, control oysters were inoculated with filtered YRW. Fifty days after the initial *P. marinus* challenge, the experiment was terminated to determine disease prevalence and intensity.

Preparation of Meront/Merozoite Suspension

Fresh meront/merozoite suspension was prepared according to La Peyre and Chu (1994). Briefly, *P. marinus*–infected oyster tissues were rinsed thoroughly with filtered (0.22 μm) YRW and subsequently homogenized in (0.22 μm) filtered YRW with a blender (Virtis, Model 23) at high speed for 2 min. The suspension was then passed through a series of screens (100, 35, 20, and 15 μm) to remove oyster tissue residues. The number of merozoites in suspension was counted with a hemacytometer and adjusted to the desired concentration.

P. marinus *Assay*

The tissue thioglycollate assay (Ray 1952, Ray 1966) was used for *P. marinus* diagnosis. Rectal tissue was removed from each oyster and incubated in fluid thioglycollate medium for 4–5 d. The intensity of infection was ranked as 0 (negative), 1 (light), 3 (moderate), and 5 (heavy), on the basis of the number of stained *P. marinus* hypnospores contained in the oyster rectal tissue smear.

Statistical Analysis

Logistic regression and log-linear modelling (Agresti 1990) were used to determine differences in infection prevalence between temperature and salinity treatments and between oyster species. Two-factor analysis of variance was used to determine differences in infection intensity between the three groups (i.e., *C. virginica*, *C. gigas* 2N and 3N) of oysters at different temperature or salinity treatments.

RESULTS

Experiment 1

Mortality

Throughout the course of the experiment, a total of 18 *C. virginica*, 38 diploid (2N) *C. gigas*, and 39 triploid (3N) *C. gigas* died. Most of the deaths occurred at 25°C during temperature adjustment (32 triploid *C. gigas*, 7 diploid *C. gigas*, and 4 *C. virginica*) (Table 1). High mortality was also noted at 25°C after oysters were challenged with freshly isolated *P. marinus*, with the

TABLE 1.

Mortality of *C. virginica* and *C. gigas* During Temperature Acclimation and After Challenge with *P. marinus* (Dermo).

Mortality	*C. virginica*			*C. gigas* (2N)			*C. gigas* (3N)		
	10°C (N = 80)	15°C (N = 80)	25°C (N = 80)	10°C (N = 79)	15°C (N = 81)	25°C (N = 85)	10°C (N = 82)	15°C (N = 82)	25°C (N = 111)
Mortality (no. of deaths) during acclimation	0	0	4	0	2	7	1	1	32
Mortality (no. of deaths) after *P. marinus* exposure	1	3	10	1	7	21	1	3	1
Total mortality (%) during experiment*	1.3	3.8	17.5	1.3	11.1	32.9	2.4	4.9	29.7

* % = no. of dead oysters/initial total number of oysters.

exception of triploid *C. gigas* (heavy mortality occurred only at the time of temperature adjustment). Although 21 diploid *C. gigas* and 10 *C. virginica* died, only one triploid *C. gigas* died at that temperature. Unfortunately, no tissue was able to be recovered from some of these mortalities for *P. marinus* diagnosis. Hence, mortalities with no meat recovered were excluded from prevalence and intensity calculations. However, for those mortalities that had tissues, it was found that one *P. marinus*–challenged *C. virginica* (N = 9) at 25 °C, one control diploid *C. gigas* (N = 4) at 15°C, and one control (N = 8) and three challenged diploid *C. gigas* (N = 7) at 25°C were infected. None of the triploid *C. gigas* (N = 2) that were examined had infections.

Prevalence and Intensity of *P. marinus* Infection

Infection prevalence (percentage of infected oysters = number of infected oysters/total number of oysters at the time of inoculation) significantly increased ($p = 0.0001$) with temperature in all *P. marinus*–challenged oysters (Fig. 1). Prevalence was higher in *C. virginica* than in the two *C. gigas* groups, with the exception of the 10°C treatment. At 10°C, 3N *C. gigas* had a higher prevalence (30%) than both 2N *C. gigas* (24%) and *C. virginica* (25%). The infection prevalences at 15 and 25°C, respectively, were 50 and 60% for *C. virginica*, 36 and 51% for 2N *C. gigas*, and 37 and 56% for 3N *C. gigas*. However, these differences were not statistically different ($p > 0.05$). Infection intensity increased significantly with increase in temperature and was significantly higher ($p = 0.001$) in *C. virginica* than *C. gigas* (2N and 3N) (Fig 2A). At 25°C, 10 (27%) of the infected *C. virginica* had moderate infections and 5 (14%) had heavy infections. There were four (11%) infected 2N *C. gigas* at 25°C and one (3%) at 10°C with moderate infections. None of the infected 3N *C. gigas* developed advanced (i.e., moderate or heavy) infections. Infection intensity expressed as weighted prevalence (= sum of disease code numbers/number of oysters) also significantly increased with increasing temperature ($p = 0.0001$). *C. virginica* had significantly

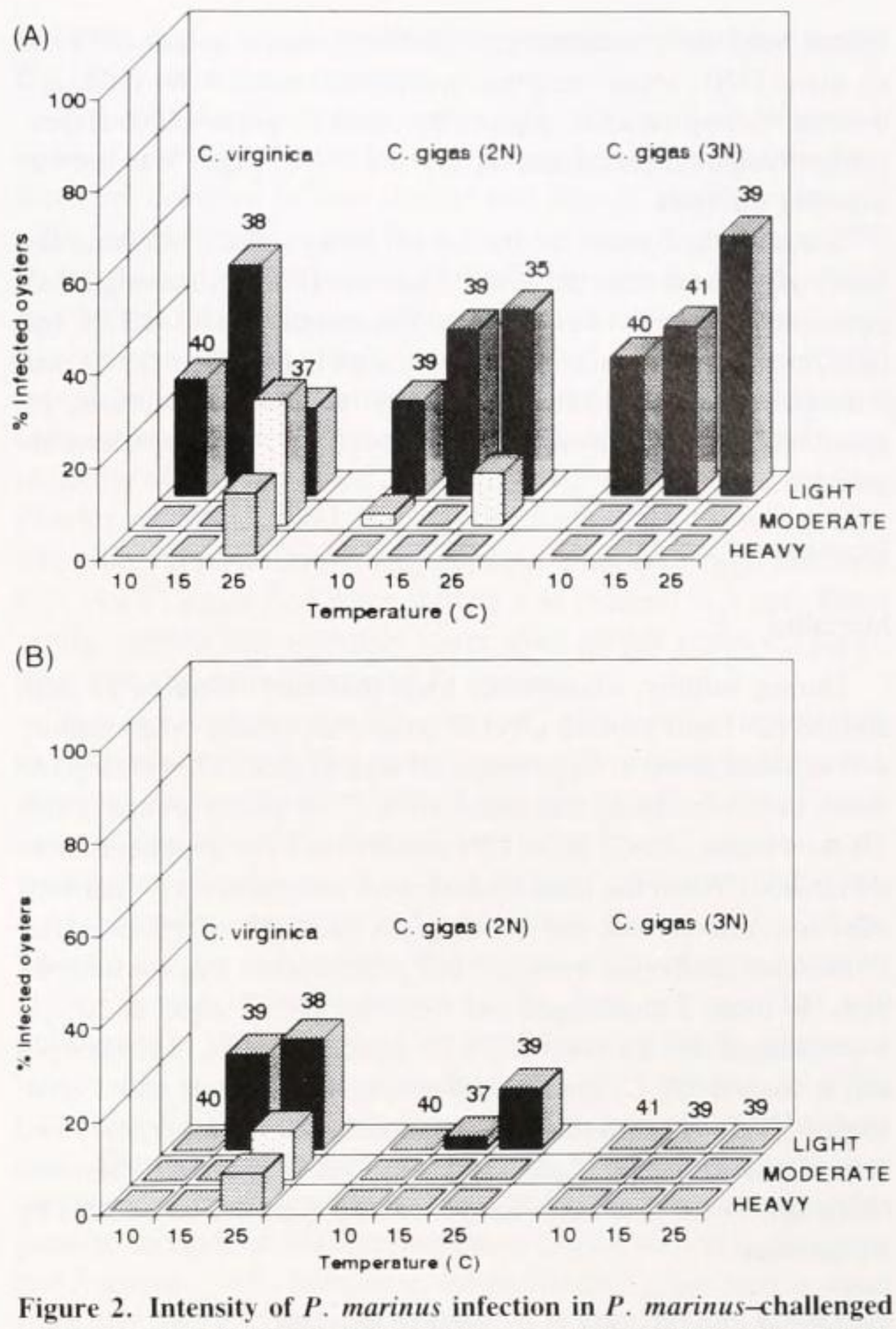

Figure 2. Intensity of *P. marinus* infection in *P. marinus*–challenged (A) and control (B) *C. virginica* and *C. gigas* (2N and 3N) oysters at 10, 15, and 25°C. Numbers above the bars represent total number of oysters in each treatment.

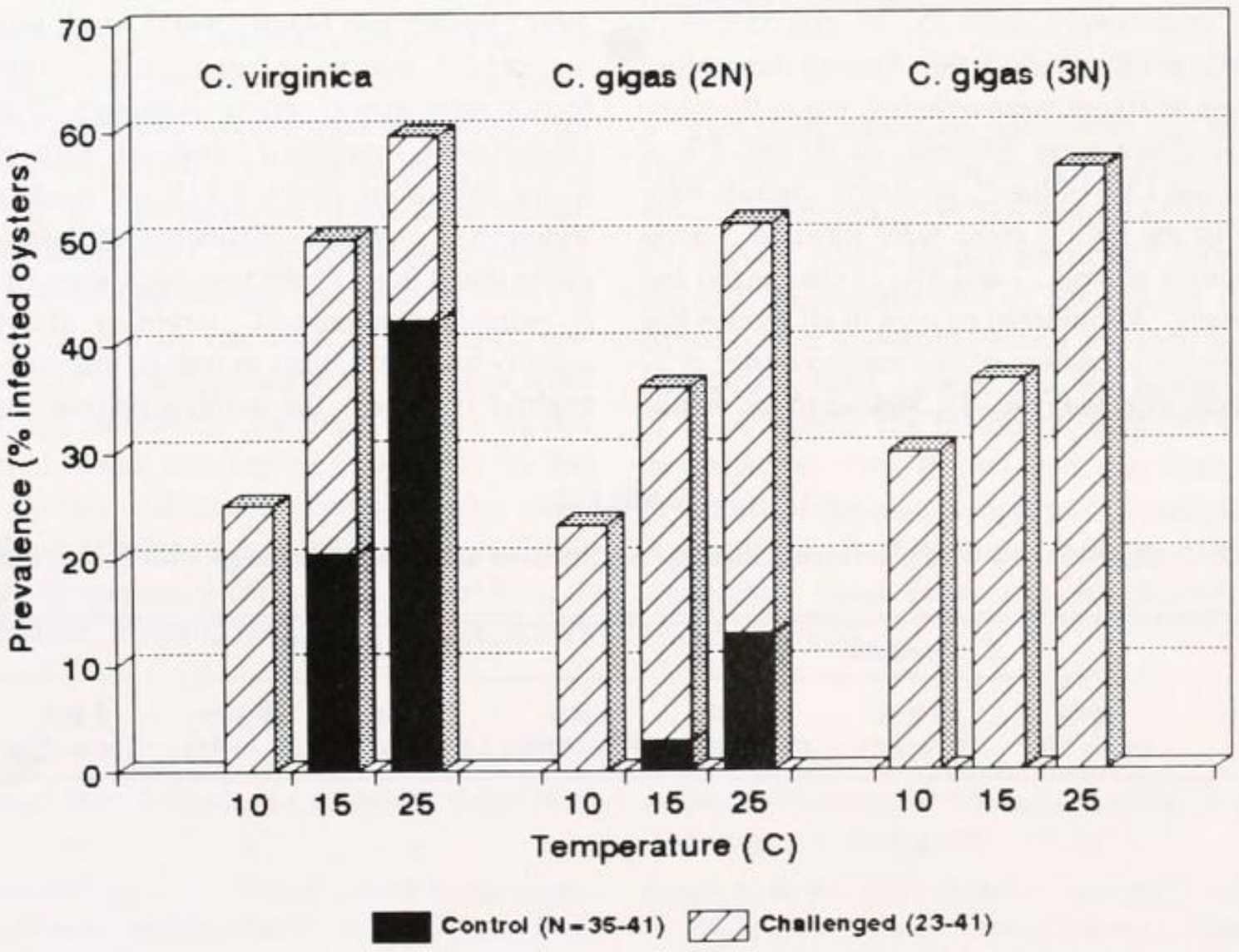

Figure 1. Prevalence of *P. marinus* infection (% infected oysters) in control and *P. marinus*–challenged *C. virginica* and *C. gigas* (2N and 3N) oysters at 10, 15, and 25°C.

higher weighted prevalence ($p = 0.0004$) than *C. gigas* (2N) and *C. gigas* (3N). Mean weighted prevalences were 0.79, 0.45, and 0.41 in *C. virginica*, *C. gigas* (2N), and *C. gigas* (3N), respectively. Weighted prevalence in 2N and 3N *C. gigas* was not statistically different.

Some of the oysters in the control groups of *C. virginica* and 2N *C. gigas* were infected with *P. marinus* (Fig. 1). Among 2N *C. gigas*, one oyster (3%) at 15°C and five oysters (13%) at 25°C had light infections. Among *C. virginica*, nine (24%), four (11%), and three (8%) oysters had light, moderate, and heavy infections, respectively (Fig. 2B). None of the control 3N *C. gigas* oysters were infected.

Experiment 2

Mortality

During salinity adjustment, high mortality occurred in both diploid (2N) and triploid (3N) *C. gigas*, especially when salinity was adjusted down to 3 ppt (44 of 80 diploid died, 37 of 80 triploid died), but no mortality was noted in the *C. virginica* groups (Table 2). As a result, the *C. gigas* (2N and 3N) at 3 ppt treatments were terminated. When the dead oysters were examined for *P. marinus* infection, one 2N and one 3N *C. gigas* had light infections. After *P. marinus* challenge, mortality in Pacific oysters was consistently high. In total, 2 challenged and 5 control 2N *C. gigas* at 20 ppt, 9 challenged and 11 control 2N *C. gigas* at 10 ppt, 9 challenged and 8 control 3N *C. gigas* at 10 ppt, and 14 control and 3 challenged 3N *C. gigas* oysters at 20 ppt perished. However, only two control and two challenged eastern oysters died after *P. marinus* challenge. None of these dead oysters were found to be infected by *P. marinus*.

Prevalence and Intensity of *P. marinus* Infection

In all salinity treatments, *C. virginica* had the highest prevalence of *P. marinus* infection ($p = 0.001$) (Fig. 3). In the *P. marinus*–challenged oysters, the prevalence in 2N *C. gigas*, 3N *C. gigas*, and *C. virginica*, respectively, were 25, 35, and 65% at 10 ppt and 25, 31, and 64% at 20 ppt (Fig. 3). Among the control oysters, no Pacific oysters at 10 ppt were infected, but at the same salinity, 7% of the *C. virginica* were infected. At 20 ppt, 5% of the 2N *C. gigas* controls and 13% of the *C. virginica* controls were infected, whereas none of the 3N *C. gigas* were infected. Prevalence was low in *C. virginica* at 3 ppt, 7 and 3%, in challenged and control groups, respectively. All infected oysters in all groups had only light infections, with the exception of one eastern oyster at 20 ppt, which was moderately infected (Fig. 4). Similar to the results

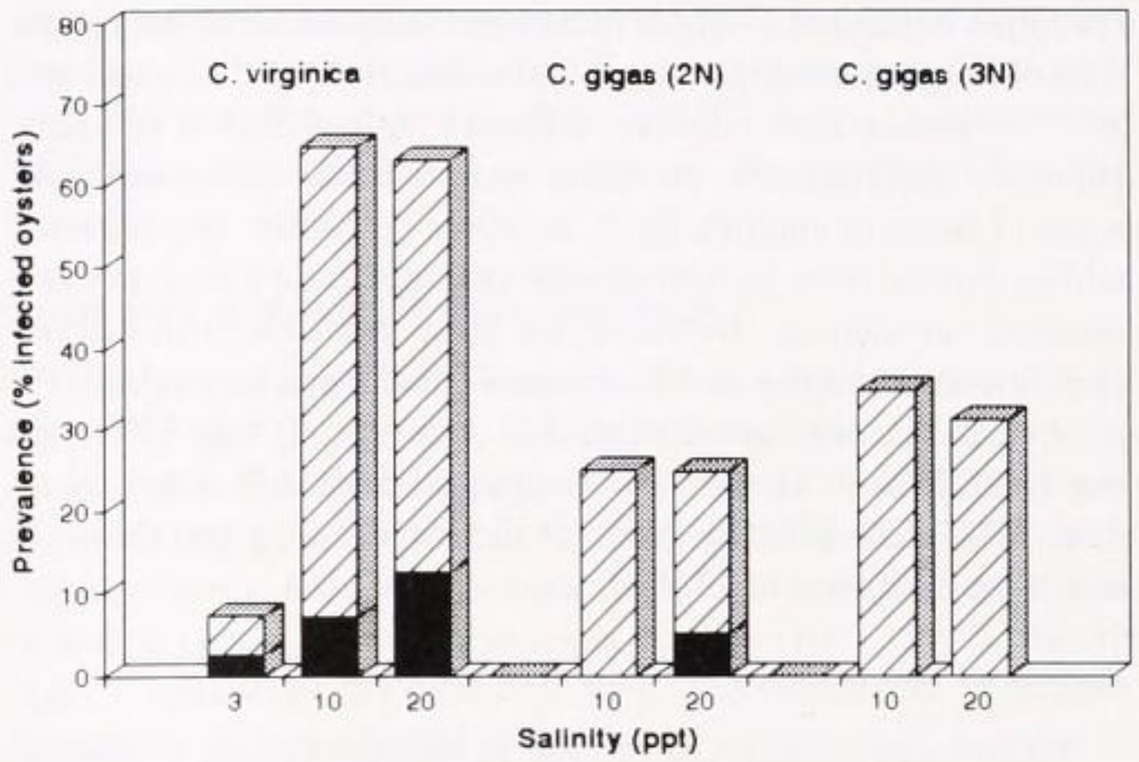

Figure 3. Prevalence of *P. marinus* infection (% infected oysters) in control and *P. marinus*–challenged *C. virginica* and *C. gigas* (2N and 3N) oysters at 3, 10, and 20 psu.

in the temperature experiment, *C. virginica* had significantly higher weighted prevalence than *C. gigas* (2N and 3N) ($p = 0.0001$). Mean weighted prevalences for *C. virginica*, *C. gigas* (2N), and *C. gigas* (3N) were 0.64, 0.25, and 0.33, respectively. Salinity (10 and 20 ppt) did not significantly affect ($p > 0.05$) the weighted prevalence. In both oyster species, no differences were observed in pooled infection intensity between salinities (10 and 20 ppt).

DISCUSSION

The results of this study revealed that *C. gigas*, both diploid and triploid, is less susceptible to *P. marinus* than is *C. virginica*. This is consistent with previous findings in experiments comparing *P. marinus* susceptibility, mortality, and growth rates between *C. virginica* and *C. gigas* challenged with the parasite (Meyers et al. 1991, Barber and Mann 1994). At all tested temperature-salinity regimes, *P. marinus*–challenged *C. virginica* suffered higher infection rates than *C. gigas*. Although 27 and 14% of *P. marinus*–challenged *C. virginica* advanced, respectively, to moderate and heavy infections, only 3–11% of moderate infections were detected in *P. marinus*–challenged diploid *C. gigas*. However, because much higher infection rates were found in the control, non-*P. marinus*–challenged *C. virginica*, at any given temperature and salinity treatment, than in non-*P. marinus*–challenged diploid and triploid *C. gigas*, the authors believe that part of the recorded

TABLE 2.

Mortality of *C. virginica* and *C. gigas* During Salinity Acclimation and After Challenge with *P. marinus* (Dermo).

Mortality	*C. virginica*			*C. gigas* (2N)			*C. gigas* (3N)		
	3 psu (N = 79)	10 psu (N = 84)	20 psu (N = 81)	3 psu (N = 77)	10 psu (N = 86)	20 psu (N = 95)	3 psu (N = 52)	10 psu (N = 78)	20 psu (N = 81)
Mortality (no. of deaths) during acclimation	0	0	0	44	6	9	37	12	10
Mortality (no. of deaths) after *P. marinus* exposure	4	0	0	—	20	7	—	17	17
Total mortality (%) during experiment*	5	0	0	—	30.2	16.8	—	37.1	33.3

* % = No. of dead oysters/initial total number of oysters, — = treatments were terminated before *P. marinus* exposure due to heavy mortalities.

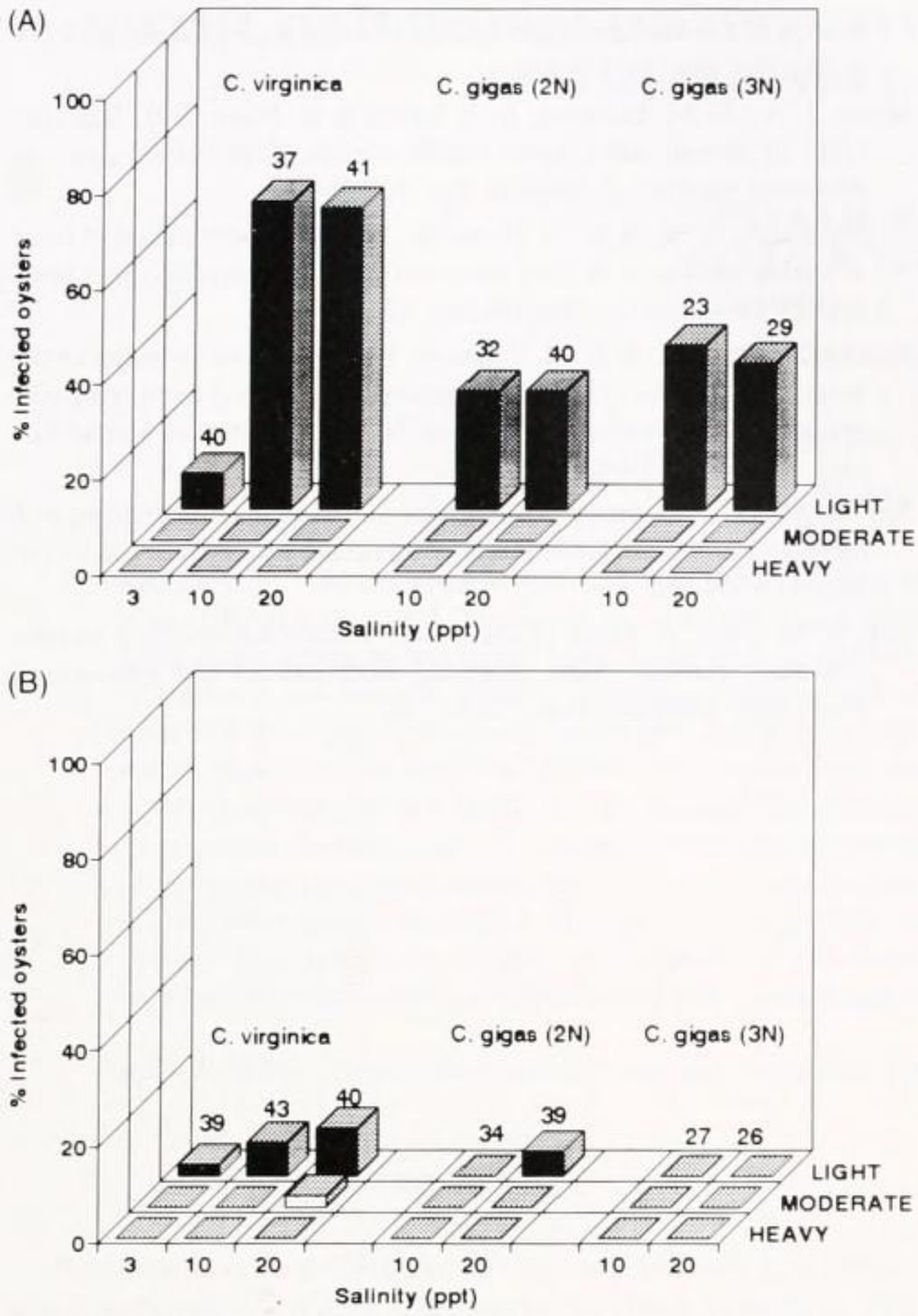

Figure 4. Intensity of *P. marinus* infection in *P. marinus*–challenged (A) control (B) *C. virginica* and *C. gigas* (2N and 3N) oysters at 3, 10, and 20 psu. Numbers above the bars represent total number of oysters in each treatment.

infection in *P. marinus*–challenged *C. virginica* was attributed to the expression of hidden infection carried over from the field. Unfortunately, the thioglycollate tissue assay used in this study for *P. marinus* diagnosis was not sensitive enough to detect cryptic infections, thus restricting the interpretation of the experimental results. However, the nonchallenged *C. virginica* showed substantially lower *P. marinus* infection prevalence and intensity than did *P. marinus*–challenged *C. virginica*. The observed increased disease prevalence and intensity in the challenged *C. virginica* must have been derived from the laboratory challenge. Future studies should use eastern oysters from an area free of *P. marinus* for this kind of study. Also, in oysters collected in winter months, overwintering infections will not develop to detectable levels until 1–2 mo post-exposure to high temperatures (i.e., 25°C). Therefore, to establish baseline information, it would be wise to expose oysters collected during winter to warm temperatures for 1–2 mo before the initial infection assessment.

Although Pacific oysters appear less susceptible than eastern oysters to *P. marinus* infection, heavy non-*P. marinus*-related mortality occurred in both diploid and triploid Pacific oysters at salinities of 10 ppt and below and temperatures higher than 15°C during the acclimation period. This indicates that the Pacific oyster may be less tolerant to high-temperature and low-salinity exposure than eastern oysters. High non-disease-related mortality (70%) was also recorded in Pacific oysters, in conjunction with salinities below 20 ppt, in a study carried out to compare the growth and mortality of *C. gigas* and *C. virginica* challenged with *P. marinus* (Barber and Mann 1994). It seems that low salinity exerts a greater effect on the physiology of this species than does high temperature. All *C. gigas* died when salinity was reduced to 3 ppt. These results suggest that salinities lower than 20 ppt stress *C. gigas*, thus reducing its resistance to *P. marinus*.

In conclusion, the Pacific oyster, *C. gigas*, is less susceptible to *P. marinus* than is the eastern oyster, *C. virginica*. However, they may not survive if introduced into Chesapeake Bay tributaries because they are unable to adapt well to the low-salinity and high-temperature conditions. The mid-Atlantic climate is relatively warm, between temperate and subtropical. The ecosystem of the Chesapeake Bay is complex. The salinity range of oyster habitats in the Chesapeake Bay varies seasonally, from as low as 0 to >20 ppt (Andrews 1988, Ragone Calvo and Burreson 1995). The water temperature of most tributaries along the bay can reach 28–29°C (Andrews 1988) and persist for more than 2 mo during the summer. The oyster pathogen, *P. marinus*, on the other hand, can survive in salinities lower than 5 ppt, and epizootics caused by this parasite increase at high temperatures (Andrews 1988, Burreson and Andrews 1988). Moreover, the shells of *C. gigas* held in water from the lower Chesapeake Bay (i.e., York River, VA) were found to be quite susceptible to invasion by the polychaete, *Polydora* sp. (Burreson and Mann 1994). Further studies are needed to ascertain the competence of *C. gigas* to support a commercial fishery in Chesapeake Bay.

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