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A physiological approach to the understanding of parasite (Perkinsus marinus) Interactions : pathological effects and disease resistance

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FINAL REPORT

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INTRODUCTION

Effects of Disease on Oyster Production

Two oyster pathogens (*Haplosporidium nelsoni*, MSX and *Perkinsus marinus*, Dermo) have contributed significantly to severely reduced oyster production along the east coast of the United States since about 1960 (see reviews by Haskin and Andrews, 1988; Andrews, 1988). In Virginia, which is reflective of Chesapeake Bay as a whole, annual production of market oysters was 7.6 million bushels in 1904 and fluctuated between 2 and 4 million bushels from 1930-31 to 1961-62 (Hargis and Haven, 1988). The first of two major declines in harvest began in 1959-60 when *H. nelsoni* began causing mortalities in the higher salinity (>20 ppt) regions of Chesapeake Bay (Hargis and Haven, 1988; Andrews, 1988.). Between 1965 and 1985, total market oyster landings ranged from about 500,000 to 1 million bushels per year. Although *P. marinus* was present in the 1950's, a combination of drought (leading to increased salinity), warmer than normal winters, and the transfer of infected seed was responsible for an increase in the pathogenicity of this disease causing a second reduction in oyster production (Andrews, 1988). Since 1986-87, oyster landings have steadily declined from over 500,000 bushels to less than 100,000 bushels (VMRC statistics). With a return to more normal rainfall and salinity regimes in Virginia since 1989, the impact of disease caused by *H. nelsoni* has been reduced, **but not the impact of the disease caused by *P. marinus***. In fact, *P. marinus* is currently resident in every oyster bed in Virginia (Burreson, 1990)

Not only have diseases contributed significantly to a decline in natural oyster production and its associated economy, diseases remain a major impediment to oyster production along the east coast of the U.S. **by any means.** Suggestions for improved management of natural oyster stocks (Hargis and Haven, 1988) have gone largely unheeded and proposed strategies for managing "around" the diseases (Ford and Haskin, 1988a; Andrews and Ray, 1988) recognize that disease pressure is unlikely to abate and the best approach is to minimize losses. Both Maryland and Virginia have large repletion programs aimed at augmenting natural oyster production. Oyster diseases have severely limited the repletion options normally utilized, namely the planting of shell and the movement of seed oysters. Severe depletion of spawning stocks has changed local spatfall patterns, making shell planting more of a gamble. If spatfall occurs, the movement of seed is risky because of the possibility of further spreading disease and the possibility of losing seed to disease by moving it to a highly diseased area. For the oyster aquaculture industry, effort at developing disease resistant strains has realized success with respect to *H. nelsoni* (Haskin and Ford, 1979; Ford and Haskin, 1987), **but not *P. marinus***. Techniques to enhance growth rate, such as off bottom culture and the use of triploid oysters, will enable a greater number of oysters to be harvested before they die from diseases.

Distribution and Epizootiology

P. marinus was first described by Mackin et al. (1950) from the Gulf of Mexico in 1948 after oyster mortalities were initially reported. Favoring warm temperatures, it occurs persistently from Tampico Bay, Mexico along the southeast coast of the U.S. to Chesapeake Bay and sporadically to Delaware Bay (Andrews, 1988). Because it is not consistently found north of Chesapeake Bay, its northern distribution is thought to be limited by low temperature in winter. In years when *H. nelsoni* is less prolific, *P. marinus* is the most prevalent oyster pathogen in Chesapeake Bay (Andrews, 1988; Burreson, 1990).

Like *H. nelsoni*, *P. marinus* is most pathogenic in higher salinity regions of estuaries. During years of drought and subsequent salinity increase, *P. marinus* has advanced farther up Chesapeake Bay and it tributaries, to the point where it is present in every public oyster bed in Virginia (Burreson, 1990). Unlike *H. nelsoni*, however, a return to normal salinity has not eradicated *P. marinus* in the lower salinity regions, indicating that once established, the parasite is quite persistent. *In vitro* studies indicate that *P. marinus* zoospores can survive in 4 ppt for up to 28 days (Chu and Greene, 1989). Even though reduced salinity may not lower the prevalence of *P. marinus*, disease proliferation and subsequent mortality in heavily infected oysters are reduced upon placement in salinity of 9 ppt and below (Ragone and Burreson, 1993). Field studies indicate that disease activity is retarded in waters below 15 ppt; based on its geographic distribution and seasonal activity, *P. marinus* favors a temperature of 20 °C or greater (Andrews, 1988). Sporulation of presporangia is inhibited at temperature below 4 °C (Chu and Greene, 1989). More detailed study of the *in vivo* temperature-salinity tolerance of *P. marinus* is warranted.

Determination of the life cycle of *P. marinus* has been aided by successful culture in the laboratory and examination of the various stages with electron microscopy (Perkins, 1988; La Peyre, 1993). The first cell type observed in oyster tissue is a uninucleate, coccoid trophozoite, often located within a phagocytic hemocyte. These immature trophozoites subsequently enlarge and acquire an eccentrically located vacuole. Successive bipartitioning of these mature trophozoites yields 8-32 cell sporangia which rupture, releasing more immature trophozoites. When placed in fluid thioglycollate, any state may enlarge to form hypnospores (prezoosporangia). Upon placement in seawater, these prezoosporangia initiate zoosporulation, yielding a mass of zoospores. Although zoospores have been shown to infect oysters in the laboratory, their importance in the life cycle of *P. marinus* is unclear as they have never been observed in nature. Thus trophozoites are probably the normal agents of disease transmission as demonstrated by the ease with which infections are induced in the laboratory in oysters free from zoospores.

The seasonal cycle of *P. marinus* disease proliferation and mortality in Virginia is reviewed by Andrews (1988). New infections are acquired either via the digestive tract (Andrews, 1988) or gill and mantle epithelium (Perkins, 1988). Invading cells are phagocytized by hemocytes which in turn facilitates the transport of the disease via the hemolymph. Multiplication of trophozoites is rapid in June at water temperature above 20 °C. Occlusion of blood sinuses and lysis of tissues preceeds mortality in late July or early August. Dying oysters release infective particles which initiate a second generation of disease resulting in further mortality from late August to early September. Mortalities decline in November when temperature decreases below 20 °C. Surviving oysters apparently harbor overwintering infections which manifest themselves the following June.

Physiological Interactions

Energetics play a fundamental role in all parasitic relationships since the survival of the parasite depends upon competing successfully with the host for available nutrients. This competition is highly complex in nature, involving both metabolic and physiological interaction (Thompson, 1983; Barber and Masso, 1987). Thus parasitism represents a stress to the host as the result of reduced amount of energy available for normal metabolic functions (Newell and Barber, 1988). In spite of the considerable knowledge that exists regarding the epizootiology of both *H. nelsoni* and *P. marinus*, very little is known about their metabolic (i.e., energetic) interactions with oysters. More specifically, we know little about the effects of these parasites on oyster energy metabolism. **This is especially true in the case of *P. marinus*.** Therefore very little can be deduced about what actually causes mortality. In addition, when disease resistance is exhibited, we know very little about the mechanisms that impart this resistance.

The limited information available indicates that the disease caused by *P. marinus* differs from that caused by *H. nelsoni*; little, however, is known about its pathological effects on oysters and the means by which it causes death. Histological observations have suggested that blood sinuses become choked with phagocytosed *P. marinus* cells, thus overwhelming the circulatory system (Andrews, 1988). *P. marinus* has been found to reduce oyster growth (Menzel and Hopkins, 1955; Paynter and Burreson, 1991) and condition index (Crosby and Roberts, 1990; Dittman, 1993). The concentration of total free amino acids in oyster mantle tissue was negatively related to the level of *P. marinus* infection, and the molar ratio of taurine-glycine (an indicator of stress) was positively correlated to level of infection (Soniat and Koenig, 1982).

No increase in resistance to the disease caused by *P. marinus* has been exhibited by *C. virginica* either in natural populations or in hatchery reared strains, and unfortunately, oysters resistant to *H. nelsoni* are not also resistant to *P. marinus* (Burreson, 1991). Phagocytosis of parasite cells is evident histologically

and has been confirmed *in vitro* (LaPeyre, 1993). Chu and LaPeyre (1989) found no relationship between hemolymph lysozyme and protein concentration and level of infection by *P. marinus*, indicating that humoral defense factors toward *P. marinus* are lacking in *C. virginica*.

It has recently been found, however, that another species of oyster, *C. gigas*, has considerable resistance toward *P. marinus* compared to *C. virginica*. After being heavily dosed with *P. marinus*, disease intensity and mortality was significantly greater in *C. virginica* than in *C. gigas* (Meyers et al., 1991). **The fact that oysters demonstrating resistance to the major oyster pathogen in the U.S. (*P. marinus*) have been identified, provided a unique opportunity for the examination of pathogenic effects and the physiological mechanisms of resistance.**

OBJECTIVES

Many gaps remain in our basic understanding of the oyster pathogen *P. marinus* and its interactions with the oyster, *C. virginica*. For example, little is known regarding the pathological effects that *P. marinus* has on oysters (other than eventually causing death) and when increased tolerance is displayed (as in the case of *C. gigas*), what physiological mechanisms are involved. This project sought to further our basic understanding of oyster disease processes and disease resistance.

The objectives of this proposed study were:

1. To determine the pathological (sublethal) effects of *P.marinus* on the growth, physiology, and biochemical composition (carbohydrate, protein, lipid, ash, and mass fractions of carbon and nitrogen) of *C. virginica*.

2. To characterize the physiological basis for resistance of *C. gigas* to *P. marinus* by comparing rates of growth, clearance, absorption, oxygen consumption, ammonia excretion, and biochemical composition between *C. virginica* and *C. gigas*.

To accomplish these objectives, oysters of both species were exposed to both light and heavy levels of *P. marinus* challenge over the course of a normal infection cycle. I.C.E.S. protocol was followed to avoid introduction of the non-indigenous *C. gigas*.

METHODS AND MATERIALS

Growth and Mortality

Broodstock of *C. virginica* was obtained from Nansemond Ridge, James River, VA. *C. gigas* broodstock was offspring of oysters imported from Washington state in 1989 (see Shpigel et al., 1992). Broodstocks were conditioned in the Virginia Institute of Marine Science (VIMS) hatchery and spawned separately. *C. virginica* broodstock (10 males; 5 females) was spawned 9 April 1991 and *C. gigas* broodstock (12 males; 3 females) was spawned 16 April 1991. Larvae were reared in 400 gal tanks. Water was changed every other day and a mixed diet of *Isochrysis galbana* (Tahitian), *Thalassiosira pseudonana* (3H), *T. weissflogii*, and *Chaetoceros calcitrans* was added 1-2 times per day (Barber and Mann, 1991). Eyed larvae were allowed to settle on crushed oyster shell, prior to transfer to upwellers. All water that came in contact with *C. gigas* in the hatchery was chlorinated and dechlorinated prior to release; water leaving upwellers containing *C. gigas* was released on land.

In July 1991, 600 oysters of each species were placed into each of two Nestier trays (total of four trays). One tray of each species was put into each of two flumes, receiving water from the York River, VA at one end and draining from a standpipe at the other end into a settling pond. Water flow into each flume was maintained as consistently as possible (about 20 l/min). Trays within each flume were rotated weekly to reduce the effect of uneven food availability within flumes. Both flumes were drained and flushed as needed to remove fouling organisms and biodeposits. Water temperature in the flumes was recorded (mercury thermometer) several times per week and salinity was continuously monitored from the VIMS pier on the York River. Weekly means for both were calculated from the daily readings.

Beginning in July 1991, and continuing through June 1992, mean shell height was obtained by measuring 100 randomly selected individuals (50 from each tray) of each species on a monthly basis. Dead oysters were counted and removed at the time of measurement. In June 1992, the number of oysters in each group was equalized at 450 oysters for subsequent experimentation. For *C. gigas*, this meant adding some oysters (reared and maintained in the same manner as the original oysters) and for *C. virginica*, this meant removing some oysters. In addition, several dozen live oysters infected with *P. marinus*, collected from Wreck Shoal, James River, VA were then added to one of the flumes to infect one group of each species. These are referred to as the "dosed" groups. The "undosed" groups in the other flume were subject only to potential infective agents entering via the influent. The goal of this approach was to have the "dosed" oysters receive a maximal *P. marinus* challenge and the "undosed" oysters a minimal *P. marinus* challenge.

From July 1992 through November 1992, the shell heights of 50 oysters from each group of both species were measured, and the number of dead oysters continued to be counted on a monthly basis. Growth of *C. virginica* and *C. gigas* was assessed for the entire study period by comparing mean monthly shell heights with t-tests (SYSTAT, Inc., 1992) and from July through November 1992 with 2-way ANOVA (SYSTAT, Inc., 1992). Finite monthly mortality rates were calculated for each species as the number of oysters that died over each monthly interval divided by the number of oysters alive at the beginning of the interval; cumulative mortality was calculated for each species as the sum of instantaneous mortality rates ($\log_e$ finite rate) (Krebs, 1972). Mortality of dosed and undosed groups of both species was compared using contingency table analysis (Zar, 1974, p. 296).

Physiological Rates

In May, June, July, August, September, and October 1992, 10 oysters from both dosed and undosed groups of each species were removed from the flumes for physiological rate measurements. Oysters were cleaned, and shell height (mm), whole weight (g), and volume (ml) were obtained. Estimates of clearance, absorption efficiency, oxygen consumption, and ammonia excretion were obtained for each individual at ambient temperature and salinity using the procedures described by Bayne et al. (1985) and utilized by Barber et al. (1991).

Clearance rate of actively filtering individuals was measured in a flow-through chamber in which the concentration of food particles in the inflowing water was kept constant (but greater than the clearance rate) and the particle concentration in the outflow was periodically measured with a Coulter Counter. Particle concentration in the outflow of a control chamber (containing shells only) was also measured. Clearance rate was then calculated as:

$$CR = \frac{C_i - C_o}{C_i} \times \text{flow } (l\ h^{-1}),$$

where C_i is concentration of particles flowing out of the control chamber and C_o is concentration of particles flowing out of chambers containing oysters. Clearance rate was expressed as l/hr/g.

Over the course of making clearance rate measurements, incoming water was filtered through a GFC filter to collect a sample of available seston (food). Feces (both feces and pseudofeces) from each oyster was removed from the clearance chamber with a Pasteur pipette and filtered onto a GFC filter and rinsed with distilled water. Absorption efficiency was calculated as:

$$e = \frac{F - E}{(1 - E)F}$$

where F is the ash-free dry weight:dry weight ratio of food and E is the ash-free dry weight:dry weight ratio of feces.

Oxygen consumption rate of actively respiring individuals was measured in closed chambers equipped with polarographic oxygen electrodes (Strathkelvin) and maintained at ambient temperature with a recirculating water bath. Electrodes were calibrated at 100% air saturation and <1% air saturation. Rate of oxygen consumption was expressed as ml O_2/hr/g.

For determination of ammonia (nitrogen) excretion rate, oysters were placed in 500 ml beakers containing 200 ml filtered (0.45μ) seawater, and held at ambient temperature for 16 hrs. 100 ml of water from each beaker was then filtered (0.45μ) and frozen prior to determination of ammonia concentration with an Auto-analyzer. Ammonia excretion rate was calculated from the difference between initial and final water ammonia concentrations, and expressed as mg NH_4-N/hr/g.

All rates were calculated on a (dry) weight specific basis. Mean rates of clearance, absorption, oxygen consumption, and ammonia excretion were compared both within and between species at each sampling date using 2 way ANOVA (SYSTAT, Inc., 1992).

P. marinus Infection and Condition Index

After making the physiological measurements, small pieces of gill and rectal tissue were removed from all remaining live oysters (some oysters died over the course of obtaining physiological measurements) for determination of *P. marinus* infection level using the thioglycollate method of Ray (1963). Disease intensity was reported as light (L), moderate (M), or heavy (H), based on the relative abundance of prezoosporangia found in the thioglycollate cultures (Barber and Mann, 1991).

Oysters were then shucked and tissues were frozen before being lyophilized. Dry tissue weight for each oyster was recorded before grinding the tissue into a powder. Shells were also dried and weighed. Condition index was calculated as:

$$\text{C.I. (WT)} = \frac{\text{Dry Tissue Wt. (g)} \times 1000}{\text{Dry Shell Wt. (g)}}$$

and

$$\text{C.I. (VOL)} = \frac{\text{Dry Tissue Wt. (g)} \times 100}{\text{Int. Shell Cavity Capacity (g)},}$$

where internal shell cavity capacity = whole live weight (g) - dry shell weight (g) (Walne and Mann, 1975; Lawrence and Scott, 1982).

Mean whole weight, dry tissue weight and condition index of all four oyster groups was compared statistically for each sample using 2 way ANOVA (SYSTAT, Inc., 1992).

Biochemical Composition

Proximate Biochemical composition of oyster tissue was determined using modifications of standard techniques employed by Barber et al. (1988b).

Total lipid was determined gravimetrically (after Barnes and Blackstock, 1973). A weighed portion of dried, ground tissue (50-75 mg) was mixed with 10 ml of a 2:1 chloroform-methanol mixture in a centrifuge tube. Aqueous sodium chloride solution (2 ml, 0.9%) was then added with further mixing and allowed to separate overnight at 4 °C. The lower phase (containing the lipid) was removed with a Pasteur pipet and placed in a tared, weighing pan. The solvent was evaporated under a fume hood and the lipid residue was weighed. The upper phase (containing the lipid-extracted tissue) was then filtered through a Whatman No. 1 filter, rinsed with 70% ethanol, and allowed to dry.

Total carbohydrate (glycogen) was determined using the phenol-sulphuric acid method (Dubois et al., 1956). A weighed portion (4-5 mg) of the lipid-extracted tissue was placed in a test tube with 3 ml distilled water and 5 ml concentrated sulphuric acid. The test tubes were covered and left at room temperature overnight to permit complete dissolution of the tissue. An aliquot of each was then transferred to another test tube and the volume adjusted to 2 ml with distilled water. To this, 1 ml 10% phenol and 5 ml concentrated sulphuric acid was added. The solution was mixed with a vortex mixer and allowed to cool for 30 min. Absorbance was then read at 490 nm and compared to a standard curve prepared with oyster glygogen.

Total protein was determined with the Folin phenol method (Lowry et al., 1951). A weighed portion of dried, ground tissue (5-10 mg) was placed in a test tube with 10 ml of 0.1 N NaOH. Tubes were covered, mixed, and allowed to stand overnight at room temperature. A 0.25-0.5 ml aliquot was then transferred to another test tube along with 5 ml Reagent C. Tube contents were mixed and allowed to stand at room temperature for 30 min. Reagent E (0.5 ml) was then added and mixed immediately. After a 2 hr period of color development,

absorbance was read at 750 nm and compared to a standard curve prepared with bovine serum albumin.

Ash was determined by placing 40-50 mg of dried, ground tissue into a tared crucible and combusting overnight at 450 °C.

The mass fractions of carbon and nitrogen were determined on a small portion of dried tissue from each oyster using a Carlo Erba NA 1500 Carbon-Nitrogen Analyzer.

Lipid, glycogen, protein, and ash levels (%DW) and mass fractions of carbon and nitrogen for each oyster were calculated on a dry weight basis. Means were compared between species and disease treatment groups for each sampling date using two-way ANOVA (SYSTAT, Inc., 1992).

RESULTS

Growth, Mortality and *P. marinus* Infection

Mean temperature in the flumes ranged from a low of 4.9 °C in January 1992 to a high of 29.5 °C in July 1991 (Figure 1). Temperature generally decreased from September 1991 to February 1992 and increased from March to August 1992. Mean salinity in the York River ranged from a low of 17.6 ppt in May 1992 to a high of 23.9 ppt in November 1991 (Figure 1). Salinity below 20 ppt was recorded from April to July 1992 and again in September 1992.

Mean shell height of *C. gigas* was significantly greater ($P \leq 0.05$) than mean shell height of *C. virginica* in all but four of the months sampled (Figure 2). Growth in both species was greatest in the fall and spring months and least in the winter and summer months. By November 1991, *C. gigas* had attained a mean shell height that was about 10 mm greater than that of *C. virginica*; this differential was maintained throughout the study. In November 1992, at age 1.5 years, *C. gigas* averaged 55.3 mm in shell height while *C. virginica* averaged 41.2 mm in shell height.

There were clear differences between oyster species in both cumulative mortality and the times at which greatest mortality occurred (Figure 3). Monthly mortality rates were greatest for *C. virginica* in September (21%), October (31%), and November (14%) 1992. Monthly mortality rates for *C. gigas* were greatest during two periods, April (12%) - May (22%) and September (24%) 1992. At the end of the study in November 1992, percent cumulative mortality of *C. gigas* was 70 % and that of *C. virginica* was 59%.

P. marinus was not detected in *C. virginica* in June 1992, prior to dosing (Figure 4). The dosed group had a 30% prevalence in July; this increased to 100% in August, September, and October. *P. marinus* was first detected in the undosed group in September (78% prevalence) and again in October (100% prevalence). Infection intensity increased rapidly in the dosed group, as heavy infections were seen in the dosed group in August (2), September (3), and October (5); no heavy infections occurred in the undosed group (Table 1). Thus *P. marinus* became readily established and infections progressed rapidly in the dosed *C. virginica* group. In the undosed group, infection occurred later (probably coming into the flumes via the influent) and did not progress to advanced stages, even by the end of the study.

In June 1992, no *P. marinus* was found in *C. gigas*, prior to dosing (Figure 4). The dosed *C. gigas* group had a 20% prevalence in July, followed by an 80% prevalence in both August and September and a 70% prevalence in October. In the undosed group, prevalence was 50% in September, but only 10% in October. All infected oysters in both *C. gigas* groups had light infections, except for one heavy infection which was found in the dosed group in August (Table 1). Thus *C. gigas* became infected with *P. marinus*, but prevalences were lower than those seen in *C. virginica* and actually decreased slightly (in both dosed and undosed groups) between September and October. At the same time, all but one infection was light, indicating that progression of the disease in *C. gigas* was limited compared to *C. virginica*.

From July through November 1992, mean shell height was significantly related ($P \leq 0.05$) to species but not to exposure to *P. marinus* (Figure 5). Thus shell height of *C. gigas* was greater than that of *C. virginica* in all months and no difference in shell height was detected between dosed and undosed groups of either species. It should also be noted, however, that no increase in mean shell height (growth) of either species occurred over this time period.

Percent monthly mortality of *C. virginica* in July, August and November 1992 was similar for dosed and undosed groups; in September and October 1992, however, mortality was significantly greater ($P \leq 0.05$) in the dosed groups than in the undosed groups (Figure 6). In September, mortality of *C. virginica* was 24% in the dosed group and 17% in the undosed group; in October, dosed mortality was 41% and undosed mortality was 21%. For *C. gigas*, percent monthly mortality was similar in dosed and undosed groups in July, August, September, and November; in October, however, mortality in the dosed group (11%) was significantly greater ($P \leq 0.05$) than in the undosed group (4%) (Figure 6).

Condition Indexes

The shell heights, volumes, whole weights, shell dry weights, and both condition (WT) and condition (VOL) for all oysters (dosed and undosed for both species) examined from May through October 1992 are listed in Table 2. The number of replicates was low for *C. gigas* in September due to mortality. Mean whole weight, tissue dry weight, and condition index (C.I. (WT) and C.I. (VOL)) were compared statistically each month for the effects of species, disease treatment, and species-disease interaction ($P \leq 0.05$). A summary of statistically significant differences is given Table 5.

Mean whole weight increased between June and July, but remained fairly constant for all goups from July through October, ranging from 15.4g (October, *C. virginica*, dosed) to 24.6g (August, *C. gigas*, undosed) (Figure 7). Mean whole weight of *C. gigas* was significantly greater than that of *C. virginica* in May, July, August, September, and October. In August and October, mean whole weight was significantly lower in dosed oysters than in undosed oysters. There were no significant species-disease interactions.

Mean tissue dry weight varied little over the course of the study. Maximum mean dry weight was 0.51g (August, *C. gigas*, undosed) and minimum mean dry weight was 0.24g (September, *C. virginica*, dosed) (Figure 8). Mean dry tissue weight of *C. gigas* was significantly greater than that of *C. virginica* in June and August. There was a significant species-disease interaction in August. Dosed oysters had a significantly lower mean dry weight than undosed oysters in October.

Mean shell dry weight for both species increased from 5-10 g in May and June to over 10 g (for most groups) in July through October (Figure 9). Mean shell dry weight of *C. virginica* was significantly greater than that of *C. gigas* in June, July, August, and October. In August and October, dosed groups had a significantly lower mean shell dry weight than undosed groups. There was a significant species-disease interaction in July.

Mean C.I. (WT) was relatively constant over the course of this study, ranging from 21.6 (October, *C. virginica*, dosed) to 44.7 (May, *C. gigas*, undosed) (Figure 10). In July, August, and October, mean C.I. (WT) was significantly greater for *C. gigas* than for *C. virginica*. Mean C.I. (WT) was significantly lower in dosed oysters than undosed oysters in October. There was a significant species-disease interaction in August.

Mean C.I. (VOL) gradually decreased from May to October. Mean C.I. (VOL) was 17.1 in May (*C. virginica*, undosed) and 2.7 in October (*C. gigas*, dosed) (Figure 11). *C. virginica* mean C.I. (VOL) was significantly greater than *C. gigas*

C.I. (VOL) in all months. Mean C.I. (VOL) was significantly lower in dosed than undosed oysters in October. Species-disease interactions were significant in August and September.

Physiological Rates

Clearance rates, absorption efficiencies, oxygen consumption rates, and ammonia excretion rates for undosed and dosed oysters of both species, from May through October 1992, are given in Table 3. The number of replicates was low for *C. gigas* in September due to mortality. Means of each were compared statistically for species, disease treatment, and species-disease interactions for each month ($P \leq 0.05$). A summary of statistically significant differences is given Table 5.

Mean clearance rates were greatest in June and July, particularly for *C. gigas*, when means were 11.3 l/hr/g (Figure 12). In June and September, mean clearance of *C. gigas* was significantly greater than that of *C. virginica*. In August, mean clearance rate of *C. virginica* was significantly greater than that of *C. gigas*. In September, mean clearance rate of dosed oysters was significantly lower than that of undosed oysters. Mean clearance of dosed oysters in October was significantly greater than that of undosed oysters.

Mean absorption efficiency was variable with no obvious seasonal component, ranging from 24% (July, *C. virginica,* dosed) to 59% (September, *C. virginica,* undosed) (Figure 13). Due to a lack of feces production, no data was obtained for the July dosed *C. gigas* group nor for any August groups. There were no differences in mean absorption efficiency between species and there were no significant species-disease interactions. The only significant difference was in July when dosed oysters had a lower absorption efficiency than undosed oysters.

Mean oxygen consumption rate increased between from about 1 ml/hr/g in May to over 4 ml/hr/g in August and decreased from August to October (Figure 14). Mean oxygen consumption was similar for both species and both disease treatments in all months. There were significant species-disease interactions in July and August, however.

Mean ammonia excretion rate was highly variable, but generally increased between May and September (Figure 15). Lowest mean ammonia excretion rate was 11.1 ug/hr/g (June, *C. virginica*, undosed) and the highest mean ammonia excretion rate was 121.2 ug/hr/g (September, *C. gigas*, dosed). In June, July, September, and October mean ammonia excretion rate of *C. gigas* was significantly greater than that of *C. virginica*. There were no differences between dosed and undosed groups, but there was a significant species-disease interaction in October.

Biochemical Composition

Percent (dry weight) lipid, glycogen, protein, ash, and mass fractions of carbon and nitrogen for all oysters sampled are given in Table 4. The number of replicates was low for *C. gigas* in September due to mortality. The mean levels of each biochemical component were compared statistically each month between species, disease treatment, and species-disease interaction ($P \leq 0.05$). A summary of statistically significant differences is given Table 5.

Mean lipid level ranged from a low of 8.7% (July, *C. gigas*, undosed) to a high of 16.6% (June, *C. virginica*, undosed) (Figure 16). In May and June, mean percent lipid was significantly greater for *C. virginica* than for *C. gigas*. In July, September, and October, dosed oysters had a significantly lower lipid level than undosed oysters. Also in July, there was a significant species-disease interaction.

Mean glycogen level gradually increased from about 12% in May, to 29.4% (October, *C. virginica*, undosed) (Figure 17). Mean percent glycogen of *C. gigas* was significantly greater than that of *C. virginica* in June, but significantly lower than that of *C. virginica* in July. In October, mean percent glycogen was significantly lower in dosed than undosed oysters. There were no significant species-disease interactions.

Mean protein level was lower in May and June (<26%) than July through October (30-40%) (Figure 18). Mean percent protein was significantly greater for *C. gigas* than *C. virginica* in May. Dosed oysters had significantly lower mean percent protein than undosed oysters in July. There were no significant species-disease interactions.

Mean ash level was generally less than 10% in all months (Figure 19). Mean percent ash was significantly greater for *C. virginica* than for *C. gigas* in May, but significantly greater for *C. gigas* than for *C. virginica* in June and July. There were no effects of disease treatment on mean percent ash but there was a significant species-disease interaction in September.

The mean mass fraction of carbon was close to 40% in all months for both species and disease treatments (Figure 20). *C. virginica* had a significantly greater mean mass fraction of carbon than *C. gigas* in all months except October. In July and August, dosed oysters had a significantly greater mean mass fraction of carbon than undosed oysters. In October, dosed oysters had a significantly lower mean mass fraction of carbon than undosed oysters. Significant species-disease interactions occurred in August and September.

The mean mass fraction of nitrogen ranged from 6.1% (May, *C. virginica*, undosed) to 8.9% (July, *C. gigas*, undosed) (Figure 21). In May and July, mass

fraction of nitrogen was significantly greater in *C. gigas* than in *C. virginica*. In July, dosed oysters had a significantly lower mass fraction of nitrogen than undosed oysters. There were significant species-disease interactions in July and August.

DISCUSSION

Growth, Mortality and *P. marinus* Infection

P.marinus infections became established in the dosed groups of both species of oysters within one month of being introduced. The undosed groups of both species became infected (to a lesser extent) three months after the dosed groups. Undoubtedly these infections resulted from infective stages of *P. marinus* entering the flume via the influent water. Infections in the undosed groups, however, never developed into heavy infections in either species, indicating that either too few infective stages got into the flume or the ones that did get in arrived too late in the summer to result in advanced cases of the disease. Thus the goal of attaining two contrasting levels of disease challenge was achieved.

This study confirms previous work demonstrating the relative tolerance of these two oyster species to intense *P. marinus* challenge (Meyers et al. 1991). *C. virginica* had 100% prevalence and heavy (fatal) infections within two months of exposure to the parasite. *C. gigas*, on the other hand, although becoming infected with *P. marinus*, had a lower maximum prevalence (80%) that actually decreased over time. Only 1 heavy infection was found in the *C. gigas* groups; all other infections were light. Obviously, *C. gigas*, even though susceptible to initial infection, is somehow able to inhibit development of the disease and even reduce infection prevalence. Differences in potential defense mechanisms between these two species are discussed by La Peyre (1993).

By age six months, *C. gigas* had attained an approximate 10 mm shell height advantage over *C. virginica*. This statistically significant size advantage was maintained throughout the remainder of the 19 month study period. Of interest is the fact that both species had very similar growth patterns. Most growth occurred in the spring and fall; almost no growth took place in the winter when water temperature fell below 10 °C and summer when water temperature rose above 25 °C. This is reflective of the optimal temperature ranges of both species (Galtsoff 1964; Mann et al. 1991).

Since a reduction in growth occurred in the summer months of July through September in both 1991(without *P. marinus* infection) and 1992 (with *P. marinus* infection), the cause of reduced growth in the summer is most likely caused by above-optimal water temperatures rather than disease. The fact that there was no

relationship between shell height or dry tissue weight and disease treatment in July, August, and September 1992 adds support to this contention. A previous study (Paynter and Burreson 1991), indicating that growth in *C. virginica* is inhibited by *P. marinus*, was not supported by the results of this study. Menzel and Hopkins (1955) also report reduced growth (based both on whole weight and shell height) in oysters heavily infected with *P. marinus.*

Growth rate of *C. virginica* in this experiment, however, was lower than that observed in trays directly in the York River by Barber and Mann (1991). After 18 months in the flumes, mean shell height of *C. virginica* was 41.2 mm, but after 18 months *in situ*, mean shell height was 64.6 mm (Barber and Mann 1991). Presumably the growth rate of *C. gigas* was similarly less than maximal in the flumes. Lower growth overall in the flumes was most likely the result of limited food availability, in spite of attempts to prevent it.

During August and September 1992, there was a major bloom of the dinoflagellate, *Cochlodinium sp.*, in the York River. Although not directly toxic to oysters, this species has questionable nutritional value (Luckenbach et al. 1993), and may have contributed at least indirectly to the lack of growth (and increased mortality, see below) seen in both species at this time.

Over the 19 month study period, cumulative mortality of *C. gigas* was 70%, compared to 59% for *C. virginica*. Greatest mortality of *C. gigas* occurred during two periods, April-May of 1991 and September 1992, while most mortality of *C. virginica* took place from September-November 1992. These differences in mortality patterns between the two species are related to the most likely causes of mortality.

As reviewed by Mann et al. (1991), the optimal salinity for growth and spawning of *C. gigas* is 20-35 ppt; the optimal salinity for *C. virginica* is 5-30 ppt (Galtsoff, 1964). In this study, salinity was below 20 ppt for an extended period, including April-May 1991. Thus the most obvious explanation for the mortality of *C. gigas* during April-May 1991 is a lack of tolerance to salinity below 20 ppt. The physiological stress experienced by *C. gigas* due to unfavorable salinity may have been exacerbated by processes related to gametogenesis. March to May is the period of gonadal maturation and spawning in *C. gigas* in this location (Barber, unpublished data). In Japan and Washington State, mortalities of *C. gigas* have been associated with periods of maximal gametogenic activity (Perdue et al. 1981; Beattie et al. 1988). Also, food coming into the flumes may have been insufficient to support both growth and gametogenesis. Thus there were several possible physiological stressors contributing to the mortality of *C. gigas* in April and May 1992, but salinity appears to be the primary factor.

The second period of mortality of *C. gigas* was in September 1992, but

given the facts that infection by *P. marinus* was light and that mortality was similar in both dosed and undosed groups, it is unlikely that this mortality was related to *P. marinus*. September 1992 was another period during which salinity in the York River dropped below 20 ppt. It is possible that less than optimal salinity, combined with the bloom of *Cochlodinium sp.*, was the primary factor causing mortality. Even though mortality in the dosed *C. gigas* group was significantly greater than in the undosed group in October 1992, it was about half that observed in September, and given the light intensities of infected oysters, probably not disease related.

Unlike *C. gigas*, mortality in *C. virginica*, greatest in September, October and November 1992, was more closely related to prevalence and intensity of *P. marinus* infections. Appreciable mortality did not occur in *C. virginica* until after the *P. marinus* became established in the flumes. Mortality in the dosed *C. virginica* group was significantly greater than in the undosed group in both September and October in conjunction with the development of moderate and heavy infections. The relatively high mortality that also occurred in the undosed groups in September and October, in spite of a lack of advanced *P. marinus* infections, could have been related to effects of the *Cochlodinium sp.* bloom in combination with maximum water temperatures. Thus it is likely this bloom was indirectly responsible for the mortalities seen in August and September in the dosed *C. gigas* group as well as the undosed groups of both species. Note that mortality in these groups was reduced in November, after the the bloom had subsided and water temperature had declined to below 20 °C.

Condition Indexes

In this study, the whole weight of *C. gigas* was significantly greater than that of *C. virginica* in 5 of the 6 months examined. There was no clear difference, however, in dry tissue weight between species, but there was a clear difference between species in dry shell weight, as *C. virginica* had a significantly greater shell weight than *C. gigas* in 4 of 6 months examined. Thus if *C. gigas* had a greater whole weight than *C. virginica* but a lower dry shell weight and a similar dry tissue weight, the difference in whole weight must be due to a greater water weight, as whole live weight includes the weight of shells, tissue, and internal water. It follows then that *C. virginica* actually had a greater dry meat weight to shell height ratio than *C. gigas.*

It is interesting to note that even though shell height of *C. gigas* was significantly greater than that of *C. virginica* virtually throughout the study period, shell weight of *C. virginica* was significantly greater than that of *C. gigas*. Thus the shells of *C. virginica* are more robust than those of *C. gigas*, at least at an early age. This would suggest that *C. virginica* might be less vulnerable than *C. gigas* to predation by oyster drills and crabs which penetrate the shells of young oysters. An experiment designed to examine the relative predation rates of the two oyster

species by major predators would be enlightening.

From a commercial standpoint, condition refers to the quality or "fatness" of an oyster (Galtsoff, 1964). Recognizing that the quality (weight or volume of oyster tissue to its internal shell volume) fluctuates between locations and times of the year led to the determination of a condition index, which could be used to rapidly assess oyster quality, either between locations or between different seasons. Several indexes have been utilized over time, with the two being employed in this study the most common variants. One (C.I. (WT)) is the ratio of dry tissue weight to dry shell weight (Walne and Mann, 1975) and the other (C.I. (VOL)) is the ratio of dry tissue weight to internal shell cavity capacity, or the difference between whole live weight and dry shell weight (Lawrence and Scott, 1982). Crosby and Gale (1990) suggest that C.I. (VOL) be adopted as a standard index of condition, as it is the easiest to use, has a lower coefficient of variation, and is the most meaningful as an index of nutritional status and recent stress.

Numerous factors, both physiological and environmental, can influence condition index. Thus condition is reduced as food becomes limiting or as metabolic demands increase (Bayne et al., 1985). Oyster condition is typically lower after spawning, but recovers as glycogen reserves are accumulated (Soniat and Ray, 1985; Barber et al., 1988a, 1988b). Pollution has been shown to reduce condition index in oysters (Scott and Lawrence, 1982). Newell (1985) and Barber et al. (1988a) demonstrated that condition index in oysters (*C. virginica*) infected with the parasite *Haplosporidium nelsoni* was significantly lower than in uninfected oysters. Similarly, a reduction in condition index of *C. virginica* infected by *P. marinus* was reported by Crosby and Roberts (1990) and Dittman (1993).

The two methods of assessing condition in this study gave different results. *C. gigas* had a significantly greater C.I. (WT) than *C. virginica* in July, August, and October 1992. This was imparted (as discussed above) by the lower dry shell weight of *C. gigas*. On the other hand, *C. virginica* had a significantly greater C.I. (VOL) than *C. gigas* in all 6 months examined, including the months in which C.I. (WT) was greater for *C. gigas* than for *C. virginica*. The greater C.I. (VOL) of *C. virginica* was imparted by the smaller whole weight and the greater shell weight which together resulted in a smaller internal shell cavity capacity.

In this study the only difference in condition attributable to *P. marinus* was in October, when both indexes were significantly lower in dosed than in undosed oysters. The lack of a significant relationship in months other than October may be the result of relatively few replicates, the varying levels of disease intensity within dosed groups, and also the fact that even the undosed groups were infected with *P. marinus* in September. More likely, however, is the possibility that since the oysters in this experiment were first exposed to *P. marinus* in June 1992, it took all summer for the disease to progress to a stage where deleterious physiological

effects were occurring. Both C.I. (WT) and C.I. (VOL) were lower in dosed than undosed *C. virginica* in September, and mortality was greatest for both species in September and October 1992. Thus it appears that *P. marinus* does negatively affect condition index, at least after an entire season of exposure; this implies that *P. marinus* has a long term negative energetic impact on infected oysters.

There were three significant species-disease interactions with respect to condition index. In August, C.I. (WT) and C.I. (VOL) of *C. virginica* were greater in dosed than undosed oysters, while C.I. (WT) and C.I. (VOL) of *C. gigas* was greater in undosed than dosed oysters. In September, C.I. (VOL) of *C. gigas* was greater in dosed than undosed oysters while that of *C. virginica* was greater in undosed oysters than in undosed oysters. Thus there was no consistent trend with respect to disease effects on condition index until October.

Physiological Rates

In this study we examined the effects of both species and disease on several physiological rates. Our rationale was that there might be observable differences between species that might explain differing levels of disease tolerance or between dosed and undosed groups that might help us understand the effects of *P. marinus* on the oyster host. The various physiological rates are essentially components of the overall energy budget of an organism. Differences in any of these between species or between disease treatments would help explain observed differences in condition index or in disease tolerance, as previously described.

Clearance rate, measured as the volume of water cleared of suspended particles per unit time per unit body weight, represents the ability of the organism to acquire food. Gill filaments, covered with cilia, create water currents that draw food particles into the mantle cavity which are then filtered from the water and funneled to the mouth. Clearance rate is affected by several factors, the most important of these being temperature and food concentration (Bayne et al., 1985).

If feeding ability is impaired, less energy would be available to the organism for normal processes, such as growth, reproduction, and defense. The mode of invasion of *P. marinus* is unknown, but may be via the gills, since the gills would be the first contact point for invading parasites. If gill function were inhibited, the oyster would be less able to feed efficiently and would thus be at an energetic disadvantage. Newell (1985) found that oysters (*C. virginica*) with systemic infections of *H. nelsoni* had significantly reduced clearance rates compared to uninfected oysters. Barber et al. (1991) noted that *C. virginica* selected for resistance to *H. nelsoni* exhibited significantly greater clearance rates than unselected oysters during the period of heaviest parasite challenge. Mackin and Ray (1954) used feces production as a measure of gill ciliary activity and concluded that *P. marinus* negatively impacts feeding ability in *C. virginica*. Newell et al.

(1994) also found that oysters with advanced *P. marinus* infections exhibit a reduced clearance rate.

With respect to this study, clearance rate of *C. gigas* was significantly greater than that of *C. virginica* in June, prior to disease infection, and in September, after *P. marinus* infections were well established. This might indicate that *C. gigas* is able to feed more efficiently than *C. virginica* prior to disease challenge and after infections have become established. In August, clearance rate was significantly ($P \leq 0.05$, t-test) lower overall (both species combined) than in July in spite of similar water temperature. This reduction was most likely due to the presence of *Cochlodinium sp.* at the time the measurements were made. More evidence that feeding was suppressed at this time is the fact that no feces was produced by either species in August (see below). In September, clearance rate was again suppressed in all groups except the undosed *C. gigas*. This resulted in a significant species effect (*C. gigas* greater than *C. virginica*), a significant disease effect (undosed greater than dosed), and a significant species-disease interaction- for *C. gigas*, clearance rate of dosed oysters was significantly ($P \leq 0.05$, t-test) lower than that of undosed oysters. In October, however, dosed oysters had a significantly greater clearance rate overall than undosed oysters, and there was no difference between the two species. Thus there is little that can be concluded regarding differences in clearance rate either between species or between disease treatments.

In filter feeding bivalves such as oysters, food particles removed from the ventilation current are transported via the mouth to the stomach and digestive gland for digestion and absorption (Bayne et al., 1985). Absorption efficiency, as determined in this study utilizing the Conover ratio, ideally provides information as to the ability of an organism to assimilate ingested food. The efficiency with which food can be absorbed is determined by several factors, including environmental factors, the type of food, the physiological condition of the animal, and the amount of food (Bayne et al., 1985).

One of the first places that *P. marinus* can be seen in histological sections is in the gut epithelium. Thus not only is it possible that the parasite invades the oyster host via the digestive tract, it is also possible that normal assimilation processes are negatively affected. If this were the case, less energy would be absorbed even if the same amount of food was ingested.

The results of this study did not reveal any differences in absorption efficiency between the two oyster species. In July, and again in September, however, absorption efficiency of *C. virginica* was significantly lower in the dosed group than in the undosed group. Thus is appears that *P. marinus* has a negative effect on the ability of *C. virginica* to assimilate food, which may eventually lead to a negative energy status and death. Due to a lack of feces production, no data

was obtained in August. This possibly was due to a decline in feeding activity caused by the *Cochlodinium sp.* bloom, as mentioned above. In September, however, when clearance rate was also suppressed, feces were produced, and assimilation efficiencies were similar to other months. Newell et al. (1994) found no difference in assimilation efficiency between *C. virginica* heavily infected and uninfected with *P. marinus*. Thus is does not appear that *C. virginica* and *C. gigas* differ in their ability to assimilate food but that *P. marinus* does negatively impact absorption efficiency in *C. virginica*.

Oxygen consumption is a measure of overall metabolic rate, or the energy required to sustain life (Bayne et al., 1985). It is influenced by environmental factors such as temperature, salinity, and dissolved oxygen, but can also be affected by other physiological processes such as gametogenesis. Presumably the increased metabolic demand imposed by a parasite would be reflected as an increase in oxygen consumption. To date, no difference in oxygen consumption rate between *C. virginica* uninfected and infected with *H. nelsoni* or *P. marinus* has been found (Newell, 1985; Newell et al., 1994). Barber et al. (1991) did note a significantly greater rate of oxygen consumption in *C. virginica* selected for resistance to *H. nelsoni* than in unselected oysters.

Oxygen consumption rate, as measured in this study, exhibited a normal seasonal trend in that overall rates were correlated with water temperature. No significant differences in oxygen consumption rate were found either between species of oyster or between dosed and undosed groups. There were, however, significant species-disease interactions in July, August, and October. In both July and August, the dosed *C. gigas* group had a considerably greater oxygen consumption rate than the undosed group while the dosed *C. virginica* group had a lower rate than the undosed group. In October, the dosed *C. virginica* mean was greater than the undosed mean while the dosed *C. gigas* mean was lower than the undosed mean. It may be noteworthy that oxygen consumption rate in dosed *C. gigas* groups in both July and August was significantly ($P \leq 0.05$, t-test) greater than in the respective undosed groups. This may indicate that active defense mechanisms were at work, especially early in the infection period, and would support the previous finding that resistant *C. virginica* have increased oxygen consumption rates when challenged by *H. nelsoni* (Barber et al., 1991). Thus an increase in oxygen consumption rate may indicate the invocation of defense related activities including an increase in the number or activity of circulating hemocytes or the production of lectins or lysozymes (see La Peyre, 1993).

A small portion of absorbed energy is excreted as metabolic waste products. In aquatic organisms, the catabolism of proteins results in the formation of nitrogenous waste products, most notably, ammonia (Bayne et al., 1985). Ammonia excretion rates vary seasonally and as a result of environmental stress. Parasitism represents a form of stress, which could alter protein catabolism and

thus ammonia excretion. Very little is known about the effect of parasitism on ammonia excretion rates, *per se*, but in general, nitrogen excretion increases and the O:N ratio decreases with increasing stress, presumably due to an increase in protein catabolism (Bayne et al., 1985).

In this study, *C. gigas* had a significantly greater ammonia excretion rate than *C. virginica* in June, July, September, and October. This indicates that *C. gigas* is catabolising more protein than *C. virginica* at these times, but there is no indication that this difference is related to parasitism by *P. marinus*. There simply may be genetic differences in nitrogen metabolism between species, or this may be indicative of a stressed condition and the catabolism of protein.

Biochemical Composition

It is widely recognized that marine bivalves, including oysters, undergo annual cycles of energy storage and utilization that can be characterized by proximate biochemical composition- the levels of lipid, carbohydrate, protein, and ash. Most often these cycles are related to gametogenic processes in that energy is stored prior to gametogenesis as glycogen, and subsequently utilized in the manufacturing of gametes (Barber and Blake, 1992). Lipid can be either polar (structural) or neutral. Neutral lipids (primarily triacylglycerols) are stored in digestive gland tissue when food is plentiful and in developing ovaries. Glycogen is the primary energy storage component of bivalves, being accumulated in mantle tissue or adductor muscle prior to gametogenesis. Protein is primarily structural, but may be catabolized when glycogen and lipid reserves are depleted. Ash is primarily inorganic material (salts) and has no established metabolic function.

When food is scarce or stressors such as parasitism divert energy resources, normal nutrient storage cycles and gametogenic processes are impaired. Thus Barber et al. (1988b) found a significant reduction in the glycogen content of oysters lightly infected with *H. nelsoni* and significant reductions in glycogen and protein contents of oysters heavily infected with *H. nelsoni*. It follows that decreased nutrient storage capability due to competition for available nutrients from the parasite, would account for the observed reduction in fecundity (Barber et al., 1988a).

In the present study, there were several noteworthy effects of either species or disease treatment on biochemical composition. Percent lipid of *C. virginica* was significantly greater than *C. gigas* in both May and June, prior to infection of either species with *P. marinus*. This may be the result of increased energy acquisition, although neither clearance rate nor absorption efficiency differed between species over this time period. More likely the difference in lipid composition is the result of differing levels of gametogenic activity at this time of the year. In Virginia, *C. virginica* undergoes gametogenesis in May and June and typically begins spawning

in late June or early July (Mann et al., 1994); the gametogenic cycle of *C. gigas* at this location was not determined as part of this study, but spawning probably occurs earlier in the year (Barber, unpublished data). With respect to disease effects, undosed oysters had significantly less lipid than dosed oysters in July, September, and October, (in July, this was entirely due to *C. gigas*). So rather than depleting lipid stores, *P. marinus* appears to augment lipid level. This is perhaps due to *P. marinus* having a higher lipid level than oyster tissue.

Percent glycogen was greater for *C. gigas* than *C. virginica* in June which corresponds to the significantly greater clearance rate exhibited by *C. gigas* at this time. In July, however, percent glycogen was greater for *C. virginica* than *C. gigas*. Otherwise, there were no obvious differences in glycogen level between species. Undosed oysters (both species) in September and October had the highest glycogen contents overall, which corresponds to normal nutrient cycling in oysters in which glycogen is accumulated in the fall after water temperatures begin to decline (Barber et al., 1988b). With respect to disease effects, dosed *C. virginica* had a significantly lower (t-test, $P \leq 0.05$) glycogen level than undosed *C. virginica* in September. In October, dosed oysters (both species) had lower glycogen levels than undosed oysters. Thus *P. marinus* has a negative effect on glycogen level, and the effect is greater for *C. virginica* than *C. gigas*. This corresponds to the greater susceptibility of *C. virginica* to *P. marinus* compared to *C. gigas*. These results suggest that *P. marinus*, like *H. nelsoni,* outcompetes the oyster host for glycogen reserves (Barber et al., 1988b). This may contribute ultimately to mortality, especially in the case of *C. virginica*.

Protein level of *C. gigas* was significantly greater than that of *C. virginica* in May, prior to dosing with *P. marinus*. In July, undosed oysters had a significantly greater protein level than dosed oysters, but there is no reason to suspect that this difference was related to parasitism, as only 5 out of 20 dosed oysters had light infections. Protein level was similar for all groups in all other months.

Ash level was significantly greater for *C. virginica* than *C. gigas* in May but significantly greater for *C. gigas* than *C. virginica* in June and July. There is no obvious reason for these species related differences. There were no differences in ash level that were related to disease treatment, although there was a significant interaction in September, with the dosed *C. gigas* group having a significantly lower (t-test, $P \leq 0.05$) ash level than the undosed group. Again, ash has no energetic value.

The mass fraction of carbon was significantly greater for *C. virginica* than *C. gigas*in all months examined except October. This indicates that in general, *C. virginica* had a higher energy content than *C. gigas* up until the metabolic effects of *P. marinus* became apparent (October was the month of greatest mortality of *C. virginica*). This agrees with the fact that C.I. (VOL) was consistently greater for *C.*

virginica than *C. gigas*. In addition, total carbon of dosed groups of both species was significantly lower than for undosed groups in October, indicating further that prolonged exposure to *P. marinus* was having a negative metabolic effect. In July and August, however, dosed oysters had significantly more total carbon than undosed oysters, with the difference in August being greater for *C. virginica* than *C. gigas*. This may be a reflection of the greater parasite burden in the dosed groups, especially if *P. marinus* contains more lipid than oyster tissue, as indicated above.

Percent total nitrogen was significantly greater for *C. gigas* than *C. virginica* in May and July. This may reflective the greater protein level observed for *C. gigas* in May. In concert with the greater ammonia excretion rate exhibited by *C. gigas*, it may also reflect a greater reliance on protein as a metabolic substrate compared to *C. virginica*. Alternatively, there simply may be a genetic difference in overall nitrogen metabilism between the two species. In July, dosed oysters had significantly lower total nitrogen than undosed oysters, but since no differences were noted later in the study, it is difficult to ascribe this difference to the exposure of dosed groups to *P. marinus*.

CONCLUSIONS

This study sought to answer questions relating to the pathological effects of the pathogen *P. marinus* on the eastern oyster, *C. virginica*. There was no evidence that *P. marinus* negatively affects clearance rate, but there was indication that *P. marinus* does negatively affect absorption efficiency. *P. marinus* also increases host lipid level, perhaps due to its own composition and after becoming moderate and heavy in intensity, results in decreased glycogen level. The lower absorption efficiency and lower glycogen level are eventually reflected in a lower mass fraction of carbon, a lower tissue weight and a lower condition index. Thus besides overwhelming the cellular defense mechanisms of *C. virginica*, as previously suggested, *P. marinus* becomes a metabolic burden to the oyster host and eventually causes mortality.

This study also sought to characterize the physiological (=energetic) basis for resistance to *P. marinus* exhibited by the Pacific oyster, *C. gigas*. Oxygen consumption rate is greater in *C. gigas* challenged by *P. marinus*, indicating an active response (perhaps defense related) to the parasite. *C. gigas* also had a greater ammonia excretion rate than *C. virginica*, but it is unclear how this may relate to parasite tolerance.

Although not a stated objective, the differences in overall growth and mortality of the two oyster species was important, given recent discussions regarding the proposed introduction of *C. gigas* to the mid-Atlantic region. Based

on these findings, *C. gigas* grows faster than *C. virginica* but also has a higher mortality rate. Most of the mortality of *C. gigas* can be attributed to a lack of tolerance of salinity below 20 ppt. There is also indication, based on the lower condition index and greater ammonia excretion rate, that *C. gigas* is environmentally stressed, even at times when salinity is above 20 ppt. In addition, this study verified the previous report of resistance of *C. gigas* to *P. marinus.*

In spite of these accomplishments, a failure to more adequately address the stated objectives was aided by at least two factors. First and foremost is the fact that oysters are inherently variable in their physiological status at any given time. Another 5-10 replicates may have reduced variability sufficiently to reveal more significant species or disease effects, but because of the scope of the project, we were unable to accomodate any more replicates. Second, the occurrence of the red tide (*Cochlodinium sp.*) was unexpected and probably affected physiological responses in August and September of 1992, when *P. marinus* infections were intensifying.

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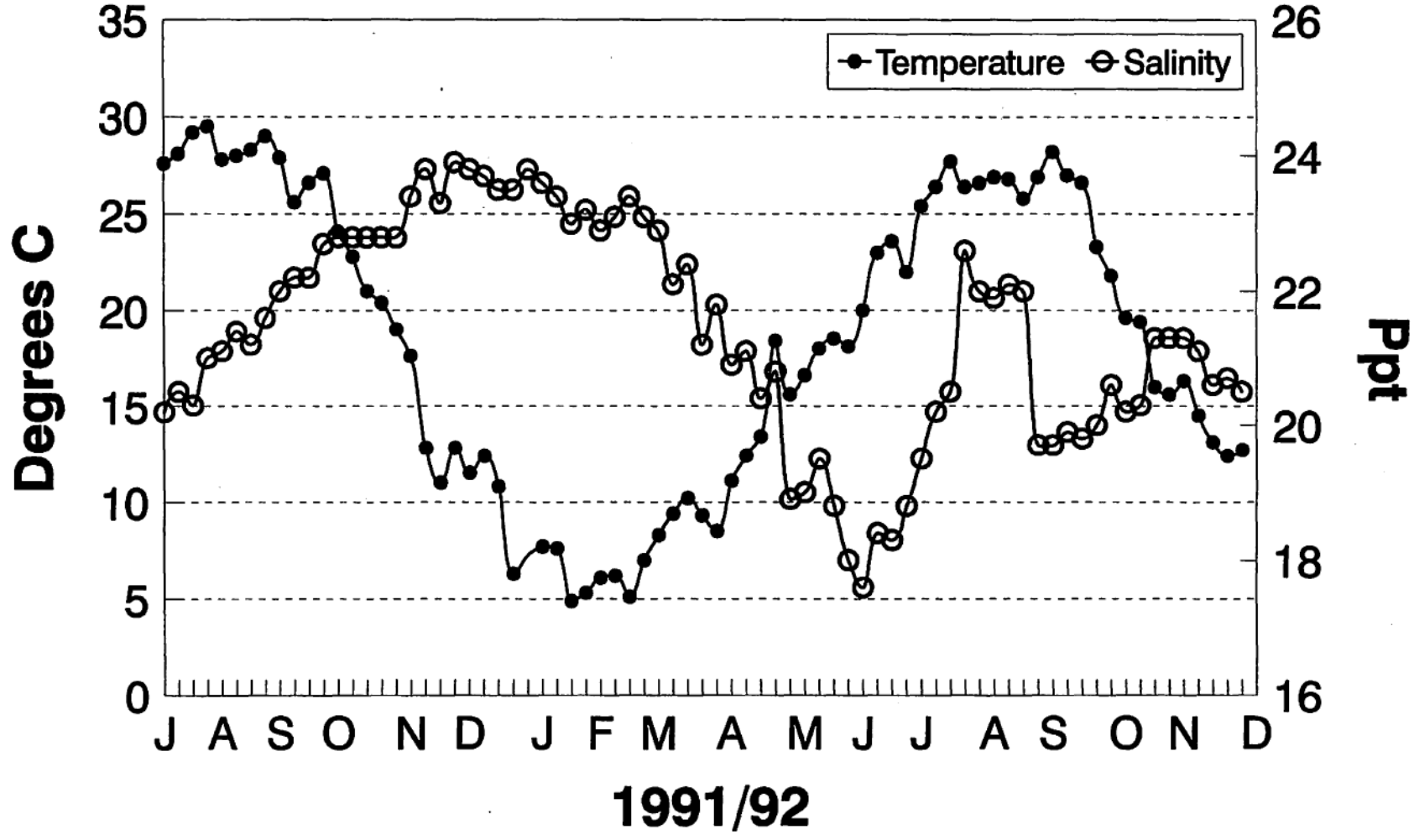

Figure 1. Weekly means of temperature (Y1) and salinity (Y2) of the York River, VA entering the flumes from July 1991 to December 1992.

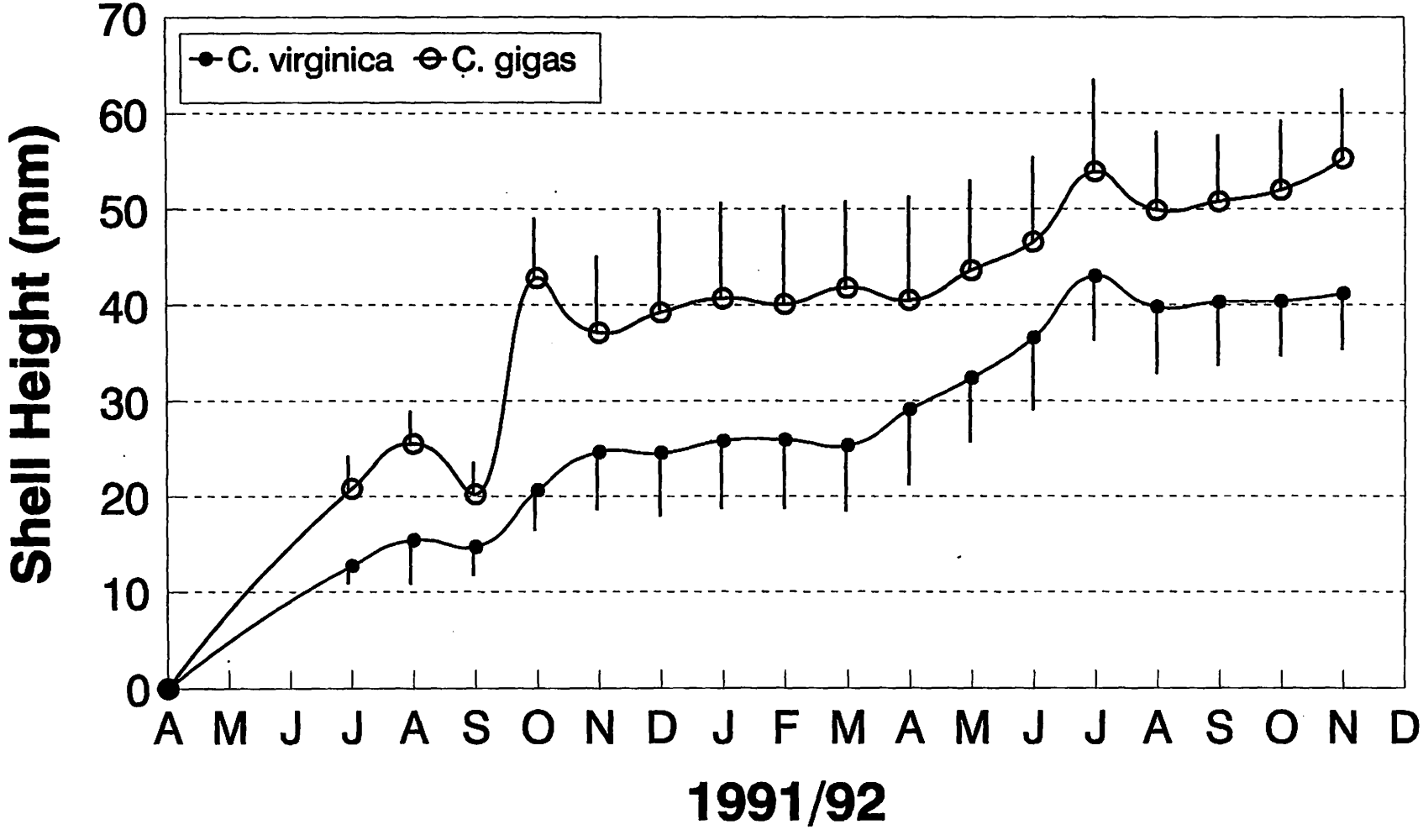

Figure 2. Mean (+/- 1SD) shell height of C. virginica and C. gigas from July 1991 to November 1992. Means after June 1992 include undosed and dosed groups; n = 100.

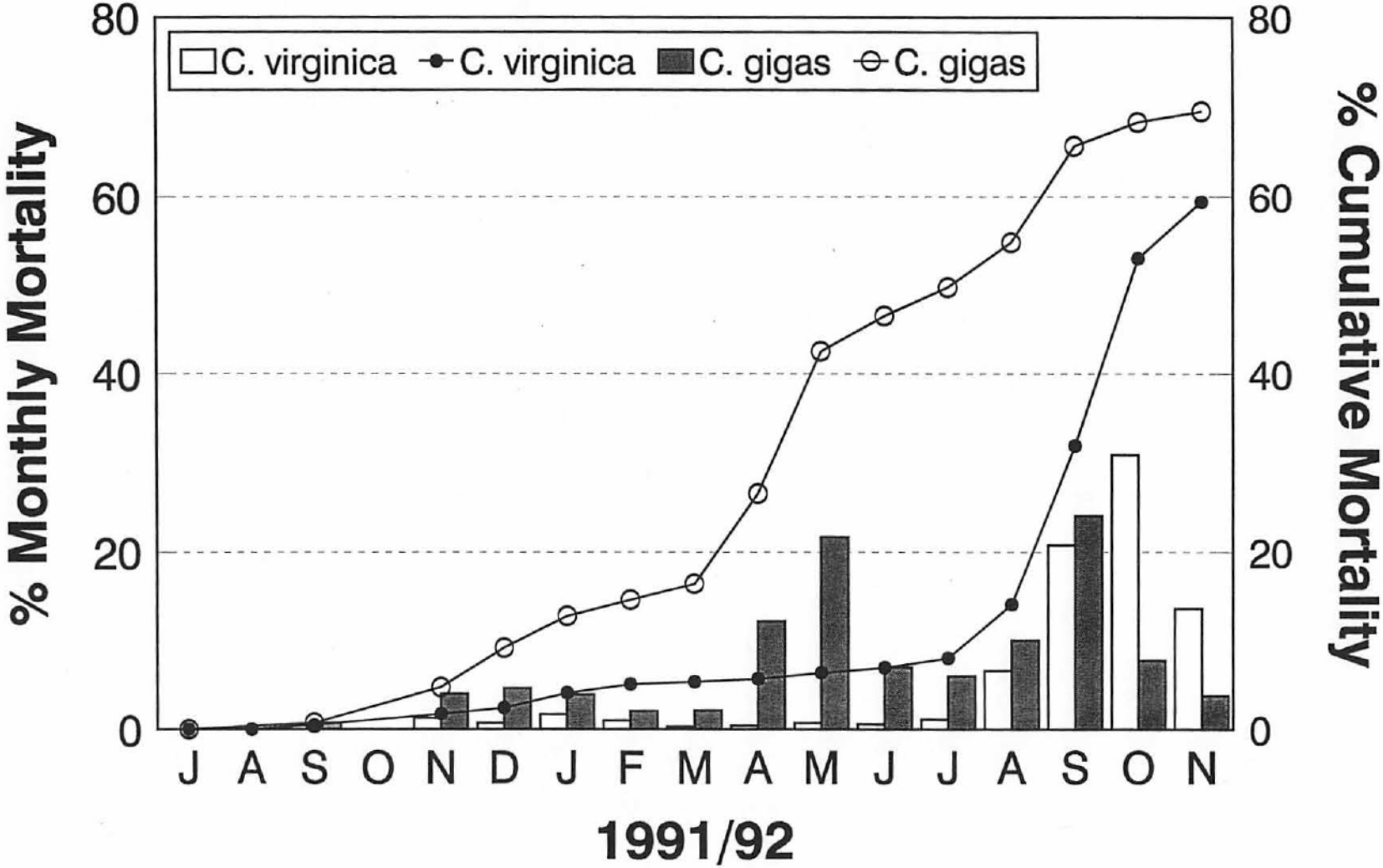

Figure 3. Percent monthly mortality (Y1) and percent cumulative mortality (Y2) of C. virginica and C. gigas from July 1991 to November 1992. Undosed and dosed groups of each species were combined after June 1992.

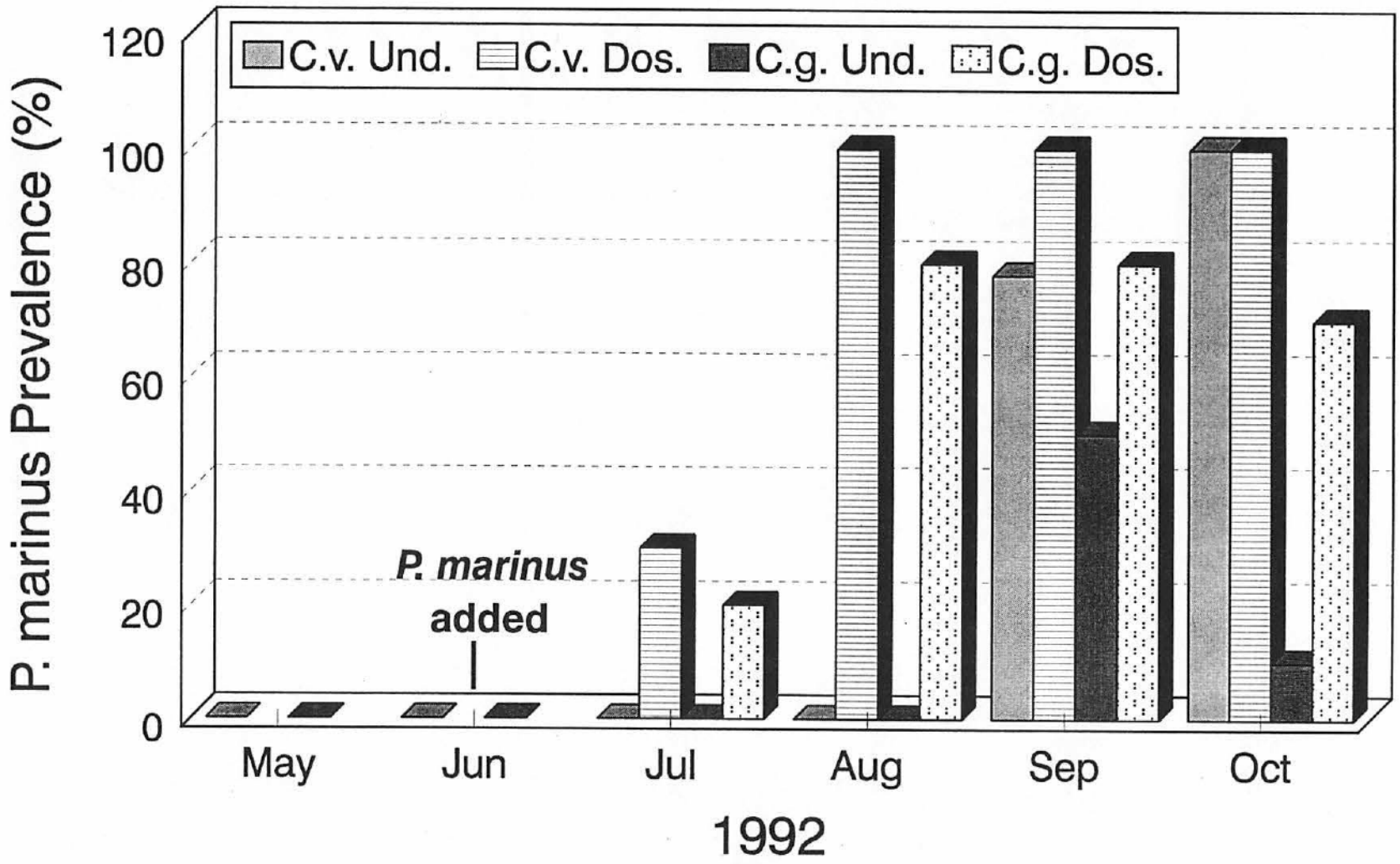

Figure 4. Percent prevalence of P. marinus in undosed and dosed flumes in months June to October, 1992. Dosed groups were challenged with addition of unfected oysters in June; undosed groups were exposed only to P. marinus entering via the influent.

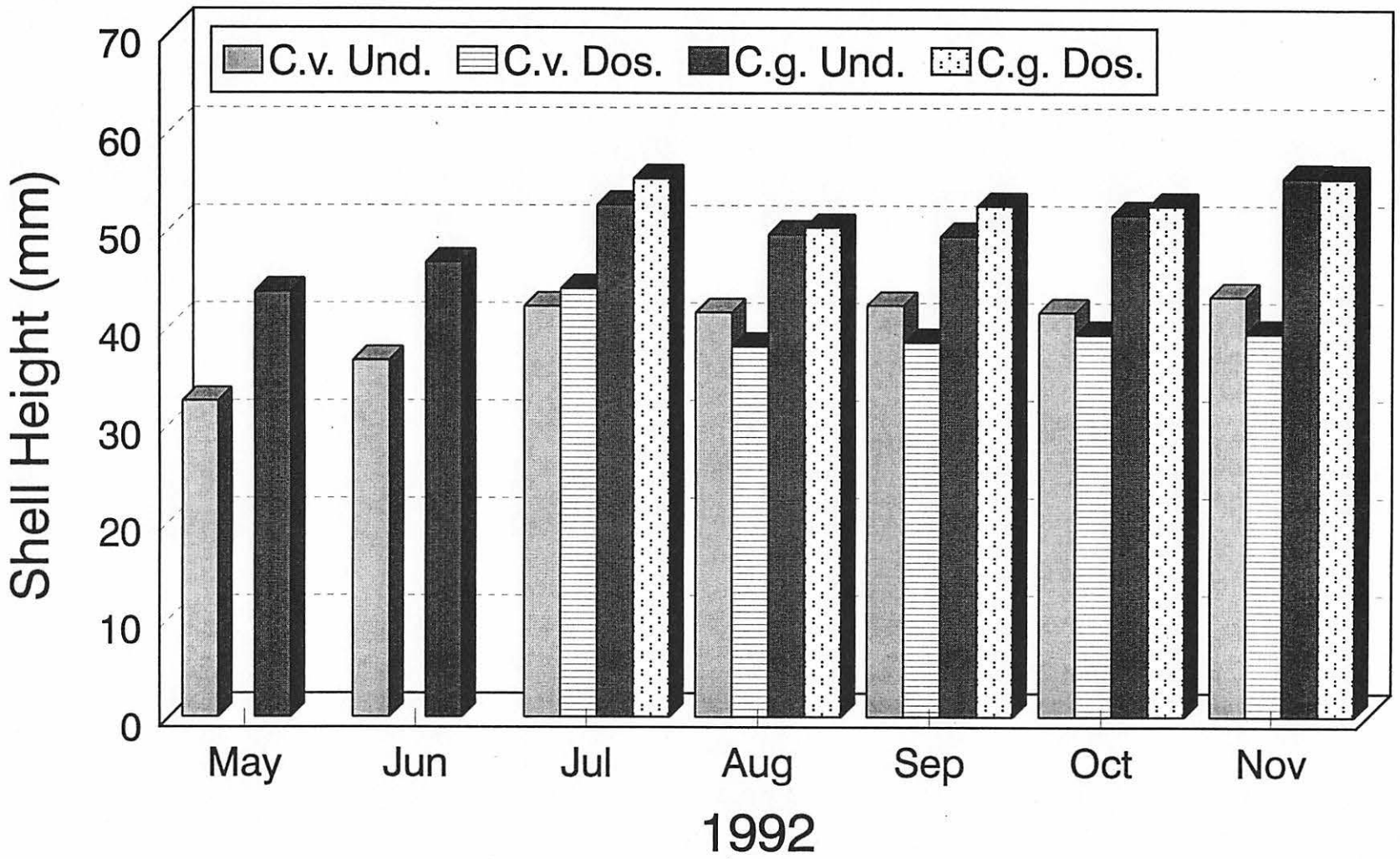

Figure 5. Mean shell height of undosed and dosed groups of C. virginica and C. gigas from May to November 1992; n = 50. The dosed groups were challenged in June.

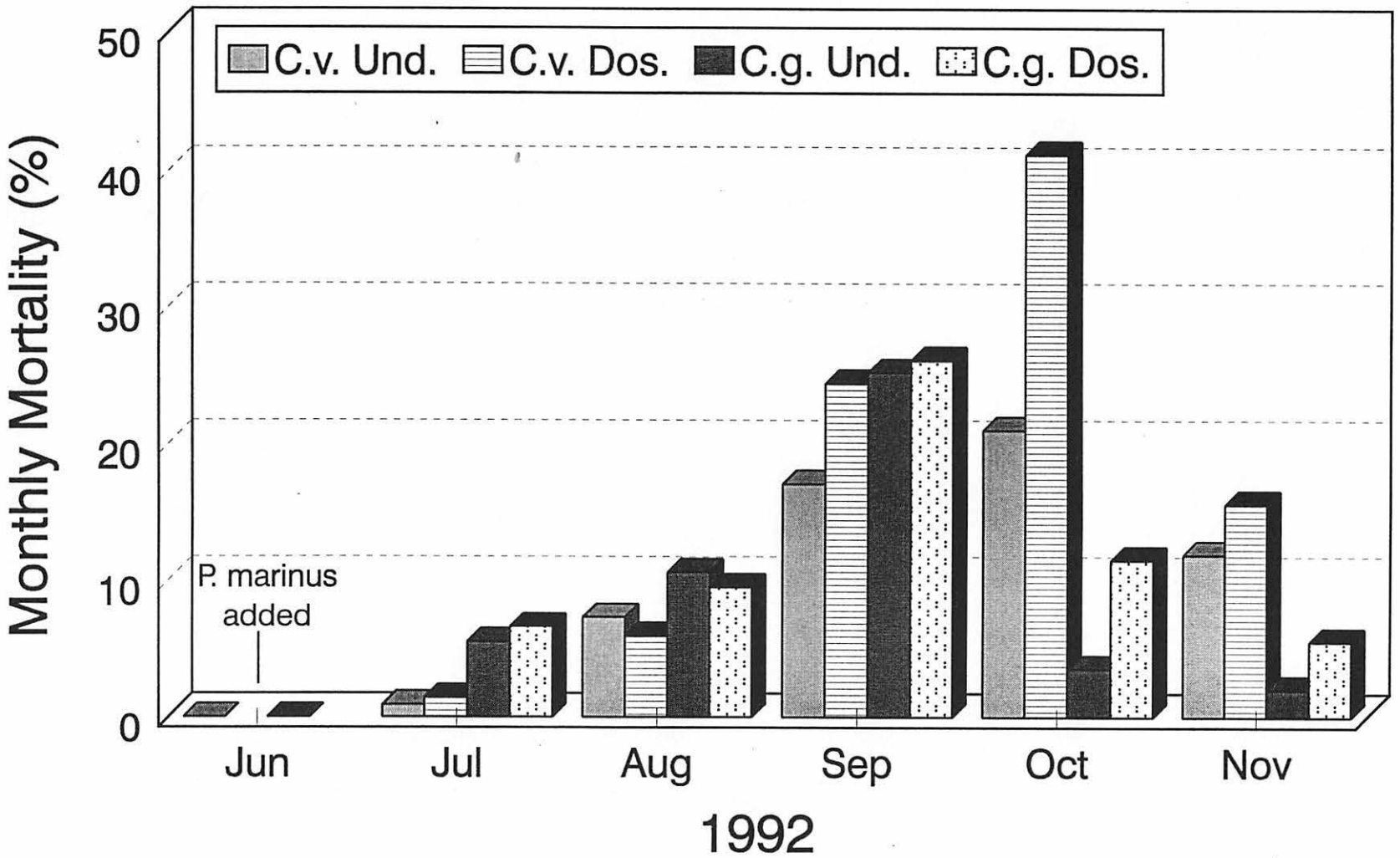

Figure 6. Percent monthly mortality of undosed and dosed groups of C. virginica and C. gigas from July to November 1992, after P. marinus was added to the dosed flume.

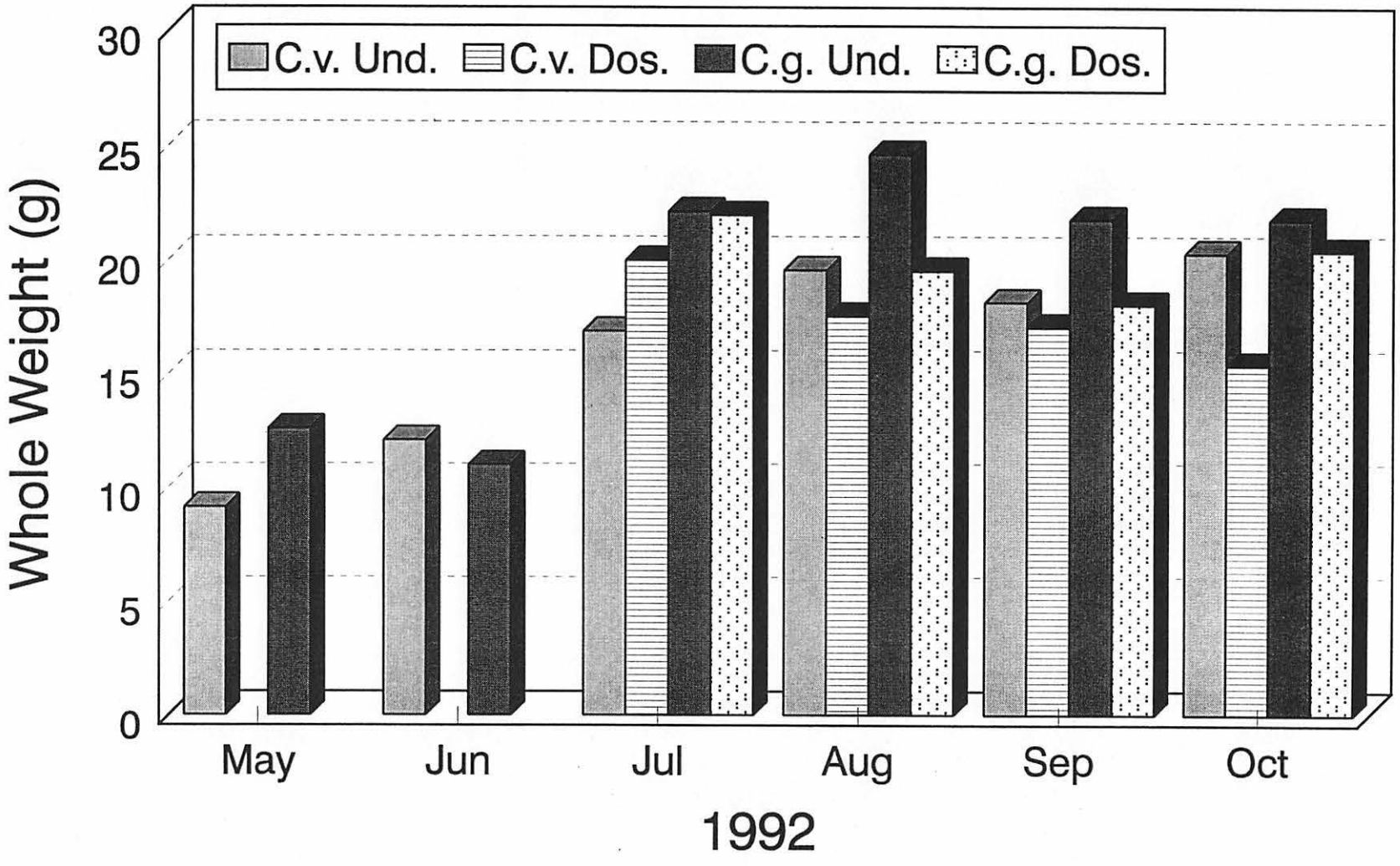

Figure 7. Mean whole weight of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 10.

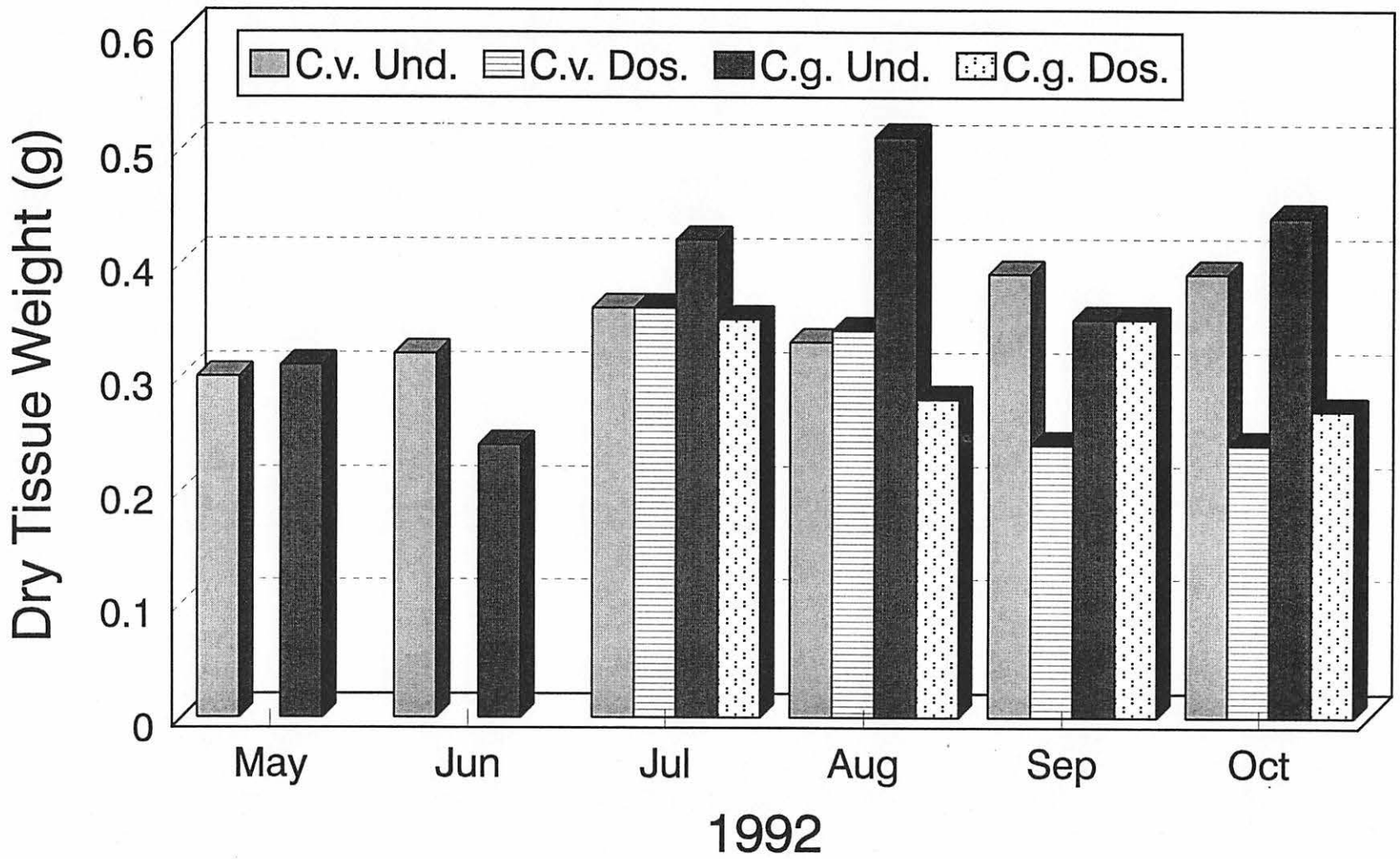

Figure 8. Mean tissue dry weight of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

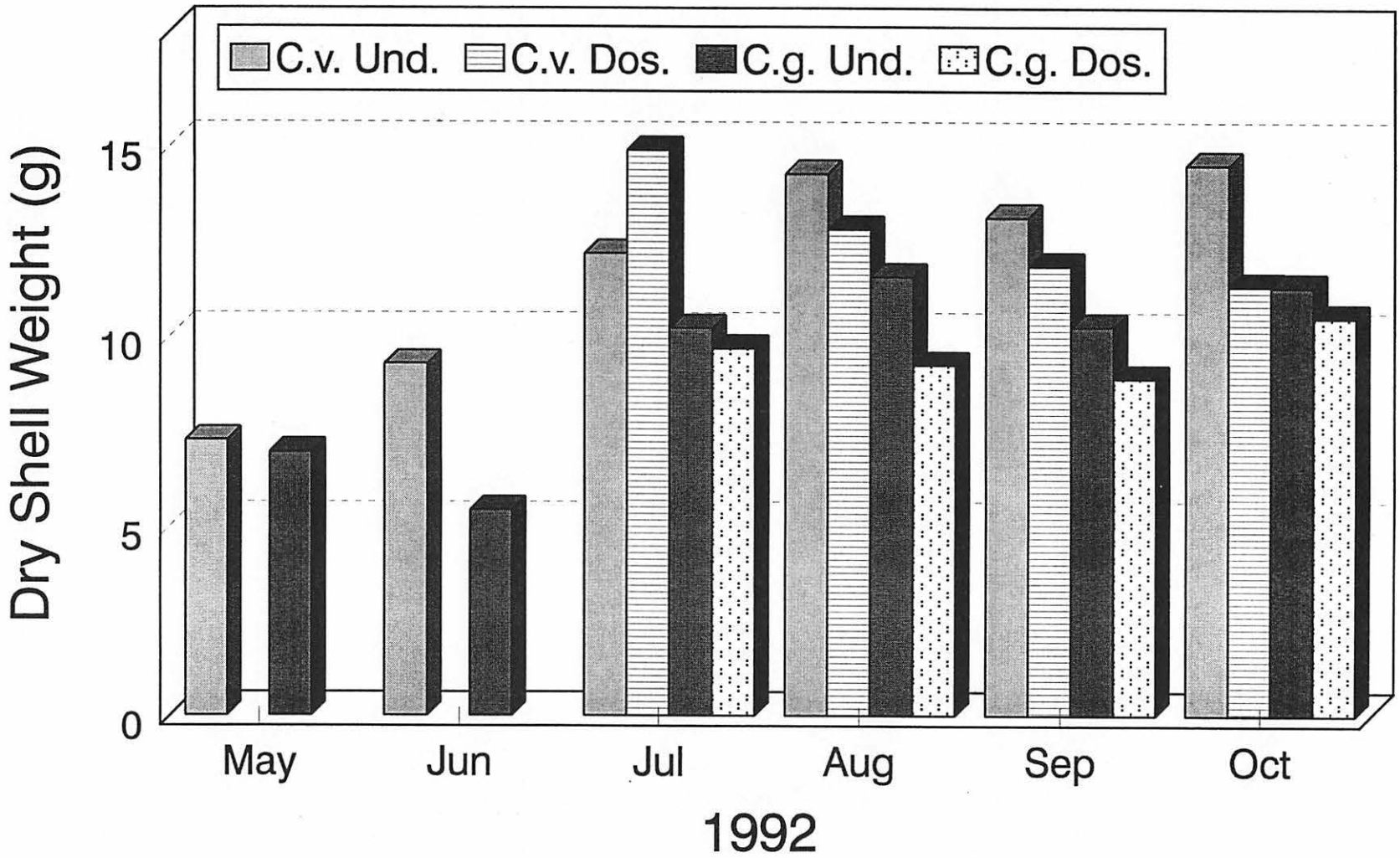

Figure 9. Mean shell dry weight of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

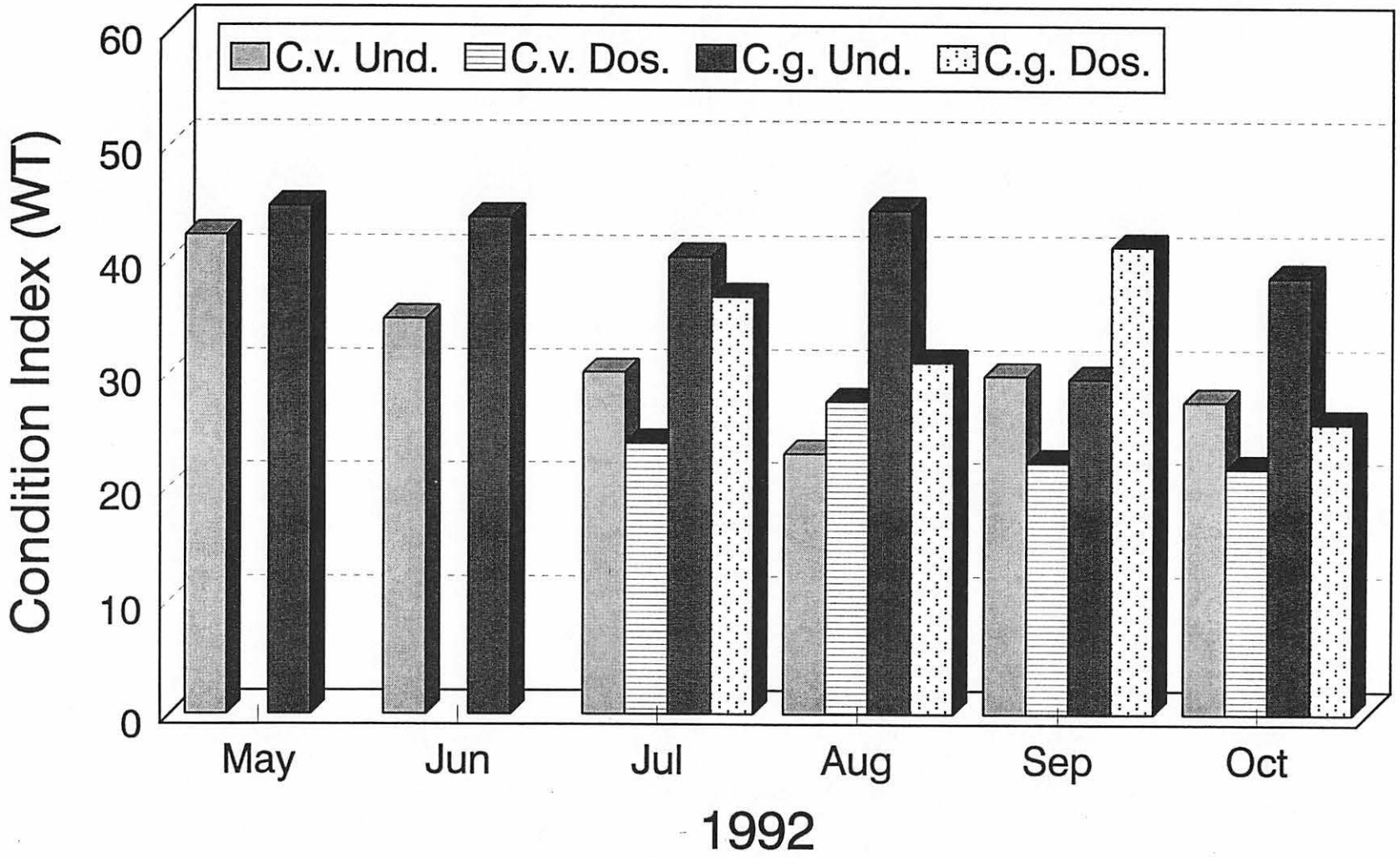

Figure 10. Mean condition index (WT) of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

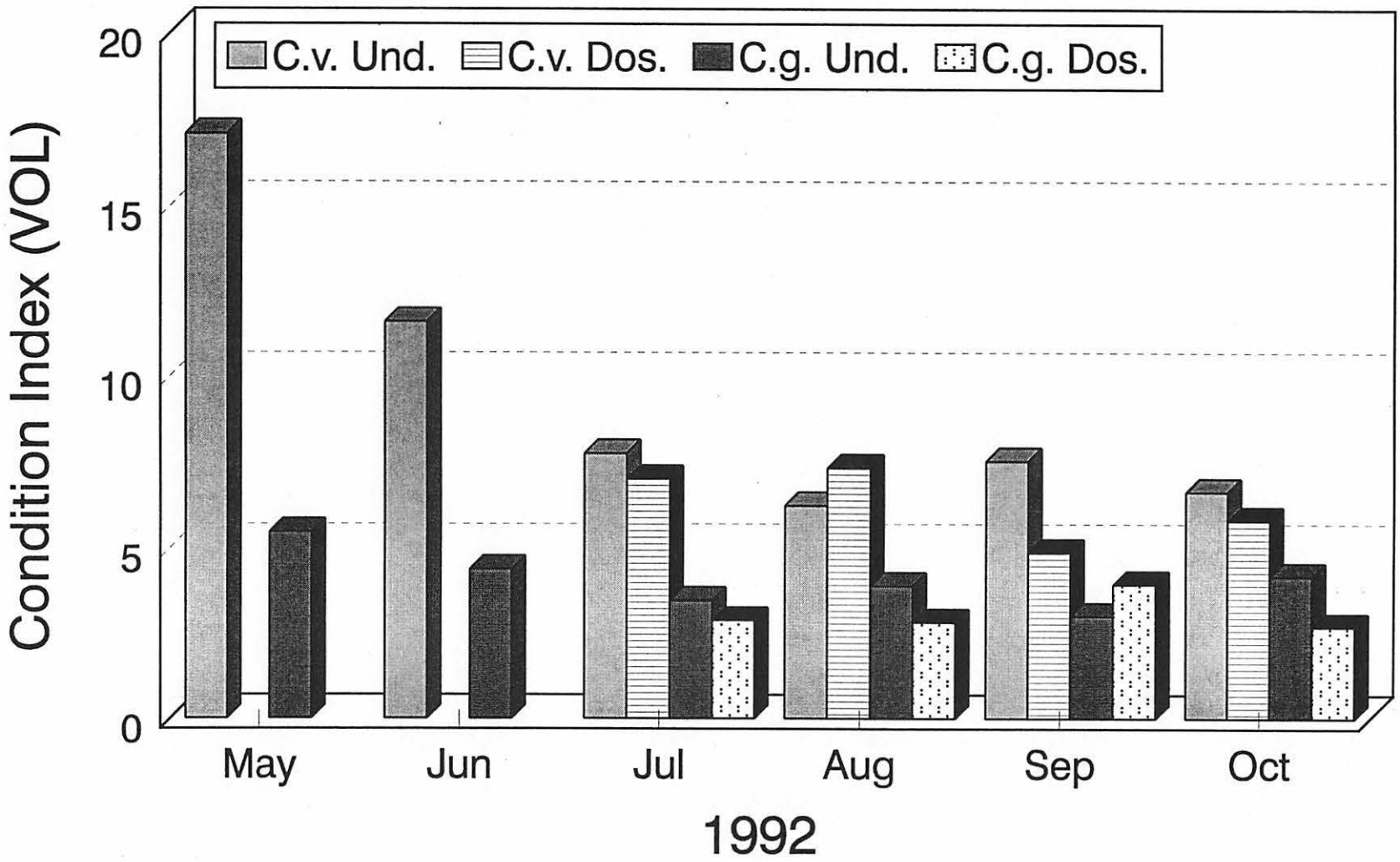

Figure 11. Mean condition index (VOL) of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

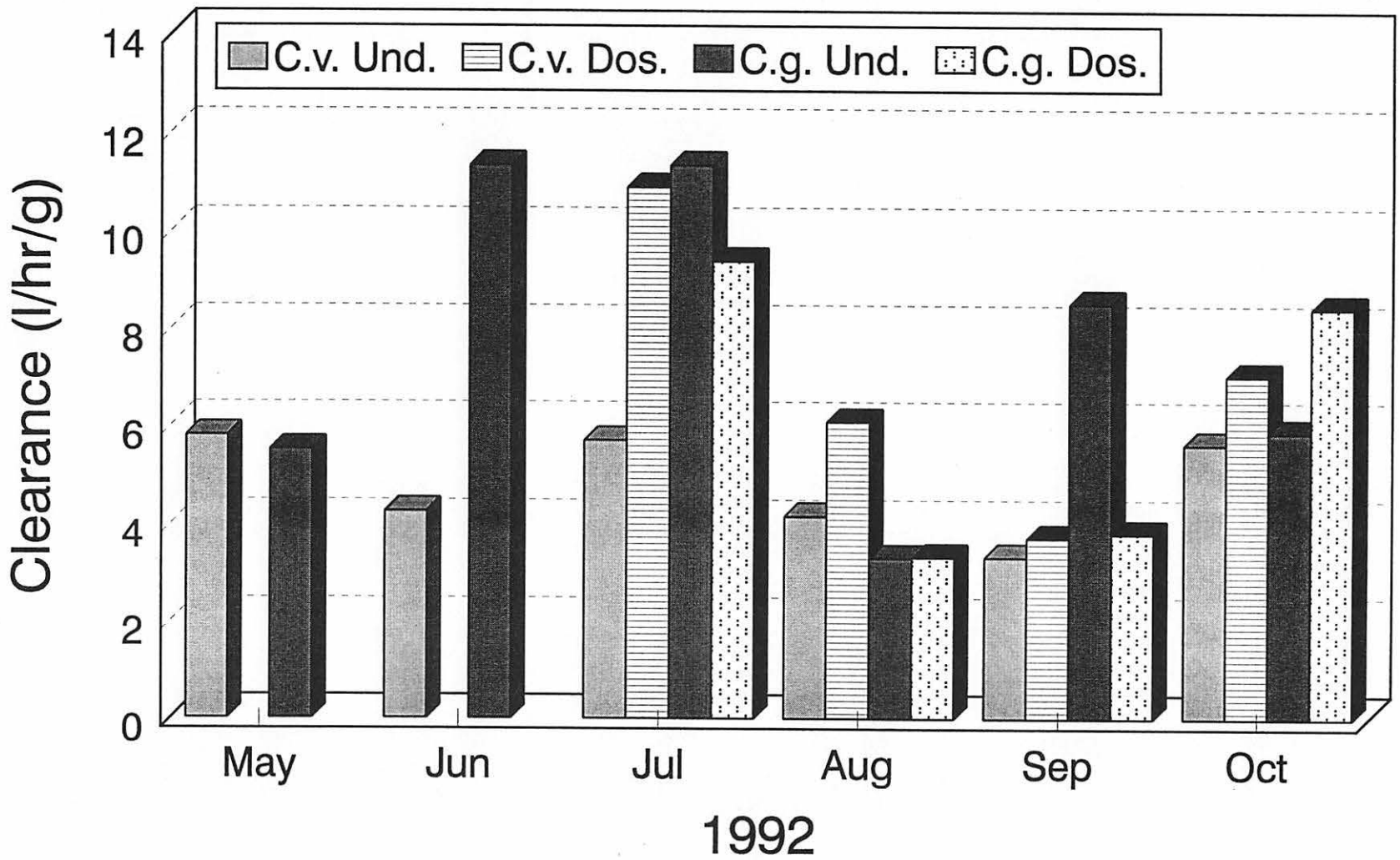

Figure 12. Mean clearance rates of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 3-10.

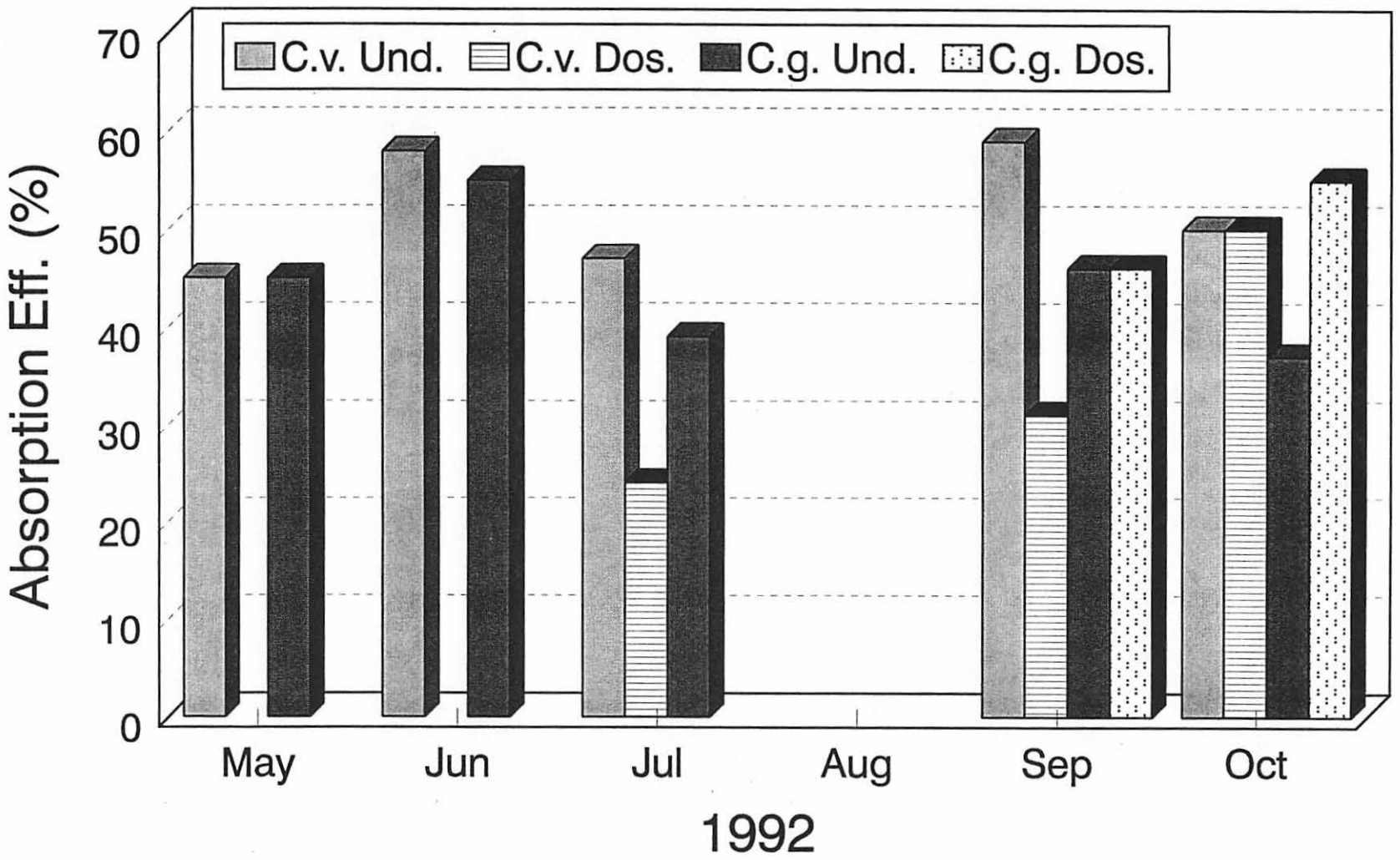

Figure 13. Mean absorption efficiencies of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n= 3-10.

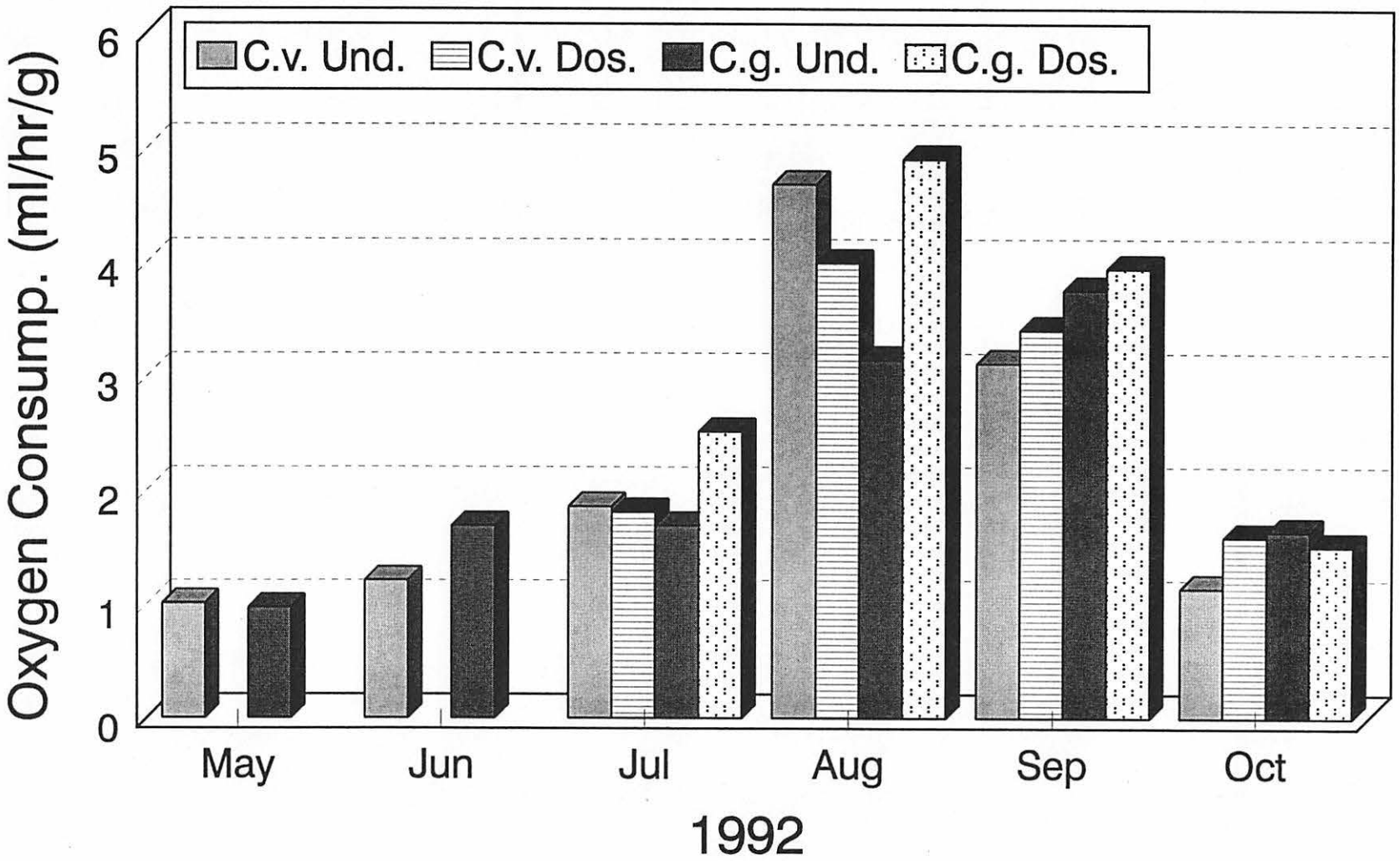

Figure 14. Mean rates of oxygen consumption of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 3-10.

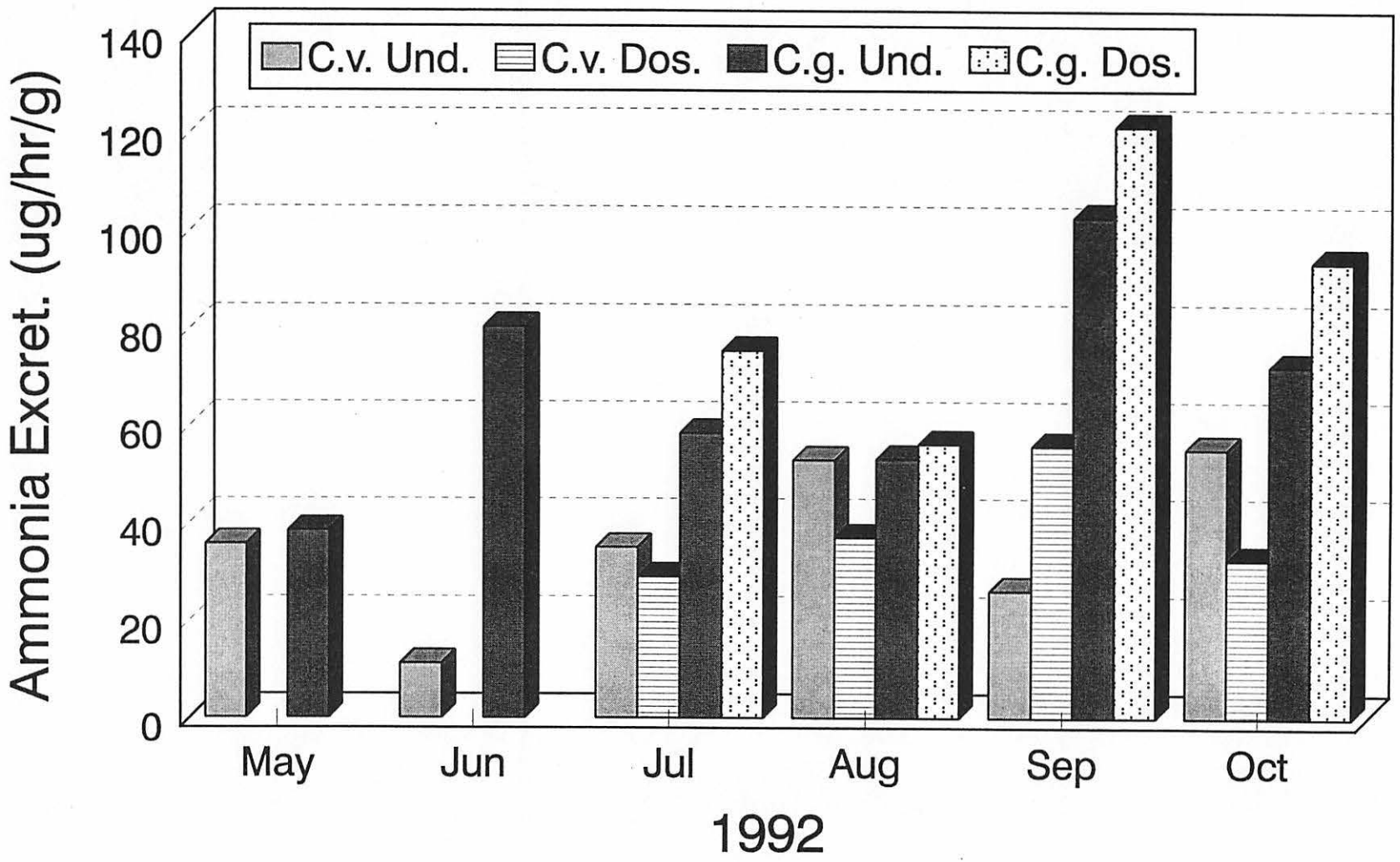

Figure 15. Mean rates of ammonia excretion of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

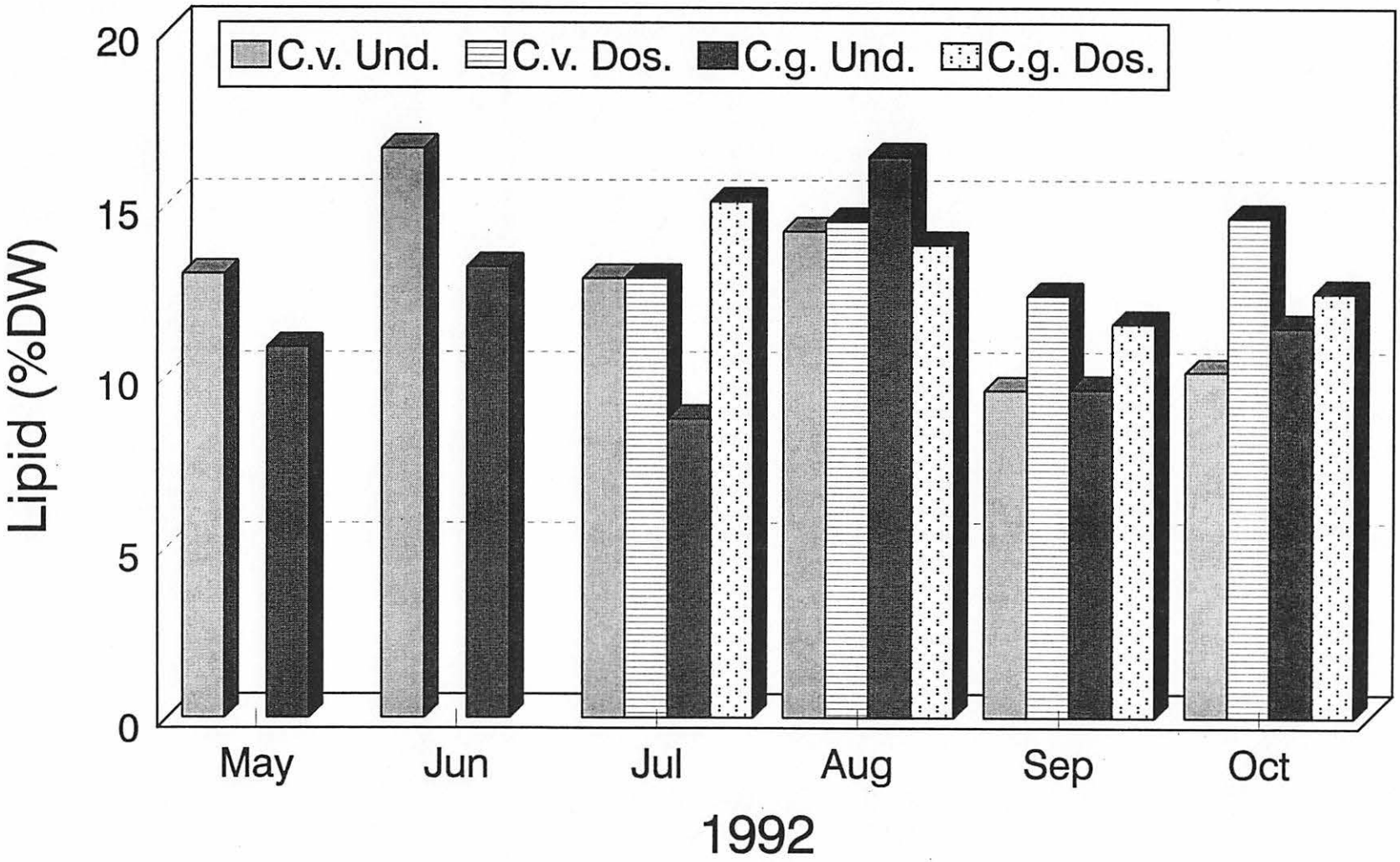

Figure 16. Mean lipid levels of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

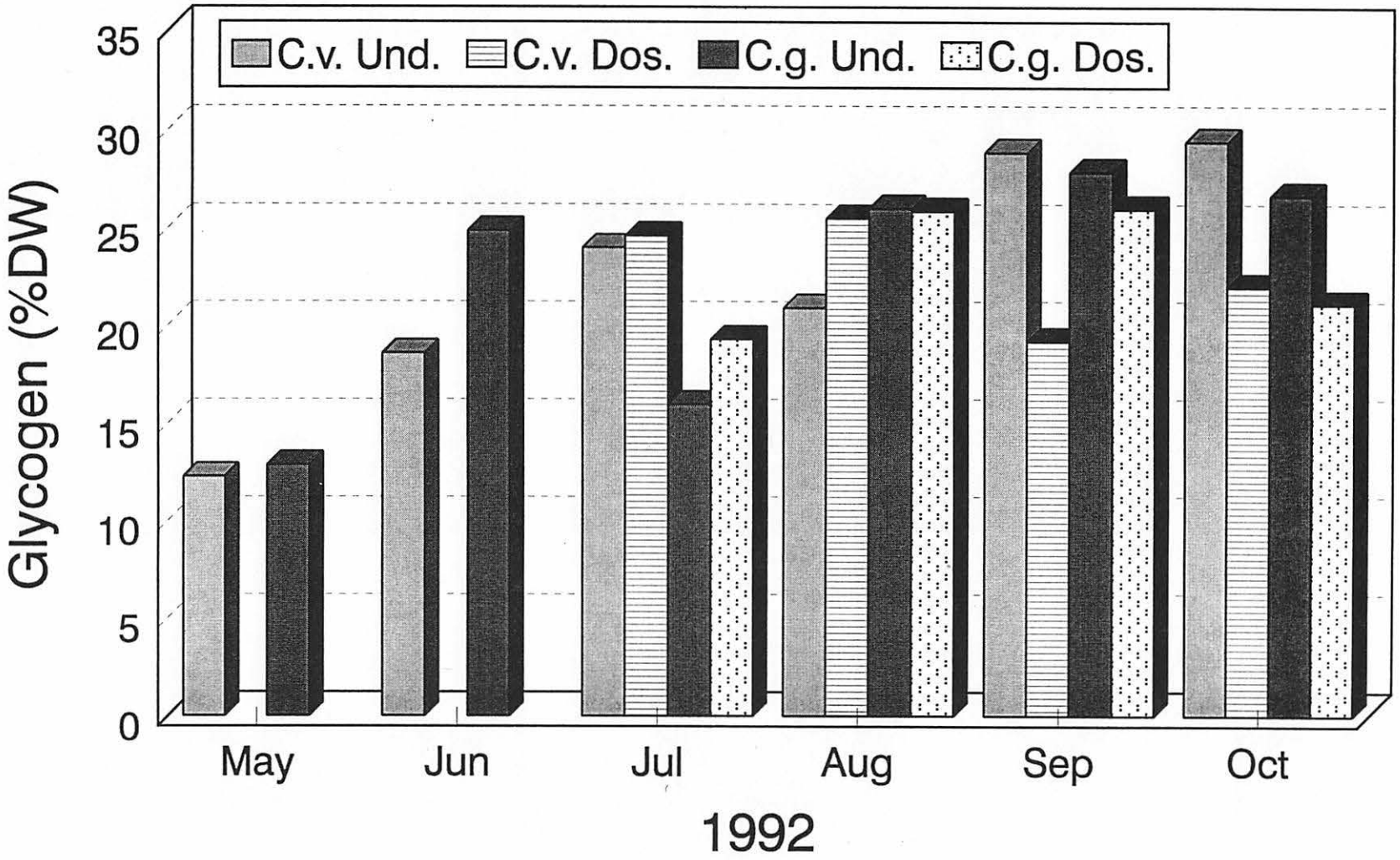

Figure 17. Mean glycogen levels of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n= 4-10.

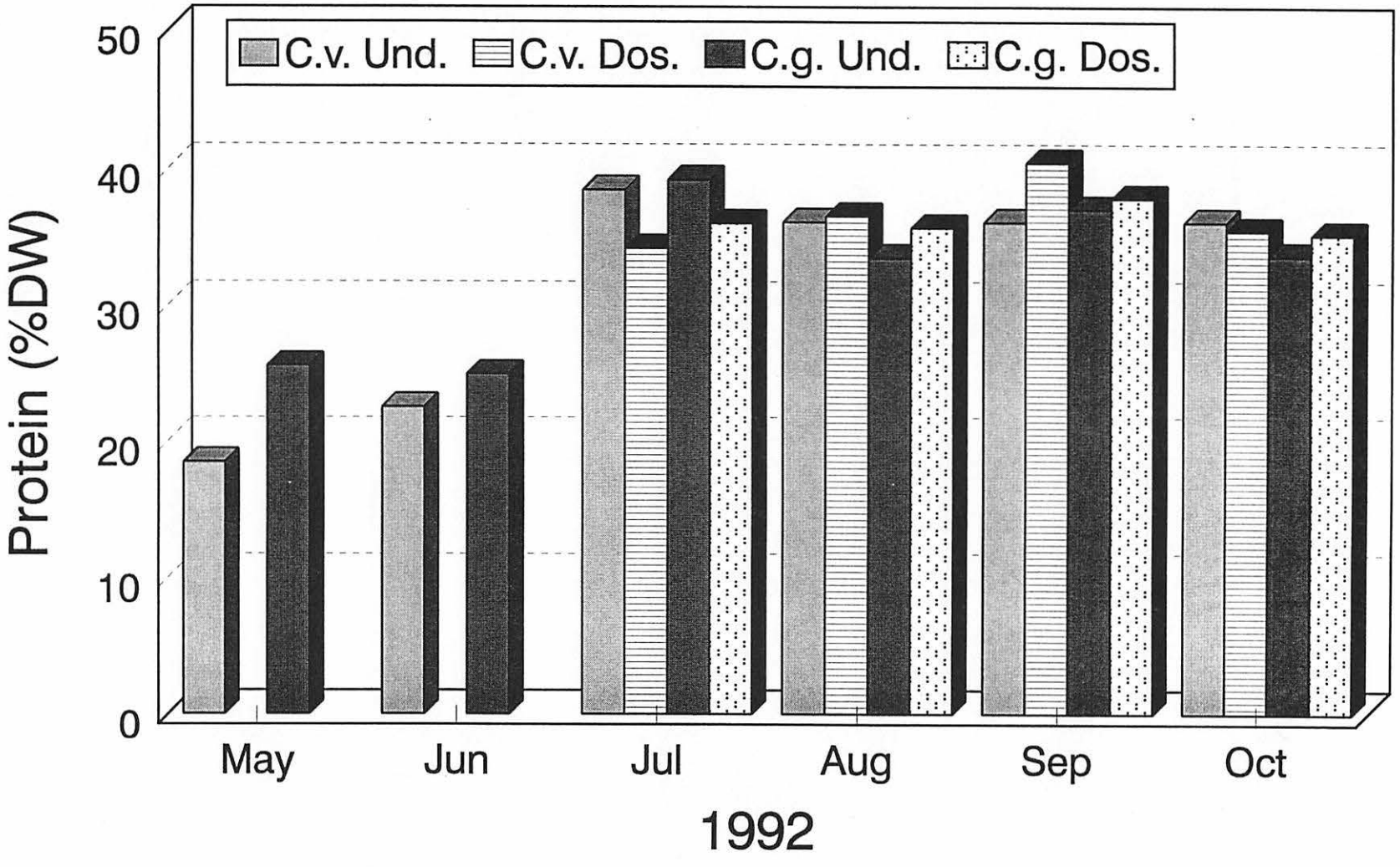

Figure 18. Mean protein levels of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

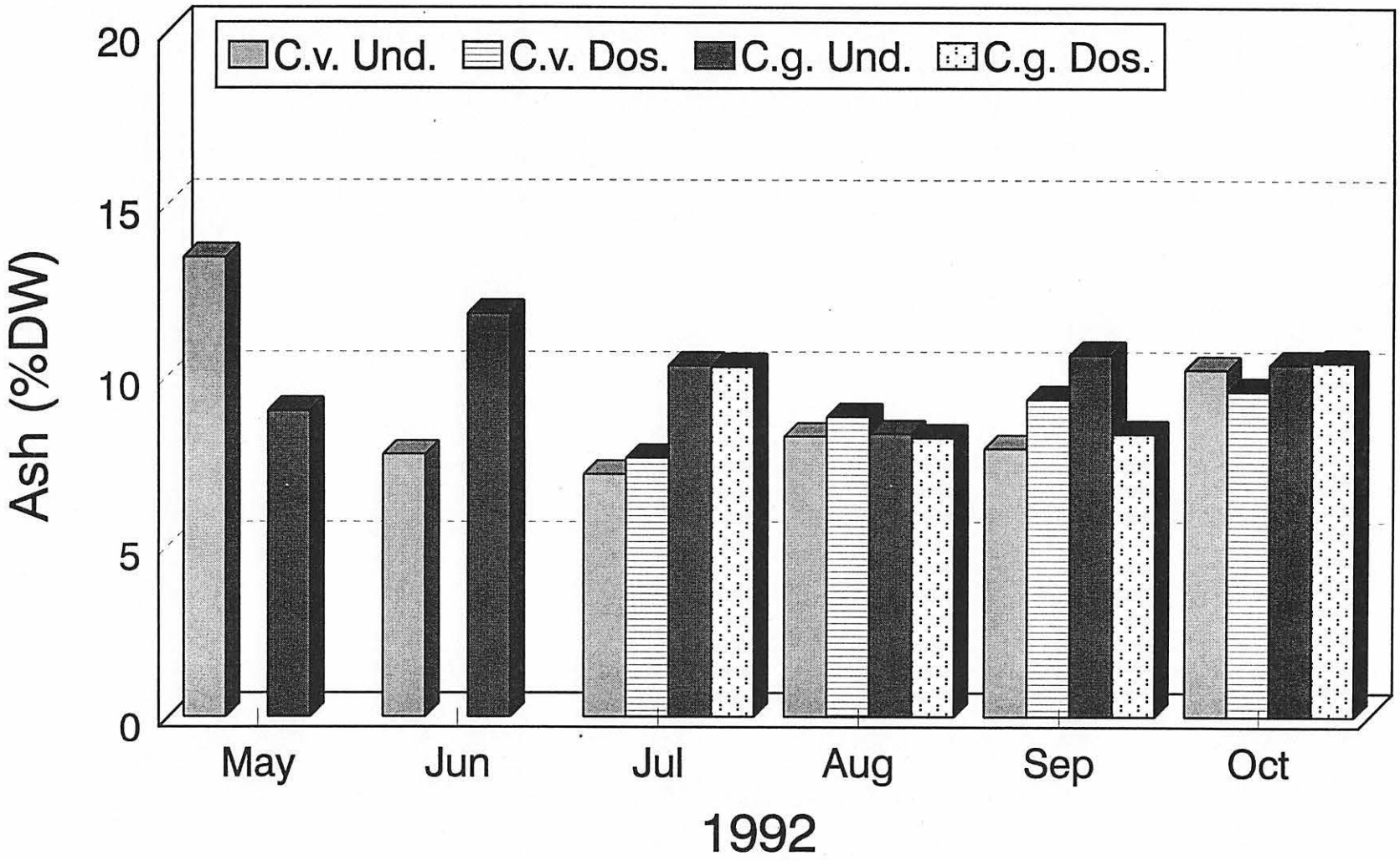

Figure 19. Mean ash levels of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

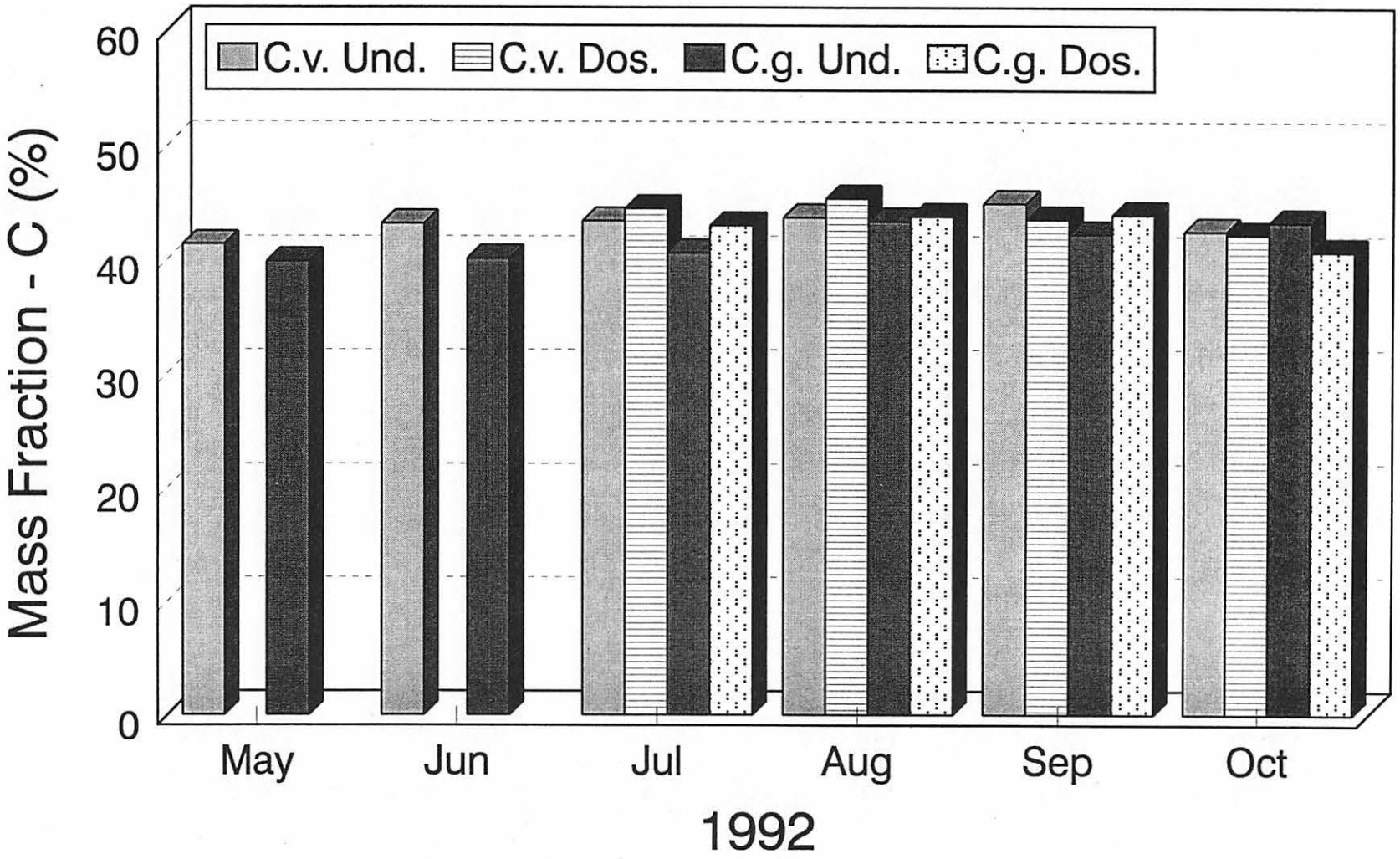

Figure 20. Mean mass fractions of carbon of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

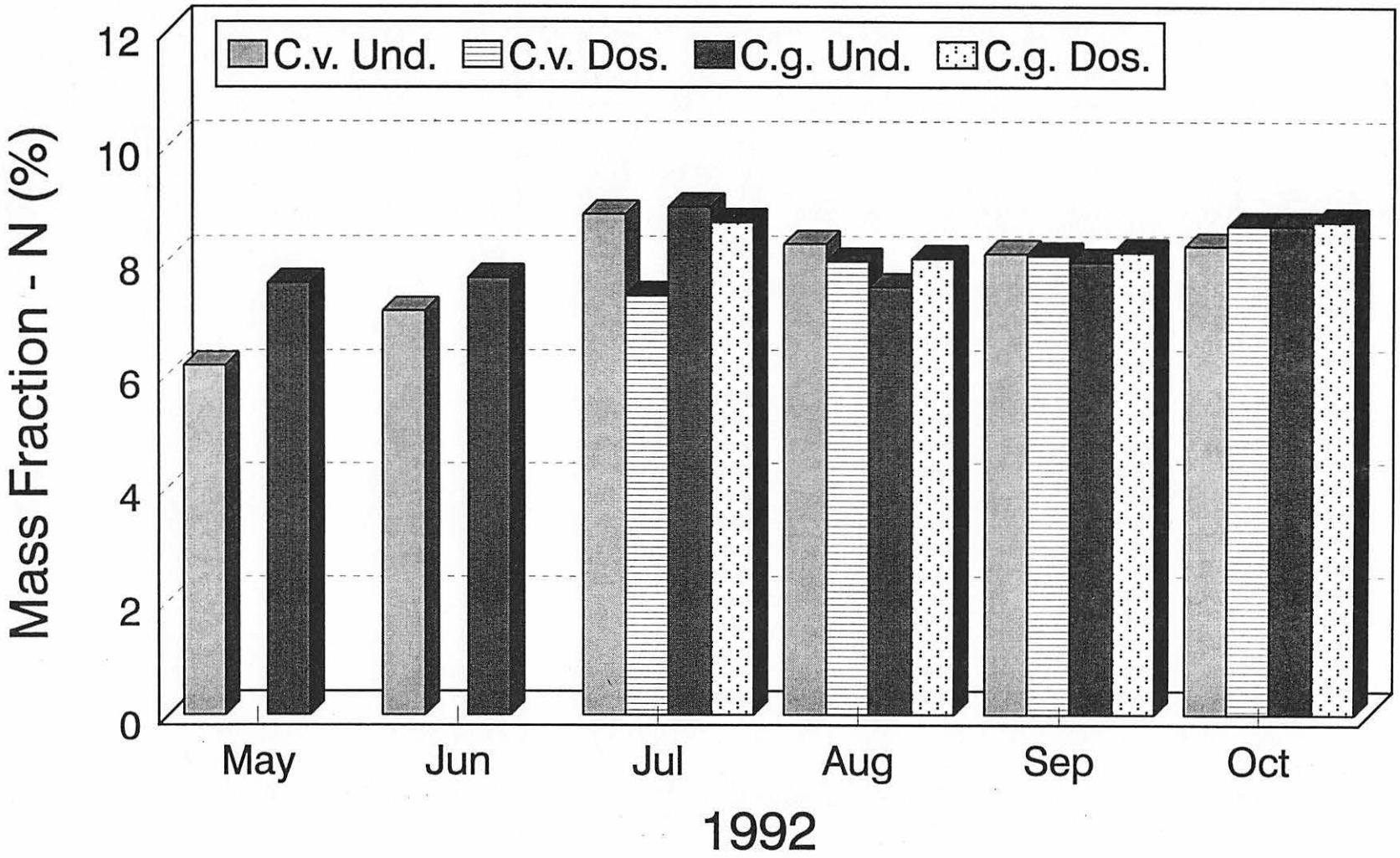

Figure 21. Mean mass fractions of nitrogen of undosed and dosed groups of oysters C. virginica and C. gigas from May to October 1992; n = 4-10.

TABLE 1

Intensity of *P. marinus* infections in undosed and dosed groups of *C. virginica* and *C. gigas* from June through October, 1992 (L=light; M=moderate; H=heavy).

Date	*C. virginica*				*C. gigas*			
	n	L	M	H	n	L	M	H
June	10	0	0	0	10	0	0	0
July								
Undosed	10	0	0	0	10	0	0	0
Dosed	10	3	0	0	10	2	0	0
August								
Undosed	10	0	0	0	10	0	0	0
Dosed	10	5	3	2	10	7	0	1
September								
Undosed	9	7	0	0	4	2	0	0
Dosed	8	4	1	3	5	4	0	0
October								
Undosed	10	9	1	0	10	1	0	0
Dosed	9	2	2	5	10	7	0	0

TABLE 2

Shell height, volume, whole weight, shell dry weight, tissue dry weight, C.I. (WT) and C.I. (VOL) of undosed and dosed oysters, C. virginica and C. gigas, from May to October, 1992. Cv = C. virginica; Cg = C. gigas

May - Undosed

Oyster	Shell Ht. (mm)	Volume (ml)	Whole Wt (g)	Shell DW (g)	Tissue DW (g)	C.I. (WT)	C.I. (VOL)
Cv1	45.2		10.25	9.12	0.336	36.84	29.73
Cv2	45.6		9.72	8.35	0.284	34.01	20.73
Cv3	48.3		11.97	10.03	0.461	45.96	23.76
Cv4	43.2		7.71	6.54	0.183	27.98	15.64
Cv5	46.5		7.69	5.43	0.274	50.46	12.12
Cv6	49.5		7.26	5.21	0.309	59.31	15.07
Cv7	45.9		10.27	7.19	0.396	55.08	12.86
Cv8	44.3		10.73	8.29	0.287	34.62	11.76
Cv9	46.6		6.51	4.8	0.2	41.67	11.70
Cv10	49.1		9.05	7.51	0.266	35.42	17.27
Mean =	46.42		9.12	7.25	0.30	42.13	17.07
SD =	2.04		1.76	1.75	0.08	10.23	6.00
Cg1	47.9		9.16	4.23	0.215	50.83	4.36
Cg2	50.5		12.48	6.56	0.366	55.79	6.18
Cg3	57.2		11.5	6.35	0.279	43.94	5.42
Cg4	48		13.96	8.5	0.287	33.76	5.26
Cg5	51.5		11.04	5.48	0.214	39.05	3.85
Cg6	52.7		11.96	7.41	0.286	38.60	6.29
Cg7	55.3		14.57	8.51	0.557	65.45	9.19
Cg8	48.2		12.42	6.41	0.252	39.31	4.19
Cg9	58.1		15.86	8.23	0.407	49.45	5.33
Cg10	47.9		12.9	7.73	0.236	30.53	4.56
Mean =	51.73		12.59	6.94	0.31	44.67	5.46
SD =	3.96		1.90	1.40	0.11	10.71	1.54

June - Undosed							
Oyster	Shell Ht.	Volume	Whole Wt	Shell DW	Tissue DW	Condition-WT	Condition-VOL
	(mm)	(ml)	(g)	(g)	(g)		
Cv1	47	9.2	16	12.71	0.368	28.95	11.19
Cv2	44.8	6.4	11.8	9.11	0.288	31.61	10.71
Cv3	48	5	9.4	7.29	0.314	43.07	14.88
Cv4	45.5	5.6	11.3	8.37	0.149	17.80	5.09
Cv5	49.6	7.5	14.1	11.2	0.406	36.25	14.00
Cv6	51	7	12.6	10.1	0.409	40.50	16.36
Cv7	47.1	7.5	11.8	8.59	0.237	27.59	7.38
Cv8	47.8	5.6	9.9	7.18	0.353	49.16	12.98
Cv9	48.4	7.5	12.9	9.85	0.325	32.99	10.66
Cv10	50.5	7.4	10.9	8.29	0.333	40.17	12.76
Mean =	47.97		12.07	9.27	0.32	34.81	11.60
SD =	2.01		1.96	1.74	0.08	8.99	3.41
Cg1	55	8.6	10.5	4.91	0.192	39.10	3.43
Cg2	57	8.2	12.5				
Cg3	51.2	9	12.2	6.7	0.204	30.45	3.71
Cg4	50	8.2	10.7	5.4	0.188	34.81	3.55
Cg5	53.3	9	10.9	5.52	0.171	30.98	3.18
Cg6	44.4	7.2	10.6	5.02	0.208	41.43	3.73
Cg7	49.7	8.2	10.2	5.57	0.414	74.33	8.94
Cg8	50	7	10.5	5.24	0.153	29.20	2.91
Cg9	56.2	10.2	11.6	5.4	0.306	56.67	4.94
Cg10	49.7	7.2	10.6	4.97	0.279	56.14	4.96
Mean =	51.65	8.28	11.03	5.41	0.24	43.68	4.37
SD =	3.79	0.99	0.79	0.54	0.08	15.47	1.85

Table 2, P. 2

July - Undosed							
Oyster	Shell Ht.	Volume	Whole Wt	Shell DW	Tissue DW	Condition-WT	Condition-VOL
	(mm)	(ml)	(g)	(g)	(g)		
Cv1	60.9	9.8	14.57	8.44	0.356	42.18	5.81
Cv2	53.9	10.4	17.9	14.13	0.35	24.77	9.28
Cv3	61.1	11	17.62	12.14	0.242	19.93	4.42
Cv4	57	8.2	14.34	9.9	0.396	40.00	8.92
Cv5	56.3	12	21.85	17.24	0.497	28.83	10.78
Cv6	54.3	12	19.98	14.21	0.578	40.68	10.02
Cv7	52.7	9.6	16.31	12.45	0.257	20.64	6.66
Cv8	52.6	10.7	16.96	12.94	0.346	26.74	8.61
Cv9	53.2	10.2	14.73	10.24	0.229	22.36	5.10
Cv10	47.2	9.6	14.67	10.22	0.353	34.54	7.93
Mean =	54.92	10.35	16.89	12.19	0.36	30.07	7.75
SD =	4.14	1.16	2.53	2.60	0.11	8.62	2.16
Cg1	58.6	13.4	16.56	7.12	0.206	28.93	2.18
Cg2	69.8	19.6	25.28	10.56	0.584	55.30	3.97
Cg3	72.4	16.8	21.47	8.9	0.142	15.96	1.13
Cg4	70.4	16.7	23.29	11.88	0.313	26.35	2.74
Cg5	68.8	17.6	24.48	12.53	0.476	37.99	3.98
Cg6	84.6	21	26.63	11.77	0.456	38.74	3.07
Cg7	66	15.4	21.7	11	0.499	45.36	4.66
Cg8	57.9	18.8	25.08	11.08	0.724	65.34	5.17
Cg9	52.1	11.2	16.42	8.01	0.164	20.47	1.95
Cg10	63.5	15.4	20.51	9.39	0.635	67.63	5.71
Mean =	66.41	16.59	22.14	10.22	0.42	40.21	3.46
SD =	9.11	2.92	3.54	1.79	0.20	18.08	1.49

Table 2, P. 3

July - Dosed							
Oyster	Shell Ht.	Volume	Whole Wt	Shell DW	Tissue DW	Condition-WT	Condition-VOL
	(mm)	(ml)	(g)	(g)	(g)		
Cv1	53.5	12.7	21.7	16.16	0.466	28.84	8.41
Cv2	54.5	11.4	18.1	13.99	0.301	21.52	7.32
Cv3	57.9	10.7	18.5	14.25	0.3	21.05	7.06
Cv4	54.8	10.6	17.9	13.44	0.271	20.16	6.08
Cv5	50	9.2	16	12.67	0.249	19.65	7.48
Cv6	51	11.6	20.9	15.97	0.251	15.72	5.09
Cv7	55.2	11.3	18.9	13.81	0.344	24.91	6.76
Cv8	53.4	11.3	20.8	16.07	0.336	20.91	7.10
Cv9	54.5	15.4	24.9	15.74	0.582	36.98	6.35
Cv10	58.5	12.4	22.4	16.81	0.473	28.14	8.46
Mean =	54.33	11.66	20.01	14.89	0.36	23.79	7.01
SD =	2.64	1.63	2.62	1.41	0.11	6.11	1.02
Cg1	81.3	21.7	28	10.65	0.476	44.69	2.74
Cg2	69.8	16.2	21.2	9.71	0.365	37.59	3.18
Cg3	77.1	16.2	21.7	7.93	0.372	46.91	2.70
Cg4	65.3	15	21.2	10	0.226	22.60	2.02
Cg5	76.7	19.1	26	11	0.432	39.27	2.88
Cg6	58	14.9	21.2	10.39	0.383	36.86	3.54
Cg7	65.9	11.5	21.5	10.84	0.387	35.70	3.63
Cg8	57.5	11.3	15.8	7.6	0.25	32.89	3.05
Cg9	60.5	14	20.3	10.46	0.221	21.13	2.25
Cg10	66.3	19.2	22.9	8.19	0.406	49.57	2.76
Mean =	67.84	15.91	21.98	9.68	0.35	36.72	2.87
SD =	8.30	3.35	3.27	1.28	0.09	9.42	0.51

Table 2, P. 4

August - Undosed							
Oyster	Shell Ht. (mm)	Volume (ml)	Whole Wt (g)	Shell DW (g)	Tissue DW (g)	Condition-WT	Condition-VOL
Cv1	54.8	10.4	18.6	13.63	0.429	31.47	8.63
Cv2	52.6	10.4	17.83	12.91	0.335	25.95	6.81
Cv3	49.5	10.2	17.61	13.2	0.433	32.80	9.82
Cv4	61.7	13.4	24.25	17.27	0.463	26.81	6.63
Cv5	55	11.1	19.33	14.31	0.384	26.83	7.65
Cv6	59	11.6	18.13	12.97	0.236	18.20	4.57
Cv7	61.5	12.4	21.17	16.2	0.147	9.07	2.96
Cv8	56.2	11.2	19.48	13.48	0.249	18.47	4.15
Cv9	51.3	9.3	17.95	12.92	0.143	11.07	2.84
Cv10	48.6	11.9	21.36	15.9	0.449	28.24	8.22
Mean =	55.02	11.19	19.57	14.28	0.33	22.89	6.23
SD =	4.66	1.19	2.11	1.60	0.12	8.25	2.46
Cg1	59.2	11.8	17	8.04	0.298	37.06	3.33
Cg2	60.2	19.9	28.14	14.15	0.496	35.05	3.55
Cg3	57.6	16.4	22.46	10.86	0.421	38.77	3.63
Cg4	57.4	14.7	20.23	9.31	0.459	49.30	4.20
Cg5	68.4	15.5	21.2	9.99	0.364	36.44	3.25
Cg6	67.7	22	35.57	17.42	0.695	39.90	3.83
Cg7	68.6	19.6	27.71	13.4	0.592	44.18	4.14
Cg8	72.3	19.7	27.55	12.19	0.7	57.42	4.56
Cg9	59.9	20.6	27.22	10.92	0.68	62.27	4.17
Cg10	54.7	13.3	19.11	9.52	0.406	42.65	4.23
Mean =	62.60	17.35	24.62	11.58	0.51	44.30	3.89
SD =	6.04	3.46	5.59	2.78	0.15	9.27	0.44

August - Dosed							
Oyster	Shell Ht.	Volume	Whole Wt	Shell DW	Tissue DW	Condition-WT	Condition-VOL
	(mm)	(ml)	(g)	(g)	(g)		
Cv1	53.7	10.7	16.86	12.57	0.328	26.09	7.65
Cv2	48.5	9.4	14.38	10.05	0.283	28.16	6.54
Cv3	52.1	10.4	18.66	14.03	0.267	19.03	5.77
Cv4	50.5	9.9	17.97	13.36	0.423	31.66	9.18
Cv5	51.7	10.6	16.54	9.43	0.446	47.30	6.27
Cv6	48	9	16.18	12.08	0.372	30.79	9.07
Cv7	53.4	9.8	17.45	13.15	0.215	16.35	5.00
Cv8	53.7	10.1	19.3	14.6	0.259	17.74	5.51
Cv9	50.7	10.7	22.64	17.66	0.537	30.41	10.78
Cv10	44.5	8.1	15.54	11.4	0.312	27.37	7.54
Mean =	50.68	9.87	17.55	12.83	0.34	27.49	7.33
SD =	2.96	0.84	2.31	2.36	0.10	8.96	1.87
Cg1	55.8	11.8	16.26	7.77	0.148	19.05	1.74
Cg2	63.1	15.4	20.42	9.23	0.138	14.95	1.23
Cg3	61.3	15.4	21.1	10.96	0.34	31.02	3.35
Cg4	61.6	16.1	25.1	11.8	0.349	29.58	2.62
Cg5	57.3	14.3	18.62	7.5	0.201	26.80	1.81
Cg6	71	12.7	19.07	8.66	0.218	25.17	2.09
Cg7	56.4	9.7	14.85	6.92	0.406	58.67	5.12
Cg8	60.2	13.5	19.39	9.75	0.328	33.64	3.40
Cg9	63.9	12.9	20.46	9.71	0.298	30.69	2.77
Cg10	61.2	13.3	20.03	10.12	0.403	39.82	4.07
Mean =	61.18	13.51	19.53	9.24	0.28	30.94	2.82
SD =	4.42	1.91	2.77	1.55	0.10	12.04	1.19

Table 2, P. 6

September - Undosed							
Oyster	Shell Ht.	Volume	Whole Wt	Shell DW	Tissue DW	Condition-WT	Condition-VOL
	(mm)	(ml)	(g)	(g)	(g)		
Cv1	54.8	9.6	20.98	15.92	0.53	33.29	10.47
Cv2	52.2	8.6	17.63	13.23	0.38	28.72	8.64
Cv3	55.6	8.2	14.59	8.98	0.348	38.75	6.20
Cv4	49	9.8	19.87	14.27	0.495	34.69	8.84
Cv5	53.9	8.4	16.51	11.57	0.22	19.01	4.45
Cv6	53.8	11.9	22.81	16.92	0.568	33.57	9.64
Cv7	50.5	8	14.61	9.6	0.341	35.52	6.81
Cv8	55.5	11.6	22.25	16.19	0.491	30.33	8.10
Cv9	49.1	9.8	17.37				
Cv10	48.4	9.2	15.05	11.59	0.16	13.81	4.62
Mean =	52.28	9.51	18.17	13.14	0.39	29.74	7.53
SD =	2.82	1.35	3.12	2.90	0.14	8.19	2.14
Cg1	64.1	17.6	21.98				
Cg2	65.9	17.6	22.79				
Cg3	68.8	20.2	24.59				
Cg4	58.1	16	22.93	10.32	0.296	28.68	2.35
Cg5	67	14.3	21.65				
Cg6	72	20.8	32.93	17.21	0.596	34.63	3.79
Cg7	60.6	12.2	18.96				
Cg8	57.7	10.8	15.94	7.98	0.228	28.57	2.86
Cg9	55.5	11.5	16.95				
Cg10	59.2	11	19.18	10.25	0.267	26.05	2.99
Mean =	62.89	15.20	21.79	11.44	0.35	29.48	3.00
SD =	5.46	3.79	4.79	4.00	0.17	3.64	0.60

Table 2, P. 7

September - Dosed							
Oyster	Shell Ht.	Volume	Whole Wt	Shell DW	Tissue DW	Condition-WT	Condition-VOL
	(mm)	(ml)	(g)	(g)	(g)		
Cv1	51	9.4	15.21	10.07	0.143	14.20	2.78
Cv2	47.1	6.5	15.05	11.33	0.227	20.04	6.10
Cv3	53.2	9.3	13.94	8.04	0.414	51.49	7.02
Cv4	52.6	8.8	17.81				
Cv5	50.4	11	22.18	16.42	0.345	21.01	5.99
Cv6	54.1	8.2	17.98				
Cv7	53.3	10.2	12.86	7.36	0.215	29.21	3.91
Cv8	51.4	9.4	19.53	14.34	0.211	14.71	4.07
Cv9	47.7	8.8	15.93	12.46	0.269	21.59	7.75
Cv10	56.6	10.2	20.16	14.88	0.067	4.50	1.27
Mean =	51.74	9.18	17.07	11.86	0.24	22.09	4.86
SD =	2.88	1.24	2.97	3.27	0.11	13.87	2.22
Cg1	64.7	10	15.8	6.88	0.368	53.49	4.13
Cg2	54.1	10	13.32				
Cg3	60.3	13.4	21.01	11.25	0.338	30.04	3.46
Cg4	60.2	10.4	15.84	7.58	0.302	39.84	3.66
Cg5	59.9	11.5	17.16				
Cg6	60.8	12.2	18.83	9.79	0.403	41.16	4.46
Cg7	58.2	12	17.2				
Cg8	65.5	18.7	25.48				
Cg9	67	13.6	20.64				
Cg10	56.4	11	15.26				
Mean =	60.71	12.28	18.05	8.88	0.35	41.13	3.93
SD =	4.06	2.59	3.54	2.01	0.04	9.61	0.45

October - Undosed							
Oyster	Shell Ht.	Volume	Whole Wt	Shell DW	Tissue DW	Condition-WT	Condition-VOL
	(mm)	(ml)	(g)	(g)	(g)		
Cv1	51	9.8	16.44	11.21	0.337	30.06	6.44
Cv2	53.6	10.8	17.41	12.54	0.257	20.49	5.28
Cv3	54.5	13	20.91	15.01	0.435	28.98	7.37
Cv4	48	10.2	20.3	15.03	0.375	24.95	7.12
Cv5	52.6	10.8	19.08	13.25	0.42	31.70	7.20
Cv6	55.2	9.9	16.89	10.04	0.404	40.24	5.90
Cv7	51.5	11.6	19.45	13.89	0.306	22.03	5.50
Cv8	55.8	11.1	21.65	15.91	0.393	24.70	6.85
Cv9	51.7	16.7	32.82	25.16	0.531	21.10	6.93
Cv10	51.3	10.9	18.08	13.01	0.397	30.51	7.83
Mean =	52.52	11.48	20.30	14.51	0.39	27.48	6.64
SD =	2.33	2.05	4.73	4.15	0.08	6.07	0.84
Cg1	58.5	12.8	17.88	9.73	0.29	29.80	3.56
Cg2	61	15.7	24.08	12.88	0.562	43.63	5.02
Cg3	66.1	17.9	26.27	13.57	0.552	40.68	4.35
Cg4	65.3	17.6	25.6	12.46	0.604	48.48	4.60
Cg5	56	13.5	20.99	11.14	0.405	36.36	4.11
Cg6	60.2	14.7	20.83	11.13	0.481	43.22	4.96
Cg7	61.5	13.1	19.51	10.52	0.455	43.25	5.06
Cg8	54	14.1	21.3	11.1	0.351	31.62	3.44
Cg9	54.4	16	22.22	10.88	0.465	42.74	4.10
Cg10	58.5	13.6	19.09	9.67	0.24	24.82	2.55
Mean =	59.55	14.90	21.78	11.31	0.44	38.46	4.17
SD =	4.14	1.83	2.78	1.29	0.12	7.51	0.81

October - Dosed							
Oyster	Shell Ht.	Volume	Whole Wt	Shell DW	Tissue DW	Condition-WT	Condition-VOL
	(mm)	(ml)	(g)	(g)	(g)		
Cv1	51.5	7.3	12.03	8.88	0.21	23.65	6.67
Cv2	46.4	8.9	15.17				
Cv3	47.9	7.3	13.97	10.25	0.172	16.78	4.62
Cv4	54	6.6	12.29	8.09	0.315	38.94	7.50
Cv5	47.4	11	19	14.59	0.351	24.06	7.96
Cv6	46.3	7	15.03	11.26	0.191	16.96	5.07
Cv7	45.8	7.6	13.82	10.27	0.186	18.11	5.24
Cv8	44.6	9.8	22.95	17.12	0.303	17.70	5.20
Cv9	47.3	8.4	15.07	10.77	0.16	14.86	3.72
Cv10	43.9	8.2	14.71	10.73	0.248	23.11	6.23
Mean =	47.51	8.21	15.40	11.33	0.24	21.57	5.80
SD =	3.08	1.37	3.27	2.82	0.07	7.34	1.39
Cg1	58.6	13.4	19.85	9.42	0.23	24.42	2.21
Cg2	55.6	14.9	23.28	13.29	0.381	28.67	3.81
Cg3	54.5	14.2	20.41	9.76	0.26	26.64	2.44
Cg4	52.9	12.1	16.88	8.56	0.099	11.57	1.19
Cg5	66.7	14.3	21.68	11.21	0.276	24.62	2.64
Cg6	59.6	15.8	22.68	11.99	0.255	21.27	2.39
Cg7	59.9	14.2	19.34	9.21	0.295	32.03	2.91
Cg8	49.7	11.2	15.27	7.89	0.298	37.77	4.04
Cg9	57.1	14.9	21.74	10.81	0.333	30.80	3.05
Cg10	63.8	16	22.93	12.84	0.227	17.68	2.25
Mean =	57.84	14.10	20.41	10.50	0.27	25.55	2.69
SD =	5.05	1.52	2.66	1.83	0.07	7.51	0.82

TABLE 3				
Clearance rate, absorption efficiency, oxygen consumption rate, and ammonia excretion rate of undosed and dosed oysters, C. virginica and C. gigas, from May to October, 1992. Cv = C. virginica; Cg = C. gigas.				
May - Undosed				
Oyster	Clearance	Absorption	Oxygen	Ammonia
	(l/hr/g)	(%)	(ml/hr/g)	(ug/hr/g)
Cv1	4.52	71%	0.86	37.38
Cv2	4.68	44%	0.92	46.34
Cv3	3.86	56%	0.67	25.51
Cv4	6.17	51%	1.31	31.48
Cv5	5.73	29%	0.91	43.65
Cv6	5.11	48%	0.68	27.70
Cv7	5.81	47%	0.68	20.61
Cv8	6.24		1.08	27.74
Cv9	9.50	19%	1.55	34.80
Cv10	6.35	39%	1.39	60.00
Mean =	5.80	45%	1.01	35.52
SD =	1.54	15%	0.32	11.77
Cg1	7.35	32%	1.07	47.26
Cg2	5.55	49%	0.85	32.68
Cg3	7.89		0.82	42.87
Cg4	6.48		1.15	38.19
Cg5	6.07	49%	1.21	57.76
Cg6	4.55		0.98	36.22
Cg7	3.14		0.66	19.32
Cg8	5.44		1.03	44.29
Cg9	3.27	32%	0.79	23.49
Cg10	5.38	63%	1.14	40.51
Mean =	5.51	45%	0.97	38.26
SD =	1.56	13%	0.18	11.23

June - Undosed				
Oyster	Clearance (l/hr/g)	Absorption (%)	Oxygen (ml/hr/g)	Ammonia (ug/hr/g)
Cv1	3.97	82%	1.03	13.34
Cv2	3.30	53%	0.83	12.05
Cv3	4.08	49%	0.92	6.46
Cv4	8.26	45%	2.28	21.14
Cv5	3.89	74%	1.08	
Cv6	2.44	66%	1.08	4.96
Cv7	2.74	21%	1.18	16.67
Cv8	2.78	45%	1.25	6.20
Cv9	7.20	78%	0.92	9.69
Cv10	3.81	70%	1.50	9.46
Mean =	4.25	58%	1.21	11.11
SD =	1.94	19%	0.42	5.31
Cg1	12.86	69%	0.94	104.74
Cg2		76%		
Cg3	11.96	38%	1.81	126.81
Cg4	12.23	28%	1.86	96.76
Cg5	16.26	28%	2.28	72.69
Cg6	10.67	52%	2.07	36.68
Cg7	7.05	80%	0.82	115.43
Cg8	14.97	98%		88.56
Cg9	8.01	52%	1.37	22.32
Cg10	8.06	28%	2.33	58.32
Mean =	11.34	55%	1.69	80.26
SD =	3.19	25%	0.58	35.60

Table 3, P. 2

July - Undosed				
Oyster	Clearance (l/hr/g)	Absorption (%)	Oxygen (ml/hr/g)	Ammonia (ug/hr/g)
Cv1	7.39	54%	1.35	41.80
Cv2	6.43	64%	2.00	33.94
Cv3	3.22	47%	2.11	65.62
Cv4	3.79	52%	2.10	24.95
Cv5	6.50	43%	1.39	17.34
Cv6	6.51	33%	1.44	18.37
Cv7	3.74	37%	2.22	39.38
Cv8	5.69	47%	1.65	22.77
Cv9	5.63	44%	2.58	47.51
Cv10	8.10	50%	1.78	36.83
Mean =	5.70	47%	1.86	34.85
SD =	1.64	9%	0.41	14.92
Cg1	14.08		2.04	132.04
Cg2			1.03	38.82
Cg3	18.73	45%	3.17	64.79
Cg4	10.70		2.46	79.23
Cg5	7.44	63%	1.34	
Cg6	10.15	27%	1.38	43.57
Cg7	8.62	18%	1.20	39.28
Cg8	6.13		0.76	34.43
Cg9	18.96	40%	2.44	
Cg10	7.26		1.12	35.48
Mean =	11.34	39%	1.69	58.46
SD =	4.85	17%	0.79	33.73

July - Dosed				
Oyster	Clearance	Absorption	Oxygen	Ammonia
	(l/hr/g)	(%)	(ml/hr/g)	(ug/hr/g)
Cv1	10.02		1.03	28.30
Cv2	11.63		2.09	33.62
Cv3	14.10		1.60	31.97
Cv4	6.20	26%	2.07	44.24
Cv5	14.82		2.53	41.16
Cv6	15.22	26%	2.31	2.07
Cv7	12.65	26%	1.45	32.53
Cv8	10.21	21%	1.64	36.88
Cv9	5.70	19%	1.67	20.14
Cv10	8.44		1.75	17.74
Mean =	10.90	24%	1.81	28.87
SD =	3.40	3%	0.44	12.54
Cg1	7.42		1.81	53.19
Cg2	9.70		3.04	55.48
Cg3	9.54		2.39	30.43
Cg4	15.27		3.41	89.60
Cg5	8.22		2.08	65.09
Cg6	6.68		3.89	94.65
Cg7	7.36		2.35	111.60
Cg8	9.28		2.84	59.68
Cg9	10.81		1.67	139.32
Cg10	9.58		1.72	54.80
Mean =	9.39		2.52	75.39
SD =	2.44		0.76	32.76

Table 3, P. 4

August - Undosed				
Oyster	Clearance (l/hr/g)	Absorption (%)	Oxygen (ml/hr/g)	Ammonia (ug/hr/g)
Cv1	1.82		2.87	52.40
Cv2	3.64		3.91	28.57
Cv3	4.23		2.68	51.62
Cv4	2.81			18.73
Cv5	3.02		4.27	57.53
Cv6	4.45		6.65	93.60
Cv7	5.10		6.67	
Cv8	5.46			95.46
Cv9	8.11		5.80	
Cv10	2.83			25.35
Mean =	4.15		4.69	52.91
SD =	1.79		1.69	29.30
Cg1	6.11		3.69	137.35
Cg2				35.18
Cg3	2.57		3.56	17.22
Cg4	3.99		2.70	
Cg5	4.95			46.87
Cg6	2.79			
Cg7	1.76			29.04
Cg8	3.54			39.63
Cg9	1.50		3.18	73.81
Cg10	2.51		2.64	46.08
Mean =	3.30		3.15	53.15
SD =	1.51		0.48	37.77

August - Dosed				
Oyster	Clearance	Absorption	Oxygen	Ammonia
	(l/hr/g)	(%)	(ml/hr/g)	(ug/hr/g)
Cv1	5.79		4.30	
Cv2	7.24		3.96	37.88
Cv3	7.34		5.28	47.98
Cv4	5.89		3.24	30.28
Cv5	4.44		3.12	45.29
Cv6	4.49		2.96	27.50
Cv7	8.42		5.67	56.14
Cv8	4.25		4.59	28.07
Cv9	4.66		2.68	32.57
Cv10	8.33		4.20	25.67
Mean =	6.08		4.00	36.82
SD =	1.64		1.01	10.72
Cg1	3.65		4.80	
Cg2	2.61		7.83	
Cg3	3.56		3.82	54.32
Cg4	2.87		3.98	37.77
Cg5	4.18		4.63	
Cg6	2.39		4.50	70.05
Cg7	2.27		4.68	
Cg8	2.96		4.94	39.05
Cg9	5.44		5.30	105.37
Cg10	3.28		4.64	30.57
Mean =	3.32		4.91	56.19
SD =	0.96		1.11	27.94

September - Undosed				
Oyster	Clearance	Absorption	Oxygen	Ammonia
	(l/hr/g)	(%)	(ml/hr/g)	(ug/hr/g)
Cv1	2.72	81%	2.19	17.08
Cv2	3.89	68%	3.76	43.55
Cv3	3.91	66%	2.56	29.60
Cv4	2.51	58%	2.32	10.46
Cv5	4.00	71%	4.41	
Cv6	2.61	62%	3.06	27.82
Cv7	4.37	37%	3.67	36.07
Cv8	2.53	53%	2.40	17.41
Cv9		37%		
Cv10			3.75	
Mean =	3.32	59%	3.12	26.00
SD =	0.79	15%	0.80	11.69
Cg1				
Cg2				
Cg3				
Cg4	8.07	45%	4.59	113.34
Cg5				
Cg6	7.05	70%	2.63	55.45
Cg7				
Cg8	8.38	24%		147.15
Cg9				
Cg10	10.60		4.04	94.76
Mean =	8.52	46%	3.76	102.68
SD =	1.50	23%	1.01	38.23

September - Dosed				
Oyster	Clearance	Absorption	Oxygen	Ammonia
	(l/hr/g)	(%)	(ml/hr/g)	(ug/hr/g)
Cv1	3.01		3.15	105.38
Cv2	4.93		4.05	52.38
Cv3	1.74		1.76	18.21
Cv4				
Cv5		43%	2.96	
Cv6				
Cv7	4.00		4.51	35.63
Cv8	3.51	31%	4.03	84.27
Cv9	5.09	20%	3.38	38.96
Cv10				
Mean =	3.71	31%	3.41	55.80
SD =	1.26	11%	0.91	32.81
Cg1		56%	2.85	94.02
Cg2				
Cg3	4.38	34%	4.53	145.89
Cg4	4.57	47%	4.37	162.09
Cg5				
Cg6	2.46		4.04	82.93
Cg7				
Cg8				
Cg9				
Cg10				
Mean =	3.80	46%	3.95	121.23
SD =	1.17	11%	0.76	38.66

Table 3, P. 8

October - Undosed				
Oyster	Clearance	Absorption	Oxygen	Ammonia
	(l/hr/g)	(%)	(ml/hr/g)	(ug/hr/g)
Cv1	4.07	61%	1.22	62.76
Cv2	8.05	30%	1.56	85.88
Cv3	4.85		1.03	40.14
Cv4	5.20	48%	0.83	58.11
Cv5	5.88	43%	0.95	40.45
Cv6	4.33	61%	0.99	54.63
Cv7	7.45		1.14	68.50
Cv8	5.88	55%	1.07	65.32
Cv9	4.76	55%	1.26	27.48
Cv10	5.74		1.31	48.61
Mean =	5.62	50%	1.14	55.19
SD =	1.29	11%	0.21	16.81
Cg1	5.79	39%	1.72	64.34
Cg2	5.00	43%	1.17	40.09
Cg3	4.86	49%	1.20	51.52
Cg4	4.77	28%	0.96	53.51
Cg5	7.85		1.85	105.78
Cg6	5.41		1.46	100.58
Cg7	4.04	35%	1.82	85.03
Cg8	6.52	30%	1.99	87.07
Cg9	5.83		1.72	54.02
Cg10	8.58		2.46	80.04
Mean =	5.87	37%	1.64	72.20
SD =	1.43	8%	0.45	22.56

October - Dosed				
Oyster	Clearance (l/hr/g)	Absorption (%)	Oxygen (ml/hr/g)	Ammonia (ug/hr/g)
Cv1	9.95	34%	2.33	59.57
Cv2				
Cv3	6.80	67%	1.98	60.70
Cv4	4.13	40%	1.16	22.44
Cv5	7.32	44%	1.23	40.17
Cv6	8.43	31%	1.52	13.46
Cv7	7.31	70%	1.77	31.94
Cv8	3.66		1.25	30.76
Cv9	6.50		1.88	17.81
Cv10	9.15	65%	1.17	14.15
Mean =	7.03	50%	1.59	32.33
SD =	2.10	17%	0.42	18.05
Cg1	7.57	76%	1.39	142.83
Cg2	7.22		0.94	83.52
Cg3	7.31	48%	0.96	84.15
Cg4		67%		
Cg5	11.01	56%	1.52	55.83
Cg6	11.61	48%	1.88	125.14
Cg7	7.66	42%	1.36	63.36
Cg8	8.22	48%		135.70
Cg9	9.61	63%	1.83	93.03
Cg10	5.64	48%	2.20	57.97
Mean =	8.43	55%	1.51	93.50
SD =	1.94	11%	0.44	33.53

Table 3, P. 10

TABLE 4						
Lipid, glycogen, protein, and ash levels (%DW) and mass fractions of carbon and nitrogen of undosed and dosed oysters, C. virginica and C. gigas. Cv = C. virginica; Cg = C. gigas						
May - Undosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	13.82	15.48	25.37	11.88	41.28	5.56
Cv2	14.98	10.70	19.77	10.81	41.64	6.00
Cv3	13.18	14.03	11.40	12.83	42.48	5.78
Cv4	13.94	7.60	27.36	11.11	41.69	6.93
Cv5	12.03	13.28	10.66	15.32	40.11	5.39
Cv6	14.36	14.25	19.94	11.59	42.93	6.52
Cv7	13.54	13.35	17.00	9.74	41.93	6.06
Cv8	12.57	12.81	13.42	13.25	41.10	6.10
Cv9	11.05	9.56		26.19	40.27	6.59
Cv10	10.46	11.65	21.24	11.68	40.10	6.52
Mean =	12.99	12.27	18.46	13.44	41.35	6.15
SD =	1.45	2.40	5.88	4.73	0.98	0.49
Cg1	13.06	17.03	12.76	17.16	40.48	7.41
Cg2	10.29	15.79	31.36	7.80	39.12	7.32
Cg3	10.38	11.54	29.02	5.95	39.27	7.16
Cg4	9.93	14.01	30.82	8.50	39.69	7.90
Cg5	10.93	9.78	34.67	13.30	38.52	7.34
Cg6	11.49	11.95	31.67	6.28	40.36	8.00
Cg7	11.86		20.47	7.14	40.51	8.12
Cg8	10.96	10.47	16.81	7.91	40.29	7.95
Cg9	9.64		30.48	10.97	40.45	6.70
Cg10	9.88	12.42	17.32	4.63	39.57	8.13
Mean =	10.84	12.87	25.54	8.97	39.83	7.60
SD =	1.06	2.54	7.83	3.82	0.70	0.48

June - Undosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	17.69	20.28	17.12	7.95	42.86	5.97
Cv2	18.74	27.25	17.33	6.83	42.60	5.78
Cv3	14.85	12.20	19.01	12.61	43.29	6.92
Cv4	11.59	21.30		8.47	41.34	7.90
Cv5	15.93	12.02	16.11	6.98	42.33	6.59
Cv6	20.78	21.68	23.11	6.21	45.10	6.51
Cv7	16.36	16.14	27.38	6.43	42.92	7.15
Cv8	15.66	18.86	23.45	5.44	43.70	6.80
Cv9	14.67	14.92	25.79	8.54	43.05	9.08
Cv10	19.77	21.11	33.17	7.47	44.48	8.35
Mean =	16.60	18.58	22.50	7.69	43.17	7.11
SD =	2.72	4.77	5.68	1.99	1.07	1.05
Cg1	10.80	29.25	25.94	10.09	40.06	7.27
Cg2						
Cg3	12.92	20.21	29.80	12.42	39.32	8.81
Cg4	9.30	23.76		14.16	39.00	7.64
Cg5	9.48	33.29	35.86	12.52	39.53	8.31
Cg6	16.42	25.65	26.39		39.83	7.67
Cg7	22.14		18.25	13.19	42.76	7.77
Cg8	12.52	24.28	24.66	11.42	38.88	7.12
Cg9	13.78		19.83	11.76	40.20	7.60
Cg10	11.33	17.59	18.62	8.83	41.13	7.06
Mean =	13.19	24.86	24.92	11.80	40.08	7.69
SD =	4.02	5.28	6.06	1.70	1.22	0.57

Table 4, P. 2

July - Undosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	6.94	31.83	26.55		42.08	7.64
Cv2	8.18	36.72	36.64	6.11	43.30	8.32
Cv3	16.44	25.98	39.11	7.82	43.96	9.14
Cv4	14.47	28.67	39.38	6.71	43.35	7.39
Cv5	10.88	18.95	42.54	6.09	44.55	9.44
Cv6	9.50	22.85	45.75	7.43	42.76	9.61
Cv7	18.86	21.00	42.20	6.43	43.85	9.77
Cv8		18.35	39.55		45.47	8.79
Cv9	17.99	21.85	27.83	7.75	41.91	8.20
Cv10	12.48	13.61	45.08	8.42	42.87	9.70
Mean =	12.86	23.98	38.46	7.10	43.41	8.80
SD =	4.33	6.91	6.57	0.88	1.09	0.87
Cg1	13.95	13.29	39.46	13.54	32.65	7.83
Cg2	8.43	15.53	42.00	9.08	42.52	10.22
Cg3	6.52	14.75	37.79	16.10	37.49	8.27
Cg4	6.84	20.10	37.95	8.96	40.69	8.86
Cg5	7.14	16.94	41.44	9.31	42.24	8.64
Cg6	9.55	19.76	37.05	10.47	41.33	9.53
Cg7	8.05	20.99	36.06	8.26	41.34	9.28
Cg8		8.76	42.50		44.81	8.57
Cg9		10.64	36.59		41.22	8.65
Cg10	9.37	18.78	41.11	6.63	41.70	9.40
Mean =	8.73	15.95	39.19	10.29	40.60	8.93
SD =	2.38	4.15	2.41	3.07	3.33	0.69

Table 4, P. 3

July - Dosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	12.77	34.38	36.45	2.83	42.90	6.87
Cv2	12.95	20.61	31.29		44.24	7.40
Cv3	14.39	31.84	30.93	12.06	44.50	7.22
Cv4	11.74	14.40	34.42	8.03	43.84	8.16
Cv5		27.77	35.17	7.13	45.02	7.12
Cv6	8.90	16.23	36.73	8.75	44.80	7.92
Cv7	13.73	24.53	48.03	6.57	45.24	6.98
Cv8	12.50	22.16	35.51	9.07	44.14	7.91
Cv9	12.10	17.37	22.95	6.66	44.89	7.54
Cv10	16.76	36.72	30.43	7.10	45.56	6.67
Mean =	12.87	24.60	34.19	7.58	44.51	7.38
SD =	2.12	7.84	6.37	2.47	0.77	0.50
Cg1	14.88	20.59	33.73	6.69	43.62	8.37
Cg2	14.99	14.93	31.60	9.08	42.30	9.73
Cg3	18.36	21.49	33.33	8.91	43.79	7.86
Cg4	11.49		43.33	10.82	42.27	9.25
Cg5	14.55	25.71	31.35	12.90	43.28	7.33
Cg6	15.84	14.18	37.41	10.27	42.02	9.31
Cg7	12.86	20.42	35.96	10.00	44.08	8.34
Cg8	20.34		39.81	10.44	42.97	9.16
Cg9	12.08	15.20	37.25	13.20	42.74	9.58
Cg10	15.38	21.83	36.47	10.25	43.01	7.68
Mean =	15.08	19.29	36.02	10.25	43.01	8.66
SD =	2.71	4.09	3.73	1.88	0.69	0.85

Table 4, P. 4

August-Undosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	14.00	24.35	31.69	6.50	44.08	7.58
Cv2	13.85	26.48	33.59	8.14	44.21	8.05
Cv3	16.05	25.66	33.21	6.31	45.62	7.53
Cv4	13.98	23.97	29.89	9.62	43.67	8.35
Cv5	13.99	26.71	30.32	7.37	43.70	7.34
Cv6	13.52	19.03	41.49	8.32	44.23	8.67
Cv7	15.02	9.24	43.91	8.93	43.10	9.56
Cv8	12.87	19.50	40.94	8.69	43.70	9.08
Cv9	13.51	9.46	41.77	9.97	40.42	9.00
Cv10	15.49	24.61	34.44		44.60	7.65
Mean =	14.23	20.90	36.13	8.21	43.73	8.28
SD =	0.98	6.62	5.32	1.28	1.35	0.77
Cg1	19.76	17.84	31.22	7.07	42.90	7.94
Cg2	13.40	24.08	37.24	9.87	42.59	7.57
Cg3	16.41	32.15	34.55	7.32	42.46	7.84
Cg4	22.29	20.46	31.04	8.98	44.76	7.70
Cg5	21.82	26.40	40.36	6.87	44.89	7.81
Cg6	14.00	26.87	37.77	9.60	43.07	7.72
Cg7	13.87	29.04	30.92		42.75	6.94
Cg8	16.35	32.73	33.81		44.24	6.51
Cg9	13.71	29.02	25.20		43.44	7.14
Cg10	12.14	21.52	32.84		42.83	8.08
Mean =	16.37	26.01	33.50	8.29	43.39	7.53
SD =	3.68	4.98	4.32	1.35	0.91	0.50

August-Dosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	14.31	29.61	35.37	10.54	45.33	7.58
Cv2	11.54	27.75	36.75	6.32	45.67	7.93
Cv3	11.68	28.29	47.55	10.81	44.82	8.32
Cv4	15.54	30.14	34.47	6.44	46.30	8.28
Cv5		25.90	31.84	7.25	44.87	7.51
Cv6	14.34	27.05	32.06	16.23	46.44	7.32
Cv7	19.53	23.64	35.20	8.37	44.09	7.74
Cv8	15.96	13.32	43.40	8.39	45.37	8.90
Cv9	14.09	22.65	32.44	6.63	45.97	7.47
Cv10	13.55	26.54	36.38	6.88	45.20	8.64
Mean =	14.51	25.49	36.55	8.79	45.41	7.97
SD =	2.41	4.89	5.11	3.08	0.72	0.54
Cg1	13.68	12.34	33.70	10.48	43.15	9.51
Cg2	17.97		32.81	5.80	44.98	7.22
Cg3	12.45	29.95	35.64	8.25	44.00	8.11
Cg4	13.36	26.65	36.35	10.73	42.74	8.52
Cg5	12.27	17.75	41.61	8.39	44.28	8.14
Cg6	13.79	28.72	35.67	9.06	43.54	8.08
Cg7	13.58	25.93	40.19	7.41	44.62	7.48
Cg8	14.84	28.32	38.22	9.66	43.78	7.74
Cg9	13.08	28.71	32.19	4.51	43.54	7.54
Cg10	13.23	34.38	30.61	7.42	43.42	7.81
Mean =	13.83	25.86	35.70	8.17	43.81	8.02
SD =	1.62	6.72	3.53	1.98	0.68	0.65

Table 4, P. 6

September-Undosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	9.68	20.53	43.07	6.91	46.71	8.12
Cv2	6.88	23.09	22.71	7.45	44.49	9.55
Cv3	6.34	32.02	38.97	8.88	44.56	7.59
Cv4	6.82	32.29		6.16	45.46	7.22
Cv5	9.23	27.57	38.97	10.65	43.26	8.86
Cv6	15.49	31.26		6.24	45.19	7.03
Cv7	10.10	25.19	41.60	9.01	44.91	8.41
Cv8	12.05	34.01	31.32	7.92	45.74	7.64
Cv9						
Cv10		33.76		7.55	44.52	8.55
Mean =	9.57	28.86	36.11	7.86	44.98	8.11
SD =	3.09	4.95	7.71	1.45	0.96	0.82
Cg1						
Cg2						
Cg3						
Cg4	9.61	33.17	42.93	10.88	42.50	8.44
Cg5						
Cg6	10.04	19.08	37.83	9.49	43.08	7.99
Cg7						
Cg8	10.66	30.28	34.20	12.09	41.17	7.66
Cg9						
Cg10	8.10	28.94	33.24	9.97	42.23	7.75
Mean =	9.60	27.87	37.05	10.61	42.25	7.96
SD =	1.09	6.12	4.39	1.14	0.80	0.35

Table 4, P. 7

September-Dosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	10.24	12.06	45.35	10.14	42.82	9.54
Cv2	16.11	16.92	37.13		44.29	8.34
Cv3	12.44	33.62	32.69	9.11	43.08	6.42
Cv4						
Cv5	13.84	11.93	46.52	11.99	43.33	7.05
Cv6						
Cv7	9.17	30.58	32.93		42.32	7.06
Cv8	14.50	10.92	51.10	9.03	44.72	9.90
Cv9	10.21	28.26	37.25	6.21	44.55	7.04
Cv10		9.18			43.36	9.23
Mean =	12.36	19.18	40.43	9.30	43.56	8.07
SD =	2.59	9.98	7.21	2.10	0.87	1.35
Cg1	10.45	29.04	38.45	7.63	44.27	7.79
Cg2						
Cg3	10.15	21.37	36.78	9.83	43.58	8.73
Cg4	13.22	22.85	38.55	8.64	43.86	8.39
Cg5						
Cg6	12.31	30.64	37.68	7.01	44.19	7.59
Cg7						
Cg8						
Cg9						
Cg10						
Mean =	11.53	25.97	37.86	8.28	43.98	8.13
SD =	1.47	4.55	0.82	1.23	0.32	0.53

Table 4, P. 8

October-Undosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1		28.84	39.08	10.20	42.15	7.99
Cv2	9.39	28.08	43.40	10.50	42.38	8.99
Cv3	10.25	32.73	32.13	7.78	43.32	7.79
Cv4	8.09	29.48	35.09	9.19	43.11	8.49
Cv5	9.10	32.01	30.78	13.72	42.10	7.56
Cv6	7.60	34.80	29.45	9.70	41.90	8.04
Cv7	10.63	27.68	41.56	12.51	41.09	8.89
Cv8	12.13	29.82	34.34	9.64	42.99	8.22
Cv9	11.94	22.77	35.09	9.60	43.65	8.33
Cv10	11.86	27.35	40.38	8.87	42.74	8.11
Mean =	10.11	29.41	36.13	10.17	42.54	8.24
SD =	1.68	3.54	4.76	1.75	0.77	0.45
Cg1	9.55	16.21	37.39	8.73	40.88	8.69
Cg2	13.40	34.49	44.39	9.86	43.88	8.05
Cg3	11.45	24.61	33.51	13.29	42.47	8.43
Cg4	12.01	30.49	25.46	8.53	43.09	7.75
Cg5	9.77	27.28	29.13	9.58	42.71	9.15
Cg6	10.81	24.54	29.76	10.59	42.86	8.10
Cg7	12.48	32.77	37.00	8.16	50.90	9.53
Cg8	10.96	24.94	37.95	9.87	42.88	8.38
Cg9	12.36	24.71	28.47	11.02	42.54	8.81
Cg10		21.96	33.33	13.58	40.99	9.12
Mean =	11.42	26.67	33.64	10.32	43.32	8.60
SD =	1.28	5.46	5.66	1.87	2.81	0.56

Table 4, P. 9

October-Dosed						
Oyster	Lipid	Glycogen	Protein	Ash	Carbon	Nitrogen
	%DW	%DW	%DW	%DW	%	%
Cv1	11.38	24.15	25.82	8.97	43.11	8.18
Cv2						
Cv3	13.01	21.07	34.05	10.39	42.56	9.01
Cv4	11.08	20.42	28.48	9.04	42.37	8.26
Cv5	15.99	27.86	31.83	6.80	43.67	7.89
Cv6	20.34	29.74	36.45	8.57	39.10	9.34
Cv7	10.58	22.16	36.64	10.09	42.36	8.82
Cv8		16.79	40.14	9.07	42.46	7.93
Cv9		17.59	43.92	13.65	41.13	9.01
Cv10	16.69	20.04	42.02	9.19	43.06	8.96
Mean =	14.62	21.96	35.48	9.53	42.20	8.60
SD =	3.75	4.60	6.08	1.85	1.36	0.54
Cg1	9.90	15.42	42.72	13.37	39.13	9.16
Cg2	7.64	24.01	32.88	11.68	40.57	8.16
Cg3	11.73	18.84	44.48	9.11	41.75	8.83
Cg4	11.09	11.24	39.81	2.97	38.67	9.05
Cg5	9.01	21.71	35.59	13.26	39.81	8.37
Cg6	8.61	18.29	31.78	6.49	40.38	8.21
Cg7	15.87	30.10	31.04	12.76	41.58	7.71
Cg8	19.97	24.07	26.25	9.45	43.04	9.27
Cg9	18.10	26.13	30.35	12.59	41.36	8.24
Cg10	14.80	15.51	37.07	12.11	40.85	9.70
Mean =	12.43	21.09	35.20	10.38	40.71	8.67
SD =	4.45	5.77	5.83	3.41	1.30	0.62

TABLE 5

Summary of significant differences (2-way ANOV, $P<0.05$) for all parameters examined. Data grouped by species, disease treatment (dosed or undosed) and species-disease interaction. Cv = C. virginica; C.g. = C. gigas; Dos = Dosed with P. marinus; Und = undosed.

Parameter		May	June	July	August	September	October
Whole Wt.	Species	Cg>Cv		Cg>Cv	Cg>Cv	Cg>Cv	Cg>Cv
	Disease				Und>Dos		Und>Dos
	Interaction						
Tissue DW	Species		Cv>Cg		Cg>Cv		
	Disease						Und>Dos
	Interaction				int.		
Shell DW	Species		Cv>Cg	Cv>Cg	Cv>Cg		Cv>Cg
	Disease				Und>Dos		Und>Dos
	Interaction			int.			
Cond- WT	Species			Cg>Cv	Cg>Cv		Cg>Cv
	Disease						Und>Dos
	Interaction				int.		
Cond- VOL	Species	Cv>Cg	Cv>Cg	Cv>Cg	Cv>Cg	Cv>Cg	Cv>Cg
	Disease						Und>Dos
	Interaction				int.	int.	
Clearance	Species		Cg>Cv		Cv>Cg	Cg>Cv	
	Disease					Und>Dos	Dos>Und
	Interaction			int.		int.	
Absorption	Species						
	Disease			Und>Dos			
	Interaction						
Oxygen	Species						
	Disease						
	Interaction			int.	int.		int.
Ammonia	Species		Cg>Cv	Cg>Cv		Cg>Cv	Cg>Cv
	Disease						
	Interaction						int.
Lipid	Species	Cv>Cg	Cv>Cg				
	Disease			Dos>Und		Dos>Und	Dos>Und
	Interaction			int.			
Glycogen	Species		Cg>Cv	Cv>Cg			
	Disease						Und>Dos
	Interaction						
Protein	Species	Cg>Cv					
	Disease			Und>Dos			
	Interaction						
Ash	Species	Cv>Cg	Cg>Cv	Cg>Cv			
	Disease						
	Interaction					int.	
Total C	Species	Cv>Cg	Cv>Cg	Cv>Cg	Cv>Cg	Cv>Cg	
	Disease			Dos>Und	Dos>Und		Und>Dos
	Interaction				int.	int.	
Total N	Species	Cg>Cv		Cg>Cv			
	Disease			Und>Dos			
	Interaction			int.	int.		