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Modelling of zinc accumulation in the American oyster, Crassostrea virginica (GMELIN)

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Modelling of zinc accumulation in the American oyster, *Crassostrea virginica* (GMELIN)

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MODELLING OF ZINC ACCUMULATION IN THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN)

A Dissertation

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LIST OF TERMS

The following terms are used as defined here unless specified otherwise in the texts.

Body Weight:	The dry weight of an organism's soft tissue, excluding shells.
Body Burden:	Total zinc content in soft tissue of an organism.
Concentration:	Soft tissue zinc content divided by body weight, *i.e.* the weight specific zinc concentration of an organism.
Uptake Rate:	The amount of zinc taken from the environment per unit time.
Depuration:	Removal of zinc from the soft tissue of an organism.

ABSTRACT

A model of zinc accumulation by the American oyster, *Crassostrea virginica*, is developed by relating in-situ zinc body burden to time-integration of uptake. Short-term uptake rates are estimated in laboratory by introducing ^{65}Zn to three groups of 12 oysters of various weights in aquaria with salinities of 18 ‰ and 12 ‰. It is found that the uptake of ^{65}Zn by an oyster (1) varies as a power function of the body weight (soft tissue dry weight) of the oyster ($\frac{dy}{dt} = kW^{b-1}$), (2) is inversely related to the salinity of ambient water, and (3) increases linearly with ambient concentration. Zinc body burdens of oysters of various weights from oyster beds with three different salinity regimes of the James River and of the Rappahannock River are measured. When the zinc body burden of oysters is fitted to a power function of body weight ($y=aW^b$), the values of power, b, are 1.33, 1.30, and 1.06 for salinities of 13, 15, and 20 ‰, respectively, in the James River and 1.16 for a nominal salinity of 18 ‰ in the Rappahannock River. The values of **b** agree with the values of power, β, derived from the ^{65}Zn uptake experiments; $b = \beta + 1$.

The model is calibrated using data for Horsehead Shoals and Nansemond Ridge, two sites in the James River having average salinities roughly the same as those used in ^{65}Zn laboratory studies. The model is verified by the use of data for Wreck Shoal, a mid-salinity sampling site of the James River, and the pooled data for the Rappahannock River sampling sites. The weight-specific zinc concentration of an oyster increases continuously, but rate of the increase is reduced as the oyster grows larger. Both uptake parameters, **k** and β vary with salinity.

It is suggested that the body weight effects, and their variation with salinity, should be incorporated in the design of monitoring programs for trace metals as well as in experimental studies.

In appendix, (1) three sources of variability in measurements that can be eliminated are identified and discussed, and (2) zinc body burden data for the hooked mussels, *Ischadium recurvum* Rafinesque, from the Rappahannock River, Virginia are compared to those for oysters.

MODELLING OF ZINC ACCUMULATION IN THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN)

I. INTRODUCTION

A. Trace metals in Aquatic Environment and Benthic Bivalves.

The American oyster, *Crassostrea virginica* (Gmelin), is one of the most widely distributed bivalve mollusks in the Chesapeake Bay and a valuable food resource. It accumulates trace metals to high concentrations, often orders of magnitude higher than those of the surrounding waters (Shuster & Pringle 1969; Huggett *et al.* 1973; Frazier 1975, 1976; Wolfe 1970a; Phelps *et al.* 1985). Concentrations of copper and zinc as high as 0.45 mg and 21 mg per gram wet weight, respectively, have been reported (Thrower & Eustace 1973). Bioaccumulation of metals is of interest for the potentially toxic effects to the organisms themselves and to humans who consume the food resources.

There are large anthropogenic sources of trace metals in the Chesapeake Bay system and high concentrations of metals have been reported in sediments (Helz *et al.* 1981; Kingston *et al.* 1982). *C. virginica* from the Chesapeake Bay are reported to have high trace metal concentrations (Gilinsky & Roland 1983; Eisenberg & Topping 1984).

It is very important to interpret the data from biomonitoring surveys carefully because many variables affect the net uptake of trace metals by bivalves. These include weight of the individual (Boyden 1974, 1977; Phillips 1976a; Davies & Pirie

1980; Popham & D'Auria 1982), season of collection (Bryan 1973; Pentreath 1973; Frazier 1975, 1976; Phillips 1976b, 1980), vertical position of the bivalve on shore-line or in the water column (Nielson 1974; De Wolf 1975; Phillips 1976b), and salinity (Huggett *et al.* 1975; Phelps *et al.* 1985). Additionally, it has been shown that age, even when it is not related to weight (Bryan & Uysal 1978; Phillips 1980; Phelps *et al.* 1985), sex of the organism (Watling & Watling 1976), growth rate (Phelps & Hetzel 1987), water content of soft tissue (Phillips 1976a, 1977b), composition of phytoplankton species in the water column (Phillips 1979), concentration of organic materials (Zamuda *et al.* 1985), and geochemical characteristics of the sediments of the area (Luoma & Jenne 1976; Luoma & Bryan 1979, 1982) affect the metal uptake of an organism.

Many authors, in an attempt to elucidate mechanisms of trace metal accumulation in bivalves, have based their explanations on a single factor. Since these data result from separate studies that have been performed independently under different ambient conditions, it is difficult to evaluate the data for different physicochemical conditions. The trace metal concentration data for estuarine and brackish-water areas are the most difficult to interpret because of the diverse and ever changing conditions in these areas.

Boyden (1977) and Simkiss & Taylor (1981) advocated a normalization procedure to eliminate the effects of weight and/or salinity. Boyden & Phillips (1981) suggested a simple statistical method to define the magnitude of residual or inherent variability when perturbations such as those noted above have been eliminated. The effects of all of the parameters, however, have not yet been defined and the

importance of each parameter is not clear. The parameters are, moreover, inter-related and affect the amounts of trace metals accumulated by bivalve mollusks and may therefore interfere with the elucidation of true location based differences in contamination (Boyden & Phillips 1981).

Large amounts of data have been accumulated in laboratory toxicity tests and in-situ monitoring of both and organisms. Questions, however, remain as to how results of those studies relate to field ecosystems (Dickson 1982; Macek 1982). For hazard assessments of metals to aquatic organisms and to humans, it is necessary to understand factors controlling transport, speciation, mode of action, and bioavailability. For all toxicology and bioaccumulation studies and for effective regulatory efforts, one also needs to understand and to define the metal pathways in oysters more precisely.

B. Study Objectives and an Overview.

Oysters have been used as an indicator species of radioactive contamination by the radionuclides of trace metals in marine environments, especially for ^{65}Zn (Chipman *et al.* 1958; Preston 1966; Romeril 1971). Oysters also have been used as an indicator species of stable elements because they accumulate trace metals much more than any other benthic invertebrates including mussels (*cf.* Preston *et al.* 1972; Frazier & George 1983; Martincic *et al.* 1984). Zinc is the metal most accumulated by oysters (*cf.* Chipman *et al.* 1958; Pringle *et al.* 1968; Kopfler & Mayer 1969, 1973; Huggett 1977; Windom & Smith 1972). Ideally one would like to know the relationship between ambient metal concentrations and tissue concentrations, but it is hard to define the relationship for oysters because soft tissue trace metal concentrations show

very wide variability among individuals and among sites (Wolfe 1970a; Boyden & Phillips 1981; Phelps *et al.* 1985). The numbers and the deviation of outliers from the mean concentrations of samples in *Crassostrea virginica,* more marked than even in the common mussel, *Mytilus edulis* (L.) (Wright *et al.* 1985), increase the difficulty in comparing similar biological systems.

Besides the above difficulties introduced by the wide variations in oysters and the fact that bioavailable zinc in the environment is seldom measured, the total concentration of zinc in the environment and that in oysters are not linearly related (Preston 1966; Boyden 1974, 1977) though some laboratory uptake and depuration studies had suggested that soft tissue metal concentration was at equilibrium with ambient concentration (Romeril 1971). This makes it difficult, if not impossible, to interpret data. The non-linear growth rate of an oyster with time, the dilution effect of growth, and the effect of stunted growth on metal accumulation (Phelps & Hetzel 1987) make the problem more complex (*cf.* Strong & Luoma 1981; Thomson 1982; Simkiss & Mason 1984).

It is appropriate to approach this complex problem with modelling, given the number of variables concerned. By setting acceptable assumptions through scientific reasoning, many components of the model can be addressed. Effects of the factors on the metal body burden should be integrated over the life span of oysters rather than relating the body burden to factors at the time of surveys. Because "understanding the factors that influence the uptake, storage and elimination of metals will be essential for developing predictive models, which will greatly assist in the structuring of realistic pollution control programs (Coombs & George 1978)," an extensive literature survey

was done first. With information from the literature survey, a conceptual framework of zinc bioaccumulation in oysters is developed and then a numerical model formulated. It simulates uptake, depuration, and accumulation to estimate the tissue concentration of zinc at a given time for given histories of factors such as salinity, temperature, growth rate, and ambient concentration of the metal.

The simple model is based on the following assumptions: (1) uptake of zinc by an oyster is a function of its body weight, (2) for a given body weight, uptake rate is inversely related to the salinity of the ambient water and increases linearly with bioavailable ambient zinc concentrations, and finally (3) the body weight and zinc body burden relationships observed in in-situ feral oysters are the time-integrated result of net uptake rate.

The model, although somewhat simple, contains a number of constants and coefficients. There have been few published works of the zinc-body weight relationship for *C. virginica* from this region except those of Huggett *et al.* (1973), Frazier (1975, 1976), Phelps *et al.* (1985) and Phelps & Hetzel (1987), but development of a model required that the relationship between soft tissue zinc concentrations and dry weights of oysters be determined quantitatively. To examine short-term kinetics, uptake was measured in the laboratory by introducing the radioactive tracer ^{65}Zn with suspended solids to oysters of various weights in aquaria with two different salinities assuming that uptake rates for stable zinc and for radioactive zinc are the same. In addition, oysters from areas with different environmental characteristics, three sites in the James River, three in the Rappahannock River, and one in the Piankatank River were collected and the

information on in-situ zinc body burdens was used to complete the calibration process and to check the parameter estimates.

Zinc was chosen in this study because (1) it is the most accumulated metal for oysters and many other organisms such as mussels, (2) its bioaccumulation process by mussels has been extensively studied by many authors, (3) its radioactive isotope, the neutron-induced radionuclide ^{65}Zn, which has a somewhat long physical (241 days) and biological (oyster soft tissues) half-life (300 to 900 days) (Wolfe 1970a; Seymour & Nelson 1972, 1973), is often released into the sea in significant quantities, and (4) it is a physiologically important element. Zinc is one of a group of bivalent metals of the transition elements that exhibit an affinity toward many organic functional groups of proteins and enzymes making it potentially toxic while it is an essential metabolic constituent of the enzyme carboxypeptidase (Vallee 1962). In most marine environments, zinc is already the most abundant trace metal and natural levels in inshore sea water range between about 0.1 and 20 micrograms per liter. It also is often found at high concentrations in sediments (Phillips 1977b; Warren 1981). The ubiquitous use of zinc as "the sacrificial anode" for crab pots, monitoring instruments, navigational structures, and boats will increase zinc concentrations in some areas where oysters grow.

Measuring zinc concentrations of oysters and the water column, moreover, is one of the most simple and easy procedures because of the high concentrations in oyster soft tissues and because zinc measurements by atomic absorption spectrophotometry are not influenced by interference of other metals or salts in the samples.

II. LITERATURE SURVEY

Oysters are directly exposed to trace metals in the water column. Many of these metals are essential elements and thus there should be some mechanisms, either active or passive, to transport those metals across cell membranes and into the organisms. When present in the water column, it is inevitable that some metals are taken into oysters accidentally by diffusion or by competition with the uptake mechanism for essential elements. Therefore, it is necessary for oysters to have some mechanisms to tolerate and/or to eliminate the unwanted metals.

Many physiological studies of metal uptake in bivalve mollusks have concentrated on *Mytilus edulis* and there is comparatively little information regarding metal metabolism in oysters. It is hard to find specific information dealing with zinc metabolism of *Crassostrea virginica*, and inferences from other metals and bivalves are often not appropriate because metals behave differently and different organisms have different metal metabolisms. Quantitative assessments of the metal uptake pathways of oysters for the modelling, however, need to be based on the most probable assumptions conferred from reports for other metals and organisms. Once those assumptions are made, it is possible to develop a logical conceptual model for metal bioaccumulation. In this chapter, initial steps toward such a model are taken; hypotheses of the model and relevant assumptions are set. The hypotheses,

assumptions, or known facts that the model is based on are stated at the beginning of each section to clarify the purpose of the subsequent literature reviews and discussions.

A. Uptake and Metabolism of Zinc in Oysters.

Oysters take up zinc from the water column through the gills and the body surface, and by ingestion of particulate materials. In this study, it is assumed that both the uptake from water and uptake from ingested suspended particulate materials are important. It is hypothesized that bioavailability is linearly related to free ion activity of the metal in water. It is assumed that bioavailability of particle associated zinc is linearly related to the amount of readily digestible zinc (in organic food particles and adsorbed to inorganic materials). It is hypothesized that an oyster takes up a constant portion of the biologically available metal associated with the particulate materials ingested.

It is also assumed that (1) there is no limit to weight-specific soft tissue metal contents, (2) oysters accumulate metals to many orders in excess of metabolic requirements without any toxic effect to the organism itself, and (3) uptake of zinc from the water column is governed by the amount of bioavailable zinc, certain metabolic rates of the oyster, and concentrations of other ions (salinity).

1. Effects of trace metals on oysters.

Generally, cells of an organism maintain a constancy of their internal environment, *i.e.* homeostasis, which allows life processes to function optimally. When cellular concentrations of essential metals are less than optimum, life processes

will not function at their maximum efficiency. Many trace metal ions (including Cu, Fe and Zn) have an essential role in the catalytic activity of enzymes. Conversely, an excess of many metals usually causes inhibition of life processes through unwanted reaction with essential functional groups of enzyme proteins and subsequent disruption of metabolic pathways. Toxicity is observed when vital processes are blocked (George 1982; George & Frazier 1982). Once the metals have been accumulated, metabolic pathways must exist either to utilize, eliminate or sequester these metals, depending upon their nutritional value or toxic potential (Engel & Brouwer 1982, 1984).

Species of oyster concentrate some required metals to levels in excess of those found in metalloenzymes, *i.e.* metal-dependent enzymes (Pequegnat *et al.* 1969; Wolfe 1970b; Coombs 1972, 1974; White & Rainbow 1985). Oysters also concentrate other metals for which no requirement has been demonstrated (Pringle *et al.* 1968; Valiela *et al.* 1974). Whether the uptake of these metals is a regulated or a non-regulated process has been disputed. Even when a certain metal is actively taken by an organism, the process could be non-regulated and a coincidental consequence of other biological functions. Korringa (1952) hypothesized that "During feeding the oysters probably cannot avoid collecting and ingesting those ions that adhere firmly to the mucous feeding sheets. Collecting these ions (trace metals) is less of a problem to the oyster than getting rid of them." The existence of metal-metal interaction in some bivalves (Romeril 1971; Jackim *et al.* 1977; Luoma & Bryan 1978; Fowler *et al.* 1981) demonstrates that element levels may be metabolically influenced under certain conditions. In *Mytilus edulis*, copper is at least partially regulated and zinc is strongly regulated (*i.e.* zinc levels show little seasonal or sample variation) (Phillips 1976a,

1976b, 1980; Davenport 1977; Davenport & Manley 1978). Oysters are much better indicator species than mussels in this aspect because they regulate metals to a lesser degree and show higher bio-concentrations than mussels.

Essential trace metals, such as copper, zinc and iron are generally used in a catalytic role either as Lewis acids, as in the case of metalloenzymes incorporating zinc, or in redox reactions where redox changes in the metal ions, as iron, catalyze valence changes in substrates as iron. Even though the physiological requirements of lamellibranch bivalves for copper or zinc are relatively small compared to many other organisms (because lamellibranch bivalves do not contain any circulating respiratory pigment), copper and zinc are essential in nutrition and growth and can limit growth if present in insufficient quantities; on the other hand, they can be toxic if present at elevated concentrations because both copper and zinc are reactive with the functional groups of amino acids (particularly -SH groups). For example, copper and zinc are toxic (with EC_{50} at 20 C°, 15.1 ppb and 205.7 ppb respectively) to embryos of *Crassostrea virginica* (MacInnes & Calabrese 1977).

Oysters are also indirectly affected by pollution of estuarine waters with metals. High concentrations of metals can decrease primary productivity (Davies 1978; Thomas *et al.* 1980; Wikfors & Ukeles 1982) and alter algal species dominance (Sanders *et al.* 1981; Wikfors & Ukeles 1982). With continued exposure to sublethal concentrations, phytoplankton species can exhibit a limited increase in tolerance and adaptation to the metals (Stockner & Antia 1976). These populations are then potentially toxic for grazing species at higher trophic levels (Wikfors & Ukeles 1982) such as oysters. Veliger larvae of *C. virginica* showed poor growth and high mortality

when fed with these phytoplankton (Calabrese *et al.* 1977; Wikfors & Ukeles 1982).

Post-settlement-oysters, however, are known to tolerate high body burdens of copper and zinc (George & Frazier 1982) and are capable of living and reproducing in highly metal-polluted environments (Thrower & Eustace 1973; Thornton *et al.* 1975). Even greatly elevated levels do not always appear to affect growth or survival of the organisms. Oysters, therefore, must have some mechanism by which they can sequester accumulated metals in non toxic forms. Membrane pumps, transport and storage proteins, and compartmentation in intracellular vesicles provides means of fine control or buffering of intracellular concentrations of essential metals and detoxify them by preventing the interactions of highly reactive metal ions with essential enzyme systems (George 1982).

2. Uptake routes.

Aquatic organisms are able to take up trace metals from the environment in at least three ways: (1) by direct assimilation of dissolved components from the surrounding water, (2) by extraction of the metals that are adsorbed onto the surface of indigestible mineral grains in gut, and (3) by ingestion of food particles. The relative importance of those uptake paths is still disputed. Goldberg (1965) stated that the assimilation of trace metals associated with ingested particles is more important than that dissolved in water for oysters. Many authors reported that the uptake of metals from food was greater than that from water for mussels (Pentreath 1973; Schulz-Baldes 1974) and for oysters (Cunningham & Tripp 1975). Alying (1974) argued that accumulation factors for *Crassostrea gigas* based upon the absorption of dissolved

metals in filtered water or the concentration of metals in sea water (Brooks & Rumsby 1965; Goldberg 1965; Riley & Chester 1971) were grossly misleading where there were high concentrations in mud and suspended particles surrounding oysters because the accumulation process associated with ingested particles was more important to oysters. Popham & D'Auria (1982) speculated that copper in *Mytilus edulis* was mostly from particulate bound because no correlation occurred between copper in the mussel and that in seawater over a wide range of total seawater copper concentrations when copper was predominantly particulate bound.

On the other hand, the uptake of metal from the water column should not be neglected even when the concentration of metals in the water column is much less than that in particulate materials. Filter-feeding organisms move large amounts of water through their filters to collect suspended particulate foods. Even if the uptake of dissolved metals in a unit volume of water would be small, oysters are exposed to large volumes of water and the total amount of the metal absorbed in a period could be large.

3. Uptake of dissolved metals.

Oysters absorb dissolved metals in sea water by active and/or passive diffusion of metal ions from the sea water across semi-permeable membranes into body fluids and subsequent distribution to other organs. The form of metals is an important factor in determining how much of that substance is available to an organism and subsequently accumulated by it. With the knowledge of the chemical speciation in the water column, derived from experiments on thermodynamics, and that of the

differentiation of metals in particulate materials, in many studies the concentrations of various forms of ambient metals have been correlated to toxicity or bioconcentrations (*e.g.*, Sunda & Guillard 1976; Anderson & Morel 1978; Luoma & Bryan 1978, 1981, 1982; Crecelius *et al.* 1982; Zamuda & Sunda 1982).

Many researchers have noted the association of metals and humic acids in natural waters (Sunda & Hansen 1979; Maest *et al.* 1984; Winner 1985). These naturally occurring metal complexing ligands and/or organic chelating agents were thought to facilitate metal uptake by oysters. Brooks & Rumsby (1965) suggested that, as one of the pathways, metals are complexed by coordinate linkages with appropriate organic molecules and then are taken up by exchange, for example, onto mucous sheets of oysters. Saltman (1965) suggested that endogenous or exogenous ligands or chelating agents combine with metal ions to form soluble low molecular-weight complexes and, in this form, are transferred into cells. The role of humic acids in bivalves, however, has not been clearly demonstrated.

George & Coombs (1977) reported an increase of uptake rate of cadmium by complexation to chelating agents in *Mytilus edulis*. Simkiss (1983) showed that group B metals (such as Cu, Zn, Cd and Hg) form inorganic complexes in saline solutions that are very lipid soluble. The electrolyte chemistry of group B metals is considerably more complicated than that of group A metals (such as Na, K, Ca and Mg) since they are able to form a wide variety of covalent compounds in solutions as complex as physiological salines or sea water where species such as $(MOH)^+$, $(MCl)^+$, $(MCO_3)^0$, $(MSO_4)^0$, $(MCl_2)^0$, $(MClOH)^0$, (MCl_3), or (MCl_4) may all be present (Ahrland 1975). These lipid soluble metal-ligand complexes may traverse membranes up to a

million times quicker than Na^+ or K^+ ions. Membranes can no longer be regarded as forming passive primary compartmental barriers and thus the control of these group B metals must be envisaged as depending on, for example, intracellular ligand binding of greater or lesser specificity (Williams 1981). The reactivity of many group B metals with proteins highlights the importance of this concept in terms of enzyme function and toxicology and raises the problem of the ways in which an organism might protect its physiological ligands from such permeable metals.

Recent uptake and toxicity studies for mussels and oysters have shown that it is the free ion concentration that is important and not the concentration of complexed ions (*e.g.*, Zamuda & Sunda 1982). Chelating agents reduce toxicity of zinc and copper suggesting these agents reduce uptake or enzyme sites. Many studies also have shown the importance of free ion activity in controlling biological availability and toxicity of trace metals (Sunda & Guillard 1976; Anderson & Morel 1978; Anderson *et al.* 1978; Jackson & Morgan 1978; Sunda *et al.* 1978; Harrison 1979; Sunda & Gillespie 1979; Zamuda *et al.* 1985). In these studies, increases in trace metal complexation decreased metal bioavailability or toxicity by reducing free ion activities. Toxicity was related only to the concentration of free cupric ion (Sunda & Guillard 1976; Anderson & Morel 1978). The accumulation of copper was related to the cupric ion activity and not to the concentration of chelated copper (Zamuda & Sunda 1982). Moreover, copper toxicity and accumulation rate are reduced by an addition of dissolved organic compounds - indicating reduced bioavailability.

Some researchers (*cf.* Frazier 1975, 1976) found that the metal body burden of *Crassostrea virginica* increased little during the non-growing winter season and

accounted this to the reduction of free ion activity of zinc during the season. This could be the result of the inactivity of oysters in low temperature (Galtsoff 1964), though. Wright & Zamuda (1987) found that salinity effect of copper accumulation by the oysters was independent of cupric ion activity and suggested that the changes in salinity may have several physiological effects on the oysters that influence the accumulation of trace metals.

Zamuda & Sunda (1982) have postulated potential mechanisms that would explain the dependence of accumulation rate on cupric ion activity: (1) copper transport directly across external cell membrane as the free cupric ion (*i.e.* $Cu(H_2O)_6^{++}$) or as inorganic complex species whose concentration covaries with that of cupric ion (*e.g.*, $CuCO_3$, $CuOH^+$, $CuCl^+$), or (2) uptake mediation by binding of copper to ligand sites at cell surfaces. The second mechanism is more probable because biological membranes (*i.e.* lipid bilayer membranes) are relatively impermeable to charged or highly polar species such as $CuCO_3$ (Finkelstein & Cass 1968). The first event in accumulation of copper is the binding with surface membrane sites (probably functional groups of proteins) exposed directly to the water with subsequent passage into the tissues. Such binding would concentrate copper at external membrane surfaces. If the rate of reaction of copper with surface sites is fast relative to the rate of passage into tissues, then the concentration of copper bound to surface sites would approach that of the external medium. The rate of passage of copper into the tissues would be proportional to the amount bound to appropriate membrane sites, and that amount, according to thermodynamics, would be directly related to cupric ion activity in the external medium. The presence of chelators would decrease the rate of

accumulation by competing with membrane ligands for available copper.

Romeril (1971) reported that ^{65}Zn was accumulated to the greatest extent in the mantle and gills of oysters but, excluding visceral mass, the concentrations in all tissues were of the same order and the biological half-lives were very similar for all tissues suggesting an effective transfer system. Intake rates for any one tissue in two oyster species (*Crassostrea angulata, Ostrea edulis*) were very similar indicating a common uptake mechanism for each particular organ (Romeril 1971).

4. Uptake of particulate associated metals.

Benthic filter-feeding organisms filter large quantities of suspended solids (Jorgensen 1960, 1975; Haven & Morales-Alamo 1970, 1972; Mohlenberg & Riisgard 1978, 1979). Sediments are a concentrated sink of trace metals in estuarine environments (Harris *et al.* 1980; Nichols *et al.* 1982, 1983). High concentrations of trace metals associated with sediment have been reported in many polluted areas (Nicholson & Moore 1981; Warren 1981). The concern about the potential hazards of dredging, such as re-mobilization of trace metals (Pheiffer 1972; Villa & Johnson 1976), has stimulated the study of bioavailability of sediment-bound contaminants (Luoma & Jenne 1976, 1977; Luoma & Bryan 1979; Phelps 1979; Rubinstein *et al.* 1983).

The high concentration of metals in oysters had been attributed once to the presence of silt and detritus particles trapped by the gill mucous feeding-sheet (Korringa 1952). This type of material would not be dialyzable, though. Coombs (1972) found that most of the metal in the oyster, *Ostrea edulis* was readily dialyzable

and concluded that the excess metals are in the oyster soft body. The mucous sheet of oysters has also been often implicated as a metal uptake site (*e.g.*, Brooks & Rumsby 1965; Romeril 1971; Simkiss 1983). Many authors view suspension feeding bivalves as mucociliary feeders, *i.e.* sediments pass through the gills of lamellibranch mollusks and are trapped by secretions of the hypobranchial glands (Barnes 1968). It is assumed that particles transferred from the through-current onto the frontal surface of the filaments become entangled in mucous and eventually enter the esophagus and stomach (Bernard & Andreae 1984); however, Jorgensen (1981) suggested that particles are retained by hydromechanical mechanisms and transferred to water currents along the frontal surface of the filaments in normal feeding, *i.e.* normal feeding depends upon hydromechanical mechanisms that produce highly concentrated suspensions of particles for ingestion, and that mucociliary mechanisms serve to clean the gills and other organs of the mantle cavity for excess particulate materials. Water currents over gills and palps are characterized by very low Reynolds numbers (Jorgensen 1981). The mucous sheets observed are thought to be a reaction to stress imposed by the experiment, such as too high concentrations of sediment, physical damage, *etc.* Ingested sediments are usually in free suspension but those rejected particles in pseudofeces are embedded in mucous (Kiorboe & Mohlenberg 1981).

Oysters accumulate trace metals such as zinc partially during ingestion of food and from coincidental ingestion of suspended sediments (Wolfe 1970a). The metals are dissociated from the particulates in the oyster gut by the digestion process and/or acidity (pH of less than 4. *cf.* Haven & Morales-Alamo 1970; Jorgensen 1975). Zinc is accumulated in the intestine and eventually dispersed by the hemolymph throughout

the body of the oyster (Galtsoff 1964).

Gibbs (1973) classified metals in river water in term of chemical mechanisms. By his classification, metals occur: (1) as dissolved ion species and inorganic associations in solution, (2) complexed with organic molecules in solution, (3) adsorbed to solids, (4) precipitated and co-precipitated (metallic coating) on solids, (5) incorporated in solid biological materials, and (6) incorporated in crystalline structure of clay minerals. Though all the particulate material associated metal may not be bioavailable to oysters, it is plausible that there is a relationship between the concentration of some part of the metals and bioavailability. Many field and laboratory studies attempting to correlate concentrations of metals in sediment to that in biota, however, have yielded ambiguous results (*e.g.*, Bryan & Hummerstone 1971, 1973; Ayling 1974; Huggett 1977; Neff *et al.* 1981; Rubinstein *et al.* 1983). Attempts to correlate the soft tissue concentration of metals in oysters and the ambient particle bound metal concentrations in phases (partitioning determined by extraction methods set by Gibbs (1973)) have shown no relationship (*e.g.*, Huggett 1977). Possible reasons for this are discussed below:

(1) A chemical extractant that removes metals from a single substrate, much less from a given form of a substrate, probably cannot be obtained (Luoma & Jenne 1976; Luoma & Bryan 1981). Luoma & Jenne (1976) reported that all of the digestion methods using weak acids, reducing agents and oxidizing agents removed significant quantities of metals from nearly every type of physicochemical sink present in natural sediments. These chemical extraction methods have been used in recent studies attempting to directly define the metal partitioning.

(2) Bottom sediments, unlike suspended particles, may have little effect on the uptake of metals by benthic filter-feeders, organisms that obtain food by pumping water through their filters - labial palps and gills in oysters. Filter-feeders are not directly exposed to bottom sediment because usually the shell separates the organisms from the sediments. For an example, chromium associated with the soluble or fine particulate phase of mud was more available to suspension feeding organisms than that associated with the dense, rapidly sedimenting particles (Carr *et al.* 1982). Similarly, when copper was added to a static laboratory system, 50% of the added copper was bound to the organic fraction of sediment, which was put at the bottom of the aquarium, and this fraction of the metal was unavailable to suspension feeding clams, *Protothaca staminea*, while, in contrast, the deposit feeding clam, *Macoma inquinata*, doubled in copper body burden within two months (Crecelius *et al.* 1982). Interstitial water could contain high concentrations of metals leached from sediments but oysters are not exposed to interstitial water.

(3) Many studies set the line between particulate and dissolved portions with the 0.45 micrometer-pore-sized membrane filter. In some estuaries, particles less then 0.45 μm in size could compose the majority of the total suspended particles (Danielsson 1982). Oysters can filter fine particles by secreting mucous sheet (*cf.* Wright *et al.* 1982). Many filter-feeders, including oysters, also have size selecting ability (Haven & Morales-Alamo 1970; Kiorboe & Mohlenberg 1981; Wright *et al.* 1982). Some bivalves have ability to select particles qualitatively (Huntley *et al.* 1983). Moreover, flocculated particles, which are abundant in estuarine environments, break down into smaller pieces during the filtering process and some of these may

pass through the filters (*cf.* Alaerts 1981; Bother & Valentine 1982; Danielsson 1982).

(4) The uptake of metals from sediments by deposit feeders differs dramatically with the nature of the sediment to which the metal is bound (Luoma & Jenne 1976, 1977; Luoma & Bryan 1982).

5. The relationship of zinc uptake with other ions.

Different metals play different metabolic roles and each metal may have a different uptake mechanism in an organism. If metal uptake is an active process and other metals share the mechanism, competition among zinc and other metal ions for selected ligand sites will be expected. The often-reported effect of salinity (low metal concentration in high salinity water oyster and *vice versa* as Huggett 1977) could be partly explained by the competition of ions. A few studies reported the change of uptake rates of metals in the presence of other metals and/or ligands. For example, suppression of zinc uptake by cobalt and iron has been reported for oysters (Romeril 1971). Those phenomena, however, could be a result of the change in bioavailable ions because of the physicochemical differences of the involved ions (the order of electronegativity is Cu>Cd>Zn>Ca; the order of stability products of the sulfides is Cu>Cd>Zn>Ca; the order of stability of chelates is Cu>Zn>Cd>Ca). The presence of the other metals (Cu, Pb, Cd) had no effect on the individual net uptake of either zinc, cadmium or lead for mussels (Phillips 1976a).

Zinc and copper seem to be most closely related in oyster metal metabolism. Neither free zinc or copper ions are present in any significant amount in oyster soft tissue, neither is bound to one specific compound, nor does there appear to be present

any chelating polypeptide such as metallothionein (Coombs 1974). There exists, however, the difference between zinc and copper. Exhaustive dialyses against distilled water removed 95% of the total zinc and but only 50% of the total copper of *Ostrea edulis* (Coombs 1972). Zinc is very weakly bound to small molecular weight compounds or it is very weakly bound by and readily dissociates from a protein moiety. Most of copper, on the other hand, is much more firmly bound and is, therefore, associated with different binding sites. There are no correlations between the levels of zinc and copper in an oyster tissue (*ibid.*). The ratio of copper: zinc is not constant in different individuals, thus indicating that these two trace metals are accumulated independently (Huggett *et al.* 1973; George *et al.* 1978).

The very high calcium levels present throughout the oyster tissue have been hypothesized as acting as a competitive factor in zinc uptake (Coombs 1972, 1974). If the competition occurs in the cellular metabolisms, an increase in the zinc concentration in an effort to overcome such competitive effects, therefore, would be a likely consequence. The zinc levels in oyster tissues are almost equimolar to the calcium levels, while, in contrast, the zinc in serum is exceeded ninefold by the calcium and the excess of calcium in sea water is an overwhelming 40000 to 1. Coombs (1972) suggested that zinc follows a metabolic pathway similar to calcium. The calcium requirements of an oyster are high, as calcium phosphate initially, for shell formation (Korringa 1952). Carbonic anhydrase and alkaline phosphatase, which are both zinc enzymes, have been implicated in shell-formation (Yonge 1966; Coombs 1974) and hence it will be vital for the oyster to ensure the full utilization of these two zinc metalloenzymes. In mantle cells, there are high concentrations of calcium-

extracellular-concretions and intracellular-calcium and the phenomenon is associated with shell formation (Thomson *et al.* 1985). To ensure the function of the enzymes in such adverse environments as the shell formation sites, the zinc concentration should be elevated. In the presence of high calcium concentrations, zinc uptake may need to be increased above normal to overcome the competitive action of calcium and thereby maintain the vital functions dependent on zinc. To overcome the 1:40000 potential, the oysters will be compelled to increase the zinc concentration to a level, which appears abnormal, but, which in fact is equimolar or near equimolar to the calcium (Coombs 1972). This is not found in other bivalves such as mussels.

There is an apparent seasonal relationship between trace-element concentration and shell-formation (Galtsoff 1953, 1964). During warm summer months, when shell deposition is greatest, the zinc concentration in *Crassostrea virginica* is high (Galtsoff 1964). The oyster shell contains a very low concentration of zinc compared to that in the tissues. Wolfe (1970b) reported that the oyster shell has an average zinc concentration of about 1/6th that of the soft tissues in the oysters. Much of the metal in the shell, moreover, could come from outside the shell by an adsorption process.

Detoxication mechanisms of *C. virginica* do not involve the elaboration of calcium concretions. The extracellular calcium granules, which are thought to be a calcium reservoir for the shell formation in many aquatic organisms, would have little value in the oyster. *C. virginica* grows enormous amounts of shell during the warm summer months. The amount of shell material, which is composed almost exclusively of calcium carbonates, produced during this growth period is 5.94 gram dry weight of shell for approximately 0.5 gram dry weight tissue (Frazier 1975). The total body

burden of about half milligram calcium is too small to be an effective reserve system. Therefore, the calcium vesicles could be thought as: (1) misplaced operations of the shell formation, (2) a very short transitional phenomenon (and therefore the concretions would have a very high turnover rate), or (3) remnants of the shell formation function that are not shut off completely during a non-shell growing season. The almost total absence of zinc in these granules and the extremely low concentrations of zinc in shells support, at least, the later part of Wolfe (1970b)'s speculations that are described later. The intracellular concretions of calcium phosphate nodules, which are thought to be the storage sites for calcium used during molting in decapode crustaceans, contain a high concentration of trace metals (George *et al.* 1978) and could be a possible detoxication system in oysters but its significance in oysters is doubted (Engel 1983).

On the uptake side, possible relationship between calcium and zinc is that zinc and calcium are taken by a common mechanism and the two metals compete for the uptake sites and, therefore, zinc is taken along with calcium coincidentally because of the non-discriminating action of the common mechanism. Oysters in low salinity (consequently a low concentration of calcium also) accumulate more zinc than those in high salinity regime. One of possible causes of the effect of salinity is as Wolfe (1970b) speculated, "the oyster has a poor ionic-discriminator capacity at its environmental interface, and efficient ion-discrimination at the mantle-shell boundary, so that calcium is assimilated and deposited in shell whereas zinc remains loosely bound to tissue protein."

B. Cellular Distribution of Metals in Oysters.

The total amount of zinc in an oyster, *Crassostrea virginica* (Wolfe 1970b) or *Ostrea edulis* (Coombs 1972, 1974), is far in excess of the amount of zinc contributed by the zinc dependent enzymes. Zinc in *C. virginica* can be almost entirely attributed to the metals in membrane-limited vesicles of specific blood cells (Ruddell& Rains 1975). The amoebocytes are rather uniformly distributed throughout the animal tissue (Wolfe 1970b; Romeril 1971). Even though this sequestered zinc is not involved in cellular metabolism, it is included in the measurements of soft tissue zinc concentrations.

1. Metalloenzymes.

In many biological systems, zinc is an integral component of carbonic anhydrase, alcohol dehydrogenase, glutamate dehydrogenase, lactic dehydrogenase, carboxy-peptidase, alkaline phosphatase and probably other pyridine-nucleotide-dependent enzymes (Vallee 1962). Wolfe (1970b) detected zinc metalloenzymes (carbonic anhydrase, alkaline phosphatase, malic dehydrogenase) but no alcohol, glutamic, and lactic dehydrogenases in the homogenate of *Crassostrea virginica*. It was suggested that alternative pathways operate for the disposition of pyruvate and acetate in the oyster. Coombs (1972) detected zinc enzymes: carbonic anhydrase, alkaline phosphatase, carboxypeptidase A, malic dehydrogenase, and zinc dependent enzyme, alpha-D-mannosidase in *Ostrea edulis*.

Metalloenzymes and the metal transporting proteins usually only contain one or two metal atoms per molecule and consequently do not account for the large amount

of metals accumulated within the oyster body. Most of the zinc accumulated by species of oysters (*e.g., C. virginica*: Wolfe 1970b; *O. edulis*: Coombs 1972) is superfluous to the animals' requirements (White & Rainbow 1985). Pequegnat *et al.* (1969) showed that oysters accumulate zinc to a level several orders of magnitude greater than that required for essential metabolic functions. Comparison of the amount of the metalloenzyme-associated metals calculated from enzyme activity determinations with the measured zinc concentrations in oysters revealed that less than 1% of the tissue zinc is associated with these enzymes (Wolfe 1970b; Coombs 1972). The excess of zinc was dialyzable (Wolfe 1970b; Romeril 1971). Dialysis of soluble tissue extracts at pH 7 - 9 removes up to 96% of the total zinc without any effect on alkaline phosphatase in *C. virginica*, indicating that most zinc accumulated is superfluous to the organism's requirement (Wolfe 1970b). Coombs (1974) found that 95% of the zinc in *O. edulis* could be removed by dialysis and 40% of the total zinc was in soluble complexes. Zinc required to satisfy the enzymes in *O. edulis* is of the order of 1×10^{-2} μg/mg protein and this is 0.1% of the total amount of zinc present (10 to 20 μg/mg protein) in the oyster (Coombs 1972). The amount of non-dialyzable zinc was of the same order as the required zinc.

2. Speciation of metals in oyster soft tissues.

A major change of speciation of trace metals occurs after transfer from sea water into the tissues (Wrench 1978). Coombs (1974) reported that in *Ostrea edulis*, free zinc and copper ions were not present in any significant amount. Nearly all (98%) the zinc in *Crassostrea virginica*, was bound either to soluble high-molecular

weight proteins or to structural cellular components such as cell membranes (Wolfe 1970b).

Tissue bound zinc contains at least two complex species: firmly-bound and less-firmly, reversibly-bound. In *O. edulis,* 40% of zinc and copper is in the soluble fraction and is weakly complexed to the small molecular weight compounds such as taurine, lysine, ATP and possibly homarine. Both soluble and tissue bound zinc are exchangeable with ^{65}Zn (Coombs 1974). The soluble complexes can act as a freely available mobile reserve of metal to ensure a constant saturation of metal-dependent enzyme systems operating under adverse environments. This would ensure a constant saturation of the enzyme-zinc ligands and, therefore, maximum activity and efficiency in adverse environments such as the shell formation sites, where a large amount of calcium as well as carbonic anhydrase and alkaline phosphatase are involved (*ibid.*).

Concentric concretions and other granules rich in heavy elements have been found in the kidneys of several mollusks (Carmichael *et al.* 1979; Carmichael *et al.* 1980; George 1980; George & Pirie 1980). These bodies have been identified in membrane-bound vesicles that are associated with and considered to be formed by lysosomes (George *et al.* 1978). Their excretion in the urine suggests that they can be a means for elimination of toxic metals (George *et al.* 1978; George & Pirie 1980). The presence of zinc and other elements in vesicles (probably lysosomes), granules, and concretions in mussel kidneys emphasized the role of the kidneys in zinc accumulation in mussels (Roesijadi *et al.* 1984) but not in oysters. Zinc is not particularly concentrated in the oyster kidney but is uniformly distributed throughout the animal tissue in *C. virginica* (Wolfe 1970b; Romeril 1971).

3. Hemolymph and amoebocytes.

In species of oyster, most of the accumulated metals are localized within certain blood cells (Ruddell 1971; George *et al.* 1978). Copper and zinc are immobilized in membrane-limited vesicles within amoebocytes (amoeboid lymphocytes) of *Ostrea edulis* (George *et al.* 1978). The amoebocytes contain more than 90% of the body copper and zinc of *Crassostrea gigas* (Thomson *et al.* 1985). Ruddell & Rains (1975) suggested that basophils of *Crassostrea virginica* and *C. gigas* contain high concentrations of both zinc and copper and that there is a correlation between the number of basophils in the mantle and the zinc concentrations in the tissue. The elevated copper and zinc levels in the tissues of the two crassostreid oysters (Ruddell 1971; Ruddell & Rains 1975) and *O. edulis* (George *et al.* 1978; George & Frazier 1982) were due almost entirely to the presence of the metal rich blood cells. In a microprobe analysis, the metals could only be detected in the amoebocytes and not in any other tissue cells (George *et al.* 1978). In *O. edulis*, the metals are localized within two distinctly different cell types, granular acidophils containing copper and granular basophils for zinc, and are associated with different chemical compounds within the vesicles (*ibid.*). In *C. virginica* and *C. gigas*, copper-containing acidophils, zinc-containing basophils and Cu/Zn-containing basophils were reported (Ruddell 1971; Ruddell & Rains 1975); however, only one type of metal-binding blood cells, which contain both zinc and copper, was found in other studies of *C. gigas* (George & Frazier 1982, Pirie *et al.* 1984).

The two types of metal containing cells had the same morphology in all tissues. The granular acidophils contain the copper that are associated with sulphur in

membrane-limited vesicles of about 0.8 μm diameter and the others, the granular basophils, contain the zinc that are associated with phosphorus in membrane-limited vesicles of about 1 μm diameter (Ruddell 1971; George *et al.* 1978). Most of the zinc in species of oyster is in tissue amoebocytes and only 5% of the zinc is in hemolymph even though the whole hemolymph represents about half of the (wet) oyster tissue mass. Of this hemolymph zinc, the amoebocytes contain 77% of the zinc (George *et al.* 1978). Since the total number of amoebocytes in the circulating fluid is very low, the concentrations in these cells are extremely high. The calculated metal concentrations in amoebocytes of oysters from polluted areas lie in the region of 1,400 to 13,000 ppm copper and 9,900 to 25,000 ppm zinc on a wet weight basis. The hemolymph fluid (serum excluding the amoebocytes) metal concentrations are some 32-fold lower for copper and 36-fold lower for zinc than those in the soft tissues. Therefore, the metals must be actively removed from serum by some energy-dependent processes. The oyster, thus, reduces the effective concentration of these metals by removing them from the fluid bathing the tissues to a level that is not toxic to the organism. These serum metal concentrations, however, are still higher than those of the surrounding estuarine water (*e.g.*, 0.065 μg Cu/ml, 0.57 μg Zn/ml: Thornton *et al.* 1975) and higher than those in the oysters from unpolluted water. The serum copper and zinc are complexed and the relative amounts of free copper and zinc ions are extremely small (Coombs 1974).

The hemolymph amoebocytes penetrate all of the soft tissues and, therefore, the amoebocytes may have a metal-transport function (George 1982). The concentrations of copper and zinc are not the same in all tissues, indicating that the metal-containing

amoebocytes are not uniformly distributed throughout the oyster body. The highest concentrations of copper and zinc in tissues are found in the gills and mantle (Ikuta 1968; Wolfe 1970b; Romeril 1971; George *et al.* 1978; Thomson *et al.* 1985). In the "green-sick" oysters, there are relatively greater increases in the copper and zinc levels in the viscera, mantle and gills, which indicates that in the muscle and kidney there is either slower rate of entry, the storage capacity is saturated, or a steady state has been reached where uptake and excretion are balanced (George *et al.* 1978).

4. Extracellular concretion.

Bivalve mollusks have to rapidly accumulate large amounts of calcium. The calcium is found in extracellular granules of calcium carbonate in lamellibranchia and in many invertebrates with exoskeletons (George 1982). The occurrence of calcium extracellular concretions and high intracellular calcium in mantle cells is associated with shell formation (Thomson *et al.* 1985). These granules are formed in the sacs of the Golgi apparatus of connective tissue cells and are in contact with the extracellular fluid via pores in the plasma membranes (George 1982). Isolated granules are readily soluble in saline solutions (Simkiss 1981) and provide a method for the storage of large quantities of calcium in a readily available form outside of the cell (George 1982). Since the extracellular fluid of most mollusks is supersaturated with CO_3, a calcium extrusion mechanism may occur. The enzyme carbonic anhydrase is associated with these granules and is presumably concerned with the carbonate metabolism. The analysis of isolated granules has shown them to be relatively pure calcium carbonate but other metals that show chemical similarities to calcium and

readily precipitate as insoluble carbonates also might be expected to occur in these cells (*ibid.*). The importance of this mechanism in oyster zinc metabolism has not been established (Engel *et al.* 1984).

C. Detoxication Mechanisms and Subcellular Distribution of Zinc.

It is clear that oysters must possess a very effective method of preventing interaction of toxic metals with essential enzymes of their cells (George *et al.* 1978). Zinc is removed from cytosol and detoxified by compartmentation and concentration within membrane-limited vesicles. Here, it is associated with some inorganic or organic complexing molecules that maintain the metals within the vesicle, thus effectively removing them from the hemolymph and cytosol (*ibid.*).

Crassostrea virginica has a cadmium-binding protein, which is different from that of vertebrates. The exact function of this metallothionein-like protein in normal metabolism and whether the induced protein is present specifically for the purpose of detoxication of trace metals is yet to be conclusively demonstrated and the presence of a low molecular weight protein that binds zinc has not been demonstrated (Engel 1983). In the present study, it is assumed that the role of metallothionein-like proteins in oysters as a storage or detoxication mechanism is of minor importance. It is also assumed that there is little utilization of the compartmented zinc, which is mostly in blood cells of *C. virginica*, and depuration is limited to the coincidental loss through shedding of epithelial cells or diffusional loss from surface tissue cells.

1. Mechanisms of tolerance of high ambient trace metals.

George & Frazier (1982) postulated three possible strategies that enable organisms to tolerate increased environmental concentrations of trace metals:

(1) Decreased uptake into cells by lower permeability to trace metals.

(2) Increased excretion. There are several routes by which excess metals can be excreted; via feces derived by excretion from the hepatopancreatic or intestinal cells and urinary excretion. In bivalve mollusks, the urine also may contain particulate metal-rich granules (George 1980). Many aquatic organisms may also excrete metals via their external epithelia either by diffusion, active secretion, or in mucous (*e.g.*, Bryan 1976).

(3) Buffering. Since it is the free metal ion that is chemically reactive, the intracellular concentration of this species may be controlled by binding or sequestering the ions by cellular ligands. Divalent metals such as cadmium, copper and zinc readily bind to many proteins in a non-specific and reversible manner, consequently, there is always a readily available pool that can satisfy metabolic requirements of copper and zinc. Specialized transport and storage proteins whose synthesis is controlled by the metal status of the cell are found in most vertebrates for Cu, Co, Fe, Ni and Zn. Some of these proteins also have been identified in invertebrates (George 1982). One of these proteins, metallothionein, also acts as a detoxication system.

2. Metallothionein.

Low molecular weight (5,000 - 15,000 dalton), sulfur-containing cadmium and zinc binding proteins have been isolated from oysters (*e.g.*, Ridlington & Fowler 1979;

Roesijadi 1981) but the role of these proteins has not been fully demonstrated. The proteins, first isolated from mammalian tissue, have the ability to bind Cd, Ag, Cu, Hg, and Zn very strongly (Kagi & Nordberg 1979; Webb 1979). They are found free in the cytoplasmic soluble fraction and characterized by their metal-binding properties, heat stability, virtual absence of aromatic amino acids and histidine, and usually high content (30 - 35 residue%) of cysteine (Kagi & Nordberg 1979). Several biochemical roles for thionein have been suggested: toxic metal detoxication, essential metal (copper and zinc) storage and regulation, and metal donation to metalloenzymes (Brady 1982). It has been characterized as the primary trace metal detoxifying protein in vertebrates (Kagi & Nordberg 1979) and speculated that marine invertebrates detoxify trace metals by producing metallothionein-like proteins that sequester metals (Roesijadi 1981).

The occurrence of the metallothionein-like (a low molecular weight, sulfur-containing metal-binding) protein has been correlated with elevated levels of trace metals in *Crassostrea virginica* (Engel & Brouwer 1984). The cadmium-binding proteins in *C. virginica* do not bind zinc (Ridlington & Fowler 1979; Squibb *et al.* 1982) and are different from those of vertebrates *e.g.,* a high percentage of dicarboxylic amino acids and lower cysteine content (Ridlington & Fowler 1979).

The exact function of these metallothionein-like proteins in normal metabolism has been disputed. The detoxication function of the metalloproteins could have developed through an evolutionary process or could be an incidental character of the proteins that have other primary functions. Engel & Brouwer (1982, 1984) suggested that the primary function of the protein was in regulating normal metal metabolism

and not in the sequestration of elevated levels of trace metals and speculated that "the function of these low molecular weight metal binding proteins in oysters is primarily storage or transport of other physiologically important elements and only secondarily act in the detoxication of trace metals (Engel & Brouwer 1982)." Their reasoning was that it was unlikely that an organism developed a detoxication mechanism for unnaturally high concentrations of metals in nature. On the other hand, the binding of trace metals to ligands has a negative heat of formation so that in the event of an oscillating environmental temperature there will be an alternating rise and fall in the concentration of cytoplasmic metal ions with subsequent stimulation of detoxication and mobilization pathway. Cytoplasmic metal pollution effects may be a daily occurrence in all animals that are subjected to such temperatures so that detoxication mechanisms would have evolved (Simkiss & Schmidt 1985). Engel (1983) suggested that these cadmium-binding proteins may also be active in the transport of other elements, such as copper, zinc, and calcium.

Whatever the primary role, the proteins have the capacity to bind large numbers of metal ions and may make a significant contribution to the total metal burden (George 1982). These transport and storage proteins enable control of the intracellular concentration of these metal ions through variation in the degree of metal saturation. Additional control or buffering of the metal concentrations is affected by protein synthesis. Thioneins are continually degraded and re-synthesized rather quickly, with a half-life of 1/2 to 5 days (*ibid.*).

Biochemical studies of oysters from a highly metal polluted environment have shown that these metals are not detoxified by binding to the cytosolic protein

metallothionein (Frazier & George 1983). The studies suggested that potentially cytotoxic concentrations of copper and zinc were detoxified by forming low molecular weight complexes. The absolute amount of zinc bound to the metallothionein-like protein of an oyster is expected to be very small anyway. Although oysters have the ability to concentrate trace metals and produce specific metal-binding proteins, only a small portion of the total metal present in the animal is in a soluble metal-protein complex (Engel 1983).

3. Intracellular granules/compartmentation.

An alternative mechanism for detoxication of metals is compartmentation within subcellular organelles, which prevents reaction with essential enzyme systems in the cytoplasm but this accumulation may, in itself, lead to toxic effects on organelle function (George 1982). In virtually every phylum of animals and in most organ systems, subcellular accumulations of metals within organelles and other membrane-limited structures have been identified (Simkiss 1981, George 1982). Once inside these structures or vesicles, the metals do not appear to be exchangeable and are therefore detoxified in a metabolically inert form. All subcellular organelles contain some metals that are either attributable to their constituent metalloenzymes or responsible for stabilization of their macromolecules (George 1982). There are two major types of metal-containing vesicles: one is predominately inorganic and is common in hepatopancreas and renal cells. These are a variable mixture of Ca/Mg phosphate/pyrophosphate containing numerous trace metals, *e.g.*, Ag, Al, Ba, Co, Fe, Mn, Pb, Sn, and Zn. The other granule is predominantly organic and is also common

in hepatic, renal, heart and brain tissues. It is derived from the lysosomal-vesicular system and is mainly composed of peroxidized lipid and partially degraded cell membranes (*i.e.* a third lysosome), which contain many trace elements including Cu, Cd, Pb, and Zn. The lysosomal-vesicular system is the major degradative system within the cell and is involved in both the degradation of food taken into the cell and the turnover of cellular proteins and granules (George 1982).

The very high copper and zinc levels in oysters are almost entirely attributed to the presence of the metals in specific blood cells (Ruddell & Rains 1975; George *et al.* 1978). The amoeboid blood cells, with accumulated copper and zinc, are present in both the hemolymph and tissues (George *et al.* 1978) and may have a metal-transport function (George 1982). They are detoxified by compartmentation within membrane-limited vesicles in these cells (George *et al.* 1978). *Crassostrea gigas* from metal-rich environments contain elevated copper and zinc concentrations in all tissues, and copper and zinc are present in the tertiary lysosomes that are accumulated in membrane-limited vesicles of blood amoebocytes (Thomson *et al.* 1985).

The calcium phosphate granules, which are intracellular concretions, from oysters exposed to trace metals have been shown to contain high concentrations of trace metals (George *et al.* 1978) and designated as a possible detoxication system. Metals could be deposited in these membrane-limited cellular inclusions after being sequestered by proteins, or they could be incorporated into the granules in a nonspecific manner. In crustaceans, these concretions are thought to be storage sites for calcium used during molting and therefore, their involvement in the sequestering of metals may be an incidental function. In *Crassostrea virginica,* however, concretions

were of limited importance in detoxication (Engel 1983).

4. Depuration mechanisms.

Another way of coping with high concentrations of unwanted trace metals is to eliminate the metals effectively, *i.e.* depuration of the metals. The high concentration of trace metals in oysters indicates that the depuration mechanism does not exist or is not effective enough to prevent the accumulation of the metals.

An oyster can eliminate unwanted materials by: (1) passing the materials to its gametes, thus eliminating the materials during gametogenesis and subsequent spawning, (2) incorporating the materials into its outside structure, *i.e.* the shell, (3) using the excretory system of the organism.

The transfer of metals, for example mercury, from adult to eggs has often been suggested as a possible depuration route. Significant gonadal mercury accumulation occurs in *Crassostrea virginica* (Cunningham & Tripp 1973, 1975) and depuration is facilitated by spawning. Greig *et al.* (1975), however, showed that eggs of *C. virginica* contained almost no detectable amount of cadmium while adult oysters contained 15.6 - 28.1 average ppm-dry-weight. Copper and zinc in adult oysters were 1260 - 2208 and 8300 - 10460 ppm-dry-weight respectively but in eggs they were 27.8 - 28.9 and 65.9 - 82.4 ppm-dry-weight respectively. It was suggested that the amount of metal transferred from adult to egg is fairly constant and not dependent on the amount of the metal accumulated in the adult oyster. The metals in the eggs, however, could arise from the contamination of the egg samples by sea water. Chromium concentration in *C. virginica* continued to increase during spawning,

whereas it decreased in *Mytilus edulis* (Zaroogian & Johnson 1983). Spawning accounted for only a small part of decrease in the concentration of trace metals in *Crassostrea gigas* and the most important factor appears to be the growth of the oyster (Thomson 1982, 1983). The tissue distribution of metals, moreover, shows lower concentrations in the gonad than in the whole oyster. In contrast to the other tissues, few membrane-limited vesicles were present in the oyster gonad and these contained very low concentration of metals (Thomson 1982). Therefore, it seems likely that oysters prevent the transfer of excess metals to eggs. The transfer during spawning and fertilization could be an accident when oysters have negligible control of the metal as in the case of mercury.

Another possible depuration route is to the shells. There have been many reports of the effects of shell growth on metal concentrations in oysters; however, the relationship could be a coincidental one that reflects the seasonal growth pattern of the oyster and not directly to the shell formation. Evidence shows that oysters transfer little trace metals into the shell during the formation. Therefore, this route of depuration is not significant, at least in oysters. In *C. virginica*, shells contained 1.5 - 8.1 ppm zinc while soft tissues contained 1200 -5700 ppm-dry-weight (Windom & Smith 1972). There was no relationship between the average shell and average soft tissue zinc concentrations either. In Wolfe's 1970 study, the soft part of the oyster, *C. virginica*, contained on the average about six times the zinc concentration of shells (24.75 ppm) in wet weights. Since the soft part accounted about for only 19.5% of the total live (wet) weight of the oyster, the shell contains nearly 45% of the total zinc of oyster; however, much of this metal could be simply temporarily adsorbed to the

surface of the shell or incorporated through adsorption processes. A firmly attached coating that contains metals could remain even after washing; scrubbing of the shell with a brush removed an additional 50% of copper and 70% of zinc in the burrowing bivalve *Scrobicularia plana* (Bryan & Uysal 1978).

In *M. edulis*, the kidney plays a major role in trace metal depuration (George 1980). The kidney of the mussel contains very high concentrations of zinc, copper, cadmium, iron, and lead. The columnar epithelial cells of the kidney contain a large number of membrane-limited vesicles. These membrane-limited granules were as much as 20% of the kidney cell volume (Pirie & George 1979). These granules and those of the podocytes are subsequently shed into the lumen of the kidney tubule and are excreted in the urine, which is therefore largely particulate. The granules in the kidney are rich in a variety of metals, including Fe, Zn, Ca, Pb and Cd (George & Pirie 1980; George & Viarengo 1985), and this could be a major way of depuration.

In *C. virginica*, the role of the kidney in depuration seems to be of minor importance. The kidney does not contain any higher concentrations of metals compared to other tissues and metal rich granules have not been reported in oyster urine. Even though both copper and zinc in oysters are readily exchangeable, they have a long half-life, *ca.* one year (Wolfe 1970a; Okazaki & Panietz 1981), which may imply the persistence of the very-low-molecular-weight complexing ligand in the tissue. The long half lives are also expected since the kidney is poorly developed and the metals accumulate in the long lived blood cells (George & Frazier 1982).

D. Factors Affecting Bioaccumulation.

Aquatic animals are exposed to metals in the medium in which they are immersed as well as metals in their diets. In aquatic habitats, the environmental variables, such as salinity, pH, redox conditions, *etc.*, can affect both the chemical speciation of the metal and the physiology of the animals (George 1982). Many authors, in an attempt to elucidate mechanisms of trace metal uptake, have based their explanations on influences such as regional differences (different sampling locations), seasonal fluctuations, salinity, temperature, pH, depth, physiological conditions of the animals, and the available chemical forms of the metal in the sea water and the sediments. Since these results come from separate studies that have been performed independently and under different ambient conditions, it is difficult to evaluate the importance of any specific physicochemical conditions. Natural variations increase the difficulty in comparing accumulated trace metal levels between similar biological systems let alone among different sites. Boyden (1977) and Simkiss & Taylor (1981) advocated a normalization procedure to eliminate the effect of size or salinity. Normalization, however, can not be justified unless one knows the relative importance of the parameter being normalized. There always will be the danger of not including some parameters that attribute the apparent difference among populations. Another approach is to standardize the sampling, for an example, a standardized collection period. It is difficult to compare samples collected at different times because seasonal effects contribute to the high level of variability (Frazier 1976); however, it would be nearly impossible to design a sampling program that eliminates all the variables reported to influence metal uptake. The effect of a factor that determines the

bioaccumulation of metals in oysters may override the effect of another factor or they may interact together. It is hard to evaluate how the change of each factor would affect bioaccumulation without using a method that integrates these effects. Therefore, the best approach appears to be using a simulation model to understand and to estimate the effects of the factors on trace metal bioaccumulation. Such a model would be a tool to analyze the relative contributions of each parameter, and thus, a method to interpret biomonitoring results.

In the present study, attempts have been made to eliminate or minimize factors and procedures that lead to ambiguous results. In particular, (1) all samplings were done in a short period of time, (2) analytical procedures have been chosen with care, (2) the study has been designed to emphasize and document weight effects, and (3) effective annual average values were used for salinity, temperature, and growth to minimize the seasonal effects. It is hypothesized that in-situ zinc body burdens of oysters reflect the integration of the temporal variations of factors that affect zinc metabolism and growth of the oysters. It is assumed that each oyster responds to the factors without any individual genetic or other intrinsic difference.

1. Deviations from mean due to analytical procedures.

Metal concentrations in individual oysters from one sampling site show large deviations from the mean value (*cf.* Gilinsky & Roland 1983). The large individual variations, by the order of two or three, in oysters with the same condition are puzzling. Much of this variation could be eliminated by proper analytical methods (Mo & Neilson 1991).

Concentrated-nitric-acid digestion has been used for the extraction of metals from the soft tissue of oysters in some experiments. Apparently, lipids left undigested by that process float to surface when the samples were cooled to room temperature. The practice of removing the lipids by filtration with glass filter may remove a considerable amount of the metal from the metal concentration measurement because zinc forms inorganic complexes in saline water that are very lipid soluble (Simkiss 1983). A digestion method that dissolves organic materials completely is preferable for the soft tissue. Another probable source of error is that some studies use wet weight while others use dry weight for the soft body. It is well known that use of wet weight would introduce errors because it is hard to prepare samples with a consistent water content. The deviations caused by these inconsistent water content will be larger for smaller organisms than for the bigger organisms because of the relative proportions to the total weight. Dry weight, which has inherent lower variability (Thomson 1983), is the preferred measurement.

Another source of error is inclusion of gut contents in the calculation of total body burden of metals. It is obvious, by common observation, that the gut of oysters contains sediment, *i.e.* clay minerals, which have metals in their structure. These metals are chemically inactive (require a rigorous digestion to extract) and therefore also biologically unavailable. Sediments, and the associated metals, would not be absorbed by the oyster and would be voided as feces. Moreover, oysters also collect and discard indigestible sediment particles as pseudofeces that do not pass through their guts. A few researchers, who apparently are aware of the possibility of this error, have attempted to eliminate it by placing the organisms in filtered sea water for

a few days after collection (*e.g.*, Boyden 1977, Cain & Luoma 1985).

In both scientific studies and regulatory monitoring of metal concentrations in oysters, it is an almost universal procedure that the whole body of the oyster, after being removed from its shell, is digested in concentrated nitric acid with heat for several hours to days. To remove the remaining residue, the sample is centrifuged (*e.g.*, Ayling 1974; Huggett 1977); or hydrochloric acid (*e.g.*, Zamuda & Sunda 1982) or H_2O_2 (*e.g.*, Zaroogian & Johnson 1983) is added. Some experimenters used hydrofluoric acid followed by nitric acid, the strongest digestion method known. Such treatments will dissolve almost all of metals associated with sediment (Gibbs 1973; De Groot & Zschuppe 1981). The total metal content of suspended sediment from Chesapeake Bay, when extracted by concentrated nitric acid, ranged from 20 to 330 μg/g dry weight for copper and 220 to 2700 μg/g for zinc (*cf.* Harris *et al.* 1980) with the averages of 127 μg/g and 750 μg/g respectively for the near bottom waters (*cf.* Nichols *et al.* 1982). The total body burdens of copper and zinc in *Crassostrea virginica* were approximately 39 and 575 μg/g (*cf.* Gilinsky & Roland 1983). The gut content varies in time with the physiological state of oyster. Feces and pseudofeces could be included in the soft body samples. If not differentiated, metals in the gut contents, the feces, and the pseudofeces will introduce variability.

The total body burden of metals, including the gut content, is, of course, what humans are exposed to when they eat oysters. Much of this metal, however, is too strongly bound to sediment material, in the crystalline structure, for example, to be of concern. A simple depuration facility to hold oysters until they void the contaminated sediment would be satisfactory for commercial distribution, if the metal concentration

in oysters is high but found to be only associated with sediments in the gut.

Another source of variable found during this study was the inclusion of pea crabs in measurement of body weights and metal body burdens. The extraneous variables in body burden measurements, the use of wet weight, the inclusion of sedimentary materials, and the inclusion of pea crabs are quantified in this study and reported in **Appendix A.**

2. Effect of body weight and growth.

It is assumed that oysters do not regulate metals to any great extent. It is, well known, however, that the total concentration of a metal in the environment and that in the organism are not linearly related, so it is not appropriate to attempt to establish a simple linear regression between the metal concentration in organisms and that in the environment even for a set of physiological and environmental conditions. The exponential growth rate of the organism and the dilution effect of tissue mass growth makes the body weight and the body burden per unit mass of tissue relationship complex (Strong & Luoma 1981; Thomson 1982; Simkiss & Mason 1984). Moreover, it has not been understood whether the metal concentration in every cell of the body tissue of oyster changes over the life time or there is a saturation concentration for each cell and the metal concentration of the cell does not increase beyond that concentration (*cf.* Simkiss & Mason 1984).

The size of an organism is one of the most important factors that influence trace metal concentrations in several species (*e.g.*, Boyden 1974, 1977; Phillips 1976a; Manly & George 1977; Davies & Pirie 1980; Popham & D'Auria 1982); however, the

correlation between the body size and metal concentration can be different among different metals in a species or among populations exposed to different environments.

Following Boyden (1974), it is assumed that individual metal body burden (Y) is related to body weight (W) as a power function $Y = A \cdot W^b$. The weight-specific concentration (C) is related to body weight as $C = \frac{Y}{W} = A \cdot W^{(b-1)}$. For a given species, different elements may display different relationships to body weight. Boyden (1974, 1977) described 3 cases. First, when the regression coefficient **b** is smaller than 1, which means **(b-1)** is negative, the smaller individuals will have a higher concentration of the metal than the larger ones. For copper, this relationship was found to be almost universal in samples of indigenous mollusks and the value of **b** was found to be 0.77. This value is close to 0.75, a value generally accepted to relate many metabolic functions to body weight in poikilotherms, such as marine invertebrates, implying a connection between accumulation and metabolic rates (Boyden 1974). In *Mytilus edulis*, zinc also showed this relationship (Boyden 1974). In short-term exposure experiments in laboratories, this relationship has been attributed to a size dependent difference in uptake rate; metal uptake by smaller individuals of many species is more rapid than that by larger individuals (Strong & Luoma 1981).

Second, if the value of **b** is 1, this means that metal content is directly related to body weight, *i.e.* the metal concentration per unit body weight is independent of body size. This suggests that equilibration of concentrations of some metals occurs in the tissues of the organism (Bryan 1976; Williamson 1980; Strong & Luoma 1981). In *Mytilus*, this relationship was maintained for cadmium even though the absolute amount of the metal within tissues differed by twelvefold between two separate

population studies (Bryan 1976). This suggests that there may be adaptive differences among populations.

Third, the value of **b** bigger than 1 suggests that a net accumulation of the metal is occurring throughout the life of the organism (Williamson 1980; Strong & Luoma 1981). Another possible explanation for this relationship is that the dilution of metals by tissue growth would have a greater effect on smaller organisms than on larger ones within the same population.

The change of the slope of the regression of the concentration per unit weight, *i.e.* **b-1**, from zero to positive has been shown in many species in metal enriched environments (Bryan 1976; Boyden 1977). This means the extent of equilibrium, and thus the relationship of concentration to size, may depend upon the level of metal exposure (Bryan & Hummerstone 1973).

In *Crassostrea gigas*, copper and chromium were absorbed up to a maximum amount that was limited by the weight of the oyster and was independent of the amount of metal in the mud while zinc and cadmium were accumulated by a process that depended primarily on the concentrations of these metals in the mud at each site (Alying 1974). Therefore, the value of **b** would be 1 for copper and chromium but probably less than 1 for zinc and cadmium.

The effect of body weight on metal concentration of oysters is hard to evaluate using field samples because metal accumulation is a complex interactive process. Environmental factors such as temperature and salinity affect chemical speciation as well as the physiological rates of oysters. The history of the environmental fluctuations for different aged organisms affects the metal body burden. Therefore, it

appears that the best approach to evaluate the effects of body weight on metabolic rates and metal uptake rates is in a controlled laboratory setting and to apply the results to the model of metal accumulation.

Information regarding body size and metal concentrations provides clues for understanding the mechanism of the bioaccumulation. The relationship between body size and metal concentrations has been studied in many species (*e.g.*, Boyden 1974, 1977; Strong & Luoma 1981; Widdows 1978a, 1978b), but there have been few studies of the relationship for *Crassostrea virginica*. Huggett *et al.* (1973) found no relationship of body size (wet tissue weight) to zinc concentration in oysters of the James River and, in a later study (Huggett *et al.* 1975), that significant concentration differences were related to salinity. Phelps *et al.* (1985) found that metal concentrations were "largely negatively correlated with body weight and with salinity." The results of both studies showed that the relationship for each population at a sampling site was not clear. Phelps & Hetzel (1987) reported that stunted oysters accumulated much more zinc than normal growth oysters of the same age (nearly twice much zinc when concentrations were normalized to weight).

Genetic differences between individuals may lead to variation in metabolic rates and other parameters resulting in some "inherent" variations between different individuals (Boyden & Phillips 1981). Because it is a frequent practice to harvest seed oysters from one place and transplant them to another both within and between Virginia estuaries, it is assumed that there are no such genetic differences among the sample oysters. Stunted oysters are treated as oysters with little or no growth in the model.

3. Salinity and temperature.

Salinity and temperature influence many bioaccumulation pathways and determine the bioavailability of a trace metal. Low salinity, and thus high free ion activity, increases the uptake rate of the metal. The salinity affects the uptake of metals by phytoplankton because the algae respond mainly to free metal ions (Sunda & Guillard 1976) solution. The transfer of metals from the phytoplankton to oysters may be an amplification step (Wikfors & Ukeles 1982) that would further accentuate the effect of salinity changes. Salinity effects on trace metal uptake could also be explained by passive osmotic effects and accidental active uptake.

Both salinity and temperature are governing parameters of not only free ion activities of trace metals but also metabolic rates and growth rates of the organisms. In *Crassostrea gigas*, increased temperature increases shell growth rates but soft tissue dry weights of the oysters are not positively related to temperature (Mann 1979). Another apparent salinity effect is that in the Virginia estuarine systems oysters grow faster in high salinity water than in low salinity water. Salinity and temperature may influence some metabolic rates that are directly involved in bioaccumulation independent of free ion activity. Changes in salinity may have several physiological effects on oysters that influence the accumulation of trace metals, for example, the filtering rate decreases at low salinity.

Salinity effects, *i.e.* higher body burden of trace metals in oysters and mussels from lower salinity regimes and *vice versa*, with estuarine systems with no gradient of the total metal concentrations, have often been reported (*e.g.*, Huggett *et al.* 1973; Phillips 1977a). There have been many attempts to correlate the phenomenon with the

gradients of: specific forms of metals, concentrations of organic materials (especially that of humic acids), sizes or concentrations of particulate materials, or other ions (*cf.* Huggett 1977). The results of most of these studies are ambiguous.

Phillips (1977a) speculated that the apparent salinity effect seen in metal accumulation in mussels may due to the ingestion of different species of plankton containing different metal concentrations. This shifts the salinity effect to a lower trophic level and this, in turn, may have physicochemical (Sunda *et al.* 1978) or physiological elements. In higher animals, salinity effects on trace metal uptake have been explained by passive osmotic effects (George *et al.* 1978) and accidental active uptake (Wolfe 1970b, Wright & Zamuda 1987).

Evidence indicates that the bioavailability of a trace metal is directly related to the free ion activity of the metal. It has been shown in both mussels and oysters and for the same total concentration of trace metal that low salinity, and thus high free ion activity, increases the toxicity (*e.g.*, Engel & Fowler 1979; MacInnes & Calabrese 1979; MacInnes 1980; Coglianese 1982) or the uptake rate (*e.g.*, George *et al.* 1978) of the metal. For example, copper accumulation by *Crassostrea virginica* is related to the cupric ion activity and not the concentration of chelated copper (Zamuda & Sunda 1982). Both copper toxicity and accumulation rate are reduced by addition of dissolved organic compounds, *i.e.* by reducing bioavailability (Zamuda *et al.* 1985).

Free ion activity (thus bioavailability), which is a function of not only total concentration but also the salinity, temperature, and organic material concentration of the water column, accounts for some part of the seasonal effects either directly or indirectly. Higher winter concentration of metals in the oysters was linked with

greater solubility (free ion activity) of metal ions in lower salinity water in *C. gigas* (Thomson 1982). The salinity affects the uptake of metals by phytoplankton because the algae respond only to metals in solution. The transfer of metals from the phytoplankton to mussels may be an amplification step that would further accentuate the effect of salinity changes (Phillips 1977b). The major portion of the total body load of metals in *Mytilus edulis* is derived from ingested phytoplankton (Phillips 1979). For the same reason, Bryan (1973) suggested that seasonal variation in the concentrations of trace metals in scallops correlates with the seasonal variation in trace metal levels found in phytoplankton. Galtsoff (1964) found that for *C. virginica* from Long Island Sound, the concentration of Fe, Zn, Cu, and Mn increased during the summer and decreased during the winter. Thomson (1982) found the reverse was true for *C. gigas* and postulated higher winter concentrations of metals in the oysters were linked with greater solubility of metal ions in lower salinity water (*cf.* Mackay *et al.* 1975) and speculated that Galtsoff's findings resulted from the difference of the amount of rainfall during the seasons, and thus, the change of salinities.

Both salinity and temperature are governing parameters of not only free ion activities of trace metals but also metabolic rates and growth rates of organisms. In *C. gigas*, increased temperature increased shell growth rates but soft tissue dry weights of the oysters were not positively related to temperature (Mann 1979). In the Virginia estuarine systems, oysters grow faster in high salinity water than in low salinity water (Haven, personal communication).

Salinity and temperature may influence some metabolic rates that are directly involved in bioaccumulation. As mentioned before, Wright & Zamuda (1987) found

that some of the salinity effects of copper accumulation by oysters are independent of cupric ion activity and suggested that the changes in salinity may have several physiological effects on oysters that influence the accumulation of trace metals. For example, the filtering rate of *M. edulis* decreased at low salinity (Bohle 1972; George *et al.* 1978).

One of these effects may override another or they may interact together. For example, low salinity did not affect the net uptake of zinc by *M. edulis* but increased the net uptake of cadmium and decreased that of lead; low temperature had no effect on the net uptake of zinc or lead; the net uptake of cadmium was unaffected by low temperature at high salinities but was decreased by low temperature at low salinities (Phillips 1976b). It is hard to evaluate how the change of each factor would affect bioaccumulation without using a method that integrates these effects. In the present study, a model is used to evaluate the relative importance of each parameter and the behavior of bioaccumulation.

4. Seasonal effects on biological parameters.

The seasonal change and the sum of the effects of factors that are associated with season, such as salinity, temperature, growth rate *etc.* are difficult to break down into individual factors and interactions. It is obvious that temperature and salinity vary with season and those parameters, in turn, affect chemical speciation of metals that determines the free ion concentration of the metals. During warm summer months, when shell deposition is greatest, the zinc concentration in *Crassostrea virginica* is high (Frazier 1976). The apparent seasonal correlation between trace-

element concentration and shell-formation can be related to any one of temperature, salinity, growth rate *etc.* In this section, the effects of season itself on biological processes affecting trace metal uptake are discussed.

Bryan (1973) suggested that the seasonality might be related to temporal changes in the trace metal concentrations present in phytoplankton. Trace metals are accumulated by phytoplankton and could show "trophic increase" (Bernhard & Andreae 1984). Another explanation is that during summer and autumn, organic substances are often accumulated in the water column due to the high primary production. Algae excrete substances to render metals in a complexed form. This results in high concentrations of dissolved-organic-metals, for example organic copper, during those seasons (Osterroht *et al.* 1985).

Seasonal effects also may be caused by the change of tissue weight or water content according to the sexual cycle. Even without any change in the total body burden, those changes will result in different metal concentrations. In *Mytilus edulis*, total metal contents remained almost constant throughout the year (Zn, Cd, Cu) but variations in wet weights of the mussel caused fluctuations in metal concentrations in soft tissues (Phillips 1976a). Samples having seasonally high wet weight are found to have seasonally low concentrations of the trace metals and vice versa. When the seasonal wet weight variation could be eliminated, seasonal fluctuations in concentrations of the trace metals become far less pronounced.

Weight fluctuations produce much of the observed seasonality of metal concentrations in *M. edulis* (Phillips 1976b; Simpson 1979). This phenomenon was related to the fluctuation in water content because mussels from the same collection

areas analyzed by dry weight concentration show no such seasonality. In *C. virginica*, two annual minima in soft tissue wet weights occur in April (owing to depletion of glycogen stores over winter) and August (subsequent to spawning). As a result, concentrations of Cd, Cu and Zn exhibit twin peaks during the year, at the same periods (Frazier 1975, 1976). *Crassostrea gigas*, in contrast, does not have the substantial increase in tissue weight in the autumn such as those ascribed to glycogen storage in *C. virginica*. Thus in *C. gigas*, tissue weight profiles are dominated by the gametogenesis-spawning cycle with a single annual maximum in element concentration (Boyden & Phillips 1981).

The use of dry weight eliminates or at least minimizes the problem of seasonal water content change of oyster tissue. The effects of growth and spawning are averaged as an effective annual average growth assuming that oysters lose little body burden of zinc during the weight reduction in winter time and spawning. The seasonal change is also averaged out by the use of annual average temperature and salinity.

E. Summary of Literature Review Covering Pathways of Zinc in Oysters.

Uptake: Reports of environmental zinc concentrations show that there are large temporal and spatial variations for both the water column and particulate materials; given this variability it is not meaningful to calculate an exact mean value or to analyze for trends. For the model, the total zinc concentration of a system is assumed to be constant throughout a year.

The partitioning between water column and particulates is a function of primarily salinity, temperature and the amount of suspended particulates. In the

model, zinc in the water column and zinc associated with particulate materials are not differentiated; it is assumed that zinc is in equilibrium between the two phases. The free ion activity of a metal is governed primarily by the total metal concentration, the temperature, and the salinity of the water. The rate of uptake of zinc from water per unit metabolism of an oyster, is linearly related to the bioavailable concentration in the environment, which is assumed to be the free ion activity of the metal in water.

The amount of zinc taken up through the water column is the amount of water pumped times the amount of bioavailable zinc. The amount of water that flows over body surfaces (especially that of the gills) by pumping is determined by body size and the metabolic rate of the organism. The metabolic rate of an oyster is in turn a function of the weight (body size) of the oyster, and the temperature and salinity of the water. Adsorption-exchange occurs on the extensively exposed surfaces of gills, mantle, and labial palps, all of which contain slightly higher concentrations of zinc than do internal tissues. The zinc uptake process itself is assumed to be independent of other metals.

Bioavailability of particle associated zinc is linearly related to the amount of readily digestible zinc (that in organic food particles and that adsorbed to inorganic materials). The model assumes that the readily digestible zinc is a constant fraction of the zinc associated with particulate materials. Zinc is dissociated from the particulates in the oyster gut by the digestion process and/or acidity. Zinc is accumulated in the intestine and eventually dispersed by hemolymph throughout the body of an oyster. The model assumes that oysters take up a constant portion of the bioavailable metal associated with particulate materials ingested. The uptake rate from particulates is,

then, a function of the bioavailable metal concentration and the amount of the ingested particulate materials. The amount of the ingestion in a given period is the sum of growth and metabolism. Metabolic rate is governed primarily by body weight, water temperature, and salinity. Elevated levels of zinc do not affect growth or the metabolic rate of the organism.

Storage: A major change in the speciation of trace metals occurs after transfer from sea water to tissues. Free zinc ions are not present in any significant amount in oyster soft tissue, are not bound to one specific compound or any polypeptides such as metallothionein. Nearly all zinc in *Crassostrea virginica* is bound either to proteins or to structural cellular components such as cell membranes, that is 98% of the zinc is associated with proteins that are non-diffusible molecules. Less than 1% of the tissue zinc is the metalloenzyme-associated metals. Metalloenzymes and metal transporting proteins usually only contain one or two metal atoms per molecule and consequently do not account for the large amount of metals accumulated within oyster bodies.

There appears to be no weight-specific limit to the metal concentration for oyster soft tissues though the rate of increase in the concentration slows down with larger weight. *C. virginica* accumulates metals far excess of its metabolic requirement (Wolfe 1970b, *cf.* White & Rainbow 1985) without any toxic effect to the organism itself.

Zinc is actively removed from serum by an energy-dependent process and this process is extremely fast and non-limiting. Serum metal concentrations, however, are still higher than those of the surrounding estuarine water. Zinc is immobilized in

membrane-limited vesicles within amoebocytes (amoeboid lymphocytes). The elevated zinc levels in the tissues of oysters are due almost entirely to the presence of the specific blood cells that contain the metal rich membrane-limited vesicles. The amoebocytes are rather uniformly distributed throughout the animal tissue. These tissue amoebocytes account for 95% of total body zinc, with the remaining 5% in hemolymph. The hemolymph amoebocytes (77% of the 5%) penetrate all of the soft tissues and, therefore, the amoebocytes may have a metal-transport function.

Both copper and zinc are removed from cytosol and detoxified by further compartmentation process of concentration within membrane-limited vesicles. Here, they are associated with some inorganic or organic complexing molecules that maintain the metals within the vesicle, thus effectively removing them from hemolymph and cytosol. Intracellular inorganic granules (calcium phosphate granules) are of limited importance in oysters.

Depuration: The high concentrations of trace metals in oysters indicate that depuration mechanisms are not effective enough to prevent the accumulation of the metals. The excretory system of oysters does not have any metal depuration function and it is contrastingly different from that of mussels, in which the kidney plays an active role in depuration (George & Pirie 1980). The rapid rate of turnover of metalliferous granules in mussel kidney may account for the very different metal contents of zinc in mussels and oysters (Rainbow *et al.* 1990). In oysters, there is little exchange of zinc in blood cells with the outside; depuration is limited to the coincidental loss through shedding of epithelial cells or diffusional loss of hemolymph

serum zinc at the surface.

In some organisms, metals are transported to their gametes, thus eliminating the materials during gametogenesis and subsequent spawning. On the contrary, it seems that oysters prevent the transfer of excess metals to gametes, except when oysters have negligible control of the metal, as in the case of mercury. Gametogenesis is, therefore, treated only as a weight change (loss) without any change of total body burden; the weight change term is averaged out as an effective annual growth for the model.

Another possible depuration route is incorporating the materials into its outside structure, *i.e.* shell, but oysters transfer little trace metals into shells during shell formation. Zinc concentration in shell is many orders lower than that of soft tissue but the amount of zinc in the shell is the same order of soft tissue zinc because of its large mass. Much of this zinc is thought to be incorporated directly into the shell surface from the water column, because there is no relationship between the average shell zinc concentration and average soft tissue zinc concentration.

III. CONCEPTUAL MODEL

The conceptual framework, and the associated mathematical expressions, of the bioaccumulation processes is presented in this chapter. In later chapters, parameters are measured or estimated, and then a numerical model developed to simulate zinc accumulation and estimate the soft tissue concentration of zinc at a given time for given histories of factors such as salinity, temperature, growth rate, and ambient concentrations of the metal.

A. Kinetics and Statistical Approaches.

Metal uptake mechanisms and rates vary among different bivalve species for a given metal and with metal for any given species, making it difficult to generalize. Efforts to conceptualize and predict have employed two approaches: the time variation in body burden and variations in body burden with body size. A simple time-based uptake-and-retention model has been used by many "kinetics" studies (*cf.* Eberhardt 1975), including the "Mussel Watch" program. Assuming first order kinetics, the uptake of metal is

$$\frac{d}{dt} y_{(t)} = k_1 C_w - k_2 y \qquad \text{--- (3-1)}$$

with the solution

$$y_{(t)} = \frac{k_1}{k_2} C_w [1 - e^{-k2t}] \text{ and } y_{(0)} = 0 \qquad \text{--- (3-2)}$$

where $y_{(t)}$ is the time variant body burden, $y_{(0)}$ is the body burden at time t = 0, k_1 is a constant uptake coefficient, C_w is the concentration of the metal in water, and k_2 is a depuration or loss constant.

At steady state, if it is ever reached, the concentration is $\frac{k_1}{k_2} C_w$, with the ratio of k_1 and k_2, commonly referred to as the bioconcentration factor (Hamelink 1977). The bioconcentration factor, however, has not been established for oysters even for a given set of physiological and environmental conditions. Field data show that the concentration of a metal in the environment and that in the organism are not linearly related and that the metal concentration in the oyster changes over its life time (*cf.* Simkiss & Mason 1984). In particular, oyster soft tissue zinc concentrations do not reach steady state, but continues to increase over the life of the oyster. Consequently we must reject eq. 3-2 as inappropriate for zinc and oysters.

The "empirical approach" is based on the observation that metabolic functions tend to vary as a power function of body weight. The relationships between body size (dry weight) and metal concentration of organisms also often fit power functions (*e.g.*, Boyden 1974, 1977; Widdows 1978; Strong & Luoma 1981; Thomson 1982; Phelps *et al.* 1985). These have the form:

$$y_{(t)} = a\ W_{(t)}^{\ b} \qquad \text{--- (3-3a)}$$

and

$$c_{(t)} = \frac{y_{(t)}}{W_{(t)}} = a\ W_{(t)}^{(b-1)} \qquad \text{--- (3-3b)}$$

where y(t) is the metal body burden, **a** and **b** are constants, $W_{(t)}$ is weight of the organism, and $c_{(t)}$ is weight-specific concentration of the metal at time **t**.

There have been few studies of the zinc-body size relationship for *Crassostrea*

virginica but development of a model requires that the relationship between soft tissue zinc concentrations and the dry weights of oysters be determined.

Clearly, body size is a function of time, so the two approaches, the "kinetics approach," with total body burdens as a function of time, and the "empirical approach," which examines the variation in body burden as a function of organism size, can be related. That transform function, however, is not likely to be a simple one for most cases, making it difficult to move directly from one approach to the other.

The purpose of this study is to develop first a conceptual framework and then a numerical model to estimate the tissue concentration of zinc at a given time for given histories of factors such as salinity, temperature, growth rate, and environmental concentrations of the metal.

B. Conceptual Model.

First, it is necessary to make some general assumptions regarding individual oysters, namely that: (1) the differences in growth rates among natural systems are caused by the combination of environmental factors, namely salinity, temperature, organic particle concentration (food resources), and (unknown) stress factor and (2) an oyster responds linearly to the combination in each subsystem without any individual intrinsic (such as genetic) difference.

When the eq. 3-3a is differentiated with respect to time,

$$\frac{dy}{dt} = abW^{b-1}\frac{dW}{dt} \qquad \text{--- (3-4)}$$

where $\frac{dW}{dt}$ is growth rate and $\frac{dy}{dt}$ is net uptake rate. The growth rate of *Crassostrea*

virginica is time variable; fast in spring and fall, negligible in winter, negative in summer due to spawning. For the numerical model, however, a daily effective growth rate (G) is defined from the net annual growth. Thus,

the net daily zinc uptake = $\frac{dy}{dt} = kW^{b-1}$ --- (3-5)

where k = a b G.

While the preceding is correct mathematically, the physiologically correct approach starts with net uptake ($\frac{dy}{dt} = kW^{b-1}$). The zinc body burden of oysters, then, is the time-integral of net uptake:

$$y = \int_0^t kW^{b-1}dt = a\ W^b \qquad \text{--- (3-6)}$$

The simple model for zinc accumulation by oysters that is proposed is based on the following assumptions: (1) the uptake rate of zinc by an oyster is a function of the body size of the oyster, (2) for a given body size of an oyster, the uptake rate is inversely related to the salinity of the ambient water, and increases linearly with the bioavailable ambient zinc concentration, and finally (3) in-situ body size and zinc body burden relationships observed in oysters are the time-integrated result of the instantaneous uptake. These assumptions will be discussed further in later sections.

For the sake of simplicity, the following working relationships were established: (1) The uptake coefficient **b** varies linearly with salinity and is dependent only on salinity, (2) The effective growth (in dry tissue weight) of an oyster is linear, at least for the first few years.

IV. MEASUREMENT OF ZINC UPTAKE PARAMETERS

The kinetics of zinc accumulation were examined by measuring laboratory uptake rates and in-situ body burdens. The uptake rates, *i.e.* the short-term uptake by oysters of differing body weight, could not be quantified by field sampling. Oysters were exposed to ^{65}Zn to determine if uptake rates varied with body size (dry weight) and salinity. The measurements provided an estimate of the uptake parameter, **b-1**, the effect of salinity on the weight-uptake rate relationship. Details of the "materials and methods" and "results" were described in V.I.M.S. Data Report No. 29 (Mo & Neilson 1988).

The effect of body weight on the metal concentration of oysters is hard to evaluate using field samples because metal accumulation is a complex interactive process (*cf.* Boyden 1974, 1977; Norstrom *et al.* 1976; Widdows 1978a; Phelps *et al.* 1985). It was believed, however, that the measurements of in-situ body burdens in this study would give insights on the bioaccumulation process because (1) oysters with wide range of weights were individually analyzed, (2) oysters were collected from different salinity regimes for each estuarine system making it possible to separate the salinity effects from the body weight effects, (3) many extraneous variabilities were eliminated by excluding gut contents and pea crabs and using dry weights, and (4) all samplings were done in a short time-span (1 week) eliminating seasonal effects.

Details of the "materials and methods" and "results" were reported in the V.I.M.S. Data Report No. 30 (Mo & Neilson 1989).

A. Zinc Uptake Measurement by the Use of ^{65}Zn.

1. Overview.

Short-term uptake kinetics of ^{65}Zn were measured in the laboratory. Three groups of *Crassostrea virginica* of various weights were held in aquaria and the radioactive tracer ^{65}Zn was introduced with suspended solids. Many prior experiments used filtered water for the incubation under the assumption that dissolved metal was the major source for metal uptake, but these were not likely to have produced results applicable to oysters in natural environments because oysters are known to detect the absence of particulate material and to change their feeding behavior (Jorgensen 1975; Jackim *et al.* 1977; Winter 1978). Short-term (108 hours) uptake of the tracer ^{65}Zn, from both water column and particulates, was measured and the difference in uptake with body size (dry weight) and salinity was determined assuming the (radioactive) metal uptake (y') would be related to (dry) body weight, W, as a power function (eq. 3-5) (*cf.* Boyden 1974).

It was assumed that: (1) Part of the newly introduced radioactive tracer would be adsorbed to sediment particles and would be bioavailable. (2) Depuration and body weight change were negligible during the experimental period. All factors, such as temperature of aquaria, other than salinity and the concentration of the tracer were kept constant.

2. Materials and methods.

Oysters, *Crassostrea virginica*, were collected near Mulberry Island in the James River, Virginia, a subestuary of the Chesapeake Bay in February 1988 (see Fig. 4-1). Typical salinity for this area for March to October was 14 ‰ (Table 4-1). Three groups of 12 oysters were selected to give wide shell-height distributions (from about 2 to 9 cm) for all groups. Bottom sediments from the same site were mixed with 1 μm filtered York River water and then filtered through a 63 μm mesh sieve to remove large particles. The sediment-water mix was stirred with a glass rod, sand and silt were allowed to settle for ten minutes, and the settled sediments were discarded. The resulting mix had a solids content of 96 g/l. The particulate carbon and nitrogen contents of the mix, measured by Carlo-Erba "CN Analyzer", were 4.35% and 0.54% of dry weight, respectively.

Two zinc tracer mixes were prepared. The stock tracer was 0.1 ml of $ZnCl_2$ in 0.5 M HCl having a purity of 0.99, with a specific activity of 1.97 mCi/mg and a concentration of 10.0 mCi/ml. The stock tracer was diluted with the sediment-water mix to give two concentrations (1.67 μCi/l and 0.84 μCi/l). York River water, which had been filtered through a 1 μm filter, and distilled-deionized water was added to the aquaria to produce salinities of 18 ‰ in two aquaria and 12 ‰ in a third aquarium; the final volume was 25 liters each. The water was aerated with an air pump. Oysters, brush-cleaned under running sea water and acclimated to the experimental salinities, temperature (20 °C), and suspended solids concentration for 7 days, were placed on racks in the aquaria. Subsequently, at the beginning of the experiment, 12.5 ml of the labeled sediment-water mix was added to each aquarium. An additional

12.5 ml was added every 12 hours in order to maintain target radioactivity concentrations of 0.5 and 1.0 μCi/l with sediment concentrations of approximately 50 mg/l, a nominal value for the near bottom waters in Chesapeake Bay estuaries (Nichols *et al.* 1983). Any dead oysters, easily recognizable because the shells were open wide and the animals did not filter solids from the water, were removed from the aquaria immediately. At the end of the experiments, 11 oysters remained in each aquarium.

After 108 hours, the experiment was ended and after 12 hours in clean filtered water, the oysters were shucked. The soft body tissues were dried at 105 C° to constant weight (about 3 to 5 days). Radioactivities then were measured by a Beckman "BioGamma II" gamma ray counter. Water samples were filtered with 0.45 μm membrane filters and the radioactivities of the filters and the filtrates were determined. Radioactivity counts were converted to tracer concentrations using the counting efficiency of the instrument that was calculated from standards. Dissolved and sediment-associated tracer concentrations were adjusted to a common unit, μCi/l. For oyster samples, the weight-specific tracer concentrations were calculated by dividing the tracer counts of soft tissue by the corresponding oyster dry weight. The tracer contents were converted to daily rates by multifying the values by 24/108).

The ^{65}Zn uptake rates ($\frac{dy}{dt}$) of an oyster was assumed to be related to body weight (W) as a power function, eq. 3-5 (Boyden 1974, 1977; Widdows 1978a). The coefficient **k** and the power, **b-1**, were fit from the least square method of logarithmic transformed values of $\frac{dy}{dt}$ and **W**.

3. Results and discussions.

To make calculations simple, it was assumed that the decrease of radioactivity in the aquarium and the uptake of the tracer by oysters were repeated on each 12 hour period of before and after the tracer introduction. When solving equations, the average values of the mean from the 12 hour periods was used. The radioactivity of the tracer decreased as an exponential function of the decay rate $e^{-\lambda t}$. For t = 108 hours, $\frac{N}{N_o}$ = 0.9873. Therefore only 1.28% of the radioactivity of the tracer was lost due to the radioactive decay. Because of the short duration of this study (t=4.5 days), relatively long radioactive half-life of ^{65}Zn ($t_{1/2}$ = 244 days), and long biological half-life of zinc in oysters (biological $t_{1/2}$ = 300 to 900 days) (Romeril 1971; Seymour & Nelson 1972, 1973), it was assumed that loss was negligible during the experiment period. Depuration and growth in the period also were assumed to be negligible.

Salinities and temperatures of the aquaria, monitored every 4 hours, were fairly constant throughout the experiment. The actual salinities (12.7 and 17.5 ‰) differed slightly from the target salinities (12 and 18 ‰) (Table 4-2). Oysters were observed to pump water actively throughout the experiment. When the sediment-water-tracer mix was added to the aquaria, the water became turbid, but twelve hours later, the water was almost clear while a control aquarium, identical with test aquaria except that no oysters were placed in this aquarium, remained turbid. Little sediment was deposited on the bottom except for faecal pellets that were not re-suspended by aeration. The suspended sediment concentrations bracketed the target concentration. Radioactivities also varied during the course of the experiment and were higher than the targets.

Concentrations of dissolved and sediment-associated tracer and of suspended solids in a control aquarium, which had no oysters, did not change significantly over 12 hours, and consequently the controls were discontinued. When total uptake and body weight were related by a power function, $\frac{dy}{dt} = k\ W^{b-1}$, the power **b-1** had value of less than 1 (Table 4-2; Fig. 4-3 & 4-4), *i.e.* metal uptake per unit biomass by smaller individuals was more rapid than that by larger individuals (Strong & Luoma 1981). This result applied to all three aquarium experiments (Table 4-2).

The regression lines for aquaria 1 and 2 had about the same slope and the two slopes became almost identical when one outlier identified in Fig. 4-3 was eliminated for aquarium 1. The lines became almost identical when the radioactivity concentrations in oysters from aquarium 1 were normalized, *i.e.* concentrations were divided two since twice as much tracer was added to this aquarium (Table 4-3). That is, the amount of tracer taken up by an oyster increased linearly as the ambient zinc concentration increased, at least for the conditions studied in these experiments. The regression line for aquarium 3 (salinity = 12 ‰) had a less steep gradient than that of aquarium 1 and 2 (salinities = 18 ‰) (Fig. 4-4). In other words, the weight effect (decreased uptake rate with increased weight) was more pronounced in higher salinity waters. If higher metal concentrations in oysters of lower salinity and vice versa were solely due to the difference in availability (free ion activity difference) (*cf.* Zamuda & Sunda 1982; Zamuda *et al.* 1985), then the slopes from the regression lines should not have changed as the salinity changed, though the amount taken up, and therefore the uptake rate, should have changed. Wright & Zamuda (1987) found salinity effects on copper accumulation by oysters that were independent of cupric ion activity. They

suggested that changes in salinity may affect several physiological processes that influence the accumulation of trace metals by oysters. It is probable that, besides the free ion activity differences (higher in lower salinity), uptake rate and growth rate differences in different salinity regimes and different estuarine systems contribute to the salinity effects and to the differences among systems.

Results indicated that larger animals would take up less per unit weight. It appeared that changes in salinity affected not only the water chemistry but also the physiological processes of the organism.

The uptake of the tracer zinc in the ^{65}Zn experiments was independent of that of stable zinc because: when the oysters were placed in the aquaria with the tracer mix, the change of the metal concentration of an oyster was, as eq. 3-1,

$$\frac{d(C_o+N_o)}{dt} = k_w(C_w+N_w) + k_p(C_p+N_p) - k_d(C_o+N_o), \text{ where}$$

C : stable zinc concentrations

N : the radioactivity concentration of tracer

and the subscripts indicate;

$_o$: in the oyster

$_w$: dissolved in water

$_p$: adsorbed on particulate materials

$$\frac{dN_o}{dt} = k_wN_w + k_pN_p - k_dN_o + [\frac{dC_o}{dt}-(k_wC_w+k_pC_p-k_dC_o)]$$

Because $\frac{dC_o}{dt} - (k_wC_w+k_pC_p-k_dC_o) = \frac{dC_o}{dt} - (k_wC_w+k_pC_p-k_dC_o)$, the bracketed term is zero and tracer uptake rate for unit body weight of the oysters is:

$$\frac{dN_o}{dt} = k_wN_w+k_pN_p-k_dN_o$$

B. Measurements of In-Situ Zinc in Feral Oysters.

1. Overview.

Zinc concentrations were measured for oysters of various weights collected from oyster beds with different salinity regimes of three Virginian coastal plain rivers. The order of soft tissue zinc concentrations (high to low) was James > Piankatank > Rappahannock River (Table 4-4). The zinc concentrations of oysters and the salinities of sites showed a strong inverse relationship in each subsystem. The relationships between the concentration and dry weight were positive (when salinity effects were eliminated) for every site, *i.e.* total zinc per unit soft tissue increased for the life time of an oyster indicating an ineffective depuration system.

The relationships were assumed to have the form: Uptake equals the product of a constant times weight raised to the power b (*e.g.*, a W^b). Values for the coefficients a and b were determined for each case. The regression of oyster body weight on zinc concentration gave powers that were high (1.30) in the low salinity area (13 ‰) and low (1.06) in the high salinity area (20 ‰) in the James River and that, at least partially accounted for the salinity effects.

Additionally, hooked mussels, *Ischadium recurvum*, of various weights and sediment samples were collected at the same time from the oyster beds. Contrast of the mussels to oysters is discussed in **Appendix B.**

2. Materials and methods.

Oysters were collected by dredge from different salinity regimes of three subsystems of the Chesapeake Bay, Virginia (Table 4-1). Nominally a dozen oysters

were selected with a shell-height distribution as wide as possible. The selected organisms were brushed under running sea water to remove adhering mud. After surface water was removed by blotting with paper towels, the oysters were placed in vinyl bags and kept in a freezer maintained at -12 °C.

Prior to analysis, oysters were transferred to a refrigerator for 6 to 12 hours until the soft tissues were partially thawed. Shells were opened and soft tissues were separated from the shells with stainless steel knives. When it was judged that an oyster had enough particulate materials to be of concern, the thinned end of a pipette was inserted into the anal opening of the oyster and gut contents were removed by flushing with deionized water.

Soft body tissue samples were dried at 105 °C to constant weight, weighed, and then digested using concentrated nitric acid. Zinc concentrations were measured by flame atomic absorption spectrophotometry.

Body burden (y) of the individual was related to body weight (W) as a power function (*cf.* Boyden 1974; Widdows 1978):

$y = aW^b$, that is, $\log y = \log a + b \log W$

The coefficients **a** and **b** were determined by regression analyses using the log-log transformed data for the oysters from each site. The significance of each regression coefficient was tested. The significance of the differences among the regressions was also tested.

3. Results and discussions.

Oysters show very wide individual differences of tissue metal concentrations.

Considerable scatter remained even after fitting regression equations to the data points. The possibility of a significant difference in model I and model II, therefore, was considered (*cf.* Laws & Archie 1981) and the need to use a Model II regression was examined. The slope (v) of functional regression was calculated from $v = + b/r$ where b was the slope of the linear regression and **r**, the correlation coefficient (Ricker 1973, 1975). Specific non-zero hypotheses about the slopes were tested and it was concluded that the differences in slopes of regression lines were statistically significant (Table 4-5). As the correlations of log-log transformed regression of body burden on body weight were extremely high, there was little difference between the geometric regression (functional regression) and the model I regression.

The oyster weights are random variables and the oysters in this study were arbitrarily selected to give as wide as possible weight ranges and to have about equal numbers of oysters in every weight class. This violates the assumption of the model I regression which requires fixed independent variables. The use of model I regression method when it is actually a model II has been argued in many fields; however, for the purpose of this study, it was assumed that, (1) for each selected size there was a normal distribution of body burdens, (2) the mean of body burdens lay on the population regression line, and (3) the standard deviation of these means was constant over body weights. Therefore the Model I regression could be used without being biased (*cf.* Snedecor & Cochran 1980; Sokal & Rohlf 1981).

The zinc concentrations in oyster shells were extremely small compared to those of oyster soft tissues (Table 4-6) suggesting that the depuration of zinc through shell formation is of minor importance.

The body burdens of zinc in oyster soft tissues apparently increased throughout the life of the organism because the total soft tissue zinc observed in this study increased with dry weight (Fig. 4-5,6,7). The soft tissue concentrations (zinc per unit dry weight) also increased with dry weight (Fig. 4-8,9,10). In the short-term laboratory exposure experiments of ^{65}Zn by oysters, it was shown that there was a weight dependent difference in uptake rate, that is, metal uptake per unit biomass by smaller individuals was more rapid than that by larger individuals. The rate of increase in body burden (the coefficient **b**) was lower in oysters from a higher salinity regime than in oysters from a lower salinity regime in the James River. This suggests that the uptake rates of oysters of higher salinity regime decrease more rapidly with weight than those of oysters in lower salinity and this agrees with the results of the short-term uptake studies. The cumulative effect of the influence of salinity on short-term uptake would result in the weight-body burden relationship mentioned above. This would contribute to the differences in the mean trace metal concentrations at different salinities (lower concentration in higher salinity and vice versa) for equal sized oysters.

In the James River, oysters that live in higher salinity waters had lower soft tissue zinc concentrations than those in lower salinity regime and *vice versa* (Table 4-4). This result agreed with that found in previous similar studies.

The mean concentration was greatest in the James River and varied as follows: James > Piankatank > Rappahannock River (Table 4-4). These differences in mean concentrations, however, were believed to be caused by the effect of weight differences among samples because the average value of the power **b** varied as

James > Piankatank > Rappahannock River.

From the results of the two experiments, it was concluded that (1) for a constant environment, tissue zinc concentration of an oyster continues to increase, but (2) the rate of the increase is reduced as the oyster grows. It is suggested that, in addition to the free ion activity differences (higher in lower salinity), uptake rate and growth rate differences in different salinity regimes and different estuarine systems contribute to the salinity effects and to the differences among systems.

The power **b** has been examined by many authors because they believe it might provide clues for understanding the uptake mechanism. When body burden is expressed as $y = a\ W^b$, then concentration is $c = \frac{y}{W} = a\ W^{b-1}$. One should differentiate between the powers for the in-situ body burden relationship, **b**, and the uptake rate relationship, **b-1.**

Boyden (1977) discussed three cases: $b = 1$, $b < 1$, and $b > 1$. A fourth case arises when the in-situ power relationship equations are differentiated with respect to weight or time for short term uptake equations because the powers are reduced by one. The change of body burden and the change of concentration are, respectively:

$\frac{dy}{dW} = a\ b\ W^{b-1}$ and $\frac{dc}{dW} = a\ (b-1)\ W^{b-2}$

and the fourth case is $1 < b < 2$. This intermediate case has unique characteristics because **b** minus 1 is greater than zero but **b** minus 2 is less than zero. The coefficients of power fit, **b** for in-situ zinc body burden data of oysters are greater than 1 but less than 2 for all of the 7 site populations (Table 4-5). As **b** is larger than 1, the oyster body burden is the same pattern (increasing with weight) as the case $b > 2$ (Fig. 4-11). Concentration also increases with body weight (Fig. 4-8,9,10) but the

patterns are different, concave downward for $0 < \mathbf{b\text{-}1} < 1$ and concave upward for $\mathbf{b\text{-}1} > 1$ (Fig. 4-12). The concentration change rate also decreases as the weight increases (Fig. 4-13), *i.e.* zinc uptake rate per unit biomass by a smaller oyster is more rapid than that by a larger oyster.

4. Seasonal changes.

Additional groups of oysters were collected from the Wreck Shoal oyster beds (June-1987) and nearby Mulberry Island oyster beds (January-1988). Unlike the differences among different sites at the same time, the relationships between zinc body burdens and oyster dry weights were similar to that of the fall-Wreck Shoal oyster data (October-1987) (Fig. 4-14). Comparing the mean concentrations without regard to weight effects is meaningless and could lead to a wrong conclusion even for the same site samples as this study pointed out. Obviously one can not follow the body burden of stable zinc in an individual oyster to monitor temporal change. It seems that one needs many data points (oysters) to improve the resolution of the relationship for any seasonal comparison.

V. NUMERICAL MODELLING OF ZINC ACCUMULATION FOR OYSTERS

A. Mathematical Expression of the Model.

The purpose of the model is to predict the soft tissue zinc concentration for each age group of oyster, so short-term variations are ignored and annual average values are used instead. The time unit for all model parameters is one day. July 15th of each year, the middle of oyster spawning season in the Chesapeake Bay area, is considered to be the starting day.

From the eq. 3-5 and eq. 3-6,

$$y_{(t)} = \int_{t=0}^{t=t} C_{env}\, a'\, b G W(t)^{b-1}\, dt \qquad \text{--- (5-1)}$$

where a is C_{env} times **a'**, and C_{env} is the bioavailable zinc, which is assumed to be the free ion fraction of total zinc ($C_{env} = C_{total}$ fraction). For the present model, bioavailability of zinc for each site is calculated from total metal concentration and hydrological data using a chemical speciation model, with salinity and temperature the primary factors governing bioavailability of dissolved (free ion) zinc.

If **dt** is approximated by daily term, **Δt**,

$$\Delta y(t) \approx C_{env}\, a'\, b \frac{\Delta W}{\Delta t} [W_{(t)}]^{b-1}\, \Delta t = C_{env} a'\, b [W_{(t)}]^{b-1} \Delta W \qquad \text{--- (5-2)}$$

and

$$W_{(t)} = \int_{t=1}^{t=t} G \;\; dt = t\, \Delta W$$

then

$$y_{(t)} = C_{env}\, a' b \Delta W \sum_{t=1}^{t=t} [t\Delta W]^{b-1} \qquad \text{--- (5-3)}$$

If rearranged, eq. 8 is the same as the finite difference solution of the eq. 3-5, which is,

$$y_{(t+1)} = y_{(t)} + C_{env}\, a' \Delta W^{b} (t+1)^{b-1}$$

The input variables to the model are total metal concentration for a site and growth rate. Effective annual average temperatures and salinity are calculated from historical monitoring data. Physiological parameters that cannot be measured directly will be estimated during calibration. Iteration and finite difference methods are used to calculate the kinetic parameters on a daily basis.

The outputs of the model, predicted zinc concentrations of oysters by weight or by time, are compared to in-situ measurements of oyster samples. After the calibration, the effects of changes of input parameters are analyzed with the model to evaluate the relative importance of each parameter.

B. Estimation of Parameters and Model Calibration.

The model, although relatively simple, contains a number of constants and coefficients. Laboratory studies were used to estimate daily uptake. Information on in-situ zinc body burden of oysters was used to complete the calibration process and to check these parameter estimates.

1. Daily uptake rate.

The short-term uptake parameter, the **b-1**, was derived from the data of the laboratory study, in which radioactive tracer ^{65}Zn was introduced with suspended

solids to oysters of various weights. It was assumed that the uptake rate constants for stable zinc and for radioactive zinc were the same. The tracer contents in oyster soft tissues measured net uptake.

It was assumed the power **b-1** holds for all temperatures. It was calculated in the model as

$$\textbf{b-1} = -0.0446 \times \text{salinity } (‰) + 0.9588 \text{ (Fig. 5-1)} \qquad \text{--- (5-4)}$$

Oysters in the ^{65}Zn uptake experiments probably exhausted the tracer before the next addition (with 12 hour interval), so a reliable net uptake parameter **k** could not be calculated from the experiments. The parameter **k** is calibrated by the use of in-situ data for the model in a later section.

2. Annual growth rate.

It is generally known that oysters grow faster (1) in the Rappahannock River oyster beds than in those of the James River, and (2) in higher salinity regime than in lower salinity regime, but there is little data regarding the in-situ growth rate of *Crassostrea virginica.* Ages of oysters were roughly estimated for each river by visual inspection of the shells and the changes in the growth rings of shells (*cf.* Carriker *et al.* 1980). The surface of an oyster shell consists of distinct growth rings (thin layers of shell material). Each year there were two rapid growth periods (Spring and Fall) and loss of weight due to spawning in Summer. It was assumed, therefore, that two distinctive marks represent the growth of one year. It was relatively easy to group the oysters by the age for the first 4 or 5 years of growth but shells of older oysters showed so much alteration due to erosion and deformation that the age determination

was impossible for those older oysters. It was assumed that the effective yearly growth of an oyster was linear until the oyster weight nears the asymptotic value. The nominal value of 5 year old oysters for the James River and for the Rappahannock River were 1.0 g and 1.2 g, respectively, and one-fifth of those values, 0.2 g/yr and 0.24 g/yr were used in the model (Fig. 5-2). Daily growth was calculated by simple division of 0.2 and 0.24 gram-dry weight/year by 365 for the James River and for the Rappahannock River, respectively.

3. Salinity.

Annual mean salinities over the natural oyster habitats sampled in this study range from 12 to 24 ‰. Freshwater discharge is a primary factor governing the salinity patterns. The greatest monthly mean freshwater discharge occurs in the early spring, and the smallest occurs in early fall. Therefore salinity is generally highest in late summer to early fall and lowest in the spring (*cf.* VIMS SRAMSOE No. 292). A nominal value of salinities (*cf.* VIMS Data Report No. 18 and 19) at seven meter depth of each of the James River oyster bed and all of the Rappahannock River oyster beds showed that the longitudinal gradient of salinity in the James River (Fig. 5-3a) was much greater than that in the Rappahannock River (Fig. 5-3b) (*cf.* Kuo & Neilson 1987).

Nominal values of the salinity at seven meter depth of each of the James River oyster beds and all of the Rappahannock River oyster beds for the period when water temperature was higher than 10 °C (from early spring to late fall) were chosen by evaluating the data (VIMS Data Report No. 18 and 19) graphically and used as an

effective average salinity for the model.

4. Temperature.

A single harmonic with a period of a year normally accounts for most of the total variance of a water temperature record. Neilson & Hsieh (1982) reported that more than 95% of the variance of daily average temperature fluctuations could be captured by a simple sine wave after analyzing the VIMS pier data. It was assumed that at the mid-depth, there was little daily temperature change and minor deviations from the sine curve

$$T = \mathcal{T} - \left\{ a \times \cos \left[\frac{2\pi\ (t\ -\ \theta)}{365} \right] \right\}$$

in which $\mathcal{T}$ is the yearly mean temperature of the area.

$$a = \frac{1}{2} \cdot [(\text{maximum daily average}) - (\text{minimum daily average})]$$

θ is the phase angle (in days).

The coldest water temperature of the area is close to February 1st. and the hottest to August 1st. in the area (θ = 30). Annual mean temperature $\mathcal{T}$ and the parameters for the James River and for the Rappahannock River were calculated by the use of the least square fit of the "VIMS slack water data report" of 1970 to 1980 data. As there was little difference of the amplitude and patterns of yearly temperature among the sampling sites in a subsystems, a single cosine curve was fit for all subsystems (Fig. 5-4). A single value, 18 °C, the average of the sine curve above 10 °C and below 25 °C, is taken as an effective annual average temperature for the model and was used for all sites. Previous studies reported that feeding and growth of oysters are inhibited below 8 °C (Galtsoff 1964) and there is little growth

out of the temperature range (Frazier 1975).

5. Major ions and zinc concentrations.

Oysters are exposed to the highly variable estuarine environment, which is subject to tidal, diurnal, seasonal cycles. The model uses the effective annual averages for its parameters assuming that in-situ soft tissue zinc concentrations reflect integrated effects of the fluctuating factors. It was assumed that the total amount of zinc (water column and particulate associated) at a location was relatively constant throughout a year; 5 µg/l and 2.5 µg/l were used for the James River and for the Rappahannock River, respectively. It also was assumed that the particulate concentration and its organic content were constant and that the metal was in equilibrium that is determined primarily by salinity.

It was assumed that the major ion composition of the sampling sites follows the "constancy of relative ionic composition of sea water". Concentrations of major elements were calculated by:

Concentration of element $M_i = \frac{salinity M_i}{35}$ x (concentr. of the M_i at salinity 35 ‰).

Compositions of sea water were quoted from Riley & Chester (1971) for the area.

A chemical speciation model, REDEQL (Morel & Morgan 1972; Ingle *et al.* 1978; Ingle *et al.* 1980) was used to calculate the percentage of the free ion for temperature of 18 °C, pH of 8.1, and the mean salinities of the sampling sites (Table 4-1). The free ion was considered as the bioavailable portion of total zinc in water column because its concentration is the most important control on dissolved metal bioavailability (Zamuda & Sunda 1982; Wright & Zamuda 1987).

C. Calibration and Verification of the Model.

1. Calibration of the model by in-situ body burden data of oysters.

Power functions were fit to the data for Horsehead Rock and Nansemond Ridge, two sites in the James River having mean salinities roughly the same as those used in ^{65}Zn laboratory studies (Fig. 5-5). Values for **b-1** (Table 4-5) are quite similar to those found in laboratory studies (Table 4-2). The smallest oyster from Nansemond Ridge (0.086g) and Horsehead Rock (0.0021g) were excluded from the fitting because those weights had a few orders higher "leverage coefficients" (see Sokal & Rohlf 1981) because of the logarithmic transform. It was decided that excluding those two weights would make the lines represent the whole weight range better.

Once the value of **a** is known, one can then calculate **a'**, if the bioavailable zinc concentration is known ($a = a' C_{env}$). C_{total} for the James River was assumed to be 5 μg/l and fraction is 0.43 and 0.34 for Horsehead Rock and Nansemond Ridge, respectively (see Table 5-1,2). When these data are substituted in the equation ($a = a' \times C_{env} = a' \times C_{total} \times$ fraction), following relationship is obtained:

$$a' = (-0.42 \times \text{salinity } (‰) + 10.33) \times 10^3 \qquad \text{--- (5-5)}$$

where C_{total} is μg zinc/liter of sea water, **t** is day, **G** is gram-growth per day (0.2/365 g/day for the James River), **W** is (dry) gram weight of oyster soft tissue, and body burden is in μg zinc.

In other words, **a'** is inversely related to salinity (Fig. 5-5); **a'** is, therefore, another salinity dependent uptake parameter that is not a power function of body weight. Note that the value of **a'** is sensitive to the units used.

2. Verification by the use of in-situ data.

The data set from the Wreck Shoal oyster beds, the mid-salinity regime in the James River and the combined data set for the three oyster beds of the Rappahannock River (see Fig. 4-1) were used to validate the model (Fig. 5-7). Unlike in the James River, which had a strong longitudinal salinity gradient, the Rappahannock River has a weak gradient and the salinities of the three Rappahannock River oyster beds were similar (*cf.* Kuo & Neilson 1987). Hence, all data for the Rappahannock oysters were pooled.

The model parameters (Table 5-2,3) were determined as follows: **b-1** interpolated from ^{65}Zn uptake measurements (Fig. 5-1), C_{total} from unpublished data, **fraction** calculated by a chemical speciation model, **a'** interpolated from values (Fig. 5-6) estimated from the power fit of the Horsehead Rock and Nansemond Ridge oyster zinc body burdens (Fig. 5-5), **G** estimated from the shell examination (Fig. 5-2). The model predictions agreed well with the field data despite all the simplications and assumptions inherent in the model (Fig. 5-7).

D. Sensitivity Analysis of Model Predictions.

The analysis of the model showed that the relationships between uptake parameters and the body burden-weight parameters are:

(1) An increase of uptake parameter **a'** linearly increases body burdens of all weights (Fig. 5-8a).

(2) An increase of uptake parameter **b-1** increases the slope of the relationship between the body burden and body weights (Fig. 5-8b).

3) A change of the growth rate of an oyster does not change the body burdens to body weights relationship, that is, the difference in growth rates does not affect the model output in body weight domain. The change of the growth rate, however, does affect the model output in the time domain. Both body burdens and body weights increased when the growth rate was increased, *i.e.* oyster weight increases faster and, subsequently, body burden increases faster per unit time for a faster growing oyster than for a slower growing oyster (Fig. 5-9).

1. Uptake parameters k and a'.

Previous uptake studies support the assumptions that zinc uptake mechanisms do not reach saturation (*cf.* Chipman *et al.* 1958; Fitzgerald & Skauen 1963; Harrison 1979) and that oysters take up zinc in proportion to the ambient concentration (Shuster & Pringle 1969). In other words, the uptake parameter **k** was linear with ambient concentration and had no upper limit.

The calibration of the model showed that uptake parameter **a'** varied with salinity (Fig. 5-6). That is, a' is an uptake parameter component that is not weight dependant. Its variation can be explained by different hypotheses of uptake mechanisms: (1) If zinc is accumulated coincidentally with calcium (Wolfe 1970b), zinc uptake would be inversely related to salinity. Ambient zinc concentration is relatively constant but the calcium concentration is proportional to salinity, so incidental zinc uptake would be inversely related to salinity for the same amount of calcium uptake. (2) If zinc and other ions compete for a common uptake mechanism, zinc uptake would be related to the ratio of zinc and other ions (Wright & Zamuda

1987). (3) Geochemical characteristics, which determine bioavailability of zinc bound to suspended sediments (Harrison 1979; Luoma & Bryan 1979), vary with salinity. (4) Winter (1973) reported that *Mytilus edulis* counterbalances a low algal concentration by a corresponding higher pumping rate; but the power coefficient **b** is independent of algal concentration. The amount of water pumped would be inversely related to the algal concentration. If oysters behaved similarly and food concentration were positively related to salinity, zinc uptake would be inversely related to salinity. Any of above hypotheses would explain the salinity effect on a' but it is also possible that more than one of the mechanisms are involved.

2. Uptake parameter b.

The values for the uptake parameter **b**, which were determined by a least squares fit of a power function to in-situ oyster body burden and body weight for oysters from all sites fell close to the line fit to the ^{65}Zn uptake measurements (Fig. 5-1).

The simple allometric equation $y = \alpha W^{\beta}$ is used often for the metabolic rate studies. The values of 0.07 to 0.37 found in this studies may look unusual at first, because many will expect β to be close to 0.75, a value related to many metabolic functions of poikilotherms. If β had been approximately 0.75, this would have implied that some aspect of metabolic process was influencing (Boyden 1974). Metal uptake from the water column is assumed to be a (facilitated) diffusion process across body surface, especially gill surface (*cf.* Pauley & Nakatani 1968; Romeril 1971; Carpene & George 1981; Phelps *et al.* 1985). Group B metals are reported to be very permeable

across lipid bilayer of cell membranes in saline solutions (Simkiss 1983), so the zinc uptake rate for the same ambient concentration is likely to be proportional to the body surface area and to the amount of water passed over the surface. If it were proportional to body surface area only, β would be approximately 0.67. It is, then, logical to relate the rate to pumping rate, the amount of water moved per unit time. Reported values of β for pumping rate range from 0.60 to 0.75 for suspension-feeding lamellibranchiate bivalves with a nominal value of particle retention rate on body weight of 0.73 (*cf.* Winter 1973, 1978; Mohlenberg & Riisgard 1978, 1979; Riisgard & Mohlenberg 1979). Widdows (1978a), however, reported the value of β as being from 0.08 to 0.51 with a nominal value of 0.38 for the mussel, *Mytilus edulis*. He attributed the discrepancy of value to the inclusion of larger animals in his study , as opposed to the weight range used in other studies. Walne (1972) reported values for the coefficient for *Crassostrea gigas* (0.27 for 0.5-2.0 gram dry-tissue weight, 0.25 for 0.5-10.0 gram wgt.), *Ostrea edulis* (0.23 for 0.5-2.0 gram wgt.), and *M. edulis* (0.11-0.34 for 0.19-4.0 gram wgt.) that are similar to the uptake parameter of the model. It is, therefore very likely that the uptake rate co-varies with pumping rate of oysters.

3. Growth term G.

Without the model, non-linear growth of the organism and the dilution effect of tissue mass growth make the interpretation of the relationship between body weights and body burdens complex (*cf.* Strong & Luoma 1981; Thomson 1982; Simkiss & Mason 1984). The model is independent of the growth function and retains the same body burden and body weight relationship, whatever the growth rate is. In the time

domain, however, both body burden and body weight would be overestimated by the model when the oyster body weight becomes larger than those of the largest oysters of this study (about from 1.2 to 1.6 gram dry weight) because the model assumes a linear growth (Fig. 5-9). The model indicates that oyster body weight will increase continuously, whereas the in-situ growth of a feral oyster is believed to slow with age as its weight approaches an asymptotic value (Fig. 5-11). A more elaborate growth term could be used in the model (instead of the linear term). Exponential growth, $W = W_0 e^{kt}$ (*cf.* Eberhardt & Nakatani 1968), the logistic equation, $\frac{dW}{dt} = k\ W(1-W)$, or the von Bertalanffy equation, $W = (1 - b\ e^{kt})^3$ could be substituted in the model. Incorporating one of above growth curves would correct the problem of the model with larger oysters in time domain studies. As an illustration, the logistic equation was applied to the Rappahannock oyster growth data (Fig. 5-11) and coefficients were fit by a trial and error method assuming the asymptotic (dry) weight to be 2.0 gram and the point of inflection at 4 years (see Ricklefs 1967):

$$W = \frac{2.0}{1 + e^{0.7(4.0-year)}} \text{ then, } \frac{dW}{dt} = 0.7\ W\ (1 - \frac{W}{2.0})$$

Applying this growth equation did not change the weight-body burden relationship as stated before, but the overshoot of body burden in older oysters in time scale was corrected (Fig. 5-12a). Linear and logistic growth did not change concentrations much (Fig. 5-12b).

Formulating the growth of organisms in mathematical terms could help "to clarify what properties of the components of a system must be known if its behavior is to be predicted; in other words, it (the formulation) tells us what we need to measure (Smith 1968)." There was, however, too little information regarding the in-situ annual

growth rate of adult *Crassostrea virginica* even to fit an annual growth equation let alone to postulate a bioenergetics growth model. Evaluating the weight of shell and examining the shell surface is admittedly a crude method of age determination for oysters. The procedure, however, provided a valuable starting base. Some bioenergetics studies have dealt with ecological energy requirements (Dame 1972), energy partitioning (Langefoss & Mauer 1975), and assimilation efficiency (Walker & Zahradnik 1975) of *C. virginica.* It may be possible to develop a growth model, such as an elaborate bioenergetics accumulation model (Norstrom *et al.* 1976) if good data for (weight) growth of area oysters were available. A bioenergetics-based model, however, generally requires many other exact parameters such as ambient food concentrations or compositions of food and usually would not work with the averaged parameters. The proposed model, on the other hand, is very robust in terms of those parameters and it might be the more realistic approach considering the temporal and spacial short-term variations of the environmental parameters of most oyster beds and the fact that the body burden of oyster itself reflects the integration of those varying parameters.

The reported inverse relationship between oyster soft tissue zinc concentration and salinity may be explained as a combined effect of salinity on either uptake and/or growth rate - which determines the body weight of an oyster at a given time and, as a consequence, affects the uptake rate - if the study did not consider the weight of organisms. For the studies of accumulation kinetics in time scale, such as long term (in months or years) uptake or depuration measurements for a given periods, one should be careful to put a true body weight change term because faster growing

oysters, for example, would uptake more metals than slower growing oysters for the same periods.

VI. DISCUSSIONS

A. Soft Tissue Concentration Change for Stunted Growth Oysters.

In the model, zinc uptake rate increases with increasing growth rate but body burden will be equal for equal weight oysters if all other factors except growth rate remain constant, *i.e.* oysters move along the body burden-weight curve, such as Fig.4-5 at different speed (growth rates), but follow the same curve if every thing else remains constant. The same relationship was observed in the mussel, *Mytilus edulis*, for zinc (Lobel & Wright 1982).

Phelps & Hetzel (1987) reported that stunted oysters had higher zinc concentrations than normal growth oysters. Stunted oysters accumulated nearly twice as much zinc as normal oysters of the same age. There was no explanation for the hyper-accumulation of metals under stunting growth conditions. These data could be interpreted as indicating that slower growing oysters have higher uptake rates but because the two populations came from different sites, the elevated body burdens could be due to environmental conditions only.

The model can be modified to reflect different growth rates on body burdens by changing **a** to $\frac{a'}{G}$, *i.e.* body burden is inversely related to the growth rate **G** ($= \frac{dW}{dt}$). For illustrations, the body burden data and parameters for Wreck Shoal oysters are used for the numerical solutions. Even for the same uptake parameter **k**, the

concentration in slower growing oyster is higher than that in faster growing oysters of the same age (Fig. 6-1; see Fig. 5-10, that is opposite to Fig. 6-1) due to less dilution effect of weight increase in slow growing oysters. The differences for different growth rates in body burden change in time are similar to those of the previous model (Fig. 5-9). The increase of body burden is higher in slower growing oysters than that of faster growing oysters in weight scales (Fig. 6-2). The concentration change is more than the body burden change in weight scales (Fig. 6-3). To confirm the validity of this alternative model, the measurements of $\frac{dy}{dW}$ for different weight oysters were required but not available.

B. Depuration.

Depuration or loss of zinc was ignored in the first stage of the model development because previous studies indicated that oysters do not regulate zinc body concentration and there is no discernable depuration mechanism found in oyster. The kidney, which plays the major role of metal depuration in mussels (George *et al.* 1978; George 1980), is poorly developed in oysters (George & Frazier 1982), zinc deposit in shell formation is incidental (Windom & Smith 1972), and little zinc is transferred to gametes (*cf.* Greig *et al.* 1975). Almost all zinc in oyster is immobilized and stored in compartments within membrane-limited vesicles of hemolymph amoeboid blood cells (Coombs 1974; Wolfe 1974; George *et al.* 1978; Engel & Brouwer 1982). The biological half-life of zinc for oysters is hundreds of days (Wolfe 1970b; Seymour & Nelson 1972, 1973; George & Frazier 1982) and depuration or loss of zinc when oysters from high ambient concentration water to low concentration water is negligible

(Greig & Wenzloff 1978; Okazaki & Panietz 1981).

The time rate of change of metal body burden is the net uptake rate. When depuration or loss of the metal is negligible, then net uptake is essentially the instantaneous uptake rate. In many respects zinc uptake is simply a side effect of calcium uptake for shell formation (Wolfe 1970), and of feeding and respiration and, therefore, it should not be surprising to find that uptake rates, like metabolic rates, vary as a power function of weight. Because body burden represents the time-integration of instantaneous uptake, it is logical that total body burden also follows a similar functional relationship when the net uptake could be approximated as the instantaneous uptake, that is, depuration is negligible.

The statistical significance of the functional relationships between body burden and body weight and the good agreement of model runs with the power fits of the field data support the hypothesis that depuration was negligible.

If we assume that depuration is proportional to body burden, a simple and frequently used first order kinetic model (Spacie & Hamelink 1982), the depuration as in eq. 3-1 would be

$$- \frac{dy}{dt} = - k_{depuration}\, y \qquad \text{--- (6-1)}$$

Seymour & Nelson(1971) reported that the biological half-life of ^{65}Zn in oyster was 300 to 850 days. If one takes the mid-value of 575 days and solves eq. 6-1, $k_{depuration} = - 1.2 \times 10^{-3}$. Incorporating this depuration into the accumulation model made the output decrease exponentially (Fig. 6-4a) from the field observations. In other words, it made the model output deviate non-linearly from the linearized lines of the log-log transformed power fits of the body burden to the body weight and any adjustments of

the uptake parameters could not correct the non-linearity (Fig. 6-4b) In other words, if depuration follows eq. 6-1, then body burdens do not vary as a power function of weight and uptake follows eq. 3-1.

Because values of the uptake parameter **b-1** were calculated, not calibrated, from the short-term uptake experiments and agreed well with the measured values of power fits of the field data (Fig. 5-1), it is more likely that the model simulates, at least, the slope of the body burden and body weight relationship well. This simulation of depuration was, therefore, concluded to be inappropriate.

One of the possible explanations of this discrepancy is that the reported value of biological half-life could be too short because of the over estimation of initial soft tissue body burden. When oysters are exposed to ^{65}Zn, considerable amount of the tracer is adsorbed to shell surface (Fitzgerald & Skauen 1963). Seymour & Nelson (1972) coated oyster shells with paraffin to prevent the adsorption but even paraffin coating might not be completely effective.

On the other hand, it is likely that the formulation of the depuration term was not correct for oysters. If one assumes that the quoted biological half-life is in correct order for an average weight oyster, it is suspected that depuration follows second order kinetics (*cf.* Spacie & Hamelink 1982). Depuration is, though not in significant amounts, probably a function of body weight, just as uptake is. There were no data to formulate the depuration parameter into a function of body weight so alternate formulas were evaluated numerically. The alternate formulations for the depuration term were: (1) Assuming that the depuration follows the same power function of weights as uptake and also first order kinetics:

$$- \frac{dy}{dt} = - k_{depuration} W^{b-1} y \qquad \text{--- (6-2)}$$

This equation was rejected for the same reason as for eq. 6-1 (Fig. 6-5).

(2) Assuming the loss was due to the shedding of body surface and, thus, the depuration parameter is proportional to the body surface area, and depuration follows the first order kinetics of concentration:

$$- \frac{dy}{dt} = - k_{depuration} W^{0.67} \frac{y}{W} \qquad \text{--- (6-3)}$$

Using the same biological half-life of 575 days for oysters of all sizes and assuming the growth term $\frac{dW}{dt}$ is a constant 0.2/365 g/day,

$$- \frac{dy}{dt} = - 1.9 \times 10^{-3} W^{-0.33} y$$

When this depuration was incorporated into the accumulation model, the model output was at least close to linear in the log-log scale but the slope was still significantly different from that of the power fit (Fig. 6-5).

Two othe alternative depuration equations that yielded similar slopes to the power fits were: (3) Assuming that depuration is proportional to uptake, the depuration term

$- \frac{dy}{dt} = - k_{loss} \times W^{b-1}$. When the depuration term is added to eq. 3-5,

$$\frac{dy}{dt} = k_{uptake} W^{b-1} - k_{loss} W^{b-1} = (k_{uptake} - k_{loss}) W^{b-1}$$

That is, a constant proportion of zinc taken up was lost immediately after uptake and the uptake parameter **k** actually represented the net uptake. In this case, the loss term does not make any difference to the model and is of no concern because the parameter **k** was calibrated by field data. The other formula was, (4) assuming the loss is the first order elimination of tissue concentrations (Fig. 6-5),

$$- \frac{dy}{dt} = - k_{depuration} \frac{y}{W} = - k_{depuration} \times C$$

where C is the metal concentration of the oysters. This $k_{depuration}$ could not be calculated analytically because there were no depuration data with body weights. Calibrating the model including this depuration term would have been meaningless because the model was calibrated already for the best fit without the term, *i.e.* $k_{depuration}$ of zero.

C. Zinc Uptake from Particulates.

Any metal uptake from particulate materials may be assumed to be proportional to particle retention, since uptake occurs through food digestion (*cf.* Pentreath 1973). Fagerstrom *et al.* (1974) expressed dietary uptake as:

$$\frac{dy}{dt} = k_1 W^{b-1} + k_2 \frac{dW}{dt}$$

where k'_1 and k'_2 are the coefficients for uptake through food utilized for metabolism and food utilized for growth, respectively. When these terms are added to eq. 3-5, then eq. 3-6 becomes

$$y = \int_0^t [(k + k'_1) W^{b-1} + k'_2 \frac{dW}{dt}] dt = a\ W^b + k'_2\ W \qquad \text{--- (6-4)}$$

The uptake parameter **k** in the model, therefore, represents the combined uptake from water column and uptake through food utilized for metabolism. To evaluate the third parameter k'_2, the estimates of in-situ body burdens of oysters were subtracted from the ovserved values (Fig. 6-6a,b). The residual values of the power fit, which would be k'_2 W, did not show any significant trends for all sites indicating that this component was of minor importance, *i.e.* k'_2 was close to 0, at least, when the power relationship between body burden and body weight is assumed to hold.

D. Comparison of Oyster Metabolism of Zinc with That of Other Metals.

Even though many metals other than zinc are accumulated by oysters, the behavior of each metal is different. Different metals play different metabolic roles in an organism, therefore, each metal may have a different uptake mechanism. Copper is the metal to which the accumulation model proposed in this study most likely can be applied. Both copper and zinc are present at elevated concentrations in oysters but the level of copper is always much lower than that of zinc. Both *Crassostrea virginica* and *Crassostrea gigas* have 10-20 times more zinc than copper (Pringle *et al.* 1968). The ratio, however, may be different for different sites (*cf.* Huggett *et al.* 1973). In addition to the large body burdens, both metals have many similarities in oyster metal metabolism: Both are essential trace metals, are used in a catalytic role, are reactive with the functional groups of amino acids (particularly -SH groups, which could cause a problem if the cellular concentrations of the metals are too high) (George 1982), are readily exchangeable but have long half lives in the oysters, are complexed and free ions are not present in any significant amount in serum (Coombs 1974), and are not bound to one specific compound nor does there appear to be present any polypeptide such as metallothionein (*ibid.*). Both copper and zinc are present in the tertiary lysosomes and are accumulated in membrane-limited vesicles of blood amoebocytes (amoeboid lymphocytes) (>90% of the body copper and zinc) in both the hemolymph and tissues and they are detoxified by compartmentation within the membrane-limited vesicles in these cells (George *et al.* 1978). Detoxication mechanisms of both zinc and copper do not involve the elaboration of calcarious concretions. The very high copper and zinc levels in oysters are almost entirely attributed to the presence of the

metals in basophil blood cells (Ruddell & Rains 1975; George *et al.* 1978) and any difference of the concentrations of copper and zinc in tissues is due to fact that the metal-containing amoebocytes are not uniformly distributed throughout the body.

However similar they are physiologically, one should be careful applying the model to other metals and should re-evaluate the assumptions and re-calculate the kinetic terms. Even zinc and copper metabolisms of oysters have differences. The two metals play differing roles in the oyster and subcellular distribution is slightly different. For example, 95% of zinc is dialyzable but only 43% of copper is (Coombs 1972) and the toxicity of copper to embryos of *C. virginica* is much higher than that of zinc (MacInnes & Calabrese 1977). Both metals are accumulated in membrane-limited vesicles of blood amoebocytes but copper is concentrated in the granules, whereas zinc is partitioned in cytoplasm and granules. In *Ostrea edulis,* the metal containing cells have the same morphology in all tissues but are of two types and the two metals are localized within different cell types: Copper is in the membrane-limited vesicles of about 0.8 μm diameter of the granular acidophils, which are associated with sulphur, and zinc is in the membrane-limited vesicles of about 1 μm diameter of the granular basophils, which are associated with phosphorus (Ruddell 1971).

Evidence from comparative studies of different metals indicates that different metals have not only different intra-organismic metabolisms but also different uptake mechanisms. Ayling (1974) reported that, in *C. gigas,* the maximum copper and chromium absorbed were limited by the weight of the oyster (200-1700 mg) and were independent of the amount of metal in the mud (3-224 ppm). Therefore copper and

chromium do not present much of a health hazard to man through accumulation by oysters. Lead was randomly incorporated in oysters grown in high concentration of sediment. Zinc and cadmium were accumulated (4.2-134 ppm Cd, 1700-14000 ppm Zn) by a process that depended primarily on the concentrations of these metals in the mud at each site (0.4-5.7 ppm Cd, 20-500 ppm Zn). Therefore, lead, zinc and cadmium could be a serious health hazard for anyone consuming oysters grown in a contaminated area (*ibid.*).

Pollutant elements such as Cd, Hg, and Pb do not have a functional biochemical role in organisms and the organisms are generally exposed to high concentrations of those elements only through anthropogenic activities since the elements have very low natural abundance. These elements exert their action through the chemical similarity to functional essential elements *e.g.*, cadmium with copper and zinc (George 1982), *i.e.* they compete with the essential elements for binding sites on ligands in active centers of enzymes. For example, mercury blocks the phosphorylation site of Na^+ K^+ ATPase by binding to its essential -SH group (Skou & Norby 1979). In a 20-week-uptake experiments, no mortality were observed for as much as 11.42 g/g-dry weight of soft tissue lead (Zaroogian 1979), though. Dry weight of the oyster had no significant relation with soft tissue lead concentration (*ibid.*) suggesting Boyden (1977)'s case (i) (see Fig. 4-11,12).

In oysters, cadmium could overwhelm the capacity of the metallothionein-like protein detoxication system and cadmium ions "spill-over" into high and low molecular weight fractions of cellular proteins and cause tissue damage (Engel & Fowler 1979) or mortalities when high dissolved cadmium concentration occurs (Engel

1983). Cadmium is a highly toxic, non-essential metal that exhibits significant interactions with the essential metals copper and zinc (George & Frazier 1982). Zinc and cadmium are known to partition in a similar fashion between the soluble and particulate fractions of receiving waters (Preston *et al.* 1972; Abdullah & Royle 1974), although cadmium may favor the soluble fraction more than zinc (Phillips 1977b). While 99% of zinc in oyster is in the sedimented material of homogenated cell, 75% of cadmium is in there. While no zinc in oysters is bound to low molecular weight protein, 23% of cadmium is in low molecular soluble proteins. Cadmium is not found in the blood cells of oysters. Cadmium in both mussels and oysters does not appears to be immobilized in membrane-limited vesicles (George *et al.* 1976) and accumulates in the digestive gland and kidney rather than gills and mantle (George & Coombs 1977). Body burdens of cadmium vary, similar to zinc in this study, a power function of body weights with a **b** value of 0.57 (Zaroogian 1980), which is Boyden (1977)'s case (ii) (see Fig. 4-11,12).

When tissue metal concentration is metabolically regulated and there is significant involvement of other metabolites such as metallothionein in the process of metal uptake, storage, or depuration, then a concentration sensitive kinetic term such as Michaelis-Menten equation should be employed for the pathway (*cf.* Spacie & Hamelink 1982).

In this model, negligible depuration was assumed and it worked reasonably well. The assumption would not be correct for many other metals, especially the pollutant elements such as Cd, Hg, and Pb mentioned above, which do not have a functional biochemical role in organisms. It is not likely that there are storage or

detoxication by compartmentalizing but more active depuration mechanisms could exist. For example, unlike zinc, depuration of mercury is facilitated by spawning. Significant gonadal mercury accumulation occurs in *C. virginica* (Cunningham & Tripp 1973).

VII. CONCLUSIONS

It is concluded that (1) uptake varies as a power function of body weight, (2) uptake increases linearly as the ambient zinc concentration increases for all weight classes, (3) uptake varies with salinity, which means that the weight effect is not constant but can vary from site to site, and (4) depuration is negligible. It also concluded that (5) body burden is the time-integration of uptake, (6) in any given period and for any weight oyster, the increase in the body burden is larger than the dilution effect of the tissue growth and, thus, weight-specific concentration of zinc continues to increase but (7) the rate of the increase is reduced as the oyster grows larger.

Pooled mean concentrations that are determined by combining many individual oysters are used in pollution monitoring studies. A difference in the mean metal concentrations of the two populations may only reflect a difference in the mean body weight of the populations. Clearly, body weight effects must be considered when monitoring programs are designed and when research is undertaken.

Chesapeake Bay area surveys of *Crassostrea virginica* trace metals (Eisenberg & Topping 1984; Phelps & Hetzel 1987) have reported high concentrations. It is recommended that tissue metal concentrations in *C. virginica* from the Chesapeake Bay system be monitored because the bay has large anthropogenic sources of trace

metals (Helz 1976; Helz *et al.* 1981; Kingston *et al.* 1982). With quantified information on the effects of salinity and body weight on metal concentrations, oysters would be a suitable organism for monitoring.

APPENDIX A.

Extraneous Variables in Metal Soft Tissue Measurements.

1. Overview.

Bivalve mollusks in general and the American oyster, *Crassostrea virginica* Gmelin, in particular concentrate metals. Many interesting physiological questions remain to be answered regarding the mechanisms and consequences of metal uptake. Similarly environmental managers need to know much more about the way that environmental factors affect metal uptake. One difficulty in answering these questions is that in-situ metal concentrations in oysters vary greatly even when collected from a single site during one sampling (Boyden & Phillips 1981; Wright *et al.* 1985). The purpose of the present study is to examine and quantify several sources of what might be called "extraneous variability" so that underlying physiological relationships and cause-and-effect mechanisms can be elucidated. Two such sources of extraneous variability are (1) inclusion of gut contents and other particulate material and (2) inclusion of pea crabs, the *Pinnotheres* species.

For the present study, dry weights were used exclusively, oysters were examined individually, and zinc associated with gut contents and other sedimentary materials and with pea crabs measured. This study was conducted concurrently with the body burden measurement studies of previous chapter. Details of the "materials and methods" were described in Chapter IV. and in the VIMS Data Report No.30 (Mo & Neilson 1989).

2. Materials and Methods.

During the preparation oysters for analyses, when it was judged that an oyster contained enough particulate material to be of concern, the thinned end of a pipette was inserted into the anal opening of the oyster and gut contents were removed by flushing with deionized water. Gut contents and any sedimentary material (mostly fecal pellets and pseudofeces) inside the cavity of the oyster shells were collected in a vial. Oysters were examined to find any female pea crabs and, when one was found, the pea crab was put into a separate vial. After dry weights were determined, samples were digested with 15 ml of concentrated HNO_3 and the zinc concentrations were measured by flame atomic absorption spectrophotometry.

3. Results and discussions.

The percentage of oysters infested with pea crabs was highly variable, ranging from 0% to 56% for some sites and dates (Table A-1). Zinc concentrations of pea crabs were lower than those of host oysters by nearly an order of magnitude (Table A-2). Zinc concentration of a pea crab was not correlated the zinc concentration of its host oyster (Fig. A-1). The zinc body burden of a pea crab appears to depend primarily on the size of the crab (Fig. A-2).

The contribution of the dried weights of the pea crabs was relatively large and accounted for 2 to 19% of total weight (Table A-2). On the average, the crabs accounted for about 9% of the weight but only 1% of the zinc for oyster and pea crab together. If the pea crab were included in the determination of the oyster soft tissue zinc concentration, therefore, the result would be lower (up to 16%) than that for the

oyster alone.

The amount of gut contents and other sedimentary materials inside of the shell cavity of an oyster also was highly variable. For individual oysters, the particulates inside shell cavities accounted for up to 14% of the dry weight and up to 24% of the zinc (Table A-3) for the eight oysters examined. The effect of including particulates was more pronounced with smaller oysters.

Pea Crabs: In some areas of Chesapeake Bay, oysters are heavily infested with the commensal pea crab, *Pinnotheres ostreum* Say (Galtsoff 1964). The crab exoskeleton has a water content much lower than that of oyster soft tissue. Zinc concentrations in pea crabs were always lower, usually by about factor of ten, than those in the host oyster.

Because the crab size is limited by the size of the cavity between the oyster shells, there is an obvious correlation between oyster size and crab size. Our data suggest that the zinc body burden of pea crab increases more or less linearly with the crab's dry weight (Fig. A-2a) while the body burden of the host oyster increases as a power function of the oyster soft tissue dry weight (Fig. 4-5,6,7) (*cf.* Boyden 1977). The effect of including pea crab in the oyster zinc concentration determinations will change with the oyster size.

Particulates: It is obvious by common observation that the gut of many oysters contains sediment, which material is likely to have metals associated with it. Fecal pellets and pseudofeces that had not been cleared out of the shell at the time of

shucking may be included in oyster soft tissue samples. One also may rupture "mud blisters" made by the *Polydora* species while shucking and let the contents contaminate the tissue sample if not careful. The amount of gut contents and other sedimentary materials inside an oyster, therefore, may vary widely among laboratory procedures and other factors. Our study demonstrates that inclusion of sedimentary materials may alter the results sometimes significantly. If the intent of a study is to measure the amount of metal incorporated in the body tissue of the oyster (as opposed to that temporarily residing in the gut) then care should be taken to exclude these sedimentary materials - whether gut contents, feces, pseudofeces, or "mud blisters" - from samples especially when dealing with small oysters. Some researchers, apparently aware of this problem, hold organisms in filtered sea water for a while (*e.g.*, Boyden 1977) to reduce the particulates in the organisms.

Wet versus dry weight: Wet weight is intrinsically more variable than dry weight because the water content of oyster soft tissues changes with the condition of the organism (Galtsoff 1964; Thomson 1983). Wet weight also varies with the laboratory method used, generally decreasing with an increase in time between shucking and weighing (Kramer *et al.* 1962). Kramer *et al.* (1962), in a study designed to determine the relationship between wet and dry weights, clearly showed that the relationship varied too widely to be quantified consistently. The average wet weight of the drained meats of the various lots varied between 38.1% and 65.4% (mean of 48.2%) of the total weight (wet weight of the meats and all liquids) whereas the dry weight varied less, between 7.7% and 12.8% (mean of 10.2%) of the wet

weight. The use of dry body weight will improve the quality of data in metal uptake studies for comparisons among different laboratory methods and investigators by reducing the variability.

Recommendations: In future studies of metal bio-concentration by oysters and similar studies, it is recommended that (1) pea crabs not be included in the weight or body burden measurements whether for single or pooled oyster samples, (2) gut contents and feces be eliminated when determining body burdens, and (3) that dry weight be used as a measure of body size, instead of, or in addition to, wet weight. All of these introduce unwanted and unnecessary errors and all can be eliminated easily.

APPENDIX B.

Comparison of Metal Accumulation by Oysters and Mussels.

Both oysters and mussels have been extensively studied for their metal bioconcentration. Some differences are expected, of course, even just for the difference in particle selection and retention efficiency (Kiorboe & Mohlenberg 1981) but the difference in metal physiology of the two organisms is also contrasting.

Because of its worldwide ubiquitous distribution, the genus Mytilus has been used extensively for biomonitoring of pollution of the marine environment (*e.g.*, Phillips 1976a 1976b, 1977b; Davies & Pirie 1978) and much more often than any other organism. Many studies have been carried out both on the uptake of ^{65}Zn by Mytilus (*e.g.*, Chipman *et al.* 1958; Brooks & Rumsby 1965; Pentreath 1973) and its loss from contaminated animals (*e.g.*, Seymour & Nelson 1973; Young & Folson 1967). As indicators of radioactive contamination by the radionuclides of such elements, mussels have been particularly used in the marine environment, for the comparison of radioactive metal distribution on a large scale (Pentreath 1973). Other studies have compared water and animal zinc concentrations at different collection sites (*e.g.*, Goldberg 1975; Phillips 1977a) or under different temperature or salinity regimes (Phillips 1976a).

Lack of the metal regulation by the organism is assumed for an indicator organism (Phillips & Yim 1981). *Mytilus edulis* is considered to be an accurate indicator of pollution by zinc and cadmium over the entire range of salinities in which

the organism can exist (Phillips 1977a). There are evidences that copper is, however, at least partially regulated (Phillips 1976a, 1976b, 1980; Davenport 1977; Davenport and Manley 1978). *M. edulis* shows highly erratic net uptake of copper in different salinity-temperature regimes atypical of the uptake of most metals by this organism, *i.e.* the physiology of copper in mussel is different from that of other metals (Phillips 1976a). The net uptake was affected by salinity and temperature changes and by the presence of the other metals (Cd, Zn, and Pb) and changes in their relative concentrations. *M. edulis* is, therefore, not reliable as an indicator of copper pollution. The kinetics of both uptake and excretion were unlike those for zinc and other metals (Phillips 1976a, 1976b). There is specific gill transport process (atypical uptake kinetics) for copper, perhaps developed because of the important physiological role of copper (a component of blood proteins) in *M. edulis* (Davies & Pirie 1980). Phillips (1976b) recommended that *M. edulis* should not be used as an indicator of copper pollution because the differences in concentrations of other metals coexisting with copper influence the net uptake of copper.

In both *M. edulis* and *Crassostrea virginica*, Cd-binding low molecular weight proteins have been isolated and identified. While the role of these proteins in oysters has not been fully demonstrated yet, it is suggested that the proteins may be active in the transport of elements, such as copper, zinc, and calcium in *M. edulis* (Engel 1983). In *M. edulis*, these metallothionein-like proteins contained some zinc and copper (George *et al.* 1982) and cadmium is primarily bound to metallothionein-like protein induced by uptake of cadmium (Noel-Lambot 1976). In the oyster it has not been shown in the presence of cadmium. These metallothionein-like proteins in *M. edulis*

are induced in the cytoplasmic solutions of the cells as a regulatory mechanism to avoid intoxication, *i.e.* a protective function against the cytotoxic effects of trace metals but the role of the metallothionein-like proteins in oysters are not proved yet.

The most striking difference between the two organisms is the metal depuration mechanism. *C. virginica* does not have any notable depuration mechanism other than immobilization of metals in hemolymph cells. Mussels, in contrast, have very effective metal regulatory mechanisms. *M. edulis*, which does not accumulate copper and zinc to the same extent as oysters, does not appear to contain very-low-molecular-weight Cu- or Cd-complexes. Mussels do not have the metal containing blood cells. Concentric concretions and other granules rich in heavy elements have been found in the kidneys of mussels (George & Pirie 1980; George *et al.* 1982). These membrane-bound vesicles are associated with and considered to be formed by lysosomes (George *et al.* 1978). The columnar epithelial cell of the kidney of *M.* contains a large number of those membrane-limited vesicles (as much as 20 % of the cell volume) (Pirie & George 1979). The presence of zinc and other elements in the vesicles (probably lysosomes), granules, and concretions in mussel kidneys emphasizes the role of the kidneys in zinc accumulation (Roesijadi *et al.* 1984). These granules and those of the podocytes are subsequently shed into the lumen of the kidney tubule and are excreted in the urine, which is therefore largely particulate and this could be a major way of depuration of toxic metals (George *et al.* 1978; George *et al.* 1980; George & Pirie 1980).

Mussels have other possible depuration mechanisms that oysters do not. Even during spawning, many metal body burdens of oysters do not change. For example,

chromium concentration in oysters, *C. virginica*, continues to increase during spawning, whereas it decreases in mussels, *M. edulis* (Zaroogian & Johnson 1983), suggesting the elimination of the metal through gametes. In oysters, the distribution of metals is uniform throughout its soft tissue while in mussel, metals are concentrated in some specific organs. The major portion of iron, for example, is transported by amoebocytes in the hemolymph and they are deposited in the byssal threads (George *et al.* 1976).

As in oysters, zinc and cadmium concentrations in mussels from low salinity areas are higher than in those from high-salinity areas. The effect of salinity on the net uptake of metals varies according to the metal concerned and the particular salinity regime to which the mussels are exposed (Phillips 1976a, 1977a, 1977b). The increase in net uptake of cadmium at low salinity, may be related to ion availability, or to a need for metabolic energy in the transport of calcium and cadmium. The decreased uptake of lead at low salinity may again reflect linkage of ion flux across the mussel gill, or possibly it is related to a lower filtration rate at the low salinity (Phillips 1976a). The filtering rate of *M. edulis* decreased at low salinity (Bohle 1972) and George *et al.* (1978) tried to explain the phenomenon of lower metal concentration of metals in the organism that were at lower salinity area than that in higher salinity.

The rapid rate of turnover of metalliferous granules in mussels explains the very different metal contents of zinc in mussels and oysters (Rainbow *et al.* 1990). The relationship between uptake and body burden should be different for oysters and mussels. The zinc body burden of the hooked mussels, *Ischadium recurvum* Rafinesque, from both the upper oyster bed, Morattico Bar (salinity *ca.* 16 ‰) and

from lower oyster bed, Parrot Rock (salinity *ca.* 14 ‰) of the Rappahannock River was almost linear with the dry weight of the organisms, *i.e.* the value of **b** was close to 1 (Table B-1a, Fig. A-1) and the metal concentration per unit body weight was independent of body weight (Fig. B-2). This indicates that equilibration of metal concentrations metal occurs in the tissues of the organism (Bryan 1976; Strong & Luoma 1981). The results suggest, however, that the body weight and concentration relationship may vary with site. The power coefficient, **b** of weights for the mussels from the higher salinity was 0.91 and for the mussels from the lower salinity, 0.98. It is probable that the relationship between metal concentration and body weight could be different from site to site because the physiology of a mussel is related to many factors, especially salinity, by the power function of body weight (Widdows 1978a) as in oysters. The longitudinal salinity gradient of the bottom water of the Rappahannock River was, however, too small (*cf.* Kuo & Neilson 1987) to be conclusive even though the regressions of each data set and the difference between the two were statistically significant (Table B-1b). At any rate, the body burden and weight relationships of mussels should be reported with salinity because the relationship may change with salinity as it does in oysters.

Table 4-1. Oyster sampling sites and collection dates. Distance is from the river mouth in km. Salinity is approximate average mid-depth salinity of March to October.

SITE	LATIT.	LONGIT.	Distance (km)	Salinity (‰)	Date (m/d/yr)
James River oyster beds:					
Wreck Shoal	37°03.2' N	76°34.6' W	30	15	6/15/87*1
Wreck Shoal					10/7/87
Nansemond Ridge	36°55.5' N	76°27.2' W	12	20	10/6/87
Horsehead	37°06.3' N	76°37.9' W	38	13	10/7/87
Mulberry Island	37°05 ' N	76°36' W	35	14	1/19/88*1
Mulberry Island					2/21/88*2
Rappahannock River oyster beds:					
Broad Creek	37°34.3' N	76°18.6' W	2	18	10/9/87
Parrot Rock	37°36.4' N	76°25.2' W	20	16	10/9/87
Morattico Bar	37°46.5' N	76°39.3' W	60	14	10/2/87
Piankatank River oyster bed:					
Ginny Point	37°32.0' N	76°24.2' W	14	15	10/12/87

*1: Oysters for seasonal difference comparisons.
*2: Oysters for ^{65}Zn uptake measurements.

Table 4-2. Power fit ($\frac{dy}{dt} = kW^{b-1}$) of total ^{65}Zn uptake (body burden) after 108 hours on body weight. One outlier of Aquarium 1 (marked in Fig. 4-2,3) was eliminated in the analyses. 1&2 represents the data set obtained when the data for Aquarium 2 are combined with the data for Aquarium 1 normalized to the added ^{65}Zn tracer concentration (divided by 2).

Aquarium	No. of Oysters	Salinity (‰) target(actual)	^{65}Zn (µg/l)	Power fit k	b-1
No. 1	10	18 (17.4)	1.0	2.32	0.187
No. 2	11	18 (17.5)	0.5	1.13	0.167
No. 3	11	12 (12.7)	0.5	1.27	0.392
No.1&2	21	18 (17.45)		1.15	0.180

Table 4-3. Regression analyses of log-log transformed daily tracer uptake per unit dry weight on dry weights [log $(\frac{y}{W})$ = log k + (b-2)log W]. One outlier of Aquarium 1 (marked in Fig. 4-2,3) was eliminated in the analyses. 1&2 represents the data set obtained when the data for Aquarium 2 are combined with the data for Aquarium 1 normalized to the added ^{65}Zn tracer concentration (divided by 2).

Aquarium	No. of Oysters	Salinity (‰) target(actual)	Analysis of Covariance	Coef.Determ. R^2	Const. log a	Slope b-2	S.E. of slope	Regress. P.
No. 1	10	18 (17.4)	p=0.012	0.660	-0.288	-0.813	0.206	0.004
No. 2	11	18 (17.5)		0.770	-0.601	-0.833	0.152	0.000
No. 3	11	12 (12.7)		0.402	-0.551	-0.608	0.247	0.036
No.1&2	21	18 (17.45)	p=0.996	0.699				

Table 4-4. Summary statistics of the oyster in-situ zinc contents data. Soft tissue dry weight, zinc body burden, and soft tissue zinc concentration are presented as Mean±Standard Deviation (range:Min.-Max.). One datum for Horsehead and one for Nansemond Ridge with weight of less than 0.01 g from the Data Report No. 30 are not included.

James:	**Horsehead**	**Wreck Shoal**	**Nansemond Ridge**
No. of oysters	23	10	15
Dry weight	0.657±0.29 (0.223-1.223)	0.503±0.294 (0.187-1.200)	0.588±0.414 (0.026-1.281)
Body Burden	6426±3752 (1318-14169)	2920±2902 (719-9254)	2096±1835 (88-5962)
Concentration	9272±2629 (4979-16508)	5254±2737 (2169-9490)	3355±1501 (2007-7680)
Rappahannock:	**Morattico Bar**	**Parrot Rock**	**Broad Creek**
No. of oyster	9	17	19
Dry weight	1.199±0.418 (0.313-1.748)	0.365±0.445 (0.025-1.507)	0.380±0.398 (0.016-1.727)
Body Burden	3160±2074 (599-8050)	942±1344 (33-4770)	828±1459 (22-6421)
Concentration	2520±993 (1464-4750)	2097±754 (1205-3737)	1619±816 (753-4123)
Piankatank:		**Ginny Point**	
No. of oysters		19	
Dry weight		0.354±0.253 (0.064-1.017)	
Body Burden		915±926 (153-3259)	
Concentration		2335±1145 (904-4825)	
The mid-reaches of the James:	**Wreck Shoal-Spring**		**Mulberry Island-Winter**
No. of oysters	12		21
Dry weight	1.385±0.503 (0.830-2.591)		0.410±0.283 (0.016-0.941)
Body Burden	8209±4163 (2948-14404)		2771±2328 (81-7268)
Concentration	5838±2397 (3414-10687)		6206±2271 (1918-10454)

Table 4-5. Regression of oyster body burden on body weight. Data are fit to the power function of body burden (μg) on body weight (g) ($y = a W^b$) by the log-log transformation, log(y) = log(a) + b log(W). One datum for Horsehead and one for Nansemond Ridge with weight of less than 0.01 g from the Data Report No. 30 are not included.

RIVER Oyster bed:	No. of Oysters	Intercept log(a)	slope b	Coef.Determn. R^2	Geometric regression*2
James River:					
Horsehead	23	4.0200	1.3276	0.8395	1.4104
Wreck Shoal	10	3.7668	1.3015	0.6085	1.6265
Nansemond Ridge	15	3.5197	1.0616	0.9127	1.1097
Rappahannock River:					
Morattico Bar	9	3.3675	1.2016	0.7654	1.3732
Parrot Rock	17	3.4023	1.1596	0.9705	1.1774
Broad Creek	19	3.2801	1.1849	0.9280	1.2446
Piankatank River:					
Ginny Point	19	3.4084	1.1584	0.7825	1.2546

*1: Salinity in ‰ is average of mid-depth monthly means from March to October.
*2: Functional relationship. Geometric regression coefficient b' = b/R

Table 4-6. Zinc in oyster shell.

Soft Tissue			Shell			Ratio*	
DryWgt. (g)	Conc. (ppm)	Total Zn (µg)	Dry Wgt. (g)	Conc. (ppm)	Total Zn (µg)	Conc.	Total
0.0316	5499	173.7834	0.4748	57	27.2451	0.0104	0.1568
0.0086	3810	32.7753	0.6009	13	7.4787	0.0034	0.2281
0.0898	934	83.8954	-	10	-	0.0107	-
0.0162	1332	21.5731	-	7	-	0.0053	-

*: Concentration (or total zinc) in oyster soft tissue divided by concentration (or total zinc) in oyster shell.
-: Missing values.

Table 5-1. The power function fits of body burden on body weight ($y = a W^b$) and regression analyses of log-log transformed data ($\log y = \log a + b \log Wgt.$) for oysters. Salinity is the average of mid-depth monthly means from March to October. 5 yr. oyster weight is the nominal soft tissue dry weight of five-year-old oysters. One datum for Horsehead and one for Nansemond Ridge with weight of less than 0.01 g from the Data Report No. 30 are not included.

River Oyster bed:	Number of Oysters	Salinity (‰)	Power fit Total $Zn=a(Wgt)^b$ a	b	Coef. of Detrm. R^2	Standard Error of b	Regress. P	5-yr. dry wgt. (g)
James River beds:								
Horsehead	23	13	10471	1.33	0.8395	0.1255	0.000	0.9
Wreck Shoal	10	15	5845	1.30	0.6086	0.2519	0.000	1.0
Nansemond Ridge	15	20	3309	1.06	0.9127	0.1637	0.000	1.1
Rappahannock River beds:								
Morattico Bar	9	14	2331	1.20	0.7654	0.1560	0.002	
Parrot Rock	17	16	2525	1.16	0.9705	0.1207	0.000	
Broad Creek	19	18	1906	1.18	0.9284	0.1681	0.000	
pooled samples of all Rappahannock:	45	18	2209	1.16	0.9467	0.1595	0.000	1.2

Table 5-2. Calibration of model parameters and input parameters for the test runs.

	Horsehead	Nansemond	WreckShoal	Rapp.-all
Input parameters				
salinity (from Brooks)	13	20	15	18
C_{total} (from various)	5	5	5	2.5
Fraction (from REDQEL.EPA)	0.430	0.342	0.403	0.364
G. (observation-Fig. 5-2)	0.2	0.2	0.2	0.24
b-1 (from ^{65}uptake-Fig. 5-1)	0.37	0.07	0.29	0.16
Calibration parameter				
a' (from power fit-Fig. 5-5)	4.87	1.93		
Verification				
a' (from eq. 5-4, Fig. 5-6)			4.03	2.77

Table 5-3. Comparisons of parameters of the model and coefficients of observed uptakes and body burdens.

	James-WS	Rapp.-all
Power fit **b**	1.30	1.16
Model **b**	1.29	1.16
Power fit **a'**	2.9	2.43
Model **a'**	4.03	2.77
Power fit **k**	3.96	1.37
Model **k**	5.74	1.92

Table A-1. Presence of pea crabs in oysters from Virginia estuaries.

River: Oyster bed	Salinity (ppt)*1	Sampling Date	No. of Oysters	No. with Crabs*2	%
James River:					
Nansemond Ridge	20	10/6/87	16	9	56
Wreck Shoal	15	6/15/87	14	3	21
Wreck Shoal	15	10/7/87	10	5	50
Mulberry Island	14	1/19/88	27	1	4
Horsehead	10	10/7/87	27	0	0
Rappahannock River:					
Broad Creek	18	10/9/87	19	2	11
Parrot Rock	15	10/9/87	19	0	0
Morattico Bar	12	10/2/87	10	0	0
Piankatank River:					
Ginny Point	15	10/12/87	26	0	0

*1: Salinity given in as approximate annual average.
*2: Female pea crab, *Pinnotheres ostreum.*

Table A-2. Potential contribution of pea crabs to dry weight and zinc concentration of individual oysters*. The weight of a pea crab is the dry weight of the whole crab and the weight of an oyster is the soft tissue dry weight of the oyster.

Pea Crab			Host Oyster			Contribution	
Wgt. (mg)	Zinc (mg)	Conc. (ppm)	Wgt. (mg)	Zinc (mg)	Conc. (ppm)	Wgt. (%)	Zinc (%)
James River - Nansemond Ridge - Fall 1987							
24.8	0.015	609	612.1	1.228	2007	3.9	1.2
53.6	0.025	470	1125.4	3.763	3344	4.6	0.7
77.5	0.052	676	1041.1	4.075	3914	6.9	1.3
101.6	0.024	241	494.0	1.199	2423	17.0	2.0
125.2	0.050	396	1280.7	3.835	2995	8.9	1.3
139.7	0.048	347	599.0	2.157	3602	18.9	2.2
141.8	0.074	521	758.0	4.225	5574	15.8	1.7
					average :	10.9	1.5
James River - Wreck Shoal - Fall 1987							
13.0	0.023	1733	556.2	1.990	3577	2.3	1.1
31.1	0.028	890	323.5	2.037	6298	8.8	1.4
39.8	0.011	265	186.6	0.733	3931	17.6	1.5
43.3	0.038	886	771.4	7.093	9196	5.3	0.5
					average :	8.5	1.0
James River - Wreck Shoal - Spring 1987							
85.5	0.015	177	1194.0	12.761	10687	6.7	0.1
96.3	0.024	251	1106.2	8.984	8121	8.0	0.3
146.3	0.033	228	1946.3	13.685	7031	7.0	0.2
					average :	7.2	0.2
Rappahannock River - Broad Creek - Fall 1987							
29.9	0.011	361	932.8	2.900	3109	3.1	0.4
46.1	0.007	156	649.6	0.791	1218	6.6	0.9
					average :	4.9	0.7
Arithmetic mean of all samples							
75.0	0.030	400	848.6	4.466	5263	8.1	1.1
					Mean of averages :	9.0	1.1
					Standard dev. :	5.25	0.62

*: The number of samples reported is less than the number indicated in Table A-1 because samples with missing values are not presented.

Table A-3. Contribution of particulates (gut contents, feces, and other sedimentary materials) to dry weight and zinc body burden of individual oysters*.

Particulates (mg)		Oyster (mg)		Contribution (% of total)	
Dry Wgt.	Zinc	Dry Wgt.	Zinc	Dry Wgt.	Zinc
21.7	0.048	129.3	0.156	14.4	23.5
12.7	0.038	173.6	0.288	6.8	11.7
28.5	0.070	235.4	0.753	10.8	8.5
34.5	0.065	381.2	3.985	8.3	1.6
74.3	0.138	648.6	2.996	10.3	4.4
60.3	0.275	758.0	4.225	7.4	6.1
27.9	0.035	863.6	2.948	3.1	1.2
		average :		8.0	7.6

Table B-1. Sampling sites and (a) the power function fits of zinc body burden (μg) on body weight (g), y= a W^b, for mussels from Rappahannock River oyster beds and (b) results of regression analysis after removing the smallest and the largest mussels for the Parrot Rock oyster bed data to make the weight ranges of two sites similar. Salinity is the average of mid-depth monthly means from March to October.

a)

Site Oyster bed:	Number of Mussels	Salinity* (‰)	Power fit a	b	Body Burden (μg Zn) mean±s.e.	Concentr. (μg/g) mean±s.e.	Dry Wgt. (mg) mean(min.-max.)
Morattico Bar	12	12	120	0.98	3.735±0.482	70.250±3.471	53.4 (25.7-95.2)
Parrot Rock	15	15	150	0.90	4.251±0.883	61.067±3.071	76.71 (3.8-285.4)

b)

Site Oyster bed:	Number of Mussels	Weight (mg)	Regression of Concentrations log a	b-1	S.E. of b	R^2	regress. P.	Analysis of Covariance P.
Morattico Bar	12	53.4 (25.7-95.2)	1.812	-0.02	0.127	0.0030	0.886	0.05
Parrot Rock	13	65.8 (13.8-116.5)	1.562	-0.17	0.056	0.4671	0.010	

*: Salinity given as approximate annual averages.

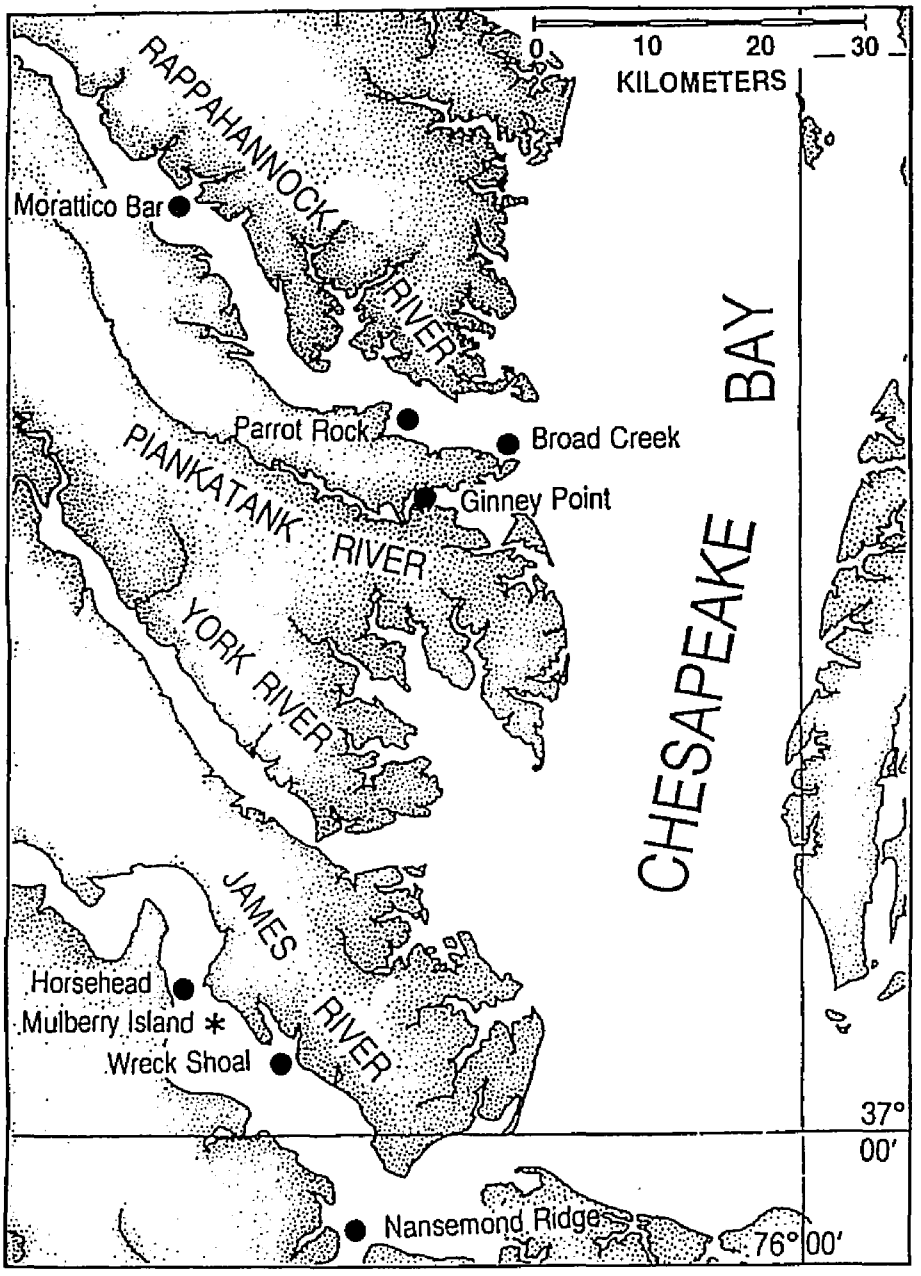

Figure 4-1. Map showing sampling sites for in-situ body burden measurements (●) and for ^{65}Zn uptake experiments (*). Mulberry Island denoted a general area of about 5 km length.

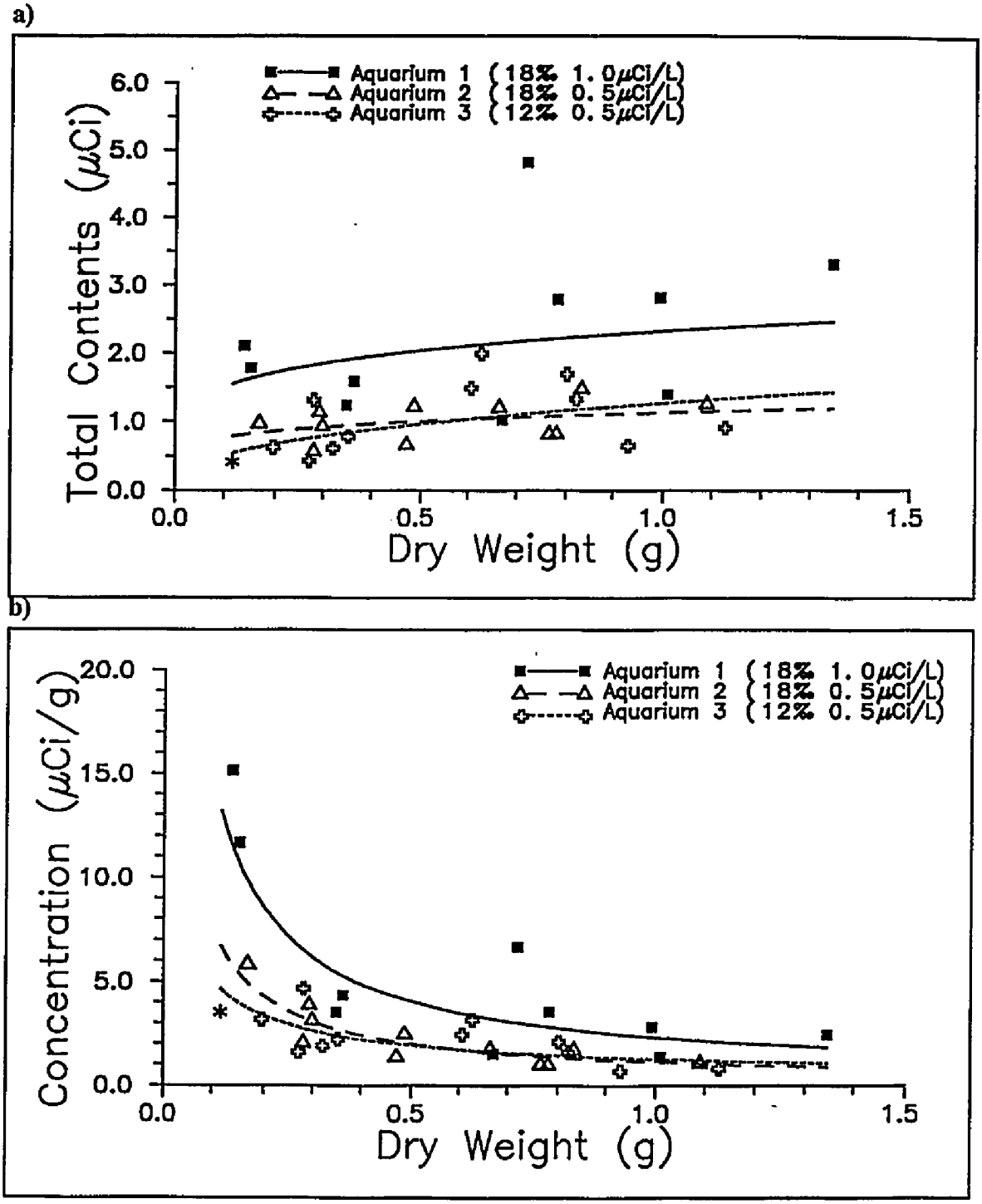

Figure 4-2. ^{65}Zn uptakes at the end of experiments (108 hours) in (a) body burden (total soft tissue contents, μCi) and (b) concentrations (per unit soft tissue dry weight, μCi/g) with oyster dry weights (g). The datum point for an oyster from Aquarium 1 that is considered to be an outlier is marked with an asterisk.

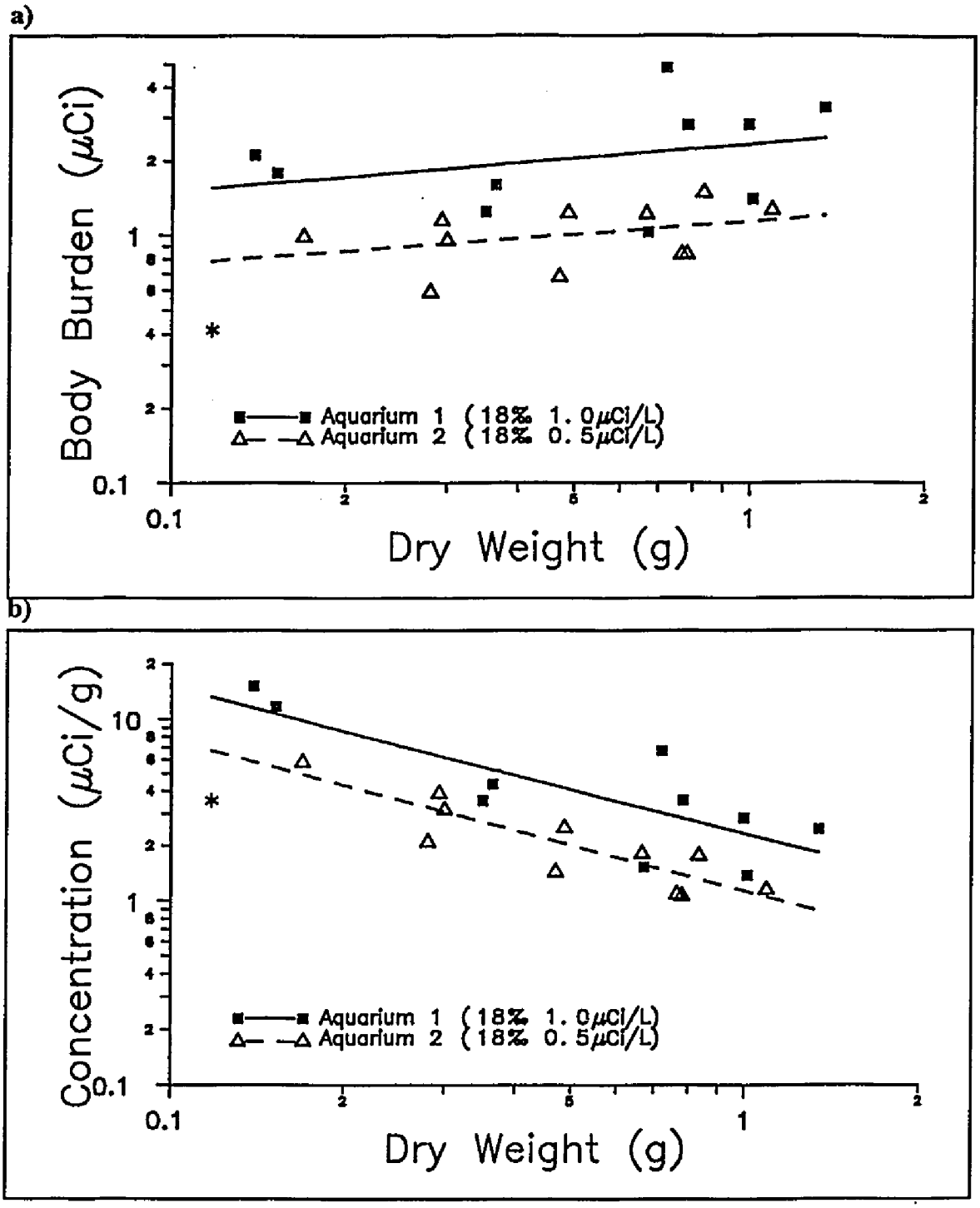

Figure 4-3. Comparison of ^{65}Zn uptakes at the end of experiments (108 hours) for Aquarium 1 and Aquarium 2 in (a) total contents (body burdens, μCi) and (b) (per unit dry weight) concentrations (μCi/g) with oyster dry weights (g). The datum point for an oyster from Aquarium 1 that is considered to be an outlier is marked with an asterisk.

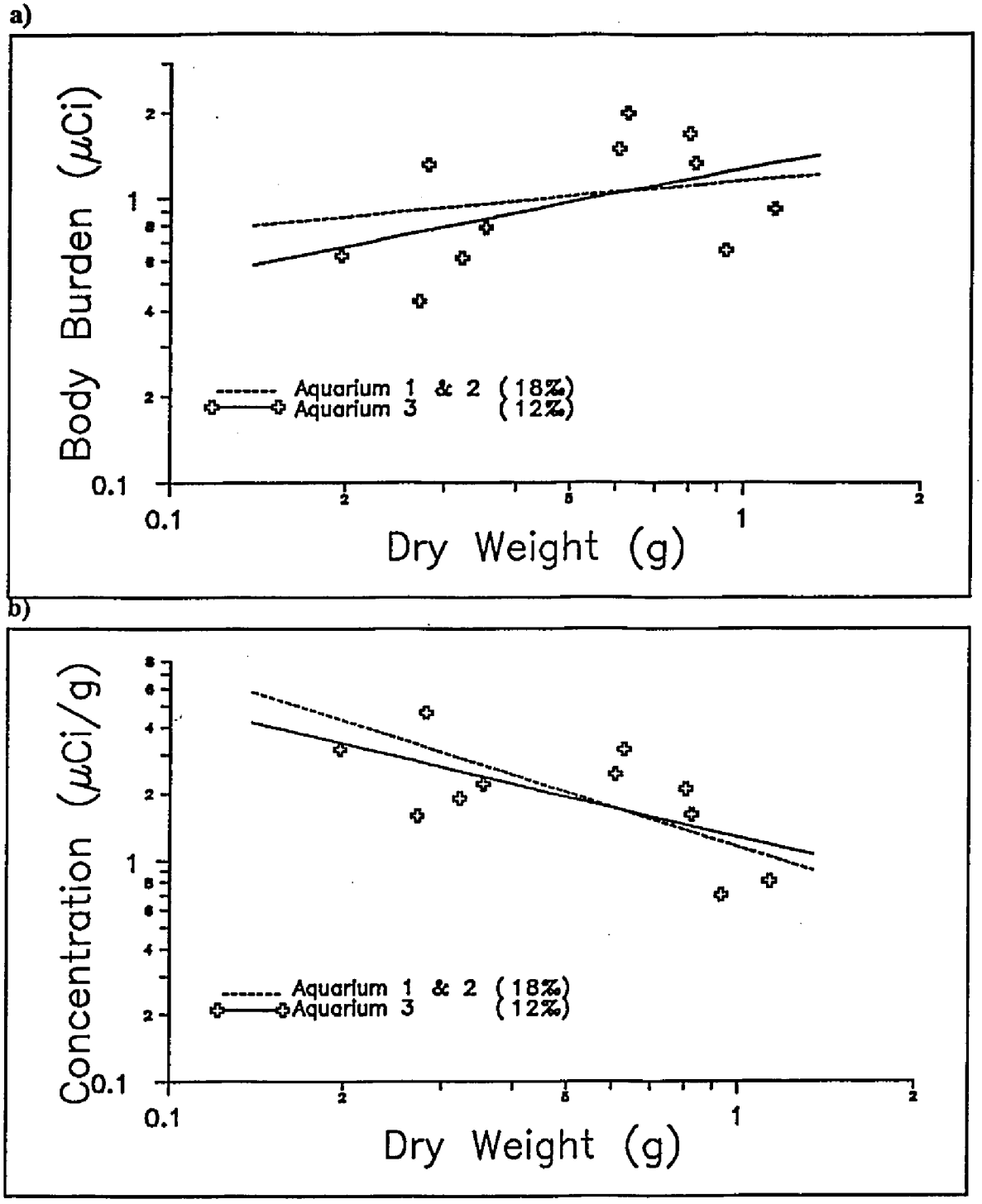

Figure 4-4. Comparison of ^{65}Zn uptakes in (a) body burdens and (b) concentrations at end of experiment (108 hours) with oyster body weights (g) for aquarium 3. Solid line is the power fit line for Aquarium 3 (salinity = 12 ‰) and dashed line is for the combined data sets for Aquaria 1 and 2 (salinity = 18 ‰) after the tracer uptake for Aquarium 2 were normalized (divided by 2).

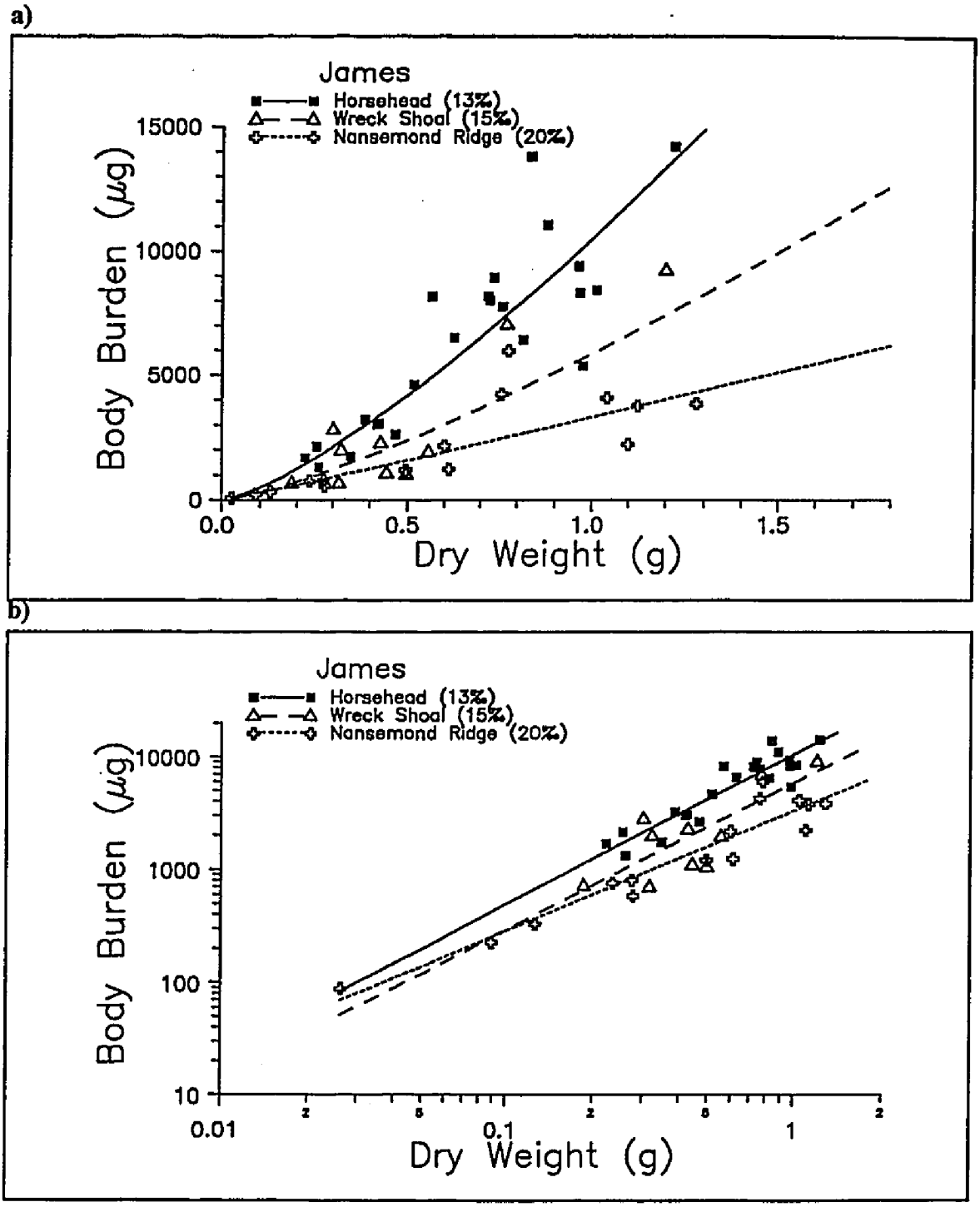

Figure 4-5. Zinc body burdens of oysters from James River oyster beds in (a) normal scales and (b) log-log scales. The lines are fit to the power function, $Y = a X^b$ by the least squares fits for the log-log transformed data.

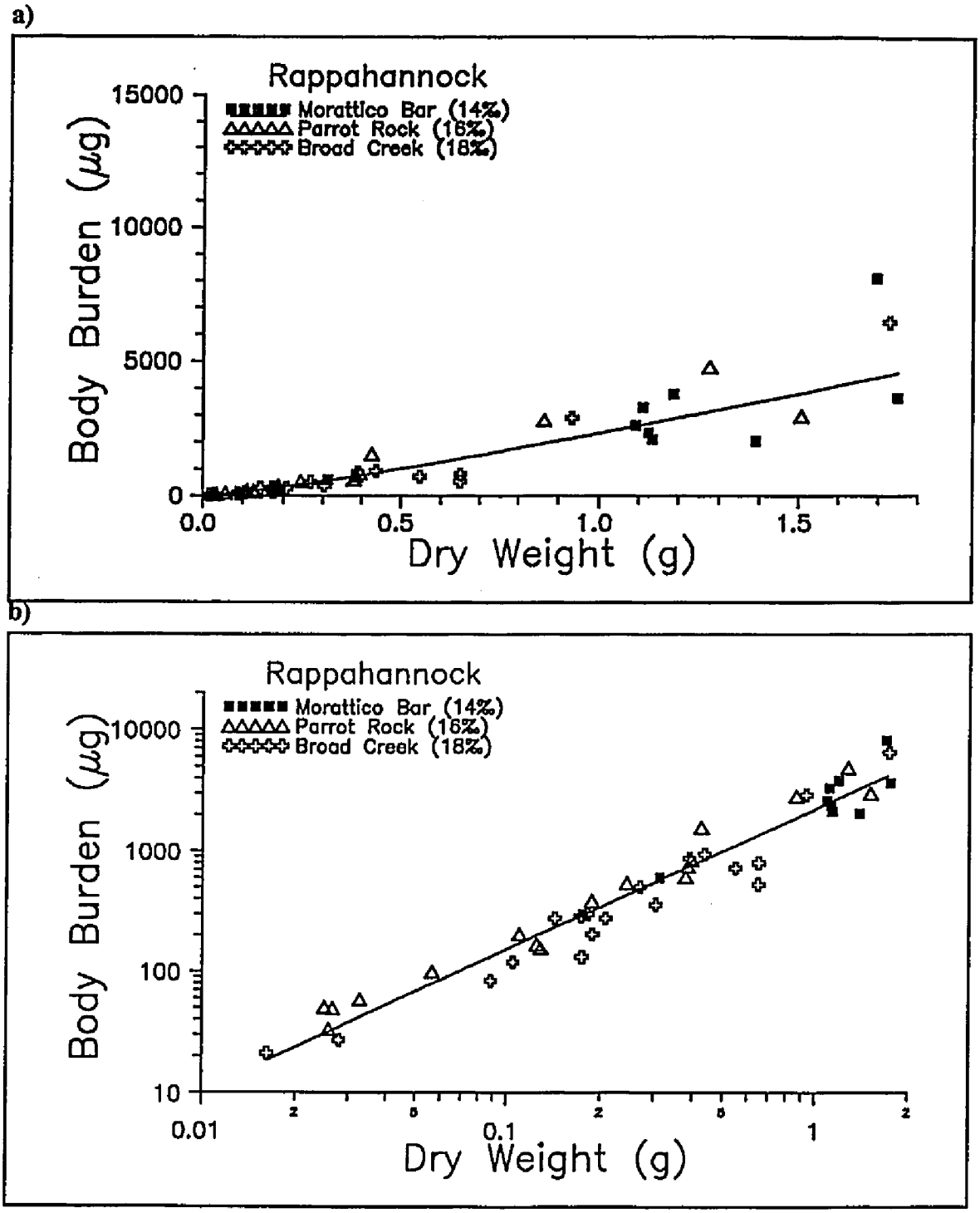

Figure 4-6. Zinc body burdens of oysters from Rappahannock River oyster beds in (a) normal scales and (b) log-log scales. The lines are fit to the power function, $Y = a X^b$ by the least squares fits for the log-log transformed data.

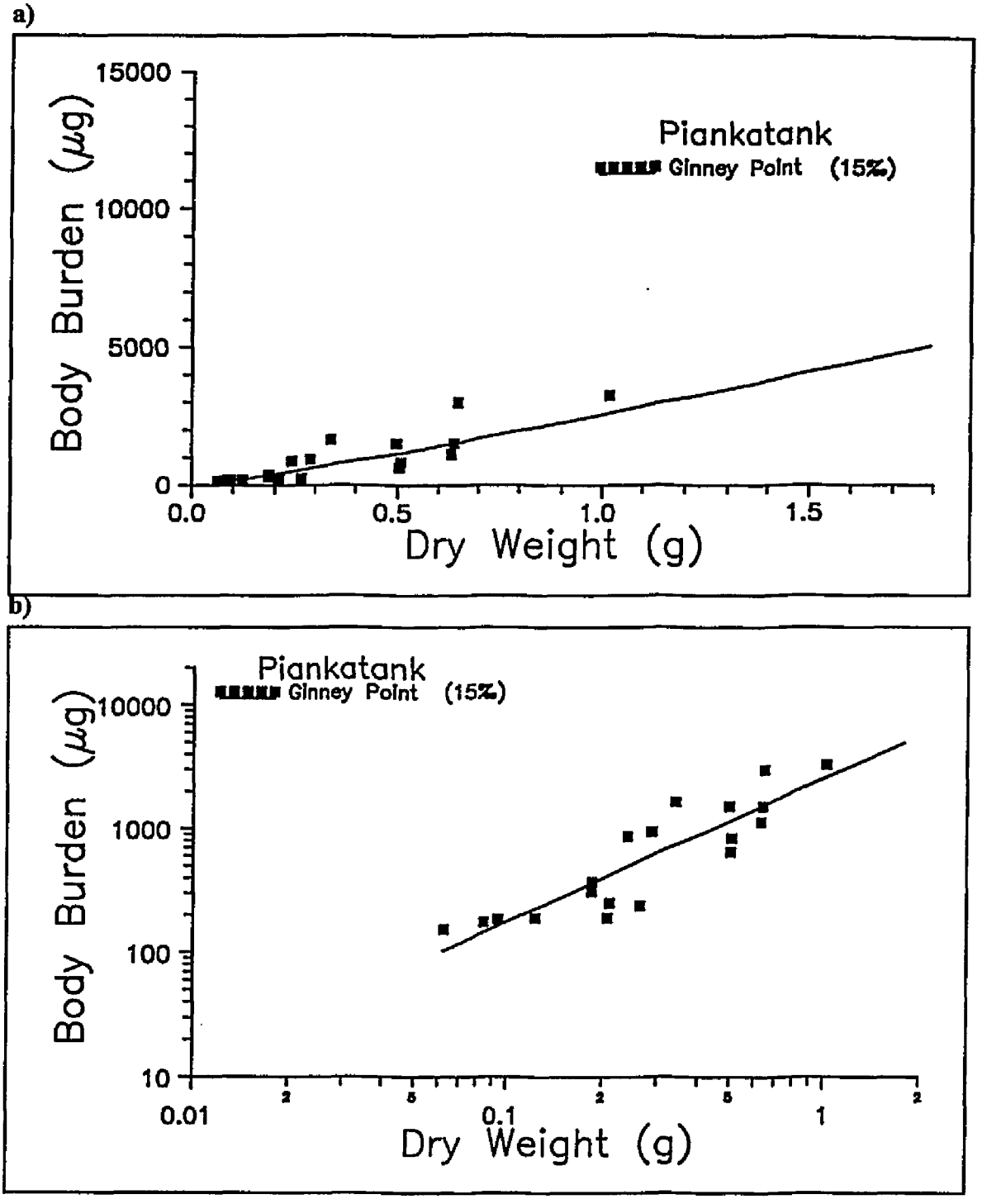

Figure 4-7. Zinc body burdens of oysters from Piankatank River oyster beds in (a) normal scales and (b) log-log scales. The lines are fit to the power function, $Y = a X^b$ by the least squares fits for the log-log-transformed data.

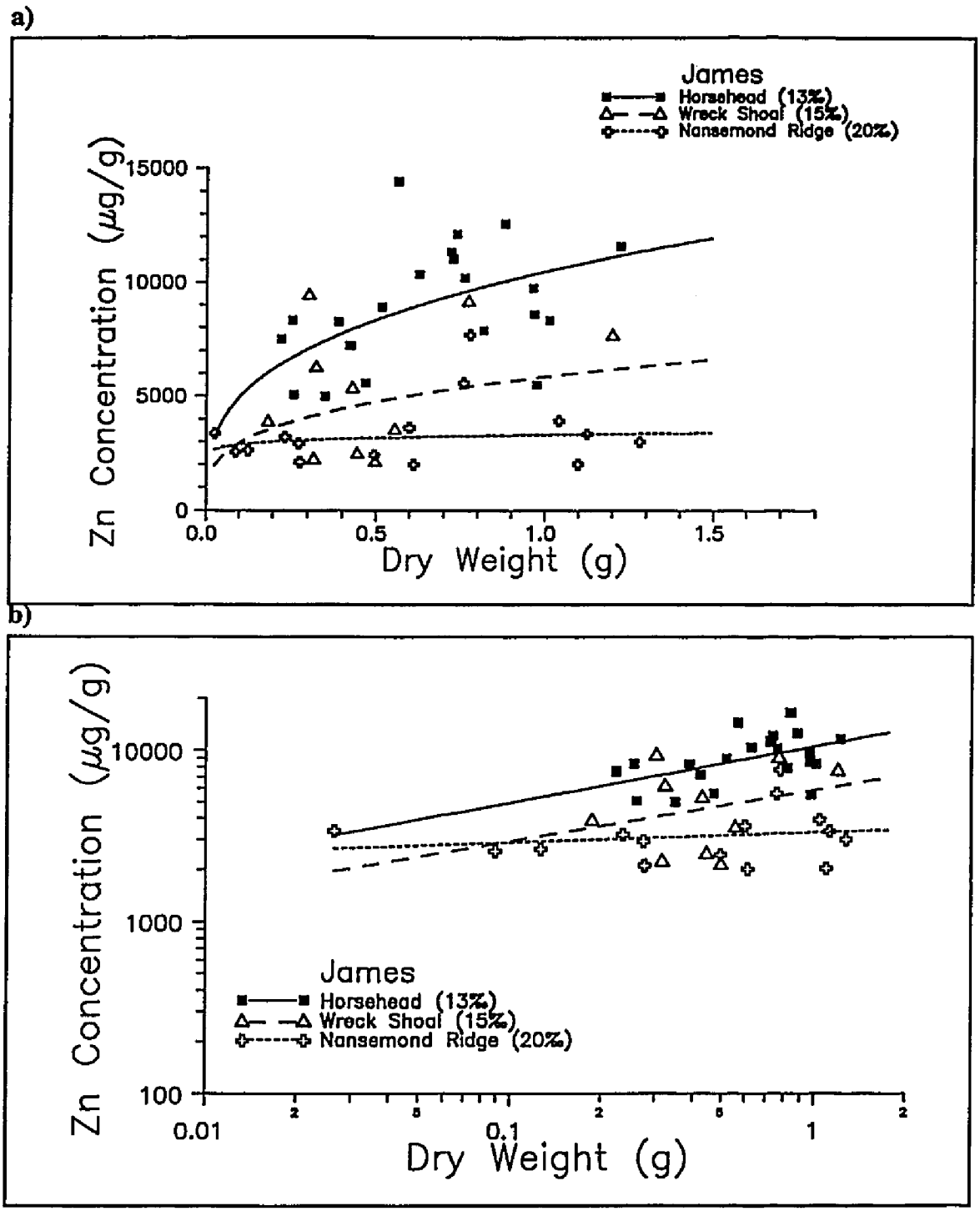

Figure 4-8. Zinc soft tissue concentrations of oysters from James River oyster beds in (a) normal scales and (b) log-log scales. The lines are fit to the power function, $Y/W = a X^{b-1}$ by the least squares fits for the log-log transformed data.

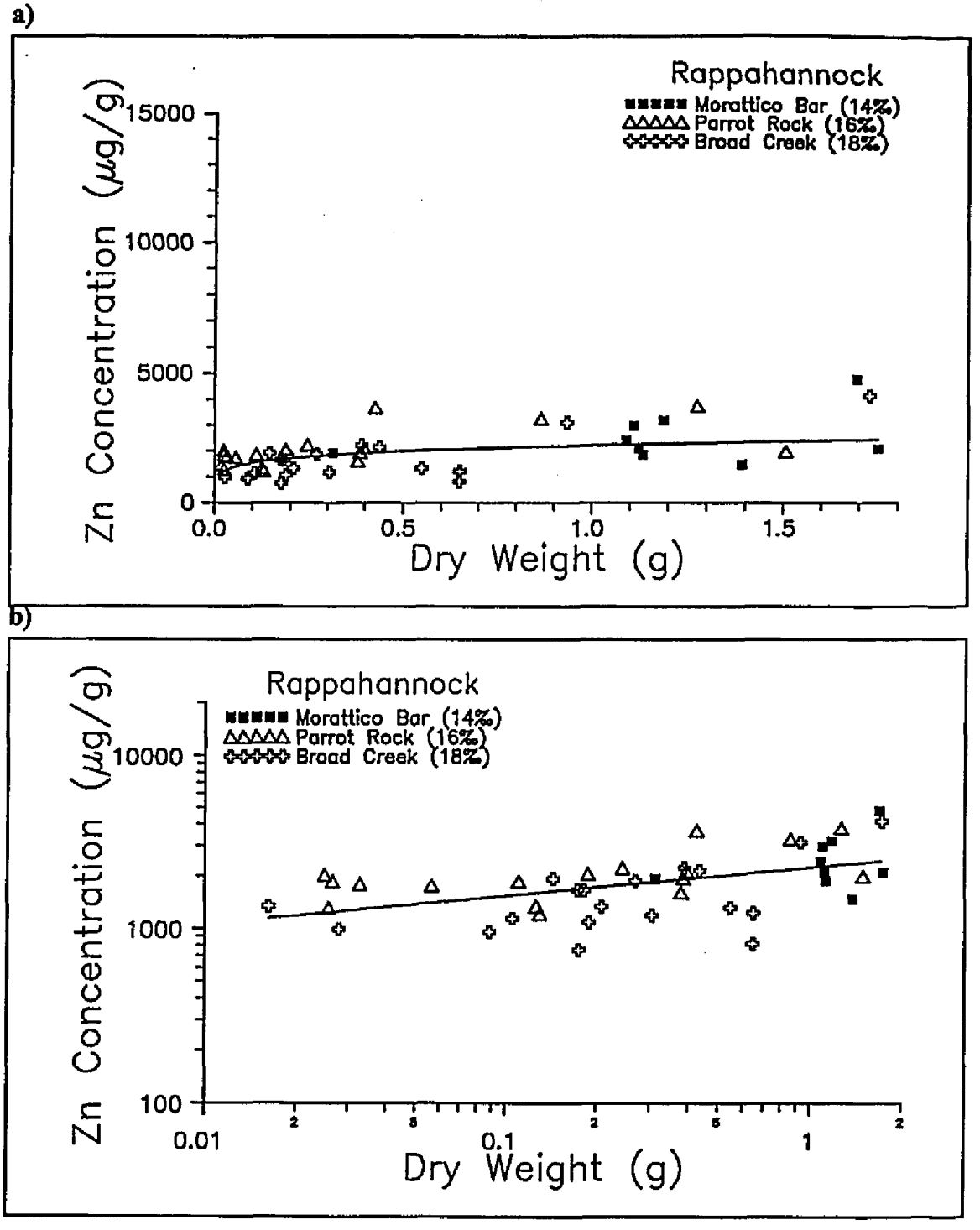

Figure 4-9. Zinc soft tissue concentrations of oysters from Rappahannock River oyster beds in (a) normal scales and (b) log-log scales. The lines are fit to the power function, $Y/W = a X^{b-1}$ by the least squares fits for the log-log transformed data.

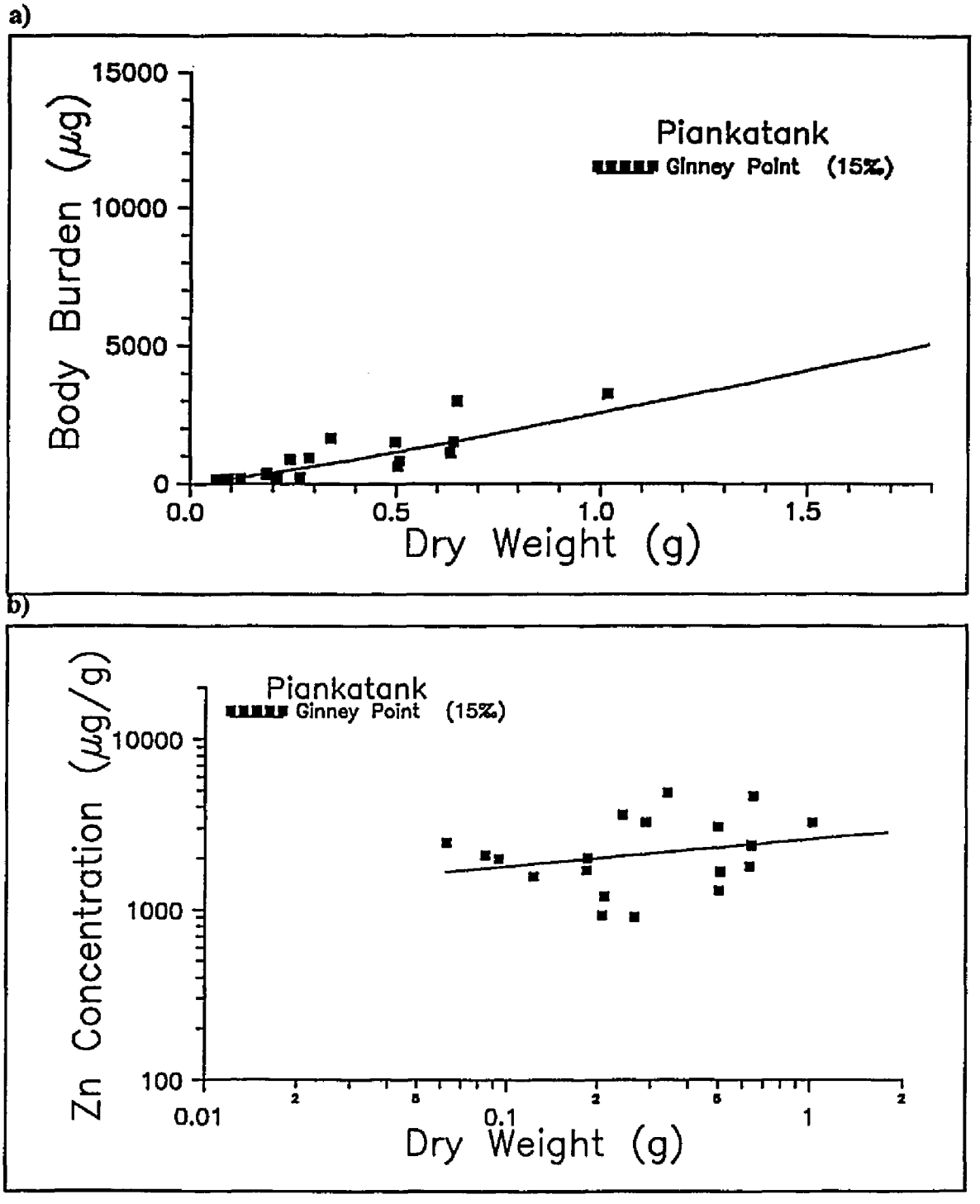

Figure 4-10. Zinc soft tissue concentrations of oysters from Piankatank River oyster beds in (a) normal scales and (b) log-log scales. The lines are fit to the power function, $Y/W = a X^{b-1}$ by the least squares fits for the log-log transformed data.

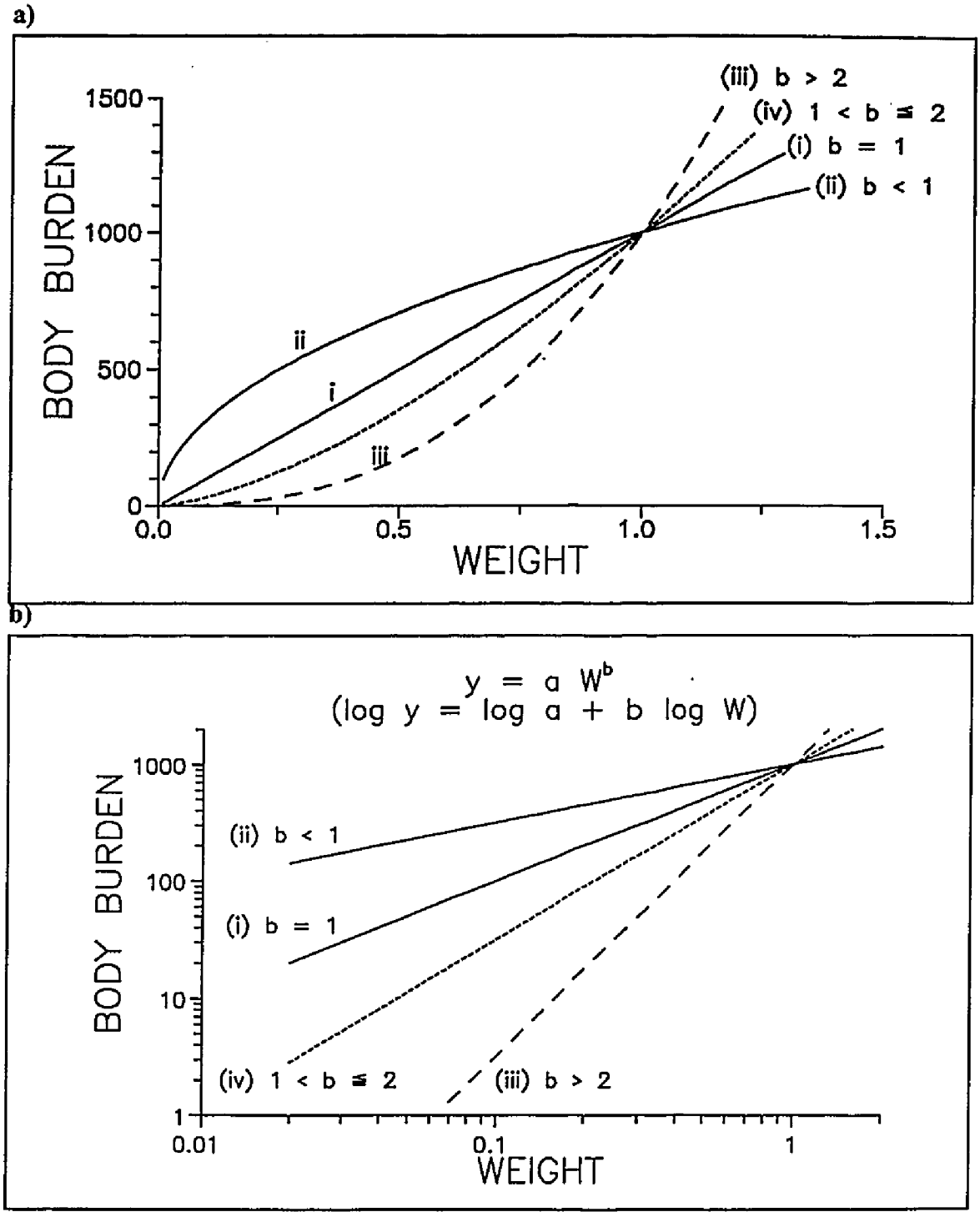

Figure 4-11. The three relationships obtained by expressing trace metal contents (body burden, μg) against body weight (g) on (a) linear and (b) logarithmic scales from Boyden (1977). Body burdens (y) are related to weights (W) by the relation $y = a W^b$: (i) $b = 1$, (ii) $b < 1$, and (iii) $b > 2$. The fourth case, (iv) $1 < b < 2$ follows the same pattern as the case (iii).

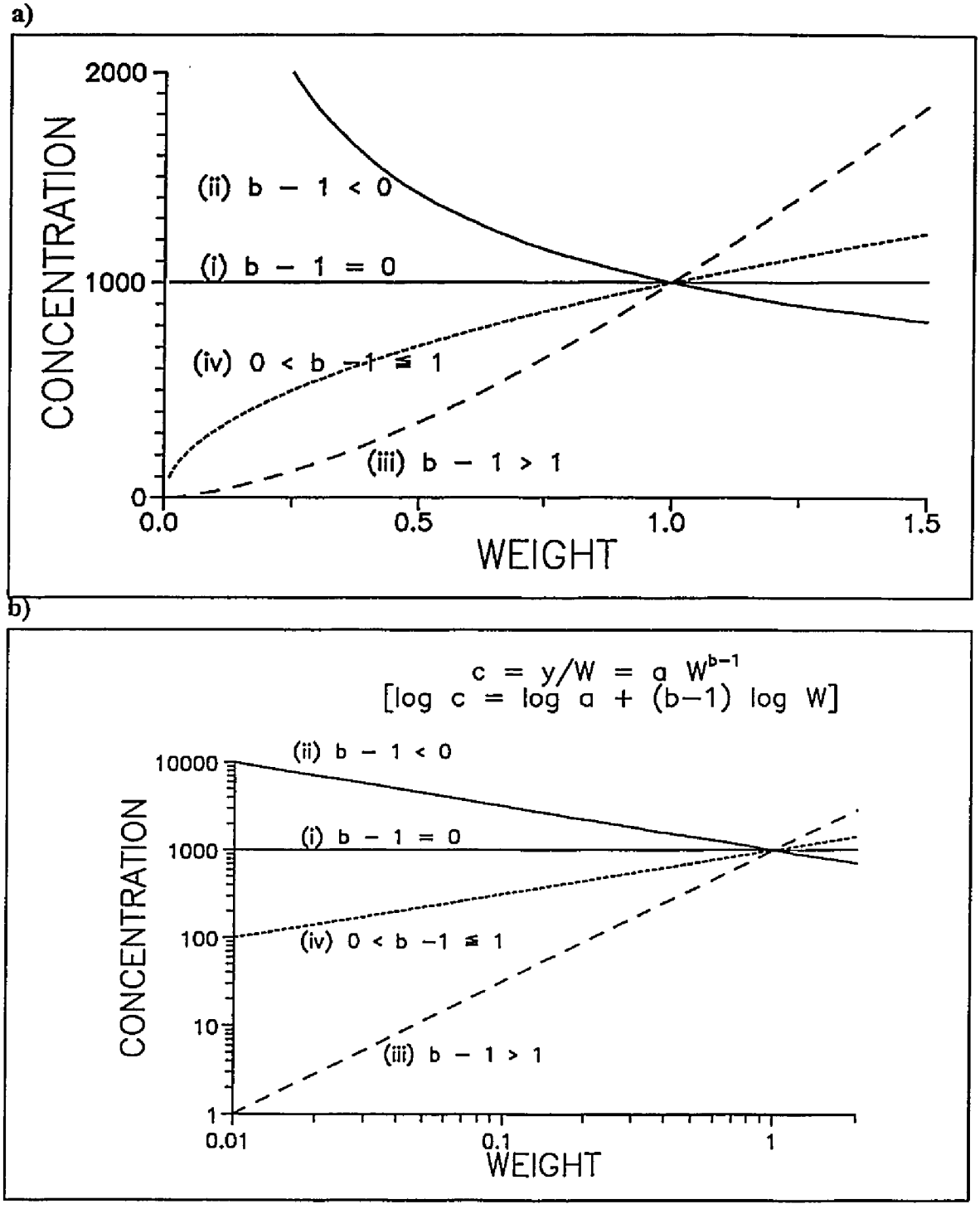

Figure 4-12. The three relationships obtained by expressing trace metal concentration (μg/g) against body weight (g) on (a) linear and (b) logarithmic scales from Boyden (1977). Concentrations (y/W) are related to weights (W) by the relation $y/W = a\ W^{b-1}$: (i) $b = 1$, (ii) $b < 1$, and (iii) $b > 2$. The fourth case, (iv) $1 < b < 2$, *i.e.*, $0 < b\text{-}1 < 1$ is different from the pattern of the case (iii) $b > 2$, *i.e.*, $b\text{-}1 > 1$.

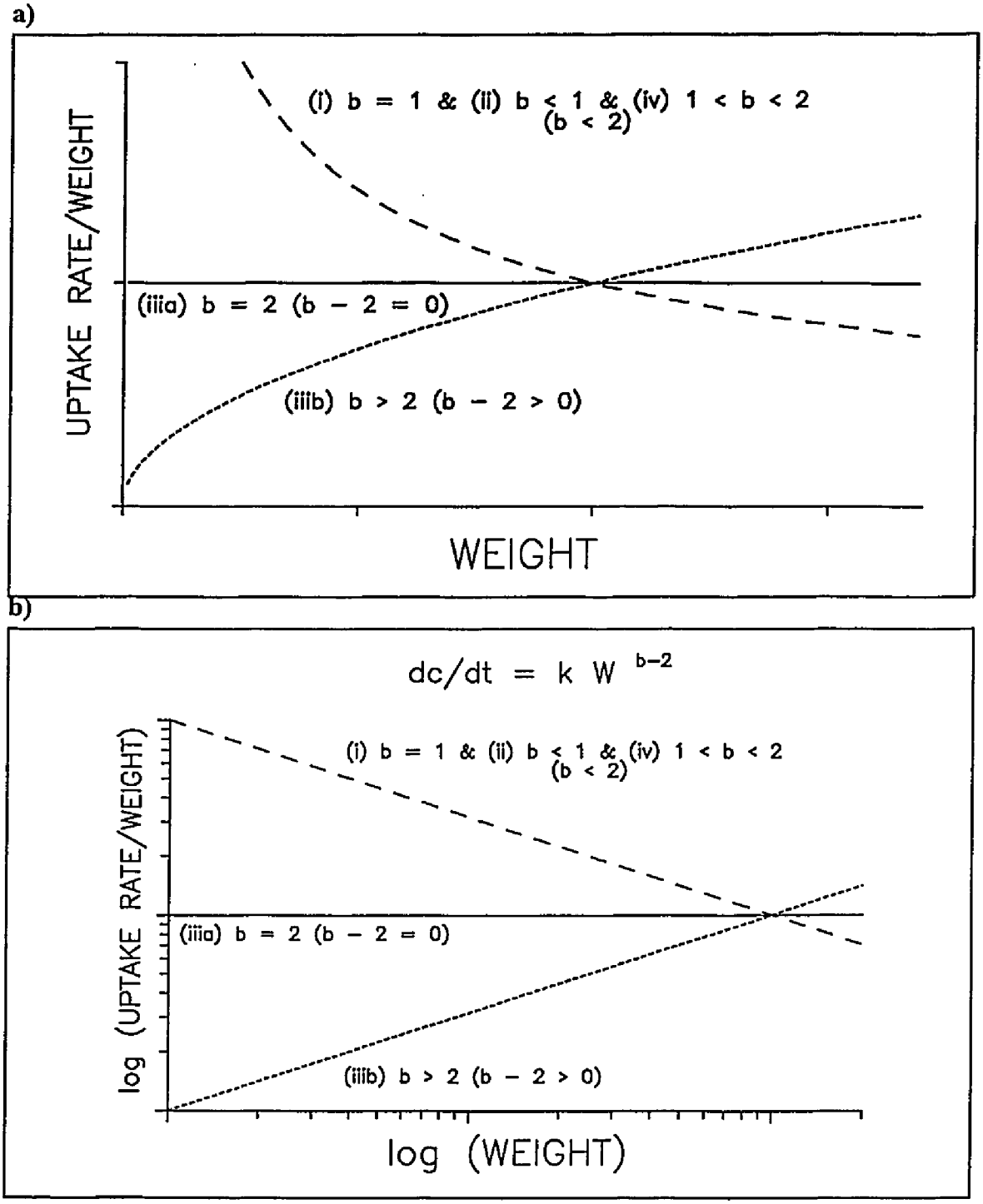

Figure 4-13. Comparison of the relationships (ii) b > 2 and (iv) 1 < b < 2 expressed in uptake rate per unit weight (µg/g/day) against body weights (g) on (a) linear and (b) logarithmic scales. $c = \frac{y}{W}$ then $\frac{dc}{dt} = k\ W^{b-2}$.

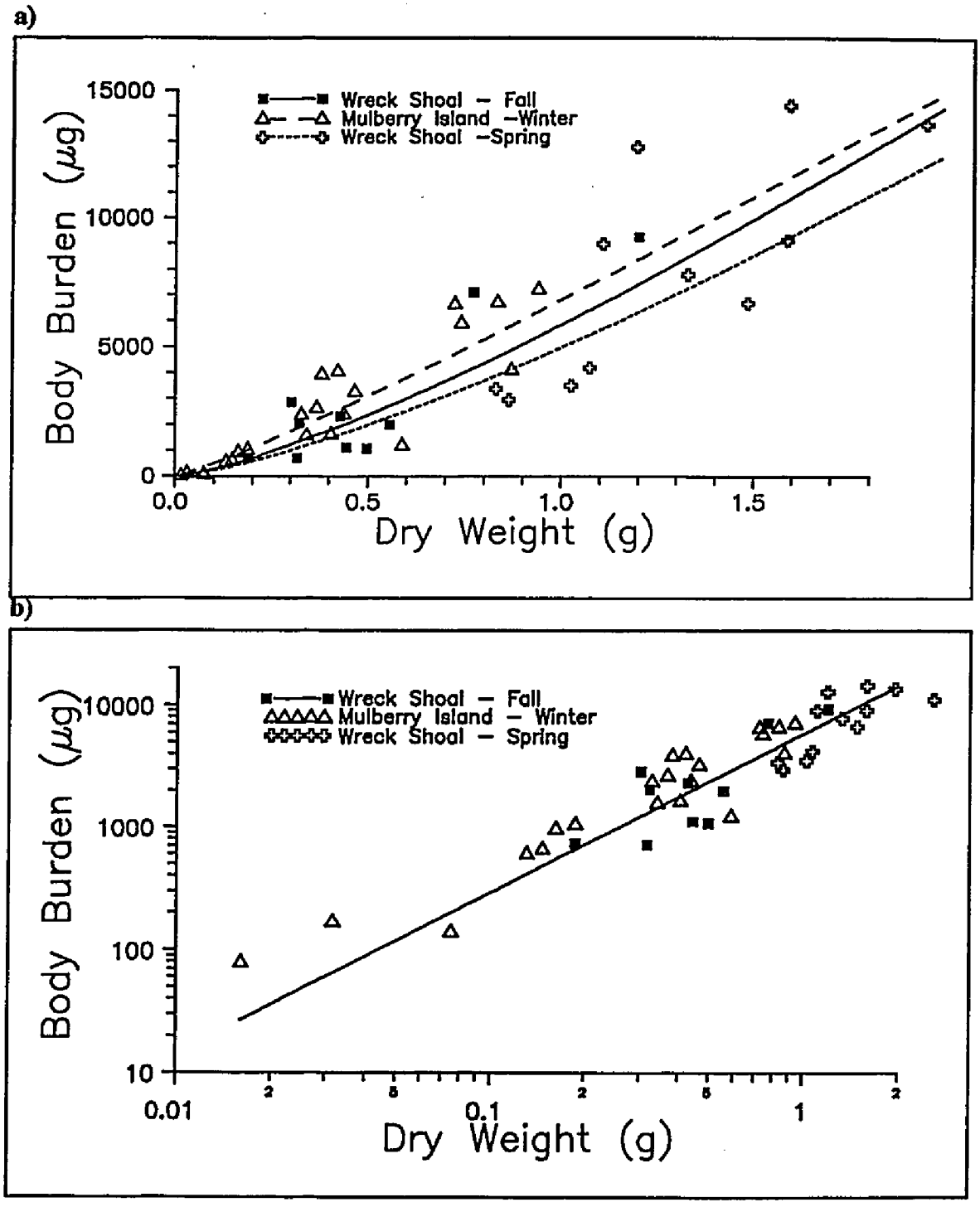

Figure 4-14. Zinc body burdens of oysters from the mid-reaches of the James River in different seasons in (a) linear scales and (b) log-log scales. The lines are fit to the power function, $Y = a X^b$ by the least squares fit.

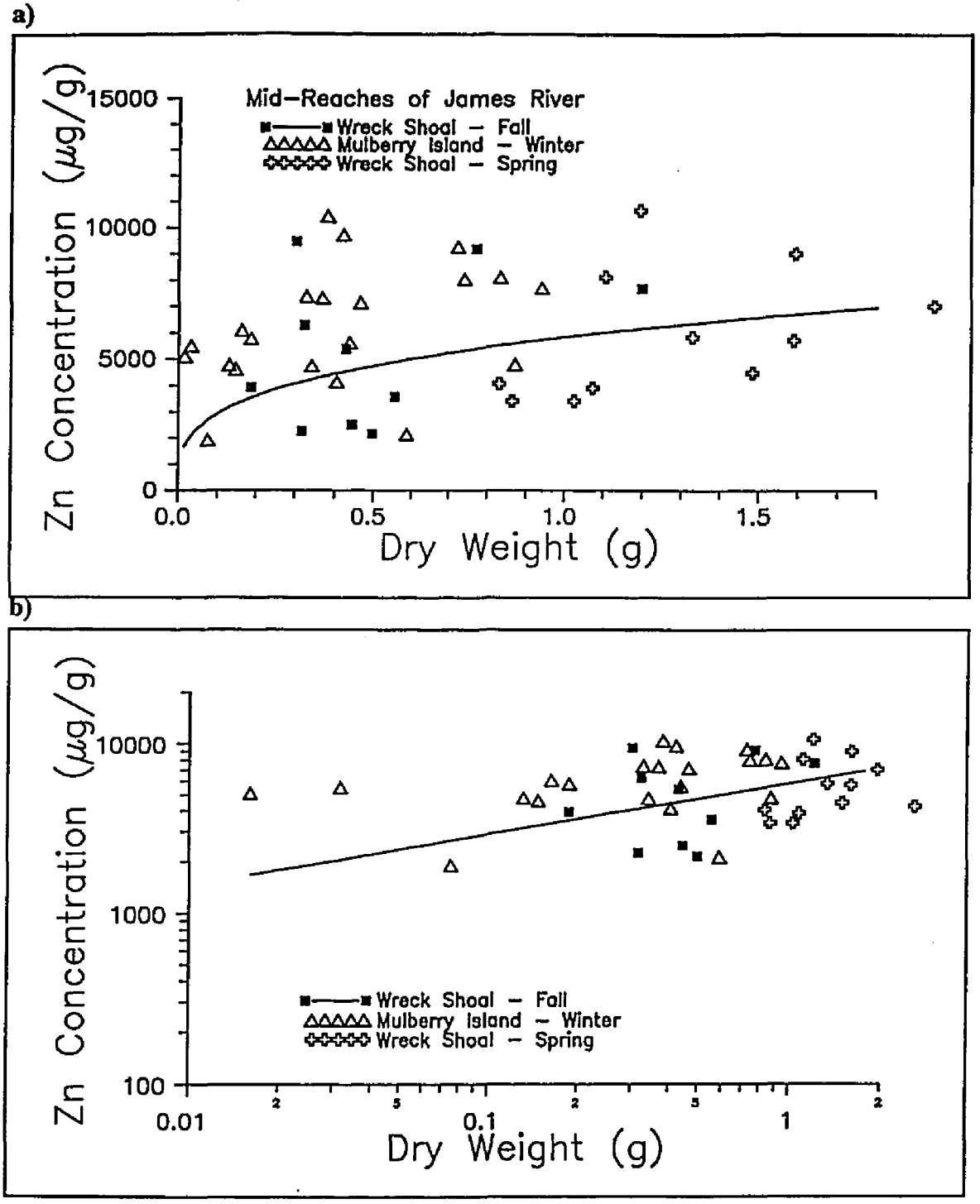

Figure 4-15. Zinc soft tissue concentrations of oysters from the mid-reaches of the James River in different seasons in (a) linear scales and (b) log-log scales. The lines are fit to the power function, $C = Y/W = a X^{b-1}$ by the least squares fit.

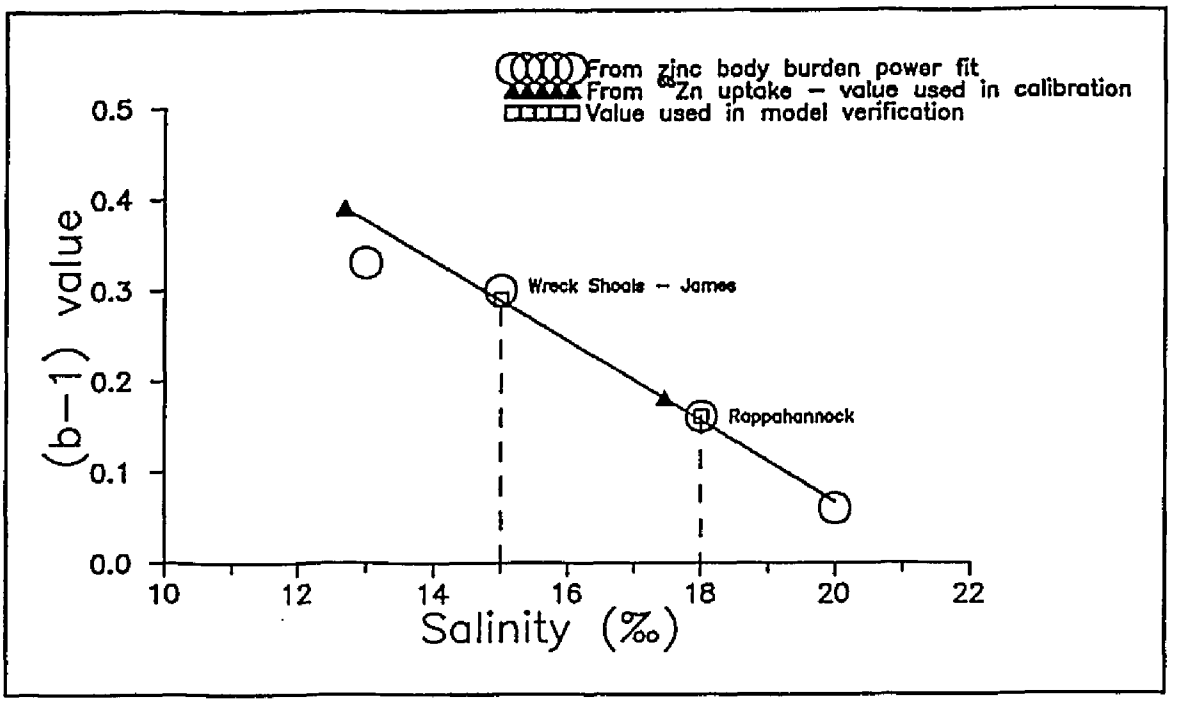

Figure 5-1. Variation of **b-1** with salinity. The line is fit to the two data points (▲) from the laboratory uptake studies. The other values (O) are obtained by fitting power function to zinc body burden data for in-situ feral oyster measurements. For verification (□), **b-1** values are interpolated using mean salinities and then used in model simulation.

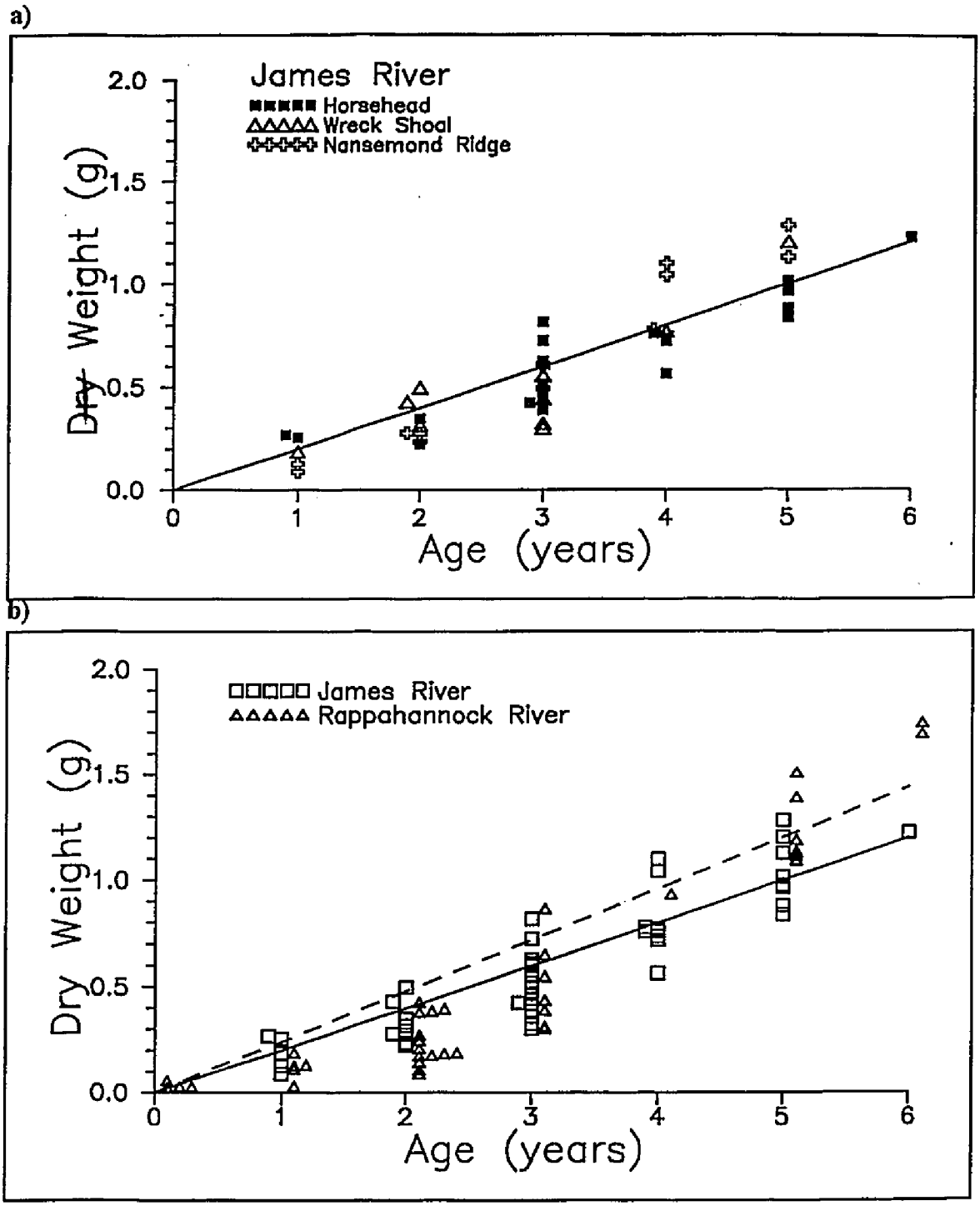

Figure 5-2. (a) Estimated effective annual soft tissue growth in dry weight (gram) for James River oysters and (b) comparison with that of Rappahannock River oysters.

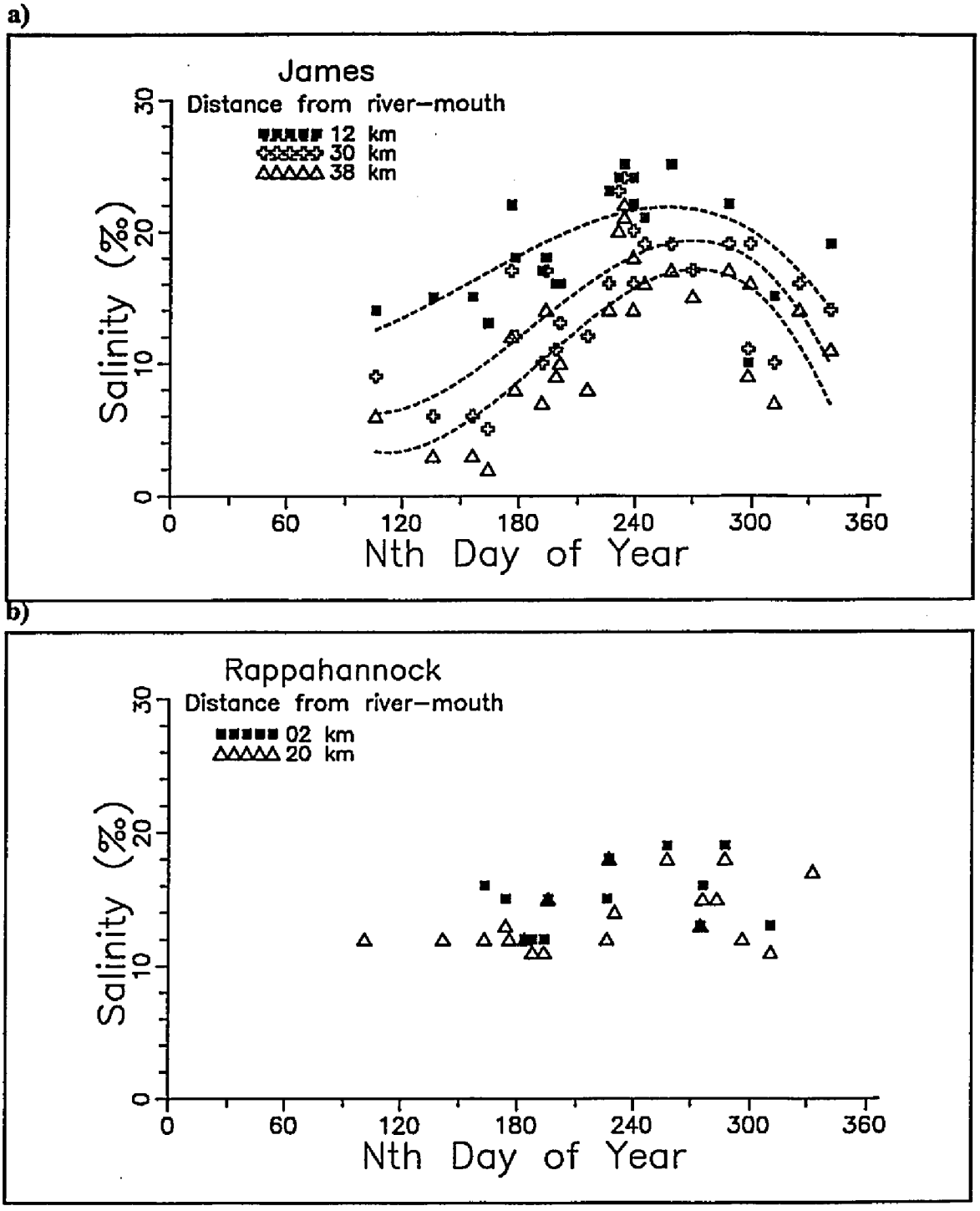

Figure 5-3. Mid-depth salinities of oyster sampling sites of (a) James River (data from Brooks & Fang 1983; Bradshow & Kuo 1987) and (b) Rappahannock River (data from Brooks 1983).

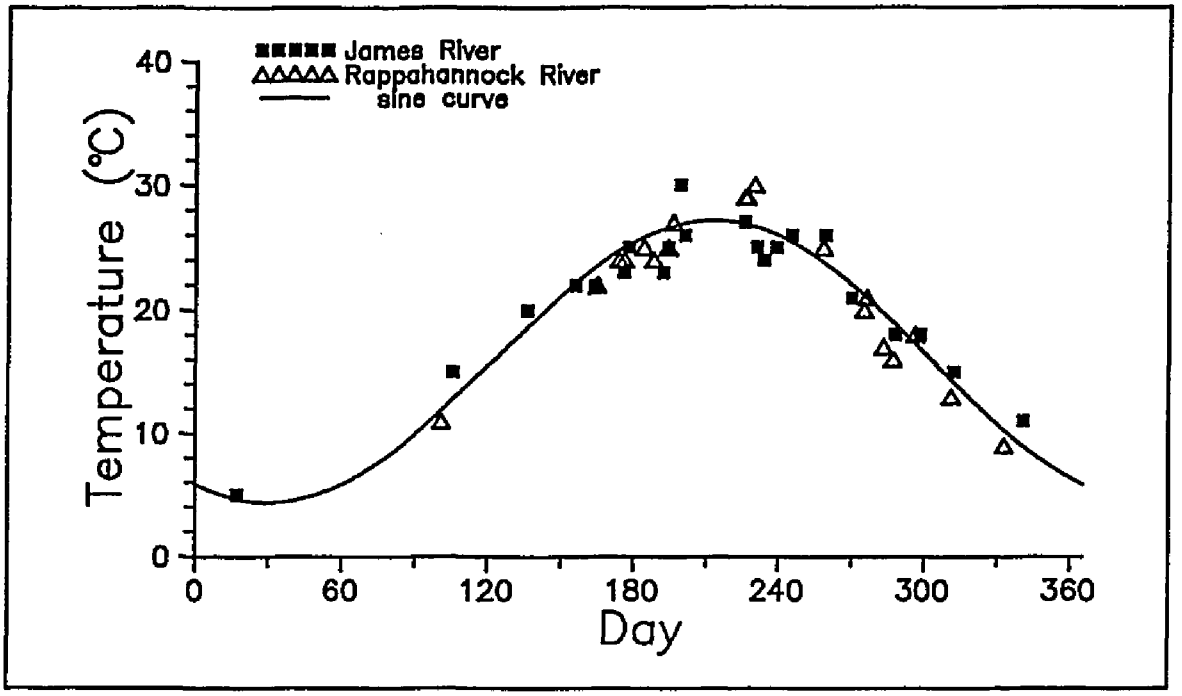

Figure 5-4. Temperature of oyster sampling sites (data from Brooks & Fang 1983; Bradshow & Kuo 1987 for James River and Brooks 1983 for Rappahannock River).

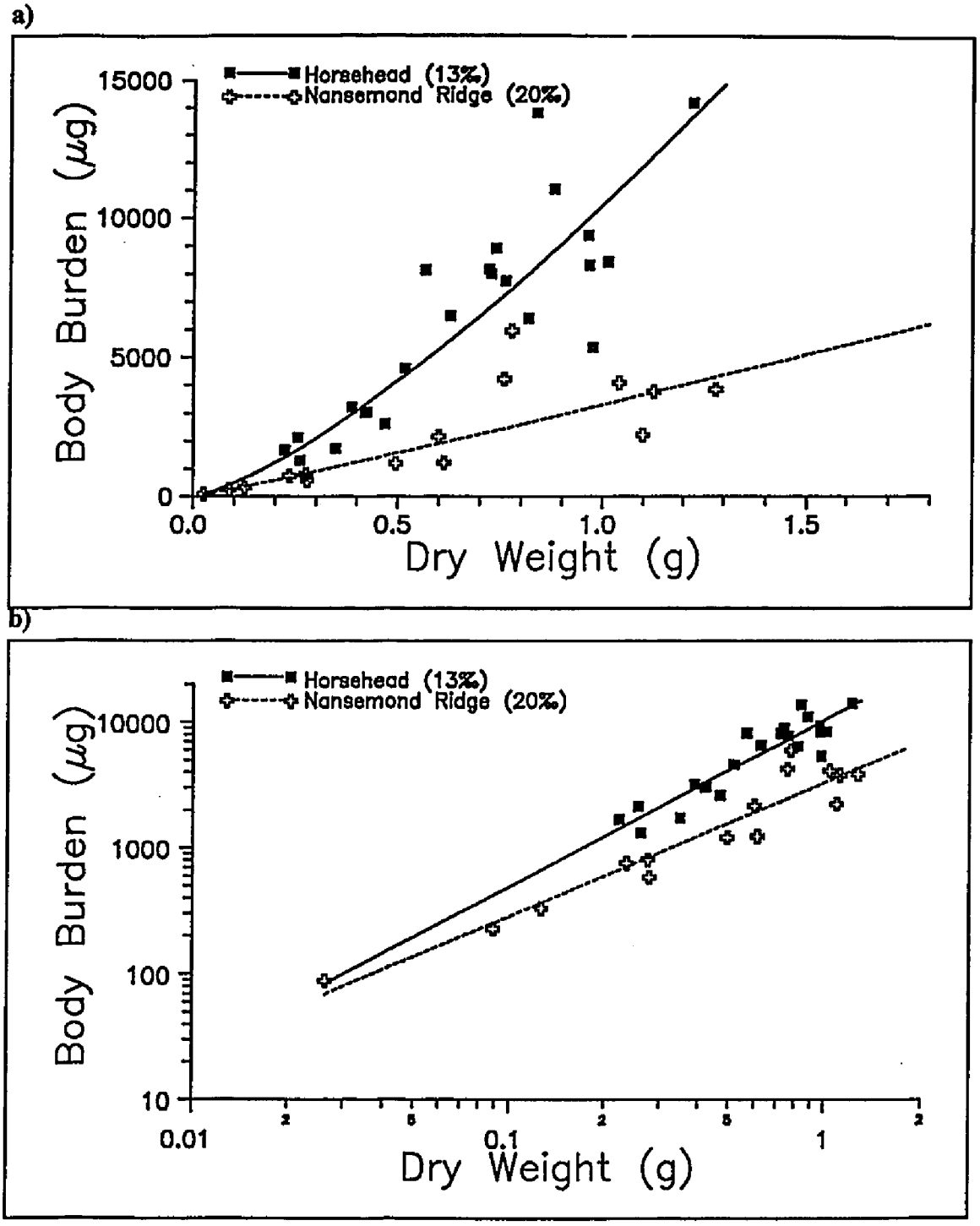

Figure 5-5. Zinc body burdens of oysters from Horsehead oyster bed (salinity 13‰) and Nansemond Ridge oyster bed (salinity 20‰) of James River in (a) linear scales and (b) log-log scales.

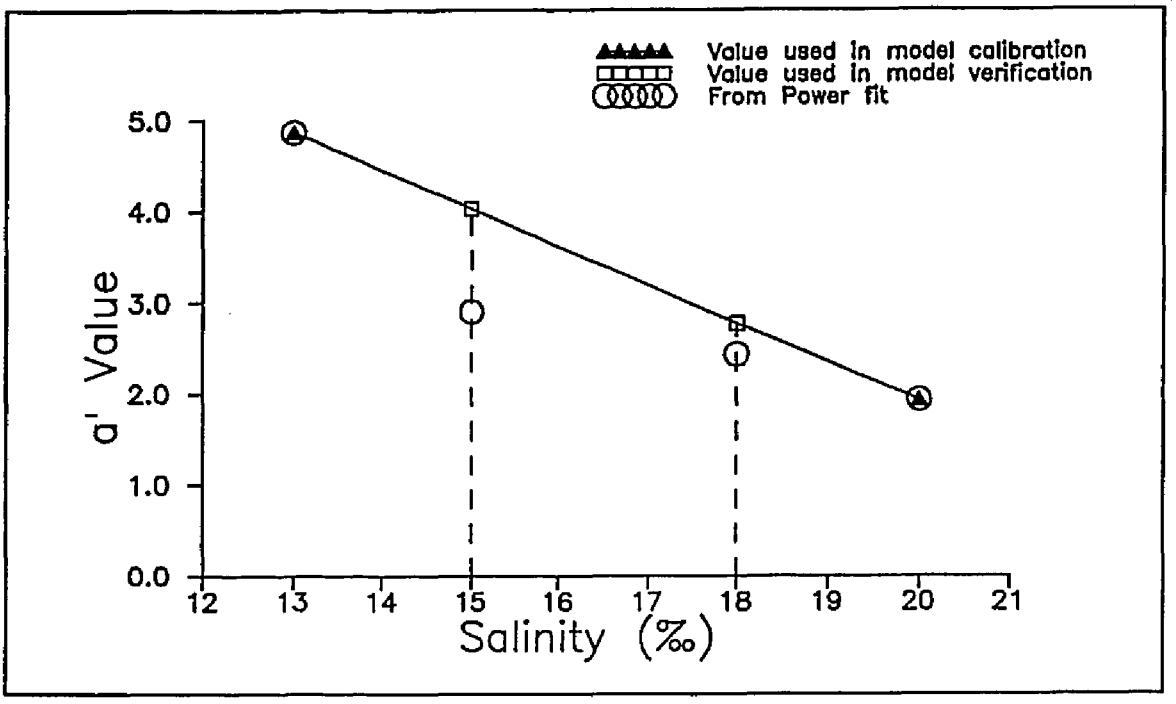

Figure 5-6. Variation of a' with salinity. The line is fit to the two data points (▲) were obtained by calibrating the model to the power function fits of Horsehead and Nansemond Ridge oysters. For verification (□), a' values are interpolated using effective average salinities and then used in model simulation.

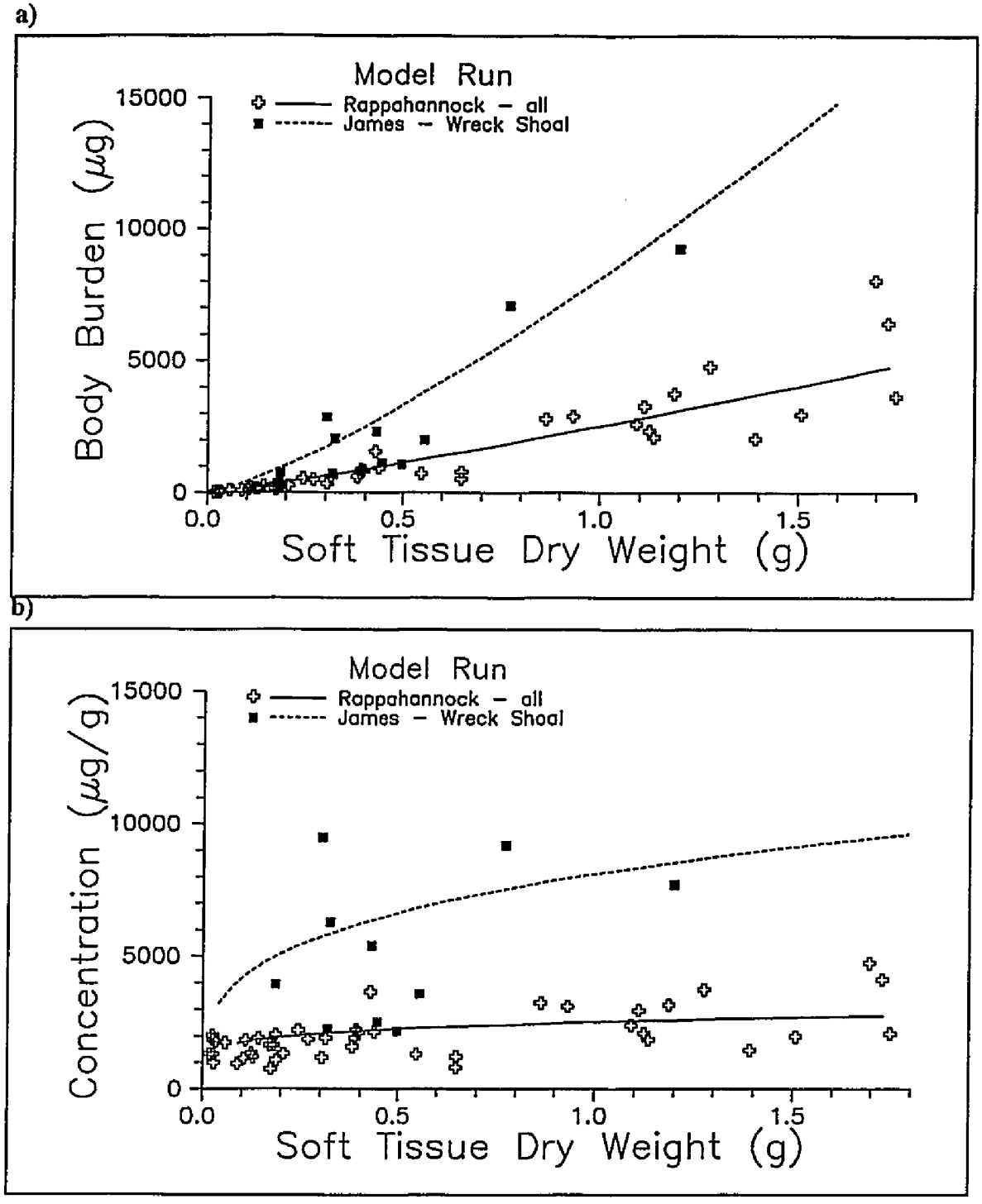

Figure 5-7. Model simulations and field measurements of zinc (a) body burdens and (b) soft tissue concentrations for oysters at Wreck Shoal in James River (solid line) and Rappahannock River (dashed line).

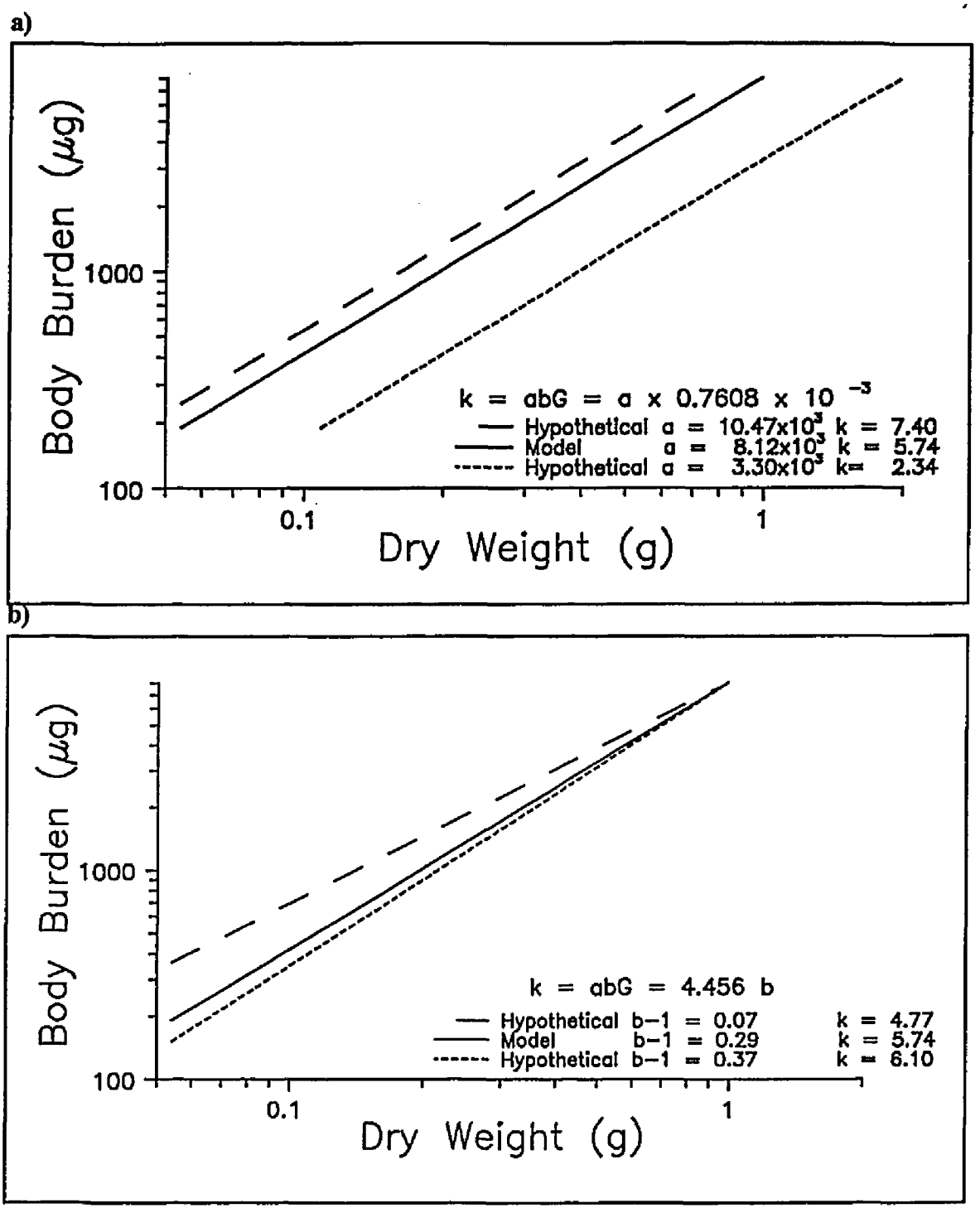

Figure 5-8. Effects of changes of uptake parameters (a) **a** and (b) **b** in k = a b G. Solid line is the model output for Wreck Shoal - James River oyster bed. Dashed lines are test runs of the model with (a) **a** values and (b) **b** values of the Horsehead and Nansemond Ridge oyster beds parameters (**b** in k = abG and **b-1** in W^{b-1}). All other parameters are kept the same as Wreck Shoal ones.

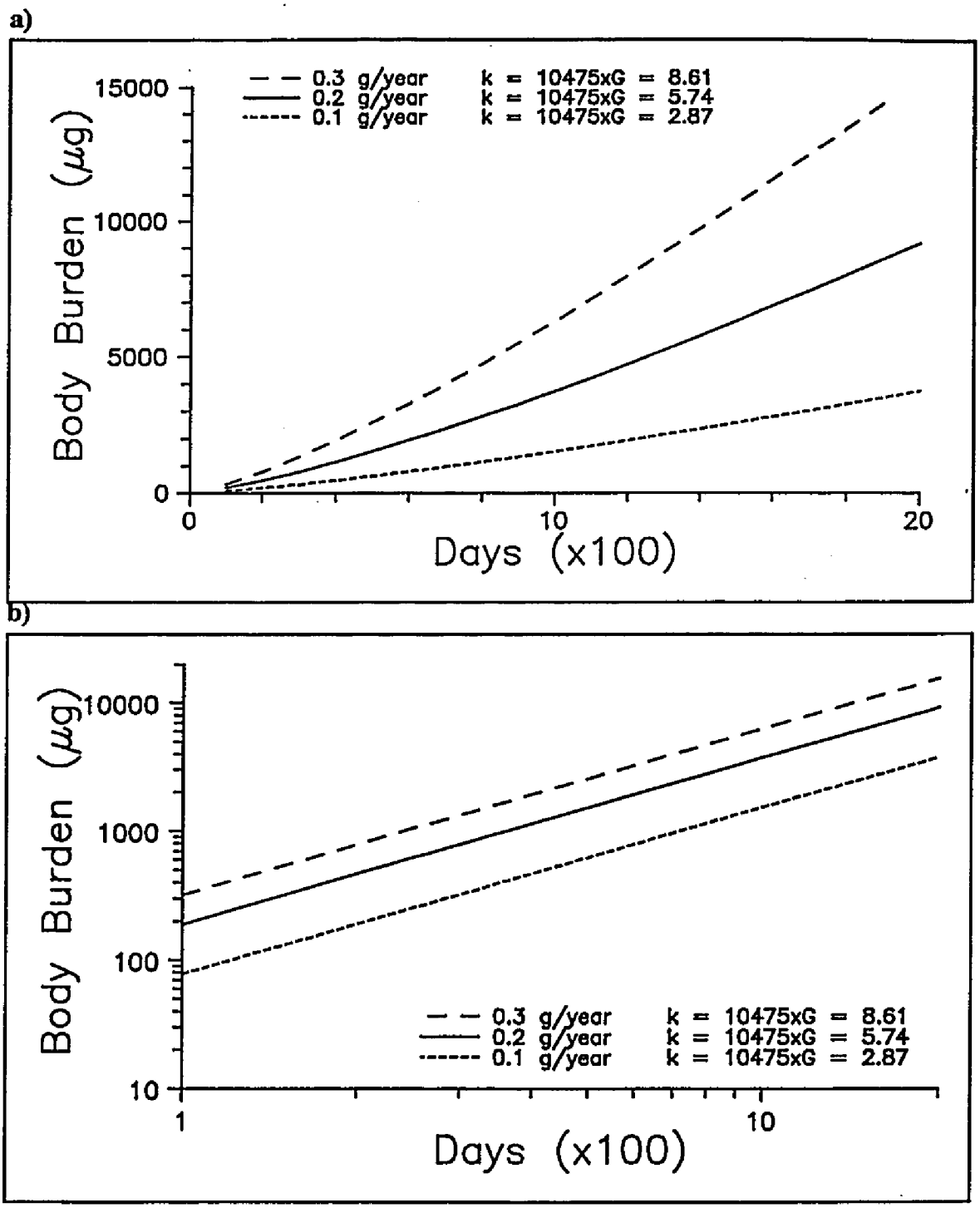

Figure 5-9. The change of zinc body burden of a Wreck Shoal oyster in time scale with different hypothetical growth rates (dry weight gram per year) in (a) linear and (b) log-log scales.

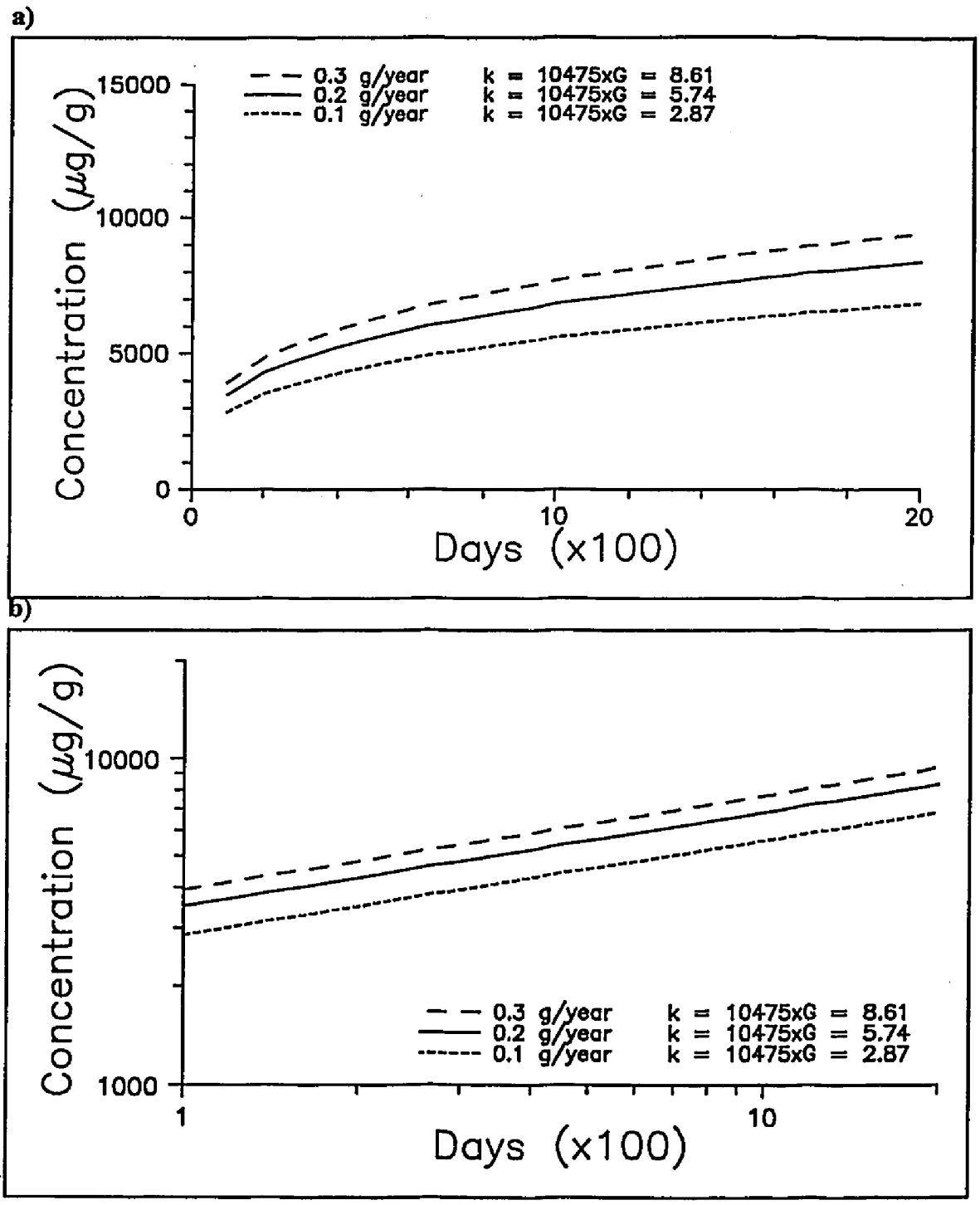

Figure 5-10. Changes of soft tissue zinc concentration for a Wreck Shoal oyster in time scale with different hypothetical growth rates (dry weight gram per year) in (a) linear scales and (b) log-log scales.

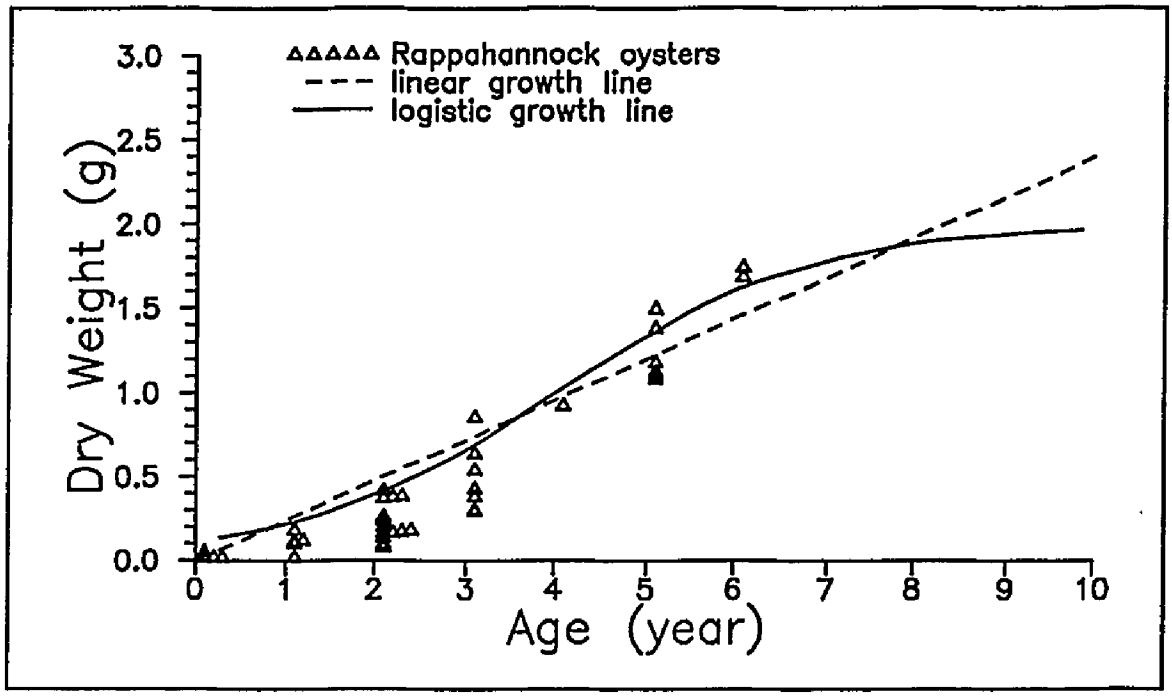

Figure 5-11. Comparison of the logistic growth curve applied to the Rappahannock oyster data (solid line) and the linear growth line used in the model (dashed line).

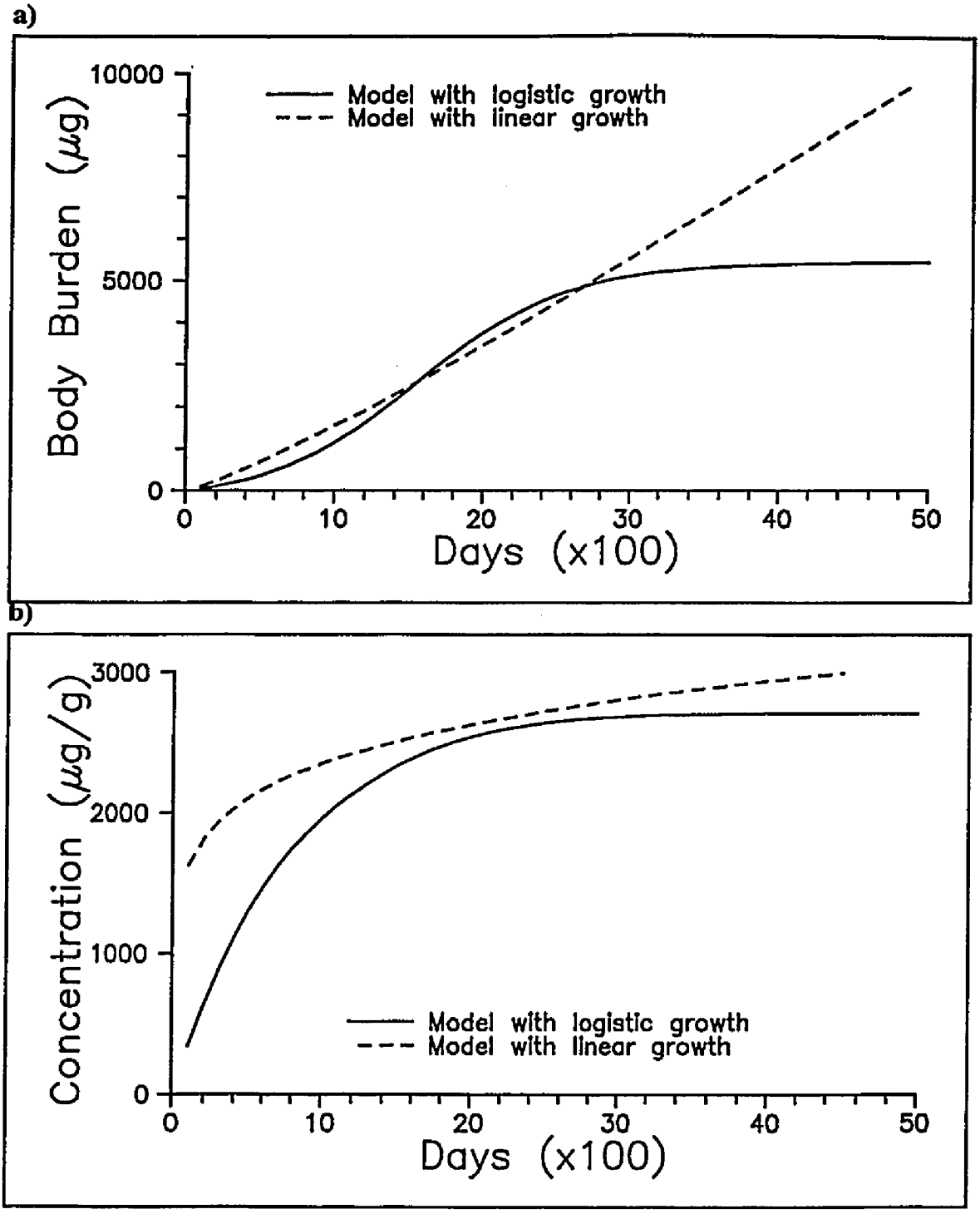

Figure 5-12. Hypothetical changes of (a) body burdens and (b) soft tissue concentrations for Rappahannock oysters in time scale for different growth equations. Solid line is when a logistic growth equation is applied and dash line is when a linear growth equation is applied.

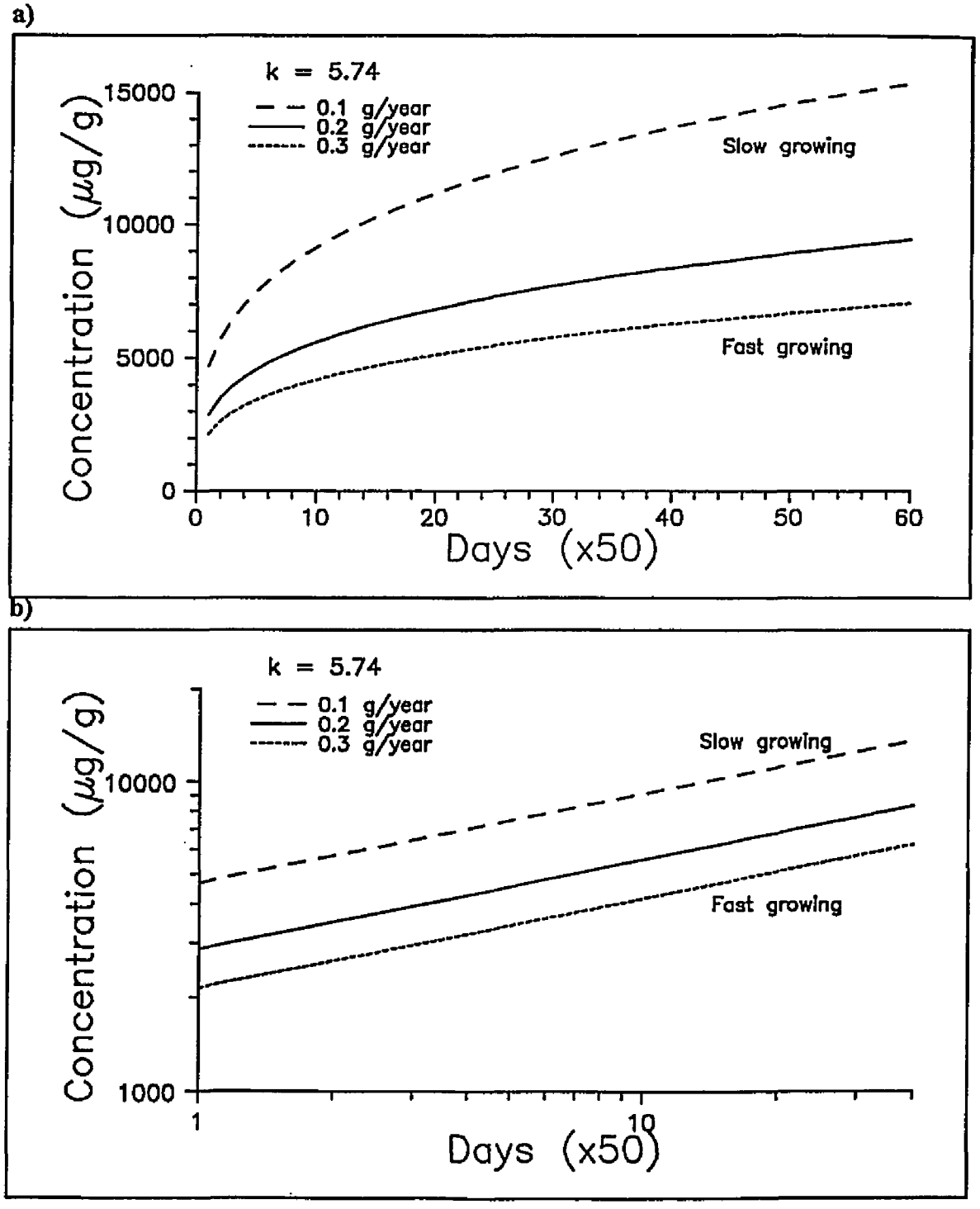

Figure 6-1. Change of zinc concentration of a Wreck Shoal oyster in time scale with different hypothetical growth rates for the alternative model.

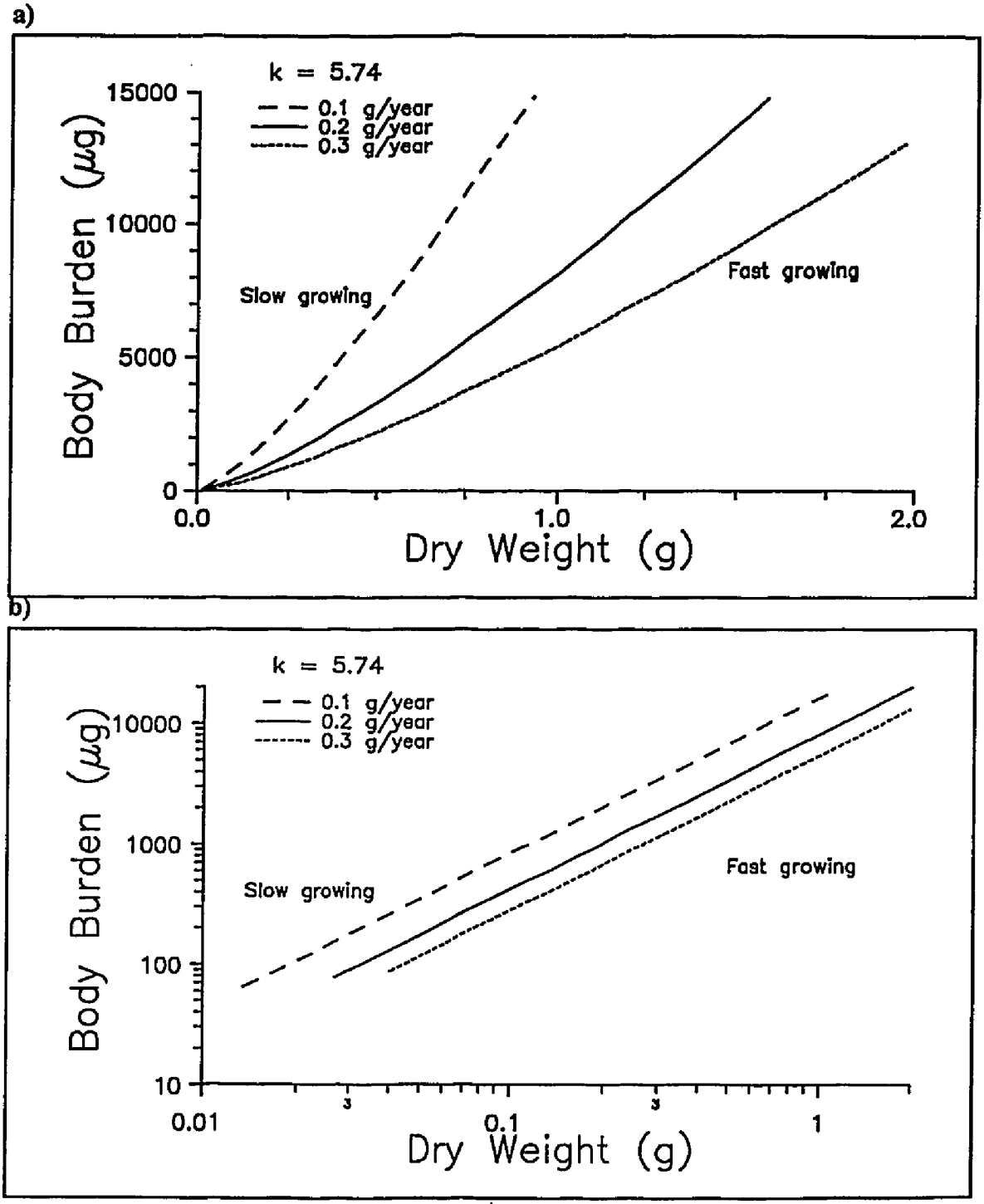

Figure 6-2. Body burden-body weight relationships of Wreck Shoal oysters for different hypothetical growth rates for the alternative model.

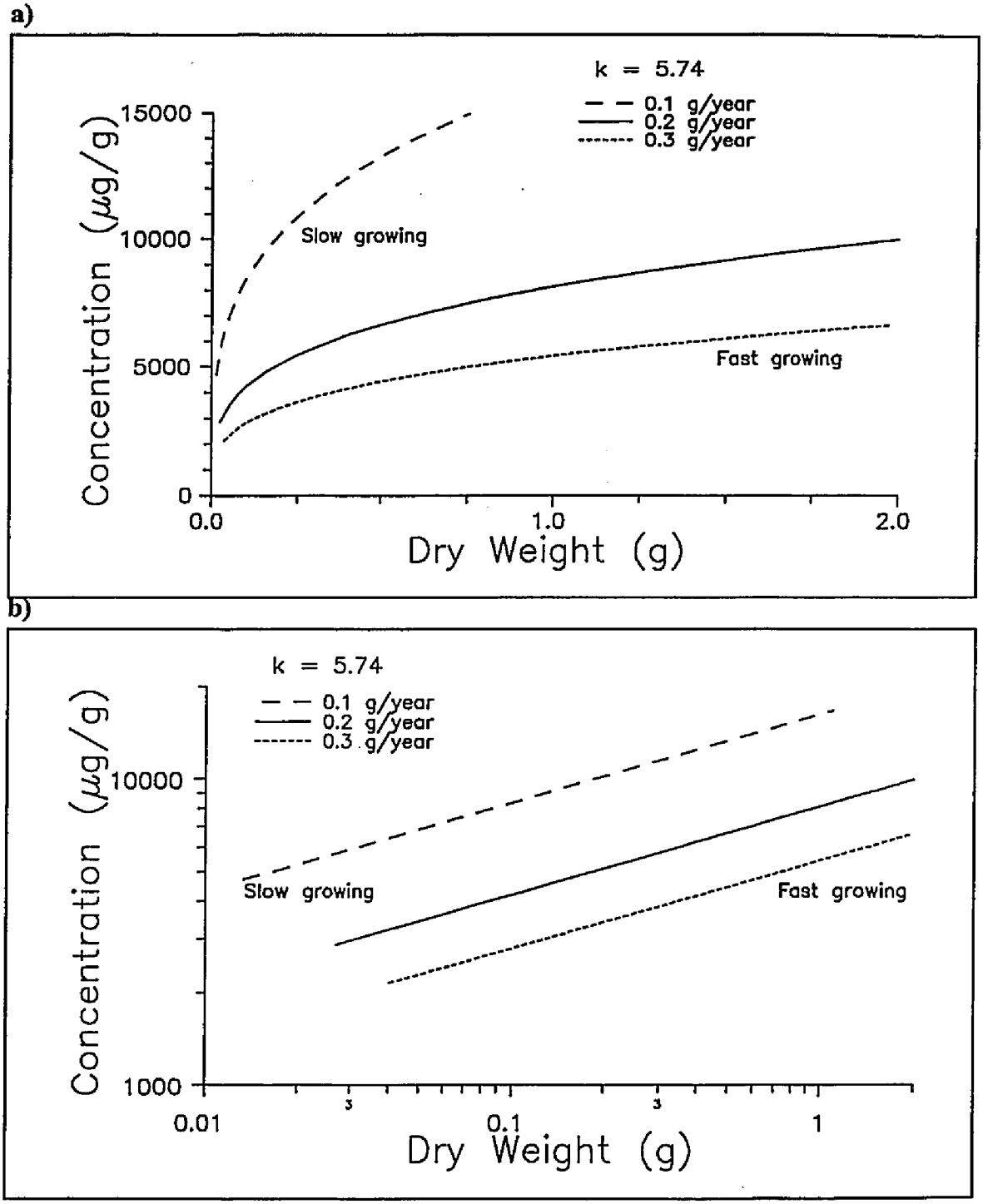

Figure 6-3. Soft tissue concentration-body weight relationships of Wreck Shoal oysters for different hypothetical growth rates for the alternative model.

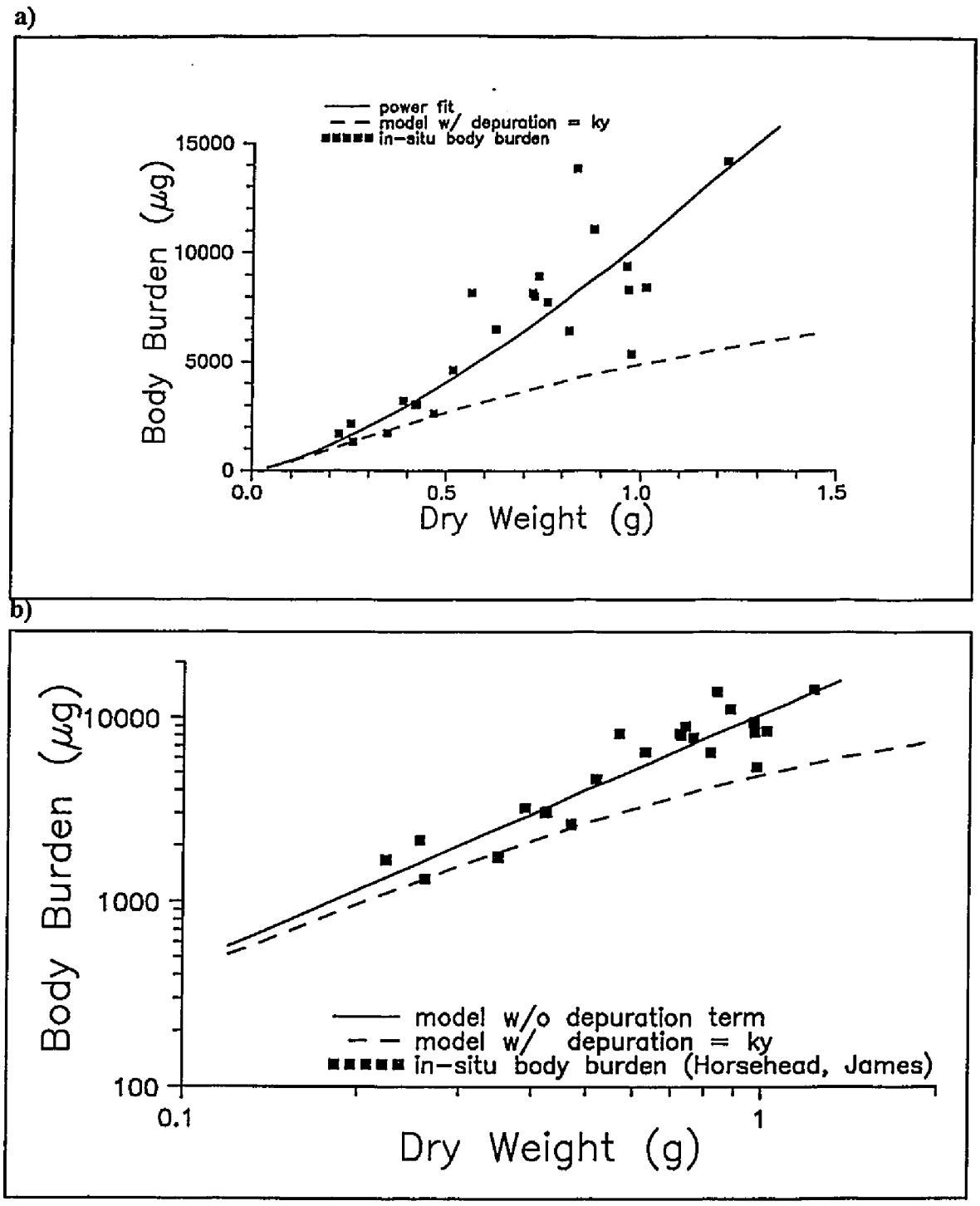

Figure 6-4. Comparison of the model output without any depuration term (solid line, which is almost identical to the power fit line for the Horsehead oyster body burdens (■) and the model output with a hypothetical depuration, the first order kinetic elimination (dashed line).

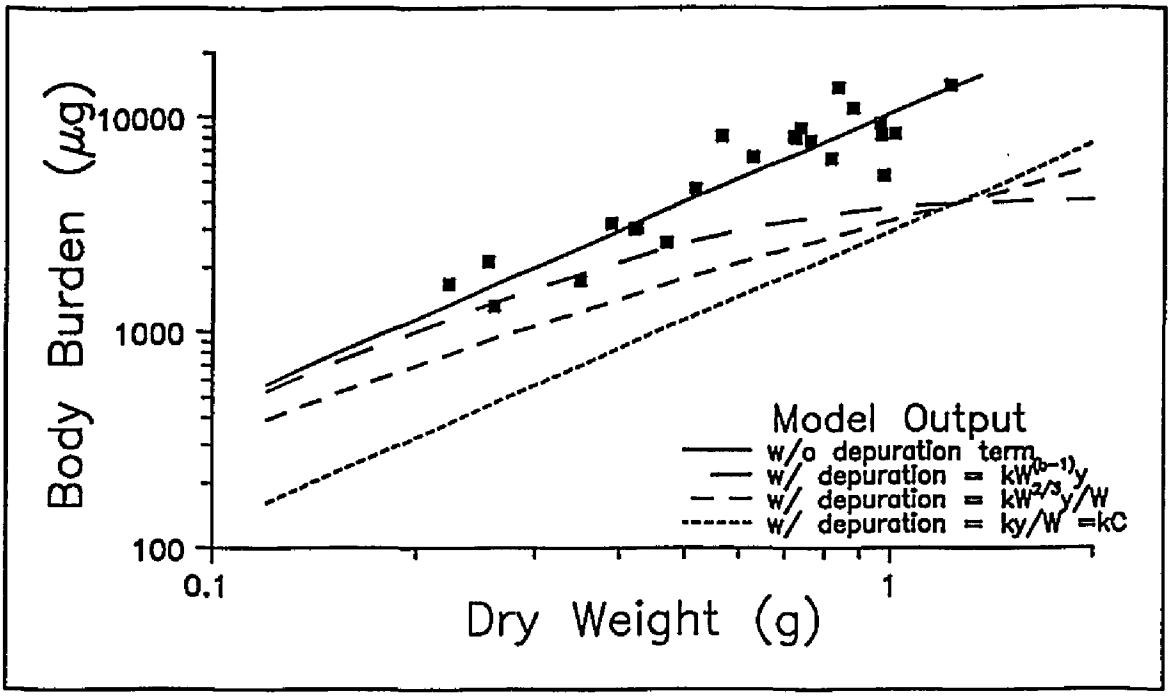

Figure 6-5. Comparison of hypothetical depuration formulas. Solid line is the model output without any depuration term for the Horsehead oyster body burdens (■). Dashed lines are the model output incorporated with hypothetical depuration terms.

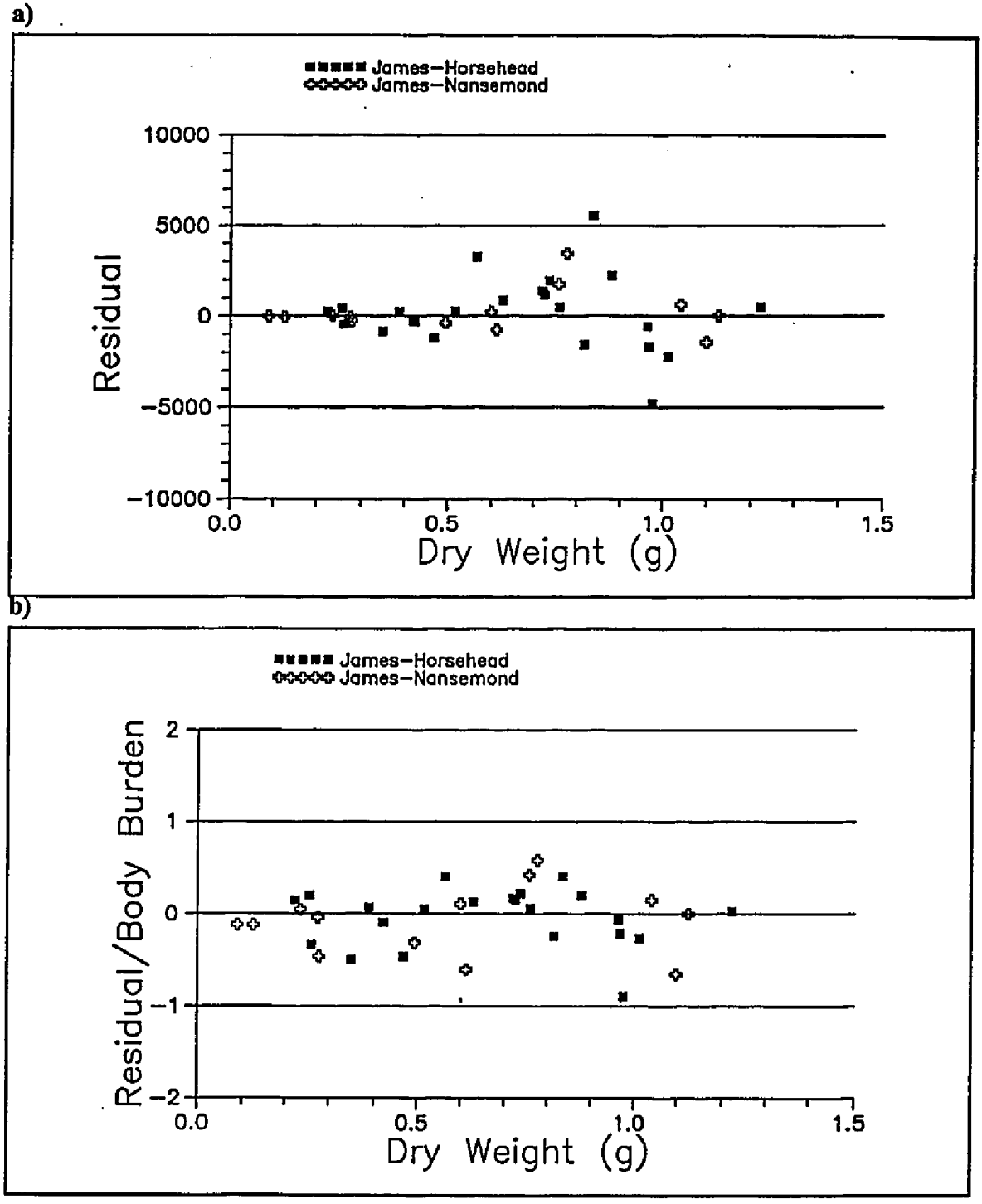

Figure 6-6. Residuals of power fits of body burdens (Fig. 5-2). (a) Body burden - power fit and (b) normalized residuals, *i.e.*, (body burden - power fit)/body burden.

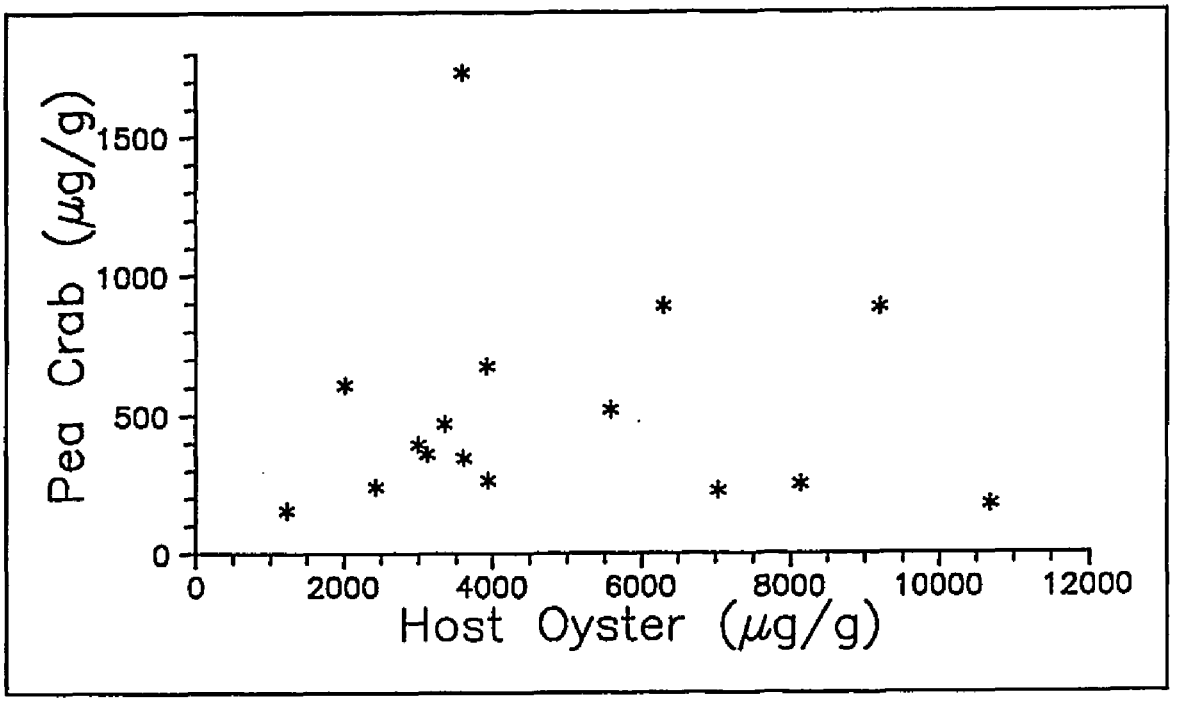

Figure A-1. Comparison of zinc concentrations of whole pea crabs and soft tissue zinc concentrations of host oysters.

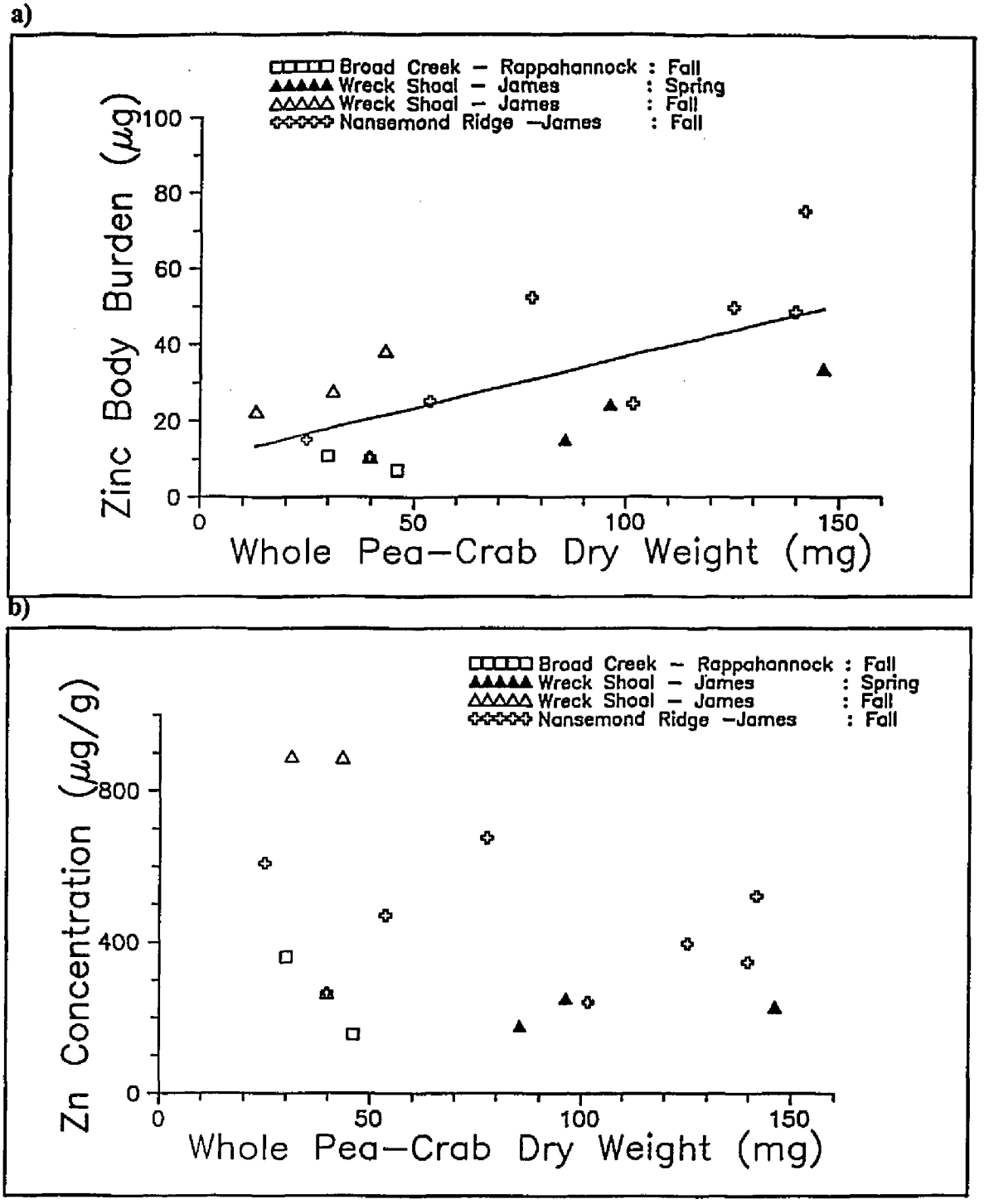

Figure A-2. Variation of zinc (a) body burdens and (b) concentrations of pea crabs. The lines are determined by the least squares fits.

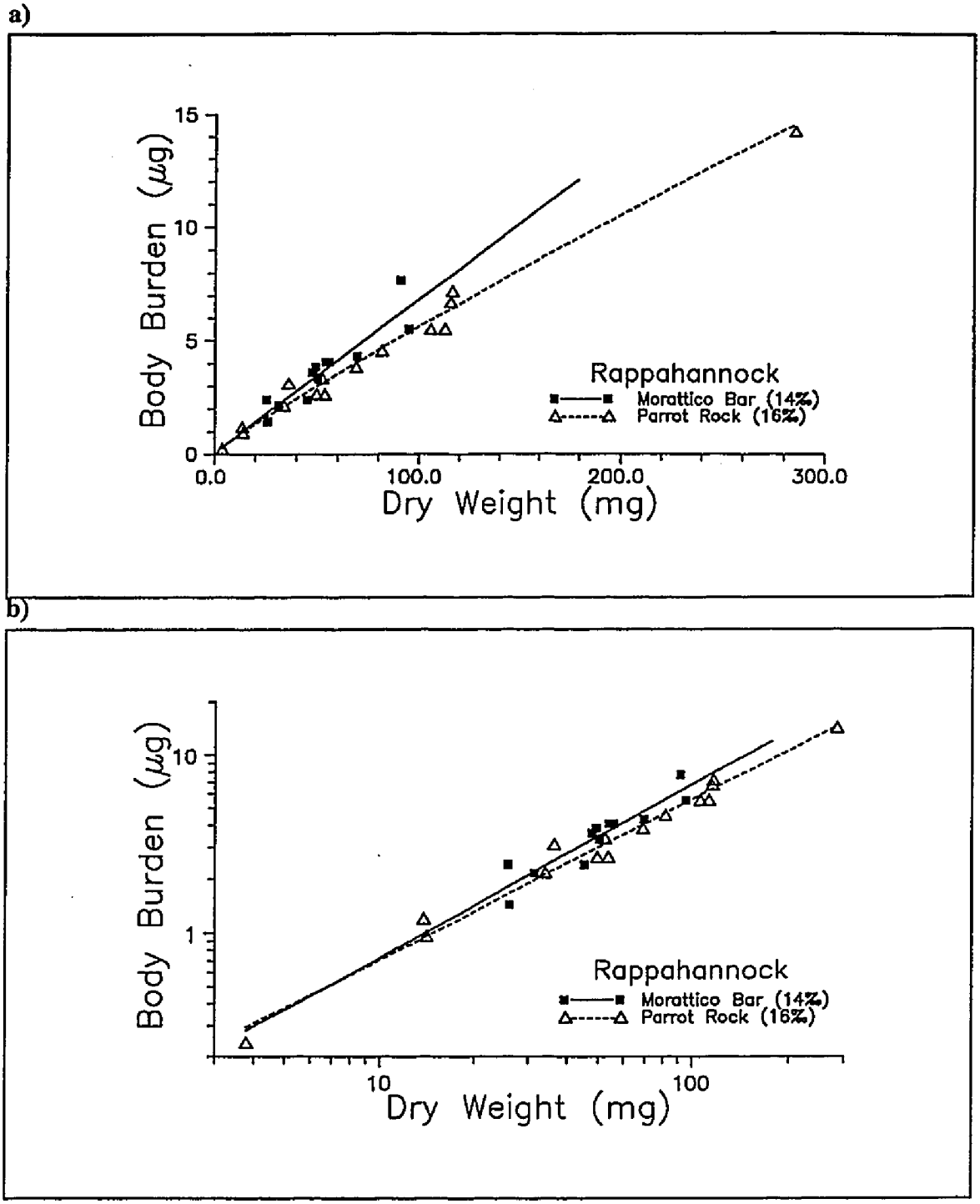

Figure B-1. Zinc body burdens of Rappahannock River hooked mussels in (a) linear scales and (b) log-log scales. The lines are the power function fits.

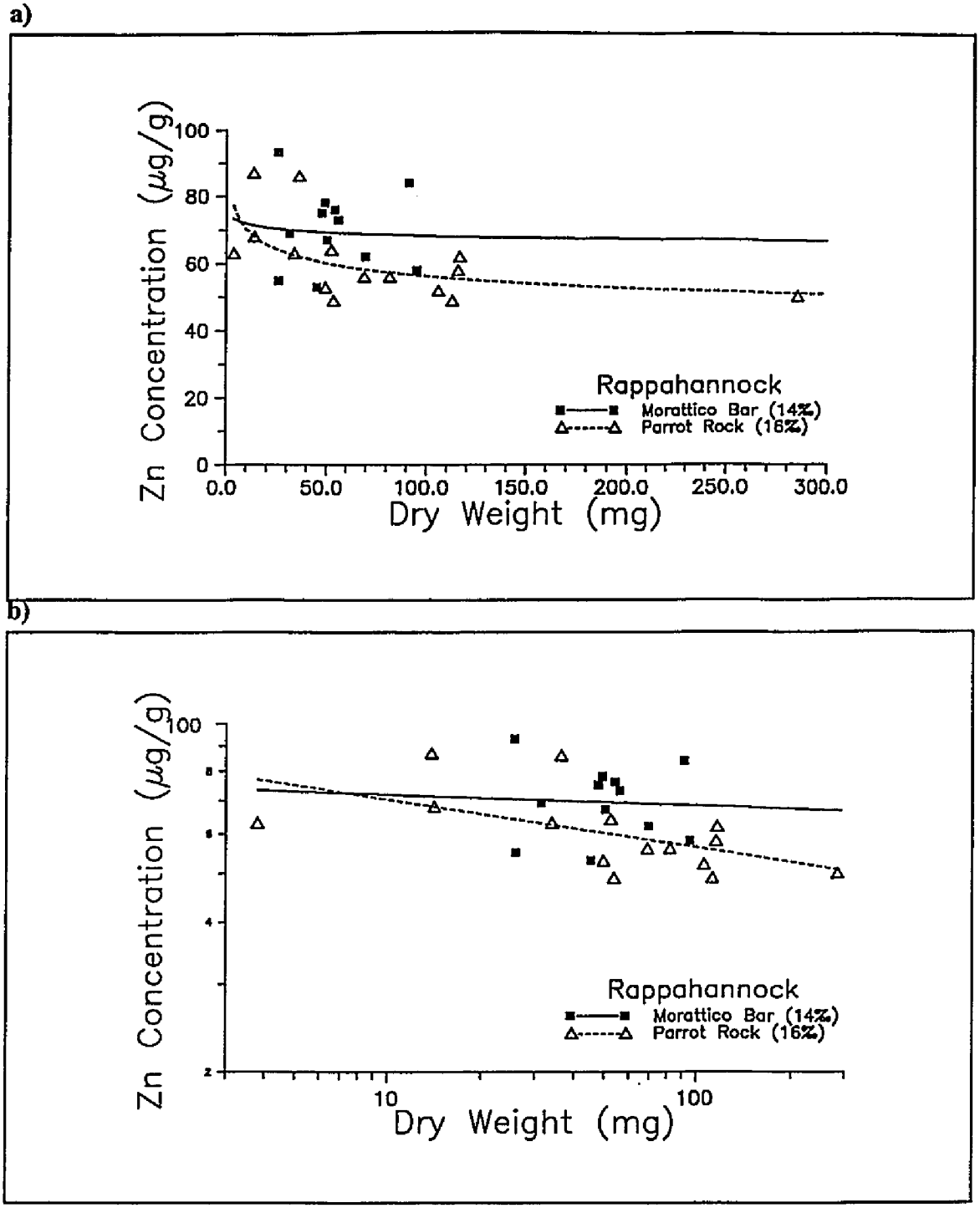

Figure B-2. Zinc concentrations of hooked mussels in (a) linear scales and (b) log-log scales. The lines are the power function fits. If the lines in Fig. B-1 were linear [b = 1, $y = aW^b = aW$], the lines in Fig. A-4 would have been parallel to x-axis [$\log (y/W) = \log C = \log a + (b-1) \log W = \log a$].

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