EFFECTS OF SALINITY AND TEMPERATURE ON THE RESPIRATORY PHYSIOLOGY OF THE DUNGENESS CRAB, CANCER MAGISTER, DURING DEVELOPMENT

by

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Cancer magister, the Dungeness crab, occurs in different habitats during its life cycle, habitats which vary widely in the magnitude of salinity and temperature changes. Cancer magister hemocyanin also changes in structure and oxygenation properties during development. The following question was considered in this thesis: what are the effects of environmental salinity and temperature on metabolic rates, ionic and osmotic regulation and hemocyanin oxygen affinity in C. magister during development.

Metabolic rates and hemolymph ionic and osmotic concentrations were measured in the megalopa, 1st juvenile, 5th juvenile and adult crab eight hours after acute exposure to 100% seawater (=32 ppt), 75% seawater and 50% seawater at both 10°C and 20°C. The oxygen binding properties of the whole hemolymph from these stages in 100% seawater at 10°C

was determined. The effects of calcium and magnesium on the oxygen affinity of purified hemocyanin from different stages were also determined.

In 100% seawater, routine metabolic rates of the four stages scale with body mass over the size range, 0.05 gm to 500 gm. The Q_{10} (10°C to 20°C) for the megalopa is higher in 75% seawater and 50% seawater than in 100% seawater. For the 1st juvenile, 5th juvenile and adult the Q_{10} values (10°C to 20°C) are independent of salinity. The megalopa, 1st juvenile and 5th juvenile are weaker regulators of hemolymph chloride, sodium and osmotic concentrations than the adult. The megalopa and adult, unlike the 1st juvenile and 5th juvenile, strongly regulate hemolymph calcium in reduced salinity. In 100% seawater hemolymph magnesium is significantly higher in the megalopa, 1st juvenile and 5th juvenile than in the adult. The oxygen affinities of whole hemolymph from the four stages are indistinguishable when adjusted for endogenous L-lactate concentrations; the Bohr coefficients are not significantly different among stages. The effect of magnesium on oxygen affinity of purified adult hemocyanin is influenced by proton concentration; the effect of calcium is independent of proton concentration. In 100% seawater, endogenous inorganic ion concentrations in the whole hemolymph of the various stages reduce the intrinsic stage specific differences in hemocyanin oxygen affinity.

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DEDICATION

To the memory of Dr. Robert C. Terwilliger: teacher, scholar and friend

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CHAPTER I

INTRODUCTION

The various processes by which organisms adapt to their environment have fascinated biologists for a long time. Contemporary studies of adaptational biology, spanning the range from molecules to organisms, are reviewed by Hochachka and Somero (1984) and by Prosser (1986). Adaptation to the environment may involve changes in gene expression, physiological processes and behavior. These responses can be caused by environmental cues or as part of a genetically determined developmental process which coincides with changes in mode of existence or habitat.

Estuarine and Ocean Habitats

Chemical and physical parameters in estuarine waters are highly variable. There are spatially and temporally predictable changes in the chemical and physical environment in the estuary related to tidal, diurnal and seasonal patterns and to the physical structure of the estuary (Newell, 1976). Morris and Taylor (1983), for example, measured salinity, temperature, pH and partial pressure of oxygen (pO_2) in a temperate intertidal area. Large diurnal fluctuations in all of these physical and chemical

parameters were observed. In contrast to highly variable estuarine areas the ocean is a more stable habitat with regard to temperature, salinity, pO_2 and pH.

Responses to Environmental Changes

General

There are many ways in which planktonic and benthic animals within the estuary cope with changes in environmental conditions, including behavioral, morphological and physiological mechanisms. Behaviorally, motile organisms can move to evade unfavorable conditions: i.e. seasonal migrations, diurnal and tidally linked activity patterns and selection of suitable microhabitats. Reynolds and Casterlin (1979) found that Homarus americanus is capable of behavioral thermoregulation in the lab. Non-motile organisms such as bivalves, snails and tube dwelling worms, can reduce or cease activity for varying lengths of time to avoid or reduce the effects of deleterious environmental conditions. These organisms can also take advantage of their relatively impermeable shells and tubes to seal themselves in when they are exposed to stressful conditions.

Physiologically, organisms have two options for dealing with environmental changes: regulating their internal conditions or conforming to whatever is changing around them. Estuarine invertebrates vary greatly in their ability

to either regulate or tolerate changes in ambient salinity. Regulation of internal ionic and osmotic concentrations can be energetically costly, requiring the active pumping of ions. However, passively letting the internal ionic and osmotic concentrations vary may exceed the tolerance of the molecular and cellular processes in the animal.

Estuarine invertebrates must conform to ambient temperature and are unable to regulate their body temperature except by behavioral mechanisms, such as moving to and selecting particular microhabitats. It is possible for a mobile animal to move to a less stressful place but the effectiveness of this is limited by how far and how fast the animal can move. By conforming to environmental temperature changes, the animal's metabolic systems may become so stressed that the ability to transport sufficient oxygen and maintain physiological processes is surpassed.

Different stages in the life cycle of a species are in many respects like separate types of animals, especially in those species with distinctly different larval types. There are many crustacean species with benthic adults and planktonic larval stages. The different stages may encounter very different magnitudes of change in environmental conditions. Survival through the larval stages has long been considered a critical point for many marine invertebrates. In this light, many researchers have examined the effects of various salinities and temperatures on the survival, growth,

development time and metabolic rates of the larvae of numerous species of crustaceans (reviewed in Sastry, 1983).

Oceanic planktonic larvae, although many are mobile and migrate vertically, tend to move within a large and relatively uniform water mass. In this situation they tend not to be exposed to large or sudden changes in either salinity or temperature. Planktonic larvae may encounter changes in salinity and temperature as they move within the water column in the estuary. Estuaries typically have an outward flowing freshwater lens at the surface and an inward flowing salt wedge at the bottom. Benthic estuarine animals are exposed to large and rapid changes in salinity and temperature with each change of the tides.

Crustaceans

There are a number of investigations of the effects of environmental change on many aspects of the biology of estuarine and intertidal animals (reviewed in Newell, 1979). A group which has been the focus of a great deal of research is the decapod Crustacea. Decapods are relatively large animals and a number of species occur in high abundance in estuaries. Many estuarine decapods are of great commercial value as well as being ecologically important species as predators and scavengers.

Two physical parameters of particular importance to estuarine organisms are salinity and temperature. Changes in

environmental temperature often have a direct effect on oxygen uptake in crustaceans (reviewed in Vernberg, 1983). A decrease in environmental temperature generally reduces the rate of oxygen consumption; conversely, increases in temperature cause an increase in metabolic rate. Changes in environmental salinity affect crustacean ionic and osmotic regulation (reviewed in Mantel and Farmer, 1983). Some species, hyperosmoregulators, are able to strongly regulate hemolymph osmolality above the ambient level in reduced salinity. Those species which maintain hemolymph osmolality below ambient levels are hypoosmoregulators. There are also species which are able to maintain hemolymph osmolality above ambient levels but as ambient salinity changes the hemolymph changes in parallel; these are hyperosmoconformers. Salinity changes also affect respiratory physiology in terms of oxygen uptake (oxygen consumption of the whole animal) via such processes as ventilation, gas exchange, permeability, oxygen transport by the respiratory protein and oxygen utilization by the tissues (reviewed in Kinne, 1964; Cameron and Mangum, 1983; Wheatly, 1988). Kinne (1964) described four types of metabolic response to changes in environmental salinity. In type 1 response, the metabolic rate increases in salinity less than the normal range and/or decreases in salinity higher than normal. In type 2 response, the metabolic rate increases in salinities above or below the normal salinity

range. In type 3 response, the rate decreases in salinities above or below the normal salinity range, and in type 4 response, the metabolic rate is not affected by changes in salinity. Temperature and salinity may have interactive effects in a variety of ways, influencing the range of tolerance to salinity and temperature, as well as survival, growth, development and metabolic rates (reviewed in Kinne, 1964; Vernberg, 1983). Typically, salinities below normal decrease the tolerance to increased temperature, and above normal salinities increase temperature tolerance (Kinne, 1964).

Hemocyanin

As described above the estuary is a stressful and variable environment. The ability to maintain adequate oxygen transport under many different environmental and physiological conditions is of primary importance for survival in any animal. The oxygen transport molecule utilized by the decapod crustaceans is the copper containing respiratory protein, hemocyanin. This extracellular protein circulates in the hemolymph. Both hemolymph and hemocyanin are at an important interface between the organism and the environment and are also important in the animal's internal transport of metabolites. The structure of the arthropod hemocyanin molecule and the reversible binding of oxygen to hemocyanin have been studied in detail (see Bonaventura and

Bonaventura, 1980; Mangum, 1980; Van Holde and Miller, 1982; McMahon, 1985; Markl, 1986 for reviews). Arthropod hemocyanins have molecular weights ranging from 450 kilodaltons (kD) to 3,200 kD. The smallest polymeric unit found in the hemolymph is a hexamer with a molecular weight of 450 kD. The hexamers are comprised of 75 kD subunits; each subunit contains two copper atoms and combines reversibly with one molecule of oxygen. The hexamers may aggregate into multiples of 2, 4, 6 and 8.

Certain subunits have been shown to play distinct structural roles within the polymeric molecules. Some are important in linking the hexamers and others appear to be important within each hexamer (Decker et al., 1989).

The hemocyanin of adult brachyuran crabs is typically composed of a two-hexamer aggregation with a molecular weight of approximately 900 kD made up of a species specific heterogeneous combination of subunits. The sedimentation coefficient of the two-hexamer is approximately 25S. A small amount of single hexamer, 16S, hemocyanin molecules is usually present in the hemolymph of adult crabs.

Interspecific comparison of adult crab hemocyanins reveals a complex pattern of hemocyanin subunit composition (Markl, 1986; Markl et al. 1986). Evidence has been presented that the differences in subunit composition in crab hemocyanin may play a role in oxygen binding function. Subunits from Cancer magister hemocyanin have been

artificially reaggregated into 25S molecules which have different oxygen binding properties than the native 25S hemocyanin (Graham, 1983). In Callinectes sapidus oxygen affinity of hemocyanin from estuarine and ocean populations is different and this functional difference appears to correlate with differences in subunit composition (Mangum and Rainer, 1988).

Not only is there variation in subunits between crustacean species, there are also ontogenic changes in hemocyanin subunit composition in those species that have been studied, Cancer magister (Terwilliger and Terwilliger, 1982; Terwilliger et al., 1986), Cancer productus (Wache et al., 1988), Hyas araneus and Carcinus maenas (Markl, 1986) and Homarus americanus (Olson et al., 1988). The changes in C. magister hemocyanin will be discussed in more detail in the next section.

Studies of the effects of hemolymph organic and inorganic factors and of salinity and temperature on the oxygen equilibrium properties of hemocyanin from a number of adult crustaceans have been reviewed recently (Mangum, 1980, 1983; Van Holde and Miller, 1982; McMahon, 1985). Hemocyanin oxygen affinity and cooperativity are dependent on pH; within the physiological pH range there is a normal Bohr shift (Mangum, 1983). The magnitude of the Bohr shift varies between species. Oxygen affinity increases as temperature decreases (Johansen et al., 1970; Truchot, 1973, 1975; Mauro

and Mangum, 1982; Burnett et al., 1988; Morris and Bridges, 1989). Environmental salinity usually affects crustacean hemolymph salinity which in turn alters hemocyanin function in the hemolymph. Oxygen affinity decreases at low salinity (Truchot, 1973, 1975; Weiland and Mangum, 1975; Mangum and Towle, 1977) and/or increases after the crustacean acclimates to higher salinity (Taylor et al., 1985). This response is variable between species and acclimation times. These salinity effects are often mediated by changes in specific ion concentrations, especially calcium and magnesium, in the hemolymph (Taylor et al., 1985; Morris et al. 1988). Calcium and magnesium have been shown to have a strong effect on the oxygen equilibrium of the hemocyanin from several crustacean species (Larimer and Riggs, 1964; Truchot, 1975). There are also several effectors or modulators of hemocyanin oxygen equilibria which are organic molecules. Truchot (1980) identified L-lactate as a factor increasing oxygen affinity in Carcinus maenas. L-lactate has subsequently been identified as a factor in a variety of crustaceans (Booth et al., 1982; Graham et al., 1983; Mangum, 1983b; Bridges et al., 1984; Taylor et al., 1985; Bridges and Morris, 1986; Morris and Bridges, 1989). Urate has also been identified as an effector of oxygen binding of hemocyanin from the crayfish Austropotamobius pallipes (Morris et al., 1985: Morris et al., 1986). Morris and McMahon (1989 a,b) describe a potentiating effect of

dopamine on oxygen affinity of hemocyanin from Cancer magister.

There are to date only two published studies of larval hemocyanin function (Terwilliger et al., 1986; Olson et al., 1989). The physiological significance and timing of the ontogenic structural and functional changes in crustacean hemocyanin are unknown.

Cancer magister

Cancer magister, the Dungeness crab, is a species which uses different portions of the estuary as well as coastal waters throughout its life cycle. The embryos, which are attached to the pleopods of the female, hatch in December through April in coastal Oregon waters. The newly hatched larvae go through five zoeal stages, all of which are planktonic in ocean waters. The final larval stage is the megalopa, which is also planktonic. The megalopa is an extremely active swimmer. From mid-April through the beginning of July megalopae enter the coastal and estuarine waters of Oregon. The precise mechanism of transport from oceanic to coastal waters is not clear (Lough, 1975). After the megalopae appear on-shore, they soon metamorphose into 1st instar juvenile crabs and join the benthic community within the bay and in the nearshore areas. The juvenile crabs molt frequently through their first summer and are found in large numbers on the tideflats. Cancer magister

reaches sexual maturity at 2-3 years, approximately 10 cm carapace width (MacKay, 1942; Butler, 1961; Wild and Tasto, 1983). The adult crabs are found mainly in the deeper channels in the estuary and in the nearshore coastal waters.

Due to their distribution and utilization of different habitat types, the megalopae, juveniles and adults of Cancer magister are exposed to different magnitudes of change in environmental salinity and temperature. Since the megalopae are planktonic and tend to move with the water mass in which they enter the bay, the changes in temperature and salinity to which they are exposed are not likely to be extreme. In contrast, the late spring and early summer low tides in Coos Bay occur in the morning and rapid solar heating of the tideflats occurs. Salinity on the tideflats is also low when the tide is out because the lens of fresh water on the surface passes down the flats as the tide recedes. Therefore the juveniles on the tideflats are exposed to extreme fluctuations in salinity and temperature. The adult crabs inhabit the subtidal portions of the bay which vary little in temperature over the tide cycle and vary far less in salinity than the tideflats in summer months. In the winter months the salinity of the channels changes widely with the tide because of the increased fresh water input from rain.

Along with the changes in mode of existence, habitat and concomitant variations in environmental conditions outlined in the preceding paragraphs there are differences

in the respiratory protein, hemocyanin, during the life cycle of C. magister (Terwilliger and Terwilliger, 1982). Adult C. magister hemocyanin is predominately in the form of 25S (two-hexamer) assemblages of 5S subunits with a trace of 16S (single hexamer) molecules. The larval and juvenile hemocyanin is predominately in the 16S form. The larval and juvenile hemocyanin is lacking one of the subunit types found in the adult. The stoichiometry of the other subunits changes as the crabs develop from the juvenile to the adult. There are also differences in hemocyanin function in purified samples from these different stages (Terwilliger et al., 1986). The oxygen affinity of the juvenile is less than that of the adult, under the same experimental conditions. Both the juvenile and adult hemocyanin have essentially the same sensitivity to L-lactate and pH (Terwilliger et al., 1986). Lactate is an end product of anaerobiosis in C. magister and has been shown to affect hemocyanin oxygen binding (Graham et al., 1983). Cancer magister hemocyanin has also been shown to be sensitive to dopamine (Morris and McMahon, 1989a,b).

Many aspects of the physiology and ecology of C. magister have been studied in depth, including respiratory physiology of the adult (Johansen et al., 1970; McDonald et al., 1980), the structure and function of adult hemocyanin (Larson et al., 1981; Ellerton et al., 1970; Wajcman et al. 1977; Graham et al., 1983), ionic and osmotic regulatory

abilities of the adult (Jones, 1941; Alspach, 1972; Engelhardt and Dehnel, 1973; Hunter and Rudy, 1975) and larval transport (Lough, 1975, 1976). Larvae have been reared in the lab and optimum salinity and temperature conditions for development and growth have been determined (Reed, 1969), however the ontogeny of ionic and osmotic regulation is unknown. In addition, the respiratory response of adult C. magister to changes in environmental salinity and temperature are unknown.

The Dungeness crab, Cancer magister, is an ideal animal in which to study the ontogeny of response to environmental change at the whole animal and at the molecular and physiological levels. Adult C. magister are large and abundant in the Coos River estuary. The reproductive cycle and life cycle of the species is well documented (MacKay, 1942; Butler, 1961; Wild and Tasto, 1983) and larval and juvenile stages are generally available in the field in a predictable seasonal pattern.

The purpose of the present study is to examine the effects of short term, tidal changes (6-8 hr) of environmental salinity and temperature on metabolic rates, ionic and osmotic regulation and hemocyanin oxygen affinity in C. magister during development. This study is unique in that not just the whole animal response of different developmental stages are examined but also some internal and molecular responses are elucidated.

CHAPTER II

OXYGEN CONSUMPTION

Introduction

Studying the rate of oxygen consumption under different environmental conditions provides insight into both the physiological capacities and limitations of a species, physiological limitations which may restrict the distribution of a species to particular habitats or geographical ranges. Measuring the rate of oxygen consumption of an organism is a method of determining its metabolic rate. Other methods include calorimetry, the measurement of total heat production of the organism, and calculations of the difference in energy in the food ingested and the energy value of the excreta (primarily feces and urine). For organisms which are primarily respiring aerobically, the measurement of oxygen consumption is a suitable estimation of metabolic rate.

Temperature and salinity are both dominant environmental factors affecting metabolism in estuarine invertebrates. Salinity and temperature change with every tidal cycle in the estuary.

There are data available on metabolic response to salinity and/or temperature for adults of several species of

decapod crustaceans. These include Hemigrapsus nudus and Hemigrapsus oregonensis (Dehnel, 1960), Panopeus herbstii (Dimock and Groves, 1975), Carcinus maenas (Taylor, 1977), Callinectes sapidus (Findley et al., 1978), Cancer magister (Prentice and Schneider, 1979), Cambarus acuminatus (Pruitt and Dimock, 1979), Palaemon elegans (Morris and Taylor, 1985) and Palaemonetes antennarius (Dalla Via, 1987). This topic has also been reviewed by Scholander et al. (1953), Kinne (1964) and Vernberg (1983). Generally, oxygen consumption increases with increasing temperature. Q_{10} values for many crustacean species range from around 1.0 to as high as 4.0. A Q_{10} of 1.0 indicates thermal insensitivity, whereas a Q_{10} of 4.0 means that the rate of oxygen consumption quadruples with a ten degree increase in temperature. Q_{10} values tend to be lower at lower temperatures, but may also vary with acclimation temperature. Kinne (1964) outlines four different metabolic responses to salinity: type 1, metabolic rate increases in salinity less than the normal range and/or decreases in salinity higher than normal; type 2, the metabolic rate increases in salinities above or below the normal salinity range; type 3, the rate decreases in salinities above or below the normal salinity range, and type 4, the metabolic rate is not affected by changes in salinity.

Data on the effects of salinity and/or temperature on oxygen consumption rates of early life stages of decapod

crustaceans are also available, including studies on Uca spp. (Vernberg and Costlow, 1966), Emerita talpoida and Libinia emarginata (Schatzlein and Costlow, 1978), Cancer irroratus (Sastry and McCarthy, 1973; Sastry, 1978, 1979), Cancer borealis (Sastry and McCarthy, 1973), Cancer productus and Panulirus interruptus (Belman and Childress, 1973), Pagurus criniticornis (Vernberg et al., 1981), Macrobrachium holthuisi (Moreira et al., 1980) and Emerita brasiliensis (Moreira et al., 1981). The temperature and salinity sensitivity of metabolic rates of different developmental stages may vary greatly. Changes in sensitivity to temperature and salinity between the life stages in a given species tend to correspond to such ecological factors as changes in habitat utilization or seasonal shifts in environmental salinity and/or temperature.

In areas with a semi-diurnal tide the duration of exposure of estuarine organisms to potentially stressful environmental conditions as a result of the tides may be as long as 6-8 hours. Cancer magister megalopae, juveniles and adults inhabit different portions of the estuary and are therefore exposed to different combinations and extremes of salinity and temperature.

The goal of this study was to determine the metabolic responses of several life stages of C. magister over the range of salinity and temperature conditions each life stage

encounters. Determining the oxygen consumption rates under naturally occurring combinations of salinity and temperature for different instars, including the adult crab, will enable us to better define ontogenic changes in physiological capacities which may relate to habitat selection and utilization throughout the life history of the species.

The rate of oxygen consumption of C. magister megalopae, 1st juveniles, 5th juveniles and adults were measured over a period of a tidal cycle, eight hours, after acute exposure to 100%, 75% and 50% seawater at 10°C and 20°C.

Materials and Methods

Animals

Cancer magister megalopae were collected from the surface waters near the mouth of the Coos River estuary with a dip net. Since the megalopae were usually in a premolt condition and would molt within 48-72 hours of being captured, they were used as soon as possible. During the brief period the megalopae were maintained in the lab they, were kept in running unfiltered aerated seawater and given no food. Seawater was pumped from near the mouth of the Coos River where salinity varies in the range of 30-33 ppt, and temperature varies from 9-15°C. The high salinities and high temperatures occur in the drier season, usually May-October. Low salinities and temperatures are typical of the rainier

season, usually November-April.

Juvenile crabs were reared from field caught megalopae in 10 gallon aquaria with running seawater and aeration. They were fed shelled mussels, pieces of fish or squid 3 to 5 times a week. Food was not given for 24 hours prior to experiments to ensure that the animals were in a post-absorptive state and to reduce fouling of the respirometers. Molt stage determination was based on time since most recent molt and hardness of the carapace. Only individuals judged to be in intermolt were used.

Adult male C. magister larger than 12 cm in carapace width were collected in the Coos River estuary next to the deep water ship channel near the river mouth. Adult crabs were maintained in large (1000 L.) holding tanks with running seawater and aeration. The crabs were fed with shelled mussels, pieces of fish or squid 2 to 3 time a week. Food was withheld for 24-48 hours prior to experiments as with the juveniles.

All stages were maintained in holding facilities exposed to natural light/dark cycles and ambient Coos Bay seawater salinity and temperature.

Measurement of Environmental Conditions

Measurements of salinity and temperature on the tideflats and in the deeper channels near the tideflats were made at different stages of the tide to determine the ranges

of salinity and temperature to use in laboratory experiments. Temperature was measured with a field thermometer (Fisher Scientific). Water samples were brought back to the lab and the osmolality determined using a Wescor 5500 vapor pressure osmometer. Throughout this thesis 100% seawater refers to Coos Bay seawater at 32 ppt or ~950 mOsm/kg.

Oxygen consumption measurements

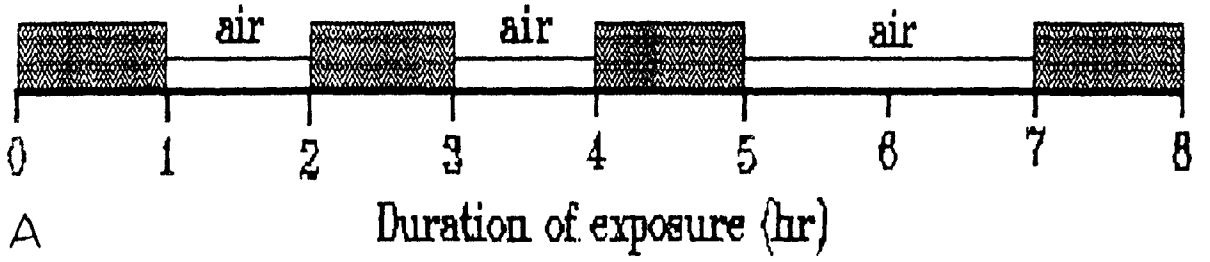
Whole animal oxygen consumption was measured in closed respirometers. Respirometers of 80 and 100 ml were used for the megalopae and 1st juveniles. Respirometers of 250 and 400 ml were used for the 5th juveniles and 5.5 L respirometers were used for the adult crabs. Seawater of the appropriate salinity, 100%, 75% and 50% seawater, was made using filtered seawater diluted with distilled water. Salinity measurements were made with a refractometer (American Optical Co.). The desired temperature, 10°C or 20°C, was maintained for the entire measurement period by immersing the respirometers in a thermostatted recirculating water bath. The rate of decrease of oxygen concentration in the sealed respirometer was measured with either a YSI (Yellow Springs Instruments) Model 5739 oxygen probe and a YSI Model 57 dissolved oxygen meter or a YSI Model 5420A oxygen probe (stirring boot removed) connected to a YSI Model 54 dissolved oxygen meter.

Animals were transferred from the holding aquaria into the respirometers immediately prior to the experiments. Animals remained in the respirometers for the entire eight hour measurement period. All measurements were made during natural daylight hours. The megalopa and 1st juvenile metabolic rates were measured for periods of one hour followed by one hour intervals to reoxygenate the water by bubbling air through the respirometers, except for the 5^{th}-7^{th} hours when there was a two hour aeration interval (Fig. 1A).

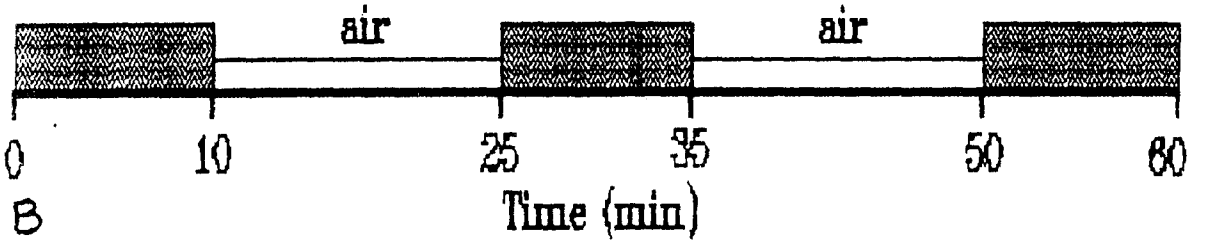
The 5th juveniles were given air breaks within each one hour measurement period in order to make sure that the oxygen level in the respirometer was not limiting. Within each one hour period there were three separate ten minute measurement periods interspersed with 15 minute intervals during which air was bubbled through the respirometers (Fig. 1B). This pattern was repeated for each one hour measurement period throughout the eight hour span, i.e. 0-1 hr, 2-3 hr, 4-5 hr and 7-8 hr as in Figure 1A.

The oxygen consumption of the adult was measured for the first and last twenty minutes within each one hour measurement period with a twenty minute aeration interval (Fig. 1C). As with the megalopa, 1st juvenile and 5th juvenile, oxygen consumption was measured for the period 0-1 hr, 2-3 hr, 4-5 hr and 7-8 hr (as in Fig. 1A) after acute exposure to the desired salinity and temperature

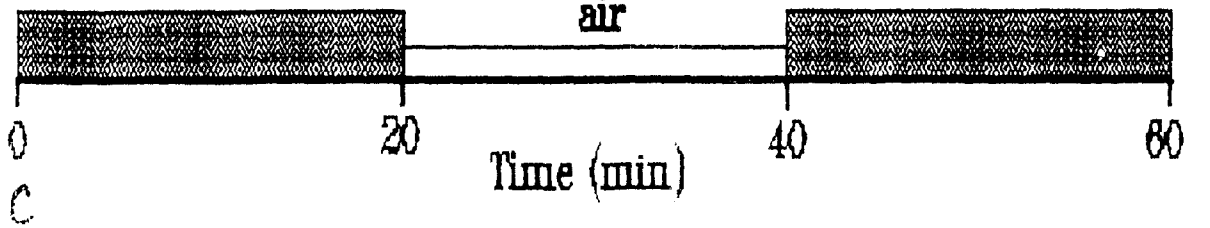
Figure 1. Schematic of the timing of measurement of oxygen consumption and aeration intervals. (A) Measurement periods from 0 to 8 hr. (B) Measurement periods within each one hour period for 5th juveniles. (C) Measurement periods within each one hour period for adults.



A Duration of exposure (hr)



B Time (min)



C Time (min)

combinations.

Within each hour of oxygen consumption measurement, the rates measured during the shorter subdivisions of the hour were pooled to give the average rate of oxygen consumption over that hour for that animal.

Wet weights for each individual were determined at the end of each eight hour experiment. They were held face down in order to drain as much water as possible from the branchial chambers and blotted with paper towels until they were no longer wet. Megalopae in the weight range of 0.033 gm to 0.055 gm (3 to 4 mm carapace width), 1st juveniles from 0.065 gm to 0.125 gm (6 to 8 mm carapace width), 5th juveniles from 2.53 gm to 5.43 gm (25 to 32 mm carapace width) and adults from 278.7 gm to 495.4 gm (120 to 144 mm carapace width) were used.

Data analysis

The effects of salinity and temperature on the different stages were tested by analysis of covariance (ANCOVA). Multiple comparison of means were made using the Tukey-Kramer method to determine the minimum significant difference (MSD). Statistical significance was accepted at $P < 0.05$. Statistical analyses were done using SYSTAT version 4.1 (SYSTAT, Inc.)

Results

Measurement of field conditions of salinity and temperature in estuarine areas where the different developmental stages of C. magister were abundant proved useful in setting the range of these parameters for laboratory studies. It was found that the mudflat environment of the juveniles in the summer is exposed to changes in temperature from 10°C when the tide is high to 25°C when the tide has receeded and the mudflats are exposed. At the same time salinity drops from 32 ppt (100% seawater) to 16 ppt (50% seawater) as the freshwater lens on the surface passes down the flats. The channels where the adults are found are much more stable with regard to summer temperature and salinity. The winter range of salinity at the bottom of the estuary varies nearly as widely as salinity on the mudflats in the summer and winter.

Figures 2 through 5 show the weight specific oxygen consumption rates of the four stages plotted against time for the eight hour exposure to each of the combinations of salinity and temperature. The Y-axis scale is different in each figure. There is no significant change in the rate of oxygen consumption during the eight hours of exposure to any one combination of salinity and temperature for the megalopa (Fig. 2) or 1st juvenile (Fig. 3). There is a small and statistically insignificant decrease in rate of oxygen consumption over time for the 5th juveniles (Fig. 4) and

adults (Fig. 5). This change in oxygen consumption is most likely related to initial excitement and handling stress from the transfer of the crabs from the aquaria to the respirometer. To avoid any complications from this handling stress effect all further comparisons of rates of oxygen consumption are made between values for the final hour in the eight hours of exposure.

The weight specific rates of oxygen consumption for the final hour of exposure are given in Table 1. There is a strong interactive effect of temperature and salinity on the rate of oxygen consumption of the megalopa (Fig. 2). At 10°C, there is no significant effect of salinity on the rate of oxygen consumption of the megalopa. At 20°C, however, the rate of oxygen consumption rises and is greater in 75% and 50% seawater than in 100% seawater. The interaction of salinity and temperature is also apparent in the Q_{10} values (Table 1) at the different salinities; the Q_{10} values are higher at lower salinity for the megalopa.

The rates of oxygen consumption of the 1st juvenile (Fig. 5), 5th juvenile (Fig. 6) and adult (Fig. 7) are not affected by salinity at either 10°C or 20°C. There is an effect of temperature on the rate of oxygen consumption in these stages. The rate more than doubles ($Q_{10} > 2$) with a ten degree increase in temperature for both the 1st juvenile and the adult (Table 1). The 5th juvenile is less sensitive ($Q_{10} < 2$) to the increase in temperature from 10°C to 20°C.

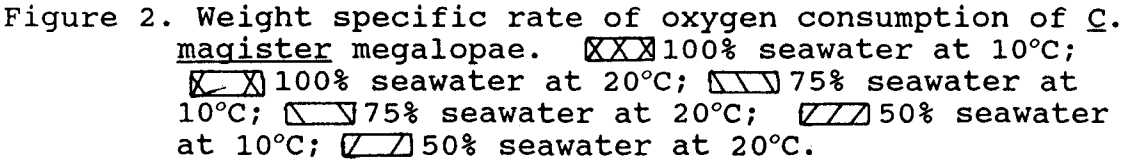
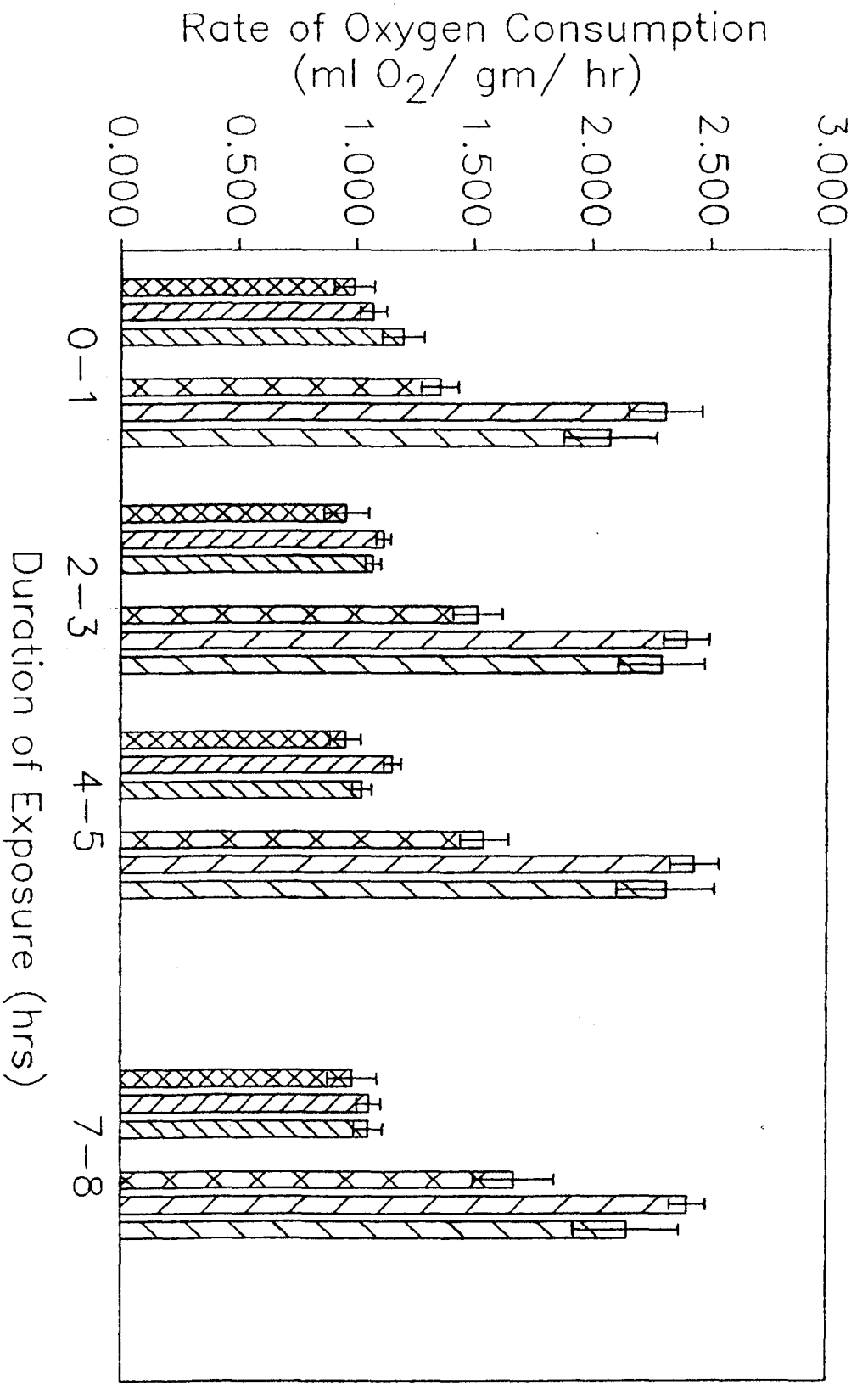


Figure 2. Weight specific rate of oxygen consumption of C. magister megalopae.


Rate of Oxygen Consumption
(ml O_2/ gm/ hr)
0.000
0.500
1.000
1.500
2.000
2.500
3.000
0–1
2–3
4–5
7–8
Duration of Exposure (hrs)

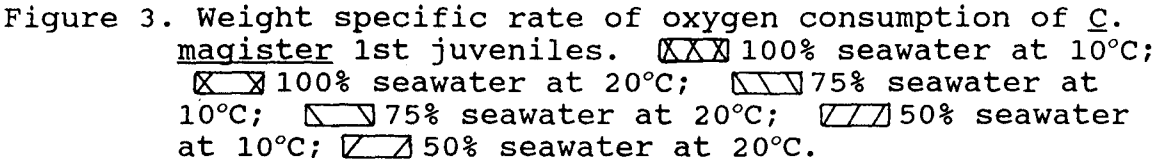


Figure 3. Weight specific rate of oxygen consumption of C. magister 1st juveniles.

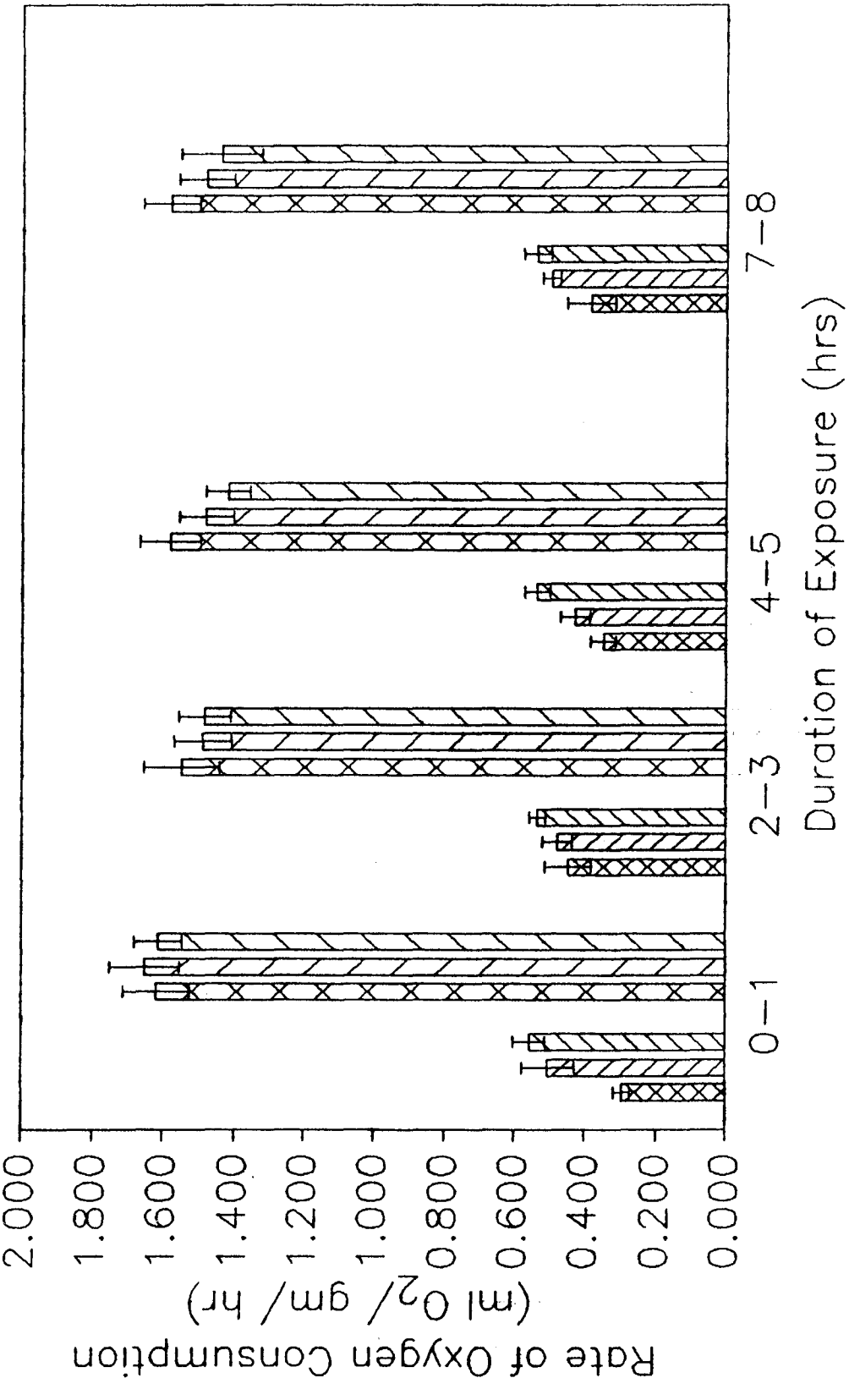
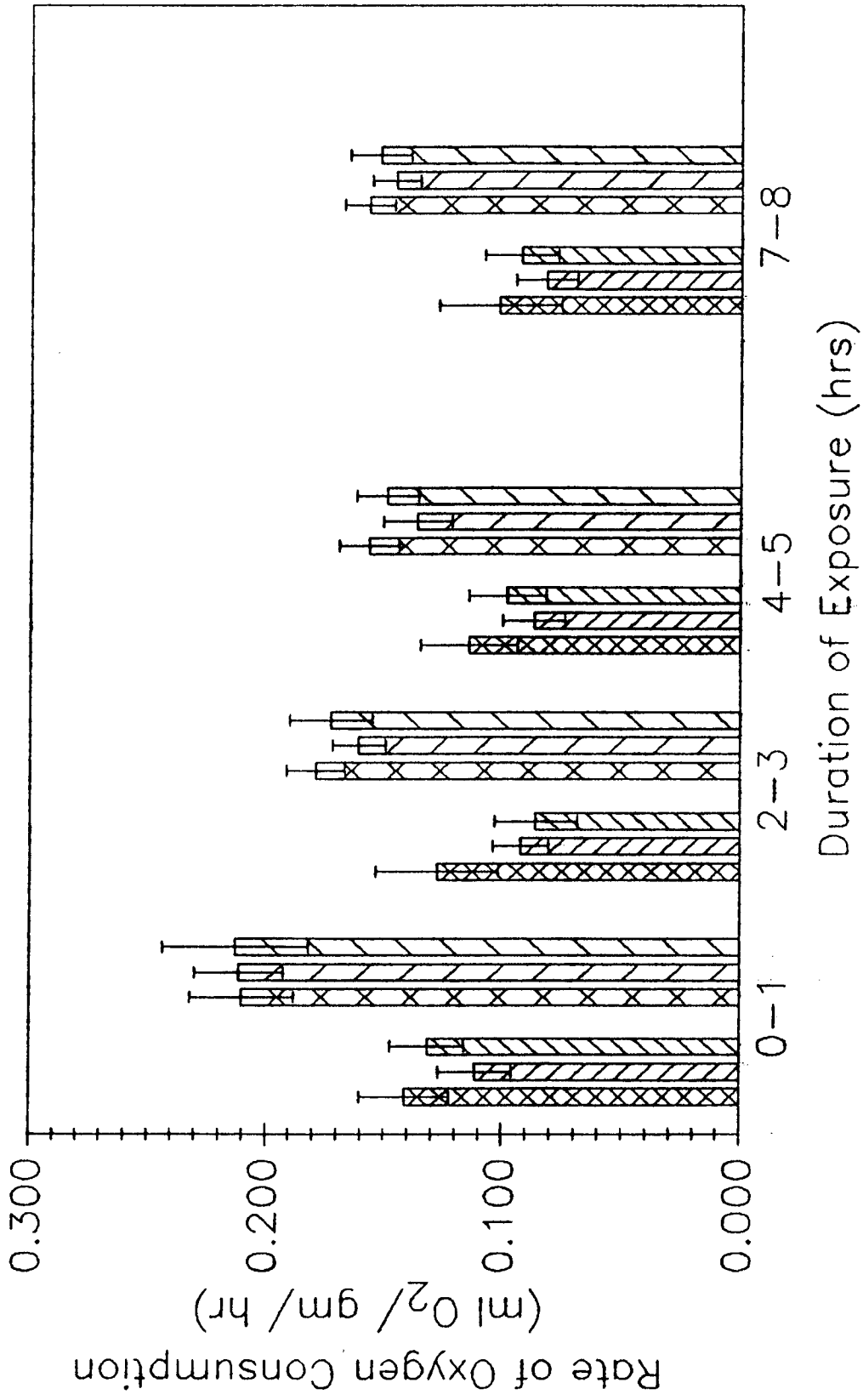

Rate of Oxygen Consumption
(ml O_2/ gm/ hr)
2.000
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0—1
2—3
4—5
7—8
Duration of Exposure (hrs)

Figure 4. Weight specific rate of oxygen consumption of C. magister 5th juveniles. XXX 100% seawater at 10°C; X X 100% seawater at 20°C; \\\ 75% seawater at 10°C; \ \ 75% seawater at 20°C; /// 50% seawater at 10°C; / / 50% seawater at 20°C.


Rate of Oxygen Consumption
(ml O_2/ gm/ hr)
0.300
0.200
0.100
0.000
0-1
2-3
4-5
7-8
Duration of Exposure (hrs)

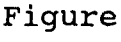


Figure 5. Weight specific rate of oxygen consumption of C. magister adults. 100% seawater at 10°C; 100% seawater at 20°C; 75% seawater at 10°C; 75% seawater at 20°C; 50% seawater at 10°C; 50% seawater at 20°C.

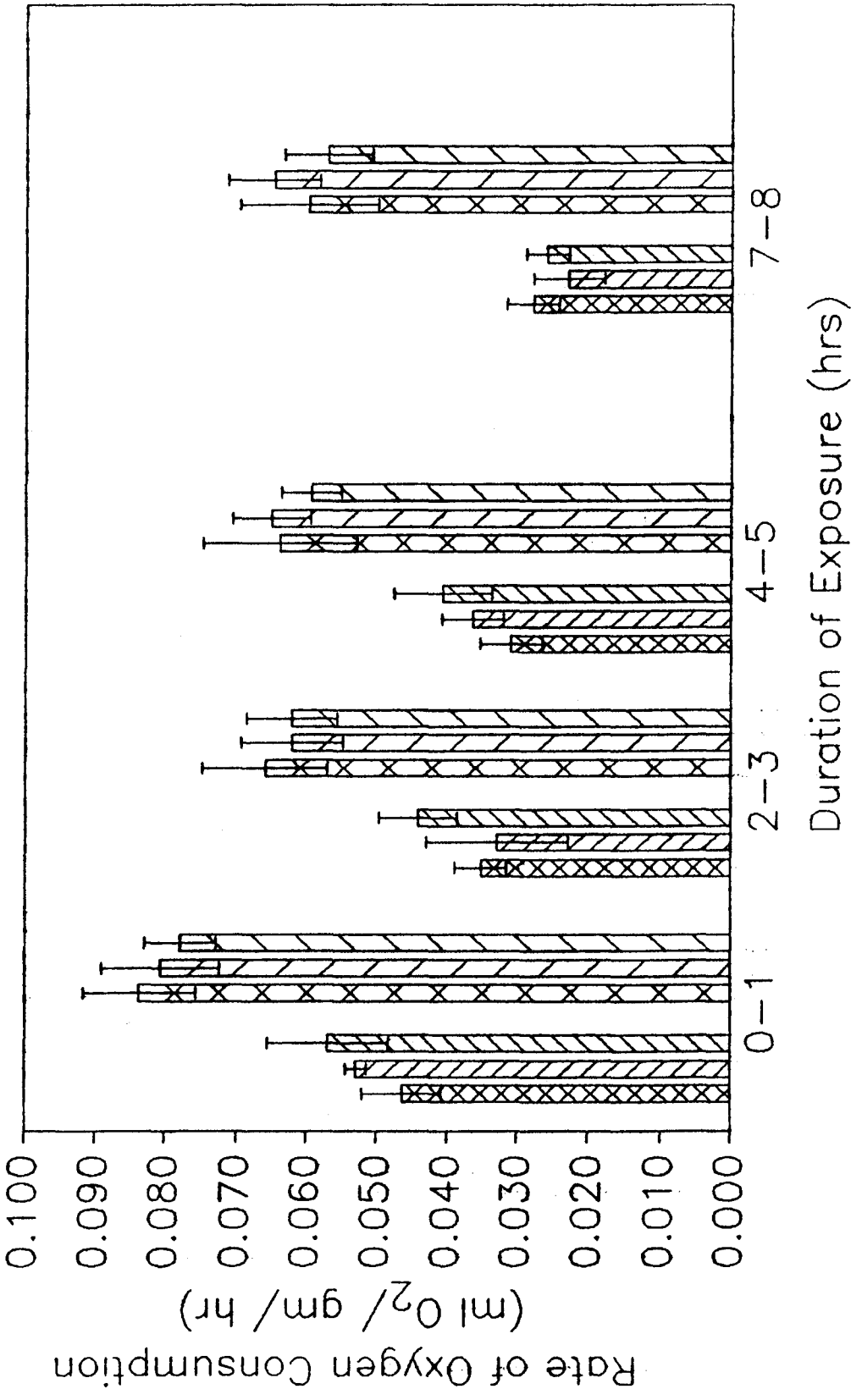

Rate of Oxygen Consumption
(ml O_2/ gm/ hr)
0.100
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0-1
2-3
4-5
7-8
Duration of Exposure (hrs)

Table 1. Weight specific metabolic rates and Q_{10} values for four life stages of C. magister

STAGE	SEAWATER	10°C Q_{02}	10°C n	20°C Q_{02}	20°C n	Q_{10}
MEGALOPA	100%	0.990 ±0.106	10	1.676 ±0.171	9	1.69
	75%	1.060 ±0.051	10	2.380 ±0.076	10	2.25
	50%	1.057 ±0.060	10	2.156 ±0.222	9	2.04
1st JUVENILE	100%	0.386 ±0.067	12	1.582 ±0.079	10	4.10
	75%	0.497 ±0.025	10	1.483 ±0.078	10	2.98
	50%	0.538 ±0.038	10	1.442 ±0.113	10	2.68
5th JUVENILE	100%	0.102 ±0.026	8	0.157 ±0.010	6	1.54
	75%	0.082 ±0.013	8	0.146 ±0.010	8	1.78
	50%	0.093 ±0.015	6	0.152 ±0.013	8	1.63
ADULT	100%	0.028 ±0.004	4	0.060 ±0.010	4	2.14
	75%	0.023 ±0.005	4	0.065 ±0.007	4	2.83
	50%	0.026 ±0.003	3	0.057 ±0.006	4	2.19

Note. Weight specific rate of oxygen consumption, Q_{02}, ml O_2/gm/hr, mean ± S.E.

Examining all four stages in different salinities at either 10°C or 20°C, the regressions of the log of weight specific metabolic rate (Q_{02}) scales linearly with the log of weight (W) (Table 2 and Fig 6).

Table 2. Regression equations for log Q_{02} vs. log W for C. magister (0.033 gm megalopae to 495.4 gm adults) at combinations of salinity and temperature

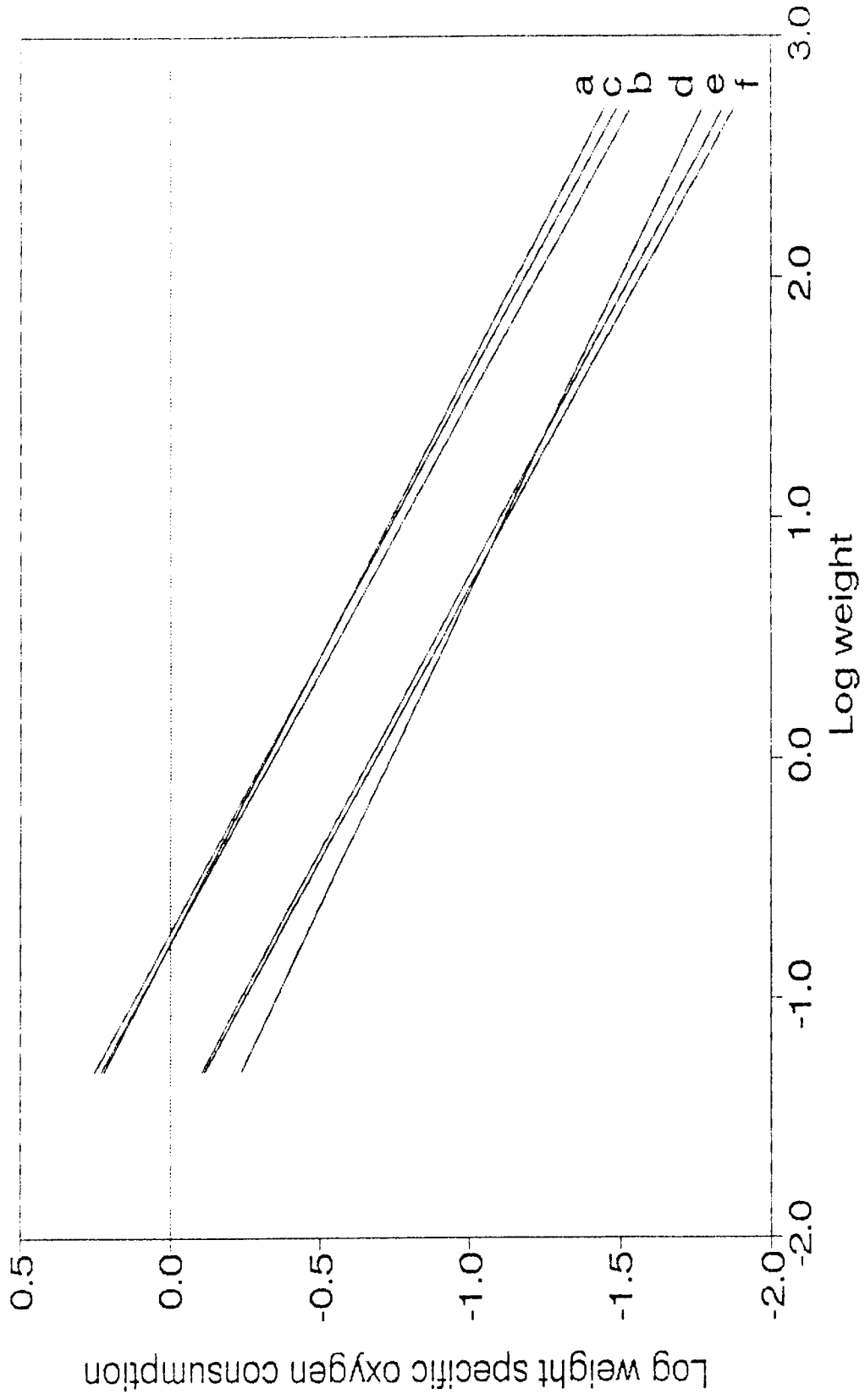
log Q_{02} = -0.383 (log W) - 0.741 (R^2: 0.767),	100% SW, 10°C
log Q_{02} = -0.440 (log W) - 0.691 (R^2: 0.921),	75% SW, 10°C
log Q_{02} = -0.433 (log W) - 0.670 (R^2: 0.937),	50% SW, 10°C
log Q_{02} = -0.416 (log W) - 0.326 (R^2: 0.935),	100% SW, 20°C
log Q_{02} = -0.435 (log W) - 0.317 (R^2: 0.921),	75% SW, 20°C
log Q_{02} = -0.440 (log W) - 0.345 (R^2: 0.922),	50% SW, 20°C.

The slopes of the regressions given in Table 2 are not significantly different. The regression constants (y-intercepts) are different between 10°C and 20°C.

Discussion

A large number of factors, such as nutritional state, culture conditions, molt stage, age, size, activity and rhythms associated with tidal, diurnal, lunar and seasonal cycles, as well as salinity and temperature, can affect the metabolic rates of crustaceans. It is therefore important to define the experimental conditions and the goals of the experiment before making comparisons with other experiments or other species. For example, Findley et al. (1973) reported the rate of oxygen consumption was less for adult

Figure 6. Relationship between log weight specific rate of oxygen consumption and log of wet weight in C. magister. (a) 100% seawater, 10°C; (b) 75% seawater, 10°C; (c) 50% seawater, 10°C; (d) 100% seawater, 20°C; (e) 75% seawater, 20°C; (f) 50% seawater, 20°C.


Log weight specific oxygen consumption
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.0
-1.0
0.0
1.0
2.0
3.0
Log weight
a
c
b
d
e
f

Callinectes sapidus exposed to cyclic changes in salinity compared to the rate of oxygen consumption for those in constant salinity exposure at a similar range of salinities. The experiments for the present study were carried out for eight hours after acute exposure. For this reason comparison to studies using cyclic or acclimated temperature and salinity designs must be made judiciously.

The rates of oxygen consumption reported in the present study would be defined as routine metabolic rates since the animals were able to move freely within the respirometers. The megalopae swam nearly continuously, and there were brief periods of locomotor activity in the other stages. In general, reported metabolic rates for crustaceans are routine metabolic rates since the measurements are on essentially unrestrained animals, in respirometers or crabs fitted with masks and free to move within a given area.

Comparisons of C. magister metabolic rates with the megalopae and juveniles of other species can not be directly made because C. magister at these stages are extremely large relative to other species for which data is available. For the extremely small larval stages of other species the regression coefficient of log Q_{O2} vs. log W is nearly -1.0 (Vernberg et al., 1981) indicating a very different relationship between metabolic rate and size than for the larger megalopae of C. magister.

The rates of oxygen consumption reported for

unrestrained adult C. magister at 8 to 10°C, range from 0.025 to 0.050 ml O_2/gm/hr (Johansen et al., 1970; McDonald et al., 1980), and correspond very closely to the values reported in the present study (Table 1). Comparisons between adults of different species of the same magnitude in size reveal similar rates of oxygen consumption to those measured in the present study. Aldrich (1975) and Aldrich and McMullan (1979) report oxygen consumption rates of 0.007 to 0.018 ml O_2/gm/hr for Cancer pagurus in the weight range of 336 to 640 gm at 10°C. Callinectes sapidus at 20°C consumes oxygen at a rate of 0.034 ml O_2/gm/hr in seawater at 30 ppt seawater while oxygen consumption is significantly higher at lower salinity, 0.053 and 0.052 ml O_2/gm/hr in 20 ppt seawater and 10 ppt seawater respectively (Findley et al., 1978). Callinectes sapidus therefore exhibits a type 1 response to salinity, as defined by Kinne (1964). In contrast to C. sapidus, the rate of oxygen consumption of adult C. magister is independent of salinity at both 10°C and 20°C and thus can be categorized as a type 4 response to salinity according to Kinne (1964). The 1st juveniles and 5th juveniles also fit the type 4 response to salinity at both 10°C and 20°C. The rate of oxygen consumption of the megalopae, however, is affected by salinity at 20°C in a type 1 fashion, an increase in metabolic rate at subnormal salinities.

Typically, the rate of oxygen consumption of animals is

proportional to body mass. Larger animals consume a greater amount of oxygen per unit time. Per unit body mass, however, the larger animal consumes less oxygen per unit time than a smaller animal. Regression coefficients of the log of weight specific oxygen consumption (Q_{02}) versus log weight (W) reported in the literature for crustaceans span a fairly broad range of values. Dehnel (1960) states that the regression coefficient depends on a number of factors including those previously mentioned at the beginning of this discussion and on the thermal and salinity history/ acclimation of the animals used. Dehnel (1960) gave regression coefficients ranging from -0.685 to -0.333 for Hemigrapsus nudus and H. oregonensis under a variety of salinity and temperature conditions. Weymouth et al. (1944) reported a coefficient of -0.2 for Pugettia producta; Roberts (1957) reported a coefficient for Pachygrapsus crassipes of -0.336. In the current study, the regression coefficients (Table 2) of the least squares regression of log Q_{02} versus log W are in the range of -0.383 and -0.440 and are similar to values reported for other crabs. The weight range of crabs used in the present study spans a much broader range than any of the other reports.

Gutermuth and Armstrong (1989) examined the extent to which oxygen consumption is temperature dependent in juvenile C. magister. Juveniles from estuarine and coastal areas were compared. The average summer estuarine water

temperature is higher than the average summer coastal water temperature. They report that 0+ year class juveniles and 1+ year class juveniles respond differently to temperature in the range encountered in coastal and estuarine waters in the summer months. The 0+ juveniles are more sensitive to temperature in the low part of the temperature range studied than the 1+ juveniles. Gutermuth and Armstrong (1989) relate this to a faster growth rate of estuarine crabs compared to coastal crabs: the higher metabolic rate of the juveniles in the warm temperature areas (i.e. the estuary) mean they can assimilate nutrients faster and take advantage of the food rich estuary. Gutermuth and Armstrong (1989) have pooled together several different instars in the juvenile development of this species by only distinguishing between year classes.

The results of the current study are not directly comparable to the results reported by Gutermuth and Armstrong (1989) for a number of reasons. First, the individual instars are pooled into year class groups by Gutermuth and Armstrong. Secondly, the crabs were acclimated to the experimental temperatures for several days prior to the experiments. Thirdly, the rate of oxygen consumption is reported per unit dry weight. Nonetheless, using the factor of dry weight equaling 20% of wet weight given by Schatzlein and Costlow (1978), the rate of oxygen consumption at 10°C, presumably in 100% seawater, of 0+ crabs, 0.145 ml O_2/gm/hr

(Gutermuth and Armstrong, 1989) and 1st juveniles or 5th juveniles at 10°C in 100% seawater, 0.386 or 0.102 ml O_2/gm/hr respectively (present study), are similar.

The differences in metabolic response between stages in the current study indicate some interesting patterns related to differences in mode of existence and habitat of the different stages of C. magister. The remarkable change in response to salinity and temperature at metamorphosis from megalopa to 1st juvenile coincides with the transition from oceanic megalopae to estuarine 1st juveniles. Upon entering the bay, from the relatively constant oceanic waters, the megalopae rapidly metamorphose into 1st juveniles. The metabolism of the 1st juveniles at high temperature is less sensititve to salinity and they may therefore be better suited to the more changable estuarine waters than the megalopae. Secondly, the high Q_{10} of the 1st juvenile (Table 1), especially in 100% seawater, would potentially enable this rapidly growing stage to readily assimilate food from the nutrient rich mudflats as suggested by Gutermuth and Armstrong (1989). Thirdly, the low Q_{10} for 5th juvenile is interesting in relationship to long duration of 5th juvenile from late summer through the winter to the following spring. The 5th juvenile is therefore exposed to a far broader range of environmental conditions than the other stages examined. A reduced sensitivity to temperature over the seasonal extremes may be important in enabling these small crabs to

spend a portion of their time hidden on the mudflats and therefore protected from most large aquatic predators.

The pattern of change in metabolic response described for C. magister can be compared with patterns observed for smaller larvae of other species. Belman and Childress (1973) conclude that the temperature sensitivities of the early larval stages of both Panulirus interruptus and Cancer productus correlate with the normal temperature range at which the larvae are usually found in the ocean. Vernberg et al. (1981) suggested that the respiratory responses to temperature of Pagurus criniticornis larvae are probably related to habitat and environment changes during development. Sastry and McCarthy (1973) discuss differences in thermal tolerance and changes in respiratory rates during development of the larvae of the sympatric species, Cancer irroratus and C. borealis. They conclude that the differences in the physiological requirements and capacities result in the temporal succession of these two species within the same area.

Given the information available regarding metabolic response to environmental parameters and how these capabilities change related to transitions from larval to adult habitats, further questions involving examination of other ontogenic changes in physiological and molecular processes present a broad potential for further study. At the physiological level, the ontogeny of ionic and osmotic

regulatory ability is the focus of Chapter III. As noted by Wheatly (1988) an increase in the rate of oxygen consumption due to salinity stress may not be due solely to an increase in energy expended in ionic and osmotic regulation, but may be partly due to increases in ventilation, perfusion and changes in other processes related to gas exchange and oxygen transport in the hemolymph. Accordingly, at the molecular level, the oxygen binding properties of the whole hemolymph and of hemocyanin from different stages is examined in Chapter IV.

CHAPTER III

IONIC AND OSMOTIC REGULATION

Introduction

Estuarine invertebrates vary greatly in their abilities to regulate hemolymph ion concentrations when exposed to changes in ambient salinity. Among the Crustacea, the species with the strongest regulatory abilities are generally the ones living in the most changable environments (Krogh, 1939; Lockwood, 1962; Prosser, 1973). There is a long history and a huge number of studies concerning the effects of changes in environmental salinity on the internal osmolality of adult decapod crustaceans (for reviews see Krogh, 1939; Beadle, 1957; Lockwood, 1962; Mantel and Farmer, 1983). Comparable information about larval and juvenile crustacean osmoregulation, however, is quite small in comparison, and there is very little data available regarding specific ion regulation in larval and juvenile stages of decapods. (for review see Gilles and Pequeux, 1983).

In the early 1970's Alspach (1972), Engelhardt and Dehnel (1973) and Hunter and Rudy (1975) all studied the osmotic and ionic regulatory abilities of the adult male

Dungeness crab, Cancer magister. They measured hemolymph osmolality and ionic concentrations when the animals had reached equilibrium, 72-96 hr after acute exposure to altered seawater. They found that C. magister is a weak hyperosmoregulator at salinities lower than that of ocean seawater. They also found that C. magister adults are able to strongly hyperregulate calcium and hyporegulate magnesium. In reduced salinity the hemolymph concentrations of sodium, potassium and chloride are all higher than the seawater concentration but are not as strongly hyperregulated as calcium. The ontogeny of osmotic and ionic regulation in C. magister was not investigated.

Studies of osmoregulation in decapod larvae are limited. Those studies that have investigated the osmoregulatory abilities of larval and post larval animals have found several differnt patterns. (See Charmantier et. al., 1988) In some cases there is no change in osmoregulatory ability with developmental stage, e.g. Sesarma reticulatum (Foskett, 1977). In Macrobrachium petersi (Read, 1984) the beginning of the adult type osmoregulation pattern is evident in the first larval stage. In Uca subcylindrica (Rabalais and Cameron, 1985), Homarus americanus and Penaeus japonicus (Charmantier et al., 1988) there is a marked change from larval to adult osmoregulatory patterns. These studies examined osmoregulation, but not the regulation of specific ions, in the hemolymph of larval or

post-larval stages.

The purpose of this study was to investigate the short-term (8 hr) osmotic and ionic regulatory abilities of selected stages in the life-cycle of C. magister.

Materials and Methods

Animals

The different life stages of C. magister were caught and maintained as previously described in Chapter II.

Protocol and Sampling

Megalopae, 1st instar juveniles (6-8 mm carapace width) 5th instar juveniles (25-33 mm carapace width) and adults (larger than 120 mm carapce width) were exposed to seawater of different temperatures and salinities for eight hours. Hemolymph samples were then taken for subsequent ionic and osmotic analysis. Experimental seawaters included 100% seawater (32 ppt seawater from the mouth of Coos Cay), 75% seawater and 50% seawater (Coos Bay seawater diluted with glass distilled water) (Table 1) at either 10°C or 20°C.

One gallon glass aquaria were used for the experimental chambers. Adults were kept one to an aquarium for the duration of the experiments. About 250 megalopae or 1st juveniles and two or three 5th juveniles were placed in each aquarium.

Table 1. Composition of seawater treatments

	TREATMENT		
	100% SW	75% SW	50% SW
Osmolality	958	773	488
Sodium	402	302	199
Chloride	535	413	268
Potassium	5.7	4.3	2.6
Calcium	7.7	6.3	4.5
Magnesium	55.2	43.0	28.0

Note. Osmolality in mOsm/kg and ion concentrations in mmol/L.

Hemolymph samples were taken from the megalopae by puncturing the heart with a glass micro-capillary pipette. The juveniles and adults were bled by puncturing the arthrodial membrane at the base of a walking leg. The 1st juveniles were bled with micro-capillary pipettes and the 5th juveniles and adults were bled with needle and syringe. The hemolymph samples from all individuals in each aquarium were pooled in order to collect a large enough volume for the ionic and osmotic analyses. In Tables 2-7 and Figures 1-6, n refers to the number of analyses of separate pooled samples. Samples were immediately frozen (-73°C) and stored for ionic and osmotic analysis. Water samples from each aquarium were also collected and frozen.

Osmotic and Ionic Analysis

Osmotic pressure of water and hemolymph samples was

measured using a Wescor 5500 vapor pressure osmometer. Chloride concentration in water and hemolymph was measured using a Buchler-Cotlove chloridometer. Hemolymph sample size was 10ul for megalopae and 1st juveniles and 40ul for 5th juveniles and adults. Magnesium concentration was measured colorimetrically after the method of Sky-Peck (1964). Samples (10ul) were deproteinized with 5% trichloroacetic acid and reacted with thiazole yellow in the presence of excess base. The absorbance at 540 nm was measured with a Beckman DU-70 spectrophotometer. Sodium, calcium and potassium concentrations were measured with ion specific electrodes. The reference electrode in all cases was an Orion 90-02 double junction reference electrode with NH_4Cl as the outer chamber filling solution and a AgCl saturated inner chamber filling solution. All measurements were made using a Radiometer Ion 83 ion meter in mV mode. Sodium concentration for adult hemolymph and water samples was measured using a Radiometer G502 sodium Selectrode. A sodium microelectrode (MI-420 from Microelectrodes, Inc.) was used to measure sodium concentration in megalopa and juvenile hemolymph and water samples. All samples were diluted 1:100 in 0.1 molar $Ca(NO_3)_2$ to adjust the ionic strength of the samples. Calcium concentration was measured using a Radiometer F2112 calcium Selectrode. The ionic strength of water and hemolymph samples was adjusted by diluting 1:100 with 0.3 molar KCl. Potassium concentration was measured

with an Orion 93-19 potassium electrode. Samples were diluted 1:100 in 0.1 molar NaCl. Prior to analysis of samples, calibration for measurement of each ion species was done with salt solutions of known concentration spanning the expected range of values.

Data analysis

Results are expressed as mean ± S.E., n is the number of observations. Three-way analysis of variance (ANOVA) was used to test for significance among treatments (developmental stage, salinity and temperature). Subsequent multiple comparisons of means were performed using the Tukey-Kramer method. Statistical significance was accepted at $P < 0.05$. Statistical analyses were done using SYSTAT version 4.1 (SYSTAT, Inc.).

Results

In the following presentation of results, all measurements were made on animals that had been exposed for 8 hr to the specified temperatures and salinities.

In 100% seawater, the megalopa, 1st juvenile, 5th juvenile and adult are isosmotic with the ambient seawater (Fig. 1 and Table 2). In 75% seawater the hemolymph osmolalities of all four stages are significantly lower than in 100% seawater, yet they are all hyperosmotic relative to 75% seawater. In 50% seawater the hemolymph osmolalities of

Figure 1. Hemolymph osmolality of C. magister. Mean ± S. E.. Salinity treatments are indicated below the x-axis. ▭ seawater, ▨ 10°C, ▨ 20°C. M, megalopa; 1J, 1st juvenile; 5J, 5th juvenile; A, adult.

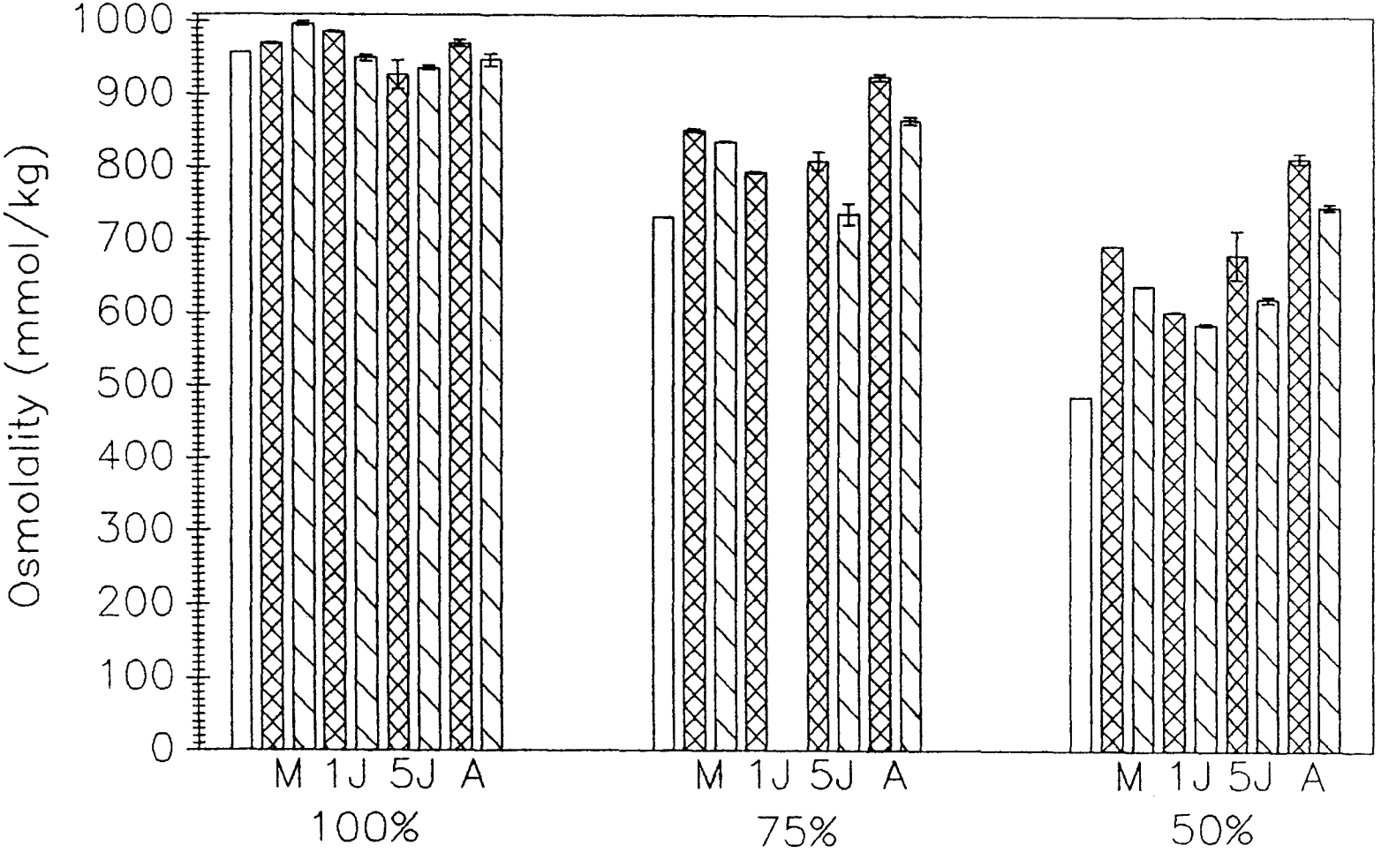

Osmolality (mmol/kg)
1000
900
800
700
600
500
400
300
200
100
0
M 1J 5J A
100%
M 1J 5J A
75%
M 1J 5J A
50%

TABLE 2. Hemolymph osmolality of megalopa, 1st juvenile, 5th juvenile and adult *C. magister*

STAGE	TREATMENT					
	100% SEAWATER		75% SEAWATER		50% SEAWATER	
	10°C	20°C	10°C	20°C	10°C	20°C
MEGALOPA	970.7	997.0	852.0	836	695	642
	±1.7	±4.3	±3.3	837		640
n =	3	3	3	2	1	2
1st	986.6	955	794.3	no	605.3	590
JUVENILE	±1.9	946	±1.2	data	±1.2	586
n =	3	2	3		3	2
5th	927.3	937.0	810.3	738.7	717	622.7
JUVENILE	±27.8	±4.5	±17.6	±20.3	650	±6.2
n =	3	3	3	3	2	3
ADULT	970.9	948.1	925.6	865.8	815.4	748.8
	±12.7	±22.0	±12.9	±13.6	±19.1	±12.4
n =	8	8	8	8	8	8

Note. Osmolality in mOsm/kg, mean ± S. E..

all four stages are significantly lower than in 75% seawater and all are significantly hyperosmotic compared to the seawater. The 1st juvenile is least able to maintain hemolymph osmolality in dilute seawater compared to the other stages examined. Water temperature affects the osmoregulatory abilities of the crabs. The hemolymph osmolality of the adult and 5th juvenile are significantly lower at 20°C than at 10°C in both 75% and 50% seawater, while the megalopa hemolymph osmolality is less at 20°C than at 10°C ($p<0.05$) in 50% seawater.

The hemolymph chloride concentration in all stages in 100% seawater is hypoionic compared to ambient water (Fig 2 and Table 3). In 75% seawater the adult becomes nearly isoionic compared to the water but has significantly lower hemolymph chloride concentration at 20°C than at 10°C. In 50% seawater the adult hemolymph chloride concentration is hyperionic and is lower at 20°C than at 10°C. The hemolymph chloride concentration of the megalopa and of the 5th juvenile are also lower at 20°C than at 10°C in 75% seawater. In 50% seawater the megalopa and 1st juvenile hemolymph chloride concentrations are the same as the ambient water chloride. In 50% seawater the hemolymph chloride concentration of the5th juvenile, however, is significantly higher than that of the megalopa and 1st juvenile and is lower than that of the adult.

Hemolymph sodium concentration (fig. 3 and table 4) in

Figure 2. Hemolymph chloride ion concentration of C. magister. Mean ± S. E.. Salinity treatments are indicated below the x-axis. ▭ seawater, ▨ 10°C, ▨ 20°C. M, megalopa; 1J, 1st juvenile; 5J, 5th juvenile; A, adult.

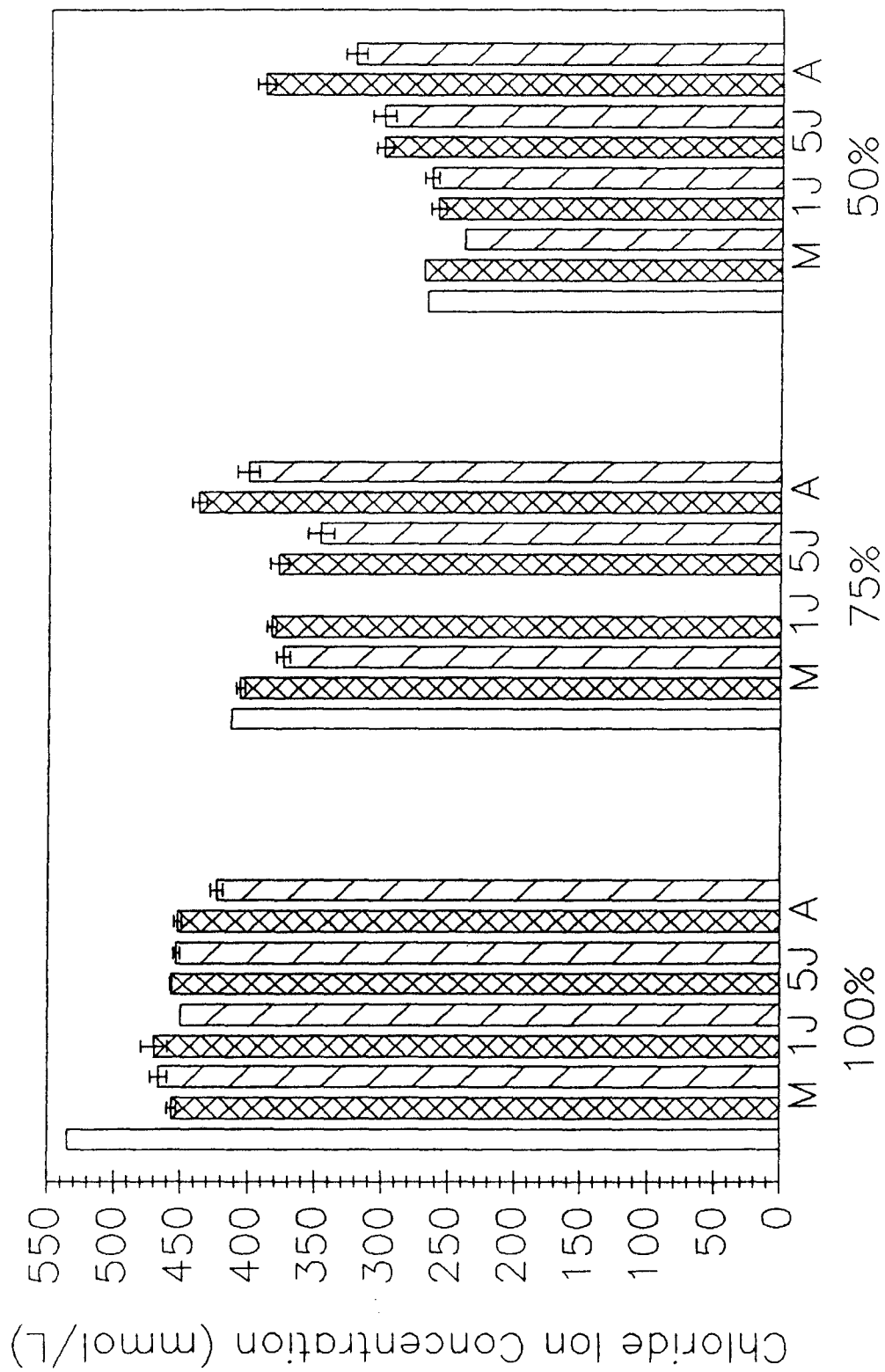

Chloride Ion Concentration (mmol/L)
0
50
100
150
200
250
300
350
400
450
500
550
M 1J 5J A
100%
M 1J 5J A
75%
M 1J 5J A
50%

TABLE 3. Hemolymph chloride concentration of megalopa, 1st juvenile, 5th juvenile and adult C. magister

STAGE	TREATMENT					
	100% SEAWATER		75% SEAWATER		50% SEAWATER	
	10°C	20°C	10°C	20°C	10°C	20°C
MEGALOPA	456.7	466.7	406.7	380	270.0	240.0
	±4.7	±9.4	±4.7	370		240.0
n =	3	3	3	2	1	2
1st	480.0	450.0	383.3	no	260.0	260.0
JUVENILE	±14.1	450.0	±4.7	data	±8.2	270.0
n =	3	2	3		3	2
5th	457.5	453.5	378.1	346.9	294.7	301.1
JUVENILE	±1.1	±3.9	±9.3	±13.4	306.8	±11.9
n =	3	3	3	3	2	3
ADULT	452.6	423.5	438.0	401.4	389.8	322.3
	±7.3	±12.4	±14.7	±21.3	±16.1	±20.6
n =	8	8	8	8	8	8

Note. Chloride ion concentration in mmol/L, mean ± S. E..

Figure 3. Hemolymph sodium ion activity of C. magister. Mean ± S. E.. Salinity treatments are indicated below the x-axis. ▭ seawater, ▩ 10°C, ▨ 20°C. M, megalopa; 1J, 1st juvenile; 5J, 5th juvenile; A, adult.

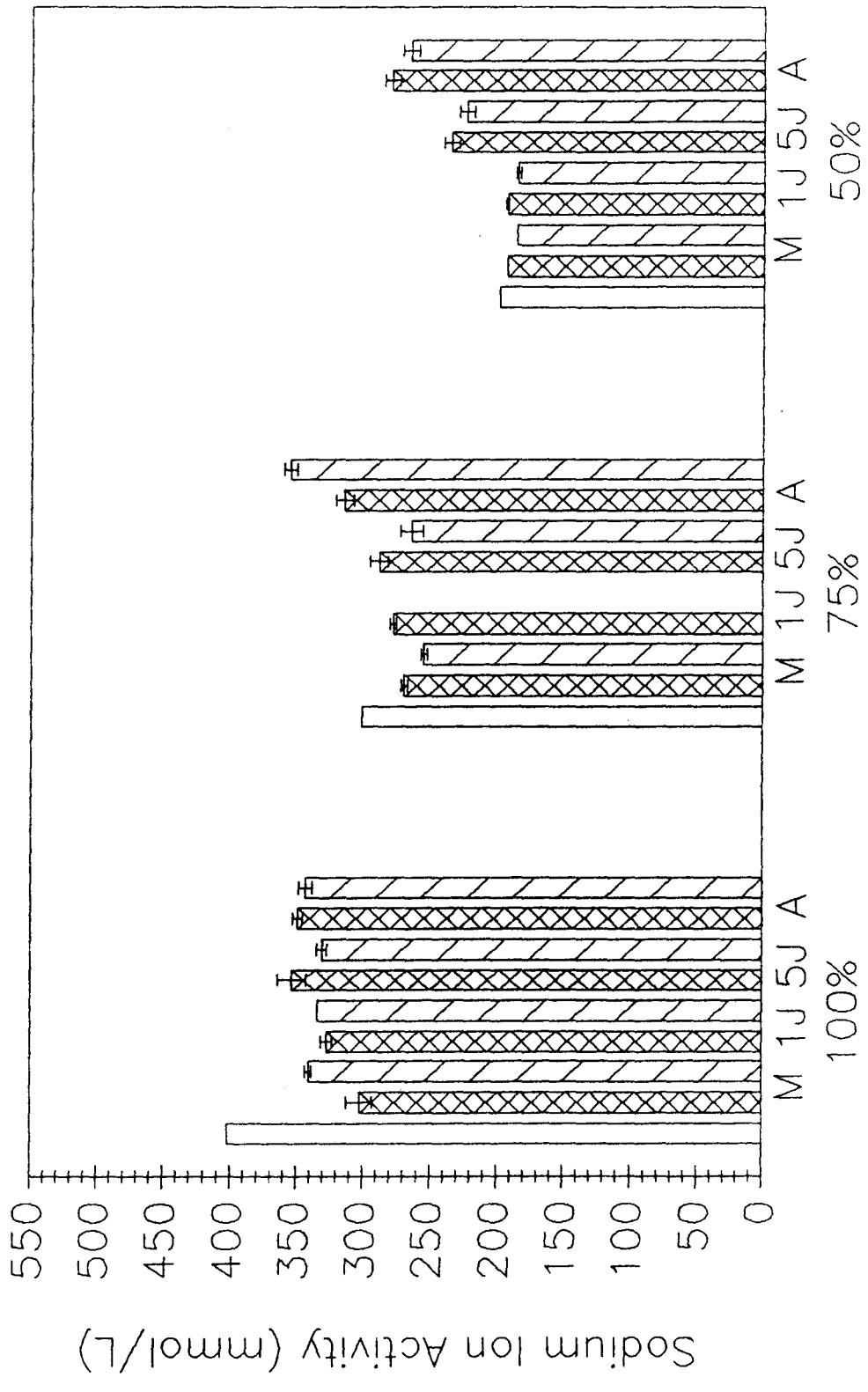

Sodium Ion Activity (mmol/L)
550
500
450
400
350
300
250
200
150
100
50
0
M 1J 5J A
100%
M 1J 5J A
75%
M 1J 5J A
50%

TABLE 4. Hemolymph sodium ion activity of megalopa, 1st juvenile, 5th juvenile and adult C. magister

STAGE	TREATMENT					
	100% SEAWATER		75% SEAWATER		50% SEAWATER	
	10°C	20°C	10°C	20°C	10°C	20°C
MEGALOPA	302.7 ±13.7	341.1 ±3.5	270.1 ±3.6	257.5 252.7	193.5	186.3
n =	3	3	3	2	1	1
1st JUVENILE	327.3 ±6.0	334.6 334.6	278.4 ±3.3	no data	193.9 ±1.6	187.2 183.7
n =	3	2	3		3	2
5st JUVENILE	353.6 ±15.4	331.1 ±5.6	289.2 ±9.2	264.9 ±12.1	229.9 241.1	224.0 ±8.0
n =	3	3	3	3	2	3
ADULT	349.2 ±9.4	343.5 ±12.8	315.2 ±17.9	355.8 ±13.8	290.3 ±15.1	266.5 ±16.0
n =	8	8	8	8	8	8

Note. Sodium ion activity in mmol/L, mean ± S. E..

all four stages shows essentially the same pattern as hemolymph chloride. In 100% seawater all four stages are hypoionic with respect to ambient seawater sodium. In 75% seawater the megalopa, 1st juvenile and 5th juvenile hemolymph sodium concentrations are significantly less than in the 100% seawater treatment, while the adult hemolymph sodium concentration is not significantly changed from the 100% seawater treatment. In 50% seawater the megalopa and 1st juvenile are isotonic to ambient sodium. The 5th juvenile hemolymph sodium concentration is significantly higher than the megalopa and 1st juvenile and less than the adult in 50% seawater. There is no effect of temperature on the hemolymph sodium concentration in any of the stages.

The hemolymph potassium concentration in all four stages in 100% seawater is not significantly different from ambient potassium concentration (fig 4 and table 5). In 75% seawater the adult hemolymph potassium concentration is not different from ambient potassium concentration but is significantly less than hemolymph potassium in 100% seawwater treatment. The 1st juvenile and 5th juvenile in 75% seawater have a significantly lower hemolymph potassium concentration than in 100% seawater. The megalopa shows no significant decrease in hemolymph potassium concentration in either 75% or 50% seawater compared to 100% seawater. The adult, 1st juvenile and 5th juvenile hemolymph potassium concentrations are not significantly different in 50%

Figure 4. Hemolymph potassium ion activity of C. magister. Mean ± S. E.. Salinity treatments are indicated below the x-axis. ▭ seawater, ▨ 10°C, ▧ 20°C. M, megalopa; 1J, 1st juvenile; 5J, 5th juvenile; A, adult.

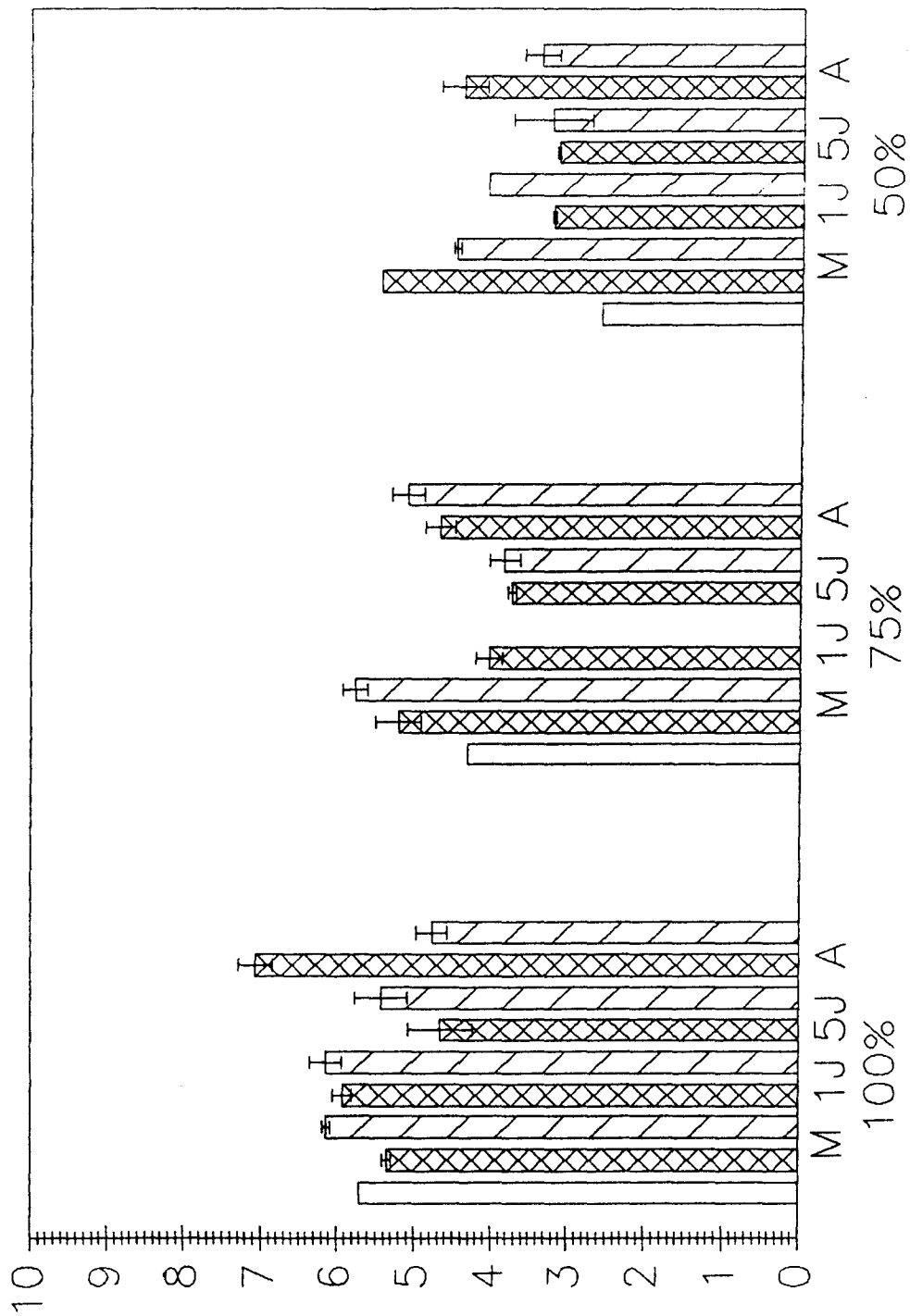

Potassium Ion Activity (mmol/L)
10
9
8
7
6
5
4
3
2
1
0
M 1J 5J A
100%
M 1J 5J A
75%
M 1J 5J A
50%

TABLE 5. Hemolymph potassium ion activity of megalopa, 1st juvenile, 5th juvenile and adult C. magister

STAGE	TREATMENT					
	100% SEAWATER		75% SEAWATER		50% SEAWATER	
	10°C	20°C	10°C	20°C	10°C	20°C
MEGALOPA	5.36	6.15	5.62	5.22	5.44	4.44
	±0.08	±0.07	5.94	±0.41		4.53
n =	3	3	2	3	1	2
1st	5.92	5.94	4.04	no	3.20	4.08
JUVENILE	±0.17	6.35	±0.25	data	±0.03	4.08
n =	3	2	3		3	2
5th	4.67	6.84	3.74	3.84	3.12	3.23
JUVENILE	±0.60	±2.01	±0.07	±0.29	3.18	±0.73
n =	3	3	3	3	2	3
ADULT	7.07	5.49	4.69	4.53	4.40	2.70
	±0.58	±0.32	±0.51	±0.14	±0.78	±0.16
n =	8	8	8	8	8	8

Note. Potassium ion activity in mmol/L, mean ± S. E..

seawater than in 75% seawater. There is no effect of temperature on hemolymph potassium concentration.

Magnesium concentration is strongly hyporegulated in adult hemolymph in all salinity treatments (fig 5 and table 6). In 100% seawater the megalopa, 1st juvenile and 5th juvenile hemolymph magnesium concentrations are significantly higher than the adult hemolymph magnesium concentration. The megalopa, 1st juvenile and 5th juvenile hemolymph magnesium concentrations are significantly lower in 75% and 50% seawater than in 100% seawater. In 50% seawater there is no difference in the hemolymph magnesium concentrations between the four stages. In 100% seawater the 5th juvenile has significantly lower hemolymph magnesium in 10°C than in 20°C. The 1st juvenile also appears to have a lower concentration at 10°C than at 20°C, however this is not significant at $P<0.05$.

In 100% seawater the hemolymph calcium concentrations in all four stages are not significantly different from the ambient water calcium concentration (fig 6 and table 7). The hemolymph calcium concentrations of the megalopa and adult do not change significantly with salinity. The 1st juvenile and 5th juvenile have significantly lower hemolymph calcium concentrations in 75% and 50% seawater than in 100% seawater; the 1st juvenile and 5th juvenile hemolymph calcium concentrations are also lower than the values in the megalopa and adult in 75% and 50% seawater. Overall there

Figure 5. Hemolymph magnesium ion concentration of C. magister. Mean ± S. E.. Salinity treatments are indicated below the x-axis. ▭ seawater, ▨ 10°C, ▱ 20°C. M, megalopa; 1J, 1st juvenile; 5J, 5th juvenile; A, adult.

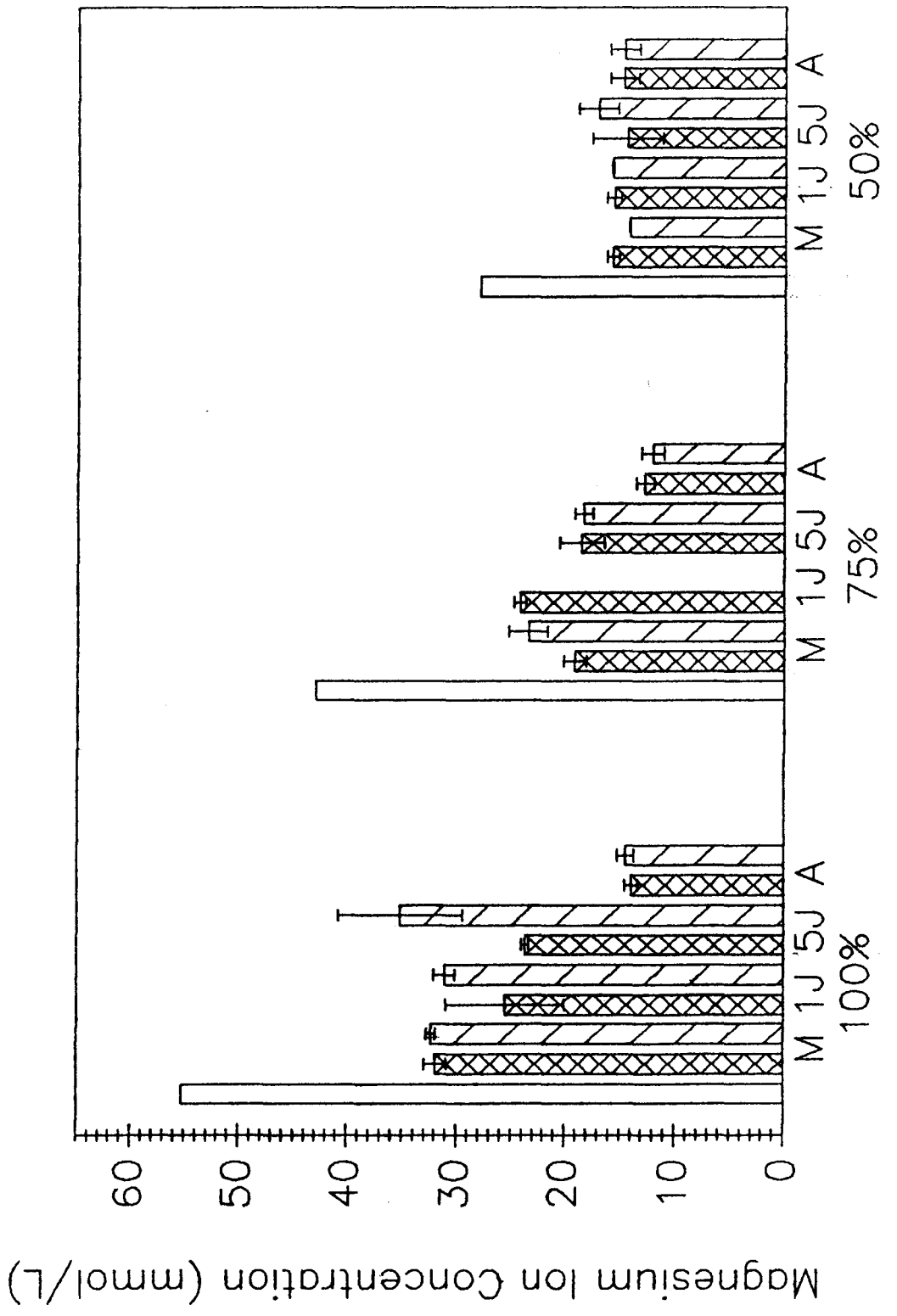

Magnesium Ion Concentration (mmol/L)
0
10
20
30
40
50
60
M 1J 5J A
100%
M 1J 5J A
75%
M 1J 5J A
50%

TABLE 6. Hemolymph magnesium concentration of megalopa, 1st juvenile, 5th juvenile and adult C. magister

STAGE	TREATMENT					
	100% SEAWATER		75% SEAWATER		50% SEAWATER	
	10°C	20°C	10°C	20°C	10°C	20°C
MEGALOPA	33.0	32.8	18.1	21.7	16.3	14.3
	30.9	31.9	20.2	25.3	15.3	14.2
n =	2	2	2	2	2	2
1st	20.1	30.2	23.7	no	16.3	15.9
JUVENILE	31.0	32.1	24.7	data	15.1	15.8
n =	2	2	2		2	2
5th	23.2	35.2	18.6	18.4	17.7	17.1
JUVENILE	±1.1	±8.0	±2.9	±1.2	11.3	±2.6
n =	3	3	3	3	2	3
ADULT	14.0	14.5	12.8	12.1	14.8	14.7
	±1.6	±2.0	±2.1	±2.8	±3.5	±3.5
n =	8	8	8	8	8	8

Note. Magnesium ion concentration in mmol/L, mean ± S. E..

Figure 6. Hemolymph calcium ion acivity of C. magister. Mean ± S. E.. Salinity treatments are indicated below the x-axis. ▭ seawater, ▨ 10°C, ▧ 20°C. M, megalopa; 1J, 1st juvenile; 5J, 5th juvenile; A, adult.

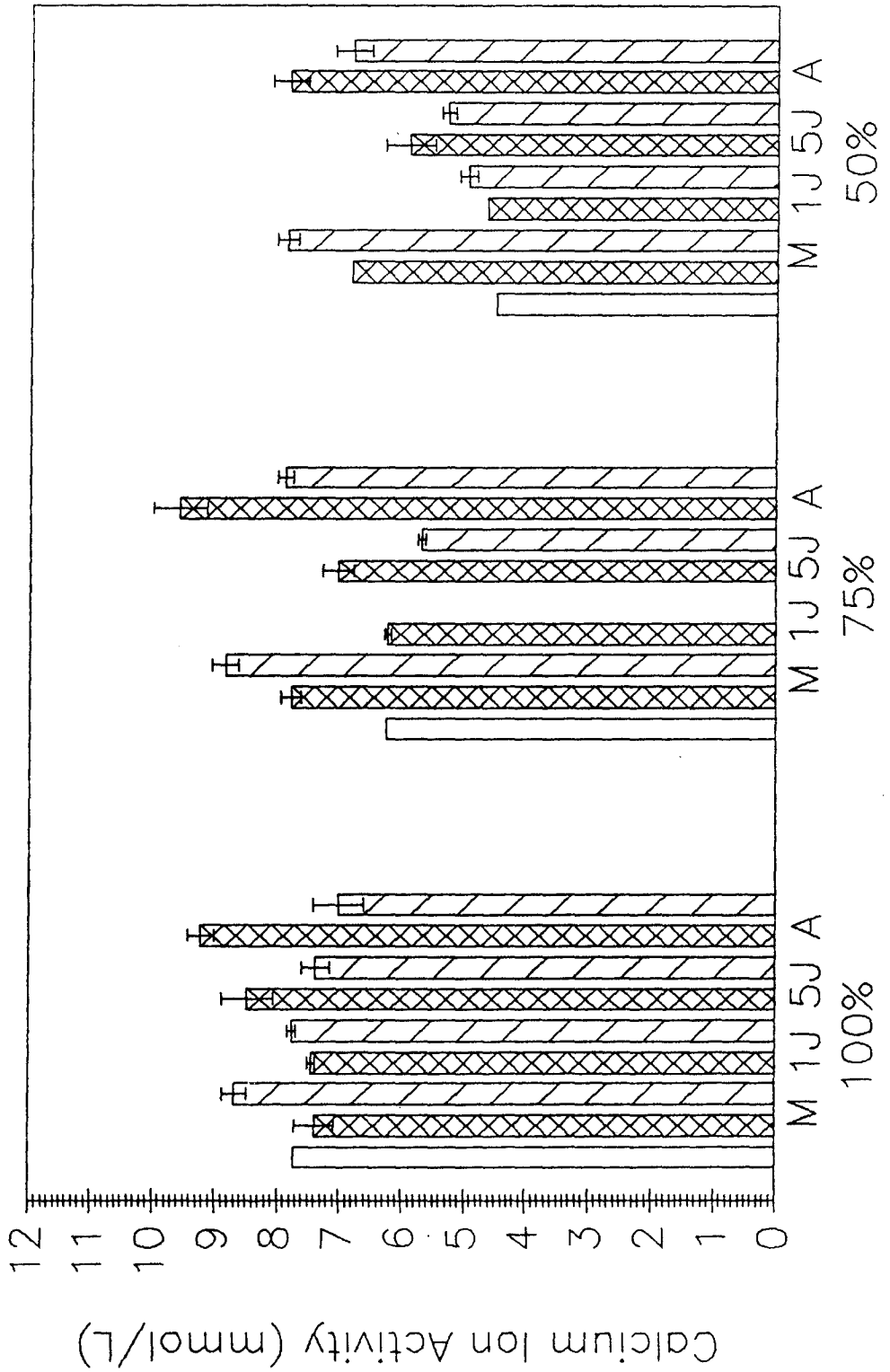

Calcium Ion Activity (mmol/L)
0
1
2
3
4
5
6
7
8
9
10
11
12
M 1J 5J A
100%
M 1J 5J A
75%
M 1J 5J A
50%

TABLE 7. Hemolymph calcium ion activity of megalopa, 1st juvenile, 5th juvenile and adult C. magister

STAGE	TREATMENT					
	100% SEAWATER		75% SEAWATER		50% SEAWATER	
	10°C	20°C	10°C	20°C	10°C	20°C
MEGALOPA	7.39	8.69	7.79	8.85	6.84	7.89
	±0.45	±0.27	±0.23	±0.30		±0.24
n =	3	3	3	3	1	3
1st	7.45	7.84	6.25	no	4.68	4.85
JUVENILE	±0.08	7.70	±0.08		4.68	5.12
n =	3	2	3		2	2
5st	8.48	7.38	7.04	5.71	6.32	5.31
JUVENILE	±0.59	±0.32	±0.35	±0.08	5.53	±0.16
n =	3	3	3	3	2	3
ADULT	9.24	7.01	9.59	7.90	7.85	6.83
	±0.56	±1.07	±1.13	±0.33	±0.77	±0.79
n =	8	8	8	8	8	8

Note. Calcium ion activity in mmol/L, mean ± S. E..

is no significant effect of temperature on hemolymph calcium concentration.

Discussion

This study shows that different developmental stages in the life cycle of C. magister have different patterns of regulation of hemolymph osmolality and ionic concentrations in reduced salinity over the 8 hr time period of a tidal cycle.

The results for ionic and osmotic regulation in adult C. magister in the present study essentially coincide with the findings of Jones (1941), Alspach (1972), Engelhardt and Dehnel (1973) and Hunter and Rudy (1975) with some differences due to experimental purpose and design. All of the previous investigations found that the hemolymph of C. magister is weakly hyperosmoregulated in water less concentrated than normal ocean seawater. The earlier studies all examined hemolymph concentrations after a state of osmotic and ionic equilibrium had been reached, approximately 72-96 hr. The values for hemolymph osmolality and ionic concentrations in the present study were obtained after an exposure time which is physiologically and ecologically important for these animals, the duration of a tide cycle. The general trends for osmotic and ionic regulation reported for long term exposure (Jones, 1941; Alspach, 1972; Engelhardt and Dehnel, 1973; Hunter and Rudy,

1975) are apparent, however, after the 8 hr exposure time used in the present study. The adult hemolymph is hyperosmotic compared to the experimental seawater osmolalities, chloride, sodium and potassium are somewhat hyperregulated in reduced salinity, calcium is strongly hyperregulated and magnesium is very strongly hyporegulated.

Studies on the larvae of a number of species of decapod crustaceans indicate that most are capable of maintaining hemolymph osmolality above that of the ambient water, either by hyperosmoconforming or by weakly hyperosmoregulating (See Charmantier et. al. 1988). Hyperosmoconformers maintain hemolymph osmolality above that of the ambient water, but as the ambient osmolality changes the hemolymph osmolality changes to the same extent. Hyperosmoregulators maintain hemolymph osmolality higher than the ambient water and as the ambient osmolality decreases the hemolymph becomes more hyperosmotic compared to the external water. Charmantier et al. (1988) note that metamorphosis often marks a profound change in osmoregulation from larval to adult type patterns, i.e., Homarus americanus, Penaeus japonicus (Charmantier et al., 1988)and Uca subcylindrica (Rabalais and Cameron, 1985). In the present study there is also a marked change in osmoregulation at metamorphosis from megalopa to 1st juvenile in C. magister. In this case, however, the juvenile is less able to regulate over the short 8 hr than are the adult and the megalopa. The direction of change in C.

magister does not parallel the pattern seen for H. americanus, P. japonicus and U. subcylindrica, since the megalopa, not the juvenile osmoregulates more like the adult, but the phenomenon of change at metamorphosis is consistent.

The hyperosmoregulation observed in the megalopa may be a result of its premolt state. Kalber and Costlow (1966, 1968) report that just prior to molting the hemolymph osmolality of the larvae of Rhithropanopeus harrisii and of the land crab Cardisoma guanhumi increases. They hypothesize that the increase in hemolymph osmolality is necessary for the uptake of water at the molt and subsequent increase in size of the animal. However Foskett (1977) found no such increase in hemolymph osmolality in the larvae of Sesarma reticulatum prior to molting.

This is the first report of the ontogenic changes in specific ion regulation in brachyuran crabs. The data show that there are differences in ion regulation between larval, juvenile and adult C. magister. The 1st and 5th juveniles studied here may be starting to show the pattern of ion regulation seen in the adult but may not yet be able to regulate to the extent of the adult. There is certainly a shift at metamorphosis in the ion regulatory pattern which is not evident by simply looking at the data on hemolymph osmolality. The ion regulation in the 5th juvenile is more like the adult than the 1st juvenile is like the adult.

Hunter and Rudy (1975) reported that smaller crabs (~30 gm) have a greater ability to hyperosmoregulate than larger crabs. They correlate this ability with a larger gill surface area to body volume ratio in the smaller animals (Gray, 1957), thereby affording a greater relative area for salt transport. The present study using crabs of approximately 0.1 gm (1st juvenile) and up to 5.0 gm (5th juvenile) indicates that the osmoregulatory ability is clearly much less than that of the adult. Also important in these considerations is the proportion of area on the gill associated with salt transport and the efficiency of that salt transport. Felder et al. (1986) have shown differences in Na+/K+ ATPase activity in the different prehatch stages of Callianassa jamaicense var. louisianensis, and have shown the presence of salt transport type tissue on the brancheostegites of the zoeae. It is possible that the different larval and juvenile stages of C. magister have different Na+/K+ ATPase activity levels as well as different relative proportions of salt transporting tissues.

There are aspects of specific ion regulation in the present study which are particularly striking. First, there are high hemolymph magnesium concentrations in megalopa, 1st juvenile and 5th juvenile in 100% seawater, when all of the other ions show no differences between stages in 100% seawater. Second, there is strong hyperregulation of calcium in megalopa and adult hemolymph compared to weak regulation

of calcium in the two juvenile stages studied.

Adults of all species of crustaceans that have been examined maintain hemolymph magnesium well below the magnesium concentration of the ambient water, except when they are in extremely dilute water. Engelhardt and Dehnel (1973) stated that 'hyporegulation of magnesium is the most universal feature of ionic regulation in crustacean blood.' Adult C. magister excrete magnesium in urine formed in the antennal gland. The urine to hemolymph ratio of magnesium is nearly 4:1 in 100% seawater (Hunter and Rudy, 1975; Holliday, 1980). The antennal gland may not be fully developed and functional inthe early stages of crustaceans (Waite, 1899; Conte, 1984). This may account for the high hemolymph magnesium in megalopa and juvenile crabs.

Hyporegulation of magnesium has often been discussed either in the light of maintaining high levels of activity or in regard to a greater extent of terrestriality. According to Robertson (1960) the decapod species with hemolymph magnesium concentrations less than 50% that of seawater are more active than those with higher hemolymph magnesium concentrations. In fact, high magnesium concentrations are often used to anaesthetize marine invertebrates. Gross (1964) discusses magnesium regulation at length in relation to the extent of terrestriality of various species of crabs. Mantel and Farmer (1983) note that grapsids and other species of semi-terrestrial and

terrestrial decapods all have low hemolymph magnesium concentrations. Neither of these hypotheses, activity level or terrestriality, proves to be helpful in understanding the role of high magnesium in the hemolymph of larval and juvenile C. magister. The totally aquatic megalopae are extremely active animals, capable of swimming very rapidly for extended periods. The juvenile crabs, although they are somewhat exposed on the mudflats at low tide, are never completely emersed and are in no sense amphibious. Their small size enables them to remain in the thin layer of water in the algae on the flats when the tide is out. The juveniles are also more active than the adults.

The relatively high magnesium concentrations in the megalopa and juvenile crabs may influence the hemocyanin oxygen binding properties in the hemolymph of these animals. Miller and Van Holde (1974) and Truchot (1975) among others have shown that magnesium concentration can affect the oxygen affinity and the cooperativity of some crustacean hemocyanins.

In addition to the stage specific magnesium levels the strong regulation of calcium in the megalopa and adult is remarkable. Calcium regulation in C. magister adults has been previously reported (Alspach, 1972; Engelhardt and Dehnel, 1973; Hunter and Rudy, 1975). The strong regulation of calcium by the megalopa and weak regulation by the juveniles is noteworthy. The observed hyperregulation of

calcium in C. magister megalopa could be the result of calcium resorbed from the carapace. Greenaway (1985), however, in a review of calcium balance during molting states theat the majority of the calcium sequestered in the hemolymph of crustaceans during premolt is likely to be in the form of bound or complexed calcium and that free or ionized calciium remains nearly constant in the hemolymph through premolt. The technique used to measure calcium in this study provided a measurement of ion activity, not total calcium in the hemolymph, therefore it would seem reasonable to assume that the high level of free calcium in megalopa hemolymph is not a result of the animals being in premolt. The functional significance of the high hemolymph calcium is not known. As with magnesium, calcium has been shown to have a strong effect on both the oxygen affinity and the cooperativity of hemocyanin from a variety of crustacean species (Larimer and Riggs, 1964; Miller and Van Holde, 1974; Truchot, 1975). The maintainance of high calcium levels in the megalopae may have a strong effect on the oxygen transport properties of the hemocyanin in the hemolymph.

Charmantier et al. (1988) propose a correlation between hyperregulation in larval and post-larval stages of Homarus americanus and Penaeus japonicus and the likelihood of those stages encountering low salinity environments. This hypothesis does not appear to hold as a possible explanation

for the pattern of osmoregulation seen for the different stages of C. magister studied here. The megalopa and adult are the best able to hyperosmoregulate over the 8 hr of exposure to low salinity. The megalopa is the stage least likely to be exposed to regular, large changes in salinity, either due to tides or rainfall. The adult crabs are found in water as low as 50% seawater and the ability to hyperregulate hemolymph osmolality and ion concentrations alleviates the stress of cell volume regulation. The juvenile stages found on large numbers on the mudflats are only slightly hyperosmotic in low salinity. In the case of juvenile C. magister it would appear that at the cellular level there is a high level of tolerance for changes in osmotic and ionic concentration. It seems that the juvenile crabs must have some mechanisn for regulating cell volume in the face of large and rapid changes in hemolymph conditions.

As mentioned above, calcium and magnesium may be involved in altering the oxygen binding function of hemocyanin. In the next chapter the experiments designed to test this hypothesis are discussed.