THE EFFECT OF HYPO-OSMOTIC STRESS ON MORTALITY AND REGULATION OF VOLUME, OSMOLALITY, AND MAGNESIUM ION CONCENTRATIONS IN THE SEA ANEMONE *METRIDIUM SENILE* IN SOUTH SLOUGH, COOS BAY, OREGON

by

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A THESIS

Presented to the Department of Biology
and the Graduate School of the University of Oregon
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"The Effect of Hypo-osmotic Stress on Mortality and Regulation of Volume, Osmolality, and Magnesium Ion Concentrations in the Sea Anemone *Metridium senile* in South Slough, Coos Bay, Oregon," a thesis prepared by Heather Lara Austin in partial fulfillment of the requirements for the Master of Science degree in the Department of Biology. This thesis has been approved and accepted by:

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An Abstract of the Thesis of

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Title: THE EFFECT OF HYPO-OSMOTIC STRESS ON MORTALITY AND REGULATION OF VOLUME, OSMOLALITY, AND MAGNESIUM ION CONCENTRATIONS IN THE SEA ANEMONE *METRIDIUM SENILE* IN SOUTH SLOUGH, COOS BAY, OREGON

Approved: _____
Dr. Craig M. Young

The sea anemone *Metridium senile* occurs along a salinity gradient in the South Slough Estuary, Oregon, where it is subjected to frequent and sometimes large fluctuations in salinity. This study determined how hypo-osmotic stress contributes to the survival and distribution of this population. In the laboratory, chronic exposure of *M. senile* to 50% and 75% seawater for twenty-eight days resulted in partial regulation of volume and magnesium ions. Anemones transplanted to the field exhibited increased mortality and partial regulation of volume, osmolality, and magnesium ions with decreased salinity during the wet season (December-February) and less regulation during the dry season (June-August). This pattern of physiological tolerance coincides with observed trends of seasonal abundance and distribution. Previous studies describe

M. senile as a marine osmoconformer, however this estuarine population is able to withstand moderate hypo-osmotic stress through partial regulation of tissue osmolality and magnesium ions.

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For my husband, who always supports my dreams, and my family, who encouraged me in the pursuit of marine biology.

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CHAPTER I

GENERAL INTRODUCTION

Sea anemones generally are considered marine, stenohaline osmoconforming organisms and currently are not regarded as euryhaline. However, a few species (*Diadumene leucolena*, *Haliplanella lineata, Nematostella* spp. and *Edwardsia* spp.) exhibit wide salinity tolerances and regularly inhabit brackish water (Shick, 1991). Recently, the actinarian species *Metridium senile* was observed at mesohaline and marine-dominated sites within the low intertidal regions of the South Slough Estuary, Coos Bay, Oregon. Within this environment, *M. senile* is subject to frequent and sometimes large decreases in salinity levels (Rumrill, 2006). This species is not estuarine, but rather marine, and does not experience drastically fluctuating salinities in its typical habitats (Deaton and Hoffmann, 1988; Shick, 1991). *Metridium senile* is exposed to hypo-osmotic stress, due to the presence of a widely variant osmotic gradient within the South Slough Estuary.

Sea anemones possess a variety of unique morphological and ionic strategies to combat hypo-osmotic stress (Shick, 1991). Morphological strategies range from equilibration with ambient seawater by means of coelenteron ventilation to creation of a mucus layer at the ectodermal surface that serves as a barrier to water and solute

movements (Bursey and Harmer, 1979; Benson-Rodenbough and Ellington, 1982; Shick, 1991). Ionic strategies vary from intracellular maintenance of the free amino acid pool (FAA) to sequestration of calcium ions in sea anemone mucus (Goreau, 1959; Deaton and Hoffmann, 1988). For example, *Metridium senile* exhibits volume regulation due to regulation of the intracellular FAA pool under dilute saline conditions which increases in concentration with increasing salinity (Deaton and Hoffmann, 1988; Shick, 1991).

However, the role of intracellular ions in osmotic regulation within sea anemones remains largely enigmatic and requires more comprehensive studies concerning the function of these ions in osmotic regulation within specific species. Furthermore, *Metridium senile* has been observed tolerating brackish conditions within the San Francisco Bay, Mersey River Estuary and South Slough Estuary, yet no relationship has been documented between the physiological tolerance limits of *M. senile* and the abundance and distribution of this species within an estuarine habitat (Rawlinson, 1934; Deaton and Hoffmann, 1988; Rumrill, 2006)

This study was undertaken to gain a more complete physiological perspective on a particular actinarian, *Metridium senile*, by studying its physiological tolerance limits in laboratory and field settings. Chapter II of this thesis describes the abundance and distribution of *M. senile* within the South Slough Estuary and relates it to mortality trends at three field sites. Chapter III describes the effect of hypo-osmotic stress on volume regulation, osmolality regulation, magnesium ion concentrations and mortality of *M. senile* in both the laboratory and within three different salinity regimes of the South

Slough Estuary. In essence, this thesis explores the physiological tolerance limits of *M. senile* under dilute saline conditions in order to gain a better physiological perspective on how *M. senile* endures hypo-osmotic conditions along an estuarine gradient. Ultimately, this information may lead to a greater understanding of physiological tolerance patterns and elucidate the ecological distribution of this organism within estuaries.

CHAPTER II

ABUNDANCE AND DISTRIBUTION OF *METRIDIUM SENILE* IN THE LOW INTERTIDAL AND SUBTIDAL ZONE OF THE SOUTH SLOUGH ESTUARY, COOS BAY, OREGON

Introduction

The sea anemone *Metridium senile* (Linnaeus 1767) is primarily circumboreal, found along the coasts of Europe, Japan, and the east and west coasts of North America (Shick, 1991; Acuña and Griffiths, 2004,); however individuals have also been documented off the coasts of Argentina and South Africa (Acuña and Griffiths, 2004). The column of *M. senile* occurs in several different color morphs, which include white, cream, tan, brown and light orange. Hundreds of short thin tentacles cover the oral disk, giving rise to its common name, the "Plumose Anemone" (Fox and Pantin, 1941; Fox *et al.*, 1967; Carlton, 2007). Typically, *M. senile* occur in dense clones of small individuals that are attached to protected pilings, rock jetties, floats and other hard substrata within the low intertidal and subtidal waters of bays and harbors (Carlton, 2007).

Clones are produced by a year-round asexual reproductive process called "pedal laceration," where little tissue fragments break off from the edge of the pedal disc as the individual either remains in one spot or moves along the substratum. These isolated tissue

fragments develop into tiny new anemones, which feed and grow to eventually produce aggregations of genetically identical individuals (clones) that encircle or trail the original individual (Kaplan, 1983; Carlton, 2007). Clones can range from a few to many hundreds of small (2-3 cm high) genetically identical individuals that are isolated from each other by anemone-free spaces (Francis, 1973; Anthony, 1997). *Metridium senile* also reproduces sexually during the summer season, from June to September (Kaplan, 1983; Anthony, 1997). Sexual reproduction commonly produces larger solitary individuals (20-30 cm high) that are found primarily in the deeper subtidal regions of bays and harbors (Hoffmann, 1976; Shick *et al.*, 1979; Anthony, 1997).

As a consequence of inhabiting the intertidal and subtidal zone of bays and harbors, *Metridium senile* can be exposed to estuarine conditions resulting in frequent and sometimes large fluctuations in salinity levels that result in hypo-osmotic stress (Deaton and Hoffmann, 1988; Rumrill, 2006). *Metridium senile* has been observed tolerating brackish conditions in a number of bays and harbors throughout the world. In the Mersey River estuary of Northwest England, *M. senile* was found in areas with salinities between 21-28 (Rawlinson, 1934). Additionally, *M. senile* has been noted in the Oosterschelde estuary and the Rhine-Meuse-Scheldt delta of the Netherlands (Braber and Borghouts, 1977; de Kluijver and Leewis, 1994). *Metridium senile* has been observed tolerating brackish conditions as low as 68% seawater within areas of the San Francisco Bay (Carlton, 2007). Laboratory experiments by Deaton and Hoffman (1988) confirm these trends. Deaton and Hoffmann (1988) determined that *M. senile* can survive 55%

seawater for at least two weeks without mortality, however animals exposed to 40% seawater died within three days. The ability to withstand fluctuating salinity and subsequent effects of hypo-osmotic stress may explain why *M. senile* has been documented within temperate estuaries throughout the world.

Recently, *Metridium senile* has been documented along a salinity gradient within the South Slough estuary in Coos Bay, Oregon. The South Slough estuary is a small and relatively shallow (mean depth of ~1 meter below Mean Lower Low Water) drowned-river estuary located in the larger Coos estuary along the southern Oregon coast. Significant freshwater inputs into the South Slough come primarily from the Joe Ney, Winchester and Sengstaken sub-systems. This estuary is influenced considerably by mixed semidiurnal tidal cycles, experiencing two high and two low tides per day. In addition, tidal currents are strong, producing average flows over 1 meter per second. Because of the shallow depth, freshwater inputs, and substantial tidal influence in the South Slough, the salinity gradient fluctuates directly with tidal phase throughout the length of the South Slough. Consequently, during one tidal cycle the upper reaches of the estuary can experience nearly fresh to full-strength seawater. Additionally, salinity patterns are highly dependent on the distinct wet and dry seasons along the Oregon coast resulting in a characteristic seasonal cycle. During the wet season (December-February), marked freshwater input results in an overall decrease in salinity. During the months of March through May, a decrease in freshwater input results in a steady increase in salinity. The dry season (June-August) is marked by high, stable salinities until they decrease in

the fall (September-November), becoming more variable (Rumrill, 2006). Thus, a strong salinity gradient exists from the lower marine-dominated region located directly inside the mouth of the estuary to the upper riverine-dominated region located along Winchester, Elliott, Talbot and John B. Creeks (Rumrill, 2006).

The South Slough estuary is therefore divided into three hydrogeomorphic zones: marine, mesohaline and riverine-dominated zones. The marine-dominated zone extends from the mouth of the South Slough southward to Long Island Point and the intersection of Winchester and Sengstacken arms consisting of high, stable salinities and cooler water temperatures. However, the area surrounding Valino Island changes its salinity profile seasonally. During the wet season, Valino Island is mesohaline (average salinity ca. 10-27); during the dry season Valino Island is polyhaline (average salinity ca. 25-33), placing its yearly salinity average ca. 18-19 (S. Rumrill, pers. comm., South Slough National Estuarine Research Reserve). Thus for the purposes of this study, Valino Island is considered a mesohaline-dominated location (Figure 2.1).

Because of increased freshwater input during the wet season, the mesohaline-dominated zone slightly overlaps the marine-dominated zone extending from just north of Valino Island southward to Talbot Creek and Danger Point, and is composed of low, variable salinities with warmer water temperatures. The third zone is riverine-dominated which extends along the length of Winchester Creek and its associated tributaries on the western side and along Talbot Creek and John B. Creek on the eastern side. This region is

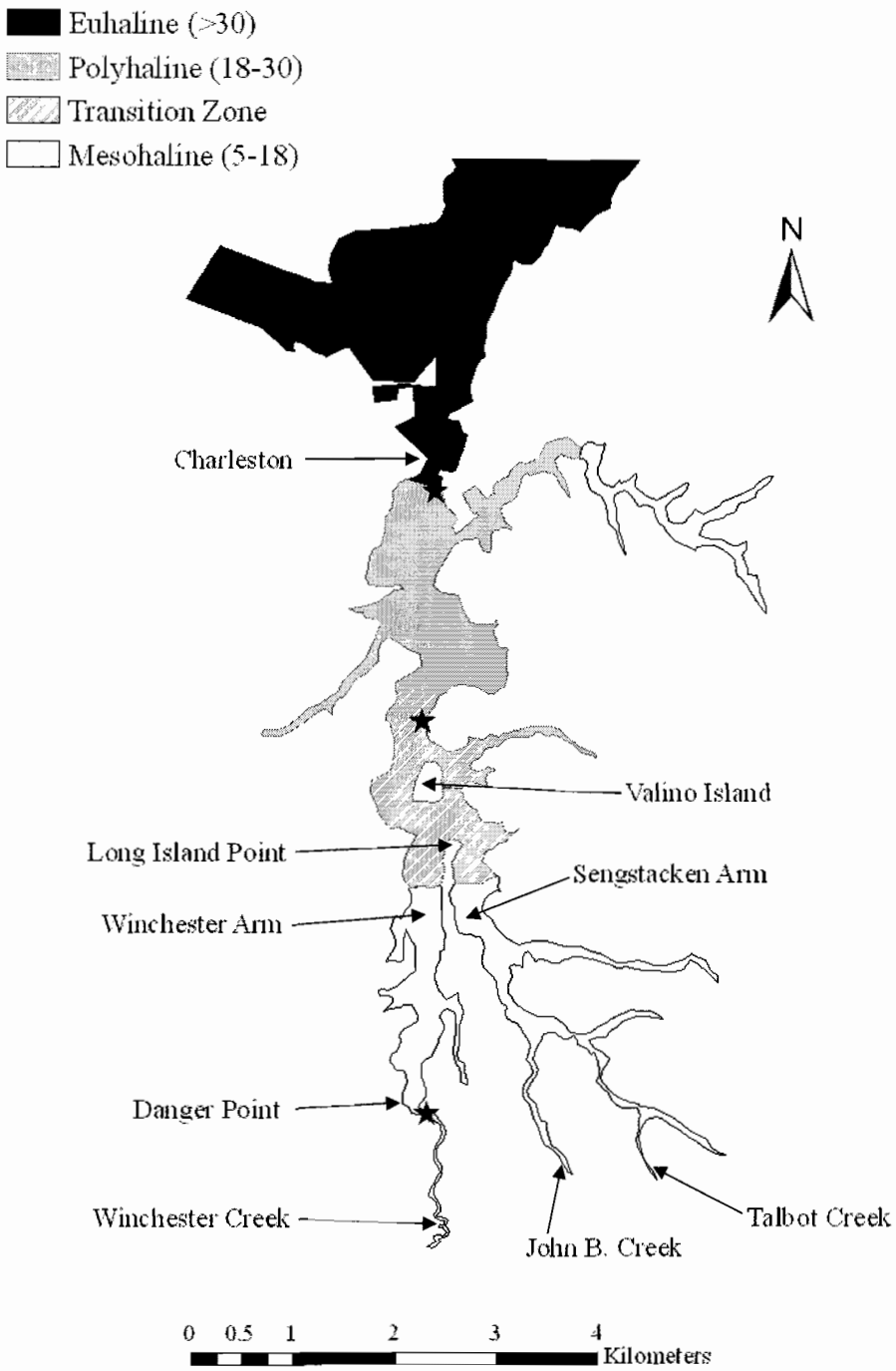

Figure 2.1. Location of field sites and key landmarks within the South Slough National Estuarine Research Reserve (SSNERR), Coos Bay, Oregon. Stars (★) indicate field transplant sites. The salinity profile is derived from Davidson (2008), Arneson (1975), ODFW and SSNERR CDMO.

composed of lower more variable salinities and even warmer water temperatures (Rumrill, 2006). These zones are not distinctly separated however, and can either move up or downstream depending on the tide or the season (Alexander *et al.*, 1932; Rumrill, 2006).

Individual *Metridium senile* were observed along this salinity gradient within marine and mesohaline-dominated zones. *Metridium senile* were seen primarily attached to floats, pilings and rock in the low intertidal within the marine-dominated zones of the Charleston Boat Basin and the Point Adams Jetty. In addition, *M. senile* was observed in the mud and sand in the low intertidal at the interface of the marine and mesohaline-dominated zones of Long Island Point, approximately 5.2 km up the South Slough estuary (Rumrill, 2006). *Metridium senile* individuals have also been observed fouling oyster shells and logs within mesohaline and marine-dominated zones of the South Slough estuary (Hewitt, 1993; Rumrill, 2006).

Within the intertidal estuarine habitat of the South Slough, organisms like *Metridium senile* are inherently susceptible to frequent and sometimes large fluctuations in salinity from a diel to seasonal basis. Additionally, the South Slough's hydrogeomorphic profile, the rate of change in salinity on a diel to seasonal basis, can be drastic (Rumrill, 2006). Therefore, the natural abundance and distribution patterns of *M. senile* may be partly determined by both the salinity gradient and the rate and magnitude of salinity change within these three hydrogeomorphic regions of the South Slough estuary (Sanders *et al.*, 1965; Rumrill, 2006).

Laboratory studies of salinity tolerance on the actinian *Bunodosoma cavernata* correlated tolerance trends to its ecological distribution in a fluctuating salinity environment along the Atlantic and Gulf coasts (Benson-Rodenbough and Ellington, 1982; Shick, 1991). Additionally, studies of ecological physiology on *Haliplanella luciae*, a colonizing actinian, have attempted to correlate physiological adaptations to distributional patterns (Shick, 1976; Benson-Rodenbough and Ellington, 1982; Shick, 1991).

I studied *Metridium senile* within the South Slough estuary, Coos Bay, Oregon with two goals in mind: 1) to determine the abundance and distribution patterns of *M. senile* in the South Slough estuary during the wet season and dry season; and 2) to measure and compare monthly mortality during the wet season and dry season at marine, mesohaline and riverine-dominated sites.

Materials and Methods

Study location and field sites

One field site was selected within each of the three hydrogeomorphic zones within the South Slough estuary. Field sites were chosen based upon location within the South Slough, accessibility, and immediate proximity to the South Slough National Estuarine Research Reserve's (SSNERR) System-Wide Monitoring Program (SWMP) stations, where real time abiotic data, including salinity, temperature and dissolved oxygen were collected (Figure 2.1).

At each field site, I deployed one floating frame for anemone containment adjacent to SSNERR's SWMP monitoring station. Each floating frame was a sealed 1 m x 1 m PVC pipe square with two mooring buoys attached to opposite corners. Floating frames were anchored in the subtidal sediment with two screw anchors placed approximately 2.5 m on either side, using twisted polypropylene rope. For mortality measurements, *Metridium senile* individuals were placed within a large 30.5 cm^3 plastic mesh flow-through container with a 14 mm mesh opening that was attached to the frame with cable ties (Figure 2.2A-C).

Because *Metridium senile* is commonly found in the low intertidal and subtidal waters of bays and harbors, the marine-dominated field site ("Charleston") served as the control site (Carlton, 2007; Figure 2.1). This site lies adjacent to the Charleston bridge (43°20'15.72 N, 124°19'13.92 W) and consists of well-mixed tidal waters with a maximum tidal amplitude of 2.6 m and monthly mean salinity range from 20 during the wet season to 31 during the dry season. Substrata around this site include cement pilings, wooden pilings, jutting bedrock, cobble, sand and mud. The mesohaline-dominated field site ("Valino") rests slightly to the north of Valino Island within the South Slough (43°19'1.98 N, 124°19'17.88 W) and consists of well-mixed tidal waters with a maximum tidal amplitude of 2.7 m and salinity ranging from 15-28 (Figure 2.1). Substrata associated with this site include eroding sand cliffs, mud, wooden pilings and some woody debris from previous logging operations. The riverine-dominated field site ("Winchester") is located within Winchester Arm across from Danger Point on the east side of the channel (43°16'56.70 N, 124°19'13.14 W) and consists of a maximum tidal amplitude of 2.0 m (Figure 2.1).

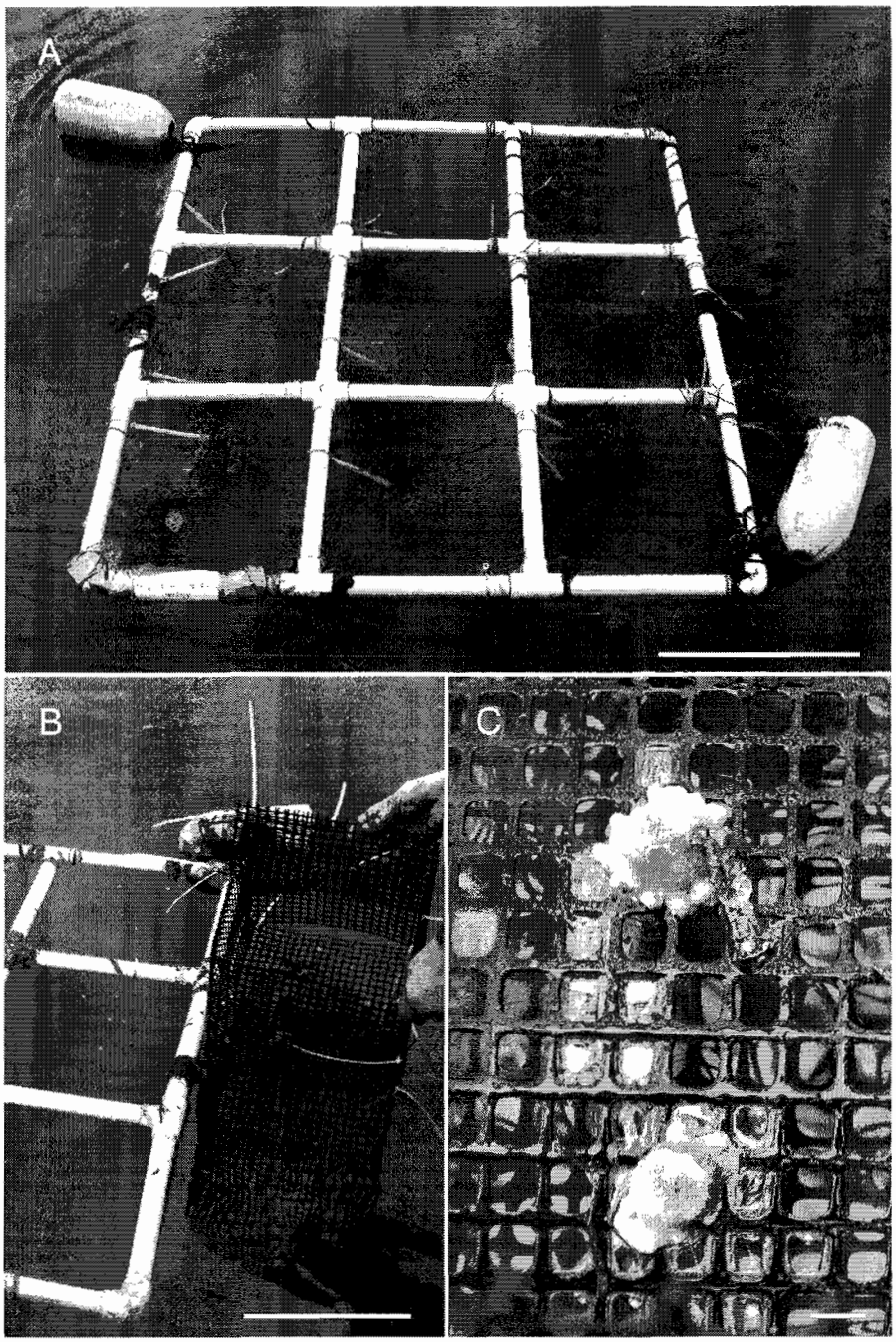

Figure 2.2. Images of (A) the floating frame with two mooring buoys, (B) large plastic mesh flow-through container attached to the floating frame with cable ties for anemone containment, and (C) anemones attached to the plastic mesh of the flow-through container. Scale bars in A and B are 30.5-cm and the scale bar in C is 1.4-cm.

During the dry season, tidal waters are characteristically well-mixed, with salinity ranging from 5-30; during the wet season however, tidal waters are partially stratified, with salinity ranging from 0-21. Available substrata consist of mud, vertical wooden pilings and associated woody debris from previous logging operations within the South Slough estuary (Rumrill, 2006).

Seasonal field surveys

Surveys of abundance and distribution were conducted from the mouth to terminal ends of the South Slough estuary in January 2009 during the middle of the wet season, and July 2009 during the middle of the dry season over two consecutive tidal cycles to determine natural abundance and distribution patterns of *Metridium senile* along an estuarine gradient within the South Slough, Coos Bay, Oregon (Rumrill, 2006). Sites were selected based on presence of available substrata and accessibility (by boat or foot). High tide surveys were executed primarily by use of boat. Low tide surveys were conducted primarily by boat or by foot for sites that were extremely shallow or difficult to access. A snorkel and mask was used to survey the subtidal regions (0-2 m below water level) of sites with available substrata. The geographical location of each site was documented using a handheld global positioning system (Garmin Geko 201 GPS unit, accuracy ± 10 meters). Available substrata at each site were then examined for approximately ten to twenty minutes. Numbers of *M. senile* individuals present on the available substrata were recorded. Each site was subsequently placed into categories of *M. senile* abundance: abundant

(>501), common (16-500), rare (1-15), or absent (0). Salinity at each site was classified as euhaline (>30), polyhaline (18-30), or mesohaline (5-18).

Salinity characteristics for each survey site were gathered from a number of sources. Davidson (2008) used salinity data from field measurements as well as field studies conducted by Arneson (1975), the Oregon Department of Fish and Wildlife and the South Slough National Estuarine Research Reserve to divide the South Slough estuary into three salinity classes: euhaline (>30), polyhaline (18-30), and mesohaline (5-18) (NERR CDMO, http://cdmo.baruch.sc.edu; Figure 2.1). Data catalogued from seasonal abundance and distribution surveys at each site were input into ArcMap GIS software and layered over an aerial photograph of the South Slough estuary obtained from Oregon Imagery Explorer (http://oregonexplorer.info/imagery/).

Seasonal abundance and distribution layers were projected using the North American datum 1983 projection in the Geographic Coordinate System. Polygons were then traced around each of the three salinity regions within the South Slough estuary based upon the salinity characteristics compiled from Davidson (2008), Arneson (1975) and the South Slough National Estuarine Research Reserve (NERR CDMO, http://cdmo.baruch.sc.edu).

Metridium senile transplants

Monthly transplant experiments were undertaken over a one-year period from July 2008 to August 2009 to determine whether observed abundance and distribution patterns

are a result of diminished survival in the upper mesohaline and riverine zones of the estuary.

At the beginning of each month, 60 individuals of approximately similar size (2.5-3.5 cm high and 1.5-3.0 cm diameter pedal discs) were manually detached from substrata adjacent to the marine-dominated site of the Charleston Bridge by scraping. Each individual was randomly selected from distinct clones determined by the presence of anemone-free spaces (Francis, 1973). Individuals were transported back to the lab where they were immediately immersed in running seawater (33 salinity) where each was allowed to naturally reattach over a 24-h period to a separate circular nylon mesh net measuring 8 cm in diameter. Once each individual was reattached, it was placed directly into a container filled with 33 salinity seawater. Twenty individuals were then transported to the field and placed within the floating frame's flow-through container at each field site (marine, mesohaline and riverine) for weekly monitoring where they remained attached to their respective mesh net.

Each individual was observed *in situ* on a weekly basis to assess condition and was given a mechanical stimulus. The criterion used to determine mortality was the inability of *Metridium senile* to respond to the mechanical stimulus at the time of observation (Benson-Rodenbough and Ellington, 1982). Cumulative weekly mortality and cumulative mortality averages at each site were determined for each month and year, respectively. *Metridium senile* individuals that were still alive at the end of each month

were gently detached from the nylon mesh net and transported back to the collection site where they were allowed to reattach to natural substrata.

Results

Seasonal field surveys

During January 2009, *Metridium senile* individuals were present in the lower estuary. Individuals were found in euhaline and polyhaline waters between the estuary mouth and Valino Island, approximately 4.5 km upriver. *Metridium senile* individuals were attached to cement pilings, wooden pilings and jutting bedrock in the euhaline region and wood pilings and woody debris in the polyhaline region. Individuals were absent from sites in the mid to upper estuary which consisted of polyhaline and mesohaline waters. However, presence of individuals primarily increased from around Valino Island northward to the mouth of the estuary. Sites near Valino Island and the Valino field site were categorized as "rare", whereas sites around the estuary mouth and the Charleston field site were predominantly categorized as either "common" or "abundant" (Table 2.1; Figure 2.3).

During July 2009, *Metridium senile* individuals were present in the lower to mid estuary, extending overall occurrence approximately 1 km upriver. Individuals were found in euhaline and polyhaline waters from the estuary mouth to just south of Long Island Point within Sengstacken and Winchester Arms, approximately 5.4 km upriver. *Metridium senile* individuals were attached to cement pilings, wooden pilings and jutting

Table 2.1. Details of sites surveyed for *Metridium senile* in the South Slough estuary, Coos Bay, OR during January 2009 and July 2009. Sites are shown in Figure 2.3, GPS coordinates and distances were collected during the seasonal surveys, salinity and abundance categories were derived from Figure 2.1. A distance upriver of "0" indicates sites that lie along the estuary mouth.

GPS Coordinates	Distance Upriver (km)	Salinity	January 2009		July 2009	
			No. of *M. senile* Individuals	Abundance Category	No. of *M. senile* Individuals	Abundance Category
N 43° 20' 56.01" W 124° 19' 11.92"	0	Euhaline	224	Common	219	Common
N 43° 20' 58.56" W 124° 19' 47.93"	0	Euhaline	5	Rare	62	Common
N 43° 20' 59.76" W 124° 19' 49.50"	0	Euhaline	2	Rare	75	Common
N 43° 20' 53.69" W 124° 19' 45.18"	0	Euhaline	0	Absent	8	Rare
N 43° 20' 43.80" W 124°19' 19.15"	0.61	Euhaline	1518	Abundant	1931	Abundant
N 43° 20' 46.22" W 124° 19' 36.67"	0.96	Euhaline	893	Abundant	1467	Abundant
N 43° 20' 18.85" W 124° 19' 11.54"	1.31	Euhaline	75	Common	253	Common
N 43° 20' 17.70" W 124° 19' 12.24"	1.33	Euhaline	33	Common	148	Common

Table 2.1. (continued).

GPS Coordinates	Distance Upriver (km)	Salinity	January 2009		July 2009	
			No. of *M. senile* Individuals	Abundance Category	No. of *M. senile* Individuals	Abundance Category
N 43°20' 21.41" W 124° 19' 23.48"	1.34	Euhaline	92	Common	146	Common
N 43°20' 21.41" W 124° 19' 23.48"	1.34	Euhaline	15	Rare	94	Common
N 43° 20' 15.87" W 124° 19' 11.58"	1.41	Polyhaline	3	Rare	8	Rare
N 43° 20' 11.41" W 124° 19' 14.33"	1.46	Polyhaline	47	Common	189	Common
N 43° 20' 11.26" W 124° 19' 10.94"	1.50	Polyhaline	2	Rare	28	Common
N 43° 20' 9.17" W 124° 19' 13.30"	1.54	Polyhaline	31	Common	175	Common
N 43° 20' 6.48" W 124° 19' 12.55"	1.63	Polyhaline	15	Rare	53	Common
N 43°19' 52.98" W 124° 19' 11.15"	2.03	Polyhaline	7	Rare	34	Common
N 43°19' 6.73" W 124° 19' 14.72"	3.53	Polyhaline	3	Rare	21	Common

Table 2.1. (continued).

GPS Coordinates	Distance Upriver (km)	Salinity	January 2009		July 2009	
			No. of *M. senile* Individuals	Abundance Category	No. of *M. senile* Individuals	Abundance Category
N 43° 18' 42.66" W 124° 19' 20.69"	4.19	Polyhaline	2	Rare	8	Rare
N 43° 18' 25.22" W 124° 19' 19.29"	4.81	Polyhaline	0	Absent	4	Rare
N 43° 18' 23.60" W 124° 19' 6.89"	5.04	Polyhaline	0	Absent	5	Rare
N 43° 18' 24.22" W 124° 19' 5.07"	5.07	Polyhaline	0	Absent	3	Rare
N 43° 18' 16.20" W 124° 19' 25.35"	5.14	Polyhaline	0	Absent	2	Rare
N 43° 18' 30.60" W 124° 18' 51.30"	5.30	Polyhaline	0	Absent	3	Rare
N 43° 18' 20.43" W 124° 18' 50.96"	5.41	Polyhaline	0	Absent	2	Rare
N 43° 18' 7.85" W 124° 19' 9.27"	5.47	Mesohaline	0	Absent	0	Absent

Table 2.1. (continued).

GPS Coordinates	Distance Upriver (km)	Salinity	January 2009		July 2009	
			No. of *M. senile* Individuals	Abundance Category	No. of *M. senile* Individuals	Abundance Category
N 43° 18' 8.64" W 124° 18' 46.81"	5.83	Mesohaline	0	Absent	0	Absent
N 43° 17' 50.72" W 124° 19' 19.78"	6.03	Mesohaline	0	Absent	0	Absent
N 43°17' 43.25" W 124° 19' 10.05"	6.22	Mesohaline	0	Absent	0	Absent
N 43° 17' 52.09" W 124° 18' 50.38"	6.45	Mesohaline	0	Absent	0	Absent
N 43° 17' 23.51" W 124° 19' 23.29"	6.94	Mesohaline	0	Absent	0	Absent
N 43° 17' 30.46" W 124° 18' 33.97"	7.23	Mesohaline	0	Absent	0	Absent
N 43° 17' 2.52" W 124° 19' 25.09"	7.53	Mesohaline	0	Absent	0	Absent
N 43° 17' 20.55" W 124° 18' 29.96"	7.54	Mesohaline	0	Absent	0	Absent

Table 2.1. (continued).

GPS Coordinates	Distance Upriver (km)	Salinity	January 2009		July 2009	
			No. of *M. senile* Individuals	Abundance Category	No. of *M. senile* Individuals	Abundance Category
N 43° 16' 56.53" W 124° 19' 11.94"	7.88	Mesohaline	0	Absent	0	Absent
N 43° 17' 7.59" W 124° 18' 29.59"	7.94	Mesohaline	0	Absent	0	Absent

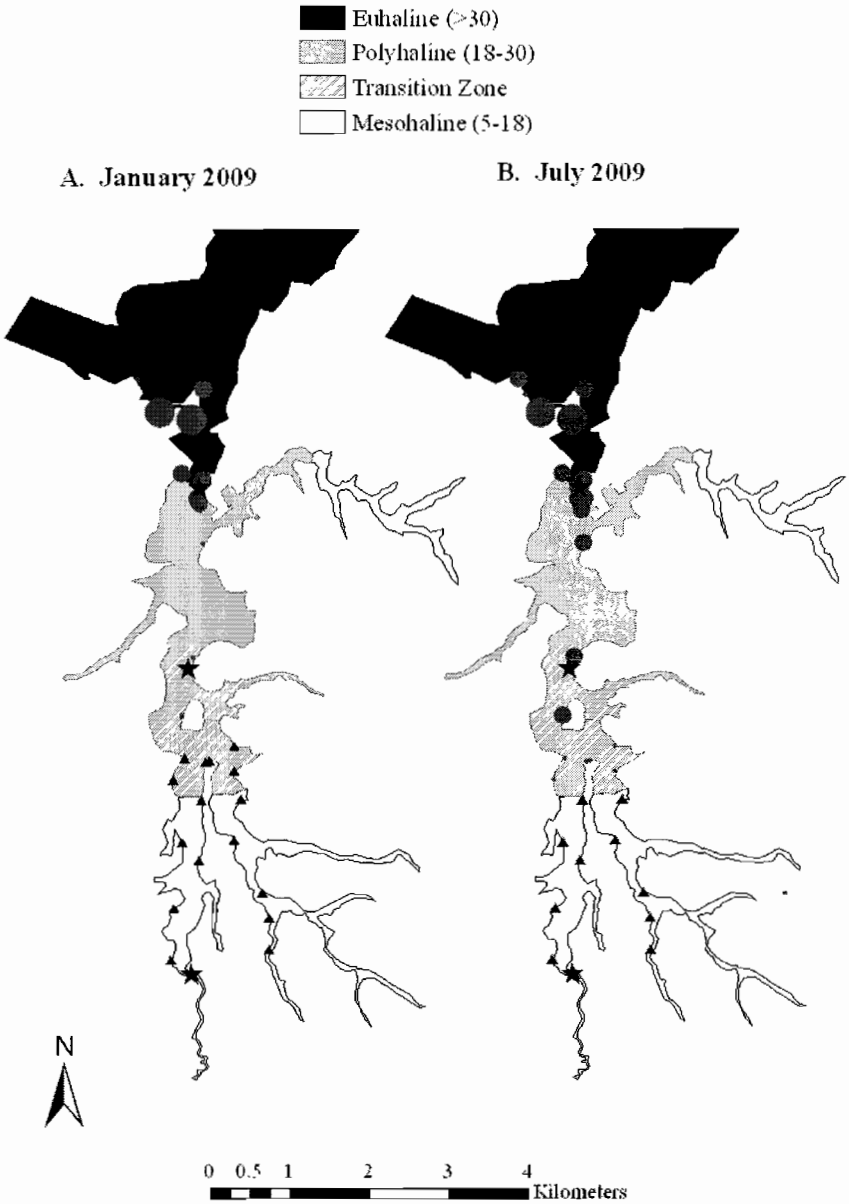

Figure 2.3. Abundance and distribution surveys of naturally occurring *Metridium senile* on various substrata in (A) January 2009 and (B) July 2009 within the South Slough. Circles represent the different levels of categorical abundance. Large circles (●) represent "Abundant" (>501) sites, medium circles (●) represent "Common" (16-500) sites, and small circles (●) represent "Rare" (1-15) sites. Triangles (▲) represent sites with no individuals. Stars (★) indicate location of field transplant sites. Coordinates for sites shown here can be found in Table 2.1. The salinity profile is derived from Davidson (2008), Arneson (1975), ODFW and SSNERR CDMO.

bedrock in the euhaline region and wood pilings and and woody debris in the polyhaline region. Individuals were absent from sites in the mesohaline region of the upper estuary. However, presence of individuals increased from around Long Island Point northward to the mouth of the estuary. Sites around Long Island Point and within Sengstacken and Winchester Arms were categorized as "rare", sites near Valino Island and the Valino field site were categorized as "common", whereas sites around the estuary mouth and the Charleston field site were predominantly categorized as either "common" or "abundant"(Table 2.1; Figure 2.3).

Seasonal differences in categorical abundance were observed at various sites within the South Slough estuary. Sites around Long Island Point and within Sengstacken and Winchester Arms lacked individuals during January 2009, yet during July 2009 a few individuals were observed as categorical abundance increased to "rare". Sites located adjacent to Valino Island and the Valino field site were categorized as "rare" during January 2009, however during July 2009 survey numbers of individuals increased, raising the categorical abundance of these sites to "common". Additionally, one site just south of the Charleston field site and the estuary mouth increased in categorical abundance from "rare" in January 2009 to "common" in July 2009 and a new site containing individuals was present near the Charleston field site during July 2009. Overall, an increase in categorical abundance was also seen in the estuary mouth around the Charleston boat basin, bridge and field site (Table 2.1 and Figure 2.3).

Metridium senile transplants

Overall, monthly mortality increased with distance from the estuary mouth during the 13-month measurement period from July 2008 to August 2009. Monthly mortality was lowest at the marine-dominated site, followed by the mesohaline and riverine-dominated sites, respectively. Seasonal differences in monthly mortality were also observed. At the marine and mesohaline-dominated site, mortality was highest in March 2009 and lowest in July 2009 showing peak survival during the dry season and lowest survival during the wet season. The riverine-dominated site exhibited highest mortality in January 2009 and lowest mortality in July 2009 showing peak survival during the dry season and lowest survival earlier during the wet season (Figure 2.4).

For the most part, cumulative weekly mortality confirmed these trends. Seasonal differences in cumulative weekly mortality were also observed at each field site. Peak survival occurred in July 2009 during the dry season for all three field sites, whereas lowest survival occurred in March 2009 for the marine-dominated site and January 2009 for the mesohaline and riverine-dominated sites (Figures 2.5, 2.6 and 2.7). In March 2009, mortality at the marine-dominated site occurred during each week, reaching a maximum of 35% mortality by the fourth week, whereas July 2009 experienced only 3% mortality by the fourth week (Figures 2.5 and 2.6). In contrast to monthly mortality trends, the mesohaline-dominated site exhibited highest weekly mortality in January 2009 exhibiting mortality each week with a maximum mortality of 100% by the fourth week (Figure 2.7). Lowest weekly mortality occurred in July 2009 with mortality occurring

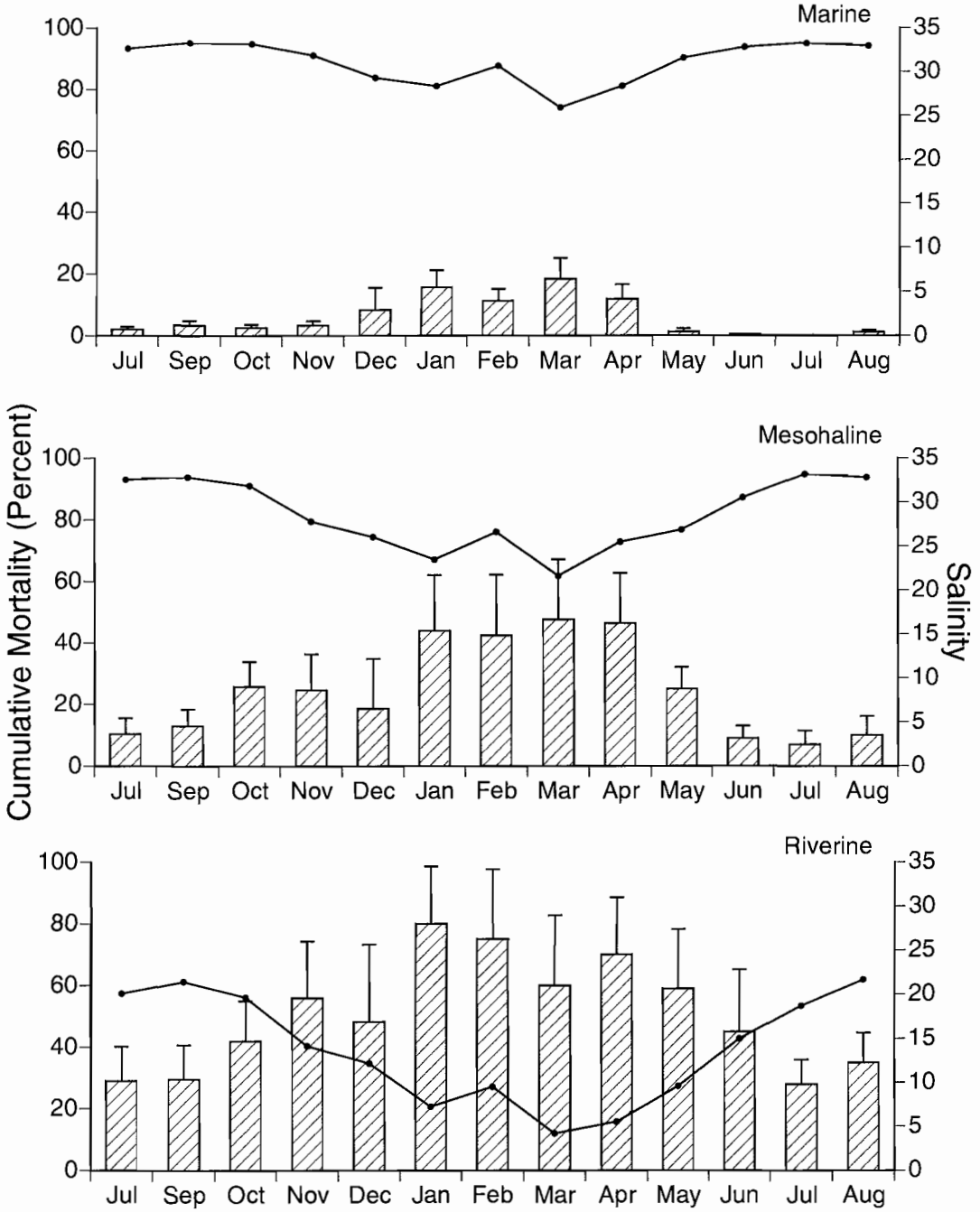

Figure 2.4. Cumulative monthly mortality of adult transplants at marine, mesohaline and riverine sites over four-week measurement periods from July 2008 to August 2009. Bars represent means with standard error (n=5, except for December (n=3) and February (n=4)). Line overlays represent changes in average salinity for each month at respective field sites.

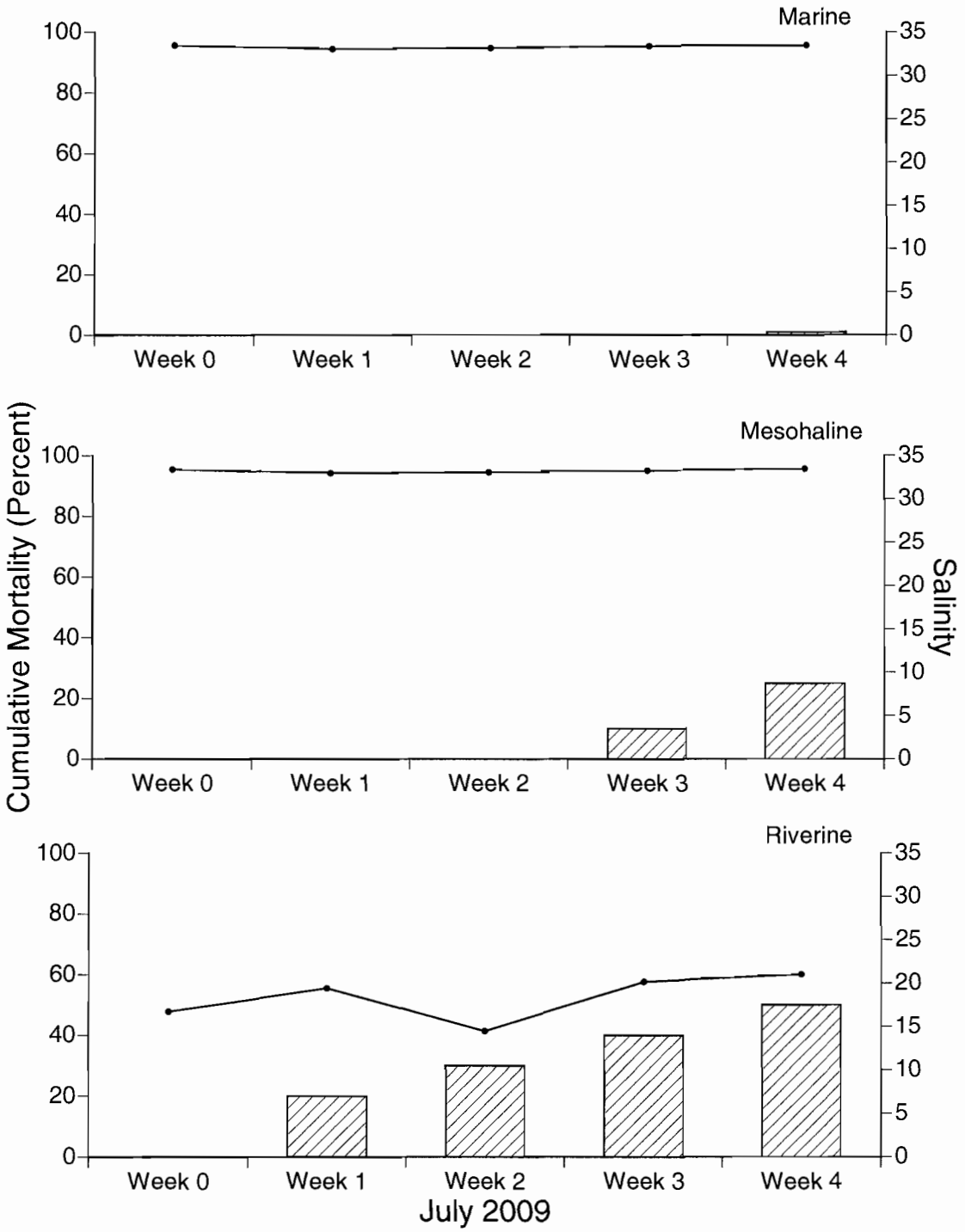

Figure 2.5. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during July 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

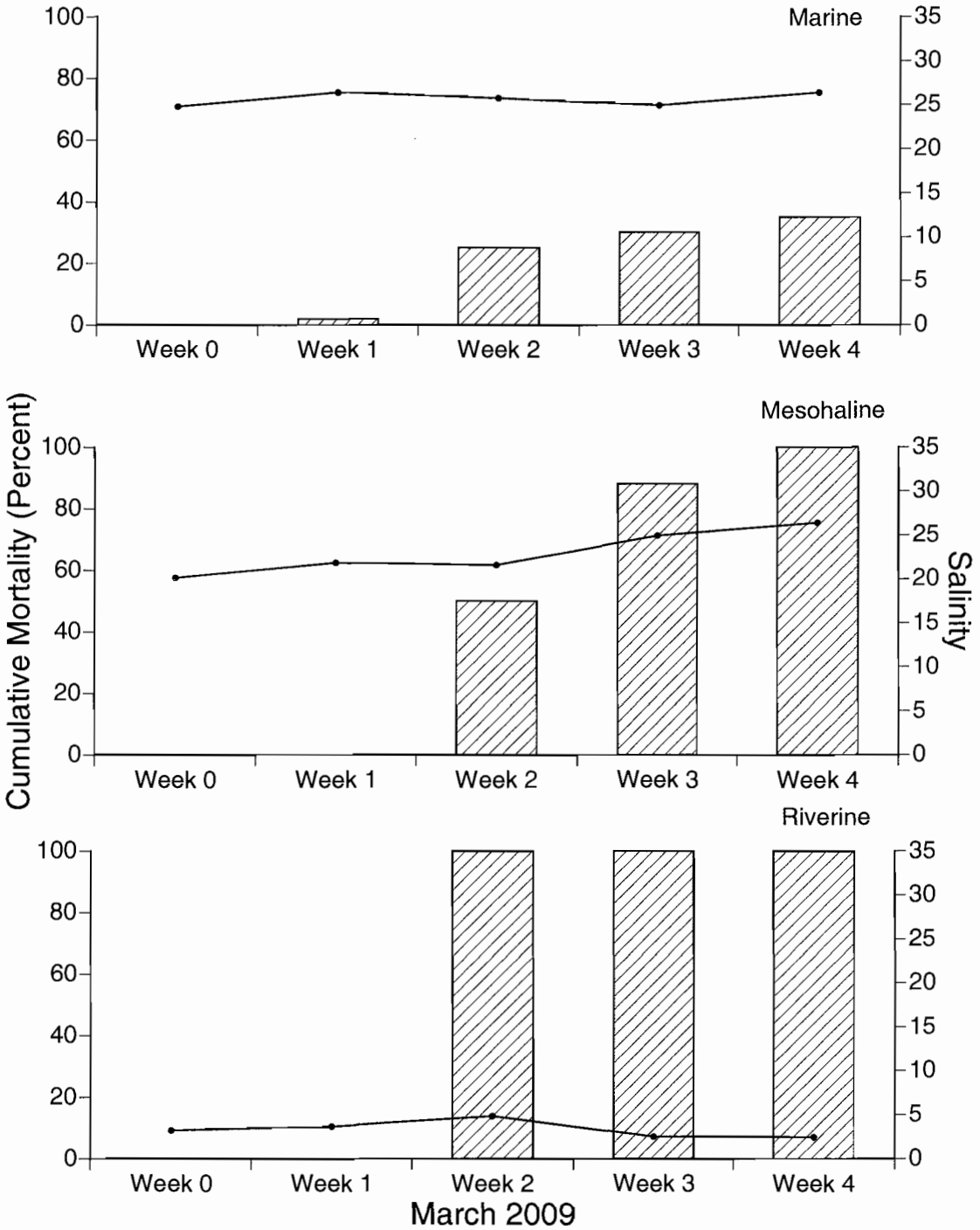

Figure 2.6. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during March 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

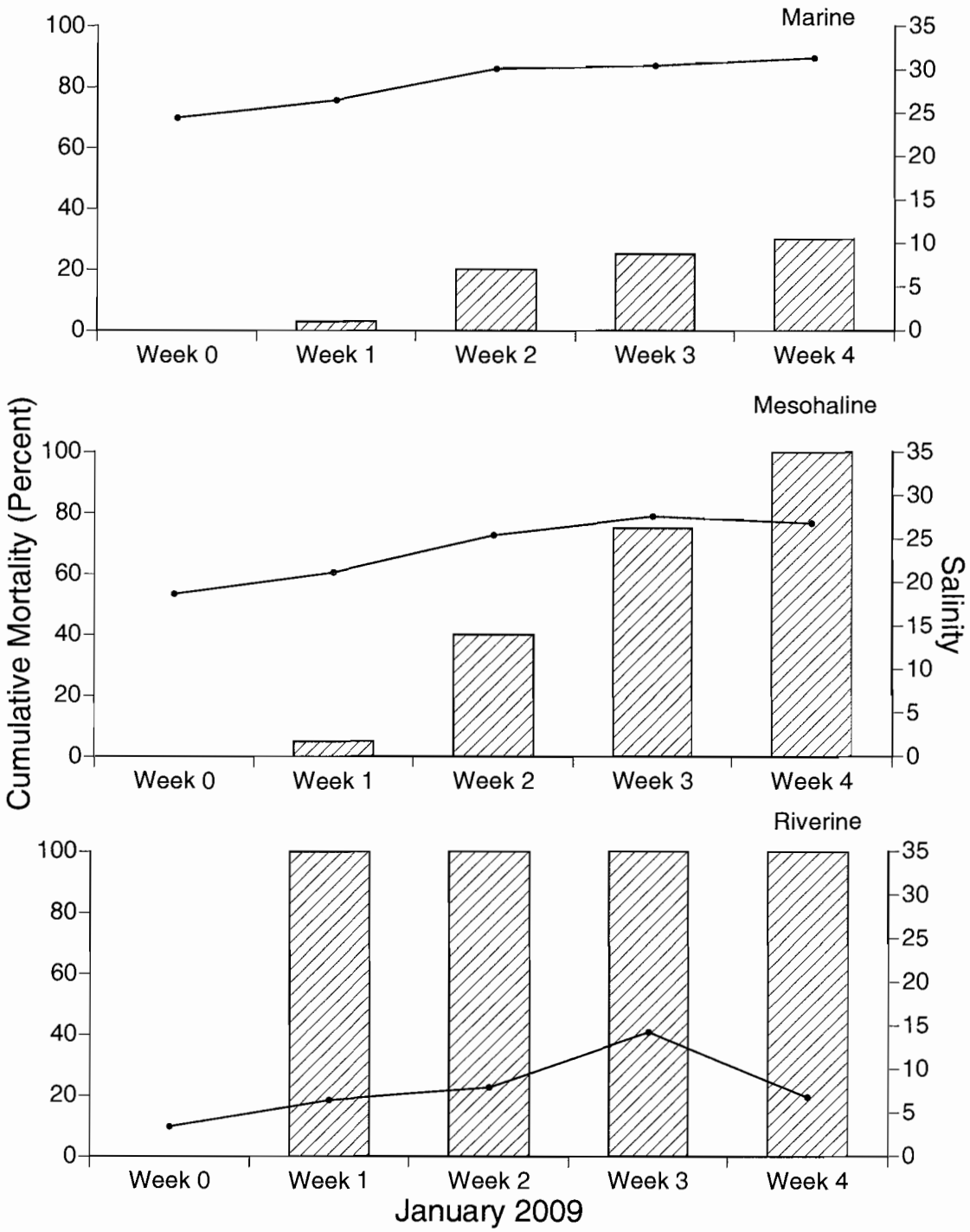

Figure 2.7. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during January 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

over the last two weeks and only 25% mortality by the fourth week (Figure 2.5). The riverine-dominated site experienced highest mortality during January 2009 with 100% mortality by the first week, whereas in July 2009 mortality was observed for the last two weeks with only 50% mortality by the fourth week (Figures 2.5 and 2.7). For additional monthly graphs of cumulative mortality refer to the Appendix Figures 28-36.

Discussion

Metridium senile exhibited discrete seasonal abundance and distribution patterns in the South Slough estuary. Individuals occupied the lower euhaline and polyhaline regions of the estuary during the winter and the lower to mid euhaline and polyhaline regions of the estuary during the summer. During the winter, the number of *M. senile* individuals present at each site was lower than in the summer, and their distributional range was smaller (Figure 2.3). This seasonal trend was also observed in the monthly transplant experiment, where individuals transplanted to the mesohaline and riverine-dominated zones showed decreased survival in the winter months of March and January respectively, and peak survival in the summer during July (Figure 2.4). Additionally, the abundance and distribution gradient observed in field surveys within winter and summer months were also supported by mortality trends observed at each of the three field sites where mortality was lowest at the marine-dominated site, followed by the mesohaline and riverine-dominated sites, respectively (Figures 2.3 and 2.4).

However, it is not appropriate to consider salinity as the sole indicator of estuarine abundance and distribution of *Metridium senile*. In addition to salinity, various other factors such as available substrata, temperature, dissolved oxygen, pH, dispersal constrictions, predation and food supply may affect the distribution and abundance of *M. senile* within the South Slough estuary. However, field surveys and experiments do suggest that the estuarine distribution of *M. senile* may be regulated by the salinity gradient present in the South Slough estuary. All of the individuals observed within the estuary were located in euhaline and polyhaline areas where there was less salinity fluctuation (mean annual salinity range: ~18 to ~33) (NERR CDMO, http://cdmo.barch.sc.edu). This trend was also supported by the lack of *M. senile* individuals in the mesohaline region of the estuary south of Long Island Point where salinity was more varied (mean annual salinity range: ~4 to ~21) because of increased domination by freshwater (NERR CDMO, http://cdmo.barch.sc.edu; Figure 2.3). Available substrata did not seem to be a contributing factor to the abundance and distribution of *M. senile* within the South Slough estuary as available substrata decreased slightly with distance upriver. Overall, docks, cement pilings, wooden pilings and rocky outcroppings, which were ubiquitous within the marine-dominated region, gave way to only slightly less available substrata, which included vertical wooden pilings and woody debris lining sections of the polyhaline and riverine regions of Winchester and Sengstacken Arms (Rumrill, 2006). A number of these pilings were encrusted with

barnacles as well. However, even with the presence of available substrata, *M. senile* has not been documented within the mesohaline regions of the estuary.

The other factors that may affect the abundance and distribution of *Metridium senile* within the South Slough estuary do not sufficiently explain the absence of individuals within the mesohaline zones of the upper estuary. In addition to salinity and available substrata, water temperature may play a role. However, it is unclear whether temperature plays a significant role in determining the abundance and distribution limit of this species in the upper region of the estuary since *M. senile* has been documented in Table Bay off of the coast of South Africa, Argentina and southern California in waters that are similar to peak temperatures in the South Slough estuary (Gates *et al.*, 1992; Acuña and Griffiths, 2004; Rumrill, 2006). Additionally, studies performed by North (1957) on the reaction time (the period between initial exposure and observed response) of *M. senile* over a temperature range of 17°C found no significant changes in average reaction time and concluded that reaction time variability was not caused by changes in physiological state. However, short-term salinity tolerance is influenced by temperature in other anemone species such as *Haliplanella luciae* in which individuals had a narrower salinity tolerance range at higher temperatures (Benson-Rodenbough and Ellington, 1982; Shick, 1976). Limitations caused by variations in dissolved oxygen and pH are unlikely since these form weaker gradients within the South Slough estuary and *M. senile* has been documented recolonizing areas that were previously depopulated by anoxic and

hypoxic waters (Wahl, 1985b; Rumrill, 2006).Individuals have also shown considerable resilience to severe hypoxia (Wahl, 1985b; Diaz and Rosenberg, 1995).

Dispersal could also influence the abundance and distribution patterns observed within the South Slough estuary. Large-scale dispersal has been documented in the planktonic larval stage and adult *Metridium senile* have been transported 0.10 to 10 km from their native substrata by water currents (Shick *et al.*, 1979, Wahl; 1985a). Additionally, rafting of *M. senile* attached to flotsam such as woody debris or litter have possibly been the means by which populations of *M. senile* were introduced into new locales such as the Flensburg Fjord in the Western Baltic (Wahl, 1985a). Therefore, *M. senile* could be transported considerable distances upriver during a flood tide by incoming water currents or on random woody material that has been observed along stretches of the South Slough estuary (Rumrill, 2006). Predation impacts on *M. senile* abundance and distribution are negligible since there is no documentation of its key predators *Aeolidia papillosa* and *Dermasterias imbricata* upriver from the Charleston Bridge within the South Slough Estuary (Francis and Kramer, 2004; Rumrill, 2006). The South Slough estuary contains a diverse supply of zooplankton that can be transported long distances by tidal advection (Rumrill, 2006). For example, I have observed crab zoea 7.7 km upriver around the Winchester field site during the summer. Therefore, it is unlikely that food supply restricts the abundance and distribution patterns of *M. senile* within the South Slough estuary. This species primarily feeds on small zooplankton (Shick, 1991).

While the observed trends in seasonal abundance and mortality could possibly be due to a variety and combination of factors that typically limit marine organisms, none explains these trends sufficiently. Seasonal field surveys and mortality trends are probably influenced by the salinity associated with the hydrogeomorphic region of the estuary where naturally occurring individuals were observed and in which transplants were placed. Decreased abundance and distribution of *Metridium senile* and increased mortality with distance from the estuary mouth as well as increased mortality during the wet season at all three field sites could be attributed to the diel and seasonal salinity flux within the South Slough estuary (Figures 2.3 and 2.4). Since diel fluctuations of salinity increase with distance from the estuary mouth, *Metridium senile* individuals are exposed to a combination of decreased ambient salinity and increased salinity variation with distance upriver (Rumrill, 2006). Seasonal precipitation during the wet season results in a substantial influx of freshwater which greatly reduces the salinity regime in each of the hydrogeomorphic regions of the estuary (Rumrill, 2006). Thus, during the wet season, *M. senile* individuals are exposed to increased salinity fluctuations.

Therefore, the abundance and distribution pattern of *Metridium senile* observed during January 2009 may be attributed to seasonal freshwater influx and increased salinity variation, which explains the absence of individuals in the mid to upper estuary (Figure 2.3). Transplant mortality in the mid to upper estuary was highest during the wet season and increased with distance from the estuary mouth (Figure 2.4). This trend mirrors the salinity profile of the South Slough Estuary during the wet season. During the

dry season however, the salinity profile changes as decreased precipitation results in a higher average salinity regime. The abundance observed during July 2009 reflects this change in the salinity profile since distribution range of *M. senile* was extended a kilometer upriver and included the lower and mid estuary regions (Figure 2.3). This extension was likely due to decreased freshwater input resulting in higher salinity within the mid estuary. Additionally, trends of transplant mortality confirm this since transplants at each of the field sites survived much better in the dry season compared to the wet season, showing lowest mortality in July 2009 and highest mortality in January and March of 2009 (Figure 2.4). Adult transplants also showed lower survival with distance from the estuary mouth during the July 2008-August 2009 measurement period, which could be attributed to the presence of a strong estuarine salinity gradient (Figure 2.4). Consequently, since the South Slough estuary inherently has a strong salinity gradient, it is likely that trends observed in seasonal field surveys and adult transplant experiments are largely governed by a salinity gradient rather than weaker gradients such as water temperature, dissolved oxygen and pH (Rumrill, 2006).

Bridge

Chapter II examined the abundance and distribution of the actinarian *Metridium senile* in a temperate estuary located along the southern Oregon coast. Abundance and distribution patterns were directly mirrored by observed trends in transplant mortality within the South Slough estuary. Trends followed a seasonal pattern and were likely regulated by the estuary's salinity profile within each of the three hydrogeomorphic regions, resulting in increased mortality with distance from the estuary mouth. While the salinity regime of the South Slough estuary is thought to govern these observed trends and inherently exposes organisms to hyposaline conditions, no evidence is presented on the effect of hyposalinity on sea anemone physiology. How do sea anemones respond to these conditions? Chapter III answers this question by examining the effect of hypo-osmotic stress on *M. senile*'s mortality and regulation of volume, osmolality, and magnesium ions. The effect of hypo-osmotic stress is examined in both a laboratory and field setting over weekly and monthly measurement periods. Finally, this chapter determines the ability of *M. senile* to regulate under hypo-osmotic stress and discusses how this ability may contribute to its survival and distribution within the South Slough Estuary.

CHAPTER III

MORTALITY AND PARTIAL REGULATION OF VOLUME, OSMOLALITY, AND MAGNEISIUM ION CONCENTRATIONS IN THE SEA ANEMONE *METRIDIUM SENILE* UNDER HYPO-OSMOTIC CONDITIONS

Introduction

Life in an estuary: a fluctuating salinity environment

Marine organisms inhabit a wide range of saline environments, from the brackish waters of estuaries to the extreme hypersaline conditions of brine pools. These habitats differ considerably in water and solute chemistry (Evans, 2009). Variations in water and solute concentrations within these habitats can impose a number of physiological stressors upon marine organisms (Rankin and Davenport, 1981; Evans, 2009). Furthermore, changes in water and solute chemistry can be augmented by a variable salinity regime or dampened by a stable salinity regime (Shumway, 1977; Rankin and Davenport, 1981). For example, estuarine habitats are inherently characterized by a variable salinity regime. This exposes organisms to a constantly fluctuating salinity environment in which they can experience nearly fresh to full-strength seawater on a diel basis (Rankin and Davenport, 1981; Rumrill, 2006). Thus, the body fluids of organisms living in this environment are either hypo-osmotic or hyperosmotic to the ambient

medium, resulting in water and salt regulation issues. However, organisms living in a stable salinity regime encounter little change in environmental salinity on either a seasonal or diel basis, resulting in little or no osmotic stress (Rankin and Davenport, 1981). Fluctuating salinity environments therefore induce a number of adaptations that aid in the maintenance of cellular salt and water balance (Rankin and Davenport, 1981; Evans, 2009).

Marine animals are divided into two groups based on their mode of physiological response to salinity stress: *osmoconformers* and *osmoregulators*. *Osmoconformers* are organisms that maintain an internal osmotic pressure that is equal to the ambient environment. This process can be active or passive. In contrast, *osmoregulators* are organisms that maintain a constant internal osmotic pressure irrespective of changes in the ambient environment. This process is primarily active since osmoregulators control salt concentrations by actively pumping salt out of the cell through specific ion channels in order to maintain cellular volume. One such mechanism is the sodium-potassium pump (i.e. Na^+/K^+-ATPase). The pump transports two potassium ions in for every three sodium ions that are pumped out of the cell and since cellular membranes are less permeable to sodium ions than potassium ions, sodium tends to remain outside the cell. This results in a continual net loss of ions out of the cell that ultimately drives water molecules out of the cell. However, it is unclear whether this mechanism is solely responsible for osmoregulation in animal cells since Ouabain, a potent metabolic inhibitor, does not inhibit the volume regulatory process. Therefore, cellular osmoregulation may be driven

by another type of sodium-potassium pump (Rankin and Davenport, 1981; Evans, 2009). Many marine species found in estuarine habitats are osmoconformers and will absorb water and lose salts until their bodies are iso-osmotic with the ambient environment. A number of these species can tolerate a wide range of salinities and are considered *euryhaline*, while other osmoconformers can only tolerate a very narrow salinity range and are considered *stenohaline*. In general, stenohaline species occur in stable salinity environments while euryhaline species can be found in either stable or fluctuating-salinity environments. Consequently, because of the range of saline environments and physiological responses to such environs, marine organisms have evolved a variety of adaptive strategies that are used in the maintenance of cellular and organismal salt and water balance (Rankin and Davenport, 1981; Evans, 2009).

Adaptive strategies across many marine invertebrate phyla have been employed to combat hypo-osmotic stress. These include excretory systems (e.g. Arthropoda, and Annelida), selective transport of ions across cellular membranes (e.g. Arthropoda, Annelida, Echinodermata, and Mollusca), selective ion secretion and uptake using antennary glands and gills (e.g. Arthropoda), regulation of the intracellular free amino acid pool (FAA) (e.g. Annelida, and Mollusca), and behavioral isolation through a shell or cuticle (e.g. Arthropoda, Annelida, and Mollusca) (Rankin and Davenport, 1981; Evans, 2009).

In contrast to arthropods, annelids, and molluscs, cnidarians lack excretory or circulatory systems, and so are without an internal environment that houses a fluid

capable of circulating and therefore regulating water and salt (Krogh, 1939; Brusca, 1980). There is, however an acellular mesoglea or partly cellular mesenchyme that separates the ectoderm and endoderm (Brusca, 1980). In comparison to other phyla, there are few studies on the osmotic conditions and osmoregulatory mechanisms involved in hypo-osmotic stress for cnidarians, since the fluid is hard to isolate and analyze (Krogh, 1939; Rankin and Davenport, 1981). Despite the difficulties of working with cnidarians, there have been studies on the physiological mechanisms and adaptive strategies of these organisms in response to hypo-osmotic stress.

Cnidarians lack an internal environment; they contain only one body cavity (the coelenteron), which has one opening and is in constant contact with the external environment (Brusca, 1980). These organisms are considered osmoconformers (Brusca, 1980). Hypo-osmotic stress causes equilibration of the body with the surrounding environment, which is achieved by absorbing water and losing salts until the body is iso-osmotic with the ambient medium. Since water movement is faster than salt diffusion, immediate exposure to dilute saline conditions causes these organisms to gain water rapidly and swell up, thereby increasing in weight (Krogh, 1939; Brusca, 1980; Rankin and Davenport, 1981). Even though cnidarians have been primarily categorized as osmoconformers, ion regulation has been documented in freshwater cnidarians such as hydroids and scyphozoans (Lilly, 1955; Fleming and Hazelwood, 1967). Even with no obvious way to excrete water, osmoregulation has also been recorded in other freshwater cnidarian species. The mechanism, however, remains somewhat of a mystery (Prusch *et*

al., 1976). In mesohaline (salinity 5-25) populations of the scyphomedusae *Chrysaora quinquecirrha*, potassium ion regulation was noted when individuals were transferred from a salinity of 20 to a salinity of 8, and other ions such as magnesium and sodium remained hyperosmotic to the ambient medium after one week (Wright and Purcell, 1997). Ion regulation in mesohaline scyphomedusae of the Chesapeake Bay may be an adaptive strategy used to combat hypo-osmotic conditions.

Another group of cnidarians, the actinarians, are generally considered marine stenohaline osmoconformers, yet a number of species are known to inhabit estuaries. The sea anemone *Diadumene leucolena* regularly inhabits brackish waters and can survive salinities ranging from 6 to 33 (Pierce and Minasian, 1974). In addition, *Haliplanella luciae* can survive indefinitely at a salinity level of 12 (Shick, 1976). To combat these hypo-osmotic conditions, both species partially regulate tissue hydration and the cellular free amino acid pool (FAA) (Pierce and Minasian, 1974; Shick, 1976). A similar trend was seen in the euryhaline anemone *Bunodosoma cavernata* which tolerated exposure for two weeks to a salinity range from 11 to 49. To acclimate to changing salinities, *B. cavernata* regulated cellular volume and in turn cellular FAA (Benson-Rodenbough and Ellington, 1982). Partial volume regulation has also been documented in the sea anemone *Metridium senile,* even though this species is primarily marine and does not normally populate estuaries (Deaton and Hoffmann, 1988; Shick, 1991). Nevertheless, *M. senile* shows an astonishing ability to survive low salinities, which may be attributed to a number of physiological and behavioral factors: retraction of tentacles, contraction of the

body wall, secretion of mucus, cellular volume regulation, or regulation of the intracellular free amino acid pool (FAA) (Shumway, 1978; Deaton and Hoffmann, 1988; Shick, 1991). Consequently, sea anemones have evolved many different behavioral, morphological, cellular and ionic adaptations to resist hypo-osmotic stress.

Retracting tentacles, contracting the body wall and secreting mucus is synonymous to changing shell gape and adducting shell valves in bivalves, and withdrawing into shells in gastropods (Shumway, 1978). These actions aid in minimizing surface area in contact with the ambient medium. Additionally, once sea anemones contract the body wall, ventilation and subsequent equilibration with the ambient medium ceases, resulting in decreased osmotic influx of water and efflux of ions and organic solutes (Shoup, 1932; Miyawaki, 1951; Shumway, 1978; Benson-Rodenbough and Ellington, 1982; Shick, 1991). Secretion of a mucus film around the anemone acts as a barrier to water and solute movements as it creates an unstirred layer to develop on the ectodermal surface (Shoup, 1932; Shick, 1976; Shumway, 1978; Shick, 1991). However, the diffusional permeability of this mucus layer has not been studied (Shick, 1991). Additional studies suggest that sea anemone mucus sequesters ions, specifically calcium ions (Ca^{2+}). Therefore, mucus secretion may provide a means to conserve calcium ions available to bind to the external membranes when ambient salinity is low, thereby reducing the efflux of free amino acids (Goreau, 1959; Pierce and Greenberg, 1973). Consequently, this mechanism may allow anemones to retain important organic osmolytes during short-term salinity fluxes (Pierce and Greenberg, 1973; Shick, 1991).

Since sea anemones characteristically exhibit re-expansion under all but extreme hypo-osmotic conditions, individual cells must regulate their volume to avoid excess cellular swelling (Shick, 1976; Kasschau *et al.*, 1984a; Deaton and Hoffmann, 1988). The process of cell volume regulation is mediated by cellular and ionic mechanisms in response to hypo-osmotic stress. Typically, osmoconforming sea anemones exhibit small variations in tissue hydration under hypo-osmotic stress which indicates that cellular volume regulation is indeed occurring (Benson-Rodenbough and Ellington, 1982; Shick, 1991). *Diadumene leucolena*, *Haliplanella lineata,* and *Metridium senile* all show increased volume regulatory ability under dilute saline conditions which may be due to a decrease in the anemones' "water permeability", (Deaton and Hoffmann, 1988; Shick, 1991). However, further studies suggest that cell volume regulation is mediated by altering concentrations of the intracellular free amino acid pool (FAA), which decreases in concentration with salinity (Lange, 1972; Gilles, 1979; Benson-Rodenbough and Ellington, 1982; Deaton and Hoffmann, 1988; Shick, 1991). Compensatory adjustments of intracellular FAA concentrations seem to be an active regulatory process since the change in intracellular FAA concentrations of *B. cavernata*, *D. leucolena*, *H. lineata*, and *M. senile* was five to eight times less than the change in FAA concentrations of the ambient environment. Both *M. senile* and *D. leucolena* have a greater ability to reduce their FAA concentrations compared to *B. cavernata,* which may contribute to their augmented volume regulatory ability at low salinity levels. One mechanism for reducing intracellular FAA concentrations in *M. senile* and *D. leucolena* is selectively increasing

the permeability of the cell membrane to available FAA, subsequently increasing their efflux. Survival of *D. leucolena* in estuarine habitats can be explained by increased volume regulatory ability. However, the ecological relevance of *M. senile*'s volume regulatory ability is largely unknown since it does not usually inhabit estuaries (Pierce and Minasian, 1974; Shick, 1976; Benson-Rodenbough and Ellington, 1982; Kasschau *et al.*, 1984a; Deaton and Hoffmann, 1988).

Some actinarians can regulate their FAA concentrations, and in turn cellular volume. Anemones may also be capable of regulating intracellular ions like their relatives, the scyphomedusae (Wright and Purcell, 1997). Regulation of intracellular ions may aid in the conservation of key metabolic processes under hypo-osmotic stress. For example, magnesium ions (Mg^{2+}) are involved in a number of enzymatic reactions as a co-factor including those involved in the transfer of phosphate groups, the stabilization of ion channels and cell membranes and most importantly the production of energy in the form of ATP (Hee Ko *et al.*, 1999). Additionally, magnesium interacts with substrates or enzymes and is sometimes required for activity as part of the active site. Consequently, because of magnesium's metabolic importance, reductions in magnesium concentrations could lead to decreased metabolic activity and eventually death (Parker *et al.*,1990; Lang, *et. al.,* 1998; Hee Ko *et al.*, 1999).

Even though actinarians generally are not considered euryhaline organisms, a few species were observed to have wide salinity tolerances (Shick, 1991). Many studies have investigated the salinity tolerances of actinarians such as *Diadumene leucolena,*

Haliplanella lineata and *Bunodosoma cavernata* and found a range of regulatory abilities for tissue hydration and the intracellular FAA (Pierce and Minasian, 1974; Shick, 1976; Benson-Rodenbough and Ellington, 1982). However, there have not been any studies that investigated the effect of hypo-osmotic stress on regulation of tissue hydration, osmolality, and intracellular ions (like magnesium) within specific actinarian species. This chapter aims to evaluate the salinity tolerance limits of the actinarian *Metridium senile* from a physiological perspective in both laboratory and field settings.

Materials and Methods

The effect of hypo-osmotic stress on mortality and regulation of volume, osmolality, and magnesium ions in *Metridium senile* was investigated at the Oregon Institute of Marine Biology (OIMB), Charleston, Oregon, and in the adjacent South Slough Estuary from 2008-2009. Laboratory studies were conducted at OIMB while field transplant studies were conducted within the estuary.

Laboratory study

During the month of February 2008, a laboratory experiment tested the effect of hypo-osmotic stress on volume regulation and Mg^{2+} concentration of *Metridium senile* subjected to 50%, 75%, and 100% seawater. At the beginning of February 2008, ninety *M. senile* individuals were manually collected from the docks in the Charleston marina by scraping. Individuals were brought back to the lab, immersed immediately in running

seawater (33 salinity), and allowed to reattach over 24-h to circular pieces of nylon mesh, measuring 8 cm in diameter. Three salinity treatments were prepared by filling three large plastic containers (46 cm x 60 cm) with one of the following: 100% SW (33 salinity), 75% SW (25 salinity) and 50% SW (16.5 salinity). The diluent was reverse osmosis water and the salinity levels of each seawater treatment were confirmed with a refractometer. Thirty anemones were placed in 100% SW, 75% SW and 50% SW, respectively. Three anemones were destructively sampled at random on a weekly basis from each treatment culminating in initial and weekly measurements of volume regulation and Mg^{2+} concentration over a one-month period.

Field study

The field portion of this study utilized the salinity gradient from the mouth (marine-dominated zone) to terminal end (riverine-dominated zone) of the South Slough estuary, a small and fairly shallow drowned-river estuary located in the larger Coos estuary along the southern Oregon coast. One field site was selected within each of the three hydrogeomorphic zones within the South Slough. Field sites were chosen based upon location within the South Slough, accessibility, and immediate proximity to the South Slough National Estuarine Research Reserve's (SSNERR) System-Wide Monitoring Program (SWMP) stations, where real time abiotic data, including salinity, temperature and dissolved oxygen were collected (Figure 2.1).

At each field site, I deployed one floating frame for anemone containment adjacent to SSNERR's SWMP monitoring station. Each floating frame was a sealed 1 m x 1 m PVC pipe square with thirty 9-cm^3 plastic mesh boxes (14 mm mesh opening), each housing one anemone. Each plastic mesh box was attached with 15 cm of thin wall PVC pipe using cable ties. Two mooring buoys were attached to opposite corners of each floating frame. Floating frames were anchored in the subtidal sediment with two screw anchors placed approximately 2.5 m on either side using twisted polypropylene rope. For mortality measurements, additional *Metridium senile* individuals were placed within a large 30.5 cm^3 plastic mesh flow-through container with a 14 mm mesh opening that was attached to the frame with cable ties (Figure 2.2B; Figure 3.1A-C).

Because *Metridium senile* is commonly found in the low intertidal and subtidal waters of bays and harbors, the marine-dominated field site ("Charleston") served as the control site (Carlton, 2007; Figure 2.1).This site lies adjacent to the Charleston bridge (43°20'15.72 N, 124°19'13.92 W) and consists of well-mixed tidal waters with a maximum tidal amplitude of 2.6 m and monthly mean salinity of 31 during the dry season and 20 during the wet season. The mesohaline-dominated field site ("Valino") rests slightly to the north of Valino Island within the South Slough (43°19'1.98 N, 124°19'17.88 W) and consists of well-mixed tidal waters with a maximum tidal amplitude of 2.7 m and salinity ranging from 15-28 (Figure 2.1). The riverine-dominated field site ("Winchester") is located within Winchester Arm across from Danger Point on the east side of the channel (43°16'56.70 N, 124°19'13.14 W) and consists of a maximum tidal amplitude of 2.0 m

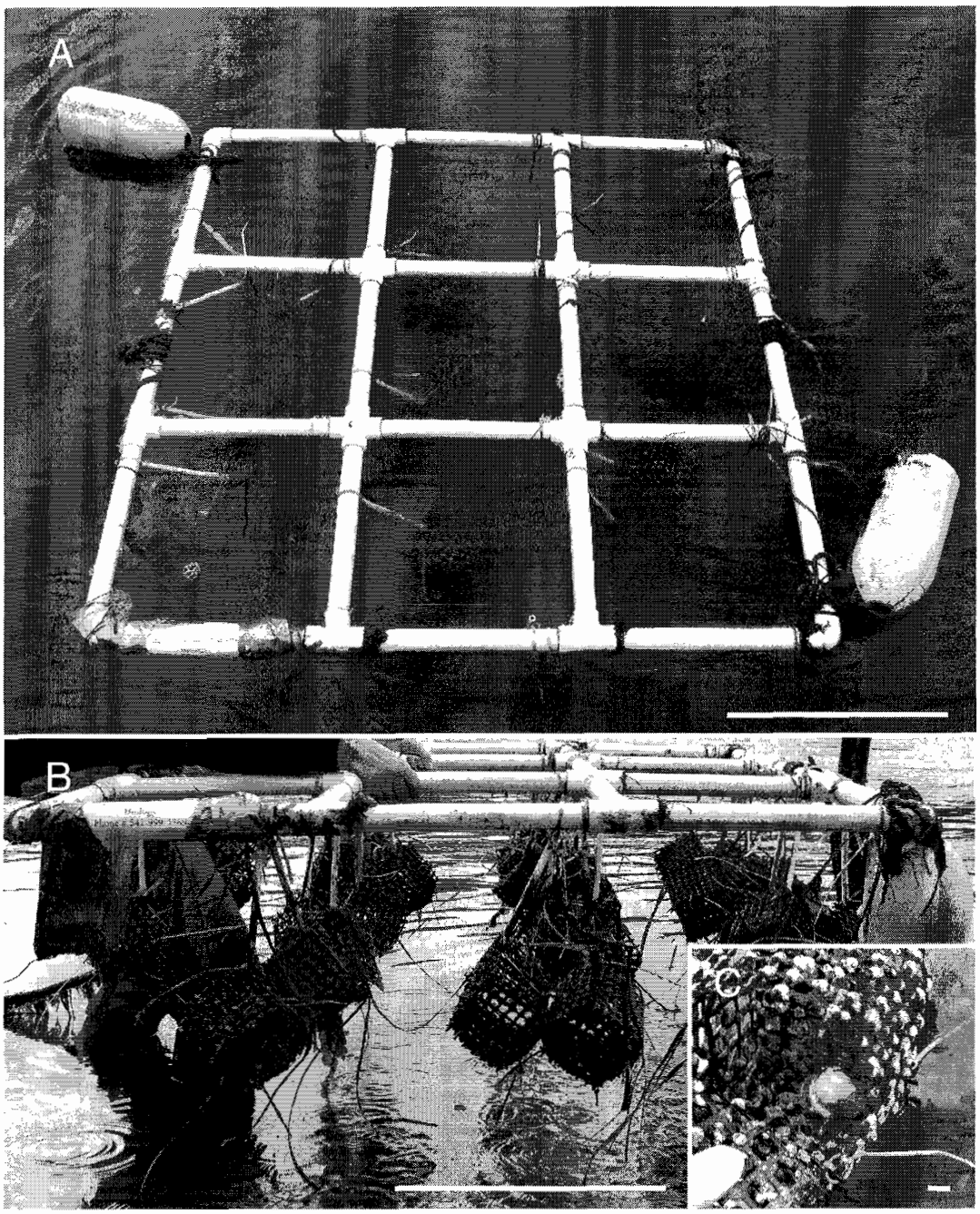

Figure 3.1. Images of (A) the floating frame with two mooring buoys, (B) plastic mesh boxes attached to the floating frame with thin wall PVC and cable ties for anemone containment, and (C) an anemone within a plastic mesh box. Scale bars in A and B are 30.5-cm and the scale bar in C is 1.4-cm.

(Figure 2.1). During the dry season, tidal waters are characteristically well-mixed, with salinity ranging from 5-30; during the wet season however, tidal waters are partially stratified, with salinity ranging from 0-21 (Rumrill, 2006).

At the beginning of each month, 150 individuals of approximately similar size (2.5-3.5 cm high and 1.5-3.0 cm diameter pedal discs) were manually detached from substrata adjacent to the marine-dominated site of the Charleston Bridge by scraping. Each individual was randomly selected from distinct clones determined by the presence of anemone-free spaces (Francis, 1973). Individuals were brought back to the lab, immersed immediately in running seawater (33 salinity), and allowed to reattach over 24-h to circular pieces of nylon mesh, measuring 8 cm in diameter. Once each individual was reattached, it was placed directly into a container filled with 33 salinity seawater. Fifty individuals were then transported and placed at each of the three field sites. Thirty of the individuals were haphazardly selected and placed within a plastic mesh box attached to the floating frame described above for weekly sampling. The remaining twenty individuals were placed within the large flow-through container for weekly monitoring where they remained attached to their respective mesh net (Figure 2.2B).

From July 2008 to August 2009 six randomly selected anemones were destructively sampled on a weekly basis over a one-month period from each field site culminating in initial and weekly measurements of regulation of volume, osmolality and Mg^{2+} concentrations for each month. Cumulative mortality measurements were also taken on a monthly basis at each field site. Results and subsequent statistical analyses are

reported on select months representative of the wet and dry seasons as well as the fall and spring transition periods within the South Slough estuary, which occurred around October 23, 2008 and March 29, 2009, respectively (www.cbr.washington.edu/data/trans.html; M. Kosro, pers. comm., Oregon State University).

Measurement of volume regulation

Percent tissue hydration was used as a proxy for volume regulation at each site along the estuarine gradient (Deaton and Hoffmann, 1988). Each individual's body wall was slit with a scalpel, allowing the coelenteron fluid to drain, and subsequently blotted with a paper towel several times for 15 seconds to remove excess fluid. Individuals were put on a piece of parafilm to see if any droplets of water formed. If water was observed on the parafilm, then individuals were blotted again (L. Deaton pers. comm., University of Louisiana). After blotting, wet weights were measured using a Mettler Toledo AT460 weighing balance. To test the accuracy of this technique, the blotting protocol was used on the same individual five times after the body wall was slit. Each individual was blotted and weighed, placed back into the water it came out of, and then blotted and weighed again to see if the same wet weight was attained after each trial (L. Deaton pers. comm., University of Louisiana). Anemones were subsequently dried at 60°C for 48 hours and then reweighed. The percentage of water in the tissues was calculated by dividing the difference between the wet and dry weights by the wet weight to determine percent tissue hydration (Oglesby, 1975; Deaton and Hoffmann, 1988).

Oglesby's (1975) equation was used to quantify volume regulation based on changes in *Metridium senile*'s tissue hydration after acclimation to marine, mesohaline and riverine-dominated sites. Oglesby (1975) defined ß "as the proportion of the maximum excess water which is actually retained after a transfer", such that ß=1.0 indicates no regulation (maximum excess water retention; organism behaves as a simple osmometer), and ß=0.0 indicates complete regulation (no excess water retention; the water content is the same in all salinities). The index of regulation (ß) is calculated by using the tissue water contents at higher (W_1) and lower (W_2) salinities:

$$W_2 = (W_{2max} - W_1)ß + W_1$$

where

$$W_{2max} = \left[\frac{C_1}{C_2}\right] W_1$$

which is the maximum excess water retained after a transfer from higher (C_1) to lower salinity (C_2) (Oglesby, 1975; Deaton and Hoffmann, 1988; Shick, 1991).

Therefore, ß-values were calculated using wet weights and ambient osmolality measurements at higher and lower salinities for each sampled anemone. Weekly and monthly percent tissue hydration and accompanying ß values were determined at the marine, mesohaline and riverine-dominated field sites for each month and year, respectively.

Measurement of magnesium ion (Mg^{2+}) concentrations

Approximately 0.5 grams of *Metridium senile* mesentery tissue (obtained by cutting longitudinally through mesentery tissue and mesogleal fluid adjacent to the pharynx) was homogenized in 200 µl of distilled water using a plastic mortar and pestle. Magnesium ion concentrations were then measured colorimetrically by deproteinizing the homogenized tissue sample with 5% trichloracetic acid and allowing the sample supernatant to react with thiazole yellow and 2N LiOH. The sample absorbance was measured immediately at 540 nm using a Beckman DU-70 spectrophotometer (Sky-Peck, 1964; Brown and Terwilliger, 1992). The concentration of magnesium for each sample was determined by comparing each sample's absorbance to a standard curve constructed of 1M, 0.5M, 0.25M, 0.125M, 0.0625M, 0.0325M, 0.0156M, 0.0078M, 0.0039M, 0.00195M $MgCl_2$ standards. Seawater samples from each of the three sites were also run in the Beckman DU-70 spectrophotometer to measure magnesium ion concentration of the seawater from each site. Weekly and monthly magnesium ion concentrations at each site were determined for each month and year, respectively.

Measurement of osmolality

Approximately 0.5 grams of *M. senile* mesentery tissue (obtained by cutting longitudinally through mesentery tissue and mesogleal fluid adjacent to the pharynx) was homogenized in 200 µl of distilled water using a plastic mortar and pestle. Tissue homogenate samples were spun at 10,500rpm for 15 minutes in an Eppendorf centrifuge

(Benson-Rodenbough and Ellington, 1982). Ten µl of the resulting supernatant was placed in a VAPRO vapor pressure osmometer to measure the tissue osmolality of each anemone. Seawater samples from each field site or laboratory salinity treatment were also quantified using the vapor pressure osmometer to measure osmolality of the seawater. Weekly and monthly osmolality at each site was determined for each month and year, respectively.

Measurement of mortality

Each individual was observed *in situ* on a weekly basis to assess its condition and was given a mechanical stimulus. The criterion used to determine mortality was the inability of *Metridium senile* to respond to the mechanical stimulus at the time of observation (Benson-Rodenbough and Ellington, 1982). Individuals that were still alive at the end of each month were gently detached from the nylon mesh net and transported back to the collection site where they were allowed to reattach to natural substrata. Cumulative weekly and monthly mortality at each site was determined for each month and year, respectively.

Statistical analyses

For the laboratory study, a two-way univariate analysis of variance (ANOVA) was used to determine differences in percent tissue hydration and magnesium ion concentrations between each day and treatment, with treatment and day as fixed factors.

For the field study, two statistical analyses were utilized: a two-way ANOVA and a two-way multivariate analysis of variance (MANOVA) to determine differences in percent tissue hydration, magnesium ion concentrations and osmolality among sites and weeks, with site and week as fixed factors. Two statistical analyses were used because of minimal variance within levels of certain factors. Factors that contained levels with minimal variance were removed from the analysis and the remaining level(s) were analyzed in each factor using a two-way ANOVA with site and week as fixed factors. A two-way MANOVA was subsequently used when variation was present within all levels of each factor. To analyze the yearly data set, similar data from adjacent months were pooled to test for seasonal differences in percent tissue hydration, magnesium ion concentration, osmolality, and mortality among sites and seasons. A two-way MANOVA was subsequently used to quantify these differences, with site and season (categorized as dry (June-August), fall transition (September-November), wet (December-February), or spring transition (March-May)), as fixed factors.

Normality and homogeneous variance assumptions were analyzed visually using scatterplots and histograms and statistically using the Levene's and Kolmogorov-Smirnov tests (Quinn and Keough, 2002; Dytham, 2003). Data were subsequently transformed using logarithmic, square root, and arcsine transformations, however all transformations were unsuccessful in normalizing and homogenizing variances within the data. Thus, all ANOVA and MANOVA tests were run with a more stringent alpha (α =

0.01) and the Pillai's Trace statistic was used in the MANOVA analysis (Olson, 1976). Violation of the equal variance assumption can increase the probability of Type I error. Therefore, the alpha adjusted to 0.01 helps lower the Type I error probability (Underwood, 1981). Therefore, all *a posteriori* comparisons were analyzed using the conservative Scheffe test when main effects were significant to help account for the departures from normality and homogeneity of variance.

Results

Overall, mean percent tissue hydration, tissue magnesium ion concentrations and tissue osmolality of *Metridium senile* transplants varied significantly among all factors and interactions in both laboratory and field studies, except for in seasonal comparisons, which revealed no significant interactions between main effects (Tables 3.1-3.6). The significant interactions were analyzed first since this can result in an invalid interpretation of the main effects (Quinn and Keough, 2002). The significant interaction between salinity treatment and day in the laboratory study reflects the similarity in the initial (day 0) percent tissue hydration and tissue magnesium ion concentration measurements prior to anemone placement in the three salinity treatments. When initial measurements were taken out of the ANOVA analysis there was no interaction between main effects. The interaction between site and week in the field study was also due to presence of a similarity in the initial (week 0) percent tissue hydration, tissue magnesium ion

Table 3.1. Results from a two-way ANOVA with treatment and day as fixed factors to test for differences in (A) percent tissue hydration and (B) magnesium ion concentrations of *Metridium senile* subjected to 100%, 75% and 50% salinity over a twenty-eight day period. Boldface indicates statistical significance.

A. Percent Tissue Hydration ANOVA

Source	df	MS	F	p
Treatment	2	32.26	179.45	**.000**
Day	4	1.68	9.33	**.000**
Treatment X Day	8	2.9	16.15	**.000**
Error	30	.18		

B. Magnesium Ion Concentrations ANOVA

Source	df	MS	F	p
Treatment	1	18.21	199.80	**.000**
Day	4	65.59	719.70	**.000**
Treatment X Day	4	18.21	16.30	**.000**
Error	19	.09		

Table. 3.2. Results from a two-way ANOVA with site and week as fixed factors to test for differences in (A) percent tissue hydration, (B) magnesium ion concentrations and (C) osmolality of *Metridium senile* subjected to marine, mesohaline and riverine sites during October 2008. Boldface indicates statistical significance.

A. Percent Tissue Hydration ANOVA

Source	df	MS	F	p
Site	2	93.94	140.54	**.000**
Week	4	8.55	12.80	**.000**
Site X Week	8	5.31	7.95	**.000**
Error	30	.668		

B. Magnesium Ion Concentrations ANOVA

Source	df	MS	F	p
Site	2	166.90	2831	**.000**
Week	4	14.92	253	**.000**
Site X Week	8	8.09	137.1	**.000**
Error	30	.059		

C. Osmolality ANOVA

Source	df	MS	F	p
Site	2	2.98E05	3111	**.000**
Week	4	2.0E04	3291	**.000**
Site X Week	8	1.9E04	4.7E04	**.000**
Error	30	6.33		

Table 3.3. Results from a two-way (A) MANOVA and (B) subsequent ANOVAs with site and week as fixed factors to test for differences in percent tissue hydration, magnesium ion concentrations and osmolality of *Metridium senile* subjected to marine, mesohaline and riverine sites during January 2009. Boldface indicates statistical significance.

A. MANOVA

Source	Pillai's Trace	F	Hypothesis df	Error df	p
Site	1.21	11.75	6	46	**.000**
Week	1.36	5	12	72	**.000**
Site X Week	1.25	3.4	15	72	**.000**

B. Percent Tissue Hydration, Magnesium Ion Concentrations and Osmolality ANOVAs

Source	Dependent Variable	df	MS	F	p
Site	% Tissue	2	44.40	26.20	**.000**
	Osmolality	2	2.25E05	22.58	**.000**
	Magnesium	2	39.63	318.86	**.000**
Week	% Tissue	4	13.34	7.87	**.000**
	Osmolality	4	8.61E04	8.65	**.000**
	Magnesium	4	13.10	105.39	**.000**
Site X Week	% Tissue	5	11.78	6.95	**.000**
	Osmolality	5	7.71E04	7.76	**.000**
	Magnesium	5	17.18	138.23	**.000**
Error	% Tissue	24	1.70		
	Osmolality	24	9.96E03		
	Magnesium	24	.124		

Table 3.4. Results from a two-way ANOVA with site and week as fixed factors to test for differences in (A) percent tissue hydration, (B) magnesium ion concentrations and (C) Osmolality of *Metridium senile* subjected to marine, mesohaline and riverine sites during March 2009. Boldface indicates statistical significance.

A. Percent Tissue Hydration ANOVA

Source	df	MS	F	p
Site	2	35.40	45.60	**.000**
Week	4	22.82	29.38	**.000**
Site X Week	4	25.69	33.08	**.000**
Error	25	.777		

B. Magnesium Ion Concentrations ANOVA

Source	df	MS	F	p
Site	2	44.82	122.33	**.000**
Week	4	18.70	51.04	**.000**
Site X Week	4	16.27	44.40	**.000**
Error	25	.366		

C. Osmolality ANOVA

Source	df	MS	F	p
Site	2	1.59E06	72320	**.000**
Week	4	1.48E05	6746	**.000**
Site X Week	4	1.06E05	4852	**.000**
Error	25	21.91		

Table 3.5. Results from a two-way ANOVA with site and week as fixed factors to test for differences in (A) percent tissue hydration, (B) magnesium ion concentrations and (C) osmolality of *Metridium senile* subjected to marine, mesohaline and riverine sites during July 2009. Boldface indicates statistical significance.

A. Percent Tissue Hydration ANOVA

Source	df	MS	F	p
Site	2	17.48	79.06	**.000**
Week	4	.508	2.30	.082
Site X Week	8	1.32	5.95	**.000**
Error	30	.221		

B. Magnesium Ion Concentrations ANOVA

Source	df	MS	F	p
Site	2	272.10	9276	**.000**
Week	4	10.43	355.60	**.000**
Site X Week	8	1815	618.60	**.000**
Error	30	.0293		

C. Osmolality ANOVA

Source	df	MS	F	p
Site	2	254900	1765	**.000**
Week	4	24990	173.10	**.000**
Site X Week	8	24740	171.40	**.000**
Error	30	144.40		

Table 3.6. Results from a two-way (A) MANOVA and (B) subsequent ANOVAs with site and season (categorized as dry, fall transition, wet, or spring transition) as fixed factors to test for differences in percent tissue hydration, magnesium ion concentrations, osmolality and mortality of *Metridium senile* during July 2008-August 2009. Boldface indicates statistical significance.

A. MANOVA

Source	Pillai's Trace	F	Hypothesis df	Error df	p
Site	.688	20.99	8	320	**.000**
Season	.524	8.53	12	483	**.000**
Site X Season	.217	1.55	24	648	.046

B. Percent Tissue Hydration, Magnesium Ion Concentrations, Osmolality and Mortality ANOVAs

Source	Dependent Variable	df	MS	F	p
Site	% Tissue	2	144.20	40.68	**.000**
	Osmolality	2	1.01E06	74.06	**.000**
	Magnesium	2	445.49	86.86	**.000**
	Mortality	2	1.61E04	24.73	**.000**
Season	% Tissue	3	64.14	18.09	**.000**
	Osmolality	3	2.64E05	19.32	**.000**
	Magnesium	3	86.56	16.88	**.000**
	Mortality	3	3.07E03	4.72	**.003**
Site X Season	% Tissue	6	8.61	2.43	.028
	Osmolality	6	1.82E04	1.33	.246
	Magnesium	6	2.14	.417	.867
	Mortality	6	481.16	.738	.619
Error	% Tissue	162	3.54		
	Osmolality	162	1.37E04		
	Magnesium	162	5.13		
	Mortality	162	651.59		

concentration and tissue osmolality measurements prior to anemone placement at the three field sites. When initial measurements were taken out of the ANOVA or MANOVA analyses there was no interaction between main effects. However, initial measurements were kept in both laboratory and field study analyses to determine if there were differences between initial and subsequent days or weeks, respectively. Furthermore, as a consequence of similar salinity profiles shared between the marine and mesohaline site during the months of October 2008 and July 2009, measurements of anemones placed at these sites were also similar resulting in a significant interaction between site and week.

Laboratory study

After exposure to 50%, 75% and 100% seawater for twenty-eight days, *Metridium senile* exhibited an increase in percent tissue hydration with decreased salinity. However, percent tissue hydration decreased slightly from day 14 until the final measurement was taken on day 28 in both 50% seawater and 75% seawater, whereas percent tissue hydration remained relatively constant in 100% seawater (Figure 3.2). This indicates that *M. senile* may exhibit increased partial volume regulation with decreased salinity during the last two weeks of exposure in 75% and 50% seawater treatments. Percent tissue hydration differed significantly between salinity treatment and day. Percent tissue hydration ranged from 76.5% to 83% in 50% seawater and 100% seawater, respectively (Figure 3.2; Table 3.1A). Furthermore, post-hoc analyses show that tissue hydration in

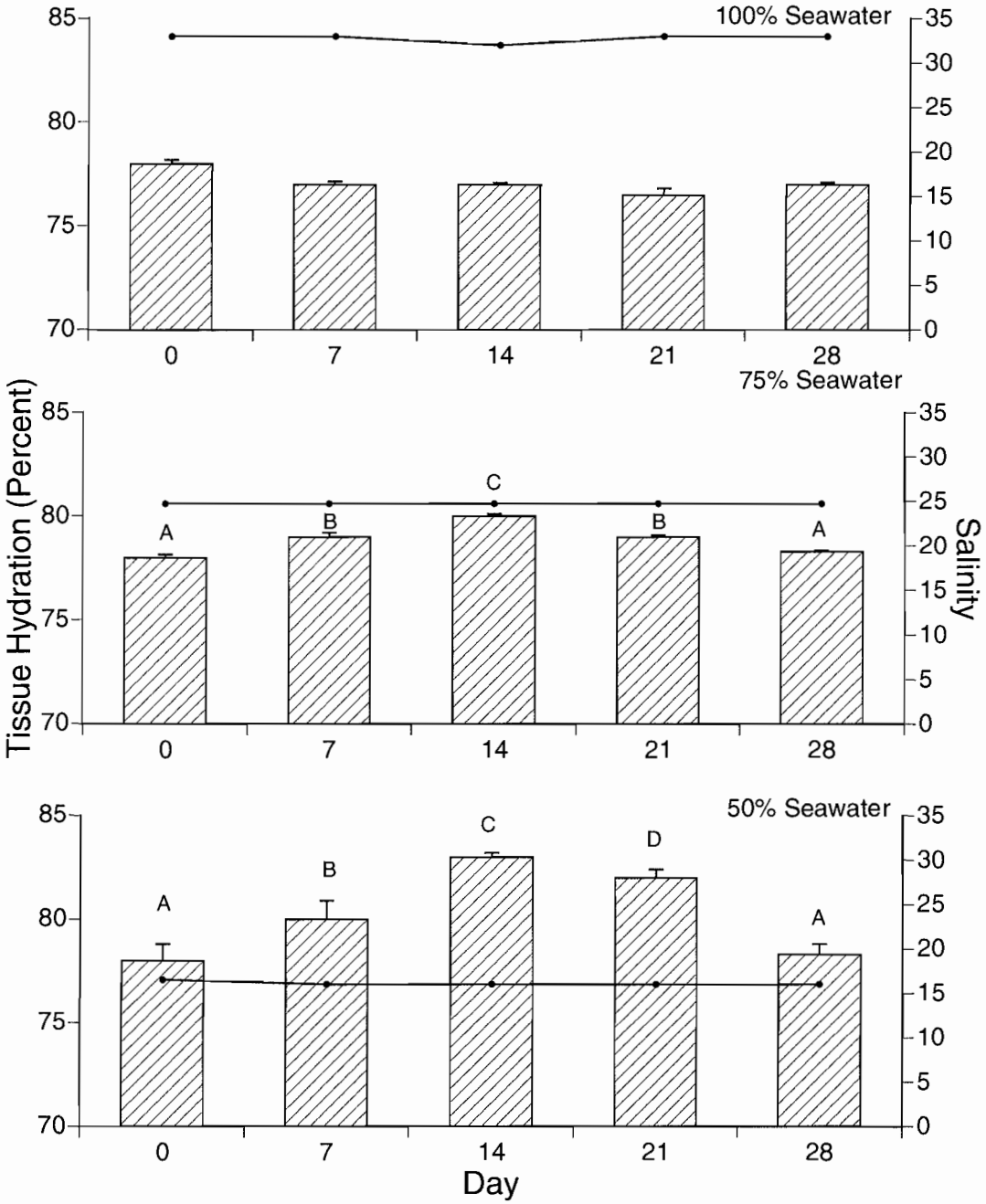

Figure 3.2. Percent tissue hydration of *Metridium senile* in 100%, 75%, and 50% seawater for 28 days. Line overlays represent changes in average salinity of each sample day in respective salinity treatments. Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days in 100% seawater ($p>0.01$).

50% seawater was significantly different between each pair of days, except for day 0 and day 28 (Figure 3.2). A similar trend was observed in 75% seawater, with day 14 significantly different from 0 and 28 and day 7 and 21; no significance was found between days in the 100% seawater treatment (Figure 3.2). Additionally, ß values ranged from 0.4 to 0.6 for individuals acclimated to 75% seawater and 0.15 to 0.3 for individuals acclimated to 50% seawater, suggesting that *M. senile* is capable of partial volume regulation and increases volume regulation with decreased salinity (Figure 3.3).

Tissue magnesium ion concentrations showed an initial decrease with decreased salinity after the first 7 days of exposure followed by a marked increase in magnesium ion concentrations. Additionally, anemones placed in 50% and 75% seawater were highly hyperionic to the ambient seawater and similar to tissue magnesium ion concentrations of control individuals in 100% seawater when the final measurement was taken on day 28 (Figure 3.4). Individuals within 100% seawater, however, remained isoionic to the ambient seawater magnesium ion concentrations over the 28-day exposure. Tissue magnesium ion concentrations in *M. senile* varied significantly among salinity treatments and days (Table 3.1B). Post-hoc analyses for 50% and 75% seawater show significant differences between days, except for days 0 and 28; no significant difference among days were found in 100% seawater (Figure 3.4). This indicates that *M. senile* may have the capacity to partially regulate tissue magnesium ions under acute salinity stress and increase hyperionic regulation as salinity decreases.

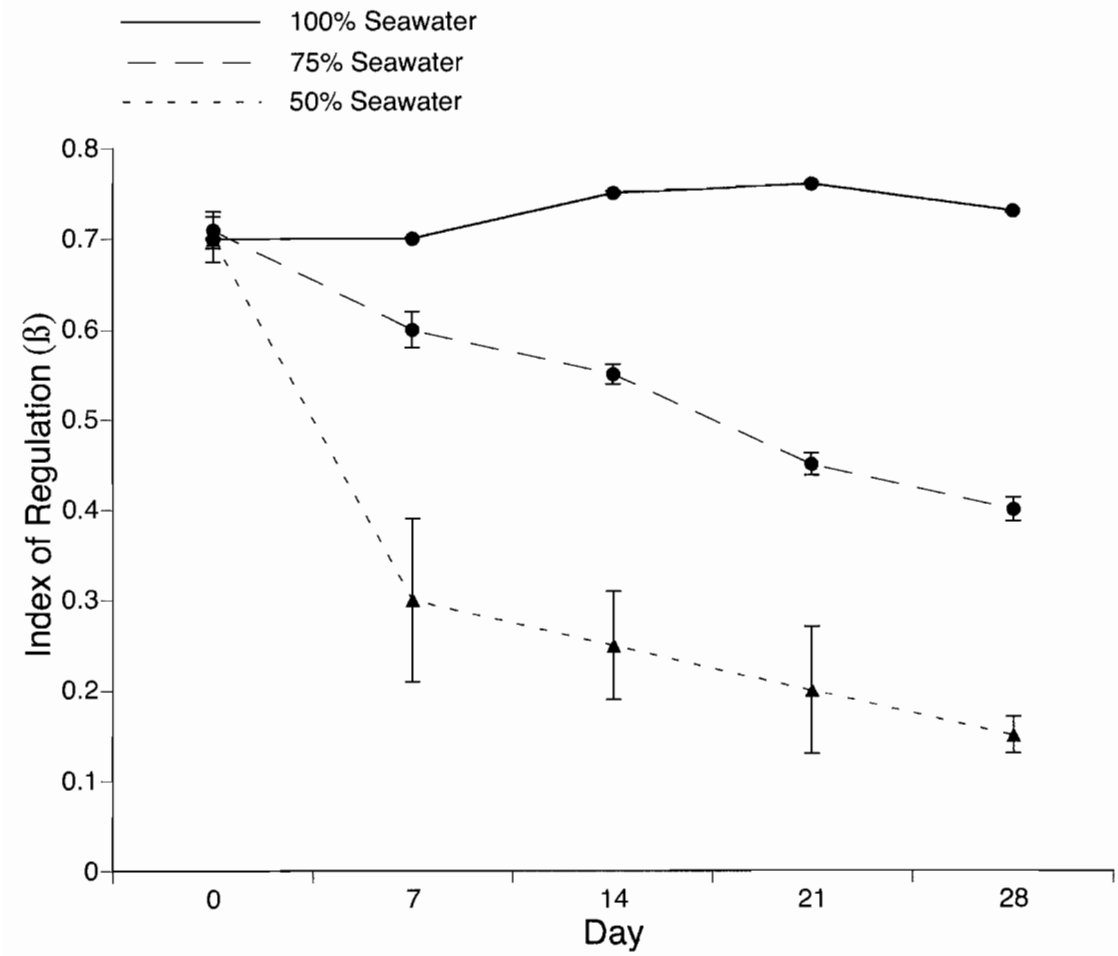

Figure 3.3. Ability of *Metridium senile* to regulate tissue water content when acclimated to 75% and 50% seawater over a 28-day period. Each point represents a mean of 3 individuals. ß value of 1 indicates no regulation; ß value of 0 indicates complete regulation. Error bars for each point indicate standard error.

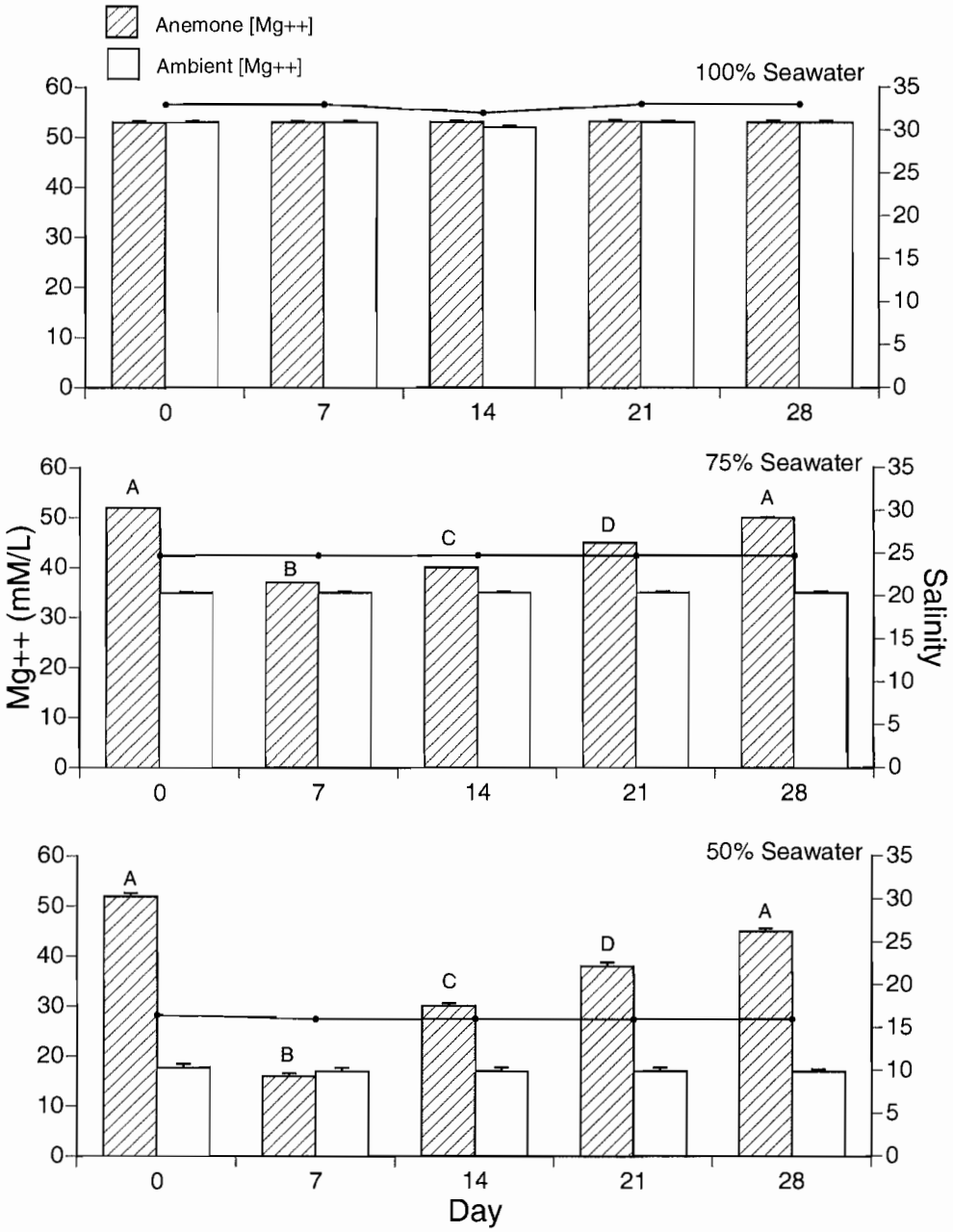

Figure 3.4. Tissue magnesium ion concentrations of *Metridium senile* in 100%, 75%, and 50% seawater for 28 days. Line overlays represent changes in average salinity of each sample day in respective salinity treatments. Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days in 100% seawater ($p>0.01$).

Field study

Fall Transition: October 2008

Percent tissue hydration was similar at marine and mesohaline sites during the fall transition period in October 2008, yielding an average tissue hydration of 77%. The riverine site, however, showed a marked increase in percent tissue hydration compared to the other field sites with a maximum tissue hydration of nearly 85% occurring during week 2 of exposure. Percent tissue hydration subsequently decreased over the following two weeks, settling at 83% in week 4 (Figure 3.5). Percent tissue hydration varied significantly between sites and weeks (Figure 3.5; Table 3.2A). Furthermore, post-hoc comparisons revealed significant differences between weeks at the riverine site but not at the marine and mesohaline sites due to the presence of similar values between weeks (Figure 3.5). At the riverine site, week 0 was significantly lower than all other weeks, and weeks 1 and 3 were significantly lower than weeks 2 and 4 indicating that anemones were able to decrease tissue hydration during weeks 1 and 3 compared to weeks 2 and 4 possibly by regulating. However, increases in tissue hydration during weeks 2 and 4 were perhaps attributed to decreased regulatory ability. In comparison, ß values for October 2008 averaged from 0.25 at the riverine site, 0.52 at the mesohaline site to 0.73 at the marine site. Because the ability to regulate water content increases with distance from the estuary mouth, this further suggests that *M. senile* is capable of volume regulation and increases such regulation as salinity in the South Slough begins to decrease at the onset of the fall transition (Figure 3.6).

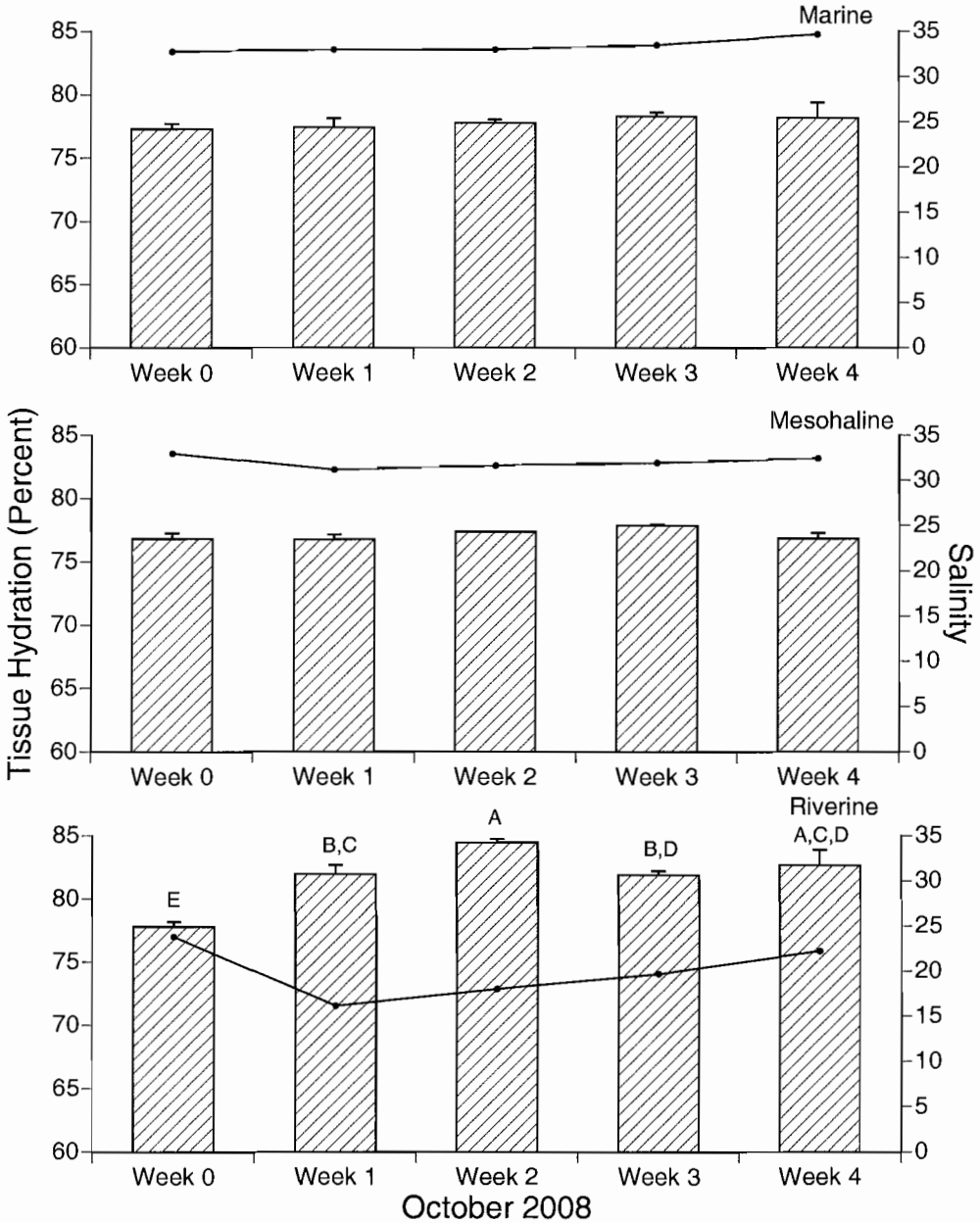

Figure 3.5. Percent tissue hydration of *Metridium senile* at marine, mesohaline and riverine sites during October 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Salinity measurements during week 1, 2, and 3 of October (October 8th, 15th, and 22nd) at the marine site were taken with a refractometer due to SSNERR CDMO datalogger malfunction. Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days at the mesohaline and riverine sites ($p>0.01$).

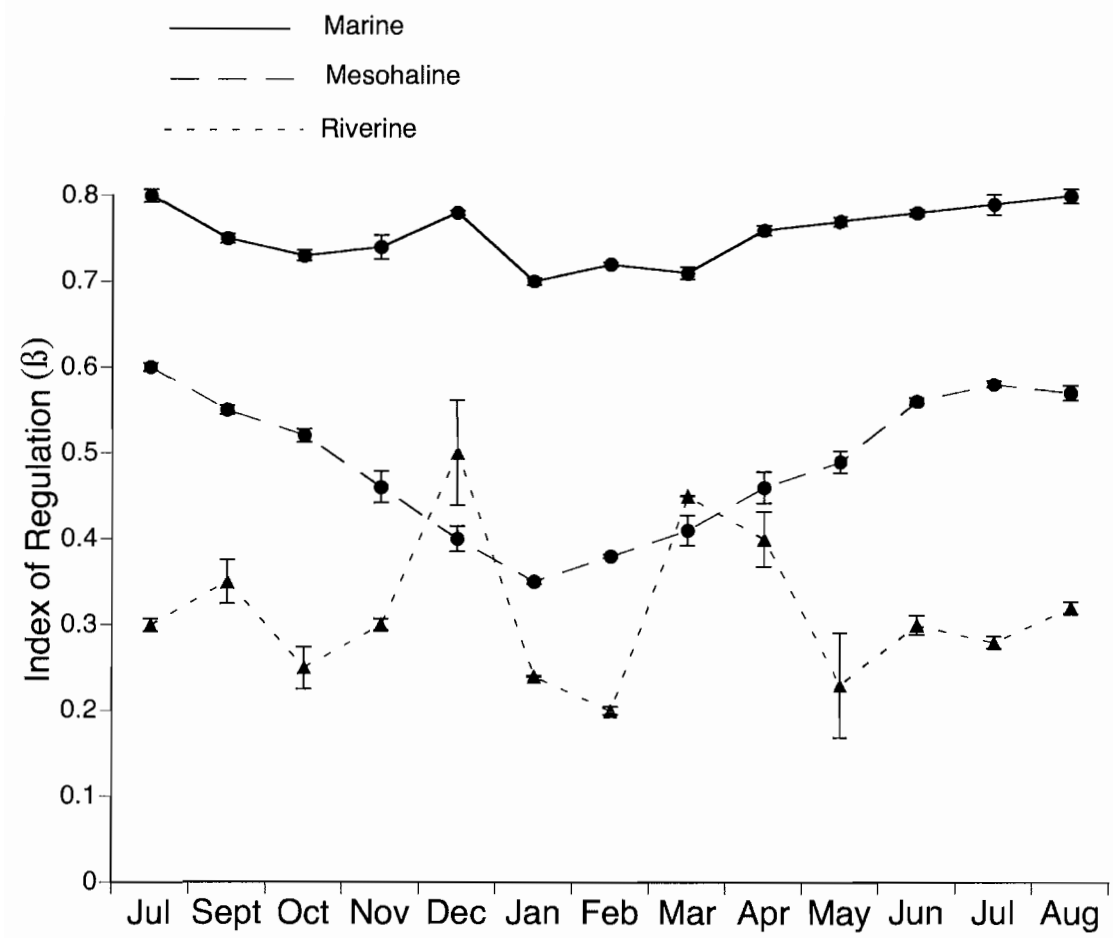

Figure 3.6. Ability of *Metridium senile* to regulate tissue water content when acclimated to marine, mesohaline and riverine sites in the South Slough estuary over a 13-month period. Each point represents monthly means. ß value of 1 indicates no regulation; ß value of 0 indicates complete regulation. Error bars for each point indicate standard error.

Tissue magnesium ion concentrations remained relatively constant for anemones placed at marine and mesohaline sites, with tissue magnesium ion concentrations within individual anemones remaining isoionic and hyperionic, respectively, to the marine and mesohaline ambient medium. However, tissue magnesium ion concentrations in anemones at the riverine site initially decreased from 51 mM/L to 21 mM/L during the first week. Anemone magnesium ion concentrations remained hyperionic to the ambient medium and steadily increased from the first week to the fourth week (40 mM/L; Figure 3.7). Tissue magnesium ion concentrations varied significantly among sites and weeks (Figure 3.7; Table 3.2B). Furthermore, post-hoc comparisons revealed significant differences among weeks at the mesohaline and riverine site but not the marine site due to the presence of similar values between weeks (Figure 3.7). Additionally, the significant decrease in anemone magnesium ion concentrations during the first week and subsequent increases at the riverine site indicates that *Metridium senile* was able to regulate magnesium ions under hyposaline conditions (Figure 3.7).

Tissue osmolality was constant throughout the month of October 2008 at the marine and mesohaline sites, with anemone osmolality remaining iso-osmotic and hyperosmotic, respectively, to the marine and mesohaline ambient medium. However, anemone osmolality at the riverine site initially decreased from 960 mmol/kg to 620 mmol/kg during the first week where it remained mostly hyperosmotic to the ambient medium and steadily increased over the remaining three weeks to week 4 (783 mmol/kg;

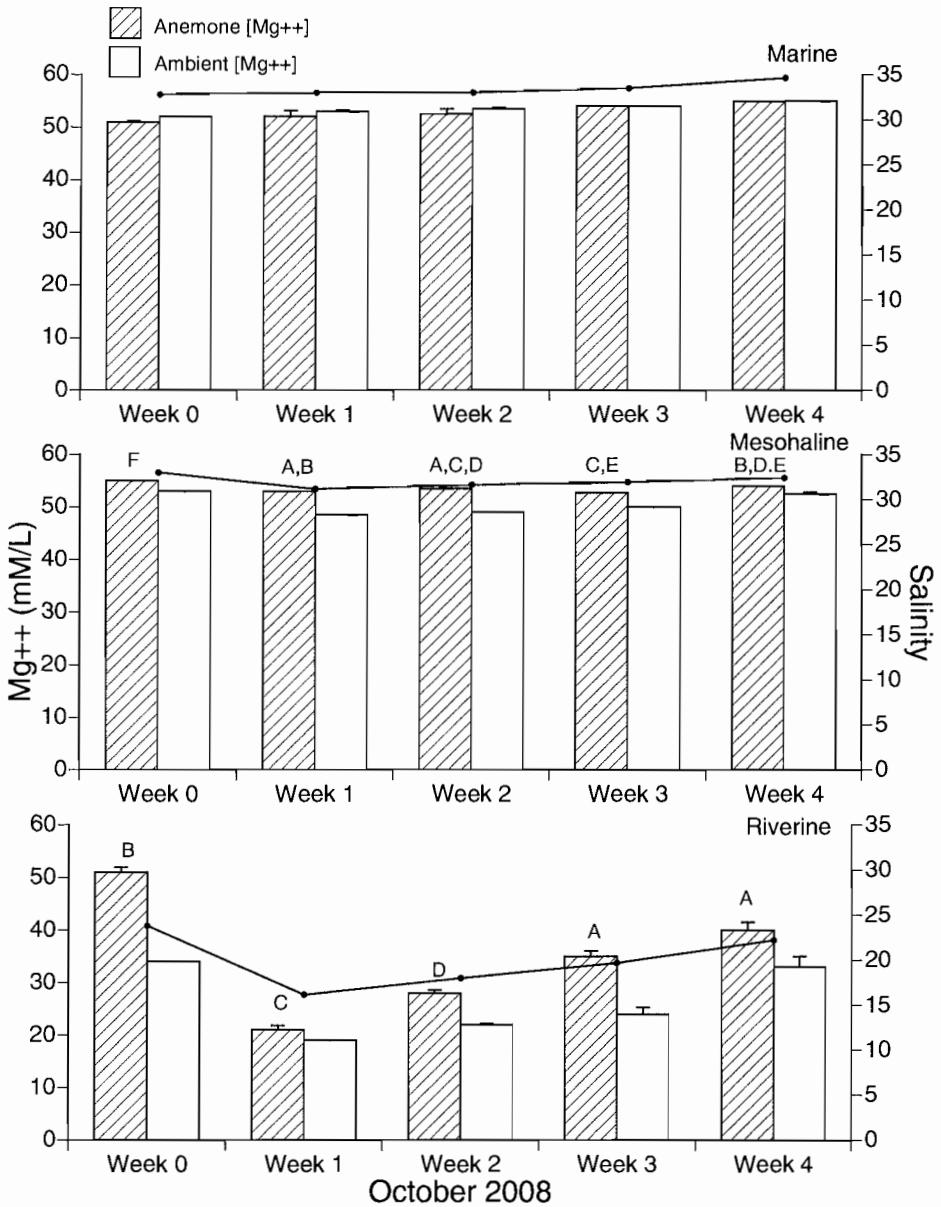

Figure 3.7. Tissue magnesium ion concentrations of *Metridium senile* at marine, mesohaline and riverine sites during October 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Salinity measurements during week 1, 2, and 3 of October (October 8th, 15th, and 22nd) at the marine site were taken with a refractometer due to SSNERR CDMO datalogger malfunction. Bars represent means with standard error (n=3). Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days at the marine site ($p>0.01$).

Figure 3.8). Tissue osmolality varied significantly among sites and weeks (Figure 3.8; Table 3.2C). Furthermore, post-hoc comparisons revealed significant differences among weeks at the riverine site but not the marine and mesohaline sites (Figure 3.8). Significant increases in tissue osmolality from week 1 to week 4 at the riverine site indicate that *Metridium senile* may be regulating in order to survive these hyposaline conditions (Figure 3.8).

Cumulative percent mortality during October 2008 exhibited a marked increase with distance from the estuary mouth. Peak mortalities of 6%, 42% and 75% occurred at marine, mesohaline and riverine-dominated sites, respectively, with 6% and 75% occurring during week 4 and 42% during week 3 (Figure 3.9).

Wet Season: January 2009

Significant multivariate effects were found among all factors and interactions were present (Table 3.3A). Percent tissue hydration varied significantly among sites and weeks and revealed similar trends in January 2009 as in October 2008 (Figures 3.5 and 3.10; Table 3.3B). *Metridium senile* showed increased percent tissue hydration with distance from the estuary mouth, with highest percent tissue water content reaching 86% at the riverine site by the end of the first week after which all remaining anemones died. Percent tissue hydration at the mesohaline site remained constant until the last week when percent tissue hydration increased to 79%. Percent tissue hydration in anemones at the marine site was approximately 77% for the entire month (Figure 3.10).

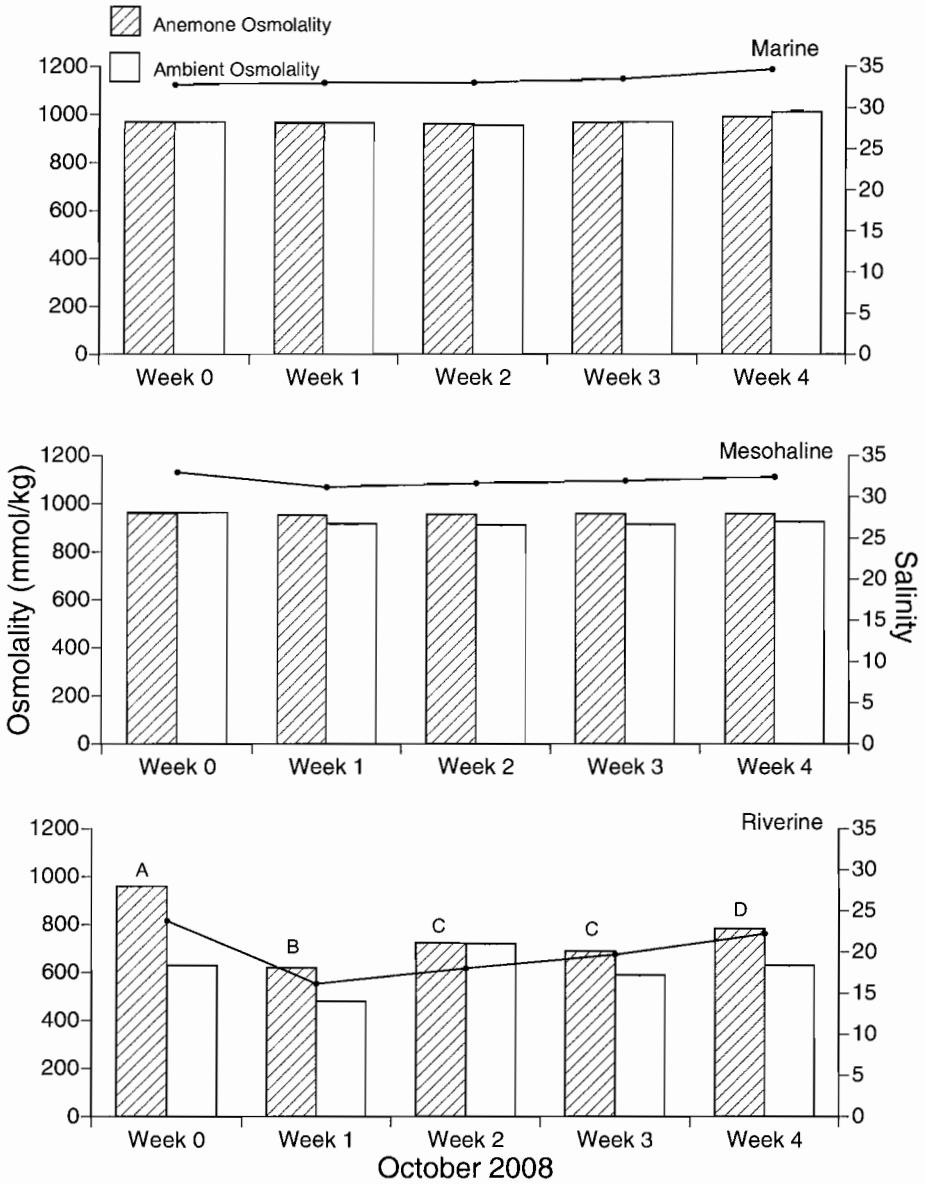

Figure 3.8. Tissue osmolality of *Metridium senile* at marine, mesohaline and riverine sites during October 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Salinity measurements during week 1, 2, and 3 of October (October 8th, 15th, and 22nd) at the marine site were taken with a refractometer due to SSNERR CDMO datalogger malfunction. Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days at the marine site ($p>0.01$).

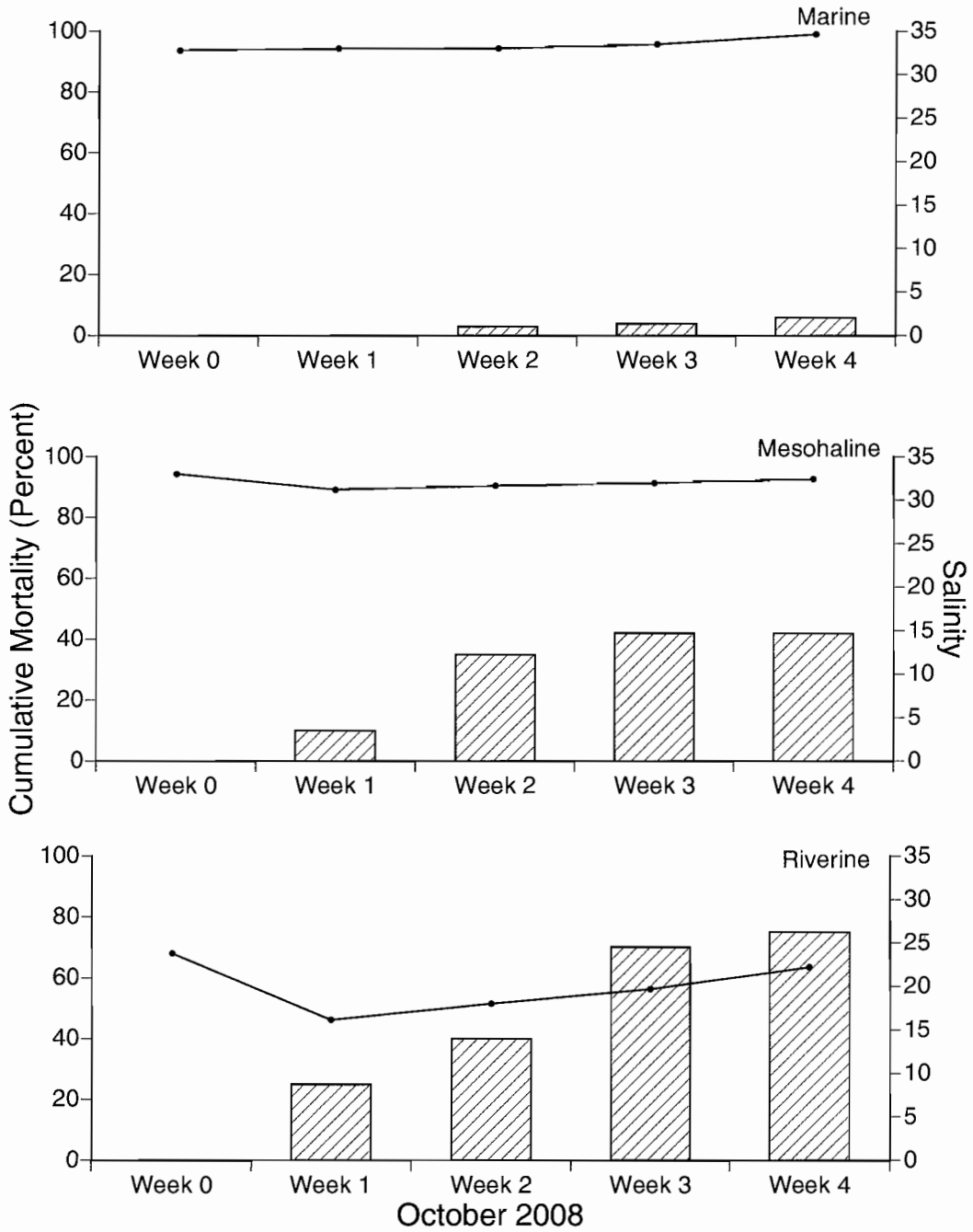

Figure 3.9. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during October 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Salinity measurements during week 1, 2, and 3 of October (October 8th, 15th, and 22nd) at the marine site were taken with a refractometer due to SSNERR CDMO datalogger malfunction.

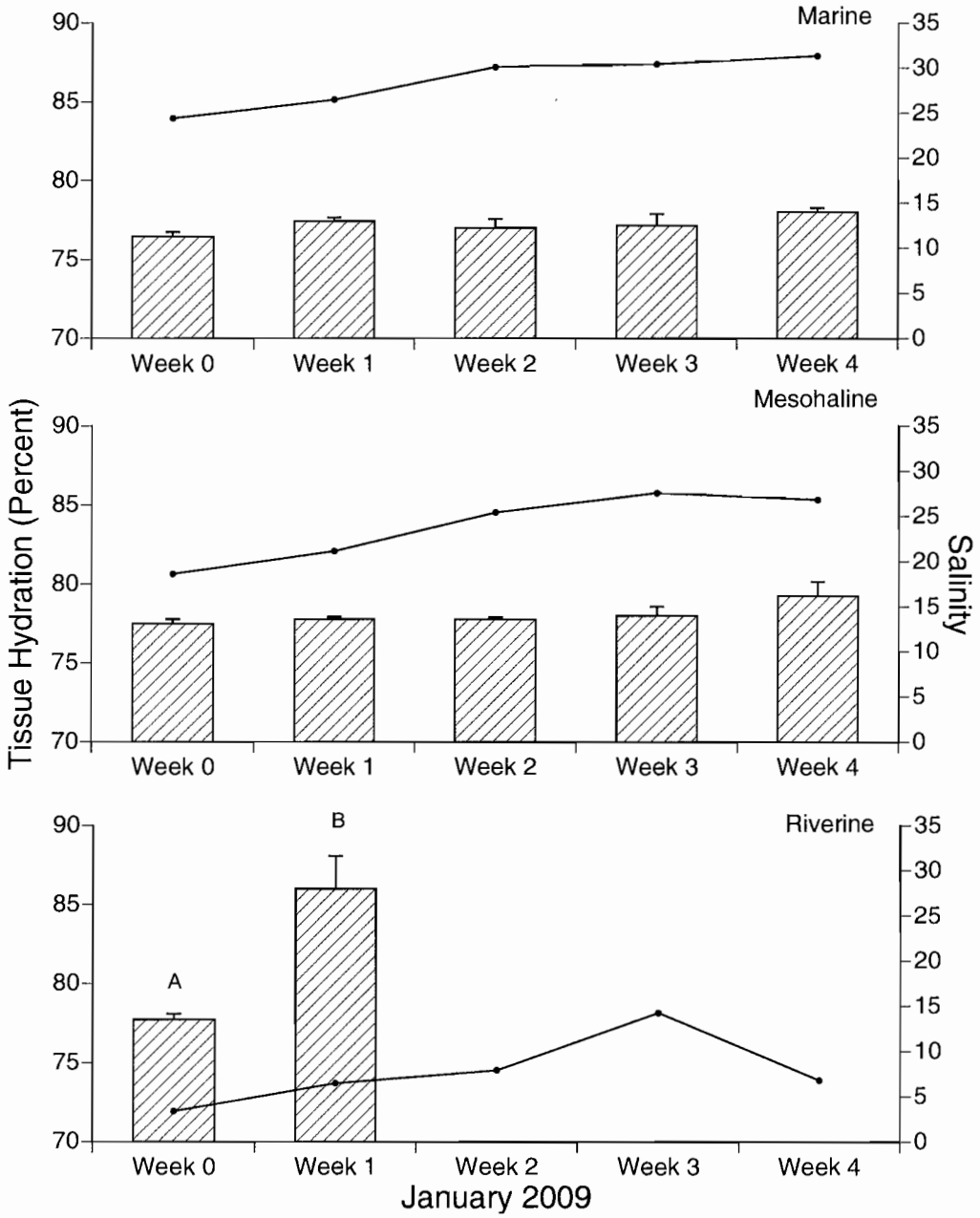

Figure 3.10. Percent tissue hydration of *Metridium senile* at marine, mesohaline and riverine sites during January 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days at mesohaline and riverine sites ($p>0.01$).

Post-hoc comparisons revealed significant differences among weeks at the riverine site but not at the marine and mesohaline sites (Figure 3.10). Resulting average ß values of 0.70 at the marine site, 0.35 at the mesohaline site, and 0.24 at the riverine site again indicates that partial volume regulation increases with distance from the estuary mouth. This suggests that *Metridium senile* is a stronger volume regulator at lower salinities (Figure 3.6).

Differences in tissue magnesium ion concentrations were significant among sites and weeks (Figure 3.11; Table 3.3B). Tissue magnesium ion concentrations remained stable and were consistently hypoionic and hyperionic to the ambient seawater at marine and mesohaline sites, respectively. However, anemones from the riverine site showed a pronounced decrease from 36 mM/L to 8 mM/L during the first week and remained slightly hyperionic to the ambient medium after which all remaining anemones died (Figures 3.11). Post-hoc comparisons revealed significant differences among weeks at the mesohaline and riverine sites but not at the marine site (Figure 3.11). The significant decrease in anemone magnesium ion concentrations during the first week at the riverine site indicates that *Metridium senile* was unable to regulate magnesium ions, resulting in complete mortality after the first week possibly because of extreme hyposaline conditions during those weeks. In contrast, significant increases in anemone magnesium ion concentrations from week 1 to week 4 at the mesohaline site indicate regulation under hyposaline conditions (Figure 3.11).

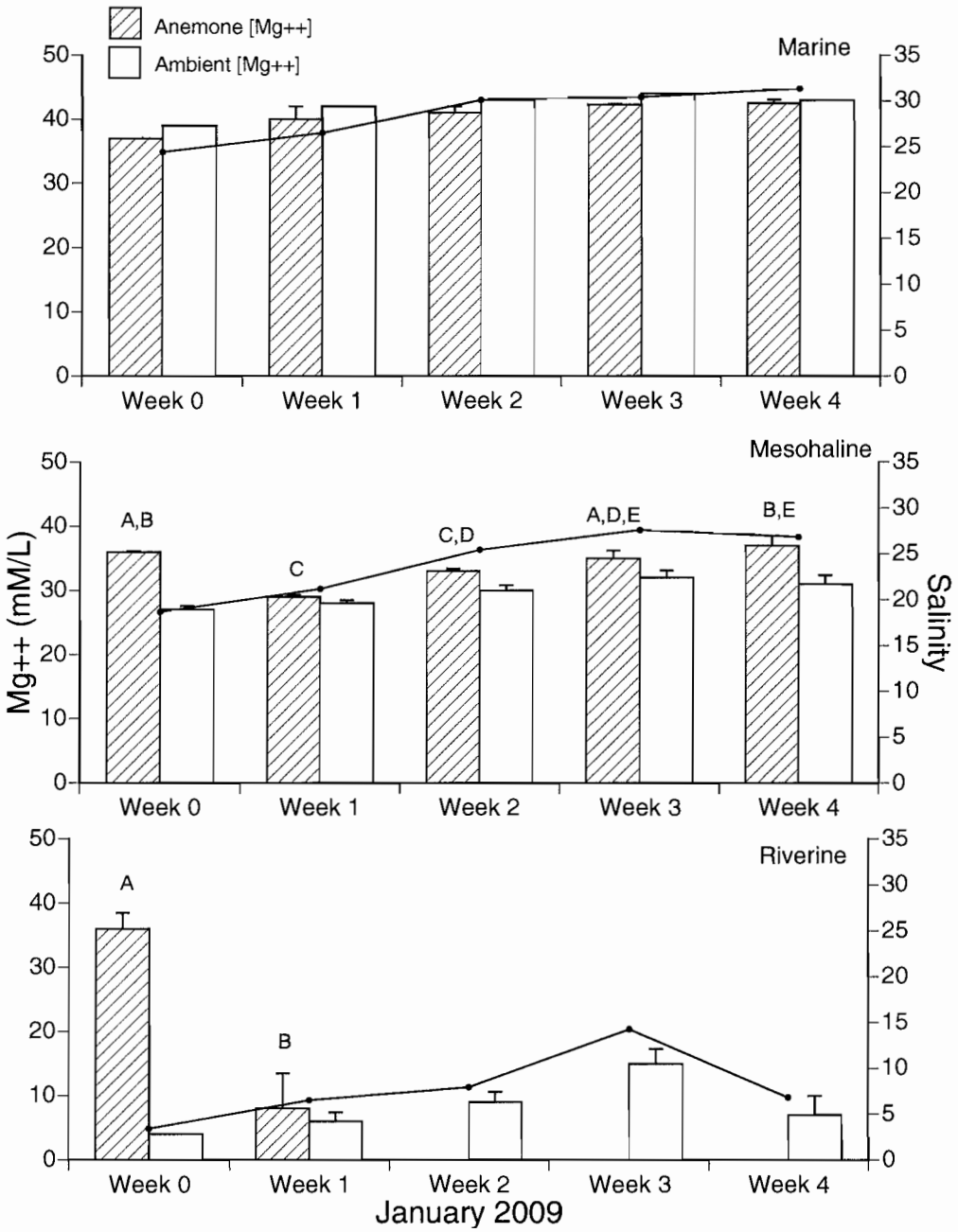

Figure 3.11. Tissue magnesium ion concentrations of *Metridium senile* at marine, mesohaline and riverine sites during January 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days at the marine site ($p>0.01$).

Tissue osmolality remained stable and iso-osmotic at the marine site. Tissue osmolality at the mesohaline site, however, initially decreased from 819 mmol/kg to 692 mmol/kg during the first week of exposure and then remained hypo-osmotic to the ambient seawater for the remaining weeks. The riverine site showed a greater decrease in anemone osmolality, declining from 733 mmol/kg to 283 mmol/kg (Figure 3.12). Tissue osmolality varied significantly among sites and weeks (Figure 3.12; Table 3.3B). Post-hoc comparisons revealed significant differences among weeks at all field sites (Figure 3.12). Additionally, the significant decrease in anemone osmolality during the first week at the riverine site indicates that *Metridium senile* was unable to regulate osmolality, resulting in complete mortality after the first week, possibly because of extreme hyposaline conditions during those weeks. In contrast, anemone osmolality significantly decreased and remained consistently hypo-osmotic from week 2 to week 4 at the mesohaline site indicating decreased regulation under hyposaline conditions (Figure 3.12). Excess mucus production was also observed on each individual at the mesohaline site.

Cumulative percent mortality at all field sites during January 2009 increased markedly compared to previous months. All anemones died during the fourth week of exposure at the mesohaline site and during the first week of exposure at the riverine site, culminating in peak mortalities of 30%, 100% and 100% mortality at marine, mesohaline and riverine-dominated sites, respectively (Figure 2.7). Consequently, the combination

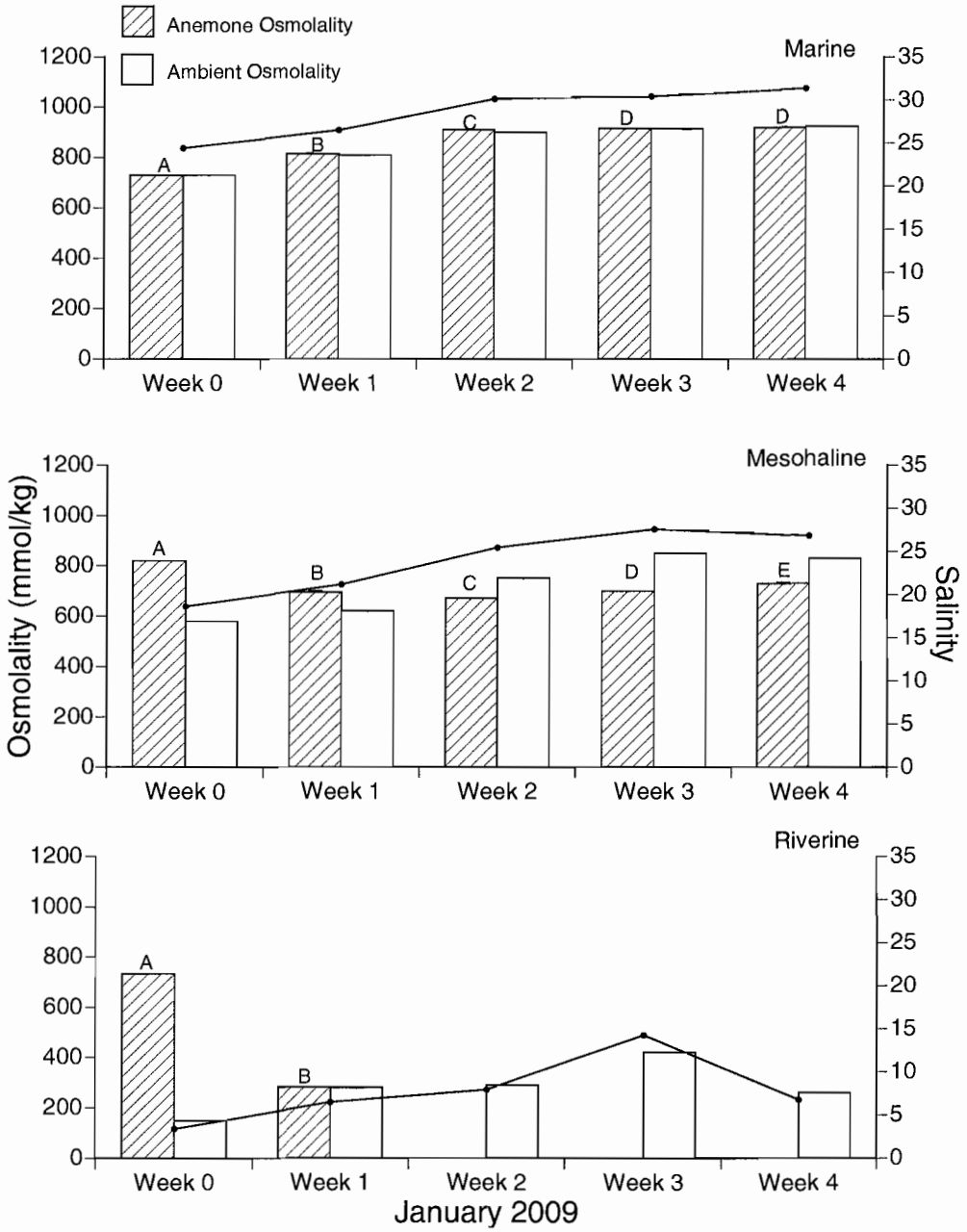

Figure 3.12. Tissue osmolality of *Metridium senile* at marine, mesohaline and riverine sites during January 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests.

of hypo-osmotic tissue, mucus production, and complete mortality at the mesohaline site suggests that hypo-osmotic tissue may be an indication of imminent death.

Spring Transition: March 2009

During March 2009, *Metridium senile* displayed a slight increase in percent tissue hydration at all three field sites compared to January 2009, with a maximum percent tissue hydration of 88% occurring at the riverine site. Percent tissue hydration was relatively constant at marine and mesohaline sites except during week 4 when tissue hydration decreased slightly at the mesohaline site (Figure 3.13). Average ß values of 0.71, 0.41 and 0.45 were observed at the marine, mesohaline and riverine sites, respectively, indicating a decrease in volume regulatory ability as compared to January 2009 (Figure 3.6). This suggests that the overall decrease in salinity between the months of January and March 2009 negatively impacted *M. senile*'s volume regulation which may indicate the presence of a salinity threshold for partial volume regulation (Figures 3.6 and 3.13). Percent tissue hydration varied significantly among sites and weeks (Figure 3.13; Table 3.4A). Post-hoc comparisons revealed significant differences among weeks at the riverine site but not at the marine and mesohaline sites (Figure 3.13). Additionally, the significant increase in percent tissue hydration between week 0 and week 1 at the riverine site indicates decreased volume regulatory ability prior to complete mortality (Figure 3.13).

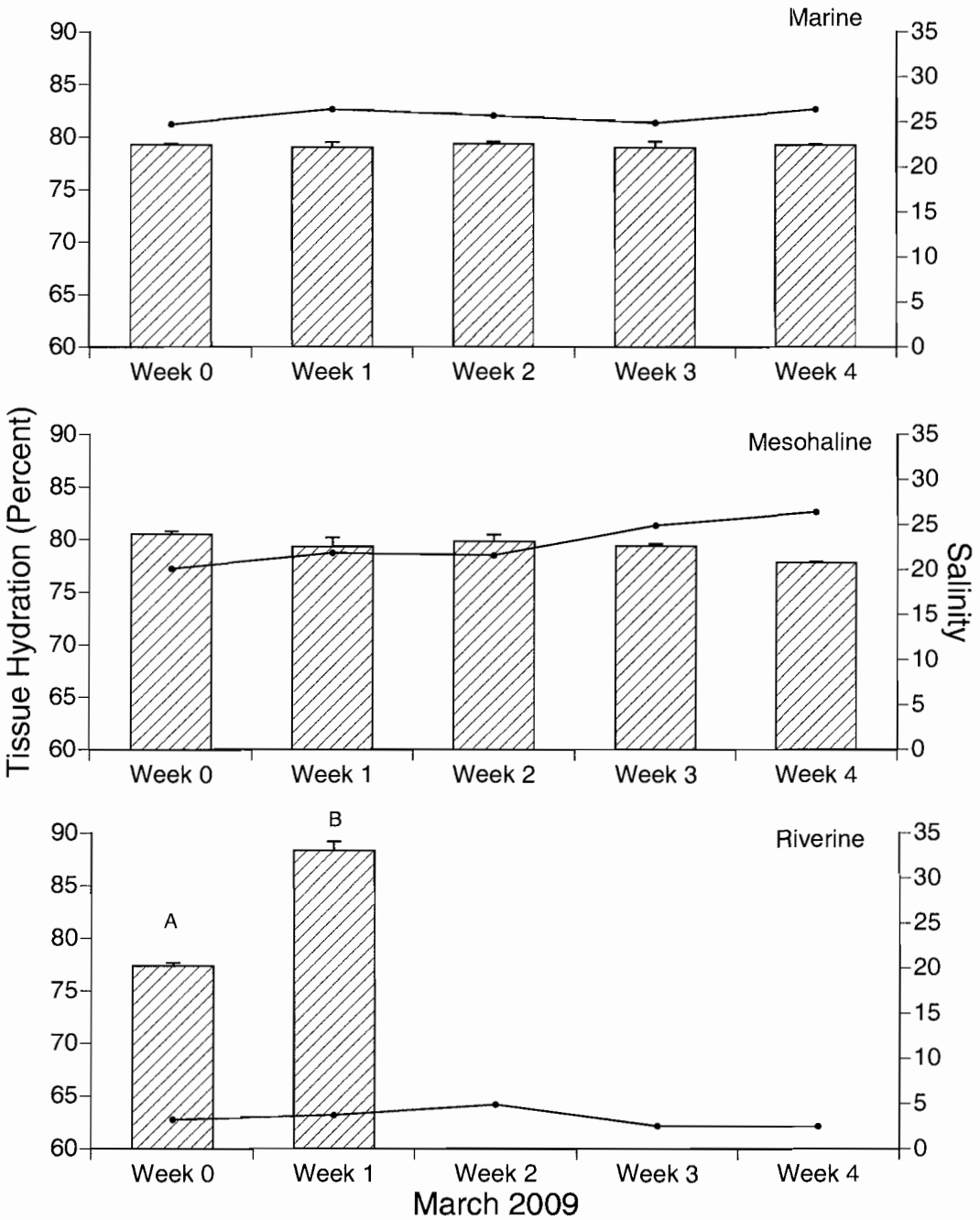

Figure 3.13. Percent tissue hydration of *Metridium senile* at marine, mesohaline and riverine sites during March 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days at the marine and mesohaline sites ($p>0.01$).

Tissue magnesium ion concentrations at the marine site remained relatively isoionic to the ambient seawater. Mesohaline and riverine sites displayed an initial decrease from 37.5 mM/L to 31 mM/L and 37 mM/L to 3 mM/L, respectively. However, individual anemones placed at the mesohaline site gradually increased to 38 mM/L after the initial decrease and remained slightly hyperionic to the ambient seawater. Anemones placed at the riverine site all died during the second week (Figure 3.14). Differences in tissue magnesium ion concentrations varied significantly among sites and weeks (Figure 3.14; Table 3.4B). Post-hoc comparisons revealed significant differences among weeks at the mesohaline and riverine sites but not at the marine site due to the presence of similar values between weeks (Figure 3.14). Furthermore, the significant decrease in anemone magnesium ion concentrations during the first week at the riverine site indicates that *Metridium senile* was unable to regulate magnesium ions, resulting in complete mortality after the first week possibly due to the extreme hyposaline conditions during those weeks. In contrast, significant increases in anemone magnesium ion concentrations from week 1 to week 4 at the mesohaline site indicate regulation under hyposaline conditions (Figure 3.14).

Tissue osmolality remained constant at marine and mesohaline sites with individual anemone osmolality remaining primarily iso-osmotic or hyperionic to the ambient medium at respective sites. However, anemone osmolality at the riverine site initially decreased from 749 mmol/kg to 209 mmol/kg during the first week, after which all anemones died (Figure 3.15). Tissue osmolality varied significantly among sites and

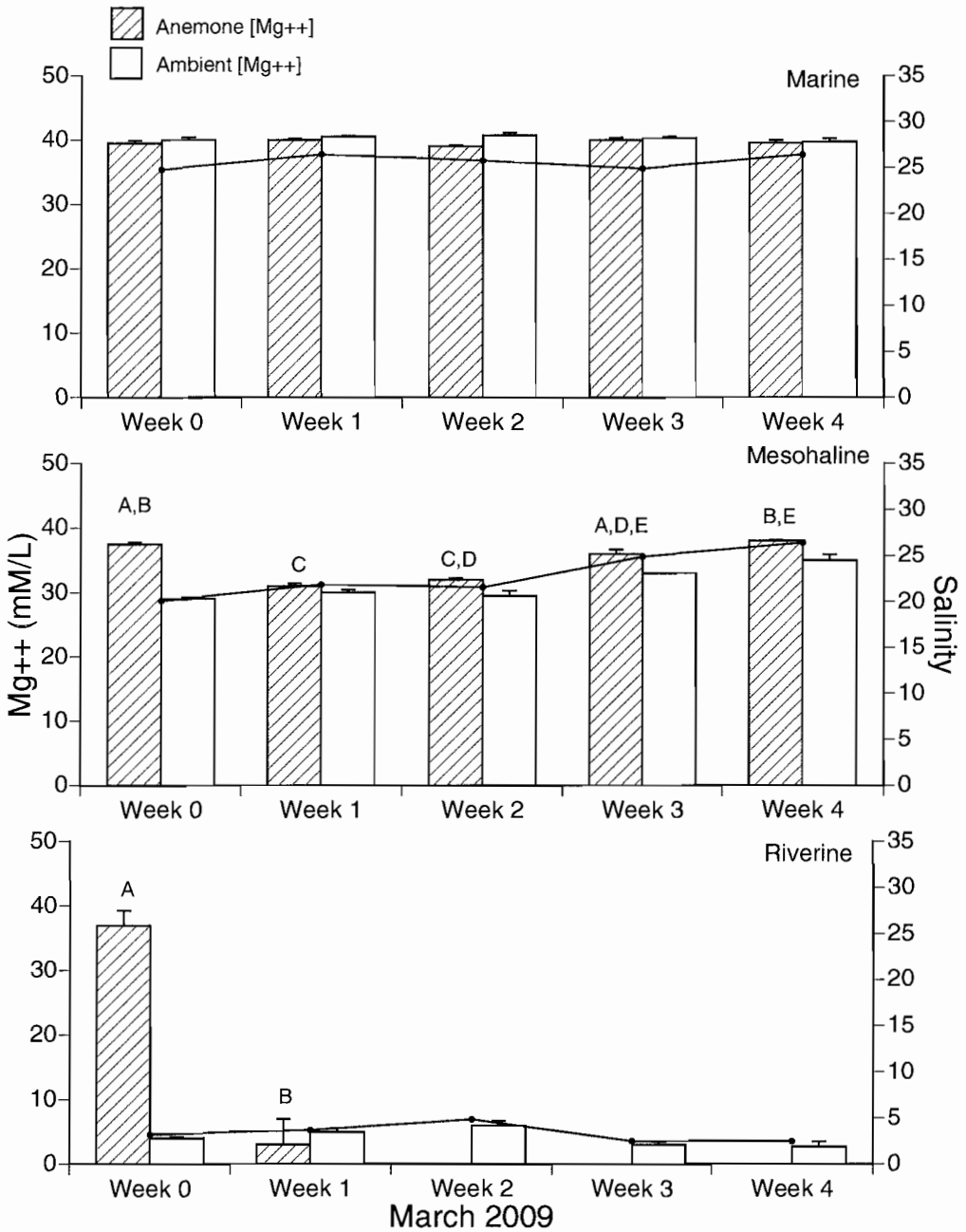

Figure 3.14. Tissue magnesium ion concentrations of *Metridium senile* at marine, mesohaline and riverine sites during March 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days at the marine site ($p>0.01$).

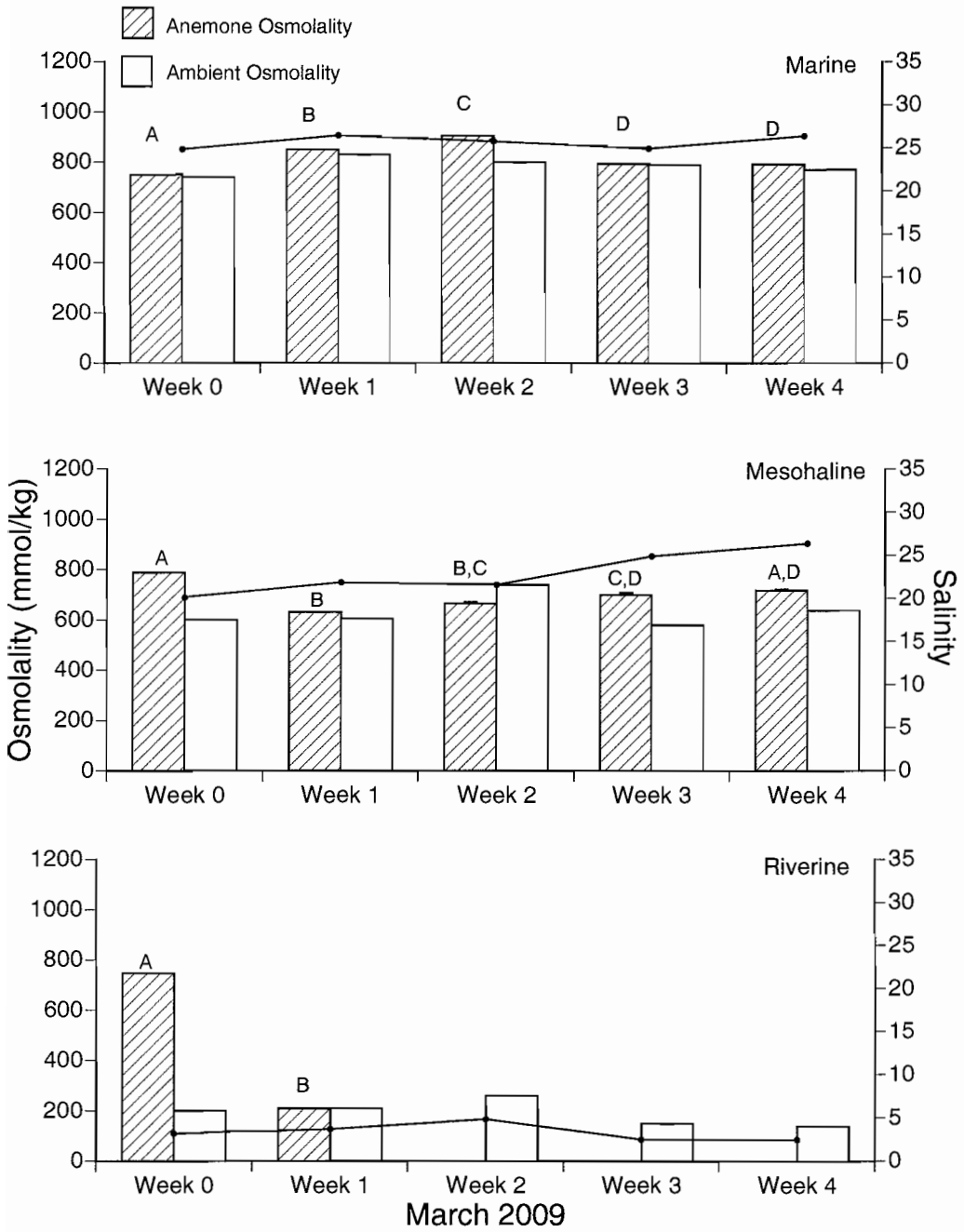

Figure 3.15. Tissue osmolality of *Metridium senile* at marine, mesohaline and riverine sites during March 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests.

weeks (Figure 3.15; Table 3.4C). Post-hoc comparisons revealed significant differences among weeks at all field sites (Figure 3.15). Additionally, the significant decrease in anemone osmolality during the first week at the riverine site indicates that *Metridium senile* was unable to regulate tissue osmolality, resulting in complete mortality after the first week, possibly because of extreme hyposaline conditions during those weeks. In contrast, significant increases in tissue osmolality from week 1 to week 4 at the mesohaline site indicate slight regulation under hyposaline conditions (Figure 3.15).

Cumulative percent mortality during March 2009 exhibited a slight increase at marine and mesohaline sites and a decrease at the riverine site as compared to cumulative mortality trends during January 2009. However, all anemones died during the second and fourth week at the riverine and mesohaline sites, respectively, yielding a peak mortality of 35%, 100% and 100% at marine, mesohaline and riverine-dominated sites, respectively (Figure 2.6).

Dry Season: July 2009

Metridium senile exhibited a slight increase in percent tissue hydration with decreased salinity during the dry season. Individuals acclimated to the riverine site showed a maximum percent tissue hydration of 78% during week 2, whereas percent tissue hydration remained at a constant 75% and 76% at the marine and mesohaline site, respectively (Figure 3.16). Average ß values for July 2009 ranged from 0.28 at the riverine site, 0.58 at the mesohaline site to 0.79 at the marine site indicating that *M. senile*

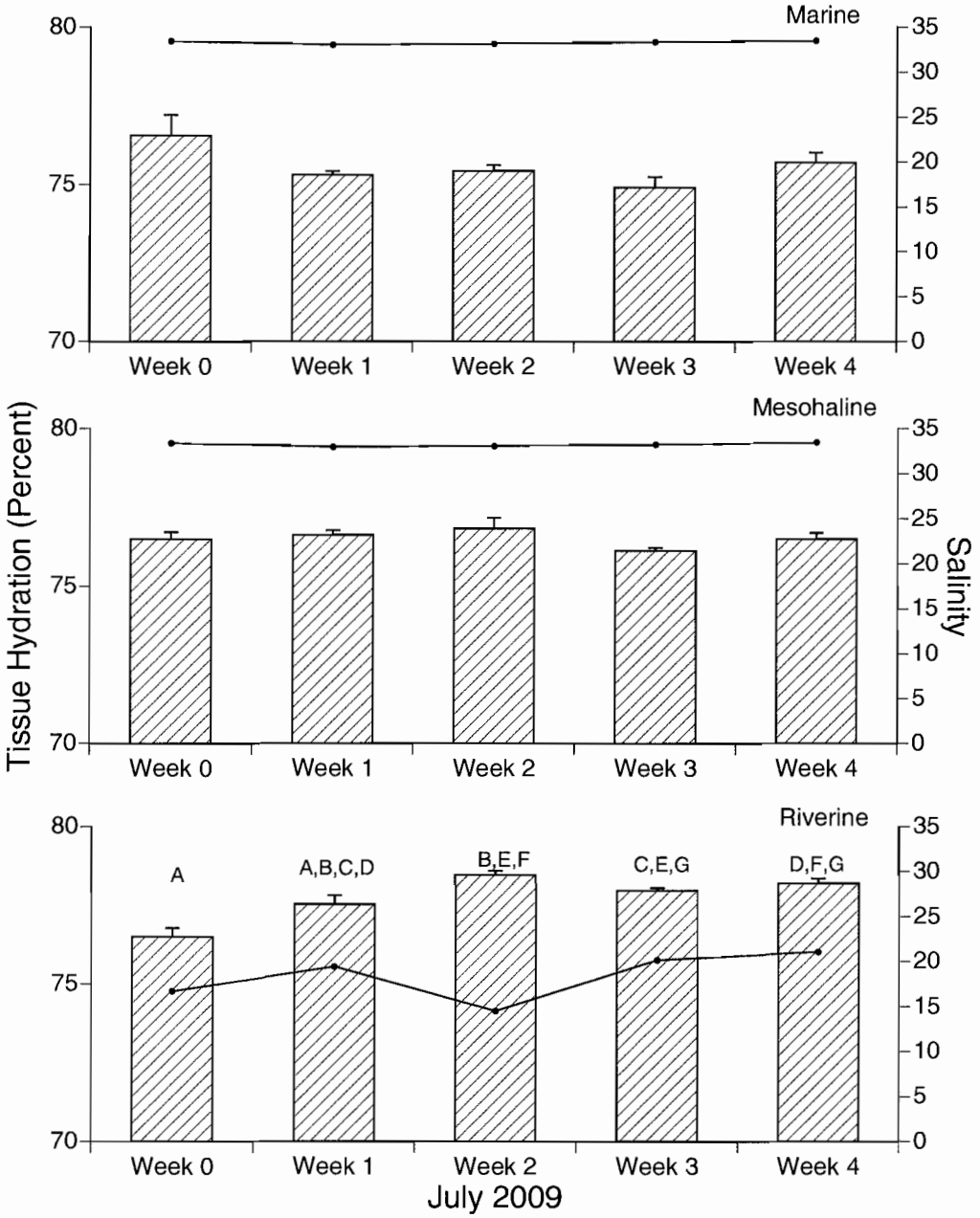

Figure 3.16. Percent tissue hydration of *Metridium senile* at marine, mesohaline and riverine sites during July 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests. No significant difference was found between days at the marine and mesohaline field sites ($p>0.01$).

is capable of partial volume regulation which increases with decreasing salinity (Figure 3.6). Percent tissue hydration varied significantly among sites and weeks (Figure 3.16; Table 3.5A). Post-hoc comparisons revealed significant differences among weeks at the riverine site but not at the marine and mesohaline sites (Figure 3.16).

Tissue magnesium ion concentrations remained relatively constant for anemones placed at marine and mesohaline sites with individual concentrations remaining isoionic and hyperionic to the respective ambient seawater. However, concentrations of tissue magnesium ions initially decreased from 49 mM/ L to 22 mM/L during the first week of exposure at the riverine site followed by a steady hyperionic increase to 39 mM/L during the fourth week (Figure 3.17). Differences in tissue magnesium ion concentrations varied significantly among sites and weeks (Figure 3.17; Table 3.5B). Post-hoc comparisons revealed significant differences among weeks at all field sites (Figure 3.17). Additionally, significant increases in anemone magnesium ion concentrations from week 1 to week 4 at the riverine site indicate regulation under hyposaline conditions (Figure 3.17). Presence of a slight hyperionic increase from week 2 to week 4 at the mesohaline site indicates partial regulation under hyposaline conditions (Figure 3.17).

Tissue osmolality also remained stable at marine and mesohaline sites with individual tissue osmolality remaining relatively iso-osmotic to the ambient medium. In constrast, anemone osmolality at the riverine site initially decreased from 928 mmol/kg to 515 mmol/kg during the first week, after which it remained hyperosmotic to the ambient medium and steadily increased from the first week the fourth week (777 mmol/kg; Figure

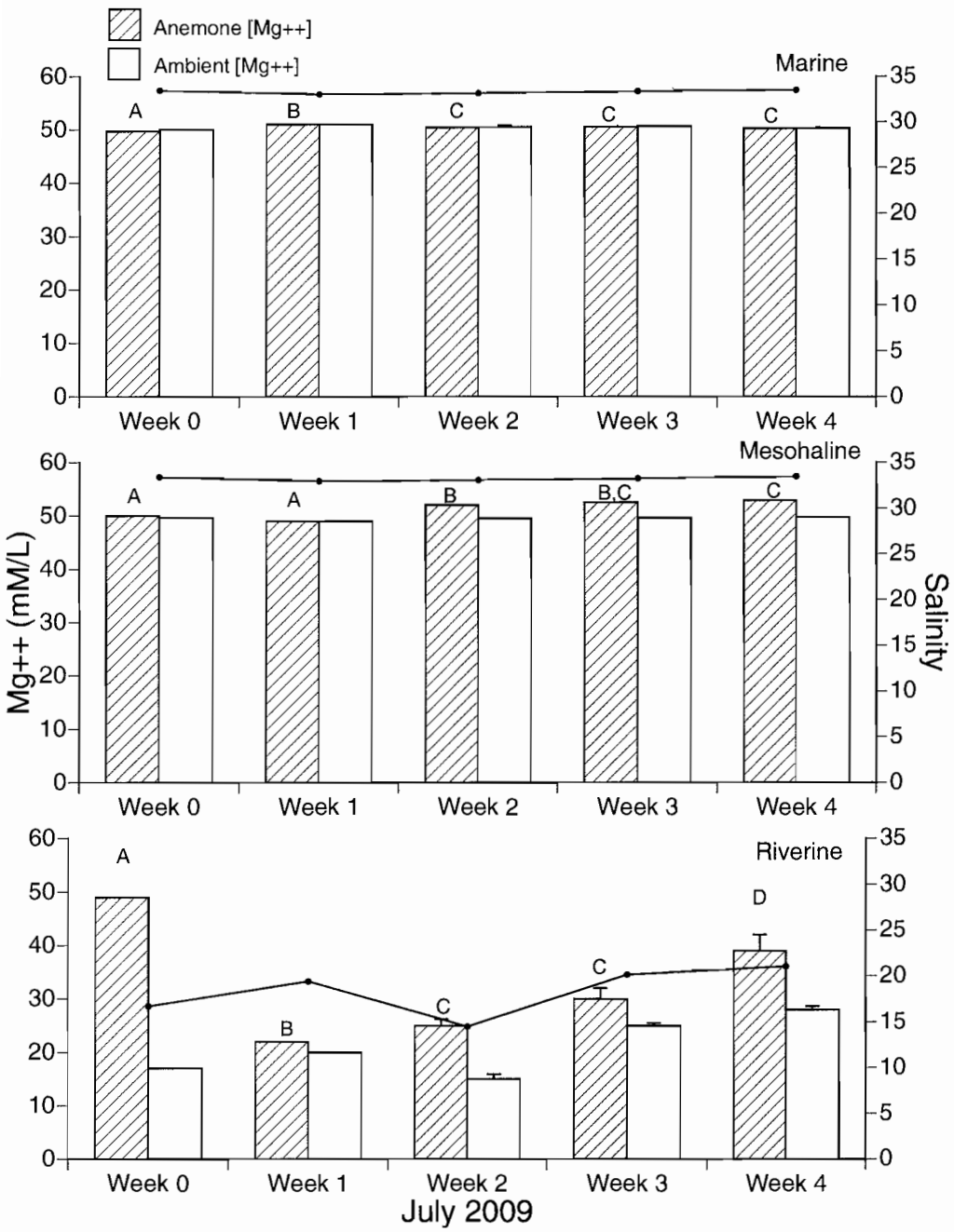

Figure 3.17. Tissue magnesium ion concentrations of *Metridium senile* at marine, mesohaline and riverine sites during July 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests.

3.18). Differences in tissue osmolality varied significantly among sites and weeks (Figure 3.18; Table 3.5C). Post-hoc comparisons revealed significant differences among weeks at the riverine site but not at the marine and mesohaline sites (Figure 3.18). Additionally, significant increases in anemone osmolality from week 1 to week 4 at the riverine site indicate regulation under hyposaline conditions (Figure 3.18).

Cumulative percent mortality exhibited increased mortality with distance from the estuary mouth but was low compared to the fall transition, spring transition and wet season. Peak mortalities of 1%, 25% and 50% occurred in concert at the marine, mesohaline and riverine-dominated sites, respectively (Figure 2.5). For additional monthly figures of percent tissue hydration, magnesium ion concentrations, osmolality and cumulative mortality refer to the Appendix Figures 1-36.

Yearly Trends

Significant multivariate effects were found among sites and seasons for all dependent variables during the yearly measurement period of July 2008 to August 2009 (Table 3.6). Percent tissue hydration varied significantly among sites and seasons (Figure 3.19; Table 3.6B). Overall, tissue hydration increased with distance from the estuary mouth with an average maximum tissue hydration of 81% and 83% occurring in November 2008 and December 2008 at mesohaline and riverine sites, respectively (Figure 3.19). These peaks were observed after the onset of the fall transition when the salinity within the South Slough decreases as a result of increased freshwater input.

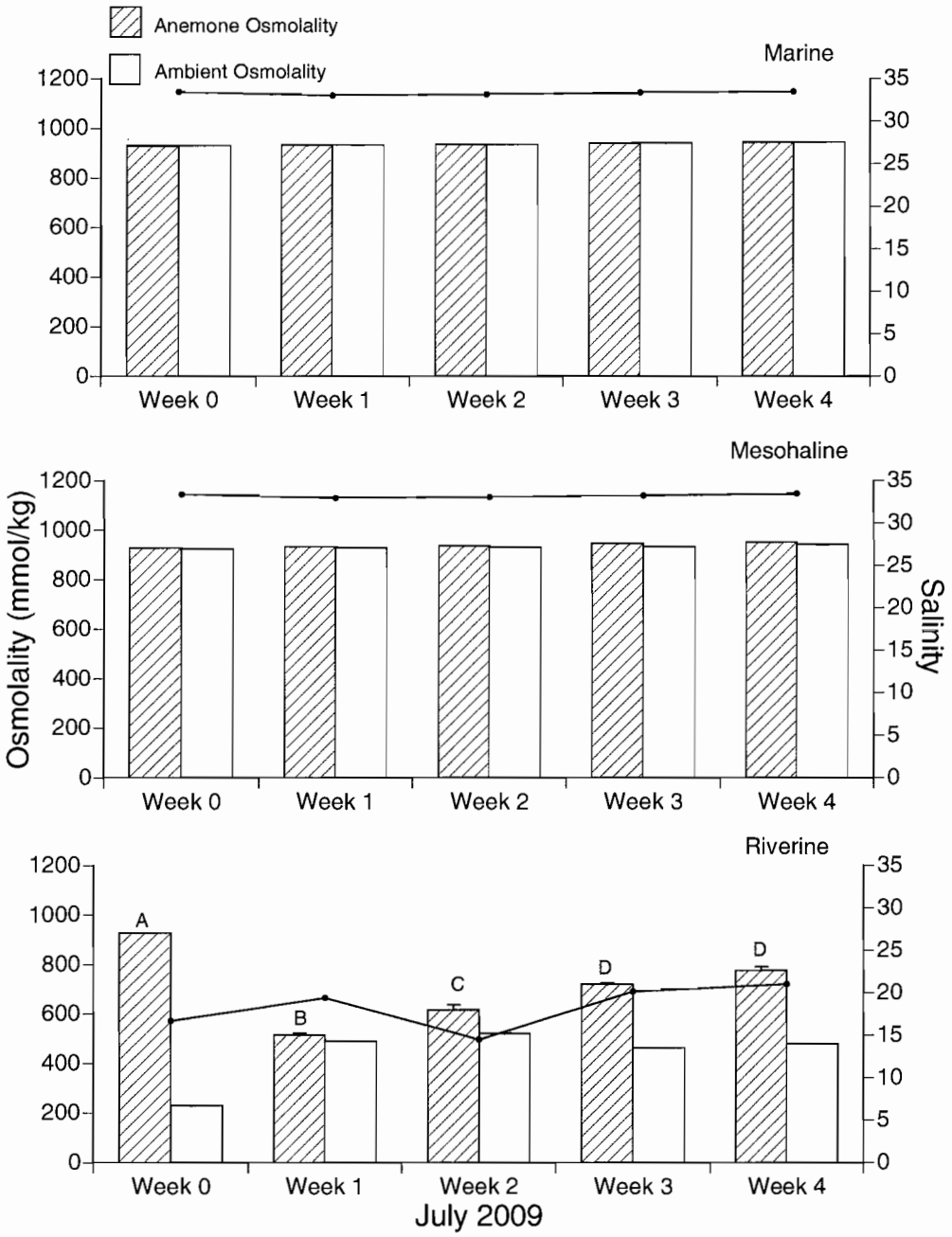

Figure 3.18. Tissue osmolality of *Metridium senile* at marine, mesohaline and riverine sites during July 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3). Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Different letters denote a significant difference ($p<0.01$) between means using Scheffe's post-hoc tests.

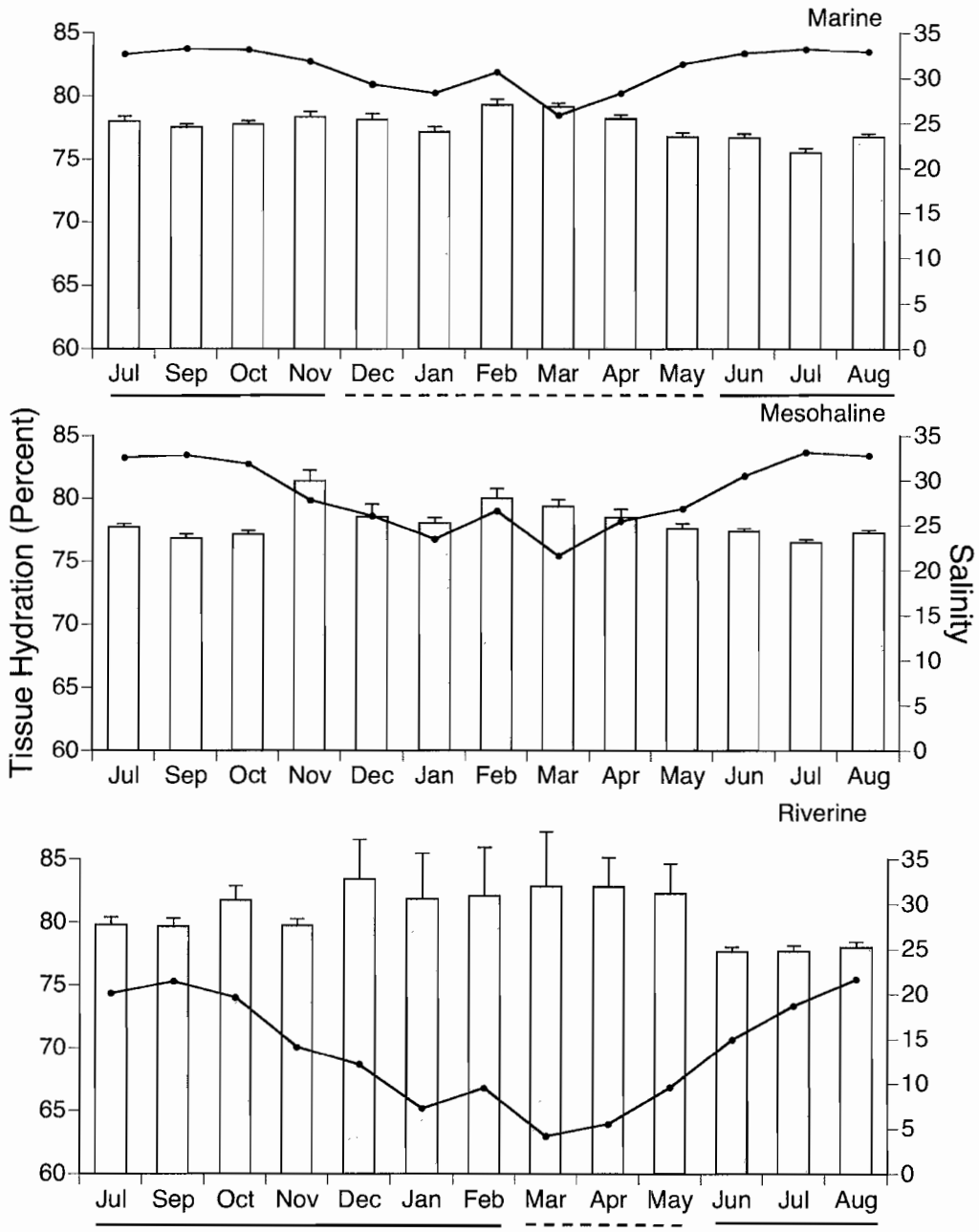

Figure 3.19. Monthly percent tissue hydration at marine, mesohaline and riverine sites over four-week measurement periods from July 2008 to August 2009. Bars represent means with standard error (n=5, except for December (n=3) and February (n=4)). Line overlays represent changes in average salinity for each month at respective field sites. Horizontal lines below months represent Scheffe's post-hoc groupings in which months covered by these lines are not significantly different from one another ($p>0.01$). No significant difference was found between months at the mesohaline site ($p>0.01$).

Conversely, percent tissue hydration decreased during the months of March through May following a decrease in freshwater input and a stable salinity increase, and remained relatively constant during the dry season from June through August (Figure 3.19). Post-hoc comparisons revealed significant differences between the dry and wet seasons and the dry and spring transition seasons at the marine site, whereas the dry and spring transition seasons were significantly different at the riverine site and no significant differences were found between seasons at the mesohaline site. This indicates that percent tissue hydration significantly increases with lower salinity during the wet and spring transition seasons because of an increase in freshwater input at marine and riverine sites (Figure 3.19).

ß values showed similar trends at mesohaline and riverine sites with increased regulation occurring during the wet season when salinity is low and variable or decreased regulation during the dry season when salinity is high and stable. However, a pronounced decrease in regulation was observed during December 2008 and March 2009 at the riverine site, which may suggest the existence of a salinity threshold for partial volume regulation following periodic freshwater pulses. This suggests that *Metridium senile* is a stronger regulator at lower salinities, up to a certain threshold salinity (Figures 3.6 and 3.19).

Tissue magnesium ion concentrations varied significantly among sites and seasons (Figure 3.20; Table 3.6B). A direct relationship was found between anemone magnesium ion concentrations and salinity as magnesium ion concentrations decreased

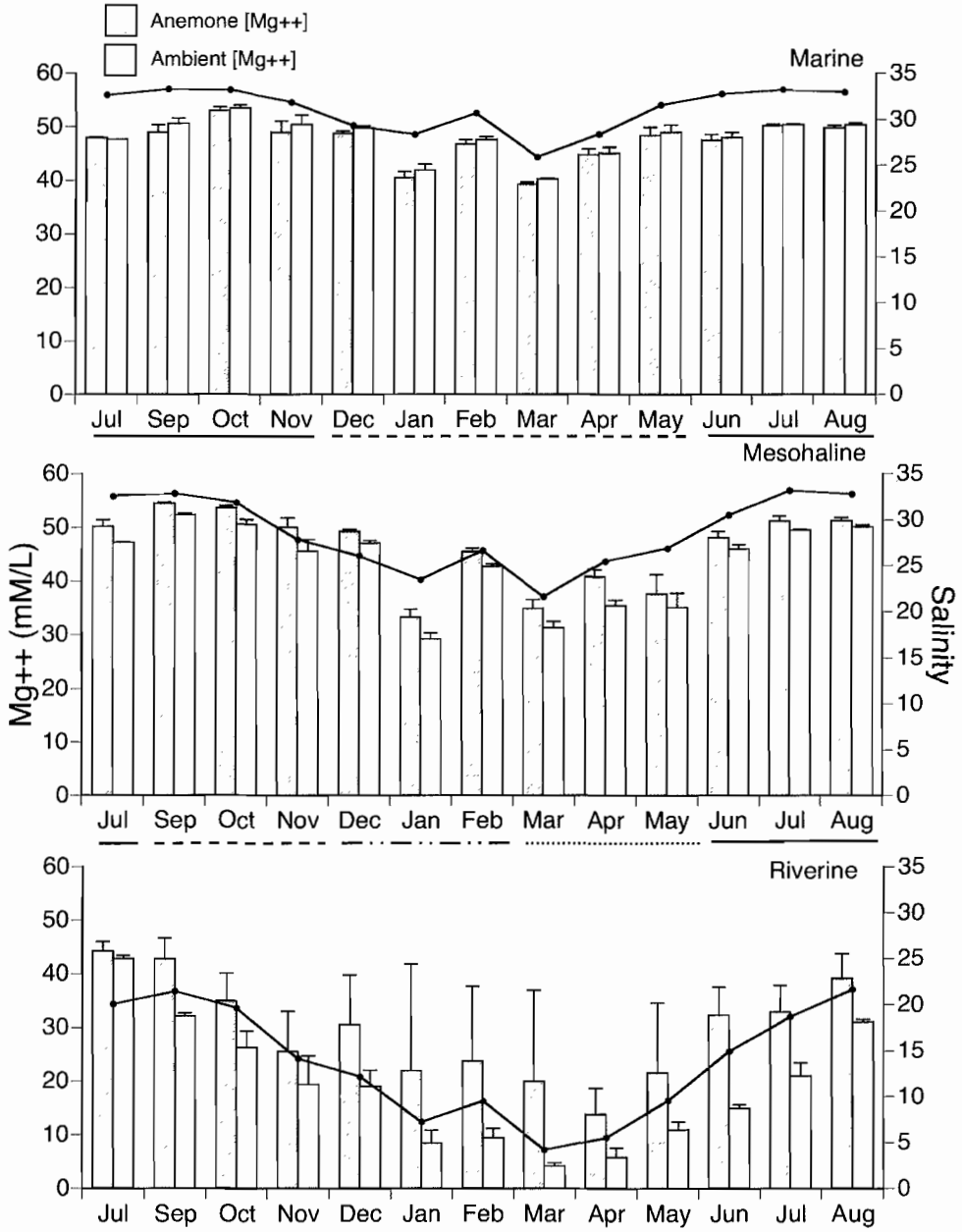

Figure 3.20. Monthly tissue magnesium ion concentrations at marine, mesohaline and riverine sites over four-week measurement periods from July 2008 to August 2009. Bars represent means with standard error (n=5, except for December (n=3) and February (n=4)). Line overlays represent changes in average salinity for each month at respective field sites. Horizontal lines below months represent Scheffe's post-hoc groupings in which months covered by these lines are not significantly different from one another ($p>0.01$). No significant difference was found between months at the riverine site ($p>0.01$).

with distance from the estuary mouth (Figure 3.20). Minimum tissue magnesium ion concentrations of 33 mM/L and 14 mM/L were found during January and April 2009 at mesohaline and riverine sites, respectively. Anemones remained relatively isoionic at the marine site and hyperionic at mesohaline and riverine sites, with greater differences between anemone and ambient magnesium ion concentrations observed at the riverine site (Figure 3.20). Furthermore, these differences observed at the riverine site increased with decreased salinity during the wet season and spring transition and decreased slightly during the dry season. Post-hoc comparisons revealed significant differences between the dry and spring transition, fall transition and wet season, and fall transition and spring transition at the marine site, whereas the mesohaline site showed significant differences among all of these seasons as well as between the dry and wet season. Due to increased variance at the riverine site, no significant differences were detected among seasons. These seasonal differences indicate that the observed changes in anemone magnesium ion concentrations at the marine and mesohaline sites are directed by seasonal changes in the ambient salinity, yielding heightened differences between anemone and ambient magnesium ion concentrations (Figure 3.20). This indicates that *Metridium senile* may be capable of partial hyperionic regulation at lower salinities (Figure 3.20).

Tissue osmolality varied significantly between site and season, revealing similar yearly trends since a direct relationship was found between anemone osmolality and salinity (Figure 3.21; Table 3.6B). Anemone osmolality decreased with distance from the estuary mouth, with lowest osmolality of 700 mmol/kg and 396 mmol/kg occurring in

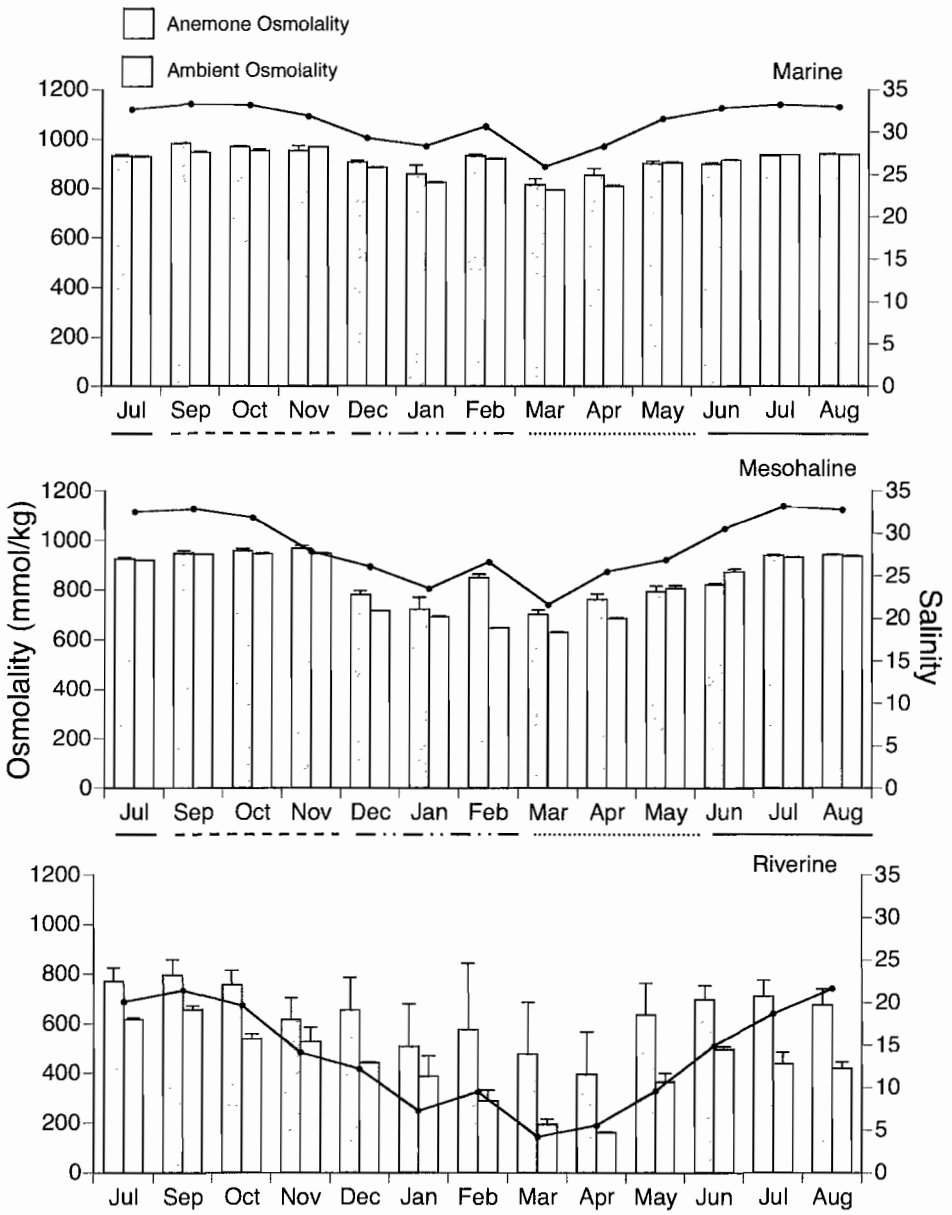

Figure 3.21. Monthly tissue osmolality of *Metridium senile* at marine, mesohaline and riverine sites over four-week measurement periods from July 2008 to August 2009. Bars represent means with standard error (n=5, except for December (n=3) and February (n=4)). Line overlays represent changes in average salinity for each month at respective field sites. Horizontal lines below months represent Scheffe's post-hoc groupings in which months covered by these lines are not significantly different from one another ($p>0.01$). No significant difference was found between months at the riverine site ($p>0.01$).

March 2009 and April 2009 at mesohaline and riverine sites, respectively. Anemones remained iso-osmotic at the marine site and generally hyperosmotic at mesohaline and riverine sites, with greater differences between anemone and ambient osmolality observed at the riverine site. Furthermore, these differences observed at the riverine site increased with decreased salinity during the wet season and spring transition. Post-hoc comparisons revealed significant differences between the dry and spring transition, fall transition and wet season, and fall transition and spring transition at the marine site, whereas the mesohaline site showed significant differences among all of these seasons as well as the dry and wet season. Because of increased variance at the riverine site, no significant differences were detected among seasons. These seasonal differences indicate that the observed changes in anemone osmolality at the marine and mesohaline sites are directed by seasonal changes in the ambient salinity, yielding heightened differences between anemone and ambient osmolality (Figure 3.21). This indicates that *Metridium senile* may be capable of partial hyperosmotic regulation at lower salinities (Figure 3.21).

Overall, cumulative percent mortality increased with distance from the estuary mouth during July 2008 to August 2009 and varied significantly among sites and seasons (Figure 3.22; Table 3.6B). At the marine and mesohaline-dominated site, mortality was highest in March 2009 and lowest in July 2009, showing peak survival during the dry season when salinity is high and lowest survival during the spring transition season when salinity was low. The riverine-dominated site exhibited highest mortality in January 2009 and lowest mortality in July 2009 again showing peak survival during the dry season and

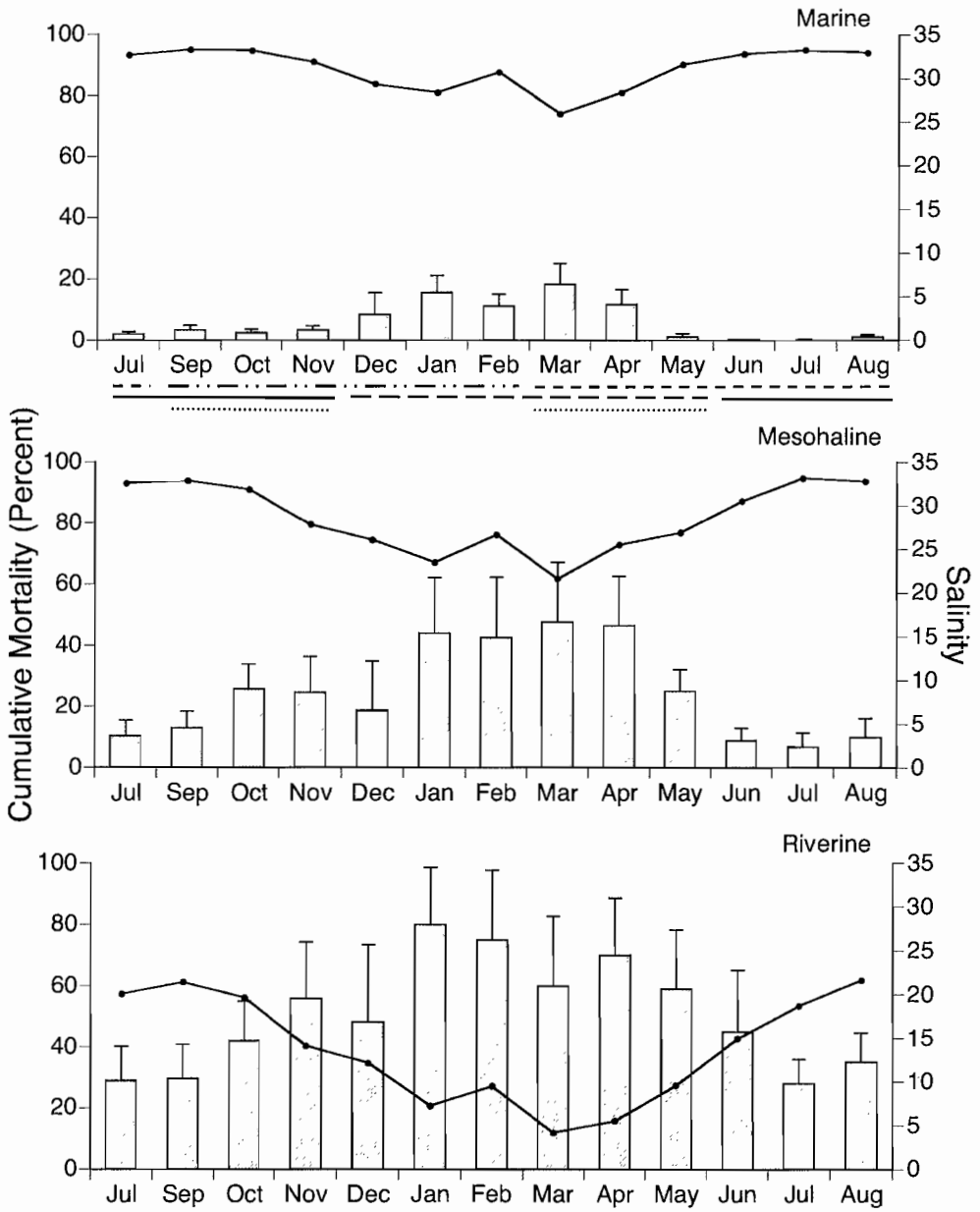

Figure 3.22. Cumulative monthly mortality of adult transplants at marine, mesohaline and riverine sites over four-week measurement periods from July 2008 to August 2009. Bars represent means with standard error (n=5, except for December (n=3) and February (n=4)). Line overlays represent changes in average salinity for each month at respective field sites. Horizontal lines below months represent Scheffe's post-hoc groupings in which months covered by these lines are not significantly different from one another ($p>0.01$). No significant difference was found between months at the mesohaline and riverine sites ($p>0.01$).

lowest survival during the wet season (Figure 3.22). However, post-hoc analyses only revealed significant differences in cumulative mortality between the wet and dry season at the marine site. No significant differences between seasons were detected between the mesohaline and riverine sites (Figure 3.22).

Discussion

Metridium senile exhibits a surprising capacity to survive at low salinities. Anemones tolerated salinities in the field as low as 6 for about a week and survived direct transfer from 100% to 75% and 50% seawater for twenty-eight days (Figures 3.2-3.4 and 3.22). The laboratory and field experiments further indicate that this remarkable hyposalinity tolerance may be attributed to the ability of *M. senile* to partially hyperosmoregulate in response to decreased salinity of 5 or 6, as the increase in percent tissue hydration is not directly proportional to the decrease in salinity (Figures 3.2, 3.3, 3.6 and 3.19). Acclimation to low salinities is also accompanied by a decreased ß value, suggesting that the ability of cell volume regulation increases with decreasing ambient salinity (Figures 3.3 and 3.6). However, *M. senile* showed an increase in tissue hydration and its accompanying ß value under extreme hyposaline conditions of around 5 or 6 in March 2009 followed by complete mortality by the second week, indicating a threshold for regulation (Figures 2.6, 3.6, and 3.19). Similar trends were noted in a laboratory study performed by Deaton and Hoffmann (1988) where acclimation to dilute salinities was also associated with a decreased ß value. These observations suggest that *M. senile* does

not act as a simple osmoconformer and may be a hyperosmoregulator within a specific range of low salinities. Consequently, other mechanisms could aid in counteracting hyposalinity stress.

Behavioral and physiological mechanisms that have been described in other actinarian species include the regulation of cellular volume by means of the intracellular free amino acid pool (FAA) (Pierce and Minasian, 1974; Shick, 1976; Benson-Rodenbough and Ellington, 1982; Kasschau *et. al.,* 1984a; Deaton and Hoffmann, 1988). Shumway (1978) discusses the ability of *Metridium senile* to retract tentacles and regulate its extension and surface area under hypo-osmotic conditions, while Bursey and Harmer (1979) noted the secretion of a "mucus sheet" which may serve as a barrier to water movement on another actinarian, *Condylactis gigantea.* However, the function of cellular ions as a mechanism for osmotic regulation has not been studied in actinarians, only in scyphomedusae, in which Wright and Purcell (1997) noted the regulation of potassium, sodium and magnesium ions. Therefore, results of the present study suggest that cellular ions and solutes may play a role in volume regulation since *M. senile* exhibits an increased capacity to partially regulate tissue osmolality and magnesium ion concentrations at lower salinities (Figures 3.20 and 3.21).

Anemone field transplants displayed an initial decrease and subsequent hyperosmotic or hyperionic increase in tissue osmolality and magnesium ion concentrations with decreased salinity during the fall transition and dry season at mesohaline and riverine sites (Figures 3.7, 3.8, 3.17, 3.18, 3.20, and 3.21). However,

during the wet season and spring transition, anemones at the mesohaline site were not as hyperosmotic or hyperionic and in some cases were hypo-osmotic to ambient salinity, while at the riverine site all anemones died during the first or second week (Figures 2.6-2.7, 3.10-3.12 and 3.14-3.15). This signifies the existence of a threshold for partial hyperosmotic and hyperionic regulation where below a certain salinity level anemones are no longer able to regulate and survive these extreme hypo-osmotic conditions (Figures 2.6-2.7, 3.10-3.12 and 3.14-3.15). Moreover, mortality occurred earlier with lower and more variable salinities, indicating that increased hypo-osmotic stress contributes to the degradation of these regulatory mechanisms (Figures 2.6, 2.7 and 3.22). Similar patterns in magnesium ion concentrations were also observed in the laboratory where anemone magnesium ion concentrations were hyperionic to the ambient medium in 50% and 75% seawater (Figure 3.4). This trend has also been documented in other cnidarians, such as the mesohaline scyphomedusae *Chrysaora quinquecirrha* which showed hyper-regulation of tissue sodium and magnesium levels under hypo-osmotic conditions (Wright and Purcell, 1997).

The hyperosmotic and hyperionic relationship between the anemone and the ambient salinity during the dry and fall transition seasons suggests that while ions and other solutes may not be actively regulated, the tissue is at least partially impermeable to them (Figures 3.7, 3.8, 3.17, and 3.18). This may be caused by the secretion of a "mucus sheet" observed on a number of individual actinarians in response to osmotic shock. This mucus sheet could act as a barrier to water and solute movements, although the

diffusional permeability has not been examined (Shoup, 1932; Shick, 1976; Shumway, 1978; Bursey and Harmer, 1979; Benson-Rodenbough and Ellington, 1982; Kasschau *et al.*, 1984a). In the field, mucus secretions were present on *Metridium senile* one week following transplant to mesohaline and riverine sites; in the laboratory, mucus secretions were observed during the first week of exposure to 50% and 75% seawater. Kirkpatrick and Bishop (1973) and Goreau (1959) suggest that sea anemone mucus a) houses phosphonic acid groups which may act as ion exchangers, and b) sequesters ions, such as calcium (Ca^{2+}). Consequently, mucus secretion may be a mechanism to aid in the conservation not only of dissolved particles important in regulating cell volume, but also of magnesium ions which are fundamental in the functioning of the sodium-potassium pump and other metabolic reactions during diel salinity fluxes within the South Slough (Rankin and Davenport, 1981; Shick, 1991; Hee Ko *et al.*, 1999; Rumrill, 2006; Evans, 2009).

The adaptive significance of conserving magnesium ions and other vital solutes has a number of important ramifications. Firstly, because the Na^{+}/K^{+}-ATPase pump may aid in cell volume regulation and utilizes the magnesium salt of ATP as a substrate to function, regulation of this vital ion could aid in conserving the operation of critical metabolic reactions necessary for anemone survival (Rankin and Davenport, 1981). Although there is some speculation as to whether this specific sodium pump is involved in cell volume regulation, magnesium ions are required for ATP hydrolysis in order to operate other ATP-controlled sodium pumps (Rankin and Davenport, 1981; Lang, 1998;

Hee Ko *et al.*, 1999). Secondly, regulation of magnesium ions may aid in the stabilization of ion channels and cell membranes (Rankin and Davenport, 1981; Lang, 1998; Hee Ko *et al.*, 1999). Consequently, hyperionic regulation of this ion could be a way for sea anemones to continue important metabolic reactions needed for survival while under hypo-osmotic stress. Thirdly, regulation of tissue osmolality may play a similar role by conserving important cellular solutes that are necessary in the maintenance of metabolic reactions. For example, regulation of sodium and potassium ions may help sustain the functioning of sodium pumps necessary for maintaining cellular homeostasis. Other solutes, like free amino acids, are also important mediators in volume regulation at low salinities within a number of actinarian species (i.e. *Metridium senile, Diadumene leucolena, Haliplanella lineata* and *Bunodosoma cavernata*) (Pierce and Minasian, 1974; Shick, 1976; Benson-Rodenbough and Ellington, 1982, Kasschau *et al.*, 1984a; Deaton and Hoffmann, 1988). Therefore, the ability to actively regulate these solutes also contributes to controlling cellular volume (Shick, 1991). Lastly, it is important to note that the hyper osmolality of anemone to ambient salinity observed in *M. senile* could possibly be explained by the increased sequestration of ions and solutes within the mucus film present on individuals exposed to dilute salinities (Figure 3.21).

The hyperosmotic and hyperionic regulation observed during the dry and fall transition seasons elucidate patterns observed in percent tissue hydration and accompanying ß values (Figures 3.6 and 3.19-3.21). Because *Metridium senile* exhibits a marked increase in tissue hydration and decrease in ß values with decreased salinity in

both the laboratory and field, it suggests that anemones increase cell volume regulation as salinity declines down to about 5 (Figures 3.6 and 3.19). However, salinity levels lower than this showed a marked increase in ß values as observed during the wet and spring transition seasons (Figure 3.6). This capacity to regulate cell volume at lower salinities could be because *M. senile* hyper-regulates magnesium ions and osmolality during the dry and fall transition seasons (Figures 3.6 and 3.19-3.21). Hyperionic and hyperosmotic regulation may partially mediate cell volume regulation as important ions and solutes are conserved to continue metabolic reactions and control cellular volume. However, a pronounced increase in hyper-regulation is observed during the wet and spring transition seasons when salinities were extremely low (~5-7) at the riverine site, yet this was accompanied by a decrease in cell volume regulation and complete mortality within the first or second week (Figures 2.6, 2.7, 3.6, and 3.19-3.22). Consequently, this suggests the presence of a salinity tolerance threshold in which partial hyper-regulation is no longer a viable physiological strategy for *M. senile* survival.

Mortality trends further corroborate the existence of a critical salinity threshold since exposure to the extreme hyposaline conditions during the wet and spring transition seasons culminated in complete mortality at riverine and mesohaline sites as compared to the dry season where mortality was minimal (Figures 2.5, 2.6, 2.7, and 3.22). Furthermore, increased mortality was accompanied by the reduced ability to regulate cell volume, magnesium ion concentrations, and tissue osmolality (Figures 2.6-2.7, 3.10-3.15, and 3.19-3.22). Therefore, partial regulation in *Metridium senile* could be a physiological

response that is initiated under hypo-osmotic stress conditions allowing individuals to survive low salinities. However, such a response may only be feasible within a certain salinity window as evinced by the seasonal and weekly trends in percent tissue hydration, magnesium ion concentrations, osmolality, and mortality at each field site (Figures 3.19-3.22).

Metridium senile exhibits a similar physiological response within the laboratory. Individuals survived salinities ranging from 100% seawater (950 mOsm) to 55% seawater (520 mOsm) for two weeks, while individuals exposed to 40% seawater (380 mOsm) died within three days (Deaton and Hoffmann, 1988). Additionally, the cell volume regulation (ß) in these individuals increased with decreased salinity and was partly mediated by intracellular FAA concentrations (Deaton and Hoffmann, 1988). Other actinarians such as *Diadumene leucolena* and *Haliplanella lineata* also show increased cell volume regulation with compensatory adjustment of FAA concentrations subsequent to acute salinity change (Pierce and Minasian, 1974; Shick, 1976; Shick, 1991). However, such physiological responses are not limited to actinarians. Regulation has been documented in other cnidarians such as scyphomedusae and the freshwater hydromedusae *Craspedacusta sowerbyi* in which potassium was hyper-regulated possibly as a means for lessening cellular hydration since it is actively expelled from cells (Fleming and Hazelwood, 1967; Hazelwood *et al.*, 1970; Wright and Purcell, 1997). Furthermore, *Chrysaora quinquecirrha* has been shown to regulate amino acids as a strategy for controlling cellular volume (Wright and Purcell, 1997). Consequently, these

physiological responses appear to be initiated by hypo-osmotic conditions as an adaptation for survival, since these species display them when experiencing dilute salinities.

In other phyla, physiological strategies for tolerating hypo-osmotic stress consist of hyper-conformation of sodium and potassium ions as found in the sea urchins *Lytechinus variegatus* and *Echinometra lucunter*, which exhibit increased regulation under hyposaline conditions (Freire and Santos-Gouvea, 2007; Freire *et al.*, 2007). Crustaceans selectively secrete ions from the blood using antennary glands and control ion uptake through the gills. This aids in dampening the rise of internal pressure as water is absorbed (Robertson, 1953; Charmantier *et al.*, 2002; Charmantier and Charmantier-Daures, 2007). In addition, there is some evidence that the shore crab *Carcinus maenas* may be able to detect changes in external osmolarity and subsequently regulate its urine production in the antennal glands (Norfolk, 1978). In contrast, molluscs regulate their intracellular FAA to withstand changes in osmotic concentration of the ambient medium. This is a fundamental process in cell volume regulation of many marine molluscs and has been documented in the gills and ventricle of the mussel *Geukensia demissa* (Baginski and Pierce, 1978; Deaton, 1987; Kube *et al.*, 2006). Certain species of polychaetes use a similar strategy. *Arenicola marina* has been shown to manage cellular amino acid concentrations by regulating their intracellular FAA (Shumway and Davenport, 1977), while other nereid polychaetes, *Nereis limnicola, N. diversicolor, N. succinea,* and *Laeonereis culveri,* were able to combat acute salinity stress through hyperosmotic

regulation of intracellular solutes (Oglesby, 1965). However, most other polychaete species are unable to tolerate low salinities for an extended period of time and respond by osmoconforming (Evans, 2009).

While sea anemones have generally been described as osmoconformers, and usually are not considered euryhaline, the results of this study suggest that *Metridium senile* is euryhaline and capable of partially regulating its cellular volume, osmolality and magnesium ion concentrations under hypo-osmotic conditions (Figures 3.2-3.4, 3.6 and 3.19-3.22). Moreover, this species exhibits a wide salinity tolerance, with salinities of 5 or 6 representing the lower limit (Figures 2.6, 2.7, and 3.22). This range is similar to other actinarians considered euryhaline. For example, *Diadumene leucolena* and *Bunodosoma cavernata* survive salinities ranging from 6 to 33 and 11 to 49 respectively, while *Haliplanella lineata* survives indefinitely at 12 (Pierce and Minasian, 1974; Shick, 1976; Benson-Rodenbough and Ellington, 1982). Since *M. senile* does not behave as a simple osmoconformer and displays partial regulation, its physiological response lies somewhere on the continuum between a perfect regulator and a perfect osmoconformer. Consequently, *M. senile*'s ability to partially regulate its cell volume, osmolality and magnesium ion concentration is a physiological strategy for resisting hypo-osmotic stress induced by dilute saline conditions commonly experienced within the South Slough Estuary.

CHAPTER IV

CONCLUDING SUMMARY

The objective of this thesis was to provide a more complete physiological perspective on the actinarian *Metridium senile* by analyzing its physiological responses and tolerance limits in a laboratory and field setting. Since this species is found along an estuarine gradient within mesohaline and marine-dominated environments, it is important to understand how *M. senile* is able to tolerate these hypo-osmotic conditions by using physiological strategies. One possible strategy is partial regulation, which may allow *M. senile* to retain physiological homeostasis and survive hyposaline conditions. Understanding these strategies may have ecological implications, as physiological resistance to hypo-osmotic stress might be a way of establishing "habitat refugia" along a salinity gradient, elucidating existing abundance and distribution patterns within the South Slough Estuary (Herbst, 2001).

Chapter II of this thesis offered a descriptive view on the abundance and distribution patterns of adult *Metridium senile* and related these to mortality trends observed at three field sites. Results presented here show that abundance and distribution patterns and trends in transplant mortality are probably driven by seasonal changes in the estuary's salinity regime. Chapter III indicates that *Metridium senile* are able to tolerate hypo-osmotic conditions by partially regulating cellular volume, tissue osmolality and

magnesium ion concentrations. The observed regulation is induced under hypo-osmotic stress conditions, and presumably enables *M. senile* to survive low salinities.

However, results described in both chapters indicate that this physiological response has limitations and is only viable within a certain salinity window as evinced by increased mortality at field sites and mere absence of individuals naturally occurring upriver during the wet season. The observed abundance and distribution of *Metridium senile* within the South Slough Estuary is likely attributed to physiological limitations. Consequently, physiological tolerance is a factor driving the distribution of *M. senile* along the South Slough's estuarine gradient.

APPENDIX

ADDITIONAL MONTHLY FIGURES OF PERCENT TISSUE HYDRATION, MAGNESIUM ION CONCENTRATIONS, OSMOLALITY, AND MORTALITY

Presented below are additional monthly figures of percent tissue hydration, magnesium ion concentrations, osmolality, and mortality from July 2008-August 2009.

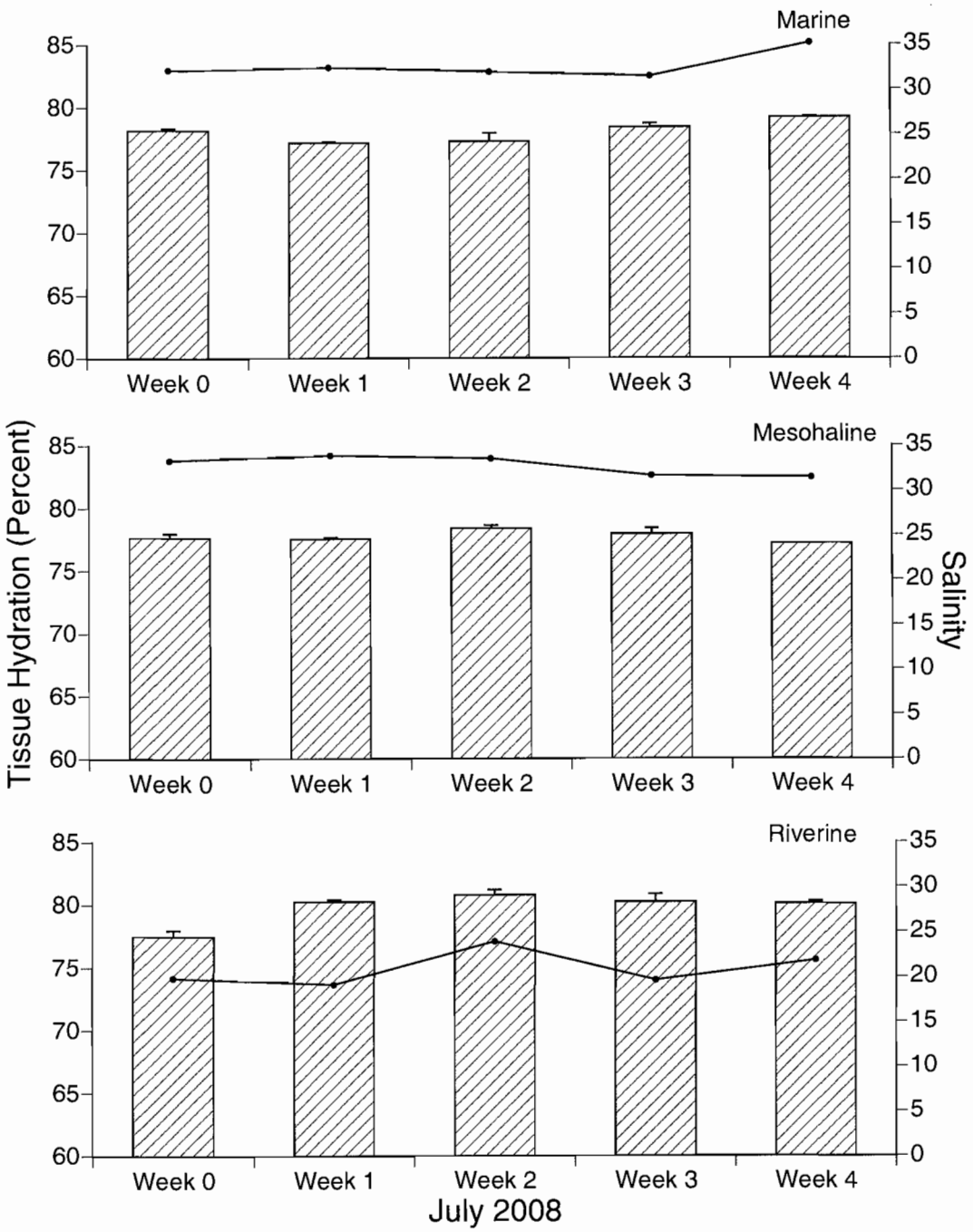

Figure 1. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during July 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

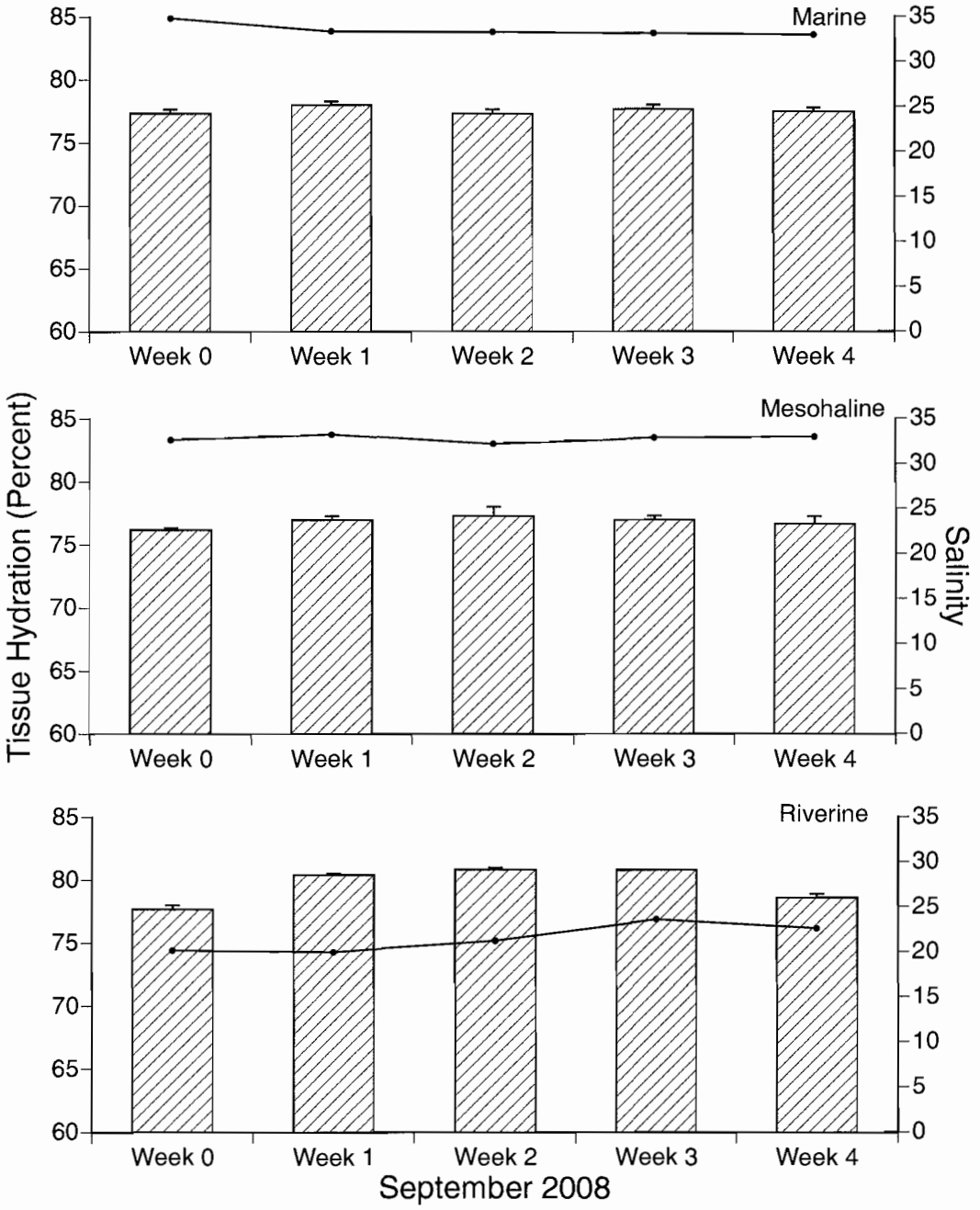

Figure 2. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during September 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

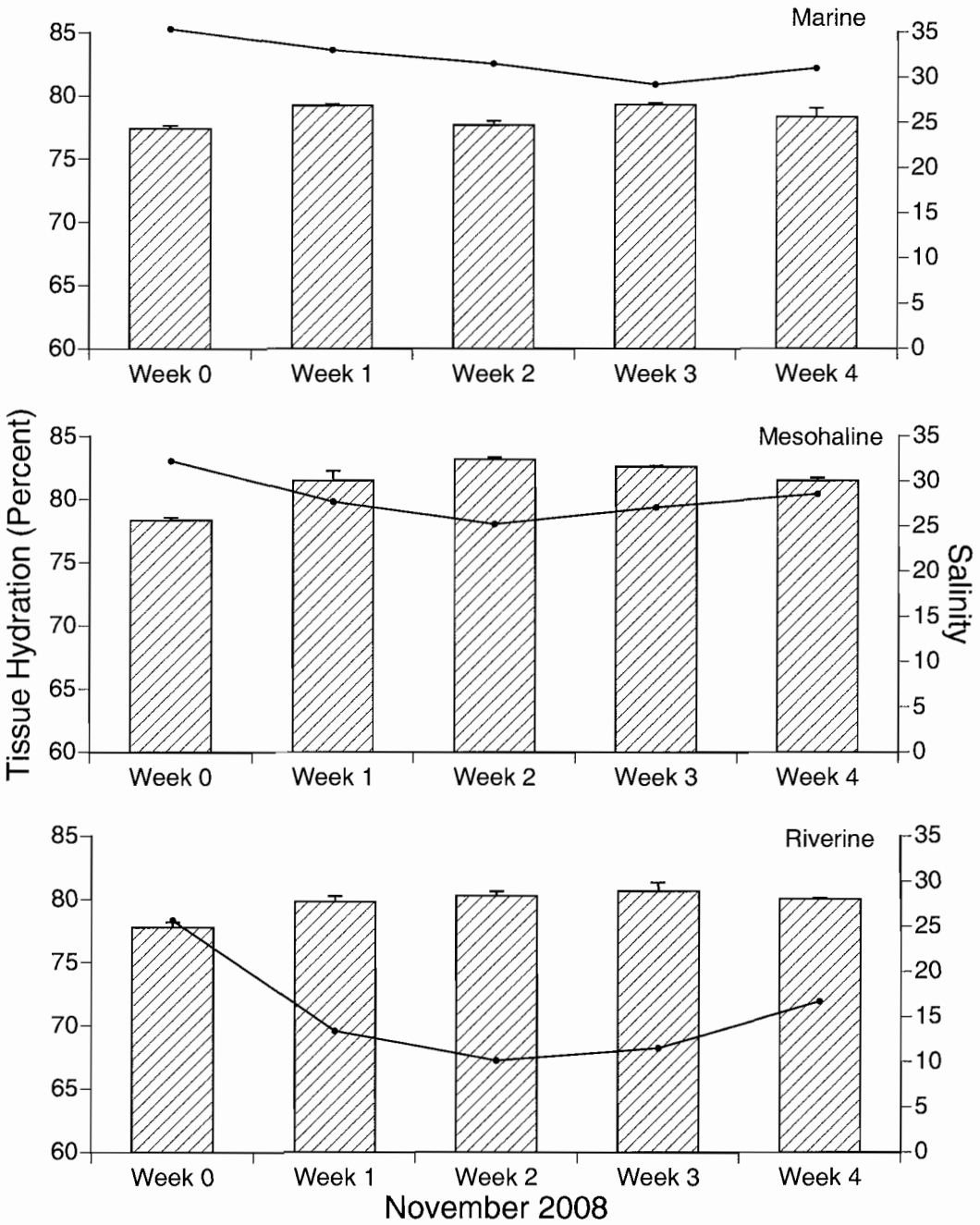

Figure 3. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during November 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

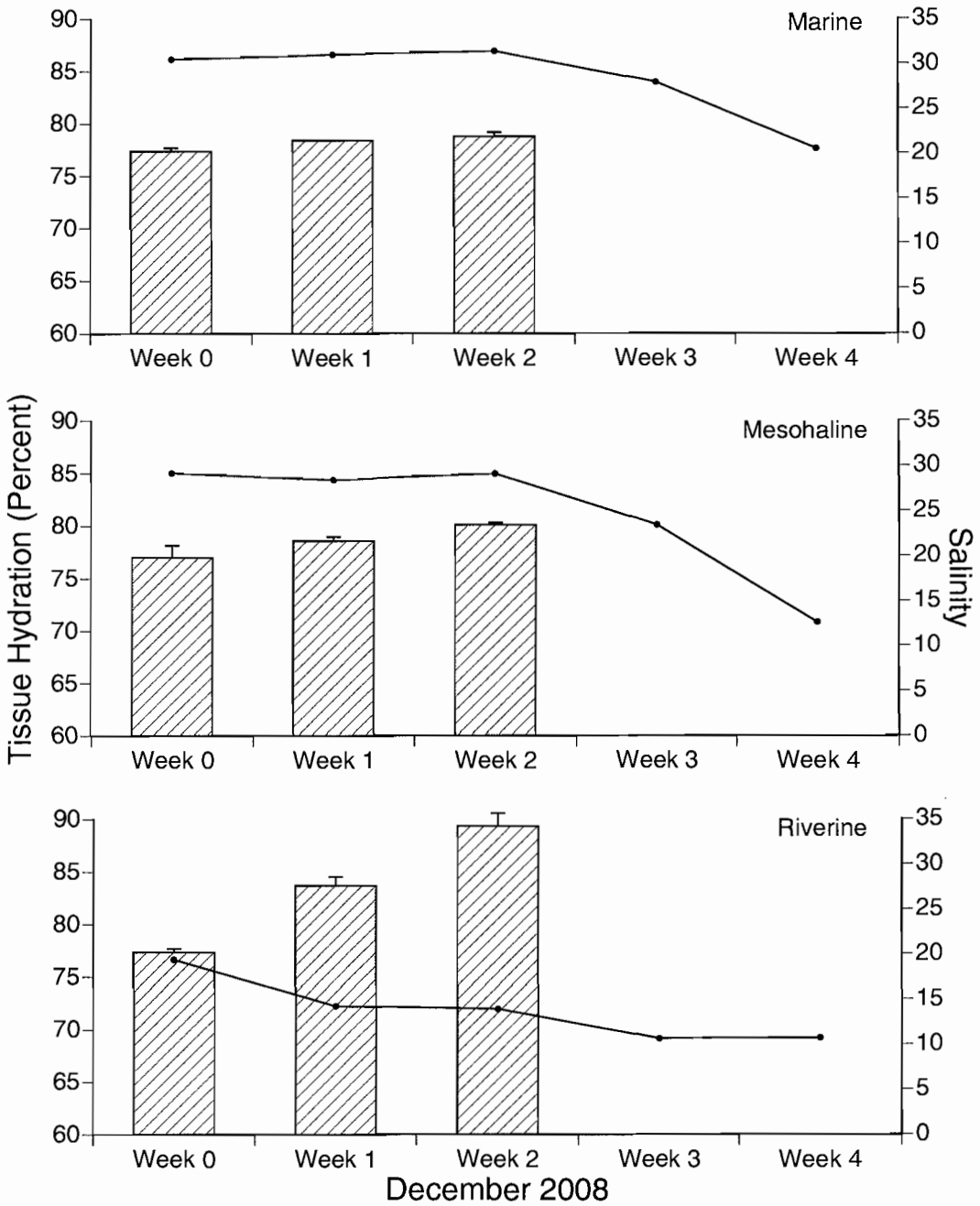

Figure 4. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during December 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

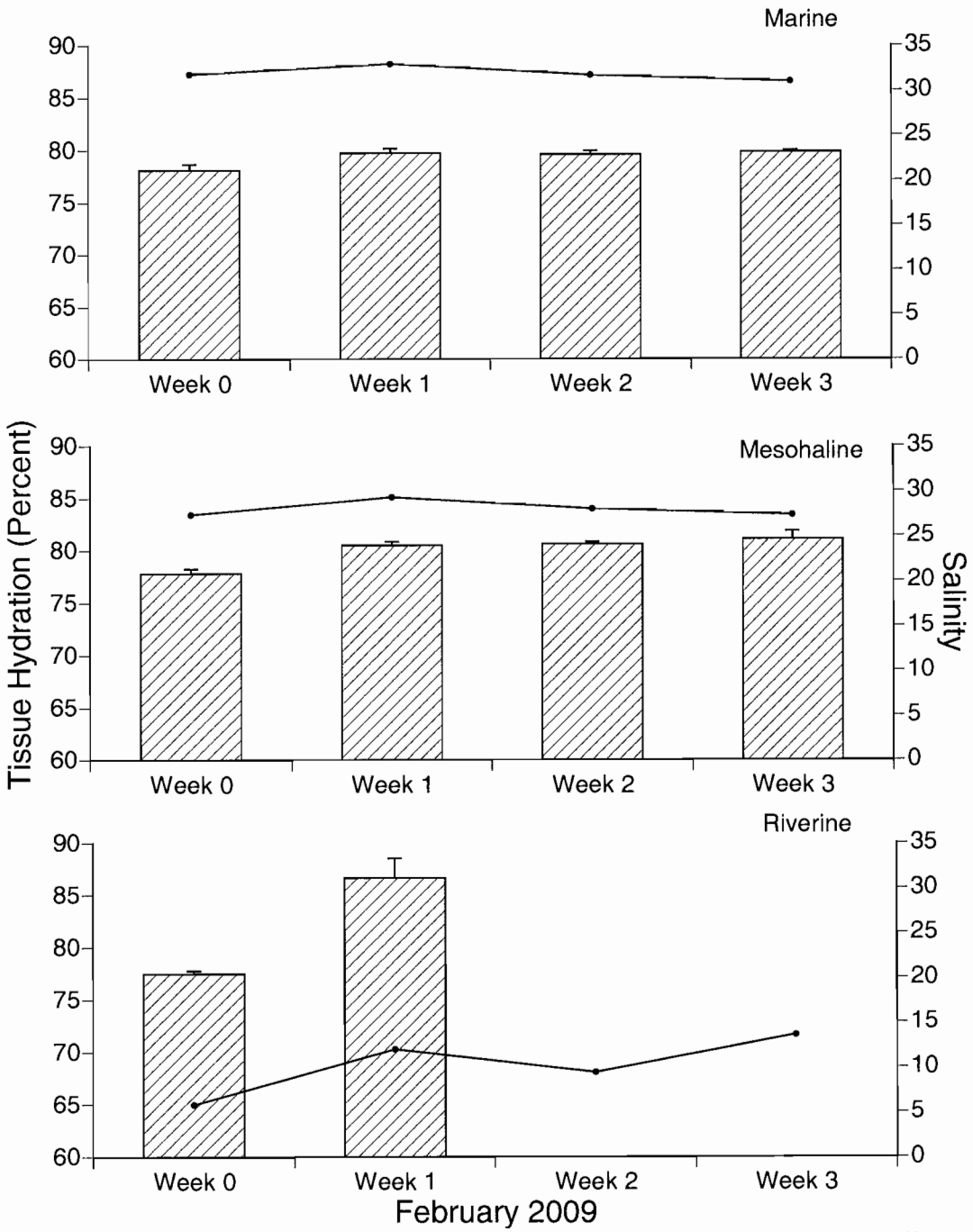

Figure 5. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during February 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

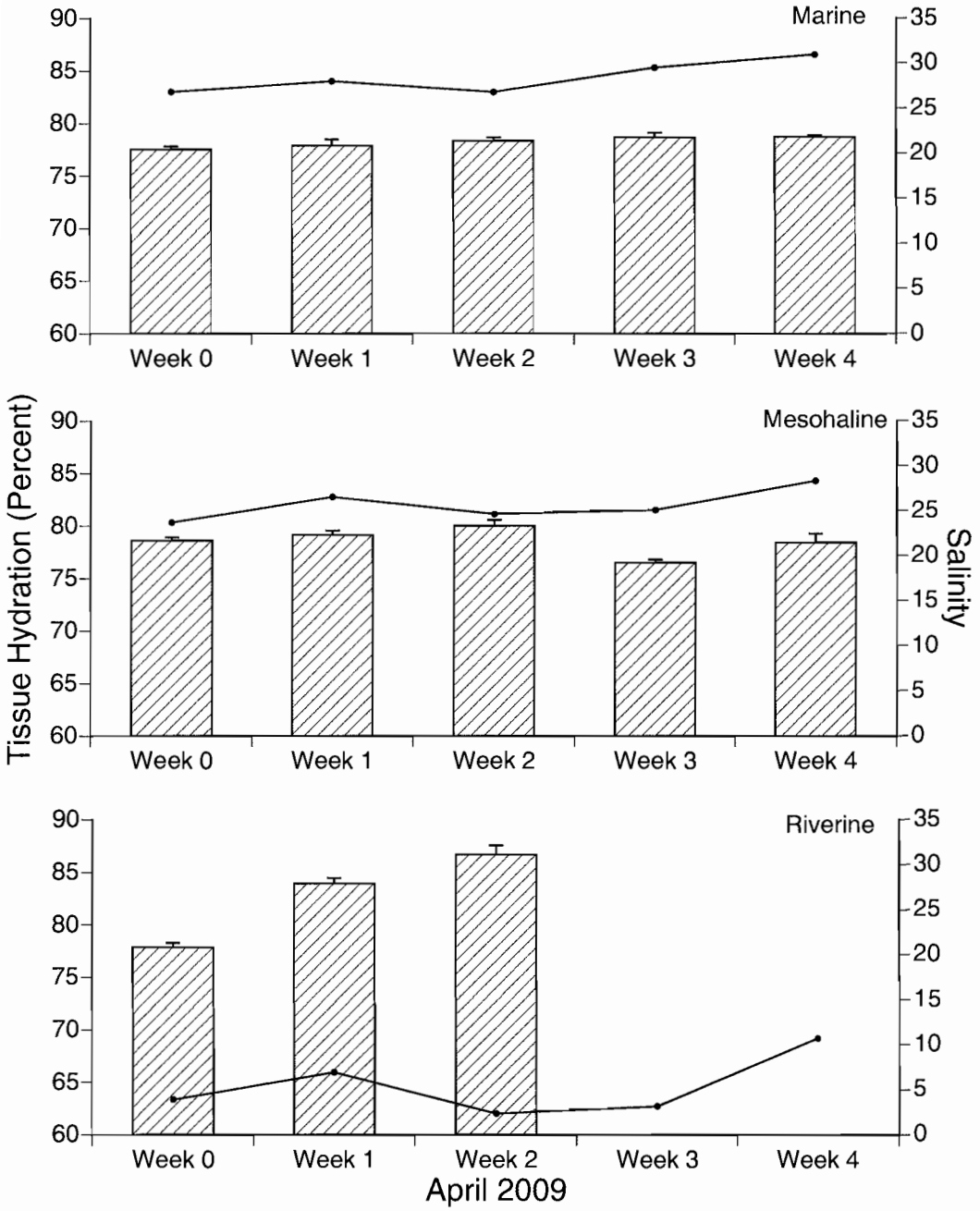

Figure 6. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during April 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

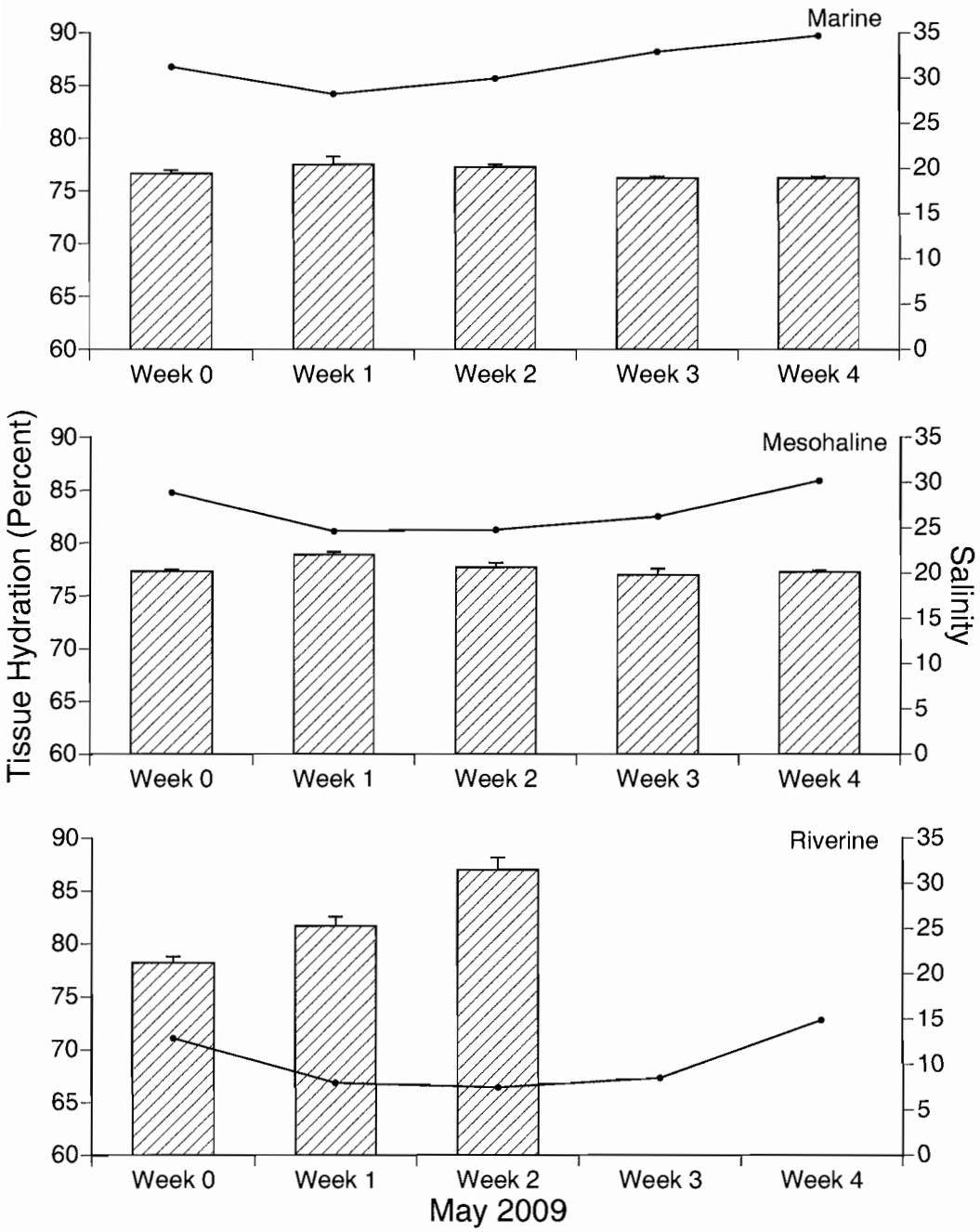

Figure 7. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during May 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

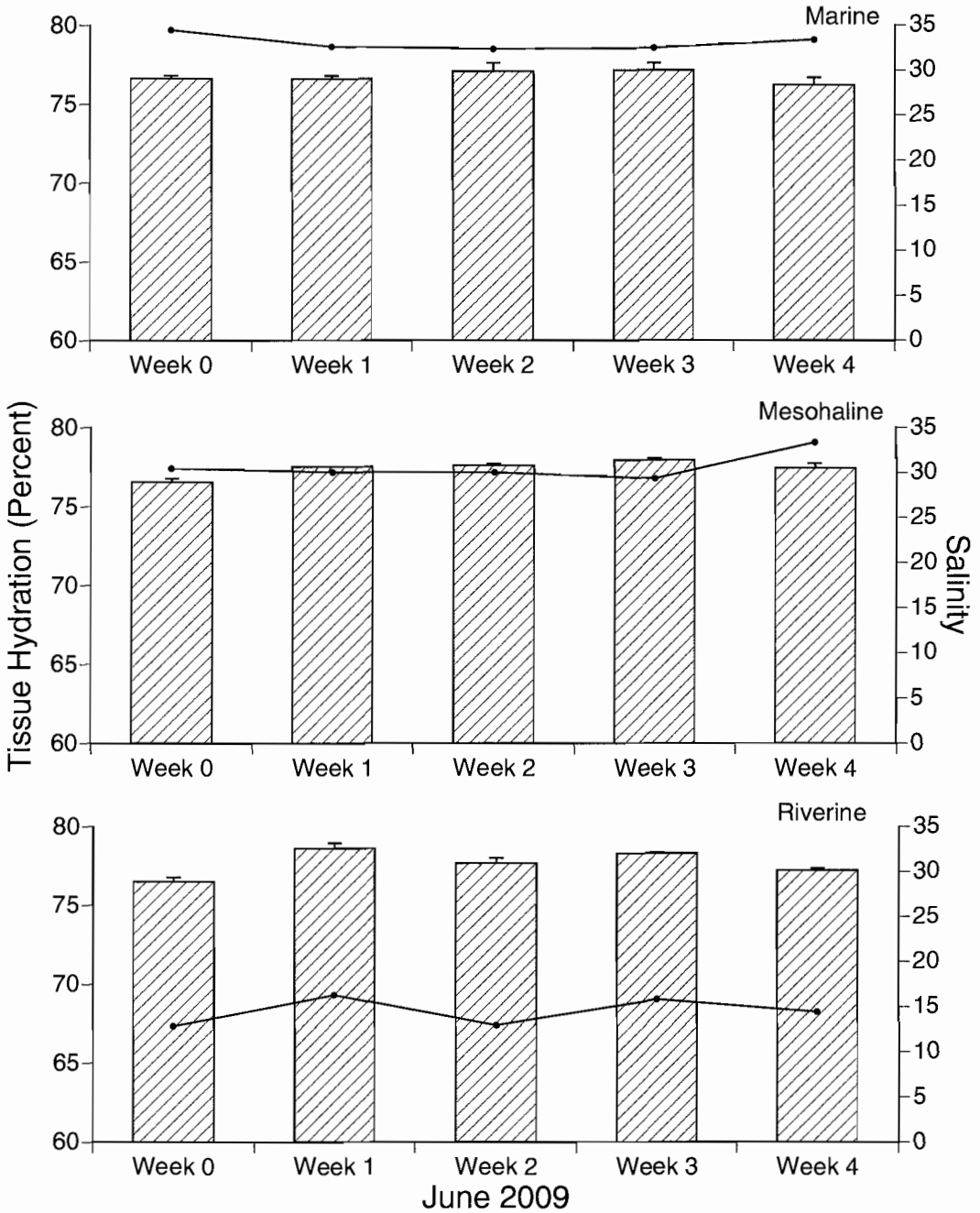

Figure 8. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during June 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

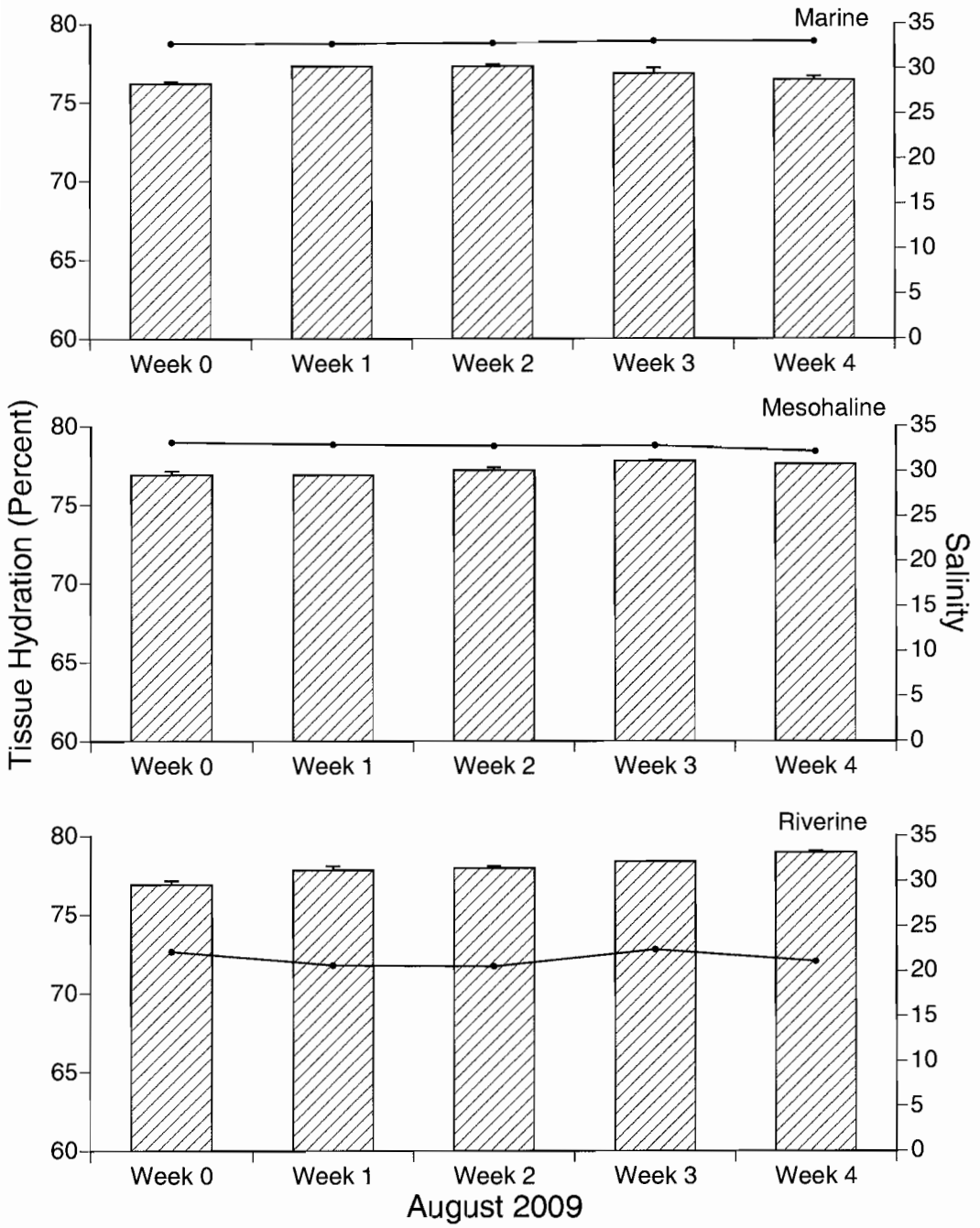

Figure 9. Percent tissue hydration of *Metridium senile* placed at marine, mesohaline and riverine sites during August 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

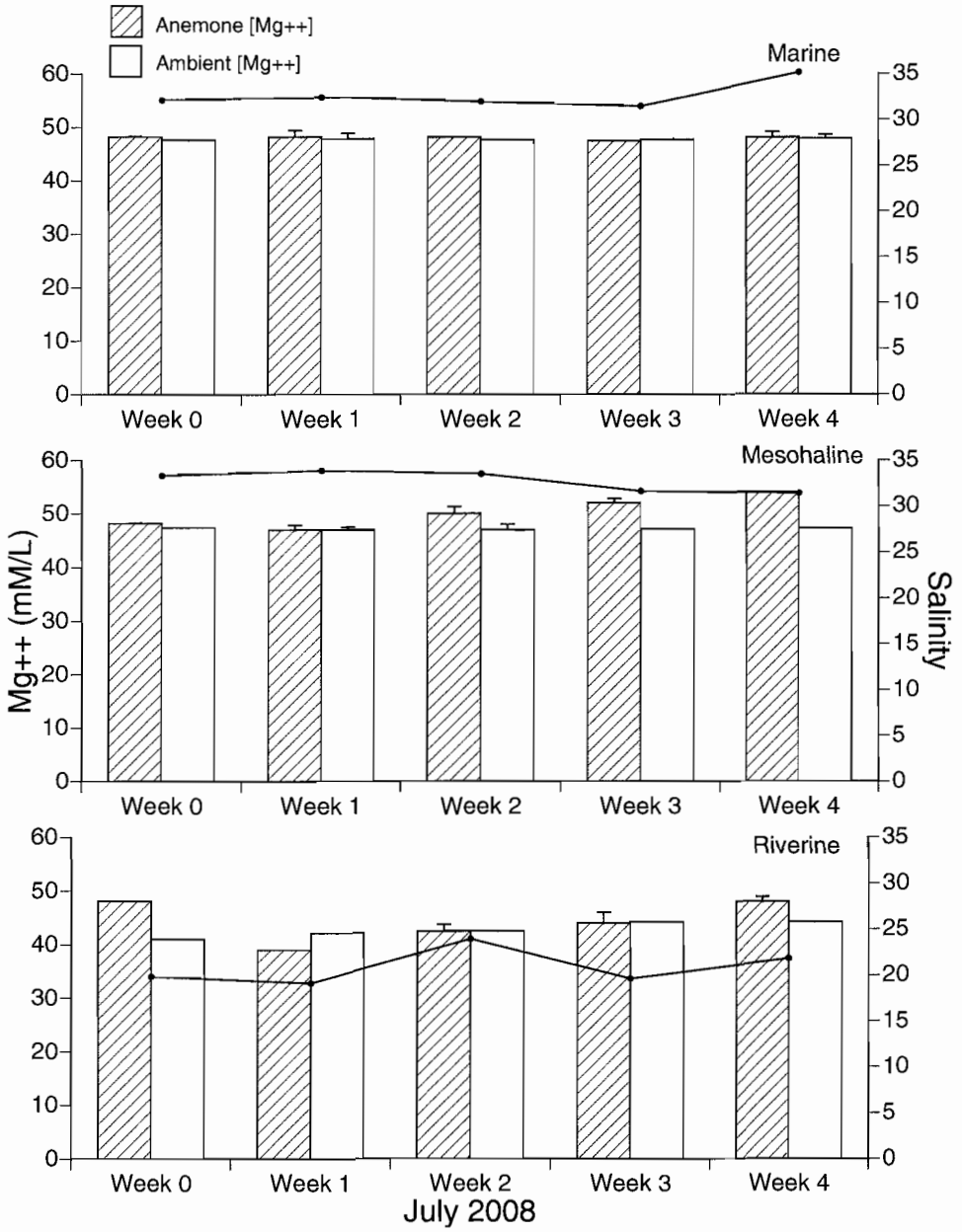

Figure 10. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaline and riverine sites during July 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

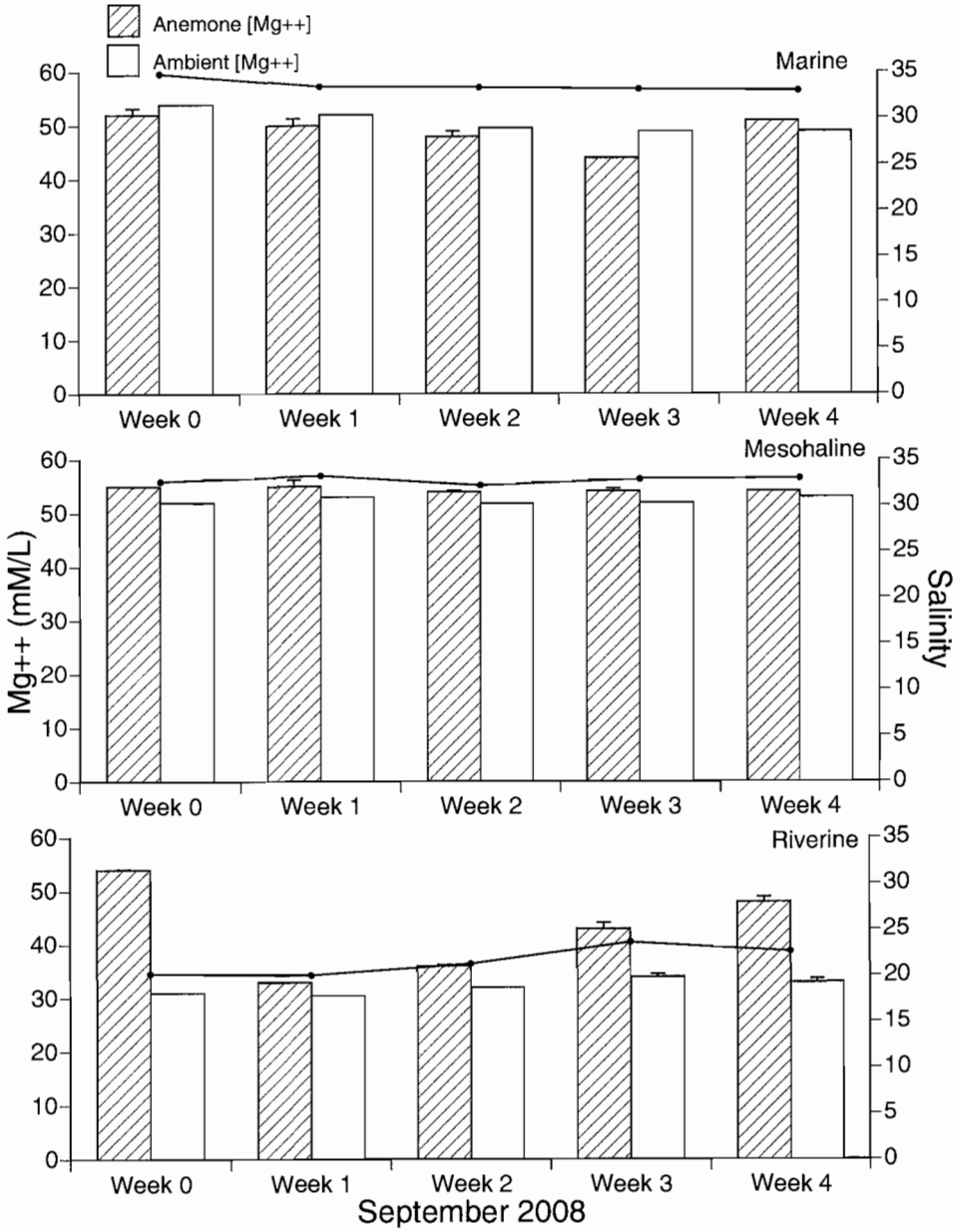

Figure 11. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaline and riverine sites during September 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

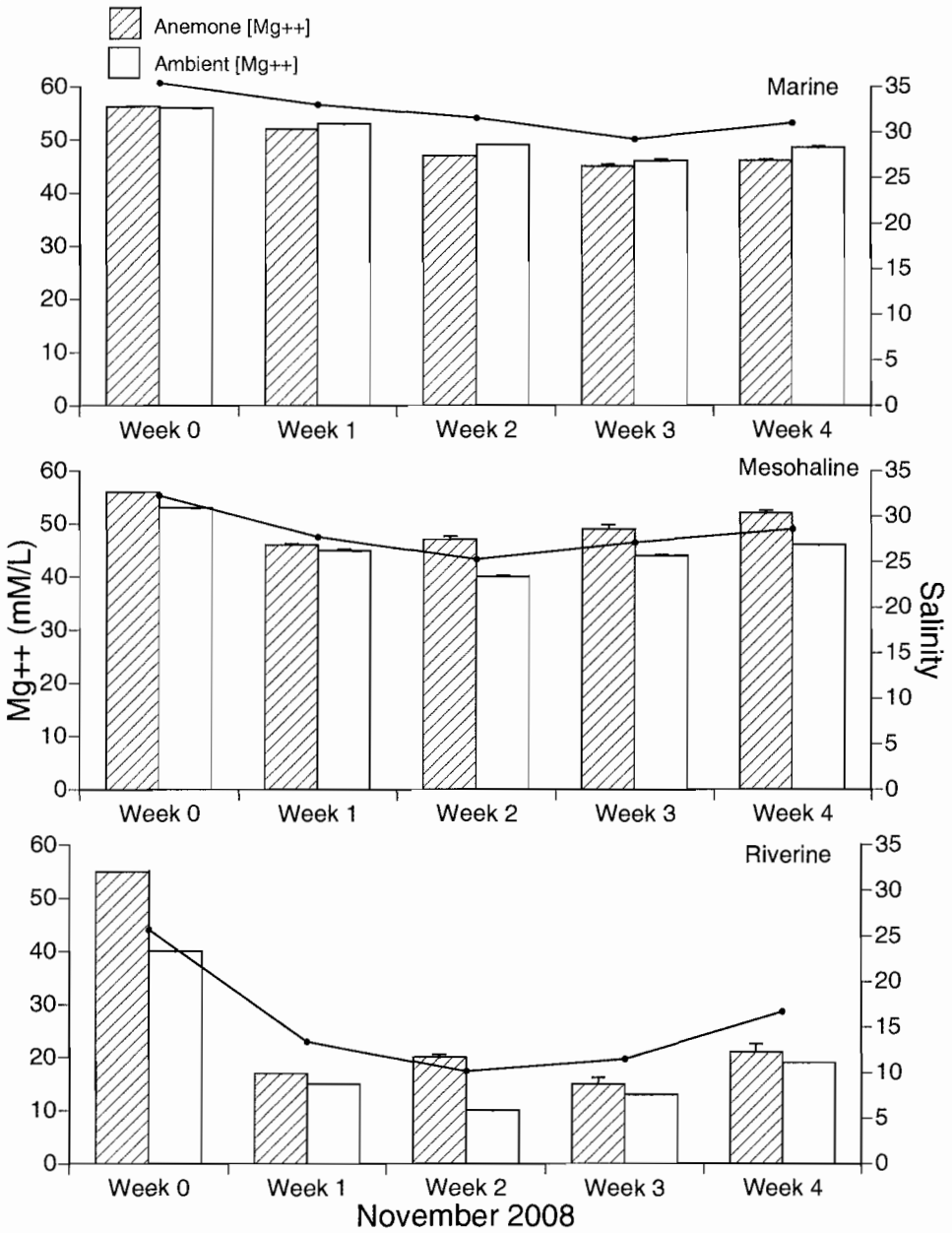

Figure 12. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaline and riverine sites during November 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

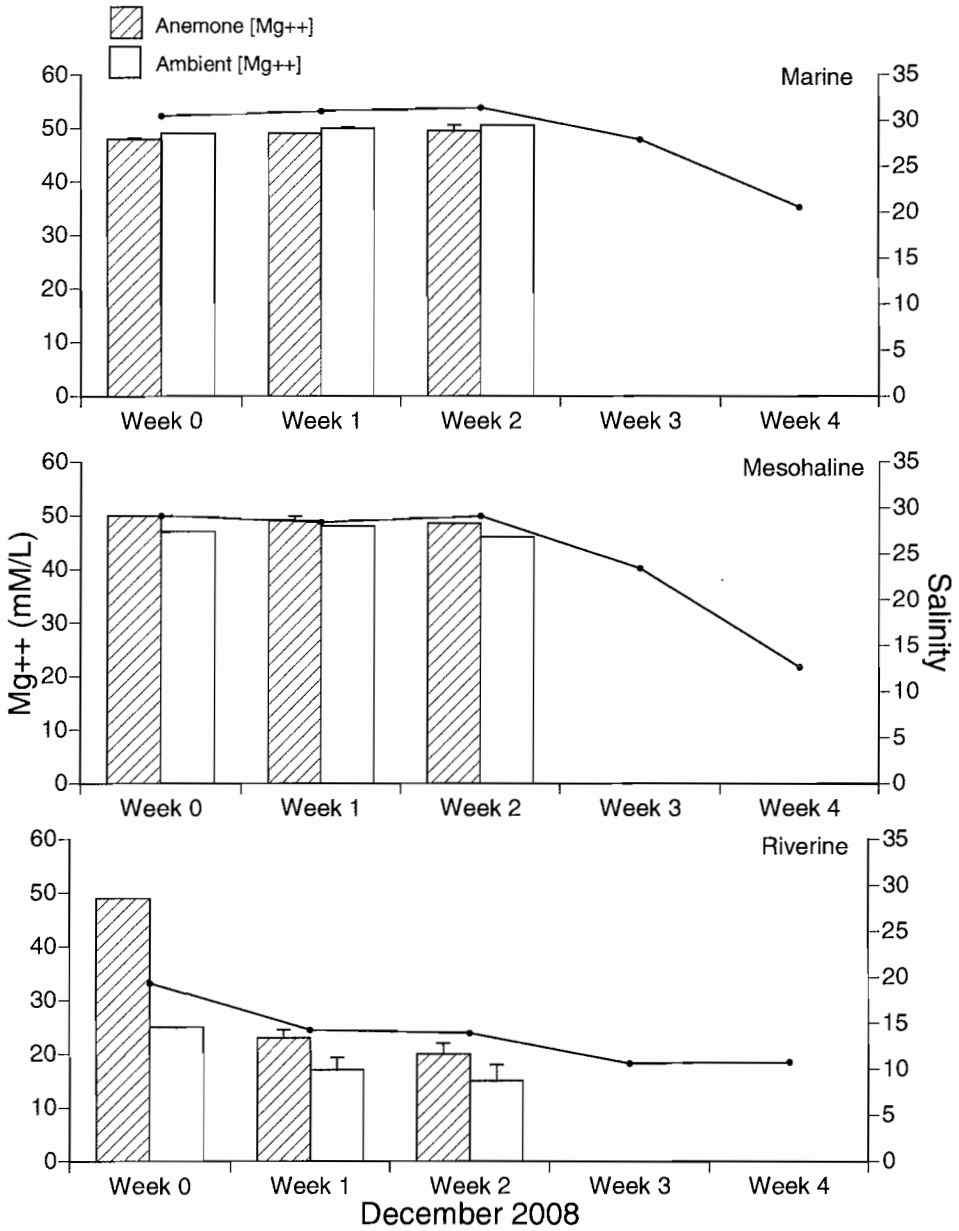

Figure 13. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaline and riverine sites during December 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

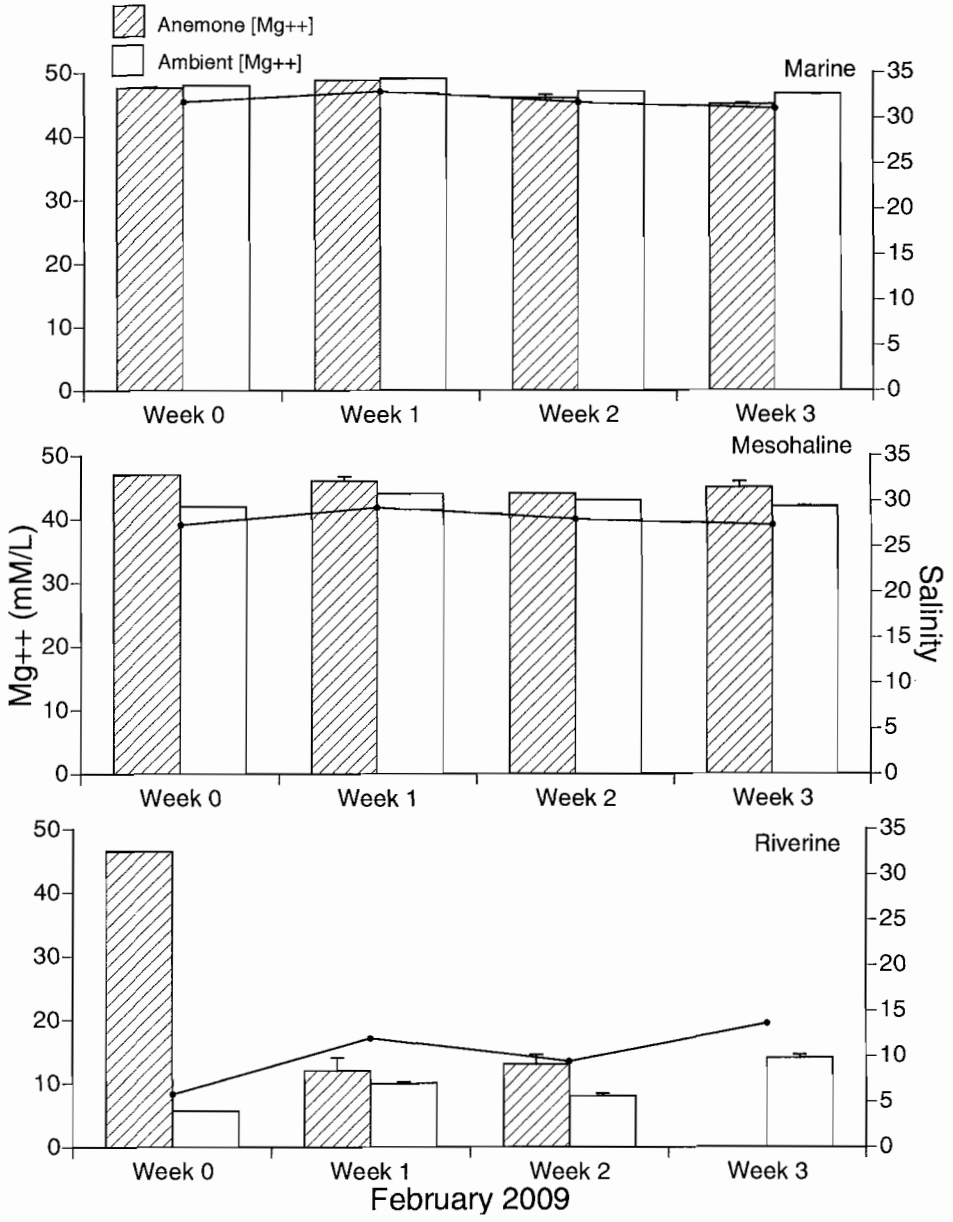

Figure 14. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaloine and riverine sites during February 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

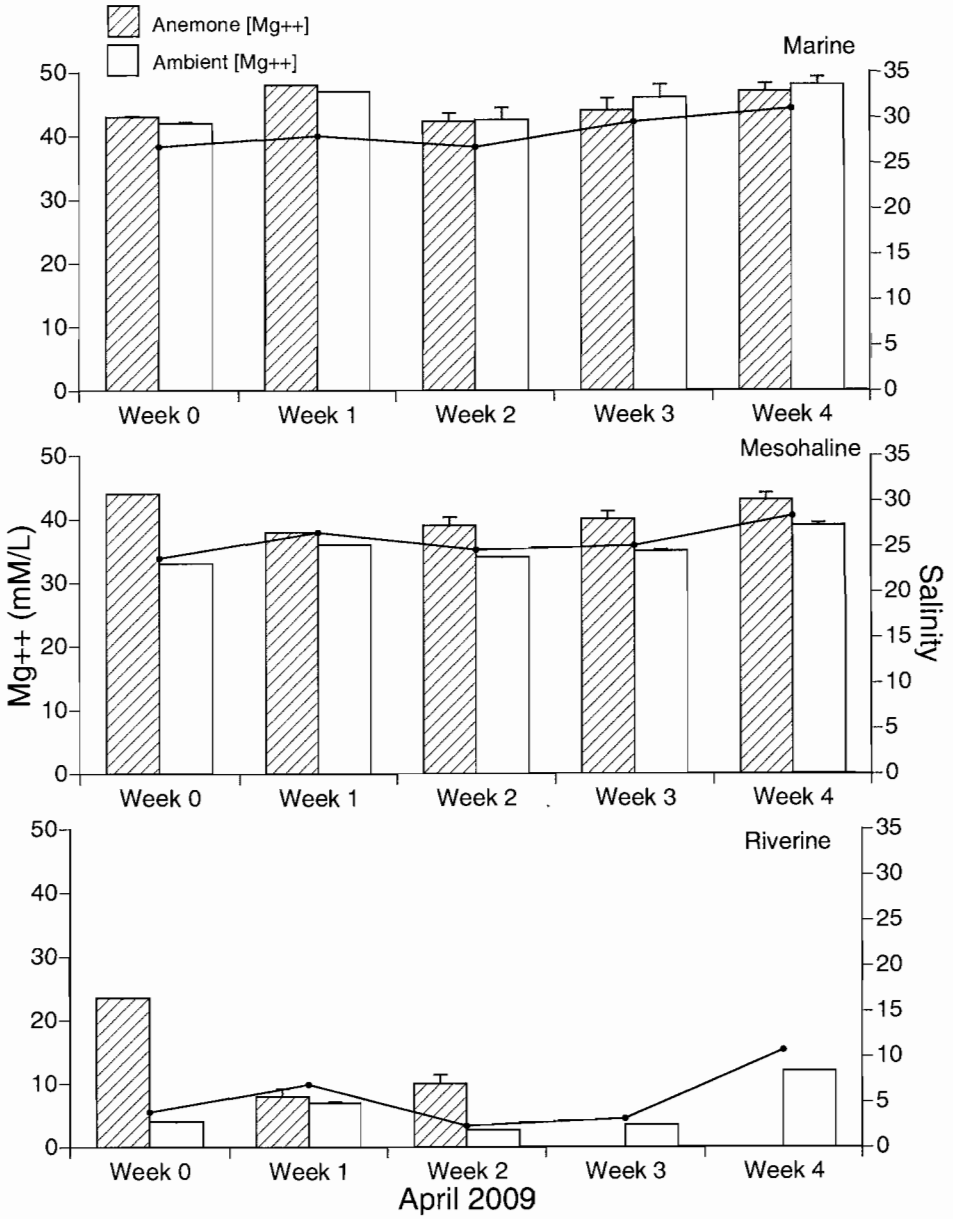

Figure 15. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaline and riverine sites during April 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

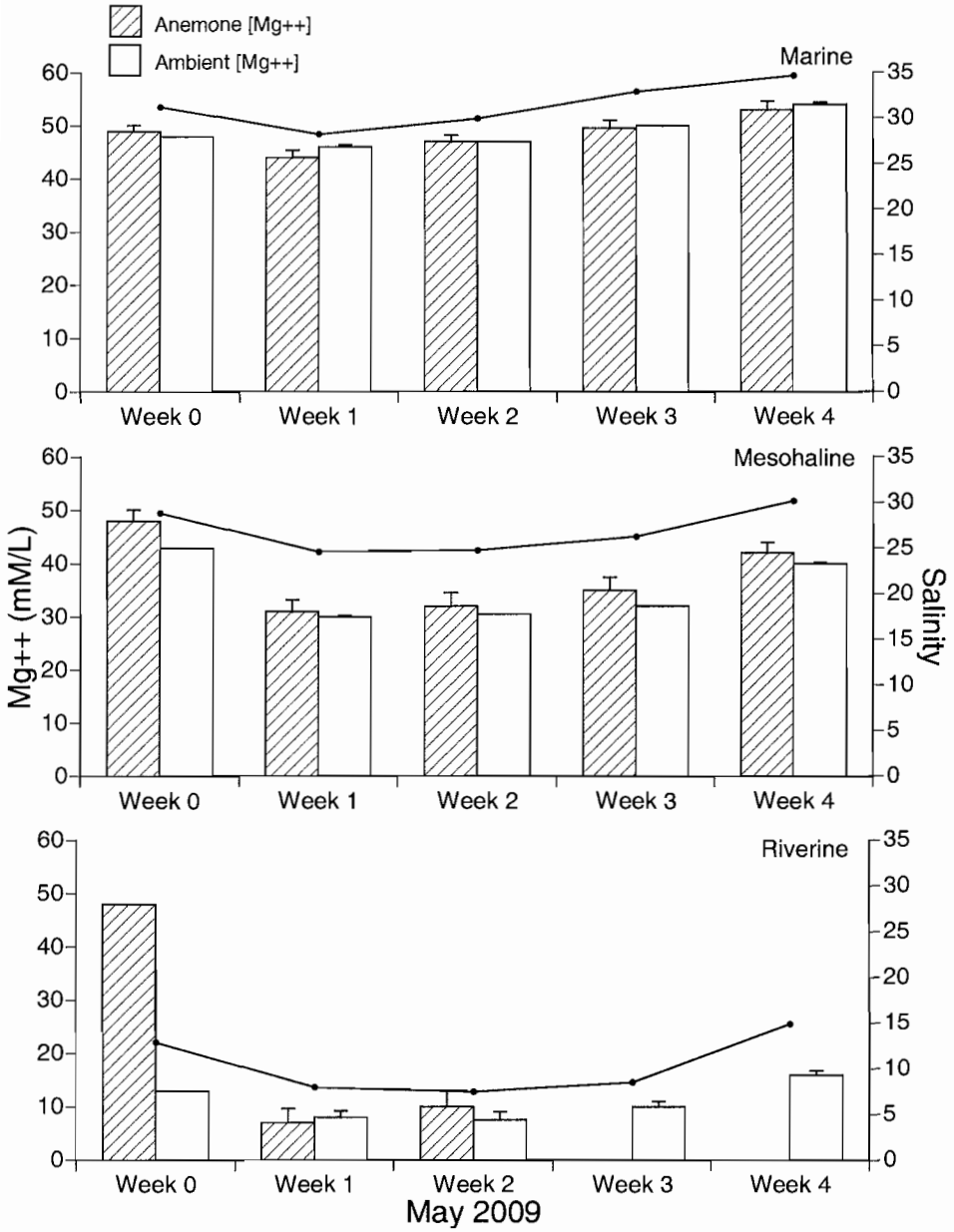

Figure 16. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaline and riverine sites during May 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

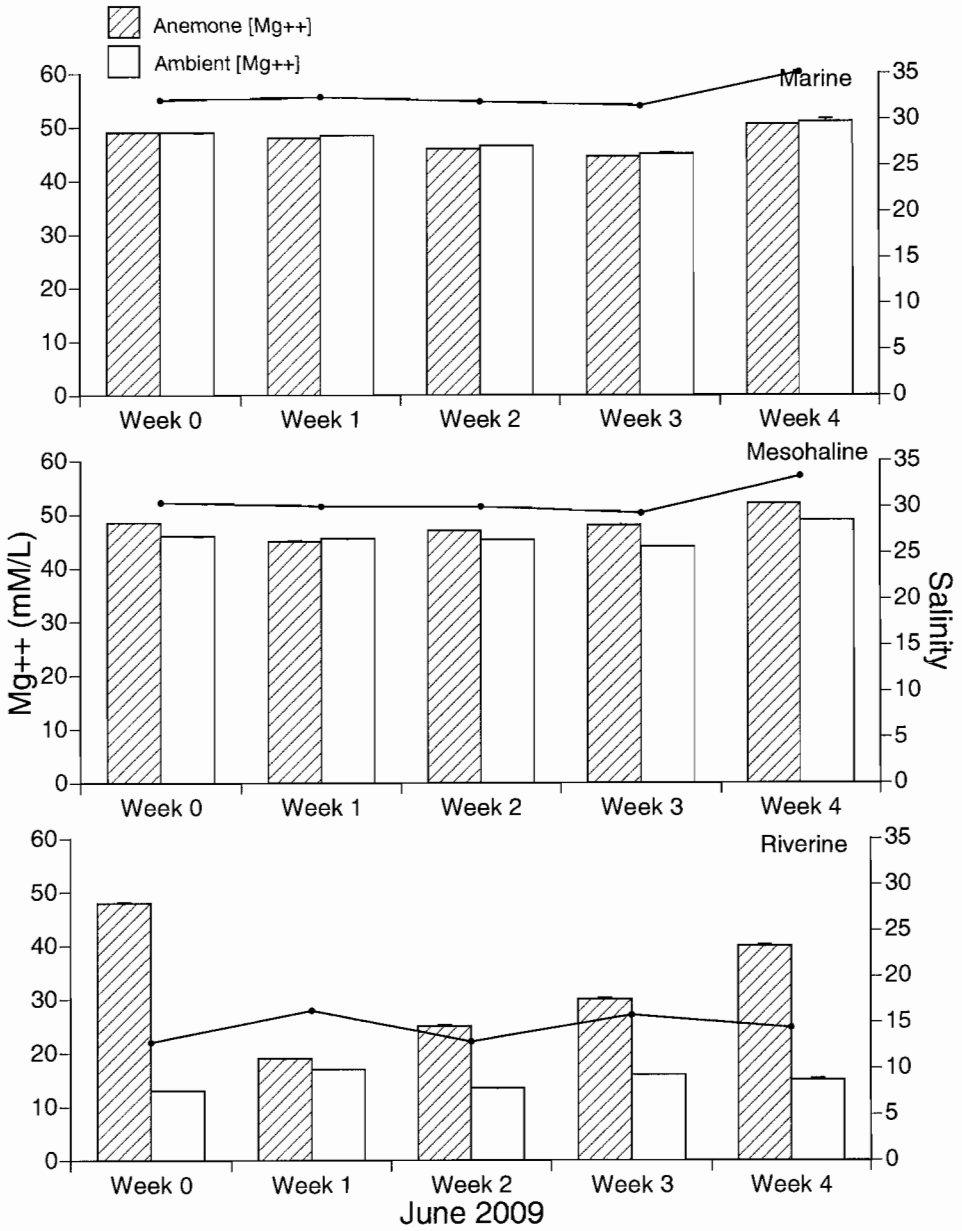

Figure 17. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaline and riverine sites during June 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

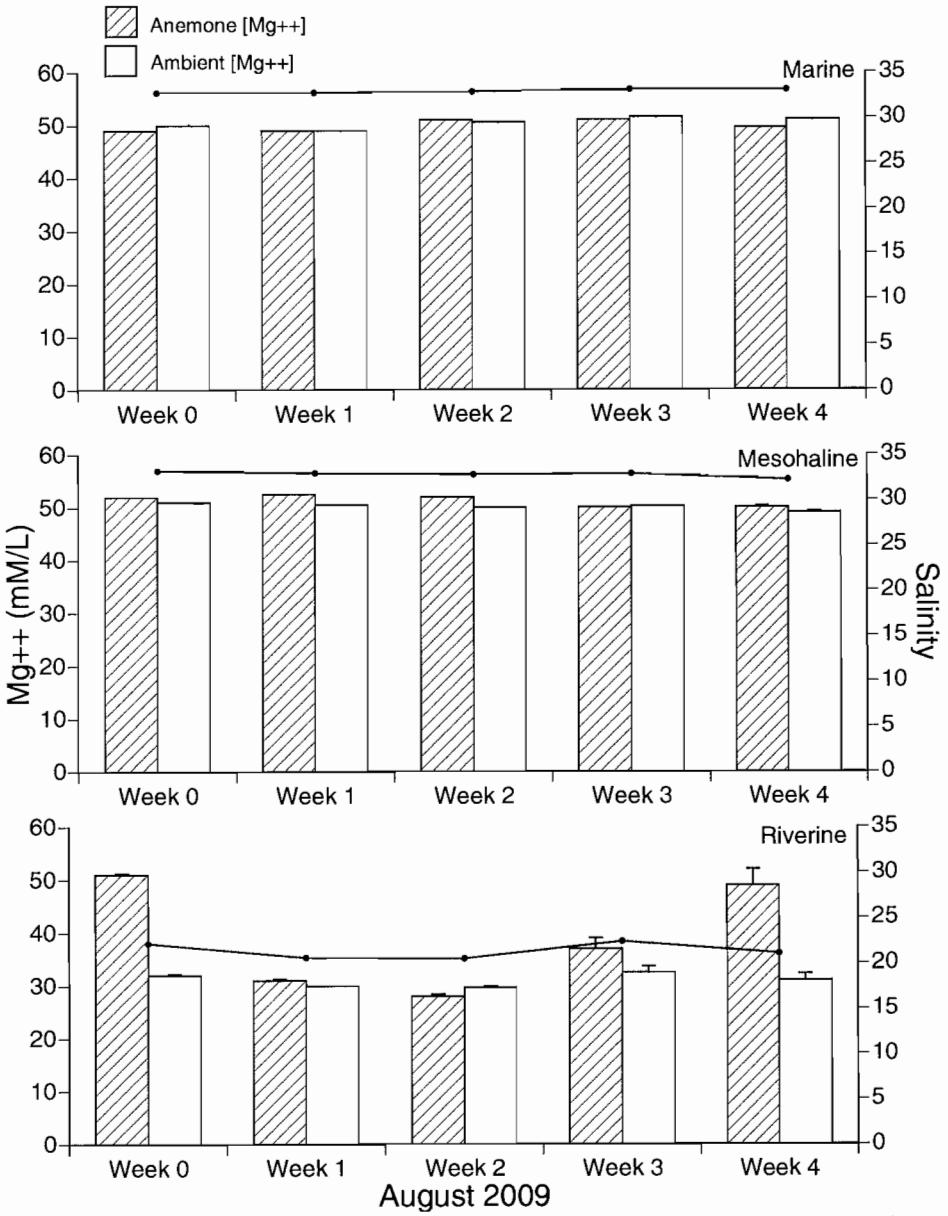

Figure 18. Tissue magnesium ion concentrations of *Metridium senile* placed at marine, mesohaline and riverine sites during August 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

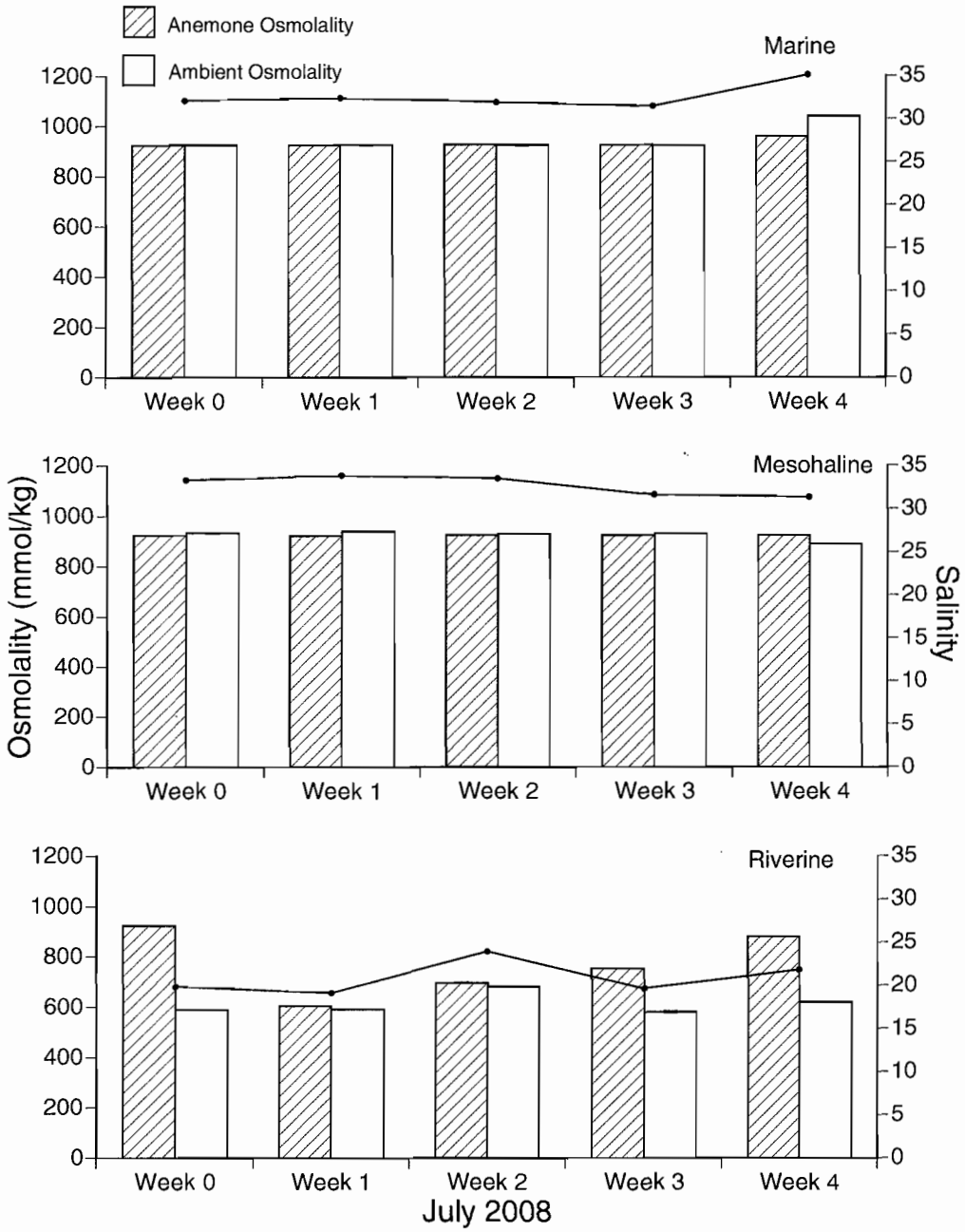

Figure 19. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine field sites during July 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

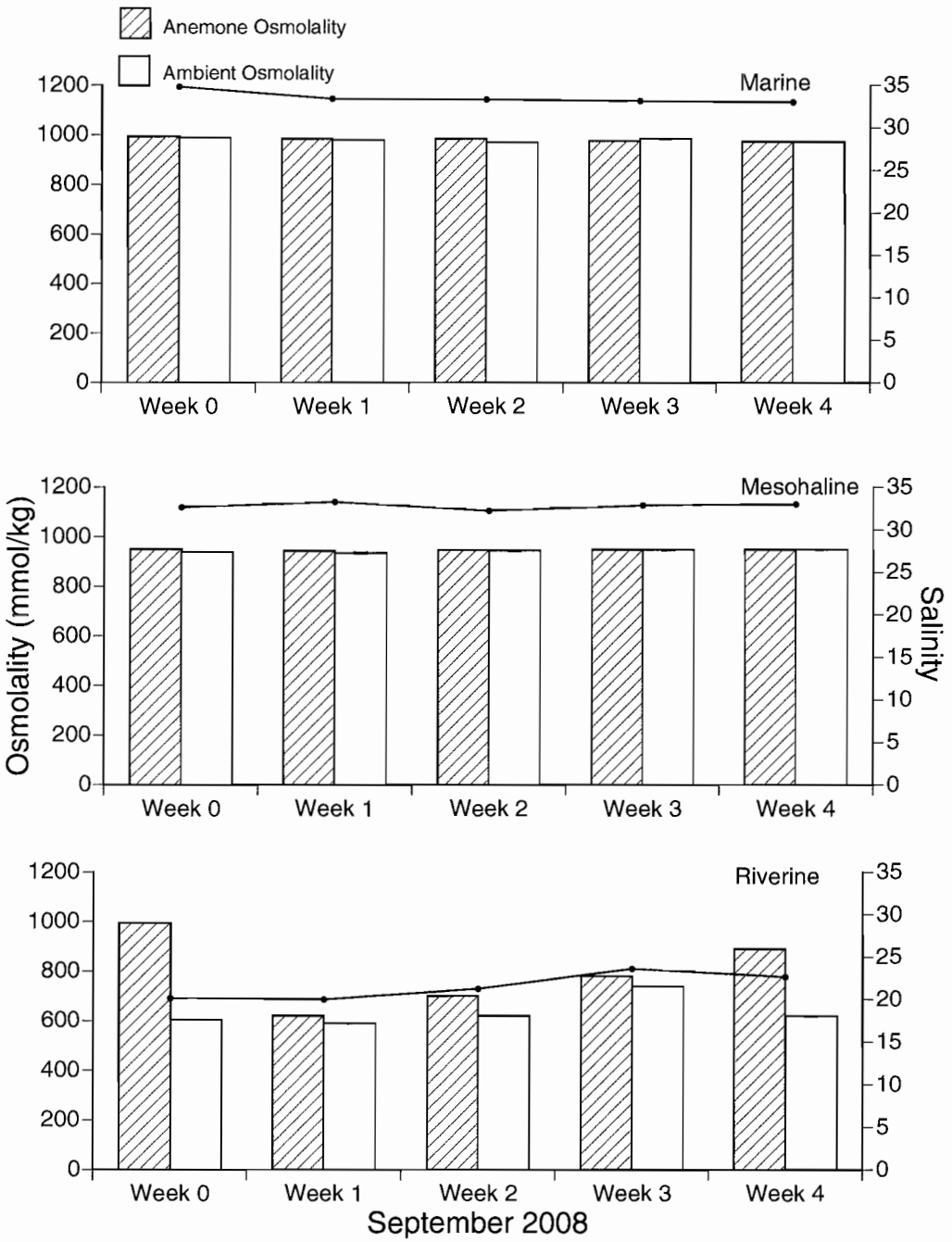

Figure 20. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine sites during September 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

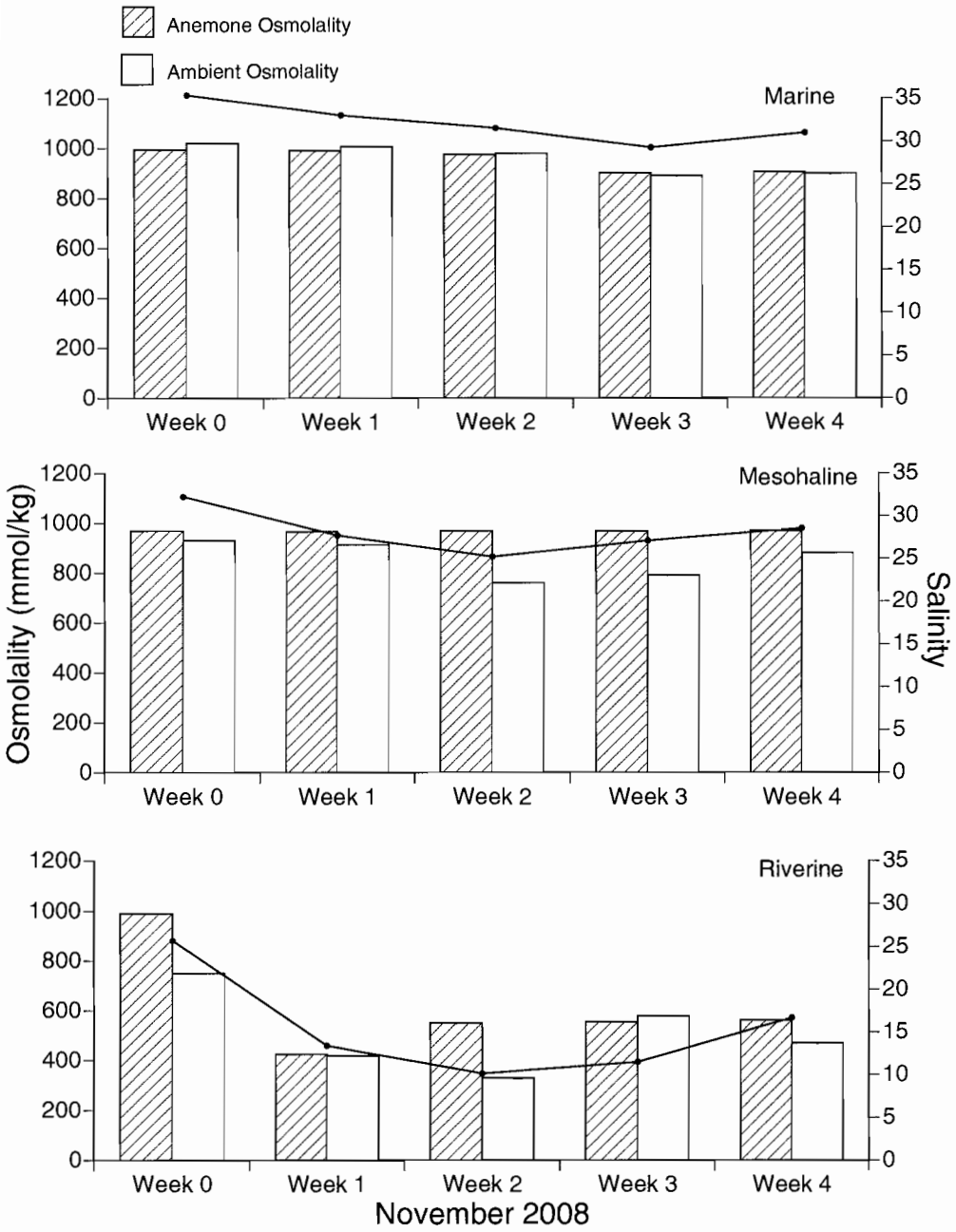

Figure 21. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine sites during November 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

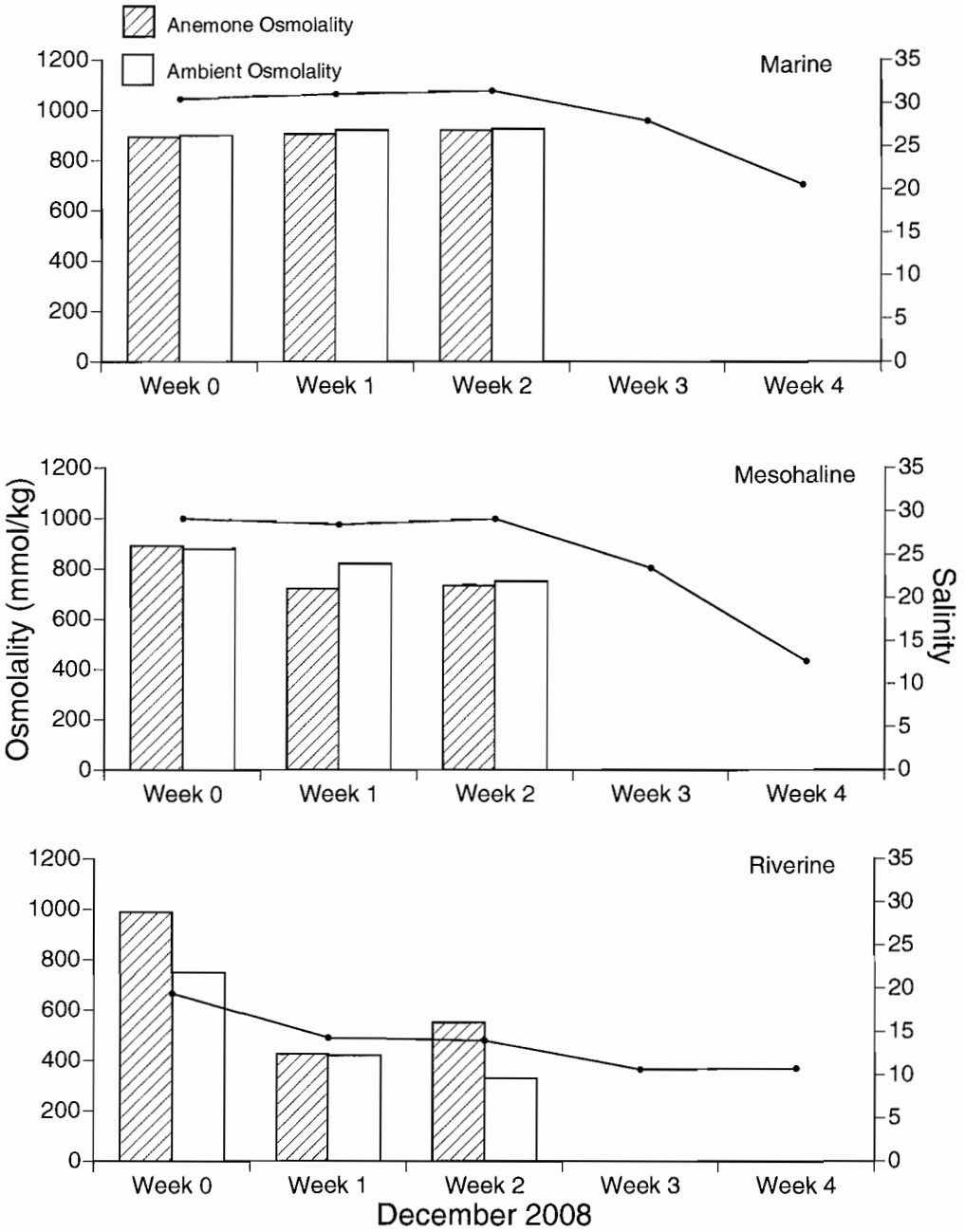

Figure 22. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine sites during December 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

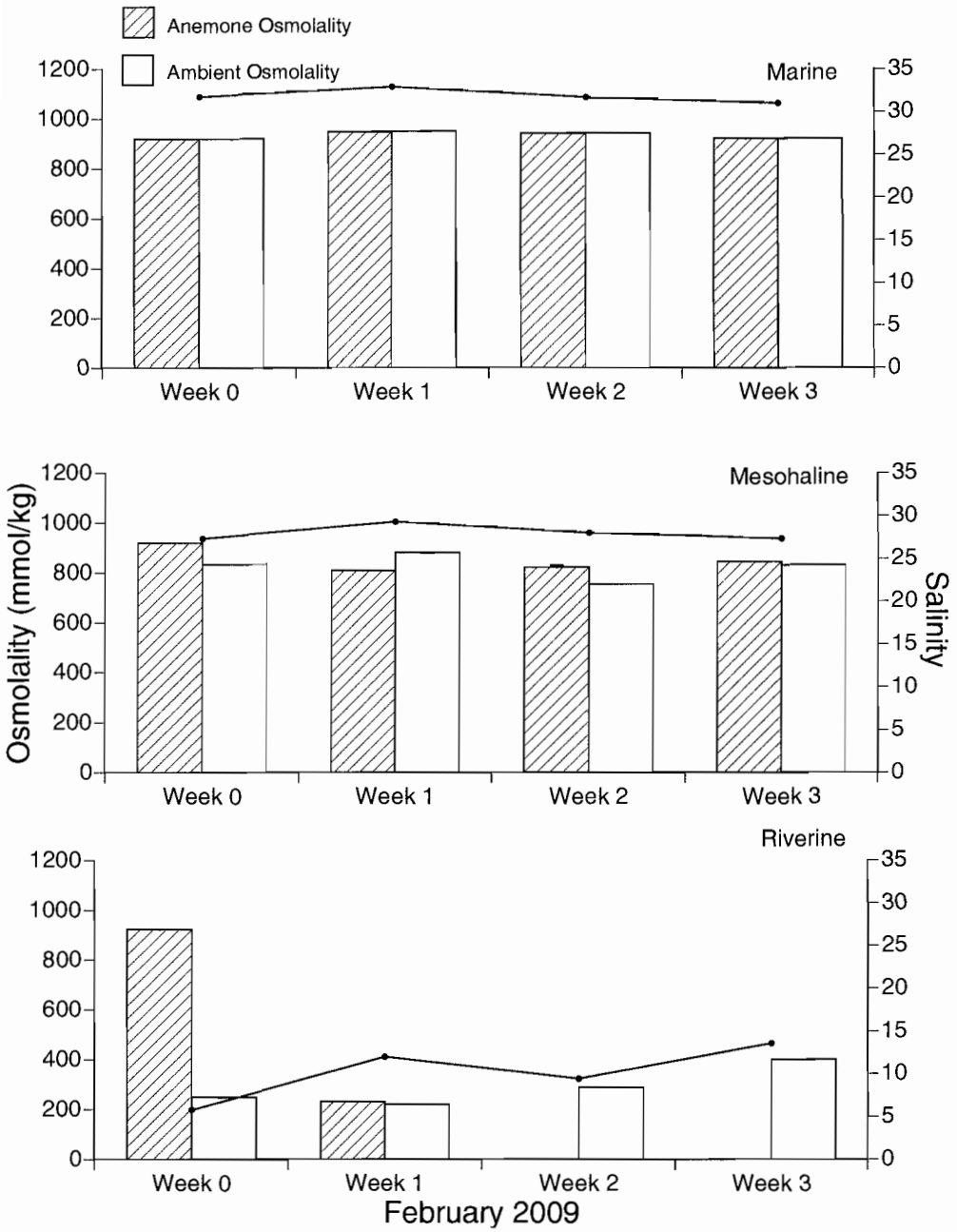

Figure 23. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine sites during February 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

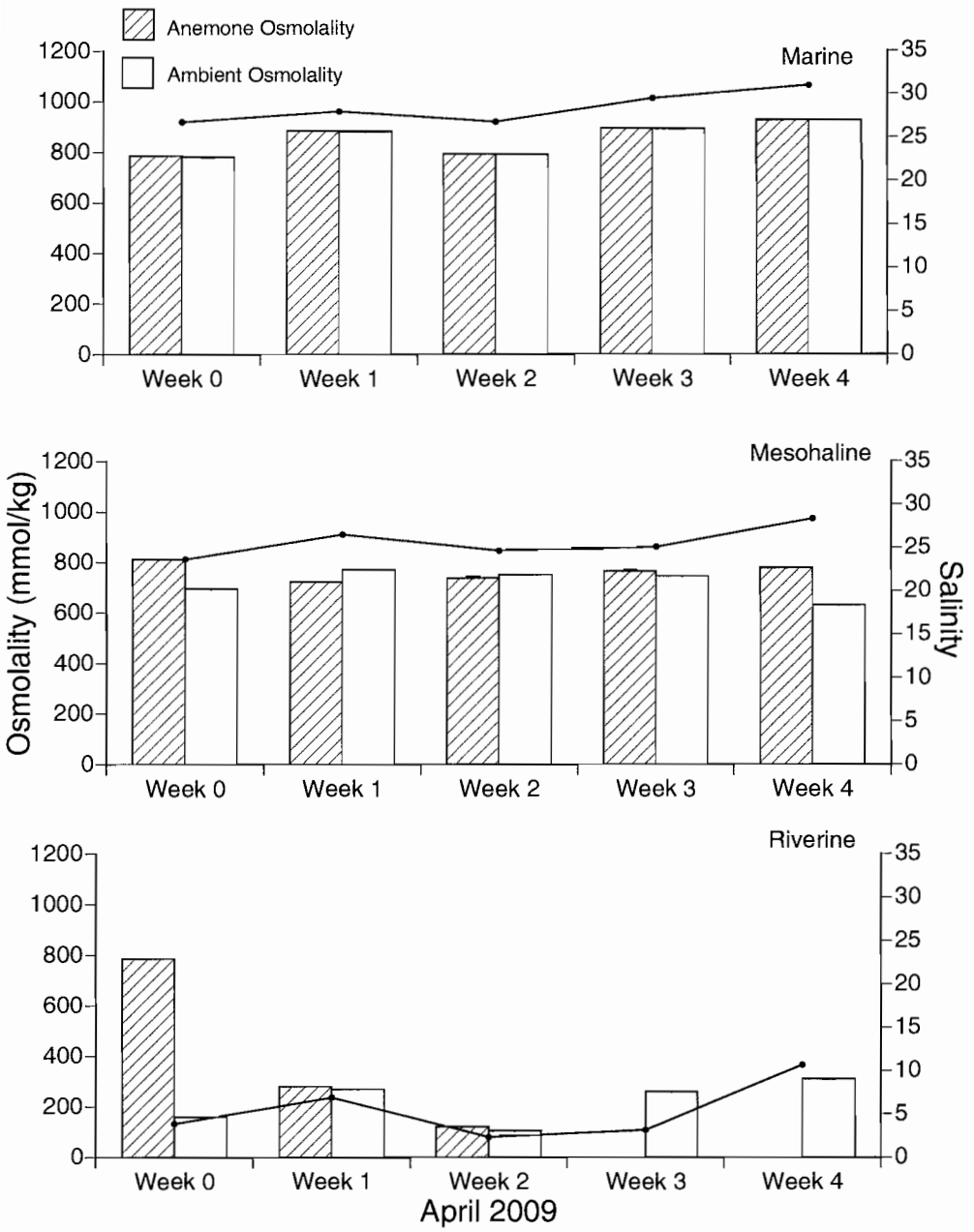

Figure 24. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine sites during April 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

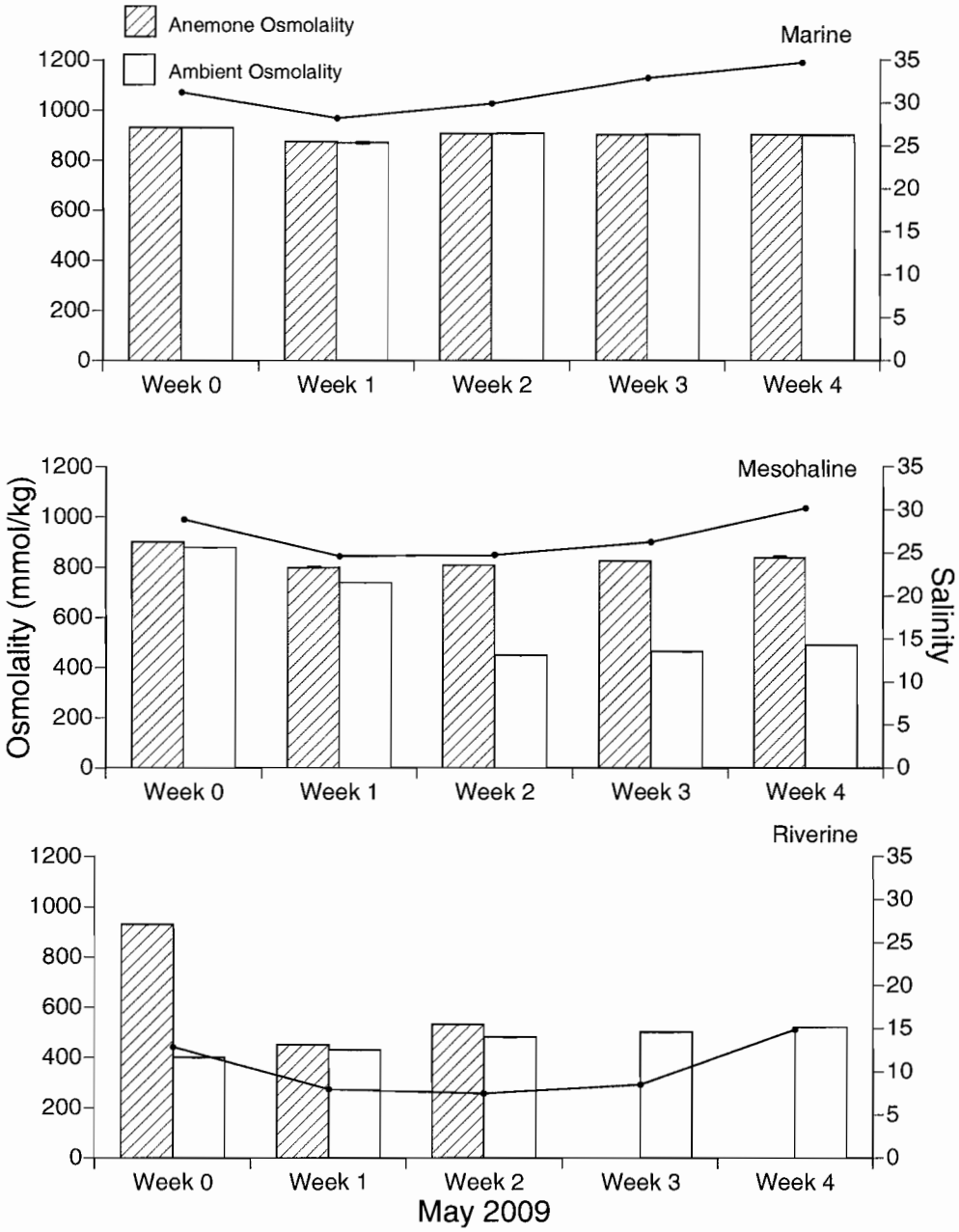

Figure 25. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine sites during May 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

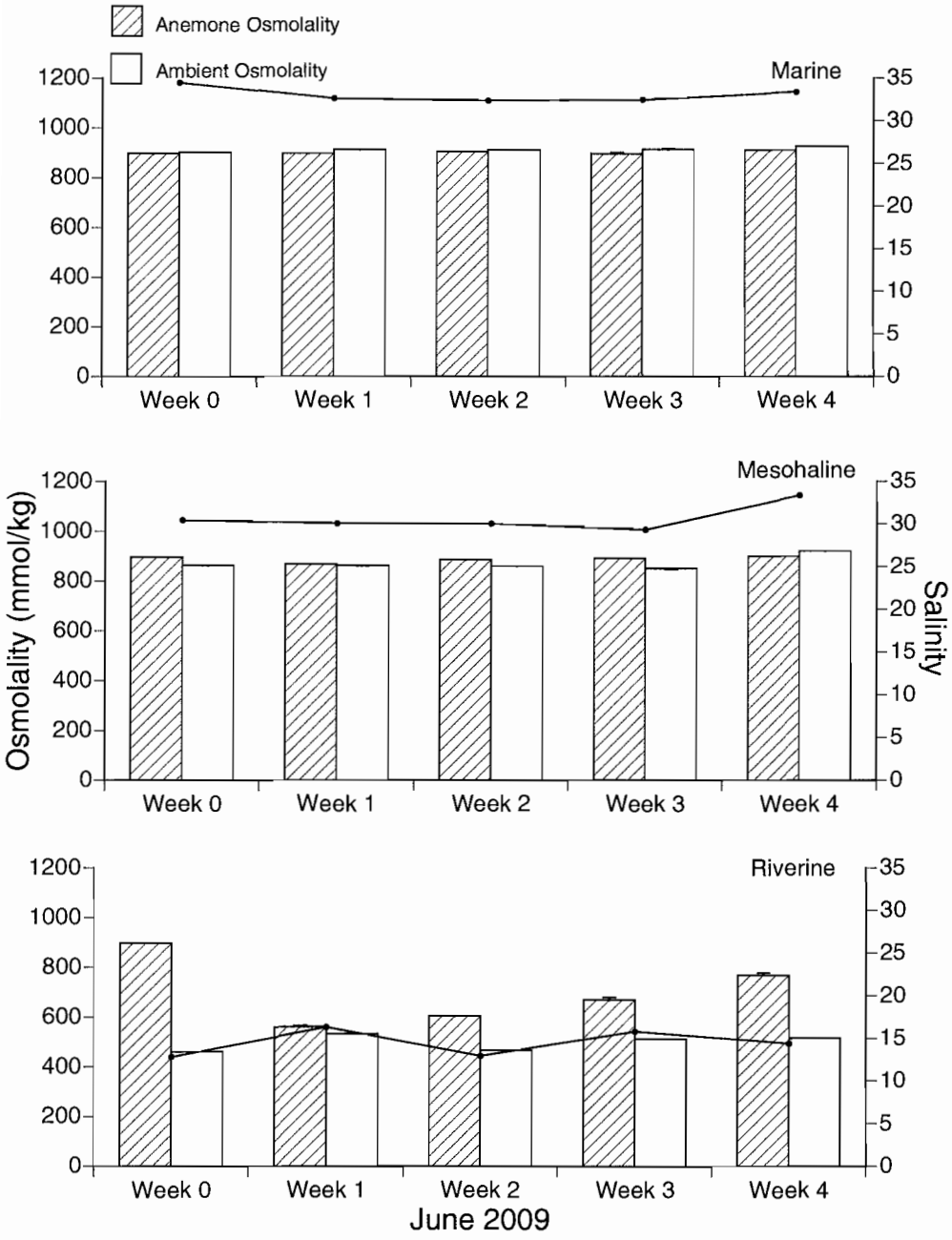

Figure 26. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine sites during June 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

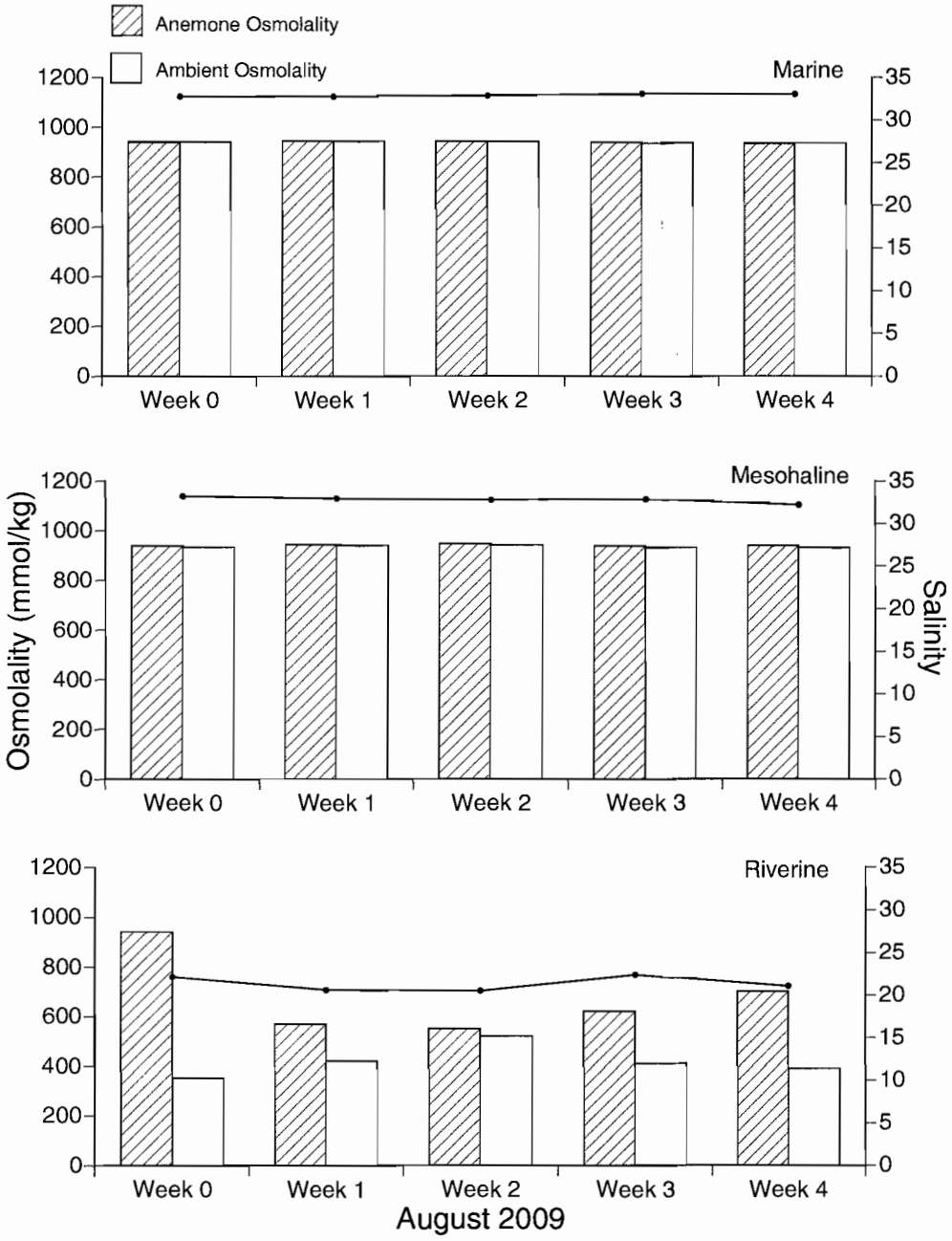

Figure 27. Tissue osmolality of *Metridium senile* placed at marine, mesohaline and riverine field sites during August 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites. Bars represent means with standard error (n=3).

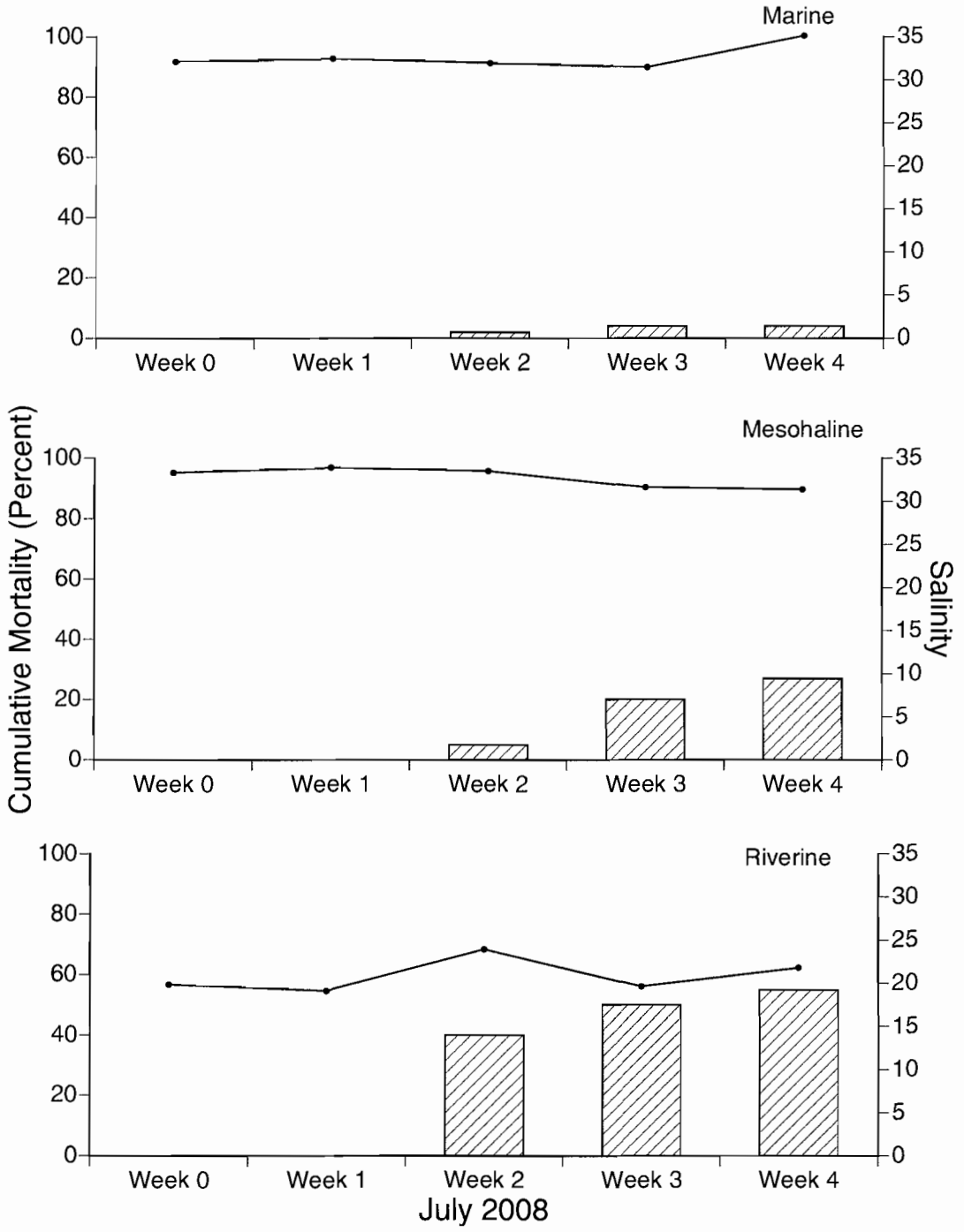

Figure 28. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during July 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

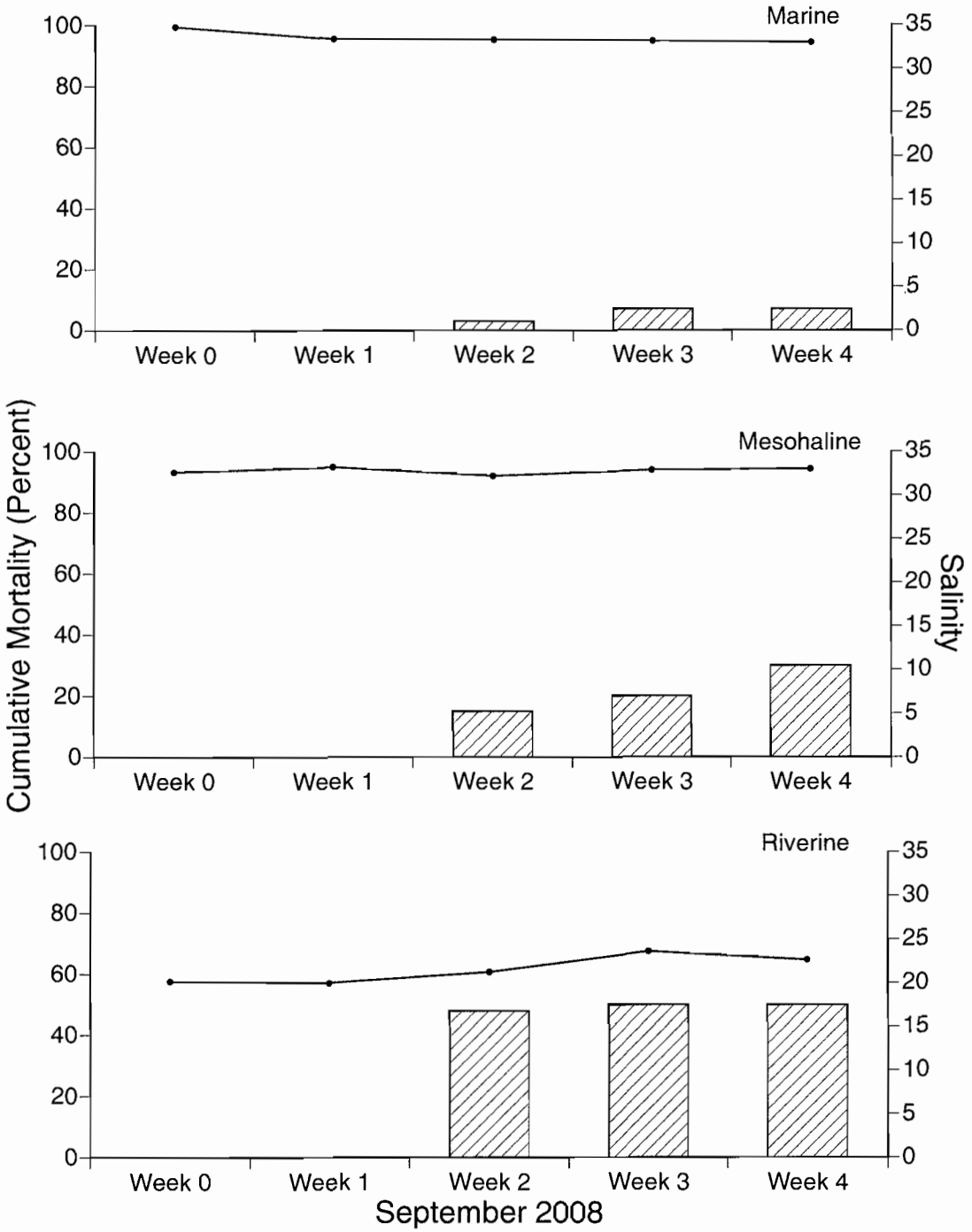

Figure 29. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during September 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

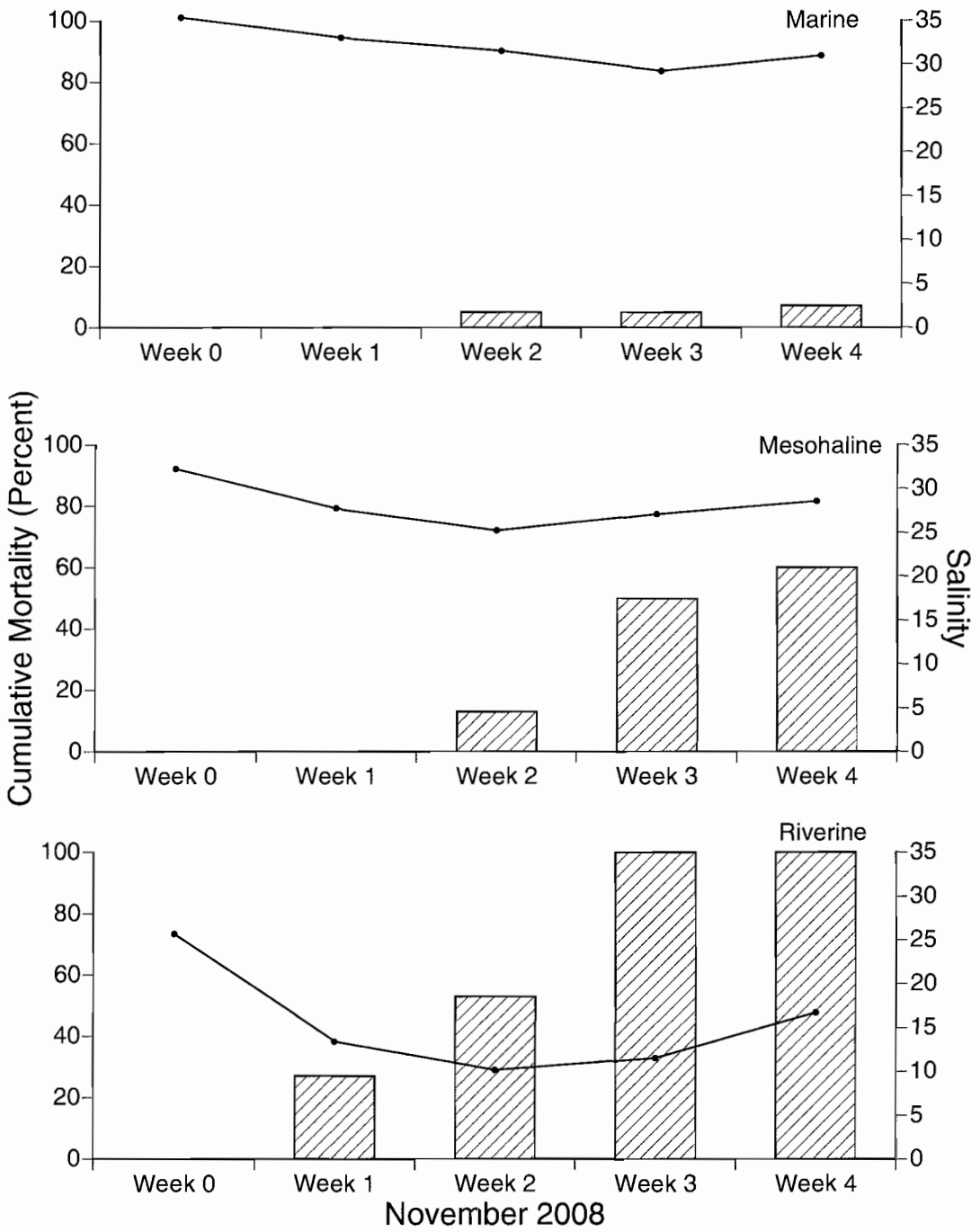

Figure 30. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during November 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

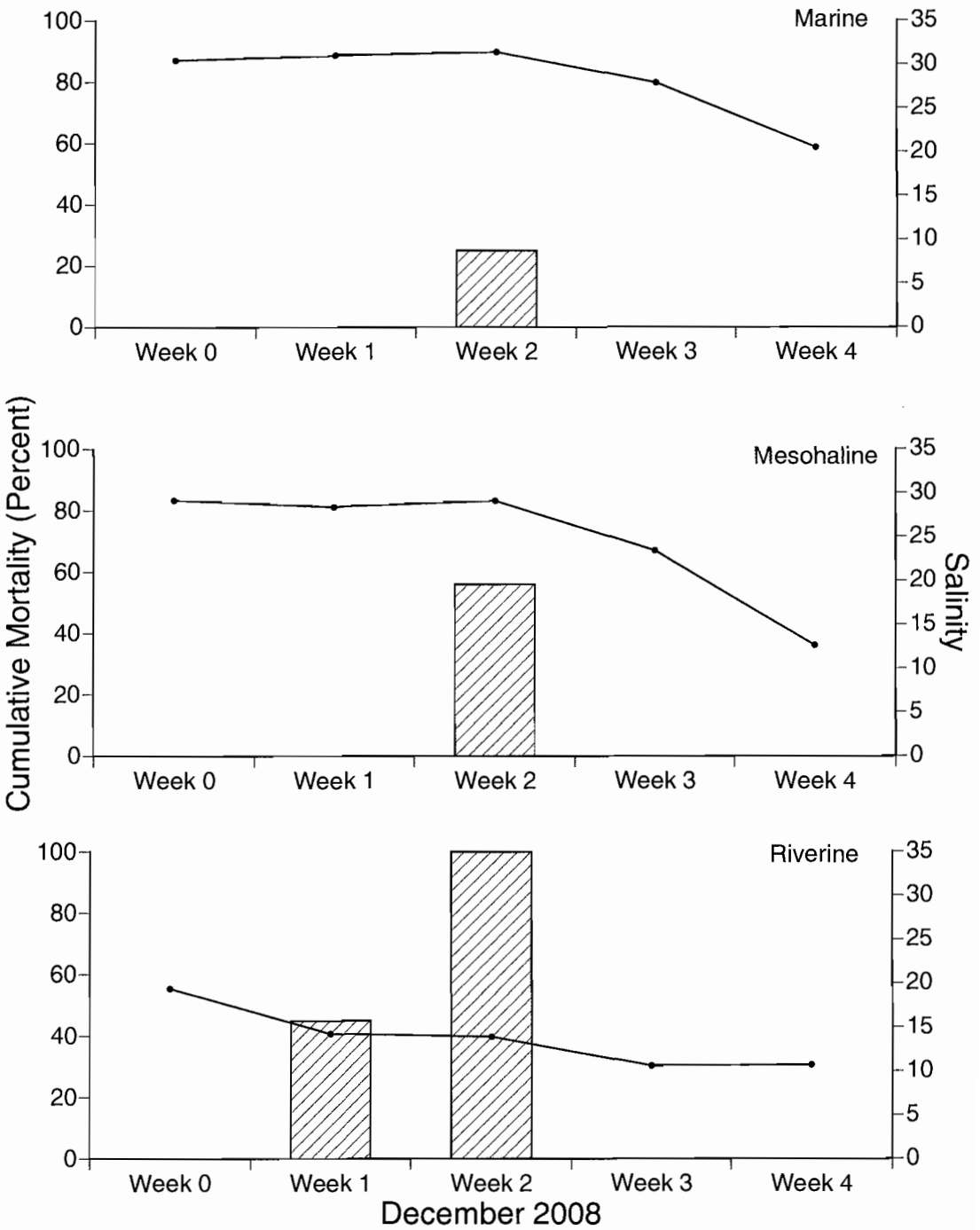

Figure 31. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during December 2008. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

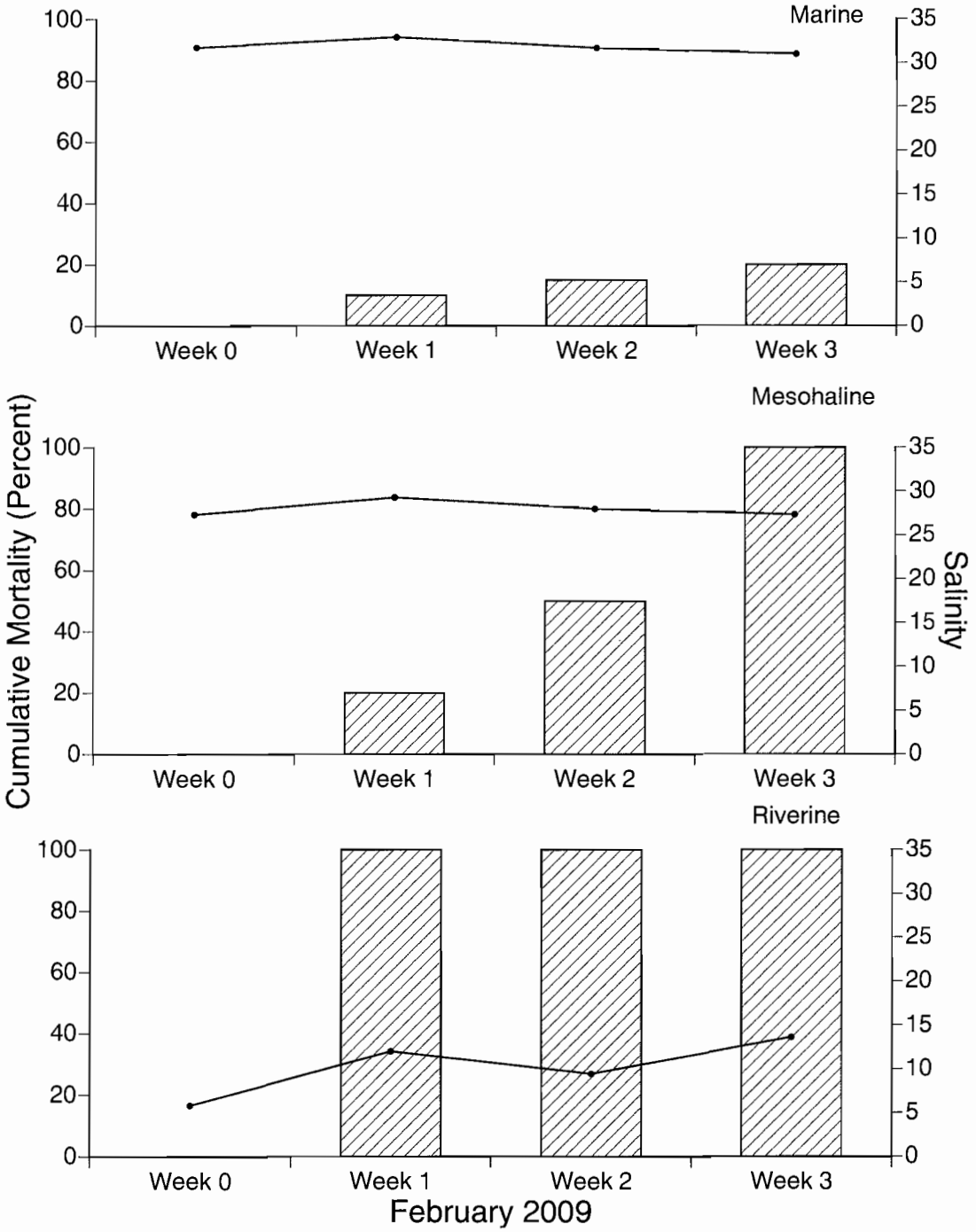

Figure 32. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during February 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

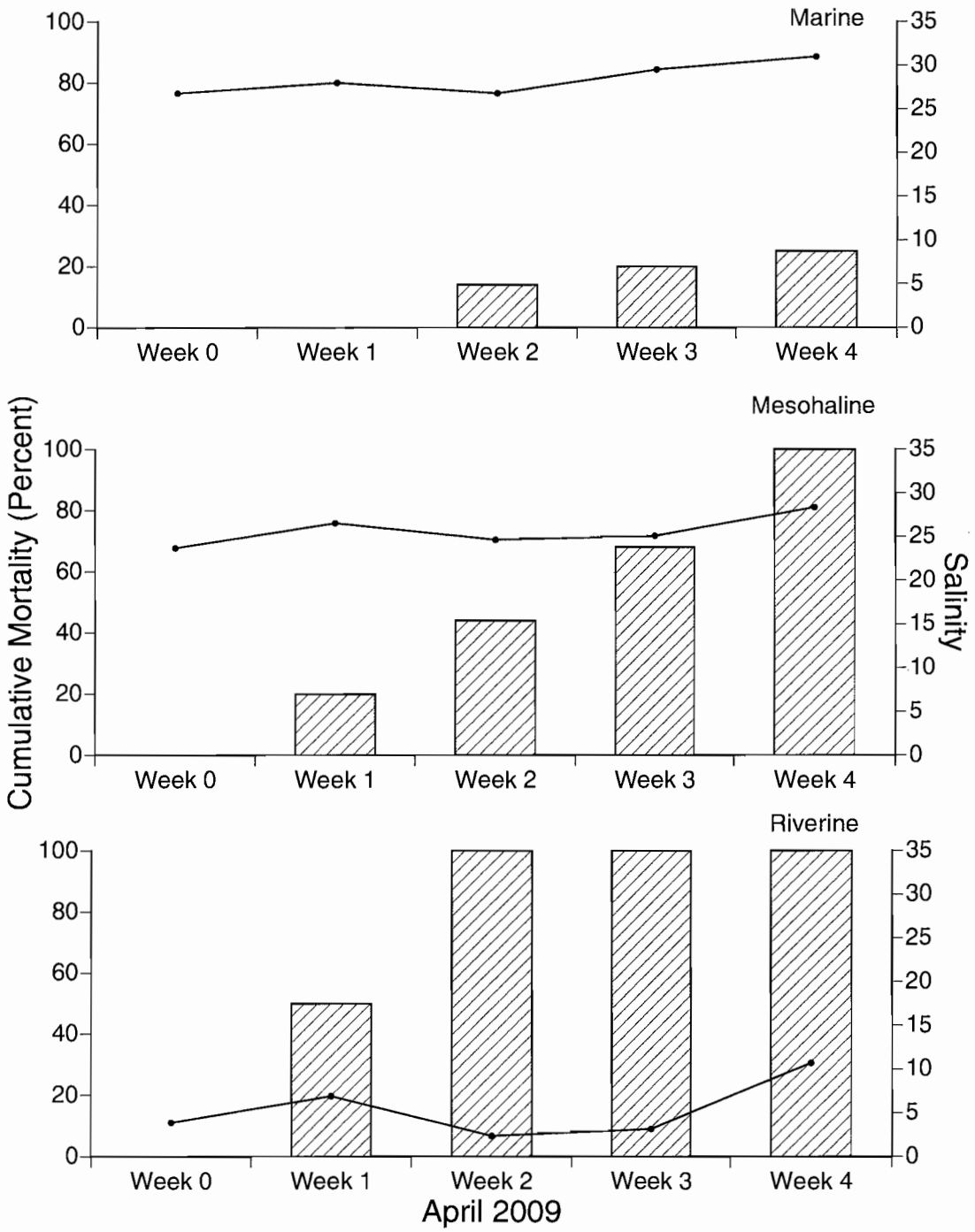

Figure 33. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during April 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

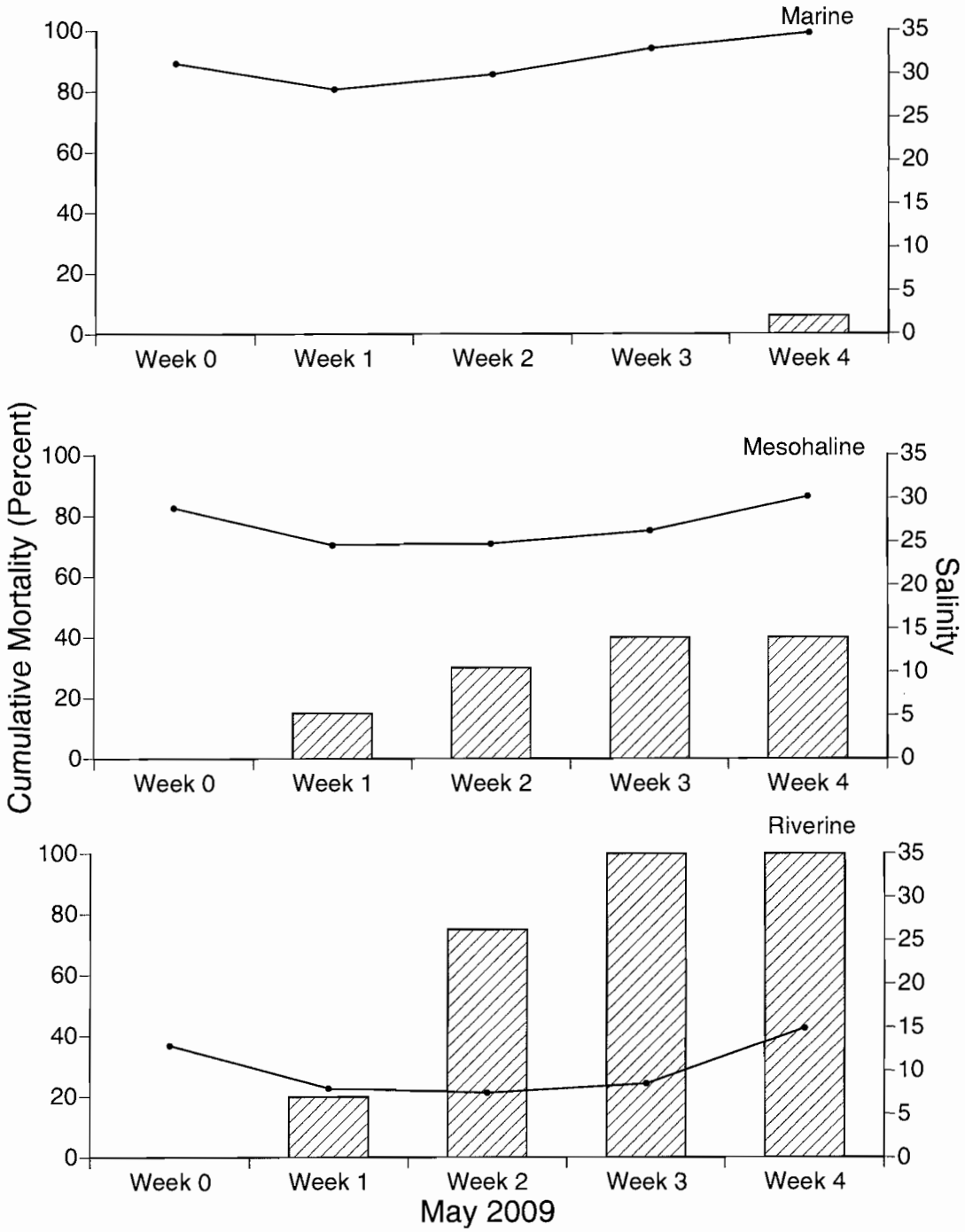

Figure 34. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during May 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

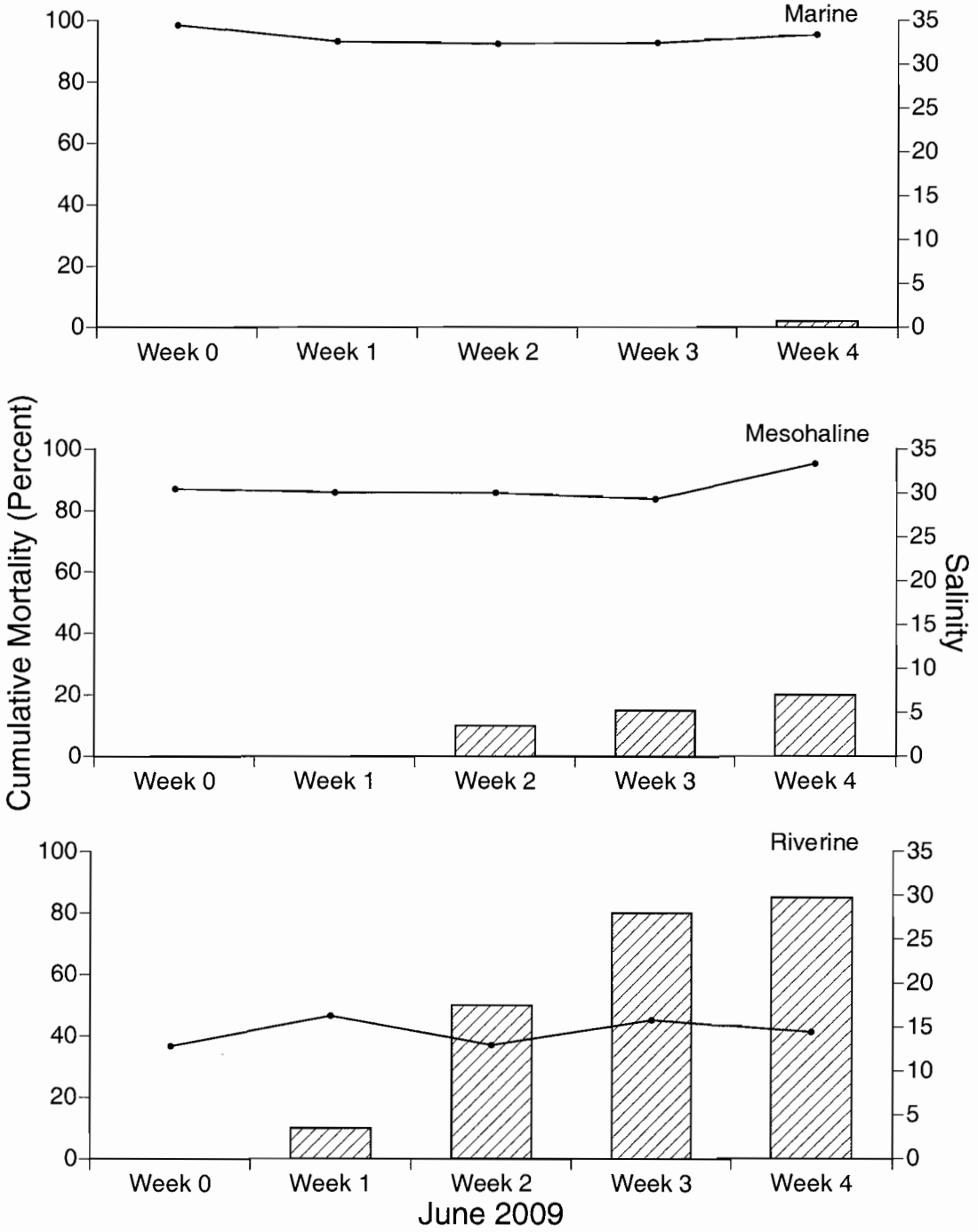

Figure 35. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during June 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

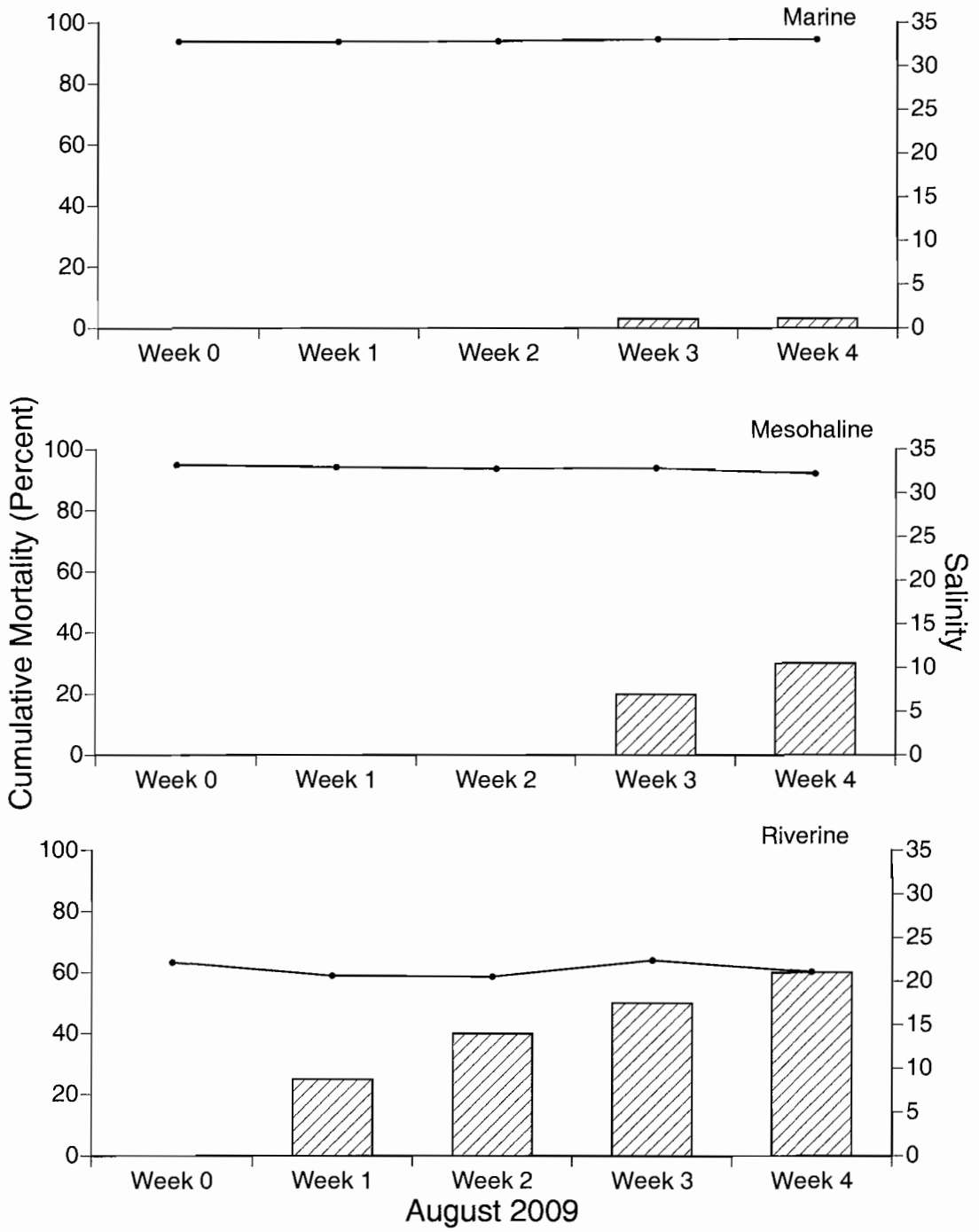

Figure 36. Cumulative weekly mortality of adult transplants at marine, mesohaline and riverine sites during August 2009. Line overlays represent changes in average salinity of each weekly sample day at respective field sites.

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