



Water for a Healthy Country

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Relations between water chemistry, otolith chemistry and salinity of a hypersaline system: implications for determining past environmental history of fish

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Foreword

The environmental assets of the Coorong, Lower Lakes and Murray Mouth (CLLAMM) region in South Australia are currently under threat as a result of ongoing changes in the hydrological regime of the River Murray, at the end of the Murray-Darling Basin. While a number of initiatives are underway to halt or reverse this environmental decline, rehabilitation efforts are hampered by the lack of knowledge about the links between flows and ecological responses in the system.

The CLLAMM program is a collaborative research effort that aims to produce a decision-support framework for environmental flow management for the CLLAMM region. This involves research to understand the links between the key ecosystem drivers for the region (such as water level and salinity) and key ecological processes (generation of bird habitat, fish recruitment, etc). A second step involves the development of tools to predict how ecological communities will respond to manipulations of the “management levers” for environmental flows in the region. These levers include flow releases from upstream reservoirs, the Lower Lakes barrages, and the Upper South-East Drainage scheme, and dredging of the Murray Mouth. The framework aims to evaluate the environmental trade-offs for different scenarios of manipulation of management levers, as well as different future climate scenarios for the Murray-Darling Basin.

One of the most challenging tasks in the development of the framework is predicting the response of ecological communities to future changes in environmental conditions in the CLLAMM region. The CLLAMMecology Research Cluster is a partnership between CSIRO, the University of Adelaide, Flinders University and SARDI Aquatic Sciences that is supported through CSIRO’s Flagship Collaboration Fund. CLLAMMecology brings together a range in skills in theoretical and applied ecology with the aim to produce a new generation of ecological response models for the CLLAMM region.

This report is part of a series summarising the output from the CLLAMMecology Research Cluster. Previous reports and additional information about the program can be found at <http://www.csiro.au/partnerships/CLLAMMecologyCluster.html>

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We also acknowledge the contribution of several other funding agencies to the CLLAMM program and the CLLAMMecology Research Cluster, including Land & Water Australia, the Fisheries Research and Development Corporation, SA Water, the Murray Darling Basin Commission's (now the Murray-Darling Basin Authority) Living Murray program and the SA Murray-Darling Basin Natural Resources Management Board. Other research partners include Geoscience Australia, the WA Centre for Water Research, and the Flinders Research Centre for Coastal and Catchment Environments. The objectives of this program have been endorsed by the SA Department of Environment and Heritage, SA Department of Water, Land and Biodiversity Conservation, SA Murray-Darling Basin NRM Board and Murray-Darling Basin Commission.

We acknowledge the assistance of Felicity McGovern in collecting fish and initial analysis of data from May and June 2007 samples, Angus Netting for assistance with LA ICP-MS analyses, and David Short for field work and providing otolith samples. We also acknowledge the useful feedback on a draft manuscript by David Paton and Scotte Wedderburn.

Executive Summary

Fish are a key species within the Coorong region and are likely to move in response to changing environmental conditions. Otolith chemistry provides an excellent natural mark to estimate past environmental history of fish and infer movement patterns but has been largely limited to freshwater and/or marine applications. For proper interpretation, variability in the mark needs to be related to environmental parameters such as water chemistry, temperature or salinity. The aim of this study was to investigate the effect of hypersaline conditions on the water chemistry of the Coorong Lagoon, the terminal estuary of Australia's largest river, and the otolith chemistry of a common fish within the system, the small-mouth hardyhead (*Atherinosoma microstoma*). Water samples and fish were collected from 10 sites along the Coorong, ranging in salinity from 5.8 to 123.4 g/L, on six occasions over 14 months. Water (Ca, Ba, Mg, Mn and Sr) and otolith (Ba:Ca, Sr:Ca, Mg:Ca, Mn:Ca, Na:Ca, Li:Ca, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) concentrations were measured. Water Sr, Mg and Ca concentrations exhibited conservative behaviour (i.e. concentrations increase with salinity), whereas Mn and Ba concentrations exhibited nonconservative behaviour along the salinity gradient. Water Ba showed a steep negative decrease from near freshwater to marine salinities followed by a gradual increase from marine to hypersaline waters, a pattern not previously reported in the literature. Four of the six otolith element:Ca ratios and $\delta^{18}\text{O}$ showed significant patterns with salinity, but the best fit model was often a segmented regression with one or two breakpoints. Positive linear relations were also found between otolith Ba:Ca and water Ba:Ca, as well as otolith Mg:Ca and water Mg:Ca. Results have implications for reconstructing past salinities inhabited by fish since they imply that several elemental and isotopic ratios will be necessary to determine if fish have inhabited or been exposed to hypersaline environments.

1. Introduction

Climate change, although occurring naturally across a range of temporal scales, is now exacerbated by human activities which will result in a variety of physical and chemical changes in estuarine and marine systems (Harley *et al.* 2006). Increased periods of drought, as well as continued regulation of freshwater inputs, is likely to see hypersaline water bodies become more prevalent. In such waters, salinities can reach extremely high levels leading to inverse estuaries where salinity is significantly higher at the head of the estuary than at the mouth. Aquatic organisms are likely to be affected by both fluctuations in salinity, as well as extremes of salinity. In response to these variations they may show changes in distribution and abundance, life history parameters and movement patterns.

Fish migrate in order to optimise life-history stages that may require specific environments, or to take advantage of a condition offered by the different water type/habitat, such as increased food resources or shelter (e.g. Limburg 2001, Secor *et al.* 2001). Traditional approaches to determining fish movement have involved conventional tags (e.g. dart tags) or more recently acoustic and radio tags (Gillanders *et al.* 2003). These methods are limited in both the information obtained and the life stage that can be tracked, suggesting an alternative method is required. Natural variation in elemental and isotopic signatures within the otoliths (ear bones) of fish are ideally suited to answering ecological questions because all fish have a complete record from early life that can be related to the age of the fish; therefore the entire life history can be characterised. The difficulty in using natural signatures to infer movement is relating variability in the signature to an interpretable pattern of movement based on environmental parameters (Elsdon *et al.* 2008).

A major assumption when describing movements of fish based on profiles of otolith signatures is that otolith chemical composition changes with environmental parameters such as water chemistry, temperature or salinity (Elsdon *et al.* 2008). It is frequently assumed that low Sr:Ca corresponds to fresh water and that increases in Sr:Ca correspond to movements to marine waters, however the effect of salinity on otolith chemistry requires careful consideration (Elsdon *et al.* 2008). Various effects of salinity on Sr:Ca (e.g. positive, negative and no relation) have been reported in the literature probably because of underlying differences in water chemistry of freshwater (Kraus and Secor 2004). A negative relation between otolith Ba:Ca and salinity has generally been reported at least up to marine salinities (40 g/L) (Elsdon and Gillanders 2005a, Dorval *et al.* 2007). However, the relation between other elements in the water and salinity requires further consideration.

To date, most studies have focused on freshwater, brackish or marine waters and there has been no comprehensive examination of the relation between water chemistry, salinity and otolith chemistry in hypersaline waters. Several studies have investigated stock structure of fish based on stable isotopes and/or Sr:Ca ratios in otoliths including sites that were slightly hypersaline (with a maximum salinity of between 42.5 and 65 g/L) (e.g. Dufour *et al.* 1998, Edmonds *et al.* 1999, Bastow *et al.* 2002). A single study has investigated the relation between Sr:Ca ratios of otoliths and salinity for fish collected from Saloum estuary (Senegal, West Africa) where salinities ranged from 32 to 58 g/L during the rainy season and from 40 to 100 g/L during the dry season (Diouf *et al.* 2006). Sr concentration in the water and its relation to salinity was also investigated within the same estuary but samples were collected two years after the fish; therefore, no direct comparison could be made between otolith Sr:Ca and water Sr (Diouf *et al.* 2006). No studies (that we are aware of) have investigated the relation between otolith chemistry, water chemistry and salinity of hypersaline waters for elements other than Sr.

Hypersaline waters where evaporation rates are not offset by freshwater input due to low rainfall and low river run-off, as well as limited mixing with oceanic waters may not show the same patterns as those found for brackish and marine waters. In particular, increases in the concentration of elements and of the heavier isotopes in evaporated water are likely (Hoeffs 1997). The objective of this study was to investigate the effect of (hyper)-salinity on the water chemistry of the Coorong Lagoon (South Australia) and the otolith chemistry of the most

numerous fish species within the system, the small-mouth hardyhead (*Atherinostoma microstoma*).

2. Methods

2.1. Study species

The small-mouth hardyhead, *Atherinosoma microstoma* (Atherinidae) is a small (<110 mm total length), short lived (~1 year) fish species that is found in shallow coastal bays, estuaries and lakes and is endemic to Australia. It feeds on plankton, and is a major food source to piscivorous fish and birds. Small-mouth hardyhead is one of the most salt-tolerant fish species worldwide. Laboratory salinity tolerances of 3.3-108 (lower-upper LD₅₀) g/L have been previously reported (Lui 1969).

2.2. Sampling site

The Coorong is a reverse estuary situated in the south-east of South Australia (Figure 1) that currently has a salinity gradient that runs from low in the north (around seawater) to extremely hypersaline in the south (148 g/L recorded at Salt Creek in March 2008). A long (140 km), narrow water body, the Coorong consists of two connected lagoons. Flows to the Coorong come from the Murray and Darling Rivers flowing from the eastern Australian states into Lakes Alexandrina and Albert before entering into the ocean. The Coorong is, therefore, directly impacted by actions throughout the Murray-Darling Basin which is significantly regulated by over 100 structures and a series of barrages which separate freshwater from marine waters close to the river mouth. The Coorong is a RAMSAR wetland of international significance due to the important habitat it provides for migratory water birds.

2.3. Field sampling

Small-mouth hardyhead were collected from the Coorong on six occasions between May 2007 and June 2008 to determine the relation between water chemistry, salinity and trace elements in otoliths. Fish were collected from ten sites along the Coorong, South Australia (Figure 1; Table 1) using a beach seine (61 m long, 2 m drop, 22 m wing mesh and 8 mm bunt mesh). Where possible, ten to twelve small-mouth hardyhead were collected from each site, cold-euthanised and stored on ice. Fish were collected from up to three seines at each site. If the fish were not obtained after three attempts, they were deemed not to be in the area.

Water temperature and salinity were measured at each site using an electronic water quality meter (TPS 90-C). Three water samples were also taken at each site using a 20 mL syringe and filtered through a 45 µm filter, preserved with 0.5 mL nitric acid (70%) and frozen before analysis. All plastic ware was washed with 10% nitric acid for at least 24 hours and rinsed with deionised water several times before use. In addition, water samples were also collected from within Lake Alexandrina at four times (exceptions May 2007 and June 2008) to provide brackish water samples.

2.4. Water analysis

Water samples were analysed for Ca concentration using a Perkin Elmer 3000 DV (Dual View) Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES), and for Ba, Mg, Mn and Sr using a Perkin Elmer Elan 6000 DRC (Dynamic Reaction Cell) Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Lutetium and indium were used as internal standards to correct for ICP-AES and ICP-MS drift respectively. During the analysis, blanks (prepared in the same

manner as the samples but with deionised water rather than seawater) and standard solutions were analysed every 10 to 15 samples and used to correct for background concentrations, assess accuracy and precision of samples and calculate concentrations in samples. The analytical accuracy of elements averaged across all samples was 94% (Ba), 98% (Ca), 99% (Mg), and 93% (Mn and Sr). The mean precision estimated from duplicate samples was less than 1.7% for all elements except Ba which was 5.9%. All water samples were analysed by National Measurement Institute, Pymble, NSW.

2.5. Otolith preparation and analysis

All fish were measured (total length, TL) and ranged in length from 34 to 92 mm TL (Table 1). The sagittal otoliths were extracted using fine forceps, and cleaned with ultra pure water, ensuring no tissue remained. Otoliths were then placed in Eppendorf tubes and allowed to dry in a laminar flow cabinet for 24 hours.

Otoliths were embedded in indium-spiked epoxy-resin (Struers) and sectioned transversely through the nucleus using a low-speed Isomet saw (Buehler Ltd). The sections were then polished to a thickness of 200 to 250 μm with 9- μm aluminium oxide lapping film followed by 3- μm lapping film. Sections were rinsed clean with ultra pure water between polishing stages and then dried in a laminar flow cabinet for 24 hours. Twelve random sections were mounted onto glass microscope slides using an indium-spiked Crystal Bond 509. Sample slides were then cleaned in an ultrasonic water bath for five minutes to remove any surface impurities, dried in a laminar flow hood for 24 hours and stored individually in sealed plastic bags until analysed.

The outside edge of all otoliths (~ 20 μm from the edge) was analysed with a 30- μm diameter beam using a Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICPMS). The edge of otoliths represents their most recent growth and is assumed to represent environmental conditions near where they were captured. Operating conditions of the laser and mass spectrometer are provided in Table 2. NIST 612 was used as a reference standard and was analysed at the beginning and end of each session, and was used in conjunction with a snapper standard to calibrate the mass spectrometer. NIST 612 samples were also analysed periodically throughout each session to correct for instrument drift. All analyses involved collecting background data for 30 seconds, followed by collection of otolith data for 70 seconds.

Mass count data were converted to concentrations (ppm) using Glitter software (<http://www.glitter-gemoc.com/>). Elemental concentrations were then converted to molar concentrations and standardised to Ca. All further data analyses were carried out on the element:Ca data.

For one sampling time (June 2007, when fish were obtained from all sampling sites), carbonate samples were milled from each otolith using a high-resolution, computerised, micromilling machine (New Wave Research MicroMill). Material was milled from the edge of the otolith, tapped onto a sheet of wax paper and transferred into a small, clean, metallic vial. After collection of each sample, the drill bit, wax paper and work surfaces were blown clear of remnant powder with compressed air. All samples were baked for ~ 4 hours at 80°C to remove any potential volatiles prior to stable isotope analysis. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotope data were acquired simultaneously on a Fisons Optima dual inlet mass spectrometer attached to an Isocarb preparation device at the University of Adelaide. $\delta^{18}\text{O}$ was corrected for equilibrium with H_2O during the reaction, by using the Craig (1957) equation and both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ samples were calibrated to Vienna peedee belemite (VPDB) using an in-house calcite standard (ANU-P3). External error (1σ) based on house standards was better than $\pm 0.05\text{‰}$ for $\delta^{13}\text{C}$ and ± 0.1 for $\delta^{18}\text{O}$. Standards were run in triplicate before each session and once after six consecutive samples. All measurements were reported in the standard δ notation, $\delta^{13}\text{C} = \left[\left(\frac{^{13}\text{C}/^{12}\text{C}}{^{13}\text{C}/^{12}\text{C}} \right)_x - 1 \right] \times 1000$, where x is the sample and s is the standard in per mil (‰).

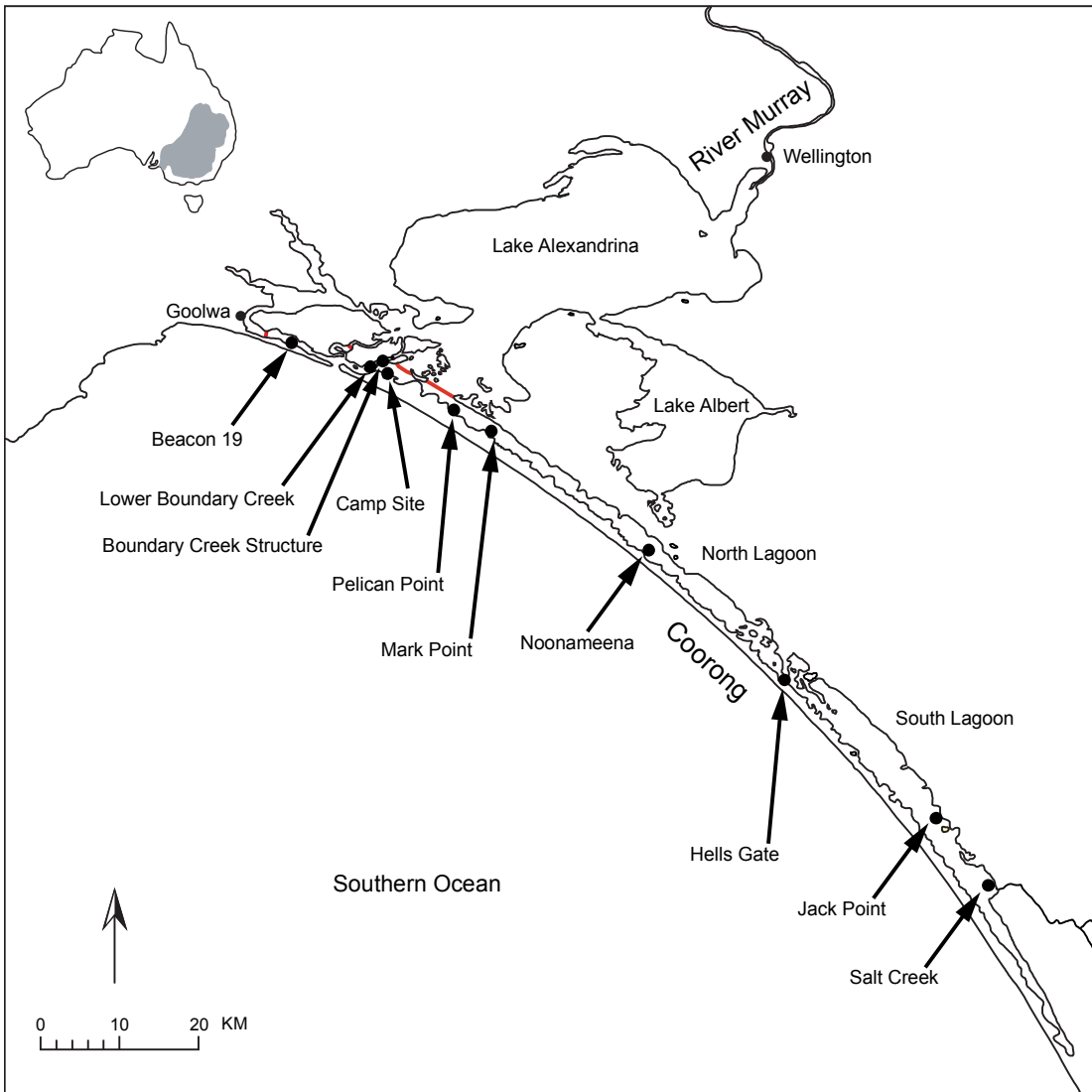


Figure 1. Coorong lagoon showing sampling locations.

Table 1. Summary of locations and sampling times when fish were obtained showing mean total length (\pm SE) and sample sizes (n). Fish were not obtained from some locations during some sampling times. Location names and codes are Beacon 19 (B19), Lower Boundary Creek (LBC), Boundary Creek Structure (BCS), Camp Site (CS), Pelican Point (PP), Mark Point (MP), Noonameena (NM), Hells Gate (HG), Jack Point (JP) and Salt Creek (SC) (see Figure 1).

Location	Time	Total length		n
		(mean \pm SE)		
BCS	May 2007	69.13	4.44	8
LBC		53.86	1.47	7
PP		48.78	2.61	9
MP		56.00	1.27	10
NM		58.50	2.09	12
HG		55.00	2.10	9
JP		45.00	1.54	7
B19	June 2007	54.91	2.38	11
BCS		49.80	2.42	10
LBC		60.25	2.98	8
CS		60.22	2.30	9
PP		52.20	3.00	10
MP		60.88	3.38	8
NM		62.30	5.17	10
HG	September 2007	48.57	2.08	7
JP		41.40	0.95	10
SC		52.00	0.97	6
BCS		54.50	2.96	4
LBC		54.44	3.91	9
CS		48.75	4.01	4
NM		67.70	3.11	10
HG	December 2007	50.50	1.97	10
JP		52.00	3.32	4
SC		52.30	1.50	10
BCS		55.60	1.83	10
LBC		49.25	3.46	8
MP		59.50	2.18	10
B19		March 2008	48.00	
LBC	48.00		3.00	2
NM	52.70		5.14	10
HG	45.00		2.12	10
B19	June 2008	56.40	3.79	5
BCS		58.75	1.92	8
LBC		64.70	2.01	10
NM		64.90	2.42	10

Table 2. Details of operating parameters of the New Wave Nd Yag 213 nm laser and Agilent 7500cs Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) used to analyse otoliths.

Parameter	Description
Laser	
Wavelength	213 nm
Mode	Q-switched
Frequency	5 Hz
Spot size	30 μm
Laser power	65%
Beam density	10-11 J/cm^2
Beam energy	0.07-0.08 mJ
Carrier gas	Ar (0.88-0.9 $\text{L}\cdot\text{min}^{-1}$)
ICP-MS	
Optional gas	He (59%)
Cone	Pt
Dwell times	^7Li , ^{25}Mg , ^{55}Mn , ^{88}Sr , ^{138}Ba : 200 msec ^{23}Na , ^{24}Mg : 100 msec ^{43}Ca , ^{44}Ca : 50 msec
Detection modes	Pulse and analogue

2.6. Statistical analysis

Univariate statistical techniques were used to test hypotheses relating to differences in salinity, as well as otolith Ba:Ca, Sr:Ca, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ along the length of the Coorong. A one factor ANOVA with location as the source of variation was used for salinity and each sampling time was used as the replicates because a single salinity measurement was taken at each site and time. A one factor ANOVA was also used for the otolith isotope data because only one sampling time was investigated. Two factor ANOVAs (location and sampling time, both random factors) were used to determine whether there were differences in otolith Ba:Ca and Sr:Ca among locations and sampling times. Because fish were not obtained from all locations and all sampling times, the analysis focused on four locations and four times (see results). Data were $\log(x + 1)$ transformed prior to analysis.

The relation between water chemistry and salinity, otolith element:Ca ratios or isotope ratios and salinity, and otolith element:Ca ratios and water element:Ca ratios was estimated using regression analyses. A range of models were used including linear, logarithmic, and segmented or broken line regression models with one or two break points (Muggeo 2003). To determine which models produced the best fits given different numbers of parameters, the model with the highest adjusted r^2 was selected. Lines of best fit were only fitted to the data if significant relations were found.

3. Results

Salinity varied significantly along the Coorong ranging from 10.4 g/L (± 3 ; mean \pm SE) at Lake Alexandrina (brackish water site) through to 123.4 g/L (± 6.1) at the southern most location, Salt Creek (Figure 2a, $F_{10,53} = 59.444$, $P < 0.001$). Locations from Beacon 19 through to Mark Point had similar salinity values (38-45 g/L) and were regarded as metahaline. Salinity then increased from Noonameena through to Hells Gate; the latter location had similar salinity values to Jack Point and Salt Creek. Little variability in salinity was seen throughout the year for locations north of Mark Point as indicated by little variation around the mean values (standard errors between 0.7 and 3 g/L), but southern locations had standard errors of between 5 and 10 g/L. Salinity tended to fluctuate depending on wind direction and whether marine or hypersaline waters were being pushed along the lagoon. Temperature showed little spatial variation along the Coorong as indicated by little variation for each temporal data point since the individual locations represented the replicates (Figure 2b). Temperature varied predictably by season with low values in the austral winter of 2007 and 2008 (June) and higher values in the austral summer (December to March) (Figure 2b).

Water samples were collected from salinities ranging from 5.8 g/L (Lake Alexandrina in September 2007) to 156.7 g/L (Jack Point in March 2008). Calcium, Mg and Sr in the water showed linear relations with salinity (Figure 3, Table 3), however at high salinities (> 80 g/L) both Sr and Ca showed greater variation than at low salinities possibly reflecting precipitation of aragonite from the water. An interesting relation was found for Ba, where high values (> 200 μ g/L) were seen at low salinity (Lake Alexandrina) which then decreased until around 37 μ g/L before increasing with increasing salinities. Thus, the best fit to the Ba-salinity relation was a segmented linear model with a break point around 37 μ g/L. No relation was found between water Mn and salinity.

When data were ratioed to Ca, similar patterns were found for Ba and Mn to that of the original concentration data (Figure 4, Table 3), although the break point for Ba:Ca was slightly lower (34.72 ± 0.26 versus 37.24 ± 0.48). For Sr:Ca and Mg:Ca, logarithmic curves best described the relation between ambient water and salinity (Figure 4, Table 3). Data for water Sr:Ca and Mg:Ca suggested that the rate of change with salinity for Sr and Mg was less than that of Ca hence the logarithmic relation.

Atherinosoma microstoma was caught at all 10 locations along the Coorong, but not necessarily at all times (Figure 5; Table 1). For example, fish were only caught from three locations in December 2007 (BCS, LBC and MP), but from all 10 locations in June 2007. The only location in which fish were caught at all six sampling times was Lower Boundary Creek. Salinities in which fish were caught ranged from 35.7 to 119.1 g/L, although it should be noted that the Lake Alexandrina location was not sampled for fish.

Because fish were not caught from all locations at each sampling time, four locations (BCS, LBC, NM and HG) and four times (May, June, and September 2007 and June 2008) in which fish were caught for all combinations were used in statistical analysis. Otolith Ba:Ca showed a significant interaction between location and sampling time largely because significant differences were found between some sampling times at each location, but the sampling times which varied changed among locations (Figure 5, Table 4). These differences may reflect differences in salinity of the water at each location for each sampling time. No significant differences were found among locations or sampling times for otolith Sr:Ca.

Significant relations were found between otolith elemental ratios and salinity for four of the six element:Ca ratios (Figure 6, Table 5). The relation between otolith Ba:Ca and salinity showed a similar relation to that found between water Ba:Ca and salinity, although the breakpoint was slightly higher for the otolith data. The best fit to the otolith Sr:Ca salinity relation was a two segment linear model where a positive linear relation was found up to a salinity of 66.75 (± 13.76) g/L after which a negative linear relation was found. Otolith Mg:Ca and Mn:Ca showed no significant relation with salinity (Figure 6). A three segment linear model best described the relation between otolith Na:Ca and salinity with break points at salinities of 54.91 (± 3.92) g/L

and $71.40 (\pm 3.92)$ g/L. Otolith Li:Ca increased linearly with salinity up to $66.86 (\pm 6.34)$ g/L after which similar values were found (i.e. slope = 0).

Oxygen isotopes showed significant variation among locations (Figure 7, $F_{9,45} = 13.156$, $P < 0.001$) which was related to water salinity. Fish from the four southerly locations (Noonameena to Salt Creek) had significantly higher oxygen isotopes, than fish from the remaining locations. No significant variation was found for carbon isotopes among locations ($F_{9,45} = 0.790$, $P = 0.627$) or with salinity. A three segment linear model best described the relation between oxygen isotopes and salinity with breakpoints at $46.02 (\pm 4.55)$ g/L and $91.51 (\pm 16.37)$ g/L (Figure 8; Table 5).

Otolith Ba:Ca and Mg:Ca both showed a significant positive linear relation with Ba:Ca or Mg:Ca in the water respectively (Figure 9, Table 5). No significant relations between otolith Sr:Ca and water Sr:Ca or otolith Mn:Ca and water Mn:Ca were found (Figure 9).

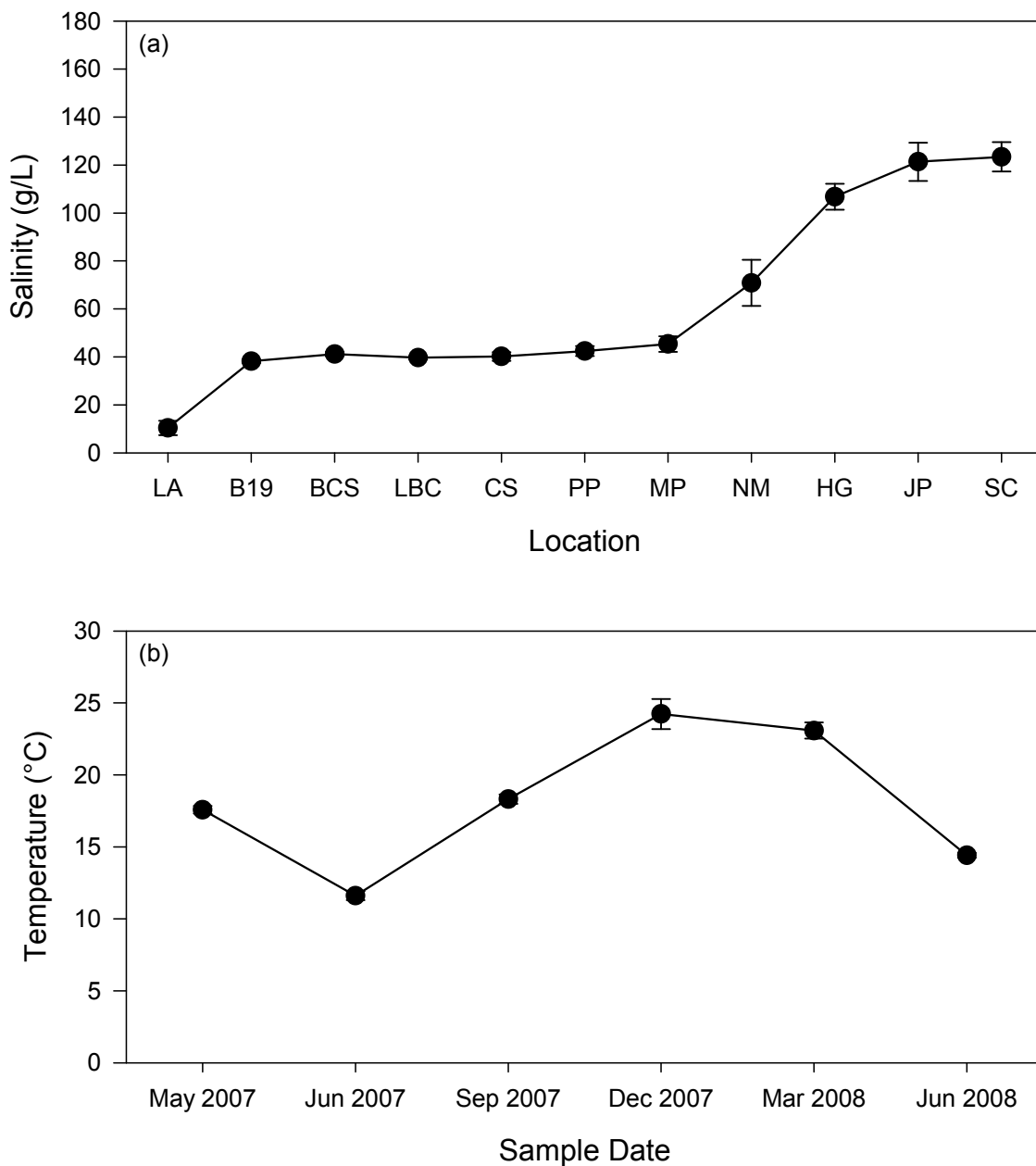


Figure 2. Summary of (a) salinity gradient along the length of the Coorong and (b) temperature over time. Replicates for salinity were each sampling period from May 2007 to June 2008, whereas replicates for temperature were measurements at each of the 10 sites. Error bars indicate ± 1 SE.

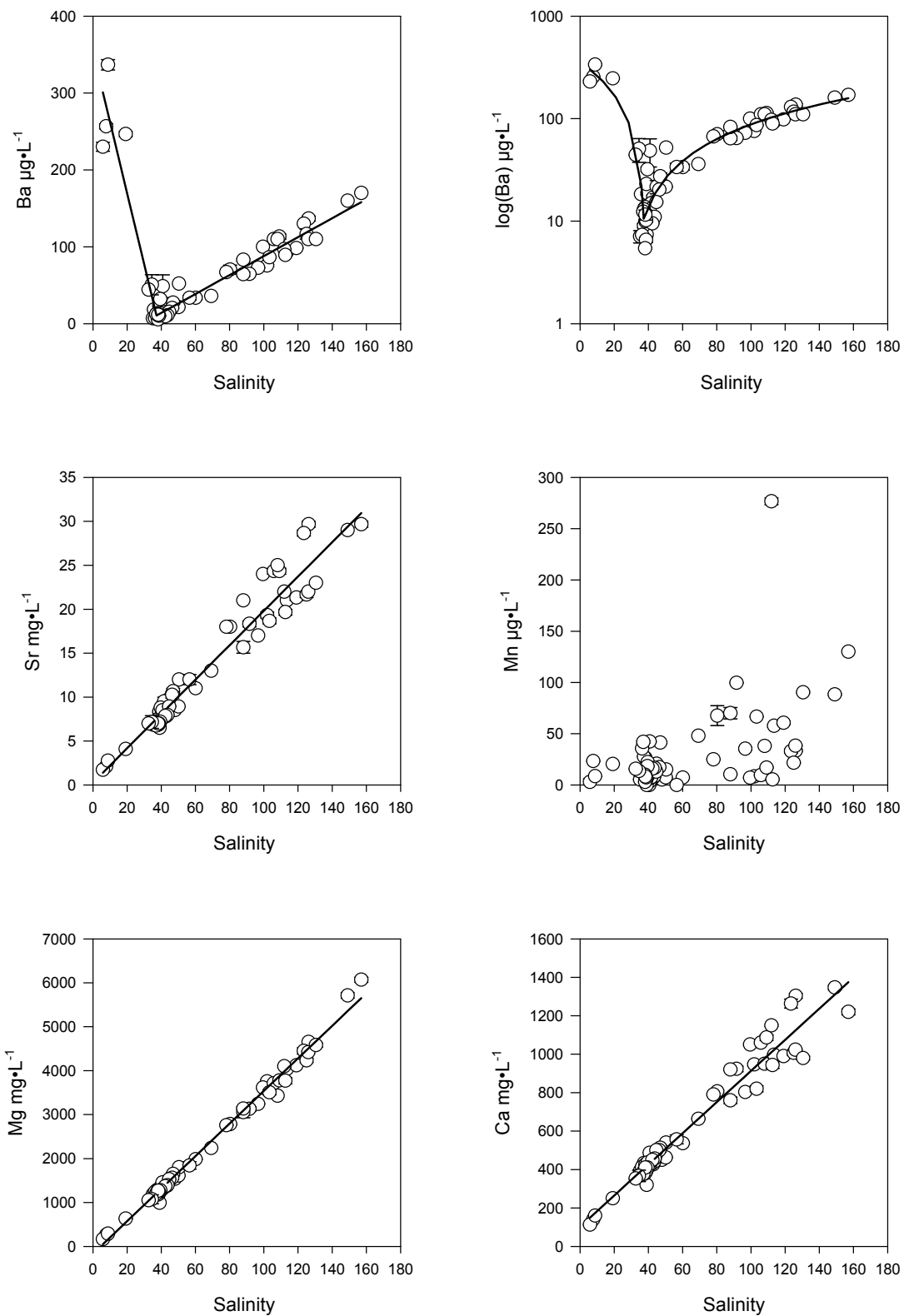


Figure 3. Mean water elemental concentrations versus salinity (g/L) for samples collected from the Coorong between May 2007 and June 2008. Error bars indicate ± 1 SE. For each data point $n = 3$.

Table 3. Relation between elements and salinity for water data unratioed and ratioed to calcium. Shown are models of best fit, equations for the model and r-squared values.

Relationship vs salinity	Model	Equation for model	r ²
Ba (µg/L)	Segmented linear model with a break point	$y = 354.4441 - 9.2339 \cdot \text{salinity} + 10.4654 \cdot (\text{salinity} - \psi)_+$, where $(\text{salinity} - \psi)_+ = \text{salinity} - 37.24$ if $\text{salinity} > 37.24$ & 0 otherwise	0.9281
Sr (mg/L)	Linear	$y = 0.2730 + 0.1954 \cdot (\text{salinity})$	0.9488
Mn (µg/L)	No relationship		0.2691
Mg (mg/L)	Linear	$y = -167.3911 + 37.0588 \cdot (\text{salinity})$	0.9919
Ca (mg/L)	Linear	$y = 102.5344 + 8.1027 \cdot (\text{salinity})$	0.9530
Ba:Ca (µmol/mol)	Segmented linear model with a break point	$y = 725.0896 - 20.6216 \cdot \text{salinity} + 20.8761 \cdot (\text{salinity} - \psi)_+$, where $(\text{salinity} - \psi)_+ = \text{salinity} - 34.72$ if $\text{salinity} > 34.72$ & 0 otherwise	0.9880
Sr:Ca (mmol/mol)	Logarithmic	$y = 4.1075 + 1.2355 \cdot \ln(\text{salinity})$	0.6197
Mg:Ca (mmol/mol)	Logarithmic	$y = 0.0616 + 1.3619 \cdot \ln(\text{salinity})$	0.8507
Mn:Ca (µmol/mol)	No relationship		0.0238

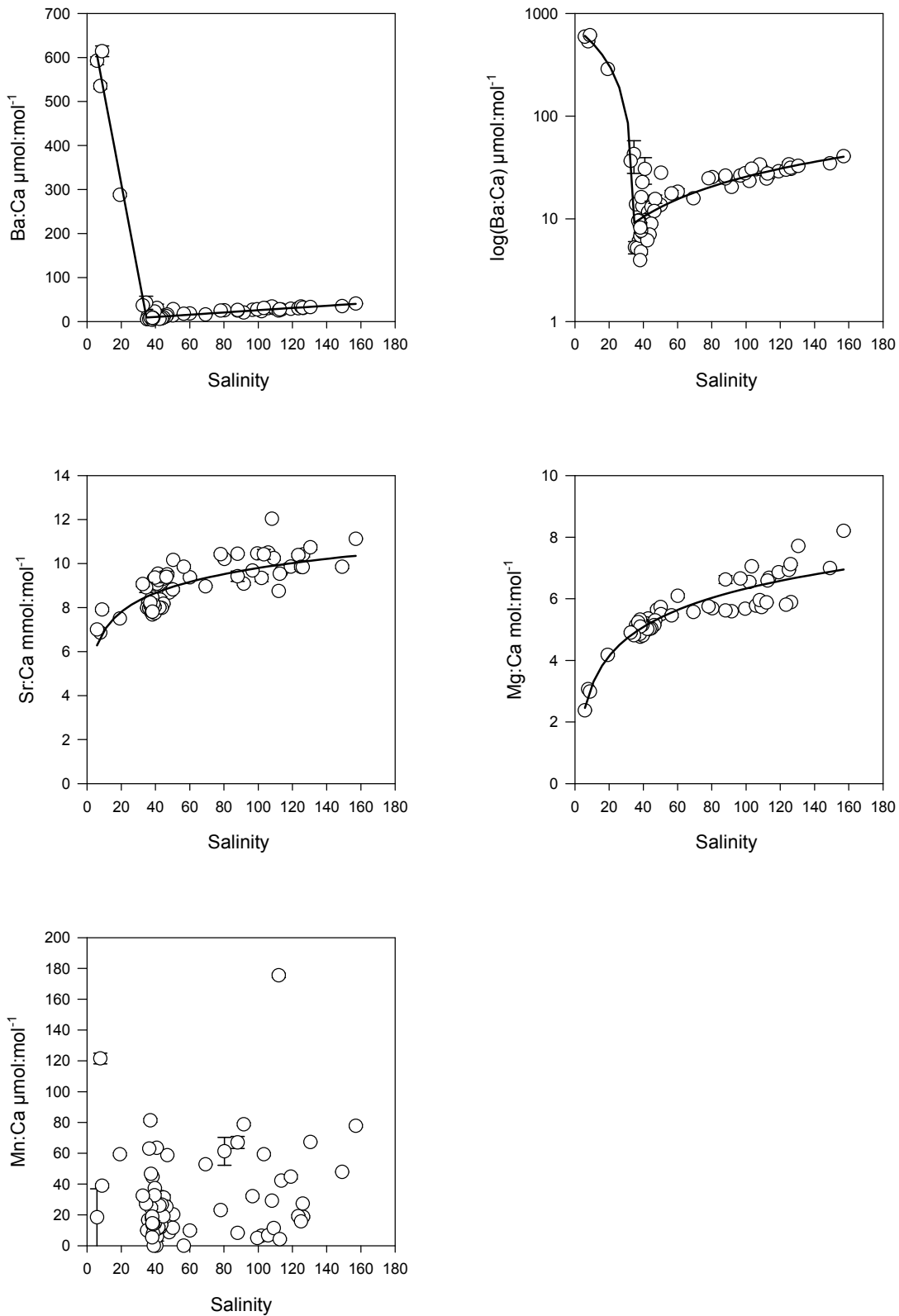


Figure 4. Mean water elemental:Ca ratios plotted against salinity (g/L) for samples collected from the Coorong between May 2007 and June 2008. Error bars indicate ± 1 SE. For each data point $n = 3$.

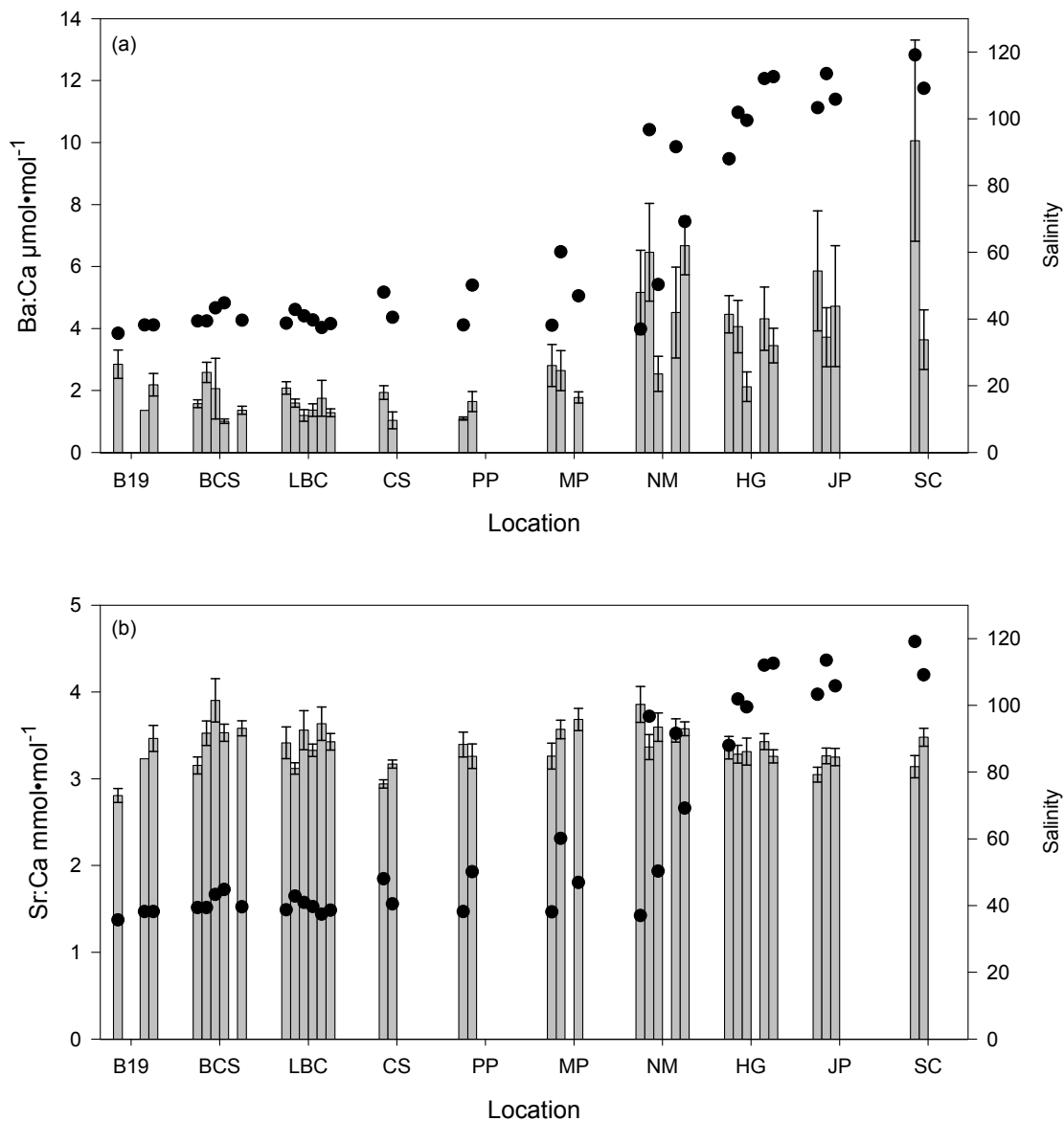


Figure 5. Mean otolith (a) Ba:Ca and (b) Sr:Ca for *Atherinosoma microstoma* collected from the Coorong during six sampling trips between May 2007 and June 2008. Data are plotted by sample location and trip. Salinity (g/L) of the sites where fish were collected is indicated by the black circles. Error bars indicate ± 1 SE. See Table 1 for full names of locations.

Table 4. ANOVA comparing otolith Ba:Ca or Sr:Ca among locations and sampling times. Because no fish were caught at some locations and times, only four locations and four times were included in the analysis. Both factors were treated as random factors. Data were $\log(x + 1)$ transformed.

Source of variation	MS	F-ratio	P
Ba:Ca			
Location	4.3756	13.6060	0.001
Sampling time	0.9179	2.8725	0.097
Loc x time	0.3255	2.3476	0.018
Residual	0.1387		
Sr:Ca			
Location	0.0317	2.0520	0.184
Sampling time	0.0171	1.1149	0.368
Loc x time	0.0156	1.6669	0.088
Residual	0.0093		

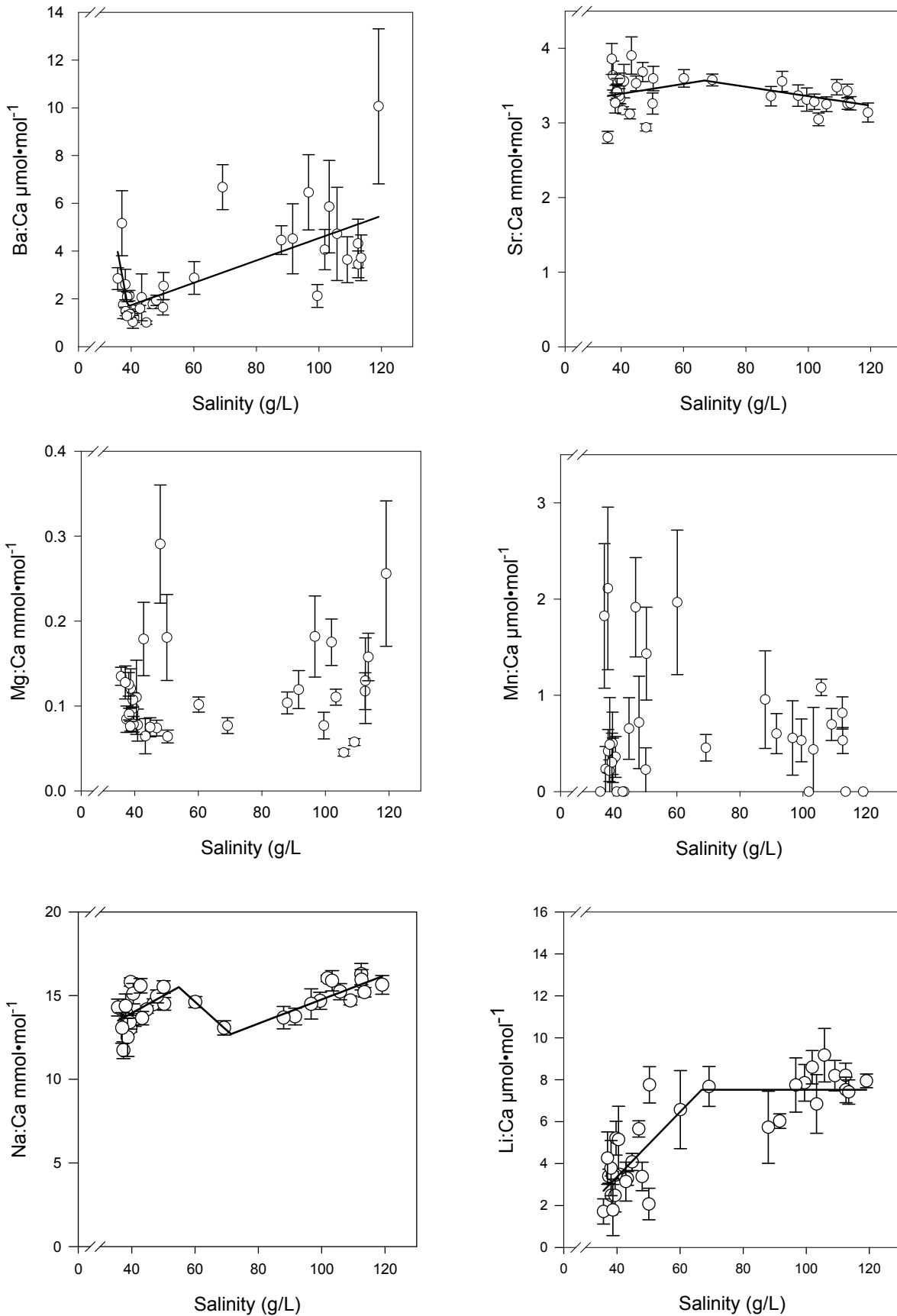


Figure 6. Comparison of mean otolith elemental ratios (Ba:Ca, Sr:Ca, Mg:Ca, Mn:Ca, Na:Ca and Li:Ca) against salinity for *Atherinosoma microstoma* collected from the Coorong during six sampling trips between May 2007 and June 2008. Error bars indicate ± 1 SE.

Table 5. Relation between otolith element ratios and either salinity or water element ratios. Shown are models of best fit, equations for the model and r-squared values.

Relationship	Model	Equation for model	r ²
vs salinity			
Ba:Ca (μmol/mol)	Two segment linear model	$y = 29.6239 - 0.7185 * \text{salinity} + 0.7652 * (\text{salinity} - \psi)_+$, where $(\text{salinity} - \psi)_+ = \text{salinity} - 38.88$ if $\text{salinity} > 38.88$ & 0 otherwise	0.1749
Sr:Ca (mmol/mol)	Two segment linear model	$y = 3.1260 + 0.0066 * \text{salinity} - 0.0129 * (\text{salinity} - \psi)_+$, where $(\text{salinity} - \psi)_+ = \text{salinity} - 66.75$ if $\text{salinity} > 66.75$ & 0 otherwise	0.0224
Mg:Ca (mmol/mol)	No relationship		0.0068
Mn:Ca (μmol/mol)	No relationship		0.0042
Na:Ca (mmol/mol)	Three segment linear model	$y = 9.8094 + 0.1037 * \text{salinity} - 0.2735 * (\text{salinity} - \psi)_{+1} + 0.2423 * (\text{salinity} - \psi)_{+2}$, where $(\text{salinity} - \psi)_{+1} = \text{salinity} - 54.91$ if $\text{salinity} > 54.91$ & 0 otherwise, and $(\text{salinity} - \psi)_{+2} = \text{salinity} - 71.40$ if $\text{salinity} > 71.40$ & 0 otherwise	0.1594
Li:Ca (μmol/mol)	Two segment linear model	$y = -2.8313 + 0.1549 * \text{salinity} - 0.1549 * (\text{salinity} - \psi)_+$, where $(\text{salinity} - \psi)_+ = \text{salinity} - 66.86$ if $\text{salinity} > 66.86$ & 0 otherwise	0.3140
δ ¹⁸ O (‰)	Three segment linear model	$y = 3.2057 - 0.0622 * \text{salinity} + 0.1412 * (\text{salinity} - \psi)_{+1} - 0.1060 * (\text{salinity} - \psi)_{+2}$, where $(\text{salinity} - \psi)_{+1} = \text{salinity} - 46.02$ if $\text{salinity} > 46.02$ & 0 otherwise, and $(\text{salinity} - \psi)_{+2} = \text{salinity} - 91.51$ if $\text{salinity} > 91.51$ & 0 otherwise	0.7183
δ ¹³ C (‰)	No relationship		0.0033
vs water			
Ba:Ca (μmol/mol)	Linear	$y = 0.6742 + 0.1401x$	0.3752
Sr:Ca (mmol/mol)	No relationship		0.0015
Mg:Ca (mmol/mol)	Linear	$y = -0.0831 + 0.0356x$	0.1873
Mn:Ca (μmol/mol)	No relationship		0.0447

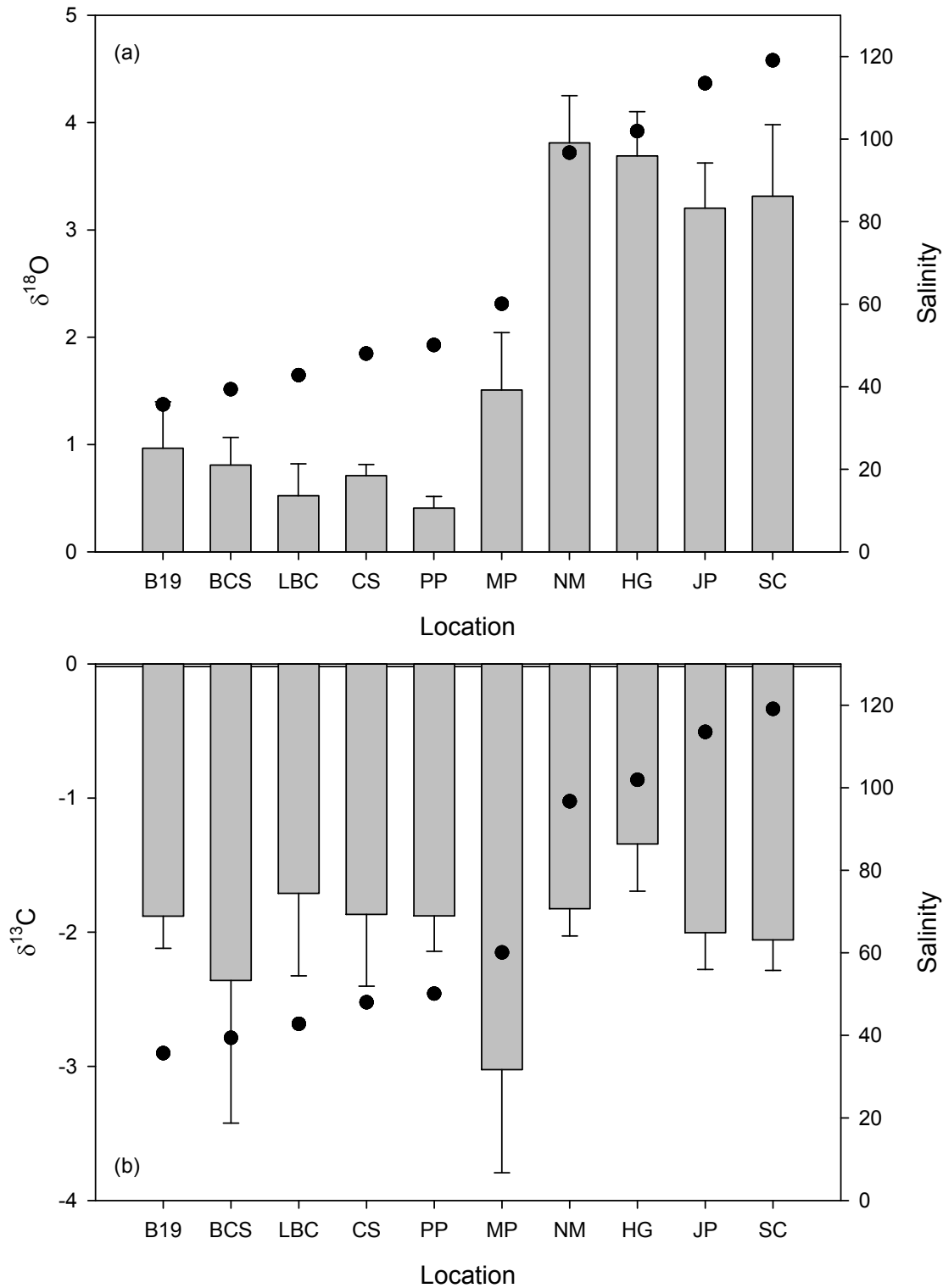


Figure 7. Mean otolith (a) oxygen isotopes and (b) carbon isotopes for *Atherinosoma microstoma* collected from the Coorong during June 2007. Data are plotted by sample location. Salinity (g/L) of the sites where fish were collected is indicated by the black circles. Error bars indicate ± 1 SE. See Table 1 for full names of locations.

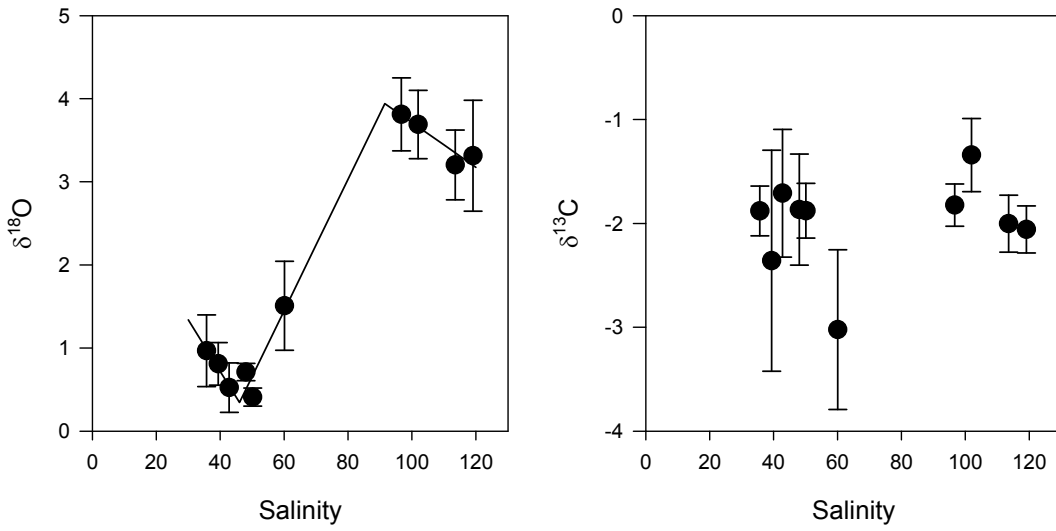


Figure 8. Comparison of mean otolith isotopic ratios ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) against salinity (g/L) for *Atherinosoma microstoma* collected from the Coorong during June 2007. Error bars indicate ± 1 SE.

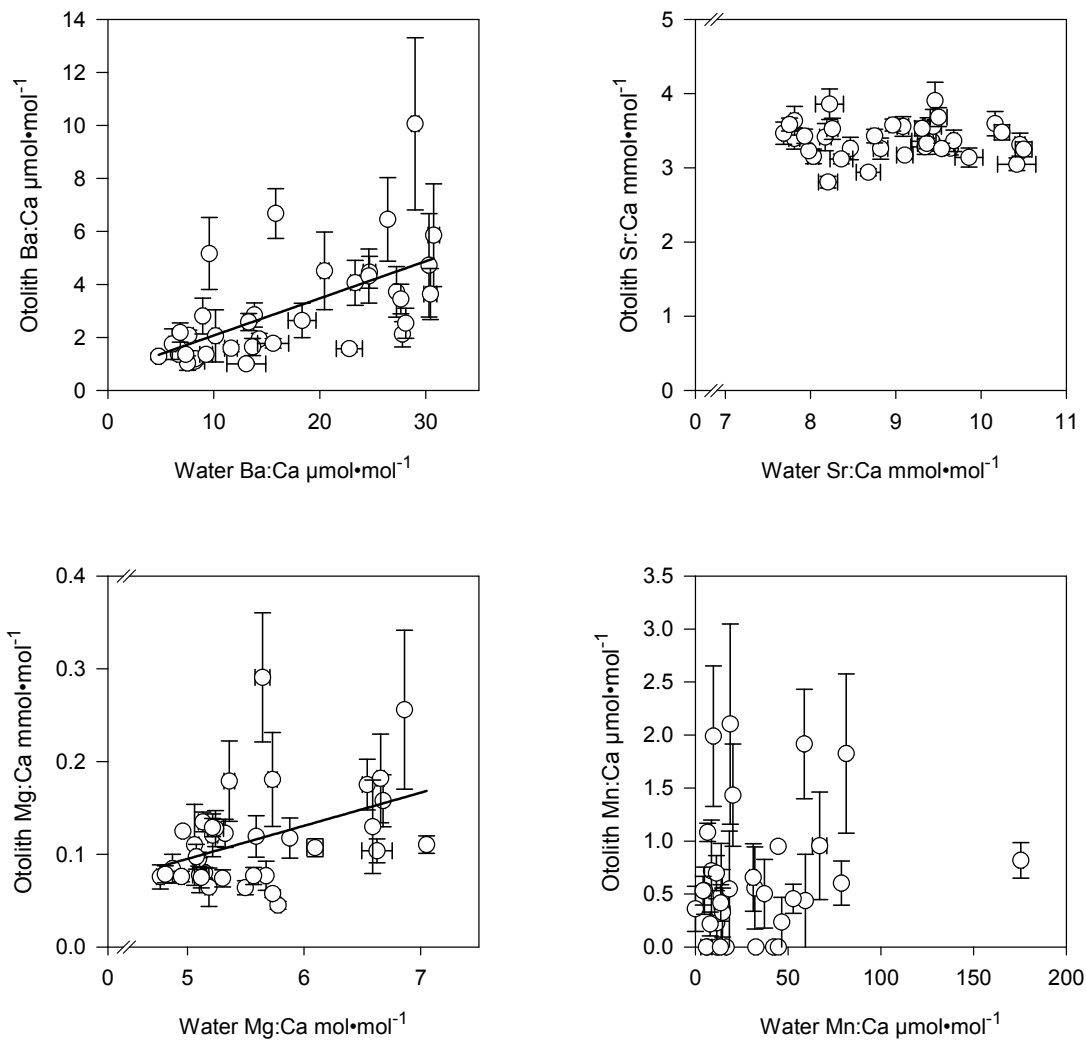


Figure 9. Relations between otolith chemistry and water chemistry for *Atherinosoma microstoma* collected from the Coorong during six sampling trips between May 2007 and June 2008. Error bars indicate ± 1 SE.

4. Discussion

The salinity of the Coorong system varied along its length ranging from brackish at Lake Alexandrina through to hypersaline in the southern reaches. These patterns were reasonably stable over the period sampled. The southern part of the Coorong system has experienced hypersaline conditions since at least the early 1980s, and even during sustained River Murray outflow had salinity around 70 (Geddes and Butler 1984, Geddes 1987). Hypersaline waters occur worldwide in estuaries and coastal lagoons around the world (Debenay *et al.* 1989, Bamber *et al.* 1992, Largier *et al.* 1997, Gordon 2000, Flecker *et al.* 2002, Vega-Cendejas and de Santillana 2004), and inland lakes (Williams 1999). For example, the St Lucia system in South Africa reaches hypersaline conditions (>70), but appears seasonally variable (Whitfield *et al.* 2006). Generally, hypersaline conditions are found within the dry summer season in Mediterranean climates.

The high salinity found in the southern end of the Coorong is largely due to restricted freshwater input. Barrages between the River Murray and the estuarine region of the Coorong were constructed in the 1940s, and since then water abstraction has reduced annual flow to 20% of natural levels (Walker 2006). There may be some variation in salinity along the Coorong due to wind tides which force hypersaline waters from the south into the mid northern Coorong during

south easterly winds. During westerly winds water is likely forced in the opposite direction. Wind driven movements of water may explain the larger variation in salinity observed between Noonameena and Jack Point.

Water chemistry also varied with salinity. Patterns for three of the five elements (Ca, Sr and Mg) showed positive linear relations across the salinity range (5-160 g/L). Other studies have shown similar patterns for salinities between 0 and 40 g/L (Surge and Lohmann 2002, Dorval *et al.* 2005) or in the case of Sr up to a salinity of 80 g/L (Diouf *et al.* 2006). Ca and Sr concentrations showed greater variation at higher salinities than at lower salinities possibly reflecting precipitation of aragonite from water which begins when the brine reaches twice the concentration of seawater (40-60 g/L) and achieves higher density than seawater (Warren 1997). Although there was some tendency for Mn to increase with salinity, this relationship was weaker than for the other elements, which is similar to other studies albeit for lower salinity waters (Dorval *et al.* 2005). Mn can show a range of patterns including conservative behaviour, mid-estuarine maxima or removal at low salinities (Colbert and McManus 2005). In general, little information is available on the behaviour of trace elements in hypersaline environments, and most of this comes from closed system conditions (laboratory or artificial salt pans) (Warren 1997).

For Sr and Mg, the rate of change with salinity in relation to Ca differs and therefore logarithmic relations were found for element:Ca ratios. Similar patterns were also observed in SW Florida estuaries (Surge and Lohmann 2002).

Ba showed the most interesting relation between elements in the water and salinity. Typically studies report a negative relation between freshwater and marine (35 g/L) salinities (e.g. Shaw *et al.* 1998, Dorval *et al.* 2005, Elsdon and Gillanders 2005a, Gillikin *et al.* 2006, Moore and Shaw 2008), and whilst our results concur with this pattern, they demonstrate that after marine salinities a positive relation was found. There can also be some variation in the salinity in which the Ba minima is found (Coffey *et al.* 1997). The slope of the positive part of the relation was considerably less than that of the negative part of the relation (1.231 vs -9.234). Martin and Wuenschel (2006) examined the relation between Ba:Ca and salinity up to a salinity of 45 g/L. Their results suggest an increase in Ba:Ca at a salinity of 45 g/L relative to 35 g/L. Ba (and Sr) was also enriched in a submarine anoxic brine pool (Orca Basin, northern Gulf of Mexico) relative to overlying seawater (Schijf 2007). We are not aware of other studies that have examined Ba in the water for salinities greater than 40 g/L. Whether a segmented relationship such as observed in the Coorong system is found in other hypersaline systems is unknown.

The increase in concentration of Ba at increased salinities is likely to reflect evaporation of water and associated concentration of Ba at higher salinities. There may also be some input of Ba from groundwater (Shaw *et al.* 1998, Jolly *et al.* 2008). Groundwater input is however more difficult to quantify because it often represents a non-point source and occurs over a broader area compared to discharge through rivers (Dowling *et al.* 2003). There may also be some desorption from intertidal sediments although this would likely be a factor throughout the system. Ba is typically desorbed under low salinity conditions particularly under high flow conditions (Coffey *et al.* 1997). Ba concentrations may therefore depend not only on salinity but also on hydrodynamics as well as transport of riverine suspended particulate matter (Coffey *et al.* 1997).

Atherinosoma microstoma were collected from all sites along the Coorong, but not necessarily at all times. The salinity where fish were caught ranged from 35 to 120 g/L. Varying salinities along the Coorong may affect energetics (e.g. feeding, metabolism, growth) of fish and while fish can survive and grow over a range of salinities there may be increased energetic costs at higher salinities especially if food is not abundant (Wuenschel *et al.* 2004). While we did not address energetic issues, growth can influence otolith chemistry of other species (Sadovy and Severin 1992, 1994, Kingsford *et al.* 2008). In addition, differences in otolith chemistry among species have been attributed to differences in metabolic rates (Hamer and Jenkins 2007) and stress (Kalish 1992).

Four of the six element:Ca ratios (Ba:Ca, Sr:Ca, Na:Ca and Li:Ca) and oxygen isotopes in the otoliths of fish showed significant patterns with salinity. All relations involved segmented or broken-line models with break points between salinities of 38.9 and 91.5 g/L. Ba:Ca in otoliths showed the same segmented relationship as that found for the water with a break point around a salinity of 38.9 g/L. Otolith Sr:Ca increased initially until a salinity of 66.7 g/L after which a negative linear relationship was found. Otolith Li:Ca increased through to a value of 7.53 $\mu\text{mol}\cdot\text{mol}^{-1}$ at a salinity of 66.9 g/L and then showed no further increase in concentration with increasing salinity. The best fit for the relationship between otolith Na:Ca or $\delta^{18}\text{O}$ and salinity involved models with two break points. The model for Na:Ca initially increased then decreased followed by an increase with increasing salinity whereas $\delta^{18}\text{O}$ showed the reverse pattern. These patterns have not previously been found for otolith element:Ca ratios probably because fish from hypersaline waters have not been investigated (but see Diouf *et al.* 2006).

Otolith Ba:Ca generally shows a negative relationship with salinity (e.g. Elsdon and Gillanders 2005a, Dorval *et al.* 2007) and has been suggested as a tracer of salinity. Likewise, Sr:Ca has often been used as a tracer of salinity because of the positive relation between otolith Sr:Ca and salinity (see review by Secor and Rooker 2000), although experimental laboratory studies report mixed results likely reflecting underlying differences in water chemistry (Gillanders 2005, Elsdon *et al.* 2008). Mg:Ca and Mn:Ca showed no relation with salinity in several controlled laboratory experiments (Elsdon and Gillanders 2002, Martin and Thorrold 2005, Martin and Wuenschel 2006). Different relations (positive and no relation) between otolith chemistry and salinity have also been found among species within the same study (Rooker *et al.* 2004) suggesting that responses are likely species-specific. With several exceptions all past studies have focused on salinities from freshwater to marine waters. Our results suggest that if fish inhabit waters with salinities greater than marine waters then caution is needed interpreting environmental histories. For example, a fish with a Ba:Ca ratio of 3 $\mu\text{mol}\cdot\text{mol}^{-1}$ may have inhabited an environment with a salinity of either 37 or 67 g/L. This will then make reconstructing past environmental salinity, whether based on fish moving from one area to another or the water in the environment around them changing, from single element:Ca ratios difficult.

Our $\delta^{18}\text{O}$ values are also higher than previously reported for fish otoliths (range -0.19 – 5.08). Previous studies have reported enrichment in ^{18}O of otoliths with increased salinity, although the upper salinity was between 8 and 65 g/L (Edmonds *et al.* 1999, Bastow *et al.* 2002, Kerr *et al.* 2007). Oxygen isotopes in otoliths are generally in equilibrium with ambient water albeit influenced by temperature and salinity. Lighter isotopes are lost to the atmosphere while the heavier isotopes become concentrated in the remaining waters from evaporation (Rohling and Bigg 1998, Edmonds *et al.* 1999). In contrast C isotopes are not deposited in equilibrium with ambient water in teleost otoliths, although a recent study suggests that otolith carbonate is mainly derived from ambient water (Tohse and Mugiya 2008).

Otolith Ba:Ca and Mg:Ca both showed positive linear relations with water Ba:Ca and Mg:Ca respectively, whereas no relation was found for Sr:Ca and Mn:Ca. Our Mg:Ca results contrast with those of several field studies that found no relation (Dorval *et al.* 2007, Hamer and Jenkins 2007, Walther and Thorrold 2008), but are similar to Mn:Ca results (Dorval *et al.* 2007, Hamer and Jenkins 2007, Walther and Thorrold 2008). Hamer and Jenkins (2007) also found no relation for Sr:Ca but examined sites with little variation in water Mg:Ca and Sr:Ca potentially limiting relations between water and otoliths. In general, positive relations are found between otolith Sr:Ca and water Sr:Ca (Bath *et al.* 2000, Elsdon and Gillanders 2003, Zimmerman 2005, Donohoe *et al.* 2008), and otolith Ba:Ca and water Ba:Ca (Bath *et al.* 2000, Elsdon and Gillanders 2003, Wells *et al.* 2003, Hamer *et al.* 2006, Hamer and Jenkins 2007), whereas no relation was found for Mn:Ca (Elsdon and Gillanders 2003). Many of the prior studies investigating otolith and water element:Ca relations have been conducted in the laboratory (Bath *et al.* 2000, Elsdon and Gillanders 2003). Some variation in Sr:Ca values between field and mesocosm-reared individuals was found (de Pontual *et al.* 2003), whereas in another study no variation was found between laboratory-reared and field collected fish for Sr:Ca but some variation was found for Ba:Ca (Elsdon and Gillanders 2005b). Field studies assume that the area of otolith sampled is representative of the area fish were collected from which may be plausible for resident species or migratory species for components of their life history. In other

cases fish have been constrained to cages in the field (e.g. Forrester 2005) to ensure this assumption is met. In addition, otolith samples generally represent a number of days, the actual number of which is dependent on the growth rate of the fish, and the ablation spot on the sample, whereas salinity or water values are generally a single point measurement.

Researchers have primarily used Sr:Ca, and to a lesser extent Ba:Ca ratios, to reconstruct environmental histories of fish based on the relations that they form with salinity (e.g. Sr: Secor *et al.* 1995, Secor *et al.* 2001, Ba: Elsdon and Gillanders 2005a, Hamer *et al.* 2006, Fablet *et al.* 2007, Hedger *et al.* 2008). We found that the relation between salinity and either Sr:Ca or Ba:Ca was not simple, and therefore it is difficult to use these simple single element relations to reconstruct environmental histories of hardyhead in the Coorong. If fish are likely to have encountered hypersaline conditions then multi-element and isotopic signatures will be needed for reconstructions (e.g. Munro and Gillanders, unpublished data). Information on changes in salinity, temperature and water chemistry at sites of interest, as well as relations with otolith chemistry is a first step towards reconstructing past environmental histories of fish. Without such information erroneous interpretations of fish movement may be obtained.

5. Summary, Conclusions & Management Implications

Using both water and otolith chemistry we showed significant relations with salinity over a wider range of salinities than previously reported. Several positive linear water chemistry-salinity relations were found (e.g. Sr, Mg, Ca), however an interesting segmented relation was found for Ba likely reflecting evaporation and associated concentration of Ba at high salinities. The otolith chemistry-salinity relations were more complex with relations suggesting that for most elements prediction of past environmental history would be difficult based on a single element reconstruction. Linear relations between otolith chemistry and water chemistry were found for two of the four elements (Ba and Mg) examined. These elemental and isotopic relations can now be incorporated into a multivariate signature that would be related to salinity. Using such an approach the environmental history of individual fish could be determined from early in its life through to when the fish was collected. The full range of salinities that a fish has experienced could then be determined as well as the amount of time it has spent in different salinity environments, although the latter also requires information on age and growth. While it will always be difficult to determine whether the fish moved to different salinity environments or whether there were changes in salinity around a stationary fish, knowledge of both spatial and temporal changes in environmental parameters and otolith chemistry helps distinguish among these possibilities. Conditions experienced by fish are important for predicting potential impacts of changing environmental flows or other management actions.

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