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Experimental Investigation of Elemental Incorporation in the Otoliths of Larval and Juvenile Fish: Implications for Use as Environmental Recorders

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**EXPERIMENTAL INVESTIGATION OF ELEMENTAL
INCORPORATION IN THE OTOLITHS OF LARVAL AND
JUVENILE FISH: IMPLICATIONS FOR USE AS
ENVIRONMENTAL RECORDERS**

by

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A Dissertation Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirement for the Degree of

DOCTOR OF PHILOSOPHY

ECOLOGICAL SCIENCES

OLD DOMINION UNIVERSITY
December 2003

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ABSTRACT

EXPERIMENTAL INVESTIGATION OF ELEMENTAL INCORPORATION IN THE OTOLITHS OF LARVAL AND JUVENILE FISH: IMPLICATIONS FOR USE AS ENVIRONMENTAL RECORDERS

Gretchen Bath Martin
Old Dominion University, 2003
Director: Dr. Mark J. Butler IV

Innovative techniques for discerning fish stocks, identifying nursery habitats, locating spawning sites, tracing larval transport pathways, and quantifying the degree of population connectivity are required to meet the goals of sustainable management of marine capture fisheries. One of the most promising techniques is the use of elemental signatures in fish otoliths (ear stones), which record valuable life history data and serve as the link between fish and their environment. To validate the assumption that otolith elemental composition is a function of water elemental concentrations, and to address the possible effects of external variables such as temperature and salinity, the composition of the ambient water must be known. Thus, three laboratory experiments were conducted using late larval to early juvenile stage spot (*Leiostomus xanthurus*) and gray snapper (*Lutjanus griseus*) to quantify the association between fish otoliths and water elemental composition, test the effects of water temperature and salinity on otolith element incorporation, and assess similarities or differences between species. Strontium/calcium (Sr/Ca) ratios in both *L. xanthurus* and *L. griseus* were significantly influenced by temperature. Sr/Ca partition coefficients (D_{Sr}) were affected by temperature and salinity in *L. xanthurus*. Magnesium/calcium (Mg/Ca) ratios and D_{Mg} were influenced by otolith

precipitation rates in *L. xanthurus*. D_{Mn} for *L. xanthurus* were significantly affected by both temperature and salinity. Although only barium/calcium (Ba/Ca) ratios in *L. griseus* otoliths were significantly affected by salinity, D_{Ba} in both *L. griseus* and *L. xanthurus* were affected by salinity. These results are independent of ontogenetic and diet effects, and represent one of the first attempts at validating minor and trace element incorporation in laboratory reared fish. This work also presents the first comparison of otolith element incorporation between fish species. The results prove that otolith element incorporation is not solely a function of water elemental composition because it is affected by both temperature and salinity and those effects varied uniquely among the elements investigated. This comparison between fish species draws attention to the necessity of validation experiments to interpret species-specific elemental signatures in otoliths.

This dissertation is dedicated to my husband Todd Martin, my son, Jake Leo Martin, my parents William and Colleen Bath, my grandmother Stella Barry, dear friends Shannon Huneke and Marion Salinger, and to my buddy Ralph. Their encouragement, support, and love enabled me to persevere.

ACKNOWLEDGMENTS

This research was supported by grants from the National Science Foundation to Cynthia M. Jones and Steven E. Campana (OCE – 9416579), Simon R. Thorrold (OCE-0134998), and to Cynthia M. Jones, Jonathan A. Hare, Simon R. Thorrold and Arnaldo Valle-Levinson (OCE-9876565). NOAA Sea Grant (to Simon Thorrold, Jon Hare, Su Sponaugle, Bob Cowen, and Luiz Barbieri) and NOS COP/SEFSC South Florida Program funded the gray snapper work. I received two grants in aid from the International Women's Fishing Association, two years of support from the Virginia S. Bagley scholarship (Department of Biological Sciences, Old Dominion University), and a PEO International Women's Organization Scholar Award. From May 1999 until the present time I have had full support from the U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service Student Career Experience Program under the direction of Donald Hoss, Jonathan Hare, and David Johnson.

My committee has dedicated countless hours to guide me through this process. I thank Mark Butler for rising to the task and for his guidance. Simon Thorrold and Jon Hare have been there for me through everything and I cannot thank them enough for all of their time, encouragement, constructive criticism, and confidence in my abilities. I am sincerely appreciative of Cynthia Jones and John Donat, for all their time, guidance, and shared knowledge.

John Burke (NOAA, National Ocean Service, CCFHR, Beaufort, NC) provided spawning and initial rearing of *Leiostomus xanthurus*. David Gray (Elemental Research Inc. Vancouver, British Columbia) performed the water ICP-MS analyses for the first

experiment. Brian Wells and Barbara McClellan volunteered additional support with the daily maintenance of the first experiment. Gary Fisher and Peter Crumley (NOAA, National Ocean Service, CCFHR, Beaufort, NC) provided technical support for the second experiment. Dr. Zhongxing Chen (LITER, Old Dominion University) assisted with the water ICP-MS analyses for the second and third experiments. Mark Wuenschel contributed significantly to the third experimental design, fish capture, and rearing, with the tireless help of Emma Jugovich. Jennifer Fitzgerald (Woods Hole Oceanographic Institution, Woods Hole, MA) assisted with sample preparation and running the laser and ICP-MS at WHOI for the third experiment.

I would like to acknowledge the coauthors of my first manuscript from Chapter II (Bath et al. 2000): Simon Thorrold, Cynthia Jones, Steven Campana, David Gray, Joseph Lam, and James McClaren. The coauthors of my second manuscript from this work but not included in this dissertation (Wells et al. 2000) are Brian Wells, Simon Thorrold, and Cynthia Jones. The coauthors of my third manuscript from Chapter III (Martin et al. *In Press*) are Simon Thorrold and Cynthia Jones.

Thank you to all my ODU support: Gail Stenberg, Frank Day, Al Savitzky, Andy Gordon, Lytton Musselman, Kevin Piner, Emmanis Dorval, Stephen Wischniowski, Sumalee Hoskin, Brian Wells, Barbara McClellan, and Julian Ashford.

The administrative, technical, and moral support from the NOAA Beaufort Lab has been phenomenal. I would especially like to thank the Directors under whom I served, Don Hoss and David Johnson. The Fisheries Oceanography and Ecology Team cheerleaders deserve a ton of praise: Elisabeth Laban, Harvey Walsh, Katey Marancik, Brian Degan, Mark Wuenschel, Frank Hernandez, Dariusz Fey, Mike Greene, Siya Lem,

Emma Jugovich, Jacqui Jenkins, Larry Settle, and Peter Hanson. Thank you to the Lab Administrative support: Jeanie Fulford, Rusty Gray, Sharon Sechler, and Dorothy Smith.

And last but not least, I could not have completed this degree without the additional moral support from my dear friends and family including Tara Cox, Leslie-Ann McGee, Colleen Condon, William Bath, Jr., Michael Bath, Stephen Bath, Frances and Paul Meyer, and Durwood and Jeannie Martin.

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CHAPTER I

INTRODUCTION AND BACKGROUND

Problems in fisheries management

Harvests from fish populations in United States waters have fluctuated substantially over the last century and in many cases have declined, creating serious economic, social, and ecological problems (NRC 1998). Out of 259 major stocks (landings greater than 90 tons) identified by the National Marine Fisheries Service, 43 are overfished and 41 are subject to overfishing (NMFS 2003). The list of overfished stocks includes some of the most valuable fishery resources, such as New England groundfish, Atlantic sea scallops, Atlantic bluefin tuna, swordfish, Chinook and Coho salmon, several Alaskan rockfish, and Alaskan king crab (NMFS 1999).

The challenge for fisheries managers is to rebuild overfished stocks, prevent further overfishing, and maintain future harvests at sustainable levels. Although the primary cause of overfishing is fishing effort and associated fishing mortality (Hutchings and Myers 1994; Myers et al. 1997), the natural variability of fish populations makes stocks more susceptible to overfishing during periods of naturally low abundance (Sissenwine 1984; Myers and Quinn 2002). Furthermore, other factors can contribute to stock declines such as habitat degradation (Murawski 2000) and fishery by-catch (Goodyear 1999). Consequently, developing population models that incorporate the complexities of natural and anthropogenic effects on fish stocks has become paramount for setting harvest levels and encouraging a precautionary approach to fisheries management.

The model journal for this dissertation is Canadian Journal of Fisheries and Aquatic Sciences.

Fishery managers responsible for managing fish stocks are often limited by an incomplete understanding of fish stock structure (NRC 1998). Typically, a fish stock is defined as an intraspecific group of randomly mating individuals with spatial or temporal integrity (Ihssen et al. 1981). Population genetic techniques using mitochondrial and nuclear DNA markers are commonly used to define stock structures, based on the premise that these tools can be used to precisely and accurately separate stocks (Park and Moran 1994; Wright and Bentzen 1994).

Recent studies have applied innovative analytical techniques to demonstrate that current stock definitions based on genetics do not necessarily reflect actual fish population structure. In a study by Thorrold et al. (2001), otolith (ear stone) chemistry was used to assess natal homing of weakfish, *Cynoscion regalis*. Thorrold et al. (2001) estimated spawning site fidelity ranged from 60 to 81%, which was comparable to natal homing estimates for birds and anadromous fish. These estimates were in contrast to genetic analyses (allozymes and mitochondrial DNA), which found no evidence of genetic differentiation between spawning sites. Because weakfish are managed as one unit stock (Vaughan et al. 1991), the evidence that weakfish may represent a metapopulation should provoke managers to reevaluate the management of this fishery.

Defining nursery habitats

Fishery managers are also faced with the largely unquantified effect of coastal habitat degradation on fisheries production. Nearshore estuarine and marine habitats including seagrass beds, marshes, and mangroves, have been identified as some of the most productive juvenile nursery habitats (Beck et al. 2001). Generally, an area is

defined as a nursery if juvenile fish occur at higher densities, avoid predation more successfully, or grow faster there than in other habitats (Beck et al. 2001). Until recently, making habitat-specific assessments of juvenile production, either quantitative or qualitative, has been extremely difficult, thereby limiting the utility of the nursery habitat concept to fisheries management. Habitat conservation and fisheries management will improve with a better understanding of the habitats that serve as nurseries for marine fish species and the factors that create site-specific variability in nursery quality and fisheries production (Beck et al. 2001).

Although estuaries and associated seagrass beds are considered important nursery habitats for many reef fish based on occurrence and abundance of juvenile fish, little direct evidence exists of movement from estuaries to reefs. In a study by Gillanders and Kingsford (1996), Australian blue groper (*Achoerodus viridis*) recruits to the offshore adult population came primarily from young that settled in offshore rocky reefs, not from the abundant young inhabiting inshore seagrass beds. Gillanders and Kingsford's (1996) data contradicted the conventional thought that seagrass beds serve an important role in the early life history stages of blue groper. Their work illustrates the fact that density estimates alone may not define nursery areas and thus highlights the importance of quantifying population connectivity rates (the exchange of individuals among geographically separated populations) to identify nursery habitats essential for fisheries productivity.

Siting Marine Protected Areas

Marine Protected Areas (MPAs) are a management approach proposed to reduce the problems of overfishing and respond to uncertainty in fisheries management (Crowder et al. 2000). The goals of MPAs in fisheries management are to control exploitation rates, protect critical life history stages, reduce secondary fishing impacts, ensure against possible failures of conventional management systems, and conserve genetic diversity (NRC 2001). MPAs, as an alternative to conventional fisheries management, also have uncertainties associated with their performance (NRC 2001). Decisions regarding location, size, and linkages between MPAs and other ecosystem components have to be made to meet management objectives (NRC 2001). MPAs will only be effective management tools if specific information about fish populations including spawning locations (source of larvae), fate of larvae, and degree of connectivity is considered for each MPA site.

The concern for effectively siting MPAs is illustrated for Nassau grouper, *Epinephelus striatus*, an historically important species in the Caribbean island fisheries. Overfishing of Nassau grouper has driven the stocks below sustainable harvest levels in many areas and has eliminated the species from portions of their historical range. Nassau grouper is especially vulnerable to overfishing because it forms spawning aggregations during one or more winter full moons (Sala et al. 2001). These spawning aggregations are subject to intensive fishing pressure because of the guaranteed landings. Protection afforded by MPAs could benefit the species if MPAs were sited in locations specifically protecting spawning aggregations. However, repatriation of species where they have been driven to commercial and biological extinction does not offer a complete solution to

the problem. Understanding the spatial connection between larval sources, transport, settlement, and degree of population connectivity would assist in the proper siting of MPAs to protect Nassau grouper stocks and thus encourage more effective stock management in the future.

Innovative techniques for discerning stocks, identifying nursery habitats, locating spawning sites, tracing larval transport pathways, and quantifying the degree of population connectivity are required to meet fishery management goals. One of the most promising techniques is the use of fish otoliths, which record valuable life history data and thus serve as the link between fish and their environment.

Otoliths as fish life history tools

Otolith development and composition

Otoliths are sensory receptors in the inner ear that enable fish to perceive frequency, amplitude, and direction of sound, as well as static and dynamic position in the water column (Mosegaard and Morales-Nin 2000). Otoliths form incrementally through the differential deposition of calcium carbonate (aragonite) and protein, influenced by both environmental and physiological factors including temperature, pH photoperiod, feeding, growth, and endogenous circadian rhythms (Simkiss 1974; Dacke 1979; Campana and Neilson 1985; Kalish 1989; Gauldie and Nelson 1990; Payan et al. 1997). These increments are formed on daily and annual growth cycles. Under conditions of food deprivation, otoliths continue to grow (Jones and Brothers 1987; Massou et al. 2002); additionally, otolith material is no longer biologically or

physiologically active when fish are stressed by exertion or by exposure to low environmental pH levels.

Otoliths are used to estimate fish ages

The daily and annual increments formed in otoliths are used to estimate fish ages in days (Panella 1971; Jones 1986) and years (Beamish and McFarlane 1983). Age estimates from annual increments in otoliths are the basis for calculating growth mortality, and are therefore an essential tool for fish stock assessments (Campana 2001). Daily ages based on otolith microstructure are used for larval and juvenile stages to address questions about recruitment, settlement, temporal and spatial patterns in pre and post-settlement growth, stage duration, and larval transport (Thorrold and Hare 2002).

Otoliths are used to describe somatic growth

Just as otolith increments are used for determining fish ages, otolith size and growth are proxies for fish size and somatic growth (Campana and Jones 1992). Fish size and otolith size are positively correlated simply because both increase in size over time (Thorrold and Hare 2002). Back-calculation methods are used to estimate individual size and growth at age (Campana 1990; Francis 1990). Thus, temporal patterns in fish growth can be elucidated from the otolith microstructure. These patterns provide yet another level of detail for understanding population dynamics and have become instrumental for improving fisheries management.

Otoliths mark life history transitions

In addition to recording age and growth information, otoliths form distinctive patterns at life history stage transitions. The first otolith increment occurs at hatching for some species, and at yolk absorption and first feeding in other species (Campana and Neilson 1985; Jones 1986). Eye migration in flatfish (*Platichthys stellatus*, *Pseudopleuronectes americanus*) and the larval-juvenile transition in bluefish (*Pomatomus saltatrix*) are marked by secondary growth centers (primordia) (Campana 1984; Sogard 1991; Hare and Cowen 1994). In many other fish species, a distinctive pattern, or settlement mark, commonly occurs in the otoliths at the time of the larval-juvenile transition (Gartner 1991; Linkowski 1991; Wilson and McCormick 1997, 1999; Searcy and Sponagle 2001). The use of larval settlement marks to back-calculate timing of settlement and larval duration offers an efficient method of evaluating temporal recruitment patterns on larger spatial scales than previously possible using field sampling alone (i.e., visual surveys, settlement traps, and mark-recapture techniques) (Wilson and McCormick 1997).

Otoliths record ambient environment

Coupled with age, size, and development, environmental history experienced by individual fish is recorded in otolith chemistry. The use of otoliths is appealing since specific questions (i.e. natal origins, spawning site fidelity, return migrations) can be addressed through otolith chemistry that cannot be addressed by other techniques. Thorrold et al. (2001) used otolith chemistry to identify separate metapopulations of weakfish, *C. regalis* that were previously managed as one unit stock. Gillanders and Kingsford (1996) used otolith chemistry to demonstrate that seagrass beds were not the

main nursery source of blue grouper, *A. viridis* juveniles recruiting to the adult, fished population, contrary to conventional thought. Otolith chemistry techniques could also be used to quantify Nassau grouper population connectivity rates and describe larval transport, which would elucidate population information for fisheries management decisions regarding Nassau grouper stocks.

Instrumentation

The use of otoliths as environmental recorders is based on the premise that the concentration of elements in the otolith can be accurately and precisely measured. Analytical techniques currently used can be categorized into those that use whole otoliths (Fowler et al. 1995a) and those that sample at specific loci within the otolith (Fowler et al. 1995b). Dissolving whole otoliths provides an integrated elemental record over a fish's life history. Sampling at specific loci in a three-dimensional otolith provides environmental information at a particular location, corresponding to a particular point in time, related to growth rate, age, ontogeny, and environmental variation.

Inductively coupled plasma mass spectrometry (ICP-MS) is a highly sensitive analytical technique that combines the detection power of mass spectrometry with the capability of near-simultaneous element analyses of dissolved otoliths (Houk 1986; Beauchemin et al. 1987; Date 1991). Because of the ability to perform rapid isotope ratio measurements, ICP-MS makes stable isotope dilution techniques possible. Isotope dilution ICP-MS is based on the addition of a known amount of an enriched isotope spike to a sample. After equilibration with the natural isotope in the sample, mass spectrometry is used to measure the altered isotopic ratios (Fassett and Paulsen 1989). In principle and

practice, isotope dilution ICP-MS provides a high degree of accuracy, precision, and sensitivity (Beauchemin et al. 1987; Fassett and Paulsen 1989; Catterick et al. 1995; Campana et al. 1997). Isotope dilution increases the accuracy of ICP-MS measurements by minimizing problems with matrix effects, plasma instability, changes in orifice geometry, and other instrument-related difficulties (Klinkhammer and Chan 1990).

Laser ablation ICP-MS is a method for the direct elemental analysis of solid samples that exposes material to a laser beam inside a cell through which an input carrier gas (typically He or Ar) passes (Wang et al. 1994). The ablated material is carried by an aerosol to the argon plasma torch and is analyzed by the mass spectrometer. Using LA ICP-MS minimizes interference, improves detection limits, and reduces sample preparation time, thus reducing a significant point of potential contamination (Wang et al. 1994). Laser ablation ICP-MS surpasses the commonly used electron probe microanalyzer for in situ analyses of otolith chemistry (Secor 1992; Kalish 1990; Gunn et al. 1992; Thresher et al. 1994; Campana et al. 1997) in terms of spatial resolution, detection limits, and sample processing efficiency (Wang et al. 1994).

Applications of otolith chemistry

Otolith elemental signatures

Differences in otolith elemental signatures are attributed to different geographical locations. Limitations of using this technique include unknown ontogenetic differences in incorporation rates, the specific behaviors of the trace elements examined, and technological limitations (i.e., precision and accuracy of technique or instrument used). Interpretation of these geochemical signatures is best applied to species that move

between or inhabit very different environments with respect to basin geology, watershed use/geology, proximity to land, and elemental composition of water. This technique has successfully been applied to specific stock identification questions on Scotian Shelf cod (*Gadus morhua*) (Campana et al. 1995). Other applications include discerning migration or transport (e.g., labrid fishes in the Great Barrier Reef, Gillanders and Kingsford 1996) and natal homing (weakfish; Thorrold et al. 2001).

Strontium/calcium thermometry

Strontium/calcium thermometry is the use of Sr/Ca ratios in biogenic calcium carbonate structures to reconstruct temperature histories of ancient and modern environments. Interest in Sr/Ca thermometry began in the 1960's with the primary objective of understanding the influence of sea level rise and fall and melting glaciers on regional and global climate change. Sr/Ca thermometry has been used in corals (Swart 1979; Smith et al. 1979; Shen et al. 1996), molluscs (Dodd and Crisp 1982; Stecher et al. 1996), foraminifera (Lea and Martin 1996; Elderfield et al. 1996; Lea et al. 1999), and more recently in fish otoliths (Thorrold et al. 1997a). Applications of this technique using otoliths include paleoclimate reconstructions as demonstrated by Kalish (1999) and more recently by Andrus et al. (2002) for climate comparisons between El Niño and non-El Niño years. Another use of Sr/Ca ratios is the reconstruction of fish migration histories of anadromous fish (Kalish 1990; Limburg 1995; Secor et al. 1995; Tzeng et al. 1997). Limitations to Sr/Ca thermometry include interspecific differences in incorporation, variable calcification and increment formation along growth axes,

variability in Sr/Ca composition of water, and technological limitations and resolution capabilities of instruments used in previous studies.

Individual fish transport pathways

Combining otolith elemental signatures and Sr/Ca thermometry techniques may allow for reconstruction of individual fish transport pathways associated with water masses. The time-keeping properties of otoliths and the high spatial resolution of LA ICP-MS may enable precise recovery of experienced environments. With the proper validation for individual species, the use of LA ICP-MS would complement tagging efforts currently conducted.

Assumptions of otolith element incorporation

The main assumption for both otolith elemental signatures and Sr/Ca thermometry is that otoliths incorporate minor and trace elements in proportion to free ion concentrations in the ambient water (Simkiss 1974). Farrell and Campana (1996) used radioisotopes of calcium and strontium to test for a relationship between water concentrations and otolith concentrations. They determined that the water contributed to 75% of the calcium and 88% of the strontium incorporated in the otoliths of Nile tilapia, *Oreochromis niloticus*. The remainder of the strontium was assumed to be from the diet. Other studies have attributed some strontium to diet (Limburg 1995; Gallahar and Kingsford 1996; Kennedy et al. 2000) yet Hoff and Fuiman (1995) suggested otolith uptake of a suite of minor elements from food was minimal. More recently, Milton and Chenery (2001) verified that otolith trace metal concentrations were related to water

concentrations, although, the otolith trace metal patterns did not extrapolate simply to the patterns measured in the natural environment, implying that the mechanisms of trace metal incorporation are more complex.

Specific to Sr/Ca thermometry, another critical assumption is that temperature affects Sr/Ca incorporation in the otolith, thereby allowing the otolith to act as a recording thermometer. Otolith Sr/Ca values have been used to examine transport pathways as a function of ambient temperature (Radtke 1989; Townsend et al. 1989, 1992, 1995; Gallahar and Kingsford 1996). The relationship between temperature and Sr/Ca ratios in fish otoliths is likely complicated because the otolith is isolated from seawater by branchial, intestinal, and the endolymphatic membranes. Proposed relationships between Sr/Ca and temperature include that Sr/Ca ratios and temperature are positively correlated (Fowler et al. 1995a; Arai et al. 1996), negatively correlated (Townsend et al. 1992), and uncorrelated (Gallahar and Kingsford 1996). These inconsistencies are quite possibly a result of inter-specific variations in Sr uptake as a function of temperature or methodological artifacts in some or all of the experiments.

Another underlying assumption in otolith chemistry studies is that there are no differences in otolith elemental incorporation among different fish species. This assumption is not stated explicitly in many studies and has not been evaluated under controlled conditions in the laboratory.

The application of otolith chemistry potentially provides tools to address a number of issues relevant to fisheries management and ecology; however, to apply the method, the assumption must be validated that minor and trace elements in otoliths are deposited in proportion to dissolved concentrations in the ambient water. To quantify the

relation between water mass elemental composition and otolith composition, and the possible effects of external variables such as temperature and salinity, the composition of the ambient water must be known. This is achieved most easily and accurately under laboratory conditions (Farrell and Campana 1996; Milton and Chenery 2001).

Dissertation research objectives

The next five chapters present the results from three laboratory experiments designed to test the relationship between otolith composition and ambient water chemistry, as well as the effects of water temperature and salinity on otolith element incorporation. Once the relation between otolith composition and ambient water chemistry is quantified, it may be possible to apply otolith elemental analyses to link fish dispersal and subsequent transport with different water masses. If successful, these techniques will provide tools for evaluating individual fish life history profiles, critical for understanding recruitment processes and habitat associations.

Outline of chapters

Chapter II examines the relation between water strontium and barium concentrations and otolith incorporation of strontium and barium using larval spot (*Leiostomus xanthurus*) in a controlled laboratory experiment. The chapter also investigates the effects of temperature on otolith Sr/Ca and Ba/Ca incorporation.

The first experiment was not designed to calibrate the temperature dependence of $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ as fish were only reared at two temperatures. To further investigate the spot otolith temperature and salinity relationship, a second experiment was performed and

reported in Chapter III. The objectives of this second experiment were to quantify the effect of temperature on $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ in spot larvae using four temperature treatments and to test the effect of salinity as a proxy for life history transitions from pelagic larvae to estuarine juveniles.

Chapter IV further analyzes the effect of temperature and salinity on barium, manganese, and magnesium incorporation in spot otoliths from the second experiment. If these elements are to be used in field applications, it is important to determine if their incorporation is effected by these environmental factors.

In the final experiment (Chapter V), juvenile gray snapper (*Lutjanus griseus*) were used to ascertain potential species differences in minor and trace metal incorporation with respect to temperature and salinity. This chapter uses a laser ablation sampling technique coupled with ICP-MS to sample specific locations in otoliths.

The final chapter is the research summary, which discusses applications of these experimental findings and identifies future directions for research.

CHAPTER II

STRONTIUM AND BARIUM UPTAKE IN ARAGONITIC OTOLITHS OF MARINE FISH¹

Introduction

Trace element studies of biogenic carbonates such as foraminiferal calcite and coral aragonite have provided a wealth of information on the physicochemical properties of modern and ancient aquatic environments. Recently, several workers have proposed that the isotopic and trace element composition of fish otoliths, or ear stones, may provide useful proxies for reconstructing temperature histories (Patterson et al. 1993; Thorrold et al. 1997a) and, perhaps, trace element concentrations in marine and freshwater systems (Thorrold et al. 1997b, 1998a). Otoliths are common in the fossil record from the late Cretaceous to the present (Nolf 1995), locally abundant in aboriginal middens (e.g., Kalish 1999), and highly resistant to diagenetic processes in sediments dating to the Jurassic Period (Patterson 1999). More importantly, in the context of oceanographic and climate proxies, otoliths form periodic rings of sufficient widths to allow sampling at a temporal resolution approaching the daily level using either micromilling (Wurster et al. 1999) or laser ablation techniques (Campana et al. 1994; Thorrold and Shuttleworth 2000). Analyses of otolith chemistry may, therefore, allow high-resolution reconstructions of temperature and water chemistry from aquatic environments where coral or sponge skeletons are not available (e.g., Patterson 1998). Trace elements and isotope values in otoliths may also serve as natural tags for

¹ Reprinted from *Geochimica et Cosmochimica Acta* Vol 64, Bath et al. Strontium and barium uptake in aragonitic otoliths of marine fish, pp 1705-1714, Copyright 2000, with permission from Elsevier.

identifying natal location and population structure of anadromous and marine fish species (Kennedy et al. 1997; Thorrold et al. 1998b). For instance, Swearer et al. (1999) have recently developed an approach for tracing the dispersal histories of larval reef fish recruits using differences in larval growth rates and otolith chemistry as a natural tag of either local retention within near-coastal waters or larval development within open ocean waters.

Before otoliths can be used to reconstruct water chemistry, it is necessary to validate the assumption that trace metals in otoliths are deposited in proportion to dissolved concentrations in the ambient environment. This assumption is controversial, with good reason (Campana 1999). Otolith aragonite crystallizes from fluid within the endolymphatic canal of the inner ear. Bicarbonate, calcium, and at least some trace metal ions in the endolymphatic fluid are derived primarily from the ambient water (Farrell and Campana 1996; Thorrold et al. 1997a). However, these ions must first pass from the water into the blood plasma via branchial or intestinal membranes, and then cross another membrane into the endolymph. There is clearly potential for decoupling of free ion concentrations across the branchial membrane, as ion barriers are essential for any organism with high osmoregulatory requirements. Variations in the levels of metal-binding proteins within the blood plasma and the endolymphatic fluid may further complicate any correlation between water and otolith chemistry (Kalish 1991).

Any relationship between seawater composition and otolith chemistry will be determined by the kinetics of ion transport from water to the precipitating surface, but will also be a function of the mechanism by which the trace elements are incorporated into otolith aragonite. Divalent metals like Sr^{2+} and Ba^{2+} that have ionic radii similar to

Ca^{2+} are generally considered to substitute for Ca^{2+} ions in the orthorhombic aragonite lattice (Speer 1983), at least in low-Sr aragonite such as fish otoliths (Gregor et al. 1997). For these elements, partitioning between aqueous and solid aragonite phases can be conveniently described by a distribution coefficient. Boyle (1988) and Lea and Spero (1992, 1994) outline an approach that uses an empirically determined distribution coefficient, termed a partition coefficient by Morse and Bender (1990), to characterize the deposition of metal cations into biogenic carbonates. The trace metal composition of otoliths ($[\text{Me}/\text{Ca}]_{\text{otolith}}$) can be related to that of the water ($[\text{Me}/\text{Ca}]_{\text{water}}$) by way of this partition coefficient (D_{Me}), where

$$(2.1) \quad \left[\frac{\text{Me}}{\text{Ca}} \right]_{\text{otolith}} = D_{\text{Me}} \left[\frac{\text{Me}}{\text{Ca}} \right]_{\text{water}}$$

This approach may be particularly useful in otolith and mollusc shell studies, where depositional surfaces are not in direct contact with the water and aragonite formation is mediated by water-soluble proteins (Asano and Mugiya 1993; Belcher et al. 1996; Falini et al. 1996).

Partition coefficients for any carbonate system may also be a function of physical parameters such as temperature and precipitation rate. Temperature is perhaps the most widely studied of these parameters in biogenic carbonates. Negative relationships between Sr/Ca ratios and temperature have been reported for coral skeletons (e.g., Beck et al. 1992; Shen et al. 1996). However, the slope of this relationship is significantly larger than that of inorganic aragonite (Kinsman and Holland 1969), suggesting that kinetic and/or vital effects must also play a role (Hart and Cohen 1996). The influence of

rate-dependent processes on Sr incorporation is well established for inorganic carbonates (e.g., Lorens 1981; Rimstidt et al. 1998), but it remains uncertain if precipitation rate is an important parameter controlling Sr/Ca ratios in biogenic aragonite (deVilliers et al. 1994; Shen et al. 1996). Less is known about the factors determining D_{Ba} in biogenic aragonite. Lea et al. (1989) and Hart and Cohen (1996) noted positive correlations between quasi-annual cycles of Sr/Ca and Ba/Ca in corals, suggesting that either temperature or a correlated variable such upwelling intensity may influence D_{Ba} to some degree. Obviously it is necessary to characterize this relationship, if indeed any relationship exists, before it will be possible to reconstruct dissolved Ba concentrations in seawater from otolith aragonite.

To calculate partition coefficients for the uptake of Sr and Ba in fish otoliths, and the possible effects of external variables such as temperature, the composition of the ambient water must be known. This is achieved most easily and accurately under laboratory conditions. Lea and Spero (1992, 1994), Mashiotto et al. (1997), and Lea et al. (1999) cultured planktonic foraminifera in the lab to calculate Mg/Ca, Sr/Ca, Cd/Ca and Ba/Ca partition coefficients for shell calcite. However, this approach has rarely been applied to the study of trace metals in otolith aragonite.

In this study, an experiment is described in which juveniles of an estuarine-dependent species of marine fish, *Leiostomus xanthurus*, were reared under controlled laboratory conditions to determine if Sr/Ca and Ba/Ca in otoliths are proportional to their concentrations in the rearing water. The effects of temperature on both Sr/Ca and Ba/Ca partition coefficients are also investigated. Finally, because fish were maintained under controlled conditions, the amount of otolith material deposited during the experiment was

quantifiable. These data provide a test of the influence of precipitation rate on the chemistry of otolith aragonite.

Materials and methods

Larval rearing

Spot (*Leiostomus xanthurus*) were spawned 22 November 1997 at the National Marine Fisheries Service, Southeast Fisheries Science Center in Beaufort, North Carolina for the experiment, assuring that the larvae were from the same brood stock and of known age. Larvae were reared in natural seawater at 30psu salinity, and in a common tank until 42 days after hatching, at which time they were transferred to the experimental tanks. Mortality rates of new-hatched larval fish are generally high (>90%), and hence by rearing the fish for a period before initiating the experiment adequate survival rates of the experimental fish were ensured. At the outset of the experiment, fish were randomly distributed among a total of 24 acid-washed 20 L high-density polyethylene tanks at a density of 2 fish·L⁻¹ and acclimated over several days to the 20psu salinity experimental conditions.

To minimize the possibility of contamination of water during the experiment, all tanks were located within a PVC frame covered with polyethylene sheeting. A continuous supply of filtered air, provided by a 0.2 µm HEPA unit, maintained positive pressure within the enclosure throughout the experiment. Room temperature was maintained at 18 °C, and aquarium heaters within each of the tanks were used to achieve desired temperatures of either 20 °C or 25 °C. The light:dark cycle was controlled at 12 hr:12 hr for the duration of the experiment. Fish were fed enriched *Artemia* for the first

two weeks of the experiment, and thereafter fed an artificial diet (Hi-Pro Starter, 0.5 and 0.7 mm, Corey Feed Mills, LTD.).

Artificial seawater (Instant Ocean®) was used as the water source throughout the experiment. Triplicate experimental tanks were randomly assigned 4 levels of Sr/Ca corresponding to ambient and then 1.2x, 1.4x and 1.8x ambient levels, and Ba/Ca corresponding to ambient and then 3x, 6x, and 10x ambient levels. The Sr and Ba spiked water was prepared by adding appropriate amounts of standard solutions (SPEX) of SrCl_2 and BaCl_2 to each of the tanks. To maintain water quality and the desired elemental concentrations in the tanks, water was changed at 50% volume daily. The new water was spiked before being added to the tanks to ensure that dissolved Sr and Ba levels were maintained at the desired levels throughout the experiment. Water samples were collected from each tank every second day of the experiment. These samples were filtered through 0.22 μm cellulose nitrate membrane filters, acidified with trace metal grade 12 N HCl to pH 2, and then stored frozen-acidified for subsequent analysis. Water temperature, salinity, and pH were also recorded daily (Table 2.1).

Otolith and water analyses

At the termination of the experiment, all remaining fish were measured, and then frozen in individual plastic bags. Sagittal otolith pairs were removed from the fish and scraped clean with acid-washed glass probes in a class-100 cleanroom. Otoliths were sonicated in Milli-Q water for 7 minutes and triple rinsed with ultrapure H_2O_2 , followed by three sequential rinses of Milli-Q water and placed on acid-washed glass slides to dry for 36 hours under a class-100 laminar flow hood. After drying, otolith pairs were

weighed to the nearest 10 μg and transferred to acid-washed 1.5 ml high-density polyethylene vials. Otoliths from a subsample of fish archived at the start of the experiment were also removed and weighed to determine the proportion of otolith material in the experimental fish deposited during the initial larval rearing. Otoliths from these fish averaged less than 50 μg and I therefore concluded that conditions during the initial rearing period had little effect on the resultant otolith chemistry of the experimental fish.

Table 2.1. Summary of mean water temperature (T), pH, and dissolved Sr/Ca ($\text{mmol}\cdot\text{mol}^{-1}$) and Ba/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) levels within each of the 24 individual tanks during the course of the experiment.

Tank #	T ($^{\circ}\text{C}$)	pH	Sr/Ca	Ba/Ca	Tank #	T ($^{\circ}\text{C}$)	pH	Sr/Ca	Ba/Ca
1	20.8	8.00	15.21	151.16	13	20.3	7.97	17.79	22.98
2	25.2	8.05	17.40	71.41	14	20.3	7.94	22.55	215.56
3	25.2	8.00	16.03	25.48	15	25.1	8.04	12.75	211.75
4	20.3	7.97	15.17	23.90	16	25.0	8.03	15.23	144.87
5	20.6	7.96	22.36	138.83	17	24.7	8.02	13.03	74.27
6	20.4	8.02	17.68	72.30	18	25.5	7.98	18.29	231.45
7	24.8	8.01	22.74	148.14	19	20.3	8.00	17.81	75.09
8	25.2	7.98	17.88	20.85	20	24.9	8.00	13.39	142.93
9	25.1	8.00	22.47	22.93	21	19.7	7.98	15.00	70.96
10	20.3	7.95	12.73	155.85	22	20.4	8.01	12.60	222.20
11	20.4	7.91	12.54	228.09	23	24.5	8.01	15.02	24.88
12	25.4	8.04	22.50	70.21	24	20.0	7.98	22.80	23.19

Otolith pairs were prepared for Sr/Ca and Ba/Ca analysis by isotope dilution inductively coupled plasma mass spectrometry (ICP-MS). Samples were dissolved in

approximately 300 μl of 10% re-distilled nitric acid solution containing the enriched isotopes of the metals targeted for isotope dilution along with the internal standard. The enriched spike solution contained ^{87}Sr and ^{137}Ba , along with an internal standard, ^{69}Ga , which was used to quantify Ca. All analyses were run on a Perkin-Elmer Elan 6000 ICP-MS equipped with a high-efficiency pneumatic nebulizer. The analyses were run in peak-hopping mode, and monitored ^{46}Ca , ^{69}Ga , ^{87}Sr , ^{88}Sr , ^{137}Ba and ^{138}Ba . The estimated limits of detection (3σ based on a 1 mg otolith mass in a 0.3 ml final volume) were 500 $\mu\text{g}\cdot\text{g}^{-1}$ for Ca, 6 $\mu\text{g}\cdot\text{g}^{-1}$ for Sr, and 40 $\text{ng}\cdot\text{g}^{-1}$ for Ba.

Analyses of water samples collected during the experiment were also conducted using isotope dilution ICP-MS. Samples were selected at weekly intervals, including the start and end of experiment, so that a total of six samples were run from each tank. All samples were spiked with a solution containing ^{86}Sr and ^{137}Ba , along with an internal standard, ^{45}Sc , which was used to quantify Ca levels. The solutions were then aspirated directly into a Turner SOLA ICP-MS, and the peak-hopping mode was again used to monitor ^{45}Sc , ^{46}Ca , ^{87}Sr , ^{88}Sr , ^{137}Ba and ^{138}Ba . Analyses of water samples were conducted in either duplicate or triplicate, and values presented here are means of the replicate analyses (Figs. 2.1, 2.2).

The use of artificial seawater presented two potential difficulties. First, Sr/Ca levels of seawater made from “Instant Ocean” salts are slightly higher, at 12 $\text{mmol}\cdot\text{mol}^{-1}$, than that found in normal seawater (typically 8.5-9 $\text{mmol}\cdot\text{mol}^{-1}$). However, little could be done to lower this value; although dilution will lower absolute Sr levels, it will not change Sr/Ca ratios in the water. The highest Sr/Ca values in this experiment were, therefore, approximately 2.5x that of normal seawater. This was not a problem with

ambient Ba/Ca levels, as spiked levels within the tanks spanned a range (23 – 230 $\mu\text{mol}\cdot\text{mol}^{-1}$), which would commonly be encountered by estuarine-dependent fish along the east coast of the United States (Coffey et al. 1997). Second, artificial seawater was used in an attempt to minimize variations in baseline Sr and Ba concentrations in the tanks. However, Sr/Ca levels fluctuated to some degree throughout the experiment (Fig. 2.1). This will have had the effect of increasing the variance of otolith Sr/Ca within individual tanks if fish were growing at different rates during the experiment. Given the coherence in Sr/Ca levels among tanks through time, mean values of otolith Sr/Ca from each of the tanks shouldn't have been unduly affected by this variability.

Fig. 2.1. Mean Sr/Ca ratios (\pm SD) at ambient (\diamond) and 1.25x (\blacktriangle), 1.5x (\blacksquare) and 2x (\bullet) ambient levels at 20°C (filled symbols) and 25°C (open symbols), from weekly sampling throughout the experiment, along with mean values (\pm SD) for each spike level/temperature combination (mean) over the duration of the experiment.

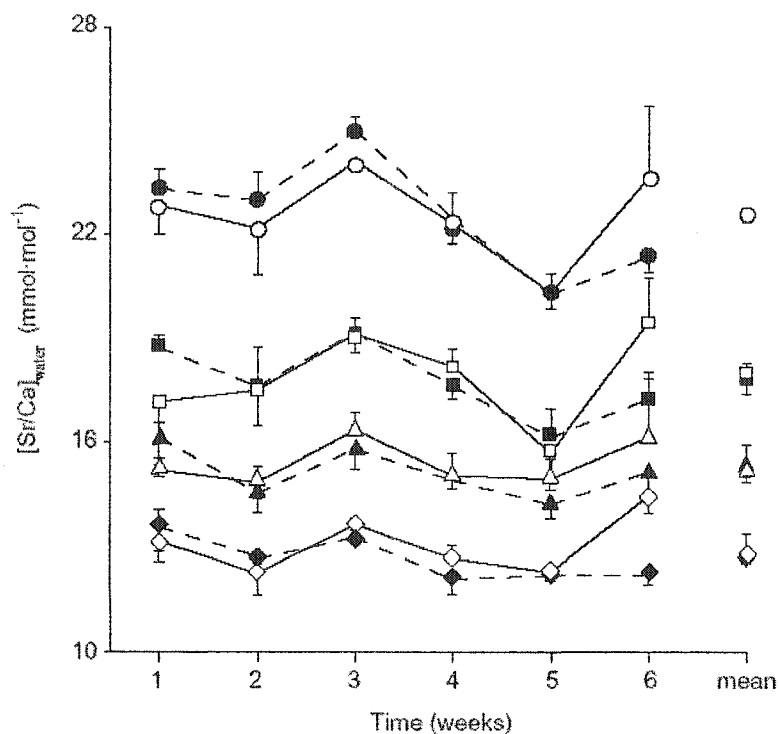
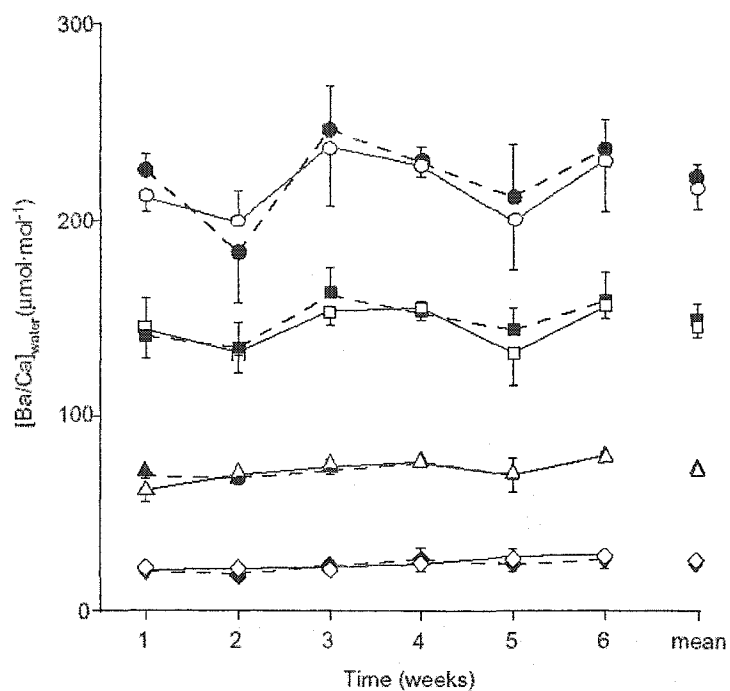


Fig. 2.2. Mean Ba/Ca ratios (\pm SD) at ambient (\diamond) and 1.25x (\blacktriangle), 1.5x (\blacksquare) and 2x (\bullet) ambient levels at 20°C (filled symbols) and 25°C (open symbols), from weekly sampling throughout the experiment, along with mean values (\pm SD) for each spike level/temperature combination (mean) over the duration of the experiment.

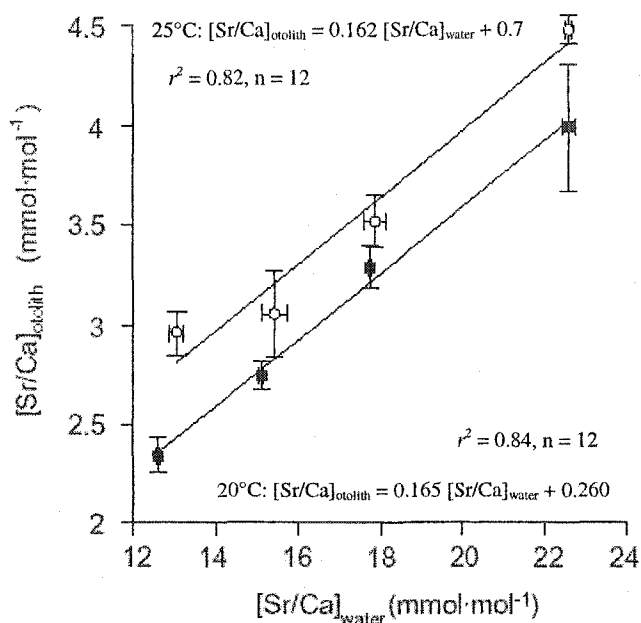


Results

Sr/Ca ratios

The Sr/Ca ratios of otoliths from 214 juvenile *L. xanthurus* ranged from 1.85 to 6.77 mmol·mol⁻¹, with an overall mean of 3.3 mmol·mol⁻¹. Using tanks as the appropriate unit of replication, Sr/Ca ratios in otoliths were directly proportional to the Sr/Ca of the water in which the fish were raised (Fig. 2.3).

Fig. 2.3. Mean Sr/Ca ratios ($[\text{Sr}/\text{Ca}]_{\text{otolith}} \pm \text{SE}$) in otoliths of lab-reared *Leiostomus xanthurus* plotted against Sr/Ca ratios of the rearing water ($[\text{Sr}/\text{Ca}]_{\text{water}} \pm \text{SE}$) at either 20 °C (■) or at 25 °C (○). Lines were fitted by linear least-squares regression for each of the temperature treatments.



Least squares regression described a linear relationship ($r^2 = 0.84$) between $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ and $[\text{Sr}/\text{Ca}]_{\text{water}}$ at 20 °C

$$(2.2) \quad [\text{Sr}/\text{Ca}]_{\text{otolith}} = 0.165 \pm 0.052 \text{ (95\% CI)} [\text{Sr}/\text{Ca}]_{\text{water}} + 0.260 \pm 0.897 \text{ (95\% CI)}$$

and a linear relationship at 25 °C ($r^2 = 0.82$)

$$(2.3) \quad [\text{Sr}/\text{Ca}]_{\text{otolith}} = 0.162 \pm 0.054 \text{ (95\% CI)} [\text{Sr}/\text{Ca}]_{\text{water}} + 0.70 \pm 0.954 \text{ (95\% CI)}.$$

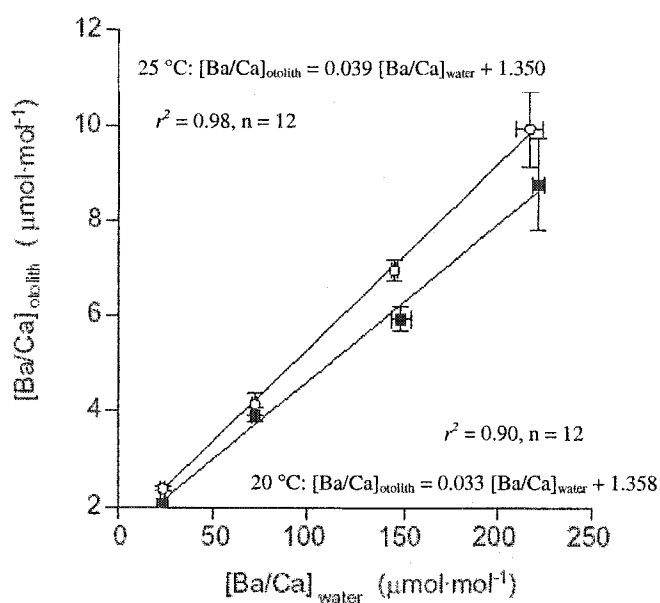
Partition coefficients (D_{Sr}) were calculated for both of the temperature treatments directly from $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ and $[\text{Sr}/\text{Ca}]_{\text{water}}$ data for each of the individual tanks. Note that this is

algebraically equivalent to constraining regression lines through a zero intercept, on the basis that fish living in seawater without Sr would be expected to have no Sr in their otoliths. Estimates of D_{Sr} were 0.182 ± 0.011 (95% CI) at 20 °C and 0.205 ± 0.04 (95% CI) at 25 °C.

Ba/Ca ratios

The Ba/Ca ratios of otoliths from juvenile *L. xanthurus* ranged from 1.7 to 15.2 $\mu\text{mol}\cdot\text{mol}^{-1}$, with an overall mean of 5.59 $\mu\text{mol}\cdot\text{mol}^{-1}$. Otolith Ba/Ca ratios were directly proportional to $[\text{Ba/Ca}]_{\text{water}}$ (Fig. 2.4) at both temperatures.

Fig. 2.4. Mean Ba/Ca ratios ($[\text{Ba/Ca}]_{\text{otolith}} \pm \text{SE}$) in otoliths of lab-reared *Leiostomus xanthurus* plotted against Ba/Ca ratios of the rearing water ($[\text{Ba/Ca}]_{\text{water}} \pm \text{SE}$) at either 20°C (■) or at 25°C (○). Lines were fitted by linear least-squares regression for each of the temperature treatments.



A linear relationship ($r^2 = 0.90$) between $[\text{Ba}/\text{Ca}]_{\text{otolith}}$ and $[\text{Ba}/\text{Ca}]_{\text{water}}$ at 20 °C was described by least squares regression as:

$$(2.7) \quad [\text{Ba}/\text{Ca}]_{\text{otolith}} = 0.033 \pm 0.007 \text{ (95\% CI)} [\text{Ba}/\text{Ca}]_{\text{water}} + 1.358 \pm 1.042 \text{ (95\% CI)}.$$

A similar linear relationship ($r^2 = 0.98$) was found at 25 °C:

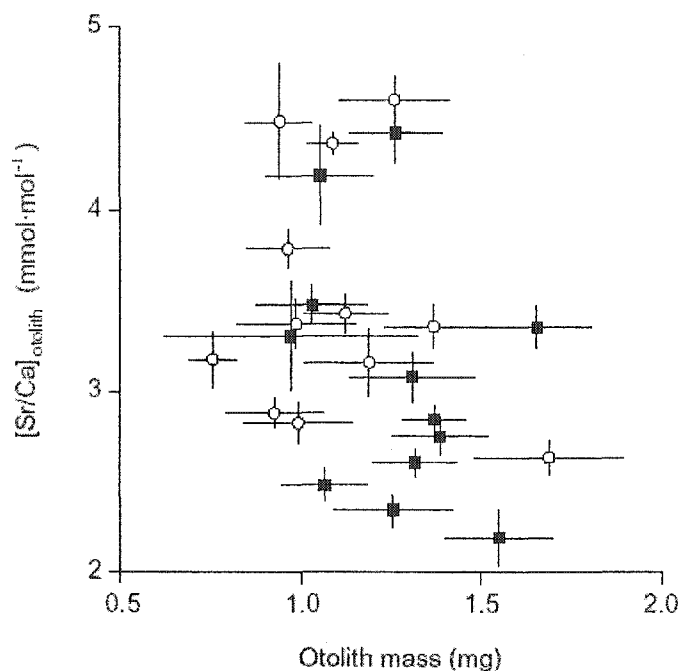
$$(2.8) \quad [\text{Ba}/\text{Ca}]_{\text{otolith}} = 0.039 \pm 0.004 \text{ (95\% CI)} [\text{Ba}/\text{Ca}]_{\text{water}} + 1.350 \pm 0.591 \text{ (95\% CI)}.$$

D_{Ba} was calculated directly from the $[\text{Ba}/\text{Ca}]_{\text{otolith}}$ and $[\text{Ba}/\text{Ca}]_{\text{water}}$ data, and found values of 0.06 ± 0.06 (95% CI) at 20 °C and 0.06 ± 0.07 (95% CI) at 25 °C.

Rate effects on otolith Sr/Ca and Ba/Ca

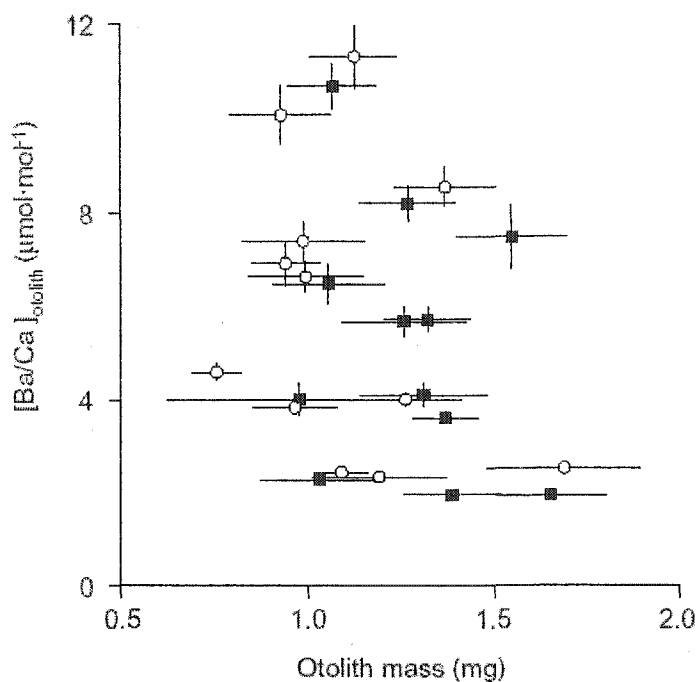
There was no significant correlation between otolith mass and Sr/Ca ratios (Fig. 2.6), averaged within each of the experimental tanks ($r = -0.314$, $p = 0.134$). There were no significant differences in otolith mass between the two temperatures ($F_{(1, 20)} = 0.022$, $p = 0.882$). There was also no relation between Sr incorporation and fish growth rate, given the high correlation between fish standard length and otolith mass ($r = 0.902$, $n = 24$). Correlations between Sr/Ca ratios and individual otolith mass within each of the tanks were also examined and 23 of the 24 correlations were negative. However, only one of the correlations was statistically significant, after adjusting the experiment-wise error to take into account the number of correlations performed.

Fig. 2.5. Mean otolith Sr/Ca values (\pm SE) from lab-reared *Leiostomus xanthurus* plotted against mean otolith mass (\pm SE) for each of 24 rearing tanks maintained at either 20 °C (■) or at 25 °C (○).



Barium incorporation into otoliths is unrelated to precipitation rate, as evidenced by a non-significant correlation ($r = 0.177$, $p = 0.4078$) between the two variables averaged within each tank (Fig 2.7). Within-tank correlations were similarly weak, with only 1 tank out of a total of 24 being statistically significant.

Fig. 2.6. Mean otolith Ba/Ca values (\pm SE) from lab-reared *Leiostomus xanthurus* plotted against mean otolith mass (\pm SE) for each of 24 rearing tanks maintained at either 20 °C (■) or at 25 °C (○).



Discussion

Sr/Ca ratios

It is apparent that Sr/Ca values in few, if any, inorganic or biogenic aragonites can be explained on the basis of thermodynamic considerations alone. Aragonite from hematypic coral skeletons typically have Sr/Ca values close to that of inorganic aragonite, with D_{Sr} values of both systems ranging from 1 to 1.2, while the theoretical $D_{\text{Sr(equil)}}$ based on thermodynamic considerations is 0.095 (Plummer and Busenberg 1987). This lack of equilibration is presumably due to kinetic processes at the crystal surface and within the solution boundary layer of inorganic aragonite, along with unknown vital effects in coral skeletons (Hart and Cohen 1996). Strontium uptake in

otolith aragonite is also out of equilibrium with the ambient water, although apparently not to the extent of either inorganic aragonite or coral skeletons. In a study of several marine fish species, Kalish (1991) estimated D_{Sr} to be 0.18 ± 0.04 , a value almost identical to that found in the present study. However, Kalish measured Sr/Ca in the endolymphatic fluid rather than seawater, implying that Sr/Ca in the endolymph accurately tracks Sr/Ca values in seawater. Partition coefficients for aragonite in mollusc shells are also lower than inorganic aragonite, ranging from 0.23 to 0.31 (Stecher et al. 1996). Otoliths and mollusc shells are similar in that the aragonite precipitates from a highly regulated internal body fluid rather than seawater. Hence, although D_{Sr} of both fish otoliths and mollusc shells are quite close to equilibrium values, it would be premature to conclude that these structures precipitate closer to thermodynamic equilibrium than inorganic aragonite and coral skeletons without more information on free ion concentrations within the endolymphatic and extrapallial fluids.

The observation that Sr/Ca values in fish otoliths were reasonably close to thermodynamic equilibrium was surprising given the potential for regulation of both Sr and Ca ions across membranes and within the blood plasma. However, the observation that Sr/Ca ratios in otoliths are deposited in direct proportion to Sr/Ca in the ambient water was more important in the context of using Sr/Ca ratios in otoliths as an environmental proxy. Although there has been growing acceptance of the observation that large differences in $[\text{Sr}/\text{Ca}]_{\text{water}}$ (i.e., from marine to freshwater systems) are faithfully recorded by otoliths (Campana 1999), this study suggests that more subtle variations will be also be recoverable. It should be noted that there was some scatter in relationship between $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ and $[\text{Sr}/\text{Ca}]_{\text{water}}$ within individual tanks. At least some

of this variance may be due to temporal changes in $[\text{Sr}/\text{Ca}]_{\text{water}}$ of individual tanks (Fig. 2.1), despite the attempts to minimize such differences.

This experiment was not designed to calibrate the temperature dependence of D_{Sr} , as fish were only reared at two temperatures. However, it is possible to get a first-order estimate of the relationship between D_{Sr} and temperature ($n = 2$) in otoliths based on these results. Least-squares regression of D_{Sr} and temperature (T) suggests a significant linear relationship

$$(2.4) \quad D_{\text{Sr}} = 0.0046 T^{\circ}\text{C} + 0.089 \quad (r^2 = 0.62).$$

The most obvious difference between the relationship and that found in corals is that temperature is positively, not negatively, correlated with D_{Sr} , although the degree of temperature dependence is similar. For instance, Shen et al (1996) found the following relationship between D_{Sr} and temperature in *Porites* corals:

$$(2.5) \quad D_{\text{Sr}} = -(0.006011 T^{\circ}\text{C}) + 1.2077.$$

Results from earlier studies on the effect of temperature on Sr/Ca ratios in fish otoliths are contradictory, in both the direction and magnitude of the temperature dependence. Negative (e.g., Radtke et al. 1990; Townsend et al. 1995), positive (Kalish 1989; Arai et al. 1996) and no relationships (Gallahar and Kingsford 1996; Tzeng 1996) between Sr/Ca and temperature have been reported in the literature. Limited data from marine mollusc shells, which like otoliths are low-Sr aragonite, suggest a positive relationship between

Sr/Ca ratios and temperature (Stecher et al. 1996; ref. 23 in Hart and Blusztajn 1998), although Buchardt and Fritz (1978) found that Sr incorporation was independent of temperature in a freshwater gastropod. Data were reanalyzed from a laboratory study on the effects of temperature and salinity on trace element chemistry of another species of sciaenid species, *Micropogonias undulatus* (Fowler et al. 1995a), assuming that there were no differences in $[\text{Sr/Ca}]_{\text{water}}$ among tanks since all had a common water source. Although only five tanks at two temperatures were available, there was a significant positive relationship between D_{Sr} and temperature,

$$(2.6) \quad D_{\text{Sr}} = 0.0086 T ^\circ\text{C} + 0.124 \quad (r^2 = 0.85).$$

The relationship for *M. undulatus* otoliths is not significantly different from that of *L. xanthurus* otoliths determined in the present study. Clearly, these data are preliminary and the temperature dependence of D_{Sr} in fish otoliths will require careful calibration for individual species of interest. However, it may be possible to reconstruct temperatures from Sr/Ca ratios in otoliths where $[\text{Sr/Ca}]_{\text{water}}$ can be adequately constrained.

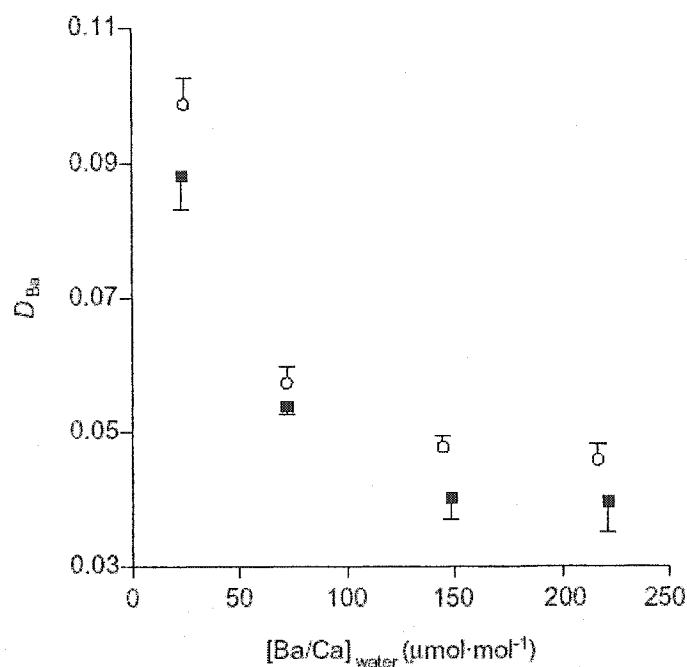
Ba/Ca ratios

The D_{Ba} estimates calculated in this chapter are significantly lower than partition coefficients for hermatypic corals (~ 1.3 , Lea et al. 1989), but are probably close to values for aragonitic mollusc shells (Stecher et al. 1996). Unlike Sr, no significant effect of temperature on D_{Ba} was found. Although Lea et al. (1989) reported quasi-periodic oscillations in coral Ba/Ca that were correlated with the seasonal temperature and

upwelling cycles, later studies have found little evidence of a temperature effect on Ba/Ca in coral aragonite in the absence of upwelling (Sinclair et al. 1998). Rather, as with Ba/Ca in foraminifera shells (Lea and Spero 1992, 1994), Ba/Ca ratios in otoliths appeared to be accurately recording changes in the Ba/Ca composition of the ambient water, and were not influenced by temperature.

The relatively large standard deviations around the estimates of Ba partition coefficients were due to a non-linearity in the relationship between D_{Ba} and $[Ba/Ca]_{water}$ at both 20 °C and 25 °C (Fig. 5). These data suggest that proportionally more Ba was incorporated in otoliths at low $[Ba/Ca]_{water}$ values when normalized to Ca. Although this does not affect our ability to recover dissolved Ba concentrations from Ba/Ca ratios in otoliths over the $[Ba/Ca]_{water}$ range in this experiment (equation 2.3), extrapolation beyond these points would not be justified without further data. It is difficult to speculate the cause of this non-linearity without information on ion transport within the fish. It may be that proportionally more Ba, relative to Ca, was transported to the endolymphatic fluid in the low ambient Ba treatments than those tanks with higher Ba levels, up to a threshold level at approximately $150 \mu\text{mol}\cdot\text{mol}^{-1}$. Non-linear uptake of potentially toxic heavy metals across the branchial membrane has been documented in freshwater fishes (Olsson et al. 1988). Alternatively, discrimination may be occurring at the crystal surface, perhaps due to saturation of kink sites suitable for Ba^{2+} attachment (e.g., Watson 1996), or some other kinetic process. Distinguishing between biological and kinetic effects should be possible by examining Ba/Ca levels in blood plasma and endolymphatic fluid, along with $[Ba/Ca]_{otolith}$ and $[Ba/Ca]_{water}$, and such experiments should be conducted in future work.

Fig. 2.7. Relation between estimates (\pm SE) of Ba partition coefficients (D_{Ba}) for otoliths of lab-reared *Leiostomus xanthurus* and Ba/Ca ratios of the rearing water ($[\text{Ba/Ca}]_{\text{water}}$) at either 20 °C (■) or at 25 °C (○).



Rate effects on otolith Sr/Ca and Ba/Ca

The effect of precipitation rate on trace metal incorporation in biogenic aragonite remains ambiguous. Rate effects have generally not been found in synthetic aragonite studies (Kinsman and Holland 1969; Zhong and Mucci 1989) although they have been widely documented in synthetic calcite precipitates (e.g., Lorens 1981; Tesoriero and Pankow 1996; Rimstidt et al. 1998). It has proved similarly difficult to document rate effects in biogenic aragonite. Several studies have found Sr/Ca ratios correlated with coral extension rates (e.g., Weber 1973; deVilliers et al. 1994, 1995), while other workers have found no such relationship (e.g., Shen et al. 1996). Data on rate effects in mollusc aragonite are sparse compared to corals, but are equally contradictory. Stecher et al. (1996) speculated that seasonal differences in shell growth rates generated quasi-periodic

cycles in Sr/Ca ratios in two species of bivalve mollusc. In contrast, Buchardt and Fritz (1978) found that Sr incorporation in the shells of a gastropod *Limnaea stagnalis* were independent of growth rate. My data allowed a definitive test of the relationship between precipitation rate and Sr/Ca and Ba/Ca ratios in otoliths, as the mass of individual fish otoliths provided an excellent proxy for average precipitation rate during the experiment.

There was no significant correlation between otolith mass and Sr/Ca ratios, averaged within each of the experimental tanks, suggesting that these data were not confounded by differences in biomineralization rates among the tanks. This conclusion was strengthened by the observation that there were no significant differences in otolith mass between the two temperatures. That is, the temperature dependence of D_{Sr} was not driven by differences in precipitation rates among tanks. This further implied that there was also no relation between Sr incorporation and fish growth rate, given the high correlation between fish standard length and otolith mass. Rate effects may be masked by temperature and water chemistry differences among tanks, so correlations between Sr/Ca ratios and individual otolith mass within each of the tanks were also examined. These data provided some evidence of a relation between Sr/Ca and otolith mass, as 23 of the 24 correlations were negative. However, only one of the correlations was statistically significant, after adjusting the experiment-wise error to take into account the number of correlations performed.

Barium incorporation into otoliths is unrelated to precipitation rate, as evidenced by a non-significant correlation between the two variables averaged within each tank. Within-tank correlations were similarly weak, with only 1 tank out of a total of 24 being statistically significant. As for Sr, this result implied that there was also no relation

between fish growth and Ba/Ca ratios in otoliths. Metabolic influences, at least as manifested by individual fish growth rates, were not a principal determinant of Sr and Ba incorporation in fish otoliths.

In summary, otolith Sr/Ca and Ba/Ca ratios are deposited in proportion to their respective ratios in ambient waters. It should be possible, therefore, to reconstruct Sr/Ca and Ba/Ca levels in environments inhabited by fish based on otolith chemistry. Evidence of a non-linearity between D_{Ba} and $[\text{Ba/Ca}]_{\text{water}}$ suggests, however, that careful calibration of the relation between Ba/Ca levels in otoliths and water will be required before extrapolating the results to lower Ba/Ca environments and to other species.

The estimates of D_{Sr} for otoliths from this study are close to the theoretical distribution coefficient for aragonite based on thermodynamic equilibrium, although this may be due, at least in part, to differential uptake of Ca relative to Sr across the membranes separating the otolith from the ambient environment.

Temperature was positively related to D_{Sr} , unlike inorganic aragonite and coral skeletons in which the temperature dependence of D_{Sr} is negative. Although an adequate temperature calibration for Sr/Ca ratios could not be provided, temperatures may be reconstructed from juvenile *L. xanthurus* otoliths once this calibration has been achieved. Temperature had no detectable influence on D_{Ba} , suggesting that most of the variation in Ba/Ca ratios in otoliths reflects concomitant variability in the Ba/Ca composition of the environment.

Effects of precipitation rate on Sr and Ba incorporation in otolith were weak and generally statistically insignificant. Metabolic effects were similarly weak, using individual fish growth rates as a measure of metabolic activity. Rather, Sr and Ba

incorporation in otoliths is primarily a function of the chemistry of the ambient environment, as modified by temperature in the case of Sr. Otoliths represent an excellent, and as yet underutilized, record of the physicochemical properties of both modern and ancient aquatic environments.

CHAPTER III
TEMPERATURE AND SALINITY EFFECTS ON STRONTIUM
INCORPORATION IN OTOLITHS OF LARVAL SPOT
(*LEIOSTOMUS XANTHURUS*)

Introduction

Inspired by Odum's (1951a,b) and Turekian's (1964) pioneering work on the biogeochemical cycling of strontium (Sr) in marine environments, scientists have used strontium concentrations of marine carbonate structures to infer temperatures of present and past marine environments. Strontium thermometry based on aragonite in coral skeletons may reveal temperature variation in marine environments over annual to millennial time scales (e.g., Beck et al. 1992; Alibert and McCulloch 1997). Several studies have also suggested that Sr deposition in the calcite tests of marine foraminifera may be temperature dependent (e.g., Lea and Martin 1996; Elderfield et al. 1996; Lea et al. 1999). Similarly, strontium/calcium (Sr/Ca) ratios in aragonite mollusc shells have been used to discern temperature and salinity variability in estuarine environments (Dodd and Crisp 1982; Stecher et al. 1996).

The geochemistry of otolith aragonite may also record temperature and elemental composition of ambient environments (Campana 1999). Otoliths are metabolically inert calcium carbonate structures that are formed by concentric daily growth increments in teleost fishes (Campana and Neilson 1985; Jones 1986). The chemical composition of otoliths may, in turn, reflect that of the surrounding water (Farrell and Campana 1996; Milton and Chenery 2001), as modified by temperature (Thorrold et al. 1997a; Bath et al.

2000). This observation has led to the development of applications using otolith chemistry in conjunction with the chronological properties of otoliths to retrospectively track larval-transport pathways through time (Tzeng and Tsai 1994; Thorrold et al. 1997a). Otolith Sr/Ca values have also been used to examine transport pathways as a function of ambient temperature (Radtke 1989; Townsend et al. 1989; Gallahar and Kingsford 1996) or salinity (Kalish 1990; Halden et al. 1995; Secor et al. 1995).

Strontium ions have the same valence and a similar ionic radius as calcium ions and are readily incorporated into aragonite by solid substitution for Ca according to the following equation:



The amount of substitution is a function of the partition coefficient (D_{Sr}) between aragonite and the fluid from which the Ca and Sr ions precipitate. In most otolith studies the partition coefficient is expressed relative to the ambient water, due to difficulties measuring concentrations in the endolymphatic fluid surrounding the otolith (Bath et al. 2000; Milton and Chenery 2001; but see Kalish 1991). Therefore,

$$(3.2) \quad [\text{Sr/Ca}]_{\text{otolith}} = D_{Sr} [\text{Sr/Ca}]_{\text{water}}$$

where D_{Sr} is the partition or distribution coefficient representing the ratio between the Sr/Ca of the calcium carbonate structure and ambient water (Morse and Bender 1990).

Strontium thermometry relies upon the observation that the Sr/Ca partition coefficient is temperature-dependent. Because biogenic aragonite is typically not in thermodynamic equilibrium, the exact form of the temperature dependence is usually determined empirically. Laboratory and *in situ* studies have been used to validate the temperature dependence of D_{Sr} in aragonitic coral skeletons (e.g., Swart 1979; Smith et al. 1979; Shen et al. 1996). However, despite a number of apparently successful validation studies, there remains vigorous debate concerning the degree to which Sr levels in coral skeletons are affected by temperature compared to biological processes [e.g., presence of algal symbionts (Cohen et al. 2002)].

The relationship between temperature and Sr/Ca ratios in fish otoliths is likely to be even more complicated than in coral skeletons because the otolith is isolated from seawater by branchial, intestinal, and the endolymphatic membranes. Proposed relationships between Sr/Ca and temperature include that Sr/Ca ratios and temperature were positively correlated (Fowler et al. 1995a; Arai et al. 1996), negatively correlated (Townsend et al. 1992), and uncorrelated (Gallahar and Kingsford 1996). It is not clear if these inconsistencies are a result of inter-specific variations in Sr uptake as a function of temperature or by unspecified methodological artifacts in some or all of the experiments.

In one of the few studies to carefully constrain water chemistry and temperature, Bath et al. (2000; Chapter II) found a positive relationship between D_{Sr} and temperature. However, their experiment was designed to test the influence of water chemistry on otolith composition, and only consisted of two temperature treatments at a constant salinity. My objectives in this chapter were to resolve the temperature dependence of the Sr/Ca partition coefficient in larval spot (*Leiostomus xanthurus*), and to examine if the

partition coefficient was also influenced by ambient salinity. Based on Bath et al.'s (2000) results, the null hypothesis is that Sr/Ca ratios in larval spot otoliths are not affected by temperature or salinity after appropriate correction for the Sr/Ca ratio of the ambient water. If the relation between Sr/Ca and temperature is sufficiently predictive, this would allow for reconstruction of temperature histories from the otoliths of individual spot larvae. Such reconstructions could, in turn, provide information on larval dispersal pathways that would be difficult, if not impossible, to gather using conventional approaches.

Materials and methods

Spot (*Leiostomus xanthurus*) were spawned and hatched on 10 December 1999 at the NOAA, National Ocean Service, Center for Coastal Fisheries and Habitat Research in Beaufort, North Carolina. Larvae were reared in a common tank in natural seawater at 34psu salinity for 42 days, at which time they were randomly distributed among 24 acid-washed 20 L high-density polyethylene tanks at a density of 2 fish•L⁻¹. Fish were acclimated to the experimental treatments for a week before initiating the experiment to ensure adequate survival of the experimental fish. The light:dark cycle was controlled at 12 h:12 h for the duration of the experiment. The fish were fed an artificial diet (Golden Pearls, 300-500 and 500-800 microns, Brine Shrimp Direct) twice daily *ad libitum*.

Experimental tanks were randomly assigned 2 salinity treatments (15 and 25psu) and four temperature treatments (17, 20, 23, and 26 °C). Three replicate tanks were used for each treatment combination (24 tanks in total). Room temperature was maintained at 16 °C, and aquarium heaters were used to regulate temperatures within individual tanks.

Artificial seawater (Instant Ocean®) mixed with deionized water was used to regulate salinity. Water was changed at 50% volume daily to maintain water quality and salinity.

Water samples from each tank were collected every third day and were filtered through 0.22 μm cellulose nitrate membrane filters, acidified with 12 N trace-metal grade HCl to pH 2, and then stored frozen for subsequent analysis. Water temperature, salinity, and pH were also recorded daily (Table 3.1). After termination of the experiment, all surviving fish were measured and frozen in individual plastic bags.

Table 3.1. Summary of mean water temperature (Temp., $^{\circ}\text{C}$, \pm standard error (SE)), salinity treatment (Sal., psu), [Sr] ($\mu\text{g}\cdot\text{g}^{-1}$, \pm SE), Ca ($\mu\text{g}\cdot\text{g}^{-1}$, \pm SE), dissolved Sr/Ca ($\text{mmol}\cdot\text{mol}^{-1}$, \pm SE), number of otoliths analyzed (n), mean otolith mass (OM [mg], \pm SE) and somatic growth rate (GR [$\text{mm}\cdot\text{d}^{-1}$], \pm SE) within each of the 24 tanks during the course of the experiment.

Tank #	Temp.	Sal.	[Sr]	[Ca]	[Sr/Ca]	n	OM	GR
9	17.5 \pm 0.1	15	7.87 \pm 0.43	234 \pm 16.9	15.5 \pm 0.35	7	0.95 \pm 0.23	0.47 \pm 0.040
11	18.1 \pm 0.1	15	6.92 \pm 1.73	199 \pm 49.4	15.8 \pm 0.28	7	0.99 \pm 0.19	0.46 \pm 0.03
20	18.5 \pm 0.1	15	6.89 \pm 0.54	188 \pm 15.6	16.8 \pm 0.85	8	0.62 \pm 0.08	0.31 \pm 0.02
24	20.1 \pm 0.1	15	6.72 \pm 1.68	200 \pm 50.5	15.6 \pm 0.43	8	0.71 \pm 0.21	0.36 \pm 0.02
5	20.8 \pm 0.1	15	7.25 \pm 0.79	203 \pm 23.8	16.5 \pm 1.02	7	0.99 \pm 0.29	0.45 \pm 0.04
18	21.2 \pm 0.2	15	8.57 \pm 0.50	253 \pm 11.8	15.5 \pm 0.30	8	0.88 \pm 0.14	0.42 \pm 0.02
10	22.7 \pm 0.3	15	6.87 \pm 0.42	199 \pm 14.2	15.9 \pm 0.44	8	1.21 \pm 0.18	0.52 \pm 0.02
21	23.4 \pm 0.2	15	8.10 \pm 0.58	235 \pm 15.3	15.8 \pm 0.41	8	1.13 \pm 0.23	0.48 \pm 0.03
22	23.8 \pm 0.4	15	7.33 \pm 0.22	216 \pm 11.9	15.6 \pm 0.39	8	0.79 \pm 0.27	0.37 \pm 0.03
4	26.1 \pm 0.2	15	7.81 \pm 0.45	223 \pm 10.8	16.1 \pm 0.69	8	1.21 \pm 0.29	0.50 \pm 0.04
14	26.2 \pm 0.2	15	8.42 \pm 0.47	246 \pm 12.3	15.6 \pm 0.21	8	0.96 \pm 0.23	0.42 \pm 0.03
16	26.8 \pm 0.2	15	7.36 \pm 1.05	215 \pm 31.1	15.7 \pm 0.16	8	1.24 \pm 0.20	0.50 \pm 0.02
1	17.5 \pm 0.1	25	10.33 \pm 0.91	334 \pm 39.1	14.4 \pm 0.67	8	0.61 \pm 0.26	0.32 \pm 0.02

Table 3.1 continued

7	17.8 ± 0.1	25	11.23 ± 0.68	366 ± 14.2	14.0 ± 0.65	4	0.79 ± 0.20	0.31 ± 0.03
15	18.7 ± 0.1	25	10.25 ± 1.04	332 ± 41.9	14.3 ± 0.60	8	0.54 ± 0.13	0.28 ± 0.02
2	19.9 ± 0.1	25	12.12 ± 1.32	382 ± 52.8	14.7 ± 0.62	8	0.69 ± 0.24	0.30 ± 0.02
8	20.2 ± 0.0	25	10.89 ± 1.01	356 ± 27.2	14.0 ± 0.71	8	0.63 ± 0.22	0.26 ± 0.03
6	20.3 ± 0.0	25	10.68 ± 0.25	349 ± 22.8	14.1 ± 0.78	8	0.70 ± 0.19	0.34 ± 0.02
23	23.2 ± 0.0	25	11.53 ± 0.83	376 ± 20.5	14.0 ± 0.70	8	0.94 ± 0.36	0.43 ± 0.05
12	23.3 ± 0.4	25	12.19 ± 0.41	382 ± 17.0	14.7 ± 0.54	7	1.03 ± 0.36	0.43 ± 0.05
13	23.5 ± 0.3	25	13.17 ± 0.66	410 ± 21.1	14.7 ± 0.37	8	0.88 ± 0.18	0.39 ± 0.02
19	25.6 ± 0.2	25	12.60 ± 0.69	406 ± 14.0	14.2 ± 0.45	8	0.91 ± 0.31	0.39 ± 0.05
3	25.8 ± 0.4	25	10.44 ± 1.64	321 ± 40.8	14.7 ± 0.55	1	0.88	0.33
17	26.3 ± 0.1	25	11.58 ± 1.36	371 ± 34.9	14.2 ± 0.52	6	1.06 ± 0.27	0.44 ± 0.04

Sagittal otolith pairs were removed from the fish and scraped clean with acid-washed glass probes in a class-100 cleanroom. Otoliths were ultrasonically cleaned in Milli-Q water for 7 minutes and triple rinsed with ultrapure H₂O₂ (Ultrex, J.T. Baker) followed by three sequential rinses of Milli-Q water. Otoliths were then placed on acid-washed glass slides to dry for 36 hours under a class-100 laminar flow hood. After drying, otoliths were individually weighed to the nearest 10 µg and transferred to acid-washed 1.5 mL high-density polyethylene vials.

Otoliths from fish archived at the start of the experiment (n = 12) were also removed and weighed to determine the proportion of otolith material in the experimental fish deposited during the initial larval rearing. Otoliths from 2 - 4 fish were pooled for each of three samples to assure adequate masses for analyses, decontaminated, and then

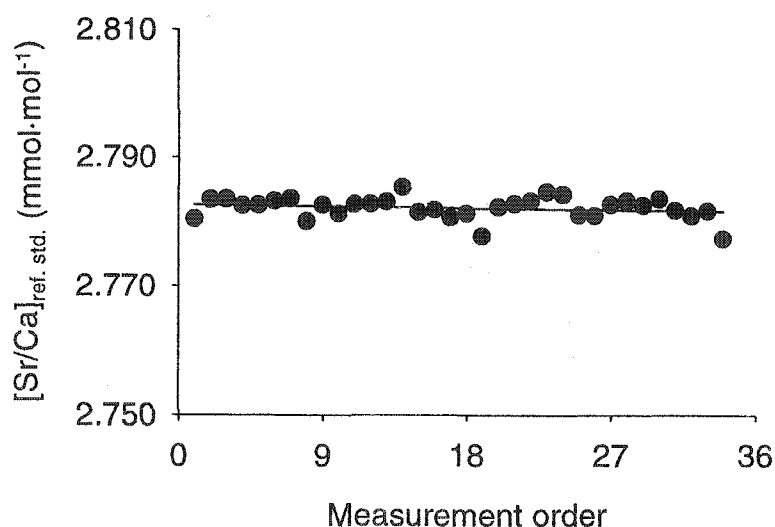
Sr/Ca ratios assayed using the approach outlined below. The mean Sr/Ca ratio from the pooled samples ($n = 3$) of pre-experiment otoliths was $3.0 \pm 0.2 \text{ mmol} \cdot \text{mol}^{-1}$.

A maximum of eight fish were randomly selected from each tank and their otoliths prepared for Sr/Ca analysis by inductively coupled plasma mass spectrometry (ICP-MS). If the total number of remaining fish in the tank after the experimental period was less than eight fish, all remaining fish were used in the tank (see Table 3.1 for fish numbers per tank). Otoliths were dissolved in 70% ultrapure nitric acid (Ultrex, J.T. Baker) and then diluted to achieve a total dissolved solid concentration of $0.1 \text{ mg} \cdot \text{g}^{-1}$ in a 1% nitric acid solution. Otolith solutions were stored at 4°C until the ICP-MS analysis. All analyses were run on a Thermo Finnigan Element 2 ICP-MS equipped with a self-aspirating ($20 \mu\text{L} \cdot \text{min}^{-1}$) PFA nebulizer and a dual-inlet quartz spray chamber. The method measured ^{48}Ca and ^{86}Sr in low resolution ($R = 300$) during a 2-minute acquisition time (a total of 126 passes). Quantification of Sr/Ca ratios followed the procedure outlined by Rosenthal et al. (1999). All samples were standardized to a dissolved solution ($0.1 \text{ mg} \cdot \text{g}^{-1}$) of an otolith reference powder with a certified Sr/Ca ratio of $2.782 \text{ mmol} \cdot \text{mol}^{-1}$ (Yoshinaga et al. 2000). The matrix of the standard was, therefore, matched to the dissolved Ca levels in the samples. An internal laboratory standard was run after each reference sample to estimate precision of the Sr/Ca method. The reference material was then treated as an unknown, and Sr/Ca values determined as for individual samples above. Measured precision (percent relative standard deviation (%RSD), $n = 34$) of the Sr/Ca method was 0.06% (Fig. 3.1).

Analyses of water samples collected during the experiment were also conducted using ICP-MS. Four samples were run from each tank including the start and end of the

experiment and two others at 11-day intervals. All samples were spiked with Indium (In) (to $4.5 \mu\text{g}\cdot\text{g}^{-1}$), which was used as an internal standard. The solutions were then aspirated into a Thermo Finnigan Element 2 ICP-MS, via a self-aspirating nebulizer ($50 \mu\text{L}\cdot\text{min}^{-1}$) and Scott's double pass spray chamber. Due to the presence of significant interferences on most of the Ca isotopes, ^{44}Ca , ^{88}Sr , and ^{115}In were measured in medium resolution (nominal $R = 4500$). Four samples from each tank were averaged and the mean values were then used in all subsequent analyses. To estimate precision of the water measurements we determined Ca and Sr values in a seawater reference material (High Purity Standards, Inc. seawater certified reference material (CRM)). The estimates of precision for both Ca and Sr concentrations in the seawater CRM were less than 2% RSD ($n = 8$).

Fig. 3.1. Solution-based inductively coupled plasma mass spectrometer (ICP-MS) measurements of Sr/Ca values in an otolith certified reference material ($[\text{Sr}/\text{Ca}]_{\text{ref. std.}}$) plotted in run order through the two days of sample analyses. Overall precision across all runs ($n = 34$) was 0.06% relative standard deviation (RSD) in the reference material with a certified value of $2.782 \text{ mmol}\cdot\text{mol}^{-1}$ (Yoshinaga et al. 2000).



Partition coefficients (D_{Sr}) were calculated by dividing the Sr/Ca ratio measured in an otolith by the Sr/Ca ratio measured in the treatment tank water. Otolith Sr/Ca values from individual fish were averaged within tanks, and then the three tank averages were used as replicates for each of the eight treatments.

Analysis of covariance (ANCOVA) was used to test the influence of otolith precipitation rate on $[Sr/Ca]_{\text{otolith}}$. The influence of otolith precipitation rate was also tested by correlating otolith mass with Sr/Ca ratios within each of the 23 tanks of sufficient sample sizes. This provided a test of rate effects on Sr/Ca ratios because all fish within the tanks have experienced identical environmental conditions (Bath et al. 2000). ANCOVAs were also used to test the influence of somatic growth rate on $[Sr/Ca]_{\text{otolith}}$. Finally, the influence of somatic growth rate on otolith precipitation rate was tested by correlating growth rate with $[Sr/Ca]_{\text{otolith}}$ within each of the 23 tanks of sufficient sample sizes. Individual growth rates were calculated as the difference between the mean standard length (SL) of pooled fish at the beginning of the experiment and the SL of individual fish at the end of the experiment divided by the number of experiment days. The means of fish-growth rates were calculated for individual tanks.

A 2 x 4 model I analysis of variance (ANOVA) was used to test for significant differences in $[Sr/Ca]_{\text{otolith}}$ and D_{Sr} among temperature and salinity treatments. Salinity and temperature were treated as independent categorical variables, and $[Sr/Ca]_{\text{otolith}}$ and D_{Sr} as dependent variables in the analyses. The assumptions of ANOVA were met: residuals were normally distributed and homogeneous among factor levels. Finally, because temperature was a quantitative variable, the significance of the relation between

both $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ and D_{Sr} with temperature was tested using both linear and quadratic functions.

Results

Conditions during the initial rearing period had little effect on the resultant otolith chemistry of the experimental fish because otoliths from these fish averaged less than 50 μg compared to a mean value of 890 μg for otoliths from fish at the end of the experiment. The Sr/Ca ratios of otoliths from 175 juvenile *L. xanthurus* ranged from 3.05 to 6.08 $\text{mmol}\cdot\text{mol}^{-1}$, with an overall mean of 4.40 $\text{mmol}\cdot\text{mol}^{-1}$. Water Sr/Ca values ranged from 11.91 to 19.36 $\text{mmol}\cdot\text{mol}^{-1}$ with an overall mean of 15.09 $\text{mmol}\cdot\text{mol}^{-1}$. Sr/Ca partition coefficients (D_{Sr}) ranged from 0.20 to 0.43 with an overall mean of 0.29.

Biom mineralization and growth rate effects

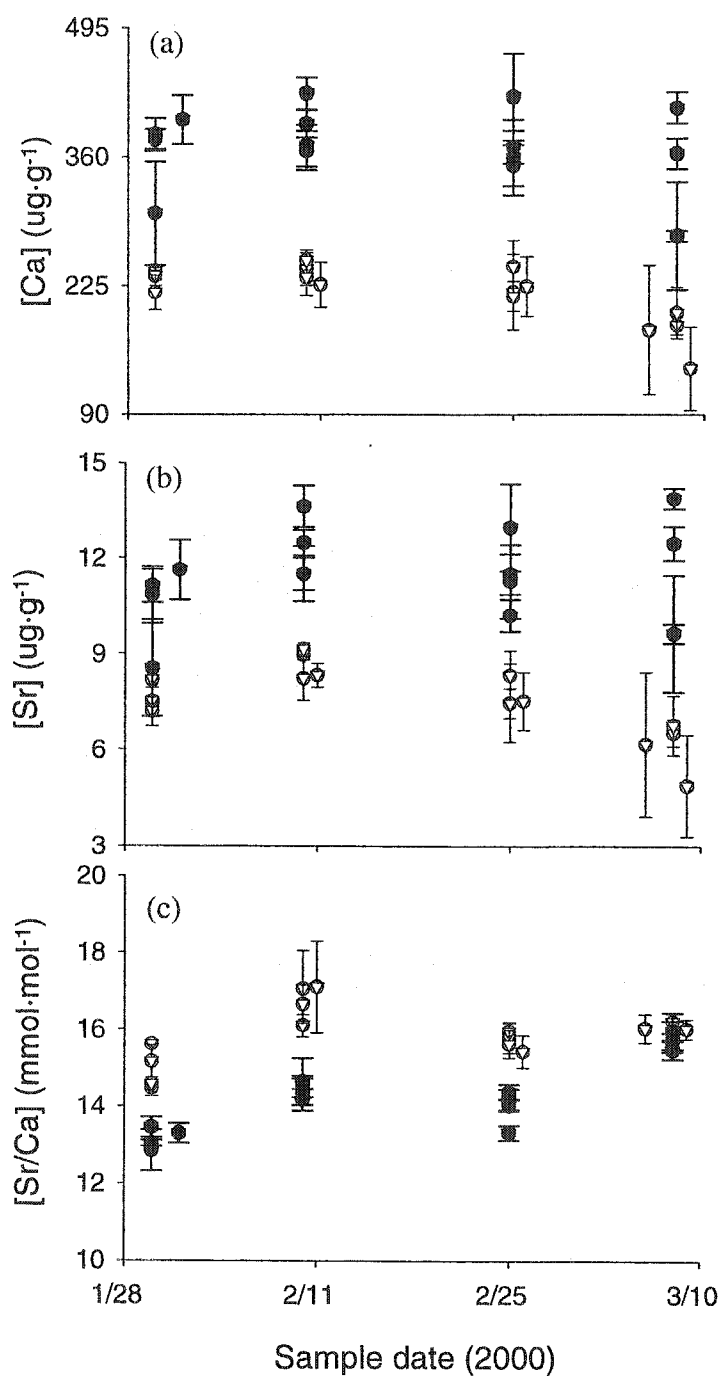
Using otolith mass as a proxy for aragonite precipitation rates, an ANCOVA demonstrated no significant effect of mean otolith mass among tanks on $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ ($F = 0.021$, $p > 0.05$, $n = 24$). There was, however, significant variability in $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ among tanks that were functions of temperature and salinity treatments, and considerable variations in otolith mass among individual fish within tanks (Table 3.1). Because individual fish within a tank had experienced identical conditions (i.e., were non-independent), we also ran Pearson correlations between otolith mass and otolith Sr/Ca on individual fish within each of 23 tanks. A total of 14 out of 23 correlations were negative, but only 3 out of 23 correlations were significant after Bonferroni correction for multiple tests. Somatic growth rates were, as expected, significantly affected by water

temperature ($F = 4.52$, $p = 0.019$, $n = 24$), and water salinity ($F = 11.43$, $p = 0.004$, $n = 24$). An ANCOVA also demonstrated no significant effect of mean growth rates among tanks on $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ ($F = 0.016$, $p = 0.900$, $n = 24$). Looking at the relation within tanks, Pearson correlations between growth rate and $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ demonstrated that although 16 out of 23 correlations were negative, only 2 out of 23 correlations were significant after Bonferroni adjustment.

Water chemistry

Elemental concentrations of ambient water in the tanks were significantly different between salinity treatments (Fig. 3.2). As expected, both Ca and Sr were higher at 25psu than at 15psu ($[\text{Ca}]_{\text{water}}: t_{0.05,(2),22} = 14.62$, $p < 0.05$; $[\text{Sr}]_{\text{water}}: t_{0.05,(2),22} = 11.85$, $p < 0.05$). Water Sr/Ca ratios did not vary among temperature treatments ($F = 0.042$, $p = 0.988$, $n = 24$) but were significantly different between salinity treatments ($F = 86.843$, $p = 0.000$, $n = 24$). Proportionately lower Ca concentrations relative to dissolved Sr in 15‰ salinity tanks led to higher Sr/Ca ratios in the low salinity treatment (Table 3.1, Fig. 3.2), presumably because Sr/Ca ratios were higher in the deionized water source than in the 25psu salinity artificial seawater.

Fig. 3.2. Measured tank water Ca (panel a) and Sr (panel b) concentrations ($\mu\text{g}\cdot\text{g}^{-1}$) \pm standard error (SE) by sample date for the duration of the experiment. Panel c is the calculated Sr/Ca ratio ($\text{mmol}\cdot\text{mol}^{-1}$) in these water samples. The black symbols represent the 25psu salinity treatment tanks and the open symbols represent the 15psu salinity treatment tanks. Each of the four temperature treatments is represented by a different symbol: 17 °C (●), 20 °C (▼), 23 °C (■), and 26 °C (◆).



Otolith [Sr/Ca]

The results of the two-way ANOVA indicate significant salinity and temperature effects on $[\text{Sr/Ca}]_{\text{otolith}}$ (Table 2). The interaction term was not significant (Table 3.2). A post-hoc Tukey multiple comparison showed that significant differences existed among all temperature treatments. There was also a significant linear, but not quadratic, relation between $[\text{Sr/Ca}]_{\text{otolith}}$ and temperature (Table 3.2, Fig. 3.3). At 25psu salinity, the linear least-squares regression equation between $[\text{Sr/Ca}]_{\text{otolith}}$ and temperature ($r^2 = 0.95$, $n = 12$) was

$$(3.3) \quad [\text{Sr/Ca}]_{\text{otolith}} = 0.154 \pm 0.012 (T \text{ } ^\circ\text{C}) + 1.16 \pm 0.256 (95\% \text{ CI}).$$

For 15psu salinity, the linear equation ($r^2 = 0.92$, $n = 12$) was

$$(3.4) \quad [\text{Sr/Ca}]_{\text{otolith}} = 0.179 \pm 0.017 (T \text{ } ^\circ\text{C}) + 0.348 \pm 0.382 (95\% \text{ CI}).$$

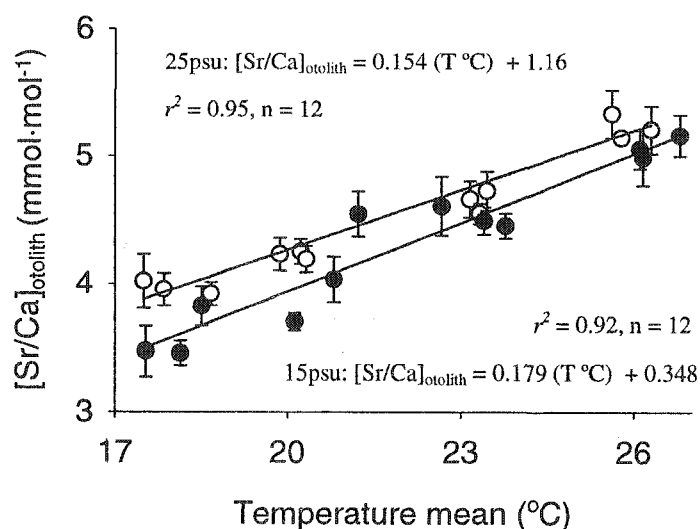
Table 3.2. *Leiostomus xanthurus*. Analysis of variance (ANOVA) table (SS – sums of squares; df – degrees of freedom; MS – mean squares) summarizing the effect of salinity and temperature treatments on otolith [Sr/Ca] and the Sr distribution coefficient (D_{Sr}), along with significance tests of linear and quadratic contrasts between temperature and otolith [Sr/Ca] and temperature and D_{Sr} .

Source	SS	df	MS	F	p < F
Otolith [Sr/Ca]					
Salinity	0.241	1	0.241	7.42	0.015
Temp	6.20	3	2.1	63.6	< 0.001
Salinity*temp	0.068	3	0.023	0.695	0.569
Error	0.520	16	0.032		
Linear	6.24	1	6.24	189	< 0.0001
Quadratic	0.048	1	0.048	1.47	0.244

Table 3.2 continued

Source	SS	df	D_{Sr}		
			MS	F	p < F
Salinity	0.011	1	0.011	61.8	< 0.0001
Temp	0.027	3	0.009	52.2	< 0.0001
Salinity*temp	0.0004	3	0.000	0.78	0.524
Error	0.003	16	0.000		
Linear	0.028	1	0.028	154	< 0.0001
Quadratic	0.0002	1	0.0002	1.18	0.294

Fig. 3.3. Sr/Ca ($\text{mmol} \cdot \text{mol}^{-1}$) ratios in otoliths of laboratory-reared *Leiostomus xanthurus* as a function of tank temperatures ($^{\circ}\text{C}$) at two salinity levels, 15psu (\bullet) and 25psu (\circ). The lines were fitted by linear least-squares regression for each of the salinity treatments.



Sr/Ca partition coefficients

I detected significant salinity and temperature effects on Sr/Ca partition coefficients (Table 3.2), with a non-significant interaction between the two factors (Table 3.2). The linear function between D_{Sr} and temperature was significant, but the quadratic

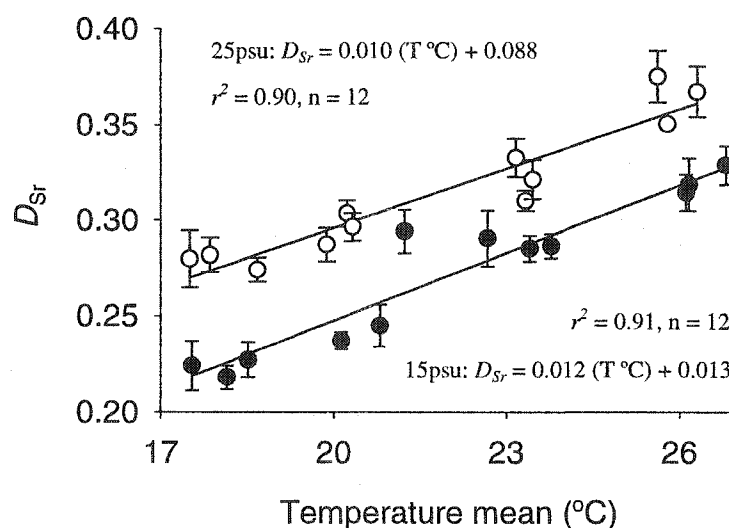
function was not (Table 3.2, Fig. 3.4). For 25psu salinity, the linear least-squares regression equation between D_{Sr} and temperature ($r^2 = 0.90$, $n = 12$) was

$$(3.5) \quad D_{Sr} = 0.010 \pm 0.001 (T \text{ } ^\circ\text{C}) + 0.088 \pm 0.025 (95\% \text{ CI}).$$

For 15psu salinity, the linear equation ($r^2 = 0.91$, $n = 12$) was

$$(3.6) \quad D_{Sr} = 0.012 \pm 0.001 (T \text{ } ^\circ\text{C}) + 0.013 \pm 0.026 (95\% \text{ CI}).$$

Fig. 3.4. Sr/Ca partition coefficients (D_{Sr}) for otoliths of laboratory-reared *Leiostomus xanthurus* as a function of tank temperatures ($^\circ\text{C}$) at two salinity levels, 15psu (\bullet) and 25psu (\circ). The lines were fitted by linear least-squares regression for each of the salinity treatments.



Discussion

Both $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ and D_{Sr} increased linearly as a function of temperature over the ranges analyzed (17 – 26 °C). The temperature effect was not a result of differences in water chemistry among temperature treatments, as elemental concentrations and Sr/Ca ratios did not differ among temperature treatments. Presumably, there was no effect of diet, as all fish were fed the same food. Moreover, several experimental studies have found little evidence for an effect of diet on otolith Sr incorporation (Hoff and Fuiman 1995; Farrell and Campana 1996; Milton and Chenery 2001). Physiological differences cannot be eliminated when such differences are correlated with growth because there was a significant effect of temperature on growth rate. However, aragonite precipitation rates and larval growth rates within individual tanks were in most cases not significantly correlated with Sr/Ca ratios in otoliths. Temperature was; therefore, the dominant variable controlling Sr/Ca ratios in the otoliths of larval spot in our experiment.

Bath et al. (2000) provided a first order approximation of the temperature-partition coefficient relation for larval spot, *L. xanthurus* (18 - 32.6 mm SL, mean = 24.2 mm SL)

$$(3.7) \quad D_{\text{Sr}} = 0.0046 T \text{ }^{\circ}\text{C} + 0.089 \text{ (} r^2 = 0.62 \text{)}.$$

Two points are worth noting with respect to the results of my earlier study. First, Bath et al. (2000) reported Sr/Ca partition coefficients that were significantly lower at a given temperature (0.18 at 20 °C, 20psu) than found in the present study (0.26 at 20 °C, 15psu; 0.30 at 20 °C, 25psu). The intercept of the linear regression between D_{Sr} and temperature

(for the 20 and 25 °C treatments) was statistically indistinguishable between the two experiments. However, the temperature sensitivity of the relation between D_{Sr} and temperature in the present study ($\sim 5\% \cdot ^\circ\text{C}^{-1}$) was approximately twice that reported by Bath et al. (2000) ($\sim 2\% \cdot ^\circ\text{C}^{-1}$). Larvae were reared at two temperatures in the earlier study (20 and 25 °C). It is possible therefore, that Bath et al. (2000) underestimated the temperature dependence due to a lack of temperature treatments. It is more difficult to reconcile the different estimates of D_{Sr} between the two studies because both were based on multiple treatments with adequate levels of replication. Apparently there are other factors influencing the magnitude of D_{Sr} in the otoliths of larval spot that remain to be identified.

The effect of temperature on Sr/Ca ratios in the otoliths of other fish species is ambiguous. Several studies have also shown positive effects of temperature on Sr incorporation into otoliths (Kalish 1989; Limburg 1995; Arai et al. 1996). Other researchers have suggested an inverse correlation between temperature and Sr/Ca ratios in otoliths (Radtke 1989; Sadovy and Severin 1992; Secor et al. 1995), or have been unable to detect any temperature dependence of $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ (Gallahar and Kingsford 1996; Tzeng 1996; Chesney et al. 1998). Methodological problems may be responsible for at least some of these discrepancies, as few studies have adequately constrained $[\text{Sr}/\text{Ca}]_{\text{water}}$. The choice of analytical instrumentation may also be a factor, as most studies have used electron probe microanalysis (EPMA). The application of Sr/Ca thermometry, at least in coral skeletons, generally requires instrument precision of better than 0.5% RSD (Lea and Martin 1996). However, instrument uncertainty of Sr/Ca measurements in otoliths using EPMA is typically on the order of 5-10% (RSD) (e.g.,

Gunn et al. 1992; Campana et al. 1997). Precision of the Sr/Ca method using sector-field ICP-MS that we employed was 0.06% RSD, matching the best precision reported for instruments routinely used to determine Sr/Ca ratios in biogenic carbonates (Rosenthal et al. 1999; Schrag 1999). The increased precision of these estimates over earlier probe-based methods certainly increased the ability to detect relatively subtle, but predictable, effects of temperature on Sr/Ca ratios. Developmental stage may also influence Sr/Ca in otoliths. Kalish (1990) suggested that variations in Ca-binding proteins caused by changes in reproductive status influenced Sr/Ca ratios in otoliths of adult fish. Such regulation may then be less important in the larval stages that we examined compared to studies on older life history stages. Finally, it is likely that Sr and Ca uptake in otoliths is species-specific, and therefore a single relationship between Sr/Ca and temperature may not apply to all marine fish species.

Considerable debate also surrounds the temperature dependence of Sr/Ca partition coefficients in other biogenic carbonates. Most work has been conducted on the aragonite skeletons of hermatypic corals that typically demonstrate an inverse relation between D_{Sr} and temperature (Smith et al. 1979; Beck et al. 1992; Sinclair et al. 1998). Positive correlations have been identified between D_{Sr} and temperature in molluscs (Buchardt and Fritz 1978; Klein et al. 1996; Vander Putten et al. 2000) and calcitic foraminifera (Elderfield et al. 1996; Lea et al. 1999). Interestingly, Sr/Ca ratios of the aragonite from studies that documented a positive relation between D_{Sr} and temperature were considerably lower ($< 2.5 \text{ mmol} \cdot \text{mol}^{-1}$) than that found in coral or sclerosponge aragonite ($8\text{-}10 \text{ mmol} \cdot \text{mol}^{-1}$). There may, therefore, be fundamentally different mechanisms generating the temperature dependence of Sr/Ca ratios between low Sr and

high Sr aragonite. Stecher et al. (1996) and Purton et al. (1999) suggested metabolic effects that were correlated with temperature led to positive correlations between Sr/Ca and temperature in bivalve and gastropod shells. Correlations between otolith Sr/Ca and several proxies of metabolic rate (somatic and otolith growth) in individual spot larvae do not seem to exist. Clearly more work in this area is needed, although there is now sufficient evidence to reject analogies between the temperature dependence of D_{Sr} in coral skeletons and fish otoliths.

More Sr was incorporated into larval spot otoliths at salinities of 25psu than at 15psu, after accounting for differences in the Sr/Ca ratios of the rearing water. Dietary effects were unlikely to have caused the differences, following the argument outlined above for temperature. Rather, absolute Sr levels appeared to influence otolith Sr/Ca values beyond that predicted by $[Sr/Ca]_{water}$. Recent studies by Chowdhury and Blust (2001, 2002) provide a potential mechanism for our observations. They found that dissolved Ca and Sr in freshwater carp mutually inhibited uptake of ions across branchial and intestinal membranes. Positive, non-linear inhibition of Ca ions by Sr ions at uptake sites on these membranes would, in turn, result in the observed effect of dissolved Sr on Sr/Ca ratios in otoliths.

The observation that Sr/Ca ratios in otoliths depend on temperature, $[Sr/Ca]_{water}$ and $[Sr]_{water}$ raises a number of potential problems for fish Sr thermometry. Both $[Sr/Ca]_{water}$ and $[Sr]_{water}$ must be constrained before temperature can be estimated from otolith Sr/Ca. Because Sr and Ca have long ($\sim 10^6$ years) residence times in the world's oceans, fish that spend their entire lives in shelf or oceanic water masses will not experience significant variations in either $[Sr]_{water}$ or $[Sr/Ca]_{water}$. However, many fish

species living in coastal waters are estuarine dependent and traverse waters of variable water chemistry and temperature. Reconstruction of temperature histories will, therefore, require independent estimates of $[\text{Sr}]_{\text{water}}$ and $[\text{Sr}/\text{Ca}]_{\text{water}}$. Temperature and salinity are easy to measure and are frequently incorporated in sampling designs. Water strontium and calcium concentrations are rarely measured yet seem to be critical for the application of strontium thermometry to field-collected fish.

In summary, Sr/Ca ratios in the otoliths of larval spot are determined primarily by the physicochemical properties of the ambient water. When combined with the results of my earlier study (Bath et al. 2000), temperature, $[\text{Sr}/\text{Ca}]_{\text{water}}$, and $[\text{Sr}]_{\text{water}}$ all have significant effects on otolith Sr/Ca ratios. In contrast, there was no consistent evidence for metabolic effects on Sr/Ca ratios in otoliths at least to the extent metabolic rates are correlated with individual growth rates. Unfortunately, this does not mean that otolith Sr/Ca thermometry will be easy in estuarine-dependent fish species because independent geochemical tracers that constrain both $[\text{Sr}/\text{Ca}]_{\text{water}}$ and $[\text{Sr}]_{\text{water}}$ will need to be developed. For instance, both stable oxygen ($^{18}\text{O}/^{16}\text{O}$) and strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotopes vary linearly with $[\text{Sr}]_{\text{water}}$ and $[\text{Ca}]_{\text{water}}$ in estuarine systems (Chesney et al. 1998; Thorrold et al. 1997c). Marine fishes that do not experience significant variations in $[\text{Sr}]_{\text{water}}$ and $[\text{Ca}]_{\text{water}}$ are logical species to test the ability to reconstruct temperature histories from Sr/Ca ratios in otoliths.

CHAPTER IV

TEMPERATURE AND SALINITY EFFECTS ON BARIUM, MANGANESE, AND MAGNESIUM INCORPORATION IN OTOLITHS OF LARVAL SPOT (*LEIOSTOMUS XANTHURUS*)

Introduction

Elemental and isotopic concentrations in fish otoliths may serve as natural tags, or signatures to differentiate fish stocks and infer movement by attributing fish to different geographically located water bodies (Edmonds et al. 1989; Campana et al. 1994; Gillanders and Kingsford 1996; Begg et al. 1998; Thorrold et al. 2001). Using elemental signatures in otoliths is based on the assumption that fish otoliths incorporate minor and trace elements from the water and thus record an environmental signature uniquely experienced by individual fish. The promise of otolith elemental signatures is the ability to examine the spatial structure of fish populations.

Several studies have used otolith minor and trace metal concentrations to successfully discriminate fish populations and assign individual fish to specific water bodies. Thorrold et al. (1998a) were able to classify estuarine dependent weakfish (*Cynoscion regalis*) to nursery areas based on their otolith elemental signatures (Mg/Ca, Mn/Ca, Sr/Ca, and Ba/Ca) and had improved accuracy once measurements of otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were added to the models. In another study by Thorrold et al. (1998b), otolith elemental signatures (Mg/Ca, Mn/Ca, Sr/Ca, and Ba/Ca) elucidated the possibility of spawning site fidelity of anadromous American shad (*Alosa sapidissima*).

Elemental signatures in otoliths have also been used as natural tags to infer juvenile nursery habitats and assess the relative contribution of juveniles from these different nurseries to the adult fished population. Gillanders and Kingsford (2000) found significant otolith metal (Sr, Ba, and Mn) concentration differences among nursery areas in estuarine dependent trumpeter (*Pelates sexlineatus*) juveniles, but also revealed that temporal variation in otolith elemental signatures complicate interpretation of nursery habitat assignments. Elements (Li, Mg, Mn, Sr, Ca) in juvenile Pacific bluefin tuna (*Thunnus orientalis*) otoliths (Rooker et al. 2001) showed clear population separation between three nursery areas over a three-year period although they did not calculate the relative juvenile contributions to the adult population.

Water bodies have different elemental compositions based on the geochemistry of the watershed, anthropogenic and natural inputs to the system, and the behavior of elemental species as influenced by complexation, biological uptake, and sorption on suspended solids (Bender et al. 1977; Turner et al. 1981; Bruland 1983; Byrne et al. 1988; Morel and Hering 1993). These compositional differences in water chemistry potentially contribute to distinct environmental signatures in otoliths. Different otolith elemental signatures may represent different locations, and similarly, indistinguishable signatures in otoliths may imply fish came from the same location based on the assumption otolith elemental composition is proportional to the ambient water elemental composition. What if otoliths record distinct signatures but in fact the fish did not come from different locations? Additionally, what if otoliths that record indistinguishable signatures, do in fact come from different locations? Otolith element incorporation may not be solely a function of the ambient water elemental composition. The factors and

mechanisms that influence element incorporation in otoliths need to be tested under controlled conditions and data must be groundtruthed in order to apply it to population-level assessments.

How the physical properties of the environment influence the uptake of minor and trace elements in otoliths is poorly understood. Each element may behave differently and the specific processes that affect otolith incorporation need to be revealed. Without testing the effects of external variables on otolith element incorporation, such as water temperature, salinity, and elemental concentrations, the measured elemental differences used to develop elemental signatures could be misinterpreted and individual fish incorrectly assigned to locations. Because fisheries management is based on the accurate classification of fish stocks, the potential for making erroneous classifications and therefore poor management decisions based on inaccurate assumptions, is increased.

Confounding the environmental effects on otolith element incorporation is the influence of aragonite precipitation rate on element incorporation in biogenic aragonite, including fish otoliths, coral skeletons, and mollusc shells. Studies on synthetic aragonite have found no effect of precipitation rate on element incorporation (Kinsman and Holland 1969; Zhong and Mucci 1989), whereas studies on biogenic aragonite have been contradictory. Some work has shown Sr/Ca in corals correlate with coral growth rates (Weber 1973; deVillers et al. 1994, 1995), although Shen et al. (1996) found no such relationship. In molluscs, Buchardt and Fritz (1978) found Sr incorporation to be independent of growth rate, but Stecher et al. (1996) speculated that there was a seasonal growth effect on Sr incorporation of two species of bivalves. In one of the first otolith studies to test these rate effects, Bath et al. (2000; Chapter II) found no effect of otolith

precipitation rate on Sr and Ba incorporation in otoliths, using otolith mass as a proxy, and no effect of metabolic rates, using somatic growth rate as a proxy.

There have been few experiments designed to address the effects of temperature and salinity on otolith element and isotope concentrations (Kalish 1989; Fowler et al. 1995a; Hoff and Fuiman 1995; Chesney et al. 1998; Milton and Chenery 2001; Elsdon and Gillanders 2002) limiting our understanding of otolith elemental signatures and their applications.

The objective of this chapter is to test the effects of temperature and salinity treatments on the incorporation of Mg, Mn, and Ba in larval spot (*Leiostomus xanthurus*) otoliths as a test for the application of otolith elemental signatures for an estuarine-dependent species. The partition coefficients D_{Me} (see Chapters II and III, Introductions) for each of these elements are also tested for temperature and salinity effects. The null hypothesis is that temperature and salinity do not have significant effects on otolith incorporation of Mg/Ca, Mn/Ca, or Ba/Ca after appropriate correction for the metal/calcium (Me/Ca) ratios of the ambient water and the precipitation rate of the otoliths. If these otolith elemental signatures in otoliths are going to be used in field applications, and subsequently used to make fishery management decisions for some species, it is important to determine if their incorporation is affected by these environmental factors, as is the case strontium as reported in Chapter III (Martin et al. *In Press*).

Materials and methods

Spot (*Leiostomus xanthurus*) were hatched on 10 December 1999 at the NOAA, National Ocean Service, Center for Coastal Fisheries and Habitat Research in Beaufort, North Carolina (USA). Larvae were reared in a common tank in natural seawater at 34psu salinity and 20 °C for 42 days, at which time they were randomly distributed among 24 acid-washed 20 L high-density polyethylene tanks at a density of 2 fish•L⁻¹. Fish were gradually acclimated to the experimental temperature and salinity treatments for a week before initiating the experiment to ensure adequate survival of the experimental fish. The light:dark cycle was controlled at 12 h:12 h for the duration of the experiment. Fish were fed an artificial diet (Golden Pearls, 300-500 and 500-800 microns, Brine Shrimp Direct) twice-daily *ad libitum*. Water was changed at 50% volume daily to maintain water quality and salinity.

Experimental tanks were randomly assigned 2 salinity treatments (15 and 25psu) and four temperature treatments (17, 20, 23, and 26 °C). Three replicate tanks were used for each treatment combination (24 tanks in total). Room temperature was maintained at 16 °C, and aquarium heaters were used to regulate temperatures within individual tanks. Artificial seawater (Instant Ocean®) mixed in deionized water was used to regulate salinity.

Water samples from each tank were collected every third day and were filtered through 0.22 µm cellulose nitrate membrane filters, acidified with 12 N trace-metal grade HCl to pH 2, and then stored frozen for subsequent analysis. Water temperature and salinity were also recorded daily (Table 4.1).

Table 4.1. Summary of mean water temperature (°C), salinity (psu), dissolved Mg/Ca ($\text{mol}\cdot\text{mol}^{-1}$), Mn/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) and Ba/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) levels, individual element concentrations, and number of otoliths analyzed (n), within each of the 24 individual tanks during the course of the experiment.

Tank #	Temp.	Salinity	[Mg/Ca]	[Mn/Ca]	[Ba/Ca]	[Mg] $\mu\text{mol}\cdot\text{L}^{-1}$	[Mn] $\mu\text{mol}\cdot\text{L}^{-1}$	[Ba] $\mu\text{mol}\cdot\text{L}^{-1}$	[Ca] $\text{mmol}\cdot\text{L}^{-1}$	n
9	17.5	15	4.57	115.86	17.72	645.83	37.30	14.13	5.83	7
11	18.1	15	4.79	108.86	14.93	577.25	30.05	9.71	4.97	7
20	18.5	15	5.19	133.26	20.07	589.03	33.86	12.85	4.69	8
24	20.1	15	4.69	115.87	17.76	559.36	29.91	11.73	4.98	8
5	20.8	15	5.03	110.52	19.12	616.72	30.33	13.26	5.06	7
18	21.2	15	4.63	111.38	17.63	710.03	39.03	15.28	6.32	8
10	22.7	15	4.81	121.25	18.12	580.61	33.13	12.37	4.96	8
21	23.4	15	4.65	100.47	20.18	661.11	32.38	16.2	5.87	8
22	23.8	15	4.72	120.37	17.70	618.93	35.1	13.13	5.4	8
4	26.1	15	4.89	100.13	18.19	661.76	30.21	13.88	5.55	8
14	26.2	15	4.61	84.60	17.21	687.50	28.40	14.49	6.14	8
16	26.8	15	4.75	84.30	17.64	612.10	23.76	12.94	5.35	8
1	17.5	25	4.76	99.86	15.01	961.36	45.3	16.91	8.32	8
7	17.8	25	4.71	87.14	10.01	1044.05	43.24	12.82	9.14	4
15	18.7	25	4.81	95.32	14.56	961.32	43.61	16.06	8.29	8
2	19.9	25	4.83	75.62	14.9	1106.77	38.84	19.77	9.53	8
8	20.2	25	4.67	109.78	16.78	1008.92	52.91	20.36	8.88	8
6	20.3	25	4.69	71.47	13.18	994.07	34.81	15.72	8.71	8
23	23.2	25	4.65	95.98	13.04	1060.17	50.32	16.16	9.39	8
12	23.3	25	4.82	72.08	9.75	1116.45	37.92	12.37	9.52	7
13	23.5	25	4.66	68.84	16.35	1156.58	39.4	22.95	10.23	8
19	25.6	25	4.63	52.6	12.97	1139.03	28.93	18.33	10.12	8
3	25.8	25	4.83	73.15	18.22	938.76	29.31	20.11	8.02	1
17	26.3	25	4.67	64.46	14.31	1045.31	30.76	17.72	9.26	6

After termination of the experiment, all surviving fish were measured and frozen in individual plastic bags. Sagittal otolith pairs were removed from the fish and scraped clean with acid-washed glass probes in a class-100 clean room. Otoliths were ultrasonically cleaned in Milli-Q water for 7 minutes and triple rinsed with ultrapure H_2O_2 (Ultrex, J.T. Baker) followed by three sequential rinses of Milli-Q water. Otoliths were then placed on acid-washed glass slides to dry for 36 hours under a class-100 laminar-flow hood. After drying, otoliths were individually weighed to the nearest $10\text{ }\mu\text{g}$ and transferred to acid-washed 1.5 ml high-density polyethylene vials.

A maximum of eight fish were randomly selected from each tank and their otoliths prepared for analysis by inductively coupled plasma mass spectrometry (ICP-MS). If the total number of remaining fish in the tank after the experimental period was less than eight fish, all remaining fish were used in the tank. Otoliths were dissolved in 70% ultrapure nitric acid (Ultrex II, J.T. Baker) and then diluted to achieve a total dissolved solid concentration of $0.1\text{ mg}\cdot\text{g}^{-1}$ in a 1% nitric acid solution. Otolith solutions were stored at $4\text{ }^\circ\text{C}$ until the ICP-MS analysis. Otolith analyses were run on a Thermo Finnigan Element ICP-MS equipped with a self-aspirating ($50\text{ }\mu\text{L}\cdot\text{min}^{-1}$) PFA nebulizer and a dual-inlet quartz spray chamber. The method measured ^{25}Mg , ^{48}Ca , ^{55}Mn , and ^{138}Ba in low resolution ($R = 300$) during a 2-minute acquisition time. Quantification of metal/calcium (Me/Ca) ratios followed the procedure outlined by Rosenthal et al. (1999). All samples were standardized to a dissolved solution ($0.1\text{ mg}\cdot\text{g}^{-1}$) of an otolith reference powder with certified Me/Ca ratios of $89.25\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$ for Mg/Ca, $0.257\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$ for Mn/Ca, and $2.174\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$ for Ba/Ca (Yoshinaga et al. 2000). The matrix of the standard was therefore matched to the dissolved Ca levels in the samples. Detection

limits were calculated as 3σ values of 1% HNO_3 sample blanks ($n = 37$) that were run throughout the analyses. These limits were 0.2% of the average sample intensity for ^{25}Mg , 0.05% for ^{48}Ca , 0.3% for ^{55}Mn , and 0.1% for ^{138}Ba . An internal laboratory standard was run after each reference sample to estimate precision of the Me/Ca method. The reference material was then treated as an unknown, and Me/Ca values determined as for individual samples above. Measured precision (% relative standard deviation (RSD), $n = 37$) of the Me/Ca method was 2.7% for Mg/Ca, 2.8% for Mn/Ca and 0.5% for Ba/Ca.

Analyses of water samples collected during the experiment were also conducted using ICP-MS. Four samples were run from each tank including the start and end of the experiment and two others at 11-day intervals. All samples were spiked with Indium (to $4.5 \mu\text{g}\cdot\text{g}^{-1}$), which was used as an internal standard. The solutions were then aspirated into a Thermo Finnigan Element 2 ICP-MS, via a self-aspirating nebulizer ($50 \mu\text{L}\cdot\text{min}^{-1}$) and Scott's double pass spray chamber. Due to the presence of significant interferences on most of the Ca isotopes, ^{44}Ca , ^{25}Mg , ^{55}Mn , ^{137}Ba , and ^{115}In were measured in medium resolution (nominal $R = 4500$). Four samples from each tank were averaged and the mean values were then used in all subsequent analyses. To estimate precision of the water measurements, Ca, Mg, Mn, and Ba values in a seawater reference material (High Purity Standards, Inc. seawater CRM) were determined. Our estimates of precision for element concentrations in the seawater CRM were 1.4% RSD for Ca, 1.4% RSD for Mg, 2.7% RSD for Mn, and 1.7% RSD for Ba ($n = 8$).

Partition coefficients (D_{Me}) were calculated by dividing the metal/calcium (Me/Ca) ratio measured in an otolith by the mean Me/Ca ratio measured in the treatment tank water (Morse and Bender 1990). Otolith Me/Ca values from individual fish were

averaged within tanks, and then the three tank averages were used as replicates for each of the eight treatments.

Analysis of covariance (ANCOVA) was used to test the influence of otolith precipitation rate on each $[\text{Me}/\text{Ca}]_{\text{otolith}}$, using otolith mass as a covariate and temperature and salinity as independent categorical variables. The influence of otolith precipitation rate was also tested by correlating otolith mass with Me/Ca ratios within each of the 23 tanks of sufficient sample sizes. This provided a test of rate effects on Me/Ca ratios because all fish within the tanks have experienced identical environmental conditions (Bath et al. 2000). ANCOVAs were also used to test the influence of somatic growth rate on each $[\text{Me}/\text{Ca}]_{\text{otolith}}$, using growth rate as a covariate and temperature and salinity as independent categorical variables. Finally, the influence of somatic growth rate on otolith precipitation rate was tested by correlating growth rate with $[\text{Me}/\text{Ca}]_{\text{otolith}}$ within each of the 23 tanks of sufficient sample sizes. Individual growth rates were calculated as the difference between the mean SL of pooled fish at the beginning of the experiment and the SL of individual fish at the end of the experiment divided by the number of experiment days. The means of fish growth rates were calculated for individual tanks.

Two-way analysis of variance (ANOVA) was used to test for significant differences in $[\text{Me}/\text{Ca}]_{\text{water}}$, $[\text{Me}/\text{Ca}]_{\text{otolith}}$, and D_{Me} among temperature and salinity treatments. Salinity and temperature were treated as independent categorical variables, and $[\text{Me}/\text{Ca}]_{\text{water}}$, $[\text{Me}/\text{Ca}]_{\text{otolith}}$, and D_{Me} as dependent variables in the analyses. The assumptions of ANOVA were met: the data was normally distributed and variances were homogeneous among factor levels. To control for experiment-wise error, the critical p

value (0.05) was adjusted (0.017) to account for the three individual ANOVAs performed.

Conditions during the initial rearing period had little effect on the resultant otolith chemistry of the experimental fish because otoliths from these fish averaged less than 50 μg compared to a mean value of 890 μg for otoliths from fish at the end of the experiment.

Results

The $[\text{Mg}/\text{Ca}]_{\text{oiliths}}$ from 172 juvenile *L. xanthurus* ranged from 0.676 to 4.74 $\text{mmol}\cdot\text{mol}^{-1}$, with an overall mean of 1.80 $\text{mmol}\cdot\text{mol}^{-1}$. Water Mg/Ca values ranged from 4.38 to 6.40 $\text{mol}\cdot\text{mol}^{-1}$ with an overall mean of 4.75 $\text{mol}\cdot\text{mol}^{-1}$. Mg/Ca partition coefficients (D_{Mg}) ranged from 0.00014 to 0.00102 with an overall mean of 0.00038.

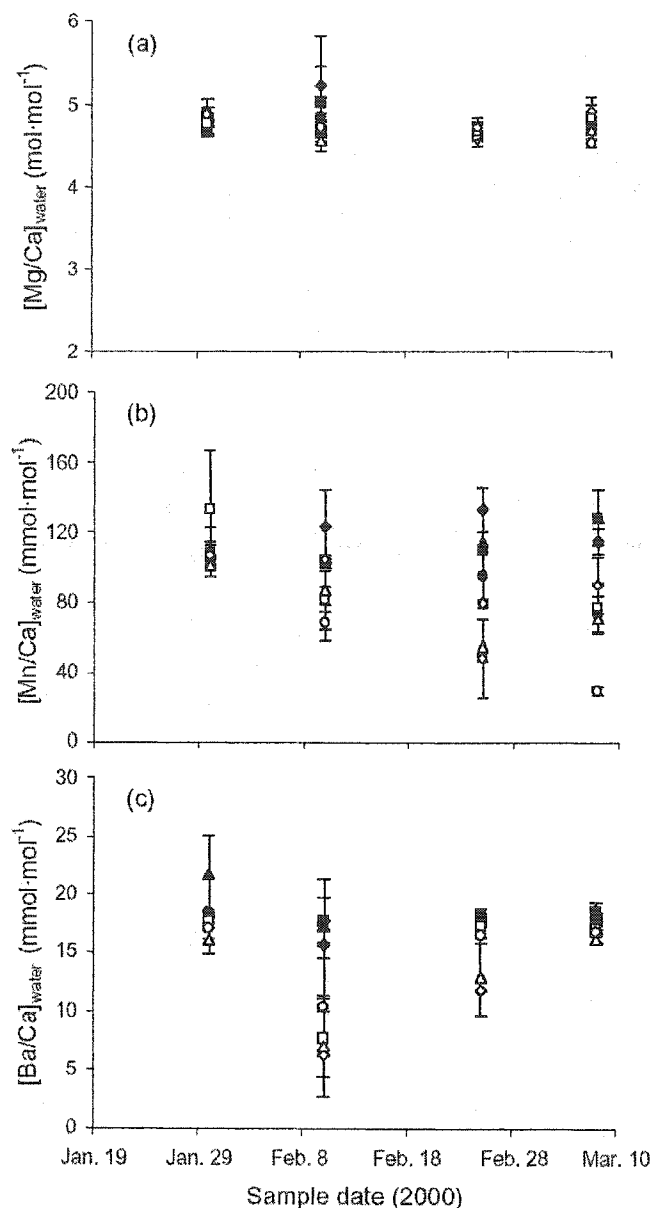
The $[\text{Mn}/\text{Ca}]_{\text{oiliths}}$ from 173 juvenile *L. xanthurus* ranged from 6.32 to 101.01 $\mu\text{mol}\cdot\text{mol}^{-1}$, with an overall mean of 18.055 $\mu\text{mol}\cdot\text{mol}^{-1}$. Water Mn/Ca values ranged from 19.71 to 200.89 $\mu\text{mol}\cdot\text{mol}^{-1}$ with an overall mean of 94.72 $\mu\text{mol}\cdot\text{mol}^{-1}$. Mn/Ca partition coefficients (D_{Mn}) ranged from 0.055 to 0.92 with an overall mean of 0.196.

The $[\text{Ba}/\text{Ca}]_{\text{oiliths}}$ from 173 juvenile *L. xanthurus* ranged from 2.02 to 15.8 $\mu\text{mol}\cdot\text{mol}^{-1}$, with an overall mean of 6.01 $\mu\text{mol}\cdot\text{mol}^{-1}$. Water Ba/Ca values ranged from 1.83 to 28.40 $\mu\text{mol}\cdot\text{mol}^{-1}$ with an overall mean of 16.08 $\mu\text{mol}\cdot\text{mol}^{-1}$. Ba/Ca partition coefficients (D_{Ba}) ranged from 0.11 to 1.23 with an overall mean of 0.37.

Water chemistry

Elemental concentrations in the tank water were significantly different between salinity treatments. As expected, Ca, Mg, Mn, and Ba concentrations in the water were higher at 25psu than at 15psu ($[Ca]_{\text{water}}$: $t_{0.05,(2),22} = 14.62$, $p = 0.000$; $[Mg]_{\text{water}}$: $t_{0.05,(2),22} = 16.57$, $p = 0.000$; $[Mn]_{\text{water}}$: $t_{0.05,(2),22} = 2.99$, $p = 0.003$; $[Ba]_{\text{water}}$: $t_{0.05,(2),22} = 4.03$, $p = 0.000$;) . $[Mg/Ca]_{\text{water}}$ were not significantly different between salinity treatments ($F = 0.565$, $p = 0.463$, $n = 24$) or temperature treatments ($F = 0.339$, $p = 0.797$, $n = 24$). $[Mn/Ca]_{\text{water}}$ were significantly different between salinity treatments ($F = 32.325$, $p = 0.000$, $n = 24$) and temperature treatments ($F = 6.648$, $p = 0.004$, $n = 24$). $[Ba/Ca]_{\text{water}}$ were significantly different between salinity treatments ($F = 19.282$, $p = 0.000$, $n = 24$) but not among temperature treatments ($F = 0.366$, $p = 0.779$, $n = 24$). Lower absolute Mn, Ba, and proportionately lower Ca concentrations in 15psu salinity tanks led to higher Mn/Ca and Ba/Ca ratios in the low salinity treatment (Table 4.1, Fig. 4.1) presumably because Me/Ca ratios were higher in the deionized water source than in the 25psu salinity artificial seawater.

Fig. 4.1. Calculated metal/calcium ratios in the tank water Mg/Ca (panel a), Mn/Ca (panel b), and Ba/Ca (panel c) concentrations \pm SE by sample date for the duration of the experiment. The open symbols represent the 25psu salinity treatment tanks and the black symbols represent the 15psu salinity treatment tanks. Each of the four temperature treatments is represented by a different symbol: 17 °C (\blacklozenge), 20 °C (\blacksquare), 23 °C (\blacktriangle), and 26 °C (\bullet).



Otolith [Me/Ca]

Temperature and salinity did not have a statistically significant effect on $[Mg/Ca]_{\text{otolith}}$ (Fig. 4.2, Table 4.2) and the interaction between the two factors was not

significant. There was no significant temperature, or salinity effect on $[\text{Mn}/\text{Ca}]_{\text{otolith}}$ (Fig. 4.3, Table 4.2) and the interaction between the two factors was not significant. There was no significant temperature or salinity effects on $[\text{Ba}/\text{Ca}]_{\text{otolith}}$ (Fig. 4.4, Table 4.2) and the interaction between the two factors was not significant.

Table 4.2. Results of three separate two-way Analysis of Variance analyses testing the effect of temperature and salinity on three elemental signatures (log-transformed) in the otoliths of juvenile *Leiostomus xanthurus* (n = 24). T = Temperature, S = salinity, and T x S = temperature salinity interaction, * = significant at $\alpha = 0.017$.

	Factor	df	MS	F	p
$[\text{Mg}/\text{Ca}]_{\text{otolith}}$	T	3	483322.15	3.660	0.035
	S	1	361225.57	2.736	0.118
	T x S	3	47976.76	0.363	0.780
$[\text{Mn}/\text{Ca}]_{\text{otolith}}$	T	3	33.497	1.766	0.194
	S	1	5.484	0.289	0.598
	T x S	3	33.343	1.758	0.196
$[\text{Ba}/\text{Ca}]_{\text{otolith}}$	T	3	2.567	0.717	0.556
	S	1	12.285	3.431	0.083
	T x S	3	2.273	0.635	0.603

Fig. 4.2. Mg/Ca ($\text{mmol}\cdot\text{mol}^{-1}$) ratios in otoliths of laboratory-reared *Leiostomus xanthurus* as a function of tank temperatures ($^{\circ}\text{C}$) at two salinity levels, 15psu (●) and 25psu (○).

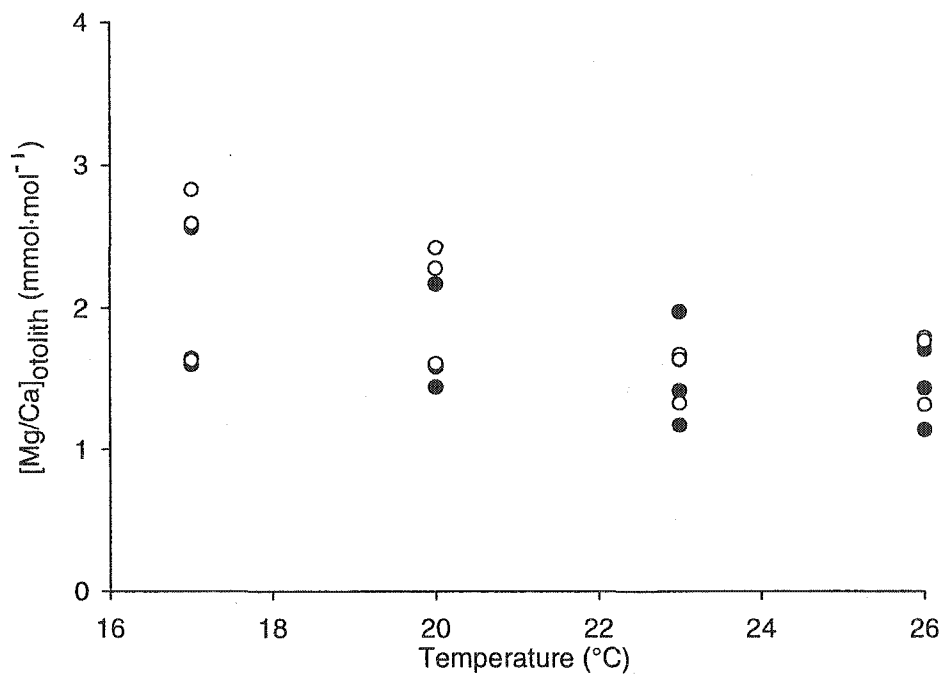


Fig. 4.3. Mn/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) ratios in otoliths of laboratory-reared *Leiostomus xanthurus* as a function of tank temperatures ($^{\circ}\text{C}$) at two salinity levels, 15psu (●) and 25psu (○).

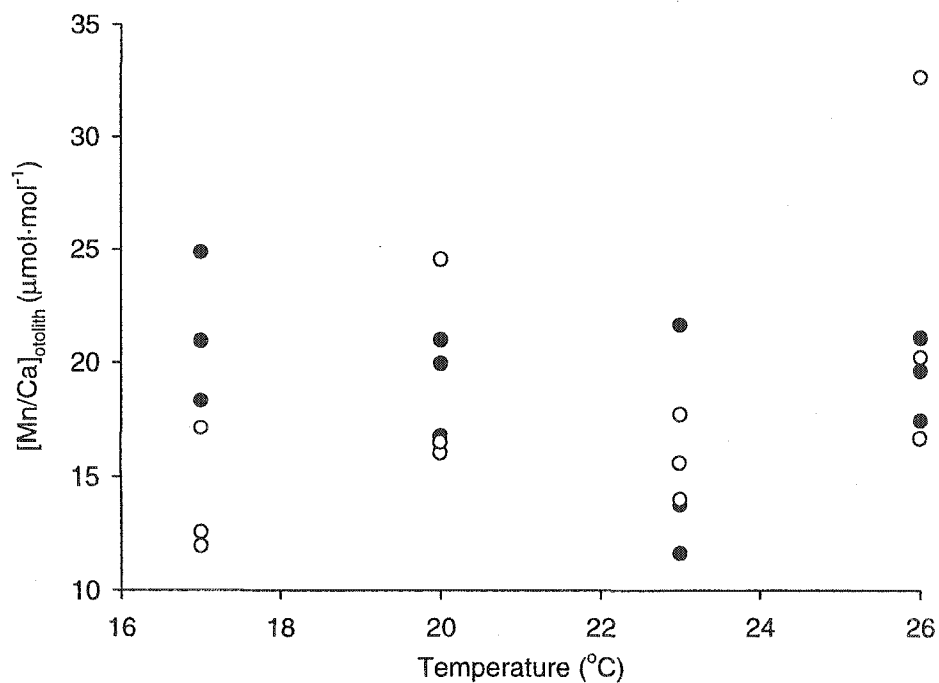
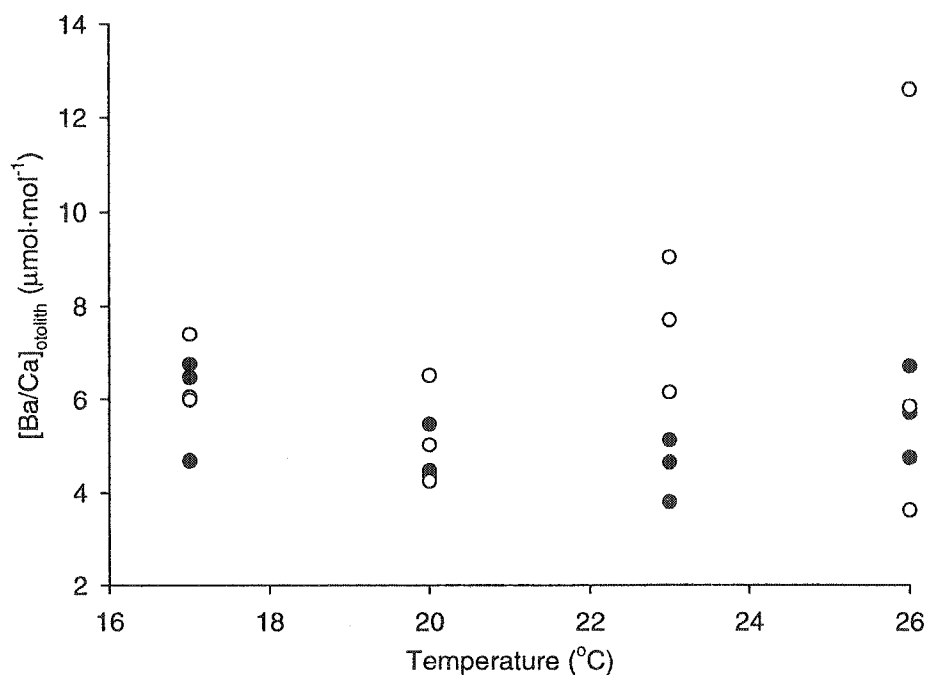


Fig. 4.4. Ba/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) ratios in otoliths of laboratory-reared *Leiostomus xanthurus* as a function of tank temperatures ($^{\circ}\text{C}$) at two salinity levels, 15psu (\bullet) and 25psu (\circ).



Me/Ca partition coefficients

Temperature and salinity did not have significant effects on D_{Mg} and the interaction between the two factors was also not significant (Fig. 4.5, Table 4.3). Temperature and salinity had significant effects on D_{Mn} , with a significant interaction between the two factors (Fig 4.6, Table 4.3). Salinity had a significant effect on D_{Ba} , with non-significant temperature effect, and non-significant interaction between the two factors (Fig 4.7, Table 4.3).

Table 4.3. Results of three separate two-way Analysis of Variance analyses testing the effect of temperature and salinity on three partition coefficients (log-transformed) in the otoliths of juvenile *Leiostomus xanthurus* ($n = 24$). T = Temperature, S = salinity, and T x S = temperature salinity interaction, * = significant at $\alpha = 0.017$.

	Factor	df	MS	F	p
D_{Mg}	T	3	0.019	3.584	0.037
	S	1	0.018	3.452	0.082
	T x S	3	0.002	0.450	0.721
D_{Mn}	T	3	0.020	13.617	0.000*
	S	1	0.020	13.316	0.002*
	T x S	3	0.008	5.187	0.011*
D_{Ba}	T	3	0.021	0.640	0.600
	S	1	0.267	8.158	0.011*
	T x S	3	0.021	0.648	0.595

Fig. 4.5. Mg/Ca partition coefficients (D_{Mg}) for otoliths of laboratory-reared *Leiostomus xanthurus* as a function of tank temperatures ($^{\circ}\text{C}$) at two salinity levels, 15psu (●) and 25psu (○).

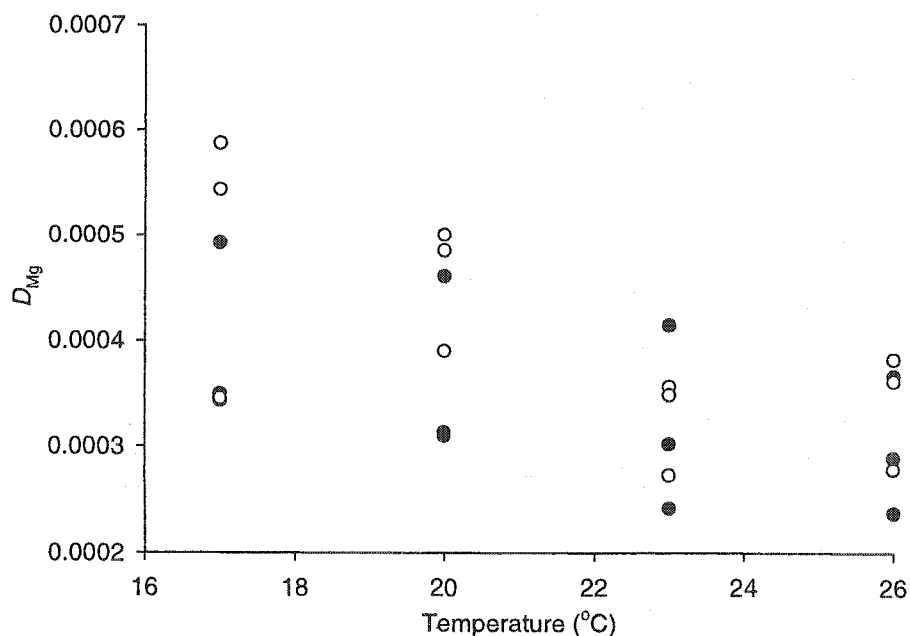


Fig. 4.6. Mn/Ca partition coefficients (D_{Mn}) for otoliths of laboratory-reared *Leiostomus xanthurus* as a function of tank temperatures ($^{\circ}\text{C}$) at two salinity levels, 15psu (●) and 25psu (○).

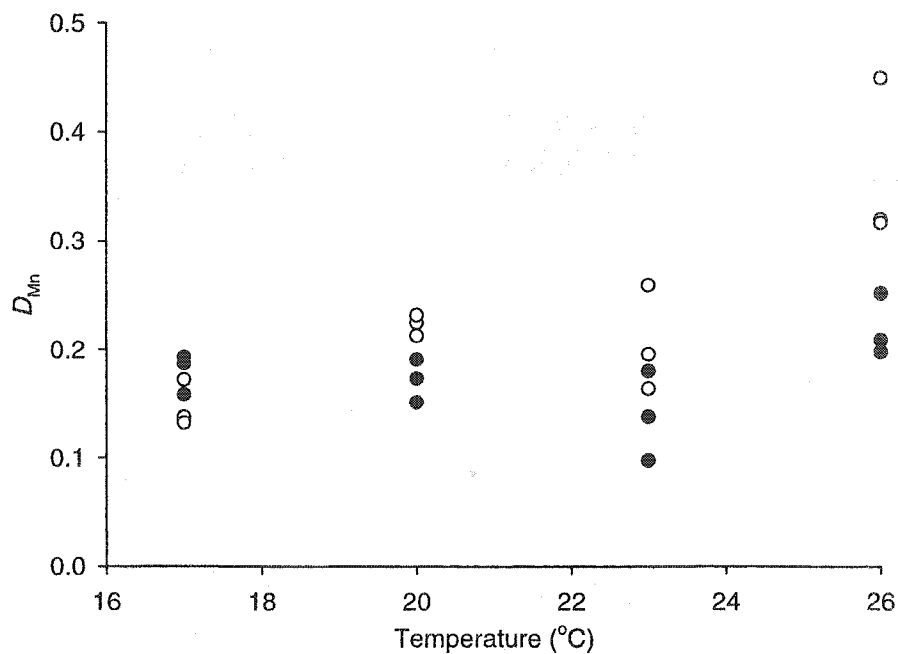
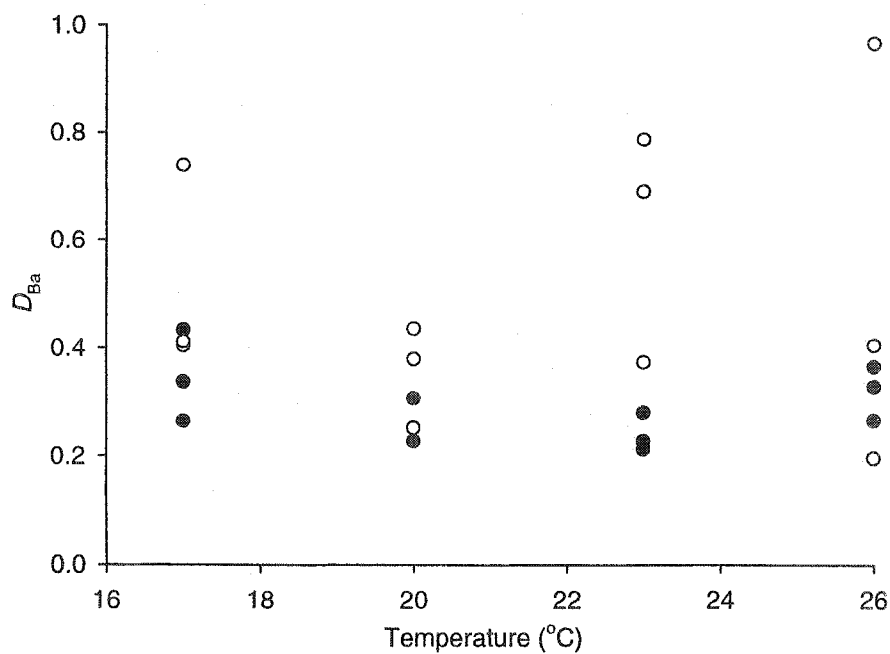


Fig. 4.7. Ba/Ca partition coefficients (D_{Ba}) for otoliths of laboratory-reared *Leiostomus xanthurus* as a function of tank temperatures ($^{\circ}\text{C}$) at two salinity levels, 15psu (●) and 25psu (○).



Biom mineralization and growth rate effects

Using otolith mass as a proxy for otolith precipitation rates, there was a significant effect of mean otolith mass among tanks on $[Mg/Ca]_{\text{otolith}}$ ($F = 40.541$, $p = 0.000$, $n = 24$), no significant effect on $[Mn/Ca]_{\text{otolith}}$ ($F = 0.295$, $p = 0.595$, $n = 24$), and no significant effect on $[Ba/Ca]_{\text{otolith}}$ ($F = 0.010$, $p = 0.923$, $n = 24$). There was, however, significant variability in $[Me/Ca]_{\text{otolith}}$ among tanks that were functions of temperature and salinity treatments, and considerable variations in otolith mass among individual fish within tanks (Table 4.1). A total of 22 out of 23 correlations between otolith mass and $[Mg/Ca]_{\text{otolith}}$ for each metal were negative, and 17 out of 23 correlations were significant after applying the Bonferroni correction for multiple tests. For $[Mn/Ca]_{\text{otolith}}$ a total of 9 out of 23 correlations were negative, and 1 out of 23 correlations were significant after applying the Bonferroni correction for multiple tests. For $[Ba/Ca]_{\text{otolith}}$ a total of 10 out of 23 correlations were negative, and none of the 23 correlations were significant after applying the Bonferroni correction for multiple tests. In summary, otolith precipitation rates significantly affected $[Mg/Ca]_{\text{otolith}}$, but did not affect $[Mn/Ca]_{\text{otolith}}$ or $[Ba/Ca]_{\text{otolith}}$.

Somatic growth rates were, as expected, significantly affected by tank temperature ($F = 4.52$, $p = 0.019$, $n = 24$), and tank salinity ($F = 11.43$, $p = 0.004$, $n = 24$). Mean growth rates among tanks had a significant effect on $[Mg/Ca]_{\text{otolith}}$ ($F = 19.261$, $p = 0.001$, $n = 24$), and no significant effect on $[Mn/Ca]_{\text{otolith}}$ ($F = 0.118$, $p = 0.736$, $n = 24$) or $[Ba/Ca]_{\text{otolith}}$ ($F = 0.143$, $p = 0.711$, $n = 24$). Looking at the relation within tanks, correlations between growth rate and $[Mg/Ca]_{\text{otolith}}$ demonstrated that 22 out of 23 correlations were negative, and 14 out of 23 correlations were significant after Bonferroni adjustment. Correlations between growth rate and $[Mn/Ca]_{\text{otolith}}$ demonstrated that 6 out

of 23 correlations were negative, and only 2 out of 23 correlations were significant after Bonferroni adjustment. Correlations between growth rate and $[\text{Ba}/\text{Ca}]_{\text{otolith}}$ demonstrated that 10 out of 23 correlations were negative, and none of the 23 correlations were significant after Bonferroni adjustment. Similar to the results for otolith precipitation rates, somatic growth rates significantly affected $[\text{Mg}/\text{Ca}]_{\text{otolith}}$ but did not effect $[\text{Mn}/\text{Ca}]_{\text{otolith}}$ or $[\text{Ba}/\text{Ca}]_{\text{otolith}}$.

Discussion

This experiment represents the first reported attempt at validating temperature and salinity effects on the incorporation of Mg, Mn, and Ba in fish otoliths. All fish were from the same brood stock and the same age, therefore limiting any possible genetic or ontogenetic effects on otolith metal incorporation. All fish were fed the same diet as well, which eliminates the effect of diet on otolith metal incorporation. The metal composition of the water was relatively constant among experimental treatments. Therefore, the metal composition of the otoliths was tested directly for temperature and salinity effects without the confounding effects of genetics, ontogeny, diet, or water compositional differences.

Water chemistry

Mg/Ca ratios were relatively constant among all treatment tanks throughout the experiment with no significant difference among salinity treatments. This result was expected since the lower salinity water was a straight dilution using deionized water. There was greater variation in Mn/Ca and Ba/Ca among treatment tanks, despite the

attempt to maintain constant concentrations. The relative differences over the course of the experiment were significantly different for Mn/Ca and Ba/Ca among salinity treatments, which was contrary to what was expected. This issue was reported in Chapter III (Martin et al. *In Press*) for strontium. Because the water for the lower salinity was a dilution of the higher salinity water, one would expect that the absolute concentrations of the metals would be different but the ratios would remain the same. The dilution source water was only deionized, and not Milli-Q water; therefore, the concentrations of Mn and Ba were higher in the deionized water, lending to higher Mn/Ca and Ba/Ca ratios in the 15psu treatment tanks. To account for any variability in water metal composition within tanks throughout the experiment, water samples were measured and partition coefficients were calculated (Morse and Bender 1990).

[Mg/Ca]_{otolith} and D_{Mg}

Although temperature did not have a statistically significant effect on [Mg/Ca]_{otolith} and D_{Mg} at $p = 0.017$, the plotted data (Fig. 4.2 and Fig. 4.5) revealed a negative influence of temperature independent of salinity on Mg incorporation in the otoliths of larval *L. xanthurus*. Assuming this trend is real, one might ask whether or not otoliths could be used as Mg/Ca thermometers given the moderate success of other biogenic Mg/Ca thermometers. Aragonitic corals (Hart and Cohen 1996; Mitsuguchi et al. 1996), molluscs (Crick and Ottensman 1983; Vander Putten et al. 2000), and calcitic foraminifera (Lea et al. 1999; Elderfield and Ganssen 2000; Rosenthal and Lohmann 2002) have been used as paleothermometers based on the Mg/Ca concentrations in their skeletal calcium carbonate. The focus of these other studies is obtaining climate data

using the organisms as passive environmental recorders, whereas, the focus on using elemental signatures in fish otoliths is to answer questions pertaining to fish stocks and fish movement (Campana and Thorrold 2001). Some research has used Sr/Ca in otolith fossils for reconstructing paleoclimates (Patterson, 1993; Kalish 1998; Patterson et al. 1999), but the majority of the otolith elemental studies are concentrating on using otoliths as tools to better understand fish populations. Thus, the use of $[\text{Mg}/\text{Ca}]_{\text{otolith}}$ concentrations as climate thermometers is not practical for questions concerning fish stocks.

Another caveat to using otoliths as Mg/Ca thermometers is that temperature also influences somatic growth rate and thus otolith precipitation rate. It would be difficult to separate the effect of temperature on Mg/Ca incorporation independent of the growth rate effect. In corals, Mg/Ca incorporation is independent of growth rate (Watanabe et al. 2001). Consequently, the relation between temperature, growth rate, and Mg/Ca incorporation should be evaluated more closely in other fish species and at different ontogenetic stages before considering otoliths for Mg/Ca thermometry. Similarly, because somatic growth rate and otolith precipitation rate influenced $[\text{Mg}/\text{Ca}]_{\text{otolith}}$ in this study, more experiments should be conducted to test these results in order for Mg to be used in otolith elemental signatures. Differences in $[\text{Mg}/\text{Ca}]_{\text{otolith}}$ may also be a result of growth rate differences and not solely environmental differences.

$[\text{Mn}/\text{Ca}]_{\text{otolith}}$ and D_{Mn}

There was no effect of temperature or salinity on $[\text{Mn}/\text{Ca}]_{\text{otolith}}$, although there was a significant effect of temperature, salinity, and a significant interaction on D_{Mn} .

These results imply $[\text{Mn}/\text{Ca}]_{\text{otolith}}$ incorporation relative to $[\text{Mn}/\text{Ca}]_{\text{water}}$ concentrations is complex. Additionally, there was a significant difference in $[\text{Mn}/\text{Ca}]_{\text{water}}$ between salinity treatment tanks, which could cause these significant results for D_{Mn} .

Magnesium, barium (nutrient-type distribution), and calcium are conservative elements in seawater with relatively long residence times (Broeker and Peng 1982). In contrast, Mn is not conservative, is weakly complexed (Byrne et al. 1988), and highly vulnerable to redox cycling (Shen et al. 1991). Therefore, Mn may not be a reliable element for otolith elemental signatures because it is unstable within a given water column.

Dissolved Mn is rapidly oxidized in estuarine waters, aided by microbial catalysis which plays a dominant role in the scavenging of Mn onto particles in the aquatic environment (Sunda and Huntsman 1987; von Langen et al. 1997; Klinkhammer and McManus 2001). Microbial-aided oxidation was likely in the experimental tanks given the high variability in Mn/Ca concentrations throughout the experiment. Bacterial growth was observed in the tanks, even though water was changed out daily and solid particles removed by siphoning. It is entirely possible that bacteria served as catalysts forming manganese oxides, which precipitated out of solution, therefore creating unstable Mn/Ca concentrations in the tanks over time. Manganese concentrations fluctuate among different geographically located water bodies and have been used successfully in combination with other metals for discriminant function analyses to differentiate fish populations by locations. However, because otolith elemental signatures rely on using multiple elements for classification, the impact of $[\text{Mn}/\text{Ca}]_{\text{otolith}}$ on the variability within the analyses remains undefined. The Mn/Ca relation between the environment and the

otolith remains unclear and for this reason the Mn/Ca values in otoliths should be interpreted with caution.

[Ba/Ca]_{otolith} and D_{Ba}

The results showed a significant effect of salinity on D_{Ba} . This salinity effect on D_{Ba} could be explained by the significant difference in the [Ba/Ca]_{water} of the two salinity treatment (15 and 25psu) tanks. If there is no significant effect of temperature or salinity on [Ba/Ca]_{otolith}, then [Ba/Ca]_{water} is possibly the main factor controlling [Ba/Ca]_{otolith}, similar to calcitic foraminifera (Lea and Spero 1992, 1994) (Bath et al. 2000).

Ba concentrations vary among estuaries as attributed to catchment rock types, weathering rates, river flows, and groundwater discharge (Coffey et al. 1997; Shaw et al. 1998). It has typically been assumed that Ba end members range from 90-634 nM in freshwater, to 36-40 nM in seawater, with a linear mixing curve between the two except for the initial decrease at salinities below 5psu (Coffey et al. 1997; Shaw et al. 1998). Shaw et al.'s (1998) work shows groundwater discharge contributes significantly to Ba concentrations along inner shelf waters of the U.S. southeast Atlantic coast, which could influence Ba concentrations along the proposed gradient between end members. This Ba input could have a significant impact on the use of [Ba/Ca]_{otolith} to describe transport or migration patterns corresponding to ambient Ba/Ca in the water. Although the end members may not change, the variability between them could be nonlinear. Therefore, water samples should be taken simultaneously to ground truth the otolith composition if Ba/Ca is used for reconstructing individual fish transport paths.

Biom mineralization and growth rate effects

Otolith precipitation rates had a significant inverse correlation with $[Mg/Ca]_{\text{otolith}}$ as there was proportionately less Mg/Ca incorporated into the otoliths as a function of otolith size; however, there was no correlation between otolith precipitation rate and $[Mn/Ca]_{\text{otolith}}$ or $[Ba/Ca]_{\text{otolith}}$. Otolith precipitation rates and somatic growth rates within individual tanks were not significantly correlated with $[Mn/Ca]_{\text{otolith}}$ and $[Ba/Ca]_{\text{otolith}}$ but were significantly correlated with $[Mg/Ca]_{\text{otolith}}$. Somatic growth rates were significantly influenced by temperature; therefore, physiological differences contributing to otolith Mg/Ca incorporation cannot be ignored.

In summary, $[Ba/Ca]_{\text{otolith}}$ seems to be indicative of the water Ba/Ca composition and not influenced by temperature or salinity. Although temperature did not have a statistically significant effect on $[Mg/Ca]_{\text{otolith}}$, the plotted data revealed $[Mg/Ca]_{\text{otolith}}$ was influenced by temperature but salinity had no effect. $[Mg/Ca]_{\text{otolith}}$ was also significantly affected by otolith precipitation rate and somatic growth rate. It is therefore difficult to separate the direct effect of temperature on otolith Mg/Ca incorporation as temperature also affected somatic growth. $[Mn/Ca]_{\text{otolith}}$ may be unpredictable for elemental signatures in otoliths since Mn concentrations are so variable in the aquatic environment at relatively short temporal and spatial scales. The results of this experiment are specific to larval *L. xanthurus* (17.3-38.0 mm SL) for the controlled conditions tested (temperature range: 17-26 °C, salinities 15 and 25psu). Based on the results from this experiment, Mg should not be used for elemental signatures in *L. xanthurus* otoliths for the fish size ranges tested because of the biomineralization and temperature effects on Mg incorporation. Manganese also should not be used because of the likelihood of

significant variability in the water composition experienced by individual fish, lending to a higher degree of erroneous elemental signatures and therefore misallocation. It may be possible to extrapolate these results to the larvae of other estuarine dependent species that have a similar life history as *L. xanthurus* (i.e., other Sciaenidae), however, not enough work has been done to say this with confidence. We still do not know what role ontogeny plays in the incorporation of elements in fish otoliths, nor do we have enough data to assess incorporation differences among species. So, both Mg/Ca and Mn/Ca may be useful as “natural” tags provided there is adequate groundtruthing. However, neither are likely to provide and estimate of Mg and Mn levels in the water column.

CHAPTER V
EFFECT OF TEMPERATURE AND SALINITY ON THE OTOLITH
CHEMISTRY OF JUVENILE GRAY SNAPPER
(*LUTJANUS GRISEUS*)

Introduction

Elemental signatures in fish otoliths have been used to discriminate fish stocks (Campana et al. 1994; Begg et al. 1998), describe natal homing (Thorrold et al. 2001), identify the relative contribution of juveniles from different nursery habitats to fished populations (Gillanders and Kingsford 1996, 2000; Rooker et al. 2001), and to detect transport or migration pathways for individual fish (Secor 1992). The assumption underlying these applications is that fish incorporate elements from their environment, and these elements are permanently recorded in their otoliths. The specific behavior of individual elements is often ignored and few studies have tested the effect of water temperature and salinity on element incorporation in otoliths (Farrell and Campana 1996; Chesney et al. 1998; Bath et al. 2000; Milton and Chenery 2001; Elsdon and Gillanders 2002; Martin et al. *In press*).

Although elemental signatures in otoliths have successfully been used as natural tags in some species, important assumptions that influence the unambiguous use of these signatures have not been validated. For example, species-specific differences in elemental incorporation have not been evaluated, and for most species, the potential effect of differences in salinity or water temperature is unknown. Although understanding

the details of elemental incorporation is not necessary for all applications of otolith elemental signatures, physical and biological factors can influence the concentrations of these elements in the otolith, rendering the interpretation of elemental signatures more complex than assumed.

The objectives of this experiment were to validate element incorporation in gray snapper (*Lutjanus griseus*) otoliths by quantifying the relation between otolith and water element concentrations (Sr/Ca, Mg/Ca, Mn/Ca, and Ba/Ca) as a function of differences in water temperature and salinity. The methods employed were similar to those used for spot (*Leiostomus xanthurus*; Chapters II – IV), and thus permit a species-specific comparison of temperature and salinity effects on otolith element incorporation.

Materials and methods

Gray snapper (*Lutjanus griseus*) were collected on a rising tide from the channel spanned by Pivers Island Bridge, near Beaufort, North Carolina (USA), approximately 2 km inside of Beaufort Inlet. Larvae were collected using a 1 x 2 m neuston net (1 mm mesh) with a floating live-box attached to the cod-end, fished at the surface (Hettler 1979). Sampling was conducted during nighttime maximum flood tides and concentrated around the days preceding full and new moons (Tzeng et al. *In Press*). Snapper were gently dipped from the live-box and transferred to 100 L holding tanks with flow-through seawater for temperature acclimation ($1\text{ }^{\circ}\text{C}\cdot\text{d}^{-1}$).

Two fully crossed, 2 x 5 factorial designs with equal replication ($n = 5$) were used to investigate the effects of 20 different temperature and salinity combinations on otolith element incorporation in juvenile gray snapper. Temperature (18, 23, 28, and 33 °C) and

salinity (5, 15, 25, 35, and 45psu) levels were chosen to represent nursery habitat conditions for gray snapper. The experiments were carried out in two separate trials due to space limitations. The first trial included the 28 and 33 °C treatment temperatures and fish collected 5-6 days preceding the September new moon (1 - 5 September, 2002). The second trial included the 18 and 23 °C temperature treatments and the fish collected in the 5 days preceding the October new moon (30 September – 5 October 2002).

Once the desired temperature treatment levels were attained in the 100 L tanks, fish were individually stocked into treatment tanks. The four salinity levels were randomly assigned to the experimental tanks and were maintained by mixing filtered sea water (30 – 35psu) with either conditioned well water or Instant Ocean® synthetic sea salt. Salinity levels were adjusted $5\text{psu}\cdot\text{d}^{-1}$ until the desired salinity treatment levels were reached. When all fish were at the desired treatment levels, they were acclimated for one week and then measured (standard and total lengths in mm and weight in mg).

Each day snapper were fed *Artemia*, a prepared gel diet, or larval fish (*Eucinostomus* sp. or guppies) prey, offered *ad libitum*. A one third volume water change was performed daily to prevent the buildup of metabolic wastes and to maintain the desired salinity levels. Tank water temperatures and salinities were measured daily. Experimental conditions were maintained for 55 days. At the end of each experimental trial, the final weights and lengths of individual fish were measured.

Water samples

Water samples ($n = 5$ per tank) were taken weekly for inductively coupled plasma mass spectrometry (ICP-MS) analysis of elemental water signatures. Samples were

collected using acid-washed 10 mL polypropylene syringes and filtered through 0.2 μm polypropylene syringe filters (Whatman) into 7 ml acid-washed polypropylene vials. Each sample was acidified to \sim pH 2 with ultrapure HCl_{conc} and stored frozen until subsequent analyses.

Otolith Analyses

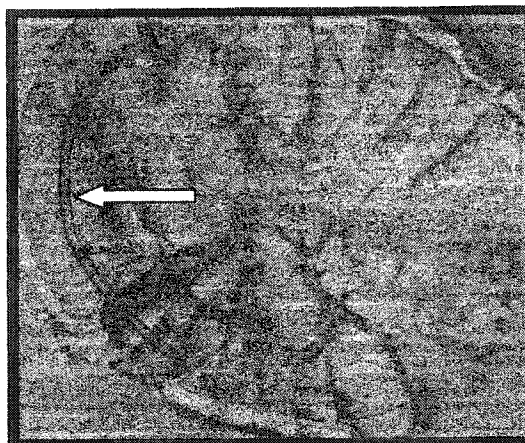
At the conclusion of each experimental trial, all of the remaining fish were measured and their sagittal otolith pairs removed for otolith analyses. Otoliths were scraped clean with acid-washed glass probes in a class-100 clean room. Otoliths were ultrasonically cleaned in Milli-Q water for 7 minutes and triple rinsed with ultrapure H_2O_2 (Ultrex, J.T. Baker) followed by three sequential rinses of Milli-Q water. Otoliths were then placed on acid-washed glass slides to dry for 36 hours under a class-100 laminar-flow hood. After drying, otoliths were stored in acid-washed 1.5 ml high-density polyethylene vials. The left otolith of each pair was mounted on a petrographic slide with superglue and polished along the sagittal plane. After polishing, the otoliths were soaked in milli-Q water, cleaned, and dried as described above. Finally otoliths were mounted on petrographic slides (21 per slide) for LA ICP-MS analyses.

Because the fish for the two trials were live-captured at different times and possibly experienced different water masses different initial otolith element compositions were assumed. For that reason, laser ablation was used to sample the portion of the otolith that corresponded in time to the period during which fish were exposed to the experimental conditions (> 30 days old). Curvilinear transects (800 μm) were ablated on each otolith (Fig. 5.1) using a New Wave UP-213 laser with a 40 μm beam width coupled

with a Thermo Finnigan Element ICP-MS equipped with a self-aspirating ($50 \mu\text{L}\cdot\text{min}^{-1}$) PFA nebulizer and a dual-inlet quartz spray chamber. The method measured ^{25}Mg , ^{48}Ca , ^{55}Mn , ^{86}Sr , and ^{138}Ba in low resolution ($R = 300$) during a 2-minute acquisition time.

Quantification of metal/calcium (Me/Ca) ratios followed the procedure outlined by Rosenthal et al. (1999). All samples were standardized to a dissolved solution ($0.1 \text{ mg}\cdot\text{g}^{-1}$) of an otolith reference powder with certified Me/Ca ratios of $89.25 \mu\text{mol}\cdot\text{mol}^{-1}$ for Mg/Ca, $0.257 \mu\text{mol}\cdot\text{mol}^{-1}$ for Mn/Ca, $2.782 \text{ mmol}\cdot\text{mol}^{-1}$ for Sr/Ca, and $2.174 \mu\text{mol}\cdot\text{mol}^{-1}$ for Ba/Ca (Yoshinaga et al. 2000). The matrix of the standard was therefore matched to the Ca levels in the samples. Detection limits were calculated as 3σ values of 1% HNO_3 sample blanks ($n = 18$) that were run throughout the analyses. These limits were 1.5% of the average sample intensity for ^{25}Mg , 0.1% for ^{48}Ca , 21% for ^{55}Mn , 0.04% for ^{86}Sr , and 0.2% for ^{138}Ba . An internal laboratory standard was run after each reference sample to estimate precision of the Me/Ca method. The reference material was then treated as an unknown, and Me/Ca values determined as for individual samples above. Measured precision (% relative standard deviation (RSD), $n = 18$) of the Me/Ca method was 0.3% for Mg/Ca, 1.2% for Mn/Ca, 0.4% for Sr/Ca, and 0.4% for Ba/Ca.

Fig. 5.1. *Lutjanus griseus* sagittal otolith polished in the sagittal plane showing the laser scar (indicated by the arrow), which sampled along growth increments corresponding to the time period during which fish experienced experimental conditions.



Water analyses

Analyses of water samples collected during the experiment were also conducted using ICP-MS. Three samples were run from each tank representing the average conditions over the course of the experiment. All samples were spiked with Indium (to $4.5 \mu\text{g}\cdot\text{g}^{-1}$), which was used as an internal standard. The solutions were then aspirated into a Thermo Finnigan Element 2 ICP-MS, via a self-aspirating nebulizer ($50 \mu\text{L}\cdot\text{min}^{-1}$) and Scott's double pass spray chamber. Due to the presence of significant interferences on most of the Ca isotopes, ^{44}Ca , ^{88}Sr , ^{25}Mg , ^{55}Mn , ^{137}Ba , and ^{115}In were measured in medium resolution (nominal $R = 4500$). Three samples from each tank were averaged and the mean values were then used in all subsequent analyses. To estimate precision of the water measurements, Ca, Sr, Mg, Mn, and Ba values in a seawater reference material (High Purity Standards, Inc. seawater CRM) were determined. The estimates of precision for element concentrations in the seawater CRM for trial 1 were 2.1% RSD for

Ca, 4.2% RSD for Sr, 1.7% RSD for Mg, 12.2% RSD for Mn, and 4.5% RSD for Ba ($n = 3$) and trial 2 were 1.4% RSD for Ca, 1.3% RSD for Sr, 1.3% RSD for Mg, 6.8% RSD for Mn, and 3.9% RSD for Ba ($n = 3$).

Partition coefficients (D_{Me}) were calculated by dividing the metal/calcium (Me/Ca) ratio measured in an otolith by the mean Me/Ca ratio measured in the treatment tank water (Morse and Bender 1990). Otolith Me/Ca values from individual fish per tank were used as replicates for each of the twenty treatments.

Statistical analyses

Two-way analysis of variance (ANOVA) was used to test for significant differences in $[Me/Ca]_{water}$, $[Me/Ca]_{otolith}$, and D_{Me} among temperature and salinity treatments for each trial. Salinity and temperature were treated as independent categorical variables, and $[Me/Ca]_{water}$, $[Me/Ca]_{otolith}$, and D_{Me} as dependent variables in the analyses. Because the $[Me/Ca]_{otolith}$ and D_{Me} data did not meet the homogeneity of variance assumption of ANOVA, various transformations of these data were attempted. Although the log-transformation did not make the variances homogeneous, it did lessen the magnitude of the differences among treatment groups. The F statistic has been proven to be very robust despite assumption violations (Lindman 1974).

Results

The means and ranges of $[Me/Ca]_{otoliths}$, and D_{Me} from 90 juvenile *Lutjanus griseus* and the tank $[Me/Ca]_{water}$, ($n = 90$) are reported in Table 5.1.

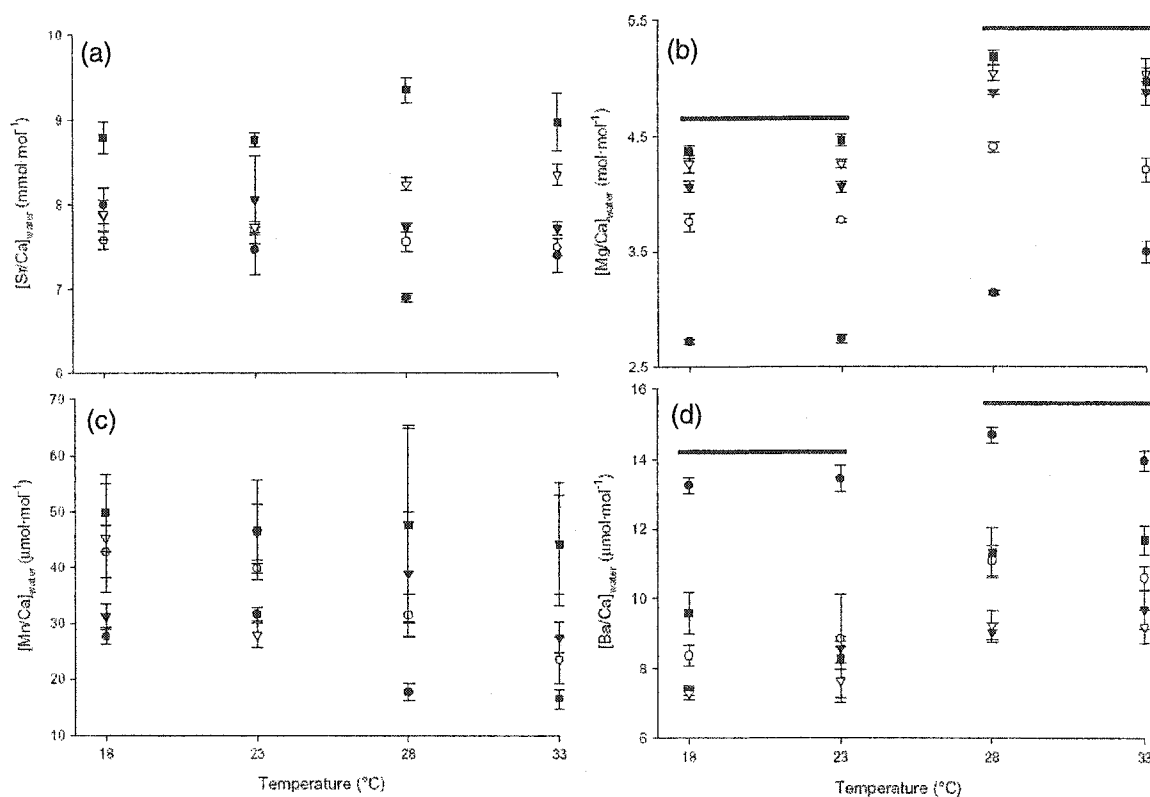
Water chemistry

As expected, elemental concentrations in the tank water were significantly affected by salinity treatments for both trials: $[\text{Sr}/\text{Ca}]_{\text{water}}$ (Trial 1: $F = 66.160$, $p = 0.000$, $n = 45$; Trial 2: $F = 6.670$, $p = 0.000$, $n = 45$), $[\text{Mg}/\text{Ca}]_{\text{water}}$ (Trial 1: $F = 166.829$, $p = 0.000$, $n = 45$; Trial 2: $F = 332.576$, $p = 0.000$, $n = 45$), and $[\text{Ba}/\text{Ca}]_{\text{water}}$ (Trial 1: $F = 47.049$, $p = 0.000$, $n = 45$; Trial 2: $F = 39.131$, $p = 0.000$, $n = 45$) (Fig. 5.2). $[\text{Mn}/\text{Ca}]_{\text{water}}$ was not significantly different at $\alpha = 0.0125$ (Trial 1: $F = 2.664$, $p = 0.049$, $n = 45$; Trial 2: $F = 3.059$, $p = 0.029$, $n = 45$).

Table 5.1. *Lutjanus griseus*. Combined trial means and ranges for each $[\text{Me}/\text{Ca}]_{\text{otolith (o)}}$ ($n = 90$), $[\text{Me}/\text{Ca}]_{\text{water (w)}}$ ($n = 90$), and D_{Me} ($n = 90$). $[\text{Mg}/\text{Ca}]_{\text{water}}$ values are $\text{mol}\cdot\text{mol}^{-1}$. $[\text{Sr}/\text{Ca}]_{\text{otolith}}$, $[\text{Sr}/\text{Ca}]_{\text{water}}$, and $[\text{Mg}/\text{Ca}]_{\text{otolith}}$ values are $\text{mmol}\cdot\text{mol}^{-1}$. $[\text{Mn}/\text{Ca}]_{\text{otolith}}$, $[\text{Mn}/\text{Ca}]_{\text{water}}$, $[\text{Ba}/\text{Ca}]_{\text{otolith}}$, and $[\text{Ba}/\text{Ca}]_{\text{water}}$ values are $\mu\text{mol}\cdot\text{mol}^{-1}$.

source	mean	range	source	mean	range	source	mean	range
$[\text{Sr}/\text{Ca}]_{\text{o}}$	2.27	1.72-2.88	$[\text{Sr}/\text{Ca}]_{\text{w}}$	7.97	6.52-9.86	D_{Sr}	0.287	0.211-0.380
$[\text{Mg}/\text{Ca}]_{\text{o}}$	0.088	0.036-0.247	$[\text{Mg}/\text{Ca}]_{\text{w}}$	7.97	2.65-5.35	D_{Mg}	2.19×10^{-5}	7.65×10^{-6} - 8.91×10^{-5}
$[\text{Mn}/\text{Ca}]_{\text{o}}$	8.22	1.38-29.36	$[\text{Mn}/\text{Ca}]_{\text{w}}$	35.98	12.54-117.94	D_{Mn}	0.285	0.0176-1.02
$[\text{Ba}/\text{Ca}]_{\text{o}}$	1.24	0.491-3.12	$[\text{Ba}/\text{Ca}]_{\text{w}}$	0.13	4.31-15.28	D_{Ba}	0.13	0.043-0.45

Fig. 5.2. Calculated metal/calcium ratios in the tank water for both trials: Sr/Ca (panel a), Mg/Ca (panel b), Mn/Ca (panel c), and Ba/Ca (panel d) concentrations ± 1 SE by temperature treatment. Each of the five salinity treatments is represented by a different symbol: 5 (●), 15 (○), 25 (▼), 35 (▽), and 45psu (■). The bars in panel b and d indicate significant differences in Me/Ca concentrations by temperatures representing the two separate trials.



Otolith [Me/Ca]

Temperature (Trial 2) and salinity (Trial 1) had significant effects on [Sr/Ca]_{otolith} for *L. griseus* with no interaction effect (Table 5.1, Fig. 5.3). Neither temperature or salinity had a significant effect on [Mg/Ca]_{otolith} and there was no interaction effect (Table 5.1, Fig. 5.4). Temperature nor salinity had significant effects on [Mn/Ca]_{otolith} and there was no interaction effect (Table 5.1, Fig. 5.5). There was a significant salinity effect (Trial 1) but no significant temperature effect on [Ba/Ca]_{otolith} (Table 5.1, Fig. 5.6) and the interaction between the two factors was not significant.

Table 5.2. Results of four separate two-way Analysis of Variance analyses for each trial testing the effect of temperature and salinity on four elemental signatures (log-transformed) in the otoliths of juvenile *Lutjanus griseus*. T = Temperature, S = salinity, and T x S = temperature salinity interaction, * = significant at $\alpha = 0.0125$.

Trial 1	Factor	df	MS	F	p
[Sr/Ca] _{otolith}	T	1	0.005	3.109	0.087
	S	4	0.010	6.666	0.000*
	T x S	4	0.004	2.844	0.038
	T	1	0.035	0.917	0.345
	S	4	0.012	0.316	0.866
	T x S	4	0.035	0.938	0.454
	T	1	0.007	0.064	0.802
	S	4	0.093	0.886	0.483
	T x S	4	0.117	1.108	0.368
[Ba/Ca] _{otolith}	T	1	0.014	0.651	0.425
	S	4	0.087	4.033	0.009*
	T x S	4	0.020	0.939	0.453
Trial 2					
[Sr/Ca] _{otolith}	T	1	0.020	10.146	0.003*
	S	4	0.003	1.509	0.221
	T x S	4	0.003	1.515	0.219
[Mg/Ca] _{otolith}	T	1	0.019	0.459	0.502
	S	4	0.044	1.065	0.388
	T x S	4	0.037	0.882	0.485
[Mn/Ca] _{otolith}	T	1	0.031	0.429	0.517
	S	4	0.037	0.520	0.721
	T x S	4	0.105	1.458	0.236
[Ba/Ca] _{otolith}	T	1	0.004	0.118	0.734
	S	4	0.037	1.009	0.416
	T x S	4	0.041	1.098	0.373

Fig. 5.3. Mean Sr/Ca ($\text{mmol}\cdot\text{mol}^{-1}$) \pm 1 SE in otoliths of *Lutjanus griseus* for both trials as a function of tank temperatures ($^{\circ}\text{C}$) at five salinity levels, 5 (\bullet), 15 (\circ), 25 (\blacktriangledown), 35(∇), and 45psu (\blacksquare).

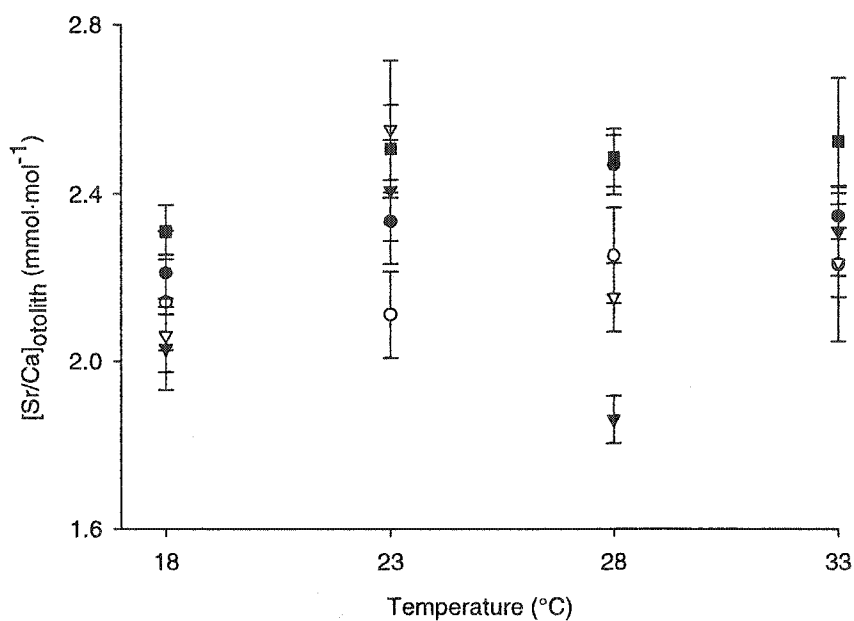


Fig. 5.4. Mean Mg/Ca ($\text{mmol}\cdot\text{mol}^{-1}$) \pm 1 SE in otoliths of *Lutjanus griseus* for both trials as a function of tank temperatures ($^{\circ}\text{C}$) at five salinity levels, 5 (\bullet), 15 (\circ), 25 (\blacktriangledown), 35(∇), and 45psu (\blacksquare).

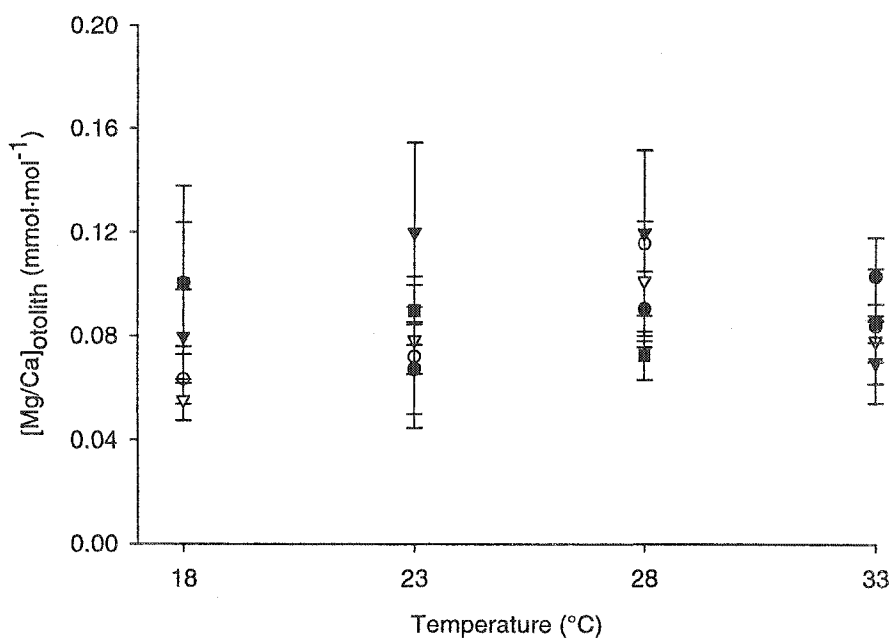


Fig. 5.5. Mean Mn/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) \pm 1 SE in otoliths of *Lutjanus griseus* for both trials as a function of tank temperatures ($^{\circ}\text{C}$) at five salinity levels, 5 (\bullet), 15 (\circ), 25 (\blacktriangledown), 35(∇), and 45psu (\blacksquare).

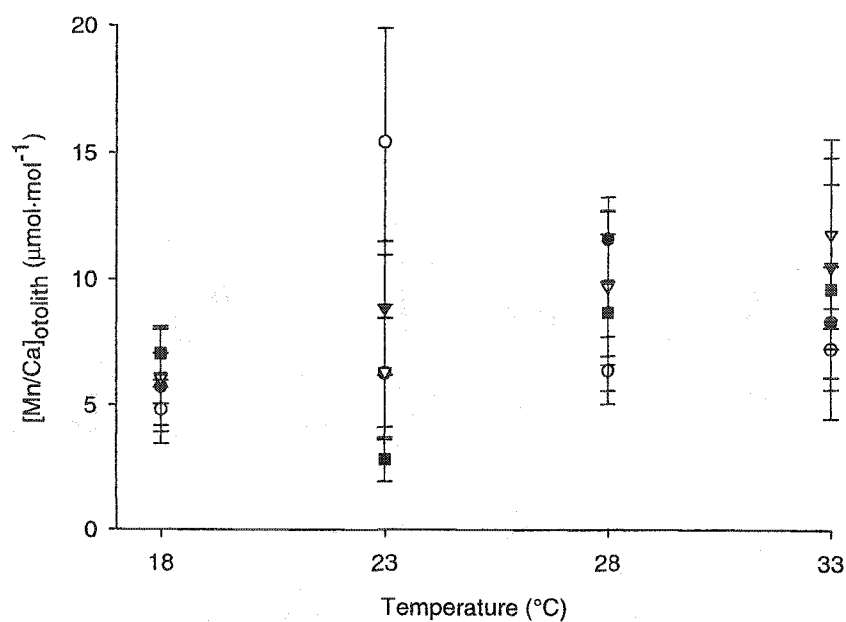
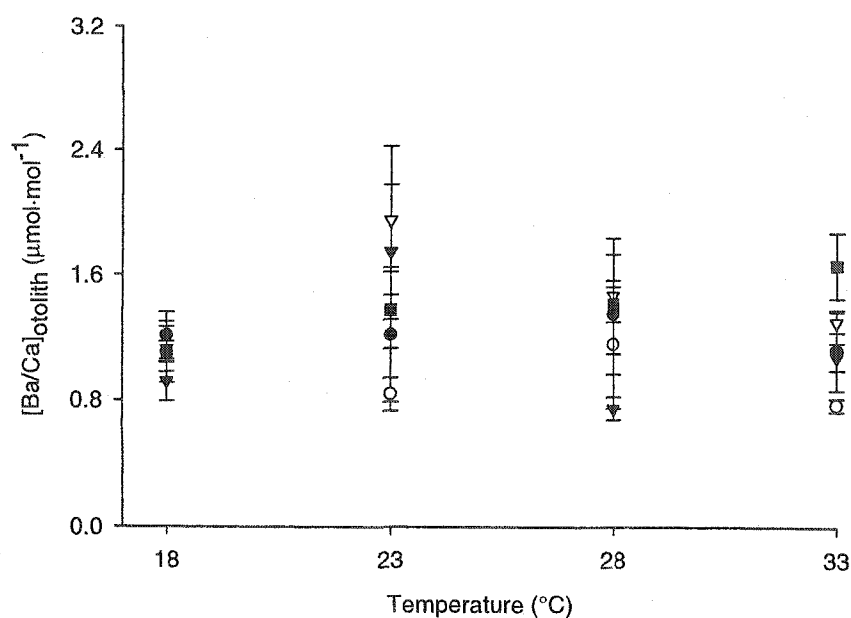


Fig. 5.6. Mean Ba/Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$) \pm 1 SE in otoliths of *Lutjanus griseus* for both trials as a function of tank temperatures ($^{\circ}\text{C}$) at five salinity levels, 5 (\bullet), 15 (\circ), 25 (\blacktriangledown), 35(∇), and 45psu (\blacksquare).



Me/Ca partition coefficients

Temperature (Trial 2) and salinity (Trial 1) had significant effects on D_{Sr} , with no interaction between the two factors (Table 5.2, Fig 5.7). The temperature effect occurred at the lower temperature treatments (18 and 23 °C), and the salinity effect occurred only at the higher temperature treatments (28 and 33 °C). If the four temperature treatments could have been included in the same analysis (could not because of time difference), this salinity effect at low temperatures would probably be manifested as a temperature x salinity interaction. There was no significant temperature, salinity effect on D_{Mg} , or interaction between the two factors (Table 5.2, Fig. 5.8). Temperature and salinity did not have significant effects on D_{Mn} , with no interaction between the two factors (Table 5.2, Fig 5.9). Salinity had a significant effect on D_{Ba} in both trials, with non-significant temperature effect, and non-significant interaction between the two factors (Table 5.2, Fig 5.10).

Table 5.3. Results of four separate two-way Analysis of Variance analyses for both trials testing the effect of temperature and salinity on four D_{Me} (log-transformed) in the otoliths of juvenile *Lutjanus griseus*. T = Temperature, S = salinity, and T x S = temperature salinity interaction. * = significant at $\alpha = 0.0125$.

Trial 1	Factor	df	MS	F	p
D_{Sr}	T	1	0.003	1.599	0.214
	S	4	0.019	9.079	0.000*
	T x S	4	0.006	3.172	0.025
D_{Mg}	T	1	0.036	0.981	0.329
	S	4	0.114	3.083	0.028
	T x S	4	0.029	0.787	0.541
D_{Mn}	T	1	0.003	0.018	0.895
	S	4	0.371	2.310	0.077

Table 5.3 continued

	T x S	4	0.104	0.645	0.634
D _{Ba}	T	1	0.017	0.743	0.395
	S	4	0.124	5.595	0.001*
	T x S	4	0.015	0.681	0.610
Trial 2	Factor	df	MS	F	p
D _{Sr}	T	1	0.025	10.865	0.002*
	S	4	0.002	0.824	0.519
	T x S	4	0.003	1.318	0.283
D _{Mg}	T	1	0.017	0.411	0.525
	S	4	0.082	2.037	0.110
	T x S	4	0.038	0.946	0.449
D _{Mn}	T	1	0.028	0.349	0.558
	S	4	0.098	1.198	0.329
	T x S	4	0.097	1.196	0.330
D _{Ba}	T	1	0.002	0.077	0.783
	S	4	0.181	5.880	0.001*
	T x S	4	0.038	1.227	0.317

Fig. 5.7. Mean Sr/Ca partition coefficients (D_{Sr}) \pm 1 SE for otoliths of *Lutjanus griseus* for both trials as a function of tank temperatures ($^{\circ}\text{C}$) at five salinity levels, 5 (\bullet), 15 (\circ), 25 (\blacktriangledown), 35(∇), and 45psu (\blacksquare).

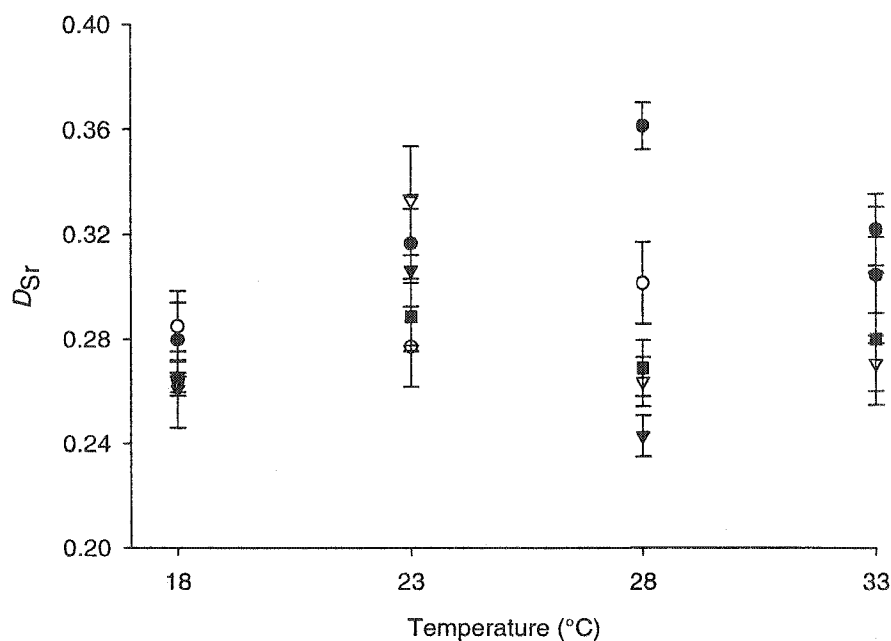


Fig. 5.8. Mean Mg/Ca partition coefficients (D_{Mg}) \pm 1 SE for otoliths of *Lutjanus griseus* for both trials as a function of tank temperatures ($^{\circ}\text{C}$) at five salinity levels, 5 (\bullet), 15 (\circ), 25 (\blacktriangledown), 35(∇), and 45psu (\blacksquare).

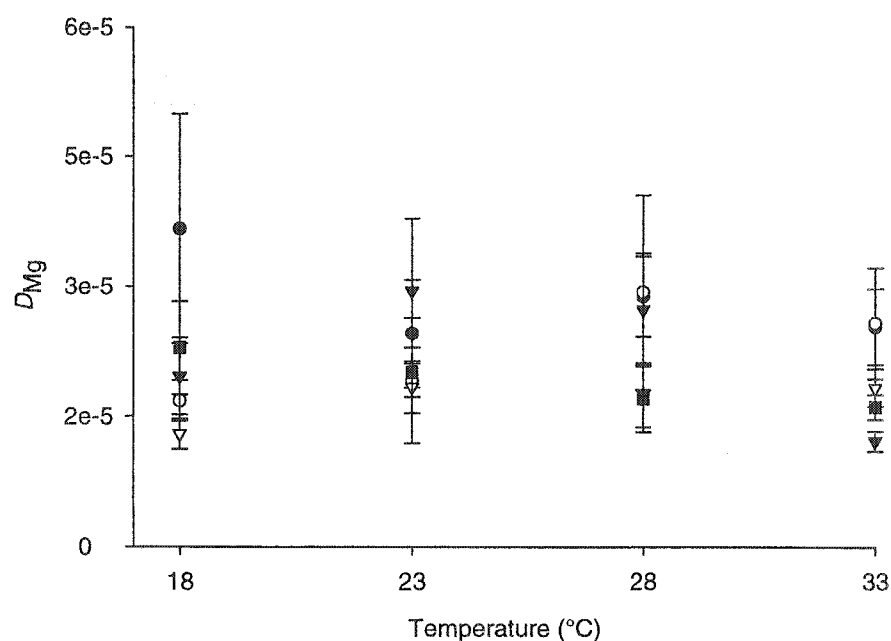


Fig. 5.9. Mean Mn/Ca partition coefficients (D_{Mn}) \pm 1 SE for otoliths of *Lutjanus griseus* for both trials as a function of tank temperatures ($^{\circ}\text{C}$) at five salinity levels, 5 (\bullet), 15 (\circ), 25 (\blacktriangledown), 35(∇), and 45psu (\blacksquare).

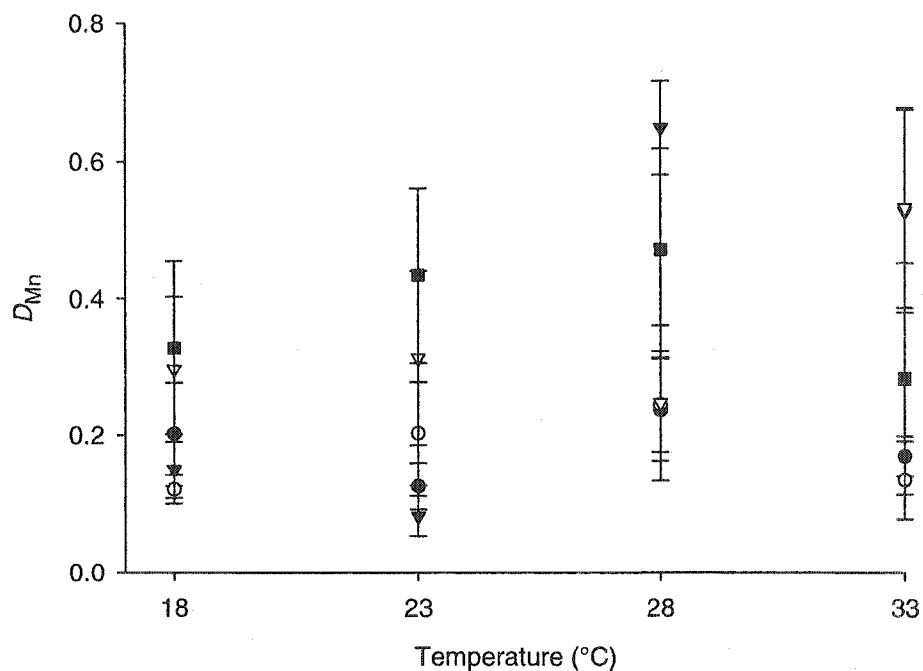
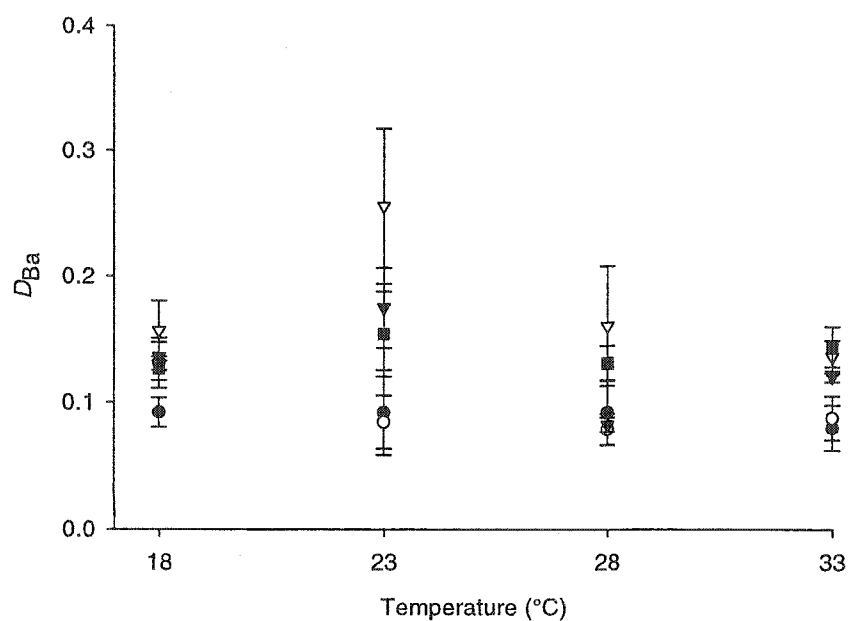


Fig. 5.10. Mean Ba/Ca partition coefficients (D_{Ba}) \pm 1 SE for otoliths of *Lutjanus griseus* for both trials as a function of tank temperatures ($^{\circ}\text{C}$) at five salinity levels, 5 (\bullet), 15 (\circ), 25 (\blacktriangledown), 35(∇), and 45psu (\blacksquare).



Discussion

The main objective of this experiment was to test the effect of temperature and salinity on element incorporation in the otoliths of juvenile gray snapper and compare the results to those from a similar experiment conducted on spot, *Leiostomus xanthurus* (Chapters III and IV). To make the comparison, I used the same temperature and salinity ranges for both species by recalculating the ANOVAS for *L. griseus*, having eliminated the 33 °C temperature treatment and the 5 and 45psu salinity treatment results ($n = 40$) which were not used in experiments with *L. xanthurus*. Temperature significantly affected $[Sr/Ca]_{\text{otolith}}$ in *L. griseus* and *L. xanthurus* within the treatment ranges I compared. There were no significant salinity or interaction effects between temperature and salinity treatments for $[Me/Ca]_{\text{otolith}}$ for either species (Table 5.4). For *L. xanthurus*, D_{Sr} was significantly affected by both temperature and salinity. The D_{Sr} for *L. griseus* was of borderline significance with an $\alpha = 0.0125$ and a $p\text{-value} = 0.019$. Temperature and salinity had significant effects on D_{Mn} for *L. xanthurus*, but not for *L. griseus*. D_{Ba} was significantly affected by salinity for both species (Table 5.4).

Differences in ontogeny do not explain the varied results of temperature and salinity effects on otolith element incorporation because both experiments were conducted with fish at the same life stage (late larval-early juvenile). The diets fed to the fish for each experiment were different, which may explain some of the variability in the relative $[Me/Ca]_{\text{otolith}}$ values, although, experimental studies have found little evidence for an effect of diet on otolith element incorporation (Hoff and Fuiman 1995; Milton and Chenery 2001).

Table 5.4. A comparison between Analysis of Variance results for the same temperature (15-28 °C), and salinity (15-35psu) ranges with the temperature-salinity interaction effects on each $[Me/Ca]_{\text{otolith}}$ and D_{Me} from *Leiostomus xanthurus* (n = 24, Chapter IV), and *Lutjanus griseus* (n = 40, Chapter V). “Y” signifies a significant result $\alpha = 0.0125$.

	Temperature		Salinity		Interaction	
	L. gris.	L. xan.	L. gris.	L. xan.	L. gris.	L. xan.
$[Sr/Ca]_{\text{otolith}}$	Y	Y				
$[Mg/Ca]_{\text{otolith}}$						
$[Mn/Ca]_{\text{otolith}}$						
$[Ba/Ca]_{\text{otolith}}$						
D_{Sr}		Y		Y		
D_{Mg}						
D_{Mn}		Y		Y		Y
D_{Ba}			Y	Y		

Few laboratory-based validation experiments have been used to address otolith element incorporation for different species. The majority of the experiments conducted looked only at Sr and Sr/Ca concentrations in otoliths, and most did not analyze water samples to calculate partition coefficients. The only other laboratory experiments to address the effect of temperature and salinity on Sr/Ca, Mg/Ca, Mn/Ca, and Ba/Ca incorporation in otoliths (Elsdon and Gillanders 2002) used both single-factor and two-factor designs. They found significant temperature and salinity interaction affects on all four Me/Ca ratios in juvenile black bream (*Acanthopagurus butcheri*). If the assumption that otolith element incorporation is proportional to the water elemental composition

were correct, then, the most appropriate way to discern interspecies differences would be to compare partition coefficients. Elsdon and Gillanders (2002) did not calculate partition coefficients, but best estimates of the ranges made by inspection of their graphs and the water data table indicate that their D_{Sr} ranges were ~ 0.42 - 0.6 , D_{Mg} ranges were ~ 0.0004 - 0.0005 , D_{Mn} ranges were ~ 0.2 - 0.55 and D_{Ba} ranges were ~ 0.26 - 0.79 . The D_{Sr} values for *A. butcheri* were higher than the values for both *L. xanthurus* and *L. griseus*. Whereas, the D_{Mn} values for *A. butcheri* were similar to *L. xanthurus*, and only slightly higher than *L. griseus*. D_{Mg} values were similar between *A. butcheri* and *L. xanthurus*, but were an order of magnitude greater than those calculated for *L. griseus*. Similarly, D_{Ba} ranges were the same for *A. butcheri* and *L. xanthurus*, but higher than D_{Ba} in *L. griseus*. These discrepancies highlight the potential for species-specific element incorporation. Whichever mechanisms controlling the uptake of individual elements into the otolith matrices are differentially influencing these partition coefficients.

In summary, *L. griseus* $[Me/Ca]_{otolith}$ and D_{Me} values were lower than *L. xanthurus* $[Me/Ca]_{otolith}$ and D_{Me} for Sr, Mg, Mn, and Ba. The effects of temperature and salinity changed between species when partition coefficients were compared (Table 5.4). There were significant differences in otolith element incorporation between species, as demonstrated for *L. griseus* and *L. xanthurus* and as compared to *A. butcheri* (Elsdon and Gillanders 2002). Otolith element incorporation is not solely a function of water elemental composition as it is influenced by temperature and salinity and affected uniquely for each element investigated. Somatic growth rate and otolith precipitation rate may also influence element incorporation as demonstrated for $[Mg/Ca]_{otolith}$ in *L. xanthurus* (Chapter IV).

Species-specific differences in elemental incorporation in otoliths make it difficult to generalize results among taxa, but perhaps of greater importance to studies using these techniques are the marked differences in element incorporation attributable to changes in temperature and salinity. For example, studies on eels (Tzeng et al. 1996; Jessop et al. 2002) and striped bass (Secor 1992; Secor et al. 1995) have used $[Sr/Ca]_{\text{otolith}}$ as proxies for salinity changes in the water. These studies interpreted fish migration paths based primarily on salinity differences from Sr/Ca signatures in the otoliths. However, conclusions about fish migrations based the $[Sr/Ca]_{\text{otolith}}$ – salinity relationship in otoliths is potentially confounded by the effect of temperature on $[Sr/Ca]_{\text{otolith}}$ incorporation. The same $[Sr/Ca]_{\text{otolith}}$ may represent both cool, salty water and warm, fresh water although, the relative magnitude of these effects may not make enough of a difference.

The interaction between temperature and salinity affects will also complicate the prospect of retrospectively identifying individual fish transport pathways as a function of their otolith elemental signatures through time. These experiments only address otolith element incorporation for two species. Clearly more research is needed to better understand the magnitude of temperature and salinity effects on element incorporation in otoliths of marine fish species. Nevertheless, this species comparison draws attention to the necessity of validation experiments to translate species-specific elemental signatures in otoliths.

CHAPTER VI

SUMMARY: APPLICATIONS AND FUTURE DIRECTIONS

Without adequate validation, otolith elemental signatures must be interpreted with caution because of interspecific or ontogenetic differences in elemental incorporation, as well as the specific behavior of metals that affects their speciation in ambient waters and their thermodynamic and physiologically mediated incorporation in otoliths. The key assumption for using otolith elemental signatures is that otoliths incorporate minor and trace elements in proportion to free ion concentrations in the ambient water. However, the relationship between $[\text{Me}/\text{Ca}]_{\text{water}}$ and $[\text{Me}/\text{Ca}]_{\text{otoliths}}$ is complicated because the otolith is isolated from the ambient water by branchial, intestinal, and endolymphatic membranes. Another underlying assumption in otolith chemistry studies is that otolith elemental incorporation is the same among species, which until this comparison, had never been evaluated.

In Chapter II, I showed that in spot (*Leiostomus xanthurus*), $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ and $[\text{Ba}/\text{Ca}]_{\text{otolith}}$ are deposited within otoliths in proportion to their respective ratios in ambient waters. Evidence of a non-linearity between D_{Ba} and $[\text{Ba}/\text{Ca}]_{\text{water}}$ (see Chapter II, Fig. 2.7) suggested that careful calibration of the relation between Ba/Ca levels in otoliths and water will be required before extrapolating the results to lower Ba/Ca environments and to other fish species. The estimates of D_{Sr} for otoliths from Chapter II are close to the theoretical distribution coefficient for aragonite based on thermodynamic equilibrium, although this may be due, at least in part, to differential uptake of Ca relative to Sr across the membranes separating the otolith from the ambient environment.

Temperature was positively related to D_{Sr} , unlike inorganic aragonite and coral skeletons in which the temperature dependence of D_{Sr} is negative. Temperature had no detectable influence on D_{Ba} , suggesting that most of the variation in Ba/Ca ratios in otoliths reflects concomitant variability in the Ba/Ca composition of the environment. Effects of precipitation rate on Sr and Ba incorporation in otolith were weak and generally statistically insignificant. Metabolic effects were similarly weak, using individual fish growth rates as a measure of metabolic activity. Thus, Sr and Ba incorporation in otoliths is primarily a function of the chemistry of the ambient environment, as modified by temperature in the case of Sr.

In the second experiment using *L. xanthurus* (Chapter III), I showed that Sr/Ca ratios in the otoliths of larval spot are determined primarily by the physicochemical properties of the ambient water. When combined with the results from Chapter II, temperature, $[Sr/Ca]_{water}$, and $[Sr]_{water}$ have significant effects on otolith Sr/Ca ratios. There was no consistent evidence for metabolic effects on Sr/Ca ratios in otoliths at least to the extent metabolic rates are correlated with individual growth rates. Unfortunately, otolith Sr/Ca thermometry may not be straightforward in estuarine-dependent fish species because independent geochemical tracers that constrain both $[Sr/Ca]_{water}$ and $[Sr]_{water}$ will need to be developed. Marine fishes that do not experience significant variations in $[Sr]_{water}$ and $[Ca]_{water}$ are logical species to test the ability to reconstruct temperature histories from Sr/Ca ratios in otoliths.

Chapter IV addressed temperature and salinity effects on Ba/Ca, Mg/Ca, and Mn/Ca in spot otoliths from the second experiment. The $[Ba/Ca]_{otolith}$ signature in *L. xanthurus* seems to be indicative of the water Ba/Ca composition and not influenced by

temperature or salinity (Chapter IV). $[Mg/Ca]_{\text{otolith}}$ was influenced by temperature but salinity had no effect. $[Mg/Ca]_{\text{otolith}}$ was also significantly influenced by otolith precipitation rate and somatic growth rate. It is therefore difficult to separate the direct effect of temperature on otolith Mg/Ca incorporation as temperature also affected somatic growth. $[Mn/Ca]_{\text{otolith}}$ may be too unpredictable for use in elemental signatures in otoliths because Mn concentrations are so variable in the aquatic environment at relatively short temporal and spatial scales. Based on the results from this experiment, Mg should not be used for elemental signatures in *L. xanthurus* otoliths for the fish size ranges tested because of the biomineralization and temperature effects on Mg incorporation. Manganese also should not be used because of the likelihood of significant variability in the water composition experienced by individual fish, lending to a higher degree of erroneous elemental signatures and therefore misallocation. It may be possible to extrapolate these results to other estuarine dependent species at the larval stage that have a similar life history as *L. xanthurus* (i.e., other Sciaenidae), however, not enough work has been done to say this with confidence.

In Chapter V, I tested the assumption that otolith element incorporation is similar between species. There were differences in otolith element incorporation between species, as demonstrated for *L. griseus* and *L. xanthurus*. The $[Me/Ca]_{\text{otolith}}$ and D_{Me} values in gray snapper (*Lutjanus griseus*) were lower than *L. xanthurus* $[Me/Ca]_{\text{otolith}}$ and D_{Me} . The differential effects of temperature and salinity on individual element incorporation changed between species when partition coefficients were compared. Somatic growth and otolith precipitation rate may also influence element incorporation as demonstrated for $[Mg/Ca]_{\text{otolith}}$ in *L. xanthurus* (Chapter IV). Thus, otolith element

incorporation is not solely a function of water elemental composition as it is affected by temperature, salinity, individual growth characteristics, taxa, and the element in question.

The interaction between temperature and salinity affects, in particular, will also complicate the prospect of retrospectively identifying individual fish transport pathways as a function of their otolith elemental signatures through time. This species comparison draws attention to the necessity of validation experiments to translate species-specific elemental signatures in otoliths.

Elemental signatures in fish otoliths provide a potentially innovative technique for discerning stocks, identifying nursery habitats, locating spawning sites, tracing larval transport pathways, and quantifying the degree of population connectivity, all of which are all required to meet fishery management goals. Applications of otolith elemental signatures have accelerated over the past decade but few validation experiments have been performed to test the effect of temperature and salinity on minor and trace metal otolith incorporation. Still, there are geographic differences in water elemental composition and fish have been accurately assigned to water bodies based on the elemental signatures in their otoliths. Experimental studies across species and ontogenetic stages will only help to improve the accuracy of these classifications. With improved accuracy and understanding of the physicochemical environmental properties that influence otolith metal incorporation, the next step is to reliably reconstruct individual fish movements by differentiating between water bodies of different temperatures and salinities through concentrations of elements and isotopes in fish otoliths.

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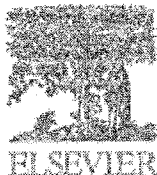
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APPENDIX I



21 October 2003

Our ref: HW/lt/Oct03.J150

Your ref:

Gretchen Bath Martin
 Email: NOAA, NOS, Center for Coastal Fisheries and Habitat Research

Dear Dr Martin

GEOCHIMICA ET COSMOCHIMICA ACTA, VOL 64, NO 10, 2000, PP 1705-1714, BATH ET AL:

"STRONTIUM AND BARIUM ..."

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Helen Wilson
 Rights Manager

APPENDIX II

----- Original Message -----

Subject: RE: permission request

Date: Mon, 27 Oct 2003 12:06:53 -0500

From: "McClymont, Paul" <Paul.McClymont@nrc-cnrc.gc.ca>

To: 'Gretchen Bath Martin' <Gretchen.Bath.Martin@noaa.gov>

CC: "Gorman, Judy" <Judy.Gorman@nrc-cnrc.gc.ca>

Dear Gretchen

Permission is granted for use of the material, as described below. We request that your dissertation not be made generally available until after publication of your article in CJFAS.

Sincerely

Paul McClymont

Business Manager

NRC Research Press

Tel: 613-993-9093

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-----Original Message-----

From: Gretchen Bath Martin [<mailto:Gretchen.Bath.Martin@noaa.gov>]

Sent: October 27, 2003 11:54 AM

To: McClymont Paul

Subject: Re: permission request

Dear Mr. McClymont,

Holly Foster is waiting for a copyright transfer agreement from one of my coauthors. Depending when she receives this last form, the volume will either be 60 (December) or 61 (January).

This is the citation information:

Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). by authors Gretchen Bath Martin, Simon R. Thorrold, and Cynthia M. Jones to be published in *Canadian Journal of Fisheries and Aquatic Sciences*.

Please let me know if you need any more information.

Best Regards,

Gretchen

VITA

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Publications

Hoss, D.E., Bath, G.E., Cross, F.A. and Merriner, J.V. 1999. U.S. Atlantic Coastal Fisheries – How effective have Management Plans been in maintaining “sustainable fisheries”? *Limnologia* 29:227-232.
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Hawn, A.T., Martin, G.B., Sandin, S.A., and Hare, J.A. (In review) Early juvenile mortality in a cohort of the coral reef fish *Chromis cyanea* (Pomacentridae): the growth-mortality hypothesis revisited. *Marine Ecology Progress Series*.
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