Strontium and barium uptake in aragonitic otoliths of marine fish

GRETCHEN E. BATH,[1,*] SIMON R. THORROLD,[1] CYNTHIA M. JONES,[1] STEVEN E. CAMPANA,[2] JAMES W. MCLAREN,[3] and
JOSEPH W. H. LAM[3]

[1]Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529 USA
[2]Marine Fish Division, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada B2Y 4A2
[3]Institute for National Measurement Standards, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6

Abstract—Minor and trace element analyses of fish otoliths (ear stones) may provide a high-resolution reconstruction of temperature histories and trace element compositions of aquatic systems where other environmental proxies are not available. However, before otoliths can be used to reconstruct water chemistry, it is essential to validate the assumption that trace metals in otoliths are deposited in proportion to dissolved concentrations in the ambient environment. We show, using a marine fish (*Leiostomus xanthurus*) reared in the laboratory under controlled experimental conditions, that otolith Sr/Ca and Ba/Ca ratios are deposited in proportion to their respective ratios in ambient waters. Temperature significantly affected Sr incorporation but did not affect Ba incorporation in otoliths. Sr/Ca partition coefficients (D_{Sr}) were 0.182 and 0.205 at 20°C and 25°C, respectively. The partition coefficients for Ba/Ca were 0.055 at 20°C and 0.062 at 25°C. A nonlinearity in the relationship between D_{Ba} and ambient Ba concentrations suggested that extrapolation beyond the Ba levels used in the experiment was not justified. On the basis of our results, it should be possible to reconstruct Sr/Ca and Ba/Ca levels in environments inhabited by fish based on otolith chemistry. Furthermore, Sr/Ca thermometry may also be possible using fish otoliths, but validation of the temperature dependence of Sr/Ca in otoliths will be required. We believe otoliths represent an excellent, and as yet underused, record of the physicochemical properties of both modern and ancient aquatic environments. *Copyright © 2000 Elsevier Science Ltd*

1. 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, b). 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 sediment dating to the Jurassic Period (Patterson, 1999). More important, 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, 1999). 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 identifying natal location and population structure of anadromous and marine fish species

(Kennedy et al., 1997; Thorrold et al., 1998a,b). 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 by way of the gills, 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, such as Sr^{2+} and Ba^{2+} that have ionic radii similar to Ca^{2+}, are generally considered to substitute for Ca^{2+} ions in the orthor-

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*Author to whom correspondence should be addressed: Gretchen E. Bath, NOAA/NOS, Center for Coastal Fisheries and Habitat Research, 101 Pivers Island Rd., Beaufort NC 28516 (Gretchen.Bath@noaa.gov).

hombic aragonite lattice (Speer, 1983), at least in low Sr aragonite such as fish otoliths (Greegor 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 ($[Me/Ca]_{otolith}$) can be related to that of the water ($[Me/Ca]_{H_2O}$) by way of this partition coefficient (D_{Me}), where

$$\left[\frac{Me}{Ca} \right]_{otolith} = D_{Me} \left[\frac{Me}{Ca} \right]_{H_2O} \qquad (1)$$

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 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). We know less about the factors determining D_{Ba} in biogenic aragonite. Lea et al. (1989) and Hart and Cohen (1996) noted positive correlations between quasiannual 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), Mashiotta et al. (1997), and Lea et al. (1999) cultured planktonic foraminifera in the laboratory 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, we describe an experiment in which juveniles of an estuarine-dependent species of marine fish, *Leiostomus xanthurus*, were reared under controlled laboratory conditions to determine whether otolith Sr/Ca and Ba/Ca is proportional to concentrations in the rearing water. We also investigate the effects of temperature on both Sr/Ca and Ba/Ca partition coefficients. Finally, by maintaining fish under controlled conditions we

were able to quantify the amount of otolith material deposited during the experiment. These data provide a test of the influence of precipitation rate on the chemistry of otolith aragonite.

2. EXPERIMENTAL METHODS

2.1. Larval Rearing

Spot (*Leiostomus xanthurus*) were spawned November 22, 1997, at the National Marine Fisheries Service, Southeast Fisheries Science Center in Beaufort, North Carolina, for the experiment, assuring the larvae were from the same brood stock and of known age. Larvae were reared in natural seawater at a salinity of 30‰ 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 we ensured adequate survival rates of the experimental fish. 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 two fish per liter and acclimated over several days to the experimental conditions of 20‰ salinity.

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 h:12 h for the duration of the experiment. Enriched *Artemia* were fed to the fish for the first 2 weeks of the experiment, and thereafter on an artificial diet (Hi-Pro Starter, 0.5 and 0.7 mm, Corey Feed Mills, LTD.).

We used artificial seawater ("Instant Ocean") as our water source throughout the experiment. Triplicate experimental tanks were randomly assigned four levels of Sr/Ca corresponding to ambient and then 1.2×, 1.4× and 1.8× ambient levels, and Ba/Ca corresponding to ambient and then 3×, 6×, and 10× 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 spike levels 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. We collected water samples 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 1).

2.2. 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 clean room. Otoliths were sonicated in Milli-Q water for 7 min and triple rinsed with ultrapure H_2O_2, followed by three sequential rinses of Milli-Q water. They were placed on acid-washed glass slides to dry for 36 h 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 sample 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 therefore, we concluded that conditions during the initial rearing period had little effect on the resultant otolith chemistry of the experimental fish.

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% redistilled nitric acid solution containing the enriched isotopes of the metals

Table 1. Summary of average water temperature (*T*), pH, and dissolved Sr/Ca (mmol/mol) and Ba/Ca (μmol/mol) levels within each of the 24 individual tanks during the course of the experiment.

Tank no.	*T* (°C)	pH	Sr/Ca	Ba/Ca
1	20.8	8.00	15.21	151.16
2	25.2	8.05	17.40	71.41
3	25.2	8.00	16.03	25.48
4	20.3	7.97	15.17	23.90
5	20.6	7.96	22.36	138.83
6	20.4	8.02	17.68	72.30
7	24.8	8.01	22.74	148.14
8	25.2	7.98	17.88	20.85
9	25.1	8.00	22.47	22.93
10	20.3	7.95	12.73	155.85
11	20.4	7.91	12.54	228.09
12	25.4	8.04	22.50	70.21
13	20.3	7.97	17.79	22.98
14	20.3	7.94	22.55	215.56
15	25.1	8.04	12.75	211.75
16	25.0	8.03	15.23	144.87
17	24.7	8.02	13.03	74.27
18	25.5	7.98	18.29	231.45
19	20.3	8.00	17.81	75.09
20	24.9	8.00	13.39	142.93
21	19.7	7.98	15.00	70.96
22	20.4	8.01	12.60	222.20
23	24.5	8.01	15.02	24.88
24	20.0	7.98	22.80	23.19

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, that 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 weight in a 0.3 mL final volume) were 500, 6, and 40 ng/g for Ca, Sr, and Ba, respectively.

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. 1 and 2).

We encountered two potential difficulties with the use of artificial seawater. First, Sr/Ca levels of seawater made up from "Instant Ocean" salts are slightly higher, at 12 mmol/mol, than that found in normal seawater (typically 8.5–9 mmol/mol). However, we could do little to lower this value, as 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 2.5× that of normal seawater. We did not face a similar problem with ambient Ba/Ca levels, and spiked levels within the tanks spanned a range (23–230 μmol/mol) that would commonly be encountered by estuarine-dependent fish along the east coast of the United States (Coffey et al., 1997). Second, we used artificial seawater in an attempt to minimize variations in baseline Sr and Ba concentrations in the tanks. However, Sr/Ca levels did fluctuate to some degree throughout the experiment (Fig. 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 should not have been unduly affected by this variability.

3. RESULTS AND DISCUSSION

3.1. Sr/Ca Ratios

The Sr/Ca ratios of otoliths from 214 juvenile *L. xanthurus* ranged from 1.85 to 6.77 mmol/mol, with an overall mean of 3.3 mmol/mol (Appendix 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. 3). Least squares regression described a linear relationship ($r^2 = 0.84$) between $[Sr/Ca]_{otolith}$ and $[Sr/Ca]_{H_2O}$ at 20°C

$$[Sr/Ca]_{otolith} = 0.165 \pm 0.052 \text{ (95\% CI) } [Sr/Ca]_{H_2O} + 0.260$$
$$\pm 0.897 \text{ (95\% CI)} \quad (2)$$

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

$$[Sr/Ca]_{otolith} = 0.162 \pm 0.054 \text{ (95\% CI) } [Sr/Ca]_{H_2O} + 0.70$$
$$\pm 0.954 \text{ (95\% CI)} \quad (3)$$

We calculated partition coefficients (D_{Sr}) for both of the temperature treatments directly from $Sr/Ca_{otolith}$ and Sr/Ca_{H_2O} 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% confidence interval [CI]) and 0.205 ± 0.04 (95% CI) at 20°C and 25°C, respectively.

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, whereas the theoretical $D_{Sr(equil)}$ 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 tracks accurately 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 obser-
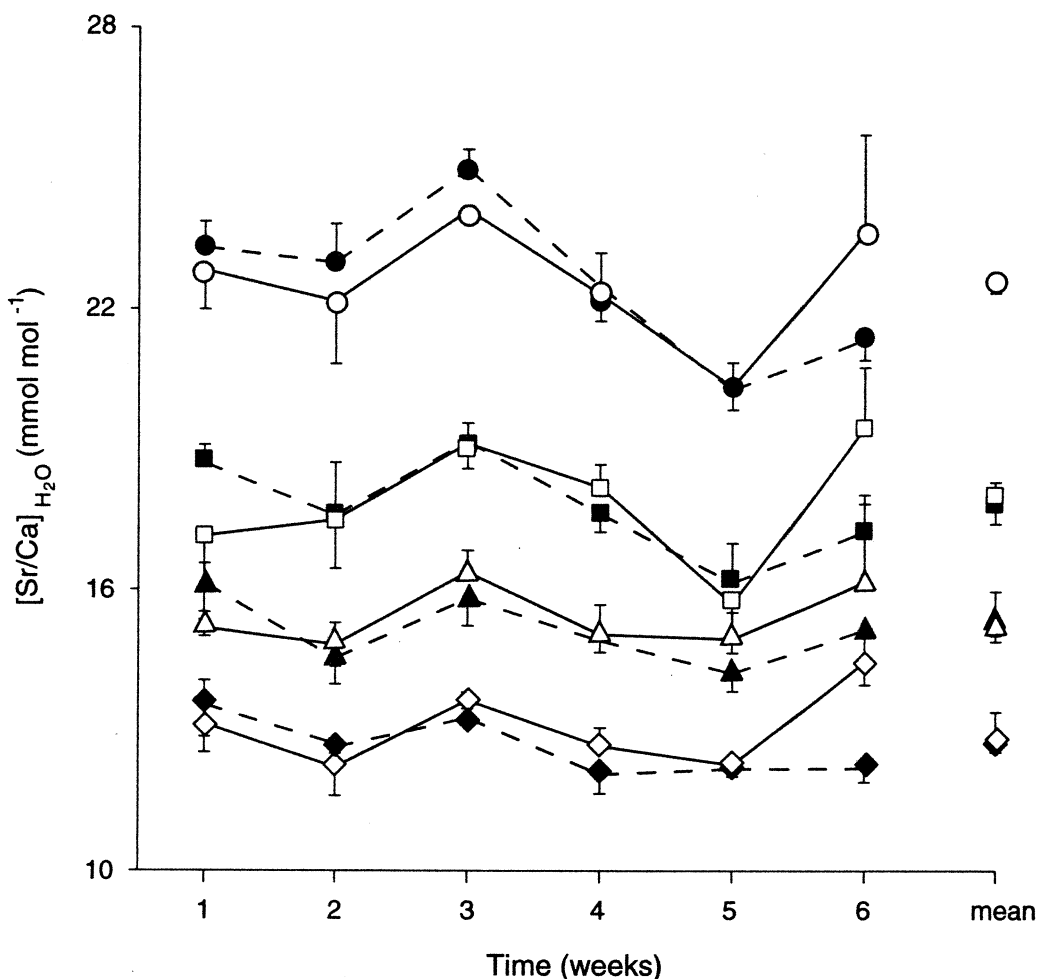
Fig. 1. Mean Sr/Ca ratios (±SD) at ambient (◆) and 1.25× (▲), 1.5× (■), and 2× (●) ambient levels at 20°C (closed symbols) and 25°C (open symbols), from weekly sampling throughout the experiment, along with mean values (±SD) for each spike level/temperature combination (mean) over the duration of the experiment.

vation 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 $[Sr/Ca]_{H_2O}$ (i.e., from marine to freshwater systems) are faithfully recorded by otoliths (Campana, 1999), our study suggests that more subtle variations will also be recoverable. It should be noted that there was some scatter in relationship between $[Sr/Ca]_{otolith}$ and $[Sr/Ca]_{H_2O}$ within individual tanks. At least some of this variance may be due to temporal changes in $[Sr/Ca]_{H_2O}$ of individual tanks (Fig. 1), despite our 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 in otoliths based on these results. Least-squares regression of D_{Sr} and temperature (T) found a significant linear relationship

$$D_{Sr} = 0.0046 \, T \, °C + 0.089 \, (r^2 = 0.62) \qquad (4)$$

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:

$$D_{Sr} = -(0.006011 \, T \, °C) + 1.2077 \qquad (5)$$

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., 1995), and no relationships (Gallahar and Kingsford, 1996; Tzeng, 1996) between Sr/Ca and temperature have been reported in the literature. Limited data from marine mollusk shells, which like otoliths are low Sr aragonite, suggest a positive relationship between Sr/Ca ratios and temperature (Stecher et al., 1996; reference 23 in Hart and Blusztajn, 1998), although Buchardt and Fritz (1978) found that Sr incorporation was independent of temperature in a freshwater gastropod. We reanalyzed data from a laboratory study on the effects of temperature and salinity on trace element chemistry of another species of sciaenid species, *Micropogonias undula-*
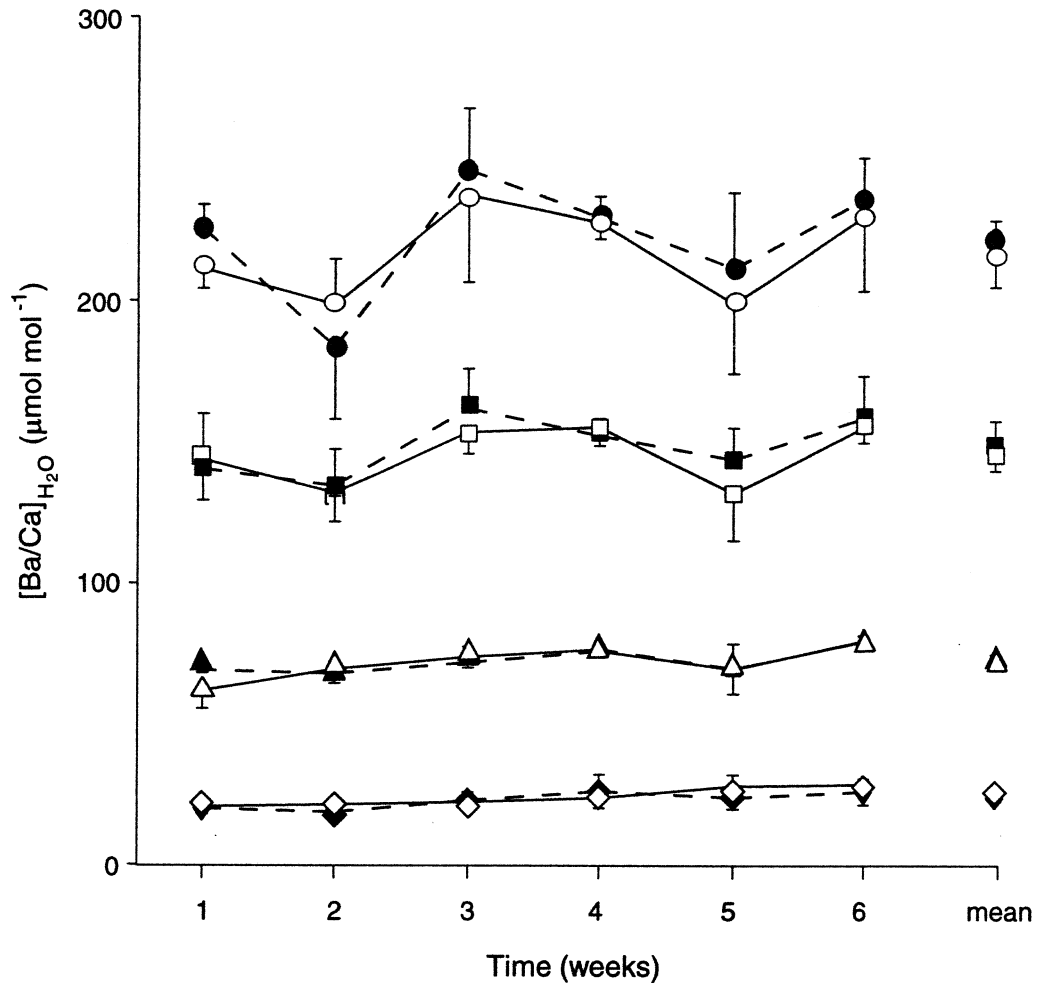
Fig. 2. Mean Ba/Ca ratios ($\pm$ SD) at ambient ($\blacklozenge$) and 1.25$\times$ ($\blacktriangle$), 1.5$\times$ ($\blacksquare$), and 2$\times$ ($\bullet$) ambient levels at 20°C (closed symbols) and 25°C (open symbols), from weekly sampling throughout the experiment, along with mean values ($\pm$ SD) for each spike level/temperature combination (mean) over the duration of the experiment.

tus (Fowler et al., 1995), assuming that there were no differences in $[Sr/Ca]_{H_2O}$ among tanks because 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,

$$D_{Sr} = 0.0086\ T\ °C + 0.124\ (r^2 = 0.85) \qquad (6)$$

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, we remain cautiously optimistic that it may be possible to reconstruct temperatures from Sr/Ca ratios in otoliths where $[Sr/Ca]_{H_2O}$ can be adequately constrained.

3.2. Ba/Ca Ratios

The Ba/Ca ratios of otoliths from juvenile *L. xanthurus* ranged from 1.7 to 15.2 μmol/mol, with an overall mean of 5.59 μmol/mol. Otolith Ba/Ca ratios were directly proportional

to $[Ba/Ca]_{H_2O}$ of the ambient water (Fig. 4) at both temperatures. A linear relationship ($r^2 = 0.90$) between $[Ba/Ca]_{otolith}$ and $[Ba/Ca]_{H_2O}$ at 20°C was described by least squares regression as:

$$[Ba/Ca]_{otolith} = 0.033 \pm 0.007\ (95\%\ CI)\ [Ba/Ca]_{H_2O}$$
$$+ 1.358 \pm 1.042\ (95\%\ CI) \quad (7)$$

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

$$[Ba/Ca]_{otolith} = 0.039 \pm 0.004\ (95\%\ CI)\ [Ba/Ca]_{H_2O}$$
$$+ 1.350 \pm 0.591\ (95\%\ CI) \quad (8)$$

We again calculated D_{Ba} directly from the $[Ba/Ca]_{otolith}$ and $[Ba/Ca]_{H_2O}$ data, and found values of 0.06 ± 0.06 (95% CI) at 20°C and 0.06 ± 0.07 (95% CI) at 25°C. These estimates are significantly lower than partition coefficients for hematypic corals ($\approx$1.3; Lea et al., 1989), but are probably close to values for aragonitic mollusc shells (Stecher et al., 1996). Unlike Sr, we found no significant effect of temperature on D_{Ba}. Although Lea et al. (1989) reported quasiperiodic oscillations in coral
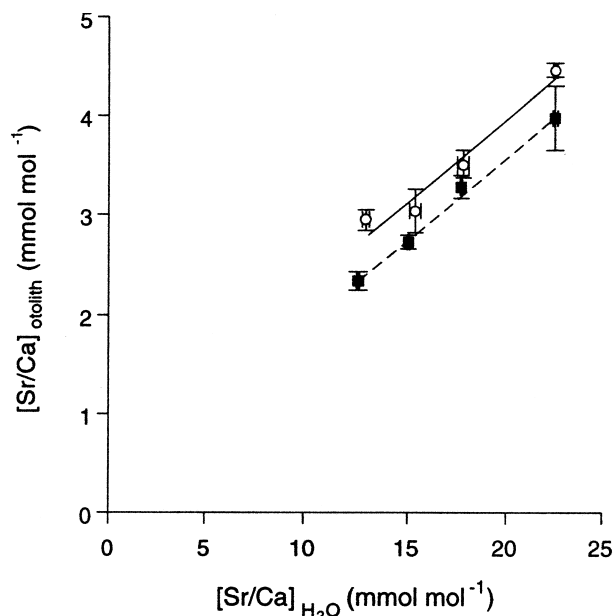
Fig. 3. Mean Sr/Ca ratios ([Sr/Ca]$_{otoliths}$ ± SE) in otoliths of laboratory-reared *Leiostomus xanthurus* plotted against Sr/Ca ratios of the rearing water ([Sr/Ca]$_{H2O}$ ± SE) at either 20°C (■, dashed line) or at 25°C (○, solid line). Lines were fitted by linear least-squares regression for each of the temperature treatments.

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 foram shells (Lea and Spero, 1992, 1994), Ba/Ca ratios in
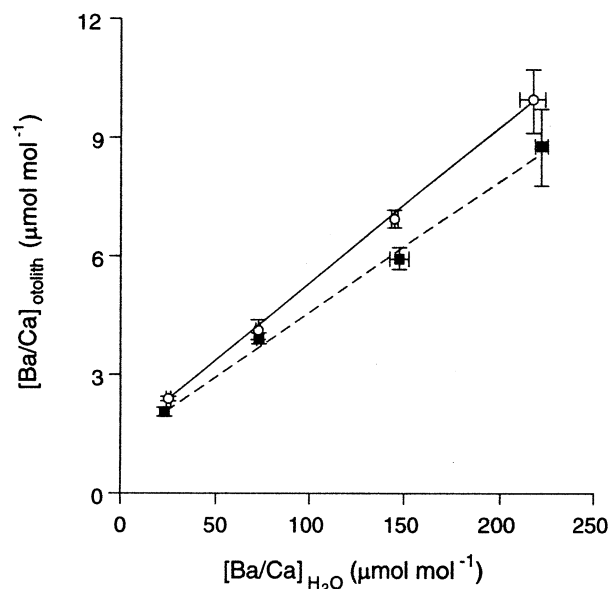

Fig. 4. Mean Ba/Ca ratios ([Ba/Ca]$_{otoliths}$ ± SE) in otoliths of laboratory-reared *Leiostomus xanthurus* plotted against Ba/Ca ratios of the rearing water ([Ba/Ca]$_{H2O}$ ± SE) at either 20°C (■, dashed line) or at 25°C (○, solid line). Lines were fitted by linear least-squares regression for each of the temperature treatments.
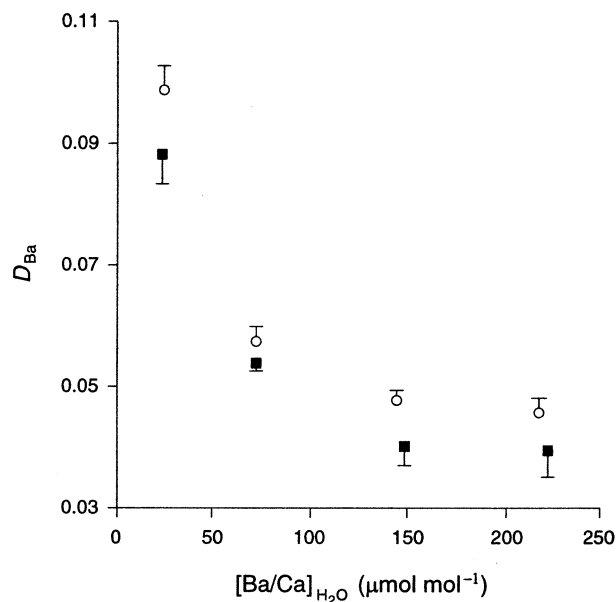

Fig. 5. Relation between estimates (±SE) of Ba partition coefficients (D_{Ba}) for otoliths of laboratory-reared *Leiostomus xanthurus* and Ba/Ca ratios of the rearing water ([Ba/Ca]$_{H2O}$) at either 20°C (■) or at 25°C (○).

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 our estimates of Ba partition coefficients were due to a nonlinearity in the relationship between D_{Ba} and [Ba/Ca]$_{H2O}$ at both 20°C and 25°C (Fig. 5). These data suggest that proportionally more Ba was incorporated in otoliths at low [BaCa]$_{H2O}$ 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]$_{H2O}$ range in this experiment (Eqn. 3), extrapolation beyond these points would be not be justified without further data. It is difficult to speculate the cause of this nonlinearity 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 μmol/mol. Nonlinear uptake of potentially toxic heavy metals across the branchial membrane has been documented in freshwater fishes (Olsson et al., 1998). 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]$_{H2O}$, and we will be pursuing such experiments in future work.

3.3. 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
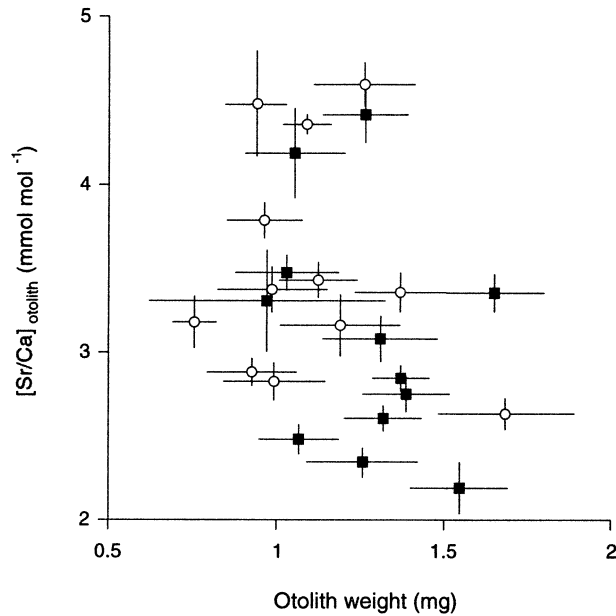
Fig. 6. Mean otolith Sr/Ca values (±SE) from laboratory-reared *Leiostomus xanthurus* plotted against mean otolith weight (±SE) for each of 24 rearing tanks maintained at either 20°C (■) or at 25°C (○).

Fig. 7. Mean otolith Ba/Ca values (±SE) from laboratory-reared *Leiostomus xanthurus* plotted against mean otolith weight (±SE) for each of 24 rearing tanks maintained at either 20°C (■) or at 25°C (○).

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), whereas other workers have found no such relationship (e.g., Shen et al., 1996). Data on rate effects in mollusk aragonite are sparse compared to corals, but are equally contradictory. Stecher et al. (1996) speculated that seasonal differences in shell growth rates generated quasiperiodic cycles in Sr/Ca ratios in two species of bivalve mollusk. In contrast, Buchardt and Fritz (1978) found that Sr incorporation in the shells of a gastropod *Limnaea stagnalis* were independent of growth rate. Our 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.

We detected no significant correlation between otolith mass and Sr/Ca ratios (Fig. 6), averaged within each of the experimental tanks ($r = -0.314$, $p = 0.134$), suggesting that our 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 ($F_{(1, 20)} = 0.022$, $p = 0.882$). 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 ($r = 0.902$, $n = 24$). Rate effects may be aliased by temperature and water chemistry differences among tanks, so we also examined cor-
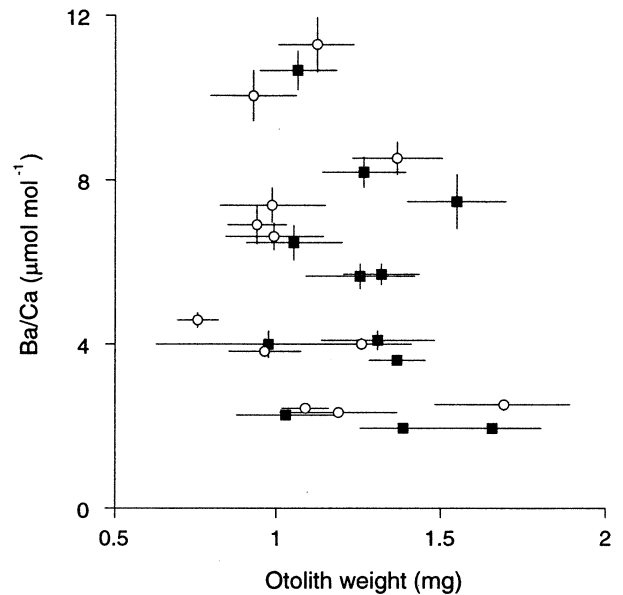
relations between Sr/Ca ratios and individual otolith mass within each of the tanks. These data provided some evidence of a relation between Sr/Ca and otolith mass, as 23 of the 24 correlations were negative. However, only one of the correlations was statistically significant, after adjusting the experiment-wise error to take into account the number of correlations performed.

Barium incorporation into otoliths is unrelated to precipitation rate, as evidenced by a nonsignificant correlation ($r = 0.177$, $p = 0.4078$) between the two variables averaged within each tank (Fig. 7). Within-tank correlations were similarly weak, with only one tank 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.

4. 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 nonlinearity between D_{Ba} and $[Ba/Ca]_{H_2O}$ 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.

Our estimates of D_{Sr} for otoliths 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 depen-

dence of D_{Sr} is negative. Although we could not provide an adequate temperature calibration for Sr/Ca ratios, we remain cautiously optimistic that temperatures can 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.

We found weak, generally statistically insignificant, effects of precipitation rate on Sr and Ba incorporation in otoliths. 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. We believe otoliths represent an excellent, and as yet underused, record of the physicochemical properties of both modern and ancient aquatic environments.

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Appendix 1. Otolith weight (mg), Sr/Ca (mmol/mol), Ba/Ca (μmol/mol) of sagittal otoliths from juvenile lab-reared *Leiostomus xanthurus*.

Tank no.	Wt (mg)	Sr/Ca	Ba/Ca	Tank no.	Wt (mg)	Sr/Ca	Ba/Ca	Tank no.	Wt (mg)	Sr/Ca	Ba/Ca
1	0.889	2.43	5.99	9	1.173	4.41	2.36	17	0.610	3.39	4.42
1	0.914	3.06	7.58	9	1.104	4.30	2.42	17	0.937	2.97	3.58
1	1.362	2.40	4.66	9	0.888	4.64	2.53	17	0.467	3.57	5.51
1	1.537	2.55	5.15	9	1.153	4.41	2.33	17	0.730	3.30	4.48
1	1.464	2.74	5.02	10	0.895	2.13	5.65	17	0.967	2.71	4.44
1	1.423	2.64	5.56	10	1.676	1.96	5.51	17	0.530	4.10	4.88
1	1.859	2.39	5.61	10	2.184	2.09	4.30	18	1.710	3.12	9.42
1	1.605	2.48	5.86	10	1.377	2.29	5.28	18	0.827	3.67	10.50
1	0.725	2.99	6.29	10	0.945	2.50	7.21	18	1.186	3.18	10.90
1	1.410	2.43	5.19	10	0.717	2.70	6.39	18	0.664	3.84	15.18
2	0.691	3.72	3.77	10	0.941	2.20	4.65	18	0.858	3.83	12.32
2	0.562	4.31	3.66	10	0.885	2.77	6.08	18	1.251	3.29	10.60
2	1.451	4.00	4.03	10	1.667	2.42	5.69	18	1.313	3.10	9.27
2	0.729	3.47	3.37	11	1.592	2.16	7.53	18	1.174	3.36	11.76
2	0.921	3.67	4.60	11	1.373	1.98	7.31	19	0.517	3.95	5.28
2	0.896	4.03	4.00	11	0.694	3.36	12.68	19	1.068	3.35	4.77
2	1.058	3.66	3.72	11	1.263	1.94	6.88	19	1.038	3.16	4.10
2	1.403	3.37	3.56	11	1.533	2.18	7.28	19	1.599	2.75	3.41
3	1.818	2.47	2.58	11	1.731	2.06	6.63	19	1.571	2.76	3.33
3	2.437	2.44	1.85	11	1.487	1.92	5.89	19	0.725	3.39	4.92
3	1.639	2.68	2.19	11	2.060	1.85	6.21	19	1.295	2.86	4.16
3	1.961	2.35	2.50	11	2.219	2.25	6.74	19	1.859	2.89	3.48
3	1.187	2.80	2.40	12	2.499	4.13	4.42	19	2.110	2.68	3.45
3	1.391	2.50	2.89	12	1.254	4.37	3.93	20	1.993	2.28	4.88
3	0.550	3.29	3.03	12	0.796	4.34	3.52	20	0.471	2.77	6.52
3	1.699	2.61	2.52	12	1.047	5.62	4.06	20	0.743	3.14	7.81
3	2.513	2.50	2.49	12	1.035	4.80	4.78	20	0.831	2.82	6.58
4	1.571	2.88	2.06	12	0.878	4.75	4.25	20	1.361	2.48	6.79
4	1.249	2.40	1.86	12	1.232	4.47	3.88	20	0.839	3.00	8.15
4	1.114	2.58	1.83	12	1.260	4.51	3.95	20	0.673	3.32	7.80
4	1.111	3.15	2.37	12	1.141	4.28	3.24	20	0.989	2.90	6.37
4	1.847	2.69	2.05	12	1.458	4.64	3.79	20	1.496	2.31	5.49
4	1.011	2.55	1.78	13	0.921	3.55	2.57	20	0.518	3.14	5.69
4	1.815	3.02	1.90	13	0.560	3.61	2.25	21	2.003	2.47	3.20
5	1.371	4.21	5.25	13	0.903	3.51	2.32	21	1.424	2.97	3.66
5	0.909	4.44	6.79	13	0.648	3.69	2.60	21	0.995	2.95	3.86
5	0.943	4.11	6.96	13	0.889	3.56	2.07	21	1.284	3.25	4.69
5	1.754	3.67	5.69	13	0.697	4.03	1.89	21	1.585	2.52	3.42
5	1.093	4.60	7.22	13	1.366	3.00	2.29	21	1.301	3.02	3.22
5	0.337	5.17	8.25	13	1.246	3.18	2.43	21	1.305	3.01	3.68
5	0.789	4.61	7.12	13	2.053	3.11	1.94	21	1.093	2.96	4.01
5	1.241	2.67	4.55	14	0.701	3.98	7.49	21	1.404	2.61	2.77
6	1.955	2.58	3.24	14	1.756	3.93	6.73	21	1.320	2.78	3.80
6	1.001	3.20	4.12	14	0.940	5.46	10.58	22	0.997	2.42	10.79
6	0.498	3.38	3.76	14	0.920	5.15	9.54	22	1.584	2.39	9.67
6	0.447	4.06	4.88	14	1.123	4.07	6.97	22	0.712	2.74	10.86
7	0.905	3.85	6.30	14	1.348	4.40	8.65	22	1.572	2.30	9.04
7	0.801	3.54	5.52	14	1.174	4.54	9.00	22	1.238	2.23	10.59
7	0.647	4.98	7.64	14	1.427	4.46	7.98	22	0.977	2.58	10.23
7	1.079	4.48	8.33	14	1.230	4.31	7.89	22	0.676	2.97	12.11
7	1.039	4.33	6.48	14	2.041	3.88	7.00	22	0.644	2.85	13.78
7	1.089	4.46	6.73	15	0.541	2.81	10.30	22	0.754	2.29	11.09
7	1.446	3.81	5.54	15	0.758	2.98	11.87	22	1.490	2.15	8.62
7	0.943	4.07	5.79	15	0.878	3.07	9.03	23	1.320	2.97	1.99
7	0.494	6.77	9.57	15	1.092	2.94	9.67	23	0.677	3.44	2.04
8	1.836	2.92	6.92	15	0.895	2.77	9.69	23	1.230	3.19	2.49
8	1.728	3.49	9.45	15	0.701	2.99	7.81	23	0.770	3.51	2.50
8	1.351	2.96	8.12	15	0.959	3.21	13.26	23	1.155	3.15	2.28
8	1.099	3.28	8.58	15	0.634	2.84	11.12	23	1.294	2.66	2.20
8	1.063	3.28	7.94	15	1.874	2.28	7.33	23	0.388	4.28	2.94
8	0.772	3.52	6.87	16	1.980	3.01	5.86	23	2.157	2.38	1.89
8	1.510	4.17	11.04	16	0.684	3.49	8.25	23	1.724	2.87	2.40
8	0.729	2.79	7.29	16	0.822	3.39	6.33	24	0.683	4.23	2.16
8	1.106	3.68	10.36	16	1.184	3.00	6.50	24	1.811	3.35	1.68
8	2.104	3.25	7.73	16	0.906	3.24	8.00	24	1.864	3.21	2.03
8	1.771	3.53	9.41	16	0.765	3.56	7.77	24	1.676	2.99	1.81
9	1.389	4.04	2.44	16	1.119	3.11	6.53	24	1.260	3.47	1.87
9	1.349	4.14	2.06	16	0.472	4.15	9.63	24	1.224	3.59	1.95
9	0.853	4.52	2.99	17	0.672	2.92	4.02	24	1.664	3.15	2.06
9	0.789	4.44	2.45	17	1.000	2.76	4.87	24	2.083	3.33	1.96
9	1.110	4.29	2.33	17	0.891	2.82	5.14	24	2.213	3.20	1.91
								24	2.070	3.01	1.98