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Effect of diet on otolith composition in *Pomatomus saltatrix*, an estuarine piscivore

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To test the hypothesis that elemental composition of otoliths (sagittae) could be influenced by differences in natural prey type, young-of-the-year bluefish *Pomatomus saltatrix* were captured immediately after their migration from oceanic waters into mid-Atlantic Bight estuaries and fed either shrimp, *Crangon septemspinosa* and *Palaemonetes* spp. or fish *Menidia menidia* under similar temperature and salinity regimes in two separate 60 day experiments. Unlimited rations of fish and shrimp prey were provided in the first experiment which led to differences in bluefish growth rate between the two prey treatments; fish prey was limited in the second experiment to ensure that growth rates of bluefish in the two prey treatments were similar. Concentrations of seven elements in bluefish otoliths were determined using solution-based inductively coupled plasma mass spectrometry (ICPMS). There was no significant effect of diet on five of the seven elements examined (Na, Mg, K, Ca and Mn). The levels of Sr and Ba in the otoliths of shrimp-fed bluefish, however, were significantly higher than fish-fed bluefish in both experiments. Concentrations of Ba in shrimp-fed bluefish otoliths were double that found in fish-fed bluefish. The results suggest that diet can explain some of the variation in otolith chemistry previously attributed to physical and chemical properties of the water.

Key words: barium; diet; otolith chemistry; *Pomatomus saltatrix*; strontium.

INTRODUCTION

Otoliths, the earstones of teleosts, are composed mostly of calcium carbonate (aragonite form) but also include protein and minor and trace elements. The concentrations of elements in otoliths have been used to reconstruct environmental histories of fishes. Examples include the use of otolith chemistry to examine questions related to temperature (Townsend *et al.*, 1992, 1995) and

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salinity history of individual fish (Radtke *et al.*, 1988; Kalish, 1990; Secor, 1992; Limburg, 1995; Secor & Piccoli, 1996; Thorrold *et al.*, 1997). Otolith chemistry has also been used for differentiating estuary of origin (Thorrold *et al.*, 1998*a*, *b*; Thorrold *et al.*, 2001) and for stock discrimination (Campana *et al.*, 1995; Secor & Zdanowicz, 1998; Thresher, 1999; Rooker *et al.*, 2001).

Otolith composition studies generally assume that elements will be incorporated into the otolith in proportion to their concentrations in the water mass that the fish occupies. Past research has shown that many factors can affect otolith chemistry. In addition to temperature and salinity, growth rate (Sadovy & Severin, 1992; Mugiya & Satoh, 1997), gonad development (Kalish, 1991), stress (Kalish, 1991; Townsend *et al.*, 1992) and diet (Limburg, 1995; Gallahar & Kingsford, 1996) are all thought to influence incorporation of elements into otoliths. The magnitude of the effect of these physiological factors, and how easily they can be accounted for, will be important in determining when and how otoliths can be used in recording environmental history.

The effect of diet on otolith chemistry is not known with certainty; of the five studies that have addressed this question, two have found a dietary effect (see Table I). Limburg (1995) found that otolith chemistry (Sr : Ca) documented a change of diet from natural freshwater zooplankton to a marine fish-derived artificial feed in young-of-the-year (YOY) American shad *Alosa sapidissima* (Wilson). Similarly, prey that was spiked with a Sr solution led to otoliths with increased Sr : Ca ratios in *Girella elevata* Macleay (Gallahar & Kingsford, 1996). Juvenile red drum *Sciaenops ocellatus* (L.) that were fed artificial diets formulated with low, medium and high levels of Mg, K, Sr, Na and Ca, however, showed no differences in otolith chemistry (Hoff & Fuiman, 1995). Farrell & Campana (1996) found that diets with differing levels of Sr and Ca did not influence concentrations of these elements in Nile tilapia *Oreochromis niloticus* (L.) otoliths. Similarly, Milton & Chenery (2001) examined the effect of enhanced levels of Cu, Sr and Pb in artificial feeds and found no effect on otolith composition. These studies have generally been limited to artificial diets and a small number of elements; of the elements studied, Sr has been examined most extensively (Table I). No one, as far as is known, has examined the effects of differences in natural prey types on otolith chemistry.

Bluefish *Pomatomus saltatrix* (L.) are an important resource species worldwide (Juanes *et al.*, 1996). On the U.S. east coast, adult bluefish spawn in offshore oceanic waters (Kendall & Walford, 1979; Hare & Cowen, 1996) and YOY bluefish move into east coast estuaries at sizes $\leq$60–70 mm total length (L_T) (Nyman & Conover, 1988; McBride & Conover, 1991). The YOY bluefish are known to include both shrimp and fish prey in their diet in mid-Atlantic Bight estuaries (Friedland *et al.*, 1988; Juanes & Conover, 1995) with the amount of shrimp in their diet dependent on the relative abundance and size of shrimp and fish prey (Juanes *et al.*, 2001). Thus, bluefish provide an opportunity to examine the effect of natural prey type on otolith chemistry using fish that have similar oceanic histories. Here, the null hypothesis that otolith chemistry of YOY bluefish is independent of diet is tested.

TABLE I. Review of studies that have examined the influence of diet on elemental composition of otoliths

Study	Family, Species-life stage	Element or ratio	Diet type	Concentration comparison in diet	Otolith effect
Hoff & Fuiman (1995)	Sciaenidae, *Sciaenops ocellatus* – juvenile		Artificial: (high		
		Mg	*v.*medium *v.* low)	1790 *v.* 812 *v.* 308 ppm	NO
		K		8160 *v.* 4470 *v.* 2410 ppm	NO
		Sr		7·5 *v.* 4·3 *v.* 1·8 ppm	NO
		Na		31 000 *v.* 4,850 *v.* 3270 ppm	NO
		Ca		4850 *v.* 5040 *v.* 3050 ppm	NO
		Mg : Ca		0·61 *v.* 0·27 *v.* 0·17 mol/mol^{-1}	NO
		K : Ca		1·72 *v.* 0·91 *v.* 0·81 mol/mol^{-1}	NO
		Sr : Ca		0·71 *v.* 0·39 *v.* 0·27 mmol/mol^{-1}	NO
		Na : Ca		11·14 *v.* 1·68 *v.* 1·87 mol/mol^{-1}	NO
Limburg (1995)	Clupeidae, *Alosa sapidissima* – juvenile	Sr : Ca	Artificial *v.* natural	3·77 mmol mol^{-1} *v.* ND	YES
Farrell & Campana (1996)	Cichlidae, *Oreochromis niloticus* – juvenile		Artificial : enhanced		
		Sr	*v.*background	280 *v.* 16 ppm	NO
		Ca		79 800 *v.* 9270 ppm	NO
		Sr : Ca		1·6 *v.* 0·79 mmol mol^{-1}	NO
Gallahar & Kingsford (1996)	Kyphosidae, *Girella elevata* – juvenile	Sr	Artificial : enhanced *v.*control	Unknown but stated that differences covered 1–11 × 10^{-3} range in Sr : Ca found in calcareous parts of marine species	YES
Milton & Chenery (2001)	Centro-pomidae, *Lates calcarifer* – juvenile		Artificial : enhanced *v.* control		
		Sr : Ca		0·22 *v.* 0·14 mmol mol^{-1}	NO
		Cu : Ca		452 *v.* 175 umol mol^{-1}	NO
		Pb : Ca		58 *v.* 6·2 umol mol^{-1}	NO
This study	Pomatomidae *Pomatomus saltatrix* – juvenile		Natural : shrimp *v.* fish		
		Sr		421·6 *v.* 110·4 ppm	YES
		Ba		3·523 *v.* 0·996 ppm	YES
		Mn		ND	NO
		Mg		1768 *v.* 2338 ppm	NO
		K		ND	NO
		Na		ND	NO
		Ca		ND	NO

ND, no data.

MATERIALS AND METHODS

STUDY PLAN

Young-of-the-year bluefish were collected immediately after entry into estuarine waters and fed either shrimp or fish prey over a 60 day period; two separate experiments were conducted under unequal (1998) and equal (2000) growth conditions. Whole otoliths were analysed at the end of the experiment and the concentrations of elements were compared between shrimp- and fish-fed bluefish. Groups of wild fish collected at the beginning and end of each year's experiment were used to test if the elemental fingerprints of wild fish were similar to those in bluefish from the laboratory and to examine if there was an effect of year on elemental signatures in wild fish.

FIELD COLLECTION AND LABORATORY EXPERIMENTS

Young-of-the-year bluefish were collected in Great South Bay, New York, U.S.A. (40°10′ N; 73°10′ W) on 25 June 1998 and in Sandy Hook Bay, New Jersey, U.S.A. (40°27′ N; 74°00′ W) on 23 June 2000 using a 30 m beach seine. Fish were transported the same day in aerated sea water to the James J. Howard Marine Sciences Laboratory, Highlands, New Jersey where laboratory experiments and otolith analyses were conducted. Upon arrival, a sub-sample (in both years) of bluefish was measured, weighed and frozen for subsequent otolith dissection and analyses (initial field sample; Table II). Total length (±1·0 mm) and wet mass (±0·01 g) were measured on the remaining fish and these were assigned randomly to either a shrimp- or fish-fed treatment. Treatments had either two (1998) or three (2000) replicate 4751 tanks (105 × 76 × 60 cm high) with four fish per replicate. Bluefish L_T and mass were measured every 20 days in 1998 and every 10 days in 2000.

The Sandy Hook Bay environment was simulated. Bluefish were held in flow-through tanks containing water from Sandy Hook Bay at ambient temperature and salinity

TABLE II. Mean ± S.D. total length, wet mass and sagittal otolith mass (pair combined) for bluefish from the field and laboratory in 1998 and 2000. Means for field fish are averages of individual fish (1998, $n = 9$; 2000, $n = 10$). Means for laboratory fish are averages of two (1998) or three (2000) tank means; there were four bluefish per tank in each year

Date	Source	L_T (mm)	Mass (g)	Otolith mass (mg)
		1998		
25 June	Initial field	70·6 ± 10·5	2·73 ± 1·35	2·20 ± 0·69
25 June	Day 0 lab-fish diet	74·6 ± 0·2	3·35 ± 0·03	NA
25 June	Day 0 lab-shrimp diet	75·3 ± 0·7	3·50 ± 0·27	NA
23 August	Day 60 lab-fish diet	160·9 ± 3·8	39·25 ± 0·49	10·04 ± 0·26
23 August	Day 60 lab-shrimp diet	136·0 ± 9·9	23·18 ± 5·98	8·13 ± 1·00
20 August	Final field	177·6 ± 25·8	56·0 ± 19·70	10·54 ± 3·62
		2000		
23 June	Initial field	68·7 ± 7·8	2·52 ± 0·81	2·17 ± 0·37
23 June	Day 0 lab-fish diet	71·0 ± 3·7	2·95 ± 0·48	NA
23 June	Day 0 lab-shrimp diet	69·3 ± 3·1	2·77 ± 0·48	NA
21 August	Day 60 lab-fish diet	138·9 ± 4·8	25·3 ± 2·91	7·81 ± 0·77
21 August	Day 60 lab-shrimp diet	139·9 ± 5·0	24·8 ± 3·35	8·69 ± 0·85
23 August	Final field	143·3 ± 8·4	24·6 ± 4·42	8·16 ± 2·04

NA, not applicable.

throughout the 60 day experiment (19–23° C, 20–25 Fig. 1) under a photoperiod of 14 L : 10 D. The flow-through system was also used to prevent any potential differences in water chemistry that might have occurred based on uneaten food. There were no significant differences in temperature or salinity between shrimp *v.* fish fed tanks in 1998 (*t*-test, temperature: d.f. = 113, $P = 0{\cdot}888$; salinity: d.f. = 113, $P = 0{\cdot}925$) or 2000 (temperature: d.f. = 114, $P = 0{\cdot}903$; salinity: d.f. = 114, $P = 0{\cdot}946$; Fig. 1).

In 1998, each experimental tank was provided with unlimited weighed amounts of food twice daily. In 2000, shrimp-fed bluefish were provided unlimited rations; however, limited rations of fish prey were provided to bluefish in an effort to achieve equal growth rates between the shrimp- and fish-fed treatments. Rations were determined based on prior laboratory feeding and growth studies with similar sized bluefish feeding on shrimp and fish prey (Juanes & Conover, 1994). Uneaten prey were removed and weighed. The fish diet was previously frozen Atlantic silversides *Menidia menidia* (L.) while the shrimp diet was previously frozen sand and shore shrimp (*Crangon septemspinosa* Say and *Palaemonetes* spp.). Fish and shrimp prey were collected in Sandy Hook Bay, where

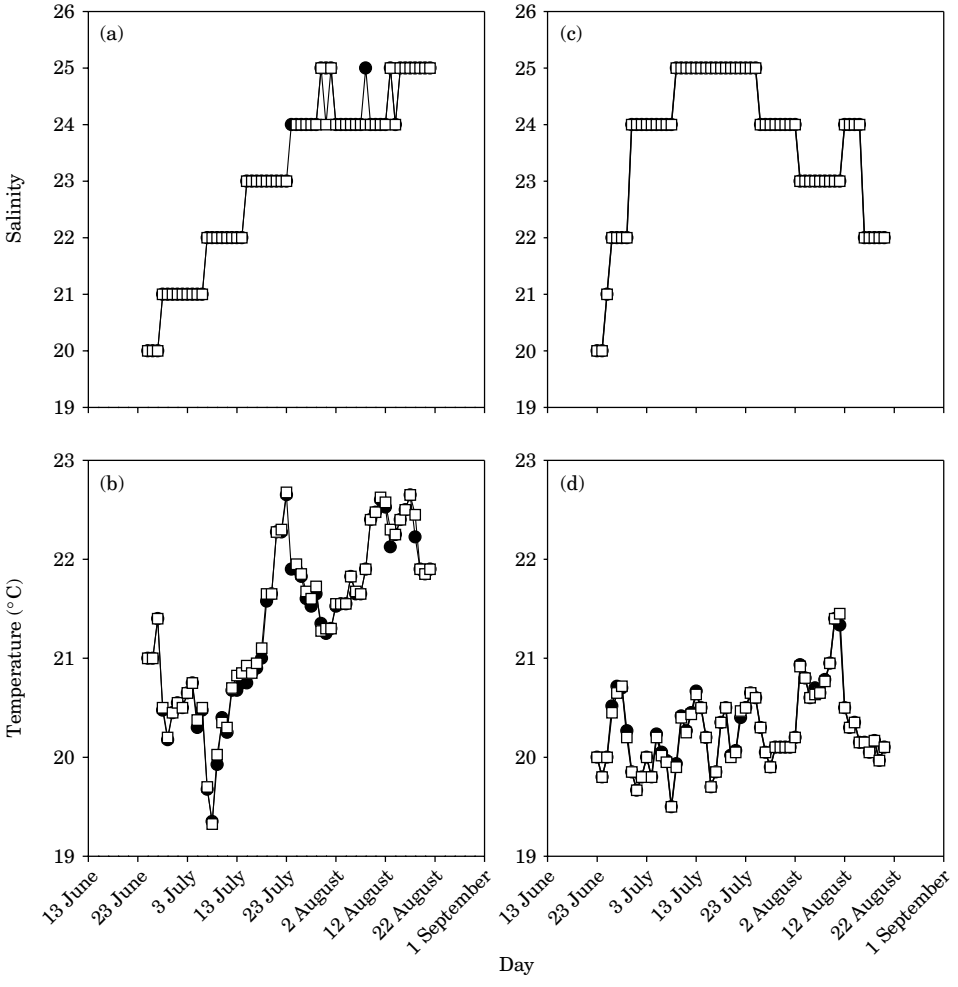

FIG. 1. Mean salinity (a) 1998 (c) 2000 and temperature (b) 1998 and (d) 2000 in laboratory experimental tanks containing young-of-the-year bluefish fed shrimp (●) or fish (□) prey.

they are both known to be important prey of YOY bluefish (Friedland *et al.*, 1988). None of the shrimp prey were in the post-molt stage.

After the final L_T and mass measurements were made at the end of the experiments (day 60: 23 August 1998; 21 August 2000; Table II), bluefish were killed with an overdose of MS-222 and immediately frozen. During these same time periods (20 August 1998 and 23 August 2000), samples of wild bluefish were collected in Sandy Hook Bay, measured, weighed and frozen (wild fish, final samples; Table II). Within 30 days of the end of the experiment, both sagittae were removed from all wild (initial and final samples) and laboratory held bluefish and stored in envelopes until analysis.

ELEMENTAL MEASUREMENTS

For elemental measurements, all reagents used were ultrapure grade and all implements and containers were cleaned with dilute nitric acid and rinsed with 18 megohm doubly deionized water ($DDIH_2O$).

Otolith samples were carefully decontaminated prior to chemical analysis. First, they were immersed in $DDIH_2O$ to hydrate any remaining biological tissue; this was removed with fine-tipped forceps. Next, they were soaked for 5 min in 3% H_2O_2 to dissolve any remaining residue. They were then soaked in 1% nitric acid for 5 min to remove surface chemical contamination, then flooded with $DDIH_2O$ to rinse off the acid, and finally dried under a HEPA Class 100 laminar flow hood. In preparation for instrumental analysis, each otolith was weighed to the nearest 0·01 mg, placed in a plastic tube and dissolved in 1% nitric acid. Procedural blanks and a standard reference material (SRM) were prepared following the same procedures. Internal standards were added to all sample solutions. Samples were analysed in random order to avoid analytical bias. Concentrations of Mg, Ca, Mn, Sr and Ba were determined using quadrupole inductively coupled plasma mass spectrometry (QICPMS). Na and K were measured using atomic absorption spectrophotometry (AAS). The SRM was NIST 915a (Calcium Carbonate Clinical Standard), obtained through the National Institute of Standards and Technology. This SRM is not certified for verification of trace metal concentrations, so only non-certified values are available for a few elements. Relevant values are (on a dry mass basis): Ca, 40·0%; Mg, 1·0 ppm ($\mu g\,g^{-1}$); Mn 0·6 ppm. Mean ± S.D. values ($n = 4$) obtained for the analysis of the SRM were Ca, 39·7 ± 0·2%; Mg, 1·02 ± 0·02 ppm; Mn, 0·63 ± 0·02 ppm.

Samples from a mixture of whole shrimp and fish prey used for the feeding experiments were taken at random. Sample digestion followed the procedures described in Zdanowicz *et al.* (1993). Briefly, *c.* 0·5 g of fish ($n = 3$) and 1·0 g of shrimp ($n = 3$) were placed in teflon vials and dried overnight at 60–65° C. Five ml conc nitric acid were added to each and they were allowed to stand at room temperature for 2–4 h. Vials were capped and placed in teflon lined bombs and the tissues were digested overnight at 120° C. After cooling, the digests were quantitatively transferred to 25 ml graduated cylinders and brought to volume using $DDIH_2O$. Concentrations of Mg, Sr and Ba were determined using QICPMS. Procedural blanks and SRM were prepared following the same procedures. Internal standards were added to all sample solutions. Samples were analysed in random order to avoid analytical bias. The SRM was NIST 1566a (Oyster Tissue), obtained through the National Institute of Standards and Technology. Certified values are (on a dry mass basis): Mg, 1180 ppm ($\mu g\,g^{-1}$); Sr, 11·1 ppm. Mean values ($n = 3$) obtained for the analysis of the SRM were Mg, 1009 ± 28 ppm; Sr, 9·27 ± 0·63 ppm; Ba, 1·77 ± 0·11 ppm.

ANALYSES

The effect of diet on bluefish growth was compared with repeated measures ANOVA for each experiment. Daily consumption rate was calculated as amount of prey consumed (g) per mean fish mass (g) per day. Mean fish mass over 20 day time periods was calculated as $[0{\cdot}5(\ln M\mathrm{t_i} + \ln M\mathrm{t_f})]$ where $M\mathrm{t_f}$ = final wet mass (g) and M_{ti} = initial wet mass (g). Consumption rate was calculated for each replicate tank.

Concentrations of elements in otoliths from shrimp- and fish-fed bluefish were determined for individual fish. Molar ratios ($element_{mol}\,Ca^{-1}_{mol}$) were calculated for Mg, Mn, Sr and Ba. Individual values were averaged by tank to obtain tank means; tank means were used as replicate values. Comparisons between shrimp- and fish-fed bluefish otoliths were made using *t*-tests for each year separately; type 2 errors were controlled by adjusting an α value of 0·05 to the number of comparisons for each element (0·05 divided by 2, 0·025).

Multivariate analysis of variance (MANOVA) was used to test if the elemental signatures in laboratory fish (shrimp- and fish-fed) were similar to wild fish collected at the end of the experiment. The effect of year on elemental signature was examined by comparing wild fish (initial and final samples were analysed separately) collected in 1998 and 2000 using MANOVA. Pillai's trace was used as the test statistic given that it is robust to violations of the homogeneity of covariance assumption (Rooker *et al.*, 2001). Data for individual fish from the laboratory and field were used in these analyses.

RESULTS

In 1998, there was no significant difference in initial size (L_T) between fish-fed and shrimp-fed bluefish (*t*-test, d.f. = 2, $P = 0{\cdot}349$). Fish sizes became significantly different, however, among treatments during the experimental period (Table III). The 1998 trial is referred to as the 'unequal growth' experiment. In the 2000 experiment, there was no significant difference in L_T between fish-fed and shrimp-fed bluefish (*t*-test, d.f. = 4, $P = 0{\cdot}583$) or in later sizes over the course of the experiment (Table III). The 2000 trial is referred to as the 'equal growth' experiment.

Fish were lost from the initial experimental design in the unequal growth experiment. During the experiment, one bluefish was partially cannibalized (day 17) in a shrimp-fed tank, while another bluefish was lost (presumably cannibalized, day 56) from a separate shrimp-fed tank. An additional shrimp-fed bluefish showed signs of illness ('popeye') at the end of the experiment and was discarded from the analysis. The remaining fish in the shrimp-fed treatment

TABLE III. Repeated measures ANOVA with treatment (diet) and time as independent variables and mass as the dependent variable for the 1998-unequal and 2000-equal growth experiments

	1998-unequal growth			
Effect	d.f.	SS	*F*	*P*
Treatment	1	231·67	14·27	0·063
Time	3	1761·71	197·08	<0·0001
Treatment × time	3	159·31	17·82	0·002
	2000-equal growth			
Effect	d.f.	SS	*F*	*P*
Treatment	1	2·60	0·21	0·670
Time	6	2477·35	361·13	<0·0001
Treatment × time	6	11·13	1·62	0·184

were in good condition with similar masses at length compared to those in the fish-fed treatment. No fish died during the experiment in 2000.

The amount of otolith deposited during the experimental period represented *c.* 75% of the final otolith mass in both years. Pairs of sagittal otoliths from shrimp- and fish-fed bluefish increased *c.* four to five-fold in mass during the unequal growth experiment (Table II). There was a similar *c.* four-fold increase in mass of bluefish otoliths during the 2000 experiment for both prey treatments (Table II).

In the unequal growth experiment, the consumption rate of fish prey was initially high, but declined as a result of allometric effects; shrimp-fed bluefish had relatively high consumption rates throughout the 1998 experiment (Table IV). Fish-fed bluefish had lower consumption rates compared to shrimp-fed bluefish for all time periods in the equal growth experiment; the magnitude of the difference in consumption between the two treatments changed from a period where shrimp consumption was double fish consumption (day 0–20) to only a one per cent difference in the last 20 days (Table IV).

In the unequal growth experiment, shrimp-fed bluefish had significantly higher levels of Sr ($P = 0{\cdot}008$) and Ba ($P = 0{\cdot}021$) compared to fish-fed bluefish (Table V). The concentration of Na in shrimp-fed otoliths was lower than fish-fed otoliths and the significance was marginal ($P = 0{\cdot}035$). No other shrimp- *v.* fish-fed elemental comparisons were significantly different in the unequal growth experiment. In the equal growth experiment, the elements that showed the same trends as in 1998 were Sr, Ba, Na and Ca; moreover, Sr and Ba were again significantly higher in shrimp-fed bluefish otoliths in 2000 (Sr, $P = 0{\cdot}001$; Ba, $P < 0{\cdot}001$) and a marginal level of significance was found in Na ($P = 0{\cdot}047$). Magnesium was marginally higher in shrimp-fed bluefish otoliths ($P = 0{\cdot}045$) in 2000 but this was opposite the pattern in 1998. Similarly, in 1998 and 2000, shrimp-fed bluefish otoliths had significantly higher Sr : Ca and Ba : Ca ratios ($P < 0{\cdot}025$) compared to fish-fed bluefish otoliths while there were no significant diet effects on Mg : Ca and Mn : Ca ratios ($P > 0{\cdot}05$) (Fig. 2).

There were significant differences in the elemental signatures between both laboratory prey treatments and field collected fish on day 60 in 1998 and 2000

TABLE IV. Mean ± S.D. mass specific consumption rate of bluefish during 1998 and 2000 laboratory experiments. Means are averages of two (1998) or three (2000) tank means; there were four bluefish per tank in each year

Time interval (days)	Consumption rate ($g\,g^{-1}\,day^{-1} \times 100$)	
	1998-unequal growth	2000-equal growth
Fish diet		
0–20	22·12 ± 0·03	12·54 ± 1·21
20–40	20·34 ± 0·15	14·86 ± 1·04
40–60	10·50 ± 6·00	12·60 ± 0·66
Shrimp diet		
0–20	18·12 ± 2·33	26·24 ± 2·93
20–40	25·59 ± 1·16	20·30 ± 0·57
40–60	21·21 ± 5·22	13·51 ± 0·53

TABLE V. Mean ± S.D. element concentration in sagittal otoliths from young-of-the-year bluefish fed shrimp or fish prey for 60 days during 1998-unequal growth and 2000-equal growth experiments. Means are averages of two (1998) or three (2000) tank means; there were four bluefish per tank in each year

Metal	Shrimp diet	Fish diet
	1998-unequal growth	
Na (ppm)	3207·3 ± 13·1	3288·3 ± 17·6
Mg (ppm)	20·4 ± 1·6	24·3 ± 1·9
K (ppm)	906·0 ± 19·5	919·2 ± 9·5
Ca (%)	36·9 ± 1·1	36·7 ± 1·3
Mn (ppm)	2·5 ± 0·6	2·6 ± 0·3
Sr* (ppm)	1644·1 ± 21·4	1354·0 ± 29·0
Ba* (ppm)	3·9 ± 0·3	2·3 ± 0·1
	2000-equal growth	
Na (ppm)	3260·3 ± 39·4	3433·7 ± 98·7
Mg (ppm)	22·9 ± 1·0	20·8 ± 0·9
K (ppm)	849·5 ± 43·6	815·4 ± 36·5
Ca (%)	37·3 ± 1·2	34·9 ± 2·6
Mn (ppm)	3·4 ± 0·7	2·5 ± 1·6
Sr* (ppm)	1490·7 ± 36·9	1069·3 ± 81·7
Ba* (ppm)	3·7 ± 0·3	1·8 ± 0·1

*, a significant effect of diet at an adjusted α level (*t*-test; $P < 0{\cdot}025$).

(MANOVA: shrimp-fed *v.* field, $P < 0{\cdot}0001$; fish-fed *v.* field, $P < 0{\cdot}0001$; Tables V and VI). Elemental signatures differed significantly between 1998 and 2000 for both initial and final field samples (MANOVA, $P < 0{\cdot}01$; Table VI); in univariate *post hoc* tests, Ca and Sr were significantly different for initial wild samples while only Sr differed for final field collections. Otoliths of bluefish from the initial wild sample in 1998 had extremely low Ca levels (mean = 25·8%) compared to otoliths from all other bluefish groups.

ELEMENTAL MEASUREMENTS OF PREY

Elemental concentrations differed between prey types (Fig. 3). Shrimp prey had significantly more Ba and Sr compared to fish prey (*t*-test, Ba: d.f. = 2, $P = 0{\cdot}0008$; Sr: d.f. = 4, $P < 0{\cdot}0001$). Ba and Sr concentrations were 250 and 280% higher in shrimp, respectively. Mean ± S.D. Mg concentration was 32% higher in fish (2338 ± 55 ppm) compared to shrimp prey (1768 ± 55 ppm); this difference was also statistically significant (*t*-test, Mg: d.f. = 4, $P = 0{\cdot}0002$).

DISCUSSION

The published literature to date has found little evidence for a major effect of diet on elemental concentrations within otoliths (Campana, 1999; Thresher, 1999; see Table I). In the present study, sagittal otoliths from shrimp-fed bluefish had significantly higher concentrations of Sr and Ba compared to otoliths

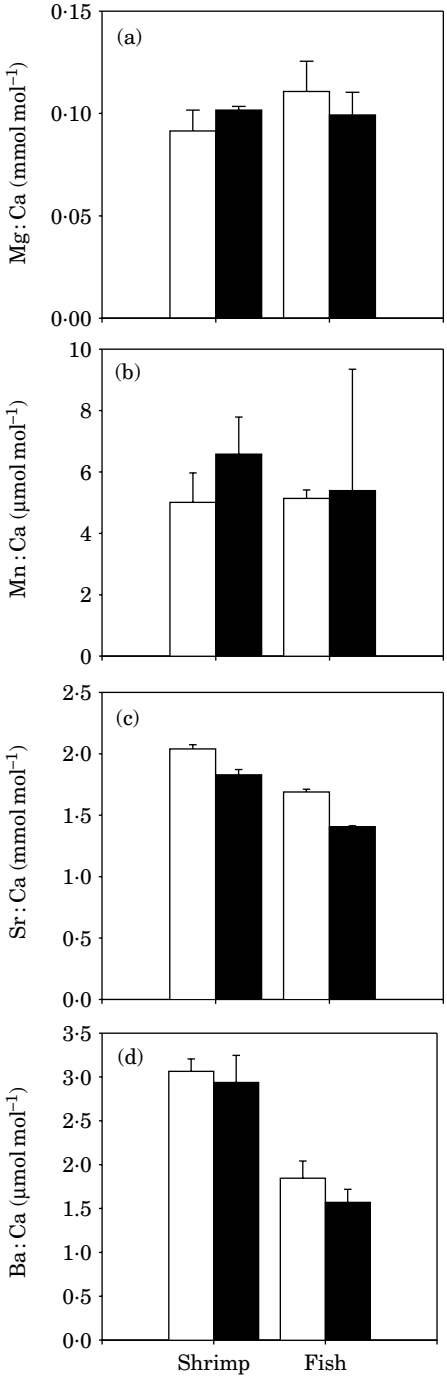

FIG. 2. Mean + S.D. ratios of molar (a) Mg : Ca, (b) Mn : Ca, (c) Sr : Ca and (d) Ba : Ca in sagittal otoliths for shrimp- and fish-fed bluefish held under laboratory conditions in 1998 (□, unequal growth; $n = 2$ replicate tanks for each treatment) and 2000 (■, equal growth; $n = 3$ replicate tanks for each treatment).

TABLE VI. Mean ± S.D. element concentration in sagittal otoliths from wild young-of-the-year bluefish captured at the beginning and end of the laboratory experiments in 1998 and 2000. Means for field fish are averages of individual fish (1998, $n = 9$; 2000, $n = 10$)

Metal	Initial field	Final field
	1998	
Na (ppm)	3376·9 ± 436·1	3472·1 ± 191·7
Mg (ppm)	39·5 ± 16·5	35·21 ± 11·9
K (ppm)	805·3 ± 182·5	827·1 ± 65·1
Ca (%)	25·8 ± 4·7	36·1 ± 3·1
Mn (ppm)	3·2 ± 2·4	2·6 ± 1·4
Sr (ppm)	941·7 ± 225·8	1478·3 ± 162·6
Ba (ppm)	3·2 ± 1·3	4·8 ± 1·4
	2000	
Na (ppm)	3483·4 ± 71·9	3308·8 ± 238·2
Mg (ppm)	33·0 ± 3·9	27·1 ± 3·1
K (ppm)	899·9 ± 69·7	881·4 ± 92·1
Ca (%)	36·9 ± 2·5	36·1 ± 3·1
Mn (ppm)	2·5 ± 0·5	1·9 ± 0·5
Sr (ppm)	1348·7 ± 95·4	1174·0 ± 141·8
Ba (ppm)	2·7 ± 1·0	3·8 ± 1·8

from bluefish that were fed fish prey in two separate experiments. The hypothesis that otolith chemistry is independent of diet was rejected for YOY bluefish.

Diet did not have a significant effect on the incorporation of Na, Mg, K, Ca and Mn in bluefish otoliths. Similar to the present study, Hoff & Fuiman (1995) found no significant dietary effect for Mg (ANOVA, $P = 0{\cdot}37$), K ($P = 0{\cdot}52$), Ca ($P = 0{\cdot}86$) and Sr ($P = 0{\cdot}25$). They also found no effect of diet on Na (Ba and Mn were not examined) although the P-value was low (0·10) suggesting that diet may have had slight effects on otolith composition for Na (Hoff & Fuiman, 1995). Similarly, a marginally significant value for Na was observed in both years of the present study. The source of the five elements in bluefish otoliths for which no dietary effect was found may be solely from water; however, an alternative explanation is that some of these elements do come from food but that the differences in elemental concentration between shrimp and fish prey are low. For Mg, the difference in concentration between fish and shrimp was statistically significant but was not as large a difference (less than two-fold) as that seen for Ba and Sr (three and a half to four-fold). Unfortunately, estimates of the other four elements in fish and shrimp prey were not obtained.

The conflicting findings (Table I) from studies that have tested for a dietary effect on otolith composition may be a consequence of the differences in elemental concentration between prey types being both small and large. Differences in Sr concentration in diets were four-fold for Hoff & Fuiman (1995) and 17-fold for Farrell & Campana (1996) (Table I). Given the dietary difference for Ba and Sr concentrations (*c.* four-fold) in the present study, dietary effects would have been expected for these studies but were not observed. The differences in Sr : Ca ratios in diets, however, were not as extreme as metal concentration in

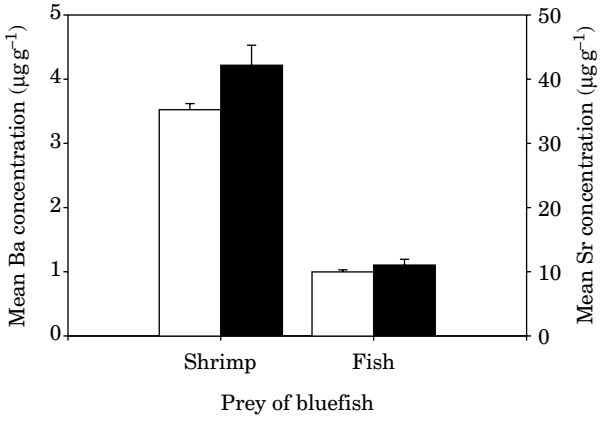

FIG. 3. Mean + S.D. concentration of Ba (□) and Sr (■) for shrimp (*Crangon septemspinosa* and *Palaemonetes* spp.) and fish (*Menidia menidia*) prey collected in Sandy Hook Bay, New Jersey in 2000.

these studies [less than three-fold for Hoff & Fuiman (1995) and two-fold for Farrell & Campana (1996)]. Similarly, Milton & Chenery's (2001) study compared diets with less than a two-fold difference in the Sr : Ca ratio and did not find a dietary effect. The differences in Sr : Ca ratios for the studies that did find a dietary effect are not known. Metal concentration and metal : Ca ratios should be used to compare diets in future studies that examine the effect of diet on elemental composition of otoliths.

These dietary effect studies (Table I) have been conducted on fishes in both fresh and sea water. It might be expected that there is a difference in elemental uptake from diet between these two groups. The elemental uptake area in freshwater fishes is the gills while for marine fishes the intestines also serve as an uptake area (Karnaky, 1998). Thus, a diet effect might be expected to be more pronounced in marine fishes. The literature does not support this argument as Hoff & Fuiman (1995) found no diet effect for red drum in sea water while Limburg (1995) found a dietary effect for American shad held in fresh water.

Future laboratory experiments examining dietary influences on otolith microchemistry should use natural prey. Additionally, elemental concentrations of prey and water should be determined to allow for mass balance calculations. In addition to Sr, a variety of elements that are prevalent in fish otoliths and that have proved to be useful in otolith research (Campana, 1999) should be examined. As far as is known, this is the first study to examine the effect of diet on the concentration of Ba in otoliths. This is surprising because Ba is often found to vary significantly between samples collected in different years (Gillanders, 2002) and habitats (Gillanders & Kingsford, 1996, 2000; Thorrold *et al.*, 1997, 1998*a*, *b*; Secor *et al.*, 2001).

Potential indirect effects of diet on otolith chemistry include growth rates, gonad development and stress; these factors can cause differences in element incorporation into the otolith. Like Juanes & Conover (1994), in 1998, bluefish fed unlimited amounts of fish were found to grow faster than those on a shrimp

diet. Sadovy & Severin (1992) for white grunt *Haemulon plumieri* (Lacepede) and Mugiya & Satoh (1997) for goldfish *Carassius auratus* (L.) found higher Sr levels in otoliths of fish exhibiting relatively low growth rates. Because shrimp-fed bluefish grew at a lower rate than fish-fed bluefish it was not clear from the 1998 data alone if the differences observed for Sr and Ba were a direct or indirect (low growth rate) result of diet. Given the similarity in results between the unequal and equal growth experiment, the potential confounding effect of growth rate was probably an insignificant source of variation in the 1998 study. Similarly, Bath *et al.* (2000) found that growth rate did not influence incorporation of Sr and Ba into the croaker *Leiostomus xanthurus* Lacepède otoliths.

Wild bluefish collected at the end of each experiment had different elemental signatures compared to laboratory held bluefish fed either shrimp or fish prey. This is not surprising given that bluefish in the wild probably experienced different conditions (*e.g.* temperature, water chemistry and diet) compared to those held in the laboratory even though attempts were made to simulate local conditions. The concentrations of Sr in field collected fish (day 60) were in between the concentrations for shrimp- and fish-fed bluefish in 1998 and 2000 while Ba concentrations in field collected fish were closer to those found in shrimp-fed bluefish in both years. Although juvenile bluefish are known to feed on both shrimp and fish prey in the estuary (Friedland *et al.*, 1988), a similar study to that of Sanchez-Jerez *et al.* (2002) [who found a significant correlation between elemental concentration (Mn and Ba) in wild trumpeters *Pelates sexlineatus* (Quoy & Gaimard) otoliths and that in its polychaete prey] is needed to confirm that diet plays a significant role in determining elemental concentration of otoliths in wild, juvenile bluefish.

Elemental signatures differed between years for both initial and final field bluefish. Although the sample size was small in each year, the results support past research that found estuarine juvenile fishes to differ in elemental signatures from year to year. One potential confounding factor in this analysis is that the initial bluefish collections were from different estuaries. These samples were of bluefish that had just recruited from the ocean so year to year differences in otolith composition are from differences experienced in the ocean and not differences in estuarine environments.

In 1998, levels of Ca in very young bluefish (those captured immediately upon entry into the estuary) were found to average *c.* 26% by mass and to increase to *c.* 36% within 60 days. Sadovy & Severin (1992) found that Ca levels in the cores of white grunt otoliths were slightly lower than outside the cores and suggested that more otolin was present in the core. There do not, however, appear to be any studies which report otolith Ca levels as low as 26%.

There are two areas of otolith research where the present results have implications. These are 'hindcasting' environmental histories and discrimination of fishes from different areas. Variations in the Sr : Ca ratios have been used to infer movements of fishes between waters of high and low salinities (Secor, 1992; Limburg, 1995; Thorrold *et al.*, 1997). There are several studies which have found three to five-fold differences in Sr : Ca ratios between lower estuary or marine and freshwater deposited otolith material (Secor, 1992; Limburg, 1995). Although statistically significant differences were found between Sr concentrations in shrimp- and fish-fed bluefish, the differences were small

(*c.* 20–40%) relative to the studies described above. Based on the present results, diet could be useful in explaining unknown sources of variation observed in Sr concentration but it will not lead to misclassification of fishes between low and high salinity environments. Diet effects, however, may influence the ability to detect smaller differences in salinity history.

Differences in otolith composition have been used to 'fingerprint' groups of the same fish species collected from different locations (Campana *et al.*, 1995; Thorrold *et al.*, 1998*a*, *b*; Gillanders, 2002). One goal is to discriminate between fishes that have originated from differing areas but have subsequently mixed (Thorrold *et al.*, 2001). In these studies, absolute differences in mean metal concentrations between groups were similar in magnitude to the differences observed in the present study based on diet alone. Thorrold *et al.* (1997) concluded that the temporal stability of elemental signatures will determine how often these signatures have to be re-measured. The variation observed by Gillanders (2002) in juvenile snapper *Pagrus auratus* (Forster) otoliths over a 3 year period in 12–15 estuaries was large and led to the conclusion that signatures would have to be generated across multiple year-classes of juveniles in order to be used successfully in identifying estuary of origin in multiple year-classes of adults. Understanding the mechanisms behind the resultant chemistry of an otolith may help determine how often signatures will have to be re-measured and increase the utility of otoliths in fisheries ecology.

Will it be possible to 'hindcast' the dietary histories of individual fish as Bell (2001) suggested? The present results suggest that a diet shift from an invertebrate to a piscivorous diet would be detectable in bluefish otoliths if their past environmental history (water temperature and chemistry) was stable. In addition to elemental data, stable isotope data may be useful in discriminating at which trophic level a fish has fed (Campana, 1999). For bluefish and other piscivores, the onset of piscivory is considered an important event during their ontogeny given the increased growth rate that results (Juanes & Conover, 1995). A determination of when the shift from invertebrate to fish diet occurred would allow the importance of the timing of the diet shift to future survival to be tested.

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