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Identifying the spawning estuaries of the tropical shad, terubok *Tenualosa toli*, using otolith microchemistry

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ABSTRACT: The tropical shad, terubok *Tenualosa toli*, is known only from 2 large estuaries and the adjacent coast of northwest Borneo. Populations of this culturally and commercially important species have fallen to dangerously low levels during the 1990s. In an attempt to conserve the species, the Sarawak state government has set up fisheries reserves to protect fish during spawning in the estuaries of the Lupar and Lassa rivers. Terubok are protandrous hermaphrodites that, at 1+ years old, migrate to the inshore areas of the adjacent coast after initially spawning as males. To assess the effectiveness of the conservation measures we had to confirm that terubok spawn only in the 2 known spawning areas. We did this by examining the elemental composition at the nuclei of otoliths of fish using the new technique of LA-ICPMS (laser ablation-inductively coupled plasma mass spectrometry). Otoliths of fish collected from 7 coastal sites and both estuarine spawning areas in 1994 had similar elemental composition. This confirmed that all fish were probably spawned in 1 of the 2 estuaries. However, otoliths of fish collected from 2 of 8 coastal sites sampled in 1995 had significantly different elemental composition from the fish from the Lupar or Lassa estuaries. These sites had not been sampled in 1994 and were at the extremes of the species' known distribution. These results indicate that the species may spawn in 1 or more other estuaries outside the known distribution. The elemental composition at the nuclei of otoliths of fish from the 2 known spawning estuaries differed little in each of the 2 years of the study, except in 1994 when ^{138}Ba concentration was higher in the Lassa. These results meant that the elemental composition of otolith nuclei could not be used to reliably estimate the relative contribution to the coastal fishery of fish from the 2 known spawning estuaries. However, elemental concentrations of 6 of the 7 elements examined differed between years for the 5 sites adequately sampled in both 1994 and 1995. These differences were not related to changes in the sex or age composition of the samples and this suggests that the water chemistry in both the spawning estuaries may have changed in a similar way during the study. Changes in otolith chemistry such as these could, in some instances, cause confusion in interpreting fish population structure from these data.

KEY WORDS: Otolith Microchemistry · Estuaries · Spawning · Tropical *Tenualosa*

INTRODUCTION

The tropical shad *Tenualosa toli* is unique among the Clupeiformes because it is a protandrous hermaphrodite (Blaber et al. 1996). The terubok is known only from the coast of Sarawak, Borneo, where it is the most commercially and culturally important estuarine fish species. The fishery has declined to historically low levels because the fishers target spawning females for their valuable ovaries (Willman et al. 1989). Recent studies of its life cycle (Blaber et al. 1996, 1997) have shown that the species is anadromous, spawning in the middle reaches of only 2 large, well-mixed estuaries. As a result of these studies, the Sarawak government has tried to conserve the species by defining fish reserves in these 2 spawning estuaries where spawning occurs.

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Little was known about the biology and life cycle of the species prior to the recent studies, which found that terubok grow to about 400 mm SL (standard length) and live from 2 to 3 yr. They mature in 1 yr as males, spawn, and then migrate to nearby coastal marine waters, where they change sex before returning to the estuary the following year to spawn as females (Blaber et al. 1996). Eggs and larvae have been collected from the middle reaches of only the 2 spawning estuaries—the Lupar and the Lassa (Pang & Ong 1990, Blaber et al. 1996)—and ripe females have also been collected from a third—the Sadong (Blaber et al. 1996). Surveys of all the rivers with estuaries in Sarawak and of the large estuaries in adjacent Sabah and Kalimantan have failed to find evidence of terubok or of estuaries that duplicate the conditions where they breed (Blaber et al. 1996, 1997).

Methods that use otolith elemental composition are being used increasingly to help researchers understand the environmental history of fish movements (e.g. Radtke & Shafer 1992, Secor 1992). Techniques that sample specific loci offer the greatest potential for gaining insight into the life history and movements of anadromous species (Kalish 1990, Coutant & Chen 1993, Secor et al. 1995) or population structure (Yamada et al. 1987, Edmonds et al. 1989, Campana et al. 1994, Thresher et al. 1994). However, most studies have used X-ray analysis with an electron microprobe, which is limited to detecting the most abundant elements (Gunn et al. 1992, Thresher et al. 1994).

Laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) is a new technique for point sampling that combines the benefits of sampling specific loci with the sensitivity of the ICPMS (Fowler et al. 1995). This method has been used to discriminate among different stocks of Atlantic cod by examining the elemental composition of their otolith nuclei (Campana et al. 1994). Given the few studies that have used point sampling methods to examine otolith nucleus chemistry, there is little data on the stability of otolith elemental composition over time. Questions such as whether stocks discriminated in one year remain discrete when sampled the following year have not been addressed to date using these types of methods.

The aims of this study were to (1) examine the elemental composition of the nuclei of terubok otoliths from the spawning estuaries and the coastal fishery in Sarawak to determine if fish caught in coastal areas come only from the estuaries where spawning is known, (2) assess whether elemental composition was similar in fish collected in successive years and (3) determine if the elemental composition of otoliths could be used to estimate the relative contribution to the coastal fishery of fish from the 2 spawning estuaries.

MATERIALS AND METHODS

Sampling and sample preparation. Fish were collected from estuaries and the coast of Sarawak (Fig. 1) between 6 and 15 August 1994, and again between 9 and 19 May 1995. All estuaries and coastal fishing villages that were accessible were visited on each sampling trip and terubok were bought from all markets where they were available. All fish were caught by commercial fishers using 75 to 100 mm mesh gillnets. Fishers were contracted to collect fish from the known spawning grounds in the Lupar and Lassa. Juvenile terubok (30 to 40 mm SL) were also collected by commercial liftnet at Pulau Seduku, a known spawning area (Fig. 1b), in the middle reaches of the Lupar estuary (Blaber et al. 1996) in August 1994.

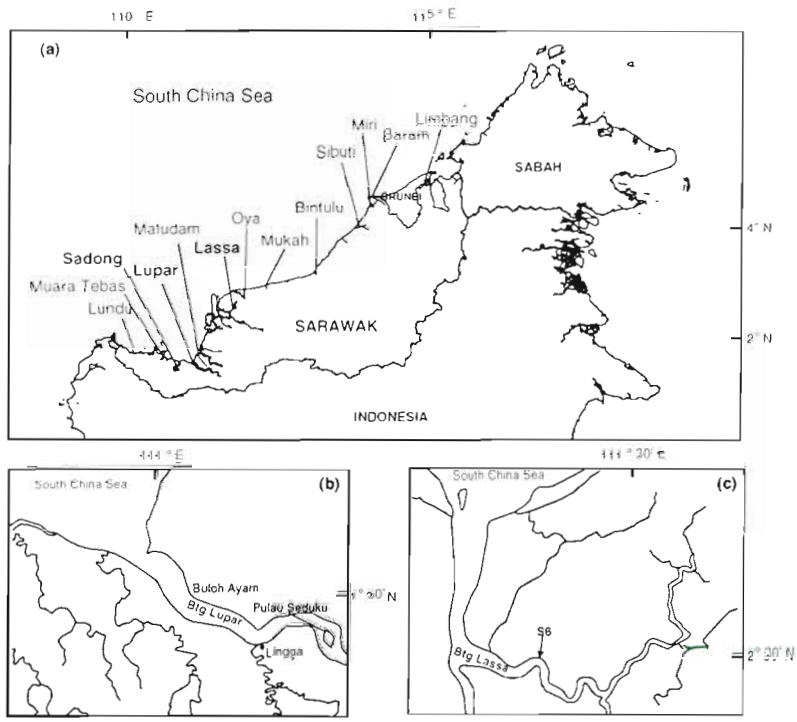

Fig. 1 (a) Sites where terubok *Tenualosa toli* were collected for otolith elemental analysis during 1994 and 1995. Estuaries where spawning has been recorded or is suspected to occur are in bold type. (b) Buloh Ayam, where spawning adults were collected, and Pulau Seduku on the Lupar River, where juveniles were collected in 1994. (c) Site S6 on the Lassa River where spawning fish were collected

All fish were measured (SL in mm) and weighed (±5 g). Their heads and viscera were removed and stored in 70% alcohol for later analysis. In the laboratory, both sagittae were removed, washed in distilled water and then dried at 60°C for 24 h in a positive air flow. Both were then weighed (±0.05 mg), and one was embedded in polyester resin (1994) or araldite 'M' (1995) blocks with the primordial surface downwards. Blocks were ground with wet and dry sandpaper (600 to 1200 grit) until the focal plane was reached. The surface was then polished with 6 µm and 1 µm pastes until the surface was smooth. Otoliths were sonicated in high quality distilled water for up to 10 min, then oven dried at 60°C in an oven with a positive air flow before being placed in individually labelled sealed plastic bags ready for LA-ICPMS. During all sample preparation, samples only came in contact with acid-washed glass equipment. Immediately prior to analysis the otolith surface was vigorously rubbed for 15 s with a tissue soaked in 1% Aristar nitric acid to ensure removal of contamination such as finger prints.

Laser ablation. During LA-ICPMS the sample (otolith) under investigation was mounted in a perspex ablation cell, which was placed under a microscope. The sample was illuminated in transmitted light to identify the growth zones of the otoliths. After we chose the area of interest (nuclei) and placed this under the crosswires, the laser was focused on the sample surface in reflected light and was fired through the microscope objective lens. The ablated material was transported from the ablation cell to the ICPMS instrument in inert argon gas flow. The laser (pulsed Nd:YAG) was run in Q-switched mode at 266 nm. The isotopes in highest concentration in the otoliths were determined from solution-ICPMS of an initial sample of 5 otoliths from the Lassa. These results were used to identify the best isotopes for analysis. For the main part of the study, the ICPMS was then operated by peak jumping mode rather than scanning mode to maximise the signal from the isotopes of interest.

Calcium was used as an internal standard to compensate for the poor precision in the analytical signal, which was caused by variations in the mass of the material ablated (Campana et al. 1994). Calcium concentration was assumed from the stoichiometry of calcium carbonate as 400 000 µg g^{-1} and the concentrations of other elements were estimated against the Ca concentration. This enabled the ICPMS to be calibrated using aqueous solutions containing all isotopes of interest (Thompson et al. 1989, Chenery & Cook 1993). The accuracy of this method of calibration has been shown to be similar to that of solution-ICPMS calibrations (Querol & Chenery 1995).

Detection limits depend on the amount of material ablated. For an ablated mass equivalent to a crater 30 µm in diameter and 10 µm deep, the following list gives the theoretical detection limits (in ppm) during our analysis for the list of elements examined: ^{7}Li: 0.2–0.5; ^{23}Na: 7–22; ^{24}Mg: 0.3–10; ^{55}Mn: 0.3–5; ^{59}Co: 0.1–1; ^{63}Cu: 0.2–5.0; ^{66}Zn: 1.0–2.0; ^{85}Rb: 0.1–0.3; ^{88}Sr: 0.1–0.6; ^{138}Ba: 0.1–0.4; ^{208}Pb: 0.6–0.8; ^{232}Th: 0.04–0.2; ^{238}U: 0.3–0.4.

At the nucleus of each otolith, 3 craters were ablated. The variability observed in the results for some trace elements confirmed that 3 samples were probably the minimum necessary to account for within-sample-craters variability. To ensure the best detection limits for the 13 elements determined, craters between 30 and 40 µm in diameter were drilled. This also ensured that as representative a sample as possible was taken. Samples collected in 1995 were embedded in Araldite 'M' resin because this was found to be less contaminated by elements that were being sampled than was the polyester resin used the previous year.

The ICPMS was alight and running all day during analysis because previous studies have shown that samples analysed at the start of instrument operations can have different readings of some elements (Campana et al. 1994). We allowed the ICPMS to warm up for at least 1 h and then calibrated using a solution of the 13 isotopes to be examined. After 3 h, and at the end of the day, the calibration solution was analysed again to test for changes in sensitivity. If there was a change greater than ±10%, then a correction has been made by interpolating with respect to time. The plasma was allowed to stabilise for 4 min after each time the ablation cell was opened to change samples. Before each group of 3 ablations of an otolith, a blank was determined for subtraction from that group of ablations. These blanks are used to calculate the detection limit for each isotope during that analytical session. At the beginning and end of every day an NIST glass reference material was analysed to check the calibration and identify any inconsistencies. In this way we minimised the possibility of systematic within-day and between-day differences in instrument operation.

All samples were randomly assigned a sequential coded label in the laboratory in Australia before they were sent to England for LA-ICPMS, and the allocation of samples among sites was unknown before analysis. Prior to laser-ablation the order of samples was further randomised to remove any possibility of operator bias. As a result, between-day effects should be noise rather than systematic errors.

Data analysis. Elemental concentration of otolith nuclei was compared by analysis of variance. The variance of the data for each element at each site on each sampling occasion was examined for homogeneity and, where appropriate, the data were transformed by

either log$x$ or $\sqrt{x}$ prior to analysis. It is difficult to avoid some contamination of the surface of otoliths by polishing media or resin. Larger otoliths can be pre-ablated to remove this surface contamination (Campana et al. 1994). For our small otoliths, if an individual determination at the nucleus of an otolith differed from the other 2 determinations at that position by more than an order of magnitude, it was discarded. The mean concentration was estimated from the 3 determinations. Data for otolith nuclei were compared between sites and years where appropriate with age [1, males (<250 mm SL); 2+, females] as a covariate. Only 5 sites were adequately sampled in both years (Sadong, Lupar, Lassa, Mukah and Bintulu). Tukey's studentised range test was used to compare each element between years and among sites within years.

Canonical discriminant analysis (CDA; Digby & Kempton 1987) was used to determine if overall elemental concentrations in otolith nuclei differed between sample sites. Data from fish collected in 1994 and 1995 were analysed separately because few sites were sampled in both years. The data were tested for multivariate normality within sites before analysis and a quadratic discriminant function was used to classify the samples (SAS Proc DISCRIM).

The elemental composition of the nuclei of otoliths of juveniles (1 mo old) caught at the spawning site in the Lupar River in August 1994 were compared with that of 1 yr old male fish caught at each site in May 1995 by Fisher's exact t-tests. These juvenile fish were the only individuals of known spawning origin. Similar elemental concentrations would support the hypothesis that the Lupar River was one of the major spawning areas for these fishes.

RESULTS

A total of 13 elements were found in detectable concentrations in terubok otoliths using LA-ICPMS. A total of 106 fish collected from 9 sites in 1994 and 139 fish from 10 sites in 1995 were examined for these elements at their otolith nuclei (Table 1). The elements in highest concentration after Ca were Na and Sr. Only 7 elements (Li, Na, Mg, Cu, Zn, Sr and Ba) were consistently recorded at levels above instrument detection limits and were thus included in the analysis (Tables 2 & 3).

Five sites were adequately sampled in both 1994 and 1995 (Sadong, Lupar, Lassa, Mukah and Bintulu). These sites were examined among sites and between years for differences in the elemental composition at otolith nuclei with analysis of variance. There were no significant Site × Year interactions or age-related (sex) effects in either year ($p < 0.3$ in all cases). The results showed that, of the 7 elements used in the analysis, only ^{23}Na concentration did not differ between years ($p < 0.005$; Table 4).

Table 1. Mean length and weight of terubok *Tenualosa toli* from each site that were used in the analysis of otolith chemical composition. n = number of fish. Sampling sites are ordered from west to east (see Fig. 1)

Site	Year	Length ± SE	Weight ± SE	n
Lundu	1995	188.8 ± 6.6	130.5 ± 12.0	13
Muara Tebas	1994	216.7 ± 34.6	311.7 ± 124.5	7
Sadong	1994	138.7 ± 3.4	59.0 ± 3.7	13
	1995	226.8 ± 34.8	453.5 ± 194.7	10
Lupar	1994	211.1 ± 12.3	248.2 ± 57.6	16
	1995	215.1 ± 10.2	298.7 ± 41.0	45
Lupar juveniles	1994	36.4 ± 0.4	0.74 ± 0.04	9
Maludam	1994	205.4 ± 26.6	296.3 ± 95.4	11
Lassa	1994	238.2 ± 18.0	344.6 ± 67.0	18
	1995	332.0 ± 11.3	872.5 ± 87.2	10
Oya	1995	216.3 ± 20.3	212.8 ± 49.4	10
Mukah	1994	252.1 ± 16.3	441.5 ± 64.0	17
	1995	201.8 ± 15.6	180.8 ± 51.6	12
Bintulu	1994	290.2 ± 7.2	542.3 ± 39.8	13
	1995	188.3 ± 13.4	141.7 ± 35.6	9
Sibuti	1994	310.0	650	1
	1995	189.2 ± 3.1	120.8 ± 7.6	9
Miri	1994	263.0	396.0	1
Baram	1995	187.9 ± 3.8	125.7 ± 10.9	10
Limbang	1995	225.8 ± 5.7	210.7 ± 16.0	12
Total		210.3 ± 3.2	208.1 ± 26.2	246

Otolith nuclei of adult fish collected in 1994

There were few overall differences among sites sampled in 1994 (Table 4). The mean ^{138}Ba concentration was higher in fish from the Lassa River than other sites ($p < 0.001$). There were few significant differences among sites in the mean concentration of other elements in the nuclei of otoliths of adult terubok.

The CDA analysis supports the analysis of variance, showing few fish outside the extent of the distribution of points from the 2 spawning rivers (Fig. 2a). The first 2 discriminant functions explained 77% of the variation among sites. Fish from the Lassa had higher values on canonical value (CV) 1, which was related to a higher concentration of ^{138}Ba than in the Lupar River (Table 5). Most fish from the coastal sites had an elemental composition similar to that of fish from the Lupar or the Sadong (Fig. 2).

Otolith nuclei of fish collected in 1995

The concentration of most elements in the nuclei of the otoliths of fish collected in 1995 differed among

Table 2. Mean concentration (ppm) ± SE of 7 elements at the nuclei of otoliths of terubok *Tenualosa toli* collected at several sites in Sarawak during the 1994 survey. n = number of otoliths

Element	Site	Nucleus	n
Lithium ^{7}Li	Muara Tebas	0.89 ± 0.11	7
	Sadong	0.95 ± 0.11	13
	Lupar	0.90 ± 0.09	16
	Lupar juveniles	0.28 ± 0.02	9
	Maludam	0.97 ± 0.12	11
	Lassa	2.10 ± 0.71	18
	Mukah	1.00 ± 0.11	17
	Bintulu	1.00 ± 0.14	13
	Sibuti	2.60	1
	Miri	1.00	1
Sodium ^{23}Na	Muara Tebas	1950.3 ± 155.2	7
	Sadong	2298.5 ± 114.4	13
	Lupar	2596.7 ± 289.5	16
	Lupar juveniles	2552.3 ± 25.7	9
	Maludam	1928.3 ± 86.0	11
	Lassa	2217.5 ± 133.50	18
	Mukah	2093.3 ± 122.70	17
	Bintulu	2175.1 ± 224.60	13
	Sibuti	1411.6	1
	Miri	2233.6	1
Magnesium ^{24}Mg	Muara Tebas	66.8 ± 11.3	7
	Sadong	184.3 ± 95.9	13
	Lupar	187.9 ± 103.2	16
	Lupar juveniles	63.4 ± 17.1	9
	Maludam	45.8 ± 11.1	11
	Lassa	46.5 ± 14.1	18
	Mukah	28.0 ± 5.4	17
	Bintulu	59.6 ± 14.6	13
	Sibuti	22.1	1
	Miri	23.8	1
Copper ^{63}Cu	Muara Tebas	4.0 ± 0.8	7
	Sadong	4.6 ± 0.8	13
	Lupar	5.9 ± 2.7	16
	Lupar juveniles	4.6 ± 4.3	9
	Maludam	2.4 ± 0.4	11
	Lassa	3.9 ± 1.4	18
	Mukah	2.7 ± 0.3	17
	Bintulu	3.6 ± 1.2	13
	Sibuti	4.8	1
	Miri	0.83	1
Zinc ^{66}Zn	Muara Tebas	11.0 ± 3.6	7
	Sadong	10.5 ± 2.0	13
	Lupar	20.1 ± 15.0	16
	Lupar juveniles	8.4 ± 3.7	9
	Maludam	5.2 ± 1.0	11
	Lassa	9.2 ± 3.7	18
	Mukah	6.5 ± 1.7	17
	Bintulu	8.1 ± 3.4	13
	Sibuti	10.4	1
	Miri	1.4	1
Strontium ^{88}Sr	Muara Tebas	1933.0 ± 28.4	7
	Sadong	2055.0 ± 65.3	13
	Lupar	1985.2 ± 40.0	16
	Lupar juveniles	1822.5 ± 43.5	9
	Maludam	1883.7 ± 52.0	11
	Lassa	2105.1 ± 40.9	18
	Mukah	2114.8 ± 54.7	17
	Bintulu	1997.6 ± 54.9	13
	Sibuti	2453.9	1
	Miri	1825.4	1
Barium ^{138}Ba	Muara Tebas	3.6 ± 1.6	7
	Sadong	3.6 ± 0.5	13
	Lupar	3.6 ± 0.6	16
	Lupar juveniles	2.2 ± 0.4	9
	Maludam	4.2 ± 1.6	11
	Lassa	11.2 ± 1.3	18
	Mukah	5.0 ± 0.9	17
	Bintulu	6.0 ± 2.0	13
	Sibuti	5.6	1
	Miri	1.0	1

Table 3. Mean concentration (ppm) ± SE of 7 elements at the nuclei of otoliths of terubok *Tenualosa toli* collected at several sites in Sarawak during the 1995 survey. n = number of otoliths

Element	Site	Nucleus	n
Lithium ^{7}Li	Lundu	0.47 ± 0.06	13
	Sadong	0.26 ± 0.08	10
	Lupar	0.42 ± 0.03	45
	Lassa	0.62 ± 0.08	10
	Oya	0.51 ± 0.06	10
	Mukah	0.45 ± 0.04	12
	Bintulu	0.51 ± 0.05	9
	Sibuti	1.2 ± 0.2	9
	Baram	1.4 ± 0.2	10
	Limbang	1.2 ± 0.1	12
Sodium ^{23}Na	Lundu	3213.6 ± 280.5	13
	Sadong	2383.6 ± 64.9	10
	Lupar	2312.4 ± 25.1	45
	Lassa	2340.2 ± 42.4	10
	Oya	2174.9 ± 53.9	10
	Mukah	2124.9 ± 41.7	12
	Bintulu	2295.7 ± 47.6	9
	Sibuti	2105.1 ± 105.5	9
	Baram	2608.6 ± 407.3	10
	Limbang	3362.1 ± 319.4	12
Magnesium ^{24}Mg	Lundu	29.6 ± 2.7	13
	Sadong	44.5 ± 6.3	10
	Lupar	20.9 ± 0.9	45
	Lassa	30.1 ± 5.1	10
	Oya	31.4 ± 8.7	10
	Mukah	34.3 ± 3.8	12
	Bintulu	27.6 ± 2.0	9
	Sibuti	23.3 ± 2.3	9
	Baram	22.0 ± 2.2	10
	Limbang	61.3 ± 15.4	12
Copper ^{63}Cu	Lundu	3.7 ± 0.8	13
	Sadong	0.3 ± 0.0	10
	Lupar	0.47 ± 0.04	45
	Lassa	1.8 ± 1.5	10
	Oya	0.77 ± 0.40	10
	Mukah	0.33 ± 0.03	12
	Bintulu	0.34 ± 0.04	9
	Sibuti	1.4 ± 0.7	9
	Baram	1.6 ± 1.1	10
	Limbang	3.6 ± 1.0	12
Zinc ^{66}Zn	Lundu	1.8 ± 0.3	13
	Sadong	2.0 ± 0.4	10
	Lupar	1.7 ± 0.1	45
	Lassa	1.6 ± 0.6	10
	Oya	4.2 ± 2.2	10
	Mukah	1.7 ± 0.2	12
	Bintulu	1.9 ± 0.3	9
	Sibuti	24.5 ± 15.3	9
	Baram	2.4 ± 1.1	10
	Limbang	1.8 ± 0.1	12
Strontium ^{88}Sr	Lundu	1776.2 ± 52.3	13
	Sadong	1737.1 ± 48.1	10
	Lupar	1967.7 ± 27.6	45
	Lassa	2006.5 ± 48.6	10
	Oya	1893.1 ± 94.7	10
	Mukah	1842.1 ± 60.8	12
	Bintulu	1813.9 ± 77.8	9
	Sibuti	1797.4 ± 71.1	9
	Baram	1823.7 ± 89.0	10
	Limbang	1768.8 ± 48.4	12
Barium ^{138}Ba	Lundu	3.1 ± 0.5	13
	Sadong	2.3 ± 0.5	10
	Lupar	2.9 ± 0.3	45
	Lassa	6.1 ± 0.7	10
	Oya	2.8 ± 0.8	10
	Mukah	2.4 ± 0.4	12
	Bintulu	2.5 ± 0.6	9
	Sibuti	2.4 ± 0.5	9
	Baram	2.1 ± 0.5	10
	Limbang	3.4 ± 0.8	12

Table 4. Results of analysis of variance of mean elemental concentration in otolith nuclei and Tukey's studentised range tests of means of each element. All significant comparisons have a probability less than $p < 0.01$; ns: not significant. Sites are arranged in sequence from south-west to north-east along the Sarawak coast (see Fig. 1 for actual position). Lun: Lundu; Mt: Muara Tebas; Sa: Sadong; Lu: Lupar adults; Ma: Maludam; La: Lassa; Oy: Oya; Mu: Mukah; Bi: Bintulu; Si: Sibuti; Mi: Miri; Ba: Baram; Li: Limbang

Element	Differences in concentration
1994 vs 1995: Sa Lu La Mu Bi	
^{7}Li	1994 < 1995
^{23}Na	1994 = 1995
^{24}Mg	1994 > 1995
^{63}Cu	1994 > 1995
^{66}Zn	1994 > 1995
^{88}Sr	1994 > 1995
^{138}Ba	1994 > 1995
Between sites (1994): Mt Sa Lu Ma La Mu Bi	
^{7}Li	All ns
^{23}Na	All ns
^{24}Mg	Mt Sa Lu > Mu
^{63}Cu	All ns
^{66}Zn	All ns
^{88}Sr	La Mu > Ma
^{138}Ba	La > Other sites
Between sites (1995): Lun Sa Lu La Oy Mu Bi Si Ba Li	
^{7}Li	Si Ba Li > other sites
^{23}Na	Lun Li > other sites
^{24}Mg	Sa Li > Ba Lu Si
^{63}Cu	Lun > Sa Lu La Oy Mu Bi Si Ba Li > Sa Mu Bi
^{66}Zn	Si > La Mu Bi Ba
^{88}Sr	All ns
^{138}Ba	La > Sa Oy Mu Bi Si Ba Li

Table 5. Standardised canonical coefficients for canonical variates (CV) 1 and 2 for the 7 elements examined in the otolith nuclei among all sites sampled in 1994 and 1995

Element	1994		1995	
	CV 1	CV 2	CV 1	CV 2
^{7}Li	0.30	−0.09	1.29	−0.53
^{23}Na	0.15	−0.08	0.41	0.68
^{24}Mg	−0.62	−0.65	0.28	0.50
^{63}Cu	−0.09	0.59	−0.16	0.43
^{66}Zn	0.17	0.23	0.19	−0.67
^{88}Sr	−0.11	1.05	−0.30	−0.23
^{138}Ba	1.20	−0.59	0.15	0.50

sites (Table 4). These overall differences were mainly attributed to fish from Limbang and to a lesser extent Lundu. Fish collected in the sea at Limbang and Lundu were apparently born in water of an elemental concentration different from that for fish from other sites. Fish from Limbang had higher concentrations of ^{7}Li, ^{23}Na, ^{24}Mg and ^{63}Cu than fish from most other sites ($p < 0.001$), especially the spawning rivers (Lupar and Lassa). Strontium concentration was also significantly lower in otoliths of fish from Limbang than in fish from the Lupar and Lassa (Table 3). Similarly, fish from Lundu had higher concentrations of ^{23}Na and ^{63}Cu than did fish from other nearby sites, and lower ^{88}Sr levels than fish from the Lupar and the Lassa.

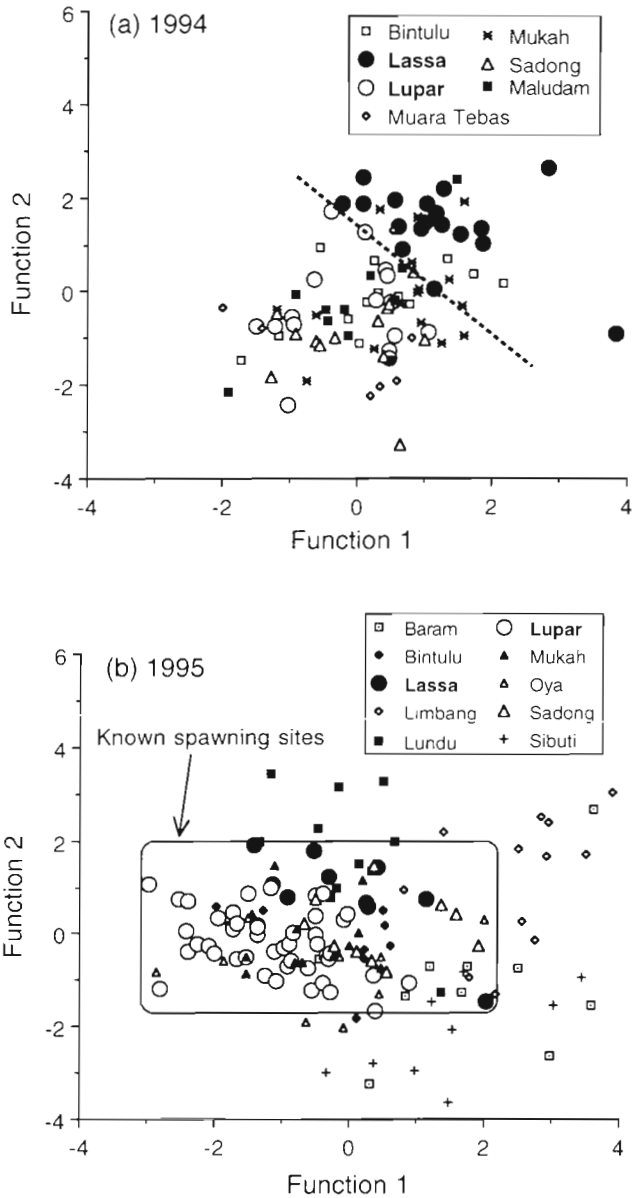

Fig. 2. Plot of the first 2 canonical discriminant functions using the elemental composition of the otolith nuclei of terubok *Tenualosa toli* adults collected in (a) 1994 and (b) 1995. Known spawning sites are shown in bold. The dotted line separates fish caught in the Lassa and Lupar Rivers

These patterns are reflected in the discriminant function analysis of all the elements (Fig. 2b). The first 2

functions explained 76% of the variation in the data. Unlike the analysis of the 1994 data, the plot of the overall elemental composition of otolith nuclei from the spawning estuaries overlapped considerably. Many more fish plotted outside the extent of the points from these estuaries. These fish were caught at sites not previously adequately sampled in 1994 and remote from the known spawning areas—Limbang, Baram and Sibuti to the north-east and Lundu to the south-west (Fig. 1). They had higher values for CV 1, which were related to ^{7}Li concentration (Table 5).

Comparison of juveniles from the Lupar River spawning area and males (1 yr old) caught the following year

Juvenile terubok (approximately 6 wk old) collected from the Lupar in August 1994 had an elemental composition at their nuclei similar to that in the nuclei of males collected the next year from the same site. Fish from other sites had an otolith nucleus chemistry similar to that of the juveniles from the Lupar. Only the Li and Na concentrations differed; fish from the northern sites (Sibuti, Baram, Limbang) had higher Li concentrations than fish from other sites, and Limbang and Lundu fish also differed from the juveniles in their mean Na concentration (Fig. 3).

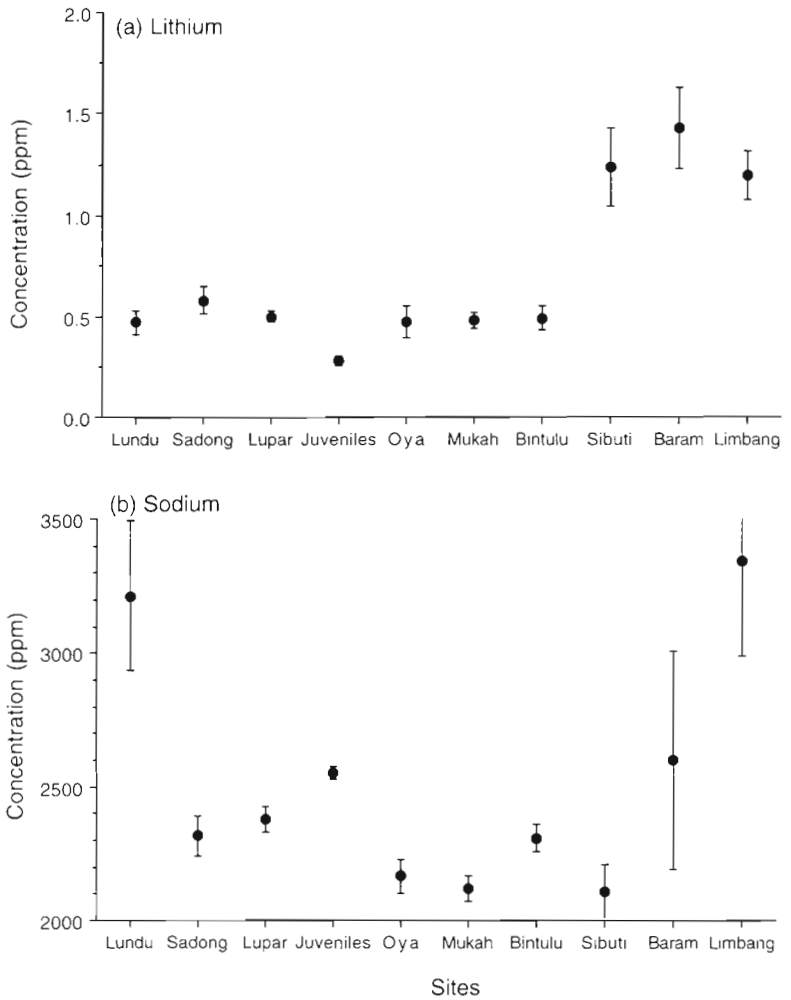

Fig. 3. Mean elemental concentration (ppm) ± SE of ^{7}Li and ^{23}Na in the nuclei of terubok *Tenualosa toli* males (1 yr old) collected in 1995 and juveniles (1 mo old) collected in 1994 at Pulau Seduku in the lower Lupar River

DISCUSSION

The elemental composition of the nuclei of otoliths of fish collected in coastal areas of Sarawak in 1994 were similar to those fish collected in the 2 estuaries where spawning has been recorded. This suggests that these fishes all spawned in similar water—probably in these 2 estuaries. Among the fish collected in 1995, those fish from the coastal sites which had also been sampled in 1994 (i.e. Bintulu and Mukah) had otolith elemental compositions similar to those of the fish collected in the spawning estuaries. However, their otolith elemental composition differed from that of fish collected at the same sites in 1994. This suggests either that the elemental composition in the spawning estuaries is changing over time, or that in 1994 all fish were spawned outside the spawning estuaries. This second alternative is unlikely, as we have strong evidence that the vast majority of the population spawn in either the Lupar or Lassa rivers (Blaber et al. 1996, 1997), but it cannot be ruled out without further work to determine whether changes do take place in the elemental composition of the spawning estuaries.

These inter-annual changes in otolith chemistry indicate that the chemistry of water in which fish are collected cannot be assumed to be stable. It suggests that we should be cautious in our interpretations of fish population structure based on the chemistry of otoliths collected during a single time period. Further sampling at a later time may give a different interpretation and thus lead to confusion about the true structure of the fish population in question. For the terubok used in this study, fish collected in 1995 were caught during May, only 9 mo after the samples collected in August 1994. However, spawning occurs throughout the dry season (April to October), with a peak in April to May (Blaber

et al. 1996). In both years, the fish examined were spawned during the same season of the year. Interannual differences in elemental compostion are unlikely to be related to seasonal changes in freshwater input.

The elemental composition of otoliths of fish from the sites sampled in 1995 was much more variable. Fish from sites that were on the extreme of the known distribution (Lundu and Limbang) had different elemental composition of their otolith nuclei. This suggests that at least some fish from these sites were not born in either of the known spawning estuaries during the 1993 or 1994 spawning seasons. It suggests that there may be other spawning estuaries for these fish, presumably in rivers not previously sampled during the 2 surveys in Sarawak (1994 and 1995) and Sabah (see Fig. 1; Blaber et al. 1996). In these surveys there were no rivers in the 2 states which have suitable conditions for terubok that were not sampled. Results from a recent trip south from Sarawak to Pontianak in Kalimantan suggest that terubok are probably not spawning in estuaries in that area (S. Blaber & J. Pang unpubl. data). This limits the possibilities for the choice of other spawning estuaries. Surveys of larger rivers in Kalimantan may find evidence of further terubok spawning areas.

The fish sampled in the spawning estuaries in both years were in spawning condition or recently spent (Blaber et al. 1996). However, we do not know if they themselves were actually spawned in that estuary. The 1994 sample of juvenile terubok were born in the lower reaches of the Lupar where they were caught (Pang & Ong 1990). The elemental composition of the nuclei of their otoliths should provide the closest approximation of the composition of fish that were born in the Lupar in 1994. The fish collected in the Lupar in 1995 had a similar elemental composition in their nuclei to that of the juveniles of the previous year, confirming that they were probably born at the same place.

The similarity in the elemental composition of the nuclei of fish collected from the 2 spawning estuaries in 1994 suggests that either the fish spawning in each river were a mixture from more than one estuary or that the elemental composition in each estuary was similar. The LA-ICPMS data could not be used to separate fish from these estuaries despite their geographic separation.

Preliminary studies of the catch and effort in the terubok fishery for spawning females found that most of the catch comes from the Lupar (Willman et al. 1989, Sarawak government unpubl. data). This suggests that most spawning probably takes place in that estuary. The similarity in the otolith chemistry of the juveniles from the Lupar and fish from nearby coastal sites whose origin is unknown supports these data. However, the evidence is not strong because of the similarity in otolith chemistry of fish from the Lassa.

Overall, the elemental data have confirmed that most of the terubok in the coastal fishery were born in 1 of the 2 estuaries where spawning is known to occur. Sarawak government efforts to conserve the species by protecting the spawning fish in these estuaries should help increase the size of the population. The data also suggest that there may be one or more other estuaries where spawning occurs. These are most likely to be in Indonesian Kalimantan and beyond the jurisdiction of the Sarawak government.

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