SPATIAL VARIATION IN OTOLITH CHEMISTRY OF *EPINEPHELUS MERRA* IN BAA ATOLL, MALDIVES

BY

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INTRODUCTION

The particular geography of Maldives archipelago induces that the economy is essentially dependant on marine resources. Despite the fact that pelagic resources are the most exploited, reef fin fishery strongly increased due to tourism and export industry development in the last decades (Sattar *et al.*, this issue). Although there are no clear signs of overexploitation and decline of fish population overall, Sattar et al. (this issue) have concerns for some groupers species in some locations. Chabanet *et al.* (this issue) also report no sighting of sharks in Baa for instance. Thus, Maldivian government and stakeholders remain vigilant, and several MPAs were established in various atolls. The goal of the Atoll Ecosystem Conservation Project (http://www.biodiversity.mv/aec/) aims to establish a network of conservation for Baa atoll for instance.

Anthropogenic activities have strongly affected coral reef ecosystems worldwide and Marine Protected Areas (MPAs) are increasingly considered as an effective tool for fishery management (Roberts *et al.*, 2001) and biodiversity conservation (Micheli *et al.,* 2004). To design MPAs and soundly manage fish stocks, it is vital to obtain information on connectivity between populations, migration and movement, and describe population dynamics. A variety of methods have been used to assess movements between populations such as population genetic (Bradbury *et al.*, 2008), hydrodynamic and lagrangian dispersal models (Cowen *et al.*, 2000), external tags (Willis *et al.*, 2001, Sattar *et al.*, this issue), acoustic telemetry (Topping *et al.*, 2005) or transgenerational marking of embryonic otolith (Thorrold *et al.*, 2006). Recently, elemental composition of fish otoliths has been successfully used to examine connectivity between populations (Milton and Cheney, 2001; Chittaro *et al.*, 2006). Elemental chemistry is used because trace elements from the environment are incorporated into the otolith during growth and the otolith is metabolically inert (Campana, 1999). According to Hamer *et al;*(2003), the success of using otolith chemistry to measure connectivity would be dependant on a detectable level of chemical variation at biologically relevant spatial scales.

The present study investigated whether otoliths of *Epinephelus merra* collected from different coral reefs in Baa Atoll could be discriminated on the basis of multielemental chemistry. The study aimed at evaluating the potential of otolith microchemistry technique to study connectivity among reefs at Baa Atoll, and brings on the long run key data for MPAs selection.

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MATERIAL AND METHODS

Study Site and Sampling

The Republic of Maldives is an archipelago of 26 atolls located in central Indian Ocean. Baa Atoll, circa 40 km long and wide, is located in the western side of the Maldives archipelago and ranges from 4°49'N and 5°23'N and 73°06'E (Fig. 1).

The survey presented here took part during a biodiversity census survey conducted in May-June 2009, on lagoon and outer slope habitats in Baa Atoll (Fig. 1). For this study, a total of 93 *E. merra* were collected at 6 stations: stations 3 and 28 (lagoon reef flat), 6 and 21 (lagoon reef slope), 17 (pass slope) and 20 (reef flat under oceanic influence). Fish were stored in ice immediately after capture and dissected within one hour of collection.
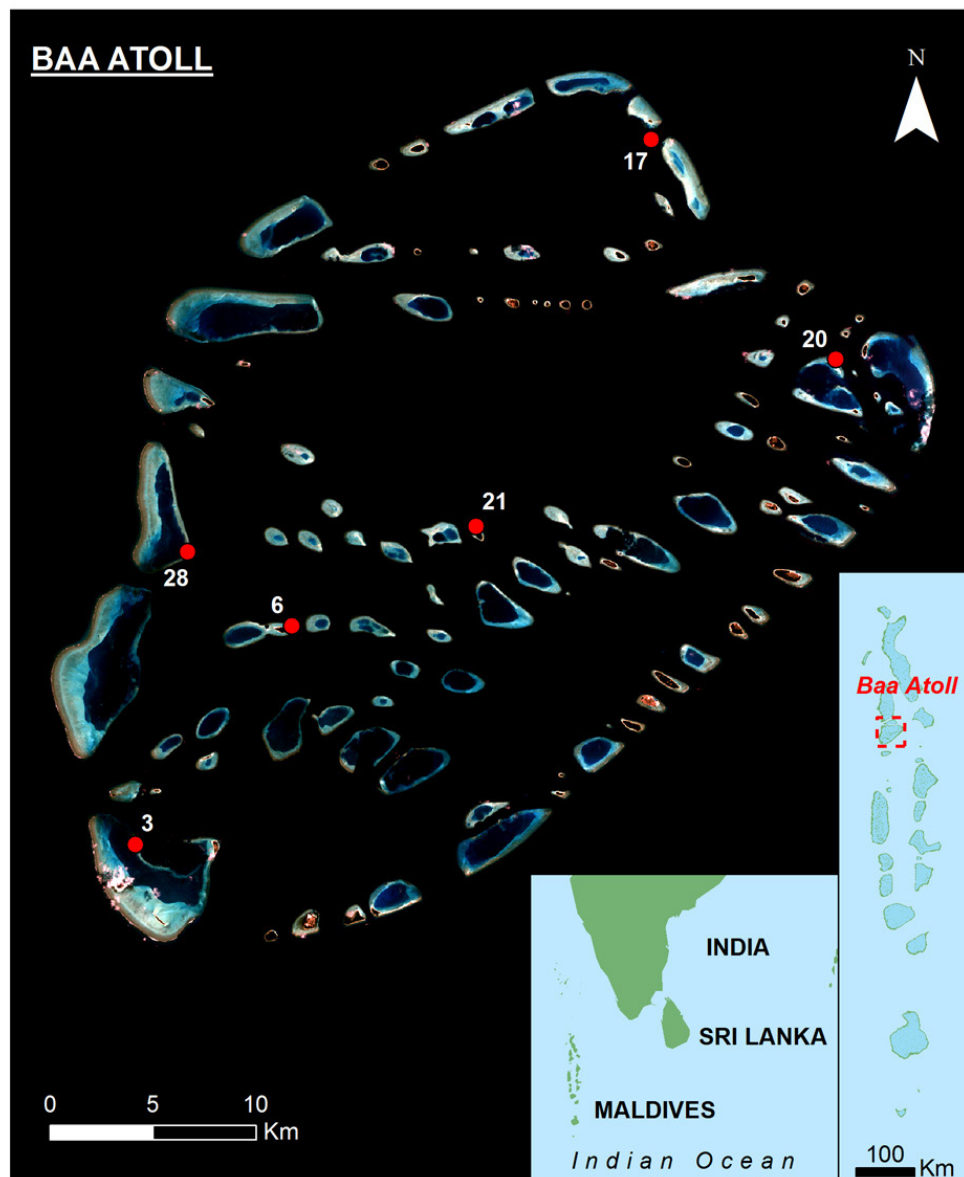
Figure 1. Map of Baa Atoll and position of *E. merra* sampling stations.

Laboratory Analysis

For each individual, total length (TL) was measured to the nearest mm and sagittal otoliths were extracted with acid-washed plastic tweezers, cleaned of adhering tissues in ultrapure water and stored dry in acid-washed eppendorf tubes. At the lab, otoliths were cleaned of organic material by soaking in an equal-volume mixture of 30% ultrapure H_2O_2 and 0.1 mol L^{-1} ultrapure NaOH for 1 hour. Each otolith was then ultrasonicated for five minutes and rinsed with ultrapure water and individually soaked in five separated 5-min baths of ultrapure water. After the fifth bath, otoliths were air-dried in a HEPA-filtered class 100 laminar flow hood. Whole otoliths were placed on double-sided tape just before laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) analysis (Warner *et al.*, 2005).

Ten otoliths per station were randomly chosen (except for station 6, N=3) and ablated with the LA-ICPMS at the edge of otolith postrostrum. All analyzes were conducted at the University of Montpellier 2 (UMR 5243 Geosciences), using a 193 nm Excimer Laser System (CompEx 102, LambdaPhysiks) coupled to a Element XR sector field ICPMS 5thermoFisher). For all otoliths, the laser beam diameter was set at 51 μm and the laser was operated with a repetition rate of 4 Hz at 15 J.cm^{-2}. Helium was used as the ablation gas, to enhance sensitivity and reduce particle condensation on the surface. An Argon gas flow was then admixed to the laser-generated aerosol, prior to introduction into the ICPMS for elemental analysis. The instrument was operated in low mass resolution and calcium was used as an internal standard using a stoichiometric value of 56% CaO. A standard reference glass material (NIST 612) was used to calibrate analyzes and to control for instrumental drift. Apart from Ca, 20 elements were measured: Li, B, Mg, Ti, Cr, Mn Co, Ni, Cu, Zn, Rb, Sr, Mo, Cd, Sn, Ba, Ce, Pb, Th and U. Calculation of drift and limits of detection (LOD) were made off-line using the Glitter software. Elements for which 25% of the measures were below LOD irrespective of fish origin were removed from further analysis. Remaining elements were: Li, B, Mg, Mn, Co, Ni, Zn, Sr, Cd, Sn and Ba. Data were expressed as ratios to calcium and element concentration below LOD were set to zero.

Statistical Analysis

We used discriminant function analyses (DFAs) to examine spatial patterns of multielemental chemical signature of otoliths. Element ratios that contributed most to the discrimination among sites (Sr/Ca, Mg/Ca, Mn/Ca and Ba/Ca) were further analyzed by non-parametric ANOVAs (Kruskall-Wallis) in order to investigate spatial differences.

RESULTS AND DISCUSSION

There was no significant difference in fish total length between sampling stations (Table 1).

DFA successfully reclassified 98% of the individuals (Cohen-kappa test) and some spatial structure was apparent (Fig. 2). The first discriminant function (Wilk's $\lambda < 0.001$, $P < 0.001$) distinguished fishes from lagoon reef flat (stations 3 and 28) to individuals from reef flat under oceanic influence (station 20) and from lagoonal and pass slope (station 21, 17 and 6). The second discriminant function (Wilk's $\lambda = 0.009$, $P < 0.001$) separated fishes from reef slope into two groups: on one hand fishes from lagoon reef slope (station 21) and on the other hand fishes from outer reef pass slope (station 17) and from lagoon reef slope (station 6).

Greatest contributors to the discrimination among sites were Sr, Mn, Mg and Ba and their ratios to calcium are given for each station in Table 2. Significant differences in concentration among sites were observed for Mg/Ca and Ba/Ca ($P < 0.05$) but not for Sr/Ca and Mn/Ca. Fish otoliths from lagoon reef flat (station 3 and 28) were characterized by a higher Mg/Ca and Ba/Ca ratios than the others. The same pattern was lightly observed for Sr/Ca and Mn/Ca..

Table 1. Results of ANOVA on fish total length. Mean size (cm) and standard error are given for each station.

Stations	Mean size	df	F	P
All stations	15.69±0.204	5	0.385	0.858
3	15.06±0.502			
6	15.86±1.004			
17	15.96±0.494			
20	15.73±0.435			
21	15.74±0.437			
28	15.79±0.456			
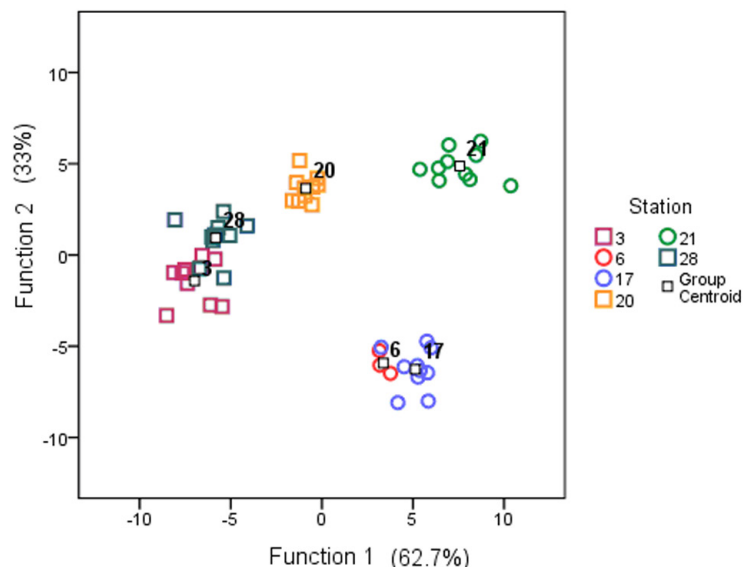
Figure 2. Discriminant function analysis (DFA) achieved with the multielemental chemical signature (Li, B, Mg, Mn, Co, Ni, Zn, Sr, Cd, Sn and Ba) of *E. merra* otoliths from different stations of Baa Atoll. Squares represent reef flat and circles represent reef slope.

Table 2. Mean and standard error of trace element ratios for each station.

	Stations					
	3	6	17	20	21	28
Sr/Ca	11.26±3.45	1.11±0.05	4.61±2.34	3.28±2.12	5.76±3.02	7.14±3.042
Mg/Ca	11.71±3.94	0.12±0.02	2.98±1.69	2.36±2.16	5.06±3.20	24.13±11.14
Mn/Ca	8.91±2.98	0.08±0.03	3.82±2.48	2.15±2.02	4.95±3.07	5.63±2.68
Ba/Ca	0.035±0.001	0.006±0.001	0.021±0.004	0.010±0.002	0.012±0.003	0.074±0.045

Our results showed that *E. merra* captured in Baa Atoll different habitats may be differentiated based on their otolith chemistry. We found a clear discrimination between otoliths of fishes from lagoon reef flat stations and lagoonal-pass slope stations, with higher trace element ratios for the first. These findings could derive from contrasting differences in environmental conditions of those stations. In fact, some environmental characteristics such as water temperature and salinity are likely to have direct effects on the concentration of some elements (Sr) in the water that affects otolith elemental composition (Gillanders and Kingsford, 2000). In the present study, we have no data on the environmental characteristics of each station (water temperature, salinity and chemistry) to support this hypothesis. Endogenous factors (diet, stress, ontogenetic effects...) can also influence otolith elemental composition, but we minimized some of these effects by analyzing otoliths of fishes with similar sizes.

The analysis of multielemental composition along *E. merra* otolith growth axis (juvenile part to adult part) may be conducted to determine the origin of fishes and to reconstruct their movement's history within Baa Atoll. However, temporal stability of elemental signature of otoliths (Gillanders and Kingsford, 2003; Ruttenberg *et al.,* 2008) of *E. merra* at Baa Atoll should first be investigated.

In conclusion, multielemental composition of fish otoliths showed a good potential to measure connectivity between populations within an atoll at local spatial scale, and the technique may be useful on the long run for the design of MPAs and the management of fisheries.

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