



# Prolonged estuarine habitat use by dusky grouper *Epinephelus marginatus* at subtropical latitudes revealed by otolith microchemistry

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**ABSTRACT:** Otolith microchemistry (Sr:Ca, Ba:Ca) was used to evaluate habitat use patterns of the endangered dusky grouper *Epinephelus marginatus* in southern Brazil. Individual Sr:Ca profiles exhibited low variation, with an overall tendency to increase with age. Interestingly, individual Ba:Ca profiles presented 3 contrasting patterns: the first comprised most sampled individuals (>80%), mostly indicative of the predominant use of marine waters throughout their life history; the second pattern (~10% of all individuals) indicated that dusky grouper may use or remain in or near estuarine waters for short time periods; finally, the third identified pattern (~5%) comprised individuals remaining in estuarine waters for long periods and provided the first evidence for this species of prolonged estuarine habitat use (over a year), in particular during juvenile life stages.

**KEY WORDS:** Epinephelidae · Fish conservation · Life-history plasticity · Movement · Natural tags · Patos Lagoon

## INTRODUCTION

Dusky grouper *Epinephelus marginatus* (Lowe, 1834) is the only large Epinephelidae species commonly found along subtropical latitudes (above 30°S) of the southwestern Atlantic Ocean, where it inhabits shelter-rich hard substrata on the continental shelf (Heemstra & Randall 1993, Sluka et al. 2001, Reñones et al. 2002). This species is highly prized by commercial fisheries and represents a significant source of income to fishermen (Condini et al. 2007, Craig et al. 2011, Sadovy de Mitcheson et al. 2013). Overfishing combined with a complex life-

history strategy (slow growth rate, late maturation, aggregative spawning behavior and sequential hermaphroditism, a trait common to several grouper species [Heemstra & Randall 1993, Craig et al. 2011]) has led to the high vulnerability of many grouper populations (Graham et al. 2009, Cheung et al. 2013, Sadovy de Mitcheson et al. 2013), and in particular to the classification of *E. marginatus* as 'Endangered' (EN a2d) in the IUCN Red List (Cornish & Harmelin-Vivien 2004).

Recent studies on *E. marginatus* in southern Brazil comparing littoral and neritic sites have suggested that Carpinteiro Bank (a neritic site) is the main

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growth and spawning ground for this species in the region (Condini et al. 2014a,b). In contrast, only immature individuals and resting mature females are found along rocky substrate in the littoral zone (Seyboth et al. 2011). Although comparative studies suggest that dusky grouper growth rates are higher in neritic habitats (Condini et al. 2014b), the relative importance of the different habitats for this endangered species remains unknown. A better understanding of habitat use patterns of marine fish populations is vital to evaluate their dynamics and resilience to harvest, ultimately supporting adequate management and conservation measures (Andrello et al. 2014, Davoren et al. 2015). This fundamental aspect of fish life history can be revealed through the evaluation of natural markers such as otolith microchemistry (Rooker et al. 2008, Reis-Santos et al. 2013a, Darnaude et al. 2014), as detailed analysis of the chemical composition of otoliths allows reconstruction of the environmental conditions experienced by individual fish throughout their entire life history (Campana 1999, Elsdon et al. 2008, Tanner et al. 2013). In general, strontium (Sr) and barium (Ba) concentrations in otoliths have a positive correlation with salinity and freshwater, respectively (Elsdon & Gillanders 2005, Sturrock et al. 2012, Reis-Santos et al. 2013b). Thus, fish living in marine waters present higher otolith strontium concentrations compared to fish living in freshwater, whereas the latter present conspicuously higher otolith Ba concentrations than fish living in marine waters.

The aim of this study was to investigate habitat use patterns of dusky grouper *E. marginatus* in subtropical latitudes (32°S) in the southwestern Atlantic Ocean. Findings may provide key insights on habitat use of different life-history stages which could be useful in the design of Marine Protected Areas and help to ensure the conservation of this endangered species at the southern limit of its distribution.

## MATERIALS AND METHODS

Sampling was conducted at 2 locations on the southern Brazilian coast (Fig. 1). The first one is located in the littoral zone (rocky jetties, <5 m in depth), at the mouth of the Patos Lagoon estuary (~1000 km<sup>2</sup>), and is constituted of a pair of 4.5 km long rocky jetties (Fig. 1; 32°09' S, 52°05' W). Due to its position, this sampling location has varying hydrological conditions and freshwater inputs that affect its salinity profiles (Moller et al. 2001). The second sampling site is the Carpinteiro Bank (Fig. 1; 32°16' S, 51°47' W), a group of submerged rocks 20 to 30 m deep in the neritic zone approximately 16 nautical miles (~30 km) from the coast.

Dusky grouper *Epinephelus marginatus* were collected from the littoral and neritic zones in collaboration with regional fishermen. Otoliths of a total of 72 individuals of *E. marginatus* were extracted for chemical analysis; 28 of these individuals, aged between 2 and 12 yr (mean ± SD: 4.8 ± 2.4 yr), were sampled in the littoral zone (rocky jetties) and 44, aged between 2 and 40 yr (11.9 ± 10.8 yr), were sampled in the neritic zone (Carpinteiro Bank). Age determination using otoliths has already been validated for *E. marginatus* in this study area (Condini et al. 2014b). All procedures for otolith chemical analysis followed Mai et al. (2014). Each otolith section was scanned from the core to the edge using a 266 nm laser ablation system (CETAC LSX 100) coupled to an ELAN 6000 (PerkinElmer—SCIEX) inductively coupled plasma-mass spectrometer (ICP-MS), and calcium (<sup>43</sup>Ca), <sup>86</sup>Sr and <sup>138</sup>Ba were measured. Blank ablations (background intensities) were measured during 50 s after every 10 otolith ablations. Strontium and calcium counts per second (cps) were subtracted from the background

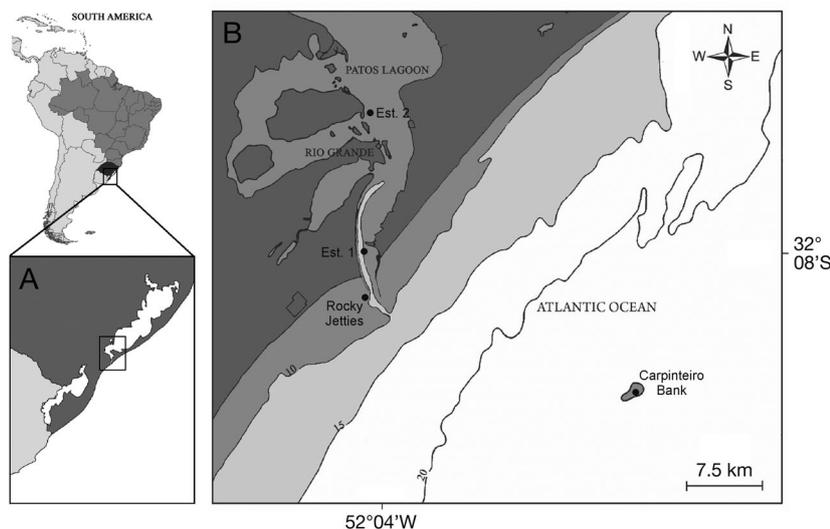
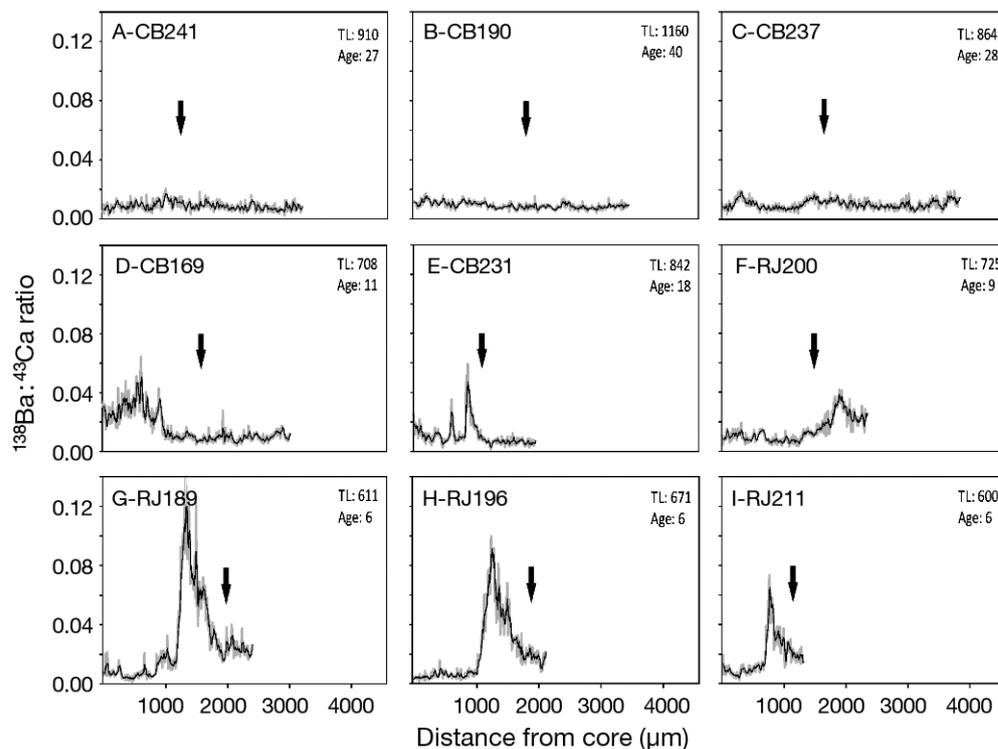


Fig. 1. Patos Lagoon (10 360 km<sup>2</sup>) and its estuarine zone in the state of Rio Grande do Sul, southern Brazil (A), showing the locations of the 2 sampling sites (rocky jetties and Carpinteiro Bank), as well as 2 sites related to salinity data (Est. 1 and Est. 2) (B). Lines along the coast denote 10 (dark gray), 15 (light gray) and 20 m (white) isobaths, respectively

Fig. 2. Ba:Ca ratio profiles along a transect from the core to the posterior edge of the otolith of *Epinephelus marginatus* collected from the littoral (RJ) and neritic (CB) zone, with age (yr) and total length (TL, mm) of each analyzed individual. All element:Ca ratios correspond to counts per second. Arrows indicate the end of the juvenile stage; before the arrow individuals are <5 yr old. The black line denotes a 3-order moving average, which was employed for better visualization of the elemental chemical profile trend exhibited by each individual otolith



level, and element:Ca ratios were then calculated for all otoliths. Before analyzing otoliths, the instrument was optimized through daily performance, with maximum analyte intensities and minimum interferences determined using oxides and double-charged ions. Repeatability was estimated as the relative standard deviation (RSD) from 10 consecutive measurements of the standard NIST 1834, resulting in relative standard deviations of 12.43, 6.91 and 3.99% for  $^{138}\text{Ba}$ ,  $^{86}\text{Sr}$  and  $^{43}\text{Ca}$ , respectively.

As the Carpinteiro Bank (a neritic zone) has been identified as an important spawning site for *E. marginatus* in southern Brazil (Condini et al. 2014a) and taking into consideration the close location of the littoral habitat (16 nautical miles), all individuals used in this study were assumed to belong to the same population; this assumption is corroborated by the long distances that dusky grouper larvae can disperse (>16 nautical miles; Andrello et al. 2014). To reconstruct and characterize habitat use patterns of *E. marginatus*, we analyzed the Sr:Ca and Ba:Ca life-history profiles of all 72 *E. marginatus* individuals collected from both littoral and neritic areas. In addition, otolith edge Sr:Ca and Ba:Ca ratios of individuals collected at littoral and neritic sites were compared using non-parametric Mann-Whitney *U*-tests to evaluate our ability to distinguish between sampled areas. For this comparison only fish from matching year classes (aged between 6 and 8 yr old and

born between 2000 and 2003) were used to avoid any potential influence of ontogenetic and temporal variations: specifically, 12 individuals from littoral ( $587.6 \pm 44.9$  mm TL) and 8 from neritic zones ( $619.0 \pm 36.1$  mm TL). Otolith edge chemical composition was defined as the 30  $\mu\text{m}$  nearest to the edge of the otolith.

Salinity data over time were analyzed from a 40 yr time series for the Carpinteiro Bank region (specifically, from 32 to 32° 50' S and 50 to 52° W) and a 20 yr time series for littoral and estuarine areas. Data were obtained from the Coastal and Estuarine Oceanography Laboratory of Rio Grande Federal University and the Ichthyology Laboratory of Rio Grande Federal University, respectively. Salinity data were averaged by month and reported with respective standard deviations.

## RESULTS AND DISCUSSION

We found 3 interesting patterns of Ba:Ca life-time profiles in *Epinephelus marginatus* that indicate differential habitat use. The first comprises most sampled individuals (>80%) and is characterized by low Ba:Ca ratios (<0.03) along an individual's entire life history (Fig. 2A–C; Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/n029p271\\_supp.pdf](http://www.int-res.com/articles/suppl/n029p271_supp.pdf)) and is mostly indicative of the predominant use of marine waters throughout their life history. The sec-

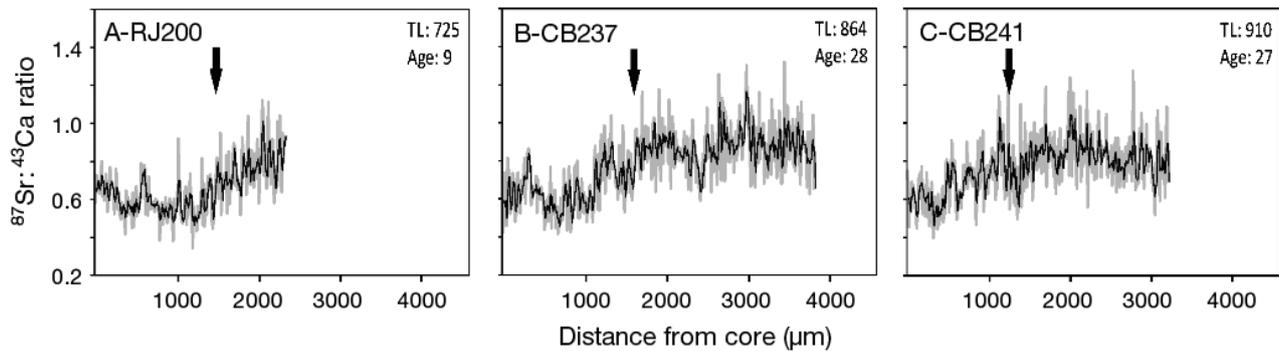


Fig. 3. Sr:Ca ratio profiles along a transect from the core to the posterior edge of the otolith of *Epinephelus marginatus* collected from the littoral (RJ) and neritic (CB) zone, with age (yr) and total length (TL, mm) of each analyzed individual. All element:Ca ratios correspond to counts per second. Arrows indicate the end of the juvenile stage; before the arrow individuals are <5 yr old. The black line denotes a 3-order moving average, which was employed for better visualization of the elemental chemical profile trend exhibited by each individual otolith

ond pattern includes juveniles and adults (~10% of all individuals) that exhibited small fluctuations in otolith Ba:Ca ratios, with peaks reaching values between 0.03 and 0.05 (Fig. 2D–F); this indicates that these fish may use or remain in, or near, estuarine waters for short time periods. Finally, the third identified pattern (~5%) comprises individuals with a steep increase in Ba:Ca ratios (up to a maximum of 0.14; Fig. 2G–I) during their juvenile stage, followed by a decrease to early life levels (<0.03). This pattern appeared mostly, though not exclusively, in fish sampled in the littoral habitat and suggests these fish remained within the Patos Lagoon estuary for extended periods of time (over 2 yr in some cases).

Our findings support the conclusions that dusky groupers have higher life-history plasticity than previously reported and that estuaries may be used as alternative habitats by some individuals during part of their lives. To our knowledge, there is no prior evidence in the literature of *E. marginatus* inhabiting brackish waters for such extended periods, though this is not entirely surprising, as juveniles of the *Epinephelus* genus (e.g. *E. striatus*, *E. coiodes*, *E. malabaricus*, *E. aeneus*, *E. itajara*) are known to use mangroves, coastal lagoons and estuaries as nursery or juvenile habitats (Heemstra & Randall 1993, Craig et al. 2011). Yet, until now, there have been only a few scattered records of occurrence of *E. marginatus* near, or within, estuarine areas (e.g. Barbanti et al. 2013, M. V. Condini unpubl. data), and no prior information on consistent use of estuaries or permanence in brackish waters.

Other studies using otolith chemistry of different species inhabiting the Patos Lagoon estuary and adjacent coastal areas (e.g. *Genidens genidens* and *Mugiliza*) showed similar ranges of otolith Ba:Ca when mi-

grating along the salinity gradient of this estuarine system. For instance, otoliths of *G. genidens* showed average Ba:Ca ratios of approximately 0.08 in the estuary, which increased to ~0.13 in the freshwater portion of the Patos Lagoon (Pereyra 2015). Ba:Ca values ranging from 0.01 to 0.14 were also reported for *Lycengraulis grossidens* and *Genidens barbuis* collected along the Patos Lagoon salinity gradient (Mai et al. 2014, Avigliano et al. 2015). We recognize that inter-specific variations in otolith microchemistry may sometimes preclude comparisons between different species (Reis-Santos et al. 2008). However, as we show here, many migrant fish species presented similar ranges of Ba:Ca ratios in their otoliths along the Patos Lagoon estuary. Also, most studies indicate that water is the major source of otolith elements and that diet has little or no effect on otolith elemental composition (Walther & Thorrold 2006, Gibson-Reinemer et al. 2009, Marohn et al. 2009), which helps to discard potential confounding effects of diet in our results. In contrast to Ba:Ca, individual Sr:Ca profiles exhibited low variation, with an overall tendency to increase with age, regardless of which site individuals were collected from (Fig. 3; Fig. S2 in the Supplement at [www.int-res.com/articles/suppl/n029p271\\_supp.pdf](http://www.int-res.com/articles/suppl/n029p271_supp.pdf)). Albuquerque et al. (2012) observed little or no variation in Sr:Ca ratios in the water between intermediate (~20) and high (~35) salinities along the freshwater-marine gradient in the estuarine system of Patos Lagoon. Similar results have also been observed by Phillis et al. (2011) in San Francisco Bay related to the Ca concentration of the freshwater end-member. However, higher than expected Sr or Ca end-member availability in the ambient water of the Patos Lagoon cannot be excluded, due to lack of available information (see Kraus & Secor 2004).

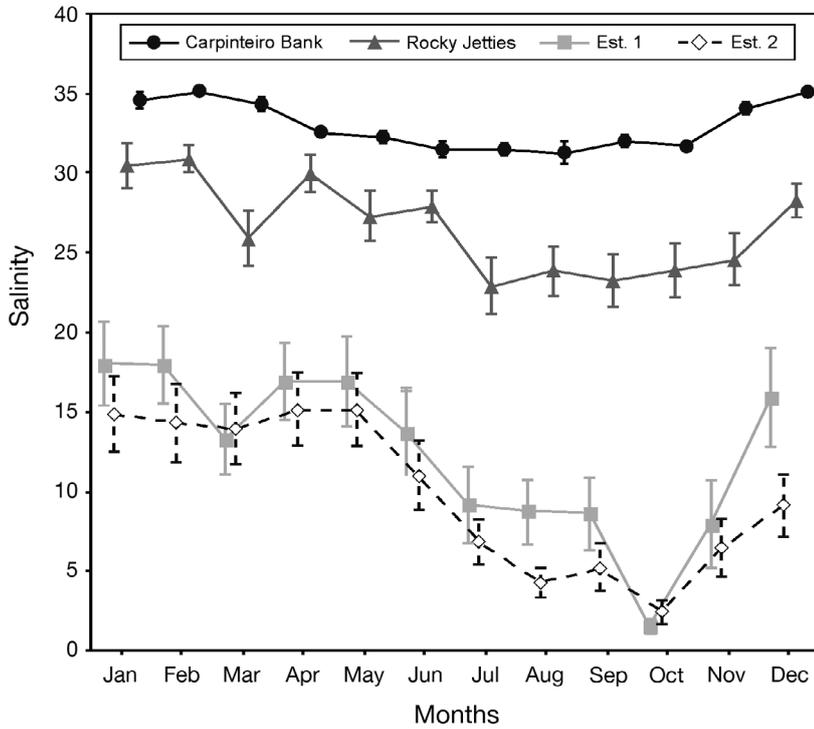


Fig. 4. Mean values ( $\pm$ SE) of salinity in Patos Lagoon estuary (Est. 1 and Est. 2) and at both marine sampling sites, the littoral (rocky jetties) and neritic zone (Carpinteiro Bank)

Climatic interannual variability is considerable in the study region. For instance, the strong El Niño Southern Oscillation (ENSO) event that occurred in 2002 (birth year of some of the individuals analyzed here), led to positive rainfall anomalies in this region that increased freshwater discharge into the lagoon, which, in turn, had significant effects on fish distribution and abundance (Garcia et al. 2003, 2004). However, no generalized substantial increases in Ba:Ca ratios in *E. marginatus* otoliths were observed, nor was it possible to link these to the increased freshwater runoff produced by this or other similar climatic events. Thus, the abrupt increase in the Ba:Ca ratio in some of the analyzed individuals (Fig. 2G–I) was not related to the ENSO phenomenon, but indicative of juvenile entrance and residency for periods of at least 2 yr in brackish estuarine waters.

Otolith edge Sr:Ca and Ba:Ca ratios from individuals collected in littoral and neritic habitats were compared to determine the discriminatory power of otolith chemical signatures. The influence of the estuarine plume of the Patos Lagoon causes average salinity at the littoral site (mean = 26.5) to be lower than that at the neritic site (mean = 32.9), as well as more variable year-round (SD = 7.0 and 2.1 in littoral and neritic zones, respectively) (Fig. 4). Although there is

a known positive correlation of Sr:Ca with salinity and Ba:Ca with freshwater (Elsdon & Gillanders 2005, Sturrock et al. 2012, Reis-Santos et al. 2013b), the mean concentrations of both element:Ca ratios in the otolith edge were not significantly different between individuals of the same year class caught in the neritic and littoral zones (Mann-Whitney  $U = 290$  and  $p > 0.05$  for Sr:Ca;  $U = 268$ ,  $p > 0.05$  for Ba:Ca). We hypothesize that the salinity difference, as well as the freshwater input, was not large enough in this case to produce distinct otolith chemical signatures between the 2 sampled habitats, hindering our ability to use otolith chemistry to disentangle movements between these 2 proximate coastal marine areas (as pointed out by Albuquerque et al. 2012 for Sr:Ca and salinity ranges of these areas).

In conclusion, the present study revealed for the first time that individuals of *E. marginatus* can remain for prolonged time periods in a brackish system, highlighting the life-cycle

plasticity of this endangered species. Future studies should actively investigate the occurrence of dusky groupers within estuarine systems, focusing on identifying key juvenile habitats for this species via combinations of other natural tags (e.g. stable isotopes of C and O) and/or telemetry, which may also aid in resolving movements between littoral and neritic habitats of adult fish. Finally, it would also be of great interest to ascertain the physiological trade-offs related to the observed differential nursery habitat use. Overall, rocky jetties and estuarine habitats within the Patos Lagoon may be considered as potential candidates for the implementation of conservation strategies to safeguard this endangered species.

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