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RELATING OTOLITH TO WATER [Sr/Ca] RATIOS: EXPERIMENTAL VALIDATION FOR SAND GOBY *POMATOSCHISTUS MINUTUS*

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Abstract

The chemical composition of fish otoliths reflects to a certain extent that of the ambient water at the time of deposition. This premise led to new applications in fisheries science such as fish stock discrimination, assessment of estuarine dependency or reconstruction of movement patterns into estuaries. The latter require prior knowledge of the variations in ambient elemental concentrations along the estuarine gradient and an accurate description of the relationship between aqueous and otolith [element/Ca] ratios. This study explored the applicability of otolith [Sr/Ca] to reconstruct sand goby *Pomatoschistus minutus* (Pallas 1770) migrations in the Scheldt estuary (Belgium – The Netherlands). Firstly, the concentration ratio of Sr/Ca over the entire salinity gradient was determined. Secondly, an experiment was conducted towards Sr incorporation in sand goby otoliths at varying ambient [Sr/Ca] levels. To this aim five experimental water mixtures of different salinity levels (3, 7, 12, 20 and 30) were prepared from seawater and water from the fresh water reaches of the estuary. The experiment was performed at two temperatures (13 and 18°C). [Sr] and [Ca] acted conservatively in the estuary resulting in a positive but nonlinear relationship between salinity and ambient [Sr/Ca], which was highly similar in the Scheldt and the experiment. Experimental results revealed positive linearity between aqueous and otolith [Sr/Ca] ($[\text{Sr/Ca}]_{\text{otolith}} = 0.31[\text{Sr/Ca}]_{\text{water}} + 0.44$). The otolith [Sr/Ca] was significantly different between each salinity level but there was no difference in otolith [Sr/Ca] between both temperature conditions. The partition coefficient (D_{Sr}) averaged around 0.38 and was slightly higher at the lowest salinity, which might indicate different incorporation mechanisms of Sr over the salinity range. Nevertheless, whether salinity per se has an effect on otolith [Sr/Ca] in sand goby or not, our results support the use of otolith [Sr/Ca] in terms of broad scale movements between marine and estuarine habitats in general, and between the North Sea and the Scheldt estuary in particular.

1. INTRODUCTION

Knowledge of how animals use habitats in space and time throughout their life history is crucial to understand their population dynamics, to determine habitat functions and to develop effective conservation efforts (Gillanders 2002). Conventional methodologies to track fish migration, such as tag recapture, telemetry and hydro-acoustics are often inappropriate on the spatiotemporal scales required to evaluate variability in seasonal and lifetime migrations (Secor *et al.* 2001; Elsdon & Gillanders 2003a). The last two decades, otolith elemental composition has increasingly been used to retrospectively describe life histories of fish and to identify the environments they have experienced. The use of trace elements in otoliths as tracers of water mass chemistry and thus habitat residency is based on the key assumption that fish incorporate elements from their environment, and that these elements are permanently deposited in their continuously growing otoliths. Hence, otolith chemistry should reflect the water chemistry experienced by fish throughout their lives (Thorrold *et al.* 2001). In particular, otolith strontium/calcium concentrations ([Sr/Ca]) have been widely applied to reconstruct the salinity history of individual fish and estuarine dependence of many species (e.g. Secor 1992, Secor & Rooker 2000, Limburg 2001, Secor *et al.* 2001, Tzeng *et al.* 2002, Rooker *et al.* 2004). These studies have been based on the assumption that otolith Sr, measured as [Sr/Ca], varies as a function of ambient salinity. Yet, there remains some disagreement on the validity of this approach, since inconsistent results were reported for the association between ambient salinity and otolith [Sr/Ca] (Secor *et al.* 1995, Secor & Rooker 2000, Elsdon & Gillanders 2003a, Rooker *et al.* 2004, Dorval *et al.* 2007). Apparently, aqueous [Sr/Ca] and not salinity is the major determinant of otolith [Sr/Ca] (Bath *et al.* 2000, Kraus & Secor 2004b, de Vries *et al.* 2005) and aqueous [Sr/Ca] in estuaries generally follows a curvilinear function with a minimum value in freshwater (Surge & Lohmann 2002). However, estuaries may contain divergent gradients in [Sr/Ca], with in some cases higher ambient [Sr/Ca] in freshwater than in marine waters (Kraus & Secor 2004b). Therefore, to interpret variation in otolith [Sr/Ca] of fish making use of estuaries, information is needed on the relationship between ambient and otolith [Sr/Ca] as well as the underlying nature of the [Sr/Ca] gradient in the surrounding water (Elsdon & Gillanders 2004, Kraus & Secor 2004b, Elsdon & Gillanders 2005a). Although critical to infer migrations over an estuarine gradient, few studies have fulfilled these requirements.

Otoliths are isolated from the external environment by successive barriers and compartments, namely gill or intestine epithelia, blood, saccular epithelium and endolymph in which they float. Consequently, trace elemental deposition does not directly reflect the elemental concentration in the water. The relationship between otolith chemistry and sea water composition is determined by the kinetics of ion transport from water to the precipitating surface and the complex chemistry of the endolymph responsible for otolith formation (Campana 1999, Elsdon

& Gillanders 2003a, Allemand *et al.* 2007). The degree of partitioning occurring between elemental concentrations in water and otoliths can be expressed by means of a partition (or distribution) coefficient (Morse & Bender 1990). For the rate of Sr incorporation into otoliths this is:

$$D_{\text{Sr}} = [\text{Sr/Ca}]_{\text{otolith}} / [\text{Sr/Ca}]_{\text{water}}$$

A distribution coefficient of 1 indicates that an element available in the ambient water is incorporated directly in the otolith without any discrimination, while a value of 0 means that the element is not incorporated at all into the otolith (Morse & Bender 1990, Campana 1999). It is a standardized measurement useful to compare the elemental discrimination during incorporation at different elemental concentrations in the water or to compare elemental discrimination between species and studies.

The purpose of this study was to examine the capacity of otolith [Sr/Ca] to measure spatio-temporal movement patterns into the Scheldt estuary of the estuarine opportunistic sand goby *Pomatoschistus minutus* (Pallas 1770) (Gobiidae, Teleostei) during its life history. The main objectives were firstly to investigate ambient [Sr/Ca] along the salinity gradient in the Scheldt estuary, secondly to experimentally establish the response of otolith [Sr/Ca] ratios to water chemistry in order to calibrate otolith [Sr/Ca] ratios across the range of salinities typically encountered by sand goby in the Scheldt estuary and thirdly to compare the Sr partition coefficient among treatments and with other published values.

Sand goby are small, bottom dwelling fish (max. length: 10 cm). It is one of the most common species along the Atlantic European coast and its estuaries (Bouchereau & Guelorget 1998), and forms an important ecological link between benthic invertebrates and larger predatory fish such as cod and whiting (Jaquet & Raffaelli 1989). The sand goby reproduces in coastal waters but shows a typical seasonal pattern of occurrence in the Scheldt and other European estuaries (Healey 1971, Maes *et al.* 2005b). However, like for many other estuarine opportunists, little is known about their individual migration patterns. The river Scheldt has a shallow, well mixed macrotidal estuary that covers a length of approximately 160 km from the mouth in the Netherlands to Ghent (Belgium) where sluices stop the tidal wave (Fig. 5.1). It is the only true estuary in the Dutch Delta area. Salt water intrudes to about 100 km inland, although this varies seasonally as the longitudinal salinity profile is partly determined by the magnitude of river discharge. The water residence time is about two to three months, depending on the river flow (Meire *et al.* 2005). The Scheldt estuary has a history of extensive anthropogenic pollution but the last decade the water quality gradually improved due to the installment of water treatment facilities. Nevertheless, the Scheldt still receives inputs from untreated domestic, industrial and agricultural activities (Van Damme *et al.* 2005).

2. MATERIALS AND METHODS

2.1. Experimental design

Juvenile sand goby *P. minutus* were collected with a beam trawl in shallow waters along the Belgian coast and immediately transported to the Laboratory of Aquaric Ecology and Evolutionary Biology (K.U. Leuven, Belgium) where the experiment was conducted. Fish were evenly distributed over four polyethylene tanks (250 l) filled with sea water (salinity: 30.5) and provided with aeration and a sand layer. Water temperature was initially set at 18°C in all tanks consistent with sea water temperature at that time, but the temperature in two tanks was gradually lowered to 13°C during the following three days, thus creating two temperature conditions for the experiment. These temperatures correspond to those in the Scheldt estuary when sand goby density is high (June and October). After a few days of acclimatization, the fish were marked with alizarin complexone $C_{19}H_{15}NO_8$ (50 ppm) to mark the start of the experiment in the otoliths. After 24 h of immersion in the alizarin solution fish, within each temperature condition, were randomly assigned to seven polystyrene aquaria (capacity 30 l) at densities of five fish per aquarium. During the translocation fish were anesthetized (MS-222) and subsequently marked with visible implant elastomers (VIE), measured to the nearest mm (standard length, SL) and weighed (fresh weight) to assess subsequent individual growth. Day of translocation was considered as day 0 of the experiment. All aquaria were initially filled with sea water, provided with a 2 cm sand layer and aeration, they were covered with a lid to prevent evaporation and escape. The light:dark regime was set at 12:12 h.

The experimental design consisted of manipulating salinity in six aquaria within each temperature condition. The fish in these aquaria experienced five salinity levels (30, 20, 12, 7, 3), starting at salinity 30 and each level lasting for 14 days. The decline in salinity was created by gradually diluting sea water with water originating from the fresh water zone of the Scheldt estuary (salinity = 0.2). Changes in salinity never exceeded 5 day^{-1} . These levels represent the range of salinities experienced by sand goby in the estuary. One aquarium in each temperature treatment served as a control and remained at salinity 30 (Table 5.1). In order to maintain water quality, 40% of the water volume in each aquarium was changed every other day, at which time the accumulated detritus was siphoned away. All water used in the experiment was filtered mechanically, aerated and treated with UV light for at least one week before usage. Sea water was trucked in weekly from the Institute of Agricultural and Fisheries Research (ILVO) in Ostend (Belgium), where it was stored in an aerated 4000 l indoor tank.

Fish were fed ad libitum twice a day with a mixture of mussels (*Mytilus edulis*) and formulated pellets based on fishmeal (producer: N.V. Joosen-Luyckx, Art 10120). Aquarium temperature and salinity were monitored every two to four days, pH was monitored occasionally (Table 5.1). Within each temperature condition water samples from three randomly chosen aquaria

and the control aquarium were collected on a weekly basis for assessment of Sr and Ca concentration. These samples were immediately filtered through a 0.45 µm polycarbonate filter and acidified with analytical HNO₃ to pH < 2 before storage in acid washed, high-density polyethylene vials at 4°C in the dark. At the end of the experiment, fish were euthanized by severing the central nervous system, measured (SL) and finally stored at -20°C.

Table 5.1. Average (\pm SD) *P. minutus* standard length (SL) and biomass per aquarium at the start of the experiment. Average (\pm SD) temperature, salinity and pH for each treatment during the experiment: 13°C and 18°C with varying salinities or at constant salinity of 30 (cont). The numbers between brackets correspond to the number of aquaria in each treatment.

Treatment	Mean SL (mm)	Biomass (g)	T (°C)	pH	Salinity level				
					30	20	12	7	3
13°C	43 \pm 3.3	5.9 \pm 0.4	13.1 \pm	8.4 \pm	30.2 \pm	19.9 \pm	12.2 \pm	7.2 \pm	3.2 \pm
(6)			0.4	0.04	0.1	0.3	0.04	0.1	0.1
13°C cont	44.7 \pm	6.1	13.4 \pm	8.4 \pm	30.2 \pm				
(1)	2.9		0.6	0.1	0.4				
18°C	44 \pm 4.1	6.3 \pm 0.5	18.4 \pm	8.5 \pm	30.5 \pm	20.0 \pm	12.1 \pm	7.2 \pm	3.2 \pm
(6)			0.6	0.1	0.1	0.2	0.1	0.2	0.1
18°C cont	44.0 \pm	5.7	18.2 \pm	8.3 \pm	30.5 \pm				
(1)	4.0		0.6	0.1	0.3				

2.2. Water samples along the estuarine gradient

In order to establish [Sr], [Ca] and [Sr/Ca] gradients in the Scheldt estuary water samples were collected at 12 stations over the entire salinity range in February, May, August and November 2003 (Fig. 5.1). These samples were taken near the bottom using the RV "Luctor" (CEME-NIOO, Yerseke, The Netherlands) during two-day cruises. Water samples were immediately treated and stored on board in exactly the same manner as the experimental water samples above.

2.3. Water analysis

Sr and Ca concentrations in Scheldt and experimental water were determined by inductively coupled plasma mass spectrometry (ICP-MS) at the Geology Department of the K.U. Leuven (Leuven, Belgium). For analysis, 200 µl of water sample was combined with 100 µl of high purity 6M HCl, 200 µl of a 1.2 µg/ml In solution and 10 ml of milli-Q water to a total of 10.5 ml. This amounted to a dilution factor of 52.5 for the water samples, which is sufficient to eliminate matrix effects due to the original salinity differences. The concentrations of Ca and Sr in the measurement solutions were several orders of magnitude above the lower limits of detection with ICP-MS. Measurement standards were prepared from analytical grade CaCO₃ and SrCl₂·6H₂O, dissolved in diluted HCl. Within-run repeatability of the measurements was checked by repeated analysis of a number of samples; the average repeatability was 2-3 % (1 SD).

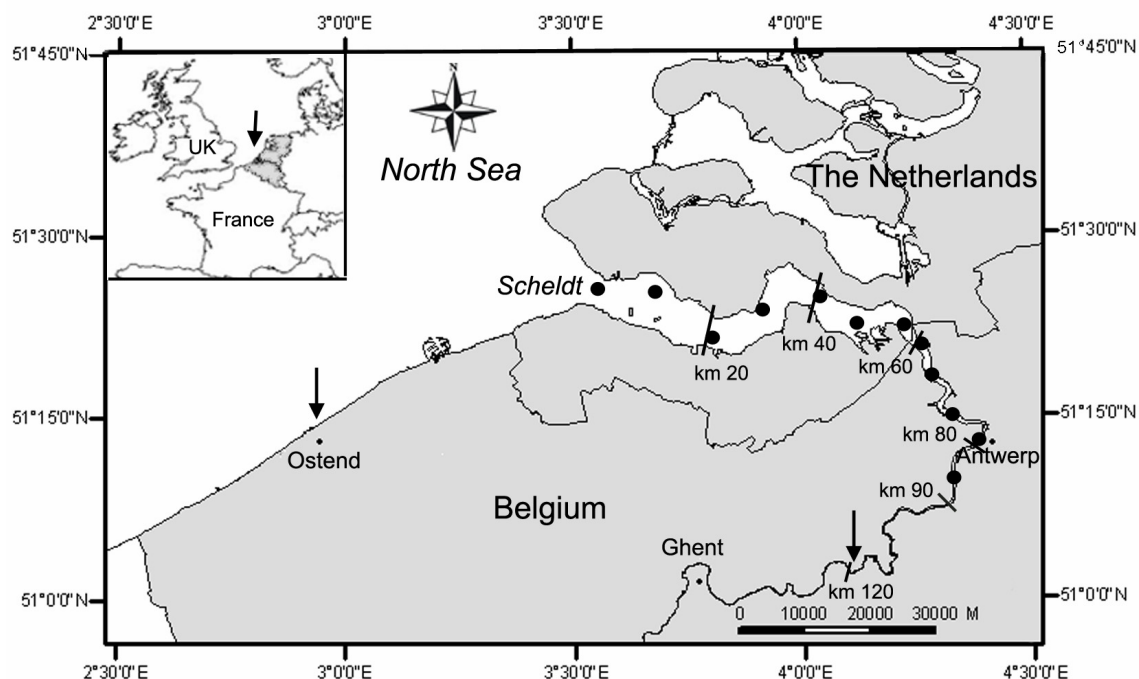


Fig. 5.1. Map of the Scheldt estuary, which discharges in the North Sea and is situated in the Dutch Delta. Dots indicate the sampling locations of the chemical water analysis. The two arrows indicate the sampling locations of the two aqueous end members in the experiment.

2.4. Otolith Analysis and D_{Sr}

Due to high mortality in the experiment eight fish from the six aquaria in the 13°C temperature treatment and five fish from five aquaria in the 18°C temperature treatment were analyzed. From each control aquarium two fish were analyzed. Sagittae were extracted, cleaned from adhering tissue and stored dry in acid rinsed eppendorfs. Right otoliths, sulcus side down, were embedded in epoxy resin (Araldite 2020) on a glass slide, then ground in the sagittal plane with progressively finer sandpapers (1200, 2000 and 4000 grit) until the pararostral (postero-dorsal side) (Fig. 5.2) was completely free of resin; otoliths were finally polished with a diamond suspension (1 μm). Automatic grinding and polishing machines (Struers Tegrapol 35 with a Tegraforce 5 head) were used in order to obtain a high quality surface state, as required for electron probe micro-analysis. Sections were ultrasonically cleaned with milli-Q water (resistivity 18.2 $\Omega\text{M.cm}$) at the end of each grinding and polishing stage. They were stored in a desiccating cabinet and carbon coated under vacuum just before analysis.

Sr and Ca concentrations were determined using a wavelength dispersive electron microprobe (WD-EM, Cameca SX50) (Ifremer, Department of Marine Geosciences, Plouzané, France) with the following beam conditions: 12 kV accelerating voltage, 12 nA beam current, 3 μm spot size, peak acquisition times of 120 s for Sr and 40 s for Ca. Strontium sulfate (SrSO_4) and calcite (CaCO_3) were used as standards for Sr and Ca, respectively. The limits of detection were 367 – 455 ppm for Ca and 244 – 275 ppm for Sr. Because Ca is substituted by Sr in

otoliths due to a similar ionic radius and because it is assumed that Sr and Ca respond similarly to changes in analytical performance (Secor & Rooker, 2000), otolith [Sr] is generally expressed relative to [Ca].

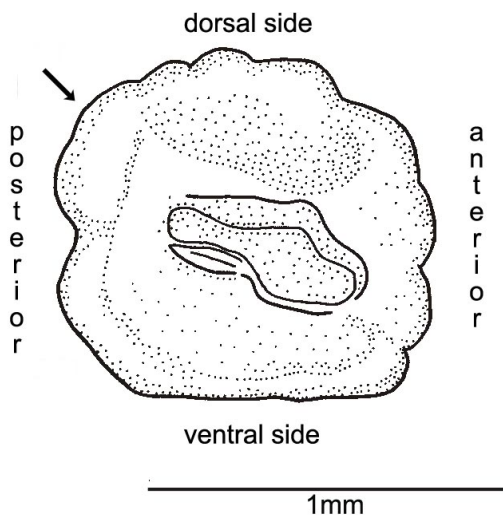
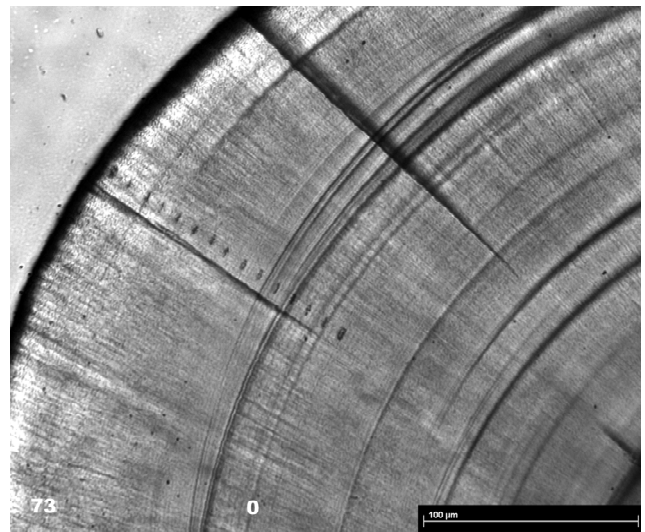
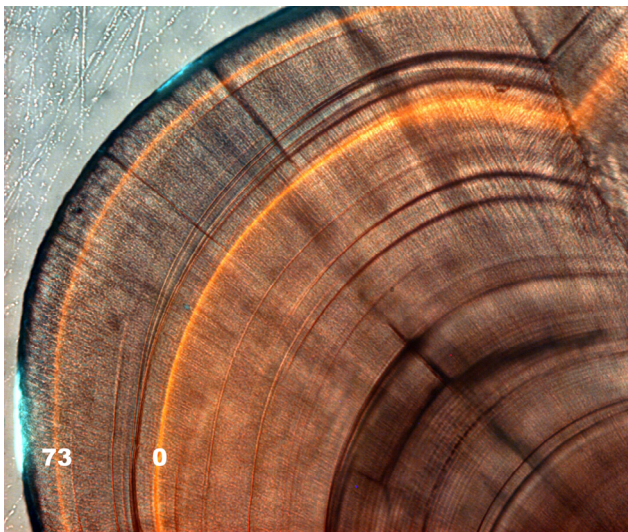


Fig. 5.2. Left: proximal side of the left sagitta of a 50 mm (standard length) sand goby. The arrow indicates the approximate position of the measurement axis on the pararostral (postero-dorsal side). Under left: sagittal cross-section of sand goby otolith under UV light to make alizarin marks visible (red). This fish was marked twice: at the beginning of the experiment (day 0) and after the experiment (day 73). The fish was killed 22 days after the second marking event. Under right: the same otolith under transmitted light and after electron microprobe analysis. The burn marks from the electron probe (\pm every 10 μ m) and several growth increments can be seen.



From the alizarin mark to the edge, linear scans were performed on the pararostral (Fig. 5.2) approximately perpendicular to the growth increments at 10 μ m intervals. For five point measurements an additional measurement was performed on each side of the measurement path. To relate measurements to salinity levels, space to time calibration was performed by daily increment counting and measuring. Arellano (1995) validated that micro-increments in sand goby otoliths were deposited daily. When increments were not clearly discernible the number of days was estimated from the average increment width in adjacent otolith zones. Only those measurements that could unambiguously be related to a salinity level were taken into account. Finally aquarium averages were calculated for each salinity level.

2.5. Data analysis

Differences in mean $[\text{Sr}/\text{Ca}]_{\text{water}}$, $[\text{Sr}/\text{Ca}]_{\text{otolith}}$ and D_{Sr} among treatments (temperature and salinity) were tested by repeated measures ANOVA as consecutive measurements were done on the same aquaria or individuals respectively. A post hoc Tukey HSD test was used to identify the significant pair-wise comparisons. The relationship between water and otolith $[\text{Sr}/\text{Ca}]$ ratios was described using a least squares linear regression. All statistical analyses were done with STATISTICA 7.0, StatSoft Inc.). To assess the predictive power of otolith $[\text{Sr}/\text{Ca}]$ on water $[\text{Sr}/\text{Ca}]$, the method of Prairie (1996) was followed to determine the categorical resolution of otolith Sr/Ca on salinity. This method essentially defines the predictive power of a regression model as the number of separate classes into which the dependent variable can be divided.

3. RESULTS

3.1. Water $[\text{Sr}/\text{Ca}]$

Over the entire salinity range (0.6 - 30.9) in the Scheldt estuary $[\text{Sr}]$ varied from 0.3 to 6.6 ppm and $[\text{Ca}]$ from 73.4 to 363.5 ppm. Although the longitudinal salinity gradient changed seasonally, with lower values during winter (Fig. 5.3), there was a strong linear relationship between salinity and $[\text{Sr}]$ (Fig. 5.4), demonstrating the conservative character of Sr in the Scheldt estuary. $[\text{Ca}]$ appears to deviate slightly from a linear relationship with salinity. $[\text{Sr}/\text{Ca}]$ showed a curvilinear relationship with salinity in the Scheldt estuary, ranging from 1.9 to 8.6 mmol mol⁻¹.

Similar relationships with salinity were found in the experiment, but the experiment had higher values for $[\text{Sr}]$ and $[\text{Ca}]$. Nevertheless, the results for $[\text{Sr}/\text{Ca}]$ of the experimental water coincide well with those of the estuarine conditions (Fig. 5.4). Ambient $[\text{Sr}/\text{Ca}]$ was found to be significantly different for each salinity level in the experiment while temperature had no effect (Table 5.2). Water chemistry in the control aquaria was not significantly different between temperature conditions or with water of experimental tanks at salinity 30.

Table 5.2 Result of the repeated measures ANOVA testing the effect of temperature and salinity of water $[\text{Sr}/\text{Ca}]$.

Source	df	MS	F	p
Temperature	1	0.020	0.59	0.524
Residual	2	0.033		
Salinity	4	12.641	370.26	< 0.001
Sal × Temp	4	0.005	0.16	0.953
Residual	8	0.034		

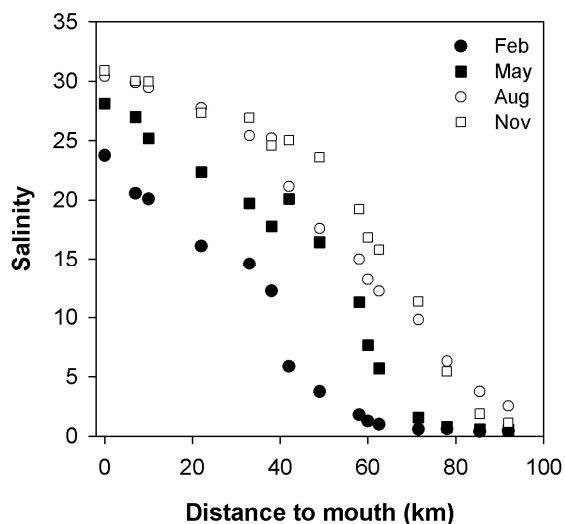


Fig. 5.3 Longitudinal salinity gradient in the Scheldt estuary in February, May, August and November 2003.

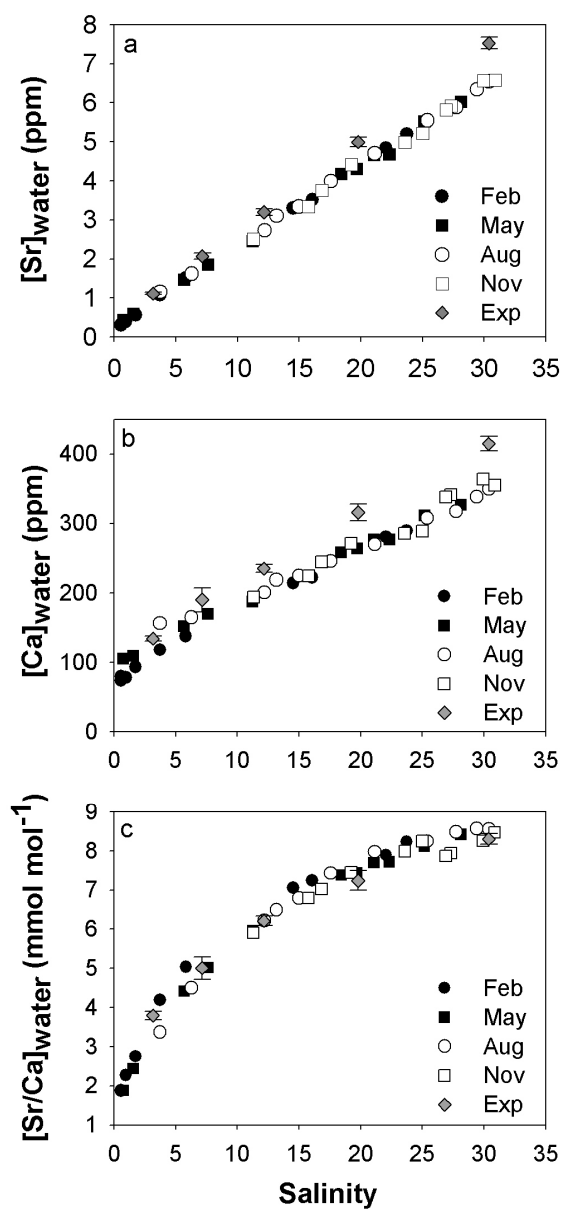


Fig. 5.4 Aqueous a) Sr concentration, b) Ca concentration and c) Sr/Ca molar ratio of the Scheldt estuary at 4 sampling occasions and of the experiment. Error bars for experimental values are standard deviations. Scheldt: $[Sr] = 0.21(\text{salinity}) + 0.26$; $[Ca] = 8.56(\text{salinity}) + 93.36$; experiment: $[Sr] = 0.23(\text{salinity}) + 0.37$; $[Ca] = 10.25(\text{salinity}) + 108.88$

3.2. Otolith [Sr/Ca] and D_{Sr}

Otolith [Sr/Ca] values in *P. minutus* varied between 1 and 3.5 mmol mol⁻¹ (Fig. 5.5). Significant differences for [Sr/Ca]_{otolith} were found among all pair-wise comparisons of salinity levels, but there was no temperature effect or interaction between salinity and temperature (Table 5.3, Fig. 5.5b). Otolith [Sr/Ca] in the control aquaria at 13°C and 18°C averaged 3.2 ± 0.4 mmol mol⁻¹ and 3.4 ± 0.4 mmol mol⁻¹ respectively and were not significantly different from each other or from [Sr/Ca]_{otolith} for salinity 30 in the experimental tanks. A positive linear relationship was found between otolith [Sr/Ca] and water [Sr/Ca] ratio ([Sr/Ca]_{otolith} = 0.31 (± 0.03) [Sr/Ca]_{water} + 0.44 (± 0.20), $p < 0.001$, $r^2 = 0.67$) (Fig. 5.5a). The predictive resolution (Prairie 1996) of water [Sr/Ca] based on otolith [Sr/Ca] ratios was estimated to be 2.04 categories, suggesting that 2 different environments can be predicted based on this relationship. A positive but non linear relationship emerged when otolith [Sr/Ca] was plotted against salinity (Fig. 5.5b).

Estimates of the partition coefficient D_{Sr} ranged from 0.28 to 0.52 and averaged around 0.38 (Fig. 5.6). The highest values were observed at the lowest salinity level and there was a statistically significant effect of salinity on D_{Sr} . However, pair-wise comparison identified that D_{Sr} was only significantly different between salinity level 3 and 12 ($p < 0.05$). There was no effect of temperature and also the interaction term was not significant (Table 5.3).

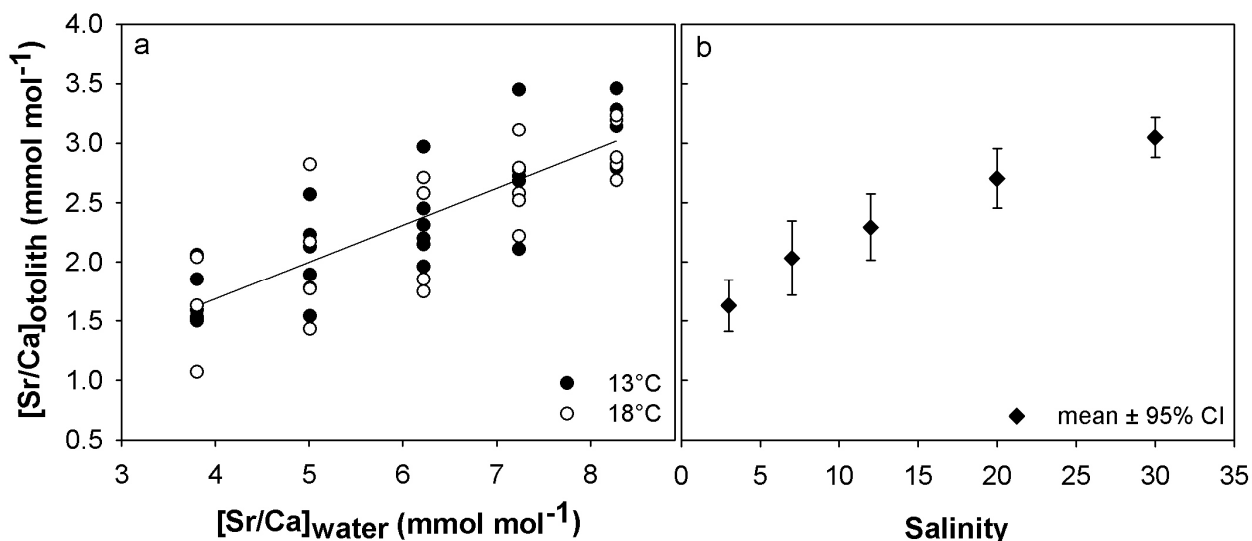


Fig. 5.5 *P. minutus*. a) Otolith [Sr/Ca] versus the average ambient [Sr/Ca] concentration. Each dot is the average value per aquarium of electron microprobe measurements taken at a given salinity level ($n = 1-8$). Except for two aquaria of which two fish were analysed, these averages come from measurements performed on one otolith (1 fish per aquarium analysed). The line represents the least squares regression over all aquaria irrespective of temperature treatment: $[Sr/Ca]_{otolith} = 0.31 [Sr/Ca]_{water} + 0.44$, $p < 0.0001$, $r^2 = 0.67$. b) Average otolith [Sr/Ca] ($\pm 95\%CI$) over all aquaria (average of dots on the left) against experimental salinity.

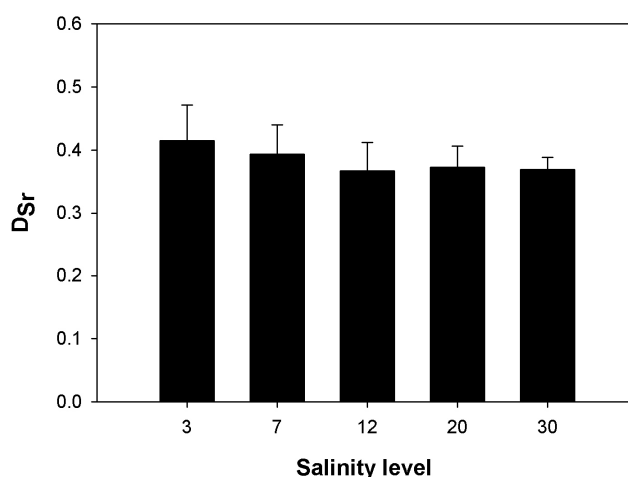


Fig. 5.6 Partition coefficient D_{Sr} (mean \pm 95% CI) for each salinity level in the experiment. Data of both temperature treatments were joined as temperature had no effect on D_{Sr}

Source	Df	MS	Sr/Ca _{ot} F	P	MS	D _{Sr} F	p
Temperature	1	0.235	0.560	0.480	0.006	0.465	0.517
Residual	7	0.422			0.013		
Salinity	4	2.757	60.670	< 0.001	0.004	3.525	0.018
Sal \times Temp	4	0.022	0.484	0.747	0.002	1.260	0.309
Residual	28	0.045			0.001		

Table 5.3 Result of the repeated measures ANOVA testing the effect of temperature and salinity on otolith [Sr/Ca] and partition coefficient D_{Sr} .

4. DISCUSSION

The changes in the ambient concentrations of Sr, Ca and Sr/Ca observed in the present study are consistent with gradients in estuaries (Ingram & Sloan 1992, Surge & Lohmann 2002, Kraus & Secor 2004b). Deviations from conservative behaviour for [Ca] might be relative small given the large Ca background concentration. Zwolsman & van Eck (1999) explained the distribution of particulate Ca within the estuary by physical mixing but they added that carbonate precipitation or dissociation within the estuary can not be ruled out. Because ambient [Ca] affects elemental uptake, the [element/Ca] ratio is a more reliable indicator of environmental availability of an element to a fish than the absolute concentration of a dissolved element itself (Campana 1999). As the ambient concentration ratios of Sr/Ca in the experiment strongly conformed with the observed change of ambient [Sr/Ca] in the Scheldt estuary (Fig. 5.4), the slight differences in ambient [Sr] and [Ca] between the experiment and the Scheldt estuary probably have no implications for our conclusions.

The concentration ratios of Sr/Ca in the experimental water were significantly different between each salinity level (Fig. 5.4c). The effect of salinity per se can not be disentangled from aqueous [Sr/Ca] in our experiment as they were strongly correlated. However, this is not

necessary for the reconstruction of migration patterns in the Scheldt because of the same relationship between ambient $[Sr/Ca]$ and salinity in the estuary (Fig. 5.4c).

The concentration of $[Sr/Ca]_{\text{otolith}}$ in *P. minutus* increased linearly with increasing $[Sr/Ca]$ of the ambient water. The positive linear relationship between $[Sr/Ca]$ in ambient water and otoliths is one of the most consistent results reported concerning the effect of an environmental variable on otolith chemistry. An overview of the reported linear equations of $[Sr/Ca]_{\text{otolith}}$ on $[Sr/Ca]_{\text{water}}$ (Table 5.4) shows that this relationship holds for a wide range of $[Sr/Ca]_{\text{water}}$ values in both laboratory and field trials. In contrast, Dorval *et al.* (2007) reported an uncoupling between water and otolith $[Sr/Ca]$ for *Cynoscion nebulosus*. This anomaly could be due to the fact that they analyzed whole otoliths from wild caught fish, thereby yielding time integrated elemental signatures over the whole life history. Additionally, all their specimens originated from polyhaline waters spanning a relatively narrow $[Sr/Ca]_{\text{water}}$ range ($\pm 7.5 - 9.5 \text{ mmol mol}^{-1}$), which was probably too small to overwrite variation in $[Sr/Ca]_{\text{otolith}}$ caused by other factors.

Table 5.4 Overview of reported linear relationships of $[Sr/Ca]_{\text{otolith}}$ on $[Sr/Ca]_{\text{water}}$ (both in mmol mol^{-1}) for various species. Temperature (*T*, °C), salinity (*sal*, psu) and approximate range of $[Sr/Ca]_{\text{water}}$ are specified for each laboratory or environmental study.

Species	Temperature and salinity	$[Sr/Ca]_{\text{water}}$ (mmol mol^{-1})	Equation: $y = [Sr/Ca]_{\text{otolith}}$; $x = [Sr/Ca]_{\text{water}}$	References
<i>Leiostomus xanthurus</i>	T: 20°C Sal: 20	13 – 22 ^s	$y = 0.165 (\pm 0.052) x + 0.260 (\pm 0.897)$ ^a	Bath <i>et al.</i> 2000
	T: 25°C Sal: 20	13 – 22 ^s	$y = 0.162 (\pm 0.054) x + 0.70 (\pm 0.954)$ ^a	
<i>Lates calcarifer</i>	T: 28-30°C freshwater	0.2 – 0.8 ^s	$y = 0.16 (\pm 0.03) x + 0.14 (\pm 0.11)$ ^b	Milton & Chenery 2001
<i>Acanthopagrus butcheri</i>	T: 20°C Sal: ± 33	10 – 130 ^s	$y = 0.0757 x + 0.1299$	Elsdon & Gillanders 2003b
<i>Oncorhynchus clarki</i>	Field: freshwater	0.5 – 3.5	$y = 0.55 x (\pm 0.02) - 0.18 (\pm 0.03)$ ^b	Wells <i>et al.</i> 2003
<i>Acanthopagrus butcheri</i>	T: $\pm 21.5^\circ\text{C}$ Sal: 5	10 – 150 ^s	$y = 0.4986 x + 1.1131$ (low)* $y = 0.4749 x + 0.4807$ (med)* $y = 0.4504 x + 0.3071$ (high)*	de Vries <i>et al.</i> 2005
<i>Acanthopagrus butcheri</i>	T: $\pm 21.5^\circ\text{C}$ Sal: 32	8 – 30 ^s	$y = 0.2028 x + 0.9740$ (low)* $y = 0.2457 x + 0.6489$ (med)* $y = 0.2600 x + 0.3731$ (high)*	
<i>Acanthopagrus butcheri</i>	Field (summer) : T: 17 - 26°C Sal: 2 - 33	6 – 24	$y = 0.154 x + 2.587$	Elsdon & Gillanders 2005a
	Field (winter): T: 14 - 18°C Sal: 0 - 37	0 – 10	$y = 0.147 x + 2.419$	
	T: 17, 20, 26°C Sal: 5, 32	7 – 33 ^s	$y = 0.157 x + 1.482$	
<i>Pomatoschistus minutus</i>	T: 13, 18°C; Sal: 3, 7, 12, 20, 30	3.8 – 8.3	$y = 0.31 (\pm 0.03) x + 0.44 (\pm 0.20)$ ^b	This study

^a uncertainty of the estimated parameters is given as 95% confidence interval

^b uncertainty of the estimated parameters is given as the standard error

^s the water was artificially spiked with strontium

* trials performed at low, medium and high ambient Ba concentrations

The slope of the equation observed for *P. minutus* is one of the highest reported (Table 5.4). Consequently, otolith [Sr/Ca] in this species increases relatively fast for the same rise in aqueous [Sr/Ca] compared to most other species evidencing less discrimination against Sr, which is also shown by the relatively high D_{Sr} values. The non zero intercept ($p = 0.029$) suggests that sources other than water contribute to Sr incorporation into otoliths. However, the influence of Sr from other sources such as diet is considered to be minor compared to sources from the ambient water (Milton & Chenery 2001, Walther & Thorrold 2006, Lin *et al.* 2007).

Due to the variation in [Sr/Ca] values of *P. minutus*, the predictive power of the equation found in the present study only allows to distinguish between two different chemical environments. This is consistent with previous studies stating that otolith [Sr/Ca] is useful for describing estuarine fish movements over larger habitat transitions between freshwater, brackish water and salt water (Secor & Rooker 2000, Rooker *et al.* 2004, Zimmerman 2005). In order to use otolith [Sr/Ca] in sand goby as a proxy for changes in water masses in the Scheldt estuary and hence to reconstruct the environmental life histories over an estuarine gradient it is of practical value to assign a threshold value separating marine from estuarine environments. As *P. minutus* does not occur in freshwater it is not necessary to establish a threshold value for the freshwater environment. Otolith [Sr/Ca] ratios higher than 2.5 mmol mol⁻¹ point to residency in polyhaline or marine waters, with values above 3.0 mmol mol⁻¹ reflecting almost exclusively marine waters (Fig. 5.5b). Otolith [Sr/Ca] ratios below 2.5 mmol mol⁻¹ rather indicate a residency in brackish waters and values smaller than 2.0 mmol mol⁻¹ strongly suggest a residency in waters having a salinity below 12. A substantial change in salinity is indeed required to produce a significant change in mean [Sr/Ca] in sand goby otoliths. This was also reported by Rooker *et al.* (2004) for *Pogonias cromis*. Our threshold values are highly consistent with characteristic values for marine and brackish waters observed in marine and estuarine fishes (Secor & Rooker 2000, Secor *et al.* 2001) and the threshold value (3 mmol mol⁻¹) employed by Limburg *et al.* (2001a) and Limburg (2001) to identify transitions of *Alosa* spp. from freshwater to marine water.

Besides the composition of the surrounding water other environmental and biological factors (e.g. growth, kinetics) affect otolith Sr/Ca (de Pontual *et al.* 2003, Elsdon & Gillanders 2003a), causing some scatter in the relationship between water and otolith [Sr/Ca]. It is unclear what caused the variability in our data. Water chemistry between aquaria was highly similar. Physiological and growth rate differences between individuals could have contributed to the observed variation. Sadovy & Severin (1994) reported that otolith [Sr/Ca] was inversely related to the growth rate of fish, suggesting that less Sr may be incorporated into the otolith during periods of higher otolith protein synthesis and higher accretion rates. Although the mean daily increment width over the duration of the experiment varied among individuals with

a significant difference between temperature treatments (mean \pm SD: 13°C: $1.3 \pm 0.3 \mu\text{m}$ and 18°C: $2.1 \pm 0.4 \mu\text{m}$), there was neither a correlation with otolith [Sr/Ca] (results not shown) nor was there a difference in otolith [Sr/Ca] between temperature conditions. So, even though some individuals in the experiment showed restricted growth in comparison to growth rates of the pararostral reported in wild caught sand goby ($1.25 - 3.5 \mu\text{m d}^{-1}$) (Arellano 1995), variations in otolith growth rate did not appear to contribute to the observed variability in otolith [Sr/Ca]. Ontogenetic factors are unlikely to have affected the variation in otolith [Sr/Ca] as all the fish were approximately the same length and age.

Part of the variation in $[\text{Sr/Ca}]_{\text{otolith}}$ may be explained by varying sensitivity to stress factors induced by capture, handling and aquarium maintenance. Irregularities (such as converging increments and discontinuities) observed in otolith microstructure indicated that fish in the experiment endured some stress and the high mortality evidenced that experimental conditions were not optimal. Stress is known to affect physiological processes which can lead to a change in endolymph composition, lower calcification rates and eventually to higher [Sr/Ca] ratios in otoliths (Kalish 1991, Campana 1999, Payan *et al.* 2004). It remains unclear how much of the variation in otolith [Sr/Ca] is caused by laboratory artifacts and extrapolation of laboratory results to the field situation should always be undertaken cautiously. However, it is reassuring that the same relationship between water and otolith [Sr/Ca] was found for laboratory reared and field collected specimens of *Acanthopagrus butcheri* (Elsdon & Gillanders 2005a). The same relationship was also found between salinity and otolith [Sr/Ca] in *Pogonias cromis* in laboratory and field trials (Rooker *et al.* 2004).

There was no difference between the two temperature treatments in our results which is consistent with other studies that reported no temperature effect on otolith [Sr/Ca] or D_{Sr} (e.g. Kawakami *et al.* 1998, Dorval *et al.* 2007). In contrast, more studies reported either negative or positive effects with variable magnitude of the temperature dependence (for a review see Secor & Rooker (2000), and Elsdon & Gillanders (2003a)). Apparently, negative effects were often reported for fish kept at lower temperatures, while positive effects were mostly observed for fish kept at higher temperatures (Campana 1999, Elsdon & Gillanders 2003a, Rooker *et al.* 2004). Based on these observations Elsdon & Gillanders (2003a) concluded that the temperature effect on otolith [Sr/Ca] may not be linear and is probably species specific. The absence of a temperature effect in this study could thus also have resulted from experimental conditions. If the experiment had been conducted over a wider range of temperatures, a temperature effect might have been revealed. However, this was beyond the scope of the present study as we aimed at testing the effects of different salinities at two temperatures, experienced by sand gobies during estuarine ingress and residency.

The partition coefficient D_{Sr} for *P. minutus* was significantly different from one, evidencing that Sr discrimination occurred between the surrounding water and the otoliths. So far it is unknown which mechanisms contribute most to this discrimination; the incorporation of elements from ambient water to the otolith matrix is a complicated multi-stage physiological process. Both the precipitation rate and the many cell boundaries Sr and Ca have to pass before being incorporated in the otolith can have an affect on otolith [Sr/Ca] (Campana 1999, Elsdon & Gillanders 2003a).

Although D_{Sr} values for *P. minutus* are within the range of the values reported (0.13 - 0.6) (for a limited overview see Martin & Wuenschel (2006) and Dorval *et al.* (2007), but also Kalish (1991), Campana (1999), Wells *et al.* (2003), de Vries *et al.* (2005), Zimmerman (2005) and Lin *et al.* (2007)), the average value (0.38) is relatively high, especially compared with other marine species. The different partition coefficients among species suggest that species vary in their mechanisms that control Sr precipitation on the otolith matrix. However, part of the reported variation in D_{Sr} is likely caused by other divergent factors in these studies such as differences in ambient conditions, stress factors, growth and ontogenetic stages.

Higher values for the partition coefficient D_{Sr} at relatively lower salinity levels (D_{Sr}) were also reported for *Acanthopagrus butcheri* (Elsdon & Gillanders 2003b, de Vries *et al.* 2005, Elsdon & Gillanders 2005a) and five salmonid species (Zimmerman 2005), though this is in contrast with Lin *et al.* (2007) who observed that D_{Sr} of *Anguilla japonica* increased with salinity. These results suggest that the degree of Sr discrimination between ambient water and the otolith surface depends on the elemental concentrations in the water. Uptake of Sr may be related to ambient [Ca]. Divalent elements such as Sr and Ca are taken up by chloride cells and Ca competitively inhibits Sr uptake, resulting in an inverse relationship between Sr uptake by fish and ambient [Ca] (de Vries *et al.* 2005). However, this effect could also be induced by ambient salinity as the pathways of elemental uptake differ between freshwater and marine environments. Depending on whether euryhaline fishes are above or below isosmotic salinity they use different tissues (e.g. intestinal wall, chloride cells in gills) to achieve osmoregulation, resulting in a combination of passive and active transport mechanisms. Consequently, the pathways and physiological barriers between the surrounding water and the crystallizing otolith differ over the entire salinity range, which may complicate the relationship between water and otolith [Sr/Ca] (Campana 1999, Wells *et al.* 2003, de Vries *et al.* 2005, Martin & Wuenschel 2006).

5. CONCLUSIONS

This work is part of a rapidly growing number of empirical studies that describe the effects of several factors such as temperature, salinity, diet and water chemical composition on otolith elemental chemistry (e.g. Elsdon & Gillanders 2003b, Elsdon & Gillanders 2005b, Zimmerman 2005, Martin & Wuenschel 2006, Dorval *et al.* 2007, Lin *et al.* 2007). The divergent results reported in these and other studies suggest that otolith elemental incorporation and the effects of influencing variables on elemental incorporation is not yet completely understood and is most likely species specific. This highlights the need to improve our understanding of the underlying processes and to conduct species specific validation experiments in order to interpret otolith elemental signatures (Secor & Rooker 2000, Elsdon & Gillanders 2003a, Martin & Wuenschel 2006).

The present study confirmed that [Sr] and [Ca] behave conservatively in the Scheldt estuary causing a positive relationship between $[\text{Sr}/\text{Ca}]_{\text{water}}$ and salinity. Secondly, a positive linearity between [Sr/Ca] in sand goby otoliths and the surrounding water was established. The predictive resolution of this regression only allowed to distinguish between two different environments (marine vs. brackish water). We could not detect any effect of temperature (13°C vs. 18°C) or growth on otolith [Sr/Ca]. The higher partition coefficient for salinity level of three suggested less discrimination of Sr at low salinities, which was also reported in other studies. Our results thus support the utility of otolith [Sr/Ca] for reconstructing salinity histories of the sand goby making large scale movements between coastal and estuarine habitats. Yet, the results also highlight the need for further research concerning the influence of growth, osmoregulation and other physiological factors on otolith microchemistry.

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