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ESTUARINE MIGRATION OF SAND GOBY

***POMATOSCHISTUS MINUTUS* EXPLORED BY MEANS OF OTOLITH [Sr/Ca]**

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Abstract

Ratios of strontium to calcium laid down as a lifetime record in otoliths are regularly used to reconstruct salinity histories of fishes. In this study, the chronologies of otolith [Sr/Ca] were qualitatively examined to chart movements of sand gobies *Pomatoschistus minutus* (Pallas 1770) in the Scheldt estuary. Variable patterns of estuarine habitat use were detected, suggesting that the migratory behaviour is probably much more diversified than assumed previously. The individuals displayed variable periods of residency in brackish water areas, with different timing of immigration. Additionally, repeated migrations between the lower and the upper estuary were detected. Consequently, it was concluded that sand gobies display a large flexibility in life histories regarding habitat choice. Furthermore, low [Sr/Ca] values near the nucleus implied that the Scheldt estuary also acts as a breeding ground for sand goby. Estuarine spawning has been detected in other estuaries but has not been observed yet in the Scheldt estuary. Finally, an elevation in otolith [Sr/Ca] occurring at a body size of approximately 15 mm is probably related to physiological stress during metamorphosis from a pelagic to a demersal life style.

1. INTRODUCTION

Migrations of marine fishes occur over a range of scales and are predominantly caused by spawning, feeding or predator avoidance cues. For a given population the scale and the timing of the migrations are generally consistent and predictable suggesting an evolutionary advantage (Campana *et al.* 2007). Unfortunately, for many species detailed knowledge of as well their movement patterns as the functionality of habitat transitions on individual, population or species level are missing. A clear view on the migratory behavior of marine fishes would contribute to a better understanding of their population dynamics and the functions provided by the specific habitats, hereby creating a basis for an efficient management (Gillanders 2002b). The absence of a sound comprehension of migration patterns for many marine animals is partly due to the technical limitations associated with tracing movements in large water bodies, particularly for (post)larval and small juvenile fish susceptible to dispersive processes and high mortality rates (Äkesson 2002, Campana *et al.* 2007).

Recently, the microanalysis of otolith chemistry has created new possibilities in fisheries research such as the reconstruction of fish movements (Arai *et al.* 2003), assessment of population connectivity (de Pontual *et al.* 2000, Thorrold *et al.* 2001, Gillanders 2002a) and the delineation of fish stocks (Edmonds *et al.* 1999, Geffen *et al.* 2003). Otoliths are considered as the fish's black box which under continuous growth permanently record the physicochemical characteristics of the surrounding environment. The physical location of analytical measurements on the otolith can be used to link environmental information to life history characteristics (Campana & Thorrold 2001). Analysis of otolith strontium, in particular, has received considerable attention for studying habitat transitions over salinity gradients (Secor *et al.* 1995, Elfman *et al.* 2000, Secor & Rooker 2000, Limburg 2001, Fablet *et al.* 2007). The technique is based on the premise that otolith strontium/calcium concentration ratios ([Sr/Ca]) mainly reflect those of the surrounding water (Campana 1999, Bath *et al.* 2000). Ambient [Sr/Ca] is positively correlated with salinity in most estuaries as a result of the conservative nature of these elements (Surge & Lohmann 2002, Kraus & Secor 2004b). Consequently, otolith [Sr/Ca] measurements along daily or seasonal increments can thus be used to reconstruct the salinity history of individual fish.

This study was initiated to obtain a better understanding of the habitat use of sand goby *Pomatoschistus minutus* (Pallas 1770) (Gobiidae, Teleostei) in the Scheldt estuary. Sand goby are small bottom dwelling fish. It is one of the most common species in the coastal areas of the eastern North Atlantic, including the Mediterranean, the Baltic, the North Sea and adjacent estuaries (Miller 1986, Bouchereau & Guelorget 1998). They form an important ecological link between benthic invertebrates and larger predatory fish such as cod and whiting (Jaquet & Raffaelli 1989, Maes *et al.* 2003, Salgado *et al.* 2004). Sand goby reproduce in the North Sea

during spring (March - June). Larvae are about 3 mm at hatching and they are pelagic for 4 to 6 weeks after which they adopt a demersal life style. Most adults die in their second summer after their first spawning (Fonds & Veldhuis 1973, Hamerlynck 1990, Pampoulie *et al.* 2004). Like many other marine species *P. minutus* exhibits a typical pattern of occurrence in the low salinity zone of North Sea estuaries. The new cohort recruits into the brackish water zone of the Scheldt at the onset of summer and a maximal density is generally reached in fall followed by low abundance during winter and spring (Healey 1971, Maes *et al.* 2005). This predictable pattern of occurrence suggests a functional significance of the estuary for this species. However, recent findings based on a carbon isotopic clock in muscle tissue demonstrated that sand gobies exhibit variable individual immigration patterns (Chapter 4). In addition, they appear to remain in the brackish water zone for a relatively brief period ($\pm 70\%$ less than one month), which questions the importance of the estuary for this species.

This study aims to explore variability in migratory behaviour by reconstructing salinity histories of sand goby caught in the Scheldt estuary by means of otolith [Sr/Ca]. The intention was to obtain a rather qualitative perspective of individual estuarine habitat use. Additionally, [Sr/Ca] measured near the otolith nucleus was used to infer the possible existence of an estuarine spawning population. The seasonal pattern of abundance parallels marine species suggesting that sand gobies spawn outside the estuary, presumably near the mouth region or on coastal habitats (Maes *et al.* 2004). However, sand goby was reported to spawn in some European estuaries (Costa 1988, Elliott & Hemmingway 2002) but spawning sites have actually never been detected in the Scheldt estuary (A. Cattrijsse pers. comm.).

2. MATERIALS AND METHODS

2.1. Study area and fish sampling

The river Scheldt has a shallow, well mixed macrotidal estuary which is approximately 160 km long from the mouth in The Netherlands to Ghent in Belgium, where sluices stop the tidal wave (Fig. 6.1). It is the last true remaining estuary in the Dutch Delta area. Salt water intrudes to about 100 km inland, resulting in relatively stable salinity zones with a brackish zone area between km 40 and 90. The water residence time varies between two to three months, depending on river discharge. Turbidity is high, especially in the upper estuary where suspended matter can reach concentrations up to 200 mg l^{-1} (Meire *et al.* 2005). There is positive but linear relationship between ambient [Sr/Ca] and salinity in the Scheldt estuary (Chapter 5).

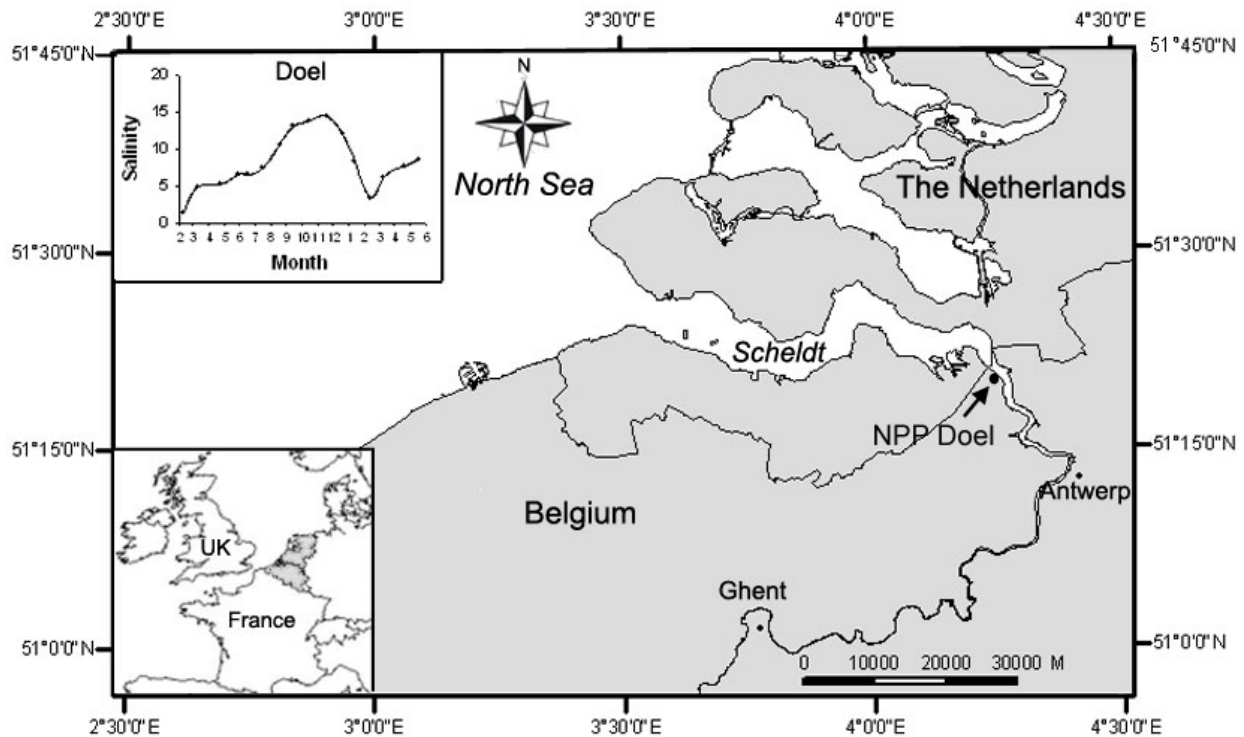


Fig. 6.1 Map of the Scheldt estuary which discharges into the Southern Bight of the North Sea. The sampling location (NPP Doel) at 61 km from the mouth is indicated with an arrow. The inset in the left upper corner shows the salinity at the sampling location between February 2003 and May 2004.

Sand gobies were obtained from the cooling water intake screens of the nuclear power plant (NPP) Doel which is located in the mesohaline zone of the Scheldt estuary at 61 km from the mouth (Fig. 6.1). Here salinity averaged 9.7 ± 3.7 (mean \pm SD) over the year. Sampling occurred when their abundance in the estuary was high (autumn 2003) and during the spawning season when abundance is low (March 2004). Fish samples were flash-frozen on dry ice for transport to the laboratory, where they were preserved at -20°C for further processing. *P. minutus* was identified according to Hamerlynck (1990).

2.2. Otolith Analysis

Sagittae were extracted under a laminar flow, cleaned from adhering tissue and stored dry in acid rinsed eppendorfs. Right otoliths were embedded sulcus side down 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 mid plane and finally polished with a diamond suspension ($1\ \mu\text{m}$). 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. Eventually 12 otoliths (Table 6.1) were prepared satisfactorily for [Sr/Ca] measurement. They were stored in a desiccating cabinet and carbon coated under vacuum just before analysis.

Table 6.1 Overview of the 12 sand gobies used for this study. *Id* is the code given to the fish during analysis. *SL*: standard length at capture, *W*: fresh weight at capture

Sample	Salinity	id	SL (mm)	W (g)	sex
October 2003	12.6	PM 50	54	2.0	f
October 2003	12.6	PM 51	52	1.6	f
October 2003	12.6	PM 52	47	1.2	m
October 2003	12.6	PM 59	44	1.3	f
November 2003	13.0	PM 37	48	1.3	f
November 2003	13.0	PM 101	43	1.0	f
March 2004	6.1	PM 26	55	2.2	f
March 2004	6.1	PM 39	48	1.2	m
March 2004	6.1	PM 40	49	1.5	f
March 2004	6.1	PM 45	60	3.3	f
March 2004	6.1	PM 47	61	3.6	f
March 2004	6.1	PM 49	46	1.5	m

Otolith Sr and Ca concentrations were determined approximately every 22 μm on a fixed axis on the pararostral (postero-dorsal side) (Fig. 6.2) from the nucleus to the edge and approximately perpendicular to the growth increments, providing a chronology of [Sr/Ca] over the entire life span of the fish. Based on the increment widths along the pararostral growth axis reported by Arellano *et al.* (1995) this corresponds to a measurement every 6 to 15 days.

Measurements 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. Given an average daily increment of $\pm 2 \mu\text{m}$ (Arellano 1995), the beam diameter integrates a signal over 2 to 3 days. Strontium sulfate (SrSO_4) and calcite (CaCO_3) were used as standards for Sr and Ca, respectively. The limits of detection were 378– 455 ppm for Ca and 245-275 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].

Residency in a marine or brackish environment can be inferred from otolith [Sr/Ca] concentrations: otolith [Sr/Ca] values higher than 3 mmol mol^{-1} indicate a marine (≥ 30 psu) or possibly a polyhaline (18 - 30 psu) environment. Values below 2 mmol mol^{-1} indicate mesohaline (5 - 18 psu) or oligohaline (0.5 - 5 psu) waters. Otolith [Sr/Ca] values between 2 and 3 mmol mol^{-1} could result from any salinity environment, yet values below 2.5 mmol mol^{-1} rather suggest brackish waters (meso- and oligohaline), while values higher than 2.5 mmol mol^{-1} suggest polyhaline or marine waters (Chapter 5).

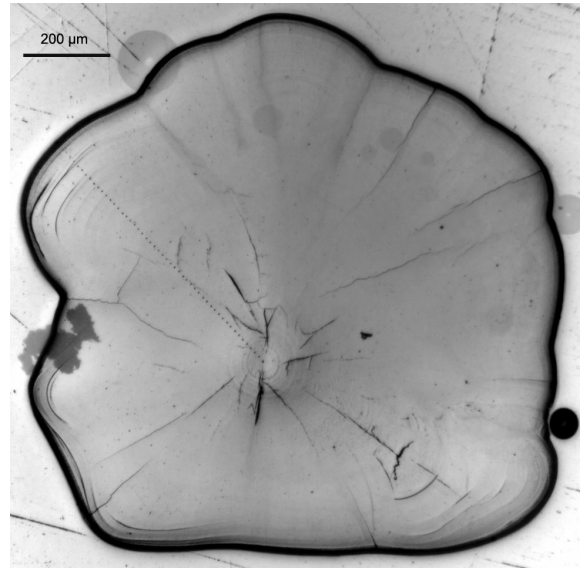
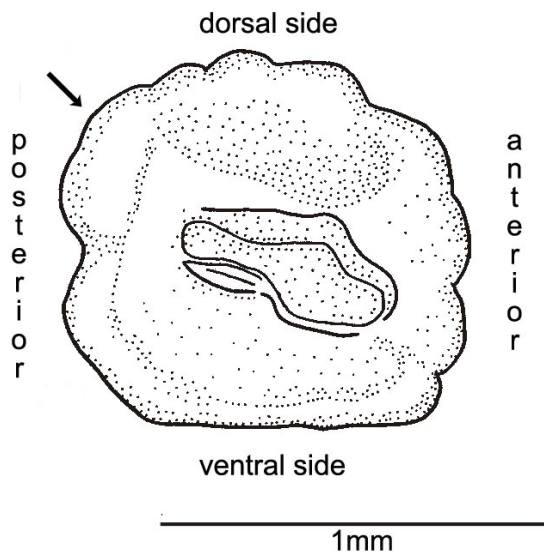


Fig. 6.2 Left: proximal side of left sagitta of a 50 mm SL sand goby. Arrow indicates the approximate position of the measurement axis on the pararostral (postero dorsal side). Right: Mid sagittal plane of analysed otolith under reflected light showing the line transect on the pararostral lobe.

2.3. Back-calculation

As daily increments could not be resolved over the whole measurement axis due to difficulties in identifying microstructures, the standard length for each measurement point was back calculated from distances to the nucleus. Many back calculation procedures have been discussed in the literature without reaching any agreement (Francis 1990, Campana & Jones 1992, Pierce *et al.* 1996, Smedstad & Holm 1996, Zivkov 1996, Klumb *et al.* 2001, Panfili & Tomas 2001, Schirripa 2002). Folkvord & Mosegaard (2002) concluded that there is no specific procedure that can be recommended as no single procedure can be regarded as better than all others in back-calculating fish sizes. Because daily growth on the pararostral growth axis is always proportional to somatic growth for sand goby (Arellano 1995), we applied the widely used Frazer Lee model, which is a linear, direct proportional back-calculation method that back-calculates the length for individual fish as:

$$L_i = c + (L_c - c)(S_i/S_c)$$

with L_i denoting the back-calculated standard length at measurement point i , L_c the standard length at capture, S_i the otolith radius to measurement i on the pararostral, S_c the total otolith radius on the pararostral, and c the a correction factor which is determined as the intercept of the least square linear regression of standard length on pararostral axis length ($c = 2.15$, $n = 26$, $R^2 = 0.79$). The Fraser Lee formula gives highly similar results as the linear formula for the body proportional hypothesis (Whitney and Carlander's model) recommended by Francis (1990) because both formulae are based on the same body length on otolith axis regression (Folkvord & Mosegaard 2002).

3. RESULTS

Otolith [Sr/Ca] chronologies are plotted against back calculated standard length for six fish sampled in October and November 2003 (Fig. 6.3) and six fish sampled in March 2004 (Fig. 6.4). The individual [Sr/Ca] patterns show considerable variation within and between sampling months suggesting high variability in estuarine and coastal habitat use within the sand goby population. This is also obvious from Fig. 6.5, which summarizes for each individual the percentage of measurements above or below specific threshold values indicative of water masses along the estuarine gradient. Individuals PM50, PM51 and PM52 resided most of their life in brackish waters, yet PM50 and PM51 moved further upstream into low salinity zones (Fig. 6.3), while PM52 appeared to remain in polyhaline or marine waters for a longer period. PM59 and PM101 resided most of their life in polyhaline or marine waters, and entered the brackish water area a relatively short period before capture. PM37 probably lived continuously in an area influenced by poly- and mesohaline waters (lower estuary) before moving further upstream just before it was caught. Different life histories along the salinity gradient were also observed for sand goby collected in March 2004 (Fig. 6.4). Otolith [Sr/Ca] values of individuals PM39, PM40 and PM49 mostly fluctuated between 2 and 3 mmol mol⁻¹ suggesting several migrations within the estuary from higher to lower salinity areas. Especially for PM 40 there seems to be a marked shift from the marine-polyhaline to the mesohaline environment at the size of about 20 mm SL. [Sr/Ca] chronologies of PM26, PM45 and PM47 suggest that they mostly preferred the brackish water area (mesohaline-oligohaline reaches).

The results indicate that most fish hatched in waters of higher salinity (>2.5 mmol mol⁻¹). This could be in the North Sea or in polyhaline reaches of the lower estuary. At least two fish appeared to have hatched in the estuary as indicated by their lower [Sr/Ca] value in the nucleus: PM59 of October 2003 and PM26 of March 2004. Nevertheless, they mostly resided in various saline environments (Fig. 6.5). Because all individuals were caught in the brackish water zone, the last measurement near the edge was expected to yield a [Sr/Ca] value smaller than 2.5 mmol mol⁻¹. This was, however, not the case for four fish: PM59 and PM101 had values slightly higher than 2.5 mmol mol⁻¹ and PM26 and PM39 showed a strong increase in otolith [Sr/Ca] near the edge up to 3.5 mmol mol⁻¹.

Several fish showed an increase in otolith [Sr/Ca] between 10 and 20 mm standard length. This temporary increase could be small (e.g. PM37, PM 45, PM52) or could be relatively large (e.g. PM40, PM59 and PM101) involving [Sr/Ca] values higher than 4 mmol mol⁻¹. These high values suggest that elevation in [Sr/Ca] results from endogenous factors rather than from emigration to fully marine waters.

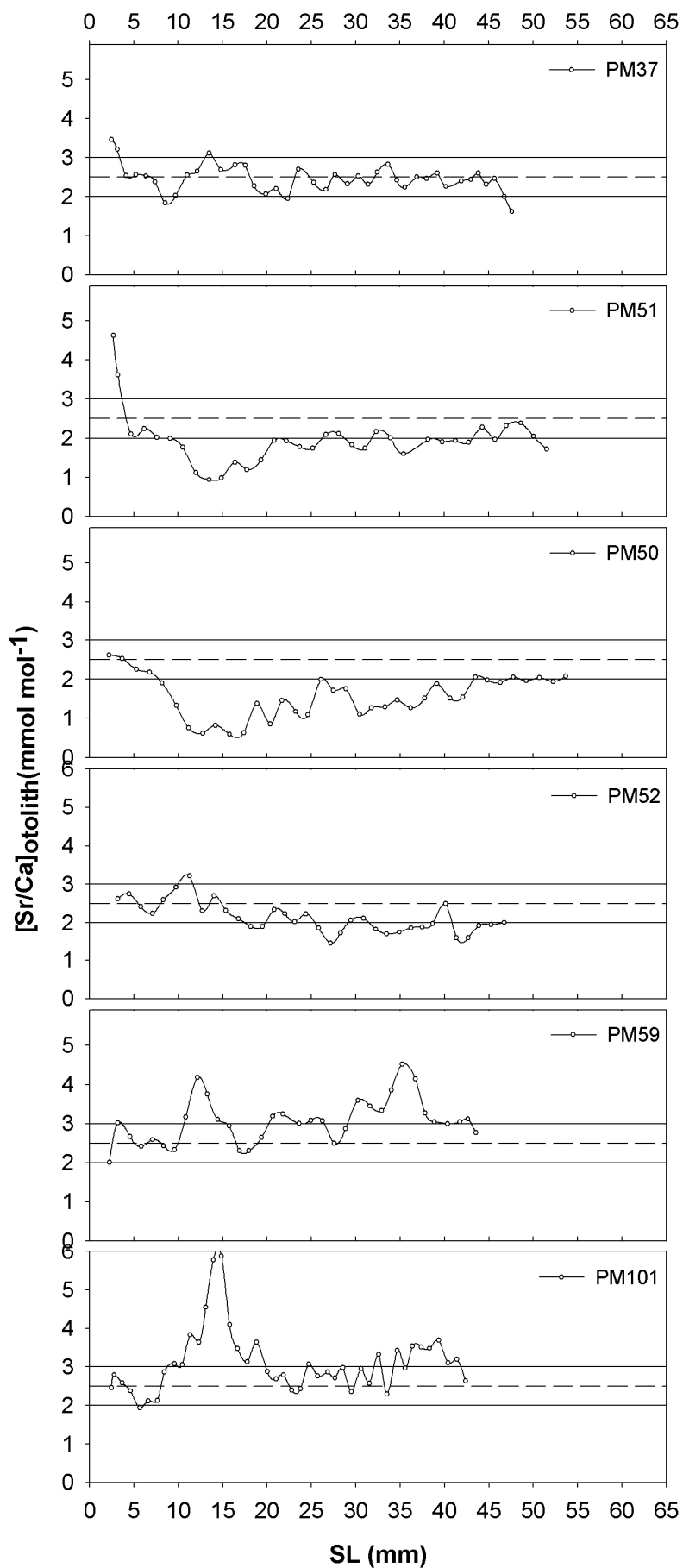


Fig. 6.3 *P. minutus* $[Sr/Ca]$ chronologies of six fish caught in October and November 2003 in the brackish water zone of the Scheldt estuary plotted against standard length.

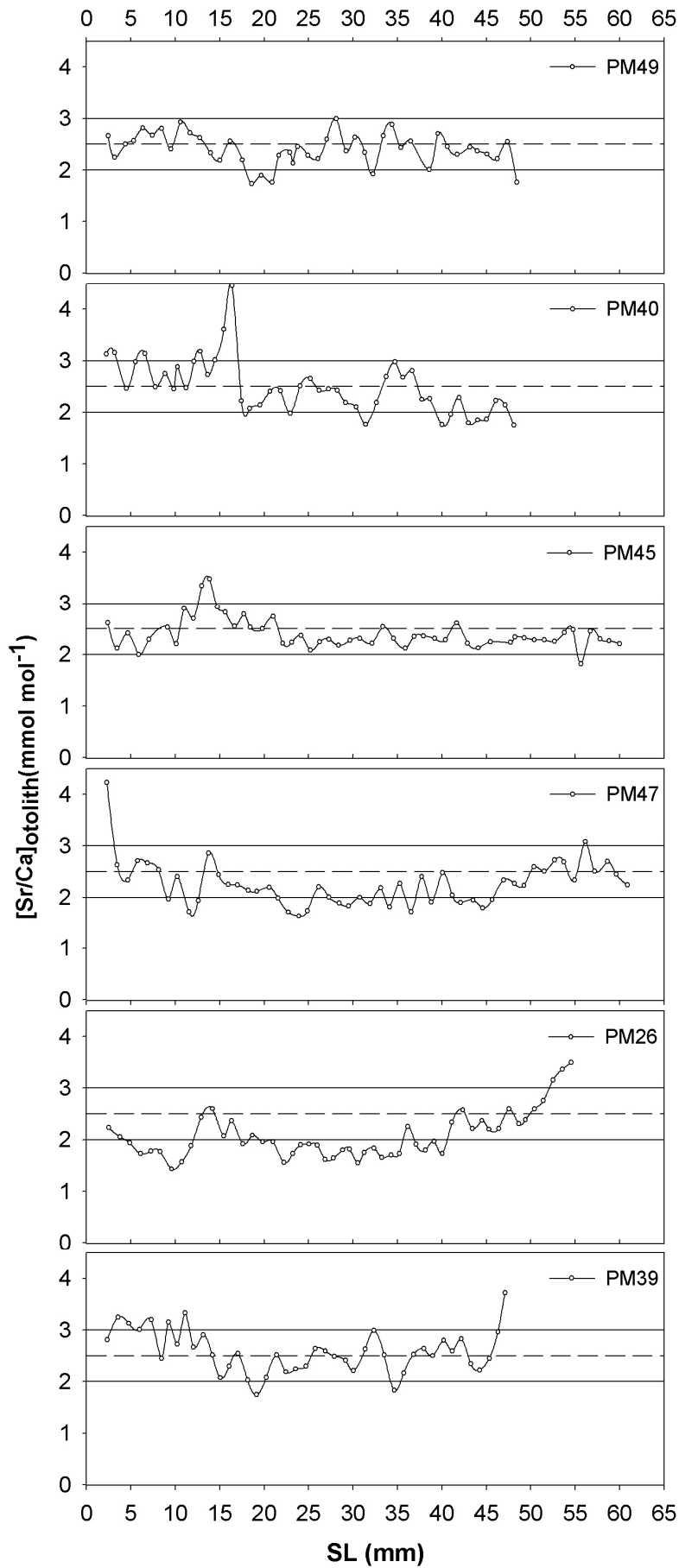


Fig. 6.4 *P. minutus*. $[Sr/Ca]$ chronologies of six fish caught in March 2004 in the brackish water zone of the Scheldt estuary plotted against standard length.

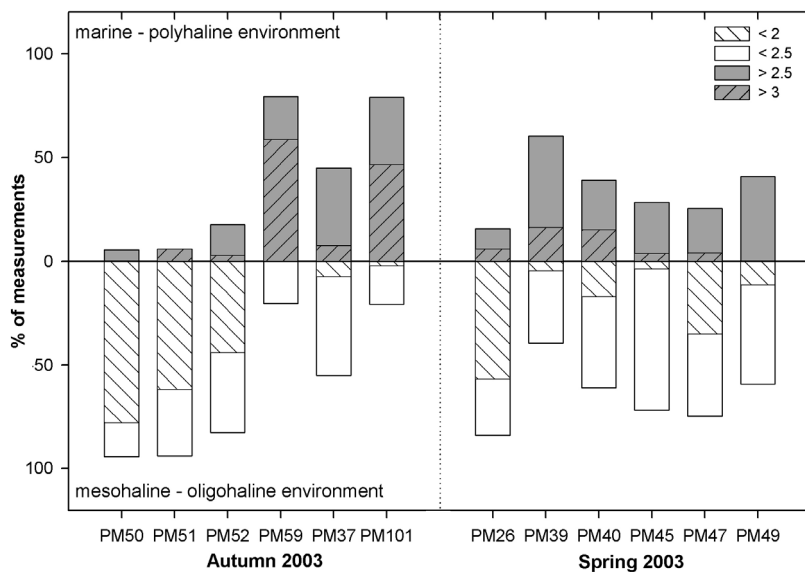


Fig. 6.5 *P. minutus*. For each specimen the percentage of measurement points classified to the marine and the brackish environment. As measurement point correspond to body size, this classification indicates in which environment most growth occurred; this can be regarded a proxy for the habitat in which fish mostly resided. Oligohaline-mesohaline waters: $< 2.5 \text{ mmol mol}^{-1}$, polyhaline - marine waters: $> 2.5 \text{ mmol mol}^{-1}$. Otolith $[\text{Sr}/\text{Ca}]$ values higher than 3 mmol mol^{-1} are typical for sea water, while values lower than 2 mmol mol^{-1} are typical for the brackish water zone. Threshold values are based on results of Chapter 5.

4. DISCUSSION

Strontium, substituting for calcium in the aragonite matrix of otoliths, is used in ichthyology to reveal salinity histories of fishes. The method has proven especially useful in tracing broad scale movements of e.g. diadromous species (Elfman *et al.* 2000, Secor & Rooker 2000), but it cannot be applied for tracing fine scale movement along a salinity gradient (Chapter 5). We applied the technique to study estuarine migrations of *Pomatoschistus minutus*, which is considered to be a marine estuarine opportunist (Thiel *et al.* 2003). Sand goby are known to spawn at sea, yet the species exhibits a typical density pattern in the low salinity area of estuaries of the eastern North Atlantic (Fonds 1973, Maes *et al.* 2005). $[\text{Sr}/\text{Ca}]$ measures were considered as proxies for habitat type (marine-polyhaline vs. mesohaline-oligohaline) for sand goby in the Scheldt estuary and the North Sea.

The diversity in individual $[\text{Sr}/\text{Ca}]$ patterns evidenced diverse uses of the Scheldt estuary. Even though only 12 fish caught in two seasons were analyzed, distinct habitat use patterns could be distinguished within each sample (Fig. 6.3, 6.4, 6.5), including residency in a specific estuarine habitat and variable migration patterns across salinities. Different estuarine habitat use patterns by sand goby corroborate earlier findings based on an isotopic clock that revealed a large variability in arrival dates in the mesohaline zone of the Scheldt as well as a large variability in body sizes at the moment of arrival (Chapter 4). The present study further supports rather short excursions into the brackish water area as was suggested in Chapter 4. Moreover, the otolith $[\text{Sr}/\text{Ca}]$ patterns implied repeated movements between polyhaline and brackish waters.

Plasticity in estuarine habitat use was recently detected for many other fish species such as American shad *Alosa sapidissima* (Limburg 1998), blueback herring *Alosa aestivalis* (Limburg

et al. 2001a), brown trout *Salmo trutta* (Limburg *et al.* 2001b), striped bass *Morone saxatilis* (Zlokovitz *et al.* 2003, Secor & Piccoli, 2007) white perch *Morone Americana* (Kraus & Secor 2004a) and three eel species (*Anguilla anguilla*, *A. japonica* and *A. rostrata*) (Tzeng *et al.* 2002, Daverat *et al.* 2006, Fablet *et al.* 2007). Contingents (groups with different migratory behaviour) seem to be present within the respective populations (Secor 1999). Fablet *et al.* (2007) even identified 37 patterns of habitat use for *A. anguilla*. Although further research is needed to elaborate and quantify the variability in estuarine habitat use for sand goby, mediated by a contingent structure or not, the high degree of flexibility in habitat use patterns within fish populations is a fact. This coexistence of different habitat use chronologies makes populations less dependent on specific habitats at certain life stages. This gives the populations the advantage of being less vulnerable to stochastic events and anthropogenic disturbances (Secor 2002).

Until now it was assumed that all sand goby in brackish waters of the Scheldt estuary migrated to these areas after hatching at sea. This assumption was based on numerous observations of sand gobies spawning in coastal areas but not in an estuarine environment (Claridge *et al.* 1985). Moreover, they seem to disappear completely from North Sea estuaries during the spawning season (Healey 1971, Fonds 1973, Claridge *et al.* 1985, Hostens *et al.* 1996, Maes *et al.* 2005). Nevertheless, the good survival of eggs in intermediate salinities suggests that sand gobies should be able to breed in estuaries (Healey 1971, Fonds & van Buurt 1974). Therefore it was stated that spawning is most likely restricted to marine areas where suitable large shells (lamellibranchs) can be found for nesting sites (Fonds 1973, Pampoulie *et al.* 1999). The present results however, suggest that natal habitats for sand goby are present in the Scheldt estuary because two out of twelve fish (PM26 and PM59) showed relatively low [Sr/Ca] values ($< 2.5 \text{ mmol mol}^{-1}$) near their nucleus. This raises the possibility of a local spawning population in the Scheldt estuary. As there are hardly large lamellibranchs living in the brackish water of the Scheldt, sand gobies might build their nest under stones. Other sand goby in this study could also have hatched in the polyhaline waters of the Scheldt estuary but it is impossible to discriminate between marine and polyhaline waters based on otolith [Sr/Ca] (Chapter 5). The presence of *Pomatoschistus* eggs and (post)larvae in hyperbenthic sledge samples from the polyhaline zone also suggested that they might spawn there (Hostens *et al.* 1996, Beyst *et al.* 1999, Hostens 2003). Finally, sand goby was reported to spawn in some estuaries although it was never specified where (Costa 1988, Elliott & Hemmingway 2002).

The last [Sr/Ca] value of PM59 and PM101 is relatively high ($>2.5 \text{ mmol mol}^{-1}$) compared to the other fish of October 2003; it does not immediately suggest a brackish water environment. This could be explained by the fact that the measurement was taken close to the otolith edge but not exactly on the edge, hence missing the last days of the fish's life. Consequently, when the fish entered the mesohaline zone just prior to capture (e.g. the last two days), the otolith

increments that recorded the brackish environment might not have been sampled by the electron probe. On the other hand, the observed variation in otolith [Sr/Ca] for sand gobies in identical ambient conditions (Chapter 5) doesn't fully exclude that these higher values do in fact come from waters with a salinity of 13 as recorded at the moment of capture. The strong increase in [Sr/Ca] observed near the otolith edge of PM26 and PM39 can probably not be explained by the reasons given above. A possible explanation could be that these elevations are induced by physiological changes correlated with gonadal development or other reproductive investments at the onset of the spawning season (Kalish 1989, 1991). It is known that besides environmental parameters and water chemistry the physiological condition of the fish can influence otolith [Sr/Ca] ratios by regulating Sr binding capacity or discrimination during uptake and precipitation onto the otoliths surface (Kalish 1989, 1991, Kawakami *et al.* 1998, Campana 1999). We suspect that the observed [Sr/Ca] elevations between 10 and 20 mm SL result from physiological changes concomitant with the shift from a pelagic habitat to a demersal habitat. This ontogenetic shift in sand goby occurs between 10 to 20 mm TL (Fonds 1973). The fact that the height of this [Sr/Ca] elevation differs among individuals or is even absent from others most likely reflects the variation by which individuals experience this developmental change. Individuals may experience life history transitions differently depending on their current physiological state, environmental conditions and/or genotype. Metamorphosis from leptocephalus to glass eel also caused variable [Sr/Ca] peaks in otoliths of *Anguilla spp* (Tzeng *et al.* 1997, Tzeng *et al.* 2002). Kawakami *et al.* (1998) reported different [Sr/Ca] elevations and number of checks induced by stress when elvers of *A. japonica* encountered fresh water. An increase in [Sr/Ca] in sole (*Solea solea*) otoliths during critical stages of first feeding and metamorphosis was also reported by de Pontual *et al.* (2003), who attributed variability in [Sr/Ca] chronologies partly to different brood stocks. As such otolith chemical composition may be affected by ontogenetic shifts in habitat or physiology, and may not be linked simply to changes in salinity or ambient [Sr/Ca]. This may severely confound interpretation of [Sr/Ca] chronologies in terms of salinity histories (de Pontual *et al.* 2003, Rooker *et al.* 2004): the elevations between 10 and 20 mm observed for sand goby probably do not represent downstream migrations.

Growth rate can also have an effect on otolith [Sr/Ca] (Sadovy & Severin 1994). This is important as sand goby are characterized by a seasonal growth difference (Doornbos & Twisk 1987, Arellano 1995). However, the results in Chapter 5 showed that variations in otolith growth rate did not appear to influence otolith [Sr/Ca] in sand goby. Even if there is an effect, it is generally relatively weak compared to the relationship between ambient and otolith [Sr/Ca] (Kraus & Secor 2004b, Elsdon & Gillanders 2005).

We related [Sr/Ca] measurements to standard lengths (SL) by means of the Frazer Lee back calculation method. It was impossible to determine fish age based on otolith increments, due to the obscurity of the counting path, visual artifacts and possibly subdaily increments.

Relating [Sr/Ca] to age would have yielded more reliable estimates of estuarine residency. The accuracy of SL estimates using back calculation methods largely depends on the characteristics of the otolith axis - fish length relationship (Folkvord & Mosegaard 2002, Schirripa 2002). Our regression was based on a limited sample representing not enough small juveniles; most likely it led to a slight overestimation of SL for these stages (Arellano 1995). Nevertheless, the overall conclusions regarding [Sr/Ca] chronologies remain.

5. CONCLUSION

Patterns of otolith [Sr/Ca] chronologies in sand goby caught in the brackish water zone of the Scheldt estuary are variable. The absence of a general trend strongly indicates that the migratory behaviour of sand gobies in estuaries is probably much more diverse among individuals than was assumed previously. The sand gobies showed varying periods of residency in brackish water reaches and different timing of migration into these areas. Based on the present results and those of Chapter 4 it was concluded that sand goby display a large flexibility in life histories regarding habitat choice. They showed highly individual movement patterns with different timing of estuarine migration at a wide range of body sizes, and variable periods of estuarine residency. Unfortunately our results do not allow to quantify the patterns in estuarine habitat use and to specify the functional significance of the estuary for sand goby. A more robust analysis might include a comparison between growth rates in the respective habitats as an indicator of habitat quality (Searcy *et al.* 2007).

Additionally, some relatively low [Sr/Ca] values near the nucleus suggested that the spawning of sand goby was not restricted to coastal areas but also occurred in the Scheldt estuary, which was never observed in the field. Some individuals exhibited [Sr/Ca] elevations at a standard length of approximately 15 mm which might be related to physiological changes concomitant with the ontogenetic habitat shift from a pelagic to a demersal way of life. This highlights the need to disentangle physiological effects from environmental influences on otolith chemical composition. This is vital for an unambiguous interpretation of the numerous microchemical analyses applied in ichthyological research (Campana 1999).

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