

Estuarine recruitment of a marine goby reconstructed with an isotopic clock

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Abstract Information on movement patterns of marine fishes between estuarine populations and stocks at sea is fundamental to understanding their population dynamics, life history tactics and behavior. Furthermore, understanding estuarine habitat use by marine fishes is crucial for their effective conservation and integrated estuarine management. Although large numbers of young marine fish make use of temperate estuaries in highly predictable abundance patterns, very little is known about how estuarine populations interact with the populations at sea. Recruitment of sand goby *Pomatoschistus minutus* (Pallas, 1770) into the low salinity zone of the Scheldt estuary (Belgium) was reconstructed over an entire year by means of an isotopic clock. These results were combined with a growth model to yield age and length at immigration. Sand gobies entered the upper Scheldt estuary almost continuously from May onwards, except in July when they appeared to avoid the estuary due to warm summer temperatures. About 70% of the fish caught in the upper

estuary resided there for less than 1 month, which indicates a strong temporal overlap of immigration and emigration. This complex migration pattern suggests that estuarine residence is caused by trade-offs made at the individual level, whereby migration is probably triggered by temperature. The high turnover of individuals in the estuarine population leads us to question the functional role of the estuary for marine fishes. Sand gobies entering the upper estuary had a wide range of ages and body sizes, although they were at least 2 months old and had a minimum standard length of ~20 mm. This study shows that the use of an isotopic clock strongly complements catch data and is useful to describe the connectivity between populations.

Keywords Nursery · Fish migration · Estuarine dependency · Carbon stable isotopes · Isotopic turnover

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Introduction

The migration of animals on any temporal and spatial scale represents a fundamental aspect of the ecology of populations and individuals. Understanding the linkage between habitats throughout the animals' life history is crucial for studying population dynamics, determining habitat function and developing effective conservation efforts (Hobson 1999; Gillanders 2002). In contrast to many animal migrations on land or bird migrations, movements of marine animals during particular periods of their life history remain largely unknown (Åkesson 2002).

Extensive research on estuarine fish communities in Europe (e.g., Elliott and Dewailly 1995; Thiel and Potter 2001; Elliott and Hemmingway 2002; Greenwood and Hill 2003; Thiel et al. 2003; Maes et al. 2005b), North America

(e.g., Hagan and Able 2003; Martino and Able 2003; Ross 2003; Able 2005; Miller and Shanks 2005), South Africa (e.g., Potter et al. 1990; Whitfield 1999) and Australia (e.g., Blaber et al. 1989; Potter and Hyndes 1999) invariantly identified marine fishes as the most important group in estuaries. These studies led to the definition of ecological guilds derived from life history strategies, such as marine juvenile migrants, marine seasonal users and marine stragglers (Elliott and Dewailly 1995; Thiel et al. 2003). Due to the high abundance of marine juveniles, estuaries are often recognized as valuable habitats (nurseries) for young-of-the-year (YOY) fish, providing abundant food resources, shelter from predation or favorable thermal conditions (Elliott and Hemmingway 2002; Greenwood and Hill 2003; Ross 2003; Attrill and Power 2004). This suggests that marine fish species depend, to some degree, on the estuary for their survival. However, the specific functional role and significance of estuaries for marine fishes remains vague and debatable (Miller and Shanks 2005), partly because migration dynamics and their underlying mechanisms are poorly understood (Rountree and Able 2007). Although the temporal distribution pattern of most marine species in estuaries is highly predictable (Thiel and Potter 2001; Greenwood and Hill 2003; Maes et al. 2004), the timing of movement between populations at sea and those in estuaries remain, on the individual level, largely unknown. For instance, it is still unknown whether estuarine immigration occurs in distinct pulses or whether it is rather individually based and dependent on the physiological state of each individual and temperature (Maes et al. 2005a). In addition, there is little information on the turnover of individuals in estuarine populations.

This gap in knowledge can be attributed to the difficulty of studying and following marine organisms from one habitat to another (Able et al. 2007). Fish movements have traditionally been inferred from spatio-temporal abundance estimates coupled with analyses of size frequency distributions and various conventional tagging methods (Herzka 2005; Able et al. 2007). There are, however, many problems associated with these techniques: the resolution is constrained by the sampling interval (e.g., Warlen et al. 2002), abundance estimates are biased by the moment of sampling (Miller and Skilleter 2006) and it is difficult to distinguish among individuals that have migrated at different times. Moreover, conventional tagging methods are not feasible for (post)larval and small juvenile fish susceptible to dispersive processes and high mortality rates (Herzka et al. 2001; Rubenstein and Hobson 2004). During the last decade, increasing emphasis has been put on natural geochemical tracers to study movement patterns. The stable isotope composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) of soft tissues has proven to be useful in examining fish migration to, from and within estuaries (reviewed by Herzka 2005) and migrations in other

aquatic and terrestrial systems (reviewed by Hobson 1999; Rubenstein and Hobson 2004). Stable isotopes can be applied to establish the timing of estuarine recruitment, provided that fish experience a shift to isotopically different food resources following the transition from marine to estuarine habitat. A diet switch to isotopically different food will gradually be reflected in the consumer's tissue, until the consumer is fully equilibrated to the new environment. The rate of this isotopic change depends on tissue growth and metabolic activity (Fry and Arnold 1982; Hesslein et al. 1993). Knowing the specific rate of isotopic change in the migrant's tissues makes it possible to determine the residence time at the sampling location and thus the arrival date (Herzka et al. 2002; Phillips and Eldridge 2006). This provides a measure with which to investigate estuarine recruitment on a finer temporal scale. The present study is the first to elaborate this for a marine species throughout an entire year using stable carbon isotopes. Estuarine recruitment is here defined as the ingress or immigration of fish from the sea to the estuary (Warlen et al. 2002).

Research effort was focused on the migration dynamics of sand goby *Pomatoschistus minutus* (Pallas, 1770) (Gobiidae, Teleostei) between the North Sea and the Scheldt estuary. Sand gobies are small bottom-dwelling fish. It is one of the most common species along the Atlantic European coast and its estuaries (Bouchereau and Guelorget 1998) and it forms an important ecological link between benthic invertebrates and larger predatory fish such as cod and whiting (Jaquet and Raffaelli 1989; Maes et al. 2003; Salgado et al. 2004). Sand gobies reproduce in the coastal waters of the North Sea during spring (March–June). Larvae are pelagic for 4–6 weeks and after metamorphosis they adopt a demersal life style. Growth rate is highest from June to October but very low during winter. Most adults die in their second summer after spawning (Fonds 1973; Hamerlynck 1990; Pampoulie et al. 2004). Like many other marine estuarine opportunists, *P. minutus* exhibits a typical pattern of occurrence in the low salinity zone of several North Sea estuaries. The new cohort recruits into the Scheldt estuary at the onset of summer and a maximal density in the brackish water zone is generally reached during fall (Healey 1971; Maes et al. 2005b). The density is generally higher in the brackish water zone than in the polyhaline zone (Hostens 2000).

The objectives were, firstly, to reconstruct the recruitment pattern of sand gobies in the upper Scheldt estuary (Belgium) during one full year using an isotopic clock; secondly, to evaluate the duration of estuarine residency and the turnover of individuals in the estuarine population and thirdly to infer fish size and age at the moment of recruitment using a commonly accepted growth model that relates length to age. This will clarify the temporal utilization of the estuary by sand gobies and help us to

understand the function of estuarine visits and life history strategies of marine fish species.

Materials and methods

Study area and fish sampling

The Scheldt river has a shallow, well mixed macrotidal estuary which is approximately 160 km long from the mouth in the Netherlands to Ghent (Belgium) where sluices stop the tidal wave (Electronic supplementary material). Salt water intrudes to about 100 km inland, resulting in a relatively stable salinity gradient with a brackish zone situated between 40 and 90 km from the mouth. The water residence time varies between 2 and 3 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). An average difference of 6‰ was demonstrated for $\delta^{13}\text{C}$ between sand goby prey items in the upper and the lower Scheldt estuary. This difference was assessed through stable isotope analysis (SIA) on gut contents derived from the same specimens used in the present study and from sand gobies collected in the lower estuary (unpublished results). For $\delta^{15}\text{N}$, no consistent difference was found between these areas, so only $\delta^{13}\text{C}$ can be used as a tracer of fish migration in the Scheldt estuary. This was also concluded by Guelinckx et al. (2006).

Between April 2003 and March 2004 sand gobies were collected on a monthly basis from the cooling-water intake screens of the Doel nuclear power plant (NPP) which is located in the mesohaline zone of the Scheldt estuary at 61 km from the mouth (Electronic supplementary material). Here, salinity averaged 9.7 ± 3.7 (mean \pm SD) during the sampling period. Sampling always started 1.5 h before the ebb tide and lasted for 3 h. Nets with a 4-mm mesh size were used for collecting fish. A technical problem in the cooling water inlet of the Doel NPP prevented sampling in December 2003. Fish samples were flash-frozen on dry ice for transport to the laboratory, where they were stored at -20°C until further processing. *P. minutus* was identified according to Hamerlynck (1990).

Sample preparation and SIA

Dorsal muscle samples of 15 randomly chosen fish were collected for SIA from each monthly catch, except for August when 14 fish were analyzed. Muscle samples were dried at 55°C to constant weight and homogenized using mortar and pestle. Aliquots (± 0.5 mg) were packed in tin containers for subsequent analysis. Dorsal muscle tissue was chosen as it has an appropriate half-life for $\delta^{13}\text{C}$

(25 days) during maximal sand goby abundance in the estuary (Guelinckx et al. 2007).

Stable isotope measurements were performed at the Laboratory for Analytical and Environmental Chemistry at the Vrije Universiteit Brussel (Belgium) on a Flash series 1112 elemental analyzer interfaced to a Delta^{plus} XL Thermo Finnigan IRMS. The working standard was high-purity CO_2 , while sucrose (IAEA-C-6: $\delta^{13}\text{C}$ 10.4‰) was used as a reference material. Stable isotopic compositions are expressed in the conventional δ notation:

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 10^3.$$

$\delta^{13}\text{C}$ values are expressed relative to the Vienna PeeDee Belemnite standard. Reproducibility for different aliquots of the reference materials was generally better than 0.3‰.

Development of isotopic clock

The isotopic signal of marine fish that arrive in the upper estuary will shift gradually towards that of estuarine food sources. The change in tissue isotopic composition over time is usually described by an exponential model (Tieszen et al. 1983), in which the isotopic composition at a given time after a diet shift (δ_t) is:

$$\delta_t = \delta_f + (\delta_i - \delta_f) \exp(-\nu t) \quad (1)$$

where δ_i is the initial isotopic value before the diet switch, δ_f the final isotopic composition in equilibrium with the new diet, t the elapsed time since the diet switch (in days) and ν a measure of the isotopic turnover rate (day^{-1}). This exponential model assumes that the incorporation of the dietary isotopic signature into an animal's tissue follows first-order, one-pool kinetics (Martínez del Río and Wolf 2005). To test whether or not multiple pools with different rate constants might be present for carbon in sand goby muscle tissue and hence also to test the appropriateness of the exponential model, we applied the reaction progress model (Aylliffe et al. 2004; Cerling et al. 2007) to our experimental data (Guelinckx et al. 2007). The reaction progress model is an alternative way to describe changes in isotopic composition of a tissue and we refer to Cerling et al. (2007) for an elaborate description. Similar to a reaction progress, the change in isotopic composition can be described as a fractional approach to equilibrium:

$$(\delta_t - \delta_f) / (\delta_i - \delta_f) = 1 - F \quad (2)$$

with $F = 0$ at the beginning of the isotope exchange and $F = 1$ at isotopic equilibrium with the new diet. Plotting the reaction progress variable $[\ln(1 - F)]$ versus time has the advantage that it permits the detection, when present, of multiple elemental pools with varying rate constants. If the isotopic incorporation follows more than one rate constant

a concave plot becomes apparent. In contrast, when only one rate constant is being followed one linear relationship is sufficient to describe the data. The intercept of the linear regression represents the fractional contribution of the pool to the whole, while the slope gives the first-order rate constant for isotope turnover (Cerling et al. 2007). The observed reaction progress variable $[\ln(1 - F)]$ for data of an experimental diet change for sand goby (Guelinckx et al. 2007) was not curvilinear in time (Fig. 1), indicating a single pool. The intercept shows that this pool contributes 98% to the total signal (Fig. 1). The exponential fit is thus satisfactory to describe the change in isotopic composition in our case. Moreover, even though the reaction progress model has several advantages over the exponential model (Cerling et al. 2007), the reaction progress model is more complicated and its model parameters are difficult to interpret (Martínez del Río and Anderson-Sprecher 2008). Hence, an isotopic clock was developed based on the exponential fit (Eq. 1).

In principle, the ν in Eq. 1 is regulated by biomass gain and metabolic turnover and can consequently be partitioned into an instantaneous rate constant for growth (k) and one for metabolic replacement (m) (Hesslein et al. 1993; Phillips and Eldridge 2006). After substituting ν by $k + m$, estuarine residence time (t_r), i.e., the time elapsed since arrival in the upper estuary, can be estimated by:

$$t_r = -\log_e[(\delta_i - \delta_f)/(\delta_i - \delta_f)]/(k + m). \quad (3)$$

The initial $\delta^{13}\text{C}$ value (δ_i) was set at -17‰ (Das et al. 2003; unpublished data of the coastal area). δ_f was

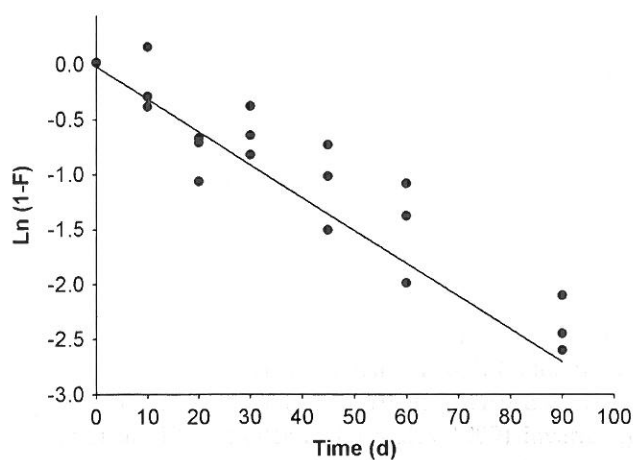


Fig. 1 Reaction progress plot $[\ln(1 - F)]$ versus time for data of an experimental diet change for sand goby (*Pomatoschistus minutus*) (data from Guelinckx et al. 2007). The data can be described by one linear relationship $[y = ax + b: \ln(1 - F) = 0.029t - 0.017]$, indicating that only one rate constant is being followed. The slope gives the first-order rate constant for isotope turnover from which the half-life can be derived $[t_{1/2} = \ln(2)/a = 24 \text{ days (d)}]$. The intercept gives the fractional contribution (f) of the rate constant to the whole ($f = e^b = 0.98$)

determined for each month by adding a trophic fractionation of 0.5‰ (Post 2002) to the $\delta^{13}\text{C}$ values of the foregut contents, collected from the same specimens that were analyzed in the present study. Instantaneous growth rates (k ; day^{-1}) for each monthly sample were calculated using $k = \log_e(W_t/W_{t-1}) \Delta t^{-1}$ (Hesslein et al. 1993; MacAvoy et al. 2006), with W_t the average fresh weight (g) of the analyzed fish per sample ($n = 14$ or 15) and W_{t-1} the average fresh weight 1 month earlier ($\Delta t = 30$ days). W_{t-1} was inferred from a seasonal von Bertalanffy growth model for *P. minutus* (Eq. 6) (Arellano 1995) and a length–weight relationship based on our field samples $[W = 4.44 \cdot 10^{-6} \text{ standard length (SL)}^{3.26}]$, which is highly consistent with the length–weight relationships reported by Doornbos and Twisk (1987) and Arellano (1995) for sand goby in the same geographical region. The metabolic turnover rate constant was experimentally determined (Guelinckx et al. 2007) but this value only applies to fish of approximately the same biomass and at the same temperature as those in the experiment. Therefore, this experimental value (m_{exp}) was adjusted to individual fish in the current study (m_s) by means of the metabolic rate (i.e., oxygen consumption) of sand gobies in the experiment and in the Scheldt estuary. Daily oxygen consumption per unit weight (r) is a function of body weight (W) and temperature (T):

$$r = R_a W^{R_b} W^{-1} \exp(R_c T) \quad (4)$$

with R_a the intercept of the allometric function relating body mass to standard respiration, R_b the slope of the allometric mass function and R_c the temperature coefficient for respiration; specific parameter values for *P. minutus* are given in Table 3 (Fonds and Veldhuis 1973, Doornbos and Twisk 1987). Oxygen consumption was calculated for fish in the experiment (r_{exp} : mean of experimental fish) and for those collected in the field (r_s for each individual) using average temperatures from the experiment (Guelinckx et al. 2007) and the Scheldt near the sampling location, respectively. The metabolic turnover constant m_s for each individual caught in the estuary was estimated as follows:

$$m_s = m_{\text{exp}} r_s / r_{\text{exp}}. \quad (5)$$

Subtracting estimated t_r from sampling dates made it possible to assess the moment of immigration and to reconstruct the estuarine recruitment pattern. However, the moment of immigration cannot be determined for fish (almost) equilibrated to estuarine food sources because their isotopic composition is nearly invariant with time as δ_i approaches δ_f asymptotically (Eq. 1). Equilibrium was assumed once a change of 90% of the difference between δ_i and δ_f was achieved. The average time ($t_{90\%}$) required to reach this isotopic composition ($\delta_{90\%}$) was calculated for each month using the appropriate values of $\delta_{90\%}$, δ_f , k and

Table 1 Upper limit of the isotopic clock ($\delta_{90\%}$) and the time required to reach this value ($t_{90\%}$) for each sampling month. Calculations are based on the specific values of δ_f , k (cf. Table 3) and monthly averages of the metabolic replacement rates (m_s)

Sampling month	$\delta_{90\%}$ (‰)	$t_{90\%}$ (days)
June 2003	−25.69	26
July 2003	−27.76	31
August 2003	−26.60	40
September 2003	−22.97	59
October 2003	−22.96	85
November 2003	−22.24	116
January 2004	−23.16	247
February 2004	−23.11	223
March 2004	−24.65	246

m_s (Table 3). Due to monthly varying isotopic turnover rates ($k + m$) the upper limit of the isotopic clock differs among the months (Table 1).

Hatching date, age and size at immigration

The age of each fish was determined using a seasonal von Bertalanffy growth model for *P. minutus* living in the coastal area close to the Scheldt estuary (Arellano 1995).

$$SL_t = SL_{\max} [1 - \exp(-K(t - t_0)) - (CK/2\pi) \times (\sin(2\pi(t - t_s)) - (\sin(2\pi(t_0 - t_s))))] \quad (6)$$

with SL_t denoting the standard body length at age t , SL_{\max} the asymptotic SL (76.2 mm), K the growth constant (1.7 year^{-1}), C the amplitude of the seasonal oscillation (1), t_0 the age at zero length (0 year) and t_s the starting point of the oscillation with respect to t_0 (0.53 year) (Arellano 1995). The hatching date of each fish was back-calculated by means of subtracting the age from the date of capture. Age at immigration was determined by the time difference between the hatching and the immigration date. Body size at immigration was inferred from the age at immigration using the seasonal von Bertalanffy growth model.

Sensitivity analysis

To assess the effects of variations in model parameters on the estimated time of residency (t_r) and age at recruitment, two sensitivity analyses were performed by varying parameters within an upper and lower limit (Hunter et al. 2000). For r_{exp} and the monthly δ_f values these limits were set by their observed SD. Based on Das et al. (2003) and field data, δ_i was varied by 1‰. Monthly growth coefficients (k) were varied by 41%, in line with the SD for k determined by Guelinckx et al. (2007). The parameters used to calculate oxygen consumption (R_a , R_b and R_c), m_{exp} (Eq. 4) and those of the seasonal von Bertalanffy growth

model (Eq. 6) were varied by 10%. Initially, we ran the model using nominal parameter values. The sensitivity analysis was then performed by running the model 500 times using a random number generator to independently select parameter values from a uniform (for δ_i) and a normal (all other parameters) probability distribution between its minimum and maximum values (Table 3). Using multiple regression, input parameters were subsequently related to a dependent variable that expressed the deviation of the sensitivity analysis from the nominal model, i.e., the squared difference between the nominal result and the result of a single sensitivity run summed over all individuals. Multiple regression estimated the amount of variance of this dependent variable explained by variation of individual parameters with the effect of other parameters statistically removed, and expresses this as the relative partial sums of squares. This way the relative contribution of each parameter to deviations of the nominal model was determined (Hunter et al. 2000; Maes et al. 2005a, 2006).

Results

$\delta^{13}\text{C}$ values

$\delta^{13}\text{C}$ values of all fish varied between −28.90 and −15.33‰ (Fig. 2a). The $\delta^{13}\text{C}$ values were less variable in June and July than during autumn and winter. Most individuals had a transitional isotopic signal between the marine and estuarine end member. Only 17 of the 134 fish were considered to be in isotopic equilibrium with the estuarine food web. The most ^{13}C depleted values were observed during summer. The lowest $\delta^{13}\text{C}$ (most estuarine) values of muscle tissue were highly consistent with the predicted estuarine end signatures (δ_f) for each month. Only three fish, caught in September and November, had much lower values. Several individuals, most of them caught in winter, had higher $\delta^{13}\text{C}$ values than the assigned marine end member (−17‰).

Estuarine recruitment and residence time

For each collection date fish were grouped into four classes based on their t_r : fish residing in the estuary for a maximum of 15 days, between 16 and 30 days, between 31 and 60 days, and more than 2 months. These classes were coded <15, 16–30, 31–60 and >60 respectively. The percentages of this classification were extrapolated to the abundance of each sampling event (Fig. 2b). More than 50% of the estuarine population every month had immigrated within the last 30 days (classes <15 and 16–30). Considering the proportion of the four classes in the estuarine population over the entire year (Fig. 2b, inset),

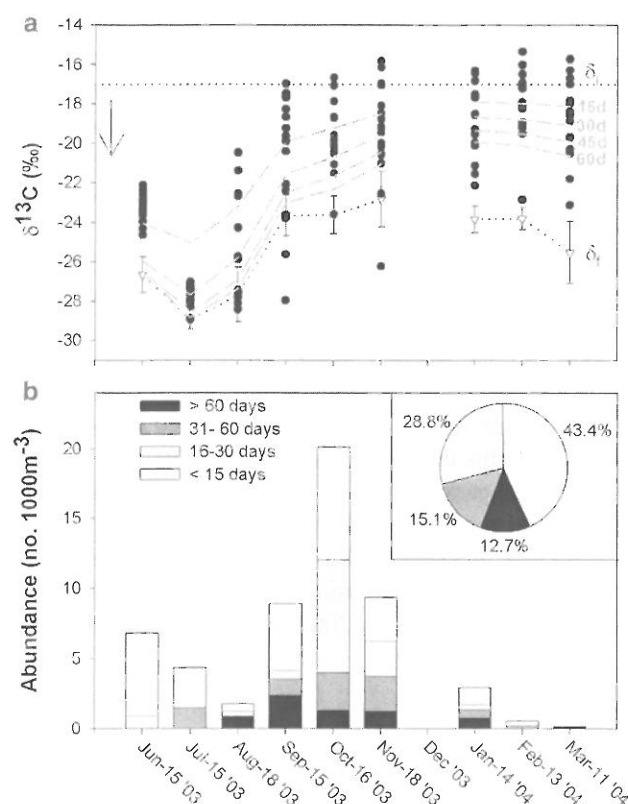


Fig. 2 **a** Isotopic composition and **b** abundance of *P. minutus* after estuarine entrance for each sampling date in the upper Scheldt estuary. **a** Individual $\delta^{13}\text{C}$ values (filled circle, $n = 134$); arrow indicates direction of $\delta^{13}\text{C}$ after estuarine entrance; $\delta_i = -17\text{‰}$ (dotted lines) and δ_f (mean \pm SD, inverted triangle) indicated. Dashed grey lines indicate the calculated $\delta^{13}\text{C}$ values (Eq. 1) for sand gobies 15, 30, 45 or 60 days after estuarine entrance. **b** Sand goby abundance (number per 1,000 m³) is partitioned into four residence classes; inset: proportion of the four residence classes in total annual catch [April (Apr) 2003 ('03)–March (Mar) 2004 ('04)]. For other abbreviations, see Table 3

roughly 30% appeared to remain in the estuary for more than 1 month (classes 31–60 and >60) with 13% classified in the >60 group. About 43% had entered the upper estuary less than 15 days before collection. These results demonstrate a relatively short stay of sand gobies in the estuary and thus a substantial turnover of individuals in the estuarine population. This also suggests emigration throughout the year and a strong interchange of individuals between the populations at sea and in the upper estuary.

Hatching mainly occurred from March to July but some hatched in August and September (Fig. 3). Juveniles were first caught in June, but their isotopic composition showed that immigration had already started in May (Fig. 3). Fish caught in June recruited into the upper estuary during a period of about 10 days. The first fish reaching the upper estuary were about 2–3 months old and measured about 22 mm (Table 2). There was almost no immigration in July as only 3 among 134 fish were found to have immigrated

Table 2 Standard length (SL) and age (mean \pm SD) of *Pomatoschistus minutus* at immigration. Individuals equilibrated to the estuarine food web could not be taken into account

Month of recruitment	<i>n</i>	SL (mm)	Age (days)
May 2003	2	21.5 \pm 0.4	68 \pm 1
June 2003	27	26.4 \pm 3.6	80 \pm 10
July 2003	3	34.6 \pm 3.7	102 \pm 10
August 2003	9	35.5 \pm 12.4	108 \pm 40
September 2003	18	41.8 \pm 10.3	126 \pm 32
October 2003	13	43.0 \pm 13.1	152 \pm 108
November 2003	8	45.2 \pm 9.6	144 \pm 49
December 2003	4	54.3 \pm 13.5	276 \pm 175
January 2004	16	48.6 \pm 11.6	188 \pm 113
February 2004	12	56.3 \pm 10.3	205 \pm 85
March 2004	3	56.7 \pm 6.7	204 \pm 45

during this month. Fish caught in July had immigrated in June, during a short period (± 10 days) that partially overlapped and followed on the immigration period of fish caught in June (results not shown). So there was no evidence of distinct pulses of immigration. About 50% of the fish caught in August had also recruited in June. Although sand goby abundance continued to decrease, their immigration resumed in early August (Fig. 2b). Subsequently, maximum sand goby influx occurred in September, which is about 4–5 months after the hatching peak (Fig. 3; Table 2), and their influx remained high during October causing their abundance to peak in October. However, at the same time considerable emigration is suggested by the strong decrease in the number of fish in consecutive recruitment groups: classes <15 and 16–30 in September become class 31–60 in October, which is substantially smaller (Fig. 2b). Sand goby abundance decreased from November onwards, yet immigration continued during

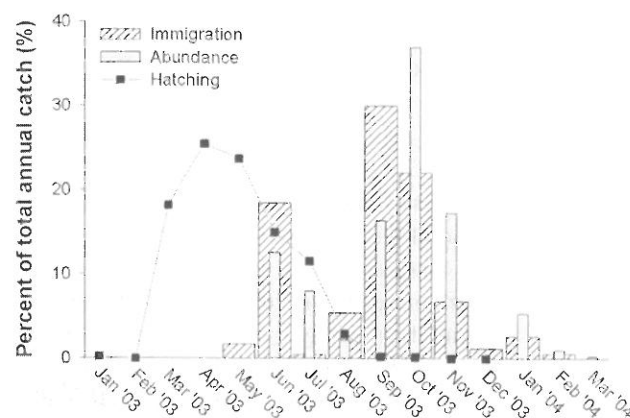
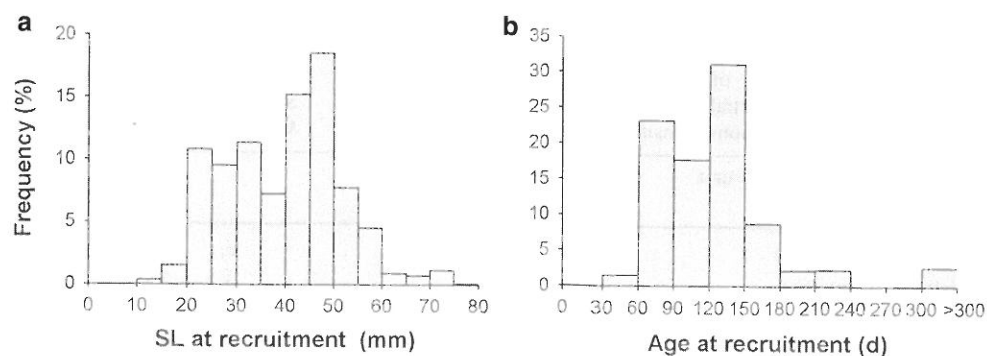


Fig. 3 Abundance, back-calculated hatching period and estuarine recruitment of *P. minutus* caught during 1 year (April 2003–March 2004). Results are shown as percentage of total catch. Six fish hatched in 2002 and are not shown here

Fig 4 Distribution of **a** standard length (SL) and **b** age of *P. minutus* at estuarine immigration. Individuals equilibrated to the estuarine food web could not be taken into account ($n = 113$). Note that the tail of the age distribution has been compressed into a single category (>300 days)



winter as proven by new arrivals in the estuary (Fig. 2). Because sampling could not take place during December 2003, the amount of recruiting fish during December and some previous months is probably slightly underestimated.

Considering age and body length of all immigrating fish over the entire year, sand gobies were found to enter the estuary over a wide range of sizes and ages (Fig. 4; Table 2). Except for one fish being 12 mm, the range in body length of immigrating fish varied from 19 to 75 mm. A peak was observed for length classes between 40 and 50 mm (35%), which corresponds to body lengths of fish entering in autumn when estuarine recruitment was high (Table 2; Fig. 3). The majority of the immigrating fish were older than 2 months (Fig. 4b). The age distribution showed roughly two peaks: one peak at age class 120–150 days and a smaller one at 60–90 days. These peaks correspond to the two periods of enhanced immigration: September–October and June, respectively (Fig. 3; Table 2).

Sensitivity analysis

The sensitivity analysis showed that δ_f seems to have the strongest influence on the calculated residence times of the fish (Table 3). The isotopic clock is relatively sensitive to the parameters m_{exp} , R_a and R_c that were used to determine instantaneous m_s (Eqs. 3, 4). Figure 5 illustrates the effect of parameter perturbations on residence time relative to the nominal results of the isotopic clock. Parameter changes (Table 3) are not likely to considerably alter the main conclusions with respect to the sand goby recruitment pattern in the Scheldt estuary. Age at recruitment and consequently also length at recruitment were most sensitive to variation in the growth constant K .

Discussion

Estuarine recruitment and residency

The hatching period of the sand gobies, back-calculated from their body length, matches their known spawning

period in the southern North Sea (Fonds 1973). Sand goby larvae are pelagic for several weeks before they shift to a demersal life style at a length of 12–20 mm (Fonds 1973). Reconstructing estuarine influx showed that sand goby individuals were at least 2 months old and had, except for one fish, a SL of at least 19 mm at the moment of estuarine entry. Bardin and Pont (2002) reported that *Pomatoschistus* spp. shift from passive (drifting) to more active migration behavior around a total length of 20 mm. Thus, sand gobies seem to attain a given ontogenetic stage first before migrating actively into the upper Scheldt estuary. It is possible, however, that our results underestimate estuarine recruitment in May and June (Fig. 3), as smaller sand goby juveniles have been sampled during these months in the inner estuary by means of a hyperbenthic sledge (Beyst et al. 1999). Because of the larger mesh size (4 mm) of the filter screens of the cooling water intake, our sampling method is inadequate for fish smaller than 30 mm.

The results demonstrate that sand gobies entered the Scheldt upper estuary almost continuously from May to March. The intensity of this continuous immigration varied strongly throughout the year resulting in periods of net immigration and net emigration producing the typical abundance pattern (Figs. 2b, 3). In July 2003 there was almost no sand goby ingress into the upper estuary, suggesting a period of only emigration as abundance also decreased. This was probably caused by the high water temperature ($\pm 22^\circ\text{C}$) at that time, making the upper estuary an unfavorable habitat for *P. minutus*, which is known to avoid temperatures above 20°C (Fonds and van Buurt 1974; Hesthagen 1979). Avoidance of the upper estuary in summer was also observed for herring and could be attributed to high water temperatures (Maes et al. 2005a).

The temporal overlap of immigration and emigration throughout the year supports the hypothesis that estuarine visits are the result of trade-offs at the individual level, rather than a fixed scheme for the whole population. Individuals may respond quickly to changes in climate condition, food availability or predation risk and shift rapidly between coastal and estuarine nursery areas in order to increase their individual state and fitness. Sand

Table 3 Sensitivity analysis of the isotopic clock and seasonal growth model. Deviation of nominal parameter values (see text) and independent contribution of the each parameter to total model uncertainty, as relative partial sum of squares (RPSS) (%) determined by multiple linear regressions. Sensitivity analyses on residence time

(t_r) and age at recruitment consisted of 500 model runs, each run with parameters randomly selected from these ranges. The most influential parameters are indicated in *bold*. Jun June, Jul July, Aug August, Sep September, Oct October, Nov November, Jan January, Feb February, March March

Parameter description and unit		Nominal value \pm deviation	t_r RPSS (%)	Age recruitment RPSS (%)
Initial $\delta^{13}\text{C}$ value (‰)	δ_i	-17.00 ± 1.00	2.8	2.4
Final $\delta^{13}\text{C}$ value (‰)	δ_f -Jun 2003	-26.65 ± 0.91	0.5	0.3
	δ_f -Jul 2003	-28.96 ± 0.46	2.0	0.0
	δ_f -Aug 2003	-27.66 ± 1.39	4.1	0.1
	δ_f -Sep 2003	-23.64 ± 1.04	0.7	2.2
	δ_f -Oct 2003	-23.62 ± 0.96	19.9	0.2
	δ_f -Nov 2003	-22.82 ± 1.17	13.3	4.2
	δ_f -Jan 2004	-23.84 ± 0.68	4.0	0.6
	δ_f -Feb 2004	-23.79 ± 0.57	6.0	0.0
	δ_f -Mar 2004	-25.50 ± 1.27	12.6	0.1
Instantaneous growth rate (day^{-1})	k -Jun 2003	0.055 ± 0.023	0.1	2.4
	k -Jul 2003	0.042 ± 0.017	2.4	0.0
	k -Aug 2003	0.030 ± 0.012	4.3	0.0
	k -Sep 2003	0.018 ± 0.007	3.1	0.4
	k -Oct 2003	0.014 ± 0.006	0.1	2.5
	k -Nov 2003	0.009 ± 0.004	0.2	4.0
	k -Jan 2004	0.003 ± 0.001	0.1	0.2
	k -Feb 2004	0.003 ± 0.001	0.0	0.2
	k -Mar 2004	0.003 ± 0.001	0.8	0.4
Instantaneous metabolic replacement in experiment ^a (day^{-1})	m_{exp}	0.017 ± 0.002	6.9	0.8
Respiration in experiment ^a ($\text{mg O}_2 \text{ g}^{-1} \text{ day}^{-1}$)	r_{exp}	3.62 ± 0.21	0.5	0.1
Intercept for the allometric mass function for respiration ^b ($\text{mg O}_2 \text{ g}^{-1} \text{ day}^{-1}$)	R_a	0.93 ± 0.09	9.3	0.0
Slope of allometric mass function for respiration ^b	R_b	0.80 ± 0.08	0.0	0.1
Temperature coefficient for respiration ^b ($^{\circ}\text{C}^{-1}$)	R_c	0.08 ± 0.01	6.1	0.2
Growth rate ^c (year^{-1})	K	1.70 ± 0.17		76.6
Amplitude of the seasonal oscillation ^c	C	1.0 ± 0.1		1.5
Starting point of the oscillation ^c (year)	t_s	0.53 ± 0.05		0.5

^a Guelinckx et al. 2007

^b Fonds and Veldhuis 1973, Doornbos and Twisk 1987

^c Arellano 1995

gobies are known to use tidal streams selectively (Bardin and Pont 2002), so despite their limited swimming performance it would take not more than a few tides to reach the lower salinity zone of the Scheldt estuary.

Estuaries are considered as beneficial areas where marine juveniles spend a substantial time to grow in a sheltered environment (Greenwood and Hill 2003; Ross 2003). Yet, the observed short estuarine residencies might compromise this view, at least for sand goby. Can short estuarine visits counterbalance the energy investment of habitat transition and provide enough surplus value to increase the fish's state and fitness considerably, therefore making the trip worthwhile? It appears that the estuary acts more as an overspill of the coastal area. Sand goby

juveniles may see the estuary merely as an extension of the coastal area, which they explore rather incidentally when estuarine conditions (e.g., temperature) allow it. After all, a wide range in length and age at recruitment was observed. However, our results on sand goby estuarine recruitment and residency are also consistent with individual-based model predictions for herring juveniles (*Clupea harengus*) (Maes et al. 2005a), and corroborate stable isotope results for this species (Guelinckx et al. 2006). Maes et al. (2005a) modeled optimal habitat selection by herring from the open sea to the upper Scheldt estuary as a function of individual fitness. During late spring, post-larval and early juvenile herring are predicted to utilize the turbid upper parts of the estuary, mainly as a shelter for predation, resulting in a

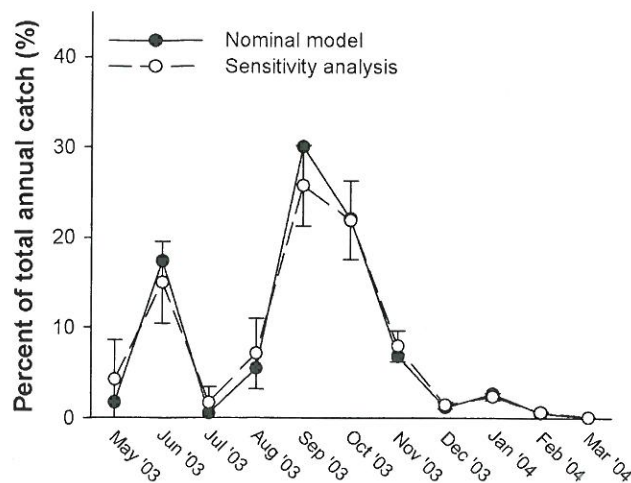


Fig. 5 Nominal model results (filled circle) of estuarine recruitment are compared to an average (\pm SD) of 500 sensitivity runs (open circle)

considerable increase in survival probability during the first year of life. During warm summer months, herring were predicted to avoid the estuary but, following this period, short in- and out-migrations may enhance both growth and survival depending on annual patterns of environmental variability. It was concluded that estuarine migration during autumn and winter by YOY herring is merely a facultative process with temperature acting as a cue. A similar scenario with short visits now seems to be true for sand goby in the upper Scheldt estuary, whereby temperature most likely regulates the temporal variation in influx. Healey (1971), Fonds (1973) and Doornbos and Twisk (1987) reported that temperature probably triggers sand goby migrations. Moreover, Hesthagen (1979) observed a seasonal temperature preference for sand goby and explained this as a behavioral thermoregulation to direct fish towards temperatures that are optimal for different physiological processes depending on the season. Consequently, as estuarine migration patterns are indeed regulated by the effect of temperature on enzymatic processes, it is to be expected that these patterns will alter due to climate change (Duarte 2007).

Although sand goby as a species is present in the upper estuary almost throughout the year, calculated residence times revealed that most individuals visit the upper estuary for less than a month, demonstrating a fast turnover in the estuarine population. Hence, we support Thiel et al. (2003) in classifying *P. minutus* as a marine estuarine opportunist instead of an estuarine resident (Elliott and Dewailly 1995). Nevertheless, about 30% of the estuarine population was observed to stay for more than a month (Fig. 2b). These different temporal usages of the upper estuary could be the result of divergent behavior expressed by two (or more) contingents in the sand goby population. Contingents, i.e.,

intrapopulation migratory groups, have already been demonstrated for a wide array of fish taxa using estuaries, although more with regard to divergent spatial patterns (Secor 1999, 2007).

Evaluation of isotopic clock

The isotopic clock (Eq. 3) is a straightforward transformation of the exponential model (Eq. 1) describing the change in isotopic composition of a tissue over time. The estimated variables t_r , age and length at recruitment depend on several parameter values (Table 3). The estuarine end member δ_f explained most of the total variation of t_r in the sensitivity analysis. Monthly δ_f values were determined from gut contents that were removed from the same specimens that were analyzed in the present study. $\delta^{13}\text{C}$ values of foregut contents are not significantly different from that of undigested food, making them reliable and easily accessible estimators of end signatures. Performing SIA on gut contents avoids additional sampling efforts for all potential prey items and eliminates the problem of identifying the food sources in their contributing proportions (Grey et al. 2002; Guelinckx et al. 2008). There was a strong similarity between the predicted δ_f values and the lowest (most estuarine) $\delta^{13}\text{C}$ values for muscle tissue in the estuary (Fig. 2a). This supports the argument that the estuarine end member signatures were well estimated. Three fish in September and November had substantially lower values than the month-specific δ_f value. These fish probably still reflected previous end signatures (e.g., August). Another explanation for these low values might be that these fish had resided further upstream where food sources are more depleted in ^{13}C (De Brabandere et al. 2002). Field surveys have shown that sand gobies can occur in these upstream areas, yet this is rather uncommon. Hypoxic conditions ($2\text{--}4\text{ mg O}_2\text{ l}^{-1}$) in the oligohaline and freshwater reaches of the Scheldt estuary severely limit migration to areas upstream from our sampling location ($5\text{--}7\text{ mg O}_2\text{ l}^{-1}$), especially during summer and early fall (Meire et al. 2005, Maes et al. 2007). Sand gobies are known to avoid oxygen levels below $4\text{ mg O}_2\text{ l}^{-1}$ (Petersen and Petersen 1990). So, the area of the sampling location can be considered as the migration end point and residency in upstream ^{13}C depleted areas was probably negligible and did not confound our results to a great extent, if any.

Based on Das et al. (2003) and isotopic results of sand goby muscle from marine waters adjacent to the Scheldt estuary, δ_i was set at -17‰ . However, this value seems to underestimate the marine end signature as some fish in the upper estuary had higher $\delta^{13}\text{C}$ values (Fig. 2a). Fish at sea did not have a higher lipid content (unpublished results) than fish in the upper estuary. The opposite could have explained

the difference as lipids are ^{13}C depleted (DeNiro and Epstein 1977). It appears that some sand gobies entering the Scheldt estuary originate from offshore waters or from the Eastern Scheldt, which is a marine bay north of the Scheldt estuary (Electronic supplementary material). Fish from these two areas showed ^{13}C enriched values (approximately -17.3 and -15.5‰ , respectively) compared to those from near-shore waters. Das et al. (2003) reported an average $\delta^{13}\text{C}$ value of $-17.1 \pm 0.5\text{‰}$ for *Pomatoschistus* spp. in the Southern Bight of the North Sea. Because it is unclear how these different areas (inshore, offshore, Eastern Scheldt) contribute to the estuarine population, a value of -17‰ was assumed for δ_i . Nevertheless, the sensitivity analysis showed that the isotopic clock was relatively insensitive to variation in δ_i . Finally, we did not characterize the entire longitudinal estuarine gradient because sand gobies preferentially aggregate either in coastal areas or in the upper estuary (Hostens 2000). In addition, carbon isotope analyses on invertebrates along the salinity gradient of the Scheldt (De Brabandere 2005) and the Thames (Leakey et al. 2008) only allowed to clearly distinguish two regions (coastal vs. brackish water) from the oligohaline zone to the sea.

v was partitioned into k and m (Fry and Arnold 1982). These parameters vary by ontogenetic stage and temperature. Consequently, the window of time over which the isotopic clock is applicable varies throughout the year (Table 1). The calculated time periods required for sand gobies to reach isotopic equilibrium ($t_{90\text{‰}}$), fits between values for rapidly growing larvae (Herzka and Holt 2000) and those of adult fish (Hesslein et al. 1993).

The average change in body weight was used to determine the k for each monthly sample (Hesslein et al. 1993; MacAvoy et al. 2006; Phillips and Eldridge 2006). This change in body mass was determined using a sand goby growth model (Eq. 6) (Arellano 1995) and was not just based on the average weight difference between consecutive field samples, in order to avoid biased results due to mortality, recruitment and migration. Future work should attempt to incorporate individual growth rates derived from otolith increments instead of using population averages. Taking the mass specific growth of the elemental pool itself into account, instead of using the change in body weight as a proxy for this, is probably the most accurate method because tissue elemental composition (e.g., C:N ratio) could vary throughout an organism's lifespan.

The experimentally obtained value for m (i.e., the incorporation and catabolism of elements in a tissue) was adjusted to field conditions for fish analyzed in this study using respiratory values (Eqs. 4, 5). Because sand goby is a sluggish species remaining most of the time inactive, except for feeding, standard metabolism of an individual in the experimental setting was probably similar to that in the field for the same temperature and biomass (Fonds and Veldhuis 1973;

Petersen and Petersen 1990). Experimental oxygen levels were kept high (Guelinckx et al. 2007) and could not have influenced the metabolic rate in the experiment. Moreover, standard metabolic rate of sand goby is not affected by acute hypoxic conditions (Petersen and Petersen 1990). Respiration is often used to measure metabolic rate or rate of energy consumption (Helfman et al. 1997). However, elemental turnover might not be the only determinant of metabolic rate and the uncoupling of metabolic rate and the rate of isotopic incorporation was demonstrated in house sparrows (*Passer domesticus*) (Carleton and Martínez del Río 2005). On the other hand, based on experiments with rodents (Muridae), MacAvoy et al. (2006) suggested the use of metabolic rate to estimate tissue turnover rate. In fishes a substantial portion of standard metabolic rate is related to the costs of protein synthesis and turnover. Cellular oxygen consumption and protein synthesis were linearly correlated in rainbow trout *Oncorhynchus mykiss* (Smith and Houlihan 1995), suggesting that metabolic rate and elemental turnover are not uncoupled in fish. In addition to the contribution of metabolic replacement to isotopic change throughout the organism's life, the relationship between elemental replacement and metabolic rate thus requires more attention. Information on these subjects is very scarce, particularly for ectotherms, though important for the use of an isotopic clock.

Conclusion

The exchange of organisms between outer coastal and estuarine areas is a key component of coastal, estuarine, and population ecology. An understanding of the spatial and temporal patterns of estuarine habitat use by marine fishes will increase the understanding of the ecological functions estuaries provide for these species (Sale et al. 2005; Rountree and Able 2007). In this study an isotopic clock was developed to back-calculate individual sand goby arrival dates in the upper Scheldt estuary and subsequently to quantify estuarine immigration throughout the year. This strongly complemented catch data and resulted in several new insights into the patterns of estuary use by *P. minutus*. Immigration in the upper estuary occurred almost continuously from May onwards at a wide range of ages and body sizes. It was assumed that emigration to sea also occurred continuously as sand gobies were found to have relatively short residence times in the upper Scheldt estuary. The temporal overlap between immigration and emigration demonstrates a strong coupling between sand goby populations at sea and in the upper estuary. Additionally, the complex migration dynamics suggest that estuarine migration is regulated on the level of the individual. Short estuarine visits as observed in this study lead us to question the functional role of estuaries for marine fishes. Brief estuarine residencies do not seem to fit with the concept of

estuaries as important feeding, growth or predator refuge areas. The possibility for marine fishes to briefly profit from estuarine areas therefore merits more research.

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