Katholieke Universiteit Leuven
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ESTUARINE HABITAT USE BY A GOBY SPECIES: A GEOCHEMICAL APPROACH

ESTUARIEN HABITATGEBRUIK DOOR EEN GRONDELSOORT: EEN GEOCHEMISCHE BENADERING

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Proefschrift voorgedragen tot
het behalen van de graad van
Doctor in de Wetenschappen

door:

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2008

Het onderzoek in dit proefschrift werd gesteund door een beurs verleend door het Vlaams Instituut voor de Bevordering van het Wetenschappelijk-Technologisch onderzoek in de Industrie (IWT) en door een K.U. Leuven onderzoekstoelage (OT/02/30).

ISBN 978-90-8649-165-0
D/2008/10.705/11

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Laboratorium voor Aquatische Ecologie en Evolutiebiologie
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"If one cares about the sand goby, this work will be very useful."

Comment of unknown reviewer on Chapter 3 of this work

"All that is now. All that is gone. All that's to come and everything under the sun is in tune but the sun is eclipsed by the moon."

Eclipse – Pink Floyd

"Il faut imaginer Sisyphe heureux."

A. Camus

Dank-Thanks-Merci

Een doctoraatsstudent is zich er van bewust dat tijdens het proces van doctoreren een 'dankwoordje' dient geschreven te worden voor zijn of haar boekje. Ondanks deze kennis gedurende enkele jaren, wordt dit schrijven echter omwille van allerlei beslommeringen uigesteld tot het aller-allerlaatste moment. Een niet al te beste zet aangezien 1) de doctorandus in spe na maanden denk- en schrijfwerk, gepaard gaande met een chronisch gebrek aan slaap, als dusdanig niet erg fit kan bevonden worden, én 2) omdat het dankwoord werkelijk door IEDEREEN wordt gelezen, stiekem hopend zijn of haar naam terug te vinden. Deze twee condities vormen een gevaarlijke combinatie: het is immers pijnlijk wanneer iemand onterecht zijn of haar naam niet terugvindt, omdat die door de mazen van het mnemotechnische net is geglipt. Nochtans wordt ook deze keer wordt de aloude traditie-van-het-allerlaatste-moment in ere gehouden. Hieronder volgt een verdienstelijke poging van mijnentwege om mijn appreciatie te uiten voor alle steun en hulp. Ik excuseer me nu reeds aan iedereen wiens naam niet wordt vermeld terwijl dit wel had moeten gebeuren. Aan alle anderen excuseer ik mij voor mogelijke typfouten ☺.

Eerst en vooral dank aan Professor Ollevier om via zijn lessen mijn interesse in de ecologie aan te zwengelen en nadien het promotorschap van dit werk op te nemen. Hierbij stelde hij steeds voldoende vertrouwen in mij om dit project tot een goed einde te brengen. Bovendien kon ik genieten van de nodige vrijheid om ideeën in de praktijk te brengen en denkpistes te verkennen. Dit gaf mij de mogelijkheid om zelfstandig te leren omgaan met alle aspecten van het wetenschappelijk onderzoek.

Onnoemelijk veel dank aan Jimi, die na het begeleiden van mijn licentiaatsthesis mij een doctoraatspositie aanbood op zijn gezamenlijk project met Prof. Volckaert: 'an offer I couldn't refuse', maar waar ik als naïeveling toch twee keer over moest nadenken. Gelukkig trok hij me over de streep met de historische woorden: 'Baat het niet dan schaadt het niet'. Jammer dat het project uiteindelijk niet de vorm heeft gekregen die je oorspronkelijk voor ogen had. Niettemin, bedankt voor de steun, de vrijheid, de efficiënte begeleiding, om in te springen waar nodig, te tonen hoe te doctoreren én ondanks je vertrek, steeds binnen enkele muisklikken bereikbaar te zijn.

Frank Dehairs, bedank ik graag voor het copromotorschap op te nemen én vooral om de zeer vlotte samenwerking te verzorgen rond het isotopenluik van dit werk. Je suggesties en opmerkingen werden ook steeds ter harte genomen. Bedankt! Filip, jou wil ik speciaal bedanken voor de stimulatie tijdens de eindfase van mijn doctoraat (ruwweg de laatste twee jaren), voor het verbeterwerk én om mij te adopteren in de genetica groep. Ook een woordje van dank voor Luc DM: niet alleen omwille van de boeiende lessen van weleer die mee mijn ecologische bagage hebben gevormd, maar ook om, na het beëindigen van mijn beurs, een sociaal plan op te richten, teneinde mij een volwaardig loon te verschaffen. Het assistentschap kwam dan ook als geroepen. Bovendien vond ik het een leerrijke ervaring: zo blijken ook Daphnia's eisen aan hun omgeving stellen!

Bij mijn weten heeft geen enkele doctoraatsstudent bij aanvang van zijn doctoraat kunnen beschikken over een 'eigen' technisch personeelslid. Deze ongebreidelde luxe is mij echter wel gegund geweest. En hoe! Meteen met mijn gat in de boter. Bram, ik zal steeds met veel plezier terugdenken aan al onze expedities aan zee of de Schelde (ongeacht de slaapplaats: appartement, camionette, illegaal in een tent, VLIZ serres of bibliotheek), het filosoferen tijdens den uitraap, het slepen in de branding, ... en uw ongelofelijke bereidheid om steeds te volgen in mijn doorgedreven staalnameplannen. Merci, zonder jou waren de afgelopen jaren niet half zoveel fun. Bij deze ook bedankt voor onze persoonlijke expeditie naar de mythische bron van de Schelde. Ik hoop dat er nog vele mogen volgen: Somme, Maas, Donau, Nijl,...

De sterkte van labo wordt voor een stuk bepaald door een bataljon aan stille werkkrachten op de achtergrond. Op dat vlak heeft de doctoraatsstudent bij het Labo van Aquatische Ecologie zeker geen klagen. Aan de dames van het secretariaat (Conny, Kim, Hilde, Sinne) bedankt om me te leiden doorheen de administratieve chaos en zoveel meer. Ria, An, Bart en Eddy bedankt voor de talrijke bestellingen vlotjes te laten verlopen en aan de mannen van 'den underground' (Stef, Ronnie en Gert) een welgemeende dank u om allerhande toestellen (o.a. bierkoelers) draaiende te houden, en specifieke experimentele opstellingen te ontwerpen en tot leven te brengen.

Het is altijd fijn te weten dat er personen zijn die, waar ook ter wereld, klaar staan om steeds je vragen te beantwoorden, teksten kritisch na te lezen en je uitdagen jezelf in vraag te stellen. Loreto, hartelijk bedankt daarvoor!

Een doctoraatsstudent moet vaak ook hulp buitenshuis de nodige ondersteuning zoeken om zijn project tot een goed einde te brengen. Bij deze bedank ik graag voor de vlotte samenwerking: het VLIZ team, de bemanning van de Zeeleeuw, Michael en Steven (VUB-ANCH), de mensen van het NIOO-CEME (thanks to the Kêête people for such a nice time), de bemanning van de Luctor, IFREMER-LASAA (Hélène, Andre, Aurélie et Marcel: merci pour tout) en Hugo (van het INBO voor de VIE).
Voor de staalnames kon ik verder steeds rekenen op een legertje enthousiastelingen die met veel overgave de grondels in het nauw dreven. The mighty goby hunters zijn: Bram, Jimi, Anton, Pim, Lies, Maarten S, Helen, Liesbeth VK, Arne, Bart L, Barbara G, Vanessa A, Stefano, Maarten L, Els C, Johan A, Bart V, Thomas en Danny (allebei van de Gentse mariene sectie), Dré en Tjess (vooral bedankt het net te komen redden in Heist), Pieter V, Sarah, Robin, Jean-Felicien, Shodja en dit aangevuld met een handvol lokale vissers: André (Blankenberge), Roland (Nieuwpoort), Jean Pierre (Oostende), de mannen van Lillo en tot slotte Gert (Yerseke).

Het zou me wat ver leiden om alle collega's van het labo bij naam te noemen. Weet dat ik jullie allemaal bedank voor de fantastische sfeer (incl. hemdjesdag, laboweekend,...) én om het werken zoveel fijner te maken. Ik kan me geen toffere mensen op de werkvloer voorstellen. Toch een speciaal woordje van dank aan de "naastere collega's'': Anton, bedankt voor het ontspannende leven op eiland 4 én voor het pdf-en in het midden van de nacht. Ik heb nu het voorbeeld gegeven en ik hoop dat je snel mag volgen; Maarten S, ik beschouw u een beetje als mijn oudere neef binnen de Schelde-familie: bedankt voor uw raad en daad; Maarten L, merci voor het oprichten en uitbouwen van het grondel-consortium. Je mag er terecht fier op zijn dat dit werk mede tot stand is gekomen door uw initiatief. Helen, bedankt voor de fijne momenten (oude markt - lunchpauzes, winterwandelen en zomers zeilen), Dragonfly Cis: merci voor

de fun en de computer depannages; Joost R, merci om met uw 60 liter en 50 stekelbaarzen mee de camionette te overladen met een 'wielei' tot gevolg, en ook bedankt om af en toe een statistisch woordje uitleg te geven; Els C, ... (zie onder).

Dank aan mijn enthousiaste thesisstudenten die zonder morren het vuile werk opknapten: het werk in Yerseke overnemen wanneer ik naar een congres moest of gewoon naar een feestje in het Leuvense (Pieter), een gigantische dataset overzichtelijk maken en het identificeren van massa's grondels (Sarah).

Naast al de werkgerelateerde mensen een woordje van dank voor alle vriendjes en vriendinnetjes (de bioloogjes, SPC-clan, Poseidoniërs,...) voor alle ontspannende momenten voor en na de werkuren van de afgelopen 5 jaren: weekendjes, reizen, feestjes, cafébezoeken, concerten,...en voor de vraag: 'en Jef, hoe is het nu met uw thesis en wanneer denkt ge af te leggen? Snel ook nog een woordje van dank voor de meisjes van het Geel Huis in de Paternosterstraat die mij getolereerd hebben toen ik tijdens de schrijffase van de afgelopen maanden mezelf heb opgedrongen als vierde bewoner (tja optimal habitat choice hé) én vooral toen ik het warm water had afgesloten.
Hier ook een heel speciaal woordje van dank voor Lies: voor de onvoorwaardelijke steun (zowel praktisch als moreel). Ik vind het meer dan uitzonderlijk dat ik altijd op jou heb kunnen rekenen en ik ben je hier dan ook enorm dankbaar voor. Merci voor alles!

Uiteraard geen doctoraat zonder de fundamentele steun van de ouders en de familie: Gijle zijt allemaal enorm hard bedankt om steeds een warm nest aan te bieden en paraat te staan. Jullie bezorgdheid voor en opvoeding van zoon- en broederlief is ongeëvenaard!

De laatste regels zijn zoals het hoort opgedragen aan het liefje. Woorden schieten helaas te kort om mijn dankbaarheid uit te drukken. Els, het is verbazingwekkend welke steun ik van jouw de afgelopen maanden heb gekregen. Je was mijn rots in de branding: je wist me steeds te motiveren en je maakte 'de laatste helse loodjes' een pak lichter. Ik ga mijn best doen om voor jou hetzelfde te betekenen als jij voor mij.

Jef Guelinckx,

18/02/08

CONTENT

1 INTRODUCTION

In this study the migration dynamics of sand goby *Pomatoschistus minutus* (Pallas, 1770) were investigated in the Scheldt estuary to obtain a better understanding of the role estuaries play in the life history of this species. To this aim two different geochemical tracers were used: the C isotope composition in muscle tissue and [Sr/Ca] chronologies in otoliths. In the following sections of this chapter we present a brief introduction to the functionality of migration in life cycles of fishes, the estuarine fish community, the Scheldt estuary, *P. minutus* and the tracer techniques used. Finally the objectives and outline of the thesis are addressed.

1. FISH MIGRATION

Habitats vary spatio-temporally and organisms are able to move between those different habitats in order to select continuously the best available combination of biotic and abiotic environmental factors for optimizing their fitness (a combined product of lifetime growth, survival and reproduction probabilities). Such movements are typically called migrations irrespective of their frequency and purpose (e.g. daily for feeding or predator avoidance, seasonal for breeding) (Moyle & Cech 1996). In past decennia the phenomenon of migrations was treated and defined as movement patterns with specific emphasis and restrictions such as periodicity, return movement, proportion of the population (Landsborough Thompson 1942, Northcote 1978) or a clear driving force (Harden Jones 1968). Baker (1978) however envisaged migration as a more general and all encompassing phenomenon and defined it as the act of moving from one spatial unit to another. In this view there are no restrictions as to return movements or confinements within time. The flexibility of the definition of a spatial unit implies that the distances covered by the migration may be small or large. The distances might be traversed by groups or by individuals (McKeown 1984). Dingle (1980) added the restriction that migration is a specialized behavior evolved for the displacement of the individual in space. This implies evolutionary adaptations leading to specific migratory patterns, including the underlying physiological mechanisms. Indeed, migrations of fishes generally seem to have evolved as a mechanism to place different life stages in optimal conditions: adult fish are present in those habitats which are favorable for breeding or feeding, and larval and juvenile fish in an environment favorable for survival and growth (Wootton 1992). Even though the

physiological and behavioral costs of a migration can be high, the benefits of the migratory behavior should eventually outweigh those costs, as movements bring fish and their progeny in habitats that are more suitable for them than the habitats they left. Moreover, fish exhibit a number of strategies to reduce energy costs during migrations such as the use of ocean currents or selective tidal stream transport. The ability of larvae to mediate transport increases with age and developmental stage (Metcalfe & Arnold 1997, Jager 1999, Leis 2006).

Classically, the migration triangle is used as a conceptual model that superimposes both the life cycle of migratory fishes and the circular route of migration connecting the different habitats (Fig. 1.1) (Harden Jones 1968, Cushing 1982). In principle, each population of a migratory fish species has a specific movement pattern (migration triangle) inherent to its life history and geographic distribution, as a result of different evolutionary selection pressures and environmental conditions experienced. Corollary to this, populations are regarded as closed entities (member-vagrant theory, Sinclair 1988) whose members exhibit an identical migration pattern. Deviations from the population's trajectory (vagrancy) would mean that they are lost to the population (Secor 2002, McDowall 2007). This paradigm of a population bound migration pattern which is obligatory for each individual, seems to contradict facultative and variable migrations patterns at individual and sub-population levels as observed for several fish species (e.g. Secor & Piccoli 1996, Elfman *et al.* 2000, Secor *et al.* 2001, Tsukamoto & Arai 2001, McDowall 2007). Variable habitat use within populations is consistent with the idea of optimal habitat choice: individuals generally select those habitats that optimize their fitness, based on intrinsic habitat quality and biotic interactions (Fretwell & Lucas 1970, Morris 2003). The abiotic environment generally sets the absolute limits of survival, growth and reproduction of an organism. Some species are tolerant to a wide range of a given factor (eurytopic species), whereas others only tolerate a narrow range (stenotopic species). The spatio-temporal distribution of fish is relatively strongly affected by abiotic factors, especially temperature, oxygen, salinity and water movement are important factors (Wootton 1992). Nevertheless the occurrence of fishes in a habitat is also influenced by biotic interactions (conspecific and heterospecific) (Begon *et al.* 1996). For example, organisms can choose to move (disperse) or be restricted to energetically less favorable habitats due to increased competition for resources or higher predation risk in the more favorable habitat (Morris 2003).

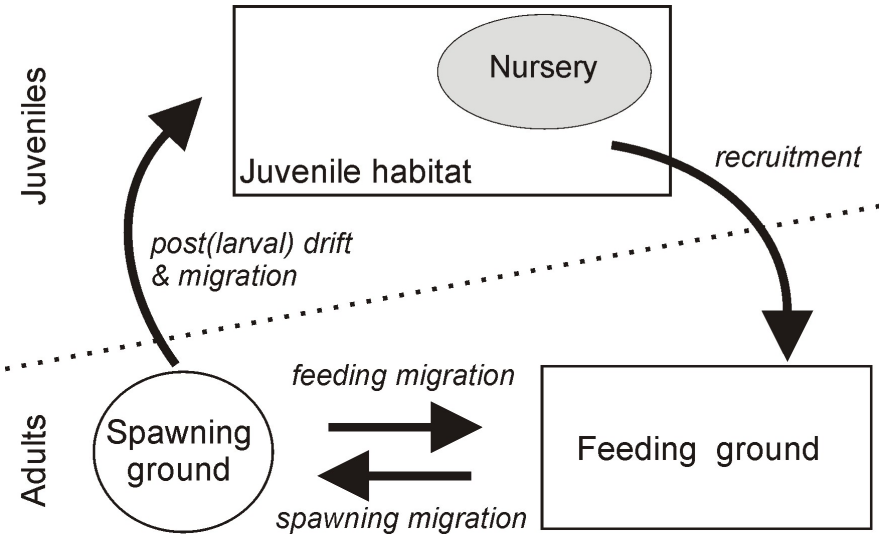

Fig. 1.1 *The classic migration triangle linking ontogenetic stages with specific habitats and demonstrating the functional role of migrations in the life history of fishes (after Cushing 1982). The dotted line separates the adult life stage from the juvenile one. A nursery area as a subset of the juvenile habitat is added (after Beck et al. 2001)*

Secor (1999) reconciled these apparently contrasting visions (population bound migration pathway vs individual habitat choice) regarding the role of migration in fish population dynamics in the contingent hypothesis, by assuming that ontogenetic migration behaviors subsume habitat choice (Fig. 1.2). The migration circuit (triangle) defines necessary ontogenetic habitat shifts and follows a mean trajectory corresponding to habitats of highest fish density (i.e. most suitable habitat according to the ideal free distribution). The trajectory itself is a path that expands or contracts depending on the energetic demands of the population: fish can diffuse from the mean trajectory to less favorable habitats. If an individual or a school deviates too far from the mean trajectory, it may not be able to rejoin the migration circuit. Whether or not these deviations from the mean migration trajectory occur during all life stages or only during particular life stages they can become a discrete ontogenetic pattern of habitat use reflecting a distinct behavior within the population. Sub-populations with divergent migration behaviors, resulting in multiple migration patterns within one population are called contingents (Clark 1968) (Fig. 1.2). Contingents may represent important phenotypic modes of response favoring population or metapopulation resiliency as divergent migrations within a population contribute to a decreased vulnerability to stochastic and anthropogenic effects (Secor 1999, 2007). Migrations within fish populations are influenced by changes in climate, flow regimes as well as degradation of migration corridors (Leggett 1977).

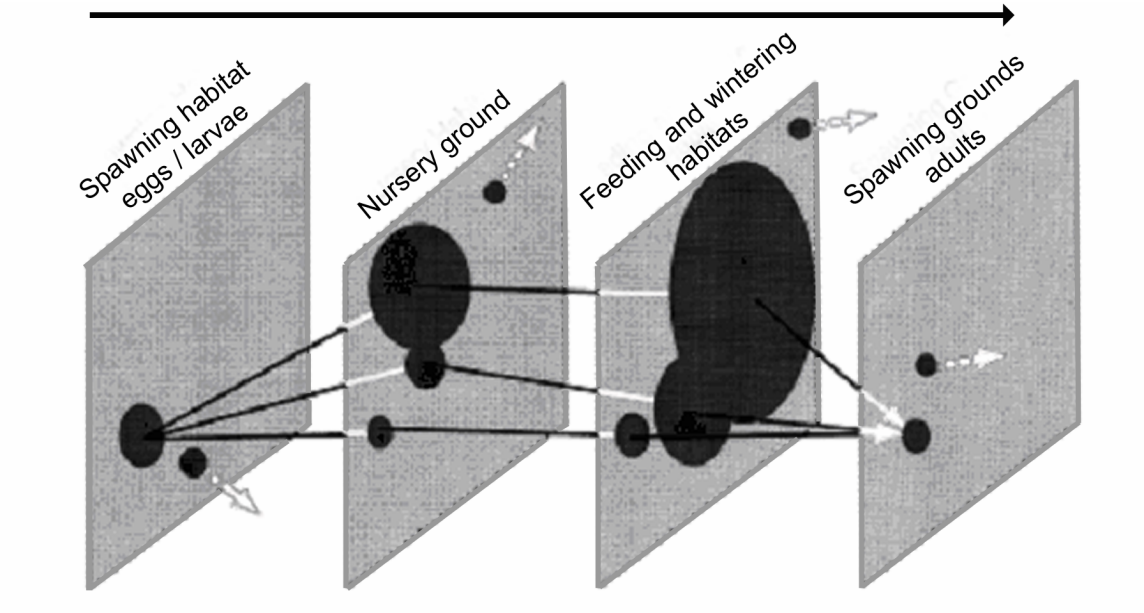

Fig. 1.2 *Three different migration patterns caused by contingent behaviors. Each pane represents a cross-sectional profile of spatial occurrence (e.g. horizontal and vertical axes of each pane are latitude and longitudes). The ontogenetic pattern of migration occurs from left to right. The three lines represent different migration circuits, while the centroids exhibit dispersion of the population aggregations. Open arrows indicate possible migration fates of vagrant aggregations, although not drawn, they can also rejoin one of the population aggregations. Note that collapse of the panes into an overall framework results in multiple migratory triangles (Secor 1999).*

Unfortunately, fish migration patterns are poorly understood for some of the most ecologically and economically important species. This is partly caused by a lack of fundamental knowledge on the variation in movement patterns, namely whether migrants respond as a single population or as multiple contingents. Describing individual movements and habitat specific residency is thus critical for understanding population structure (Able & Grothues 2007).

Note that the terms 'migration' and 'dispersal' could be confounding as the former is often regarded as the mass directional movement of large numbers of a species (Northcote 1978) and the latter as the spreading of individuals (actively or passively) away from others. Yet, on the level of the individual there is no sharp distinction between 'migration' and 'dispersal' as it is the individual that actually moves (Begon *et al.* 1996). As this study mainly focuses on individual movement patterns of fish we do not make a distinction between both terms.

2. ABOUT (EUROPEAN) ESTUARIES AND THEIR ROLE IN THE LIFE CYCLE OF (MARINE) FISHES

Being transition zones between salt and fresh water, estuaries are characterized by a measurable dilution of sea water with fresh water, derived from tidal action and land drainage (Fairbridge 1980, Elliott & McLusky 2002). This makes them very dynamic with a wide variety of rapidly changing physical, chemical and biological gradients, mainly based on hydrodynamics, salinity, oxygen concentration, turbidity and temperature (Haedrich 1983, McLusky 1993). This highly variable environment in space and time imposes considerable physiological and physical stress on an organism, so that most species simply cannot survive there. The brackish water area where strong salinity changes occur and turbidity is maximal, is therefore characterized by low species diversity. However, the high input of nutrients and organic matter allow high densities of the few species which adapted to this harsh environment (Day *et al.* 1989, Mees *et al.* 1993).
In general, two food chains are considered in estuaries. They are basically fuelled by either phytoplankton and suspended benthic diatoms (microphytobenthos) or by detritus as a source of energy (de Sylva 1975, Elliott & Hemmingway 2002). Detritus originates from autochthonous material, i.e. organic matter produced in the estuary itself and allochtonous material which comes from upstream reaches, tributaries, the sea, land run-off and sewages. The bed dominated detrital food chain and the phytoplankton based food chain are linked by several energy transfers, especially in estuaries with a well mixed water column. In turbid systems, however, primary production is often light limited and the high organic loads that estuaries receive predominantly fuel the higher trophic levels through bacterial production and detrivorous invertebrates. Because of a continuous input and recycling of organic loads, a high productivity is maintained in estuaries (Day *et al.* 1989, Soetaert *et al.* 1994, Heip *et al.* 1995, Fockedey & Mees 1999, Soetaert *et al.* 2000, Elliott & Hemmingway 2002, De Brabandere 2005). As respiratory processes often exceed the in situ autotrophic production, estuaries are known to be net heterotrophic. Yet, there is a gradient in the degree of heterotrophy, with the highest degree in the maximum turbidity zone. In strongly eutrophic estuaries the respiratory processes in the brackish and fresh water zones can cause severe oxygen declines (Soetaert & Herman 1995a, Frankignoulle *et al.* 1998, Herman & Heip 1999, Marchand *et al.* 2002).

Despite the fact that estuaries are energetically demanding, a large number of fish species spends at least part of their lives in estuaries. The estuarine fish assemblage typically comprises marine species which invariantly form the majority, next to the freshwater species, the estuarine resident species and the diadromous species (Haedrich 1983, Day *et al.* 1989). The use of the estuary by these different species and their relative abundance is very dynamic and varies among species, estuaries, localities, years, seasons and even among individuals of the same species (Claridge *et al.* 1986, Day *et al.* 1989, Henderson 1989, Potter *et al.* 1990,

Elliott & Dewailly 1995, Potter *et al.* 1997, Thiel & Potter 2001, Pihl *et al.* 2002, Thiel *et al.* 2003, Maes *et al.* 2005b, Fablet *et al.* 2007). Despite this very fluctuating composition the basic structure of the estuarine fish community is rather stable and predictable in terms of species specific responses. This stability is the result of (1) regular spatial distribution of species along physicochemical gradients: mainly salinity, turbidity and oxygen content (Blaber & Blaber 1980, Henderson 1989, Cyrus & Blaber 1992, Maes *et al.* 1998b, Whitfield 1999, Thiel & Potter 2001, Maes *et al.* 2007), (2) the predictable seasonal movements of fishes in and out of the estuaries which have been associated with the species specific spawning periods and temperature (Claridge *et al.* 1986, Power *et al.* 2000, Thiel & Potter 2001, Attrill & Power 2004, Maes *et al.* 2004, Maes *et al.* 2005b); (3) the dominance of eurytopic species (Whitfield 1999); and (4) the robust nature of estuarine food webs containing abundant food resources for fish (Day *et al.* 1989, Moyle & Cech 1996, Pihl *et al.* 2002). Large scale patterns in the structure of the estuarine fish assemblage are predominantly a result of individual species responses to dominant abiotic gradients, as well as ontogenetic migrations, while smaller scale patterns appear to be the result of species interactions (foraging, competition and/or predator avoidance) (Day *et al.* 1989, Martino & Able 2003).

In order to study the structure of estuarine fish assemblages more appropriately, to denote the primary habitat use made by different species, to classify and compare estuarine systems and to evaluate their ecological quality, functional guilds were defined. The ecological guild based on the life cycle of fishes is probably the most widely used, as the different categories imply not only different habitat functions but also a certain degree of estuarine dependence (Haedrich 1983, Elliott & Dewailly 1995, Potter *et al.* 1997, Whitfield 1999, Mathieson *et al.* 2000, Thiel & Potter 2001, Pihl *et al.* 2002). There is however some disagreement about the different life history categories and the designation of the different fish species found in estuaries to these categories (Whitfield 1999, Thiel *et al.* 2003, Ray 2005, Elliott *et al.* 2007). Recently, Elliott *et al.* (2007) revised the ecological guild approach and reconsidered the different ecological categories in a global context proposing the following main categories:

- Marine stragglers — Species that spawn at sea and typically enter estuaries in low numbers mostly restricted to lower reaches of estuary.
- Marine migrants — Species that spawn at sea and enter estuaries in large numbers, often as juveniles; these species are highly euryhaline.
- Estuarine species — This category is further subdivided in species capable of completing their entire life cycle within the estuarine environment (estuarine residents) and species that have larval stages of their life cycle completed outside the estuary or are also represented by discrete marine or freshwater populations (estuarine migrants).

- Freshwater stragglers — Freshwater species found in low numbers in estuaries and whose distribution is usually limited to low salinity.
- Freshwater migrants — Freshwater species found regularly and in moderate numbers in estuaries and whose distribution can extend further beyond oligohaline sections.
- Diadromous species — Species that undergo their greatest growth at sea but spawn in freshwater (anadromy) or species that mainly live and grow in rivers to grow but spawn at sea (catadromy). Elliott *et al.* (2007) further recognize semi-anadromous, semi-catadromous and amphidromous species.

In correlation with the ecological guild classification five different functions that estuarine habitats may fulfill for fishes are generally considered: nursery area, spawning area, feeding area, refuge area and a pathway for diadromous species (Haedrich 1983, Pihl *et al.* 2002). Based on the number of species involved for each function Pihl *et al.* (2002) concluded that feeding is the most frequent use overall in European estuaries, followed by the use as a nursery. Some species use estuaries for spawning and just a few species are recorded as diadromous. The use as a refuge is often mentioned but it is hard to separate it from the other uses and it is considered to be an integral part of the nursery function (Pihl *et al.* 2002).
A nursery is defined as a valuable component within the juvenile habitat where juveniles aggregate in relatively higher densities and where fitness is enhanced through better feeding conditions, faster growth, refuge opportunities and a high connectivity with adult habitats, ultimately leading to higher contribution to the adult population than other juvenile habitats (Beck *et al.* 2001, Dahlgren *et al.* 2006). Because estuarine fish communities are typically dominated by young-of-the-year (YOY) fish, predominantly belonging to marine species, estuaries are often referred to as (important) nursery grounds where juveniles (Pleuronectidae, Gadidae, Clupeidae) can profit from the abundant food resources, shelter from predation (due to the high turbidity) and/or favorable thermal conditions. Juveniles would then leave the estuary after attaining a critical threshold length (Day *et al.* 1989, Elliott & Dewailly 1995, Potter *et al.* 1997, Maes *et al.* 1998a, Thiel & Potter 2001). Besides juveniles, estuarine communities mainly consist of older age classes of marine species that visit the estuary on a seasonal basis (Clupeidae, Mugilidae). Although these fishes might seek some refuge, they are known to profit from the highly productive estuarine areas (Haedrich 1983). In addition to these marine species occurring in high densities, there are marine species that only occur in low numbers (marine stragglers) (Callionymidae) and freshwater species (Cyprinidae, Percinidae). Although these fishes also feed in the estuary, their occurrence there is considered to be rather accidental. The fact that freshwater species mainly occur in low numbers in European estuaries is because tidal fresh water areas are minor habitats in European estuaries and also due to the extensive human impact on these areas (Elliott &

Hemmingway 2002). Not many fishes in the northern hemisphere spawn in estuaries and can be considered as estuarine resident, yet these species are often very abundant (Sygnathidae, Gobiidae). Estuarine spawners are mostly small species with adapted reproductive features (Haedrich 1983, Claridge *et al.* 1986, Whitfield 1999, Pihl *et al.* 2002). Characteristic to these species is the production of benthic, adhesive eggs, necessary to prevent the eggs from being swept away by the currents. Diadromy (Anguillidae, Salmonidae) forces fish to migrate between marine waters, brackish and freshwater areas for spawning (McDowall 1997) and consequently they use the estuary as a migration route.

Fishes using estuaries can be grouped into obligate and facultative users. This was often based on the life history categories, although recently this distinction is used to further subdivide categories (e.g. marine estuarine-dependent vs. marine estuarine-opportunist) (Elliott *et al.* 2007). The widespread use of estuaries by juveniles and adults of so many species on a seasonal basis has led to the concept of estuarine dependence implying that the estuary is required for some part of the life cycle of these organisms (Day *et al.* 1989, Elliott & Dewailly 1995, Potter *et al.* 1997, Able 2005). However, this often cited estuarine dependence of many species is in fact an intuitive observation and some species formerly considered to be estuary dependent for their existence appear to use estuaries rather facultative. The term 'estuarine opportunist' might thus be more appropriate (Potter *et al.* 1997, Tsukamoto & Arai 2001, Ray 2005). The question whether or not populations are truly dependent on estuaries to complete their life cycles made fisheries research recently focusing on estuarine habitat use by individuals and on the connectivity between estuarine juvenile habitats and the adult spawning populations in order to identify true nurseries (de Pontual *et al.* 2000, Beck *et al.* 2001, Gillanders *et al.* 2003, Kraus & Secor 2004a, Able 2005, Ray 2005, Dahlgren *et al.* 2006). This knowledge is pivotal because estuarine fish communities can change dramatically (and permanently) in response to severe anthropogenic disturbances, despite the robustness and flexibility of fish in estuaries. This is clearly evidenced by an impoverished diversity in many urban estuaries today (Able *et al.* 1999, Elliott & Hemmingway 2002, Breine *et al.* 2007, Maes *et al.* 2007). In order to develop restoration and preservation programs for estuarine and marine habitats as well as effectively manage fish populations using estuaries, it is necessary to understand the processes that control estuarine habitat use by fishes as well as the role of estuaries in the life history of these fishes. However, to understand processes in ecology the patterns should be understood first (Underwood *et al.* 2000).

3. THE SCHELDT ESTUARY

The river Scheldt is a rain fed lowland-river, which originates in the northern part of France (St-Quentin) and flows through Belgium to discharge into the North Sea near Vlissingen (The Netherlands). The total catchment area is about 21 800 km² and the total length of the river is 355 km, with a fall of 110m. Approximately 10.4 million people live in the entire Scheldt river basin, forming a dense population of about 477 inhabitants km^{-2} (Meire *et al.* 2005).

The river Scheldt has a shallow, well mixed macrotidal estuary which is approximately 160 km long from the mouth in the Netherlands to Ghent (Belgium) where a complex of sluices stop the tidal movement. The lower and middle estuary is called the Westerschelde (the Dutch part) and is characterized by a complex morphology of tidal flood and ebb channels, surrounding several large intertidal mud and sand flats. The average channel depth is about 15-20 m. Near the Dutch-Belgian border the estuary narrows. The river, here called Zeeschelde, is characterized by a single channel. Due to the funnel-shaped morphology of the estuary, the mean vertical tidal range is maximal (5.2 m) in the freshwater tidal reaches (90 km). The vertical range is 3.8 m at the mouth and 1.9 m at the head of the estuary. The mean river discharge amounts to 180 m^3s^{-1} during winter, while average summer values decrease to 60 m^3s^{-1}. The water residence time varies by season, with about two months in winter and three months in summer (Soetaert & Herman 1995b, Baeyens *et al.* 1998).

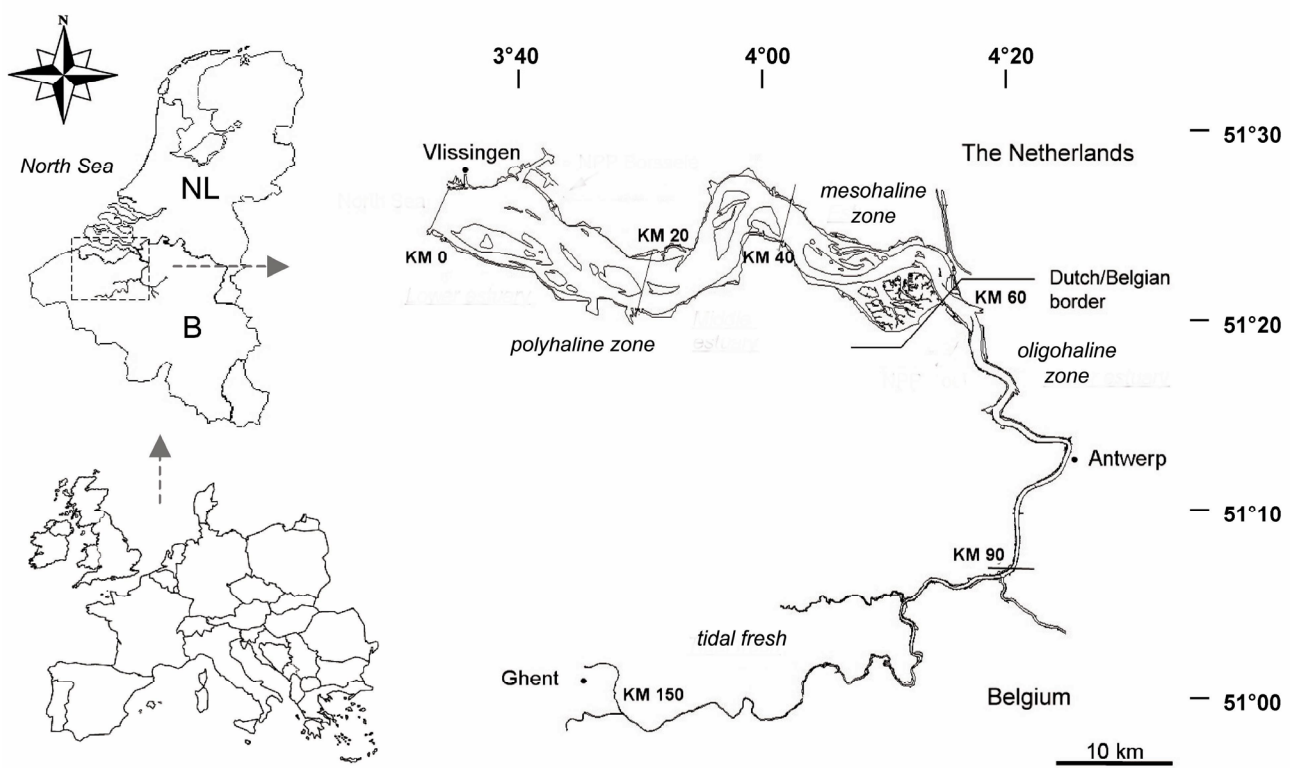

Fig. 1.3 *Map of the Scheldt estuary with indication of the major salinity zones. The kilometers represent the distance to the mouth near Vlissingen.*

The Scheldt estuary is the only remaining true estuary in the Dutch Delta area with a clear salinity gradient. The polyhaline zone (salinity 18-30) ranges from the mouth of the estuary to approximately 40 km upstream. The mesohaline zone (brackish: salinity 5-18) is generally situated between 40 km upstream and the Dutch-Belgian border at 60 km from the mouth while the oligohaline zone (brackish: salinity 0.5-5) stretches to about 90 km. The remainder is a tidal fresh water area. As the longitudinal salinity profile is partly determined by the magnitude of the river discharge the transition zone between fresh and salt water can shift seasonally over a distance of 20 km. Vertical salinity gradients are small or negligible (Meire *et al.* 2005, Van Damme *et al.* 2005). Turbidity is high, especially in the upper estuary where suspended matter can reach concentrations up to 200 mg l^{-1}. The location of the maximum turbidity zone depends on the freshwater discharge and is situated at about 110 km from the mouth during dry periods and at about 50 km during wet periods. Two maximum turbidity zones might be observed: one at the freshwater/seawater interface and a second resulting from tidal asymmetry (Baeyens *et al.* 1998, Herman & Heip 1999, Meire *et al.* 2005).

The Scheldt estuary is subject to severe eutrophication as it receives high inputs from untreated domestic, industrial and agricultural activities. The enormous input of allochtonous organic matter and nutrients supports high activities of heterotrophic and nitrifying bacteria. These oxygen consuming processes can cause anoxic conditions in the upper and freshwater tidal area. Because of increased waste water treatment, dissolved oxygen concentrations gradually improved in the last two decades. A spectacular improvement of the water quality occurred in 2007 after a new sewage treatment facility in Brussels was brought into use. Nevertheless low oxygen levels still occur in the fresh water part, especially during summer. The Scheldt estuary is a net heterotrophic ecosystem in which the annual bacterial production exceeds the primary production. However, there is a gradient in the degree of heterotrophy, with the lowest degree found near the sea and the highest in the high turbidity region (Soetaert & Herman 1995c, Goosen *et al.* 1999, Gazeau *et al.* 2005, Van Damme *et al.* 2005, Soetaert *et al.* 2006).
Since centuries, the estuary also suffered substantial losses of intertidal habitat due to dike building, land reclamation through embankments and dredging. These changes, together with the low water quality, contributed to the impoverishment of the biotic communities in the Scheldt estuary (Meire *et al.* 2005, Van den Bergh *et al.* 2005).

Different aspects of the ecosystem of the Scheldt estuary have been well studied as proven by an abundant literature (e.g. Heip (1989) and Hydrobiologia vol. 540: Special issue on the ecological structures and functions in the Scheldt Estuary). The estuarine phytoplankton community was investigated by Muylaert *et al.* (2000), while the zooplankton is described by Soetaert & Van Rijswijk (1993) and Tackx *et al.* (2005). Ysebaert *et al.* (2003) and Seys *et al.* (1999) confirm a clear shift in macrobenthic species and functional groups along the salinity

gradient, while Ysebaert *et al.* (2000) report such a change for birds. The fish communities in the different parts of the Scheldt estuary are also well documented. The spatio-temporal patterns of the fish community in the Westerschelde was described by Hostens & Mees (1999) and Hostens (2000), while Beyst *et al.* (1999) reported on postlarval fishes in this part of the estuary. Nekton communities in marshes along the salinity gradient were described by Hampel *et al.* (2004). The composition and structure of the fish community in the Zeeschelde was extensively studied by Maes *et al.* (1998a, 1998b, 2004, 2005b). These authors recorded during a period of ten years (1991–2001) 62 fish species in the brackish water area of which 17 marine estuarine opportunists, 23 marine stragglers, 16 freshwater and 4 diadromous species. Three goby species represented about 68% of the total catch in numbers. Besides a mathematical model to predict optimal habitat use by herring along a marine-estuarine gradient (Maes *et al.* 2005a), no study has explicitly investigated the migration dynamics of marine fishes in the Scheldt estuary.

4. THE SAND GOBY *POMATOSCHISTUS MINUTUS* (PALLAS, 1770)

The Gobiidae comprising about 200 genera and an estimated 1800 species, is the largest family of marine fishes. They are relatively small, demersal fishes occurring mainly in (sub)tropical coastal areas, although a considerable number of species are present in fresh and brackish water as well. At least 52 species of Gobiidae are recorded from the north-eastern Atlantic and Mediterranean coasts. Gobiidae have a typical moderately elongated cylindrical body form and dorsolateral eyes. The pelvic fins are thoracic and fused, forming an adhesive disk. This allows them to remain stationary on the substrate in relatively strong currents. They have intermittent swimming behavior with short darting movements (Miller 1986). It is assumed that the group has evolved in estuarine or shallow marine habitats, which is supported by the fact that most species have a form of reproductive behavior that is typical for estuarine and shallow water species (Dando 1984). Their capacity to colonize riverine habitats of recent origin such as exist on Pacific Islands is also suggestive of a euryhaline past. Many riverine gobies have retained amphidromous characteristics but other species complete their life cycle in fresh water (Moriyama *et al.* 1998, Huyse *et al.* 2004, Chang *et al.* 2006, McDowall 2007).

The genus *Pomatoschistus* comprises 11 species, which are very abundant along the Atlantic European coast (Fonds 1973, Miller 1986), but only four of them are found in the Dutch and Belgian coastal areas. These are the sand goby *P. minutus* (Pallas 1770), painted goby *P. pictus* (Malm, 1865), the common goby *P. microps* (Krøyer, 1938), and Lozano's goby *P. lozanoi* (de Buen, 1923) (Fonds 1973, Hamerlynck 1990). *P. minutus* is morphologically often difficult to distinguish from *P. Lozanoi* and *P. microps*, but they differ in size, body pigmentation pattern and the arrangement of papillary pattern of the head lateral system. The

latter is the most reliable morphological criterion between *P. lozanoi* and *P. minutus* (Webb 1980, Hamerlynck 1990). Additionally these *Pomatoschistus* species can be distinguished based on allozyme markers (Wallis & Beardmore 1984) and recently a faster new molecular technique was developed based on PCR-RFLP (Larmuseau *et al.* 2008).

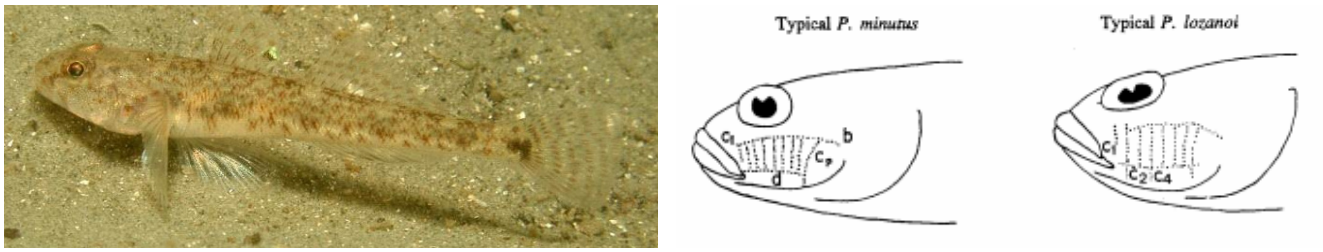

Fig. 1.4 *Sand goby* Pomatoschistus minutus *(Pallas, 1770) on the left and the papillary pattern of the head lateral system to distinguish P. minutus from P. lozanoi on the right (Miller 1986).*

P. minutus geographic distribution encompasses the eastern Atlantic from the north of Norway and the Faroe Islands to the south of Spain, the Baltic Sea, the Mediterranean Sea and the Black Sea. The sand goby prefers sandy and muddy sediments, to depths of about 30m (Miller 1986). This species usually spawns at sea (Fonds 1973), although spawning in lagoons and estuaries has been reported as well (Costa 1988, Arruda *et al.* 1993, Elliott & Hemmingway 2002). Because of this the sand goby is considered to be an estuarine resident (Elliott & Dewailly 1995), although this is contested by Thiel *et al.* (2003), who categorize it as a marine-estuarine opportunist.

Like all species of the genus *Pomatoschistus* the sand goby displays a courtship behavior in which the male establishes a territory, builds the nest under an empty bivalve shell (e.g. *Ostrea edulis*, *Mya arenaria*) or under a rock and courts females. The female attaches her eggs at the ceiling of the nest and these are subsequently fertilized by the male, who protects and fans them until they hatch (Fonds 1973, Nellbring 1993). Larvae are about 3 mm at hatching and they are pelagic for at least one month before they become demersal after metamorphosis at about 12 mm. Females are repeat spawners, producing several batches of eggs per spawning season and males can guard batches of eggs from different females at the same time (Fonds 1971, 1973, Miller 1986).

The sand goby is a generalist and opportunistic consumer feeding on endo- and epibenthic prey, yet zooplankton and hyperbenthic prey items also occur in its diet. As they grow the diet becomes more diverse and consists of amphipods, mysids, polychaetes, shrimps, copepods and siphons of bivalves, but during their (post)larval stage they predominantly depend on zooplankton. They are known to use intertidal areas (e.g. estuarine mud flats) for feeding (Fonds 1973, Doornbos & Twisk 1987, Gee 1987, Jaquet & Raffaelli 1989, Delnortecampos &

Temming 1994, Hamerlynck & Cattrijsse 1994, Hostens & Mees 1999, Maes *et al.* 2003, Salgado *et al.* 2004, Leitão *et al.* 2006). On the other hand *P. minutus* is quite important in the energy budget of YOY-gadoids (Salvanes & Nordeide 1993). In addition they are preyed upon by a spectrum of fish species such as *Gobius niger*, *Anguilla anguilla*, *Trigla lucerna*, *Ciliata mustela*, *Echiichthys vipera*, *Scophthalmus* spp., but also by piscivorous birds and young harbour seals (Hamerlynck & Hostens 1993, Hamerlynck & Cattrijsse 1994). Therefore they are considered to be an important link between benthic invertebrates and higher trophic levels. Due to the abundance of food in estuarine and coastal areas it was concluded that they have relatively little impact on the prey community and that goby populations are probably top-down regulated (Doornbos & Twisk 1987, Jaquet & Raffaelli 1989, Ehrenberg *et al.* 2005).

Considerable intraspecific differences in life history traits have been recorded for distinct geographical populations of the sand goby. As temperature is important for spawning (Fonds & van Buurt 1974) the breeding period changes gradually with latitude: early spawning (december-april) in southern Europe (the Mediterranean, Iberian Peninsula) and delayed spawning (May-July) at the Norwegian coast (Hesthagen 1977, Bouchereau & Guelorget 1998). In the North Sea spawning occurs from February to June (Healey 1971, Fonds 1973). Furthermore, the life cycle of the sand goby in the Mediterranean region is rather 'contracted' with fast growth and rapid maturity, increased spawning effort and shorter life spans (8-14 months). In contrast, in Northern Europe (above the English Channel) they have a more 'protracted' life cycle with shorter spawning seasons and longer life spans (20-24 months) (Fonds 1973, Bouchereau & Guelorget 1998, Pampoulie *et al.* 1999, Ehrenberg *et al.* 2005). The Portuguese populations showed life history characteristics situated in between those of the Mediterranean and the North Atlantic (Arruda *et al.* 1993, Leitão *et al.* 2006, Dolbeth *et al.* 2007). Sand gobies in northern Europe are considered to make thermic migrations from estuaries and shallow waters into deeper warmer waters during winter and subsequent spawning migrations to shallow coastal waters after winter (Healey 1971, Fonds 1973, Hesthagen 1977, Claridge *et al.* 1985, Doornbos & Twisk 1987, Ehrenberg *et al.* 2005). In addition Hesthagen (1977) reported avoidance of waters above 20°C in Norway. Emigration from estuaries and lagoons during winter in southern Europe is most likely for the purpose of reproduction (Pampoulie 1999, Dolbeth *et al.* 2007).

Genetic differentiation was found among several regions of the sand goby's distributional range. Based on allozyme, microsatellite and mitochondrial DNA markers a strong divergence was observed between sand gobies in the Adriatic Sea (eastern Mediterranean) on the one hand and populations in the western Mediterranean Sea and the Atlantic on the other hand (Stefanni *et al.* 2003, Gysels *et al.* 2004) and it was suggested to consider sand gobies of the Adriatic Sea as a distinct species (Huyse *et al.* 2004). The populations in the eastern Mediterranean and the Atlantic Ocean appeared to have a limited genetic diversity. However, this is contradicted by (Larmuseau *et al.* in prep.) who recently found evidence of more genetic

diversity. According to this study there is substantial genetic difference between western Mediterranean (Gulf of Lyon) and Atlantic sand goby populations. Additionally within the Atlantic group, the populations along the Iberian Peninsula are historically separated from the populations north of the Gironde and form a separate Evolutionarily Significant Unit (ESU). Moreover, limited gene flow was found among the populations of the North Atlantic (Southern Bight of the North Sea, Baltic Sea and Irish Sea). Pampoulie *et al.* (2004) reported population differentiation over a small geographical scale and suggested the existence of two spatially separated breeding units in the southern bight of the North Sea, namely in the Oosterschelde (a marine bay north of the Scheldt estuary) and the Belgian coastal area. These results however were not supported by Larmuseau *et al.* (in prep).

5. TWO BIOGEOCHEMICAL MARKERS TO STUDY FISH MOVEMENTS

Nowadays natural biogeochemical markers are used to infer geographical origins of animals (e.g. Hobson *et al.* 1999, Kelly *et al.* 2002), to differentiate among populations (e.g. Campana *et al.* 2000) or to reconstruct individual movements of organisms (e.g. Limburg 1998, Arai *et al.* 2003, Chang *et al.* 2006). We relied on stable isotopes in muscle tissue and otolith [Sr/Ca] to study sand goby migratory behaviour. Here we present an introduction about the specific terminology, the application of both techniques in animal migration studies and corresponding conditions.

5.1. Stable isotopes

Isotopes are any of the different forms of a chemical element each having a different atomic mass (mass number) caused by a different number of neutrons in the nucleus, yet with the same number of protons and electrons. In contrast to radioisotopes which have an unstable nucleus, stable isotopes are not radioactive. In nature, there are two naturally occurring stable isotopes of carbon and nitrogen. For carbon, about 98.9% of the carbon is ^{12}C, while about 1.1% is ^{13}C and for nitrogen, about 99.64% is present as ^{14}N and 0.36% as ^{15}N (Peterson & Fry 1987, Gannes *et al.* 1998).

Because absolute abundances of stable isotopes cannot be measured with great precision the isotopic composition of a sample is measured as the relative difference of the heavy to light isotope ratio between the sample and a common standard (Lajtha & Michener 1994). Small differences in the ratio between two compounds can actually be accurately measured using a mass spectrometer. This measurement of the difference relative to a standard is typically expressed in the δ-notation in units per mil (‰):

$$\delta X\ (‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3,$$

with X denoting ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, which is the ratio of the fractional abundances of heavy to light isotope. The standards for $\delta^{13}C$ and $\delta^{15}N$ measurements are VPDB (Vienna PeeDee Belemnite) and atmospheric N_2, respectively. A negative δ-value means that the isotope ratio in the sample is lower than in the standard, or in other words is depleted in the heavy isotope. Samples with higher δ-values are relatively enriched in the heavy isotope (Peterson & Fry 1987, Fry 2006).

The tiny mass differences due to a difference in the number of neutrons cause isotopes to behave differently in physical processes and (bio)chemical reactions. In general, the mass differences make the formation and destruction of bounds between heavy atoms more difficult than for light ones or make the heavy isotopes move slower than light isotopes in e.g. diffusion reactions. As a consequence the abundance of stable isotopes of an element will vary between substrate and product. This process of isotope redistribution is termed (isotope) fractionation and refers to a depletion or enrichment of the heavy isotope. Opposite to the process of fractionation is the mixing process which combines isotopes from different sources. Fractionations can occur during thermodynamic equilibrium processes as well as during kinetic processes (time dependent) and the effect of isotope fractionation on the isotopic composition of the substrate and product differs between open and closed systems (Peterson & Fry 1987, Farquhar *et al.* 1989, Lajtha & Michener 1994, Gannes *et al.* 1997, Fry 2003, 2006). Several types of fractionation factors (e.g. $\alpha = R_{source}/R_{product,}$ Farquhar *et al.* 1989) have been developed to describe the isotopic changes occurring between products and reactants in different systems and we refer to Peterson & Fry (1987), Farquhar *et al.* (1989), Martínez del Rio & Wolf (2004) and Fry (2006) for elaboration of these specific fractionation effects and factors. Given the scope of this work we restrict this introduction to the discrimination value 'Δ' which is an approximation for the extent of discrimination against an isotope when small fractionation effects occur between isotopes in natural abundances.

$\delta_{product} = \delta_{source} - \Delta.$

This equation applies exactly if a large supply of reactant is available (open system) and does not limit the reaction rate, and it expresses the isotopic discrimination expected at an instant in time (Peterson & Fry 1987, Fry 2003).

So, without fractionation effects the isotopic composition of the product is similar to that of the source, but if fractionation occurs the δ-value of the product also reflects information about the process. For example, the $\delta^{13}C$ values of a C3 plant (-28‰) reflect both the signal of the source (i.e. atmospheric CO_2 at -8‰) as the magnitude of the discrimination against heavier $^{13}CO_2$ (Δ = 20‰) during CO_2 assimilation (Peterson & Fry 1987, Farquhar *et al.* 1989).

The isotopic composition of animals strongly reflects that of their food and environment as stable isotopes are incorporated directly from the diet into the consumer's body. Nevertheless,

there are small differences in isotopic composition between a consumer and its diet. These differences are often called trophic fractionation although trophic shift or trophic discrimination would be more correct ($\Delta_{trophic} = \delta_{tissue} - \delta_{diet}$) (Martinez del Rio & Wolf 2004). Organisms in steady state are generally slightly enriched in ^{13}C relative to their diet by about 0-1‰ (DeNiro & Epstein 1978, Post 2002) and for $\delta^{15}N$ this averages around +3.4‰ (Fry & Sherr 1984, Minagawa & Wada 1984, Owens 1987). These values are actually generalized values based on a wide variety of animals and can be quite variable between species, diets, trophic levels or even between tissues of one individual (Adams & Sterner 2000, Vander Zanden & Rasmussen 2001, Post 2002, McCutchan *et al.* 2003, Vanderklift & Ponsard 2003) as they are the result of different fractionating processes and stoichiometric effects (DeNiro & Epstein 1978, Gannes *et al.* 1998). In principle, the difference between an animal's tissue and its diet reflects a dynamic equilibrium between discrimination vectors associated with assimilation and excretion of elements (Olive *et al.* 2003). The isotopic composition of an animal's tissue in equilibrium with the ambient food web can thus be described by a mass balance model in which inputs balance outputs (Fig 1.5) (Fry 2003):

$$\delta_{input} = \delta_{diet} - \Delta_{input} = \delta_{tissue} - \Delta_{output} = \delta_{output}$$

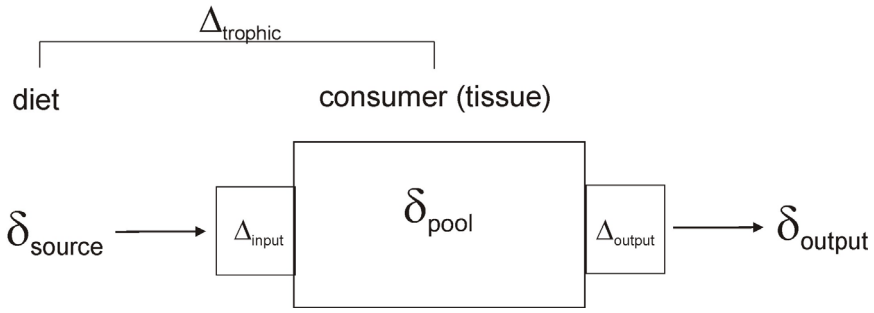

Fig. 1.5 *A steady state model describing the isotopic shift ($\Delta_{trophic}$) between an internal pool (e.g. animal tissue) and its source (e.g. prey item). While input δ-values must equal output δ-values, the value of the internal pool is determined as a balance between discrimination Δ_{input} and Δ_{output} occurring in uptake (e.g. assimilation) and loss (e.g. excretion), respectively (after Fry 2003).*

This is however a simple steady-state model with only one source contributing to the diet. In reality the isotopic composition of animal's body is mostly the result of different food sources or isotope mixtures. Different mass balance mixing models are available that incorporate the proportion of each food source in the animal's diet and take multiple outputs, fractionation, differences in food's stoichiometry and assimilation efficiency into account. For a description of these different models and their assumptions we refer to Phillips & Koch (2002), Fry (2003) and Martínez del Rio & Wolf (2004).

The application of stable isotopes to study animal migrations is based on the fact that stable isotope signatures in animal tissue predictably agree with that of local food webs and that isotopic 'signals' of food webs (habitats) can vary spatially. Due to fractionation and mixing processes the isotope abundance for any element varies naturally in the environment yielding distinct isotopic landscapes and striking isotopic gradients in nature (Hobson 1999, Rubenstein & Hobson 2004) such as the H isotopic distribution in meteoric water and food webs across North America (Hobson *et al.* 1999); C isotope distribution in terrestrial C3 versus C4 food webs (Peterson & Fry 1987), marine versus terrestrial/freshwater food webs (Fry & Sherr 1984) and benthic versus pelagic food webs (France 1995); N isotope distribution in terrestrial/freshwater versus marine food webs (Owens 1987) and undisturbed versus eutrophicated aquatic food webs (Schlacher *et al.* 2005) or S isotope distribution in rooted marsh plants versus phytoplankton based food webs (Krouse & Grinenko 1991). Given that these isotopic patterns in nature are understood they can be exploited to monitor movements of individuals traveling among them. When an animal switches to an isotopically different food source or location the isotopic composition of each tissue will change until equilibrium with the new isotopic environment is obtained (Martinez del Rio & Wolf 2004). The rate at which the isotopic signal of a diet is incorporated into an animal's tissues is an important variable as it determines how long an animal can retain information of a previous diet. For inert tissues like feathers, the isotope composition reflects the environment in which they were formed whereas the rate of isotopic change in other tissues depends on their metabolic activity (Fig. 1.6) (Tieszen *et al.* 1983, Hobson 1999). Thus prior to investigating animal movements it is crucial to first establish if the species of interest is moving between isotopically different environments and feeds in the local food webs, and to use a tissue with the proper rate of isotopic change (Rubenstein & Hobson 2004, Herzka 2005).

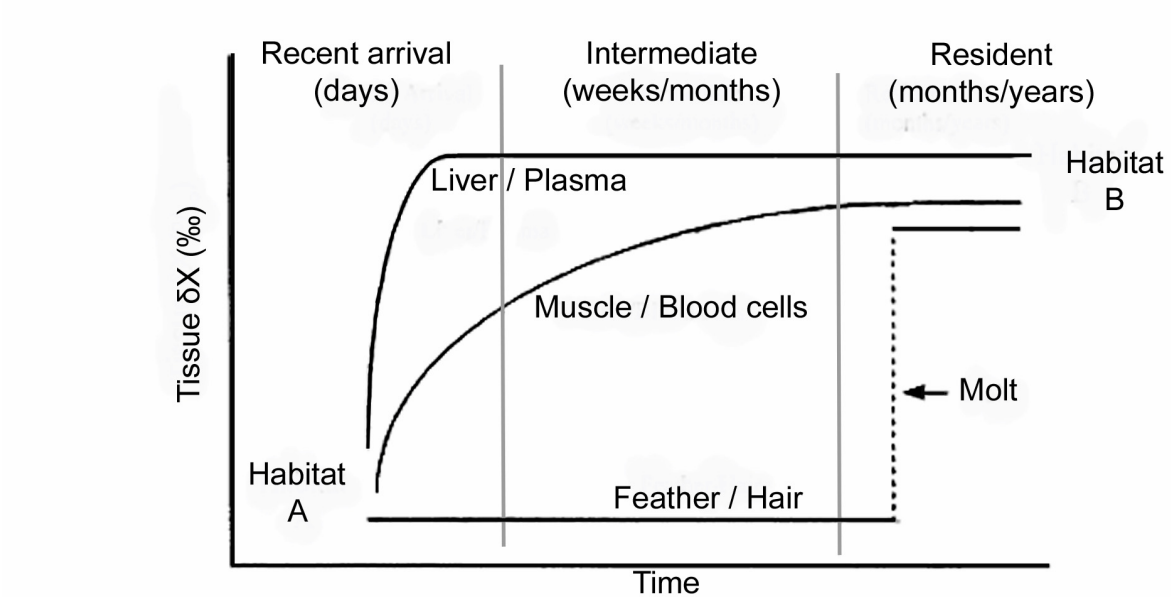

Fig. 1.6 *The expected change in isotopic composition for different tissues of an animal (bird or mammal), when it migrates between two isotopically distinct habitats A en B (Hobson 1999).*

5.2. Otoliths are the fish's black box

Otoliths or 'ear bones' are 3-D calcium carbonate structures in the inner ear that are used for balance and orientation in teleost fish. There are three pairs of otoliths, namely the sagittae which are the biggest, followed by the lapilli and the asterisci. They float in the endolymph of the sac-like structures under the semi-circular canals (Secor *et al*. 1992), where they are formed as a result of a biomineralization process of $CaCO_3$ onto an organic matrix template (Degens *et al.* 1969). $CaCO_3$ can mineralize in three different crystal polymorphs: aragonite, vaterite and calcite. Aragonite is the norm for sagittae and lapilli while most asteriscii are composed of vaterite. Otoliths develop in the later part of the egg stage and grow (or accrete) continuously throughout the lifetime of the fish. This growth follows an endogenous circadian rhythm linked with successive depositions of an organic rich layer (D-zone) and a mineral rich layer (L-zone) providing daily chronologies. On a yearly scale variation in seasonal accretion rate produces alternatively opaque and translucent zones. Additional growth patterns in the otolith structures are discontinuities (or 'check marks'). These correspond to various stresses that are not necessarily regular during the life history of the individual (Panella 1971, Campana & Neilson 1985, Wright *et al.* 2002) and they include important events in the life history of a fish like hatching, metamorphosis, settlement and egg deposition. Additionally these check marks are formed during stress periods, e.g. when water temperature is high or during starvation. Checks are composed of hyaline matter and are mostly not taken into consideration for age determination (Campana & Neilson 1985, Wright *et al.* 2002, Payan *et al.* 2004). The existing relationship between somatic and otolith growth although very complex (Francis 1990, Folkvord & Mosegaard 2002) means that endogenous factors (e.g. hormone levels) and exogenous factors (e.g. temperature, food availability, stress, salinity, temporary hypoxia, tidal rhythms) influencing somatic growth also influence otolith growth (Campana & Neilson 1985). However, otoliths continue to accrete even when somatic growth slowed down naturally and processes governing accretion rate appear also to be related to metabolic rate (Wright 1991, Wright *et al.* 2001).

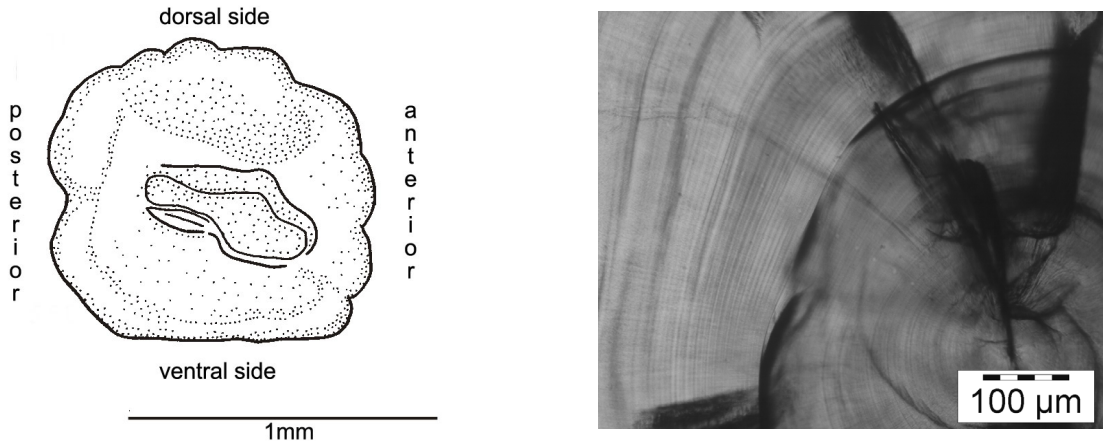

Fig 1.7 *Left: proximal side of the left sagitta of a 50 mm (standard length) sand goby. The shoe-like feature on the surface is the sulcus acusticus, which is closely associated with sensorial epithelium. Right: Detail of sagittal cross section of sand goby otolith. Nucleus and growth increments are visible.*

The macro- and microstructure of otoliths have several applications in fisheries research. The external morphology of the otoliths of teleost fish is complex and species-specific due to genetic and environmental influences. Interspecific differences in shape have been found useful in taxonomy (Nolf 1993) and in diet studies. Similarly, otoliths from archaeological and paleontological finds have been used in the reconstruction of paleoenvironments and paleofauna (Girone *et al.* 2006). The external morphology of the otoliths can be used for identification of species and stocks (Tuset *et al.* 2003). Validated growth increment counts can be used to infer age (Geffen 1992). In particular the 'annuli' which are formed on a yearly basis and the 'daily growth increments' are most important in growth and life history studies (Campana & Neilson 1985). The otolith increment technique is commonly used for the study of early life history, growth, length at age keys, recruitment, migration, mortality, stock assessment and fisheries management (Francis 1990, Stevenson & Campana 1992, Kritzer 2002, Panfili *et al.* 2002, Begg *et al.* 2005). However, because of their small size, otolith examination and reading can be quite difficult and time consuming, especially in smaller stages: correct identification of the location of its nucleus, the interpretation of checks and the counting of the rings at the outer margin require a lot of practice and expertise. Moreover there are currently no objective criteria for identifying secondary structures (checks) (Stevenson & Campana 1992).

Otoliths are relatively pure compounds with a total of inorganic impurities of less than 1% of the otolith weight (Campana *et al.* 1997, Thresher 1999, de Pontual & Geffen 2002). Campana (1999) reported that a total of 31 elements have been detected in otoliths (excluding radioisotopes as Th and Ra). However, due to improvements of analytical detection limits, more elements have been detected recently (de Pontual *et al.* 2000, Geffen *et al.* 2003, Limburg *et al.* 2007). Ca, C and O are the major elements, while Sr, Na, K, S, N, Cl, and P are minor elements which occur in concentrations above 100 ppm. All other elements (e.g. Cd, Pb, Zn, Cu, Ba, Si) are only present at trace or infra-trace levels and can only be assayed by means of very sensitive analytical techniques (de Pontual & Geffen 2002). The exact location of elemental impurities in the otolith matrix is not known but three possibilities were put forward: within the crystal lattice as a substitute for Ca, as an inclusion in interstitial spaces or in association with the proteinaceous matrix (Campana 1999). Although a strong physiological regulation exists during elemental uptake onto the otolith, many elements (trace elements, Sr) are incorporated reflecting the physical and chemical condition of the ambient water. This entails that they may serve as useful environmental markers. Given that otolith growth is continual and that otolith material is neither resorbed nor reworked after deposition, otoliths have the potential to store a complete age-structured record of exposure history to the environment (Kalish 1989, Fowler *et al.* 1995, Campana & Thorrold 2001, Panfili *et al.* 2002). The extent to which this chemical record can be considered to be an undistorted log of the past environments experienced by a fish, is still an open debate (Campana 1999, Tresher 1999).

The translation of environmental factors into otolith composition is a complex process which involves four compartments: (1) the external medium, where variations in abiotic factors occur, (2) the blood plasma which responds to the external medium but exhibits at the same time endogenous variations, (3) the endolymph which modulates the various signals and regulates the biomineralization of the otolith, and (4) the otolith itself, which integrates and records a response to all these signals. Although the inorganic content in blood plasma is mainly derived from surrounding water by uptake through the branchial (freshwater fish) or the intestinal (marine fish) epithelium, a proportion of otolith elements is assimilated from food sources (Farrell & Campana 1996, Campana 1999, de Pontual & Geffen 2002). Elemental discrimination can occur at any of the three interfaces between the four compartments. Discrimination is greatest for major and physiologically regulated ions and least for trace elements, but the site of maximum discrimination is often unpredictable. Very few studies have examined the relationships between the chemistries of these compartments (but see Kalish (1991), Payan *et al.* (1999), Payan *et al.* (2004)). Consequently, there is limited knowledge with regard to the physiological processes of elemental uptake and transport and specifically to the mechanisms of calcification and the relationships between the chemical composition of the otolith and the endolymphatic fluid surrounding it (Allemand *et al.* 2007). However, due to empirically derived relationships between otolith composition and environmental factors it is possible to infer environmental histories from otolith chemistry to a certain extent (Campana & Thorrold 2001, Thorrold *et al.* 2001, Panfili *et al.* 2002). For example, stable isotopes of oxygen are deposited in otoliths close to isotopic equilibrium and with a well defined fractionation effect due to ambient temperature. Consequently otolith $\delta^{18}O$ is not only specific for the ambient water mass, it is also a good proxy of water temperature (Thorrold *et al.* 1997, Radtke *et al.* 1998). Otolith chemistry is also an accurate proxy for concentrations of at least some trace elements (e.g. Sr, Ba, Hg, Pb) in the ambient environment. Elemental concentrations are hereby referenced to Ca as the ambient [element/Ca] ratio is more relevant than the absolute concentrations in the water (Geffen *et al.* 1998, Bath *et al.* 2000, Elsdon & Gillanders 2005). Differences in elemental fingerprints in otoliths have been applied to estimate the contribution of different habitats to fish stocks (e.g. Gillanders & Kingsford (1996), de Pontual *et al.* (2000), Thorrold *et al.* (2001) and Gillanders (2002)). Some of these elements (e.g. Hg and Pb) are hereby characteristic for pollution (Dove & Kingsford 1998, Geffen *et al.* 2003). There is also a strong relationship between $^{87}Sr/^{86}Sr$ in otoliths and in the ambient water as isotopic fractionation during Sr incorporation is negligible. Given that the isotopic composition of dissolved strontium in rivers depends on the local geology, otolith $^{87}Sr/^{86}Sr$ can be used as a location (stream) specific indicator (Kennedy *et al.* 1997, Kennedy *et al.* 2000). Otolith [Sr/Ca] has also been related to ambient temperature, although inconsistent relationships have been found (Secor & Rooker 2000, de Pontual & Geffen 2002). Of all environmental tracers in otoliths, Sr/Ca is probably the most widely used as it appears to serve as an excellent proxy for ambient salinity making it a valuable tracer to reconstruct

diadromous migrations (Secor 1992, Limburg 2001, Rooker *et al.* 2004). Moreover, being a minor element Sr occurs in relatively high concentrations in otoliths and therefore it can be measured more accurately and precisely by most instruments than trace elements (Campana *et al.* 1997). This higher Sr concentration is probably due to the fact that Sr (as a Ca analog) can move from the ambient environment to the endolymphatic fluid across the membranes via para-or transcellular Ca channels, and because Sr substitutes for Ca in aragonite due to a similar ionic radius (Kalish 1991). Thus, contrary to other minor elements that are present in relatively high concentration, it appears that Sr is not under strict physiological regulation (Campana 1999). Although otolith Sr/Ca concentration is often used as a proxy for salinity, aqueous [Sr/Ca] is the major determinant of otolith [Sr/Ca] (Bath *et al.* 2000, Payan *et al.* 2004). To interpret variation in otolith [Sr/Ca] of fish migrating between environments of different salinity, information is thus needed about the relationship between ambient and otolith [Sr/Ca] as well as the underlying nature of the ambient differences in [Sr/Ca] (Elsdon & Gillanders 2004, Kraus & Secor 2004b, Elsdon & Gillanders 2005).

Thus, unlike other hard parts of fishes such as scales and bones, otoliths are chemically stable and gross remodelling of otolith structure is limited, even under severe stress conditions (e.g. exposure to low pH, exertion or food deprivation) (Campana & Neilson 1985). Otoliths thus preserve a permanent record of age, daily growth, life-history changes and the thermal and chemical environment of the fish (Panfili *et al.* 2002). When combining the information obtained from the growth structure and the chemical composition, otoliths can provide a detailed view on individual movement patterns between bodies of water. However, care must be taken to validate chronologies and calibrate chemical analyses. Having accomplished these crucial tasks, fascinating ecological and evolutionary studies become possible (Bell 2001).

6. AIMS AND OUTLINE OF THE THESIS

Fish communities in temperate estuaries show a strong seasonal pattern in species composition and abundance. This cyclicity in estuarine fish communities is mainly caused by marine species that use estuaries in high numbers during a particular period in their life cycle. This suggests that estuarine habitats are valuable areas for these species, providing abundant food resources, shelter from predation or favorable abiotic conditions. Consequently these species were often considered as estuary dependent for their survival, although the functional significance of the estuary for these species was never assessed (Day *et al.* 1989). However, by means of modern techniques that allow studying fish movement on a fine spatio-temporal scale, it was observed that marine fish populations use estuaries rather facultatively. In order to study processes controlling migratory behavior and to evaluate habitat functions it is necessary to first completely understand the movement patterns within a population (Able 2005). This thesis focuses on the estuarine migration dynamics of the sand goby (*Pomatoschistus minutus*). The sand goby has a typical and predictable seasonal peak of occurrence in estuaries of the northeast Atlantic coast. Although the species is present in the upper Scheldt estuary during most of the year, with a remarkably high density in September-October (Maes *et al.* 2005b), it is unknown how individual sand gobies use the estuary in space and time or whether there is a threshold age or size for immigration. The species is considered to be an estuarine resident (Elliott & Dewailly 1995), although Thiel *et al.* (2003) suggested to classify it as a marine estuarine opportunist. In order to obtain a better understanding of its life history with respect to habitat use, we studied individual movements of the sand goby in the Scheldt estuary using two geochemical tracers: stable C isotopes in dorsal muscle tissue and otolith [Sr/Ca].

Chapter 1 gives an introduction about the different aspects of this study. First the role of migration in animal life histories is addressed, followed by the estuarine fish community composition with emphasis on different life cycle categories, then a general description of the Scheldt estuary and the sand goby. Subsequently, both tracing techniques are introduced by providing the necessary background information, terminology and prerequisites.

The next five chapters comprise the six papers which are the result of this study. They can be grouped according to the technique used: Chapters 2, 3 and 4 deal with the stable isotope technique, while chapters 5 and 6 concern otolith [Sr/Ca] chronologies. Prior to application of bot tracers (Chapters 4 and 6), the techniques needed to be calibrated to our system and conditions needed to be verified (Chapters 2, 3 and 5).

Unravelling migratory connectivity using stable isotopes involves in the first place isotopic characterization of the habitats (food webs) between which the migration occurs. As we depend on sand goby gut contents for the accurate establishment of the isotopic differences between the upper and lower estuarine food sources and also for predicting end member values in these habitats, we first discuss the use and limitations of stable isotope analysis on

gut contents (**Chapter 2a**). This was done by means of an experiment towards the isotopic effects that might occur during digestion and assimilation. Furthermore, this experiment offered empirical insight in the mechanisms leading to isotopic shifts between a consumer and its diet. **The second part of Chapter 2 (2b)** reports then on the C and N isotope differences between sand goby food sources in the lower and upper Scheldt estuary over an entire year based on their gut contents. Additionally, we assessed the spatial variability in the C and N isotope composition of the coastal sand goby population adjacent to the estuary. **Chapter 3** elaborates on an experiment towards the isotopic turnover rate of C and N in sand goby muscle, liver and heart tissue. Knowing the incorporation rate of an element into a tissue is indeed pivotal to understand the time window over which the 'isotopic signature' of a previous location is retained in a migratory animal. Based on the accumulated knowledge from chapters 2 and 3 an isotopic clock is developed in **Chapter 4** and subsequently used to assess the timing of estuarine recruitment for individual sand goby in the Scheldt during an entire year. This enabled us to evaluate the duration of estuarine residency and the turnover of individuals in de estuarine population. Furthermore, we inferred fish size and age at the moment of recruitment using a commonly accepted growth model that relates length to age.

In **Chapter 5** we discuss the use of otolith [Sr/Ca] for studying migrations over salinity gradients and specifically test this for sand goby movements in the Scheldt estuary. To this aim we firstly investigated changes in ambient Sr, Ca and Sr/Ca concentrations along the longitudinal salinity gradient in the Scheldt; secondly, we assessed in an experiment the relationships of otolith [Sr/Ca] versus ambient [Sr/Ca] and salinity. These relationships are used to infer threshold values which delineate the marine environment from the brackish water area. In **Chapter 6** spatio-temporal movement patterns of 12 sand goby in the estuary were reconstructed from otolith [Sr/Ca] chronologies, based on the results of Chapter 5. Furthermore [Sr/Ca] values near the otolith nucleus were used as a tracer of sand goby spawning grounds.

Finally, **Chapter 7** forms the general discussion. The results are summarized and their implications discussed both for our understanding on estuarine habitat use by fishes as for the applications of the different methodologies. Additionally, recommendations for future studies on these subjects are presented.

2a

EFFECT OF DIGESTION ON THE $\delta^{13}C$ AND $\delta^{15}N$ OF FISH GUT CONTENTS

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Journal of Fish Biology (2008) 72:301-309

Abstract

Gut contents of sand goby *Pomatoschistus minutus* (Pallas) showed higher C and N isotope values than the food before consumption. This enrichment was more pronounced in the hindgut than in the foregut, probably because of preferential assimilation of ^{12}C and ^{14}N along the gastro-intestinal tract. The results indicate that the shift towards higher values in the alimentary canal occurs in the first two hours after feeding.

1. INTRODUCTION

Stable isotope techniques are being routinely used in the study of trophic relationships and animal migrations in aquatic and terrestrial ecosystems (Gannes *et al.* 1998, Hobson 1999, Thompson *et al.* 2005). Use of stable C and N isotopes to elucidate trophodynamics requires a priori estimates of the alteration in $\delta^{15}N$ and $\delta^{13}C$ values between diet and consumer (known as trophic fractionation). The stable isotopic composition of a consumer was commonly accepted to be enriched in ^{13}C by about 0-1‰ and in ^{15}N by about 3.4 ± 1.1‰ relative to its diet (DeNiro & Epstein 1978, Minagawa & Wada 1984, Owens 1987). However, considerable variation in the trophic shift for $\delta^{13}C$ and $\delta^{15}N$ has been observed between and within trophic groups or even among conspecifics. Multiple potential sources for this variation have been recognised, such as taxon identity (Minagawa & Wada 1984), main biochemical form of the nitrogenous waste (Vanderklift & Ponsard 2003), dietary differences (Adams & Sterner 2000, Vander Zanden & Rasmussen 2001, McCutchan *et al.* 2003, Robbins *et al.* 2005), ration size, temperature (Barnes *et al.* 2007), variable tissue composition (Focken & Becker 1998, Pinnegar & Polunin 1999), isotopic routing (Gannes *et al.* 1997) and physiological stresses such as starvation (Hobson *et al.* 1993). Nevertheless, the real underlying physiological and biochemical mechanisms responsible for the change in isotopic ratios between food source and consumer remain poorly understood (Gannes *et al.* 1997, Sponheimer *et al.* 2003).
Ponsard & Averbuch (1999) proposed that the isotopic composition of a whole organism is the result of a dynamic equilibrium between isotopic discrimination during food digestion and incorporation on the one hand and isotopic discrimination associated with the discharge of excretion products on the other hand. The ratio of fractionation during assimilation (including digestion and absorption) to fractionation during excretion thus determines the direction and magnitude of the trophic shift (Ponsard & Averbuch 1999, Olive *et al.* 2003). The lighter isotope is preferentially used in biochemical reactions, due to its weaker bonds. Consequently the consumer's body should become isotopically lighter relative to the diet during food assimilation, while the unabsorbed food in the gut becomes more enriched in the heavier isotope (Fry *et al.* 1984). However, catabolic reactions also favour the lighter isotope (DeNiro & Epstein 1978, Minagawa & Wada 1984) and the consumer must become enriched in the heavier isotope as excretory products are eliminated (Olive *et al.* 2003).

Stable isotope analysis (SIA) on stomach or gut contents may be a powerful tool in food web and animal migration studies. SIA on gut contents can provide direct information about the diet of consumers and subsequently also about the food sources of their prey (Grey *et al.* 2002). This way some of the uncertainty associated with trophic fractionation can be circumvented. Additionally, it may contribute to establish an appropriate isotopic base line (Post 2002) in food web studies. In order to study animal movement patterns using stable isotopes, it is necessary that the locations between which migration occurs are isotopically

different (Hobson 1999, Herzka 2005). As migrating animals gradually adapt their isotopic composition to that of the new feeding ground (Hobson 1999), their tissues can not be used to test this prerequisite, especially if the timing of arrival and the isotopic turnover rate of the tissues is unknown. Gastro-intestinal contents can then provide a solution to establish isotopic differences between target locations and to predict completely adapted isotope values of migrants at specific locations (Guelinckx *et al.* 2006).
Using SIA on gut contents may avoid additional sampling efforts towards prey items and eliminate the problem of investigating and identifying the food sources in their contributing proportions (Peterson 1999). This is all the more important when the species under study has an unknown preference for one or a few prey items within the available prey spectrum (Jardine *et al.* 2005).

Although Tieszen *et al.* (1983) and Peterson (1999) suggested performing SIA on stomach or gut contents only a few studies actually did so (Fry *et al.* 1984, Fry 1988, Peterson *et al.* 1993, Grey *et al.* 2002, Page & Lastra 2003, Yatsuya *et al.* 2004, Jardine *et al.* 2005, Guelinckx *et al.* 2006). Only two studies reported possible effects on the stability of stable isotope ratios of ingested food due to digestion or fractionation during absorption and excretion (Fry *et al.* 1984, Grey *et al.* 2002). These effects may complicate the interpretation of isotope measurements on ingested food items.

This study is part of a research project that uses stable C and N isotopes to investigate recruitment of the marine sand goby *Pomatoschistus minutus* (Pallas, 1770) to the Scheldt estuary (Belgium). Gut contents are analysed to determine the C and N isotopic gradient between the marine and the brackish water food web and to predict estuarine end member signatures for fish muscle tissue (Chapter 2b and 4). A major concern here is the degree and timing of the change in the isotopic composition of the ingested food due to digestive and assimilation processes. In addition, isotope measurements on gut contents can help to understand the mechanisms controlling trophic fractionation. Thus the aims of the present study were twofold: to test for any change in diet $\delta^{13}C$ and $\delta^{15}N$ in the gastro-intestinal tract of the sand goby as a function of time and to assess the isotopic differences among foregut content, hindgut content and the food prior to ingestion. It was expected that the gut content would become enriched in the heavy isotope over time and that this effect would be stronger in the hindgut than in the foregut.

2. MATERIALS AND METHODS

A feeding experiment was conducted in which gut contents were collected for SIA at regular time intervals after foraging. The experimental design was constrained by the limited number of available fish in a laboratory stock of a larger experiment (Chapter 3). Fifteen specimens were distributed in equal numbers over three 20 l aquaria, which were installed in a continuous flow through system. At least 20% of the water volume was changed on a daily basis. Temperature was preset on 17°C and the light-dark regime on 12-12 h. Twice a day, fish were fed ad libitum a formulated pellet food (Table 2a.1) and were observed to forage well after four days. On the fifth day the fish were fed in excess during 30 min. The remaining food was then removed and 1, 2, 4, 6 and 8 h later one fish from each aquarium was killed by severing the central nervous system and immediately stored at –20°C. This resulted in three replicates for each sampling event. The experimental design did not include any measurement after 8 h, since calculations of the depletion rate of gastro-intestinal content, based on the model of Andersen (1984), predicted that only 9 % of the ingested food remained 9 h after feeding. For additional details on aquarium set up and maintenance see (Chapter 3).

Whenever possible, food samples from the foregut (oesophagus and proximal region of the intestines) as well as from the hindgut (distal region of the intestines without the rectum) were collected from the same fish for isotope ratio measurements. Surprisingly, several gastrointestinal tracts were empty even though the fish were observed to forage on the pellets. In order to obtain more samples the whole experiment was immediately repeated with an additional 9 fish. They were killed 2, 4 and 6 h after foraging on the pellet food. Results of both experiments were treated together for statistical analysis. Dorsal muscle tissue of one randomly chosen fish from each aquarium was also collected for analysis (n = 6).

Gut content and muscle tissue samples were dried at 55°C to constant weight, homogenized using mortar and pestle, and aliquots were weighed into tin containers. Stable C and N isotope ratio measurements were performed at the Laboratory for Analytical and Environmental Chemistry (ANCH) at the Vrije Universiteit Brussel (Belgium) on a Flash series 1112 elemental analyzer interfaced to a Delta^{Plus} XL Thermo Finnigan IRMS. The working standards were high-purity N_2 and CO_2, while IAEA-C-6 (-10.4 ± 0.1 ‰) and IAEA-N2 (20.4 ± 0.1 ‰) were used as reference materials. Stable isotopic compositions are expressed in the conventional δ-notation:

$$\delta X\ (‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3,$$

where *X is* ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

$\delta^{13}C$ values are expressed relative to the VPDB (Vienna PeeDee Belemnite) standard, while $\delta^{15}N$ values are expressed to atmospheric N_2. Reproducibility for different aliquots of the reference materials was generally better than 0.3‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

Linear regressions were used to examine temporal trends in the $\delta^{13}C$ and $\delta^{15}N$ data of ingested food in the gut contents. Subsequently, the Mann-Whitney U test was applied to test for isotopic differences between the diet and gut contents, while the Wilcoxon matched pairs test was used to evaluate whether significant differences occurred between foregut and hindgut contents (STATISTICA 7.0, StatSoft Inc., Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

Assuming a preferential assimilation of ^{12}C and ^{14}N, it was expected that gastric $\delta^{13}C$ and $\delta^{15}N$ values would become enriched relative to the pellet diet (Olive *et al.* 2003). The results seem to confirm this, though quite a lot of variation was observed and interpretation should be done with caution. Mean $\delta^{13}C$ and $\delta^{15}N$ values (± S.D.) for the pellets, dorsal muscle tissue, foregut and hindgut contents are summarized in Table 2a.1. For $\delta^{15}N$, both foregut and hindgut contents differed significantly from the pellets (+ 1.3 ‰ and + 1.9 ‰, respectively) and for $\delta^{13}C$ there was an enrichment of + 1.2 ‰ in the hindgut content, but no significant difference was detected in the foregut compared to the pellets. Although this enrichment was probably caused by differential absorption of the lighter isotope, mixing of the undigested food with digestive tract material such as intestinal cells, digestive enzymes and mucus cannot be ruled out completely (Ponsard & Averbuch 1999, Sponheimer *et al.* 2003, Jardine *et al.* 2005). Goby muscle tissue was about 6.6 ‰ enriched in ^{13}C and ^{15}N relative to the pellet food (Table 2a.1). However, if only contamination with ^{13}C and ^{15}N enriched endogenous material (e.g. mucus, sloughed cells) was responsible for this isotopic difference between pellets before ingestion and gut contents, we would expect equal shifts for $\delta^{13}C$ and $\delta^{15}N$ under the assumption of a similar relative contamination for C and N. Yet, the isotopic shift appeared to be higher for $\delta^{15}N$ than for $\delta^{13}C$ in foregut and hindgut. This would mean that for N a relative stronger contamination of the ingested pellets by endogenous material has occurred, but this is not likely as the pellets are N rich. Moreover, the significantly higher $\delta^{13}C$ and $\delta^{15}N$ values of the hindgut samples relative to the foregut samples also suggest a preferential digestion and (or) uptake of ^{12}C and ^{14}N along the digestive tract.

Table 2a.1. *Mean (± S.D.) $\delta^{13}C$ and $\delta^{15}N$ of the pellet feed, muscle tissue, foregut and hindgut content samples. Mann-Whitney U tests were used to test for differences between the isotopic composition of pellets and gut content samples (Fgut: foregut; Hgut: hindgut) and Wilcoxon matched pairs tests for differences between foregut and hindgut samples.*

	Pellet	**Muscle**	**Foregut**	**Hindgut**	**Δ Fgut-pel**	**Δ Hgut-pel**
$\delta^{13}C$ (‰)	-23.3 ± 0.6 (n = 7)	-16.6 ± 0.8 (n = 6)	-22.8± 0.4 (n = 12)	-22.0 ± 0.4 (n = 9)	0.4	1.2
			n = 9, p < 0.05		p = 0.17	p < 0.01
$\delta^{15}N$ (‰)	9.1 ± 0.2 (n = 7)	15.7 ± 0.6 (n = 6)	10.5 ± 0.4 (n = 12)	11.0 ± 0.5 (n = 9)	1.3	1.9
			n = 9, p < 0.01		p < 0.001	p < 0.001

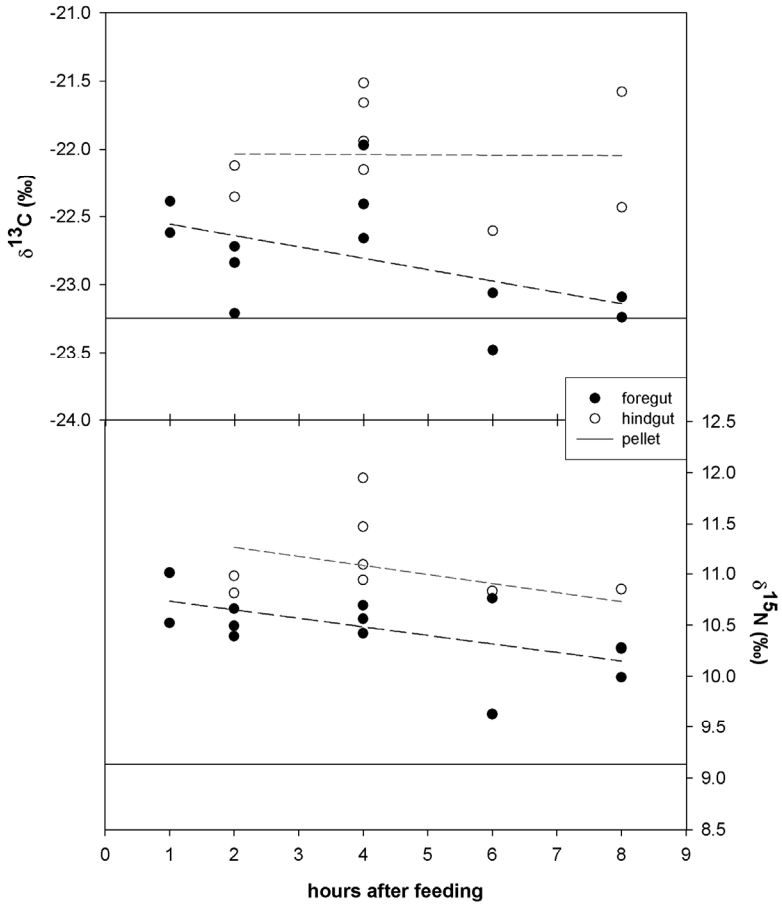

Fig 2a.1 *$\delta^{13}C$ and $\delta^{15}N$ of foregut and hindgut contents sampled 1, 2, 4, 6, and 8 hours after feeding. Dashed lines are best-fit linear regressions (y = ax + b) through the data (foregut: $\delta^{13}C$: a = -0.08; p = > 0.05; $\delta^{15}N$: a = -0.08, p > 0.05; hindgut: $\delta^{13}C$: a = 0.001; p > 0.05; $\delta^{15}N$: a = -0.09, p > 0.05). The full line represents the average value of the formulated pellet feed. There were no samples for hindgut one hour after feeding.*

As a result of discrimination against heavy isotopes during assimilation, the consumer's body is expected to become depleted in heavy isotopes relative to the diet. This contradicts the fact that consumers are generally enriched compared to their diet (DeNiro & Epstein 1978, Minagawa & Wada 1984, Vander Zanden & Rasmussen 2001, Vanderklift & Ponsard 2003). Consequently, the fractionation against heavy isotopes must be larger during processes leading to excretion than during the assimilation processes in order to account for trophic enrichment of animals (Ponsard & Averbuch 1999, Olive *et al.* 2003).

The higher values for $\delta^{13}C$ and $\delta^{15}N$ in the hindgut and for $\delta^{15}N$ in the foregut contrast with results reported by Grey *et al.* (2002), who observed that the $\delta^{13}C$ and $\delta^{15}N$ composition of crustacean zooplankton and Arctic char *Salvelinus alpinus (L.)*, both derived from fish gut samples, were within the inherent natural variability exhibited by local specimens. They concluded that the effects of digestive processes on $\delta^{13}C$ and $\delta^{15}N$ of ingested food were negligible, yet it might well be that differences between food sources in the natural environment and the gut contents remained undetected due to a higher natural variability. Fry (1981) (see Fry *et al.* 1984) reported no influence of digestion on $\delta^{13}C$ of food recovered from the stomachs of benthic crustaceans. This is in agreement with the foregut $\delta^{13}C$ data of sand goby. Fry *et al.* (1984) on the other hand showed that midgut and fecal samples of benthic

shrimps became actually depleted in ^{13}C relative to foregut samples during assimilation. In contrast to the latter study, there are many reports showing isotopically enriched faeces (e.g. DeNiro & Epstein (1978), Steele & Daniel (1978) and Sponheimer *et al.* (2003) and references herein) confirming the observed enrichment in sand goby guts. The present study is, to our knowledge, the first to present data on the rate of this enrichment. The shift towards higher $\delta^{13}C$ and $\delta^{15}N$ values for ingested food seems to occur within the first two hours of feeding (Fig. 2a.1) as $\delta^{13}C$ and $\delta^{15}N$ of foregut and hindgut did not show an increasing trend between 1 and 8 h after foraging. On the contrary, the $\delta^{13}C$ and $\delta^{15}N$ data suggested decreasing trends over time, although none of slopes were significantly different from zero (Fig. 2a.1).
The relatively fast isotopic shift might be explained by high initial absorption rates (Goldstein & Elwood 1971, Andersen 1984) and the preferential digestion and uptake of the lighter isotope. The absence of a persisting $\delta^{13}C$ and $\delta^{15}N$ increase over time probably results from the decreasing overall absorption rate of nutrients and a relatively greater uptake of the heavier isotope which is expected to become more available as digestion proceeds.

Although the experiment was limited in its design and the results showed considerable variation, this study provides insight in processes leading to trophic fractionation. The results seem to support the model proposed by Ponsard & Averbuch (1999), stating that trophic fractionation derives from a combination of isotopic fractionation during assimilation and synthesis, as well as during catabolic processes and excretion. Additionally, with respect to applications of SIA on gut contents, this study suggests that the moment of sampling after feeding time is relatively unimportant and does not confound isotopic values. This confirms that gut contents can be applied to investigate isotopic gradients in the environment, i.e. the relative differences in isotopic composition between habitats.
Nevertheless, care should be taken in determining absolute isotopic signatures for prey items or trophic fractionation based on gut contents. Differences in isotopic composition between food and gut contents were observed, especially for the hindgut. More elaborate studies are needed to identify the influencing factors such as temperature, diet quality, food ration and consumer's physiological status, and ultimately the underlying physiological mechanisms behind diet - tissue fractionation. Efforts should hereby be made to perform (compound specific) SIA on excretion products and on gut contents free of adhering digestive tract fragments, in order to assess the degree of isotopic fractionation during catabolic and assimilation processes, respectively.

Acknowledgments. We thank L. De Brabandere, F. Volckaert and J. Maes for their suggestions on this manuscript, P. Van Den Driessche and B. Geysen for preparing the samples and A. Ghesquiere for improving the manuscript linguistically. Special thanks to the Centre of Estuarine and Marine Ecology (NIOO-CEME, Yerseke, The Netherlands) and M. Korntheuer (ANCH, Vrije Universiteit Brussel) for their logistic support. The first author acknowledges a Ph.D grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). Two anonymous reviewers provided useful feedback, which helped us to improve the manuscript.

2b

SPATIAL VARIABILITY IN $\delta^{13}C$ AND $\delta^{15}N$ OF ESTUARINE AND COASTAL BENTHIC CONSUMERS DERIVED FROM SAND GOBY GUT CONTENTS AND MUSCLE TISSUE

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Unpublished manuscript

Abstract

A prerequisite to trace animal movements between two areas using stable isotopes is that food sources of the species under study are isotopically different between both areas. Additionally, for clear interpretation, it is important that the migratory source population is relatively homogeneous in isotopic composition. This preliminary study examined both conditions with the prospect of investigating sand goby *Pomatoschistus minutus* (Pallas, 1770) immigration patterns from the North Sea to the upper Scheldt estuary (Belgium and The Netherlands) by means of C and N isotopes ($\delta^{13}C$ and $\delta^{15}N$). Stable isotope analyses on monthly gut contents (June 2003 - March 2004) demonstrated that the $\delta^{13}C$ value of sand goby prey items was on average 6 ‰ higher in the lower estuary than in the upper estuary. From June until November, $\delta^{15}N$ was higher in the upper estuary than in the lower estuary, but this pattern reversed during winter and early spring. Sand goby muscle tissue showed no spatial $\delta^{13}C$ variability along the Belgian coast, but our data revealed that coastal $\delta^{13}C$ values were depleted relative to those offshore. Coastal $\delta^{15}N$ values, on the other hand, increased considerably with increasing distance from the estuary during summer and autumn, but an inshore-offshore $\delta^{15}N$ gradient was not detected. In contrast to $\delta^{13}C$, $\delta^{15}N$ is not an appropriate tracer to study fish migration into the Scheldt estuary due to the alternating $\delta^{15}N$ gradient in the estuary and the spatial heterogeneity of $\delta^{15}N$ along the Belgian coast.

1. INTRODUCTION

Stable isotopes can provide a powerful means to define habitat usage by migratory fauna and to acquire insight in their natural history given that the species move between isotopically different habitats. The isotopic composition of local food webs can differ due to varying primary producers or different physicochemical environments in the respective habitats (Fry & Sherr 1984, Peterson & Fry 1987, Hobson 1999, Herzka *et al.* 2001). Ideally, before investigating animal movements between habitats the stable isotope composition of the food sources at the target locations as well as the temporal variation herein should be assessed (Hobson 1999, Herzka 2005).

In aquatic systems primary producers and detrital energy sources can exhibit high temporal variation in isotopic composition. This complicates their direct use as reliable isotopic indicators (baseline) for secondary consumers that integrate isotopic variation over much longer time periods resulting in strongly reduced variations at these higher trophic levels (Fry *et al.* 1984, Cabana & Rasmussen 1996, Peterson 1999, Herman *et al.* 2000). In fact, assessing the appropriate isotopic baseline in food web or migration studies can be troublesome because a good baseline (1) integrates isotopic changes at a time scale near that of the species under study, (2) is sampled during the same time period and (3) captures the spatial variability relevant for the species under study (Post 2002). To meet these conditions when determining habitat specific isotopic signatures for migratory organisms it can be useful to analyze their gastro-intestinal content, which represents just one trophic level lower than the species under study. Compared to sampling primary producers as a baseline for estimating the isotopic signal of higher trophic levels, a great deal of uncertainty related to the increased isotopic variability at lower trophic levels, food web complexity and the variable trophic fractionations herein is avoided when using gastro-intestinal contents (Fry *et al.* 1984, Cabana & Rasmussen 1996, Hansson *et al.* 1997, Vander Zanden & Rasmussen 2001). Additional benefits from using stomach or gut contents are that sampling of prey organisms in the field is circumvented and the correct prey items are immediately analyzed in the right contributing proportions. This is all the more important when the species exact dietary items are not known (Jardine *et al.* 2005). Sufficient sampling can counteract the snapshot nature of stomach contents.

However little is known about the effect of digestion and assimilation on the stable isotopic composition of prey items in animal gastro-intestinal tracts. Yet, results from chapter 2a show that the carbon isotope ratios do not differ between foregut contents of sand goby (*Pomatoschistus minutus*) and the diet before ingestion. For $\delta^{15}N$ an enrichment of ± 1.3 ‰ was observed from diet to foregut content. This enrichment occurred in the first hour after feeding after which the isotopic composition of the foregut content remained stable (Chapter 2a).

Based on clupeoid stomach contents, Guelinckx *et al.* (2006) observed for $\delta^{13}C$ in 2000-2001 an average difference of about 7 ‰ between pelagic primary consumers in the lower

(polyhaline) and upper (oligohaline) Scheldt estuary (Fig 2b.1). For $\delta^{15}N$, however, the isotopic difference was highly variable and even reversed, with higher estuarine values from June until November 2000 and lower estuarine values from January until March 2001 (Fig 2b.1). That study was the first to report monthly C and N isotopic differences between the upper and lower estuarine food web of the Scheldt. Moreover the alteration of the $\delta^{15}N$ gradient has, to our knowledge, not yet been reported for the Scheldt or other estuaries. Hence, these observations needed to be confirmed primarily to increase the general understanding of the estuarine ecosystem. Secondly, the isotopic C and N differences had to be verified in the prospect of studying sand goby estuarine migrations.

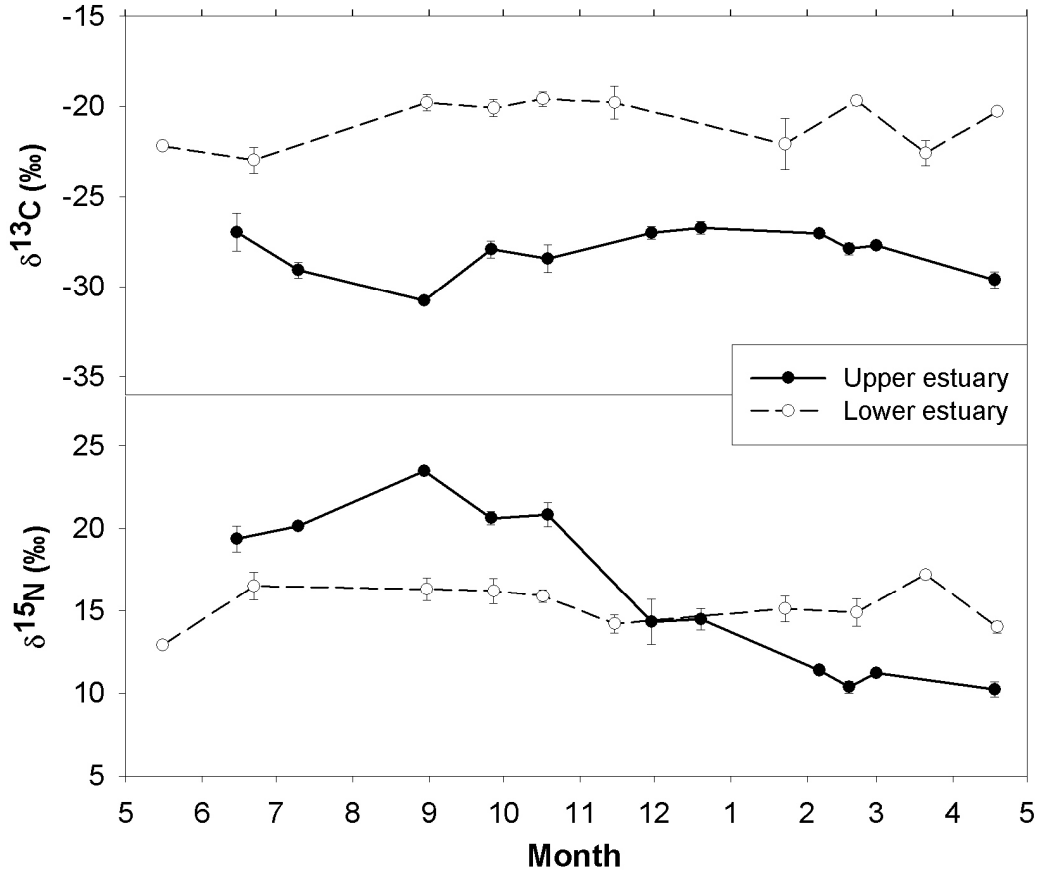

Fig. 2b.1 *Results from Guelinckx et al. (2006): isotopic characterization (mean ± SE, n = 1-6) of clupeid food sources in lower and upper estuary from May 2000 to April 2001 based on stomach contents. Tick marks on X-axis ('Month') indicate the first day of each month.*

The present paper is framed in a study towards the connectivity of sand goby *Pomatoschistus minutus* (Pallas, 1770) populations in the North Sea and Scheldt estuary. *P. minutus* is a relatively small demersal species that is very abundant along the Atlantic European coasts and its estuaries. It is a generalist feeding on epibenthic prey, mainly harpacticoid copepods, mysids, polychaetes and amphipods (Fonds 1973, Maes *et al.* 2003, Salgado *et al.* 2004). Like

many other marine fish species, *P. minutus* exhibits a typical seasonal pattern of occurrence in the North Atlantic estuaries. For the Scheldt estuary, highest sand goby densities are yearly recorded in September - October (Maes *et al.* 2004), but little is known about these estuarine migrations on an individual level. In order to describe their immigration pattern using stable C and N isotopes, it is necessary that the isotopic difference between the upper Scheldt estuary and the marine environment is firstly assessed. An additional requirement to use stable isotopes more precisely in migration studies is that the isotopic composition of potential migrants in the migratory source populations exhibits minimal variability (Herzka *et al.* 2001). Too much heterogeneity in the C and N isotopic composition of the sand gobies at sea would hamper the assessment of a characteristic marine end member, necessary to identify unambiguously new immigrants in the estuary. Thus, the objectives of the present study were (1) to assess the C and N isotopic differences between sand goby food sources in the lower and upper Scheldt estuary and (2) to verify the spatial homogeneity in C and N isotopic composition of sand goby muscle tissue in the coastal waters adjacent to the estuary.

2. MATERIALS AND METHODS

2.1. Study Area

The Scheldt estuary (Fig. 2b.2) is approximately 160 km long from the mouth in the Netherlands to Ghent (Belgium) where sluices stop the tidal wave. Salt water intrudes to about 100 km inland, resulting in a relatively stable salinity gradient with a brackish zone situated between km 40 and km 90. The water residence time varies from about 50 days in winter to 70 days in summer. Turbidity is high, especially in the upper estuary where suspended matter can reach concentrations up to 200 mg L^{-1}. The Scheldt estuary is subject to severe eutrophication as it receives high inputs from untreated domestic, industrial and agricultural activities. The huge amounts of anthropogenic organic loads and nutrients fuel bacterial production leading to a net CO_2 production in the estuary (Frankignoulle *et al*. 1998, Middelburg & Nieuwenhuize 1998, Hellings *et al.* 2001, Boschker *et al.* 2005, Soetaert *et al.* 2006).

The Belgian continental shelf and the Dutch continental shelf off Zeeland are situated in the Southern Bight of the North Sea. The Belgian continental shelf is on average 30 m deep and has a 67 km long sandy coastline that merges eastwards into the Scheldt estuary (Maes *et al.* 2000) (Fig. 2b.2).

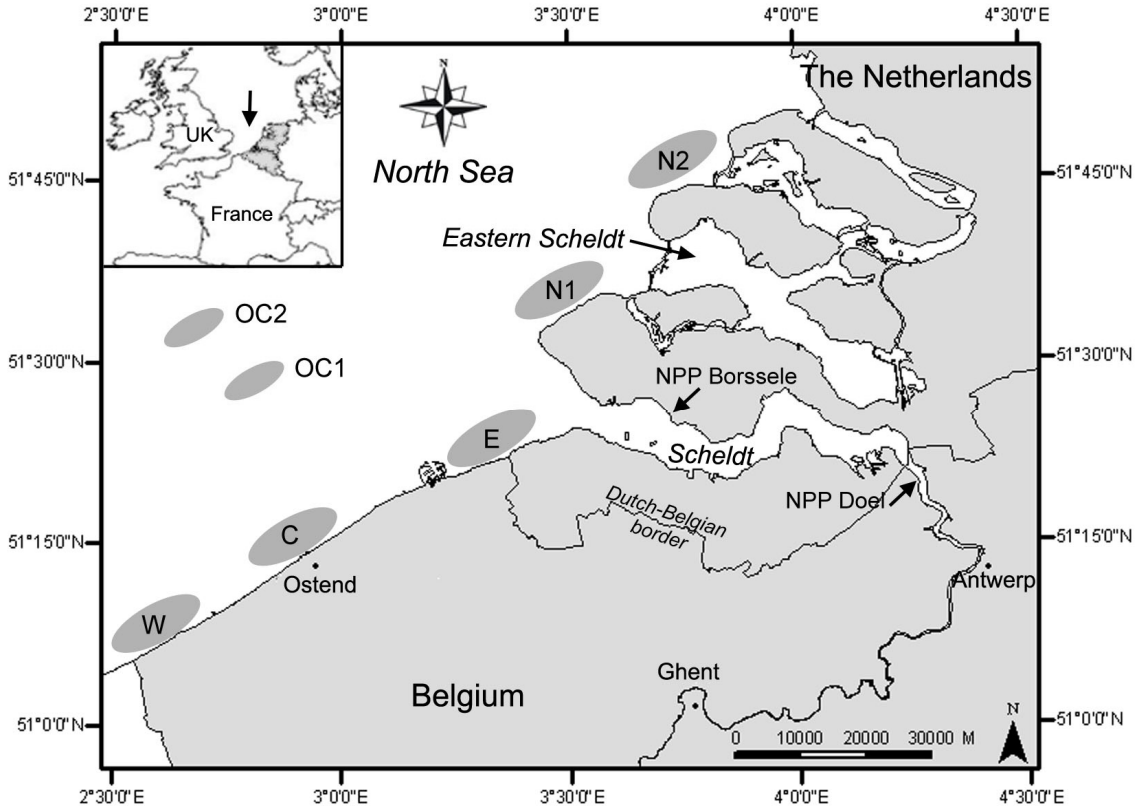

Fig 2b.2 *Map of the study area with indication of the sampling locations in the upper and lower estuary (NPP Doel and NPP Borssele, respectively) and the North Sea: W, C, E, OC1 and OC2 on the Belgian Continental Shelf; N1 and N2 are located on the Dutch Continental Shelf.*

A saline water mass enters the area from the Atlantic via the English Channel by the NE directed residual current and meets the SW oriented Scheldt outflow. Water masses entering the North Sea from the English Channel need ca. 4 months to reach the northern end of the North Sea. This water is transported in a narrow zone along the continental coast. Three main rivers influence the Belgian coastal waters and the southern Dutch coastal waters: the Rhine, the Meuse and the Scheldt (Megens *et al.* 2001, Dewicke *et al.* 2003). The current regime is macro-tidal and keeps the water column well mixed.

2.2. Field sampling

Between April 2003 and March 2004 sand gobies were collected on a monthly basis in the lower and upper Scheldt estuary using the cooling water intake of the nuclear power plant (NPP) Borssele and NPP Doel, respectively (Fig. 2b.2). NPP Borssele is located 8 km upstream from the mouth and NPP Doel at 62 km from the mouth. Salinity averaged 28.7 ± 1.3 and 9.7 ± 3.7 (psu) during the sampling period in the lower and upper estuarine sampling location, respectively. Nets with a 4 mm mesh size were used for collecting fish. A technical problem prevented sampling in December 2003.

To assess the spatial heterogeneity of muscle tissue $\delta^{13}C$ and $\delta^{15}N$ along the coast, sand gobies were obtained seasonally from sampling campaigns on the Belgian Continental Shelf and Dutch Continental Shelf off Zeeland. These fish were sampled in July 2003, October 2003, December 2003 and March 2004 in nearshore areas along the Belgian-Dutch coast (W, C, E, N1 and N2 on Fig. 2b.2) using beam trawls operated from the research vessels 'Zeeleeuw' and 'Belgica'. Sand gobies caught in September 2005 in one nearshore (C) and two offshore (OC1, OC2) zones were used to examine the existence of an inshore-offshore gradient for $\delta^{13}C$ and $\delta^{15}N$ of sand goby muscle tissue.

Fish samples were immediately frozen (dry ice or –20°C) for transport to the laboratory, where they were preserved at -20°C for further processing. *P. minutus* was identified according to Hamerlynck (1990).

2.3. Sample preparation and stable isotope analysis

Foregut contents from fish caught monthly in the lower (n = 1-6) and upper Scheldt estuary (n = 5-6), were excised with forceps under a stereomicroscope. Gobies do not have a functional pyloric sphincter at the gastrointestinal junction so a real stomach could not be distinguished. Consequently, prey items were collected from the oesophagus to the proximal intestine (foregut). When the state of digestion had progressed too far gut contents were not retained. Initially we aimed to analyse six specimens from each sampling event, but this was not always possible due to empty guts and/or small catches, especially in the lower estuary.
Dorsal muscle tissue from sand goby collected at sea was removed to monitor the marine isotopic signal (n = 6).

Muscle and gut content samples were dried at 55 °C to constant weight and homogenized using mortar and pestle. Aliquots (± 0.5 mg) of muscle samples were put in tin containers for stable isotope analysis. Aliquots of gut contents, however, were weighed into silver containers, acidified with 5% HCl to remove inorganic carbon and then dried again at 50 °C prior to analysis. A posteriori, no significant differences in $\delta^{13}C$ and $\delta^{15}N$ were found between gut content samples with or without acidification.
Stable isotope measurements were done 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 standards were high-purity N_2 and CO_2, while sucrose (IAEA-C-6: $\delta^{13}C$ -10.4 ± 0.1‰) and ammoniumsulfate (IAEA-N2: 20.4 ± 0.1‰) were used as reference materials. Stable isotopic compositions are expressed in the conventional δ-notation:

δX (‰) = $[(R_{sample} - R_{standard})/R_{standard}] \times 10^3$,
where *X is* ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

$\delta^{13}C$ values are expressed relative to the VPDB (Vienna PeeDee Belemnite) standard, while $\delta^{15}N$ values are expressed relative to atmospheric N_2. Reproducibility for different aliquots of the reference materials was generally better than 0.3‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

2.4. Statistical analysis

Differences in isotopic composition between North Sea zones were tested using one-way ANOVA within each month. When the assumptions were not fulfilled the non-parametric Kruskall Wallis test was applied. Subsequently post hoc Tukey HSD or Mann-Whitney U tests were used to identify the significantly differing groups (STATISTICA 6.0, StatSoft Inc.).

3. RESULTS

3.1. Gut contents from lower and upper Scheldt estuary

Fig. 2b.3 shows the $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) of the sand goby foregut contents in the lower (NPP Borssele) and upper estuary (NPP Doel) from May 2003 until March 2004. No fish were caught in May, June and July 2003 in the lower estuary and in May 2003 in the upper estuary. In general, less fish were caught in the lower estuary due to the sheltered position of the cooling water intake of NPP Borssele.

Although there was considerable temporal variability in $\delta^{13}C$ for the upper estuary (mean ± SD: -25.7 ± 2.1 ‰), with lower values during summer, prey items in the lower estuary (-20.1 ± 0.8 ‰) were always more enriched in ^{13}C than those in the upper estuary. Throughout the year there was a mean difference of about 6 ‰ between both locations. The $\delta^{15}N$ signal for the lower estuarine environment was relatively stable (15.9 ± 1.2 ‰), except for the higher value in August, which was based on only one sample. The $\delta^{15}N$ signal in the upper estuary had higher values from June until October (20.3 ± 1.5 ‰) and lower values from November until March (14.4 ± 0.7 ‰). The strong temporal variability in the upper estuary resulted in an alternating $\delta^{15}N$ difference between the lower and upper estuary.

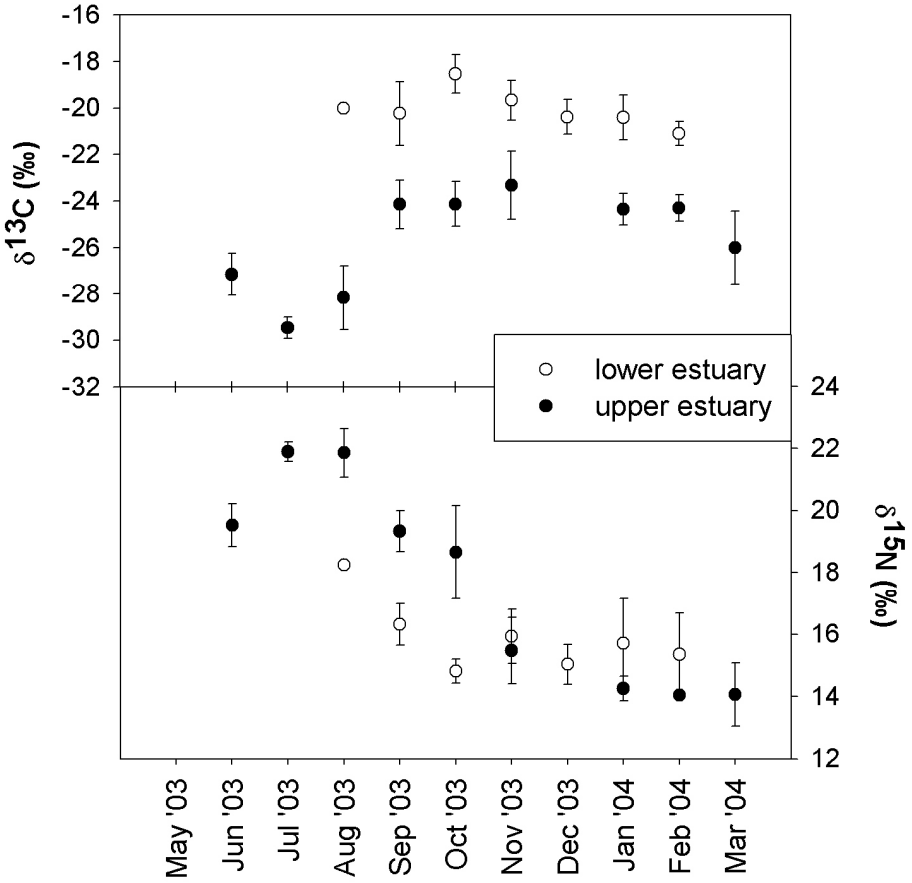

Fig. 2b.3 *$\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) of sand goby foregut contents in the lower and upper estuary from May 2003 to March 2004. A technical failure at NPP Doel (upper estuary) prevented sampling in December 2003. No fish were caught in May, June and July 2003 in the lower estuary and in May and December 2003 in the upper estuary.*

3.2. Muscle δ13C and δ15N variability in coastal waters

$\delta^{13}C$ of sand goby muscle tissue was rather homogenously distributed along the Belgian coast within each month. Only in July, zone E (-20.9 ± 1.2 ‰) was slightly depleted relative to zone C (-19.4 ± 0.6 ‰) and W (-19.3 ± 0.5 ‰) ($p < 0.05$) (Fig. 2b.4). There were no significant $\delta^{13}C$ differences between the zones in October 2003 (mean $\delta^{13}C$: -19.0 ± 0.9‰) and March 2004 (mean $\delta^{13}C$: -17.5 ± 0.6 ‰). However, there seems to be an inshore-offshore gradient for $\delta^{13}C$ as we found significantly different values between C (-19.9 ± 0.8 ‰), OC1 (-17.8 ± 0.3 ‰) and OC2 (–17.3 ± 0.2 ‰). Also, a temporal trend with increasing $\delta^{13}C$ values from July to March can be detected (Fig. 2b.4). For $\delta^{15}N$, there was considerably more spatial variation along the coast (Fig. 2b.4). In July sand gobies in zone E (20.2 ± 0.4 ‰) were highly ^{15}N enriched relative to those in C (15.2 ± 0.1 ‰) and W (14.4 ± 0.3 ‰). In October zone W (15.5 ± 0.6‰) was significantly different from C (16.5 ± 0.9 ‰) and E (17.3 ± 0.9 ‰). There were no spatial differences along the Belgian-Dutch coast in March 2004 (mean $\delta^{15}N$: 16.3 ± 0.8 ‰). There seems to be no inshore-offshore gradient for $\delta^{15}N$ (C: 15.9 ± 0.5 ‰; OC1: 15.7 ± 0.4 ‰; C2: 16.0 ± 0.1 ‰), nor was there a temporal trend in the coastal samples.

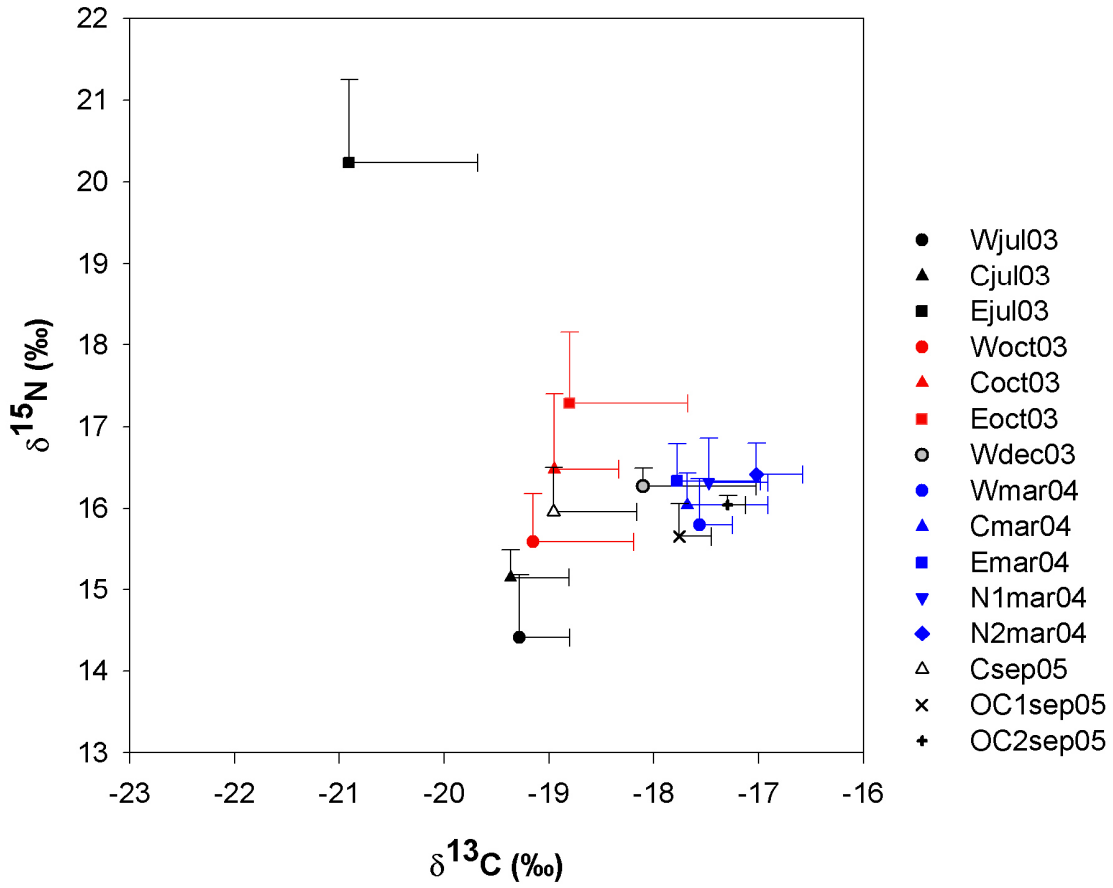

Fig. 2b.4 *δ13C and δ15N (mean ± SD) in sand goby muscle tissue on the Belgian Continental Shelf and for the southern Dutch coast. Fish were collected along the coast in July 2003 (zones: W, C, E), October 2003 (zones: W, C, E), December 2003 (zone: W) and March 2004 (W, C, E, N1, N2). During September 2005 an inshore (C) and two offshore locations (OC1 and OC2)were sampled.*

4. DISCUSSION

4.1. Variability in δ13C and δ15N of upper and lower estuarine gut contents

Current results based on sand goby gut contents support the previously observed trends for 2000-2001 based on clupeid stomachs (Fig 2b.1), with a seasonally alternating difference for $\delta^{15}N$ and a consistent difference for $\delta^{13}C$ between the marine and the upper estuarine food web. This suggests that these patterns are yearly recurrent in the Scheldt estuary. For $\delta^{13}C$ the results follow the general phenomenon in temperate estuaries consisting of a gradual enrichment of $\delta^{13}C$ with increasing salinity. This is mainly caused by the gradual increase in the $\delta^{13}C$ of inorganic carbon sources (DIC) of photoautotrophs and variable composition of suspended particulate organic matter (SPOM) along the estuarine gradient. Freshwater systems are impacted by input of ^{13}C depleted organic matter consisting of domestic sewage, run-off water and riparian vegetation. Bacterial respiration of this organic matter results in lower $\delta^{13}C$ values for DIC relative to DIC of seawater (± 0 ‰). The ^{13}C-depleted CO_2, which dissolves and dissociates in water is subsequently used for photosynthesis leading to ^{13}C-

depleted SPOM. These bottom-up geochemical influences are then recorded in estuarine food webs and isotopic composition of animals (Fry & Sherr 1984, Lajtha & Michener 1994, Middelburg & Nieuwenhuize 1998, Hellings *et al.* 2001, Fry 2002). For clupeid stomach contents, the $\delta^{13}C$ values in the upper estuary were about 7.2 ‰ lower than those from the lower estuary. In the present study the difference between both sampling stations was smaller (± 5.8 ‰). This smaller difference is caused by the higher mean value of gut contents in the upper estuary (mean ± SD: -25.7 ± 2.1 ‰ in 2003-2004 versus -28.1 ± 1.3 ‰ in 2000-2001) as there was no difference in the average marine signal between both studies (-20.1 ± 0.9 ‰ in 2003-2004 vs. -20.9 ± 1.3 ‰ in 2000-2001).

There was considerable temporal variability in the $\delta^{13}C$ values of sand goby gut contents in the upper estuary, with lower values during summer (± -28 ‰) relative to the rest of the year (Fig. 2b.3). The lower value in March 2003 suggests that the prey items of sand goby display a consistent seasonal trend with ^{13}C-depleted values during spring and summer. This could indicate that ^{13}C depleted phytoplankton is processed in the benthic food web. Phytoplankton $\delta^{13}C$ values in the Scheldt estuary are relatively low throughout the year due to continuously supersaturated CO_2 levels (Hellings *et al.* 2001, Boschker *et al.* 2005, Soetaert *et al.* 2006). External CO_2 concentrations are known to have a strong effect on phytoplankton $^{13}C/^{12}C$ distributions and isotope discrimination, with the largest isotope discriminations found in high CO_2 conditions (Fry 1996). During the bloom period, increased phytoplankton contribution to SPOM is known to decrease average SPOM $\delta^{13}C$ values (Hellings *et al.* 1999). The highest chlorophyl a biomass in the estuarine sediment has been found during early summer implying an increased algae contribution to the benthic food web and the herein concomitant lower $\delta^{13}C$ values during and after the bloom period (Herman *et al.* 2000, Widdows *et al.* 2004). After summer the phytoplankton contribution to the benthic detrital food web decreases, resulting in less ^{13}C depleted values of benthic organic matter (Middelburg & Nieuwenhuize 1998) and consumers, which explains the higher $\delta^{13}C$ values of sand goby gut contents during the rest of the year (Fig. 2b.3). The $\delta^{13}C$ values for clupeoid stomach contents remained consistently low throughout the year (–28.1 ± 0.4 ‰) (Fig. 2b.1). The divergence in the $\delta^{13}C$ patterns of clupeoid and sand goby gastro-intestinal contents can be attributed to the different habitat they occupy, with clupeoid being pelagic and sand goby being epibenthic feeders. Clupeiods predominantly rely on calanoid copepods (Maes *et al.* 2003), which can feed very selectively on phytoplankton in a turbid environment (Tackx *et al.* 2003). Around a size of 30 mm sand gobies are known to undergo an ontogenetic diet switch from planktonic to benthic prey items (Hostens & Mees 1999, Salgado *et al.* 2004). This diet switch might also contribute to the temporal variation in $\delta^{13}C$ of sand goby stomach contents. This temporal variability should be taken into account when estimating end member values for sand goby in the estuary.

$\delta^{15}N$ exhibits major temporal variation in the upper Scheldt estuary resulting in an alternating gradient between the marine and the upper Scheldt estuary (Fig. 2b.3), which corroborates

earlier the findings from 2000-2001 (Fig. 2b.1). From June until October, stomach content $\delta^{15}N$ values are higher in the upper estuary than in the lower estuary. These high $\delta^{15}N$ values can be explained by an increase in the $\delta^{15}N$ of phytoplankton which becomes enriched in ^{15}N during the growth season when nitrification and microbial NH_4^+ uptake gradually deplete the NH_4^+ pool and enrich it in ^{15}N. This enrichment reflects a Rayleigh-like fractionation (De Brabandere *et al.* 2007). Persisting high $\delta^{15}N$ values during summer and early fall in the upper estuary can be attributed to nitrification and the presence of enriched material, mainly imported from upstream reaches (De Brabandere *et al.* 2002). In autumn, $\delta^{15}N$ of sand goby gut content in the upper estuary falls back to lower values. These ^{15}N depleted values are induced by an increase of the apparent fractionation during N uptake by phytoplankton and bacteria as a result of higher NH_4^+ concentrations in the water caused by enhanced mineralization, less nitrification during winter and increased run off (Mariotti *et al.* 1984, Montoya *et al.* 1990, Middelburg & Nieuwenhuize 1998, De Brabandere *et al.* 2002, Fry 2002).

4.2. $\delta^{13}C$ and $\delta^{15}N$ variability on the Belgian Coastal Shelf

Muscle $\delta^{13}C$ showed no or little spatial heterogeneity along the Belgian coast within each month. Only in July 2003 was there a slight ^{13}C depletion noticeable in zone E. Since this station is located closest to the mouth, such a low $\delta^{13}C$ value is likely caused by the presence of organic matter advected from the Scheldt estuary, of which the plume extends southwestwards along the coast. Marine regions close to river mouths are isotopically influenced by riverine output (Fry *et al.* 1984, Peterson 1999). Another explanation might be that these fish in zone E came from the estuary, as we suspect net emigration out of the estuary during July based on density patterns and muscle isotopic compositions in the upper estuary (Chapter 4).

Most of our observed $\delta^{13}C$ values for sand goby muscle along the coast (sampling locations W, C, E, N1 and N2) were depleted relative to reported values for fish species in the North Sea. Das et al. (2003) found $\delta^{13}C$ values between –15.9 ‰ and –17.2 ‰ for different benthic fish species (Pleuronectiformes and Perciformes) in the Southern Bight of the North Sea, with a mean value of –17.1 ± 0.5‰ for Pomatoschistus spp. This is supported by our data for September 2005, with ^{13}C depleted muscle values inshore (C: ± -20‰) and enriched values offshore (OC2: ± -17.3‰) (Fig. 2b.4), suggesting an inshore-offshore gradient for $\delta^{13}C$. Inshore waters probably have a DIC pool depleted in ^{13}C due to terrestrial and riverine sources and due to relatively more respiratory processes than in offshore waters, resulting in lower $\delta^{13}C$ values of organic matter along the coast. In addition, the larger DIC pool in the proximal coastal areas compared to offshore regions results in a stronger discrimination against ^{13}C during microbial CO_2-uptake leading to lower $\delta^{13}C$ values in primary producers. A decrease in partial pressure of CO_2 towards offshore regions on the Belgian Continental Shelf throughout the year was shown by Borges & Frankignoulle (2002).

The temporal variation in coastal $\delta^{13}C$ values, with a 2-3‰ increase from July to March, was also noticed in mantle tissue of *Mytilus edulis* in zone E (Gillikin *et al.* 2006). This can be explained by the variable contribution of phytoplankton, which is ^{13}C depleted, as an indirect C source for sand goby. During the bloom period (summer) this contribution is highest, but it gradually decreases because phytoplankton biomass decreases and also because sand gobies shift from a pelagic to a hyperbenthic feeding mode. Furthermore the fractionation between DIC and phytoplankton probably decreases as more CO_2 is taken up by phytoplankton (Boschker *et al.* 2005). The samples in March had the highest $\delta^{13}C$ values, as they were taken just before the phytoplankton bloom.

Contrary to $\delta^{13}C$ data, a strong gradient for $\delta^{15}N$ was found along the Belgian coast during July 2003 and to a lesser extent during October 2003, with the lowest values in W and highest values in E (Fig. 2b.4). Because of the clear correlation with distance to the estuarine mouth we suspect that mixing of seawater coming from the English Channel and the Scheldt estuarine plume causes this gradient. Especially zone E in July 2003 seems to be strongly influenced by the estuarine outflow, which was also noticed for $\delta^{13}C$, yet to a minor degree. However, if there was such a strong effect of the Scheldt plume, an inshore-offshore gradient for $\delta^{15}N$ could also be expected, but this was not detected in our data. This might indicate that other factors influence the isotopic N composition along the coast. Das *et al.* (2003) reported similar $\delta^{15}N$ values for benthic invertebrate feeding fish in the Southern Bight of the North Sea (15.2 ‰ – 17.8 ‰), with *Pomatoschistus* sp. having the highest values and also the largest standard deviation (17.8 ± 1.9 ‰). This high standard deviation supports the $\delta^{15}N$ variability for sand goby muscle tissue observed in the present study. Further research on the spatial and temporal variation of $\delta^{13}C$ and $\delta^{15}N$ on various trophic levels is needed to fully interpret the $\delta^{13}C$ and $\delta^{15}N$ distribution on the continental shelf.

5. CONCLUSIONS

Based on sand goby gut contents it was demonstrated for the Scheldt estuary that the $\delta^{13}C$ value for food sources in the lower estuary were on average about 6 ‰ higher than those of the upper estuary. The $\delta^{15}N$ gradient between upper and lower estuary, however, switched during autumn. These findings for benthic consumers are largely in agreement with those for clupeoid stomach contents sampled in 2000-2001. Therefore, it was concluded that the observed $\delta^{13}C$ and $\delta^{15}N$ patterns between lower and upper Scheldt estuary are probably yearly recurrent and generally valid. The consistent isotopic difference for $\delta^{13}C$ between food webs in the upper and lower estuary makes this tracer suitable for studying fish migration. However, the temporal variability observed in sand goby gut contents in the upper estuary can not be ignored and should be taken into account. This study also confirmed that $\delta^{15}N$ in the upper Scheldt estuary is highly variable over the seasons. This phenomenon might also occur in other eutrophic estuaries, due to increased microbial activity and nitrification. The absence of a clear $\delta^{15}N$ gradient makes this tracer not suitable for studying estuarine fish migration. Moreover, $\delta^{15}N$ showed considerable spatial heterogeneity in fish muscle tissue along the Belgian coast, which complicates its use as a tracer for estuarine migration even more. $\delta^{13}C$ showed little or no spatial variability along the coast, but an inshore-offshore gradient was observed for this tracer.

Acknowledgements. We are grateful to Electrabel Nuclear Production Zone Doel and EPZ Borssele who kindly gave permission to collect fish at the cooling water inlet. We thank P. Van Den Driessche for preparing the samples, M. Kornthueur for his technical assistance on the IRMS, J. Maes for his remarks on this manuscript and A. Ghesquiere for improving it linguistically. Special thanks to the crew of R.V. Zeeleeuw (managed by VLIZ, Flanders Marine Institute) and R.V. Belgica (managed by MUMM, Management Unit of the North Sea Mathematical Models). The first author acknowledges a Ph.D grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). The study was funded by the Belgian Science Policy grant (TROPHOS, EV/01/25C) and by a K.U.Leuven-OT grant (OT/02/30).


C: mossel
E: pellet

3

CHANGES IN $\delta^{13}C$ AND $\delta^{15}N$ IN DIFFERENT TISSUES OF JUVENILE SAND GOBY *POMATOSCHISTUS MINUTUS*: A LABORATORY DIET SWITCH EXPERIMENT

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Marine Ecology - Progress Series (2007) 341:205-215

Abstract

Studies on diet or migration of organisms based on stable isotopes require precise estimates of how quickly stable isotope ratios change in the investigated tissues. Isotopic turnover rates in fish, however, are poorly understood. Prior to field applications of the stable isotope technique for investigating sand goby *Pomatoschistus minutus* (Pallas 1770) migrations, a laboratory diet switch experiment was conducted to: (1) determine C and N isotopic turnover rates in sand goby muscle, liver and heart tissue and (2) evaluate the relative contribution of growth and metabolic replacement to the total change in isotopic composition. Both time-based and growth-based models adequately described the carbon and nitrogen isotopic change in each tissue. The variation in isotopic turnover rates among the tissues and elements could be attributed to differences in metabolic activity. Muscle tissue had the slowest turnover rates, with half-lives of approximately 25 and 28 days for $\delta^{13}C$ and $\delta^{15}N$, respectively. The shortest half-life value for $\delta^{15}N$ was found in liver tissue (three days) and for $\delta^{13}C$ in heart tissue (six days). The rate of isotopic change in goby muscle tissue was mainly regulated by somatic growth, but metabolic replacement significantly accelerated the turnover rate for $\delta^{13}C$. In liver and heart tissue, basal metabolism contributed considerably to the isotopic shift. As a result, effects of short-term food deprivation were only found in liver and heart tissue. Although the observed trophic fractionation factors were within reported ranges, they were exceptionally large for $\delta^{13}C$ in muscle and liver tissue.

1. INTRODUCTION

Stable C and N isotope ratios are widely used for reconstructing diets and elucidating migration dynamics. Coupling known spatial variation of stable C and N isotopes with tissue specific temporal variation allows researchers to track animal movement (Hobson 1999, Kurle & Worthy 2002, Herzka 2005). The principle is based on the fact that stable isotopic signatures of animal tissues reflect those of their diets. When an animal switches to an isotopically different food source the isotopic composition of its tissues will change as a consequence of two processes. For growing animals isotopic turnover rate is predominantly regulated by simple dilution effects. As a result of switching to an isotopically different diet the initial isotopic composition of the animal (δ_i) will change with growth to a final value (δ_f), which is in equilibrium with the new diet. In this case the old δ_i is only diluted by addition of new tissue synthesized from the new diet during growth. Isotopic turnover rates, however, can be accelerated by the additional effect of tissue specific maintenance metabolism, i.e. the metabolic breakdown of old tissue synthesized during feeding on a previous diet and its subsequent replacement by tissue made from the new diet (Fry & Arnold 1982, Tieszen *et al.* 1983, Hobson & Clark 1992). Although the contribution of metabolic replacement to the total isotopic turnover rate has generally been considered to be negligible or of minor importance in ectotherms, contrasting results have recently been found for fish tissues (Hesslein *et al.* 1993, Herzka & Holt 2000, MacAvoy *et al.* 2001, Bosley *et al.* 2002, Sakano *et al.* 2005, Suzuki *et al.* 2005, Logan *et al.* 2006, McIntyre & Flecker 2006).

Time lags associated with the change in stable isotope ratios are essential information for quantitatively analyzing shifts in food habits and habitats. Because isotopic turnover rates are specific to taxon, type of tissue being analyzed, ontogenetic stage and environmental conditions (Tieszen *et al.* 1983, Frazer *et al.* 1997, Bosley *et al.* 2002, McIntyre & Flecker 2006) it is crucial that studies using stable isotopes to infer dietary information and migration dynamics, take tissue specific turnover rates for the organism and the system under study into account. Unfortunately, studies focussing on the rate of change in isotopic composition of various fish organ tissues after switching to a different food source are very scarce, especially for slow growing fish or short-lived species. Recently, however, this particular research topic became increasingly more popular. See Herzka (2005) and McIntyre & Flecker (2006) for a review of isotopic turnover rate studies on fishes but also Suzuki *et al.* (2005), Miller (2006) and Logan *et al.* (2006).

When in equilibrium with the local food web, the isotopic composition of any organism closely resembles that of its food. Typically the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of a consumer are slightly higher than in its diet, mainly due to the fact that the lighter isotope (^{12}C and ^{14}N) is preferred in enzymatic processes. In general, the stable C isotopic composition of the whole body of an

animal is enriched in ^{13}C relative to its diet by about 0-1‰ (DeNiro & Epstein 1978). Corresponding trophic enrichment values for $\delta^{15}N$ are more variable but average around +3.4‰ (Fry & Sherr 1984, Minagawa & Wada 1984, Owens 1987). Although these trophic enrichments are commonly used in ecological studies they are actually mean values of a wide variety of animals. Isotopic shifts associated with trophic level can be quite variable and may depend on several possible factors, such as taxon, main biochemical form of the nitrogenous waste and type of diet. Even within one individual there are considerable differences in trophic shift among tissues (Focken & Becker 1998, Vander Zanden & Rasmussen 2001, McCutchan *et al.* 2003, Vanderklift & Ponsard 2003). Taking these tissue or component specific differences into account can greatly improve the accuracy when predicting trophic levels or stable isotopic signatures of organisms based on known isotopic data.

This study is framed in a metapopulation research towards the migration dynamics of sand goby *(Pomatoschistus minutus)* between the North Sea and the Scheldt estuary. The sand goby is one of the most common fish species along the European Atlantic coast and its estuaries. They spawn in the North Sea between February and June. Growth rate is highest from July to October and negligible during winter. The sand goby has a short life span; most adults die in their second summer right after their first spawning (Fonds 1973, Hamerlynck 1990). As a marine species they presumably use the Scheldt estuary as a nursery. Due to immigration, maximum densities are generally reached in the upper estuary during September-October. Although this abundance pattern is highly consistent and predictable (Maes *et al.* 2004), the utilization of stable isotopes as tracers of individual recruitment to the estuary will reveal their migration dynamics on a finer temporal scale, thereby improving our understanding of the life history strategies of marine fish species (Herzka & Holt 2000).
The main purpose of this study was to experimentally determine the isotopic turnover rate in dorsal muscle, liver and heart tissue of juvenile *P.minutus* and to identify the most appropriate tissue for subsequent use in a migration study. We hypothesised that the three goby tissues would have different isotopic turnover rates, although contrasting results for fish tissue have been published (e.g. Hesslein *et al.* 1993, MacAvoy *et al.* 2001, Suzuki *et al.* 2005, Logan *et al.* 2006). As a centre of metabolism, the liver is likely to be characterized by a more dynamic change in its biochemical composition than muscle tissue and possibly also heart tissue. When multiple tissues with different turnover rates are measured, $\delta^{13}C$ and $\delta^{15}N$ can provide both short-term and long-term dietary information (Tieszen *et al*. 1983), ultimately increasing the temporal resolution of this technique in migration studies (Hobson 1999). Secondly, the relative contribution of growth and metabolic replacement to the total change in isotopic composition was considered. The observed isotopic change can be better understood with knowledge about the underlying physiological mechanisms. Thirdly, the influence of short-term food deprivation was investigated to infer possible confounding effects of fasting in the field.

Finally, this experiment was used to shed some light on the differences in trophic enrichment among the three tissues.

2. MATERIALS AND METHODS

2.1. Experimental design

On August 24 2004, sand gobies were sampled with a 2-meter beam trawl in the Eastern Scheldt (a marine bay north of the Scheldt estuary), close to the Centre for Estuarine and Marine Ecology (NIOO-CEME) where this experiment took place in a climate test chamber. Within the hour sampled individuals where transferred to three large polyethylene containers to acclimatize to laboratory conditions. Seven fish were randomly selected, measured and sacrificed to determine the initial isotopic composition (δ_i) for each tissue before the diet switch. After two days of acclimatization, fish were randomly assigned to aquaria (capacity 30 L), at densities of six fish per aquarium. Aquaria were installed in a continuous flow through system of filtered (to 45µm) Eastern Scheldt water. At least 20% of the water volume was changed on a daily basis. Temperature was preset on 17°C and the light-dark regime on 12-12 h. The aquaria were provided with a 2 cm sand layer for the gobies to hide in and fine meshed gauze to prevent them from leaping out. During the translocation fish were anesthetized (MS-222) and subsequently marked individually with visible implant elastomers (VIE), measured to the nearest mm (standard length, SL) and weighed (fresh weight) to assess individual growth afterwards. Only fish within a certain length range, corresponding to the average length (± SD) of the population in the Scheldt estuary at that time, were considered for the experiment.

The aquaria were then randomly assigned to a pellet (PEL) and a starvation (STARV) treatment (Table 3.1). The PEL group consisted of 18 aquaria and received a pellet diet based on fishmeal (producer: N.V. Joosen-Luyckx, Art. 10120). The $\delta^{13}C$ value of this food differs by approximately 6 ‰ from that of the initial goby muscle (see Table 3.3); this resembles the isotopic difference between the marine and oligohaline zone of the Scheldt estuary (Guelinckx *et al.* 2006, Chapter 2b). Feeding was twice a day, similar to the natural frequency of sand goby (Healey 1972). Rations were applied in such a way that food was always visible. This corresponded to portions that were approximately 3% of fish body weight. During the experiment, the rations were adapted to increasing fish biomass. The pellet feed was initially selected from a range of feeds based on their isotopic composition and sand goby feeding preference, which was a priori tested in a pilot study. The STARV group consisted of three aquaria in which fish were deprived of any food for 20 days.

Table 3.1. Pomatoschistus minutus*. Overview of experimental design with mean (± SD) initial biomass per aquarium and fish length for the 2 treatments (PEL: pellet food; STARV: starvation). There was no significant difference between treatments for biomass or fish length (Student's t-test, p > 0.05). Biochemical composition and energy content of dried pellets are also shown. Energy content values (± SD) are based on 3 measurements.*

Parameter	PEL	STARV
number of aquaria	18	3
number of fish per aquarium	6	6
initial biomass per aquarium (g)	5.9 ± 0.4	6.3 ± 0.8
mean initial standard length (mm)	42.8 ± 1.4	43.5 ± 0.9
sacrificed on day	10, 20, 30, 45, 60, 90	10, 20
% ash	7.8	
% crude protein	61.7	
% carbohydrate	14.2	
% lipid	13.6	
energy content (kcal/g)	4.62 ± 0.05	

The actual experiment (feeding) started the day after allocation to the aquaria, namely August 28, which was appointed day 0, and lasted 90 days. At specific time intervals, all fish of three randomly chosen aquaria were killed (Table 3.1) to document the change in isotopic composition. In the STARV group only two fish per aquarium were killed on day 10, all the others were killed on day 20. Fish were measured and weighed again before storage at –20°C. Every two days faeces and the remaining food were siphoned away. Dead and sick fish were immediately removed and replaced by marked fish from a reserve stock in order to maintain the same density in each aquarium. These specimens were not considered for further analysis. Aquarium temperature was monitored three times a week and pH, oxygen and salinity twice a week. NO_2^- and NH_3-NH_4^+ were monitored occasionally. All variables were found to be stable (Table 3.2).

Table 3.2. *Mean (± SD) abiotic conditions of the experiment and of the Eastern Scheldt.*

	Temperature (°C)	Salinity	Oxygen saturation (%)	pH	NH_3-NH_4^+ (mg/l)	NO_2^- (mg/l)
aquaria	16.9 ± 0.5	31.8 ± 0.1	92.9 ± 4.1	7.9 ± 0.1	0.46 ± 0.28	0.06 ± 0.05
Eastern Scheldt	14.2 ± 4.2	31.7 ± 0.3	91.7 ± 13.4	8.1 ± 0.4	0.3	/

2.2. Stable isotope analysis

To reduce ecological variability (Gearing 1991), dorsal muscle, liver and heart tissue of two specimens from each aquarium were used for stable isotope measurements. Consequently, tissues from six individuals from each sampling event were analysed resulting in three replicas. The two fish with the largest increase in biomass per aquarium were selected for analysis because we assumed they were most at ease during the experiment. Within the STARV group fish were chosen randomly for isotope analyses.

All tissue samples were dried for two days (55°C) to constant weight and ground with a mortar and pestle. An aliquot (0.5-0.6 mg) was subsequently packed in tin containers for isotope analysis (for heart tissue this often meant the entire sample). Lipids were not removed from our samples prior to analysis to avoid dispersion of $\delta^{13}C$ and $\delta^{15}N$ (Pinnegar & Polunin 1999). Stable isotope measurements were done 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 standards were high-purity N_2 and CO_2, while IAEA-C-6 and IAEA-N2 were used as reference materials. Stable isotopic compositions are expressed in the conventional δ-notation:

δX (‰) = $[(R_{sample} - R_{standard})/R_{standard}] \times 10^3$,
where *X is* ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

$\delta^{13}C$ values are expressed relative to the VPDB (Vienna PeeDee Belemnite) standard, while $\delta^{15}N$ values are expressed to atmospheric N_2. Reproducibility for different aliquots of the reference materials was generally better than 0.3‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

Differences in isotopic composition among tissues before the diet switch and within the same tissue among sampling events in the STARV group were tested using one-way ANOVA. When the assumptions were not fulfilled the non-parametric Kruskall-Wallis test was applied. Consequently, post hoc Tukey's HSD or Mann-Whitney *U* tests were used to identify the significantly differing groups (STATISTICA 6.0, StatSoft).

2.3. Turnover modeling

Data processing was done with the mean values (two fish) of each aquarium (three replica) resulting in three values per sampling event for each tissue. Single-pool models as a function of time and growth were used to describe the shift in $\delta^{13}C$ and $\delta^{15}N$ within each tissue.

Model as a function of time:

The following exponential model describes the change in isotopic composition as a function of time (Tieszen *et al*. 1983):

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

where δ_t is the stable isotopic composition of a tissue at the time of fish collection from the aquaria, δ_i is the initial value before the diet switch, δ_f is the final isotopic composition equilibrated to the new diet, t is the time that fish were in the experiment (in days), v is a measure of the turnover rate and has unit time^{-1}. δ_f and v were determined by fitting (least squares method) the model to the data using Sigma Plot 2000 6.0 (SPSS Inc.). During the model fitting procedure, the value of δ_i was fixed to the mean value of the seven individuals sampled from the Eastern Scheldt.

A more conventional way to express the isotopic turnover rate is by the half-life value ($t_{1/2}$), i.e. the amount of time required to reach the midpoint value of the initial (δ_i) and final values (δ_f). By transforming equation 1 the half-life value for each tissue was calculated as follows (Tieszen *et al*. 1983):

$$t_{1/2} = \ln (2) / v \quad (2)$$

Model as a function of growth:

The relative contributions of growth and metabolic turnover to the observed changes in $\delta^{13}C$ and $\delta^{15}N$ were examined using the following equation (Fry & Arnold 1982, Herzka *et al.* 2001):

$$\delta_t = \delta_f + (\delta_i - \delta_f) (W_t/W_i)^{c} \quad (3)$$

where δ_t, δ_i and δ_f have the same meaning as in the time model above, W_i and W_t indicate the fresh weight of an individual before the experiment and at the moment of collection respectively, c is the exponent of metabolic decay and is a measure of the relative contribution of growth and metabolic activity to the isotopic turnover rate. When c = -1, the rate of change in isotopic composition is mediated by growth alone and the dilution model prevails. When c < -1, metabolic replacement increases the rate of isotopic change (Fry & Arnold 1982, Herzka *et al*. 2001). δ_f and c were estimated by the Levenburg-Marquardt iterative, non linear, least squares fitting algorithm using STATISTICA 6.0 (StatSoft). Again, for the curve fitting procedure, δ_i was set to the mean initial value.

3. RESULTS

3.1. $\delta^{13}C$ and $\delta^{15}N$ before the diet switch

Mean $\delta^{13}C$ and $\delta^{15}N$ values (δ_i) of the three tissues before the diet switch (August 24) are presented in Table 3.3. Liver (L) was significantly depleted in ^{13}C relative to muscle ($\Delta\delta^{13}C_{L-M}$ = 4.9 ‰) and heart tissue ($\Delta\delta^{13}C_{L-H}$ = 4.8 ‰) (Tukey's HSD test, $p < 0.001$), while muscle and heart tissue had very similar $\delta^{13}C$-values. For $\delta^{15}N$, the three tissues differed significantly from

each other, although these differences were small. Muscle tissue was more enriched in ^{15}N than heart ($\Delta\delta^{15}N_{M-H}$ = 1.7 ‰) and liver tissue ($\Delta\delta^{15}N_{M-L}$ = 2.9 ‰) (Tukey's HSD test, $p < 0.001$) while heart tissue was also slightly more enriched than liver tissue ($\Delta\delta^{15}N_{H-L}$ = 0.6 ‰) (Tukey's HSD test, $p < 0.01$). Table 3.3 also displays the mean isotopic composition of the pellet diet and the expected isotopic composition of the tissues when equilibrated to the pellet diet. Pellet samples were taken every two weeks confirming that there was no change in diet isotopic composition during the experiment. The expected muscle values were predicted from the mean pellet values by assuming a trophic enrichment factor of +1.0‰ for $\delta^{13}C$ and +3.4‰ for $\delta^{15}N$ (DeNiro & Epstein 1978, Minagawa & Wada 1984, Owens 1987). For liver and heart tissue the deviation of these tissues from muscle before the experiment was additionally taken into account. The expected final values differed about 6.2‰ for $\delta^{13}C$ and 3.6‰ for $\delta^{15}N$ from sand goby values before the diet switch.

Table 3.3 *$\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) of the pellet diet, sand goby muscle, liver and heart tissue in the Eastern Scheldt and values expected for the three tissues when in equilibrium with the pellet diet. Fish were sampled on July 20, 2004 (preliminary study) and August 24, 2004 (start of experiment). For muscle tissue, expected values were calculated by adding +1.0 and 3.4 ‰ to pellet $\delta^{13}C$ and $\delta^{15}N$, respectively; for liver and heart tissue, isotopic differences from muscle tissue were taken into account.*

Tissue	**$\delta^{13}C$ (‰)**			**$\delta^{15}N$ (‰)**		
	July 20	August 24	expected	July 20	August 24	expected
muscle	-16.1 ± 0.1	-16.2 ± 0.6	-22.4	15.8 ± 0.4	16.1 ± 0.4	12.5
liver	-20.9 ± 0.5	-21.2 ± 0.5	-27.3	13.9 ±0.3	13.7 ± 0.2	10.1
heart	-16.0 ± 0.6	-16.4 ± 0.8	-22.6	14.4 ± 0.2	14.4 ± 0.2	10.5
pellet diet		-23.4 ± 0.4			9.1 ± 0.2	

3.2. Turnover modeling as a function of time

During the three months of the experiment the goby tissues shifted towards a new equilibrium as a result of the pellet diet (Fig. 3.1). All estimates and models were significant ($p < 0.05$) and explained between 81 % and 95 % of the variation. The rate of isotopic change differed among tissues and between C and N. Muscle tissue had the slowest turnover with similar half-lives of 24.7 and 27.8 days for $\delta^{13}C$ as $\delta^{15}N$, respectively. For $\delta^{13}C$ the shortest half-life was found in heart tissue (6.1 days), while liver had an intermediate half-life of 9.1 days. For $\delta^{15}N$, the highest isotopic turnover rate was recorded in liver tissue (2.8 days). Surprisingly, heart $\delta^{15}N$ had a similar turnover rate (27.6 days) to muscle $\delta^{15}N$. The dashed lines in Fig. 2 represent the expected final values for each tissue (cfr. Table 3.3). Our data approached the expected values in 4 of 6 tissue-isotope combinations with exceptions for $\delta^{13}C$ in muscle and liver. There was a discrepancy of 3.2‰ for muscle and 2.1‰ for liver between the expected value and δ_f estimated by the model.

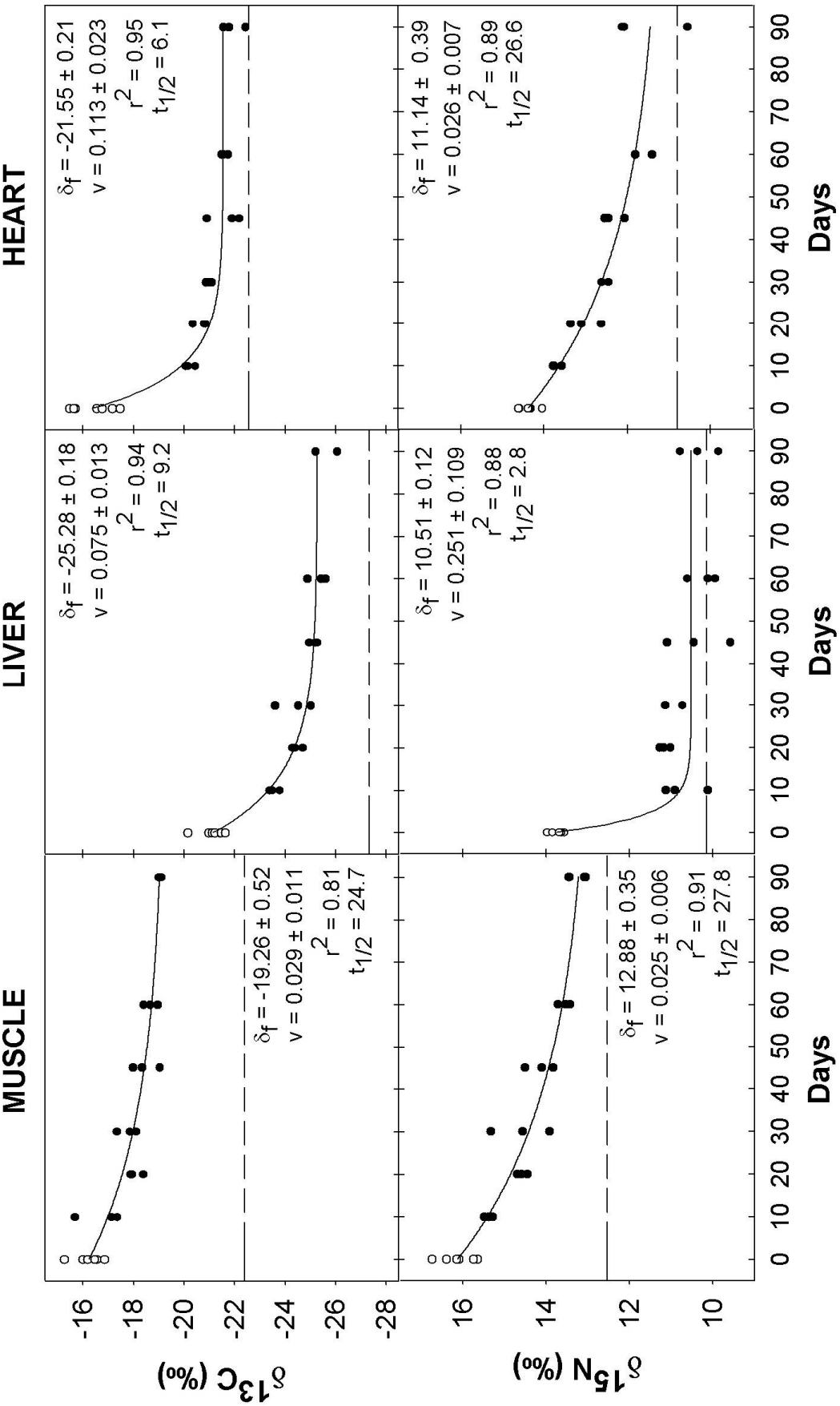

Fig. 3.1. Pomatoschistus minutus. *Changes in $\delta^{13}C$ and $\delta^{15}N$ as a function of time for muscle, liver and heart tissue in the PEL group. ●: δ_t mean value of 2 fish (1 aquarium). ○: δ_i (1 individual). Full lines represent best fit through the data following $\delta_t = \delta_f + (\delta_i - \delta_f)exp(-vt)$; δ_f (‰) and v were estimated by the model (± SE); r^2 and $t_{1/2}$ (days) are also shown. Dashed lines: expected final values when in equilibrium with pellet diet (cf. Table 3.3).*

3.3. Turnover modeling as a function of growth

On average, fish in the PEL group had doubled their biomass by the end of the experiment (Fig. 3.2). Throughout the experiment the mean growth rate k (= $\log_e [W_t/W_i] \times t^{-1}$) in the PEL group was 0.012 day^{-1}. The change of $\delta^{13}C$ and $\delta^{15}N$ in the three tissues was also described as a function of biomass increase (Fig. 3.3). Again, all estimates were significant and the models explained between 79 and 90 % of the variation. The exponent of metabolic decay c was not significantly different from -1 for $\delta^{15}N$ in muscle and heart tissue. However, metabolic activity seemed to become increasingly more important in muscle $\delta^{13}C$, liver $\delta^{13}C$, heart $\delta^{13}C$ and liver $\delta^{15}N$, in which the lowest c value was observed (-15.9). The dotted lines in Fig. 3.3 represent simple dilution models where c assumes a value of -1.

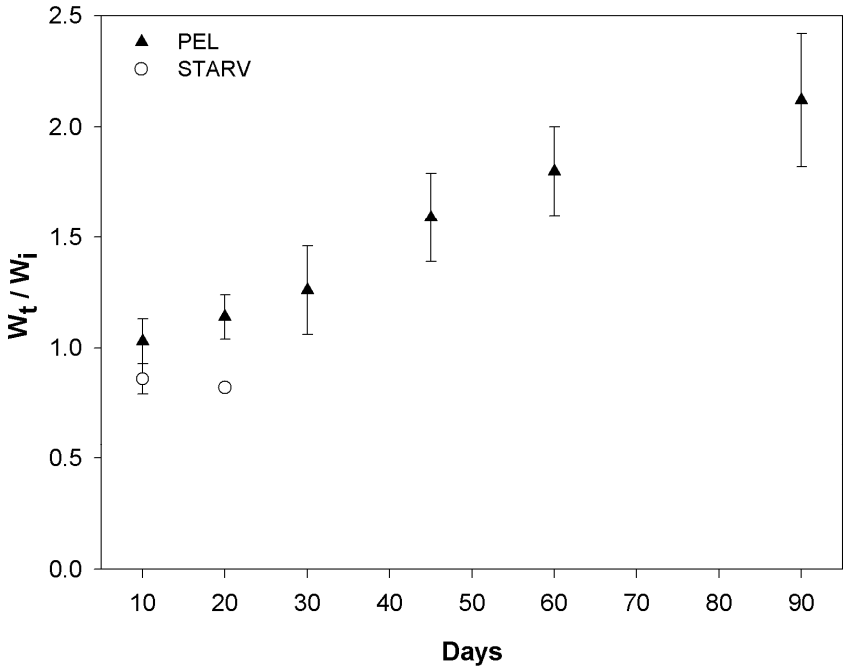

***Fig. 3.2**:* Pomatoschistus minutus. *Average (± SD) change in biomass, expressed as W_t/W_i, for fish in the pellet-fed (PEL) and starved (STARV) groups. Error bars are standard deviations. Data are based on all surviving individuals from Day 0.*

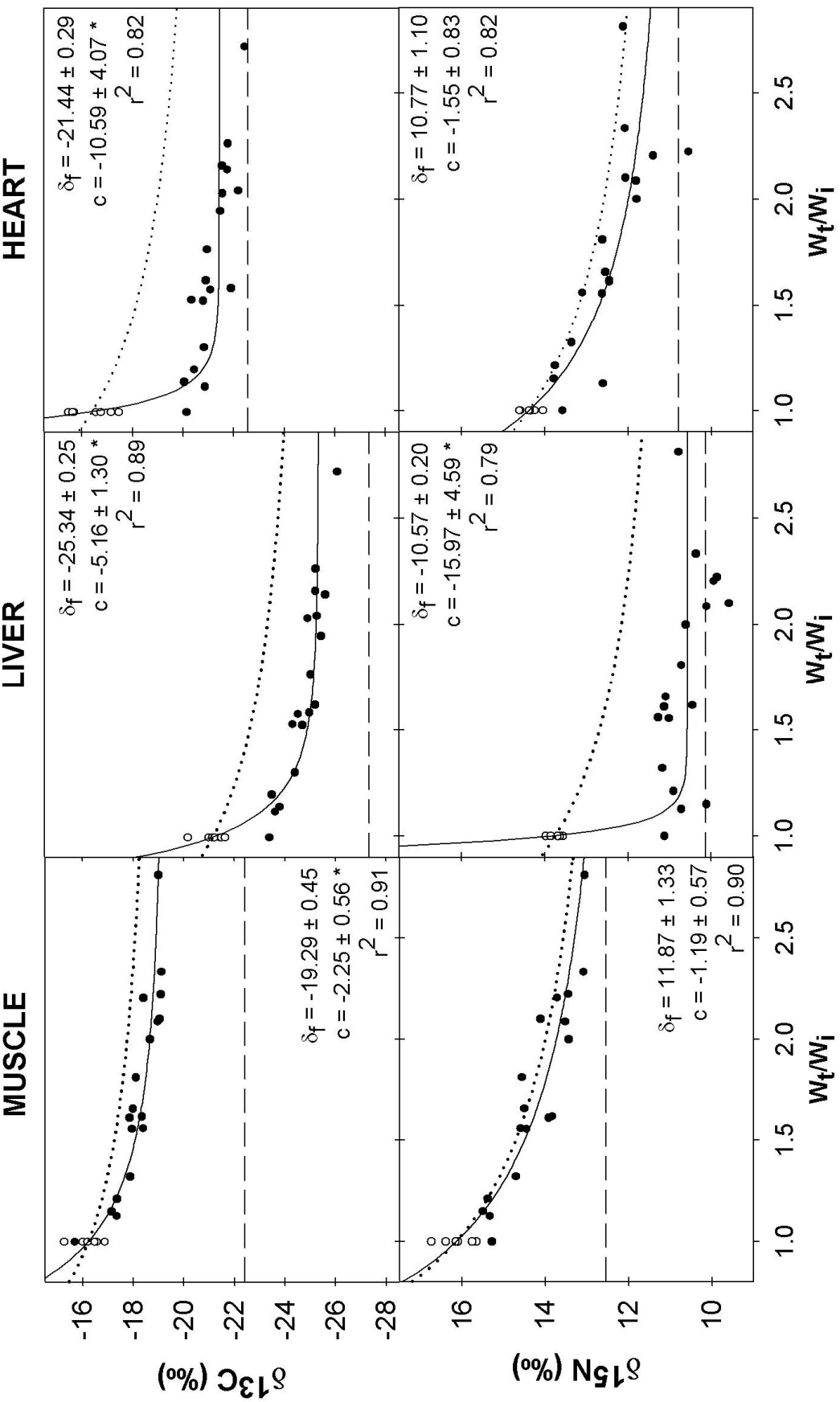

Fig. 3.3. Pomatoschistus minutus. *Changes in $\delta^{13}C$ and $\delta^{15}N$ as a function of fish biomass for muscle, liver and heart tissue in the PEL group. (●) δ_t mean value of 2 individuals (1 aquarium); (○) δ_i (1 individual). Continuous lines: best fit through the data following $\delta_t = \delta_f + (\delta_i - \delta_f)(W_t/W_i)^c$, where δ_f (‰) and c were estimated by the model (± SE); * indicates when c is significantly different from -1; dotted lines: change in isotopic composition due to dilution only (c = -1); dashed lines: expected signatures when in equilibrium with the pellet diet (cf. Table 3.3)*

3.4. Effect of short term fasting

Fish in the STARV group had decreased in biomass by approximately 14 % after 10 days and 20 % after 20 days (Fig. 3.2). No change in muscle $\delta^{13}C$ and $\delta^{15}N$ or in liver $\delta^{13}C$ was observed after 20 days of fasting (Fig. 3.4). However, liver $\delta^{15}N$ and heart $\delta^{15}N$ increased while heart $\delta^{13}C$ decreased as a result of food deprivation. For liver $\delta^{15}N$, the effect already occurred after 10 days; for heart $\delta^{13}C$ and $\delta^{15}N$ the effect of starvation was only detectable on day 20.

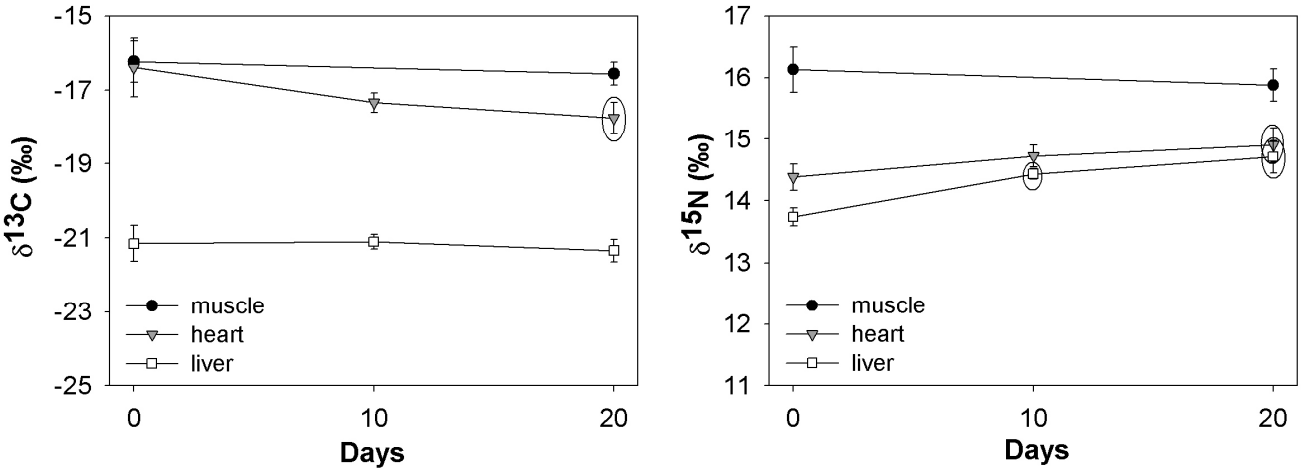

Fig. 3.4 Pomatoschistus minutus. *STARV group: effect of food deprivation on $\delta^{13}C$ and $\delta^{15}N$ of muscle, liver and heart tissue (mean value ± SD) after 10 and 20 days. δ-values significantly different ($p < 0.05$) from those before the diet switch are encircled.*

4. DISCUSSION

4.1. Mortality and growth

Fish mortality was highest during the first two weeks during which several fish died. Mortality was the same in both treatments and eventually never exceeded 20 %. It could not be attributed to one specific cause. We observed that some fish did not forage on the pellet diet and they became feeble and died. Some suffered visibly from bacterial and fungal infections. Marine gobies are highly susceptible to mycosis and bacterial infections and this causes a high mortality at sea. Such infections probably affected some of our freshly caught gobies, and were enhanced by external stress occasioned by capture and captivity (M. Fonds, pers. comm.). The remaining fish were probably resistant to the infections and their average growth rate (PEL group) equalled that of their natural habitat for the same period (J. Guelinckx unpublished results), indicating that the experimental conditions were satisfactory for *P. minutus* and that the experimental results can be applied to field data.

4.2. Tissue specific $\delta^{13}C$ and $\delta^{15}N$ values

The initial $\delta^{13}C$ and $\delta^{15}N$ values were tightly grouped in each tissue and correspond strongly (Mann Whitney *U* tests, $p > 0.05$) to values of sand gobies sampled one month earlier (July 20 2004) in the Eastern Scheldt during a preparatory study. These results are also shown in Table 3 for comparison. The consistency in isotopic composition of both sampling events strongly suggests that the tissues were in equilibrium with the local marine food web on day 0. The significant differences among the tissues indicate tissue specific trophic shifts. The same ranking in $\delta^{13}C$ and $\delta^{15}N$ values for muscle, liver and heart tissue was also found in other studies, e.g. for rainbow trout *Oncorhynchus mykiss* (Pinnegar & Polunin 1999). These isotopic variations result partly from differences in biochemical composition. The lipid content in particular seems to be important for $\delta^{13}C$, with lipids being depleted in ^{13}C relative to the other biochemical components (DeNiro & Epstein 1977, Tieszen *et al*. 1983, Gearing 1991). Liver, which has a high lipid content, generally has lower $\delta^{13}C$ values (Focken & Becker 1998, Pinnegar & Polunin 1999, Lorrain *et al.* 2002). Hesslein *et al.* (1993) found for broad whitefish (*Coregonus nasus*) that liver $\delta^{13}C$ was on average 4.1 ± 0.5 ‰ more depleted than muscle tissue, which is highly consistent with our results. However, they also found that lipid removal from liver tissue could only account for a small portion (0.7 ± 0.3‰) of the difference between liver and muscle. This, together with an increased variability in isotopic composition after lipid removal (Pinnegar & Polunin 1999), made us decide not to extract lipids from our samples.

The tissues are also distinct in terms of $\delta^{15}N$ and this is probably caused by the relative abundance of different amino acids in the tissues. The isotopic composition of essential amino acids exhibits little change during assimilation. Amino acids that are wholly synthesized or at least partly modified however, may undergo shifts in $\delta^{15}N$ of varying magnitude depending on the biosynthetical pathway (Pinnegar & Polunin 1999). Liver protein is known to have a greater proportion of essential amino acids, which might explain the lower $\delta^{15}N$ value observed for liver (Pinnegar & Polunin 1999, Kurle & Worthy 2002, McClelland & Montoya 2002). Thus, in addition to the different biochemical composition of tissues, secondary fractionation during physiological processes contributes to the isotopic variation among tissues. Also, lipid synthesized from dietary carbohydrate is relatively enriched in ^{12}C (DeNiro & Epstein 1977) in comparison to fat deposited directly from dietary lipid. Due to metabolic activity and/or biochemical components these secondary fractionation effects governing nitrogen and carbon assimilation are tissue specific (Kurle & Worthy 2002).

Furthermore, differences in isotopic composition among organs could also reflect the phenomenon of isotopic routing, which means that dietary nutrient components are allocated differentially to specific tissues and tissue components. Consequently, a tissue often reflects

the isotopic composition of the nutrient component of the diet from which the tissue was synthesized and not the isotopic composition of the whole diet (Gannes *et al.* 1997).

We suspect that the nutrient components of the formulated pellets were not homogeneous in C isotopic composition, and that differential assimilation and routing of these components led to the observed discrepancy between the model predicted $\delta^{13}C$ value (δ_f) and the expected $\delta^{13}C$ value in muscle and liver tissue (Fig. 3.1, 3.3). The other models, especially those for $\delta^{15}N$, predicted a final value, δ_f, close to expected values supporting the applied classical trophic enrichment factors and the observed differences among the tissues before the diet switch.

The carbon trophic enrichment factor in marine vertebrates is generally very small (Kurle & Worthy 2002). Post (2002) reviewed the trophic fractionation within aquatic organisms and found an average increase of 0.4 ± 1.3 ‰ (n = 107) for $\delta^{13}C$. There was, however, a relatively large variation around this value ranging from approximately –3 ‰ to +4 ‰. Peterson & Fry (1987) and Vander Zanden & Rasmussen (2001) reported similar values. High trophic enrichment factors for $\delta^{13}C$ have been reported for muscle tissue of brook trout (*Salvelinus fontinalis*) (+3.3 ± 0.29 ‰) (McCutchan *et al*. 2003) and of juvenile mado (*Atypichthys strigatus*) (+3.7 ‰) reared on a diet of commercial flake for 12 months (Gaston & Suthers 2004). It remains unclear as to what the most important underlying biochemical and physiological mechanisms are that control the isotopic differences among tissues. It is obvious that more field and laboratory investigations are required to elucidate the sources of variation in tissue isotopic composition and specific trophic shifts. This is all the more important since isotopic variability among organs may reflect their different metabolic activity as well as their isotopic turnover rates (Gannes *et al*. 1997, Gannes *et al.* 1998, Lorrain *et al*. 2002).

4.3. Change in isotopic composition following a dietary change

The isotopic turnover rates vary widely among different tissues and organs in endothermic vertebrates (e.g. Tieszen *et al.* 1983, Hobson & Clark 1992, Hobson *et al.* 1993, MacAvoy *et al.* 2005), but this has not yet been unambiguously established for poikilothermic fish. C, N and S turnover rates for liver and muscle tissue were similar in broad whitefish, suggesting that the large differences in turnover rates among tissues of endotherms might not occur in fishes (Hesslein *et al*. 1993). This was among others supported by Herzka & Holt (2000), who stated that the high basal metabolism of endotherms, that probably results in tissue specific turnover rates does not occur in ectotherms, explaining the negligible effect of metabolic replacement to isotopic change often found for ectotherms. The half-life periods found in this study, however, clearly show that different fish organ tissues can vary in isotopic turnover rate. Different $\delta^{13}C$ and $\delta^{15}N$ isotopic turnover rates were found for goby muscle, liver and heart tissue, with muscle having the slowest turnover rate (Fig. 3.1). Moreover, our results indicate that metabolic activity is not always negligible in fish tissue and can play a dominant role in isotopic change for tissues other than muscle (Fig. 3.3). Similar results were recently reported for whitefish (*Coregonus Lavaratus*) (Perga & Gerdeaux 2005), Japanese temperate bass

(*Lateolabrax japonicus*) (Suzuki *et al.* 2005) and juvenile mummichogs (*Fundulus heteroclitus*) (Logan *et al.* 2006).

The exponent of metabolic decay, c allows interpretation of the isotopic turnover rate in terms of growth and metabolic replacement. A decrease in c corresponds with faster isotopic turnover rates in our results (Fig. 3.1, 3.3) and this implies an increase in the relative contribution of metabolic replacement to isotopic change. c was significantly different from -1 for $\delta^{15}N$ in liver and for $\delta^{13}C$ in muscle, liver and heart tissue, demonstrating that metabolic replacement accelerates the rate of isotopic change and causes tissue specific isotopic turnover rates. The liver is a regulatory tissue with a continuous protein turnover, which exceeds the one in muscle tissue (de la Higuera *et al.* 1999). This explains the shorter half-life of $\delta^{13}C$ and $\delta^{15}N$ in liver compared to muscle. Muscle is the most representative tissue of growth when protein synthesis and deposition are considered, but at the same time it has a relatively low protein turnover in fish (de la Higuera *et al*. 1999). The $\delta^{13}C$ in goby heart tissue had a relatively high turnover rate. To some extent, this could have been caused by the adhering blood plasma fractions, which have a short $\delta^{13}C$ half-life period (Hobson & Clark 1993).

Metabolic activity was found not to be important for nitrogen isotopic change in goby muscle, with the estimated c not significantly different from -1 (95% CI: -2.37, -0.01). For carbon, however, -1 just fell outside the estimated 95% CI for c (-3.45, -1.07). We support the general idea that mainly growth is responsible for isotopic change in fish muscle tissue. Nevertheless, a refinement of the part of metabolic replacement seems necessary. Metabolic activity is most likely negligible for the isotopic turnover rate in early life stages or fast growing fish and a simple dilution model is adequate to describe the change of isotopic composition of the whole body (Hesslein *et al*. 1993, Herzka & Holt 2000, MacAvoy *et al*. 2001, Bosley *et al*. 2002), However, metabolic replacement cannot be ignored in muscle tissue of almost full-grown fish like the gobies in this study. The exponent c for muscle $\delta^{13}C$ had a relatively low value (-2.26) with respect to other published metabolic decay values for larvae and juvenile fish (Herzka & Holt 2000, Herzka *et al*. 2001, Bosley *et al*. 2002) and was indeed different from -1, indicating a significant contribution of metabolic replacement. So, in tissues of moderate to slow growing fish both metabolic turnover and growth are likely to play an important role in isotopic changes (Suzuki *et al.* 2005, Logan *et al.* 2006, Miller 2006) as was already hypothesized by Fry & Arnold (1982). Sakano *et al.* (2005) showed for sockeye salmon (*Oncorhynchus nerka*) that the degree of metabolic contribution became increasingly more important with age as growth rate decreased.

4.4. Isotopic composition during starvation

Fasting animals tend to have stable isotope ratios that are distinct from those fed ad libitum. The effects of food deprivation are especially expected to manifest in metabolically more active tissues (Hobson *et al*. 1993) or tissues for which a low exponent of metabolic decay is detected.

In this study, no effects were indeed found for muscle tissue but significant changes occurred in liver $\delta^{15}N$ and heart $\delta^{13}C$, consistent with their low exponent of metabolic decay, c. Liver $\delta^{13}C$, however, exhibited no change although its c was rather low and the ^{15}N enrichment in heart was not expected based on its c close to –1 (Fig. 3.3, 3.4).

The tissues of starving animals often show a progressive increase in the $^{15}N/^{14}N$ ratio as body mass decreases (Hobson *et al*. 1993, Oelbermann & Scheu 2002, Olive *et al.* 2003). The mechanisms by which their tissues become enriched in ^{15}N are partly the same as those causing trophic fractionation. The catabolized and excreted lighter ^{14}N is not replaced by dietary protein, therefore the animal becomes progressively more ^{15}N enriched during starvation (Gannes *et al*. 1997). The rate of increase in the animal's ^{15}N concentration happens faster when the ratio of the excretion rate of ^{14}N to ^{15}N is high and N turnover is large (Ponsard & Averbuch 1999). An alternative explanation for the ^{15}N enrichment involves possible changes in amino acid composition (Hobson & Clark 1992).

The decrease in $\delta^{13}C$ noted in heart tissue is quite remarkable, since most other starvation experiments do not report a $\delta^{13}C$ change and if so, the change is toward ^{13}C enrichment (Hobson & Clark 1992, Frazer *et al.* 1997, Gorokhova & Hansson 1999, Oelbermann & Scheu 2002, Olive *et al.* 2003, Tominaga *et al.* 2003). A ^{13}C enrichment can be explained by the preferential loss of ^{12}C during oxidation of acetyl groups derived from catabolism of lipids, proteins and carbohydrates (Hobson *et al*. 1993). Depleted $\delta^{13}C$ values as a result of food deprivation have to our knowledge not yet been reported. One explanation for the lower values in heart tissue could be that blood components, "contaminating" the heart tissue, contained catabolized products enriched in ^{12}C. Another explanation is that ^{13}C depleted lipids are used as fuel in heart muscle tissue. It is evident that more research is needed for complete understanding of the change in isotopic composition and the origin and fate of mobilized biochemical components during catabolism.

4.5. Comments on the experimental design

The experiment was carefully designed to resemble natural conditions as much as possible. The start of the experiment coincides with the start of sand goby migration into the Scheldt estuary. Consequently, sampled individuals had approximately the same age and length as those from the estuarine population under study. In addition, when transferring the fish to aquaria, all specimens not corresponding to the average length ± SD of the estuarine population at that time were excluded from the experiment. Temperature and photoperiod corresponded to average conditions for the Scheldt estuary during the specific period. Despite the fact that experimental conditions mimicked natural conditions, care should be taken when applying the experimental results to field data, especially due to the high estimated trophic shifts for $\delta^{13}C$ in muscle and liver. It would have been better to prolong the experiment until all tissues reached isotopic equilibrium with certainty and to use a natural diet. This was however not possible in our study. A shorter sampling interval during the first 20 days would also have

improved our experimental design. Nevertheless, our study provided a useful first insight into stable isotopic turnover rates in juvenile sand goby tissues but further research is required to better understand how variations in metabolic activity (e.g. due to temperature, body size, stress, etc.) influence isotopic turnover rate in tissues.

Finally, we highly encourage the use of a control treatment to detect shifts in isotopic composition not caused by a new diet. A control treatment based on mussel meat (*Mytilus edulis*) was *a posteriori* rejected from our experiment because, after accounting for trophic enrichment, mussel $\delta^{13}C$ and $\delta^{15}N$ values deviated from those of goby muscle before the diet switch, a result in contrast to that of a preliminary study. In addition, from day 45 onwards there was a difference in growth rate between fish fed the pellet or the mussel diet. When selecting diets for an isotopic turnover study care should be taken to not only consider the isotopic compositions but also to confirm that the nutritional composition and the food quality of the respective diets are equal to each other and to that of the food sources of the species under study.

5. CONCLUSIONS

The results of this study encourage the use of stable C and N isotopes for analyzing sand goby migrations in terms of several days to several weeks. The controlled laboratory conditions resulted in clear changes in the isotopic compositions of sand goby muscle, liver and heart tissue. Observed isotopic turnover rates were specific for tissue type and element. Muscle tissue was identified to have an appropriate temporal resolution ($t_{1/2}$ = ± 26 days) to determine residence times of individuals caught on a monthly basis in the upper Scheldt estuary. Moreover, muscle is to be preferred since it is not influenced by short term fasting, in contrast to metabolically more active liver and heart tissue. The isotopic turnover rate in muscle is predominantly controlled by somatic growth, although we found a significant contribution of metabolic replacement for $\delta^{13}C$. The relative contribution of basal metabolism is likely to increase in older, slow growing (sub)adult fish and/or during warm periods. The isotopic turnover rate for liver and heart tissue was quite high and can probably be explained by the important role of metabolic replacement in these tissues. This feature is generally accepted for endotherms but not for ectotherms like fish. The observed fractionation factors for $\delta^{13}C$ in muscle and liver emphasize the need for further research towards the physiological and biochemical mechanisms that control the isotopic shift between an organism and its diet.

Acknowledgements. We thank the staff of the Centre of Estuarine and Marine Ecology (NIOO-CEME) in Yerseke (The Netherlands) for cooperating and making their infrastructure available. Similarly we would like to thank the Research Institute for Nature and Forest (INBO) for placing the VIE marking equipment at our disposal. Special thanks to G. Westrate, E. Cuveliers, A. Ghesquiere for their assistance in collecting, measuring and marking the fish and to A. Van de Putte for the energy measurements. We are also grateful to L. De Brabandere and C. Martínez del Rio whose comments greatly improved this manuscript. The first author acknowledges a Ph.D grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). The study was funded by a K.U.Leuven-OT grant (OT/02/30).

4 ESTUARINE RECRUITMENT OF A MARINE GOBY RECONSTRUCTED WITH AN ISOTOPIC CLOCK

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Oecologia (in press)

Abstract

Information on movement patterns of marine fishes between estuarine populations and stocks at sea is fundamental to understand their population dynamics, life history tactics and behaviour. 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 on how estuarine populations interact with the population at sea. Immigration 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. About 70% of the fish caught in the upper estuary resided there for less than one 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 questions 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 two 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.

1. 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 (Äkesson 2002).
Extensive research on estuarine fish communities in Europe (e.g. Elliott & Dewailly 1995, Thiel & Potter 2001, Elliott & Hemmingway 2002, Greenwood & Hill 2003, Thiel *et al*. 2003, Maes *et al*. 2005b), North America (e.g. Hagan & Able 2003, Martino & Able 2003, Ross 2003, Able 2005, Miller & Shanks 2005), South Africa (e.g. Potter *et al*. 1990, Whitfield 1999) and Australia (e.g. Blaber *et al*. 1989, Potter & Hyndes 1999) invariantly identified marine fishes as the most important group in estuaries. These studies led to defining ecological guilds derived from life history strategies, such as marine juvenile migrants, marine seasonal users and marine stragglers (Elliott & 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 & Hemmingway 2002, Greenwood & Hill 2003, Ross 2003, Attrill & 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 & Shanks 2005), partly because migration dynamics and their underlying mechanisms are poorly understood (Rountree & Able 2007). Although the temporal distribution pattern of most marine species in estuaries is highly predictable (Thiel & Potter 2001, Greenwood & 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 & 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 & Hobson 2004). During the last decade, increasing emphasis has been put on natural geochemical tracers to study movement patterns. The stable isotope composition ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) 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, and Rubenstein & 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 & 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 & Eldridge 2006). This provides a measure 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 C 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 & Guelorget 1998) and it forms 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 gobies reproduce in the coastal waters of the North Sea during spring (March - June). Larvae are pelagic for 4 to 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 de 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 to understand the function of estuarine visits and life history strategies of marine fish species.

2. MATERIALS AND METHODS

2.1. 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 (Fig. 4.1). 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 two and 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). An average difference of 6 ‰ was demonstrated for $\delta^{13}C$ 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 (unpubl. results). For $\delta^{15}N$, no consistent difference was found between both areas, so only $\delta^{13}C$ 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 nuclear power plant (NPP) Doel which is located in the mesohaline zone of the Scheldt estuary at 61 km from the mouth (Fig. 4.1). Here, salinity averaged 9.7 ± 3.7 (mean ± SD) during the sampling period. Sampling always started 1.5 h before ebb tide and lasted for 3 h. Nets with a 4 mm mesh size were used for collecting fish. A technical problem 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).

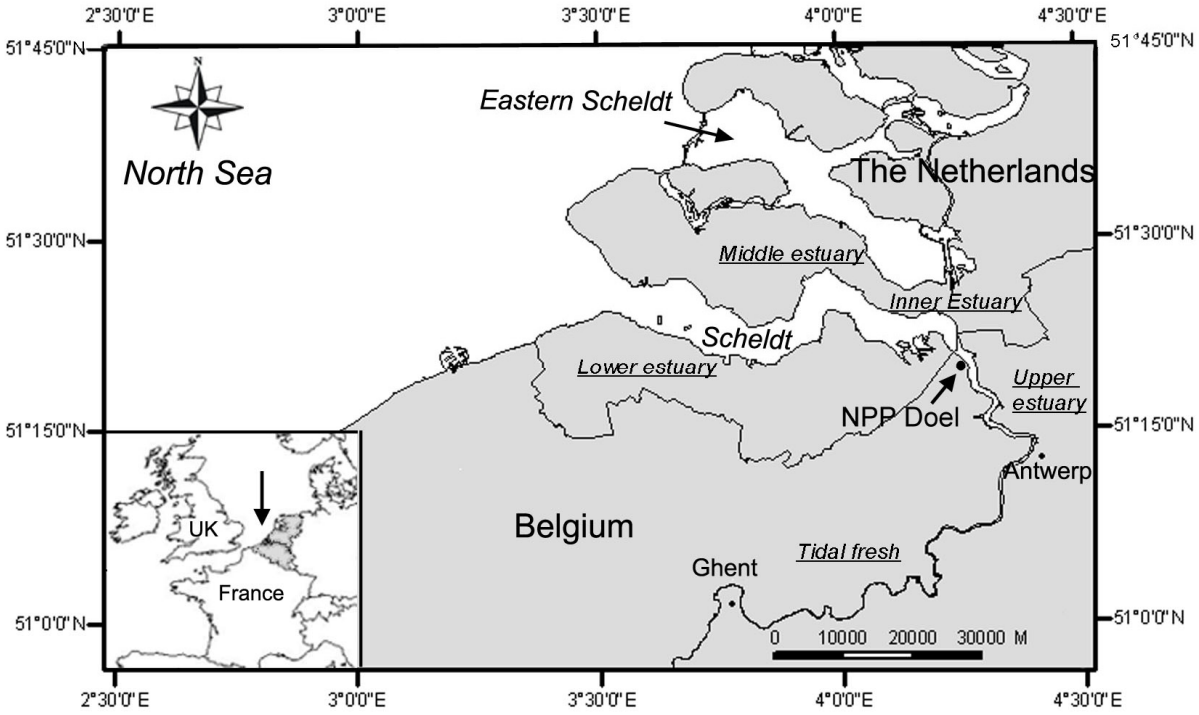

Fig. 4.1 *Map of the Scheldt estuary, which discharges in the North Sea and is situated in the Dutch Delta. The sampling location (NPP Doel) is located in the upper estuary at 61 km from the mouth.*

2.2. Sample preparation and stable isotope analysis (SIA)

Dorsal muscle samples of 15 randomly chosen fish were collected for SIA from each monthly catch, except from August of which 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 $\delta^{13}C$ (25 days) during maximal sand goby abundance in the estuary (Chapter 3).

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, $\delta^{13}C$: -10.4 ± 0.1 ‰) was used as a reference material. Stable isotopic compositions are expressed in the conventional δ-notation (‰):

$$\delta^{13}C\ (‰) = [(^{13}C/^{12}C_{\ sample} - {}^{13}C/^{12}C_{standard}) / (^{13}C/^{12}C_{\ standard})] \times 10^3$$

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

2.3. Development of the 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:

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

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 v 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 Rio & Wolf 2005). To test whether or not multiple pools with different rate constants might be present for C in sand goby muscle tissue and hence also to test the appropriateness of the exponential model, we applied the reaction progress model (Ayliffe *et al*. 2004, Cerling *et al*. 2007) to our experimental data (Chapter 3). 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:

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

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 to detect, when present, 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 (Chapter 3) was not curvilinear in time (Fig. 4.2), indicating a single pool. The intercept shows that this pool contributes 98 % to the total signal (Fig. 4.2). 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 Rio & Anderson-Sprecher, submitted). Hence, an isotopic clock was developed based on the exponential fit (eq. 1).

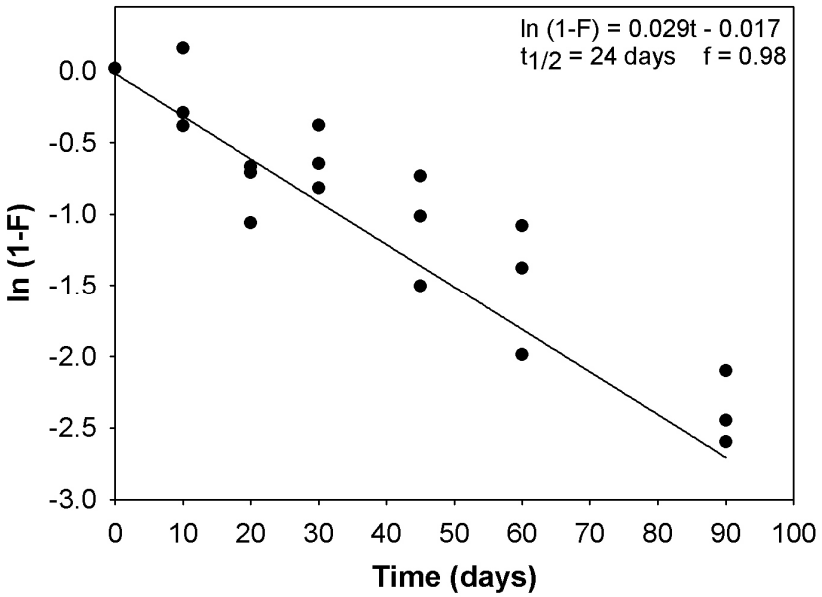

Fig. 4.2 *Reaction progress plot [ln (1-F) vs. time] for data of an experimental diet change for sand goby (Guelinckx et al. 2007). The data can be described by one linear relationship (y = ax + b) indicating that only one rate constant is being followed. The slope gives the first order rate constant for isotope turnover from which the half life can be derived ($t_{1/2}$ = ln(2)/a). The intercept gives the fractional contribution (f) of the rate constant to the whole ($f = e^b$).*

In principle, the isotopic turnover rate v 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 & Eldridge 2006). After substituting v by k + m, estuarine residence time (t_r), i.e. the time elapsed since arrival in the upper estuary, can be estimated by:

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

The initial $\delta^{13}C$ value (δ_i) was set at -17 ‰ (Das *et al*. 2003, unpublished data of the coastal area). δ_f was determined for each month by adding a trophic fractionation of 0.5 ‰ (Post 2002) to the $\delta^{13}C$ 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 one month earlier (Δt = 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 ($W = 4.44\ 10^{-6} SL^{3.26}$), which is highly consistent with the length-weight relationships reported by Doornbos & Twisk (1987) and Arellano (1995) for sand goby in the same geographical region. The metabolic turnover rate constant was experimentally determined (Chapter 3) 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 water temperature (T):

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

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* in Table 4.3 (Fonds & Veldhuis 1973, Doornbos & 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). Herefore, average temperature values from the experiment (Chapter 3) and the Scheldt near the sampling location were used. The metabolic turnover constant m_s for each individual caught in the estuary was estimated as follows:

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

Subtracting estimated residence times (t_r) from sampling dates made it possible to asses 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_t approaches δ_f asymptotically (eq. 3). Equilibrium was assumed once a change of 90% of the difference between δ_i and δ_f was achieved. The average time ($t_{r90\%}$) required to reach this isotopic composition ($\delta_{90\%}$) was calculated for each month using the appropriate values of $\delta_{90\%}$ δ_f, k and m_s (Table 4.3). Due to monthly varying isotopic turnover rates (k + m) the upper limit of the isotopic clock differs among the months (Table 4.1).

Table 4.1 *Upper limit of the isotopic clock ($\delta_{90\%}$) and the time required to reach this value ($t_{r90\%}$) 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	$\delta_{90\%}$ (‰)	$t_{r90\%}$ (days)
June '03	-25.7	26
July '03	-27.8	31
August '03	-26.6	40
September '03	-23.0	59
October '03	-23.0	85
November '03	-22.2	116
January '04	-23.2	247
February '04	-23.1	223
March '04	-24.7	246

2.4. 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). This growth model was based on otolith microstructure analysis.

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

with SL_t denoting the standard body length at age t, SL_{max} the asymptotic standard length (76.2 mm), K the growth constant (1.7 year), C the amplitude of the seasonal growth 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.

2.5. 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 standard deviation. 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 standard deviation for k determined in Chapter 3. The parameters 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 4.3). Using multiple regression, input parameters were subsequently related to a dependent variable which 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 the dependent variable explained by each parameter 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).

3. RESULTS

3.1. $\delta^{13}C$ values

$\delta^{13}C$ values of all fish varied between -28.9 ‰ and -15.3 ‰ (Fig. 4.3a). The $\delta^{13}C$ 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 $\delta^{13}C$ (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 $\delta^{13}C$ values than the assigned marine end member (-17 ‰).

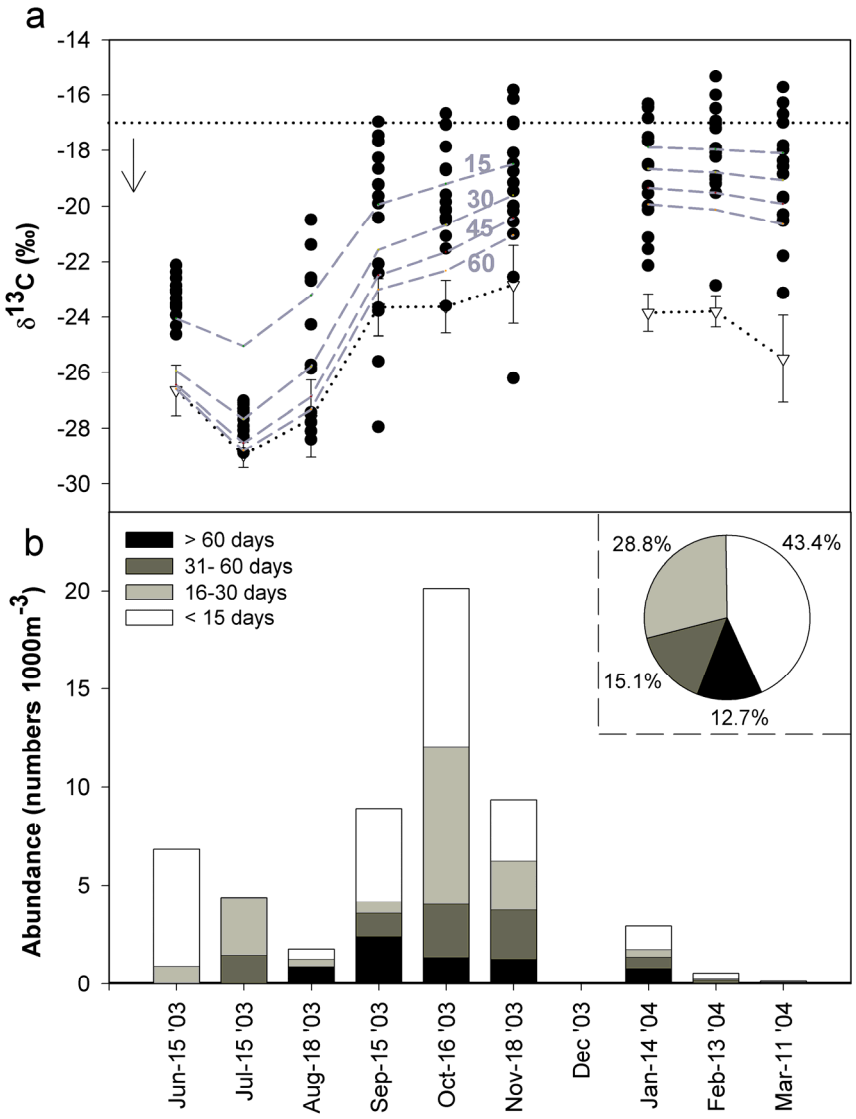

Fig. 4.3 *Isotopic composition and residence times in the upper Scheldt estuary of* P. minutus *for each sampling date. a) black dots: individual $\delta^{13}C$ values (n = 134). The arrow indicates the direction of the $\delta^{13}C$ change after estuarine entrance. Straight dotted line: δ_i (-17 ‰), triangles with dotted line: δ_f (mean ± SD). Dashed grey lines represent calculated $\delta^{13}C$ values (eq. 1) sand goby would have 15, 30, 45 and 60 days after arrival. b) Sand goby abundance was partitioned into 4 residence classes: <15 days, 16-30 days, 31-60 days and > 60 days. Inset: proportion of residence classes in total annual catch.*

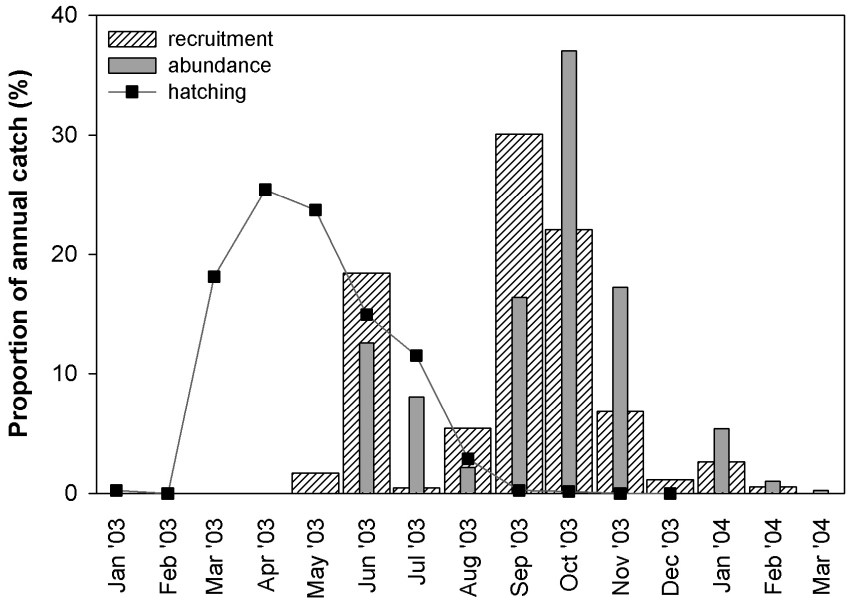

Fig. 4.4 *Abundance and back-calculated hatching period and estuarine recruitment of* P. minutus *caught during one year (April 2003 to March 2004). Results are shown as percentage of total catch. 6 fish hatched in 2002 and are not shown here.*

3.2. Estuarine recruitment and residence time

For each collection date fish were grouped into four classes based on their residence time (t_r): fish residing in the estuary a) for maximum 15 days, b) between 16 and 30 days, c) between 31 and 60 days and d) 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. 4.3b). 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 4.3b, inset), roughly 30% appeared to remain in the estuary for more than one month (classes '31-60' and '>60') with 13% classified in the '>60' group. About 44% 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. 4.4). Juveniles were first caught in June, but their isotopic composition showed that immigration had already started in May (Fig. 4.4). Fish caught in June recruited into the upper estuary during a period of about ten days. The first fish reaching the upper estuary were about two to three months old and measured about 22 mm (Table 4.2). There was almost no immigration in July as only three among 134 fish were found to have immigrated 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. 4.3b). Subsequently, maximum sand goby influx occurred in September, which is about four to five months after the hatching peak (Fig. 4.4, Table 4.2), 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. 4.3b). Sand goby abundance decreased from November onwards, yet immigration continued during winter as proven by new arrivals in the estuary (Fig. 4.3). 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. 4.5, Table 4.2). 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 4.2, Fig. 4.4). The majority of the immigrating fish were older than two months (Fig. 4.5). 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. 4.4, Table 4.2).

Table 4.2 *Standard length (SL) and age (mean + SD) of* P. minutus *at immigration. Individuals equilibrated to the estuarine food web could not be taken into account.*

Month of recruitment	n	SL (mm)	Age (days)
May '03	2	21.5 ± 0.4	68 ± 1
June '03	27	26.4 ± 3.6	80 ± 10
July '03	3	34.6 ± 3.7	102 ± 10
August '03	9	35.5 ± 12.4	108 ± 40
September '03	18	41.8 ± 10.3	126 ± 32
October '03	13	43.0 ± 13.1	152 ± 108
November '03	8	45.2 ± 9.6	144 ± 49
December '03	4	54.3 ± 13.5	276 ± 175
January '04	16	48.6 ± 11.6	188 ± 113
February '04	12	56.3 ± 10.3	205 ± 85
March '04	3	56.7 ± 6.7	204 ± 45

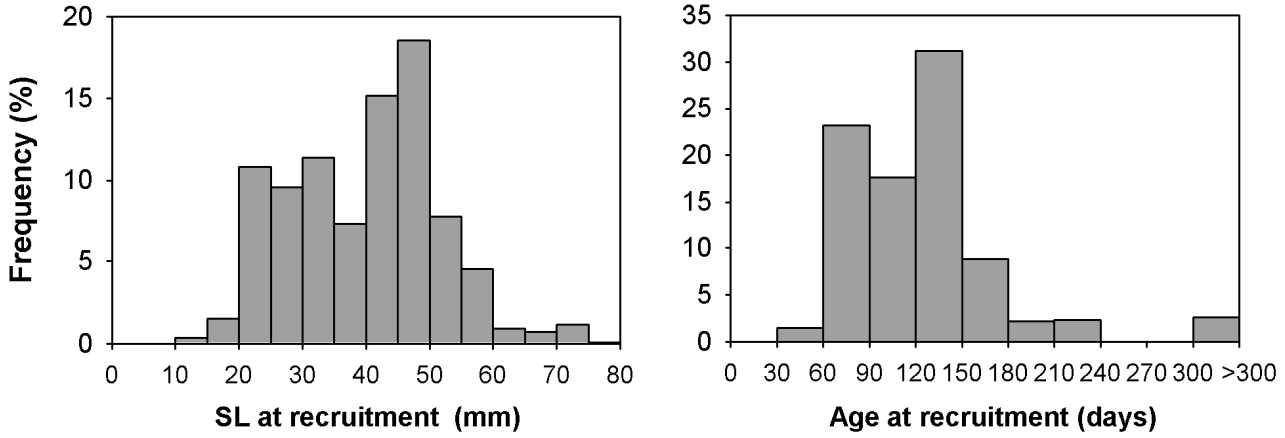

Fig 4.5 *Distribution of standard length (SL) and age of immigrating* P. minutus*. Individuals equilibrated to the estuarine food web could not be taken into account. Note that the tail of the age distribution has been compressed into a single category (>300 days).*

3.3. Sensitivity analysis

The sensitivity analysis showed that δ_f seems to have the strongest influence on the calculated residence time of the fish (Table 4.3). The isotopic clock is relatively sensitive to the parameters m_{exp}, R_a and R_c that were used to determine instantaneous metabolic replacement m_s (eq. 3, 4). Fig. 4.6 illustrates the effect of parameter perturbations on residence time relative to the nominal results of the isotopic clock. The results of the sensitivity analysis support the nominal model results. Parameter changes (Table 4.3) are not likely to 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.

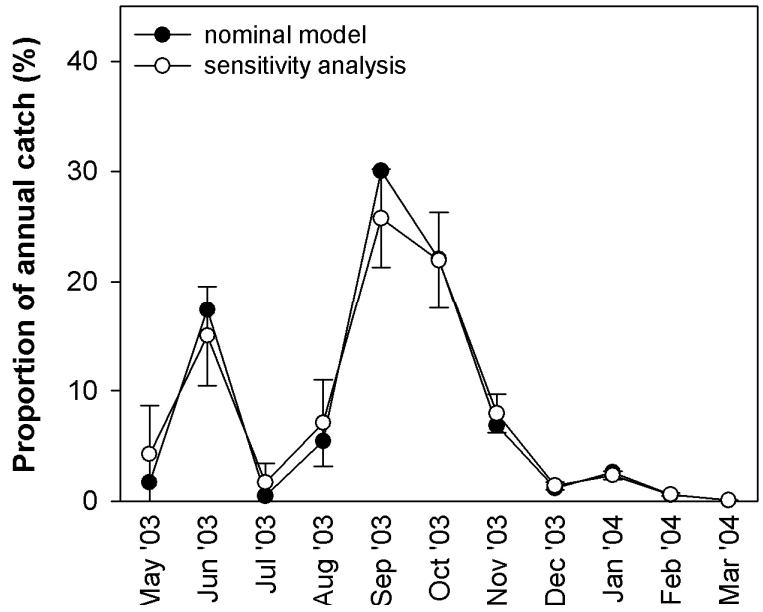

Fig. 4.6 *Sensitivity analysis of the isotopic clock. Nominal model results of estuarine recruitment are compared to an average (± SD) of 500 sensitivity runs.*

Table 4.3 *Sensitivity analysis on 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 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.*

Parameter description and unit		**Nominal value ± deviation**	**t_r RPSS (%)**	**Age recr RPSS (%)**
Initial $\delta^{13}C$ value (‰)	δ_i	-17.0 ± 1.0	2.8	2.4
Final $\delta^{13}C$ value (‰)	δ_f-Jun '03	-26.7 ± 0.9	0.5	0.3
	δ_f-Jul '03	-28.9 ± 0.5	2.0	0.0
	δ_f-Aug '03	-27.7 ± 1.4	4.1	0.1
	δ_f-Sep '03	-23.6 ± 1.0	0.7	2.2
	δ_f-Oct '03	-23.6 ± 1.9	**19.9**	0.2
	δ_f-Nov '03	-22.8 ± 1.2	**13.3**	4.2
	δ_f -Jan '04	-23.8 ± 0.7	4.0	0.6
	δ_f-Feb '04	-23.8 ± 0.6	6.0	0.0
	δ_f-Mar '04	-25.5 ± 1.3	**12.6**	0.1
Instantaneous growth rate (day^{-1})	k-Jun '03	0.055 ± 0.023	0.1	2.4
	k-Jul '03	0.042 ± 0.017	2.4	0.0
	k-Aug '03	0.030 ± 0.012	4.3	0.0
	k-Sep '03	0.018 ± 0.007	3.1	0.4
	k-Oct '03	0.014 ± 0.006	0.1	2.5
	k-Nov '03	0.009 ± 0.004	0.2	4.0
	k -Jan '04	0.003 ± 0.001	0.1	0.2
	k-Feb '04	0.003 ± 0.001	0.0	0.2
	k-Mar '04	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] (mg O_2 g^{-1} d^{-1})	r_{exp}	3.62 ± 0.21	0.5	0.1
Intercept for the allometric mass function for respiration[b] (mg O_2 g^{-1} d^{-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] ($°C^{-1}$)	R_c	0.08 ± 0.01	6.1	0.2
Growth rate[c] (y^{-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]	t_s	0.53 ± 0.05		0.5

[a] Chapter 3
[b] Fonds & Veldhuis 1973, Doornbos & Twisk 1987
[c] Arellano 1995

4. DISCUSSION

4.1. Estuarine recruitment and residency

The hatching period of the sand gobies, back-calculated from their body length, matches with its 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 10 to 20 mm (Fonds 1973). Reconstructing estuarine influx showed that sand goby individuals were at least two months old and had, except for one fish, a standard length of at least 19 mm at the moment of estuarine entry. Bardin & Pont (2002) reported that *Pomatoschistus* spp. shift from passive (drifting) to more active migration behaviour 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. 4.4), 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 (Fig. 4.3b, 4.4). 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 (± 22 °C) at that time, making the upper estuary an unfavourable habitat for *P. minutus,* which is known to avoid temperatures above 20°C (Fonds & van Buurt 1974, Hesthagen 1979). Avoidance of the upper estuary in summer was also observed for herring and was attributed to higher water temperatures (Maes *et al*. 2005a). It is not clear whether temperature per se is important or the mismatch between an increased oxygen demand and the capacity of oxygen supply to tissues (Pörtner & Knust 2007). The metabolic rate (oxygen requirements) of fish increases with increasing temperature, while dissolved oxygen concentrations decrease with increasing temperature. Fish are known to escape from areas with limited oxygen levels.

The individual immigration patterns and 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 gobies are known to use tidal streams selectively (Bardin & 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. Note that it is impossible to disentangle the effects that mortality and emigration have on the estuarine population. Here, we assume emigration to be mainly responsible for the loss of individuals from the estuary (Fig. 4.3b) for there is no reason to assume an increase in mortality rate in the estuary.

Estuaries are considered as beneficial areas where marine juveniles spend a substantial time to grow up in a sheltered environment (Greenwood & 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) modelled 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 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 cue. A similar scenario with short visits now seems to be true for sand goby in the upper Scheldt estuary, whereby temperature might regulate the temporal variation in influx. Healey (1971), Fonds (1973) and Doornbos & 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 behavioural 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 and oxygen demand, it is to be expected that these patterns will alter due to climate change (Duarte 2007, Pörtner & Knust 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 & Dewailly 1995). Nevertheless, about 30 % of the estuarine population was observed to stay for more than a month (Fig. 4.3b). 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).

4.2. Evaluation of the 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 4.3). 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 (Chapter 2b) that were removed from the same specimens that were analyzed in the present study. $\delta^{13}C$ values of foregut contents are not significantly different from that of undigested food, making them reliable and easily accessible estimators of end signatures. A trophic shift of 0.5 ‰ was taken into account to predict $\delta_{f,}$. This value corresponds to an average enrichment between diet and consumer (Post 2002). We did not use the large trophic enrichment factor (+3.2 ‰) that was observed in chapter 3 as this was considered to be an anomaly. Formulated diets are more likely to cause anomalous trophic shifts due to heterogeneity in isotopic composition, differential assimilation of dietary components and/or isotopic routing (Gaston & Suthers 2004, B. Fry pers. comm.). Moreover, adding 0.5 ‰ to gut content $\delta^{13}C$ values from the lower estuary results in $\delta^{13}C$ values consistent with sand goby muscle values from the coastal area (Chapter 2b). This is, however, not the case when 3.2 ‰ is added. Finally, if 3.2 ‰ was used to predict estuarine end member signatures, monthly δ_f values would be larger than most muscle $\delta^{13}C$ values in the upper estuary (Fig 4.3a). This would then suggest that these fish were in equilibrium with estuarine food resources. However, the variation in muscle $\delta^{13}C$ values clearly shows that this is not correct. Using 0.5 ‰ results in a strong similarity between the predicted δ_f values and the lowest (most estuarine) $\delta^{13}C$ values for muscle tissue in the estuary (Fig. 4.3a). 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 (2-4 mg O_2/l) in the oligohaline and freshwater reaches of the Scheldt estuary severely limit migration to areas upstream from our sampling location (5-7 mg O_2/l), especially during summer and early fall (Meire *et al*. 2005, Maes *et al*. 2007). Sand gobies are known to avoid oxygen levels below 4 mg O_2/l (Petersen & 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 $\delta^{13}C$ values (Fig. 4.3a). Fish at sea did not have a higher lipid content (unpubl. results) than fish in the upper estuary. The opposite could have explained the difference as lipids are ^{13}C depleted (DeNiro & Epstein 1977). It appears that some sand gobies entering the Scheldt estuary originate from offshore waters or from the Eastern Scheldt (Fig. 4.1). Fish from these two areas showed ^{13}C enriched values (approximately -17.3 ‰ and -15.5 ‰, respectively)

compared to those from nearshore waters. Das *et al*. (2003) reported an average $\delta^{13}C$ value of -17.1 ± 0.5 ‰ 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, C isotope analyses on invertebrates along the salinity gradient of the Scheldt (De Brabandere 2005) and the Thames (Leakey *et al*. 2007) only allowed to clearly distinguish two regions (coastal vs. brackish water) from the oligohaline zone to the sea.

The isotopic turnover rate constant v was partitioned into an instantaneous rate constant for growth (k) and one for metabolic replacement (m) (Fry & Arnold 1982, MacAvoy *et al.* 2006). 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 4.1). The calculated time periods required for sand gobies to reach isotopic equilibrium ($t_{r90‰}$), fits between values for rapidly growing larvae (Herzka & Holt 2000) and those of adult fish (Hesslein *et al*. 1993).

The average change in body weight was used to determine the growth rate constant (k) for each sample (Hesslein *et al*. 1993, MacAvoy *et al*. 2006). This change in body mass was determined using a sand goby growth model (eq. 6) (Arellano 1995) and 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 life-span.

The experimentally obtained value for the metabolic turnover rate 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 (eq. 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 (Fond & Veldhuis 1973, Petersen & Petersen 1990). Experimental oxygen levels were kept high (Chapter 3) 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 & 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 & Martínez

del Rio 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 & 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 organisms' 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.

5. CONCLUSIONS

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 & 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.* Sand gobies migrated to the upper estuary almost continuously from May onwards and immigration occurred 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 periods 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 at the level of the individual. Short estuarine visits as observed in this study question the functional role of estuaries for marine fishes. Brief estuarine residencies do not seem to fit in 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.

Acknowledgements We thank C. Martínez del Rio, L. De Brabandere and F. Volckaert for their valuable comments on this manuscript, P. Van Den Driessche for preparing the samples and A. Ghesquiere for improving the manuscript linguistically. C. Martínez del Rio kindly provided us with an unpublished manuscript. Electrabel Doel gave permission to sample the cooling water. Special thanks to F. Dehairs and M. Korntheuer (ANCH, Vrije Universiteit Brussel) for their logistical support. The first author acknowledges a Ph.D grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). The study was funded by a K.U.Leuven-OT project (OT/02/30).

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

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Submitted to Journal of Experimental Biology and Ecology

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] ($[Sr/Ca]_{otolith} = 0.31[Sr/Ca]_{water} + 0.44$). 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:

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

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) were 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 HNO_3 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 (± SD)* P. minutus *standard length (SL) and biomass per aquarium at the start of the experiment. Average (± 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.*

					Salinity level				
Treatment	**Mean SL (mm)**	**Biomass (g)**	**T (°C)**	**pH**	**30**	**20**	**12**	**7**	**3**
13°C (6)	43 ± 3.3	5.9 ± 0.4	13.1 ± 0.4	8.4 ± 0.04	30.2 ± 0.1	19.9 ± 0.3	12.2 ± 0.04	7.2 ± 0.1	3.2 ± 0.1
13°C cont (1)	44.7 ± 2.9	6.1	13.4 ± 0.6	8.4 ± 0.1	30.2 ± 0.4				
18°C (6)	44 ± 4.1	6.3 ± 0.5	18.4 ± 0.6	8.5 ± 0.1	30.5 ± 0.1	20.0 ± 0.2	12.1 ± 0.1	7.2 ± 0.2	3.2 ± 0.1
18°C cont (1)	44.0 ± 4.0	5.7	18.2 ± 0.6	8.3 ± 0.1	30.5 ± 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 $CaCO_3$ and $SrCl_2.6H_2O$, 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).

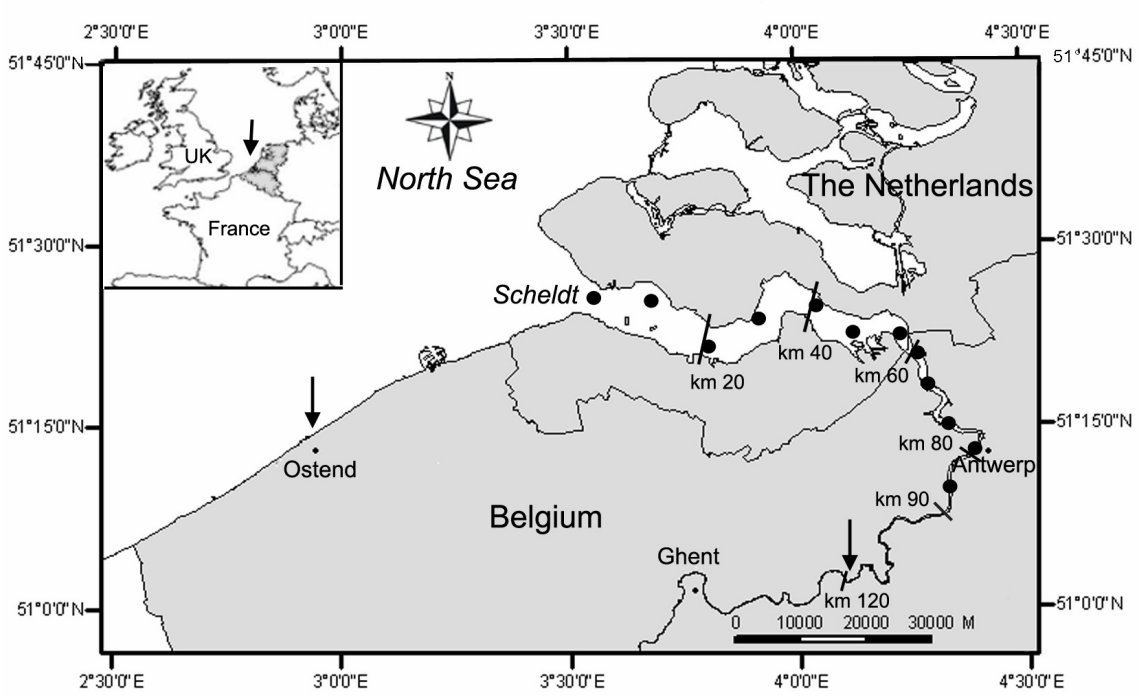


Fig. 5.1. *Map of the Scheldt estuary, which discharges in the North Sea and is situated in the Dutch Delta. Dots indicate the sampling locations of the chemical water analysis. The two arrows indicate the sampling locations of the two aqueous end members in the experiment.*

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 ΩM.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].

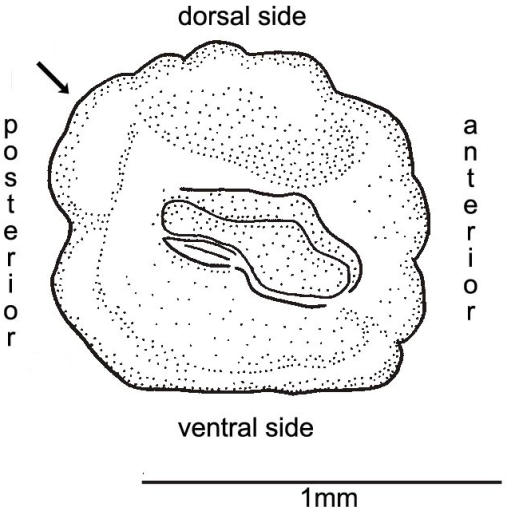
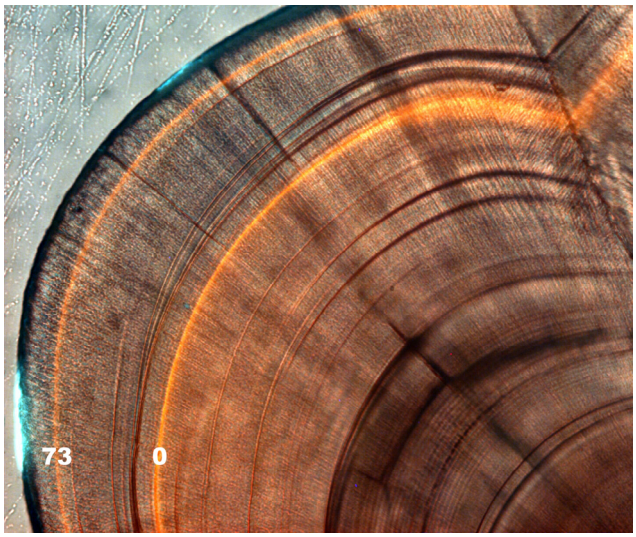
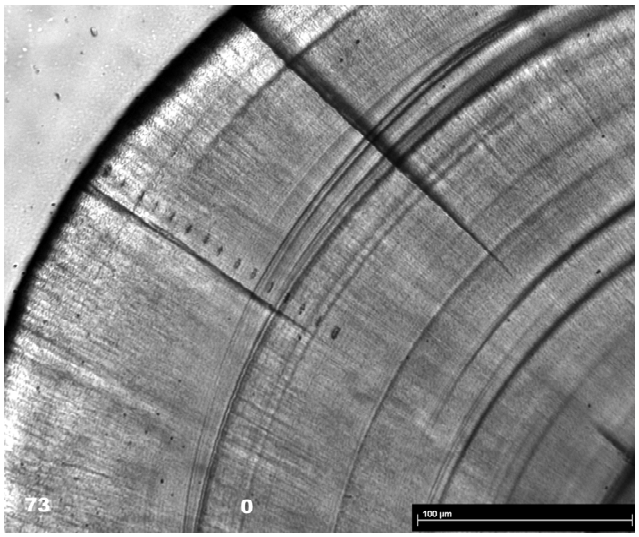


Fig. 5.2. *Left: proximal side of the left sagitta of a 50 mm (standard length) sand goby. The arrow indicates the approximate position of the measurement axis on the pararostral (postero-dorsal side). Under left: sagittal cross-section of sand goby otolith under UV light to make alizarin marks visible (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 (± every 10 µm) 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 µm 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 $[Sr/Ca]_{water}$, $[Sr/Ca]_{otolith}$ 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 [Sr/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 [Sr/Ca] on water [Sr/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 [Sr/Ca]

Over the entire salinity range (0.6 - 30.9) in the Scheldt estuary [Sr] varied from 0.3 to 6.6 ppm and [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 [Sr] (Fig. 5.4), demonstrating the conservative character of Sr in the Scheldt estuary. [Ca] appears to deviate slightly from a linear relationship with salinity. [Sr/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 [Sr] and [Ca]. Nevertheless, the results for [Sr/Ca] of the experimental water coincide well with those of the estuarine conditions (Fig. 5.4). Ambient [Sr/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 [Sr/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		

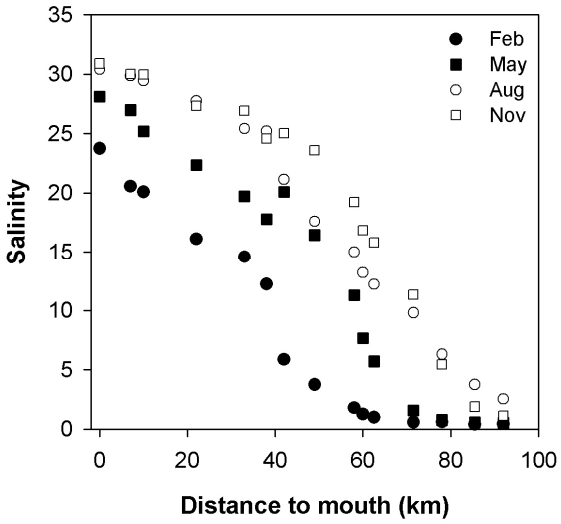


Fig. 5.3 *Longitudinal salinity gradient in the Scheldt estuary in February, May, August and November 2003.*

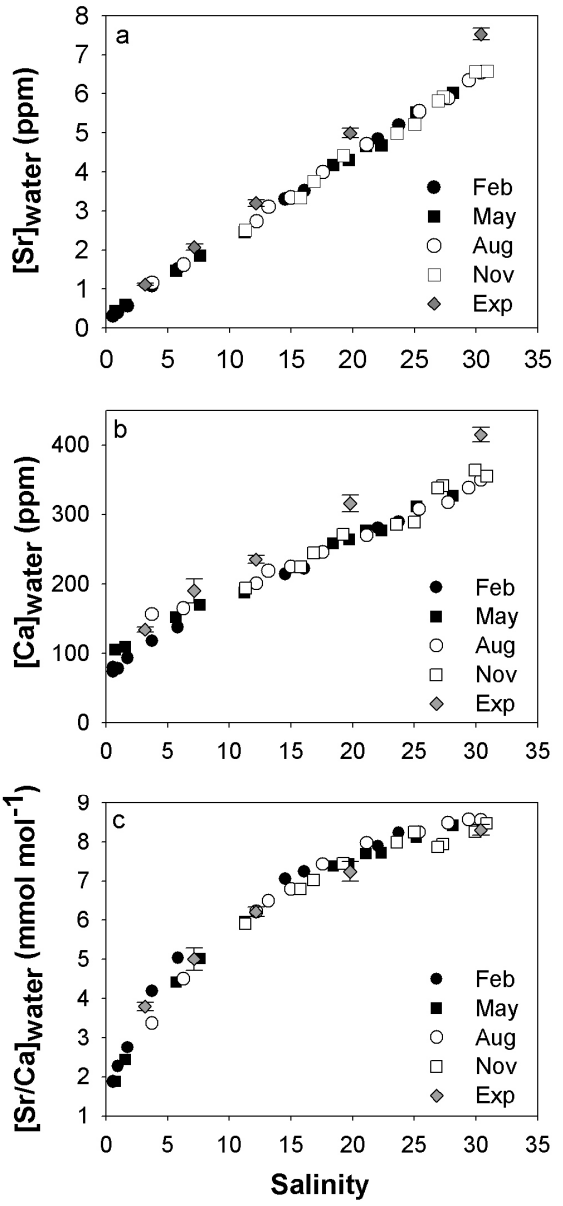


Fig. 5.4 *Aqueous a) Sr concentration, b) Ca concentration and c) Sr/Ca molar ratio of the Scheldt estuary at 4 sampling occasions and of the experiment. Error bars for experimental values are standard deviations. Scheldt: [Sr] = 0.21(salinity) + 0.26; [Ca] = 8.56(salinity) + 93.36; experiment: [Sr]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

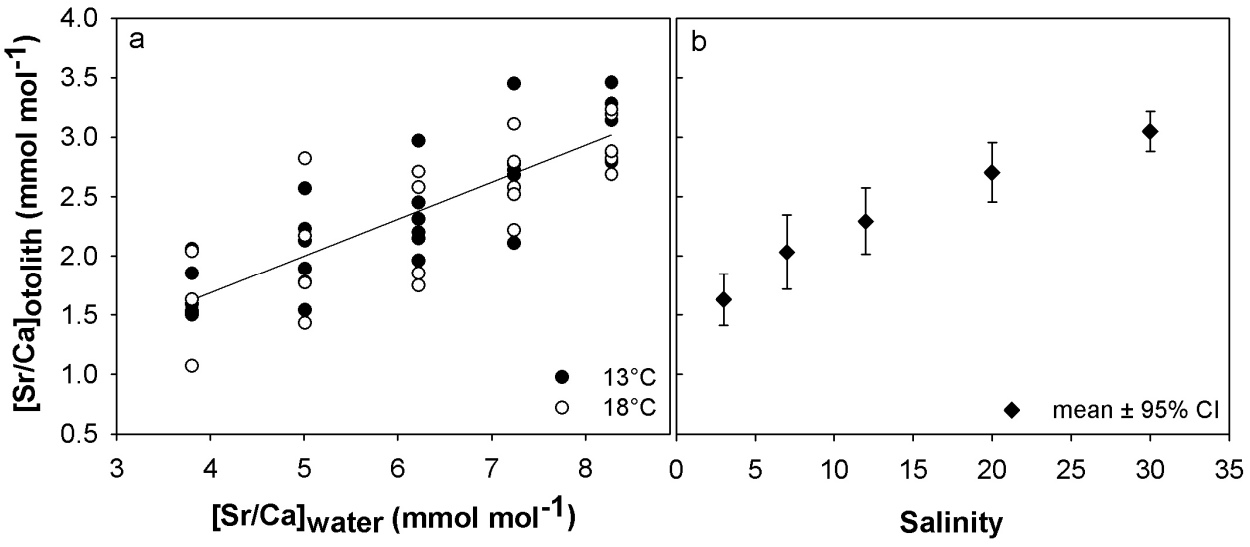


Fig. 5.5 P. minutus*. a) Otolith [Sr/Ca] versus the average ambient [Sr/Ca] concentration. Each dot is the average value per aquarium of electron microprobe measurements taken at a given salinity level (n = 1-8). Except for two aquaria of which two fish were analysed, these averages come from measurements 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] (±95%CI) over all aquaria (average of dots on the left) against experimental salinity.*

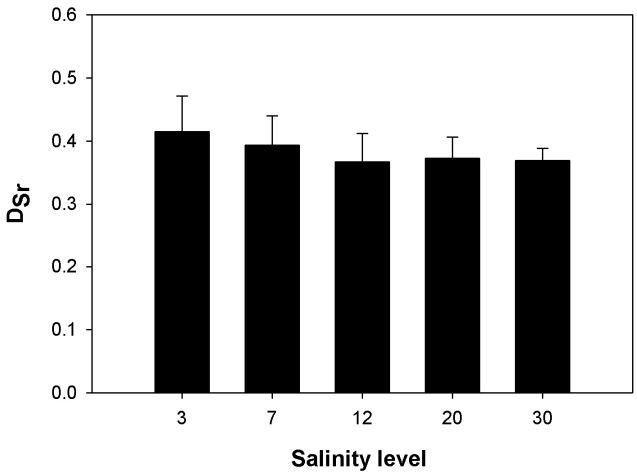


Fig. 5.6 *Partition coefficient D_{Sr} (mean ± 95% CI) for each salinity level in the experiment. Data of both temperature treatments were joined as temperature had no effect on D_{Sr}*

Source		Sr/Ca_{ot}			D_{Sr}		
	Df	**MS**	**F**	**P**	**MS**	**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 × 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 $[Sr/Ca]_{otolith}$ 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 $[Sr/Ca]_{otolith}$ on $[Sr/Ca]_{water}$ (Table 5.4) shows that this relationship holds for a wide range of $[Sr/Ca]_{water}$ 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 $[Sr/Ca]_{water}$ range (± 7.5 – 9.5 mmol mol^{-1}), which was probably too small to overwrite variation in $[Sr/Ca]_{otolith}$ caused by other factors.

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

Species	Temperature and salinity	$[Sr/Ca]_{water}$ (mmol mol^{-1})	Equation: y =$[Sr/Ca]_{otolith}$; x = $[Sr/Ca]_{water}$	References
Leiostomus xanthurus	T: 20°C Sal: 20	13 – 22 [s]	y = 0.165 (± 0.052) x + 0.260 (± 0.897) [a]	Bath *et al.* 2000
	T: 25°C Sal: 20	13 – 22 [s]	y = 0.162 (± 0.054) x + 0.70 (± 0.954) [a]	
Lates calcarifer	T: 28-30°C freshwater	0.2 – 0.8 [s]	y = 0.16 (± 0.03) x + 0.14 (± 0.11) [b]	Milton & Chenery 2001
Acanthopagrus butcheri	T: 20°C Sal: ±33	10 – 130 [s]	y = 0.0757 x + 0.1299	Elsdon & Gillanders 2003b
Oncorhyncus clarki	Field: freshwater	0.5 – 3.5	y = 0.55 x (± 0.02) – 0.18 (± 0.03) [b]	Wells *et al.* 2003
Acanthopagrus butcheri	T: ± 21.5°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 *et al.* 2005
Acanthopagrus butcheri	T: ± 21.5°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)*	
Acanthopagrus butcheri	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	
Pomatoschistus minutus	T: 13, 18°C; Sal: 3, 7, 12, 20, 30	3.8 – 8.3	y = 0.31 (± 0.03) x + 0.44 (± 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 ± SD: 13°C: 1.3 ± 0.3 µm and 18°C: 2.1 ± 0.4 µm), 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 µm 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 $[Sr/Ca]_{otolith}$ 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 & Gillianders (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 & Gillianders (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 $[Sr/Ca]_{water}$ 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.

Acknowledgements. We are grateful to E. Cuveliers and B. Geysen for their assistance during the experiment, A. Jolivet for her assistance in the otolith analyses and D. Nolf for otolith drawings. The institute of Agricultural and Fisheries Research (ILVO), unit Fisheries (Ostend, Belgium) kindly provided filtered sea water. We thank the crew of the RV "Luctor" (CEME-NIOO, Yerseke, The Netherlands) for logistic support and the Research Institute for Nature and Forest (INBO) for making the VIE marking equipment available. The first author acknowledges a PhD grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). The study was funded by K.U.Leuven-OT (OT/02/30).

6 ESTUARINE MIGRATION OF SAND GOBY *POMATOSCHISTUS MINUTUS* EXPLORED BY MEANS OF OTOLITH [SR/CA]

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Unpublished manuscript

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 (± 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).

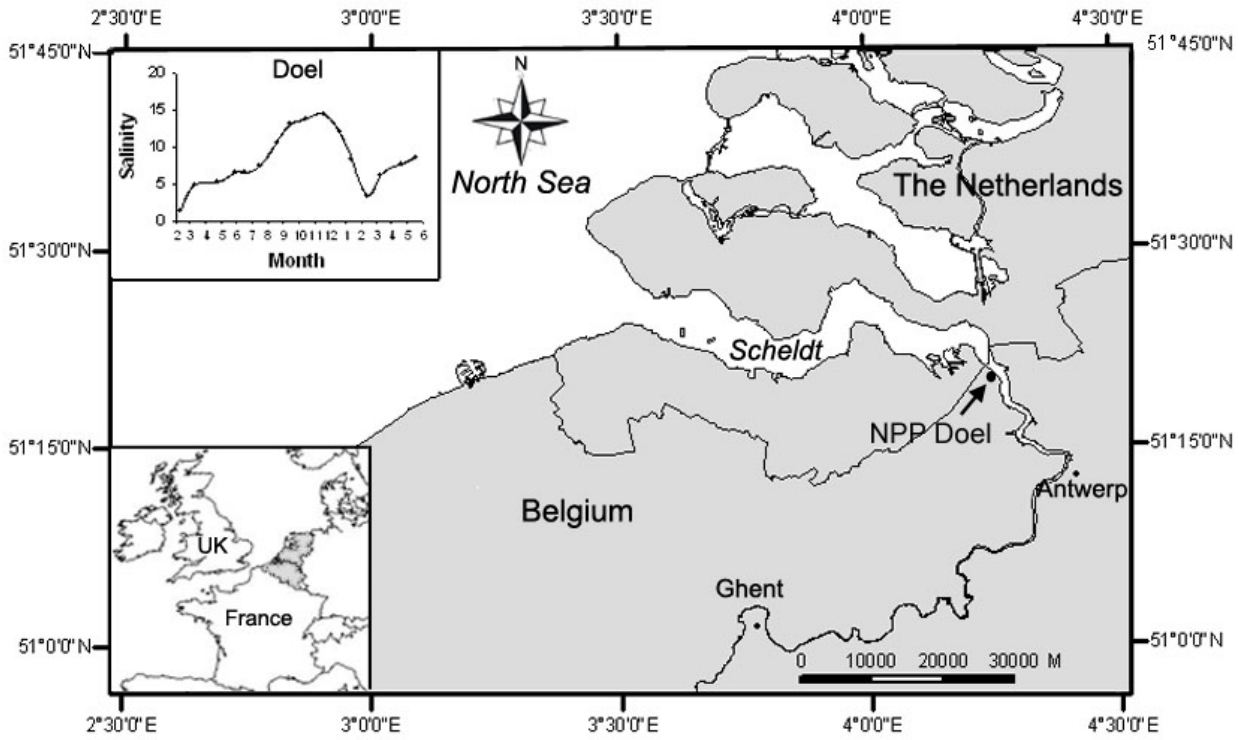

Fig. 6.1 *Map of the Scheldt estuary which discharges into the Southern Bight of the North Sea. The sampling location (NPP Doel) at 61 km from the mouth is indicated with an arrow. The inset in the left upper corner shows the salinity at the sampling location between February 2003 and May 2004.*

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 ± 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 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 ΩM.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 ± 2 µm (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).

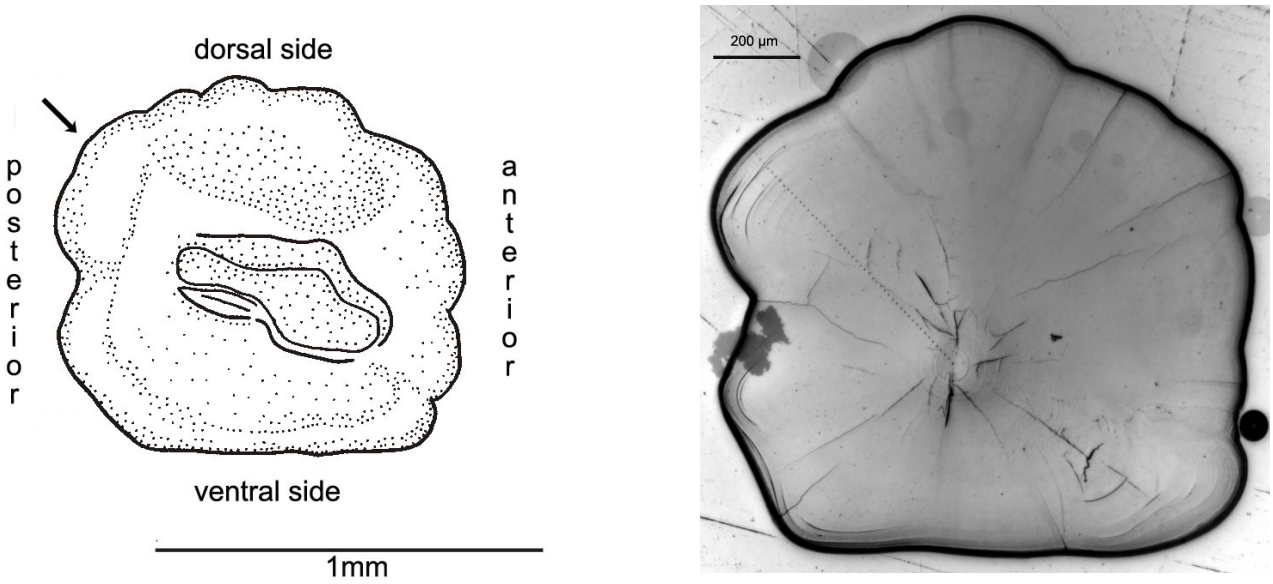

Fig. 6.2 *Left: proximal side of left sagitta of a 50 mm SL sand goby. Arrow indicates the approximate position of the measurement axis on the pararostral (postero dorsal side). Right: Mid sagittal plane of analysed otolith under reflected light showing the line transect on the pararostral lobe.*

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.

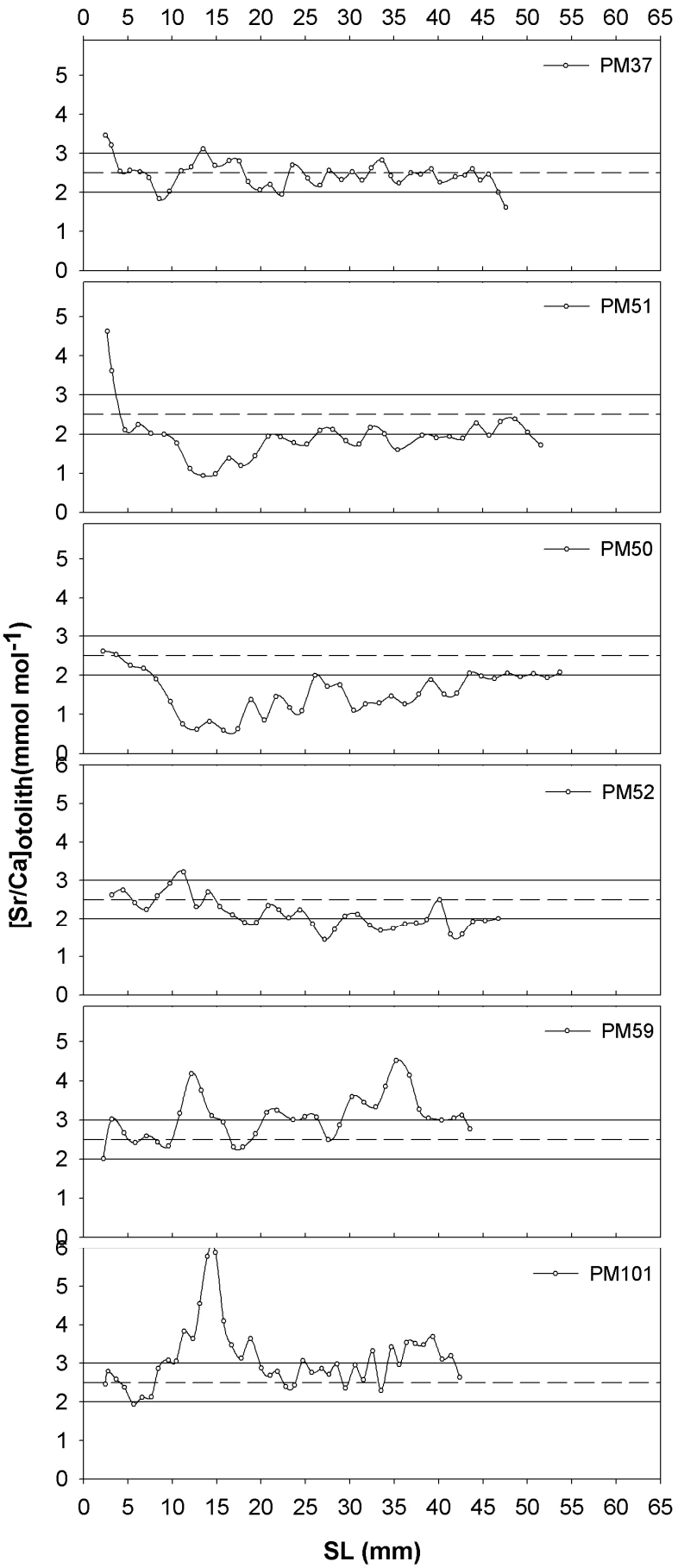

Fig. 6.3 P. minutus *[Sr/Ca] chronologies of six fish caught in October and November 2003 in the brackish water zone of the Scheldt estuary plotted against standard length.*

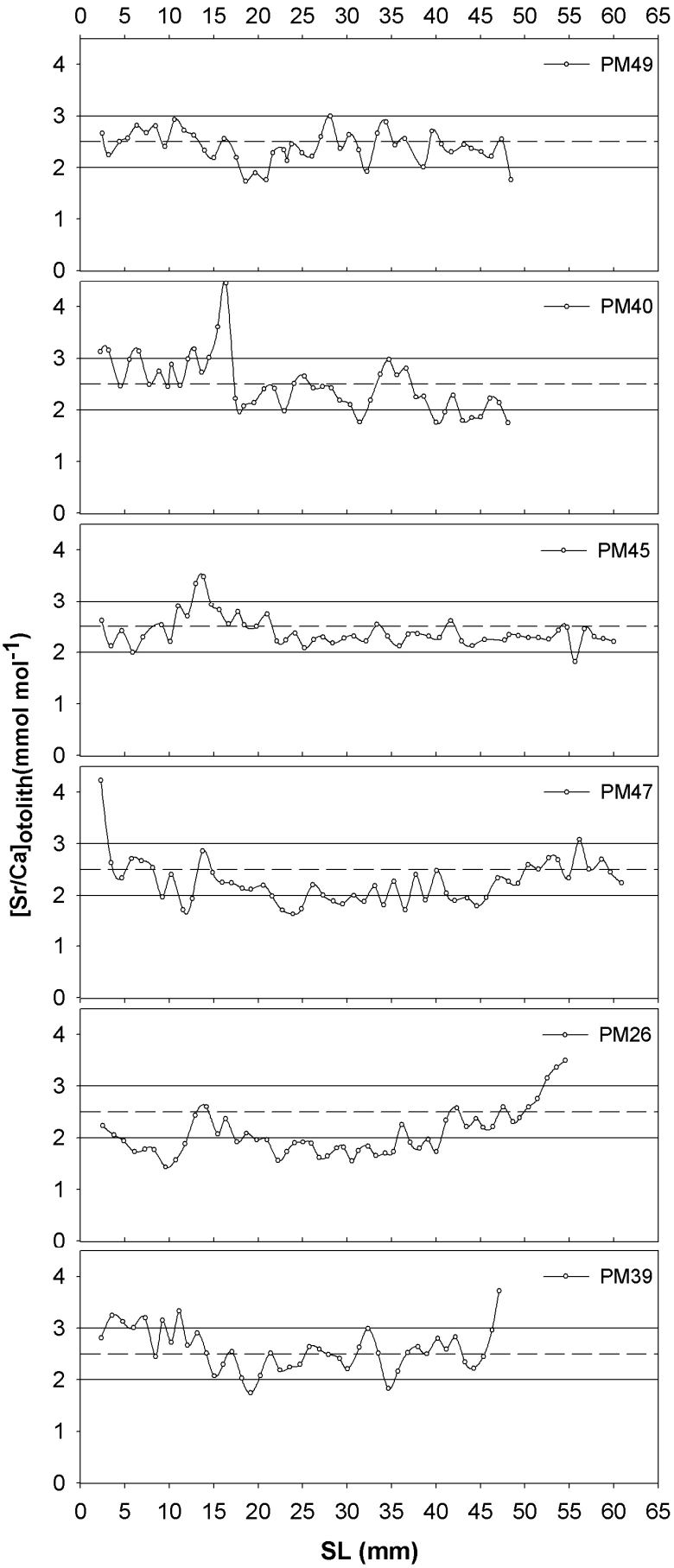

Fig. 6.4 P. minutus*. [Sr/Ca] chronologies of six fish caught in March 2004 in the brackish water zone of the Scheldt estuary plotted against standard length.*

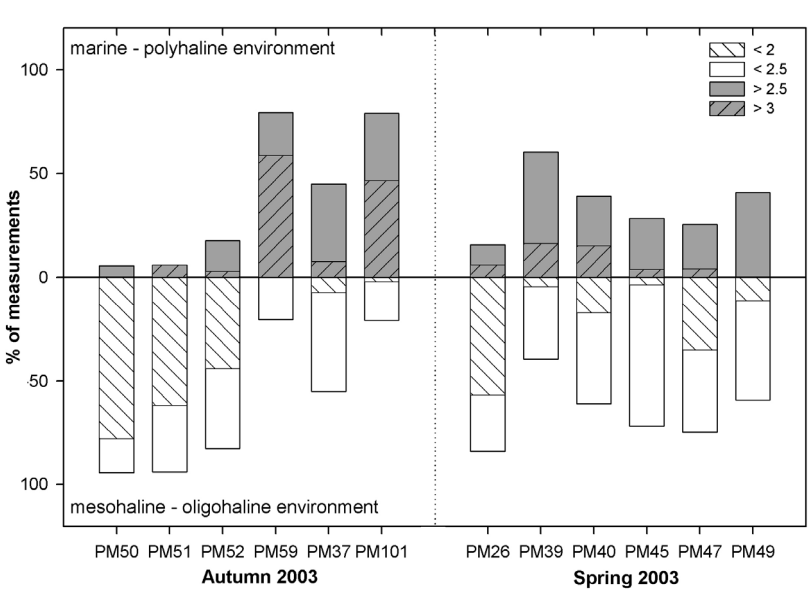

Fig. 6.5 P. minutus*. For each specimen the percentage of measurement points classified to the marine and the brackish environment. As measurement point correspond to body size, this classification indicates in which environment most growth occurred; this can be regarded a proxy for the habitat in which fish mostly resided. Oligohaline-mesohaline waters: < 2.5 mmol mol^{-1}, polyhaline - marine waters: > 2.5 mmol mol^{-1}. Otolith [Sr/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). [Sr/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 [Sr/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 [Sr/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 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 mmol mol) 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. The 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).

Acknowledgments. We thank B. Geysen for collecting the fish samples, A. Jolivet (LASAA-Ifremer, Plouzané, France) for her assistance in the otolith analyses and A. Ghesquiere for improving the manuscript linguistically. Electrabel NPP Doel gave permission to sample the cooling water. The first author acknowledges a PhD grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen).

7 GENERAL DISCUSSION

Animal movement patterns and connectivity between populations are essential to understand behavioral and population ecology. Moreover, detailed knowledge on how animals use habitats in space and time is not only necessary to comprehend habitat functioning, it is also fundamental to conservation and integrated management of ecosystems (Rubenstein & Hobson 2004, Sale *et al.* 2005). Patterns of migrations in coastal and estuarine fishes are now known to be variable at the individual and subpopulation level (e.g. Able & Grothues 2007b). However, measuring the migration variability within fish populations is complex because methodologies (e.g. tag-recapture, telemetry, hydroacoustics, biochemical markers) are usually applied at the population level and are rarely applied at the temporal and spatial scales necessary to evaluate variability in seasonal and lifetime migrations (Secor & Rooker 2000, Äkesson 2002).

This work explored the migration dynamics of the sand goby *Pomatoschistus minutus* in the Scheldt estuary by means of stable C isotopes in dorsal muscle tissue and otolith [Sr/Ca]. Yet, for successful application of both geochemical tracers in our study system, the techniques had to be calibrated and conditions verified. Consequently, a substantial part of this work deals with adjusting the methodologies. This work contributes to a better understanding and application of both methodologies and yields new insights in the life history of sand goby with respect to its migratory behavior as well. This final chapter summarizes the main findings concerning estuarine habitat use of the sand goby and the techniques used. Implications of these results are addressed and recommendations for future research are presented.

1. ESTUARINE HABITAT USE BY SAND GOBY

Individual behavior

For the first time sand gobies of the river Scheldt are shown to have a high variability of movement patterns within the estuary. This was demonstrated by means of two biogeochemical techniques namely an isotopic clock in muscle tissue and otolith [Sr/Ca] chronologies. Carbon stable isotopes in muscle tissue mainly demonstrated a high turnover of individuals in the brackish water population (Chapter 4). More specifically, it was observed that **(1)** immigration in the upper estuary persisted almost throughout the entire year, even when densities were decreasing or low. There was **(2)** almost no immigration in July when temperature was high or in April when gobies were considered to spawn at sea. **(3)** About 70% of the sand goby seem to remain in the upper estuary for less than one month, suggesting a strong temporal overlap between immigration and emigration. Moreover, **(4)** estuarine recruitment was found to occur over a wide range of body sizes and ages. Otolith [Sr/Ca] chronologies (Chapter 6) were consistent with these results. Otolith analyses supported individual movement patterns in space and time over the estuarine salinity gradient with variable timing of immigration and different periods of residency in the upper estuary. Complementary to the isotopic clock, the otolith analyses suggested **(5)** repeated movements between marine-polyhaline and brackish waters. Low $\delta^{13}C$ and $[Sr/Ca]_{otolith}$ values suggest **(6)** that some sand gobies move further upstream into lower salinities.
The strong variability in estuarine usage, evidences that no standard scheme is valid for the whole population. Such a scheme, with a distinct period of immigration into and emigration from the brackish water area, could easily be assumed based on the species abundance pattern in the estuary, as put forward by Maes & Ollevier (2002) for herring (*Clupea harengus*). However, $\delta^{13}C$ values demonstrated a similar immigration pattern for herring as for sand goby, with an equally high turnover of individuals in the brackish water area (Guelinckx *et al.* 2006).

Facultative vs. opportunistic use?

The novel observations of estuarine habitat use seriously question our view on the role of estuaries in the life cycle of marine fishes, specifically for sand goby. Estuaries are generally considered as valuable areas where marine fishes seasonally spend a substantial period to feed, to avoid predation or to profit from beneficial abiotic conditions in order to enhance fitness (Greenwood & Hill 2003, Le Pape *et al.* 2003, Ross 2003, Attrill & Power 2004). The results of this study strongly suggest a facultative use of the estuary by sand goby, indicating that there is no obligate estuarine stage. This is supported by the simultaneous occurrence of sand goby in the estuary and adjacent marine habitats during the different life stages (Hostens

2003, Vanden Eede 2006). The continuum in estuarine usage patterns in terms of timing of immigration and body size at immigration could suggest that sand goby explore the estuary rather coincidentally when estuarine conditions (e.g. temperature and dissolved oxygen concentration) allow for. The estuary can be regarded as an overspill of the coastal area. There are, however, indications for an underlying ecological mechanism inducing an optimal habitat choice based on trade-offs made at the individual level. This would result in an opportunistic and "motivated" use of the estuarine environment. First of all, they seem to aggregate preferentially in the brackish water area because sand goby densities were reported to be higher in brackish area than in the polyhaline zone (Hostens *et al.* 1996, Hostens 2000). Secondly, the high $\delta^{13}C$ values detected in the upper estuary indicate that there are individuals that migrate directly from (offshore) marine areas into the brackish water area without residing first in the lower estuary or coastal waters (which are characterized by relatively lower $\delta^{13}C$ values) (Chapter 2b and 4). Thirdly, sand goby in the brackish water area were observed to be significantly smaller than sand goby in adjacent coastal areas, suggesting a nursery function (Vanden Eede 2006). Finally, female sand goby appeared to dominate over males in the samples taken in the brackish zone, especially during summer and autumn, indicating sex related differences in habitat use (Vanden Eede 2006).

Based on dorsal muscle $\delta^{13}C$ values we found consistent results for herring (Guelinckx *et al.* 2006) and sand goby immigration in the Scheldt upper estuary. We therefore discuss in the following sections the potential functional role of the estuary for sand goby (and marine fishes in general) starting from model predictions for estuary use by herring (Maes *et al.* 2005a).

Functional role of the estuary

One of the underlying questions related to the migration studied, is whether or not short estuarine visits counterbalance the energy investment of habitat transition and provide sufficient surplus value to increase the fish's state and fitness to make the trip rewarding. At least for herring, the answer seems to be positive. A dynamic state variable model was used to predict optimal habitat selection by herring from the open sea to the upper 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 considerable increase in survival probability during their first year. During warm summer months, herring was predicted to avoid the estuary but after this period, short in and out migrations between the North Sea may result in a lower predation risk and enhanced growth, depending on the environmental variability. These short visits during autumn and winter are regarded as a facultative mechanism triggered by temperature (Maes *et al*. 2005b).
A similar scenario with facultative short visits seems to be true for sand gobies in the upper Scheldt estuary, where water temperature might regulate the temporal variation in

immigration rates. Healey (1971), Fonds (1973) and Doornbos & Twisk (1987) reported that temperature variation causes sand goby migrations to deeper waters during winter in the North Atlantic. Moreover, Hesthagen (1979) observed a seasonally varying temperature preference for sand goby and explained this as a behavioural thermoregulation to direct fish towards temperatures that are optimal for specific physiological processes depending on the season. Attrill & Power (2004) found a temperature abundance model for sand goby (non linear quadratic model, $r^2 = 0.46$) as well as for 14 other fish species in the Thames estuary. They suggested that selective movement into estuaries to maximise potential fitness through exploitation of thermal resources, might be a driving force for seasonal migration patterns of marine fish. It is however our conviction that within a season the relatively small temperature differential between the estuary and the sea is not likely to direct the eurytopic sand goby between coastal and estuarine habitats. Immediate effects of temperature and salinity only come into play if the species' tolerance limits are approached. Thermal limits of fishes are probably not even the result of temperature per se. A mismatch between oxygen demand and the capacity of oxygen delivery to tissues is the first mechanism to restrict animal tolerance to thermal extremes. It was shown for eelpout *Zoarces viviparus* that thermally limited oxygen supply closely matches environmental temperatures beyond which growth performance and abundance decrease (Pörtner & Knust 2007). Fish are known to escape from low oxygen conditions. It is however possible, that abiotic factors such as temperature might indirectly affect estuarine migrations through a triggering effect or by influencing prey and predator distribution.

The herring model predicted a lower predation risk for young of the year during summer but also during the facultative short winter migrations (Maes *et al.* 2005a). It is generally accepted that one of the key functions of estuaries, particularly the lower salinity regions, is to increase juvenile survival, by providing a refuge from piscivorous predators (Elliott & Hemmingway 2002, Ross 2003). The ruling turbid conditions reduce effectiveness of visual predation (Blaber & Blaber 1980, Cyrus & Blaber 1992) and/or estuaries provide habitats with relatively few predators (Ruiz *et al.* 1993, Cattrijsse *et al.* 1997, Paterson & Whitfield 2000). Turbidity gradients are hypothesized to act as an orientation cue for juveniles migrating into estuaries (Cyrus & Blaber 1987). Predation risk and food availability are considered to be the most important factors determining habitat quality for juvenile and small fishes (Gibson 1994). For gobies, which are considered to be top down regulated (Doornbos & Twisk 1987, Jaquet & Raffaelli 1989), differences in predation risk are probably more important than in food abundance. Although the density of prey in the brackish water area of the Scheldt is far higher than in the lower estuary and the adjacent coastal area (Hamerlynck *et al.* 1993b, Mees 1994, Hostens 2003), this does not seem to be the main reason for sand goby migration into the brackish water area. First of all, this species is a highly opportunistic and generalist feeder and therefore not dependent on a specific prey type or species (Hamerlynck & Cattrijsse 1994,

Salgado *et al.* 2004). Additionally, even though the sand goby reaches high abundances, it was suggested that it has a rather limited impact on its prey community in coastal areas (Hamerlynck & Cattrijsse 1994) and that interspecific competition with *P. lozanoi* is absent (Hamerlynck *et al.* 1986). Being a small species however, sand goby suffers from a higher predation risk (Fonds 1973, Doornbos & Twisk 1987, Arellano 1995). Sand gobies form an important energy source for bib (*Trisopterus luscus*), whiting (*Merlangius merlangus*) and cod (*Gadus morhua*) in coastal areas, and it is preyed upon by a large spectrum of species (Hamerlynck & Hostens 1993, Salvanes & Nordeide 1993). Sand goby have developed several behavioural responses to avoid predation. They are known to bury themselves in the sediment or to group in the presence of a predator (Magnhagen & Forsgren 1991). In the spawning season females are less selective in choosing a male when under predation risk (Forsgren 1997). Moreover, gobies occur in very shallow habitats (< 1 m), which is generally regarded as a predator avoidance mechanism (Paterson & Whitfield 2000). Contrary to burial in the sediment, predator avoidance behaviour by seeking shelter in the estuary has the advantage that foraging is possible under a strongly reduced predation risk. This enables fish to maximize their feeding behaviour and feeding rates, especially in combination with a higher food supply in the estuary.

It remains unclear however, how these habitat functions could significantly contribute to an increase in an individual's fitness or state, taking into account that the periods of estuarine residency, as observed in the present study, are brief. In this perspective another explanation for estuarine migration arises. *Pomatoschistus* spp. are known to be important hosts and transmitters of parasites (Hamerlynck *et al.* 1989, Zander *et al.* 1993, Geets 1998). However, many parasites are stenotopic and cannot osmoregulate. In particular ectoparasites are sensitive to salinity changes. Stenohalinity of parasites and their host are considered as the main reasons for a low diversity of fish parasites in low salinity areas (Malmberg 1970, Zander & Reimer 2002). Möller (1978) transplanted flounder infested with two ectoparasitic copepod species (*Acanthochondria depressa* and *Lepeophteirus pectoralis*), from sea water to various salinities. His experiment showed that at salinity 12, the parasitic load was reduced by 50 % after 15 days, and at salinity 8 and 4 after 10 and 5 days respectively. Flounder specimens at salinity 12, 8 and 4 lost all copepods after respectively 30, 25 and 10 days. A restricted marine parasite spectrum was also observed for *Pomatoschistus* spp. in brackish water of the Baltic (Zander & Reimer 2002, Zander 2004). An excursion into brackish water areas might thus be a response to reduce the parasite load or the risk of such infections, and consequently optimize associated fitness traits. Moreover, the highest density of sand gobies in the estuary in autumn appears to coincide with the highest population density of mesoparasitic copepods on sand goby in adjacent marine areas (Van Damme *et al.* 1997) and with an infection peak of the ectoparasitic monogonean (*Gyrodactylus* sp.) (Geets 1998). The distribution of parasites is usually not taken into account when evaluating habitat quality for fish. However, such

hypothesis deserves further attention, especially when investigating estuarine habitat functions that sustain the possibility for fishes to briefly take advantage from estuaries.

To sea or not to sea?

Because the immigration patterns of herring (Guelinckx *et al*. 2006) and sand goby (Chapter 4) appear similar, it is tempting to assume that the model predictions for herring (Maes *et al.* 2005a) are also valid for sand goby. However, the comparison between sand goby and herring or any other marine species does not hold fully; different mechanisms might regulate sand goby migrations into the brackish water area. First of all, sand goby is a small demersal species displaying an intermittent swimming behavior with short darting movements (Miller 1986). Contrary to pelagic species like herring, it is not adapted to migrate long distances. On the other hand larvae and juveniles of marine fish are known to use tidal currents selectively to reach or remain in favourable habitats (Jager 1999, Leis 2006). Bardin & Pont (2002) reported that *Pomatoschistus* spp. shift from passive (drifting) to more active migration behaviour from about a total length of 20 mm on. We observed that sand goby entering the upper estuary were at least 2 months old and measure minimum ~20 mm meaning they probably used selectively tidal currents to reach the Scheldt upper estuary. The residual velocity of the bottom layer in the lower estuary (Westerschelde) is directed upstream. Moreover, with a maximum tidal velocity during an average tidal cycle of about 0.9 m s^{-1} (Baeyens *et al.* 1998) it would take not more than a few tides to reach the brackish water area, when using tidal transport.

Secondly, in contrast to marine opportunists that are clearly coming from the sea (e.g. *Clupea harengus*, *Solea solea*, *Pleuronectes platessa*), we found confounding results about the geographical origin of sand goby in the Scheldt estuary. The typical seasonal abundance pattern that sand goby display in the brackish water zone is similar to that of marine opportunistic species and suggests that spawning takes place either at downstream sites or at sea. Fish probably originate from the known coastal spawning areas adjacent to the mouth (areas A in Fig. 7.1). The relatively high ^{13}C values for fish caught in the upper estuary suggest that some fish might originate from offshore areas or from the Eastern Scheldt (a marine bay north of the Scheldt estuary) (Fig. 7.1). Similarly enriched ^{13}C values were indeed found in these areas for sand goby muscle tissue, whereas more depleted values were observed along the Belgian-Dutch coast (Chapter 2b and unpublished data). Fish from the Eastern Scheldt might arrive via the North Sea or through a 9 km long canal (Kanaal door Zuid-Beveland) that connects the Eastern Scheldt with the Scheldt estuary (Fig 7.1). The canal enters the Scheldt estuary at 40 km from the mouth in the mesohaline zone. Turbidity in the Scheldt estuary is much higher than in the Eastern Scheldt where the fish community is most likely much more predator controlled. Hamerlynck *et al.* (1993a) attributed the very low numbers of *P. lozanoi* in

the Eastern Scheldt to predation. Hostens *et al.* (1996) suggested fish migration through the canal to explain the occurrence of species that are more specific for the Eastern Scheldt (e.g. *Zoarces viviparus*) in the Scheldt estuary. However, the canal is provided with sluices to prevent major tidal currents and these open only for shipping traffic. Consequently, fish migration through the canal is probably not significant but has unfortunately never been studied. It is obvious that fish migration between both water bodies merits a profound study, especially now the Dutch government is initiating trials to open the sluices more often to enhance fish migration. [Sr/Ca] values near the otolith nucleus suggest that spawning might also take place in the polyhaline or brackish water area of the Scheldt (Chapter 6). Although sand goby is able to spawn in brackish waters (Fonds & van Buurt 1974), there are probably no suitable nesting sites in this area of the Scheldt to sustain a substantial spawning population. This is also evidenced by the lack of spawners in the brackish area from April to June (Hostens 2003, Maes *et al.* 2005b). If spawning occurs in the estuary it probably occurs in the polyhaline zone (Hostens *et al.* 1996). Sand goby larvae have been observed in the lower estuary, though they may have been advected from coastal areas (Beyst *et al.* 1999).

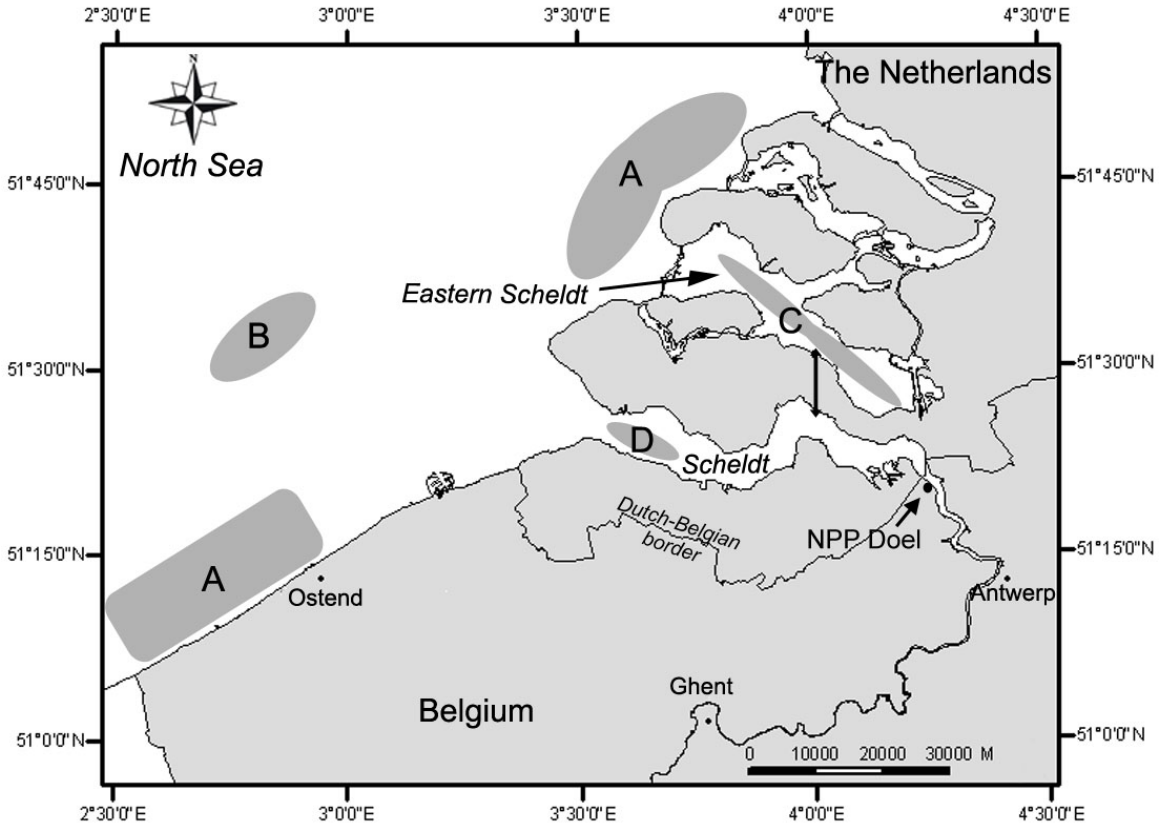

Fig. 7.1. *Map with potential source populations of sand goby in the Scheldt estuary. Spawning occurs in coastal areas (A) and the entire Eastern Scheldt (C), though it may also occur more offshore (B) and in the lower estuary (D).The main sampling location in de upper estuary is indicated (NPP Doel), and the double arrow locates the canal between the Eastern Scheldt and the Scheldt estuary.*

Our results evidence a wide diversity in life histories regarding estuarine habitat choice. It is consistent with the different patterns of estuarine habitat use within populations of many other fish species such as American shad *Alosa sapidissima* (Limburg 1998), blueback herring *Alosa eastivalis* (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 2004), sole *Solea solea* (Leakey *et al.* 2007) and three eel species (*Anguilla anguilla, A. japonica* and *A. rostrata)* (Tzeng *et al.* 2002, Daverat *et al.* 2006, Fablet *et al.* 2007). The occurrence of divergent habitat use patterns within fish populations is considered a 'bet-hedging' strategy against adverse events in specific habitats. Facultative estuarine residence by marine fishes would serve as a strategy to hedge bets across predictable less productive coastal habitats versus unstable productive estuarine habitats (Kraus & Secor 2004, Daverat *et al.* 2006).

The life history trait related to habitat use is often strongly related to other life history traits such as growth rate or physiological processes such as metabolic rate. For example, migratory individuals usually grow larger and have a higher reproductive potential but lower survival than resident conspecifics (Jonsson & Jonsson 1993). The coexistence of different life history tactics within populations might result from discrete genetic entities, environmental influences or interactions between both. Different tactics related to the choice or a shift of habitat within populations could thus result from and be maintained by different sub-populations separated by a degree of reproductive isolation, an evolutionary stable polymorphism or a heterogeneous distribution of habitats (Gross 1985, Tsukamoto 1987, Jonsson & Jonsson 1993, Secor 1999, Kraus & Secor 2004). Demonstrating different migratory behaviors is nevertheless insufficient to support the predominance of a given mechanism (Jonsson & Jonsson, 1993). Yet, we may exclude that genetically distinct sand goby populations cause the wide variability observed in estuarine habitat use. Although small scale genetic structure in the Dutch Delta was suggested by Pampoulie *et al.* (2004), the results were not reproducible, probably because their study suffered from technical limitations and poor genome sampling. According to the latest genetic analyses there is no evidence of genetic structure along the Belgian and southern Dutch coast (Larmuseau *et al*. in prep). We suspect that the observed variability in estuarine habitat use by sand goby is mainly caused by phenotypic plasticity. The plasticity of habitat use patterns at the individual level is a mechanism by which this species occupies habitats during their life cycle. Moreover, this plasticity is a strategy that allowed sand gobies to colonize different ecosystems across their distributional range. The strong phenotypic plasticity is probably also reflected in the divergent life history traits observed within and among populations (Gibson & Hesthagen 1981, Bouchereau & Guelorget 1998, Pampoulie *et al*. 1999, Dolbeth *et al*. 2007), but this remains to be confirmed. This plasticity could eventually lead to local adaptations and separate genetic units (Jokela *et al.* 2003, Huyse *et al.* 2004, Larmuseau *et al.* in prep.). The

high plasticity observed in sand goby habitat use patterns might thus be illustrative for the mechanism leading to speciation and the wide radiation of Gobiidae in the world (Miller 1986).

To conclude…

It has been known for a long time that fish have complex life histories and that the ontogenetic habitat use varies among species. The occupation of estuarine and oceanic habitats by different fish species is a continuum due to multiple sources caused by biotic and abiotic factors varying over a spatiotemporal scale (Whitfield 1999, Able 2005, Elliott *et al.* 2007). With the use of new techniques it became clear that different patterns of estuarine habitat use exist among and within populations, as was shown for sand goby here. Especially facultative estuarine fishes may vary in their use of this habitat on varying geographic, ontogenetic, annual, cohort-specific and individual scales. Consequently, the ecological guild classification and the estuarine dependency of several species is being reconsidered (Lenanton & Potter 1987, Thiel *et al.* 2003, Ray 2005). Eels (*Anguilla spp.*) for example have long been regarded as catadromous, although now it appears that freshwater residency is rather facultative, and they should be regarded as semi-catadramous or facultative catadromous (Tsukamoto & Arai 2001, Daverat *et al.* 2006). Fablet *et al.* (2007) even reported 37 different patterns of habitat use for *Anguilla anguilla*. Whether a species is estuarine dependent or not for a given life stage should be put in an evolutionary context. Estuary-dependent fishes are those that are 'obligate' in an evolutionary adaptive sense. That is, if estuaries were removed, these species would be at risk of significant depletion. Facultative users are species that 'may' use estuaries as they are not dependent of estuarine habitats for their survival. "Dependency'' is thus different from ''occurrence'' or even ''abundance'' (Ray 1997, 2005). The best way to assess the significance of an estuarine habitat for a marine species in a given area is to quantify its contribution to the spawning population (Beck *et al.* 2001, Dahlgren *et al.* 2006).

Elliott & Dewailly (1995) classified sand goby as 'estuarine residents' because this species is capable of completing its entire life cycle within the estuarine environment. Estuarine spawning has indeed been reported for several European estuaries (Pihl *et al.* 2002), yet they are particularly known to spawn in marine areas (Healey 1971, Fonds 1973, Claridge *et al.* 1986). In southern Europe (Mediterranean and Iberian Peninsula) emigration from estuaries and lagoons during winter is for the purpose of spawning (Bouchereau & Guelorget 1998, Pampoulie *et al.* 1999, Leitão *et al.* 2006). Based on the sand goby literature and especially on the observed variability in estuarine habitat use, including the high turnover of individuals in the brackish water zone, we conclude that sand goby should be considered as a marine-estuarine opportunist. Estuarine residency is probably not obligatory for any life stage of the sand goby. Their short residence and individual behavior in the estuary severely questions the functional significance of the estuary for sand gobies. Their behavior in the Scheldt estuary

contrasts with the concept that estuaries are important areas, offering abundant food supply, favorable abiotic conditions and shelter from predation. Of these features, predator avoidance is probably the most important for sand gobies. However, to yield enhanced growth and better survival probabilities from these beneficial conditions, a relatively long period in the estuary seems required. Given the observed movement patterns we suspect that sand gobies visit the estuary rather incidentally when abiotic conditions (temperature) allow for. If their behaviour is indeed deterministic, estuarine migration is probably found in other mechanisms such as parasite shake off.

Although estuarine residency is not obligatory and rather short, the estuarine habitat could still influence the dynamics of sand goby populations at sea significantly, despite its relatively small surface. Therefore we compiled the following recommendations to fully understand the functional significance of the estuary and the underlying mechanisms of estuarine migration. These recommendations can be extrapolated to other taxa and geographical areas.

(1) Connectivity between the estuarine habitat and sand goby spawning populations should be understood to establish the contribution of estuarine migrants to each cohort (Beck *et al.* 2001, Dahlgren *et al.* 2006). Especially the dynamics of migration patterns and the underlying contingent structure, if existing, should clarify the role of estuaries.

(2) Long term research will clarify the contributions of contingents, which vary over the years. Kraus & Secor (2005) have shown for white perch *Morone americana* that the contribution per unit area of two juvenile habitats to the production of adults differs between years depending on year-class strength. This could have severe implications to prioritize habitats for management and conservation.

(3) It is still not clear what the underlying genetic structure represents. Especially, adaptation is probably a common structuring factor of marine fish populations with low levels of neutral genetic divergence (Larsen *et al.* 2007).

(4) Simultaneous and comparable studies over the sea-estuary ecotone are in short supply. These studies should assess and compare life stage specific characteristics in each environment (e.g. density, growth and mortality). Focus should be put on the specific subhabitats within estuaries, instead of the estuary as a whole. Pihl *et al.* (2002) described nine habitats in European estuaries which are important for fishes (e.g. salt marshes and intertidal soft substratum).

(5) As females seem to dominate the brackish water samples of sand goby (Vanden Eede 2006) sex biased dispersal should be tested in the studies recommended above.

(6) These quantitative analyses of migration could then be implemented in an overall dynamic population model.

(7) Inherent to the discussion of estuarine habitat functions and dependency for fishes is the definition of an estuary. Nevertheless, there is not a single and suitable definition which covers all types of estuaries worldwide (Elliott & McLusky 2002, Elliott *et al.* 2007). The treatment of the seaward limits of estuaries varies among authors and thus confounds the concept of estuarine dependency (Able 2005). A consensus on this is necessary within fisheries research. Note that, although we recognize that the coastal area is partly "estuarinized", here we did not include the estuarine plume as part of the estuary.

2. ASSESSING THE CONDITIONS

2.1. C and N isotope gradients in the Scheldt

Based on sand goby gut contents it was demonstrated for the Scheldt estuary that the $\delta^{13}C$ value for food sources in the lower estuary was on average 6 ‰ higher than in the upper estuary. However, a large temporal variation of $\delta^{15}N$ in sand goby gut contents was observed in the brackish water area, probably caused by natural changes in isotopic composition of the inorganic N pool (Chapter 2b). It leads to seasonally alternating $\delta^{15}N$-differences between the upper estuary and the North Sea. These findings are largely in agreement with those of clupeoid stomach contents sampled in 2000-2001 (Guelinckx *et al.* 2006). The consistent isotopic difference for $\delta^{13}C$ between food webs in the upper and lower estuary makes this tracer suitable for studying fish migration between the sea and brackish water reaches. The absence of a clear $\delta^{15}N$ difference makes $\delta^{15}N$ not a useful tool for studying estuarine migration by marine fishes. This is in contrast with other studies which successfully applied $\delta^{15}N$ as a tracer of migration (e.g. Hansson *et al.* 1997, McCarthy & Waldron 2000). However, these studies were conducted in systems with only a minor temporal variation in $\delta^{15}N$ and $\delta^{13}C$ at the base of the food web. In eutrophic systems, enhanced microbial activity (e.g. nitrification) results in highly variable isotope composition of the substrates of primary producers and as a consequence $\delta^{15}N$ and $\delta^{13}C$ are less useful to study fish migration (De Brabandere 2005). During our study period the Scheldt received untreated sewage material. Raw sewage water is depleted in $\delta^{15}N$ relative to seawater (Owens 1987), but the inorganic N pool becomes strongly enriched due to the microbial activity in the Scheldt and its tributaries. Treated sewage effluent tends to be enriched relative to seawater, due to the microbial processes in the treatment plants (Gaston *et al.* 2004, Savage 2005). We expect that with enhanced waste water treatment $\delta^{15}N$ might become a useful tracer to investigate marine fish migration in the Scheldt estuary. An efficient treatment of waste water would lead to a consistent $\delta^{15}N$ gradient in the Scheldt, decreasing from the brackish water area to the sea. This was recently demonstrated for the Thames estuary by Leakey *et al.* (2007).

We sampled sand goby gut contents in the upper and lower estuary to isotopically characterize their food sources and to assess the isotopic difference between both environments. This methodology is supported by recent studies in other estuaries. It is neither necessary to sample other trophic levels (Vinagre *et al.* 2008), nor to sample more locations (Leakey *et al.* 2007). This is especially the case for $\delta^{13}C$ that shows a steep transition between the upper and lower estuary. However, Leakey *et al.* (2007) also confirmed that a multiple-isotope approach is valuable in improving the resolution with which isotope end-members can be distinguished (Peterson 1999). They showed that besides $\delta^{13}C$ and $\delta^{15}N$, $\delta^{34}S$ with ^{34}S enrichment towards the mouth, can be a useful tracer of fish migration.

2.2. Different C and N isotopic turnovers in fish tissues

The diet switch experiment (Chapter 3) resulted in clear changes in the isotopic composition of sand goby muscle, liver and heart tissue. Observed isotopic turnover rates were specific for tissue type and element involved, with muscle tissue having the slowest turnover rates. These differences were explained by differential metabolic activity in the respective tissues. Additionally, we even found a statistically significant contribution of metabolic replacement in muscle tissue. A high metabolic activity in tissues leading to among tissue differences for isotopic turnover rate is generally accepted for endotherms but not for ectotherms like fish. This feature holds great promise for the use of multiple tissues to estimate the timing of diet shifts of fishes (Phillips & Eldridge 2006). The use of multiple tissues and/or markers could also lead to a higher temporal resolution of the technique in general (Hobson 1999, Kurle & Worthy 2002). In addition non-lethally sampled tissues such as scale, fin tissue or blood plasma provide substitutes for lethally sampled tissues, especially for investigation of rare and endangered species (Hobson & Clark 1993, Kelly *et al.* 2006). However, probably the most promising application in isotope ecology is compound (e.g. fatty acids, cholesterol, and amino acids) specific isotope analysis. This will greatly advance our understanding of observations made on bulk material or tissues. Different compounds within tissues have differential sources, turnover rates and isotopic discrimination due to specific metabolic pathways. For example, essential amino acids undergo no or minimal isotope fractionation during incorporation, whereas, a trophic enrichment occurs in non-essential amino acids (Pinnegar & Polunin 1999, McClelland & Montoya 2002, Popp *et al.* 2007). This difference allows to acquire information about both trophic level and nutrient sources at the base of the food web from single samples of consumer tissues (McClelland & Montoya 2002, Graham *et al.* 2006). Graham *et al*. (2006) analysed the N isotope composition of essential and non essential amino acids to distinguish between effects of migration and trophic dynamics in bulk tissue of yellowfin tuna *Thunnus albacares* in the Pacific. Another example is compound-specific ^{13}C analysis of phospholipid-derived fatty acids used to reveal carbon energy sources of species and communities (Boschker *et al.* 2005).

2.3. Otolith [Sr/Ca] as a tracer for estuarine migration

A positive linear relationship between [Sr/Ca] in sand goby otoliths and this concentration ratio in the surrounding water was experimentally assessed (Chapter 5). Due to the interindividual variability in otolith [Sr/Ca] values, the predictive resolution of this regression only allowed for distinguishing between two environments (marine vs. brackish water). It is however unclear what caused this scatter in the relationship between water and otolith [Sr/Ca]. Besides the composition of the surrounding water other environmental (e.g. temperature) and biological factors (e.g. growth, ontogenetic stage, kinetics and stress) affect otolith [Sr/Ca] (Campana 1999, de Pontual *et al.* 2003, Elsdon & Gillanders 2003). Nevertheless, our results are consistent with Secor & Rooker (2000), Rooker *et al.* (2004) and Zimmerman (2005) who also reported that otolith [Sr/Ca] is useful for describing estuarine fish movements over larger habitat transitions between fresh water, brackish water and salt water. So although otolith [Sr/Ca] could yield a high temporal resolution due to the daily increments, the geographical resolution remains relatively low. A considerable shift in ambient salinity (> 10 psu) (Zimmerman 2005, chapter 5) appears necessary to induce a distinguishable habitat specific otolith [Sr/Ca] mark. Thus, this technique is not very adequate to investigate movement patterns of opportunistic or obligate users if they make restricted migrations in estuaries, to study estuarine excursions of stenohaline stragglers (marine or freshwater species) or to study relatively small scale movements of estuarine species. Similarly, the quantification of estuarine migrants or residents in a population will be biased when fish migratory pathways are rather restricted along the salinity gradient. Hence, otolith [Sr/Ca] is particularly useful to study chronologies in habitat use of diadromous fishes (Elfman *et al.* 1999, Secor & Rooker 2000). A better spatial resolution might be achieved by using multiple tracers as habitat signatures. Besides Sr, trace elements such as Ba and Mn can be particularly useful to trace estuarine fish migration (Gillanders 2002). New techniques lower the limits of detection, such that more trace elements can (simultaneously) be measured on a finer spatial scale on the otolith. This could then result in high resolution maps of otolith sections for multiple elements. Combination of these elemental maps could consequently reveal more detail about habitat use throughout the fish's life (Limburg *et al.* 2007). Moreover different parts in otoliths may incorporate elements at different rates. Consequently entire maps of otoliths are more reliable than line transects (Radtke *et al.* 1999, Limburg *et al.* 2007). It is however also of atmost importance to improve our understanding of the underlying processes of elemental incorporation into otoliths. This would allow an enhanced interpretation of otolith elemental measurements in relation to changes in various environmental (e.g. ambient concentration, temperature, salinity) and biological factors (e.g. growth, ontogenetic stage) (Martin & Wuenschel 2006).

3. IT IS A MERIT FOR AN ECOLOGIST TO BE ALSO A PHYSIOLOGIST

The data presented in this work underscore the necessity for more biochemical analyses and models based on physiology to provide the underlying mechanisms that enable full exploitation of biochemical tools in ecology and to explain important ecological patterns at varying spatiotemporal scales.

The analysis of stable isotope composition and otolith chemistry has become an important tool in animal ecology. These techniques have been used in a wide array of applications such as the reconstruction of animal diets and environmental histories, migration studies and studies of food web interactions (Campana 1999, Post 2002, McKechnie 2004, Rubenstein & Hobson 2004, Begg *et al.* 2005). Animal ecologists have mainly adopted a phenomenological approach and relied on observational patterns when using these tools. For example the approximate shift in isotopic composition between a consumer and its resource or the relationship between otolith [Sr/Ca] and ambient salinity. Because these patterns appear fairly robust, the approach has been very useful. However without a mechanism which allows to explain them, we cannot fully comprehend the generic character of the detected patterns or the boundaries of their usefulness (Martínez del Rio & Wolf 2004). To this aim models in animal isotope ecology (Phillips & Koch 2002, Fry 2003, Martínez del Rio & Wolf 2004) and otolith studies (Kalish 1991, Borelli *et al.* 2001, Payan *et al.* 2004, Allemand *et al.* 2007) have been developed for a mechanistic and hence predictive foundation for these techniques. Nevertheless the impact of many factors remains unresolved and the models require experiments that entail physiological measurements.

We observed trophic shifts for sand goby (Chapter 3) that were relatively large but still within reported ranges for $\delta^{13}C$ and $\delta^{15}N$ trophic shifts. Unfortunately, the physiological or metabolic processes that determine tissue specific trophic enrichment are poorly understood. The results of Chapter 2a support the model proposed by Ponsard & Averbuch (1999), stating that isotopic enrichment stems from a combination of isotopic discrimination during assimilation and synthesis as well as during catabolic processes and excretion. Research to this variety of processes firstly demands detailed tissue specific budgets of elements and isotopes for animals under a variety of conditions (e.g. differing temperatures, diet quality, food ration and consumer's physiological status and ontogenetic stage). Secondly, the details of the biochemical processes that lead to differences in the isotope composition of what animals eat and what they defecate and excrete need to be unraveled. Measuring compound specific isotopic composition could avoid the problem of nutrient routing and reveal biochemical pathways (Gannes *et al.* 1997, Martínez del Rio & Wolf 2004). Determining the origin and fate of mobilized biochemical components during catabolism will also provide insight in observed isotopic shift during starvation (Chapter 3).

The newly developed isotopic clock (Chapter 4) relies on an exponential model that presumes the incorporation of the dietary isotopic signature into an animal's tissue follows first order, one-pool kinetics. As tissues may have different isotopic turnover rates dependent on their biochemical composition (Tieszen *et al.* 1983, Lorrain *et al.* 2002) and exchange materials among each other, several elemental pools probably exist. Moreover, different proteins are known to have widely diverging half lives (Martínez del Rio & Wolf 2004). Ayliffe *et al.* (2004), proposed a multi-compartment approach to describe tissue isotopic change and this approach deserves more attention, especially for understanding elemental replacement. Furthermore, the development of the isotopic clock strongly highlighted the need to assess the relationship between metabolic rate and the rate of elemental replacement in tissues and to establish the effect of growth and temperature on this relationship.

Otoliths are seperated from the external environment by successive physiological barriers and compartments, namely gill or intestine epithelia, blood, saccular epithelium and endolymph in which they bath. Consequently, elemental deposition does not directly reflect the elemental concentrations in the water. The relationship between otolith chemistry and ambient 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, de Pontual & Geffen 2002, Allemand *et al.* 2007). Our results (Chapter 5 and 6) support the view that element incorporation and the influence of various (a)biotic factors on such incorporation differ among fish species and even among conspecifics. This limits the development of generalized models aimed at reconstructing environmental conditions from otolith chemistry (Elsdon & Gillanders 2003, Martin & Wuenschel 2006). Validation experiments to translate species specific elemental signatures in otoliths should take otolith growth rate, ontogenetic stage, temperature and salinity into account. Furthermore, future work and analytical techniques should focus on simultaneous measurements of elemental concentrations in the ambient environment, blood plasma, endolymph and otolith surface to reveal elemental kinetics along the cell boundaries and during precipitation (Kalish 1991, Allemand *et al.* 2007).

Finally, knowing physiological adaptations and metabolic processes within individuals helps to understand ecological patterns and mechanisms on varying spatial and temporal scale, including habitat use chronologies. For example: regional endothermy, an elevated metabolism and increased heart rate contribute to continuous, relatively fast swimming by tunas and minimize thermal barriers to habitat exploitation, permitting niche expansion into high latitudes and to ocean depths (Graham & Dickson 2004). Graham *et al.* (2007) propose a critical mass threshold for yellowfin tuna *Thunnus albacares* that enables sufficient endothermic capability to allow this species to access prey in deeper, colder water. Another

example can be found in the growing evidence that glass eels' first habitat selection might be influenced by thyroid hormones which affect individual migration ability (Daverat *et al.* 2006).

Recently, a metabolic theory of ecology was presented in which the metabolic rate of organisms scales with body size and temperature (West *et al.* 1997, Clarke & Johnston 1999, Brown *et al.* 2004). This metabolic theory predicts how metabolic rate regulates biological processes at all levels of organization, by setting the rates of resource uptake from the environment and resource allocation to survival, growth, and reproduction (Brown *et al.* 2004). The theory is still at its initial phase but it seems to be a unifying ecological principle (Duarte 2007), that provides a conceptual foundation and framework for much of ecology (e.g. carrying capacity of a system (Savage *et al.* 2004, Stevens 2006). An example in marine ecology is given by O'Connor *et al.* (2006) who demonstrated that the temperature dependency of planktonic larval duration time of fishes is consistent with the expectations of the metabolic theory. Consequently, the theory could be applied to predict planktonic dispersal distances (Duarte 2007).
Elaboration of such mechanistic models might hold the answer for the anomalous patterns of estuarine habitat use by sand gobies. At least, the avoidance of the upper estuary during warm summer months seem to agree with the metabolic theory of ecology. The metabolic rate of fish increases with increasing temperature, while dissolved oxygen concentrations decrease with increasing temperature. This mismatch between oxygen demand and the capacity of oxygen delivery to tissues, is probably the first mechanism to restrict fish tolerance to thermal extremes (Pörtner & Knust 2007). Following the metabolic theory, it is to expect that bigger fish, having a higher metabolic rate, will leave the estuary sooner than smaller fish when temperatures are high. This might explain why the smaller common goby (*Pomatoschistus microps*) tolerates higher temperatures than the sand goby, and reaches high abundances in the Scheldt in summer. The common goby is also known to move further upstream, into the hypoxic zones of the Scheldt estuary. Their small size and lower metabolic rate probably contribute to enable this behaviour.

EN

SUMMARY

Many marine fish species are known to seasonally enter estuaries in large numbers during a period of their juvenile life stage. Yet, very little is known about the interaction between the estuarine populations and the population at sea or about the use of estuaries on a spatio-temporal scale by individual fish. Such knowledge is however fundamental to understand population dynamics, life history tactics and behaviour of marine fishes. Moreover, detailed knowledge of habitat use patterns is necessary to comprehend habitat function and forms the basis of efficient conservation and integrated management plans. The functional significance of estuarine visits at the level of the individual, the population and the species is still debatable for most marine fishes. This gap in knowledge can be attributed to the complexity of studying and following marine organisms from one habitat to another. Conventional methodologies often suffer from a constrained spatio-temporal resolution. Furthermore, many of those methods are not applicable for (post)larval and small juvenile fish susceptible to dispersive processes and high mortality rates. The migration dynamics of sand goby *Pomatoschistus minutus* (Pallas, 1770) between the North Sea and the Scheldt estuary (Belgium and The Netherlands) was investigated in this study by means of two biogeochemical tracers, namely stable carbon isotopes in dorsal muscle tissue (Chapter 4) and otolith [Sr/Ca] (Chapter 6). Prior to the application of both geochemical tracers in our study system, the techniques needed to be calibrated and conditions verified (Chapter 2, 3 and 5).

We depended in this study on sand goby gut contents for determining the isotopic difference between food sources in the upper and lower estuary, and for the prediction of an upper estuarine end member signal. Therefore, we first describe an experiment towards the isotopic effects that might occur during digestion and assimilation (***Chapter 2a***). Gut contents of sand goby showed higher $\delta^{13}C$ and $\delta^{15}N$ isotope values than the food before ingestion. This enrichment was more pronounced in the hindgut than in the foregut, probably because of preferential assimilation of ^{12}C and ^{14}N along the gastro-intestinal tract. There was however no statistically significant difference for $\delta^{13}C$ between the food source and the foregut content.
A prerequisite to trace animal movements between two areas using stable isotopes is that food sources of the species under study are isotopically different between both areas. Additionally, for clear interpretation, it is important that the source population is relatively homogeneous in isotopic composition (***Chapter 2b***). Stable isotope analyses on monthly gut contents demonstrated that the $\delta^{13}C$ value of sand goby prey items was on average 6 ‰ higher in the

lower estuary than in the upper estuary. From June until November, $\delta^{15}N$ was higher in the upper estuary than in the lower estuary, but this pattern reversed during winter and early spring. Sand goby muscle tissue showed no spatial $\delta^{13}C$ variability along the Belgian coast. Our data however, revealed that coastal $\delta^{13}C$ values were depleted relative to the offshore values. Coastal $\delta^{15}N$ values, on the other hand, increased considerably with increasing distance from the estuary during summer and autumn, but an inshore-offshore $\delta^{15}N$ gradient was not detected. These results confirm that $\delta^{13}C$, in contrast to $\delta^{15}N$, is an appropriate tracer to study fish migration into the Scheldt estuary.

Studies on diet or migration of organisms based on stable isotopes require precise estimates of the rate at which the isotopic composition changes in the investigated tissues. Isotopic turnover rates in fish, unfortunately, are poorly understood. A laboratory diet switch experiment (***Chapter 3***) was conducted (1) to determine C and N isotopic turnover rates in sand goby muscle, liver and heart tissue and (2) to evaluate the relative contribution of growth and metabolic replacement to the total change in isotopic composition. This experiment showed that isotopic turnover rates varied among tissue types and elements, with dorsal muscle having the slowest turnover rates. These differences were attributed to the different metabolic activity in the respective tissues. In liver and heart, metabolic turnover of elements contributed considerably to the isotopic shift. A high metabolic activity in tissues leading to among tissue differences for isotopic turnover rate is generally accepted for endotherms but not for ectotherms like fish. With a half life of about 25 days, $\delta^{13}C$ in dorsal muscle tissue was identified as the most appropriate tracer to study sand goby immigration in the Scheldt estuary.

Recruitment of sand goby into the upper Scheldt estuary was reconstructed over an entire year using an isotopic clock (***Chapter 4***). These results were combined with a growth model to yield age and length at immigration. Sand goby 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 throughout the year in the upper estuary resided there for less than one month, which indicates a strong overlap between immigration and emigration. 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 length of ~20 mm. The results showed that the use of an isotopic clock strongly complements catch data and that it is useful to describe the connectivity between populations.

Ratios of strontium to calcium laid down as a lifetime record in otoliths are regularly used to reconstruct salinity histories of fishes. This technique requires prior knowledge of the differences in ambient Sr/Ca concentrations along the estuarine gradient and an accurate description of the relationship between aqueous and otolith [Sr/Ca]. To this aim, the changes in [Sr/Ca] over the entire salinity gradient of the Scheldt estuary were determined for each season, and an experiment was conducted towards Sr incorporation in sand goby otoliths at

five salinity levels (3, 7, 12, 20 and 30) (***Chapter 5***). The experiment was conducted at two temperatures (13 and 18°C). [Sr] acted conservatively in the estuary, while [Ca] deviated slightly from a conservative trend. This resulted in a positive but nonlinear relationship between salinity and ambient [Sr/Ca]. Experimental results revealed a positive linear relationship between aqueous and otolith [Sr/Ca]. Otolith [Sr/Ca] was significantly different between each salinity level but there was no temperature effect. Due to the variability in otolith [Sr/Ca] values among individuals of the same treatment, the predictive resolution of this regression allows to distinguish between only two different environments (marine vs. brackish water).

Otolith [Sr/Ca] chronologies were eventually examined to chart movements of twelve sand gobies in the Scheldt estuary (***Chapter 6***). Variable patterns of estuarine habitat use were detected, corroborating the results of the isotopic clock. The individuals displayed varying periods of residency in brackish water areas with different timing of immigration into these areas. Additionally, repeated migrations between the lower and the upper estuary were detected. This shows that the migratory behaviour of sand goby in estuaries is probably much more diversified than previously assumed. Consequently, it was concluded that the sand goby population displays a large flexibility in life histories regarding habitat choice. Based on these estuarine habitat use patterns, the sand goby should be considered as a marine-estuarine opportunist.

The observed variability in migration patterns indicate that estuarine residency of sand goby is the result of an optimal habitat choice based on trade-offs made at the individual level, rather than a standard migration scheme that is valid for the whole population. The short estuarine residencies resulting in a high turnover of individuals in the brackish water zone severely challenges the functional role of estuaries for the sand goby. Brief estuarine residencies do not seem to fit in the general concept that estuaries are important feeding, growth or predator refuge areas for marine fishes. The observed movement patterns suggest that sand gobies visit the estuary rather incidentally. If their behaviour is deterministic, estuarine migration is probably found in other mechanisms such as parasite shake off. The possibility for marine fishes to briefly profit from estuarine areas definitely merits further research.

NL

SAMENVATTING

Mariene vissoorten zijn gekend om seizoenaal in grote getale te verblijven in estuaria. Voor veel soorten is dit het geval tijdens de juveniele levensfase. Nochtans is er weinig geweten over de interacties tussen de respectievelijke populaties in estuaria en deze op zee. Evenzeer is geweten hoe mariene vissen op individueel niveau gebruik maken van estuaria in tijd en in ruimte. Dergelijke kennis is echter onontbeerlijk om de populatiedynamica, de levensgeschiedenisstrategieën en het gedrag van mariene vissen te begrijpen. Bovendien draagt een gedetailleerde kennis voor het habitatgebruik bij tot een beter begrip van habitatfuncties. Dit vormt immers de basis voor een integraal gebiedsbeleid en efficiënt beheer van de betrokken soorten. Het functionele en kwantitatieve belang van estuaria op het niveau van de soort, de populatie en het individu staat voor mariene vissen nog steeds ter discussie. Dit is deels te wijten aan de moeilijkheidsgraad om migraties van mariene vissen te bestuderen. Zo hebben klassieke methodes om migraties te bestuderen vaak een vrij lage ruimtelijke en temporele resolutie. Bovendien zijn veel methoden niet geschikt voor (post)larvale stadia en kleine vissen, gekenmerkt door een hoge graad van dispersie en mortaliteit.
Deze studie onderzocht de migratiedynamica van het dikkopje *Pomatoschistus minutus* (Pallas 1770) tussen de Noordzee en het Schelde-estuarium (België) met behulp van twee geochemische merkers, nl. De verhouding van stabiele koolstofisotopen ($\delta^{13}C$) in rugspierweefsel (Hoofdstuk 4) en de Sr/Ca concentratieverhouding in otolieten (Hoofdstuk 6). Vooraleer beide merkers in ons systeem konden toegepast worden, dienden beide technieken gekalibreerd te worden én werd nagegaan of de specifieke voorwaarden voldaan waren (Hoofdstukken 2, 3 en 5).

Voor het bepalen van het isotopenverschil tussen voedselbronnen nabij de monding en het brakwatergebied, en in tweede instantie voor de voorspelling van de isotopenwaarde van dikkopjes in het brakwatergebied waren we afhankelijk hun darminhoud. Hierom werd eerst een experiment uitgevoerd naar het isotoopeffect dat zich eventueel voordoet ten gevolge van de vertering en assimilatie van prooien (***Hoofdstuk 2a***). De darminhoud van het dikkopje toonde hogere $\delta^{13}C$ en $\delta^{15}N$ waarden dan het voedsel vóór consumptie. Deze verhoging was meer uitgesproken in de einddarm dan in de voordarm. Dit heeft waarschijnlijk te maken met een preferentiële opname van ^{12}C en ^{14}N langsheen het darmkanaal. Er werd voor $\delta^{13}C$ echter geen significant verschil waargenomen tussen het voedsel vóór consumptie en in de voordarm.

Het is een noodzakelijke voorwaarde voor het aanwenden van stabiele isotopen in migratiestudies is dat de voedselbronnen van de bestudeerde soort een andere isotopensamenstelling hebben op de respectievelijke plaatsen waartussen de migratie plaatsvindt. Voor een duidelijke interpretatie is het bovendien belangrijk dat de isotopensamenstelling van de bronpopulatie zich binnen zeer nauwe grenzen bevindt (***Hoofdstuk 2b***). Isotoopanalyses op maandelijkse stalen van darminhouden toonden aan dat de $\delta^{13}C$ waarde van de voedselbronnen van het dikkopje gemiddelde 6 ‰ hoger lagen nabij de Scheldemonding dan in het brakwatergedeelte (Beneden-Zeeschelde). Voor $\delta^{15}N$ werden in het brakwatergebied van juni tot november hogere waarden opgetekend dan in het mondingsgebied, maar lagere waarden tijdens de winter en de lente. Spierweefsel van het dikkopje toonde langsheen de Belgische kust geen ruimtelijke variabiliteit voor $\delta^{13}C$. Er werden wel significant lagere $\delta^{13}C$ waarden opgetekend in weefselstalen van vissen gevangen langsheen de kust ten opzichte van stalen verder op zee. Voor $\delta^{15}N$ werden geen verschillen waargenomen in spierweefsel tussen kust- en offshore gebieden. Er werd voor $\delta^{15}N$ echter wel een sterke gradiënt waargenomen langsheen de kust in functie van de afstand tot de Scheldemonding tijdens de zomer en de herfst. Deze resultaten tonen aan dat, in tegenstelling tot $\delta^{15}N$, $\delta^{13}C$ een geschikte merker is om vismigratie in het Schelde-estuarium te bestuderen.

Migratie- en voedselwebstudies op basis van stabiele isotopen vereisen dat de snelheid waarmee isotopensamenstellingen wijzigt in de organismen gekend is. Er is echter weinig geweten omtrent de omzetsnelheid van isotoopsignalen in visweefsel. Daarom werd een laboratoriumexperiment (***Hoofdstuk 3***) uitgevoerd (1) om de omzetsnelheden van C en N isotoopsignalen te bepalen in spier-, lever- en hartweefsel van het dikkopje en (2) om de relatieve bijdrage in te schatten van weefselgroei en metabole omschakeling tot de totale verandering in isotoopsignaal. Dit experiment toonde aan dat de veranderingssnelheid van isotopensignaal afhankelijk is van het type weefsel en het specifieke element, waarbij spierweefsel de traagste omzetsnelheid vertoonde. De verschillen waren te wijten aan de intensiteit van de metabolische activiteit in de verschillende weefsels. Vooral in lever- en hartweefsel droeg de hogere metabolische vervanging van elementen aanzienlijk bij tot de verschuiving in isotoopsignaal. Een weefselafhankelijke metabolische omzetting die leidt tot weefselspecifieke verschillen in omzettingsnelheden van de isotopensignaal was reeds aanvaard voor endotherme dieren, maar niet voor poikilotherme dieren zoals vissen. Met een halfwaardetijd van 26 dagen kan $\delta^{13}C$ in spierweefsel beschouwd worden als een geschikte merker om de migratie van het dikkopje te bestuderen.

De immigratie van het dikkopje naar de Beneden-Zeeschelde werd voor een heel jaar gereconstrueerd met behulp van een isotopenklok (***Hoofdstuk 4***). Deze resultaten werden gecombineerd met een groeimodel om zowel de leeftijd als de lengte van de vissen te bepalen op het moment van de immigratie. Hieruit komt naar voor dat dikkopjes vanaf mei bijna continu migreerden naar de Beneden-Zeeschelde. In juli echter leken ze het estuarium even te mijden vermoedelijk ten gevolge van te hoge watertemperaturen. Ongeveer 70 % van de

dikkopjes bleken minder dan 30 dagen voor hun vangst naar de Schelde geïmmigreerd te zijn. Dit duidt op een sterke temporele overlapping van immigratie en emigratie. Dikkopjes die het brakwatergebied introkken waren minstens twee maanden oud en 20 mm groot. De isotopenklok levert dus veel meer informatie op dan wat kan afgeleid worden uit de vangstgegevens alleen. Deze extra informatie is enorm bruikbaar om uitwisselingen tussen populaties te beschrijven.

De weerspiegeling van de actuele strontium/calcium concentratieverhouding ([Sr/Ca]) die gedurende de hele levensloop in otolieten wordt opgeslagen, vormt een veelgebruikte merker om de levensgeschiedenis van vissen over een saliniteitsgradiënt te reconstrueren. Dergelijke reconstructies vereisen in de eerste plaats informatie over de [Sr/Ca] wijziging in de omgeving langsheen een saliniteitsgradiënt, én in de tweede plaats een accurate kennis van de relatie tussen [Sr/Ca] in de omgeving en in de otolieten van de betrokken vissoort (***Hoofdstuk 5***). Hiertoe werden de veranderingen in [Sr/Ca] over de hele saliniteitsgradiënt in de Schelde in iedere seizoen vastgesteld. Verder werd er een experiment uitgevoerd naar Sr incorporatie in de otolieten van het dikkopje bij vijf verschillende zoutgehaltes (3, 7, 12, 20 and 30) en bij twee verschillende temperaturen (13 en 18°C). Een positieve maar niet-lineaire relatie tussen de saliniteit en [Sr/Ca] in het water werd vastgesteld. De experimentele resultaten toonden een positief lineair verband aan tussen [Sr/Ca] in het water en in de otolieten. De [Sr/Ca] in de otolieten was significant verschillend tussen ieder zoutgehalte, maar er werd geen temperatuurseffect waargenomen. Omwille van de individuele variabiliteit in de otoliet [Sr/Ca] heeft de gevonden regressie een vrij lage resolutie. Hierdoor kunnen slechts twee verschillende omgevingen onderscheiden worden, nl. marien water en water met een relatief laag zoutgehalte.

Het [Sr/Ca] patroon opgeslagen in otolieten van twaalf dikkopjes uit de Beneden-Zeeschelde werd geanalyseerd teneinde hun migratiepatroon in functie van de saliniteitsgradiënt op te stellen (***Hoofdstuk 6***). In deze groep grondels werden verschillende migratiepatronen geobserveerd. Deze variabiliteit in estuariumgebruik sluit aan bij onze resultaten bekomen met de isotopenklok. De individuen vertoonden niet alleen andere residentietijden in het brakwatergebied, maar ook de tijdstippen van immigratie vertoonden duidelijke verschillen. Bovendien suggereerden de resultaten heen- en weermigraties tussen het mondingsgebied (zee) en de Beneden-Zeeschelde. Hieruit blijkt dat de migratiepatronen van dikkopjes in estuaria veel complexer zijn dan tot nu toe werd aangenomen. Dit leidt tot de conclusie dat binnen de dikkopjespopulatie een grote flexibiliteit aanwezig is aangaande habitatgebruik doorheen de levenscyclus.

De geobserveerde variabiliteit in migratiepatronen suggereert dat de estuariene residentie van het dikkopje veeleer een gevolg is van ruilfuncties gemaakt op het niveau van het individu, dan wel een gevolg van een vast migratiepatroon dat geldt voor de hele populatie. De korte verblijftijden in het estuarium, die resulteren in een hoge wissel van individuen in het

brakwatergebied, stellen opnieuw de klassiek toebedeelde functionele rol van deze gebieden ernstig in vraag voor het dikkopje in het bijzonder maar in tweede instantie ook voor de andere betrokken mariene vissoorten. Onze resultaten lijken immers niet te passen in het algemene concept dat estuaria belangrijke gebieden zijn die mariene vissen gebruiken om vlotter op te groeien of om hun overlevingskansen te vergroten. Uit deze studie blijkt dat het dikkopje kan beschouwd worden als een mariene soort, die opportunistische gebruik maakt van estuaria, dan wel deze soort te klasseren als een estuariene resident. Het is duidelijk dat de achterliggende mechanismen die mariene vissen toelaten om gedurende een korte periode te profiteren van estuariene habitatten nog verder onderzocht dienen te worden. De rol bij het reduceren van de parasietenlading verdient hierbij zeker aandacht. Bovendien moeten de kwantitatieve bijdragen van estuariene migranten aan de adulte populaties op zee gekwantificeerd worden, zodat het ultieme belang van estuariene habitatten voor deze migrerende vissoorten, zoals het dikkopje, kan bepaald worden.

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