

ESTUARINE HABITAT USE BY A GOBY SPECIES: A GEOCHEMICAL APPROACH

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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 by individual fish on a spatio-temporal scale. 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 migration patterns of marine organisms. 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 present study focussed on the migration dynamics of sand goby *Pomatoschistus minutus* (Pallas, 1770) between the North Sea and the Scheldt Estuary (Belgium and The Netherlands). The sand goby is a small bottom-dwelling marine fish. As it is one of the most common species along the Atlantic European coast and its estuaries, it forms an important ecological link between benthic invertebrates and larger predatory fish such as cod and whiting. Like many other marine fish, *P. minutus* exhibits a typical pattern of occurrence in the low salinity zone of North Sea estuaries every year. We investigated this estuarine migration on the level of the individual fish using two biogeochemical tracers, namely stable carbon isotopes in dorsal muscle tissue (Ch. 4) and otolith [Sr/Ca] (Ch. 6). Prior to the application of both geochemical tracers in our study system, the techniques needed to be calibrated and the conditions to be verified (Ch. 2, 3 and 5).

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 (Ch. 2b). Stable isotope analyses on monthly gut contents demonstrated that the $\delta^{13}\text{C}$ value of sand goby prey items was on average 6‰ higher in the lower estuary than in the upper estuary. From June until November, $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ variability along the Belgian coast. Our data however, revealed that coastal $\delta^{13}\text{C}$ values were depleted relative to the offshore values. Coastal $\delta^{15}\text{N}$ values, on the other hand, increased considerably with increasing distance from the

estuary during summer and autumn, but an inshore-offshore $\delta^{15}\text{N}$ gradient was not detected. These results confirm that $\delta^{13}\text{C}$, in contrast to $\delta^{15}\text{N}$, is an appropriate tracer to study fish migration into the Scheldt Estuary.

Because we depended on sand goby gut contents for determining the isotopic difference between food sources in the upper and lower estuary (Ch. 2b), we performed an additional experiment towards the isotopic effects that might occur during digestion and assimilation (Ch. 2a). Gut contents of sand goby showed higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ between the food source and the foregut content.

Stable isotope studies on diet or migration of organisms 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 (Ch. 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, $\delta^{13}\text{C}$ in dorsal muscle tissue was identified as the most appropriate tracer to study sand goby immigration in the Scheldt Estuary.

Based on the results of chapter 2 and 3 an isotopic clock was developed to reconstruct the recruitment of sand gobies into the upper Scheldt Estuary over an entire year (Ch. 4). These results were combined with a growth model to yield age and length at immigration. Sand gobies entered the upper Scheldt Estuary almost continuously from May onwards, except in July when they appeared to avoid the estuary due to warm summer temperatures. About 70% of the fish caught 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) and two temperatures conditions (13 and 18°C) (Ch. 5). [Sr] acted conservatively in the Scheldt

Estuary, while [Ca] deviated slightly from a conservative trend; this resulted in a positive but nonlinear relationship between salinity and ambient [Sr/Ca] in the estuary. 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 of twelve sand gobies were eventually examined to chart their movements in the Scheldt Estuary (Ch. 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 indicates 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 it is the result of 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 might be found in other mechanisms such as parasite shake off. The possibility for marine fishes to briefly profit from estuarine areas definitely merits further research.

Chapters

CH. 1	Introduction
CH. 2A	Effect of digestion on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fish gut contents (Journal of Fish Biology 72: 301-309)
CH. 2B	Spatial variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of estuarine and coastal benthic consumers derived from sand goby gut contents and muscle tissue
CH. 3	Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in different tissues of juvenile sand goby <i>Pomatoschistus minutus</i> : a laboratory diet switch experiment (Marine Ecology Progress Series 341:205-215)
CH. 4	Estuarine recruitment of a marine goby reconstructed with an isotopic clock (Oecologia 157: 41-52)

- CH. 5 Relating otolith to water [Sr/Ca] ratios: experimental validation for sand goby *Pomatoschistus minutus*
(Submitted to Journal of Experimental Marine Biology and Ecology)
- CH. 6 Estuarine migration of sand goby *Pomatoschistus minutus* explored by means of otolith [Sr/Ca]
- CH. 7 General Discussion