Microchemical analyses of otoliths in Baltic Sea fish

-Possibilities and limitations of otolith elemental analysis to describe individual life history and stock characteristics of fish in the Baltic Sea-

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SUMMARY

In this thesis otolith microchemistry analyses were used to gain insights into the individual life history and stock characteristics of three fish species from the Baltic Sea - the European eel *Anguilla anguilla*, the Atlantic cod *Gadus morhua* and the thicklip grey mullet *Chelon labrosus*. The special hydrographic environment of the world's largest brackish water system provide promising conditions for the use of otolith elemental analysis to investigate individual migration patterns and stock structures of fish. Here, it was used to gain information with relevance for stock management of fish species that differ widely in their biology, ecology and stock structure.

In **chapter I** the influence of continental migratory behaviour on health and spawner quality of the European eel was analysed. Otolith strontium (Sr) composition was used to identify characteristic migration patterns. Results show that the muscle fat contents of silver eels with strictly catadromous life cycles are significantly reduced compared to silver eels that never entered freshwaters. Furthermore, prevalence and infection intensities of the swimbladder nematode *Anguillicoloides crassus* are highly increased in catadromous silver eels. Both, a reduced accumulation of fat reserves and intense *A. crassus* infestations are assumed to impair the successful reproduction of *A. anguilla*. These results highlight the importance of brackish water habitats for the production of high quality spawners and question the benefit of restocking measures into inland waters.

In order to investigate the influence of water temperature and feeding behaviour on the element incorporation into *A. anguilla* otoliths, two experimental studies were conducted (**Chapter II & III**). It was investigated whether experienced temperature history and individual food preferences can be reconstructed by otolith microchemistry analysis. It was further tested if temperature or diet affect the incorporation of Sr into otoliths to such a degree that the validity of otolith Sr based migration studies is impaired. Therefore, juvenile eels were reared for 15 weeks at three different water temperatures (14°C, 19°C, 24°C) and the elemental composition of the newly grown aragonite was subsequently analysed. Although the otolith composition differed significantly among treatments, detected differences were low and an *in situ* reconstruction of temperature history seems challenging. Similar results were obtained in the feeding experiment. Feeding of eight different diets during eight weeks caused no detectable differences in otolith elemental composition among treatments. Hence, the use of otolith microchemistry to determine individual food preferences of European eels seems to be unfeasible. However, the results presented indicate that neither temperature changes nor individual dietary behaviour impair the use of otolith Sr concentrations as a tracer of diadromy. Thus the reliability of such migration studies is fundamentally increased.

In **chapter IV** it was investigated whether multi-element otolith analysis can be used to discriminate Atlantic cod individuals according to their origin. Therefore, the concentrations of 16 elements were determined along the growth axis of otoliths from adult individuals caught at spawning grounds in the North Sea, the western Baltic Sea and the eastern Baltic Sea. Furthermore, the multi-element composition of the core region of juvenile cod otoliths from western and eastern Baltic areas was analysed. Multivariate statistical analyses successfully discriminated between adults from different stocks as well as between western and eastern Baltic Sea juveniles. Significant differences between the eastern Baltic spawning grounds, however, were not detected. These results demonstrate the potential of otolith microchemistry analysis to investigate the structure and connectivity of *G. morhua* stocks in the Baltic Sea.

In **chapter V** the migratory behaviour of thicklip grey mullet was examined for the first time on individuals caught in the Baltic Sea. By detecting *C. labrosus* otolith Sr concentrations, this study aimed to gain first insights into preferences and whereabouts of individuals in the Baltic Sea. Results confirmed a preference of brackish habitats for all analysed specimens and suggest a high plasticity of *C. labrosus* migratory behaviour. Some individuals seem to undertake annual migrations to marine waters, while others only sporadically left brackish waters. Although the behaviour of Baltic Sea *C. labrosus* could not be conclusively clarified, the results support the assumption that a considerable fraction of individuals entering the western Baltic Sea regularly migrate to the North Sea.

The results presented in this thesis confirm the feasibility of otolith microchemistry analyses for the investigation of a broad range of questions on Baltic Sea fish. Knowledge about investigated species was expanded and opportunities for future studies were indicated.

ZUSAMMENFASSUNG

In dieser Arbeit wurden anhand mikrochemischer Otolithenanalysen Erkenntnisse über die individuelle Lebensweise und die Bestandsstruktur von drei Fischarten aus der Ostsee gewonnen – dem Europäischen Aal *Anguilla anguilla*, dem Atlantischen Kabeljau (Dorsch) *Gadus morhua* und der Dicklippigen Meeräsche *Chelon labrosus*. Die hydrographischen Besonderheiten der Ostsee als weltgrößtes Brackwassersystem bieten vielversprechende Voraussetzungen für die Anwendung von Otolithen-Elementanalysen zur Untersuchung des individuellen Wanderverhaltens von Fischen und deren Bestandsstrukturen. Hier wurden sie genutzt, um managementrelevante Fragestellungen von Fischarten zu untersuchen, die sich hinsichtlich ihrer Biologie, Ökologie und Bestandsstruktur sehr unterscheiden.

In **Kapitel I** wurde der Einfluss des kontinentalen Wanderverhaltens auf die Gesundheit und die Laicherqualität des Europäischen Aals untersucht. Dazu wurden anhand der Otolithen-Strontiumverteilung charakteristische Wandermuster ermittelt. Die Ergebnisse zeigen, dass der Muskelfettgehalt von Blankaalen mit einem strikt katadromen Wanderverhalten signifikant geringer ist als der von Blankaalen, die niemals ins Süßwasser eingewandert sind. Darüber hinaus sind Prävalenz und Befallsintensität mit dem Schwimmblasen-Nematoden *Anguillicoloides crassus* bei katadromen Blankaalen stark erhöht. Sowohl eine verringerte Einlagerung von Fettreserven als auch der intensive Befall mit *A. crassus* stehen im Verdacht, den Reproduktionserfolg von *A. anguilla* zu vermindern. Die vorliegenden Ergebnisse heben die Bedeutung von Brackwasserhabitaten für die Produktion von gesunden Laichtieren hervor und stellen den Nutzen von Besatzmaßnahmen in Binnengewässer in Frage.

Um den Einfluss von Wassertemperatur und Futterverhalten auf die Elementeinlagerung in *A. anguilla*-Otolithen zu prüfen, wurden zwei experimentelle Studien durchgeführt (**Kapitel II & III**). Es wurde untersucht, ob erlebte Temperaturveränderungen und individuelle Futterpräferenzen anhand der im Otolithen gespeicherten Elemente rekonstruiert werden können. Ferner wurde geprüft, ob Temperatur oder Diät die Einlagerung von Strontium (Sr) in einem Maße beeinflussen, das die Aussagekraft von Otolithen-Sr basierten Migrationsstudien einschränken könnte. Dazu wurden juvenile Aale für 15 Wochen bei drei unterschiedlichen Temperaturen gehalten (14°C, 19°C, 24°C). Anschließend wurde die Elementzusammensetzung des hinzu gewachsenen Aragonits analysiert. Zwar wurden statistisch signifikante Unterschiede in der Elementzusammensetzung verschiedener Temperaturbehandlungen festgestellt, diese waren jedoch so gering, dass eine Rekonstruktion erlebter Temperaturänderungen *in situ* schwierig erscheint. Zu einem ähnlichen Ergebnis kam auch der Futterversuch. Die Fütterung von acht verschiedenen Diäten über einen Zeitraum von acht Wochen führte zu keinen statistisch

signifikanten Unterschieden in der Elementeinlagerung. Futterpräferenzen lassen sich demnach offenbar nicht anhand der Otolithen-Elementzusammensetzung nachverfolgen. Allerdings zeigen die vorliegenden Ergebnisse, dass beim Europäischen Aal weder Temperaturschwankungen noch unterschiedliches Futterverhalten die Verwendung von Sr zur Rekonstruktion diadromen Verhaltens einschränken. Damit wird die Aussagekraft solcher Migrationsstudien deutlich erhöht.

In **Kapitel IV** wurde untersucht, ob Otolithen-Multielementanalysen dazu geeignet sind, *G. morhua*-Individuen ihrer Herkunft zuzuordnen. Dazu wurden die Konzentrationen von 16 Elementen entlang der Wachstumsachse von Otolithen adulter Dorsch von Laichplätzen aus der Nordsee, der westlichen und der östlichen Ostsee untersucht. Des Weiteren wurde die Multielementzusammensetzung der Kernbereich von Otolithen juveniler Dorsche aus der westlichen und der östlichen Ostsee analysiert. Multivariate statistische Analysen konnten sowohl die adulten Bestände erfolgreich trennen, als auch die Juvenilen aus der westlichen und der östlichen Ostsee unterscheiden. Signifikante Unterschiede zwischen den drei östlichen Laichgebieten konnten aber nicht detektiert werden. Die Ergebnisse zeigen das Potential von Otolithen-Multielementanalysen zur Untersuchung der Struktur und Konnektivität von *G. morhua*-Beständen.

In **Kapitel V** wurde das Wanderverhalten der Dicklippigen Meeräsche *C. labrosus* erstmals an Individuen aus der Ostsee untersucht. Ziel der Studie war es, mittels der Analyse von Sr-Konzentrationen in *C. labrosus*-Otolithen grundsätzliche Informationen über Aufenthaltsorte und Habitatspräferenzen der Ostsee-Meeräschen zu erlangen. Die Ergebnisse bestätigen die Präferenz von Brackwasserhabitaten und lassen eine hohe Plastizität des Wanderverhaltens vermuten. Einige Individuen scheinen jährlich periodische Wanderungen in voll marine Gewässer zu unternehmen, während andere nur sporadisch das Brackwasser verlassen. Obwohl das Verhalten der Ostsee-Meeräschen nicht abschließend erklärt werden konnte, stützen die vorliegenden Ergebnisse die Vermutung, dass ein erheblicher Teil der in die Ostsee einwanderenden Individuen regelmäßig in die Nordsee zurückkehrt.

Die Ergebnisse dieser Arbeit bestätigen die gute Eignung mikrochemischer Otolithenanalysen zur Untersuchung unterschiedlichster Fragestellungen an Fischarten aus der Ostsee. Das Wissen über die untersuchten Arten wurde erweitert und neue Wege für zukünftige Studien wurden aufgezeigt.

CONTENT

GENERAL INTRODUCTION

Otoliths – function, composition and microchemistry applications

Otoliths, so called earstones, are calcified structures composed mostly of the calcium carbonate ($CaCO_3$) polymorph aragonite which is embedded in an organic matrix (Degens *et al.* 1969). They are located in the inner ear of all teleost fish and are major part of the hearing and equilibrium organ. Three pairs of otoliths (sagitta, lapillus, asteriscus) are located in compartments of the labyrinth system (saccule, utricule, lagena) where they are placed on sensory tissue, the macula (Figure 1). They are surrounded by an acellular medium, the endolymph, which is enclosed by the inner ear epithelium.

Reibisch (1899) was the first who discovered annual rings in otoliths. He distinguished between translucent winter bands, containing low amounts of $CaCO_3$ and a higher fraction of organic material, and opaque summer bands with high amounts of aragonite. This discovery enabled precise age determination of fish and made otolith age reading a central instrument in fisheries science (Figure 2). After Pannella (1971) revealed the existence of daily increments and therewith showed that otoliths are built by daily growth throughout the entire life of fish, the use of otoliths for scientific purposes emerged rapidly, especially in the field of stock characterisation and population dynamics. Inspired by the use of microchemical analyses in other biogenic carbonates like mollusc shells and corals, which were mostly related to palaeooceanographic questions (e.g. Dodd 1965; Weber 1973), first studies on the elemental composition in otoliths were conducted by Robert W. Gauldie, Adrienne Nathan, Richard L. Radtke and John M. Kalish. They investigated the heavy metal concentrations of Tarakihi (*Cheilodactylus macropterus*) (Gauldie & Nathan 1977) and published their pioneering studies on otolith chemistry of Painted notie (*Nototheni larseni*) (Radtke & Targett 1984), Mummichog (*Fundulus heteroclitus*) (Radtke 1989), Australian salmon (*Arripis trutta*) and Blue grenadier (*Macruronus novaezelandiae*) (Kalish 1989).

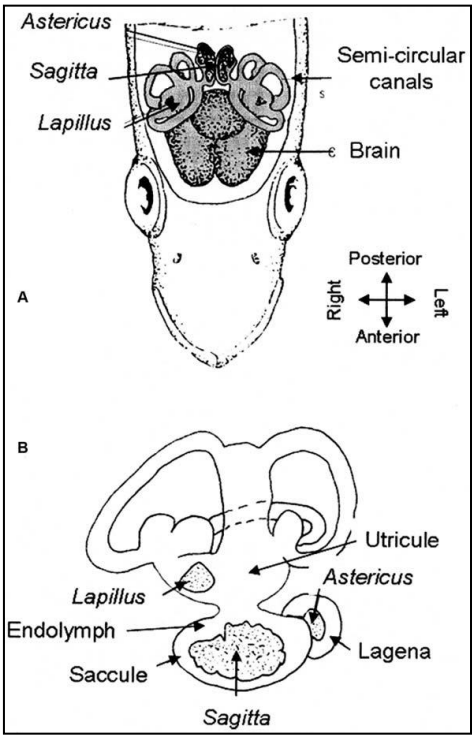

Figure 1: A-Dorsal view on the labyrinth organ. B- Labyrinth organ with otoliths (Source: Secor *et al.* 1992 - modified by Payan *et al.* 2004a)

Around 96% of the otolith is composed of $CaCO_3$, while another 3-4% is made of organic matrix. Less than 1% consists of minor elements (sodium (Na), strontium (Sr), potassium, sulphur, nitrogen, chlorine and phosphor) and a high number of trace elements (Campana 1999). Minor and trace elements are either incorporated into the $CaCO_3$ lattice as a substitute for calcium, with calcium (Ca) being replaced by other divalent ions like strontium, barium or magnesium. Alternatively they are incorporated into interstitial regions or crystal defects, where sodium, chlorine, zinc or potassium incorporation is increased. Such inclusions are rather weakly bound and might therefore leach out (Campana 1999; Elsdon *et al.* 2008).

Otoliths provide information that is achieved neither by tagging devices nor by population genetics or parasite community analysis. Life history information is stored throughout the entire life span of the fish from the larval phase, represented by the centre of the otolith, the primordium, until its death, represented by the outer edge of the otolith. Between these two points information about the entire lifespan is chronologically stored. Because otoliths are metabolically inert the quality of stored information is usually not affected by time (Campana & Neilson 1985).

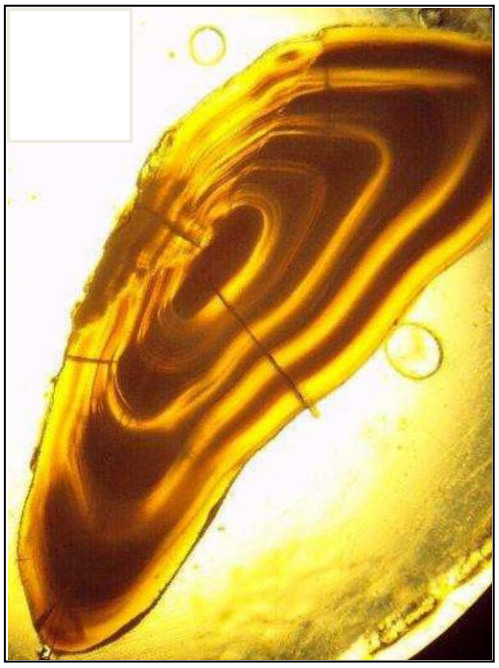

Figure 2: Opaque and translucent ring structures in a *Gadus morhua* otolith (Photo: F. Heidemann)

Accompanied by a fast improvement of analytical tools and a massive reduction of detection limits, a wide range of applications of otolith microchemical analyses was developed and applied to a variety of fish species. The incorporation of a number of minor and trace elements is affected by environmental and endogenous factors like e.g. water elemental composition, temperature, pollution, diet and growth rate (reviewed in Campana 1999). Concentrations of such elements are therefore widely used to reconstruct life history traits and migration patterns (e.g. Secor *et al.* 1995; Tzeng *et al.* 2000; Walther *et al.* 2011). Multi elemental approaches further provide so called elemental fingerprints that can help identify and separate stocks (e.g. Edmonds *et al.* 1991; Campana *et al.* 2000), assess the connectivity among populations (e.g. Gillanders 2002; Standish *et al.* 2008) and identify natal origins (e.g. Thorrold *et al.* 1998; Pangle *et al.* 2010). Element concentrations in otoliths are further used to indicate water pollution (e.g. Grady *et al.* 1989; Geffen *et al.* 1998,

2003) or physiological events like metamorphosis, stress or spawning (e.g. Arai *et al.* 2000; Kalish 1992; Fuiman & Hoff 1995).

Reconstruction of movements through different environments and the determination of migration strategies within a population

The reconstruction of movements through different environments is a well established application of otolith microchemistry analyses. Variations in water salinity are particularly well reflected by changes in otolith strontium/calcium (Sr/Ca) ratios (e.g. Secor *et al.* 1995; Secor & Rooker 2000), due to a five-fold increase of Sr/Ca ratios of marine waters compared to freshwaters (Campana 1999). This relation and the high otolith Sr concentrations, which make it comparably easy to detect and reduce analytical requirements, led to its successful use for the detection of diadromy (e.g. Kalish 1990; Tzeng *et al.* 1997). Beyond that, otolith element distribution along the growth axis was also used to detect movements through water bodies with minor salinity differences (e.g. migrations between spawning areas and estuarine nursery habitats (Thorrold *et al.* 1997)) or to reconstruct individual temperature history (e.g. Townsend *et al.* 1995).

It has to be considered that endogenous factors like feeding behaviour, growth or age might influence the incorporation of trace elements into otoliths (Campana 1999) and changing water properties at a single location could also cause element fluctuations, which can easily be confounded with active movements of the analysed specimen. It was also reported that freshwaters might have Sr/Ca ratios that exceed marine levels due to special geological conditions (Kraus & Secor 2004). In general, the precise reconstruction of life history traits requires the evaluation of all possible influences on the element incorporation into otoliths to ensure that water chemistry is truly reflected. Furthermore, huge interspecific differences make it necessary to assess all influences for the species of interest (reviewed in Campana 1999 and Elsdon & Gillanders 2003).

However, the determination of individual migratory behaviour allows the characterization of different migration strategies within a population. Although it is difficult to localize a fish at a certain time point, the differentiation between individuals according to their migration history reveals basic knowledge about the plasticity of migration strategies within a population and their importance for the spawning population. It further allows an evaluation of the importance of different habitats for a fish stock and can help to improve stock management.

Stock discrimination, connectivity among groups and the detection of natal origins

A major application of otolith microchemistry is the discrimination between stocks and the assessment of connectivity between populations. For this, the multi-elemental composition of otoliths has to be analysed and it is required that environmental conditions produce different chemical signatures in otoliths of individuals that spend times of their lifes in different environments. In other words: environmental conditions must leave characteristic site specific elemental fingerprints in otoliths. This is complicated by the fact that physicochemical properties of marine areas are rather unstable and unchanged conditions persist hardly longer than a few months (Campana 1999). Nevertheless, otolith multi-element approaches were frequently and successfully used to identify and discriminate fish stocks (reviewed in Thresher 1999). They allow determining the contribution of different stocks or sub-populations to a mixing adult population and provide important information for stock management and fishery regulations.

Multi-element otolith analyses are further used to identify natal origins and nursery areas of fish from freshwater and marine environments (e.g. Thorrold *et al.* 1998, 2001; Rooker *et al.* 2003; Warner *et al.* 2005). If environmental conditions at different spawning grounds or nursery areas result in different otolith element incorporation among recruits, elemental fingerprints can be used to assess the contribution of specific spawning grounds and cohorts to the adult stock and can help to quantify the mixing between populations.

The interpretation of elemental fingerprints is simplified by the fact that reasons for differing otolith element concentrations between sites not need to be identified. Hence, experimental work on environmental influences as required for migration studies is not necessary.

Beside the above mentioned variety of influencing factors, the high physiological regulation of ion transportation further complicates the interpretation of otolith elemental composition. Otoliths are completely surrounded by endolymph, whose element composition strongly deviates from water concentrations due to several barriers of element discrimination. The first barrier from water into blood plasma is via gills or intestine, with the gills being the major entry organ of most elements in freshwater fish, while the continuous drinking of marine fish leads to a high uptake via the intestine (Olsson *et al.* 1998). Campana (1999) identified these barriers as the most important for element discrimination due to their regulative function in osmoregulation. It was also reported that a minor fraction of elements originate from food (e.g. Limburg 1995; Buckel *et al.* 2004) but this is far less than the amount derived from water. Further element discrimination takes place at the barrier from blood plasma to the endolymph, which is secreted by the inner ear epithelia surrounding each otolith (Payan *et al.* 2004a). These

epithelia control the otolith composition by secreting the appropriate macromolecules for the organic matrix and providing the ionic environment for controlled mineralisation (Payan *et al.* 2004a). The fluid contains calcium, carbonate, bicarbonate, trace metals and proteins (Payan *et al.* 1997). Mechanisms and constraints of transport across the inner ear epithelia are rather unknown. Ionocytes seem to participate in endolymphatic pH regulation and ion transport (Shiao *et al.* 2005). Payan *et al*. (2004a) considered Ca^{2+} channels, Na^{+}/Ca^{2+} exchange and an ATP-dependent Ca^{2+} pump to participate in a transcellular Ca^{2+} transport. Other studies support a passive paracellular Ca^{2+} (Ibsch *et al.* 2004; Payan *et al.* 2002) and Sr^{2+} transport (Payan *et al.* 2002). The last barrier of discrimination is the crystallization process itself. Campana (1999) assumed this to be the main location of Sr discrimination. The calcification process depends on endolymph properties like pH and protein composition. If and to which amount minor and trace elements are incorporated into otoliths could be influenced by precipitation rate and by temperature sensitive partition coefficients (Campana 1999). The degree of discrimination varies widely among elements.

Suitable indicators of environmental conditions have to be carefully selected regarding their physiological regulation. Especially the intake of elements that are of high physiological importance like sodium, magnesium, phosphor, sulfur, clorine, potassium, calcium and copper are under osmoregulatory control to maintain blood concentrations that are required for physiological processes (Campana 1999). Other elements like lithium, manganese, iron, nickel, zinc, strontium, cadmium, barium or lead seem to be less regulated. Although plasma element/Ca concentrations are usually higher than otolith concentrations (Campana 1999) these elements are of particular interest for the use in life history studies.

The physiological regulation of the uptake of elements impedes the establishment of general conclusions from the impact of certain environmental or endogenous factors. Furthermore, their effect on otolith microchemistry seems to vary considerably between species. Among the factors influencing element incorporation into otoliths, elemental composition of the surrounding water, water temperature and interactions between these variables are assumed to have the highest impact. Nevertheless, factors of comparably little importance like diet, growth, age, pH or oxygen concentrations should never be neglected. The diversity of responses and interactions among species requires experimental work on the species of interest to reconstruct reliable life history traits.

Analytical methods

Various analytical methods are used to assess the elemental composition of biogenic carbonates. Among others, synchrotron X-ray fluorescence analysis (SYXRF) (e.g. Tsukamoto *et al.* 1998), solution based and laser ablation inductively coupled plasma mass spectrometry ((LA-) ICPMS) (e.g. Fowler *et al*. 1995; Campana *et al.* 1994), proton induced X-ray Emission (PIXE) (e.g. Elfman *et al.* 1999) and electron microprobe analysis (EMPA) (e.g. Limburg *et al.* 2003) were frequently applied in otolith science. They vary in spatial resolution and in detection limits and must be chosen according to the study objectives. In the here presented study LA-ICPMS and EMPA were used and shall be described in more detail.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS)

Inductively coupled plasma mass spectrometry provides a rapid simultaneous multi-element determination with detection limits at ng g^{-1} level. Prior to the use of laser technology ICPMS was used for analysis of solutions only. Materials were dissolved and sprayed into the ICPMS for elemental analysis. The application of lasers allowed a less destructive analysis of solids combined with a high spatial resolution (Figure 3). The focused laser radiation evaporates a microscopic amount of material which is transported by carrier gases (Helium and Argon) into the plasma ion source. The positively charged ions are further transported into the high vacuum of the mass spectrometer and detected according to their mass and charge.

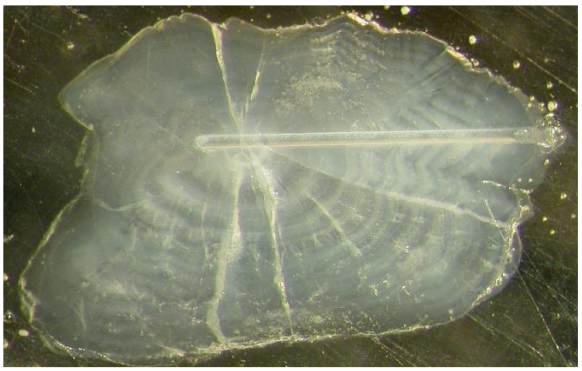

Figure 3: Polished *Anguilla anguilla* otolith with laser groove along the growth axis (Photo: K. Zumholz)

This technology is particularly suitable for the spatial analysis of element distributions on sample surfaces. Therefore, nowadays it is one of the most applied methods to detect the multi-element composition of fish otoliths. It is also used to follow life history traits along the growth axis and to reveal the element incorporation at certain life time points. Figure 4-A depicts Sr concentrations along transects from the core to the edge of three European eel (*Anguilla anguilla*) otoliths with different diadromous migration behaviours.

Electron microprobe analysis (EMPA)

Electron microprobe analysis allows the non-destructive detection of elements with concentrations above 100 µgg^{-1} at a very high spatial resolution of approximately 1 µm.

An electron beam of around 15kV is focussed on the surface of a solid sample, which re-emits element specific X-rays. These X-rays carry information in their wavelength and energy, which can be detected by either wavelength dispersive spectrometry (WDS; used in the present thesis) or energy dispersive spectrometry (EDS).

EMPA is well suited for a high spatial resolution analysis of highly concentrated elements along a transect, at a spot or on the entire surface of an object. Detailed surface maps allow outstanding insights into the element distribution within the object of interest. In otolith science this feature is often used to detect ring structures and to identify regions of differing concentrations (Figure 4-B).

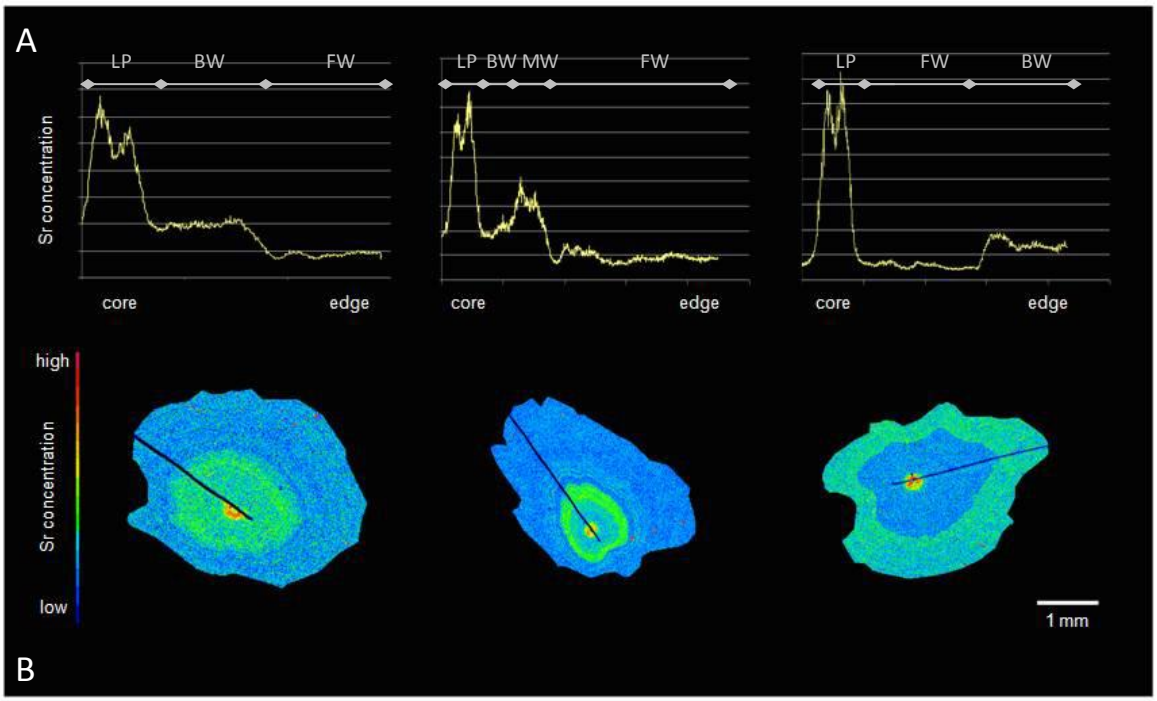

Figure 4: Strontium concentrations in three *Anguilla anguilla* otoliths detected by LA-ICPMS (A) and EMPA (B) showing different migratory patterns. LP = larval phase; FW = freshwater; BW = brackish water; MW = marine water

Investigated species – biology, gaps in knowledge and open questions

This thesis focuses on the otolith microchemistry of the European eel *Anguilla anguilla*, which was examined in two experimental and one field study. Further, the otolith composition of the Atlantic cod *Gadus morhua* and the thicklip grey mullet *Chelon labrosus* were analysed in two field studies. These three species differ widely in their life strategies, reproduction behaviours and distribution areas and provide a broad field to study the different applications of otolith microchemistry.

The European eel - *Anguilla anguilla* (Anguilliformes – Anguillidae)

One of the most impressive life cycles among fish species is undergone by the European eel (Figure 5). It includes two transatlantic journeys and a continental life phase covering a wide range of habitats. In 1923 the Danish marine biologist Johannes Schmidt discovered the Sargasso Sea as the putative spawning ground of *A. anguilla* (Schmidt 1923). Despite neither adult European eels nor their eggs were caught in the presumed area in the subtropical north-western Atlantic, repeated catches of eel larvae (leptocephali) reinforced Schmidt's hypothesis. Hatched leptocephali are assumed to drift with oceanic currents for about two to three years to the European and northern African coasts (Munk *et al.* 2010), where they distribute randomly. Although the European eel was historically often regarded as a textbook example for panmixia, some first genetic studies detected weak temporal differences (Dannewitz *et al.* 2005; Palm *et al.* 2009) without further identifying potential population structures. In the so far most comprehensive investigation, Als *et al.* (2011) recently found no evidence for genetic differentiation in eel larvae from the Sargasso Sea and glass eels from all over Europe and therefore strongly supported the panmixia hypothesis.

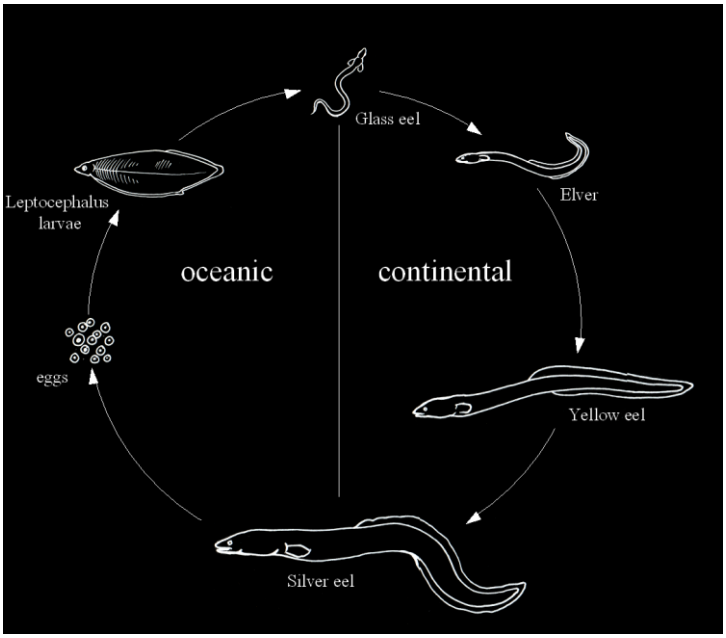

Figure 5: Lifecycle of the European eel (Source: Dekker 2000 - modified)

After arriving in continental waters during winter and spring leptocephali metamorphose into transparent glass eels and immigrate into coastal waters and estuaries where they become pigmented. In recent years a number of studies revealed a high plasticity of habitat choice and continental migratory behaviour of European eels (Limburg *et al.* 2003; Daverat *et al.* 2006; Tzeng *et al.* 2000). The traditional point of view of obligatory catadromous behaviour was refuted by Tsukamoto *et al.* (1998), who caught migrating silver eels in the North Sea which had never entered freshwater during their life.

The duration of the continental phase depends on growth rate rather than on age (Svedäng *et al.* 1996) and varies widely between habitats and sexes, with males leaving continental waters earlier and with a shorter body length than females (males: 2-15 years, 29-54 cm; females 4-20

years, 46-100 cm (Tesch 1999)). Prior to migration, *A. anguilla* transform into so called silver eels accompanied by extensive morphological changes like the enlargement of eyes and pectoral fins, a whitening of the belly and the reduction of the alimentary tract (Pankhurst 1982; Pankhurst & Sorensen 1984; Durif *et al.* 2005), which are preparatory adaptations to the oceanic environment and maturation. Larsson *et al.* (1990) assumed the beginning of the spawning migration to be triggered by the amount of stored energy reserves (minimum = 28% muscle fat content), while Svedäng *et al.* (1997) caught silver eels with lower fat contents and concluded that maturing eels are able to interrupt spawning migration and resume feeding. However, fat stores of silver eels that leave continental waters have to suffice for a transoceanic migration of about six months to the Sargasso Sea (Palstra & van den Thillart 2010) and are therefore a fundamental requirement of migrating silver eels to complete their semelparous life cycle.

The European eel stock has been dramatically declining since the 1970's. Current recruitment of glass eels is around 1% of former levels and catches are decreasing (ICES 2010a) (Figure 6). Consequently, *A. anguilla* is listed in the International Union for Conservation of Nature (IUCN) Red list as "critically endangered" and in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). This decline appears to be caused by a combination of overexploitation and various other anthropogenic as well as climatic and oceanographic impacts. Great effort has been invested during recent years to identify and quantify possible reasons including continental and oceanic issues.

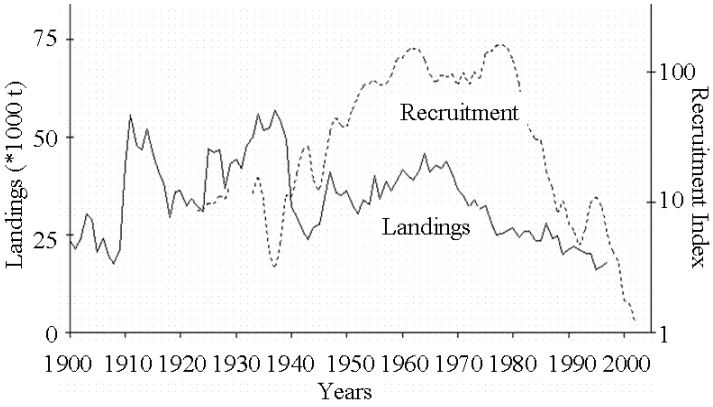

Figure 6: Landing and recruitment of *A. anguilla* during the 20th century (Source: ICES)

- The massive construction of hydropower stations and dams and the reclamation of wetlands led to habitat loss and high mortalities. Obstacles impair or inhibit the access of yellow eels into rivers and lakes, while conversely turbines cause massive injuries and mortalities during downstream migration of silver eels (Feunteun 2002).
- Numerous industrial pollutants were introduced into inland waters during the last decades among which several are known to impair reproduction success and harm embryonic development of eels (e.g. polychlorinated biphenyls (PCBs), dioxins, pesticides, heavy metals) (reviewed in Robinet & Feunteun 2002). Among these,

lipophilic compounds that interfere with reproduction and egg survival, like PCBs, were reported to be especially harmful (EELREP 2005; Palstra *et al.* 2006). The high muscle fat content of *A. anguilla* and its semelparous lifecycle lead to high body concentrations of such contaminants. They accumulate in fat stores, which are metabolized during the spawning migration, resulting in high pollutant blood concentrations during this crucial life phase (Robinet & Feunteun 2002). Besides affecting reproduction and embryonic development, several compounds disturb lipogenesis resulting in reduced fat accumulation and consequently in reduced long-term swimming performance (Belpaire *et al.* 2009).

- The introduction of the swimbladder nematode *Anguillicoloides crassus* from Asia to European freshwaters in the early 1980's further deteriorated health of *A. anguilla* (Kirk 2003). The parasite inhabits swimbladders of European eels in much higher densities than in Japanese eels due to a lack of adaptations for resistance. This causes a variety of pathological effects in addition to seriously affected swimbladder functions (Kirk 2003; Kennedy 2007). During their spawning migration eels undergo dial vertical migrations during which an efficient buoyancy control is essential (Aarestrup *et al.* 2009). Swimming efficiency of highly infected eels was shown to be reduced and energy demand increased by 20% (Palstra *et al.* 2007). Though usually not a direct cause for mortality, *A. crassus* is thought to severely affect eel reproduction by reducing the ability of long-term migration.
- *Herpesvirus anguillae* (HVA) and Eel Virus European X (EVEX) are also considered serious threats to European eels (van Ginneken *et al* 2005; Davidse *et al.* 1999). Infections can cause pathological alterations and reduce stress tolerance and migration abilities (Haenen *et al.* 2009; van Ginneken *et al.* 2005).
- The increased predation pressure through the Great cormorant *Phalacrocorax carbo* is assumed to considerably affect the European eel stock size (Brämick & Fladung 2006). However, the lack of reliable studies complicates the quantification of eel consumption through cormorants and a severe impact of the opportunistic feeding cormorant on the eel stock at today's low stock size is not clarified.
- Beside these continental threats, oceanic and climatic aspects mainly caused by global warming are also discussed to negatively affect *A. anguilla* recruitment. Changes in the North Atlantic Oscillation Index (Knights 2003, Durif *et al.* 2011), ocean-atmospheric changes (Friedland *et al.* 2007) and rising water temperatures in the Sargasso Sea

(Bonhommeau *et al.* 2008a; Durif *et al.* 2011) were shown to correlate with low glass eel recruitment.

It has to be considered that a combination of the above mentioned factors together with high fishing pressure is responsible for the eel stock decline. *A. anguilla* is exploited at all continental life stages from glass eel to silver eel and is the target of usually small scaled coastal and inland fisheries. Glass eel fisheries are limited to the coasts of Morocco, Portugal, Spain, France, the United Kingdom, Ireland and Italy (ICES 2006; Dekker 2003). They are caught for direct consumption and as supply for eel farms and restocking measures. Yellow eels are fished over almost the entire distribution area, while silver eel fisheries are predominant in northern areas (Dekker 2003). Knowledge of the stock size is lacking and the complex lifecycle complicates the implementation of measures for its recovery.

In 2007, the European Union developed a regulation that obligated member states to prepare national management plans at river basin level that, amongst others, take measures to allow a silver eel escapement of 40% of pristine levels (EC 2007). The appropriate measures specified are the reduction of fisheries, the reconstruction and elimination of river obstacles, the catch and transport of silver eels from enclosed inland waters to the sea and the restocking of young eels into suitable inland waters. Although of fundamental importance for the management plans of several EU states, the sustainable impact of restocking measures is questionable. Beside the deteriorating effects of low habitat quality on spawner quality (e.g. Feunteun 2002; ICES 2008; Belpaire *et al.* 2009) it is yet not clarified if *A. anguilla* requires an imprinted olfaction clue for orientation. Westin (1990, 2003) assumes that eels restocked into tributaries of the Baltic Sea will not find the way to the Atlantic Sea because of a lack of imprinting. In addition, it is debated whether translocation of eels enhances the spread of diseases and parasites and reduces genetic variability (ICES 2008).

The Atlantic cod - *Gadus morhua* (Gadiformes – Gadidae)

The Atlantic cod is widely distributed along the northeast and northwest Atlantic shelf. It is found from North America, Greenland and Iceland to Norway, the North Sea and the Baltic Sea (Cohen *et al.* 1990). The economically most important stocks are the Arcto-Norwegian stock and the Icelandic stock, while most of the stocks in the northwest Atlantic collapsed due to overexploitation in the early 1990's (Hutchings & Myers 1994).

Traditionally, the Baltic Sea cod is divided into two separately managed stocks which are distributed to the west (*Gadus morhua morhua*) and to the east (*Gadus morhua callarias*) of Bornholm Island (ICES 2007). Spawning of these stocks is separated by space and time. While

main spawning in the western Baltic takes place during March and April along a widespread area in the western Baltic, the eastern stock spawns mostly from May to July in the eastern Baltic Basins (Bornholm Basin, Gdansk Deep and Gotland Basin) (Bleil *et al.* 2009; Wieland *et al.* 2000) (Figure 7). It is assumed that some mixing between both stocks occurs in the Arkona Basin (reviewed in Hüssy 2011).

Gadus morhua is a traditional target of the Baltic Sea fishery and has suffered from strong fishing pressure during the last decades. The eastern Baltic cod spawning stock declined strongly from approximately 665,000 t in 1982/83 to constant levels below 100,000 t between 2002 and 2006 (ICES 2007). Besides high fishing pressure the decline is thought to be potentiated by bad spawning conditions in the eastern Baltic due to a reduction of inflow events of saline oxygen rich North Sea water and high eutrophication (Bagge & Thurow 1994; Köster et al. 2005). Almost any recruitment from Gdansk Deep and Gotland Basin is impeded by a lack of oxygen in the deep layers of these Basins where spawning takes place (Köster & Möllmann 2000). The stock size is further influenced by the abundance of Baltic sprat (*Sprattus sprattus*). *S. sprattus* feeds on cod eggs and high abundances negatively affect cod recruitment (Köster & Möllmann 2000). A combination of bad spawning conditions, high fishing pressure and high spat abundances in the 1990's (Bagge *et al.* 1994; Köster *et al.* 2003) has reduced the Baltic cod stock to current low levels. However, between 2005 and 2009 the spawning stock biomass increased again to 350,000 t (ICES 2010c), which is assumed to be the consequence of a drastic reduction of fishing pressure and points out fishery as the main driving force for Baltic stock population dynamics (Cardinale & Svedang 2011).

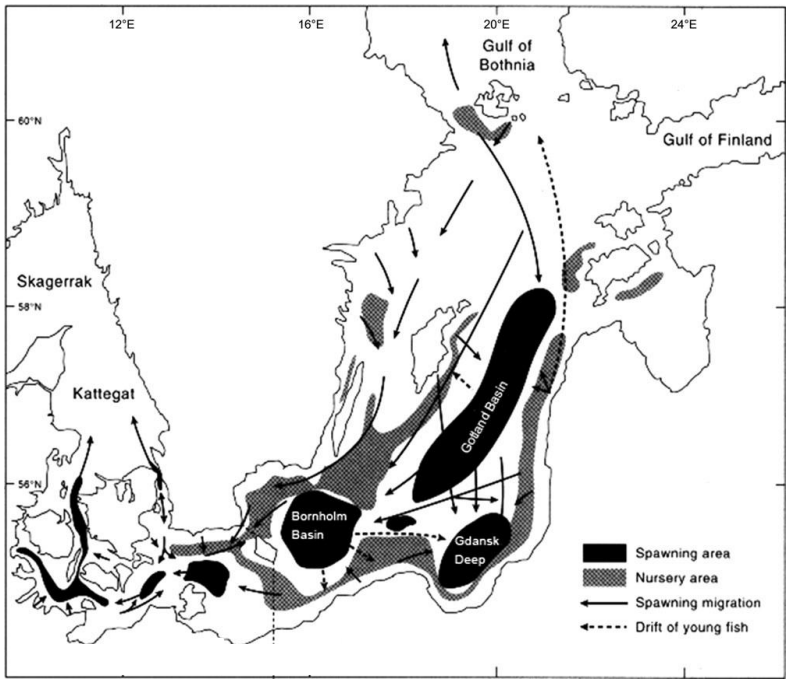

Figure 7: *Gadus morhua* spawning areas in the Baltic Sea (Source: Bagge *et al.* 1994)

To optimize stock management within the Baltic Sea and to allow a sustainable fishery at a maximum yield it is of fundamental importance to separate between stocks, to quantify stock

exchange and to evaluate the importance of the different spawning grounds for recruitment. In addition, little is known yet about natal homing behaviour and spawning site fidelity within the Baltic Sea.

The thicklip grey mullet - *Chelon labrosus* (Mugiliformes – Mugilidae)

The thicklip grey mullet is distributed along the eastern Atlantic coasts from Senegal and Cape Verde to Norway and in the Mediterranean Sea (Muus & Nielsen 1999). This highly euryhaline species spawns during winter at sea and inhabits a wide range of water salinities (McDowell 1988).

Since the 1970´s observations of *C. labrosus* in the western Baltic Sea are increasing (Meixner 1978; Mohr 1986). It is caught from May to October as bycatch in gillnet and trawl fisheries and by recreational fisheries (BLE 2009). Reasons for increasing abundances of *C. labrosus* in the western Baltic Sea seem to be diverse, reaching from increasing food availability and high stock numbers in adjacent waters to raising water temperatures (Mohr & Horn 1977; Mohr 1986; Vorberg *et al.* 2005). A growing importance of *C. labrosus* as an additional target for the western Baltic Sea fisheries can therefore be expected.

So far, nothing is known about the origin and migratory behaviour of specimens in the Baltic Sea and their whereabouts during winter. Such information is of high importance to successfully and sustainably manage the Baltic Sea *C. labrosus*. The identification of spawning grounds and migration routes can help to understand stock structures and habitat preferences of *C. labrosus* in the Baltic Sea and discover connectivity of populations to provide scientific advices for a sustainable fishery.

AIM AND OUTLINE OF THIS THESIS

The Baltic Sea is an important habitat for many euryhaline fish species. During the last decades anthropogenic influences strongly modified this unique brackish water system. High fishing pressure, increased eutrophication and the introduction of non native species have altered the assemblage and distribution of fish communities (Thiel *et al.* 1996). Because of the wide range of water salinities in the Baltic Sea and the strong influence of river outflows from different geological origins on the water chemistry of certain areas (Andersson *et al.* 1992), otolith elemental analysis provides a promising tool to investigate ecological and biological questions of fish species in this unstable environment.

Using three species of fish found in the Baltic Sea with very different life history characteristics different applications of otolith microchemistry were examined. In **chapter I**, it was investigated whether migration strategy and habitat choice of *A. anguilla* have an impact on its condition and reproductive capacity in order to evaluate the importance of certain ecophenotypes and the contribution of brackish environments to a healthy development of eels. To test the traceability of temperature history and feeding behaviour and to validate the findings from chapter I, the influence of both factors on otolith microchemistry was experimentally tested (**Chapter II & III**). In **Chapter IV** the connectivity of Baltic Sea cod stocks was analysed in a multi-element approach and **Chapter V** deals with the distribution and whereabouts of thicklip grey mullets in the western Baltic Sea, a summer guest, whose invasion is facilitated by current hydrographic conditions and which might become increasingly important for the Baltic Sea ecosystem.

Chapter I

Released into a perilous environment? - The dilemma of mass-restocking of eels to freshwaters

This study aimed at assessing the influence of continental migratory behaviour and habitat choice on the condition and reproduction capacity of the European eel. Diadromous migration behaviour was analysed by LA-ICPMS and specimens were classified accordingly. Individuals with different migration strategies were tested for variations in fat contents, *A. crassus* infection levels and condition factors. Our results help evaluate the importance of inland and coastal habitats for the production of high quality spawners and question restocking as an appropriate measure for stock recovery.

Chapter II

Temperature dependency of element incorporation into European eel (*Anguilla anguilla*) otoliths

Temperature is known to be a major factor influencing otolith elemental composition of several fish species. In this experimental approach it was investigated whether and to which amount water temperature influences the multi-element composition of European eel otoliths. Traceable effects would allow the reconstruction of experienced temperature history and provide insights into the behaviour of this endangered species. The experiment was aimed to further clarify whether influences of water temperature on Sr incorporation might cause misinterpretations of migration studies based on otolith microchemistry.

Chapter III

Dietary effects on multi-element composition of European eel (*Anguilla anguilla*) otoliths

In order to quantify the influence of diet on otolith microchemistry of eels and to evaluate whether feeding behaviour could influence the outcome of migration studies, the influence of different diets on the multi-element incorporation into *A. anguilla* otoliths was experimentally tested. Beside the validation of migration studies it should further been investigated whether dietary behaviour is reflected by otolith microchemistry and could be used to reveal individual food preferences.

Chapter IV

Evaluating the suitability of otolith microchemistry for stock separation of Baltic cod (Gadus morhua)

This multi-element approach aimed at the detection of characteristic elemental fingerprints in otoliths of *G. morhua* from different areas and spawning grounds. LA-ICPMS was used to analyse the multi-elemental composition of cod otoliths from the North Sea, the western Baltic Sea and from three eastern Baltic Sea spawning grounds. Furthermore, the core regions of otoliths from young-of-the-year cod from spawning grounds in the western and the eastern Baltic Sea were analysed. Results should help identify the origin of individual Baltic Sea cod and assess the connectivity between stocks and spawning grounds.

Chapter V

Newcomers in the Baltic Sea: an attempt to trace the origins and whereabouts of thicklip grey mullet *Chelon labrosus*

Chelon labrosus is a new summer guest in the Baltic Sea. Little is known about its whereabouts during winter and its migration routes. In the field study presented, otolith elemental analysis was performed for the first time on otoliths from thicklip grey mullets caught in the Baltic Sea. By analysing Sr concentrations along the otolith's growth axis it was aimed to reconstruct the migration routes of *C. labrosus* through waters of different salinities and to gain knowledge about its habitat preferences and annual migrations patterns.

CHAPTER I Released into a perilous environment? – The dilemma of mass restocking of eels to freshwaters

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Abstract

European eel (*Anguilla anguilla*) recruitment is declining dramatically since the 1970´s. The causes for this decline are ambiguously explained either by a variety of anthropogenic impacts during the continental phase or by environmental changes basically affecting oceanic larval stages as well as a combination of both. Recently, European Union member states elaborated management plans for a recovery of the stock. The translocation of juvenile eels for restocking purposes into suitable inland waters is therein regarded as one of several appropriate management tools. However, scientifically accepted site-selection criteria for restocking programs are lacking and habitat quality continues to be neglected as a basic requirement for reasonable restocking measures. The present study tested the influence of migration behaviour and habitat choice on the condition of European eels. Individual migration history was examined by otolith microchemistry analysis using strontium as a proxy for water salinity. Our study showed that individuals that exclusively inhabited freshwaters have significantly lower muscle fat contents and are more seriously infected with the introduced swimbladder nematode *Anguillicoloides crassus* than eels that never entered freshwaters. Since low *A. crassus* infection intensities and high fat contents are considered as prerequisites for a successful transoceanic spawning migration of eels, the translocation of eels from coastal habitats into more perilous inland waters might impair reproductive success and further deteriorate the stock situation.

Keywords: *Anguilla anguilla*, habitat quality, restocking, otolith microchemistry, fat content, *Anguillicoloides crassus*

Introduction

The European eel (*Anguilla anguilla*) stock has experienced a sharp decline. Recruitment of glass eels dropped to historically low levels of 1% to 7% of the average values of the 1960s and 1970s (ICES 2010a). Reasons for this decline appear to be diverse. Beside exploitation, the loss of habitats and increased mortality due to river obstacles (ICES 2006) as well as possible climatic and oceanic changes (Knights 2003; Friedland *et al.* 2007; Bonhommeau *et al.* 2008b; Durif *et al.* 2011), the discussion is currently focusing on habitat and spawner quality as possible major influences (Belpaire *et al*. 2009; Geeraerts & Belpaire 2010; Clevestam *et al.* 2011). Due to its complex life cycle *A. anguilla* is specifically vulnerable to environmental changes that potentially impair its ability for long-distance migration, a prerequisite for successful reproduction. To reach its spawning area in the Sargasso Sea (Schmidt 1923), mature *A. anguilla* have to migrate distances of 5000 up to 7000 km, known as the longest spawning migration within the genus *Anguilla* (Aoyama 2009) and estimated to last between 3.5 and 6 months of continuous swimming (Palstra & van den Thillart 2010). To successfully spawn after such a long journey excellent health conditions and a good swimming performance are required, which were shown to be severely impaired by a variety of environmental factors like contaminant loads (Palstra *et al.* 2006; van Ginneken *et al.* 2009; Geeraerts & Belpaire 2010), infection with the introduced swimbladder nematode *Anguillicoloides crassus* (Kirk 2003; Palstra *et al.* 2007; Clevestam *et al.* 2011) and a lack of energy resources (Svedäng & Wickström 1997).

A crucial factor for spawning success is the accumulation of fat reserves. During spawning migration *A. anguilla* ceases feeding (e.g. Durif *et al.* 2005), hence it exclusively depends on energy reserves stored during the continental life phase. Energy reserves of silver eels comprise up to 80% of fat (Boëtius & Boëtius 1985) resulting in total body fat concentrations of up to 40% (Tesch 1999; Andersson *et al.* 1991). Fat mobilization starts with silvering and provides the energy for routine metabolism, swimming activity and maturation, which is completed during migration. The minimum fat content required for successful spawning was examined by several authors; however proposed numbers differ between studies. Van den Thillart *et al.* (2007) determined around 20% body fat as minimum energy requirement for the completion of migration and successful reproduction, whereas Palstra *et al.* (2007) suggest 13.5% to be sufficient, while Larsson *et al.* (1990) conclude that a minimum of 28% in yellow eels is necessary to initiate silvering. Despite this diverging information the importance of an undisturbed fat accumulation for spawning success is undisputed.

Beside high contaminant loads, the quality of freshwater habitats is further deteriorated by parasite pressure (Jakob *et al.* 2009a). Within the diverse parasite community in eels of

European inland waters, the swimbladder nematode *A. crassus* is especially harmful. Introduced from Asia in the early 1980´s as a result of uncontrolled intercontinental transfer of life eels (Køie 1991), *A. crassus* successfully spread all over Europe (reviewed in Kirk 2003 and Jakob *et al.* 2009b). Highly infected swimbladders have various dysfunctions (reviewed in Kirk 2003). Additionally, an increase of energy costs during swimming was observed causing a loss of long-term swimming ability (Sprengel & Lüchtenberg 1991; Palstra *et al.* 2007).

During recent years the use of otolith microchemistry delivered new insights into the continental life phase of *A. anguilla*. It was shown that its life cycle is not obligatory catadromous, but that a significant number of individuals never enters freshwaters or repeatedly shifts between freshwater and saline waters (e.g. Tzeng *et al.* 2000; Limburg *et al.* 2003; Daverat *et al.* 2006). Tsukamoto *et al.* (1998) caught migrating silver eels in the North Sea that obviously lived in coastal waters during their entire growth phase without entering freshwaters. The authors concluded that individuals from freshwater do not participate in reproduction, a hypothesis which was later on refuted by Limburg *et al.* (2003). Regardless of possible predetermined habitat preferences of eels (Côté *et al.* 2009) and often unclear or doubtful habitat quality of inland waters, stocking intensity of eels into freshwater remains high (ICES 2010a) and is often even claimed as the only suitable measure to sustain and recover the European eel stock.

To efficiently and sustainably manage the European eel it is of high importance to validate the contribution of different habitats to the spawning stock. In the present study we examined the impact of individual migratory behaviour on the condition of *A. anguilla* in order to determine the influence of the migration strategy on spawner quality. We analysed the otolith strontium (Sr) concentrations of individuals from 11 sampling stations in Germany, Denmark and Finland, covering marine, brackish and freshwater habitats. Microchemical otolith analysis was carried out by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) and delivered individual migration patterns for all examined eels, which were classified accordingly. As indicators for spawner quality we chose muscle fat content, infection with *A. crassus*, gonadosomatic index (GSI), hepatosomatic index (HIS) and Fulton's condition factor.

Otolith Sr profiles were further used to identify potentially restocked individuals, in order to roughly estimate the possible influence of stocking on our results.

Materials and Methods

Sampling and dissection

287 *A. anguilla* were sampled at 11 stations in the Baltic and North Sea proper or their tributaries (Figure I-1). All eels except those from Helgoland Island, river Eider and river Oder

were caught by commercial fishermen either by fyke nets or stow nets. Sampling details are listed in table I-1. In total 104 individuals were caught in freshwater, 140 in brackish water and 43 in marine waters. Immediately after catch eels were frozen at -40°C until further examination.

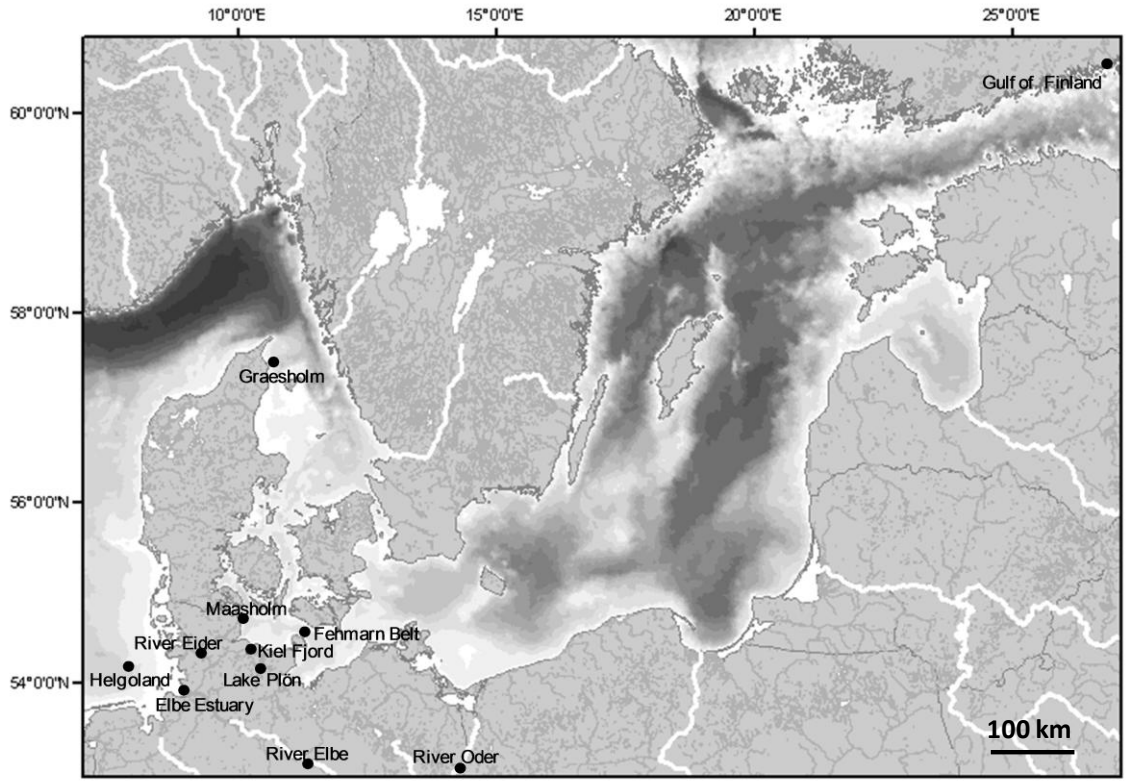

Figure I-1: Sampling stations

After thawing, total length (L_T) and body mass (M) were measured to the nearest mm and g, respectively. Pectoral fin length and eye diameter were measured to the nearest 0.1 mm to classify eels into six maturation stages according to Durif *et al.* (2009) (undifferentiated growth phase SI, female growth phase SFII, female premigrant stage SFIII, female migrant stages SFIV and SFV and male migrant stage SMII). Swimbladders were removed for assessing *A. crassus* infestation. Fulton's condition factor was calculated ($K=10^5\ ML_T^{-3}$) and gonad (M_G) and liver (M_L) were weighted to the nearest 0.01 g to determine gonadosomatic ($I_G=100\ M_G\ M^{-1}$) and hapatosomatic indices ($I_L=100\ M_L\ M^{-1}$). Sagittal otoliths were extracted and stored dry for microchemical analyses.

Table I-1: Sampling details and conducted analyses

station	Lake Plön	River Eider	River Elbe	River Oder	Gulf of Finland	Fehmarn Belt	Kiel Fjord	Maasholm	Elbe Estuary	Graesholm	Helgoland
water	freswater	freswater	freswater	freswater	brackish	brackish	brackish	brackish	brackish	marine	marine
N	25	23	30	26	27	29	25	29	30	27	16
fishing gear	fyke net	electrofishing	stow net	electrofishing	fiyke net	fyke net	fyke net	fyke net	stow net	fyke net	fyke net
fat contant	yes	yes	no	yes	yes	yes	no	no	yes	yes	yes
A. crassus	yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes
Fulton's cf	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
GSI	yes	yes	no	no	yes	yes	yes	yes	yes	yes	yes
HSI	yes	yes	yes	no	yes	yes	yes	yes	yes	yes	yes

Microchemical otolith analysis

Sagittal otoliths were extracted, rinsed with distilled water and stored dry in 1.5 ml plastic vials (Eppendorf; Hamburg, Germany). They were embedded in thermo epoxy (Buehler; Düsseldorf, Germany) on glass slides and polished from the proximal side, using lapping film of 30, 12 and 3 µm (3M; Neuss, Germany) until the core was exposed.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) was performed with a spot size of 75 µm along the growth axis from the otolith core to its anterior edge with a scan speed of 3 μms^{-1} using a UP193 solid-state laser (New Wave Research; Fremont, USA) coupled to a Finnigan Element2™ (Thermo; Waltham, USA). Irradiance and pulse rate were set to approximately 1 GW cm^{-2} and 10 Hz, respectively. In order to clean the otolith surface, transects were preablated prior to measurement (spot size: 120 µm, scan speed: 100 μms^{-1}). NIST612 was measured as external calibration standard following every second transect. For quantification GeoPro™ software was used. Further details of measurement procedure and quantification of element concentrations are found in Marohn *et al.* (2009). For concentrations above 0.5-1 μgg^{-1} a precision of better than 3% is indicated by our data and for concentrations below 0.01 and above 13% for 0.5 μgg^{-1}.

Individual movements between waters of differing salinities were reconstructed based on measured Sr/calcium ratios. As reported by Marohn *et al.* (2011, 2009) the influence of water temperature and dietary behaviour on Sr/Ca ratios in *A. anguilla* otoliths is marginal. Therefore, potential fluctuations of these factors will not lead to misinterpretations of migration studies based on otolith Sr/Ca ratios. Sr/Ca ratios below 1 mmol mol^{-1} indicate freshwater conditions, whereas Sr/Ca ratios up to 2.7 mmol mol^{-1} are incorporated in brackish waters and values exceeding 2.7 mmol mol^{-1} are regarded to reflect fully marine waters (Tzeng *et al.* 1997; Daverat *et al.* 2006; Shiao *et al.* 2006; Limburg *et al.* 2003). According to their otolith Sr profiles specimens were classified into five groups, representing different migration types. Freshwater residents were defined to ascend or to be anthropogenically transferred to freshwaters directly after they reach coastal waters. They remain in freshwaters until they leave for reproduction. Brackish and marine residents in contrary do never enter freshwater during their entire life span.

The remaining three groups contain individuals that inhabit waters of different salinities during their continental life phase. Upstream shifter spend a significant amount of time in coastal waters before they enter freshwater, which they do not leave again until spawning migration, while downstream shifter stay in saline waters after an initial freshwater phase. Individuals changing more than once between freshwaters and saline waters were defined as interhabitat shifter.

Natural movement patterns in European eels are presumed to be substantially masked by anthropogenic restocking activities. To obtain a rough estimation of the resulting systematic bias due to restocking, Sr profiles were checked for the presence of brackish water signals to reflect estuarine passages of elvers at least for Baltic Sea tributaries. Since it may take up to 20 days in a new environment before the corresponding Sr signal is fully reflected in otoliths (Elsdon & Gillanders 2005a), differentiation of stocked versus naturally ascended eels is not feasible for North Sea tributaries.

Sagittal otoliths of eels might partially consist of vaterite instead of aragonite. The element incorporation into these calcium carbonate polymorphs deviates from each other and might lead to misinterpretations of migration pathways. In order to prevent the use of measurements in vaterite structures Sr, sodium, barium, magnesium and manganese concentrations were checked for characteristic values reported for vaterite (Tzeng *et al.* 2007).

Fat determination

Muscle fat content of 196 eels from eight locations was analysed (Table I-1). Bone free white muscle tissue was taken from a cross section anterior to the anus and frozen at -40°C. After thawing, the tissue was homogenised using a hand blender (Waring; Torrington, USA). 36 ml of a isopropanol:cyclohexan (16:20) solution (Carl Roth; Karlsruhe, Germany) was added to 5 g of the muscle homogenate and mixed in an ultra turrax (IKA; Staufen, Germany) for 2 minutes. Thereafter 20 ml of distilled water was added and the solution was centrifuged for 5 min at 2000 rpm. The organic phase was filtered through washed cotton in order to remove tissue particles. 20 ml of 2-propanol:cyclohehan (13%) (Carl Roth; Karlsruhe, Germany) was added to the aqueous phase and homogenised in the ultra turrax for 1 min and centrifuged at 2000 rpm for another 5 min. The organic phase was removed again and added to the supernatant of the first centrifuge process. For the drying process the solution was first put into a rotary evaporator (IKA; Staufen, Germany) (51°C, 234 mbar) and thereafter into a round bottomed flaskfor 1 h at 105°C. It was finally cooled in a desiccator and weighted.

$$\text{fat content (\% of muscle)} = \frac{end\ weight}{start\ weight}\ x\ 100$$

Parasitological examination

Swimbladders of 261 eels from 10 stations were examined for *A. crassus* infestation. Detailed information about the parasitological examination is given in Jakob *et al.* (2009a).

Statistical analyses

To test for differences in fat content and *A. crassus* infection intensities between migration groups analysis of variances (*ANOVA*) was performed followed by Tukey's *HSD* multiple comparison tests. If values were not normally distributed (Shapiro-Wilk test) or homogeneity of variances was not given (Levene's test) the non parametric Kruskal-Wallis *H* test was used. Fat data were arcsin transformed for statistical analyses.

A significance level of $P \leq 0.05$ was used for all tests.

Results

Migratory history and habitat use

Analyses of the migratory history of 287 eels of different developmental stages by otolith microchemistry revealed 31.3% of all individuals to be freshwater residents and another 31% to be brackish or marine residents (Figure I-2, Table I-2). The remaining eels were distributed over the three shifter groups.

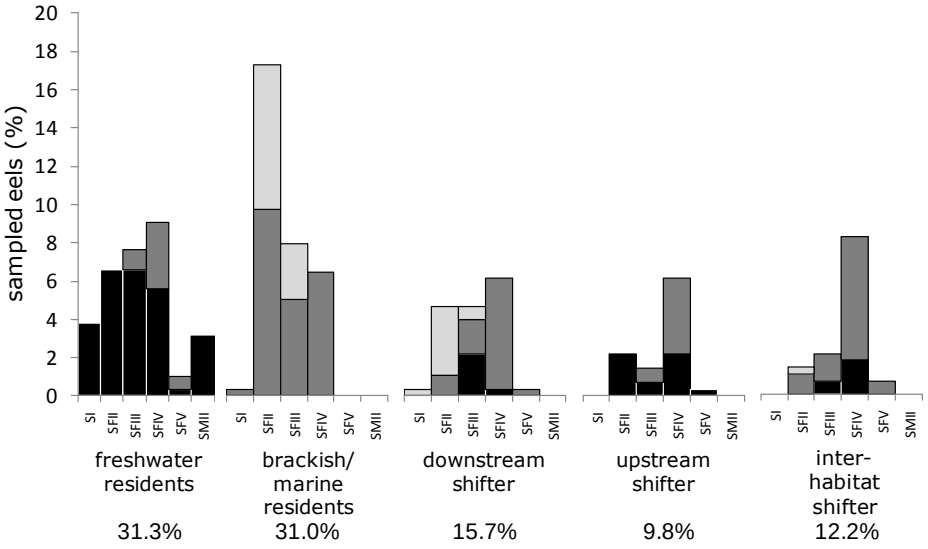

Figure I-2: Fraction of migration groups (%) by stages. Different colours represent water salinities at sampling stations (freshwater = black, brackish = dark grey, marine = bright grey)

A. anguilla caught in freshwaters were found to be considerably more stationary than individuals caught at brackish or marine stations (Table I-2). 72.2% of individuals caught in freshwater did

not change their salinity preferences during the continental growth phase, whereas only 42.9% of the eels caught in brackish waters consisted of permanent brackish residents, which never entered freshwaters. Eels caught in fully marine waters mostly spent their entire life in brackish or marine waters and never enter freshwater (67.4%). Figure I-2 depicts the development stages within the different migration groups. A closer look on SFIV and SFV individuals allows further conclusions, as these individuals presumably were about to finish their continental life phase and started their spawning migration to the Sargasso Sea. Silver eels from freshwater stations also mainly consisted of freshwater residents (56.7%), followed by upstream shifter (23.3%) and interhabitat shifter (16.7%) (Figure I-2). In brackish waters silver eels were almost equally distributed between interhabitat shifter (25.6%), brackish/marine residents (23.1%) and downstream shifter (21.8%).

Table I-2: Distribution of migration groups at sampling stations (%)

station	freshwater residents	brackish/marine residents	downstream shifter	upstream shifter	interhabitat shifter	*N*
all	31.4	31.0	15.7	9.8	12.2	287
freshwater	72.1	0.0	6.7	14.4	6.7	104
brackish	10.7	42.9	17.9	9.3	19.3	140
marine	0.0	67.4	30.2	0.0	2.3	43

Fat content

The fat content of muscle tissues increased with developmental stage (Figure I-3). Kruskal-Wallis *H* test revealed SI and SFII individuals to be significantly lower in fat content than eels of stages SFIII, SFIV and SMII ($p \leq 0.001$). Differences between SI and SFII were considerably high, but sample size was too small to test for statistical differences. The same trend is indicated by figure I-3 for differences between SFV and SI and SFII, respectively. The highest average fat content was detected in males of stage SMII, most of which exceeded muscle fat contents of 30%. Sample size for stages SI, SV and SMII were too low to be considered for a proper interpretation of condition parameters. Therefore, all further analyses are exclusively based on eels of developmental

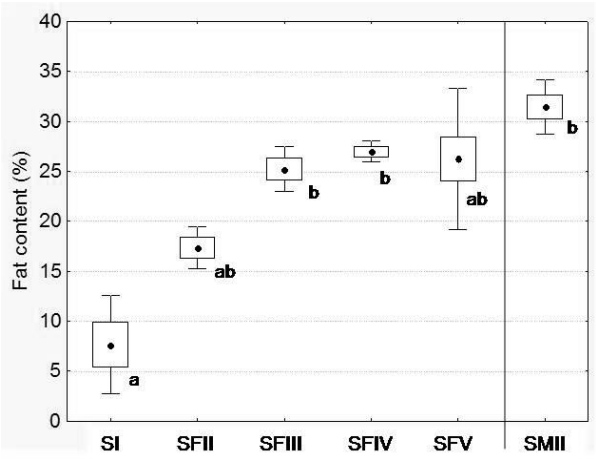

Figure I-3: Fat content (%) at development stage. Different characters indicate statistical difference. Points and error bars represent mean values and 95% CI, boxes represent SE

stages SFII, SFIII and SFIV.

Analysis of variance (*ANOVA*) revealed muscle fat contents to significantly differ among migration groups (Figure I-4, Table I-3). At developmental stage SFII freshwater residents had significantly lower fat contents compared to brackish/marine residents (p=0.026) and at SFIV the difference between both groups was even more pronounced (p=0.001). Freshwater residents again differed from downstream shifter (p=0.003). The Kruskal-Wallis *H* test could not detect any statistical significance at SFIII despite a similar tendency towards lower fat contents in freshwater eels (Figure I-4).

Indices and condition factors

Gonad weights of 213 individuals were measured in order to calculate the GSI (Table I-3, Table I-4). At SFII freshwater residents had significantly lower GSI (0.13) than brackish/marine residents (0.59; p=0.000), downstream shifter (0.51; p=0.008) and interhabitat shifter (0.62; p=0.006). It has to be taken into account that SFII freshwater residents were caught at a single station (river Eider). At SFIII no differences between migration groups were detected, while at stage SFIV downstream shifter had lower GSI (1.18) than upstream shifter (1.41; p=0.047).

Table I-3: Results of *ANOVA* and Kruskal-Wallis *H* tests. Significant results are highlighted

	F	*H*	*p*	*df*
fat content vs. stage	-	80.259	**0.0000**	190
fat content vs. migration groups				
SFII	3.519	-	**0.0138**	46
SFIII	-	6.525	0.1632	23
SFIV	5.645	-	**0.0004**	87
GSI vs. migration groups				
SFII	-	25.516	**0.0000**	74
SFIII	1.789	-	0.1518	41
SFIV	2.143	-	0.0819	95
HSI vs. migration groups				
SFII	-	3.960	0.2658	76
SFIII	-	1.378	0.8480	63
SFIV	-	2.936	0.5685	99
Fulton`s condition factor vs. migration groups				
SFII	-	12.333	**0.0150**	89
SFIII	-	14.540	**0.0058**	66
SFIV	-	8.782	0.0668	100
A. crassus intensity of infection vs. migration groups				
SFII	-	9.324	**0.0253**	76
SFIII	-	4.071	0.2539	63
SFIV	-	20.307	**0.0004**	100

The hepatosomatic index (HSI) of 241 specimens ranged between 1.14 and 2.30 and did not significantly differ among migration groups (Table I-3, Table I-4). On the contrary, Kruskal-Wallis *H* test of 258 individuals revealed Fulton's condition factor of freshwater residents to be elevated compared to brackish/marine residents at SFII p=0.006) and SFIII (p=0.013) and to downstream shifter at SFIII (p=0.028) (Table I-3, Table I-4). At stage SFIV no significant differences could be detected. All values ranged between 0.16 and 0.23.

Table I-4: *Anguillicoloides crassus* prevalence, GSI, HSI and Fulton's condition factor in different migration groups at development stages SFII, SFIII and SFIV

stage	migration group	*A. crassus prevalence*		GSI		HSI		Fulton's condition factor	
		(%)	*N*	mean (95%CI)	*N*	mean (95%CI)	*N*	mean (95%CI)	*N*
SFII	freshwater residents	83.3	12	0.13 (±0.05)	10	2.30 (±1.52)	12	0.19 (±0.01)	19
	brackish/marine residents	22.9	48	0.59 (±0.07)	48	1.41 (±0.09)	48	0.17 (±0.01)	48
	downstream shifters	7.7	13	0.51 (±0.13)	13	1.33 (±0.20)	13	0.16 (±0.01)	13
	upstream shifters		0		0		0	0.16 (±0.04)	6
	interhabitat shifters	25.0	4	0.62 (±0.16)	4	1.19 (±0.22)	4	0.17 (±0.01)	4
SFIII	freshwater residents	75.0	20	0.95 (±0.28)	7	1.19 (±0.11)	20	0.21 (±0.01)	22
	brackish/marine residents	31.8	22	0.86 (±0.15)	22	1.32 (±0.15)	22	0.18 (±0.01)	22
	downstream shifters	30.8	13	1.06 (±0.25)	7	1.24 (±0.20)	13	0.18 (±0.01)	13
	upstream shifters	66.7	3	1.37 (±0.84)	2	1.14 (±0.19)	3	0.20 (±0.01)	4
	interhabitat shifters	66.7	6	1.22 (±0.20)	4	1.29 (±0.26)	6	0.19 (±0.02)	6
SFIV	freshwater residents	96.2	26	1.28 (±0.09)	24	1.34 (±0.08)	26	0.22 (±0.01)	26
	brackish/marine residents	38.9	18	1.34 (±0.12)	18	1.24 (±0.09)	18	0.23 (±0.01)	18
	downstream shifters	47.1	17	1.18 (±0.10)	16	1.36 (±0.17)	16	0.21 (±0.01)	17
	upstream shifters	64.7	17	1.41 (±0.14)	16	1.33 (±0.16)	17	0.22 (±0.01)	17
	interhabitat shifters	60.9	23	1.31 (±0.10)	22	1.40 (±0.14)	23	0.21 (±0.01)	23

Anguillicoloides crassus infestation

Anguillicoloides crassus prevalence of infection was highest in freshwater residents, with 75% (SFIII) and 96% (SFIV), respectively (Table I-4). It was lowest for brackish/marine residents (23-39%) and downstream shifter (8-47%). The intensity of infection of SFIV was significantly higher for freshwater residents compared to brackish/marine residents (p=0.001) and downstream shifter (p=0.004) (Table I-3, Figure I-5). At SFII a trend towards differences in *A. crassus* infestation between single migration groups was detectable (p=0.0253), but Kruskal-Wallis H test could not identify the causative groups. However, intensity of infection of freshwater residents was increased compared to brackish/marine residents and downstream shifter. At SFIII no significant effects could be detected.

Restocking

Otolith Sr determination revealed at least 41.1% of all freshwater residents having derived from restocking (Table I-5). A fraction of 17.9% of all upstream shifter and another 14.3% of the interhabitat shifter were stocked. The number of potentially restocked individuals is even higher including all downstream shifter.

Discussion

Catadromy, by definition the movement of fishes from marine areas to freshwater to feed and grow combined with a subsequent downstream migration back to the sea to spawn, is a phenomenon more widespread at low latitudes. This is most likely an adaptation to pronounced productivity gradients between freshwaters and marine environments in the tropics (Inoue *et al.* 2010). In contrast, temperate zones generally provide productive coastal areas with rich feeding conditions (Gross *et al.* 1988), which makes it more difficult to understand the evolution of catadromous behaviour in high latitude freshwater eels like European, American and Japanese

eels. Inoue *et al.* (2010) hypothesized predator avoidance and reduced intraspecific competition as possible additional selection criteria to explain accordant life strategies. However, after microchemical analyses of Japanese eel (*Anguilla japonica*), Tsukamoto & Arai (2001) concluded that anguillid eel migrations into freshwater are clearly not an obligatory migratory pathway, and this form of diadromy should be defined as facultative catadromy, with the purely marine freshwater eel as one of several ecophenotypes. While the origination of freshwater eels from marine midwater ancestors and their habitat shift into rivers and lakes seems fairly well understood from an evolutionary point of view (Inoue *et al.* 2010), it is questionable if recent environmental conditions in temperate latitudes are still favouring freshwater penetration of European eels. Compared to coastal waters, European freshwaters presently represent a rather perilous environment for eels. High mortalities caused by fisheries and migration barriers (ICES 2006), contaminant loads (Belpaire *et al.* 2009; Geeraerts & Belpaire 2010; Palstra *et al.* 2006; van Ginneken *et al.* 2009) and the presence of the anthropogenically introduced parasite *A. crassus* (Kirk 2003; Palstra *et al.* 2007) in most European rivers and lakes may significantly impair migration capacities and reduce individual fitness. Furthermore, it was repeatedly reported that growth is slower in freshwater compared to saline conditions (Edeline *et al.* 2005; Melia *et al.* 2006). Concerns about less favourable living conditions for European eels in freshwater are corroborated by significantly reduced fat accumulation and drastically increased *A. crassus* prevalence and intensities of freshwater residents and downstream shifter compared to saline ecophenotypes in the here presented study.

Various studies aimed at determining critical fat values for a successful spawning migration and reproduction of European eels (e.g. van den Thillart *et al.* 2007; Larsson *et al.* 1990; Palstra *et al.* 2007). Fat contents of female silver eels in the present study averaged at 27.1% (SFIV) and 26.3% (SFV) (Figure I-3) and therefore exceeded most literature values regarded as minimum fat contents for a successful spawning migration and maturation. However, a closer look at the SFIV individuals reveals large differences between migration groups. Mean values of saline residents (29.8%) and downstream shifter (29.5%) clearly exceeded values from freshwater residents (24.0%) by about 6% (Figure I-4) or in other words: freshwater residents lack a fifth of the fat reserves available for migration, maturation and reproduction compared to eels that never entered freshwaters or moved from freshwater to coastal waters. These results contradict the findings of Limburg *et al.* (2003), who detected the highest fat content in upstream shifter.

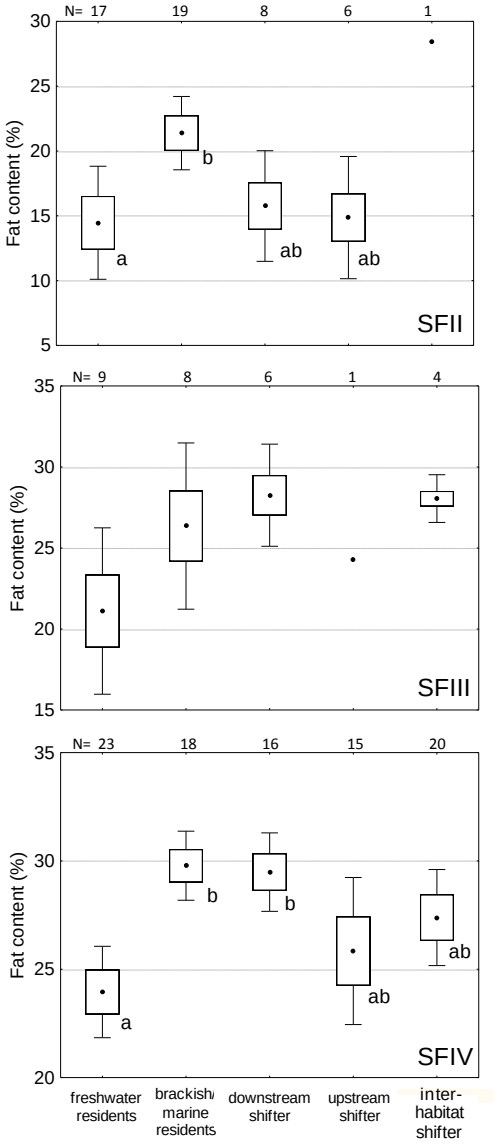

Figure I-4: Muscle fat content of *A. anguilla* with different migration histories at development stages SFII, SFIII and SFIV. Points and error bars represent mean values and 95% CI, boxes represent SE. Significant differences are indicated by different characters. Numbers of individuals per group (N) are noted above each graph

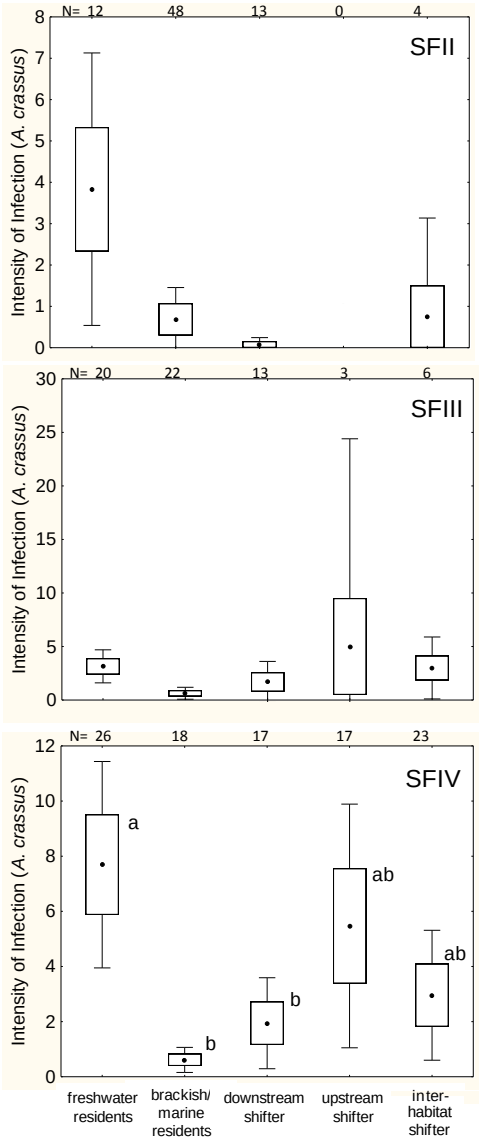

Figure I-5: Mean intensity of infection with *A. crassus*. Points and error bars represent mean values and 95% CI, boxes represent SE. Significant differences are indicated by different characters. Numbers of individuals per group are noted above each graph (N)

Prevalence of *A. crassus* was highest for freshwater residents, where at stage SFII already 83.3% of all analysed individuals were infected. At SFIV the fraction of infected eels rose to 96.2%. In contrast, *A. crassus* prevalence of saline residents and downstream shifter at SFIV reached maximum values of 38.9% and 47.1%, respectively. These results indicate that more than one half of the silver eels inhabiting coastal waters are free of *A. crassus*, while nearly all individuals that exclusively inhabited freshwaters were infected. Furthermore, there is an evident difference between freshwater residents and upstream and interhabitat shifter, which show prevalences of

Table I-5: Fractions (%) of stocked, potentially stocked and non stocked eels within the migration groups at different migration stages

stage	migration group	stocked (%)	potentially stocked (%)	non stocked (%)	*N*
	freshwater residents	41	59	0	90
	brackish/marine residents	0	0	100	89
all	downstream shifter	0	100	0	45
	upstream shifter	18	7	75	28
	interhabitat shifter	14	63	23	35
fat analysis					
	freshwater residents	41	59	0	17
	brackish/marine residents	0	0	100	19
SFII	downstream shifter	0	100	0	8
	upstream shifter	0	17	83	6
	interhabitat shifter	0	0	100	1
	freshwater residents	67	33	0	9
	brackish/marine residents	0	0	100	8
SFIII	downstream shifter	0	100	0	6
	upstream shifter	100	0	0	1
	interhabitat shifter	0	75	25	4
	freshwater residents	52	48	0	23
	brackish/marine residents	0	0	100	18
SFIV	downstream shifter	0	100	0	16
	upstream shifter	33	0	67	15
	interhabitat shifter	25	60	15	20
A. crassus analysis					
	freshwater residents	0	100	0	12
	brackish/marine residents	0	0	100	48
SFII	downstream shifter	0	100	0	13
	upstream shifter				0
	interhabitat shifter	0	50	50	4
	freshwater residents	20	80	0	20
	brackish/marine residents	0	0	100	22
SFIII	downstream shifter	0	100	0	13
	upstream shifter	0	0	100	3
	interhabitat shifter	0	83	17	6
	freshwater residents	50	50	0	26
	brackish/marine residents	0	0	100	18
SFIV	downstream shifter	0	100	0	17
	upstream shifter	29	0	71	17
	interhabitat shifter	22	57	22	23

64.7% and 60.9%, respectively. Hence, a permanent residence in coastal waters or even a temporal leave of freshwaters reduces *A. crassus* prevalence by almost 30%.

Mean infection intensity of SFIV freshwater residents was 7.7 *A. crassus* per host versus 0.6 in saline residents (Figure I-5). Clevestam *et al.* (2011) defined 7 nematodes per eel as a heavy infection, a number reached by 42% of all SFIV freshwater but by none of the saline residents in the present study. High infection intensities greater than 10 *A. crassus* per host, which apply to 31% of SFIV freshwater residents in the present study, are reported to cause substantial losses in swimming speed of up to 18% (Sprengel & Lüchtenberg 1991) as well as additional energy demands for swimming of up to 20% (Palstra *et al.* 2007).

The Baltic Sea as a brackish water system resembles an enlarged estuary and might represent a very special habitat for eels. Less pronounced salinity gradients at the transition from the saline environment to freshwater, limited abundance of large predators and rich feeding conditions seem to repress strictly catadromous behaviour and favour less pronounced migration strategies. As already observed in other studies (Tzeng *et al.* 2000; Limburg *et al.* 2003; Shiao *et al.* 2006) silver eels caught in the Baltic Sea are often difficult to assign to a typical migratory history pattern. In the present study almost every 4th silver eel caught in brackish waters had no contact to freshwater during its entire life, making (low) saline residency a significant life history strategy. Given an average age of >10 years of female silver eels leaving the Baltic Sea (Clevestam *et al.* 2011), it is debatable if this pattern is simply a consequence of the historically low recruitment during the last years (ICES 2010a). However, since the dispersion rate of eels into freshwater seems to be a density-driven process (Ibbotson 2002), reduced intraspecies competition in times of low recruitment might reduce the absolute number of *A. anguilla* naturally ascending freshwaters, resulting in a diminishing importance of freshwaters as eel habitats.

Silver eels caught in freshwater could more easily be assigned to a specific pattern of habitat use with freshwater residents and upstream shifter accounting for 56.7% and 23.3%, respectively. However, the reconstruction of migration patterns from eels caught in freshwaters was heavily biased, with at least 76.7% of all silver eels caught in rivers and lakes showing clear evidence for anthropogenic translocation through restocking activities (Table I-5).

The influence of additional deviations from natural conditions, especially the abundance of migration barriers like hydropower stations and other obstacles was not specifically investigated in this study, but can be assumed to further increase the percentage of stationary individuals under present freshwaters conditions compared to a pristine environment. The rationale behind the restocking of wild-caught juvenile eels is that stocked eels face higher habitat quality and/or lower anthropogenic mortalities at their final destination compared to their waters of origin. But the scientific evidence that this prerequisite is fulfilled for European river basin districts is scarce leading to the often referred assumption that restocking of eels is rather a measure to support fisheries than to sustain the European eel population. Significantly reduced fat reserves and drastically increased *A. crassus* infections as proxies for spawner quality of freshwater residents and downstream shifter compared to saline ecophenotypes further corroborate the assumption that a large-scale anthropogenic translocation of juvenile eels from estuaries to freshwaters might be less beneficial or even counteractive for the presently intended eel stock recovery programs. Although there is little doubt that freshwaters are important habitats for European

eel, the common practice of restocking large quantities of wild caught juveniles should be critically evaluated against alternative management options, like the removal of migration barriers and the reduction of direct anthropogenic mortality.

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CHAPTER II Temperature dependency of element incorporation into European eel (*Anguilla anguilla*) otoliths

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Abstract

The present study experimentally tested the influence of water temperature on the inclusion of 15 elements into juvenile European eel (*Anguilla anguilla*) otoliths in freshwater. It should be investigated (1) if temperature effects on otolith Sr/Ca might impair the interpretation of migration studies and (2) if the elemental composition of otoliths can be used to reconstruct experienced temperature histories of eels. Therefore, eels were kept under full experimental conditions at three different water temperatures (14°C, 19°C, 24°C) for 105 days. Thereafter, laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) was conducted on the outer edge of their otoliths. Our analyses revealed significant temperature effects on otolith Na/Ca, Sr/Ca, Mg/Ca, Mn/Ca, Ba/Ca, Zr/Ca and Y/Ca ratios. Variations of Sr/Ca caused by temperature were far below those used to detect eel movements between waters of different salinities and will therefore not affect the interpretation of migration studies. Elemental fingerprints of Sr/Ca, Mg/Ca, Mn/Ca and Ba/Ca ratios resulted in clearly separated groups according to temperature treatments, indicating that changes in water temperature might lead to characteristic changes in otolith elemental composition. However, the successful application of elemental fingerprints to reconstruct moderate changes of water temperature seems doubtful because the influence of somatic growth on otolith microchemistry still remains unclear, and temperature-induced variations could be overlaid by changes of water element concentrations during growth periods. Nevertheless, our results contribute to the completion of knowledge about factors influencing element incorporation and help to explain variations in element composition of fish otoliths.

Keywords: *Anguilla anguilla*, otolith, microchemistry, LA-ICPMS, temperature effect

Introduction

Microchemical otolith analysis is widely used to track individual migration behaviour of fish. It is a well established powerful tool to reconstruct individual movements between distinct water bodies commonly based on the positive correlation of otolith strontium/calcium (Sr/Ca) ratios and water salinities and therefore especially applicable for diadromous species (e.g. Radtke *et al.* 1988; Halden *et al.* 1995; Secor *et al.* 1995; Tzeng *et al.* 1997). Furthermore, trace and minor element analyses in fish otoliths were successfully used for stock discrimination (Edmonds *et al.* 1989; Campana 1999; Gillanders & Kingsford 2000; Rooker *et al.* 2003) and serve as indicators for changes in physiological conditions during e.g. metamorphosis, reproduction and stress (Kalish 1989, 1992; Otake *et al.* 1997; Tzeng *et al.* 2002).

The interpretation of otolith elemental composition as a record of individual environmental history and migration behaviour is based on the assumption that element incorporation into otoliths is directly influenced by physicochemical conditions of the surrounding water. This supposition is supported by numerous studies, which clearly indicate effects of water salinity (Secor *et al.* 1995; Kalish 1990; Tzeng 1996) and water element composition (Bath *et al.* 2000; Elsdon & Gillanders 2006) on otolith element composition. An unambiguous, generalized interpretation of microchemical data as a reflection of the environmental history of individual fish is nonetheless hindered by species-specific differences in the underlying processes of otolith element incorporation. They result in e.g. varying sensitivity for water element composition (Hamer & Jenkins 2007) or differences in strength and direction of temperature effects (Campana 1999; Elsdon *et al.* 2008). Hence, findings gained for single species cannot be used to draw general conclusions. These interspecific variations might be triggered by species-specific differences in physiological processes. The inner ear of fish is filled with an acellular medium directly surrounding the otoliths, the so-called endolymph, whose ion composition is of major importance for the element incorporation into otoliths (Payan *et al.* 2004a). Elemental compositions of otoliths and surrounding water are commonly not identical because element passage into the endolymphatic fluid and otolith crystallisation itself depend on physiological processes including active ion transport through different barriers. Water-borne elements pass either branchial or intestinal membranes as well as the inner ear epithelium (Campana 1999; Payan *et al.* 2004a), usually resulting in lower element concentrations in otoliths compared to the surrounding water (Campana 1999). Additionally, endogenous factors like age (Kalish 1989; Hoff & Fuiman 1993), metamorphosis (Otake *et al.* 1994, 1997; Tzeng *et al.* 2002), otolith crystal formation (Tzeng *et al.* 2007) as well as other parameters like diet (Limburg 1995; Farrell & Campana 1996; Buckel *et al.* 2004) or individual growth rate (Sadovy & Severin 1992, 1994) and

water temperature (Secor *et al.* 1995; Radtke 1989; Townsend *et al.* 1992; Hoff & Fuiman 1995) affect and possibly enhance interspecific variations.

Temperate eel species (*Anguilla anguilla*, *Anguilla japonica* and *Anguilla rostrata*) have been subject of several life history investigations based on otolith element composition (e.g. Tzeng *et al.* 1997, 2002; Tsukamoto *et al.* 1998; Jessop *et al.* 2002; Limburg *et al.* 2003; Arai & Hirata 2006; Daverat *et al.* 2006). A clearly positive relation between water salinity and otolith Sr/Ca ratios was confirmed for wild-caught individuals, while only a few studies have tested potential environmental influences on otolith microchemistry under experimental conditions. Among these, a study of Marohn *et al.* (2009) demonstrated that feeding behaviour does not affect otolith microchemistry of *A. anguilla*. Kawakami *et al.* (1998) and Tzeng (1996) investigated the influence of temperature on Sr incorporation of *A. japonica*, without finding any effect, while the latter showed an inverse relationship between otolith Sr/Ca and water temperature for the same species two years before (Tzeng 1994). The present study is the first to investigate the effect of water temperature on the multi-element composition of *A. anguilla* otoliths. Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) was used to perform a multi-element analysis (lithium (Li), sodium (Na), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn), rubidium (Rb), strontium, yttrium (Y), zirconium (Zr), niobium (Nb), cadmium (Cd), barium (Ba), lead (Pb) and uranium(U)) of otoliths of individuals kept at different water temperatures (14°C, 19 °C, 24°C) under full experimental conditions.

A better understanding of the temperature impact on element incorporation processes could significantly improve the interpretation of otolith element concentrations for life history studies of *A. anguilla* by examining if changes in otolith Sr concentrations are solely caused by diadromy or if other factors cause, enhance or reduce Sr fluctuations. By performing a multi-element analysis, this study further aims at discovering potential candidate elements as tracers for the reconstruction of experienced temperature histories of individual eels. Elemental fingerprints could deliver information to deepen knowledge about the ecology of this endangered species, which like other temperate eel stocks around the world is suffering from a strong decline during the last decades (Dekker *et al.* 2003; ICES 2009). Increased knowledge about their migratory behaviour and habitat preferences are of fundamental importance for a better management with regard to stock recovery.

The experimental setup was designed to provide stable conditions and to ensure temperature to be the only variable between treatments. Nevertheless, a clear distinction between temperature and growth effect is challenging, due to the close relation between water temperature and body growth in fish. In most of the published studies, it remains unclear whether changes in otolith

composition are due to water temperature or to differences in growth rates. Those who differentiated between the major influencing components found conflicting results. Sadovy & Severin (1994) showed that Red hind (*Epinephelus guttatus*) Sr incorporation exclusively depends on growth rate, not on water temperature, while Martin *et al.* (2004) reported opposite results for larval spot (*Leiostomus xanthurus*), where Sr incorporation was exclusively affected by temperature. We compared element incorporation of differently grown individuals within temperature treatments to address this problem.

Materials and Methods

Alizarin marking

Otoliths were marked with Alizarin red S (Merck; Darmstadt, Germany) prior to the experiment to ensure that only aragonite grown under controlled temperature conditions was later on analysed. Alizarin was used by Simon & Dörner (2005) to mark glass eel otoliths, but to our knowledge, this method was not successfully adopted to elvers so far. Tests in salt water conducted prior to our experiment convinced us to change the procedure from fresh water to salt water conditions. It took place in a 200L water tank at 25°C and a salinity of 38. Eels were acclimated to the tank conditions for 7 days before Alizarin red S (150 mgL^{-1}) and Tris-buffer (1 gL^{-1}) (Carl Roth; Karlsruhe, Germany) were added. Eels were kept in the staining bath for 23 h.

Experimental design

The experimental setup consisted of nine tanks, three different temperature regimes (14°C, 19°C, 24 °C) over three replicates. All treatments were designed as flowthrough systems and fed by untreated tap water. Plastic tubes were provided for shelter, and grids were embedded into the tanks to enlarge the available surface area. Light regime was set to a 10h light/14h dark period.

Pigmented juvenile *A. anguilla* with an initial mean weight of 9.2 g (SD 2.4) were obtained from a commercial fish farm (Aalversandstelle; Halstenbek, Germany). After acclimatisation, 20 specimens were placed into each tank and fed three times daily with commercial eel pellets (Skretting; Stavanger, Norway). After 105 days, they were measured, weighed and sacrificed. Sagittal otoliths were extracted and stored dry in Eppendorf caps. Otoliths of the five best grown individuals of each tank were used for further analysis to ensure the growth of sufficient otolith material under experimental conditions.

Otolith processing and elemental analyses

Dried sagittal otoliths were embedded in thermo-epoxy resin (Buehler; Düsseldorf, Germany) on glass slides. They were polished from the proximal side down to the nucleus using lapping film of 30, 12 and 3 µm (3 M; Neuss; Germany).

Concentrations of 15 isotopes (^{7}Li, ^{23}Na, ^{25}Mg, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{111}Cd, ^{138}Ba, ^{208}Pb and ^{238}U) were determined along transects of 600 µm length at the anterior edge of the proximal side of the otolith.

Analyses were carried out by LA-ICPMS using a NewWave UP193 solid-state laser coupled to a Thermo-Finnigan Element2™ at the Department of Geosciences, University of Bremen. Analytical conditions included a pulse rate of 10 Hz, irradiance of ca. 1 GWcm^{-2}, a spot size of 75 µm and a transect scan speed of 3 µms^{-1}. Prior to measurement, transects were preablated with a 120 µm spot at 100 µms^{-1} scan speed in order to clean the otolith surface. Helium (0.4 lmin^{-1}) was used as sample gas, and argon (0.8 lmin^{-1}) was subsequently added as make-up gas; plasma power was 1,200 W. All isotopes were analysed at low resolution with five samples in a 20% mass window and a total dwell time of 50 ms per isotope. Blanks were measured during 20 s prior to ablation.

Following every second transect, a glass reference material (NIST612) was analysed as external calibration standard using the concentrations of Pearce *et al.* (1997). We note that the Mg concentration suggested by these authors (77.4 µgg^{-1}) appears to be too high and may be closer to 64 µgg^{-1} as obtained by Gao *et al.* (2002). As internal standard, we used Ca with an assumed concentration of 38.8 wt% (similar to the NIES22 otolith standard; (Yoshinaga *et al.* 2000). All element concentrations were expressed as element/Ca ratios to account for the substitution for Ca by divalent elements like i.e. Sr or Ba or the coprecipitation of other carbonates like $MgCO_3$ (Campana 1999). For data quantification, the Cetac GeoPro™ software was used.

The data quality was assessed by regular analyses of a pressed pellet of the NIES22 otolith standard (Table II-1). For Na, Mg, Sr, Ba, Cu and Zn, there is good to excellent agreement with the certified values, which suggests that NIST612 is well suited as a calibration standard for carbonate analyses despite its strongly different matrix (Na silicate glass) and its low Sr concentration (76 µgg^{-1}) compared to otoliths. The accuracy for Mg is even better if 64 µgg^{-1} rather than 77.4 µgg^{-1} is used as calibration value. Because the variations of our NIES22 analyses include heterogeneities within the tablet, the actual analytical precision of the laboratory setup is better than the relative standard deviations shown in table II-1; for concentrations above 0.5–1 µgg^{-1}, we estimate a precision of better than 3%.

Table II-1: Average and relative standard deviation (RSD) of 19 analyses of a pressed pellet from NIES22 otolith (National Institute for Environmental Studies, Yoshinaga *et al.* 2000) conducted in 2007 through 2009

		Na	Mg	Cu	Zn	Sr	Ba	Pb
average	($\mu g\ g^{-1}$)	2270	25	0.807	0.511	2273	2.66	0.037
RSD	(%)	3.6	12	22	20.8	3	4.4	39.5
reference value	($\mu g\ g^{-1}$)	2230	21	0.74	0.47	2360	2.89	0.023

As reported by Tzeng *et al.* (2007), eel otoliths might contain areas consisting of vaterite instead of aragonite, with extensive impacts on the incorporation of several elements. To avoid the use of data collected in vaterite, concentrations of Sr, Na, Ba, Mg and Mn were crosschecked for characteristic values published for vaterite (Tzeng *et al.* 2007).

Water analyses

Water samples of all treatments were bottled into sterile polyethylene bottles at the beginning and the end of the experiment. 14 low concentrated elements (Ba, Li, P, Zn, Rb, Mn, Cu, Zr, U, Pb, Y, Cd, Nb and Cs) were analysed by ICP-MS (ThermoFinnigan Element2™), higher concentrated elements (Ca, Na, Mg, K, Sr and B) by ICP-AES (Perkin-Elmer Optima 3300), both at the Department of Geosciences, University of Bremen.

Statistical analyses

Analysis of variances (*ANOVA*) was performed using Statistica 8 to test the effect of water temperature on element incorporation into otoliths. Element/Ca ratios were calculated and averaged for each specimen. Replicate values did not differ significantly among each other and were pooled for further analyses. *ANOVA* was followed by Tukey's *HSD* multiple comparison test or in case variances were not distributed homogeneously among factor levels a Kruskal–Wallis *H* test was performed.

The effect of water temperature on body growth was tested with *ANOVA*. Individual growth was expressed in percentage of mean starting weight of all eels. Growth values were square root transformed to meet assumptions for parametric statistics.

To test growth effects on element incorporation, regression analyses of element concentrations against growth were performed within single temperature treatments.

Primer 6.0 was used to carry out an analysis of similarities (*ANOSIM*) on Bray–Curtis matrix including element/Ca ratios of Sr, Mg, Mn and Ba. For individual comparison, data were square root transformed. Similarities were visualized with multidimensional scaling (*MDS*).

A significance level of P=0.05 was used for all tests.

Results

Water chemistry

Water concentrations of 20 elements were analysed (Ca, Na, Mg, K, Sr, B, Ba, Li, P, Zn, Rb, Mn, Cu, Zr, U, Pb, Y, Cd, Nb and Cs). A detailed view of the results is presented for Ca, Na, Mg, Sr, Ba, Pb, Cd, Mn, Cu, Zr and Y (Table II-2), as otolith concentrations of these elements were suitable for statistical analyses. Water concentrations of the remaining elements were not used for further analyses because their concentrations in otoliths were below limits of detection or inconsistent between replicates.

Out of the tested elements, variations of Mn/Ca, Cu/Ca, Pb/Ca and Cd/Ca between and/or temporarily within treatments were found, while Sr/Ca, Ba/Ca, Na/Ca, Y/Ca, Zr/Ca and Mg/Ca remained stable (Table II-2).

Table II-2: Water element/calcium ratios measured by ICP-AES or ICPMS. Numbers represent mean values of 3 or 5 measurements ± standard deviations (SD)

Element		lowest (±SD)	highest (±SD)	*N*	Method
Ca	(mmol L^{-1})	1.947 (±0.003)	1.927 (±0.006)	3	ICP-AES
Na/Ca	(mol mol^{-1})	0.298 (±2*10^{-4})	0.300 (±2*10^{-4})	3	ICP-AES
Mg/Ca	(mol mol^{-1})	0.083 (±3*10^{-4})	0.085 (±7*10^{-4})	3	ICP-AES
Sr/Ca	(mmol mol^{-1})	1.043 (±0.000)	1.075 (±0.006)	3	ICP-AES
Ba/Ca	(mmol mol^{-1})	0.247 (±0.000)	0.273 (±0.003)	3	ICP-AES
Pb/Ca	(µmol mol^{-1})	19.76 (±0.00)	82.03 (±1.11)	5	ICPMS
Cd/Ca	(µmol mol^{-1})	20.01 (±2.50)	31.58 (±3.19)	5	ICPMS
Mn/Ca	(µmol mol^{-1})	1.03 (±0.02)	33.11 (±0.60)	5	ICPMS
Cu/Ca	(µmol mol^{-1})	1.57 (±0.04)	29.24 (±0.08)	5	ICPMS
Zr/Ca	(µmol mol^{-1})	0.12 (±0.004)	0.125 (±0.004)	5	ICPMS
Y/Ca	(nmol mol^{-1})	58.0 (±3.60)	64.7 (±0.00)	5	ICPMS

Otolith analysis

Alizarin marking

No fluorescent alizarin mark could be detected in otoliths. In the meantime, Neukamm (2009) developed a method to mark elvers with Alizarin red S. It turned out that low water conductivity is required to successfully stain the otoliths of pigmented eels. In the present study, marking took place in salt water, whose high conductivity probably impaired the incorporation of Alizarin red S. Nevertheless, a clear ring like check was visible in most of the otoliths, which was assumed to originate from stress during the marking procedure (Campana 1983; Payan *et al.* 2004b). Otoliths were laser ablated beyond this stress mark to ensure element measurement in aragonite that had grown during the experiment.

No mortality occurred during the marking procedure.

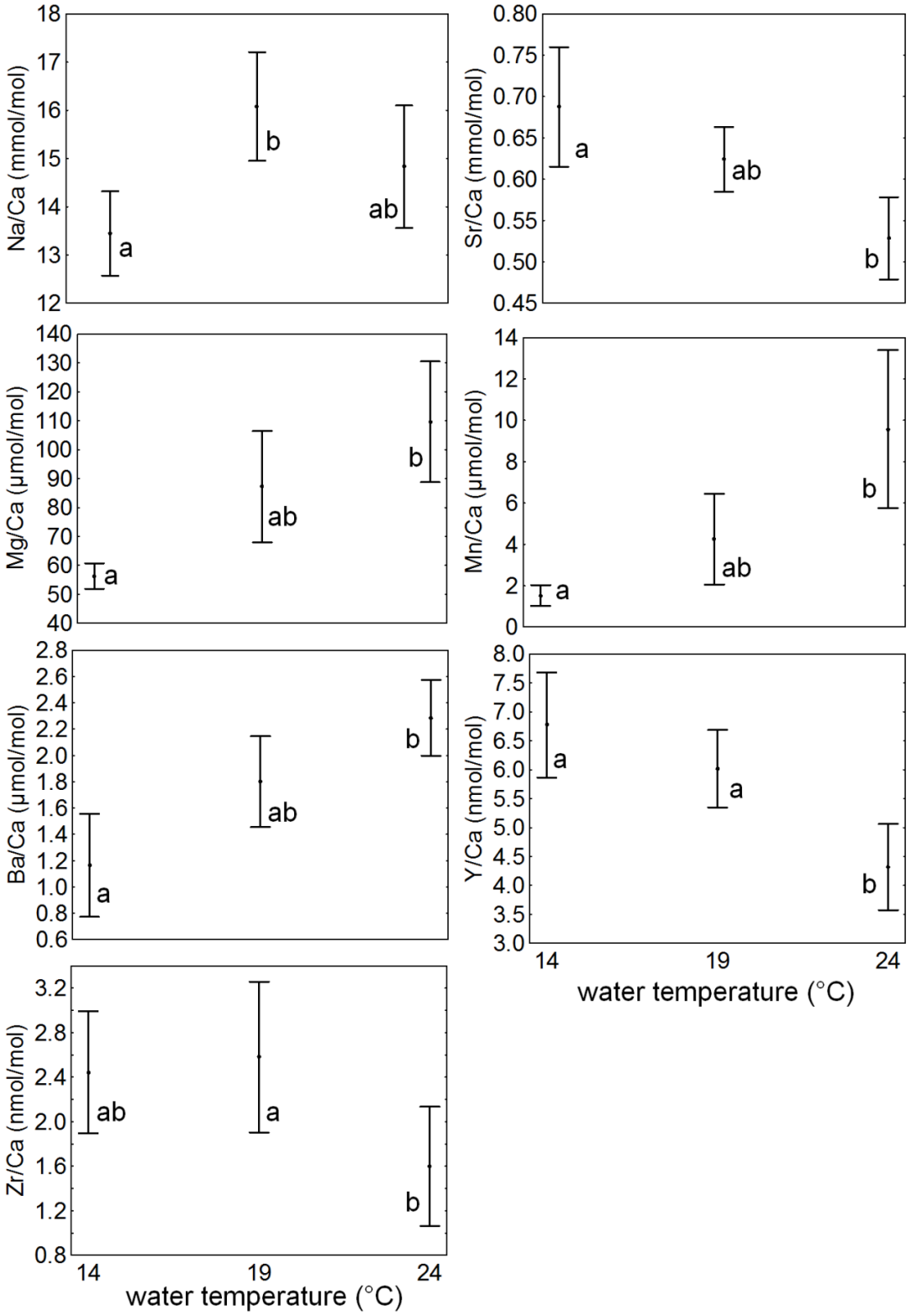

Figure II-1: Otolith element/calcium ratios (molar) at different temperatures. Points and error bars represent mean values of 5 specimens and 95% confidence intervals, respectively. Different letters indicate statistical significant differences

Otolith elemental composition

Out of the 15 analysed elements, only Na, Sr, Mg, Mn, Ba, Cu, Y, Cd, Zr and Pb could be used for statistical analyses. Zn, U, Rb, Nb and Li were excluded because measured values were inconsistent between replicates (Rb and Zn) or because concentrations were below limits of detection (Li, Nb and U).

Table II-3: Results of analysis of variance (*ANOVA*) and Kruskal–Wallis *H* test summarising the effect of three different temperatures on otolith element/calcium ratios. Significant results are highlighted

	F	*H*	*p*	*df*
Na/Ca	6.5737		**0.0033**	2, 42
Sr/Ca	9.5689		**0.0004**	2, 42
Mg/Ca		19.140	**0.0001**	2, 45
Mn/Ca		20.977	**0.0000**	2, 45
Ba/Ca	12.236		**0.0001**	2, 42
Cu/Ca		4.810	0.0903	2, 45
Y/Ca	11.945		**0.0001**	2, 42
Cd/Ca	0.3753		0.6894	2, 42
Zr/Ca	3.7349		0.0321	2, 42
Pb/Ca		2.301	0.3165	2, 45

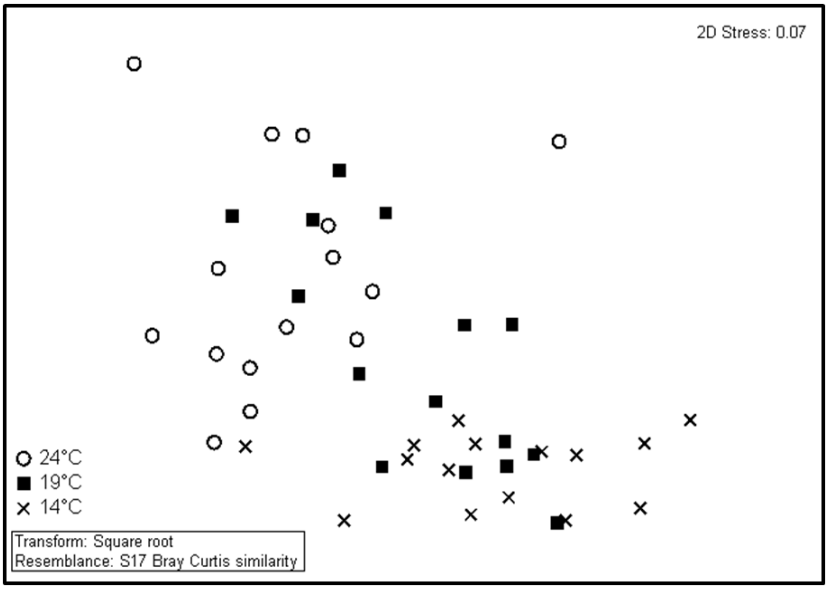

Figure II-2: Multidimensional scaling plot containing element/calcium ratios (molar) of Sr, Mg, Mn and Ba in different temperature treatments

Significant differences between treatments were found for Na, Sr, Mg, Mn, Ba, Zr and Y incorporation (Table II-3, Figure II-1). While Sr/Ca (p=0.000) and Y/Ca (p=0.000) ratios decreased with increasing water temperature, positive relations between temperature and Mg/Ca (p=0.000), Mn/Ca (p=0.000) and Ba/Ca (p=0.000) incorporation were found (Figure II-1).

Despite being obviously affected by water temperature, Na (p=0.003) and Zr (p=0.032) incorporation did not show an unambiguous temperature-dependent trend. Na significantly increased from 14°C to 19°C, but the 24°C treatment did not differ significantly from either 14°C or 19°C treatment. The only significant difference found for Zr was between intermediate and high temperature treatments, while both did not significantly differ from the 14°C group (Figure II-1). For Cd, Cu and Pb, no significant temperature-dependent variations were detected.

In order to test the potential of elemental fingerprints as a tool to recover individual temperature histories of eels, an *ANOSIM* was performed containing Sr/Ca, Mg/Ca, Mn/Ca and Ba/Ca ratios. It revealed highly significant differences between treatments (p<0.01). The MDS plot (Figure II-2) illustrates a clear separation of individuals from 14°C and 24°C. Only two individuals from 24°C are placed outside the group, while one individual from 14°C is located close to the 24°C group. Eels from19°C were placed intermediately, overlapping the other groups.

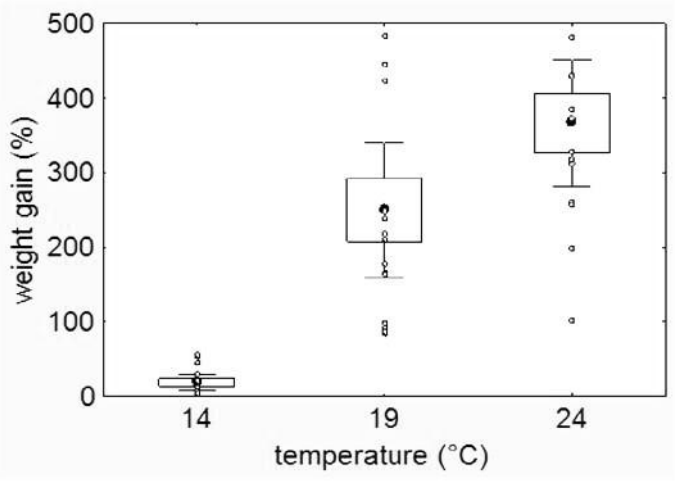

Figure II-3: Body growth (% of initial weight) at different water temperatures. Closed circles (●) represent mean values, boxes represent standard error and error bars are indicating 95% confident intervals. Raw data are represented by open circles (○)

The growth performance of eels during the experiment depended on water temperature ($F(2,42) = 58.276$, p=0.000). At a water temperature of 14°C, eels gained between 1.5% and 54% of their initial weight, while eels at 19°C and 24°C gained between 85% and 606% and between 101% and 661%, respectively (Figure II-3), hence growth rate has to be considered to possibly affect element incorporation into otoliths.

Regression analysis showed significant relations between somatic growth and Sr/Ca (p=0.000), Mg/Ca (p=0.039), Mn/Ca (p=0.000) and Y/Ca (p=0.032) ratios at 19°C (Table II-4). No significant effects were detected at 14°C and 24°C and for Na/Ca, Ba/Ca and Zr/Ca at any temperature.

Discussion

Of all elements found in otoliths, Sr is of outstanding importance concerning migration studies of diadromous fish due to its well-documented correlation with water salinity. Since the late 1990´s, it has widely been used to track fish movements between salt, brackish and freshwater

(Campana 1999), but until today, potential regulatory effects of extrinsic and intrinsic factors influencing Sr incorporation remain largely untested. The present study confirmed a significant impact of water temperatures on otolith Sr incorporation at least for freshwater conditions. However, despite being statistically significant, the range of temperature-induced changes of Sr/Ca ratios from about 0.7 to 0.5 mmol mol^{-1} at 14°C versus 24°C is far below the resolution limit of most migration studies and therefore does not raise doubts about their validity.

Table II-4: Results of regression analysis. Element/calcium ratio (molar) vs. body growth (% of initial weight). Significant results are highlighted

	temperature	R^2	*p*	*df*
Na/Ca	14°C	0.07	0.330	13
	19°C	0.12	0.212	13
	24°C	0.01	0.780	13
Sr/Ca	14°C	0.03	0.560	13
	19°C	0.56	**0.000**	13
	24°C	0.03	0.520	13
Mg/Ca	14°C	0.00	0.920	13
	19°C	0.29	**0.039**	13
	24°C	0.12	0.210	13
Mn/Ca	14°C	0.00	0.899	13
	19°C	0.73	**0.000**	13
	24°C	0.25	0.054	13
Ba/Ca	14°C	0.03	0.850	13
	19°C	0.04	0.500	13
	24°C	0.00	0.860	13
Zr/Ca	14°C	0.24	0.065	13
	19°C	0.13	0.188	13
	24°C	0.24	0.066	13
Y/Ca	14°C	0.01	0.683	13
	19°C	0.31	**0.032**	13
	24°C	0.09	0.290	13

Literature values of Sr/Ca ratios in *A. anguilla* otoliths range from 0.5 to 1.4 mmol mol^{-1} in freshwater to above 2.7 mmol mol^{-1} in fully marine waters. Sr/Ca ratios incorporated in brackish waters rank in between those of fresh and marine waters (Daverat *et al.* 2006; Shiao *et al.* 2006). Hence, Sr/Ca fluctuation amplitudes of 0.2 mmol mol^{-1} as detected in our study would not be regarded sufficient to validate individual migration behaviour of eels.

Previous investigations dealing with temperature effects on otolith microchemistry of various fish species often resulted in conflicting findings. While otolith Sr incorporation in some studies correlated positively with water temperature (e.g. Bath *et al.* 2000; Limburg 1995; Martin *et al.* 2004; Elsdon & Gillanders 2004), others revealed an inverse relation (e.g. Secor *et al.* 1995; Radtke 1989) or no effect at all was detected (e.g. Tzeng 1996; Gallahar & Kingsford 1996; Chesney *et al.* 1998). A more complex behaviour of Sr incorporation was found by Elsdon & Gillanders (2002), who worked on Black bream (*Acanthopagrus butcheri*) at water temperatures between 12°C and 28°C and reported a negative correlation of otolith Sr concentration and temperature below 20°C and a positive correlation above 24°C. It was also reported that the impact of water temperature on Sr incorporation might vary gradually with changes of

surrounding water salinities (Secor *et al.* 1995; Elsdon & Gillanders 2002). Although no clear interdependency of temperature and salinity was found for a number of species (e.g. Chesney *et al.* 1998; Yamashita *et al.* 2000) including *A. japonica* (Tzeng 1996; Kawakami *et al.* 1998), it cannot be excluded that the impact of temperature on element incorporation into *A. anguilla* otoliths may change with rising water salinities or rising water Sr contents. Since the present study was performed in freshwater, an extrapolation of the obtained results to saltwater conditions or to freshwaters of special geological origin resulting in elevated Sr concentrations, as reported by Limburg (1995) and Kraus & Secor (2004) might be premature. Tzeng *et al.* (1999) reported periodical shifts of Sr concentrations in otoliths of *A. anguilla* caught in fully marine waters, where narrow bands of elevated Sr concentrations correspond with hyaline zones and alternate with broader bands of lower Sr contents. This might be caused by seasonal temperature changes or by changes of temperature-correlated physiological processes and suggests that the extent of temperature effects depends on gradual changes in water salinity.

Most previous studies that detected temperature effects on Ba incorporation support our findings of positive correlations between water temperature and otolith Ba contents (*A. butcheri* (Elsdon & Gillanders 2002, 2004) and *Sebastes melanops* (Miller 2009)). However, Bath *et al.* (2000), Martin & Thorrold (2005) and Martin & Wuenschel (2006) did not find any temperature effect on Ba incorporation into otoliths of *L. xanthurus* and *Lutjanus griseus*, respectively. Mg and Mn were analysed in only a few studies. Unlike our findings for the European eel, a negative correlation of Mn and temperature was found by Miller (2009) for black rockfish (*S. melanops*). Elsdon & Gillanders (2002) concluded neither Mn nor Mg to be suitable for the use of temperature studies due to large variations between replicate tanks and between individuals reared in the same tank. We cannot support these findings as our results deliver reliable values that suggest a temperature dependency of both elements. To our knowledge, no study has analysed temperature effects on Y incorporation into fish otoliths so far. In the present study, otolith Y concentrations decreased with increasing water temperatures. The relation between Na and Zr incorporation and water temperature, however, is ambiguous (Figure II-1).

Combined elemental fingerprints of Sr/Ca, Mg/Ca, Mn/Ca and Ba/Ca showed clear temperature-dependent differences (Figure II-2). Elsdon & Gillanders (2002) detected significant temperature effects on *A. butcheri* otoliths using fingerprints of the same elements. Thus, this element combination seems to be the most promising for the reconstruction of experienced temperature history. Due to the above-mentioned effects of water salinity on otolith chemistry and the possible interaction of temperature and salinity, such temperature history studies require environments with constant salinities.

To ensure temperature and its related physiological processes to be the only variables between treatments, the experimental setup provided identical conditions for all tanks. Eels were fed the same food to avoid possible food effects, which in the meantime was proved to have no detectable impact on *A. anguilla* otoliths microchemistry anyway (Marohn *et al.* 2009). Water element composition, the major driver of element incorporation into fish otoliths (Farrell & Campana 1996; Elsdon & Gillanders 2003, 2004; Walther & Thorrold 2006), was held constant and equal between treatments for most element/Ca ratios and, therefore, was not responsible for otolith element variations. Only Mn/Ca, Cu/Ca, Cd/Ca and Pb/Ca ratios varied between treatments or during experimental time, presumably caused by initial tank contamination. However, despite significant differences in water concentrations of Cu, Cd and Pb, their otolith concentrations were strikingly constant. Also for Mn, variations in water concentrations did not proportionally affect otolith element incorporation. Highest Mn water concentrations were measured at 19°C, corresponding to intermediate otolith concentrations, and lowest water concentrations were found at 24°C, corresponding to highest Mn concentrations in otoliths. For this reason, we conclude that the here observed differences in otolith Mn concentrations between treatments are not due to changing water concentrations. In fact, it seems more likely that they were caused by temperature differences between treatments. Nevertheless, it cannot be excluded that changing Mn, Cu, Cd and Pb concentrations in tank water probably have affected otolith microchemistry to a minor extent.

As introductorily described, the impact of body growth on otolith microchemistry is controversially discussed and appears to vary between species. Not surprisingly, we found a positive correlation between somatic growth and water temperature, complicating the identification of a single factor responsible for changes in otolith chemistry. To determine whether differences in otolith chemistry are caused by water temperature or by growth performance, we tested otolith element composition within single temperature treatments. Particularly at 19°C and at 24°C, individual growth rates varied widely. According to their initial weight, eels gained from 85% to 606% and from 101% to 661%, respectively, during the experimental time of 105 days. Results showed no significant relations between any of the tested elements and growth within the 24 °C treatment, but at 19°C, significant relations were found between growth rate and the incorporation of Sr, Mg, Mn and Y. Variations of Na, Ba and Zr concentrations were interpreted to be exclusively caused by variations in water temperature, not by growth rate. A growth effect on Sr, Mg, Mn and Y incorporation is not confirmed by individuals kept at 24 °C, where no significant relations between element incorporation and body growth could be found, even though individual growth rates differed as much as at 19 °C. It

has to be borne in mind that each analysis consists of only 15 individuals, resulting in a rather small statistical power. Small effect sizes might therefore remain undetected. Nevertheless, our findings suggest interactive effects of somatic growth rate and water temperature on otolith element incorporation, with higher importance of body growth at intermediate and no influence at higher water temperatures.

The present study revealed a significant effect of water temperature on the elemental composition of *A. anguilla* otoliths. However, the practical use of otoliths to reconstruct moderate changes of experienced water temperatures of European eels seems rather unlikely. The relatively wide error limits of the determined relations would lead to uncertain estimations of water temperatures. Furthermore, temperature effects might be superposed by the influence of growth rate on incorporation processes and by changes of water elemental concentrations. A possible interaction of temperature and salinity should be investigated in future experiments to examine the importance of water temperature in brackish and marine waters.

Nevertheless, despite all difficulties of using experimentally gained results to comprehend processes occurring in the wild, our results contribute to the completion of knowledge about factors influencing the incorporation of elements into European eel otoliths. They help to explain variations in specific elements and achieve a more comprehensive interpretation of otolith elemental composition.

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CHAPTER III Dietary effects on multi-element composition of European eel (*Anguilla anguilla*) otoliths

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Abstract

Otolith microchemistry is widely used as a tool to track individual migration pathways of diadromous fish under the assumption that the elemental composition of fish otoliths is directly influenced by the physicochemical properties of the surrounding water. Nevertheless, several endogenous factors are reported to affect element incorporation into fish otoliths and might lead to misinterpretations of migration studies. This study experimentally examined the influence of eight different diets on the microchemical composition of European eel (*Anguilla anguilla*) otoliths using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Seven natural prey types and one artificial diet were fed during eight weeks in freshwater circuits. Results show for the first time that food has no significant influence on the incorporation of Na, Sr, Ba, Mg, Mn, Cu and Y into European eel otoliths. This indicates that the incorporation of elements usually chosen for migration studies is not affected by diet and that individual feeding behaviour of *A. anguilla* will not lead to any misinterpretation of migration pathways.

Key words: *Anguilla anguilla*, otolith, microchemistry, diet, food effect, LA-ICPMS

Introduction

Otoliths, the calcified earstones of bony fish, are mainly composed of aragonite, otolin and different minor and trace elements. The aragonite structure can be locally substituted by vaterite (Strong *et al.* 1986; Tzeng *et al.* 2007). In recent years microchemical analyses primarily focusing on strontium (Sr) concentrations were frequently used to track individual migration pathways of wild caught eels (e.g. Tzeng *et al.* 1997; Tsukamoto *et al.* 1998; Limburg *et al.* 2003; Arai *et al.* 2004; Daverat *et al.* 2005; Jessop *et al.* 2006; Shiao *et al.* 2006). The correlation between Sr/calcium ratios and ambient salinities is well established and the Sr content in otoliths is comparatively high and therefore easy to measure. Element composition of biogenic aragonites is thought to depend mainly on physicochemical properties of the surrounding water (Campana 1999). Nevertheless, it has been repeatedly reported that endogenous factors such as diet might have an effect on the element compositions of fish otoliths (Limburg 1995; Farrell and Campana 1996; Gallahar and Kingsford 1996; Buckel *et al.* 2004) as well as cephalopod statoliths (Zumholz *et al.* 2006), since cations entering the inner ear endolymph via the blood circuit can originate either from branchial or intestinal uptake (Campana 1999). Previous investigations on the importance of food on element incorporation into otoliths were highly ambiguous. Walther & Thorrold (2006) concluded that Sr and barium (Ba) contents in marine fish otoliths clearly reflect ambient Sr and Ba concentrations, while Kennedy *et al.* (2000) reported 70% of the Sr isotopic signature in freshwater Atlantic salmon (*Salmo salar*) otoliths to originate from food. Significant dietary effects on Sr incorporation have been demonstrated for American shad (*Alosa sapidissima*) (Limburg 1995) and Black bream (*Girella elevata*) (Gallahar & Kingsford 1996) and for Sr and Ba incorporation in bluefish (*Pomatomus saltatrix*) (Buckel *et al.* 2004). No effects were detected for the uptake of magnesium (Mg), potassium (K), Sr, sodium (Na), and calcium (Ca) in Red drum (*Sciaenops ocellatus*) (Hoff and Fuiman 1995), for Sr, copper (Cu) and lead (Pb) in barramundi (*Lates calcarifer*) (Milton & Chenery 2001), for Na, Mg, K, Ca and manganese (Mn) in bluefish (*Pomatomus saltatrix*) (Buckel *et al.* 2004) and for Sr in Japanese eel (*Anguilla japonica*) (Lin *et al.* 2007a). Lin *et al.* (2007a) examined the influence of two different diets on Sr contents of *A. japonica* otoliths. It was shown that neither formulated feed nor tubifex had a measurable effect on Sr incorporation. This study performed a multi-element analysis to investigate the influence of a broad range of diets on the chemical composition of European eel (*Anguilla anguilla*) otoliths under fully controlled experimental conditions. In order to embrace the wide scope of eel nutrition, seven different limnic, brackish and marine species known as potential prey for eels in their natural environment and one artificial aquafeed were chosen as diets.

Different analytical methods are commonly used for microchemical studies of fish otoliths: Synchrotron X-ray fluorescence analysis (SYXRF) (Tsukamoto *et al.* 1998), particle induced x-ray emission (PIXE) (Elfman *et al.* 1999), electron microprobe analysis (EMPA) (Hoff and Fuiman 1995), solution based inductively coupled plasma mass spectrometry (ICPMS) (Buckel *et al.* 2004) and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) (Walther and Thorrold 2006). In the present study LA-ICPMS was chosen to measure feed-dependency on the incorporation of 16 elements because of its high precision in multi-element analysis and good spatial resolution.

Materials and Methods

Animal husbandry and experimental design

96 pigmented juvenile eels (*A. anguilla*) were obtained from a commercial fish farm (Fischzucht Reese; Sarlhusen, Germany). Before the start of the experiment, eels were acclimated to water conditions for eight weeks during which they were fed with commercial pellets (A 0.7 Perle Eel; Skretting; Stavanger, Norway) every second day. They were kept in a freshwater recirculation system in a temperature-controlled room at 20°C with a 12h/12h light regime. The system consisted of 32 plastic tanks (eight litres each) connected to a biofilter with a total volume of 60 litres. Inflow rates were adjusted to a three- to fourfold water exchange per day. Water lost by evaporation was refilled with freshwater every day. Shelter was provided in form of plastic tubes. Eels were divided into 32 groups of three individuals each. With eight different diets allocated to the 32 tanks, each treatment had four replicates. The experimental feeding period was set to 56 days. To document individual growth, each eel was marked with an individual code (visible implant elastomer system (Northwest Marine Technology Inc; Shaw Island, WA, U.S.A.)).

Experimental diets

Considering the facultative catadromous lifecycle of the European eel, fresh-, brackish and saltwater organisms were chosen as experimental diets (Table III-1). All food organisms were stored at –20°C and fed to the eels *ad libitum* in bite-sized pieces or as whole organisms once a day. Remaining food was removed every 24 hours.

As a piscine freshwater diet we chose roach (*Rutilus rutilus*) caught in Lake Dörp near Kiel, Germany, of which small pieces (approximately 0.5 cm^3) of filet were fed. Freshwater amphipods (*Gammarus pulex*) were chosen to represent a freshwater crustacean. Gammarids are considered a major contribution to the diet of small eels in freshwater habitats (Mann and Blackburn 1991). *G. pulex* was caught in River Eider (Kiel, Germany) and fed as a whole. As an

insect diet Chironomid larvae, obtained frozen (Claudia Erdmann GmbH; Ritterhude, Germany), were fed as a whole. Herring (*Clupea harengus)* represented a potential marine piscine diet of European eel. *C. harengus* were obtained frozen from a local commercial fishery (Wiese Eduard & Kruse Ivens GmbH; Kiel, Germany) and fed as small pieces (approximately 0.5 cm^3) of filet. Marine crustaceans were represented by Pacific krill *Euphausia superba* and mysids, both obtained frozen from an aquarium food manufacturer (Claudia Erdmann GmbH; Ritterhude, Germany) and fed as whole organisms. Brown shrimp (*Crangon crangon*), caught in the Kiel Bight, was chosen as a potential marine/brackish crustacean prey, due to the hydrological properties of the western Baltic. Small pieces (approximately 0.5 cm^3) of *C. crangon* were fed. Commercial pellets were fed as an artificial reference diet. Pellets (A 0.7 Perle Eel) were obtained from a fish feed producer (Skretting; Stavanger, Norway) and stored at 4°C.

Table III-1: Mean element concentrations ± standard deviation (SD) of different diets and their origin

Food origin	*Clupea harengus*[b] [saltwater]	*Mysis* sp.[b] [saltwater]	*Euphausia superba*[b] [saltwater]	*Crangon. crangon*[a] [brackish]	*Rutilus rutilus*[a] [freshwater]	Chironom. larvae[b] [freshwater]	*Gammarus pulex*[a] [freshwater]	Pellets[c] [undefined]	*N*
Ca (ppt)	7.2 ± 6.5	29.6 ± 29.1	24.0 ± 3.4	81.8 ± 11.0	27.2 ± 23.4	*2.0* ± 22.0	*112.6* ± 30.0	*31.5 ± 0.5*	3
Na (ppt)	*1.9* ± 0.8	4.9 ± 1.2	9.7 ± 7.1	*13.2* ± 2.6	3.6 ± 0.5	12.4 ± 0.2	4.5 ± 0.2	*11.7 ± 0.2*	3
Mg (ppt)	*1.6* ± 0.1	3.1 ± 0.1	*4.8* ± 0.7	3.1 ± 0.2	2.2 ± 0.2	2.1 ± 0.0	2.8 ± 1.9	*2.6 ± 0.1*	3
Sr (ppm)	13.9 ± 9.0	344.8 ± 24.2	349.7 ± 76.5	*918.1* ± 63.4	21.4 ± 18.1	4.7 ± 0.1	331.5 ± 265.6	*95.4 ± 1.2*	3
Zn (ppm)	*43.1* ± 14.5	88.3 ± 1.5	68.4 ± 24.1	147.7 ± 72.4	94.6 ± 27.0	96.1 ± 0.1	81.3 ± 6.7	*268.1 ± 5.6*	3
Mn (ppm)]	*4.5* ± 3.5	90.2 ± 8.9	11.7 ± 1.0	45.9 ± 13.7	5.2 ± 2.8	64.3 ± 0.8	*140.7* ± 55.0	*52.6 ± 2.3*	3
Ba (ppm)	*1.0* ± 0.6	42.7 ± 4.1	8.4 ± 1.1	17.6 ± 5.0	5.0 ± 2.1	12.8 ± 0.3	*85.9* ± 42.5	*11.4 ± 0.9*	3
Cu (ppm)	4.8 ± 1.9	29.5 ± 4.3	59.2 ± 9.4	47.9 ± 9.3	*2.3* ± 0.5	12.8 ± 0.3	*72.4* ± 10.3	*13.0 ± 0.6*	3
Rb (ppm)	*1.1* ± 0.7	3.6 ± 1.2	2.2 ± 1.5	6.6 ± 0.4	*20.1* ± 4.7	14.0 ± 0.2	4.2 ± 1.2	*2.5 ± 0.1*	3
Y (ppb)	6 ± 4	170 ± 4	163 ± 29	128 ± 74	*3* ± 1	48 ± 6	*612* ± 83	*81 ± 5*	3

Concentrations were determined by solution ICPMS and represent weight fractions of the freeze-dried samples prior to dissolution

[a] Caught and immediately frozen

[b] Bought frozen

[c] A 0.7 Perle Eel, Skretting

Lowest and highest element concentrations are italicised

Prey and water analyses

Prey analyses were made by solution ICPMS at the Institute of Geosciences, University of Bremen, using a Thermo Element2™. Organisms and fish pieces used for feeding were freeze-dried and pulverised. Between 100 and 200 mg of dry samples were mixed with 10 ml of concentrated ultrapure-grade nitric acid in PTFE digestion vessels. Samples were heated to 200°C within 10 min in a MLS Ethos™ microwave and kept at 200°C for another 20 min prior to pressure digestion. After cooling, the sample solutions were filled with deionized water to 35 ml. For ICPMS analyses the solutions were diluted (1:20) and spiked with indium used as internal standard. A PEEK cyclonic spray chamber with a micro-flow nebulizer operating in self-aspirating mode was used for sample introduction. Mass interferences were avoided by measuring ^{23}Na,

^{25}Mg, ^{55}Mn, ^{63}Cu and ^{66}Zn at medium resolution (4,000) and all other elements (^{7}Li, ^{43}Ca, ^{85}Rb, ^{86}Sr, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{111}Cd, ^{138}Ba, ^{208}Pb, ^{238}U) at low (300) resolution. Internal precision as expressed by the relative standard deviation of nine analytical passes was typically less than 4% for concentrations above 0.1 $\mu g g^{-1}$ and increased to 13% for lower concentrations.

Water analyses were carried out at the beginning and at the end of the experiment by ICP-AES (inductively coupled plasma atomic emission spectroscopy; Institute of Geosciences, University of Bremen) for ^{43}Ca, ^{23}Na, ^{25}Mg and ^{86}Sr and by ICP-MS for all other elements (^{7}Li, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{111}Cd, ^{133}Cs, ^{138}Ba, ^{208}Pb, ^{238}U).

Otolith processing and elemental analyses

Sagittal otoliths were dried in air and embedded in thermo-epoxy (Buehler; Düsseldorf, Germany). Embedded otoliths were polished from the proximal side until the core was exposed. LA-ICPMS analyses were performed with a NewWave UP193 solid-state laser coupled to a ThermoFinnigan Element2™ at the Institute of Geosciences, University of Bremen.

The analytical setup provided the determination of 16 isotopes (^{7}Li, ^{23}Na, ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{111}Cd, ^{138}Ba, ^{208}Pb, ^{238}U) recorded along a transect of 600 µm length at the anterior edge of the proximal side of the otolith (Figure III-1). Prior to measurement, the transect was preablated at 100 $\mu m s^{-1}$ scan speed and a spot size of 120 µm in order to clean the surface. The analyses were performed at 3 $\mu m s^{-1}$ scan speed and a spot size of 75 µm with a pulse rate of 10 Hz and an irradiance of approximately 1 $GW cm^{-2}$. Helium was used as sample gas (0.4 $L min^{-1}$) and Argon was subsequently added (0.8 $L min^{-1}$) to the gas flow.

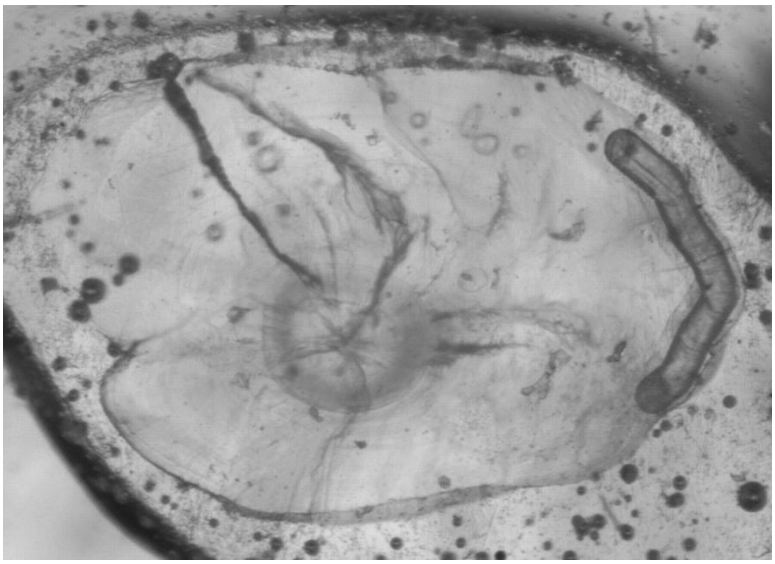

Figure III-1: Photograph of a polished eel otolith after LA-ICPMS. The laser groove is located at the outer edge of the anterior side of the otolith

Following every 2nd transect a glass reference material (NIST612) was measured as external calibration standard. For quantification the concentrations of Pearce *et al.* (1997) were selected. GeoPro™ software was used for quantification. Prior to ablation a blank of 20 seconds duration was measured and subtracted from a signal period of approximately 200 seconds. As internal

standard we used Ca with an assumed concentration of 38.8 wt% (similar to the NIES22 otolith standard; National Institute for Environmental Studies, Yoshinaga *et al.* 2000). Our data indicate a precision of better than 3% for concentrations above 0.5–1 $\mu g g^{-1}$ and up to 13% for concentrations between 0.01 and 0.5 $\mu g g^{-1}$.

As recently reported, the local formation of vaterite instead of aragonite can affect element incorporation into eel otoliths (Tzeng *et al.* 2007). To avoid the use of data collected in vaterite structures, examined concentrations of Sr, Na, Ba, Mg and Mn were checked for values characteristically found in vaterite as described in Tzeng *et al.* (2007).

Statistical analyses

Element/Ca ratios of otoliths were calculated and values were averaged for each specimen. We compared otolith element/Ca ratios using analysis of variance (*ANOVA*) to test for the hypotheses of no overall effect of diet on element incorporation into otolith aragonite followed by Tukey's *HSD* multiple comparison tests. In case variances were not distributed homogeneously among the factor levels, a Kruskal-Wallis *H t*est was used. The possible influence of growth rate on element incorporation was tested with a regression analysis. A significance level of $p \leq 0.05$ was used for all tests.

Results

Prey composition and water chemistry

Element concentrations differed largely among different prey types. Results showed a wide range of element concentrations covered by the different preys. Lowest and highest element concentrations are highlighted in table III-1.

Table III-2: Water element/calcium ratios at beginning and end of the feeding period (means ± SD (mol)) determined by solution ICPMS or ICP-AES

Element		beginning	end	*N*	method
Na/Ca	(mol mol^{-1})	0.714 (± 0.005)	0.334 (± 0.002)	5	ICP-AES
Mg/Ca	(mol mol^{-1})	0.385 (± 0.005)	0.182 (± 0.002)	5	ICP-AES
Sr/Ca	(mmol mol^{-1})	4.604 (± 0.026)	2.498 (± 0.015)	5	ICP-AES
Ba/Ca	(mmol mol^{-1})	0.399 (± 0.002)	0.224 (± 0.001)	5	ICP-AES
Cu/Ca	(µmol mol^{-1})	34.277 (± 0.524)	68.929 (± 0.565)	5	ICPMS
Zn/Ca	(µmol mol^{-1})	5.161 (± 0.092)	255.73 (± 1.43)	5	ICPMS
Rb/Ca	(µmol mol^{-1})	18.548 (± 0.177)	9.367 (± 0.082)	5	ICPMS
Mn/Ca	(µmol mol^{-1})	6.374 (± 0.02)	4.430 (± 0.297)	5	ICPMS
Y/Ca	(nmol mol^{-1})	74.227 (± 1.418)	43.307 (± 0.466)	5	ICPMS

Element concentrations in rearing water were quantified at the beginning and at the end of the experiment. All analysed elements except Nb and Cd could be measured in water samples (^{7}Li,

^{23}Na, ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{89}Y, ^{90}Zr ^{133}Cs, ^{138}Ba, ^{238}U). No significant changes in the concentrations of most elements were detected, except for Ca which had doubled throughout the experiment and Zn which had changed strongly. Most of the differences found in table III-2 are caused by the increase in Ca content, since values are expressed as element/Ca ratios.

Otolith analyses

Eels fed with *Gammarus pulex* and *Euphausia superba* did not grow at all or even lost weight during the eight weeks of feeding and were therefore excluded from further analyses.

We were able to quantify the following elements in the eel otoliths: ^{23}Na, ^{25}Mg, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{89}Y and ^{138}Ba. For some unknown reason Rb values scattered over a much wider range than other elements and had to be removed from further statistical analyses. Zn had to be excluded as well, since Zn concentrations differed widely along single transects, altering between high and low content regions without any hint of a biological cause.

^{23}Na, ^{25}Mg, ^{55}Mn, ^{65}Cu, ^{88}Sr, ^{89}Y and ^{138}Ba concentrations did not differ between feeding groups (*p* ranged from 0.14 for Mn/Ca to 0.92 for Y/Ca) (Table III-3, III-4, Figure III-2).

Effects of growth rate on element incorporation could not be observed for any of the analysed elements (*p* ranged from 0.251 for Na/Ca to 0.857 for Sr/Ca) (Table III-5).

Table III-3: Mean element/calcium ratios ± SD (mol) in otoliths of different feeding groups determined by LA-ICPMS

Food	*Clupea harengus*	*Mysis* sp.	*Crangon crangon*	*Rutilus rutilus*	Chironomidae larvae	Pellets	*N*
Na/Ca (mmol mol^{-1})	11.26 ± 1.21	11.11 ± 0.79	10.93 ± 1.15	10.99 ± 1.10	11.38 ± 1.16	10.81 ± 1.32	12
Sr/Ca (mmol mol^{-1})	0.65 ± 0.14	0.63 ± 0.17	0.66 ± 0.13	0.60 ± 0.16	0.69 ± 0.32	0.59 ± 0.13	12
Mg/Ca (µmol mol^{-1})	44.71 ± 23.01	44.23 ± 16.07	37.24 ± 16.89	69.20 ± 83.25	60.28 ± 35.58	48.35 ± 17.20	12
Ba/Ca (µmol mol^{-1})	1.26 ± 0.44	1.42 ± 0.40	1.07 ± 0.31	1.15 ± 0.36	1.30 ± 0.54	1.45 ± 0.46	12
Mn/Ca (µmol mol^{-1})	1.70 ± 0.57	2.29 ± 0.74	1.82 ± 0.48	1.45 ± 0.41	2.25 ± 1.32	2.21 ± 1.31	12
Cu/Ca (µmol mol^{-1})	0.12 ± 0.06	0.08 ± 0.05	0.15 ± 0.09	0.09 ± 0.05	0.11 ± 0.08	0.16 ± 0.14	12
Y/Ca (nmol mol^{-1})	3.33 ± 2.00	3.74 ± 1.79	3.79 ± 1.33	3.12 ± 1.95	3.60 ± 2.01	3.29 ± 1.18	12

Discussion

Our results demonstrate that diet has no significant effect on the incorporation of trace and minor elements into European eel otoliths. Although element composition of food items differed widely (Table III-1) no significant food effect on otolith microchemistry was detected. Na, Sr, Mg, Mn, Ba, Cu and Y maintained rather constant concentrations across all feeding groups (Table III-3, Figure III-2).

Significant effects on Sr and Ba uptake into otoliths were found in bluefish, *Pomatomus saltatrix* (Buckel *et al.* 2004) fed on fish versus shrimp diets. Contents of Sr and Ba reached values from 110.4 ppm Sr and 0.996 ppm Ba in fish to 421.6 ppm Sr and 3.523 ppm Ba in shrimp diet. In our study Sr contents in the selected prey organisms ranged from 4.7 ppm in chironomidae larvae to 918.1 ppm in *C. crangon*, Ba contents from 1.0 ppm in herring to 42.7 ppm in mysids (Table III-1). Surprisingly, except for bluefish (Buckel *et al.* 2004), no other published study that successfully detected dietary effects also compared element content of diets.

The absence of any significant dietary effect in our experiment and the conflicting results from similar investigations on the influence of natural and artificial prey on otolith microchemistry in salt- and freshwater suggest species specific physiological processes to be responsible for interspecies differences of element uptake into otoliths. The uptake of elements into otoliths strictly depends on the element composition of the endolymph, an acellular medium, which is secreted by the inner ear epithelium (Payan *et al.* 2004a). Active element and ion discrimination at several barriers along the way from the environment to the endolymph causes a decoupling of otolith increment from ambient conditions or food composition (Payan *et al.* 2004; Campana 1999). Food-carried elements are selected at intestine/blood, blood/inner ear epithelium as well as inner ear epithelium/endolymph barriers (Payan *et al.* 2004). Selective processes like cellular transport and crystallisation additionally modify element concentrations (Campana 1999). Precise species specific knowledge about physiological processes at these barriers is required to fully understand the interspecific differences of element uptake into otoliths as recently reported by Hamer & Jenkins (2007) for the seabream *Pagrus auratus* and the sand flathead *Platycephalus bassensis*.

Table III-4: Results of analysis of variance (*ANOVA*) and Kruskal-Wallis *H* test summarising the effect of six different diets on otolith element/calcium ratios

	df	*F*	*H*	*p*
Na/Ca	5	0.39	-	0.856
Sr/Ca	5	-	5.49	0.359
Mg/Ca	5	-	6.74	0.241
Ba/Ca	5	1.52	-	0.196
Mn/Ca	5	-	8.34	0.138
Cu/Ca	5	-	7.22	0.205
Y/Ca	5	0.29	-	0.914

Table III-5: Results of regression analysis summarising the effect of specific growth rate (% weight day^{-1}) on otolith element/calcium ratios

	R^2	*p*	*N*
Sr/Ca	0.0005	0.857	72
Ba/Ca	0.0020	0.707	72
Mn/Ca	0.0102	0.399	72
Mg/Ca	0.0079	0.457	72
Na/Ca	0.0188	0.251	72
Cu/Ca vs	0.0142	0.320	72
Y/Ca	0.0148	0.308	72

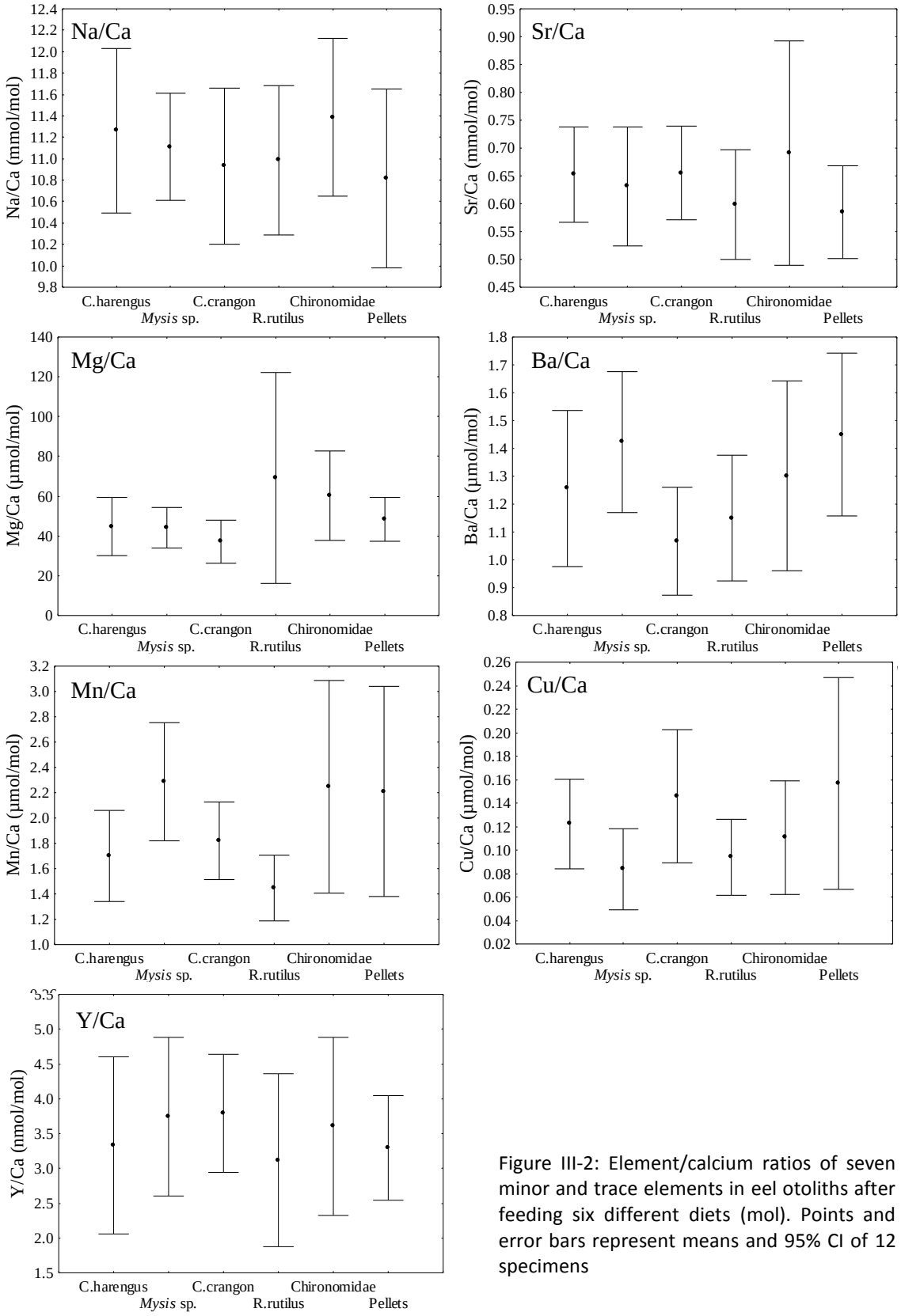

Figure III-2: Element/calcium ratios of seven minor and trace elements in eel otoliths after feeding six different diets (mol). Points and error bars represent means and 95% CI of 12 specimens

Our experimental setup intended to exclude every potential influence on otolith chemistry except feeding and we assume food to be the only variable between feeding groups. A laser

diameter of 75 µm was chosen to ensure measurements within the otolith increments grown during the experiment. Umezawa & Tsukamoto (1991) reported a daily growth rate of about 1.97 µm (±0.433) day^{-1} in *A. japonica* elver otoliths. Assuming a similar growth rate in *A. anguilla,* otolith increments would amount to 86 and 135 µm during the experiment. Water temperature and chemistry were equal for all treatments and growth rate had no effect on element incorporation (Table III-5). The circulation system and the high water exchange rate of three to four per day provided constant and equal water conditions for each treatment. Concentrations of most elements in water remained largely constant during the experiment, except for Ca and Zn (Table III-2). The use of tap water to replace evaporation loss could have caused a notable enrichment of these elements, but cannot fully explain the extreme increase of Zn. Nevertheless, we consider the changes of these two elements to have no impact on overall results since all treatments were carried out in the same circuit. Changes in water composition affect all treatments in the same way and food still remains the only variable between feeding groups. Therefore, Zn was removed from otolith analyses due to strong variations of concentrations within the same otolith increments.

We conclude feeding behaviour not to substantially contribute to otolith element composition in *A. anguilla* and consequently to have no disturbing effect on tracking migration through microchemical analyses, since widely used proxy elements like Sr or Ba remained unaffected by diet even at high resolution analytics ensured through LA-ICPMS measurements. Nevertheless, additional investigations are required to further unambiguously exclude effects on element incorporation into eel otoliths caused by exogenous and endogenous variables like temperature, stress and growth. The continuous enhancement of analytic methods like e.g. the detection of isotopic signatures with multi collector LA-ICPMS (Fietzke *et al.* 2008) might help to gain further information about environmental influences on otolith microchemistry.

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CHAPTER IV Evaluating the suitability of otolith microchemistry for stock separation of Baltic cod (*Gadus morhua*)

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Abstract

Microchemical otolith analysis provides information on life history, dispersal, migration and stock characteristics of teleost fish species. The present study examined the suitability of otolith microchemistry of Atlantic cod *Gadus morhua* L. to identify the origin of individuals from Baltic Sea spawning grounds. Specimens from one western and three eastern Baltic spawning grounds and from one spawning ground in the North Sea were compared according to the chemical composition of otoliths using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Since the Baltic Sea is a brackish water system with a wide range of environmental parameters, we expected otolith elemental composition to leave characteristic fingerprints according to the ambient environmental condition of individual fish. Additionally, elemental fingerprints from the core region of juvenile cod otoliths of two different Baltic Sea regions were compared. The main result from this study is that microchemical analyses of Baltic cod otoliths are applicable to differentiate between individuals of different stocks. Despite the good discrimination between North Sea, western Baltic and eastern Baltic cod, a separation of the three eastern Baltic spawning grounds was not possible. However, the detection of consistent differences in otolith elemental fingerprints between cod stocks in adults and juveniles shows the potential of this method to provide more information about migration behaviour and stock connectivity of *G. morhua* in the Baltic Sea.

Keywords: *Gadus morhua*, Baltic Sea, otolith microchemistry, stock discrimination

Introduction

The Atlantic cod (*Gadus morhua)* is distributed over almost the entire Baltic Sea. Genotypic and phenotypic characteristics indicate a separation into a western (ICES subdivisions (SD) 22-24) and an eastern stock (SD 25-32), only overlapping in a relatively narrow zone around the island of Bornholm (Bagge *et al.* 1994; Nielsen *et. al.* 2001, 2003).

Both stocks are subject to large fluctuations in spawning stock biomass and recruitment (Hüssy 2011; Eero *et al.* 2011). Beside the influence of fisheries, the eastern Baltic stock dynamics appear to be mainly driven by changes in salinity and oxygen, due to a special set of environmental conditions required for successful spawning (MacKenzie *et al.* 2000). Historically, there have been three main spawning areas for the eastern Baltic stock: the Bornholm Basin, the Gdansk Deep and the Gotland Basin. Analyses of the spatial and temporal heterogeneity of the spawning environment have recognised that beneficial conditions for egg survival are most likely to be found in the Bornholm Basin (MacKenzie *et al.* 2000). Due to a lack of oxygen combined with low salinities, the more eastern spawning grounds in the Gdansk Deep and Gotland Basin do often not provide environmental conditions required for egg survival, especially since the early 1980's (Köster *et al.* 2005; Nissling *et al.* 1994). As obtained from egg abundances (Hinrichsen *et al.* 2007) and single fish detection data obtained from hydroacoustics (Schaber *et al.* 2009), a clear preference for spawning of eastern Baltic cod at locations in the deep basins below the permanent halocline was observed, while preference for spawning in well-oxygenated water masses above the halocline was not detected. Oxygen levels below the halocline can become extremely low due to aerobic metabolism and the decomposition of organic matter sinking down from the surface layer (Stigebrandt & Wulff 1987) resulting in high egg mortalities (Hinrichsen *et al.* 2007). For the western Baltic cod the number of spawning grounds is less limited. Good spawning conditions are found in several areas from the Kattegat to the Arkona Basin (Hüssy 2011). In contrast to the eastern Baltic cod the western stock is assumed to be less influenced by hydrographic conditions, but more by the proportion of female spawners and the abundance of clupeid prey (Hüssy 2011).

Baltic cod is distributed over a large area and may perform wide and variable feeding and spawning migrations through different environments (Otterlind 1985). The younger age groups are usually found in coastal areas (Bagge & Steffensen 1989), while horizontal movements of adult individuals in the distribution area are not clearly directed and could be seen as random migrations (Bagge *et al.* 1974). In the transition zone some exchange between both stocks occurs. Tagging studies revealed that individuals from the Arkona Basin can undertake long eastward migrations (Otterlind 1985) and a genetic study suggests that even interbreeding

between both stocks occurs in that area (Nielsen *et al.* 2003). However, to what extent migrations and interbreeding contribute to the mixing between stocks remains to be quantified. A successful management of Baltic cod stocks requires knowledge about their connectivity and about the exchange between North Sea and the western Baltic Sea stocks. Furthermore, an identification of natal origins of individuals would allow a quantification of the contribution of each spawning ground to the spawning stock biomass and would improve the understanding of stock dynamics.

Otolith microchemistry analysis provides a promising tool to answer some of these questions. It was used in many studies to separate between fish stocks (e.g. for pink snapper *Chrysophrys auratis*: Edmonds *et al.* 1989; orange roughy *Hoplostethus atlanticus*: Edmonds *et al.* 1991; jackass morwong *Nemadactylus macropterus*: Thresher *et al.* 1994; Spanish mackerel *Scomberomorus* spec.: Begg *et al.* 1998) including the Atlantic cod (Campana *et al.* 2000; Higgins *et al.* 2010). The salinity gradient within the Baltic Sea and the strong regional influence of river discharge on the water composition (e.g. Andersson *et al.* 1992; Wachniew 2006; Maksymowska *et al.* 2000) provide appropriate conditions for the incorporation of site specific elemental fingerprints into otoliths from different Baltic stocks.

The objective of the present study was to test the potential of otolith microchemistry to discriminate between cod stocks by analysing the multi-element composition of otoliths from adult individuals caught in the North Sea, the western Baltic Sea as well as the three eastern Baltic spawning grounds using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Additionally, the elemental composition in the core regions of juvenile cod otoliths from the eastern and the western Baltic Sea were examined.

Materials and Methods

Sample material and preparation

84 sagittal otoliths from adult *G. morhua* and 20 otoliths from juvenile specimens were sampled. Adult individuals were caught in the first half of 1998 in different regions of the Baltic Sea and at one North Sea station (Figure IV-1, Table IV-1). Removed otoliths had been stored in paper bags until preparation. 11 juvenile cod otoliths from the western Baltic and 9 from the Bornholm Basin were sampled (Table IV-1).

Adult cod otoliths were embedded in a mixture of GTS polyester casting resin and MEKP-hardener (both Voss Chemie GmbH; Uetersen, Germany) and thin-sectioned across the center of the otolith by usage of a half-automated mineralogy sawing machine (Conrad; Clausthal-Zellerfeld, Germany). Cross sections and juvenile otoliths were mounted to glass slides with

thermoplastic glue (Crystalbond Type 509, Kager; Dietzenbach, Germany) and subsequently polished with lapping film (30, 12 and 3 µm, 3M; Neuss, Germany) until the core was exposed.

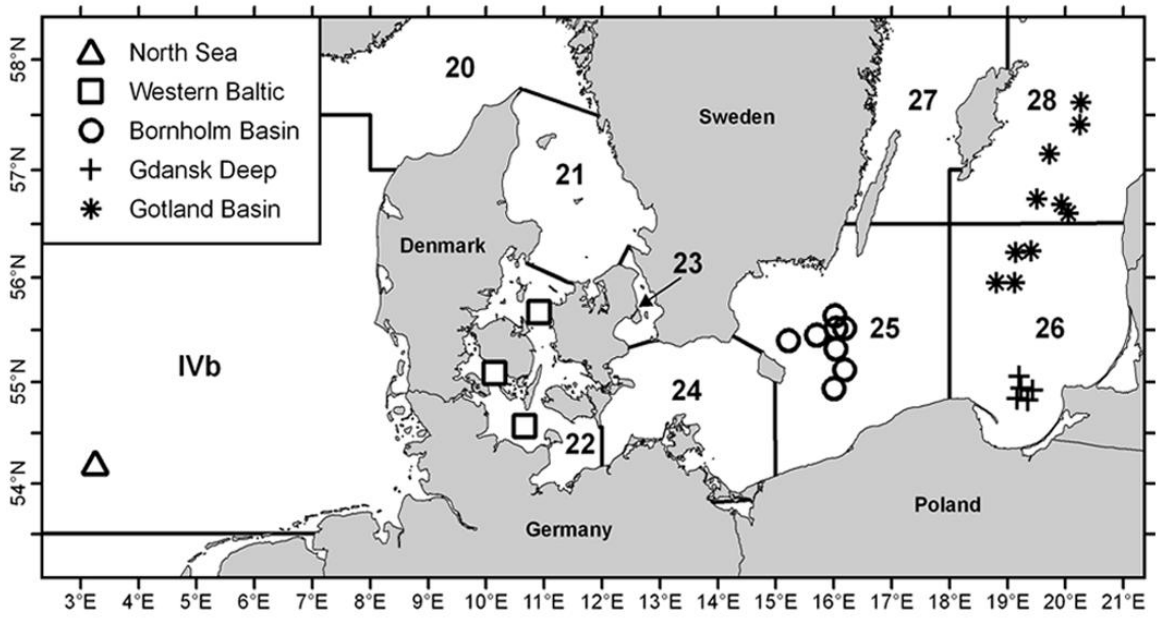

Figure IV-1: Map of sampling areas. Numbers represent ICES subdivisions

Table IV-1: Sampling data. *N* = Number of samples, TL = Total length of sampled individuals; NS = North Sea, WB = western Baltic, BB = Bornholm Basin, GD = Gdansk Deep, GB = Gotland Basin, SD = ICES Subdivision

	Region	Catch date	*N*	TL (cm)	
				Range	Mean
Adults	NS	Feb 1998	13	30 - 49	35.7
	WB	Jan – Mar 1998	19	51 - 60	55
	BB	May 1998	24	46 - 76	53
	GD	May 1998	9	50 - 61	54.2
	GB	May 1998	19	48 - 67	56
Juveniles	SD 22 (WB)	Jun 1998	11	2.6 – 4.4	3.5
	SD 25 (BB)	Nov 1998	9	3.4 – 4.5	3.8

Otolith analysis

Concentrations of trace elements were determined by LA-ICPMS using a NewWave UP193 solid-state laser coupled to a ThermoFinnigan Element2™ at the Department of Geosciences, University of Bremen. Element composition in adult cod otoliths was measured along transects from the core to the dorsal edge of the otolith sections (Figure IV-2). Otoliths were ablated with an irradiance of ca. 1 GW cm^{-2}, a pulse rate of 10 Hz, a spot size of 75 µm and a line scan speed of 4 μms^{-1}. Otoliths of juveniles were ablated in the core region for 60 s with a pulse rate of 5 Hz

and a spot size of 50 µm. A preablation was carried out prior to every measurement in order to clean the surface. Helium was used (0.4 $Lmin^{-1}$) as sample gas and argon (0.8 $Lmin^{-1}$) as make-up gas. Plasma power was 1200 W. 16 isotopes (lithium (Li), sodium (N), magnesium (Mg), calcium (Ca), manganese (Mn), copper (Cu), zinc (Zn), rubidium (Rb) strontium (Sr), yttrium (Y), zirconium (Zr), niobium (Nb), cadmium (Cd), barium (Ba) lead (Pb) and uranium (U)) were analysed at low resolution with five samples in a 20% mass window and a total dwell time of 50 ms per isotope. Blanks were measured during 20 s prior to ablation.

For external calibration the NIST612 standard reference material (SRM), a Na silicate glass, was analysed after each transect (standard bracketing). Ca was used as internal standard with an assumed concentration of 38.8 wt% for the otoliths (similar to the NIES22 otolith SRM; Yoshinaga *et al.* 2000). For data quantification the Cetac GeoPro™ software was used with the concentrations for NIST612 of Pearce *et al.* (1997). The Mg concentration provided by these authors (77.4 $\mu g g^{-1}$), however, significantly differed from the newly determined value of 68 $\mu g g^{-1}$ (Jochum *et al.* 2011).

Figure IV-2: Ablation line after LA-ICPMS measurements along the growth axis of an adult cod otolith

The data quality was assessed by repeated analyses of a pressed pellet of NIES22 otolith powder (Table IV-2) and of BCR2G basaltic glass (United States Geological Survey). For Na, Mg, Sr, Ba, Cu and Zn there is good to excellent agreement with the certified values, which indicates that NIST612 is well suited as a calibration standard for carbonate analyses. The accuracy for Mg improves significantly if the Jochum *et al.* (2011) rather than Pearce *et al.* (1997) value for NIST612 is used for calibration. Because the variations of our NIES22 analyses include heterogeneities within the pellet, the actual analytical precision of the laboratory setup is better than the relative standard deviations shown in table IV-2; based on our BCR2G data the overall precision is better than 5% for most elements at concentrations above 0.5-1 $\mu g g^{-1}$.

Table IV-2: Average and relative standard deviation (RSD) of nine analyses of a pressed pellet from NIES22 otolith powder conducted in 2008 through 2009. Reference values are from Yoshinaga *et al.* (2000); the Mg value in parentheses is obtained when using the Jochum *et al.* (2011) rather than the Pearce *et al.* (1997) value for calibration

	Na	Mg	Cu	Zn	Sr	Ba	Pb
Average [µg/g]	2270	25.8 (22.7)	0.796	0.531	2302	2.7	0.042
RSD [%]	5.1	14	30	23	2.8	2.6	46
Reference Value [µg/g]	2230	21	0.74	0.47	2360	2.89	0.023

Statistical analysis

Statistical evaluation was conducted using the software STATISTICA (Version 6.1, StatSoft Inc. 2003; Hamburg, Germany) and PRIMER 6 (Version 6.1.9, PRIMER-E Ltd., Plymouth, UK (Clarke & Gorley 2001)).

For statistical analysis, transect data of every otolith from adult cod were combined to mean values for single elements. Means were grouped according to the sampling region (NS, WB, BB, GD, GB) and tested in univariate analyses of variance (*ANOVA*) for every single element. In order to assign differences between sampling regions *ANOVA* was followed by Tukey's *HSD* multiple comparison tests. In case variances were not distributed homogeneously among factor levels a Kruskal–Wallis *H* test was performed. *ANOVA* and Kruskal-Wallis *H* test were conducted using a Bonferroni corrected level of significance (p=0.004).

Combination of transect means of all analysed elements form an elemental fingerprint of the otolith or the respective individual. To test whether these elemental fingerprints differ significantly between sampling areas four-root transformed data were used to perform an analysis of similarities (*ANOSIM*) (significance level p=0.05). In addition, a discriminant analysis was conducted using the transformed data to evaluate the contribution of single elements to the differentiation between sampling areas (significance level p=0.05). For graphical representation a *MDS* plot (Non-metric Multidimensional Scaling) was created on basis of a Bray-Curtis-similarity matrix.

Differences of element concentrations of juvenile cod from the western and eastern Baltic Sea were analysed using *t*-tests (Bonferroni corrected level of significance p=0.004). Differences in otolith elemental fingerprints of juveniles were visualized by a *MDS* plot.

Results

Elemental fingerprints of adult cod

An overview of mean values of all measured otolith element/calcium (El/Ca) ratios is presented in table IV-3, separated according to sampling areas. Elements with concentrations below

specific detection limits were obtained for Cd, Nb and U. Therefore these three elements were excluded from statistical evaluation.

Highly significant differences between sampling areas were found for all elements (Table IV-4). Individual elemental fingerprints including 12 representative elements (Li, Na, Mg, Mn, Cu, Zn, Rb, Sr, Y, Zr, Nb and Ba) are visualized by the *MDS* plot in figure IV-3. Samples from the North Sea are divided from all other samples and the western Baltic samples are separated from the cluster of eastern Baltic samples. *ANOSIM* revealed significant differences between sampling areas (global R=0.574, $p<0.001$). Pairwise a-posteriori tests showed significant differences based on elemental composition between the North Sea samples and all other stations (Table IV-5) and between western Baltic and the others. Within the eastern Baltic only Bornholm Basin and Gotland Basin differed significantly.

Table IV-3: Mean element/calcium values of adult cod otoliths ± standard deviation for different sampling areas. NS = North Sea, WB = western Baltic, BB = Bornholm Basin, GD = Gdansk Deep, GB = Gotland Basin

	NS	WB	BB	GD	GB
Na/Ca (mmol/mol)	16.98 (±0.58)	17.44 (±1.01)	16.52 (±0.75)	16.10 (±0.44)	16.13 (±0.67)
Sr/Ca (mmol/mol)	2.38 (±0.28)	2.18 (±0.22)	1.70 (±0.21)	1.54 (±0.11)	1.45 (±0.14)
Mg/Ca (µmol/mol)	161.89 (±22.40)	130.53 (±13.62)	129.34 (±21.45)	129.28 (±16.01)	123.35 (±14.65)
Mn/Ca (µmol/mol)	13.65 (±7.89)	37.56 (±22.52)	24.00 (±7.94)	21.37 (±6.45)	27.10 (±12.75)
Ba/Ca (µmol/mol)	2.21 (±0.60)	4.88 (±1.14)	6.80 (±1.56)	6.70 (±1.67)	6.60 (±1.16)
Li/Ca (µmol/mol)	8.40 (±5.00)	2.57 (±0.97)	1.31 (±0.88)	1.50 (±0.52)	1.67 (±0.84)
Zn/Ca (µmol/mol)	1.21 (±0.30)	2.72 (±0.91)	2.00 (±0.97)	2.01 (±0.78)	1.41 (±0.61)
Cu/Ca (µmol/mol)	0.53 (±0.20)	2.19 (±0.81)	1.48 (±1.27)	1.99 (±1.36)	0.88 (±0.60)
Rb/Ca (µmol/mol)	0.23 (±0.03)	0.41 (±0.08)	0.34 (±0.12)	0.44 (±0.15)	0.29 (±0.07)
Zr/Ca (nmol/mol)	15.93 (±6.70)	42.23 (±29.01)	112.94 (±96.60)	59.20 (±88.55)	95.06 (±88.79)
Pb/Ca (nmol/mol)	6.43 (±3.46)	46.20 (±30.57)	36.34 (±29.77)	24.42 (±17.14)	22.12 (±17.93)
Y/Ca (nmol/mol)	29.41 (±3.74)	20.64 (±2.47)	16.98 (±3.52)	20.47 (±1.70)	18.43 (±1.93)
Cd/Ca (nmol/mol)	13.29 (±12.15)	14.73 (±14.70)	20.73 (±18.52)	21.07 (±21.43)	19.43 (±11.22)
Nb/Ca (nmol/mol)	2.40 (±0.47)	0.50 (±0.96)	0.22 (±0.84)	1.43 (±0.29)	0.70 (±0.66)
U/Ca (nmol/mol)	0.22 (±0.24)	0.10 (±0.04)	-0.01 (±0.25)	0.08 (±0.07)	0.07 (±0.02)

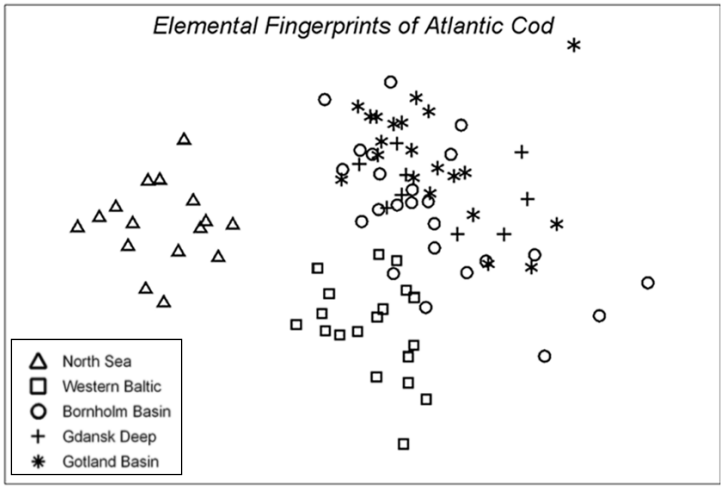

Figure IV-3: *MDS* plot of otolith elemental fingerprints of adult cod. Every data point represents one elemental fingerprint including 12 El/Ca ratios of the respective otolith

According to the discriminant analysis Sr, Ba, Y, Mg, Zr and Mn had the strongest influence on the differentiation between the sampling areas (Table IV-4). Tukey's *HSD* multiple comparison tests revealed that concentrations of these elements differ between North Sea samples and some Baltic Sea sampling areas (Figure IV-4). Furthermore, Sr and Ba concentrations diverge between western and eastern Baltic samples and Sr and Y differ at least between two of the three eastern Baltic areas.

Elemental fingerprints of juvenile cod

In case of core concentrations of juvenile cod otoliths Pb, Nb and U fluctuated strongly around zero. These three elements were therefore excluded from statistical analyses. Results of *t*-tests between otoliths from the western Baltic Sea (SD 22) and those from the Bornholm Basin (SD 25) are listed in table IV-4 for each element. The core concentrations of juvenile cod otoliths differed

Table IV-4: Results of *ANOVA* (*F* test) and Kruskal-Wallis *H* test, summary of discriminant analysis in otoliths of adult cod among sampling areas and results of *t*-tests for El/Ca ratios in core region of juvenile cod otoliths. Significant results are italicized

	ANOVA & Kruskal-Wallis *H*-Test				discriminant analysis		T-test juvenile cod		
	F	*H*	*p*	*df*	*F*	*p*		*T*-value	*p*
Sr/Ca	-	66.61	*0.000*	4	40.09	*0.000*	Sr/Ca	3.60	*0.002*
Ba/Ca	-	52.69	*0.000*	4	27.66	*0.000*	Ba/Ca	-4.49	*0.000*
Y/Ca	-	51.78	*0.000*	4	14.86	*0.000*	Y/Ca	1.98	0.063
Mg/Ca	11.92	-	*0.000*	4	6.98	*0.000*	Mg/Ca	3.83	*0.001*
Zr/Ca	-	37.30	*0.000*	4	6.28	*0.000*	Zr/Ca	-0.77	0.452
Mn/Ca	-	32.14	*0.000*	4	5.66	*0.001*	Mn/Ca	2.24	0.038
Na/Ca	9.94	-	*0.000*	4	4.09	*0.005*	Na/Ca	2.22	0.039
Rb/Ca	-	41.72	*0.000*	4	2.16	0.082	Rb/Ca	1.28	0.217
Cu/Ca	-	40.00	*0.000*	4	1.57	0.193	Cu/Ca	-1.36	0.192
Li/Ca	-	53.57	*0.000*	4	1.31	0.273	Li/Ca	-1.01	0.325
Pb/Ca	-	36.58	*0.000*	4	0.86	0.490	Cd/Ca	1.81	0.088
Zn/Ca	-	31.65	*0.000*	4	0.34	0.851	Zn/Ca	-0.24	0.812

significantly between sampling areas for Ba, Mg and Sr (Table IV-4, Figure IV-5). Other elements showed no significant differences when Bonferroni corrected level of significance was used. Elemental fingerprints of juvenile cod, including all 12 El/Ca ratios, were able to distinguish samples according to their sampling areas (p=0.002, Figure IV-6). Test statistic of a conducted *ANOSIM* was significant (p=0.002, Global R=0.506).

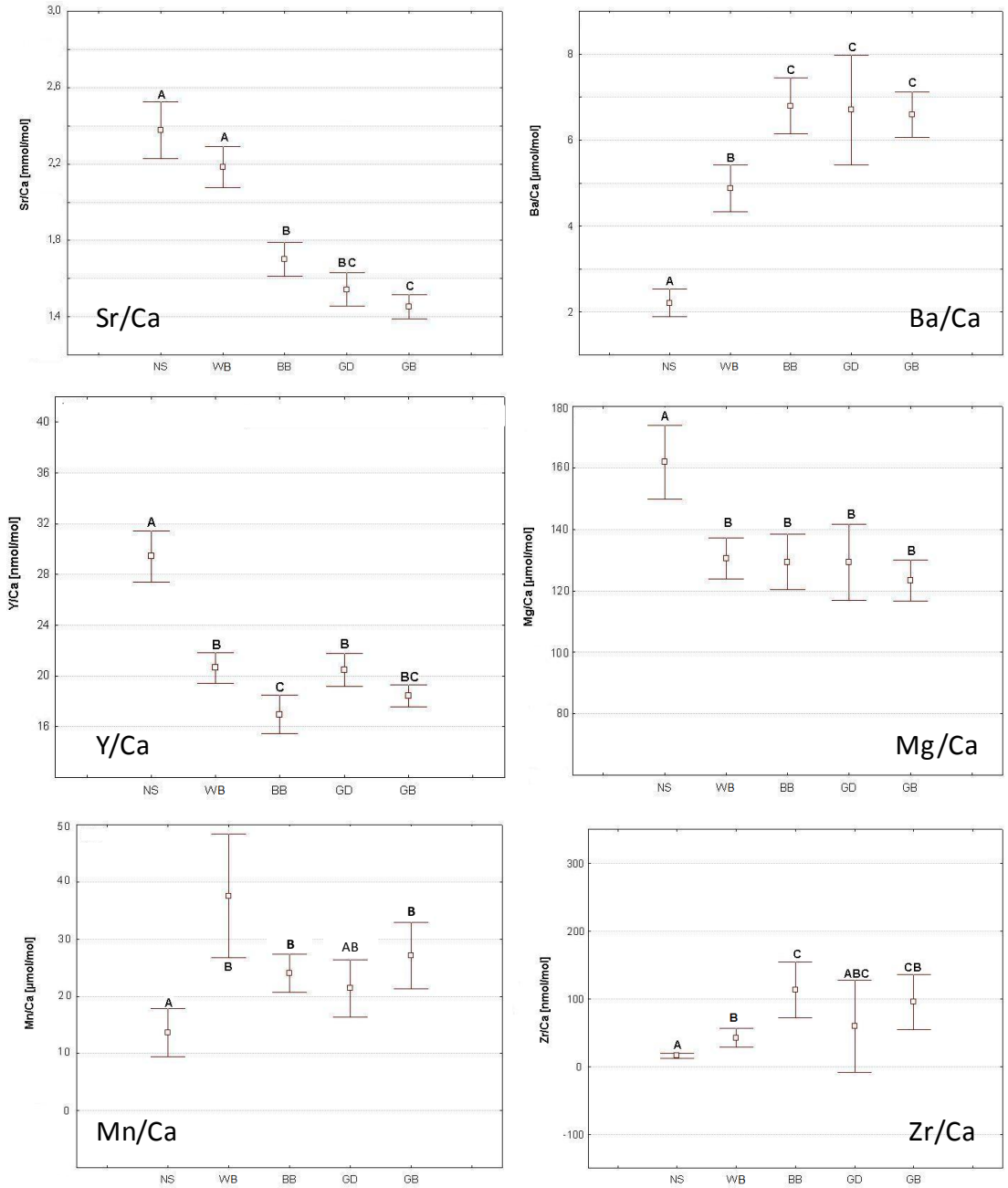

Figure IV-4: El/Ca ratios of elements with highest potential to distinguish between sampling areas. Squares represent overall mean values, error bars the 95% confidence interval. Groups without significant difference are labeled by same letters. NS = North Sea, WB = western Baltic, BB = Bornholm Basin, GD = Gdansk Deep, GB = Gotland Basin

	NS	WB	BB	GD
WB	0.960**	-	-	-
BB	0.881**	0.453***	-	-
GD	0.985***	0.736***	-0.036	-
GB	0.946***	0.781***	0.090**	0.133

Table IV-5: Results of pairwise tests of similarity analysis (*ANOSIM*). Values=R-statistic
Significance level:
**: $p \leq 0.01$
***: $p \leq 0.001$

Discussion

The use of otolith multi-element analyses allowed the discrimination of *G. morhua* individuals according to their sampling area. Otolith elemental fingerprints from adult cod clearly separated the North Sea from all Baltic Sea sampling areas as well as the western from the eastern Baltic individuals. The same was obtained for elemental fingerprints of the core region of juvenile cod otoliths from the western Baltic and the Bornholm Basin. However, no differences between the three eastern Baltic Sea basins were detected, except for a slight separation between Bornholm Basin and Gotland Basin.

The incorporation of different elemental fingerprints of western and eastern Baltic sampling areas support the assumption that individuals from these areas are generally separated. Reasons for the establishment of such differences are diverse and cannot be conclusively clarified in the present study. They might be caused by a reduced exchange of individuals between the sampling areas as reported before by e.g. Otterlind (1985) and Neuenfeldt *et al.* (2007) or could be the consequence of an annual homing performance at certain spawning areas. Most of the examined animals were caught during spawning season. For the eastern Baltic stock it is assumed that cod returns to the same spawning area every year where they spend spring and summer (Wieland *et al.* 2000). Thus specific hydrographic conditions within the spawning areas could cause distinguishable otolith elemental fingerprints. The use of different feeding grounds could also lead to the results obtained here. However, differences in Sr and Ba concentrations between individuals indicate differing salinity preferences for western and eastern Baltic cod and suggest migrations between both areas to be limited in time.

Despite the clear discrimination between sampling areas, it is not possible to allocate adult individuals to a certain natal origin. The here compiled elemental fingerprints cover the entire life and early life history signals are overlaid by subsequent years. Consequently, initial migrations of juvenile cod and mixing between stocks at young age cannot be excluded by the

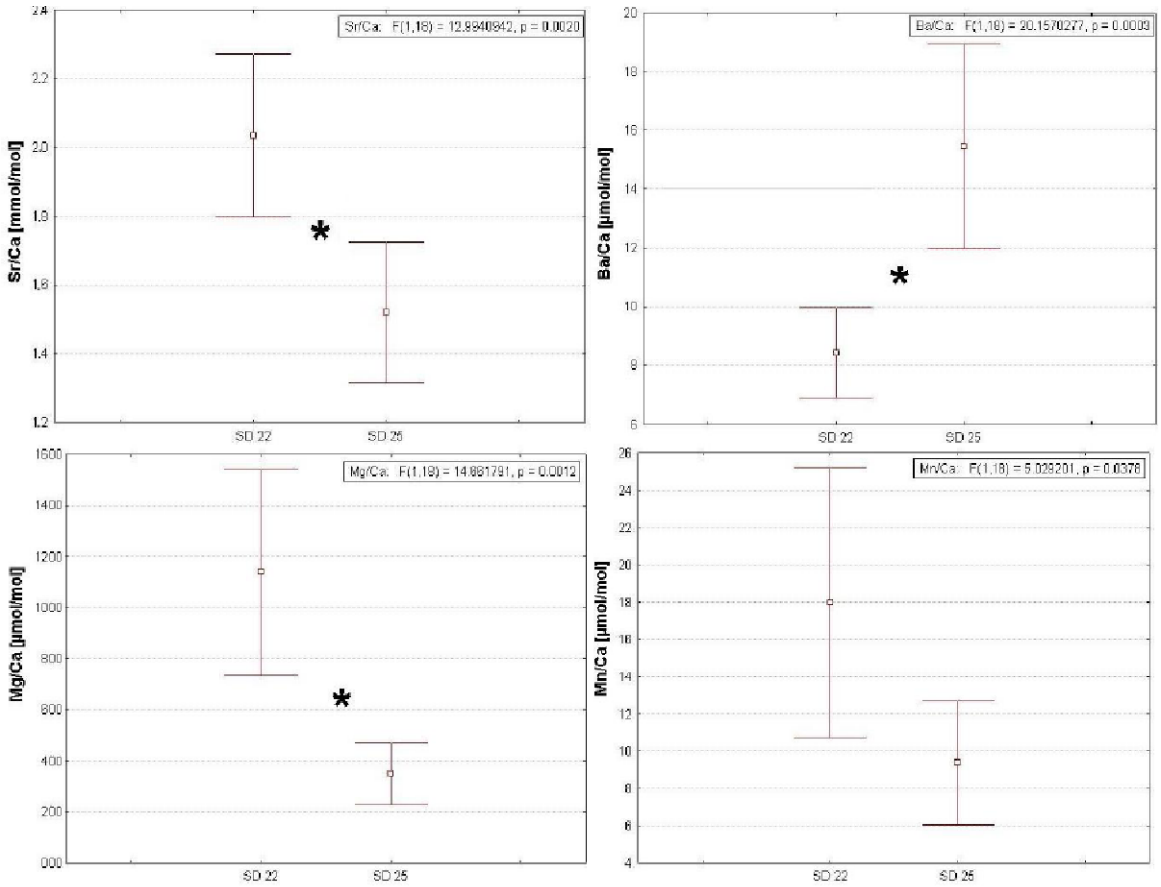

Figure IV-5: El/Ca ratios in the core region of juvenile cod otoliths for Sr, Ba, Mg and Mn. Significant differences between sampling areas are labeled with a star. Squares represent mean values. Error bars show the 95% confidence interval. Significance values (*p*) derive from *t*-tests. SD 22 = western Baltic, SD 25 = Bornholm Basin

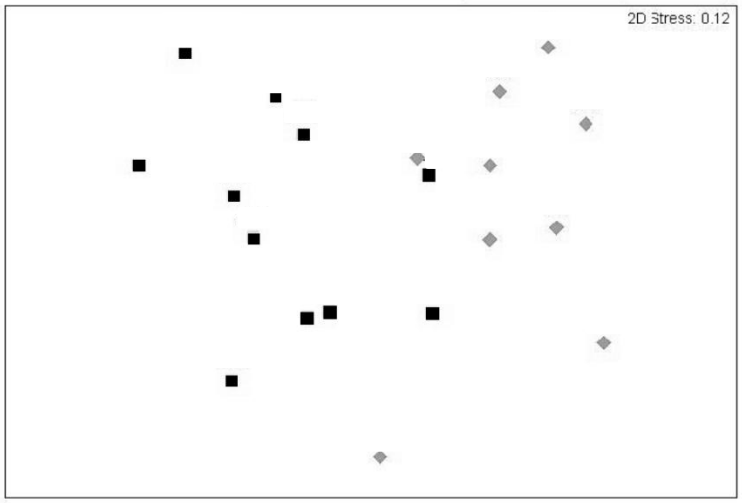

Figure IV-6: *MDS* plot of elemental fingerprints in the core region of juvenile cod otoliths, grouped according to sampling areas. Black squares = western Baltic (SD 22), grey diamonds = Bornholm Basin (SD 25)

present study. Nevertheless, the possible separation of juveniles from different areas strengthens the assumption that core regions of Baltic cod otoliths might store specific hydrographic conditions of different spawning grounds. Presuming these conditions to be stable, site specific otolith core concentrations could help identify the natal origin of individual cod. However, it has to be considered that water element composition may vary in time and differences between regions might change among years (e.g. Gillanders & Kingsford 2000). Campana *et al.* (2000) reported elemental compositions of cod otoliths from the same area only to be stable over short periods up to one year. This might impede the development of site-specific elemental fingerprints in the Baltic Sea.

The present study demonstrates that the Baltic Sea hydrography provides good conditions for the use of otolith microchemistry as a potential discriminator of cod stocks. Hydrographic conditions change over comparably short distances. Water salinity ranges from almost fully marine in the Kattegat to almost freshwater in the innermost part of the Gulf of Bothnia and the local influence of river discharge is strong (Andersson *et al.* 1992). It can be assumed that most of the discrimination between areas detected in the present study is caused by differences in water salinity, which was shown to be positively correlated with otolith Sr/Ca ratios for several fish species (e.g. Secor *et al.* 1995; Secor & Rooker 2000). However, the Sr/Ca ratio alone could not be used for the separation between North Sea and western Baltic. Hence, consistent variations in additional elements are responsible for the separation of these groups. Besides Sr, Ba incorporation is known to vary with water salinity as well (de Vries *et al.* 2005). It is the second strongest discriminator between sampling locations in adult cod with concentrations that increase eastwards inversely to water salinity. In juvenile cod, Ba even revealed the strongest impact to separate between sampling areas, supporting the assumption of water salinity to mainly influence the element incorporation into cod otoliths in the Baltic Sea.

Although most pronounced between North Sea and western Baltic Sea elements with high discriminatory power also differed between western and eastern Baltic samples. Beside these comparably high concentrated elements, some heavy metals showed elevated concentrations in Baltic cod otoliths (Zn, Cu, Pb and Cd) potentially reflecting higher water concentrations caused by pollution (Pohl & Hennings 2009; BLMP 2002). Although values fluctuated largely among individuals from the same sampling areas, heavy metals might provide potential candidate elements in future studies. In contrast, Y concentrations remained very stable for each sampling area. Although they could only be used to distinguish between adult cod sampling areas and showed no differences between juveniles, the potential of Y should be investigated in future studies.

Despite the good discrimination potential of many of the here analysed elements, it was not possible to determine characteristic elemental fingerprints that clearly differ between the three eastern Baltic spawning grounds. Although Sr content decreased from Bornholm Basin over Gdansk Deep to Gotland Basin and Y as well differed between these locations, the combination of all analysed elements did not lead to clear discriminating patterns. This might be caused by equal water composition within the eastern Baltic Sea or by high connectivity between spawning areas, as reported for cod from Gotland Basin that leaves endemic spawning areas to migrate westwards when spawning conditions suffer from a lack of oxygen or from high cod densities (Baranova 1989, 1995).

Despite the problem regarding small-scale separation of the eastern Baltic cod stock, many of the drawn conclusions obtained from elemental fingerprints of otoliths are suitable to more effectively provide information to enhance the management of the Baltic Sea cod. The combination of biological markers with otolith microchemistry as done by Higgins *et al.* (2010) could further improve the separation of Baltic cod stocks. Because the inter-relationships between the western and the eastern Baltic cod stock are presently only poorly understood, the utilization of otolith microchemistry for adult and juvenile fish otoliths may be helpful as a management tool for quantifying the importance of east-westward-oriented migration and hence mixing of the different stocks: a topic which is presently still under debate.

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CHAPTER V Newcomers in the Baltic Sea: an attempt to trace the origins and whereabouts of thicklip grey mullet *Chelon labrosus*

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Abstract

In recent years, thicklip grey mullet *Chelon labrosus* has shown increasing expansion of its native habitats in the north-eastern Atlantic into northerly adjacent areas including the North Sea and the brackish Baltic Sea. Despite the regular annual and seasonal occurrence of *C. labrosus* in the western Baltic during the warm months, nothing is known of the origin or whereabouts of the mullet during the cold season. As different possible migration scenarios can be considered, we performed otolith microchemistry analyses on specimens from the western Baltic Sea to identify the origin of this nonindigenous species. Comparison with North Sea samples revealed common habitat preferences and underlined the highly euryhaline nature of *C. labrosus* in different recently occupied habitats. Occasional fluctuations of Sr/Ca ratios along the growth axis suggest periodical migration between waters of different salinities but did not reveal distinct migration pathways.

Keywords: migration behaviour, non-indigenous species, otolith microchemistry, Sr/Ca ratio

Introduction

The family Mugilidae consists of 72 species with a worldwide distribution (Nelson 2006). They play a relatively important role in commercial and artisanal fisheries and are used for aquaculture production (Harrison 2003). Especially in eastern Asia, mullets are commercially important for both capture fisheries and aquaculture and many recent studies have been conducted on the migration behaviour of these species (e.g. Chang *et al.* 2004; Hsu *et al.* 2009; Wang *et al.* 2010). In northern Europe, three species—thicklip grey mullet *Chelon labrosus*, thinlip mullet *Liza ramada*, and golden grey mullet *Liza aurata*—are common in the north-eastern Atlantic, distributed in the Bay of Biscay, the English Channel, and along the North Sea coasts (Thomson 1966; Muus & Nielsen 1999). An expansion of *C. labrosus* from the southern North Sea towards the south-western Baltic Sea is documented in many cases. At the beginning of records, mugilids were generally considered exotics along German coasts, especially in the Baltic Sea, with the appearance of even solitary specimens considered worth documenting (Heincke 1894; Mohr 1928; Meyer 1935). Despite general expansion of the distribution range into the western Baltic, reports of catches of *C. labrosus* were restricted to mainly solitary specimens until the mid 1970´s (Mohr 1988), when numbers started to increase (Meixner 1978; Mohr 1986). Nowadays *C. labrosus* is regarded as a regular summer guest along the North Sea coasts and the Danish Belt Sea (Muus & Nielsen 1999). According to commercial catches, the periodical presence of *C. labrosus* in the western Baltic meanwhile is most pronounced in the warmer months from May to October (BLE 2009). Different assumptions have been raised for the expanding propagation of *C. labrosus* in these newly colonized areas. These range from enhanced food availability (Mohr & Horn 1977) to increased stock numbers in adjacent, native habitats (Mohr 1986) and also increased water temperature due to climate change (Vorberg *et al.* 2005). So far, nothing is known on the origin of *C. labrosus* in the Baltic Sea and their whereabouts after disappearance in autumn with decreasing water temperatures.

Overall, the North Sea and the brackish Baltic Sea differ distinctly in hydrography, with salinity pronouncedly decreasing in the transition zones between the two areas. While surface salinity in North Sea coastal waters usually ranges from 32 to 34.5 psu, salinities in the Skagerrak and further southeast in the Kattegat decrease to levels between 34 and 25 and 25 and 10 psu, respectively (OSPAR commission 2000). Surface salinities in the western Baltic (Kiel Bight) usually range from 15 to 20 psu in summer (Siedler & Hatje 1974). The Wadden Sea area of the North Sea in several regions receives freshwater input from river discharge. In these regions, salinities are lower than open-sea surface salinities and underlie seasonal variations. Principal rivers influencing the salinity in the German and Danish parts of the Wadden Sea are—amongst

others—Weser, Elbe, Eider and Varde Å (Postma 1982). Despite reduced salinities in the Wadden Sea parts, overall salinities in these North Sea regions are still pronouncedly higher than coastal salinities in the adjacent Baltic Sea. Both average summer surface salinities as well as main rivers influencing salinities in the North Sea/Wadden Sea are depicted in figure V-1.

To gain information about migration patterns of *C. labrosus*, microchemical otolith analyses were performed using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Due to the positive relation of otolith strontium (Sr) content and water salinity shown for a number of species (Campana 1999; Elsdon *et al.* 2008), otolith microchemistry has turned out to be an excellent tool to recover migratory behaviour of species moving between waters of different salinities (e.g. Kimura *et al.* 2000; Shen *et al.* 2009; Tzeng *et al.* 1997; Limburg 1995). However, water salinity is not the only factor influencing Sr incorporation into otoliths. Beside water Sr concentrations and temperature, also growth, development stage and age were shown to have potential effects (reviewed in Campana 1999). Hence, interpretation of otolith Sr composition should always consider potential disturbing environmental and physiological influences. Nevertheless, recent studies (Chang *et al.* 2004; Hsu *et al.* 2009; Wang *et al.* 2010;

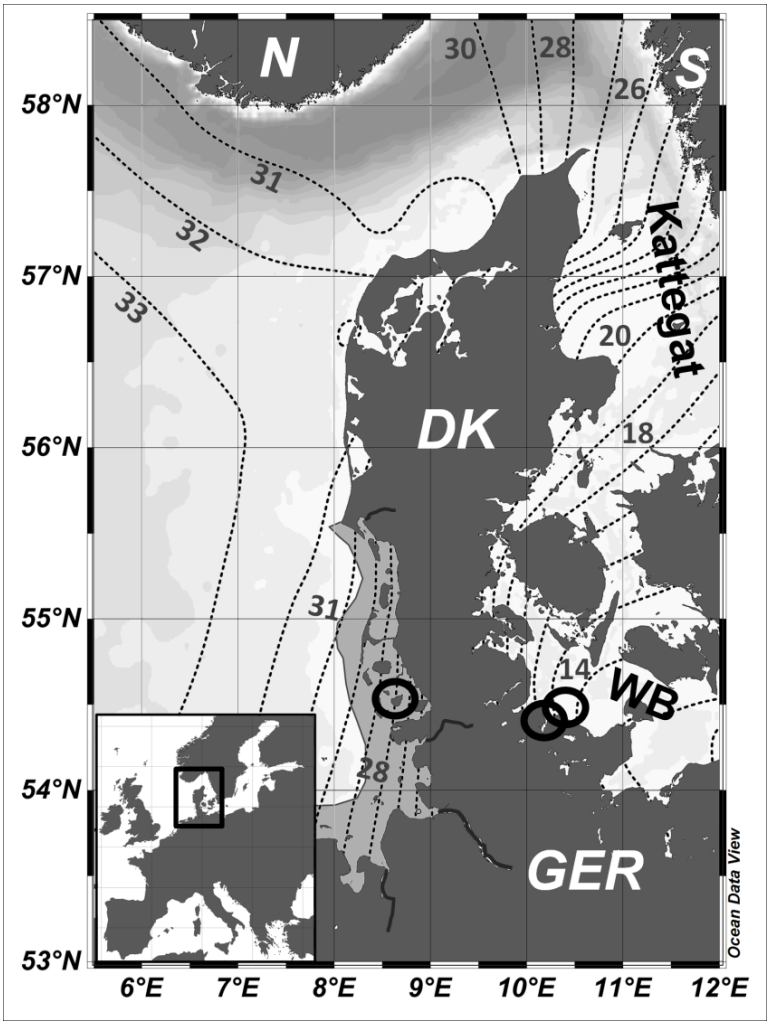

Figure V-1: Area in focus (see inset). Black circles indicate sampling areas in the North Sea (Pellworm Island) and the western Baltic (WB, inner and outer Kiel Fjord). DK Denmark, GER Germany, N Norway, S Sweden. Grey-shaded area depicts Wadden Sea region of North Sea. Isohalines (dashed) and corresponding numbers indicate average summer sea surface salinities (from World Ocean Atlas 2009 (Antonov *et al.* 2010)). Rivers discharging fresh water into Wadden Sea are highlighted (north to south: Varde Å, Eider, Elbe, Weser)

Miles *et al.* 2009) showed a clear impact of water salinity on the Sr concentration of otoliths of eastern Asian mugilid species. These promising results obtained in closely related species led to the assumption that otolith microchemistry might also deliver substantial information about migratory movements of *C. labrosus* in newly colonized areas, between the fully marine North Sea and the brackish western Baltic Sea, which is characterized by considerably lower water salinities.

We used otolith microchemical analyses: to (1) identify the origin of *C. labrosus* specimens found in the western Baltic Sea and (2) discover possible annual migration patterns.

Materials and Methods

C. labrosus specimens were caught either by rod and line or by gill and fyke nets in the Kiel Fjord area (Baltic Sea, n = 10) between 2005 and 2007. Individuals from the North Sea (n=5) were caught in 2008 near Pellworm Island in the German Wadden Sea (Figure V-1). Fish were measured (total length, cm), weighed (wet weight, g) and dissected. Additionally, scale samples were taken from under the base of the first dorsal fin (if still present) for age estimation (Hotos 2003). Detailed information on recorded individual parameters is given in table V-1.

Table V-1: Origin, sampling gear, and biological data of *Chelon labrosus* measured and analysed in this study

ID	Area	ICES	Location	Gear	Date	TL (cm)	WW (g)	Age (y)
B1	WB	IIIc 37G0	Inner Kiel Fjord	Rod and line	13.07.2005	59	n.a.	n.a.
B2	WB	IIIc 37G0	Inner Kiel Fjord	Gill net	01.09.2006	58	2273	10
B3	WB	IIIc 37G0	Inner Kiel Fjord	Gill net	01.09.2006	62	2956	10
B4	WB	IIIc 37G0	Inner Kiel Fjord	Gill net	14.06.2007	59	2590	11
B5	WB	IIIc 37G0	Inner Kiel Fjord	Gill net	14.06.2007	62	2485	11
B6	WB	IIIc 37G0	Outer Kiel Fjord	Gill net	09.10.2007	57	2720	9
B7	WB	IIIc 37G0	Outer Kiel Fjord	Gill net	09.10.2007	61	3105	11
B8	WB	IIIc 37G0	Outer Kiel Fjord	Gill net	09.10.2007	58	2300	9
B9	WB	IIIc 37G0	Outer Kiel Fjord	Gill net	09.10.2007	52	1950	8
B10	WB	IIIc 37G0	Inner Kiel Fjord	Fyke net	13.08.2007	40	788	5
N1	NS	IVb 38F8	Pellworm (WS)	Gill net	11.08.2008	62	3254	12
N2	NS	IVb 38F8	Pellworm (WS)	Gill net	11.08.2008	59	2695	10
N3	NS	IVb 38F8	Pellworm (WS)	Gill net	11.08.2008	63	3056	10
N4	NS	IVb 38F8	Pellworm (WS)	Gill net	11.08.2008	59	2827	8
N5	NS	IVb 38F8	Pellworm (WS)	Gill net	11.08.2008	58	2506	9

Age reading was conducted on scales (Hotos 2003)
ID specimen identifier, WB western Baltic Sea, NS North Sea, WS Wadden Sea, TL total length, WW wet weight

Sagittal otoliths were extracted, cleaned with distilled water, dried in air, and stored in Eppendorf caps. For analysis they were ground by hand from dorsal and ventral sides using abrasive paper (1913 siawat fc, grit: p600, Sia; Frauenfeld, Switzerland) until they were sufficiently thin to be embedded into thermo-epoxy (Buehler; Düsseldorf, Germany) on glass slides. Thereafter, lapping films 30, 12, and 3 MIC (3M; Neuss, Germany) were used for further polishing. LA-ICPMS analysis was performed at the Institute of Geosciences, University of Bremen using a UP193 solid-state laser (New Wave Research; Fremont, USA) coupled to a Finnigan Element2TM (Thermo; Waltham, USA). Otolith Sr content was recorded along a transect from the core to the edge following the anterior– posterior growth axis. Spot size of 75 µm and scan speed of 3 μms^{-1} were chosen. The pulse rate was set to 10 Hz, and irradiance was about 1 GW cm^{-2}. Sr was analysed at low resolution. Prior to ablation, blanks were measured for 20 s. A glass reference material (NIST612) was analysed as external standard after every measurement using the concentrations given by Pearce *et al.* (1997). Helium (0.4 $Lmin^{-1}$) and argon (0.8 $Lmin^{-1}$) were used as sample gas and make-up gas, respectively. To clean the surface, transects were preablated with spot size of 120 µm and scan speed of 100 μms^{-1} previous to measurements. All results are expressed as strontium/calcium (Sr/Ca) ratios. Further details about the measurement procedure, element quantification, and analytic precision can be found in Marohn *et al.* (2009). To validate that Sr concentrations measured by LA-ICPMS represent consistent ring structures throughout the entire otolith, wavelength-dispersive X-ray spectrometry was carried out on the entire surface of one exemplary otolith using electron microprobe analysis (EMPA). This was accomplished by using a JXA-8200 Superprobe (Jeol; Tokyo, Japan) at IFM-GEOMAR, Kiel. Prior to measurement, otoliths were evaporated with carbon to improve conductivity. The map analysis program was used, and diameter of 1 µm at dwell time of 250 ms μm^{-2} was chosen. Accelerating voltage was set to 15 kV, and the intensity of current was approximately 30 nA. Though not particularly prepared for age reading, all otoliths were checked by stereomicroscopy for occurrence of annuli to specify annual otolith growth and to detect potential seasonal Sr fluctuations.

The deduction of surrounding water salinities from otolith Sr/Ca ratios required the development of a relationship between these two parameters. Fisheries landing statistics show annual occurrence of *C. labrosus* in the western Baltic starting from May (BLE 2009). We therefore assumed that fish sampled from June onwards had entered western Baltic waters several weeks prior to catch. Hence, local water salinities as experienced until catch are reflected by the Sr/Ca ratios at the edge of the otoliths. To intercalibrate otolith Sr/Ca ratios and water salinity, we analysed the Sr/Ca ratios of the latest grown otolith material (50 µm) of

individuals from Kiel Fjord and compared it with a time series of local water salinities (C. Clemmesen, unpubl. data, 2010; Figure V-2). Annual increment widths measured at least 190 µm, of which we assume the majority to be grown from spring to autumn, since most of the annual otolith growth usually takes place during higher water temperatures (e.g. Mosegaard *et al.* 1988). We therefore presume the daily growth rate from spring to autumn to exceed 1 µm day^{-1} and assume 50 µm of otolith material to reliably reflect the final growth period directly prior to catch. Salinity was measured weekly from October 2004 from 2 m below surface to close to the seafloor (15 m) and was integrated over a period of 2 months prior to catch.

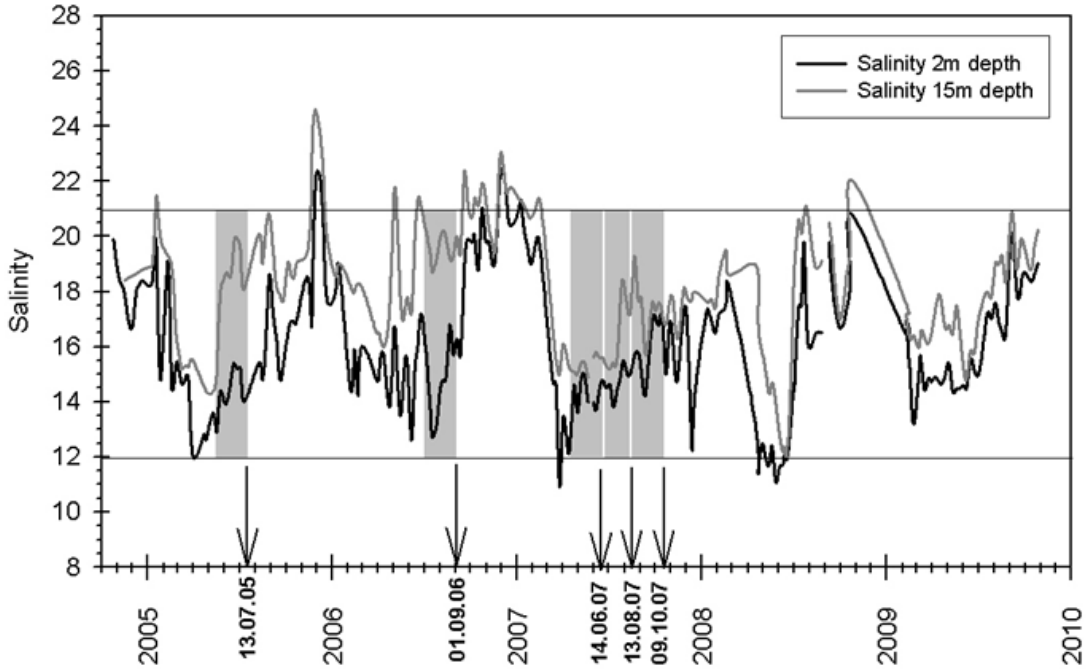

Figure V-2: Weekly resolved time series of inner Kiel Fjord salinity (psu) at 2 and 15 m depth (maximum depth 17 m) from October 2004 to October 2009. Arrows indicate catch dates of *Chelon labrosus* individuals from each respective year (compare with details in table V-1). Grey bands reflect salinity conditions 2 months before catch, which were used to intercalibrate local salinity conditions with Sr/Ca ratios of the latest grown otolith material (50 µm)

Results

Hydrography and calibration of measurements

Salinities in Kiel Fjord varied considerably during the measurement period, depending on the occurrence and magnitude of inflow events of high-saline waters from adjacent North Sea/Skagerrak/Kattegat through the Belt Sea (Figure V-2). During the period chosen for calibration, salinities ranged from 12 psu (2 m depth) to 21 psu (15 m depth). Corresponding mean Sr/Ca ratios incorporated into otoliths during this period varied between 4.81 x 10^{-3} and 7.10 x 10^{-3} (95% confidence interval (CI) 4.62 x 10^{-3}– 7.59 x 10^{-3}). Accordingly, grey bands in the Sr/Ca profiles depicted in figure V-3 highlight otolith Sr/Ca ratios between 4.60 x 10^{-3} and 7.60 x

10^{-3} that represent the salinity range measured. Further interpretation of otolith Sr/Ca ratios was made according to this relation.

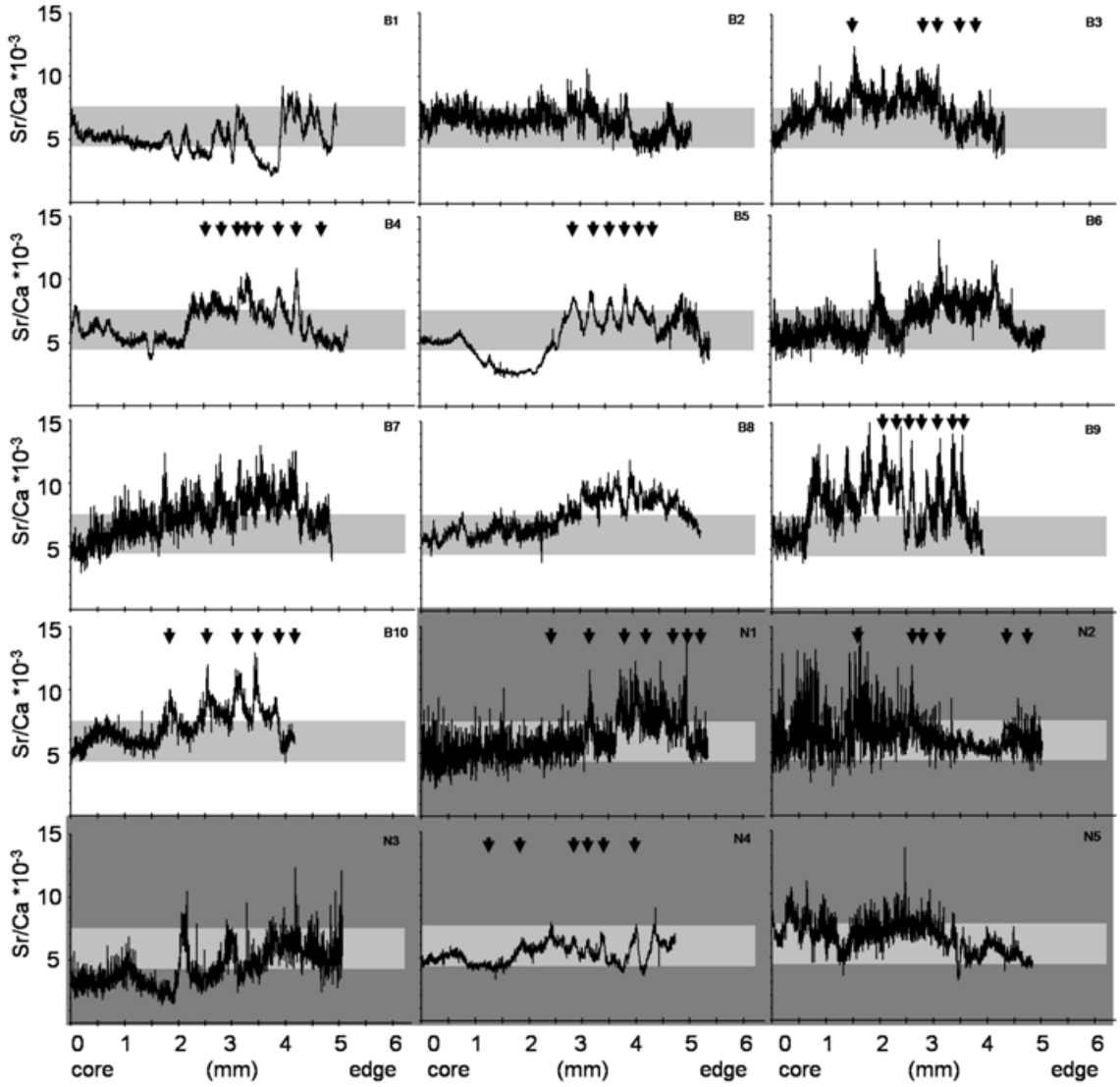

Figure V-3: Strontium/calcium ratios in thicklip grey mullet *Chelon labrosus* otoliths from the western Baltic Sea and from the North Sea (*shaded*). Profiles were measured from otolith core to edge along anterior–posterior axis. Grey bands indicate Sr/Ca ratios incorporated during water salinities between 12 and 21 psu (see text for further explanation). *Arrows* indicate positions of microscopically identified annuli

Biological measurements and otolith microchemistry

The length range of all specimens sampled from the Baltic Sea was 40–62 cm, and of North Sea specimens was 58–63 cm. Corresponding ages as derived from scale readings were 5–11 and 8–12 years, respectively (Table V-1). Otolith microchemistry analysis revealed core Sr/Ca ratios between 3.0 x 10^{-3} and 8.0 x 10^{-3} with most common values around 5.0 x 10^{-3}–6.0 x 10^{-3}, independent of sampling site. Generally, a characteristic pattern of Sr/Ca ratios along the otolith growth axis of specimens caught in the western Baltic was observed. Individuals spent a first life phase in brackish water followed by a second life phase at higher salinities before entering

brackish waters again (Figure V-3). This migratory pattern was found in 9 of 10 individuals (except B2), though the time spent in different habitats and the extension of Sr/Ca fluctuations varied among individuals. B5, for example, initially spent a long time period in brackish waters including waters of relatively low salinity (<12 psu), while B9 spent nearly all its lifetime at salinities greater than 21 psu. Sr/Ca ratios of several specimens showed clear periodical fluctuations during certain life phases (e.g. B5 and B10; Figure V-3). If possible, otoliths were checked for correspondence between Sr/Ca peaks and winter annuli identified by stereomicroscopy. For many peaks, a corresponding annulus could be identified, suggesting annual periodicity of Sr incorporation. Some of the peaks exceeded values expected for the Baltic Sea.

In contrast to the Baltic Sea, no typical Sr/Ca pattern was found for North Sea samples. Altogether, values detected in *C. labrosus* caught in the North Sea varied within the same range as samples from Kiel Fjord (Figure V-3). Except for sporadic peaks, no Sr concentrations corresponding to marine water conditions could be detected in otoliths from North Sea samples. Clear ring structures of elevated Sr concentrations could be detected by EMPA (Figure V-4). Rings corresponded to Sr peaks measured by LA-ICPMS and to winter annuli.

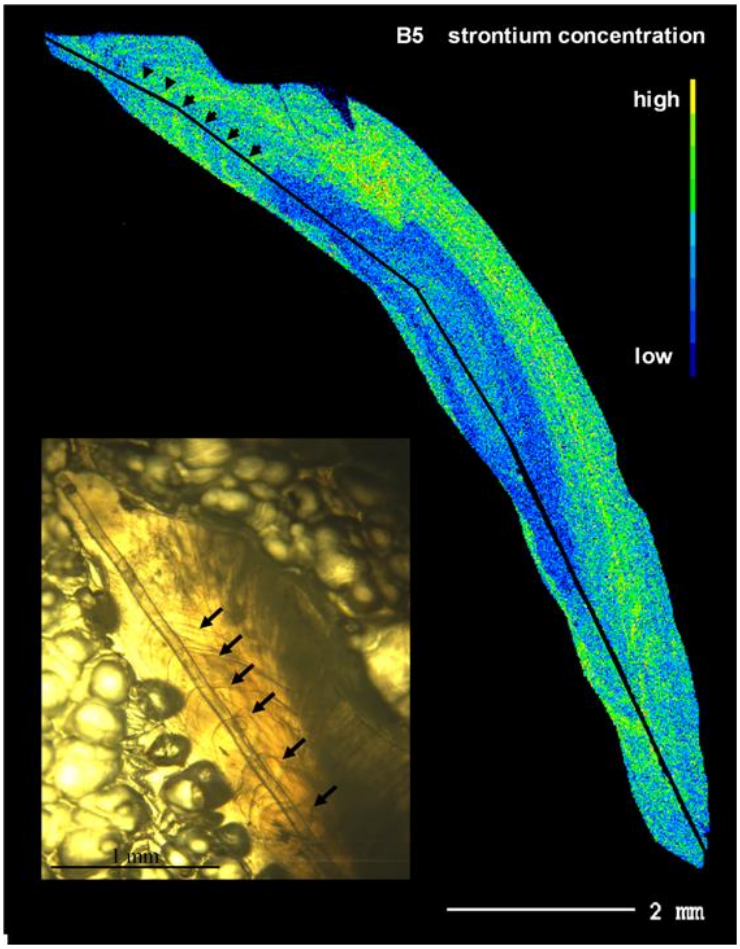

Figure V-4: Relative strontium distribution in otolith B5 determined by EMPA. The groove from prior laser ablation is visible. Inverted triangles indicate strontium peaks corresponding to winter annuli observed by stereomicroscopy. A photograph of the same otolith shows the identified annuli (arrows). Refer to text for further information

Discussion

Considering the possible scenarios concerning origin and fate of *C. labrosus* observed in the Baltic Sea during the warm season, three patterns seem likely: (1) *C. labrosus* seasonally migrate between their ‘‘native’’ habitats in the adjacent North Sea and the western/southwestern Baltic Sea along the North Sea coast. This would imply that they enter the Baltic Sea via the Skagerrak and Kattegat with warming water in the late spring months and migrate back with cooling waters in autumn. (2) *C. labrosus* individuals irregularly or nonrecurringly enter the Baltic Sea from adjacent waters, stay during the warm season and leave in autumn. (3) *C. labrosus* individuals enter the Baltic Sea in a singular event and remain in that area, undertaking extensive feeding migrations along the coastline in the warm season and retreating into deeper, warmer areas during the cold season, where they stay until littoral waters start to heat up again in spring.

Otolith microchemistry analyses performed in this study on sample specimens collected from both the North Sea and the Baltic Sea did not reveal a clear pattern that could be allocated to any of the scenarios described above. The results were unexpected insofar as the North Sea specimens—sampled from a habitat considered as fully marine (compared with the Baltic Sea)—showed no clear Sr/Ca signal that could be related to corresponding conditions. This might change with increasing sample size, as the North Sea samples consisted of only five specimens and thus cannot be considered fully representative.

C. labrosus is known to spawn offshore, with juveniles appearing in inshore areas and river mouths just a few months after hatching (Hickling 1970). Therefore, a signal referring to higher salinity levels would have been expected near the core of the otoliths. However, as the main goal of this study is to identify possible migration pathways and habitat preferences in newly colonized areas rather than to resolve the early life history pattern of individuals and as the corresponding area on the otoliths is minute, the core area was not covered sufficiently in this study to reveal this high-salinity signal. The laser might have missed the primordium in some otoliths, hence the present study does not include any information about the whereabouts of early life stage *C. labrosus*.

Generally, there are several possible explanations for the similarity of Sr/Ca ratios from both North Sea and Baltic Sea samples. *C. labrosus* in the coastal regions of the North Sea could avoid fully marine conditions by remaining in coastal areas under influence of freshwater discharge by rivers (Postma 1982; Ehlers 1994). Another possible explanation could be reduced growth during marine phases. There are indications that *C. labrosus* tend to migrate further offshore towards deeper waters during the cold season and additionally cease feeding during this phase (Hickling

1970; Le Dantec 1955). This might lead to reduced or inhibited growth, which could result in no otolith formation during that time of the year. It also has to be taken into account that incorporation of Sr into otoliths does not increase simultaneously with increasing Sr concentration in surrounding waters. Elsdon & Gillanders (2005a) showed that it may take up to 20 days until Sr incorporation into otoliths is saturated. Short-term stays in marine waters might therefore not be fully reflected by otolith microchemistry.

Our results nevertheless underline the highly euryhaline behaviour of *C. labrosus* with its preference for inshore areas, estuaries and river mouths (Hickling 1970; Ben-Tuvia 1996). Individuals analysed in this study mostly spent the majority of their lifetime in an environment with reduced salinities as compared with fully marine levels. However, distinct changes between brackish signals and higher salinities as identified between annuli on several otoliths indicate seasonal or annual migration patterns and could reflect movements between Baltic Sea and North Sea. Referring to Chang *et al.* (2004), who suggested values between 3.0×10^{-3} and 7.0×10^{-3} to correspond to brackish water conditions, our results would imply the possible interpretation that the individuals caught in the brackish Kiel Fjord had spent a significant amount of time in fully marine areas, which required them to leave the (western) Baltic Sea towards North Sea regions (OSPAR commission 2000; Siedler & Hatje 1974). The otolith Sr/Ca range for the intercalibration in the present study (4.62×10^{-3}–7.59×10^{-3}) was chosen more conservatively to account for uncertainties regarding the whereabouts of the individuals prior to catch and otolith growth rates. Despite these uncertainties, we decided to use the intercalibration as an orientation mark. It does not provide precise thresholds between different water bodies. Though most of the identified annuli correspond to regions of elevated Sr content, this is not the case for all of them (Figure V-3). However, this does not imply that individuals without periodical Sr/Ca fluctuation do not undergo annual migrations, as an inshore migration route through the Wadden Sea along the German and Danish coasts of the North Sea would cover vast areas under fresh/brackish water influence, e.g. the Weser and Elbe River estuaries and, further north and to a lesser extent, the Eider and Varde Å River mouths, etc. (Postma 1982; Ehlers 1994). Use of this migration route would reduce temporal residence in waters of higher salinities during migration and could therefore probably not be detected by otolith microchemistry. Whether this applies or whether individuals without any changes in Sr concentrations are rather stationary has to be evaluated by further studies.

It must be noted though that changes in otolith Sr content might also be caused by other factors beside salinity. Water temperature and its correlated physiological processes potentially produce Sr changes equivalent to those caused by diadromy (Campana 1999). It can only be

speculated if this is also true for *C. labrosus*, as direction and strength of these impacts vary between species (Campana 1999). It therefore remains unclear if the peaks observed are caused by active migration to waters of higher salinities or if they are due to enhanced Sr incorporation during slow growth phases as described for various fish species (Sadovy & Severin 1994; Secor *et al.* 1995). Thus, the actual origin and fate of western Baltic *C. labrosus* still remain puzzling.

Additional experiments are needed, assessing the impacts of abiotic (salinity, temperature) and biotic (food, growth rate, gonad development, and spawning cycle) factors on the microchemical deposition proportions and rates in *C. labrosus* otoliths. Furthermore, future studies should include analysis of otolith barium concentration and ^{87}Sr:^{86}Sr isotope ratios, which might provide additional information about migration pathways (e.g. Elsdon & Gillanders 2005b; Milton & Chenery 2003). Ideally, studies should merge otolith microchemistry with tagging methods, e.g. data storage tags, to follow large-scale seasonal or spawning migrations as well as small-scale locally restricted movements via stationary hydroacoustics or telemetry (Gehrke *et al.* 2001; Butler *et al.* 2009). Recently, implanted acoustic transmitters have successfully been applied to follow small-scale movement patterns of *C. labrosus* at Mediterranean fish farms (Arechavala-Lopez *et al.* 2010) and one tagging study is currently ongoing in the Dutch Wadden Sea (F. Quirijns, personal communication, 2010). Genetic analyses (e.g. Heras *et al.* 2009) provide further tools to identify the population of origin and, given a highly resolved sample structure, may contribute to the detection of migration routes and the annual whereabouts of *C. labrosus* from the western Baltic Sea.

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GENERAL DISCUSSION

Otolith microchemistry analyses in the Baltic Sea

The Baltic Sea is the world's largest brackish water system with a salinity gradient from almost fully marine in the northern Kattegat to nearly freshwater in the outermost eastern regions (Andersson *et al.* 1992). The influence of North Sea water is reduced by the narrow and shallow Belt Sea and the Oresund and beside occasional inflow events of marine water eastern parts are predominantly influenced by river discharge (Matthäus 1996; Andersson *et al.* 1992). This thesis highlights the feasibility of otolith microchemistry analysis for the use on Baltic Sea fish. The characteristic hydrography of this almost enclosed brackish water system provides a perfect environment for elemental analyses of fish otoliths to answer a broad range of questions. However, so far it has been of minor interest in the Baltic Sea. Only a few studies investigated otolith elemental composition of Baltic Sea fish (*Salmo trutta*: Limburg *et al.* 2001; *Esox lucius*: Westin & Limburg 2002; *Coregonus lavaretus*: Limburg *et al.* 2007; *Gadus morhua*: Higgins *et al.* 2010) with most of them focusing on the European eel (Tzeng *et al.* 2000; Limburg *et al.* 2003; Shiao *et al.* 2006; Lin *et al.* 2007b, 2009).

The present thesis brings together diverse information on migratory behaviour and stock characteristics of fish species in the Baltic Sea. It provides insights with fundamental relevance for stock management. Despite their high ecological and economical importance, little is known about the migratory behaviour of *Anguilla anguilla* and the stock structure of *Gadus morhua* in the Baltic Sea. Furthermore, nothing is known about origin, migration routes and whereabouts of the new regular summer guest *Chelon labrosus*, although it might become increasingly important for the western Baltic ecosystem and the coastal fisheries. In this thesis, the influence of different migration strategies on the condition and spawner quality of *A. anguilla* was investigated, *G. morhua* stocks were separated and migration routes of *C. labrosus* were followed by using different approaches of otolith microchemistry analysis.

Methodology – possibilities and constraints

By analysing otolith strontium/calcium ratios the movements of fish through waters of different salinities can be reconstructed. Sr/Ca ratios are frequently used to investigate the migration behaviour of diadromous fish, including different eel species around the world (Tzeng *et al.* 2000; Tsukamoto & Arai 2001; Daverat *et al.* 2006). In **chapter I** LA-ICPMS was used to quantify the Sr/Ca ratios in European eel otoliths to reconstruct individual diadromous behaviour. LA-ICPMS is well suited for the detection of Sr fluctuations along transects. A measurement precision of better than 3% allows to accurately trace movements between water bodies of

different salinities. However, when analysing transects the spatial resolution of this method is comparatively low (laser diameter in the present study: 75µm). It therefore has to be considered that short term stays in different water bodies might not be detected. Furthermore, it takes up to 20 days for the surrounding Sr concentration to be completely reflected in the otoliths (Elsdon & Gillanders 2005a), which questions the feasibility of otolith microchemistry analyses for the precise detection of short term movements. Nevertheless, for the reconstruction of general migration patterns and the determination of habitat preferences it provides an invaluable tool.

For the same reason LA-ICPMS analysis of Sr/Ca ratios was performed on *C. labrosus* otoliths (**Chapter V**). In general, otolith Sr concentrations hardly reflect movements through brackish waters of different salinities, which are rather small and do not leave distinguishable Sr marks. However, movements between brackish and marine waters were assumed to be detectable in *C. labrosus* otoliths as promising results have previously been obtained for different Asian mugilid species (Chang *et al.* 2004; Hsu *et al.* 2009; Wang *et al.* 2010). In the present study the interpretation of Sr patterns were complicated by the preference of brackish water habitats and the apparent synchronisation of movements to higher salinities and slow growth phases in winter. Due to the comparatively low resolution (75 µm), annual short term migrations during slow growth phases were probably not detected. However, EMPA analysis confirmed that Sr fluctuations along the LA-ICPMS transects represent ring structures and therefore certain time periods, which is a fundamental requirement for the use of Sr for life history reconstruction.

The combination of multiple elements and the use of multivariate statistics increase the possibility to separate individuals from different treatments or groups. Therefore, LA-ICPMS was used to analyse the multi-elemental composition of European eel otoliths from temperature and diet experiments (**Chapter II & III**). The simultaneous and precise measurement of up to 16 elements and detection limits in the ng g^{-1} concentration range make LA-ICPMS the method of choice to determine otolith elemental fingerprints. The spatial resolution was sufficiently high to ensure that only otolith material grown under experimental conditions was analysed.

For the same reasons a multi-element approach was also chosen to gain insights into the distribution of *Gadus morhua* in the Baltic Sea (**Chapter IV**). Under the assumption that different hydrographic conditions cause distinguishable otolith elemental fingerprints, it was investigated whether differences between stocks or spawning grounds are reflected in otoliths of adult and juvenile cod.

The role of additional factors

The incorporation of elements into otoliths is influenced by a variety of environmental and endogenous factors (reviewed in Campana 1999), which have to be considered when using otolith element composition to reconstruct the life history. Among these, temperature is known to heavily influence element incorporation due to its high impact on physiological processes (Campana 1999; Secor & Rooker 2000; Elsdon & Gillanders 2003). To investigate whether Sr/Ca ratios in eel otoliths primary reflect movements through waters of different salinities, the influence of temperature was quantified under experimental conditions. Although a significant temperature effect on Sr incorporation was detected, results suggest that the effect was too low to be confounded with fluctuations caused by diadromy. According to the results presented, the reconstruction of the individual migration history of European eels is not disturbed by changes in water temperature and the reliability of *A. anguilla* migration studies based on otolith microchemistry is fundamentally increased.

Other factors like growth, diet or age were shown to affect element incorporation into otoliths as well (reviewed in Campana 1999 and Secor & Rooker 2000). Although their effects on Sr incorporation seem to be less pronounced than that of salinity, they remain untested for many species. In the present thesis the influence of dietary behaviour on the composition of eel otoliths was experimentally tested. The absence of any statistically significant effect under experimental conditions makes it unlikely that individual food preferences cause differences in otoliths of wild individuals. However, it has to be considered that the experiments presented were conducted on juvenile specimens in freshwater. It can therefore not be excluded that the impact of temperature and diet increases with increasing age or water salinity (Kalish 1989; Elsdon & Gillanders 2004).

In case of the thicklip grey mullet, such experimental studies are lacking. It is therefore possible that Sr fluctuations as detected in this study might not reflect migration patterns, but are caused by drastic changes of water temperature. Nevertheless, the absence of annual Sr fluctuations in several of the analysed otoliths suggests that temperature plays a minor role, since otherwise such fluctuations should be detected more consistently. As mentioned above, the influence of other environmental and endogenous factors is assumed to be low. However, to conclusively clarify their impact on *C. labrosus* otoliths experimental studies are needed.

The use of elemental fingerprints to discriminate between groups of fish does not require background information on factors influencing otolith element incorporation. Such studies merely look for differences between groups that are sufficiently consistent to distinguish between individuals. The identification of reasons for site or treatment specific element

incorporation might be interesting, for the discrimination between groups, however, it is not necessary.

Investigated species – new insights

Anguilla anguilla

In the present study *A. anguilla* individuals showed a high plasticity of migration behaviour, which has already been shown in previous studies (Tzeng *et al.* 2000; Limburg *et al.* 2003; Daverat *et al.* 2006). Furthermore, the results highlight the importance of the Baltic Sea as a habitat for the European eel. Due to its biology and high adaptability to different environments, hydrographical changes within the Baltic Sea do not severely affect the condition of *A. anguilla*, which is threatened by fisheries and human impacts on its freshwater habitats (e.g. with the construction of hydropower stations and dams (ICES 2010b)). However, according to catch data the eel abundance in the Baltic Sea is declining (ICES 2010b). Although partly compensated by massive anthroprogenic restocking measures in many of the Baltic Sea countries, it has to be considered that nowadays the distribution area of the European eel in the Baltic Sea and its tributaries might be reduced due to the historically low recruitment (ICES 2010a).

Our results revealed for the first time, that individuals that never entered freshwater during their life have higher fat contents and lower *Anguillicoloides crassus* infection levels, both prerequisites for successful spawning (e.g. van den Thillart *et al.* 2007; Larsson *et al.* 1990; Palstra *et al.* 2007), than eels with a strictly catadromous lifecycle. These findings support previous studies that suggest reduced spawner quality of eels from different freshwaters due to high contaminant loads (Belpaire *et al.* 2009), parasites infestations (Jakob *et al.* 2009a) and food availability (Svedäng & Wickström 1997). Consequently, the use of restocking measures into freshwaters in times of low stock sizes has to be questioned. The catch of glass eels for restocking purposes is usually justified by high density driven glass eel mortalities in Atlantic estuaries. However, current recruitment is far below former levels (ICES 2010a) and density dependent mortalities should therefore have diminished as well. It must be questioned whether the translocation of eels from Atlantic estuaries into freshwaters of potentially reduced habitat quality can be considered a stock sustaining measurement under such conditions. The present study underlines the importance of careful habitat quality assessments prior to restocking. It has to be ensured that restocking measures, if ever needed, primarily support the European eel stock, and not the local fisheries.

The experimental studies revealed that changes in water temperature and dietary behaviour do not reduce the reliability of *A. anguilla* migration studies. In a reverse conclusion that means that neither the experienced temperature history nor individual food preferences can be

reconstructed by this method. It must be assumed that differences, if present, are too low to be detected *in situ*, where numerous factors influence the incorporation process of elements.

Gadus morhua

Gadus morhua is one of the ecologically and economically most important fish species in the Baltic Sea. It suffers strongly from high fishing pressure and bad environmental conditions, which led to low stock sizes (ICES 2010c). The anthropogenic impact is especially strong on the eastern Baltic Sea cod, where adequate spawning conditions are already reduced in some of the spawning areas (Köster *et al.* 2005). Therefore, the characterisation of stock dynamics, including the assessment of stock connectivity and the contribution of spawning grounds to the spawning stock biomass is a fundamental requirement for a successful management of Baltic Sea cod. The results presented here confirm former findings that suggest limited exchange between western and eastern Baltic cod stocks (Otterlind 1985; Neuenfeldt *et al.* 2007). Although connectivity between stocks due to egg drift and migration during juvenile stages cannot be excluded, the present results suggest that the distribution area of adults do not overlap substantially. It was further demonstrated that the elemental composition of the core region of cod otoliths potentially reflects site specific hydrographic conditions and might be used in future studies to identify the natal origin of individual cod. We conclude that otolith microchemistry analysis provides a feasible tool to separate between Baltic cod stocks. The discrimination between individuals from the three eastern spawning grounds, however, was not possible. Nevertheless, single elements like Sr, Y and some heavy metals differed between the eastern basins and provide possible candidate elements to distinguish between individuals from these regions.

Chelon labrosus

The appearance of the thicklip grey mullet is increasingly reported in the western Baltic Sea (Meixner 1978; Mohr 1986), which is assumed to be facilitated by eutrophication, rising temperatures and high abundances in the adjacent North Sea (Mohr & Horn 1977; Vorberg *et al.* 2005). Nothing is known so far about its migratory behaviour and its habitat preference in this newly invaded environment. Knowledge on the distribution and stock dynamics of *C. labrosus* is required to predict its further propagation and estimate its impact on the ecosystem. The study presented here provides first insights but could not conclusively clarify the migration behaviour of *C. labrosus* in the Baltic Sea. The preference of *C. labrosus* for brackish water habitats (Hickling 1970; Ben-Tuvia 1996) was confirmed by the microchemical analyses performed in the present study. All individuals, including five from the North Sea, spent most of their life in brackish waters, while some showed periodical ring structures of elevated Sr concentrations that

correspond to winter annuli suggesting annual migration into waters of higher salinities. In other specimens, such fluctuations were less pronounced, which might imply that these individuals do not migrate annually into marine waters. Another possibility is that the comparatively high resolution of the performed analysis could have missed short term migrations into waters of higher salinities. However, the results obtained suggest that future otolith microchemistry studies could help shed light on the remaining questions of the whereabouts of *C. labrosus* in the Baltic Sea.

OUTLOOK

The improvement of analytical methods will further enlarge the scope of otolith microchemistry analyses (e.g. Fietzke *et al.* 2008). Otolith isotope analyses might enhance knowledge about distribution and habitat choice of *Anguilla anguilla* in the Baltic Sea. The Baltic Sea is surrounded by two major bedrock units from different eons: the Phanerozoic along the southern coasts and the Precambrian in Scandinavia. They differ in geology and age and consequently also in their isotopic composition (Andersson *et al.* 1992), which is reflected by the water composition of rivers originating in these bedrocks (Andersson *et al.* 1992, 1994). It might therefore be possible to identify river specific isotope ratios in eel otoliths (e.g. ^{87}Sr:^{86}Sr) and to extend the life history reconstruction to the identification of certain freshwater systems. Such analyses could further be used to identify specific regions of the Baltic Sea. ^{87}Sr:^{86}Sr decreases with water salinity (Reinhardt *et al.* 1998) and big rivers like the Oder, Vistula, Daugava, Neva or Torne transport high amounts of water into the Baltic Sea and strongly influence its chemical composition locally (Andersson *et al.* 1994; Wachniew 2006; Maksymowska *et al.* 2000). These conditions could be used to establish site specific characteristic isotopic fingerprints in the Baltic Sea. If reflected in otoliths they could be used to obtain more detailed knowledge about the whereabouts of individual fish.

Otolith isotope analyses can also be used to reconstruct temperature history (^{18}O:^{16}O) (Kalish 1991; Høie *et al.* 2004; Thorrold *et al.* 1997). As otolith multi-element analyses apparently are not well suited for the detection of temperature changes in eel otoliths, the application of oxygen isotope analyses might be a feasible method to gain additional knowledge about the individually experienced environmental history of the European eel. In contrast, a successful use of the otolith isotope analysis in European eels to reconstruct dietary behaviour seems rather unlikely due to their opportunistic feeding behaviour (Tesch 1999), although previous studies on other species showed that this is in principle possible (using ^{13}C:^{12}C; ^{34}S:^{32}S) (Radtke *et al.* 1996; Weber *et al.* 2002),

The potential of otolith microchemistry to separate between Baltic cod stocks seems promising. However, in combination with additional biological markers like e.g. genetics, body morphometrics or parasite communities, as done by Higgins *et al.* (2010), the discriminatory power of otolith microchemistry analyses could further improve. Based on the successful discrimination of juveniles from different spawning grounds in the present study, future work should test whether adult individuals can be allocated to their natal origins. To enable this, it has to be investigated whether spawning grounds produce perennial site specific elemental fingerprints in cod otoliths.

Further studies on *C. labrosus* otoliths should consider the application of analytical methods that provide a higher spatial resolution. Detailed analyses with e.g. EMPA on a µm scale could possibly detect less pronounced Sr fluctuations and identify migratory patterns that allow general conclusions on the behaviour of *C. labrosus* in the Baltic Sea. It also has to be considered that experimental work similar to that for the European eel is the basis for all otolith microchemistry studies that aim to reconstruct the environmental life history of fish. Such experiments would help identify the driving forces for Sr incorporation into *C. labrosus* otoliths and facilitate the interpretation of the patterns seen.

It seems promising that the continuous analytical development will continue to improve the explanatory power of otolith elemental analysis and further increase its applicability especially in hydrographical divers waters like the Baltic Sea.

REFERENCES

Aarestrup, K, Økland F, Hansen MM, Righton D, Gargan P, Castonguay M, Bernatchez L, Howey P, Sparholt H, Pedersen MI & McKinley RS (2009) Oceanic Spawning Migration of the European Eel (*Anguilla anguilla*). *Science* 325:1660

Als TD, Hansen MM, Maes GE, Castonguay M, Riemann L, Aarestrup K, Munk P, Sparholt, Hanel R & Bernatchez L (2011) All roads lead to home: panmixia of European eel in the Sargasso Sea. *Mol Ecol* 20: 1333-1346

Andersson J, Sandström O & Hansen HJM (1991) Elver (*Anguilla anguilla* L.) stockings in a Swedish thermal effluent—recaptures, growth and body condition. *J Appl Ichthyol* 7:78–89

Andersson PS, Wasserburg GJ & Ingri J (1992) The sources and transport of Sr and Nd isotopes in the Baltic Sea. *Earth Planet Sc Lett* 113:459-472

Andersson PS, Wasserburg GJ, Ingri J & Stordal MC (1994) Strontium, dissolved and particulate loads in fresh and brackish waters: The Baltic Sea and Mississippi Delta. *Earth Planet Sc Lett* 124:195-210

Antonov JI, Seidov D, Boyer TP, Locarnini RA, Mishonov AV, Garcia HE, Baranova OK, Zweng MM & Johnson DR (2010) *World Ocean Atlas 2009*. Salinity. NOAA Atlas NEDSIS 69, vol 533 2. US Government Printing Office, Washington

Aoyama J (2009) Life history and evolution of migration in catadromous eels (Genus Anguilla). *Aqua-BioSci Monogr* 2:1–42

Arai T, Otake T & Tsukamoto K (2000) Timing of metamorphosis and larval segregation of the Atlantic eels *Anguilla rostrata* and *A-anguilla*, as revealed by otolith microstructure and microchemistry. *Mar Biol* 137:39-45

Arai T, Kotake A, Lokman PM, Mille MJ & Tsukamoto K (2004) Evidence of different habitat use by New Zealand freshwater eels *Anguilla australis* and *A. dieffenbachii*, as revealed by otolith microchemistry. *Mar Ecol Prog Ser* 266:213–225

Arai T & Hirata T (2006) Differences in the trace element deposition in otoliths between marine- and freshwater-resident Japanese eels, *Anguilla japonica*, as determined by laser ablation ICPMS. *Environ Biol Fish* 75:173–182

Arechavala-Lopez A, Uglem I, Sanchez-Jerez P, Fernandez-Jover D, Bayle-Sempere JT & Nilsen R (2010) Movements of grey mullet *Liza aurata* and *Chelon labrosus* associated with coastal fish farms in the western Mediterranean Sea. *Aquacult Environ Interact* 1:127-136

Bagge O, Tiews K, Lamp L & Otterlind G (1974) German, Swedish and Danish cod tagging experiments in the Baltic 1968–69. *Rap Proces* 166:22–39

Bagge O & Steffensen E (1989) Stock identification of demersal fish in the Baltic. *Rap Proces* 190:3–16

Bagge O, Thurow F, Steffensen E & Bay J (1994) The Baltic cod. Dana 10:1-28

Bagge O & Thurow F (1994) The Baltic cod stock: Fluctuations and possible causes. *ICES Mar Sci Symp* 198:254-268

Baranova T (1989) Some parameters of the eastern Baltic cod population in Subdivision 26 and 28: growth and ripening in connection with abundance dynamics. *Rap Proces* 190:97-101

Baranova T (1995) The structure of spawning cod stock in the eastern Baltic during 1972–1995. ICES CM/J:9

Bath GE, Thorrold SR, Jones CM, Campana SE, McLaren JW & Lam JWH (2000) Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochim Cosmochim Acta* 64:1705–1714

Begg GA, Cappo M, Cameron DS, Boyle S & Sellin MJ (1998) Stock discrimination of school mackerel, *Scomberomorus queenslandicus*, and spotted mackerel, *Scomberomorus munroi*, in coastal waters of eastern Australia by analysis of minor and trace elements in whole otoliths. *Fish Bull* 96:653-666

Belpaire CGJ, Goemans G, Geeraerts C, Quataert P, Parmentier K, Hagel P & de Boer J (2009) Decreasing eel stocks: survival of the fattest? *Ecol Freshw Fish* 18:197–214

Ben-Tuvia A (1996) Mugilidae. In: Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E (eds) *Fishes of the North-eastern Atlantic and the Mediterranean* Vol. 3. UNESCO, Paris, pp 1197-1204

BLE (2009) Catches and landings from German commercial fisheries. Bundesanstalt für Landwirtschaft und Ernährung (Federal Institute for Agriculture and Food), Ref. 522, Hamburg, Germany

Bleil M, Oeberst R & Urrutia P (2009) Seasonal maturity development of Baltic cod in different spawning areas: importance of the Arkona Sea for the summer spawning stock. *J Appl Ichthyol* 25:10–17

BLMP (2002) *Meeresumwelt 1997-1998*. Bundesamt für Seeschifffahrt und Hydrographie (BSH), Hamburg und Rostock

Boëtius I & Boëtius J (1985) Lipid and protein content in *Anguilla anguilla* during growth and starvation. *Dana* 4:1–17

Bonhommeau S, Chassot E & Rivot E (2008a) Fluctuations in European eel (*Anguilla anguilla*) recruitment resulting from environmental changes in the Sargasso Sea. *Fish Oceanogr* 17:32-44

Bonhommeau S, Chassot E, Planque B, Rivot E, Knap AH & Le Pape O (2008b) Impact of climate on eel populations of the Northern Hemisphere. *Mar Ecol Prog Ser* 373:71–80

Brämick U & Fladung E (2006) Quantifizierung der Auswirkungen des Kormorans auf die Seen- und Flussfischerei Brandenburgs am Beispiel des Aals. *Fischer und Teichwirt* 57:8-11

Buckel JA, Sharack BL & Zdanowicz VS (2004) Effect of diet on otolith composition in *Pomatomus saltatrix*, an estuarine piscivore. *J Fish Biol* 64:1469–1484

Butler GA, Mackay B, Rowland SJ & Pease BC (2009) Retention of intra-peritoneal transmitters and post-operative recovery of four Australian native fish species. *Mar Freshwater Res* 60:361–370

Campana SE (1983) Calcium deposition and otolith check formation during periods of stress in coho salmon, *Oncorhynchus kisutch*. *Camp Biochem Physiol* 75:215–220

Campana SE & Neilson JD (1985) Microstructure of fish otoliths. *Can J Fish Aquat Sci* 42:1014-1032

Campana SE, Fowler AJ & Jones CM (1994) Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. *Can J Fish Aquat Sci* 51:1942-1950

Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar Ecol Prog Ser* 188:263–297

Campana SE, Chouinard GA, Hanson JM, Frechet A & Brattey J (2000) Otolith elemental fingerprints as biological tracers of fish stocks. *Fish Res* 46:343-357

Cardinale M & Svedäng H (2011) The beauty of simplicity in science: Baltic cod stock improves rapidly in a 'cod hostile' ecosystem state. *Mar Ecol Prog Ser* 425:297-301

Chang CW, Iizuka Y & Tzeng WN (2004) Migratory environmental history of the grey mullet *Mugil cephalus* as revealed by otolith Sr:Ca ratios. *Mar Ecol Prog Ser* 269:277-288

Chesney EJ, McKee BM, Blanchard T & Chan LH (1998) Chemistry of otoliths from juvenile menhaden *Brevoortia patronus*: evaluating strontium, strontium:calcium and strontium isotope ratios as environmental indicators. *Mar Ecol Prog Ser* 171:261–273

Clarke KR & Gorley RN (2001) *Primer v5: user manual/tutorial*. Primer-E, Plymouth

Clevestam PD, Ogonowski M, Sjöberg NB & Wickström H (2011) Too short to spawn? Implications of small body size and swimming distance on successful migration and maturation of the European eel *Anguilla anguilla*. *J Fish Biol* 78:1073–1089

Cohen DM, Inada T, Iwamoto T & Scialabba N (1990) Gadiform fishes of the world (Order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date. *FAO Fish Synop* 10 442 pp

Côté CL, Castonguay M, Verreault G & Bernatchez L (2009) Differential effects of origin and salinity rearing conditions on growth of glass eels of the American eel *Anguilla rostrata*: implications for stocking programmes. *J Fish Biol* 74:1934–1948

Dannewitz J, Maes GE, Johansson L, Wickström H, Volckaert FAM & Järvi T (2005) Panmixia in the European eel: a matter of time. *P Roy Soc B-Biol Sci* 272:1129– 1137

Daverat F, Tomas J, Lahaye M, Palmer M & Elie P (2005) Tracking continental habitat shifts of eels using otolith Sr/Ca ratios: validation and application to the coastal, estuarine and riverine eels of the Gironde–Garonne–Dordogne watershed. *Mar Freshw Res* 56:619–627

Daverat F, Limburg KE, Thibault I, Shiao JC, Dodson JJ, Caron FO, Tzeng WN, Iizuka Y & Wickström H (2006) Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla*, *A-japonica* and *A-rostrata*. *Mar Ecol Prog Ser* 308:231–241

Davidse A, Haenen OLM, Dijkstra SG, van Nieuwstadt AP, van der Vorst TJK, Wagenaar F & Wellenberg GJ (1999) First isolation of herpesvirus of eel (*Herpesvirus anguillae*) in diseased European eel (*Anguilla anguilla*) in Europe. *B Eur Assoc Fish* Pat 19:137–141

de Vries MC, Gillanders BM, Elsdon TS (2005) Facilitation of barium uptake into fish otoliths: Influence of strontium concentration and salinity. Geochim Cosmochim Ac 69:4061-4072

Degens ET, Deuser WG & Hardrich RL (1969) Molecular structure and composition of fish otoliths. *Mar Biol* 2:105-113

Dekker W 2000 The fractal geometry of the European eel stock. *ICES J Mar Sci* 57:109-121.

Dekker W (2003) On the distribution of the European eel (*Anguilla anguilla*) and its fisheries. *Can J Fish Aquat Sci* 60:787–799

Dekker W, Casselman JM, Cairns DK, Tsukamoto K, Jellyman D, Lickers H *et al.* (2003) Québec declaration of concern—worldwide decline of eel resources necessitates immediate action. *Fisheries* 28:28–30

Dodd JR (1965) Environmental control of strontium and magnesium in *Mytilus*. *Geochim Cosmochim Ac* 29:385-398

Durif C, Dufour S & Elie P (2005) The silvering process of *Anguilla anguilla*: a new classification from the yellow resident to the silver migrating stage. *J Fish Biol* 66:1025–1043

Durif C, Guibert A & Elie P (2009) Morphological discrimination of the silvering stages of the European eel. *Am Fish S S* 58:103-111

Durif CMF, Gjosaeter J & Vollestad LA (2011) Influence of oceanic factors on *Anguilla anguilla* (L.) over the twentieth century in coastal habitats of the Skagerrak, southern Norway. *P Roy Soc B-Biol Sci* 278:464-473

EC (2007) Council Regulation (EC) No. 1100/2007 of 18 September 2007 establishing measures for the recovery of the stock of European eel. *Official Journal of the European Union* L248:17–23

Edeline E, Dufour S & Elie P (2005) Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. *Mar Ecol Prog Ser* 304:191–199

Edmonds JS, Moran MJ, Caputi N & Morita M (1989) Trace element analysis of fish sagittae as an aid to stock identification—pink snapper (*Chrysophrys auratus*) in Western Australian waters. *Can J Fish Aquat Sci* 46:50–54

Edmonds JS, Caputi N & Morita M (1991) Stock discrimination by trace-element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. *Aust J Mar Fresh Res* 42:383–389

EELREP (2005) Estimation of the reproduction capacity of European eel. Final report. http://www.fishbiology.net/eelrepsum.html.

Eero M, MacKenzie BR, Köster FW & Gislason H (2011) Multi-decadal responses of a cod (*Gadus morhua*) population to human-induced trophic changes, fishing, and climate. *Ecol Appl* 21:214–226

Ehlers J (1994) Geo-morphology and hydrography of the Wadden Sea. In: Lozán JL *et al.* (eds) *Warnsignale aus dem Wattenmeer*. Blackwell, Berlin, pp 1-10

Elfman M, Limburg KE, Kristianssona P, Malmqvista K & Pallona J (1999) Application of micro-PIXE to fish life history analyses: trace element analysis of otoliths. *Nucl Instrum Methods Phys Res B* 150:272–286

Elsdon TS & Gillanders BM (2002) Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. *Can J Fish Aquat Sci* 59:1796–1808

Elsdon TS & Gillanders BM (2003) Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. *Rev Fish Biol Fish* 13:219–235

Elsdon TS & Gillanders BM (2004) Fish otolith chemistry influenced by exposure to multiple environmental variables. *J Exp Mar Biol Ecol* 313:269–284

Elsdon TS & Gillanders BM (2005a) Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. *Mar Ecol Prog Ser* 285:233-243

Elsdon TS & Gillanders BM (2005b) Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. *Can J Fish Aquat Sci* 62:1143-1152

Elsdon TS & Gillanders BM (2006) Temporal variability in strontium, calcium, barium, and manganese in estuaries: implications for reconstructing environmental histories of fish from chemicals in calcified structures. *Estuar Coast Shelf Sci* 66:147–156

Elsdon TS, Wells BK, Campana SE, Gillanders BM, Jones CM, Limburg KE, Secor DH, Thorrold SR & Walther BD (2008) Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. *Oceanogr Mar Biol* 46:297–330

Farrell J & Campana SE (1996) Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comp Biochem Physiol* 115:103–109

Feunteun E (2002) Management and restoration of European eel population (*Anguilla anguilla*): an impossible bargain. *Ecol Eng* 18:575-591

Fietzke J, Liebetrau V, Günther D, Gürs K, Hametner K, Zumholz K, Hansteen TH & Eisenhauer A (2008) An alternative data acquisition and evaluation strategy for improved isotope ratio precision using LA-MC-ICP-MS applied for stable and radiogenic strontium isotopes in carbonates. *J Anal At Spectrom* 23:955–961

Fowler AJ, Campana SE, Thorrold SR & Jones CM (1995) Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS. *Can J Fish Aquat Sci* 52:1421-1430

Friedland KD, Miller MJ & Knights B (2007) Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. *ICES J Mar Sci* 64:519–530

Fuiman LA & Hoff GR (1995) Natural variation in elemental composition of sagittae from red drum. *J Fish Biol* 47:940-955

Gallahar NK & Kingsford MJ (1996) Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: an experimental investigation. *J Fish Biol* 48:174–186

Gao S, Liu X, Yuan H, Hattendorf B, Günther D, Chen L & Hu W (2002) Determination of forty-two major and trace elements in USGS and NIST SRM glasses by laser ablation-inductively coupled plasma-mass spectrometry. *Geostand Newsl* 26:181–196

Gauldie RW & Nathan A (1977) Iron content of the otoliths of tarakihi (Teleostei: Cheilodactylidae). *New Zeal J Mar Fresh* 11:179-191

Geeraerts C & Belpaire C (2010) The effects of contaminants in European eel: a review. *Ecotoxicology* 19:239–266

Geffen AJ, Pearce NJG & Perkins WT (1998) Metal concentrations in fish otoliths in relation to body composition after laboratory exposure to mercury and lead. *Mar Ecol Prog Ser* 165:235-245

Geffen AJ, Jarvis K, Thorpe JP, Leah RT & Nash RDM (2003) Spatial differences in the trace element concentrations of Irish Sea plaice *Pleuronectes platessa* and whiting *Merlangius merlangus* otoliths. *J Sea Res* 50:247-256

Gehrke PC, Gilligan DM & Barwick M (2001) Fish communities and migration in the Shoalhaven River – before construction of a fishway. NSW Fish Fin Rep Ser 26, New South Wales Fisheries, Port Stephens

Gillanders BM & Kingsford MJ (2000) Elemental fingerprints of otoliths of fish may distinguish estuarine 'nursery' habitats. *Mar Ecol Prog Ser* 201:273–286

Gillanders BM (2002) Connectivity between juvenile and adult fish populations: do adults remain near their recruitment estuaries? *Mar Ecol Prog Ser* 240:215-223

Grady JR, Johnson AG & Sanders M (1989). Heavy metal content in otoliths of king mackerel (*Scomberomorus cavalla*) in relation to body length and age. *Contrib Mar Sci* 31:17-23

Gross MR, Coleman RM & McDowall RM (1988) Aquatic productivity and the evolution of diadromous fish migration. *Science* 239:1291–1293

Haenen O, van Ginneken V, Engelsma M & van den Thillart G (2009) Impact of Eel Viruses on Recruitment of European Eel. In: *Spawning migration of the European Eel*. Eds: G. van den Thillart, S. Dufour, J. C. Rankin. Fish Fisheries Serie. 30:387-399

Halden NM, Babaluk JA, Campbell JL & Teesdale WJ (1995) Scanning proton microprobe analysis of strontium in an arctic charr, *Salvelinus alpinus*, otolith—implications for the interpretation of anadromy. *Environ Biol Fish* 43:333–339

Hamer PA & Jenkins GP (2007) Comparison of spatial variation in otolith chemistry of two fish species and relationships with water chemistry and otolith growth. J Fish Biol 71:1035–1055

Harrison IJ (2003) Mugilidae. In: Carpenter KE (ed) The living marine resources of the western central Atlantic, FAO species identification guide for fishery purposes. Vol. 2. FAO, Rome, pp 1071-1085

Heincke F (1894) Fishes of Helgoland. *Wiss Meersunters Abt Helgoland* 1:100-120

Heras S, Roldán MI & Castro MG (2009) Molecular phylogeny of Mugilidae fishes revised. *Rev Fish Biol Fish* 19:217-231

Hickling CF (1970) A contribution to the natural history of the English grey mullets (Pisces, Mugilidae). *J Mar Biol Assoc UK* 65:609-633

Higgins RM, Danilowicz BS, Balbuena JA, Daníelsdóttir AK, Geffen, AJ, Meijer WG, Modin J, Montero FE, Pampoulie C, Alonso DP, Schreiber A, Stefánsson MÖ & Wilson B (2010) Multi-disciplinary fingerprints reveal the harvest location of cod *Gadus morhua* in the northeast Atlantic. *Mar Ecol Prog Ser* 404: 197–206

Hinrichsen HH, Voss R, Wieland K, Köster F, Andersen KH & Margonski P (2007) Spatial and temporal heterogeneity of the cod spawning environment in the Bornholm Basin, Baltic Sea. *Mar Ecol Prog Ser* 345:245–254

Høie H, Otterlei E & Folkvord A (2004) Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua* L.). *ICES J Mar Sci* 61:243-251

Hoff GR & Fuiman LA (1993) Morphometry and composition of red drum otoliths—changes associated with temperature, somatic growth-rate, and age. *Comp Biochem Physiol A* 106:209–219

Hoff GR & Fuiman LA (1995) Environmentally induced variation in elemental composition of red drum (*Sciaenops ocellatus*) otoliths. *Bull Mar Sci* 56:578–591

Hotos GN (2003) A study on the scales and age estimation on the grey golden mullet, *Liza aurata* (Risso, 1810), in the lagoon of Messolonghi (W. Greece). *J Appl Ichthyol* 19:220-228

Hsu CC, Chang CW, Iizuka Y & Tzeng WN (2009) A growth check deposited at estuarine arrival in otoliths of juvenile flathead mullet (*Mugil cephalus* L.). *Zool Stud* 48:315-324

Hüssy K (2011) Review of western Baltic cod (*Gadus morhua*) recruitment dynamics. *ICES J Mar Sci* 68:1459-1471

Hutchings JA & Myers RA (1994) What can be learned from the collapse of a renewable resource? Atlantic cod, *Gadus morhua*, of Newfoundland and Labrador. *Can J Fish Aquat Sci* 51:2126-2146

Ibbotson A, Smith J, Scarlett P & Aprhamian M (2002) Colonisation of freshwater habitats by the European eel *Anguilla anguilla*. *Freshwater Biol* 47:1696–1706

Ibsch M, Anken R, Beier M & Rahmann H (2004) Endolymphatic calcium supply for fish otolith growth takes place via the proximal portion of the otocyst. *Cell Tissue Res* 317:333-336

ICES (2006) Report of the 2006 session of the joint EIFAC/ICES working group on eels. CM2006/ACFM:16, 352p

ICES (2007) Report of the ICES Baltic fisheries assessment working group. CM2007/ACFM, 15

ICES (2008) Report of the 2008 session of the joint EIFAC/ICES working group on eels. CM2009/ACOM:15, 192 p

ICES (2009) Report of the 2009 session of the joint EIFAC/ICES working group on eels. CM2009/ACOM:15, 540p

ICES (2010a) Report of the 2010 session of the joint EIFAC/ICES working group on eels. CM2010/ACOM:18, 721p

ICES (2010b) Report of the workshop on Baltic eel (WKBALTEEL). CM2010/ACOM:59

ICES (2010c) Report of the ICES Advisory Committee, ICES Advice 2010, Book 8. ICES, Copenhagen

Inoue JG, Miya M, Miller MJ, Sado T, Hanel R, Hatooka K, Aoyama J, Minegishi Y, Nishida M & Tsukamoto K (2010) Deep-ocean origin of the freshwater eels. *Biol Letters* 6:363-366

Jakob E, Hanel R, Klimpel S & Zumholz K (2009a) Salinity dependence of parasite infestation in the European eel *Anguilla anguilla* in northern Germany. *ICES J Mar Sci* 66:358–366

Jakob E, Walter T & Hanel R (2009b) A checklist of the protozoan and metazoan parasites of European eel (*Anguilla anguilla*): checklist of *Anguilla anguilla* parasites. *J Appl Ichthyol* DOI:10.1111/j.1439-0426.2009.01345.x

Jessop BM, Shiao JC, Iizuka Y & Tzeng WN (2002) Migratory behaviour and habitat use by American eels *Anguilla rostrata* as revealed by otolith microchemistry. *Mar Ecol Prog Ser* 233:217–229

Jessop BM, Shiao JC, Iizuka Y & Tzeng WN (2006) Migration of juvenile American eels *Anguilla rostrata* between freshwater and estuary, as revealed by otolith microchemistry. *Mar Ecol Prog Ser* 310:219–233

Jochum KP, Weis U, Stoll B, Kuzmin D, Yang Q, Raczek I, Jacob DE, Stracke A, Birbaum K, Frick DA, Günther D & Enzweiler J (2011) Determination of Reference Values for NIST SRM 610-617 Glasses Following ISO Guidelines. *Geostand Geoanal Res* 35:1-33

Kalish JM (1989) Otolith microchemistry—validation of the effects of physiology, age and environment on otolith composition. *J Exp Mar Biol Ecol* 132:151–178

Kalish JM (1990) Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. *Fish B-NOAA* 88:657–666

Kalish JM (1991) Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). *Mar Biol* 110:37-47

Kalish JM (1992) Formation of a stress-induced chemical check in fish otoliths. J *Exp Mar Biol Ecol* 162:265–277

KawakamiY, Mochioka N, MorishitaK, Tajima T, NakagawaH, Toh H & Nakazono A (1998) Factors influencing otolith strontium/calcium ratios in *Anguilla japonica* elvers. *Environ Biol Fish* 52:299–303

Kennedy BP, Blum JD, Folt CL & Nislow KH (2000) Using natural strontium isotopic signatures as fish markers: methodology and application. *Can J Fish Aquat Sci* 57:2280–2292

Kennedy CR (2007) The pathogenic helminth parasites of eels. *J Fish Dis* 30:319–334

Kimura R, Secor DH, Houde ED & Piccoli PM (2000) Up-estuary dispersal of young-of-the-year bay anchovy *Anchoa mitchilli* in the Chesapeake Bay: inferences from microprobe analysis of strontium in otoliths. *Mar Ecol Prog Ser* 208:217-227

Kirk RS (2003) The impact of *Anguillicola crassus* on European eels. *Fish Manage* 10:385–394

Knights B (2003) A review of the possible impacts of longterm oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. *Sci Total Environ* 310:237–244

Køie M (1991) Swimbladder nematodes (*Anguillicola* spp.) and gill monogeneans (*Pseudodactylogyrus* spp.) parasitic on the European eel (*Anguilla anguilla*). *J Cons Int Explor Mer* 47:391–398

Köster FW & Möllmann C (2000) Trophodynamic control by clupeid predators on recruitment success in Baltic cod? *ICES J Mar Sci* 57:310-323

Köster FW, Hinrichsen HH, Schnack D, St John MA, Mackenzie BR, Tomkiewicz J, Mollmann C, Kraus G, Plikshs M, Makarchouk A & Aro E (2003) Recruitment of Baltic cod and sprat stocks: identification of critical life stages and incorporation of environmental variability into stock-recruitment relationships. *Sci Mar* 67:129-154

Köster FW, Möllmann C, Hinrichsen HH, Wieland K, Tomkieviscz J, Kraus G, Voss R, Makarchouk A, MacKenzie BR, St. John MA, Schnack D, Rohlf N, Linkowski T & Beyer JE (2005) Baltic cod recruitment – the impact of climate variability on key processes. *ICES J Mar Sci* 62:1408-1425

Kraus RT & Secor DH (2004) Incorporation of strontium into otoliths of an estuarine fish. *J Exp Mar Biol Ecol* 302:85–106

Larsson P, Hamrin S & Okla L (1990) Fat content as a factor inducing migratory behavior in the eel (*Anguilla anguilla* L.) to the Sargasso Sea. *Naturwissenschaften* 77:488-490

Le Dantec J (1955) Quelques observations sur la biologie des muges des reservoirs de Certes (Audenge). *Rev Trav Inst Pech Marit* 19:93-112

Limburg KE (1995) Otolith strontium traces environmental history of subyearling American shad (*Alosa sapidissima*). *Mar Ecol Prog Ser* 119:25–35

Limburg KE, Landergren P, Westin L, Elfman M & Kristiansson P (2001) Flexible modes of anadromy in Baltic sea trout: making the most of marginal spawning streams. *J Fish Biol* 59:682–695

Limburg KE, Wickström H, Svedäng H, Elfman M & Kristiansson P (2003) Do stocked freshwater eels migrate? Evidence from the Baltic suggests “Yes”. *Am Fish Soc Symp* 33:275–284

Limburg KE, Huang R & Bilderback DH (2007) Fish otolith trace element maps: new approaches with synchrotron microbeam x-ray fluorescence. *X-Ray Spectrom* 36:336–342

Lin SH, Chang CW, Iizuka Y & Tzeng WN (2007a) Salinities, not diets, affect strontium/calcium ratios in otoliths of *Anguilla japonica*. *J Exp Mar Biol Ecol* 341:254–263

Lin YJ, Ložys L, Shiao JC, Iizuka Y & Tzeng WN (2007b) Growth differences between naturally recruited and stocked European eel *Anguilla anguilla* from different habitats in Lithuania. *J Fish Biol* 71:1773–1787

Lin YJ, Shiao JC, Ložys L, Plikšs M, Minde A, Iizuka Y, Rašals I & Tzeng WN (2009) Do otolith annular structures correspond to the first freshwater entry for yellow European eels *Anguilla anguilla* in the Baltic countries? *J Fish Biol* 75:2709–2722

MacKenzie B, Hinrichsen HH, Plikshs M, Wieland K & Zezera AS (2000) Quantifying environmental heterogenity estimating the size of habitat for successful cod egg development in the Baltic Sea. *Mar Ecol Prog Ser* 193:143-156

Maksymowska D, Richard P, Piekarek-Jankowska H & Riera P (2000) Chemical and isotopic composition of the organic matter sources in the Gulf of Gdansk (southern Baltic Sea). *Estuar Coast Shelf S* 51:585–598

Mann RHK & Blackburn JH (1991) The biology of the eel *Anguilla anguilla* (L.) in an English chalk stream and interactions with juvenile trout *Salmo trutta* (L.) and salmon *Salmo salar* L. *Hydrobiologia* 218:65–76

Marohn L, Prigge E, Zumholz K, Klügel A, Anders H & Hanel R (2009) Dietary effects on multi-element composition of European eel (*Anguilla anguilla*) otoliths. *Mar Biol* 156:927–933

Marohn L, Hilge V, Zumholz K, Klügel A, Anders H & Hanel R (2011) Temperature dependency of element incorporation into European eel (*Anguilla anguilla*) otoliths. *Anal Bioanal Chem* 6:2175-2184

Martin GB, Thorrold SR & Jones CM (2004) Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). *Can J Fish Aquat Sci* 61:34–42

Martin GB & Thorrold SR (2005) Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*. *Mar Ecol Prog Ser* 293:223–232

Martin GB & Wuenschel MJ (2006) Effect of temperature and salinity on otolith element incorporation in juvenile gray snapper *Lutjanus griseus*. *Mar Ecol Prog Ser* 324:229–239

Matthäus W (1996) Ozeanographische Besonderheiten. In: Lozán JL *et al.* (eds) *Warnsignale aus der Ostsee*. Blackwell, Berlin, pp 17-24

McDowall RM (1988) Diadromy in fishes: migrations between freshwater and marine environments. Timber Press, Portland, Oregon

Meixner M (1978) Grey mullets presently are not considered as rare vagrants anymore. *Inf Fischwirtsch* 25:95-96

Melia P, Bevacqua D, Crivelli AJ, Panfili J, de Leo GA & Gatto M (2006) Sex differentiation of the European eel in brackish and freshwater environments: a comparative analysis. *J Fish Biol* 69:1228–1235

Meyer PF (1935) Marine and brackish fishes of Mecklenburg. *Arch Ver Freunde Naturgesch Mecklenburg* 9:59-98

Miles NG, West RJ & Norman MD (2009) Does otolith chemistry indicate diadromous lifecycles for five Australian riverine fishes*? Mar Freshwater Res* 60:903-911

Miller JA (2009) The effects of temperature and water concentration on the otolith incorporation of barium and manganese in black rockfish *Sebastes melanops*. *J Fish Biol* 75:39–60

Milton DA & Chenery SR (2001) Sources and uptake of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). *J Exp Mar Biol Ecol* 264:47–65

Milton DA & Chenery SR (2003) Movement patterns of the tropical shad hilsa (*Tenualosa ilisha*) inferred from transects of Sr-87/Sr-86 isotope ratios in their otoliths. *Can J Fish Aquat Sci* 60:1376-1385

Mohr EW (1928) Teleostei Physoclisti: 5. Atheriniformes. In: Die Tierwelt der Nord- und Ostsee, 4. Akademische Verlagsgesellschaft, Leipzig, Germany, pp 36-44

Mohr H & Horn W (1977) Trials in catching grey mullets in the Wadden Sea. *Inf Fischwirtsch* 24:174-176

Mohr H (1986) The grey mullet - a new fish species in the Wadden Sea. *Seevögel* 7:63-65

Mohr H (1988) On the biology of the Grey Mullets and their importance for fisheries at the German coast. *Protok Fisch Tech* 15:163-175

Mosegaard H, Svedäng H & Taberman K (1988) Uncoupling between somatic and otolith growth in arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. *Can J Fish Aquat Sci* 45:1514-1524

Munk P, Hansen MM, Maes GE, Nielsen TG, Castonguay M, Riemann L, Sparholt H, Als TD, Aarestrup K, Andersen NG & Bachler M (2010) Oceanic fronts in the Sargasso Sea control the early life and drift of Atlantic eels. *P Roy Soc B-Biol Sci* 277:3593-3599

Muus BJ & Nielsen JG (1999) *Sea fish*. Scandinavian fishing year book, Hedehusene, Denmark

Nelson JS (2006) *Fishes of the world*. 4th ed. John Wiley & Sons, Inc., Hoboken, New Jersey, USA

Neukamm R (2009) Final report, FIAF Pilotprojekt zur Förderung des Aales in den Gewässersystemen Nord-Ostsee-Kanal und Elbe-Lübeck-Kanal. Available at http://www.lsfv-sh.de/downloads/sonstiges/fiaf-aalprojekt-abschlussbericht-2006-2008/view.html

Neuenfeldt S, Hinrichsen HH, Nielsen A & Andersen KH (2007) Reconstructing migrations of individual cod (*Gadus morhua* L.) in the Baltic Sea by using electronic data storage tags. *Fish Oceanogr* 16:526–535

Nielsen EE, Hansen MM, Schmidt C, Meldrup D & Grønkjær (2001) Population of origin of Atlantic cod. *Nature* 413:272

Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D & Grønkjær P (2003) Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. *Mol Ecol* 12:1497–1508

Nissling A, Kryvi H & Vallin L (1994) Variation in egg buoyancy of Baltic cod *Gadus morhua* and its implications for egg survival in prevailing conditions in the Baltic Sea. *Mar Ecol Prog Ser* 10:67-74

Olsson PE, Kling P & Hogstrand C (1998) Mechanisms of heavy metal accumulation and toxicity in fish. In: Langston WJ, Bebianno MJ (eds) *Metal Metabolism in Aquatic Environments. Chapman and Hall*, London, p 321-350

OSPAR Commission (2000) Quality status report 2000, Region II – Greater North Sea. OSPAR Commission, London

Otake T, Ishii T, Nakahara M & Nakamura R (1994) Drastic changes in otolith strontium/calcium ratios in leptocephali and glass eels of Japanese eel *Anguilla japonica*. *Mar Ecol Prog Ser* 112:189–193

Otake T, Ishii T, Ishii T, Nakahara M & Nakamura R (1997) Changes in otolith strontium: calcium ratios in metamorphosing Conger *Myriaster leptocephali*. *Mar Biol* 128:565–572

Otterlind G (1985) Cod migration and transplantation experiments in the Baltic. *J Appl Ichthyol* 1:3–16

Palm S, Dannewitz J, Prestegaard T & Wickstrom H (2009) Panmixia in European eel revisited: no genetic difference between maturing adults from southern and northern Europe. *Heredity* 103:82–89

Palstra AP, van Ginneken VJT, Murk AJ & van den Thillart GEEJM (2006) Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? *Naturwissenschaften* 93:145

Palstra AP, Heppener DFM, van Ginneken VJT, Szekely C & van den Thillart GEEJM (2007) Swimming performance of silver eels is severely impaired by the swimbladder parasite *Anguillicola crassus*. *J Exp Mar Biol Ecol* 352:244–256

Palstra AP & van den Thillart GEEJM (2010) Swimming physiology of European silver eels (*Anguilla anguilla* L.): energetic costs and effects on sexual maturation and reproduction. *Fish Physiol Biochem* 36:297–322

Pangle KL, Ludsin SA & Fryer BJ (2010) Otolith microchemistry as a stock identification tool for freshwater fishes: testing its limits in Lake Erie. *Can J Fish Aquat Sci* 67:1475-1489

Pannella G (1974) Fish otoliths: daily growth layers and periodical patters. *Science* 173:1124-1127

Pankhurst NW (1982) Relation of visual changes to the onset of sexual maturation in the European eel *Anguilla anguilla* (L.). *J Fish Biol* 21:127–140

Pankhurst NW & Sorensen PW (1984) Degeneration of the alimentary tract in sexually maturing European *Anguilla anguilla* (L.) and American eels *Anguilla rostrata* (LeSueur). *Can J Fish Aquat Sci* 62:1143-1149

Payan P, Kossmann H, Watrin A, Mayer-Gostan N & Boeuf G (1997) Ionic composition of endolymph in teleosts: origin and importance of endolymph alkalinity. *J Exp Biol* 200:1905-1912

Payan P, Borelli G, Priouzeau F, De Pontual H, Boeuf G & Mayer-Gostan N (2002) Otolith growth in trout *Oncorhynchus mykiss*: supply of Ca2+ and Sr2+ to the saccular endolymph. *J Exp Biol* 205:2687-2695

Payan P, De Pontual H, Boeuf G & Mayer-Gostan N (2004a) Endolymph chemistry and otolith growth in fish. *C R Palevol* 3:535–547

Payan P, De Pontual H, Edeyer A, Borelli G, Boeuf G & Mayer-Gostan N (2004b) Effects of stress on plasma homeostasis, endolymph chemistry, and check formation during otolith growth in rainbow trout (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci* 61:1247–1255

Pearce NJG, Perkins WT, Westgate JA, Gorton MP, Jackson SE, Neal CL & Chenery SP (1997) A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. *Geostand Newsl* 21:115–144

Pohl C & Hennings U (2009) Trace metal concentrations and trends in Baltic surface and deep waters. HELCOM Indicator Fact Sheets 2009. Online. http://www.helcom.fi/BSAP_assessment /ifs/ifs2010/en_GB/HeavyMetalsWater/_text/

Postma H (1982) Hydrogaphy of the Wadden Sea: Movements and properties of water and particulate matter. In: Wolff WJ (ed) Ecology of the Wadden Sea Vol. 1. A. A. Balkema, Rotterdam, pp 2/1-2/75

Radtke RL & Targett TE (1984) Rhythmic structural and chemical patterns in otoliths of the Antarctic fish *Notothenia larseni*: Their application to age determination. *Polar Biol* 3:203-210

Radtke RL, Kinzie RA & Folsom SD (1988) Age at recruitment of Hawaiian fresh-water gobies. *Environ Biol Fish* 23:205–213

Radtke RL (1989) Strontium calcium-concentration ratios in fish otoliths as environmental indicators. *Comp Biochem Physiol A* 92:189–193

Radtke RL, Lenz P, Showers W & Moksness E (1996) Environmental information stored in otoliths: insights from stable isotopes. *Mar Biol* 127:161-170

Reibisch J (1899) Über die Eizahl bei *Pleuronectes platessa* und die Altersbestimmung dieser Form aus den Otolithen. Wissenschaftliche Meeresuntersuchungen (Kiel) 4:233-248

Reinhardt EG, Stanley DJ & Patterson RT (1998) Strontium isotopic-paleontological method as a high-resolution paleosalinity tool for lagoonal environments. *Geology* 26:1003-1006

Robinet TT & Feunteun EE (2002) Sublethal effects of exposure to chemical compounds: a cause for the decline in atlantic eels? Ecotoxicology 11:265-277

Rooker JR, Secor DH, Zdanowicz VS, De Metrio G & Relini LO (2003) Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. *Fish Oceanog*r 12:75–84

Sadovy Y & Severin KP (1992) Trace-elements in biogenic aragonite—correlation of body growth-rate and strontium levels in the otoliths of the white grunt, *Haemulon plumieri* (Pisces, Haemulidae). *B Mar Sci* 50:237–257

Sadovy Y & Severin KP (1994) Elemental patterns in red hind (*Epinephelus guttatus*) otoliths from Bermuda and Puerto-Rico reflect growth-rate, not temperature. *Can J Fish Aquat Sci* 51:133–141

Schaber M, Hinrichsen HH, Neuenfeldt S & Voss R (2009) Hydroacoustic resolution of small-scale vertical distribution in Baltic cod *Gadus morhua*—habitat choice and limits during spawning. *Mar Ecol Prog Ser* 377:239–253

Schmidt J (1923) Breeding places and migrations of the eel. *Nature* 111:51–54

Secor DH, Dean JM & Laban EH (1992) Otolith removal and preparation for microstructural examination. In *Otolith microstructure examination and analysis*. Stevenson DK & Campana SE, ed. pp 19-57. Ottawa, Canada: Canadian special publication of Fisheries and Aquatic Science 117

Secor DH, Henderson-Arzapalo A & Piccoli PM (1995) Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *J Exp Mar Biol Ecol* 192:15–33

Secor DH & Rooker JR (2000) Is otolith strontium a useful scalar of life-cycles in estuarine fishes? *Fish Res* 46:359–371

Shen KN, Chang CW, Iizuka Y & Tzeng WN (2009) Facultative habitat selection in Pacific tarpon *Megalops cyprinoides* as revealed by otolith Sr:Ca ratios. *Mar Ecol Prog Ser* 387:255-263

Shiao JC, Lin LY, Horng JL, Hwang PP & Kaneko T (2005) How can teleostean inner ear hair cells maintain the proper association with the accreting otolith? *J Comp Neurol* 488:331-341

Shiao JC, Lozys L, Iizuka Y & Tzeng WN (2006) Migratory patterns and contribution of stocking to the population of European eel in Lithuanian waters as indicated by otolith Sr:Ca ratios. *J Fish Biol* 69:749–769

Siedler G & Hatje G (1974) Temperature, salinity and density. In: Maagard L, Rheinheimer G (eds) *Meereskunde der Ostsee*. Springer-Verlag, Berlin, Heidelberg, pp 43-60

Simon J & Dörner H (2005) Marking the European eel with oxytetracycline, alizarin red and coded wire tags: an evaluation of methods. *J Fish Biol* 67:1486–1491

Sprengel G & Lüchtenberg H (1991) Infection by endoparasites reduces maximum swimming speed of European smelt *Osmerus eperlanus* and European eel *Anguilla anguilla*. *Dis Aquat Org* 11:31–35

Standish JD, Sheehy M & Warner RR (2008) Use of otolith natal elemental signatures as natural tags to evaluate connectivity among open-coast fish populations. *Mar Ecol Prog Ser* 356:259-268

Stigebrandt A & Wulff F (1987) A model for the dynamics of nutrients and oxygen in the Baltic Proper. *J Mar Res* 45:729–759

Strong MB, Neilson JD & Hunt JJ (1986) Aberrant crystallization of Pollock (*Pollachius virens*) otoliths. *Can J Fish Aquat Sci* 43:1457–1463

Svedäng H, Neuman E & Wickström H (1996) Maturation patterns in female European eel: age and size at the silver eel stage. *J Fish Biol* 48:342–351

Svedäng H & Wickström H (1997) Low fat contents in female silver eels: indications of insufficient energetic stores for migration and gonadal development. *J Fish Biol* 50:475–486

Tesch FW (1999) *Der Aal*. Blackwell Wissenschaftsverlag, Berlin

Thiel R, Winkler H & Urho L (1996) Zur Veränderung der Fischfauna. In: Lozán JL *et al.* (eds) *Warnsignale aus der Ostsee*. Blackwell, Berlin, pp 181-188

Thomson JM (1966) The grey mullets. *Oceanogr Mar Biol Ann Rev* 4:301-335

Thorrold SR, Jones CM & Campana SE (1997) Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). *Limnol Oceanogr* 42:102-111

Thorrold SR, Jones CM, Campana SE, McLaren JW & Lam JWH (1998) Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). *Limnol Oceanogr* 43:1826-1835

Thorrold SR, Campana SE, Jones CM & Swart PK (1997) Factors determining δ13C and δ18O fractionation in aragonitic otoliths of marine fish. *Geochim Cosmochim Ac* 61:2909-2919

Thorrold SR, Latkoczy C, Swart PK & & Jones CM (2001) Natal Homing in a Marine Fish Metapopulation. *Science* 291:297-299110

Thresher RE, Proctor CH, Gunn JS & Harrowfield IR (1994) An evaluation of electronprobe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae). *Fish Bull* 92:817-840

Thresher RE (1999) Elemental composition of otoliths as a stock delineator in fishes. *Fish Res* 43:165-204

Townsend DW, Radtke RL, Corwin S & Libby DA (1992) Strontium-calcium ratios in juvenile atlantic herring *Clupea harengus* L otoliths as a function of water temperature. *J Exp Mar Biol Ecol* 160:131–140

Townsend DW, Radtke RL, Malone DP & Wallinga JP (1995) Use of otolith strontium:calcium ratios for hindcasting larval cod *Gadus morhua* distributions relative to water masses on Georges Bank. *Mar Ecol Prog Ser* 119:37-44

Tsukamoto K, Nakai I & Tesch WV (1998) Do all freshwater eels migrate? Nature 396:635–636

Tsukamoto K & Arai T (2001) Facultative catadromy of the eel *Anguilla japonica* between freshwater and seawater habitats. *Mar Ecol Prog Ser* 220:265-276

Tzeng WN (1994) Temperature effects on the incorporation of strontium in otolith of Japanese eel *Anguilla japonica*. *J Fish Biol* 45:1055–1066

Tzeng WN (1996) Effects of salinity and ontogenetic movements on strontium:calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica* Temminck and Schlegel. *J Exp Mar Biol Ecol* 199:111–122

Tzeng WN, Severin KP & Wickström H (1997) Use of otolith microchemistry to investigate the environmental history of European eel *Anguilla anguilla*. *Mar Ecol Prog Ser* 149:73–81

Tzeng WN, Severin KP, Wickström H & Wang CH (1999) Strontium bands in relation to age marks in otoliths of European eel *Anguilla anguilla*. *Zool Stud* 38:452–457

Tzeng WN, Wang CH, Wickström H & Reizenstein M (2000) Occurrence of the semi-catadromous European eel *Anguilla anguilla* in the Baltic Sea. *Mar Biol* 137:93-98

Tzeng WN, Shiao JC & Iizuka Y (2002) Use of otolith Sr:Ca ratios to study the riverine migratory behaviors of Japanese eel *Anguilla japonica*. *Mar Ecol Prog Ser* 245:213–221

Tzeng WN, Chang CW, Wang CH, Shiao JC, Iizuka Y, Yang Yj, You CF & Lozys L (2007) Misidentification of the migratory history of anguillid eels by Sr/Ca ratios of vaterite otoliths. *Mar Ecol Prog Ser* 348:285–295

Umezawa A & Tsukamoto K (1991) Factors influencing otolith increment formation in Japanese eel, *Anguilla japonica*, elvers. *J Fish Biol* 39:211–223

van den Thillart G, Palstra AP & van Ginneken V (2007) Simulated migration of European silver eel; swim capacity and cost of transport. *J Mar Sci Technol* 15:1–16

van Ginneken V, Ballieux TB, Willemzer R, Coldenhoff K, Lentjes E, Antonissen E, Haenen O & van den Thillart G (2005) Hematology patterns of migrating European eels and the role of EVEX virus. *Comp Biochem Physiol Part C* 140:97-102

van Ginneken V, Palstra A, Leonards P, Nieveen M, van den Berg H, Flik G, Spanings T, Niemantsverdriet P, van den Thillart G & Murk A (2009) PCBs and the energy cost of migration in the European eel (*Anguilla anguilla* L.). *Aquat Toxicol* 92:213–220

Vorberg R, Bolle L, Jager Z & Neudecker T (2005). Wadden Sea quality status report 2004. *Fish Wadden Sea Ecosystem* 19:219-236

Wachniew P (2006) Isotopic composition of dissolved inorganic carbon in a large polluted river: The Vistula, Poland. *Chem Geol* 233:293–308

Walther BD & Thorrold SR (2006) Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. *Mar Ecol Prog Ser* 311:125–130

Walther BD, Dempster T, Letnic M & McCulloch MT (2011) Movements of diadromous fish in large unregulated tropical rivers inferred from geochemical tracers. *PLoS ONE* 6: e18351

Warner RR, Swearer SE, Caselle JE, Sheehy M & Paradis G (2005) Natal trace-elemental signatures in the otoliths of an open-coast fish. *Limnol Oceanogr* 50:1529-1542

Wang CH, Hsu CC, Chang CW, You CF & Tzeng WN (2010) The migratory environmental history of freshwater resident flathead mullet *Mugil cephalus* L. in the Tanshui river, northern Taiwan. *Zool Stud* 49:504-514

Weber JN (1973) Incorporation of strontium into reef coral skeletal carbonate. *Geochim Cosmochim Ac* 37:2173-2190

Weber PK, Hutcheon ID, McKeegan KD & Ingram BL (2002) Otolith sulfur isotope method to reconstruct salmon (*Oncorhynchus tshawytscha*) life history. *Can J Fish Aquat Sci* 59:587-591

Westin L (1990) Orientation mechanisms in migrating European silver eel (*Anguilla anguilla*): Temperature and olfaction. *Mar Biol* 106:175-179

Westin L & Limburg KE (2002) Newly discovered reproductive isolation reveals sympatric populations of *Esox Indus* in the Baltic. *J Fish Biol* 61:1647-1652

Westin L (2003) Migration failure in stocked eels *Anguilla anguilla*. *Mar Ecol Prog Ser* 254:307-311

Wieland K, Jarre-Teichmann A & Horbowa K (2000) Changes in the timing of spawning of Baltic cod: possible causes and implications for recruitment. *ICES J Mar Sci* 57:452-464

Yamashita Y, Otake T & Yamada H (2000) Relative contributions from exposed inshore and estuarine nursery grounds to the recruitment of stone flounder, *Platichthys bicoloratus*, estimated using otolith Sr:Ca ratios. *Fish Oceanogr* 9:316–327

Yoshinaga J, Nakama A, Morita M & Edmonds JS (2000) Fish otolith reference material for quality assurance of chemical analyses. *Mar Chem* 69:91–97

Zumholz K, Hansteen TH, Klügel A & Piatkowski U (2006) Food effects on statolith composition of the common cuttlefish (*Sepia offcinalis*). *Mar Biol* 150:237–244

ANNEX

List of figures

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LIST OF PUBLICATIONS

The chapters of this thesis are partly published (Chapter II, III & V), submitted (Chapter I) or prepared for submission (Chapter IV) to peer reviewed scientific journals

Chapter I
Released into a perilous environment? – The dilemma of mass restocking of eels to freshwaters

Authors: Lasse Marohn, Eva Jakob, Reinhold Hanel

Manuscript prepared for submission

Chapter II
Temperature dependency of element incorporation into European eel (*Anguilla anguilla*) otoliths

Authors: Lasse Marohn, Volker Hilge, Karsten Zumholz, Andreas Klügel, Heike Anders, Reinhold Hanel

Published in *Analytical and Bioanalytical Chemistry* 399:2175–2184 (2011)

Chapter III
Dietary effects on multi-element composition of European eel (*Anguilla anguilla*) otoliths

Authors: Lasse Marohn, Enno Prigge, Karsten Zumholz, Andreas Klügel, Heike Anders, Reinhold Hanel

Published in *Marine Biology* 156: 927-933 (2009)

Chapter IV
Evaluating the suitability of otolith microchemistry for stock separation of Baltic cod (*Gadus morhua*)

Authors: Franziska Heidemann, Lasse Marohn, Hans-Harald Hinrichsen, Bastian Huwer, Karin Hüssy, Andreas Klügel, Uwe Böttcher, Reinhold Hanel

Manuscript prepared for submission

Chapter V
Newcomers in the Baltic Sea: an attempt to trace the origins and whereabouts of thicklip grey mullet Chelon labrosus

Authors: Matthias Schaber, Lasse Marohn, Christoph Petereit, Jan P. Schröder, Karsten Zumholz, Reinhold Hanel

Published in *Fisheries Science* 77: 757-764 (2011)

CONTRIBUTIONS OF AUTHORS

Chapter I

Released into a perilous environment? – The dilemma of mass restocking of eels to freshwaters

The investigation was designed by Reinhold Hanel. Otolith analyses, data analysis, and graphics were done by Lasse Marohn. Eel sampling and dissection were performed by Lasse Marohn and Eva Jakob. Parasitological examinations were conducted by Eva Jakob. The manuscript was written by Lasse Marohn and Reinhold Hanel. Eva Jakob provided helpful comments to improve the manuscript.

Chapter II

Temperature dependency of element incorporation into European eel (*Anguilla anguilla*) otoliths

The experiment was designed by Lasse Marohn, Karsten Zumholz and Reinhold Hanel and performed by Lasse Marohn, who also conducted eel dissection, otolith preparation and otolith analysis. Andreas Klügel and Heike Anders adjusted the analytical setup for the LA-ICPMS and supported during analyses. Water element analysis was conducted by Heike Anders. Data processing, first manuscript writing and graphics were done by Lasse Marohn. All co-authors provided helpful input to improve the manuscript.

Chapter III

Dietary effects on multi-element composition of European eel (*Anguilla anguilla*) otoliths

The experiment was designed by Karsten Zumholz, Enno Prigge and Reinhold Hanel and performed by Enno Prigge, who was also responsible for eel dissection and measurements. Otolith processing and analyses were done by Lasse Marohn. Andreas Klügel and Heike Anders supported during otolith elemental analyses and adjusted the analytical setup. Analyses of water and food items were conducted by Heike Anders. Data analyses, writing and all graphics were made by Lasse Marohn. All co-authors provided important support to improve the manuscript.

Chapter IV

Evaluating the suitability of otolith microchemistry for stock separation of Baltic cod (*Gadus morhua*)

The manuscript was written by Franziska Heidemann and Lasse Marohn. Graphics were made by Franziska Heidemann and Bastian Huwer. Otolith preparation, otolith analyses and data analyses were performed by Franziska Heidemann under the supervision of Lasse Marohn. Andreas Klügel

adjusted the analytical setup and supported during measurements. All co-authors provided helpful input to improve the manuscript.

Chapter V

Newcomers in the Baltic Sea: an attempt to trace the origins and whereabouts of thicklip grey mullet *Chelon labrosus*

The manuscript was written by Matthias Schaber, Lasse Marohn and Christoph Petereit, including all graphics. Sampling and dissection was done by Christoph Petereit, Jan Schröder and Matthias Schaber. Otolith preparation was conducted by Lasse Marohn, Christoph Petereit and Jan Schröder. Otolith and data analyses were made by Lasse Marohn. The manuscript was improved by comments of all co-authors.

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CURRICULUM VITAE

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Education

07/2007 – 10/2011	PhD-Student at the Leibniz Institute of Marine Sciences IFM-GEOMAR, Kiel, Germany. Title: "Microchemical analyses of otoliths in Baltic Sea fish - Possibilities and limitations of otolith elemental analysis to describe individual life history and stock characteristics of fish in the Baltic Sea"
04/2007	Diploma thesis "Differential Gene Expression Analysis of European Eel (*Anguilla anguilla*) after PCB Contamination" at Leibniz Institute of Marine Sciences, IFM-GEOMAR, Kiel, Germany and Max Rubner Institute, Hamburg, Germany
10/2001 – 04/2007	Student in Biology at the Christian Albrechts University, Kiel, Germany
06/1994	School leaving examinations (Abitur) at the Freie Waldorfschule, Kiel, Germany

Scientific work experience

03/2009 – present	Scientist at the Leibniz Institute of Marine Sciences, IFM-GEOMAR, Kiel, Germany Project "Quantification of the mortality of European eels in German inland waters"
07/2007 – 02/2009	Scientist at the Leibniz Institute of Marine Sciences, IFM-GEOMAR, Kiel, Germany Project "Habitat selection of the European eel"
2002 – 2006	Assistant at the Aquarium at the Leibniz Institute of Marine Sciences, IFM-GEOMAR, Kiel, Germany

Research cruises

06/2005 – 07/2005	RV "Alkor"; pelagic trawl fishery, German Bight
05/2004	RC "Littorina"; dredge fishery, Baltic Sea
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Workshops and Conferences

11/11	Bio-Resources from Oceans. Interdisciplinary workshop. Berlin, Germany

11/11	21st Biennial Conference of the Coastal and Estuarine Research Federation. Daytona Beach, USA
03/2011	2nd ICES/ACOM Workshop on Age Reading of European and American Eel. Bordeaux, France
07/2010	10th European Workshop on Laser Ablation. Kiel, Germany
09/2009	ICES Annual Science Conference. Berlin, Germany
08/2009	4th International Otolith Symposium. Monterey, USA
04/2009	1st ICES/ACOM Workshop on Age Reading of European and American Eel. Bordeaux, France
10/2008	3rd International student conference. Biodiversity and functioning of aquatic ecosystems in the Baltic Sea region. Klaipeda (Juodkrante), Lithuania
11/2006	AQUALAB Student Conference. Galway, Ireland

Peer reviewed articles

Heidemann F, Marohn L, Hinrichsen HH, Huwer B, Hüssy K, Klügel A, Böttcher U & Hanel R (in prep) Evaluating the suitability of otolith microchemistry for stock separation of Baltic cod (*Gadus morhua*).

Marohn L, Jakob E & Hanel R (in prep) Released into a perilous environment? – The dilemma of mass restocking of eels to freshwaters.

Schaber M, Marohn L, Petereit C, Schröder JP, Zumholz K & Hanel R (2011) Newcomers in the Baltic Sea: an attempt to trace the origins and whereabouts of thicklip grey mullet *Chelon labrosus*. *Fish Sci* 77:757-764

Marohn L, Hilge V, Zumholz K, Klügel A, Anders H & Hanel R (2011) Temperature dependency of element incorporation into European eel (*Anguilla anguilla*) otoliths. *Anal Bioanal Chem* 6:2175-2184

Marohn L, Prigge E, Zumholz K, Klügel A, Anders H & Hanel R (2009) Dietary effects on multi-element composition of European eel (*Anguilla anguilla*) otoliths. *Mar Biol* 156:927-933

Marohn L, Rehbein H, Kündiger R & Hanel R (2008) The suitability of cytochrome-P4501A1 as a biomarker for PCB contamination in European eel (*Anguilla anguilla*). *J Biotechnol* 136:135-139

Oral presentations and poster

Marohn L, Jakob E, Klügel A & HanelR. The impact of continental migratory behavior and habitat choice on the spawner quality of European eels. 21st Biennial Conference of the Coastal and Estuarine Research Federation. Daytona Beach, USA. November 2011

Marohn L, Prigge E, Hilge V, Zumholz K, Klügel A & Hanel R. Effects of water temperature and diet on the elemental composition of European eel (*Anguilla anguilla*) otoliths. 10th European Workshop on Laser Ablation. Kiel, Germany. June/July 2010

Marohn L, Prigge E, Zumholz K, Klügel A & Hanel R. Effects of diet and water temperature on the multi-element composition of European eel (*Anguilla anguilla*) otoliths. 4th International Otolith Symposium, Monterey, USA. August 2009

Marohn L, Zumholz K, Klügel A, Anders H & Hanel R. Microchemical analyses of European eel (*Anguilla anguilla*) otoliths. 9th European workshop on laser ablation in elemental and isotopic analysis, Prague, Czech Republic. July 2008 (Poster)

Marohn L, Zumholz K, Jakob E & Hanel R. Migratory behaviour and habitat choice of the European eel (*Anguilla anguilla*). Microchemical otolith analyses provide an insight into the environmental history of an endangered species. 3rd International student conference. Biodiversity and functioning of aquatic ecosystems in the Baltic Sea region, Klaipeda (Juodkrante), Lithuania. October 2008

ERKLÄRUNG

Hiermit erkläre ich, dass die vorliegende Dissertation selbständig von mir angefertigt wurde. Die Dissertation ist nach Form und Inhalt meine eigene Arbeit und es wurden keine anderen als die angegebenen Hilfsmittel verwendet. Diese Arbeit wurde weder ganz noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Fischereibiologie erfolgen.

Kiel, den 10.07.2011

Lasse Marohn