行政院國家科學委員會專題研究計畫 成果報告

遺傳及耳石微化學標記在兩側洄游型及瀕危經濟性魚類族群管理的應用(3/3)

研究成果報告(完整版)

計畫類別：個別型
計畫編號：NSC 95-2313-B-002-016-
執行期間：95年08月01日至96年09月30日
執行單位：國立臺灣大學漁業科學研究所

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報告附件：國際合作計畫研究心得報告

處理方式：本計畫可公開查詢

中華民國 96年09月30日

PROGRAM ON MUTUAL FUNDS FOR THE SCIENTIFIC CO-OPERATION OF REPUBLIC OF LITHUANIA AND REPUBLIC OF LATVIA WITH
REPUBLIC OF CHINA (TAIWAN)

FINAL REPORT

On

Application of Genetic and Microchemical Markers as Implements for Diadromous and Endangered Commercial Fish Species Populations Management

Submitted to: National Science Council, Bonn office, Bonn, Germany

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May 2007

APPLICATION OF GENETIC AND MICROCHEMICAL MARKERS AS IMPLEMENTS FOR DIADROMOUS AND ENDANGERED COMMERCIAL FISH SPECIES POPULATIONS MANAGEMENT

YEAR 2004 - 2007

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CONTENT

APPLICATION OF GENETIC AND MICROCHEMICAL MARKERS AS IMPLEMENTS FOR DIADROMOUS AND ENDANGERED COMMERCIAL FISH SPECIES POPULATIONS MANAGEMENT
YEAR (2004 - 2007)

I. GENERAL PART

1.1 Summary of GMM project

This research project is aimed at demonstrating the effective use of the genetic and microchemical markers analysis as a fundamental scientific basis upon which advice could be given to the interested stakeholders: fisheries managers, conservationists, fishermen, socio-economists and etc. on a local, national or international scale. By analyzing microsatellite DNA in the tissues and Sr/Ca ratio in the otoliths of fish, chosen as model species, i.e. the facultatively catadromous eel (*Anquilla anquilla*), as well as the freshwater and the amphidromous-like behaving pikeperch (*Sander lucioperca*) and the perch (*Perca fluviatilis*), the project results should provide a set of answers to crucial questions of the stock management. Problems such as absence of knowledge of natural recruitment, stocking effectiveness of the endangered European eel and general lack of understanding of the genetic structure, a spatial and temporal migratory behavior of the amphidromous perch and pikeperch in the region impede management of the species. The project should assess:

- the population genetic structure and stock boundaries of the targeted fish species in the South-Eastern Baltic Sea region based on the investigations into polymorphic microsatellite loci;
- migration patterns of the targeted fish species between fresh and brackish water bodies, as well as the eel recruitment and stocking success by means of microchemical otolith analysis.

Cooperation between specialists from Lithuania, Latvia and Taiwan, R.O.C. should lead to an efficient combination of facilities, knowledge and expertise, which are adequate to obtain new important and reliable results in the field of diadromous fish population management, i.e. genetic structuring and tracing of the migratory behavior. One of the most important objectives of the project is to provide a good opportunity to transfer knowledge from the high level research centre of Taiwan, R.O.C. to those in the Baltic countries. Joint efforts and knowledge will lead to an innovative approach to management-related problems and expand our understanding of life history, behavior and the population structure of fish species.

The implementation of the project is expected to contribute to integration and promotion of cooperation between the regions. The project will be implemented in accordance with the relevant international and national legal and ethical requirements.

1.2 Objectives and Tasks within GMM Project

Many interpretations of phylogeographical and population genetic studies of fish suffer from uncertainty arising from artificial stocking or due to lack of information about the migrating behaviour of species of interest. Successful fish stock management is not effective without a complete understanding of the population genetic structure, stock boundaries and spatial and temporal migratory patterns. In recent years, advances in analytical techniques have led to the use of otolith microchemistry as an aid to stock discrimination in fishes. Genetic markers are widely used as implements for acknowledging potential stock differences. The recent development of such investigation methods as the assay of microsatellites provided us with the opportunity to evaluate relationships between populations and subpopulations. By combining two modern techniques, i.e. the otolith microchemical and microsatellite DNA analysis, we intend to demonstrate the effectiveness of such an approach to the diadromous fish stocks management. The techniques applied will present an innovate approach to address the management-related problems. Therefore, combining the microchemical otolith analyses with the genetic analyses will enable us to expand our knowledge of the migration pattern and population/subpopulation-related evaluation of diadromous fish.

The purpose of the present research proposal is to demonstrate benefits of the presented techniques in outlining guidelines for commercially important fish species management based on the data derived from the investigations into the subpopulations genetic structure and seasonal migration behaviour. During the GMM project implementation the catadromous[1] eel (*Anquilla anquilla*), as well as both freshwater and amphidromous[2]-like behaving subpopulations of the pikeperch (*Sander lucioperca*) and perch (*Perca fluviatilis*) will be investigated as model species to demonstrate effectiveness of our approach.

The main research objectives of the project are as follows:

1. Evaluation of the population genetic structure and stock boundaries of the targeted fish species in the South-Eastern Baltic Sea region based on the investigations into polymorphic microsatellite loci.
2. Estimation of migration patterns of the targeted fish species between fresh and brackish water bodies, as well as eel recruitment and stocking success by means of the microchemical otolith analysis.

To achieve the above–stated objectives of the proposed project a number of tasks are defined:

- to collect field material from the South-Eastern part of the Baltic Sea (coastal fresh and brackish waters of Latvia and Lithuania) for both the genetic and microchemical analysis. Samples of the targeted species representing spawning and summering (summering in different water sites in terms of water salinity) should be collected during the first and second years of research;
- to screen available microsatellite primers for assessing the genetic structure of the pikeperch and perch originating from Latvian and Lithuanian coastal water areas of the Baltic Sea. To identify species-specific microsatellite primers for the eel based on screening the primers developed for taxonomically related species;
- to evaluate the genetic structure of targeted species based on the microsatellites assay;
- to perform the microchemical analysis of the Sr/Ca ratio of the material collected to estimate spatial and temporal migratory patterns of the perch and pikeperch, as well as

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[1] - fish species spending most of their adult lives in freshwater, and migrating to saltwater to spawn, subdivision of diadromous.

[2] - fish species migrating between sea and fresh water for feeding, subdivision of diadromous

- to evaluate the eel natural recruitment and stocking success;
- to integrate the data obtained and to determine the following:

  -whether subpopulations of the pikeperch and perch from Latvia and Lithuania are isolated by means of the distance between the inhabited water areas (level of genetic isolation);

  -whether the amphidromous and non-migrating pikeperch and perch subpopulations have different spawning sites and possess significant genetic differences;

  -whether the amphidromous *Percids* migrate yearly;

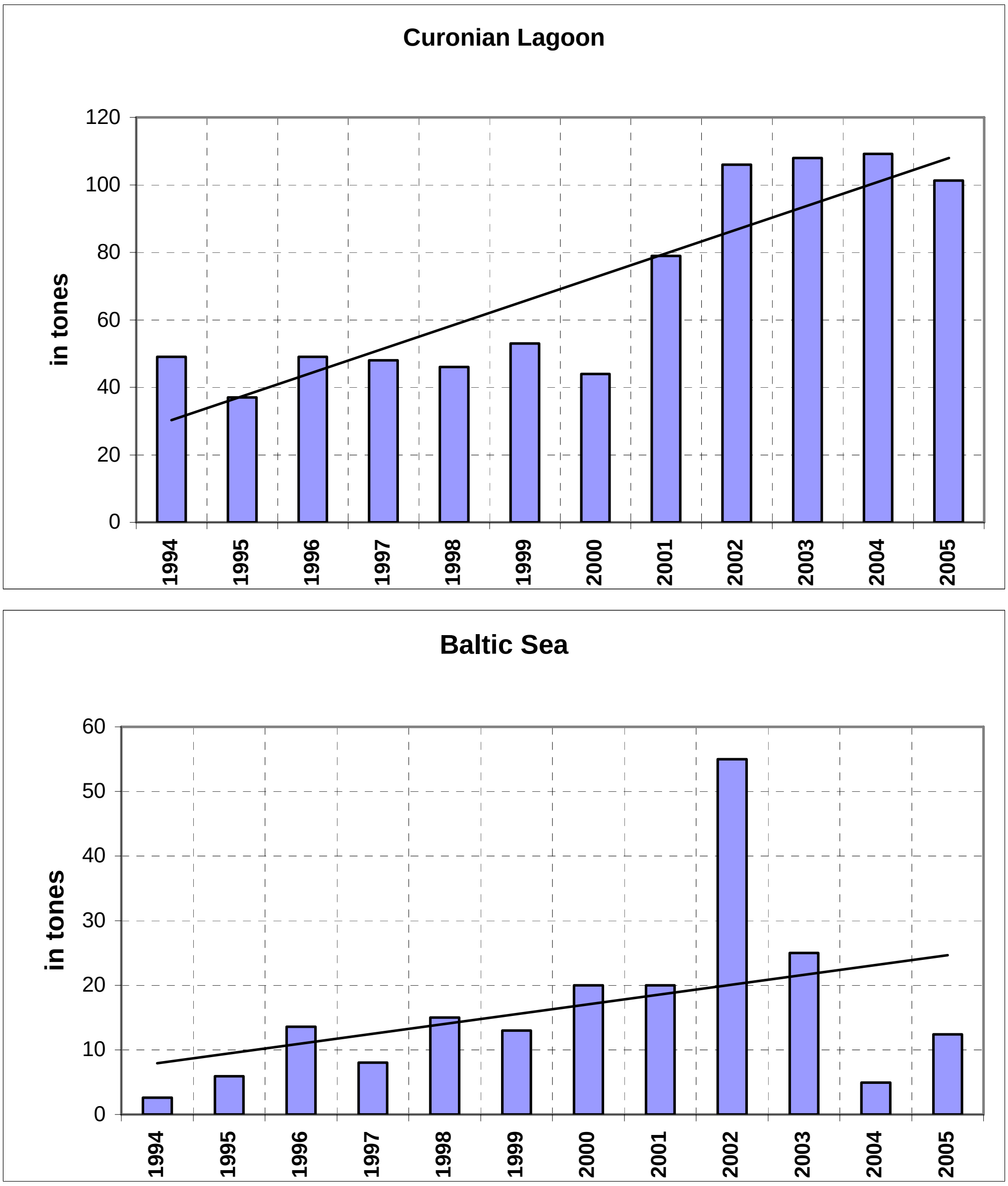
  -whether the catadromous eel exhibits features of amphidromous behaviour;

  -whether the stocked or naturally recruited eel prevail in the coastal waterbodies of Lithuania and Latvia;

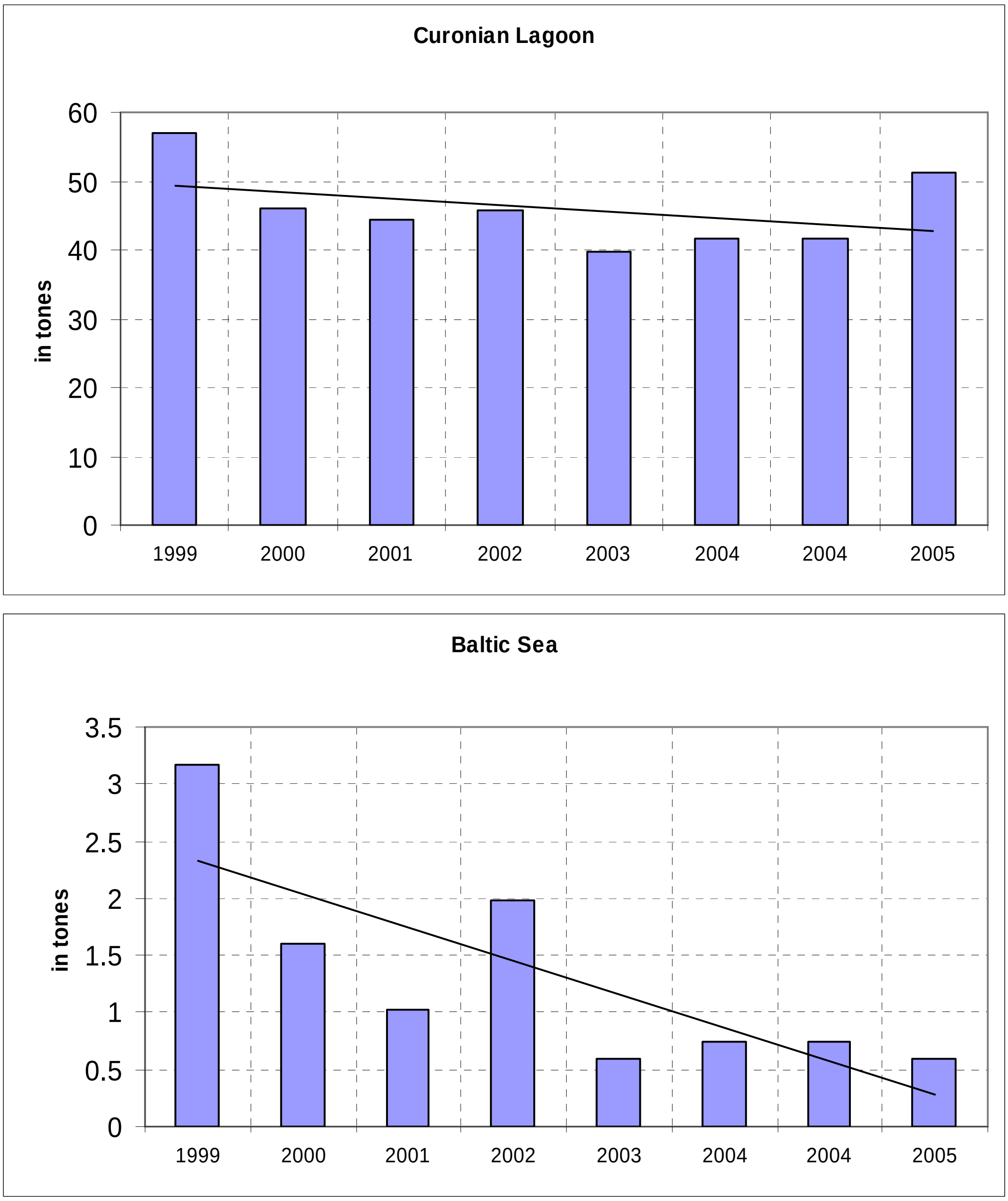
  -whether the stocked and recruited eel (if any) has detectible genetic differences.
- Summing up the data obtained to prepare recommendations for fisheries managers, conservationists and other stakeholders.

1.3 Introduction

Perch and pikeperch belong to the freshwater fish family Percidae (Collette et al., 1977). The pikeperch and the perch are common in the inland waters of Europe and are primary freshwater fish species with a long evolutionary history in freshwaters. (Collette et al., 1977; Lehtonen et al., 1996). However, they also live in brackish waters (Karås, 1996; Lehtonen et al. 1996) and both have some tolerances to water salinity. As the result in some localities both species inhabit brackish water sites instead freshwater. Seasonal amphidromous migrations between fresh and brackish waters in both species are documented as well (Skora, 1996, Gyllensten et al., 1985). Amphidromous migrations are typical behavior for *Percids* inhabiting the South-Eastern part of the Baltic Sea. However, only some part of *Percids* population migrates for a summer to the Sea. In Lithuania, each year, after over-wintering and spawning in the Curonian Lagoon, some part of populations of these typical fresh water fish species migrates to the brackish coastal waters of the Baltic Sea, while in the autumn returns back to the Lagoon. It is proved that *Percids* benefit faster growth rates do to such migrations to the brackish environment (Ložys, 2004). However, currently there is a general lack of information on *Percids* migrations in the South-Eastern region of the Baltic Sea basin. As a result, without exact answers to the population structure or a spatial and temporal migratory pattern, management of the valuable *Percid* fish species is based only on the unconfirmed hypothesis. In Lithuania coastal waters *Percids* fishery is not quoted assuming that coastal fishermen exploit the same populations as in the Curonian Lagoon. Fisheries managers and conservationists on a local scale (in Lithuania) support the hypothesis that pikeperch spawning in the Eastern part of the freshwater Curonian Lagoon (the Nemunas River delta) is subpopulation “not migrating” to the Baltic Sea; whereas pikeperch spawning in northern part of the Lagoon (in quite close distance from the Sea) migrates for summer to the brackish Baltic Sea. Similar behavior of *Percids* was observed in brackish Vistula Lagoon (territorial waters of Kaliningrad region (Russia) and Poland): pikeperch spawn in the Lagoon, whereas a greater part of the population migrates to the Baltic Sea for a summer after spawning. Russia, Lithuania and Latvia managers assume that in the Baltic Sea they exploit the pikeperch originating from the Vistula and Curonian Lagoon, or even from the Gulf of Riga. However, exploitation of the mixed stocks (Russian-Lithuanian-Latvian) can not be denied, since Lehtonen (1983) surveying conventionally tagged pikeperch populations demonstrated that pikeperch migration distances could reach as many as 200 km. To sum up the *Percids* management in the South-Eastern part of the Baltic Sea is based on the unconfirmed hypothesis. The definition of the population genetic structure of the perch and the pikeperch and their temporal and spatial migration patterns could serve the improvement of sustainable exploitation and management of this fish stocks in the coastal waters of the Baltic Sea and the adjusted freshwater basins. The perch and the pikeperch are one the most valuable fish species and as a result their landings determine a large part of fishermen incomes in the region. Landings of both Percids in the Curonian Lagoon are rather stabil (Figs.1-2), however landing trend of perch in Lithuania coastal waters raise concern. Pikeperch landings during the last two years are also very small. An improper approach in fisheries management (absence of fishing limits in coastal waters) could cause the “migrating” *Percid* stock crash in the coastal waters of the Baltic Sea as it happened to the amphidromous-behaving bream (*Abramis brama*) subpopulation in the coastal waters of Lithuania in the 1990s after coastal trawl fishery on bream was set up. Therefore, the knowledge obtained within GMM project should support the *Percid* fisheries management by knowledge of primary importance.



1 Fig. Pikeperch landings in the Curonian Lagoon and Baltic Sea, Lithuania during 1994-2005



2 Fig. Perch landings in the Curonian Lagoon and Baltic Sea, Lithuania during 1999-2005

In Latvia pikeperch is important commercial species with high market price around 3,5 USD per kg for fresh fish. Pikeperch are caught mainly in coastal waters of Gulf of Riga where landings make more than 90 % from total annual catches. Pikeperch is fished mainly with fykenets. Catches in other types of gears are insignificant. The great interest of fishermen has resulted in overfishing of this species and significant decrease in pikeperch landings not only in Latvian part of Riga Gulf (Fig. 3), but also in Estonian waters (Ojaveer et al 2003). The landings have decreased from around 45 tons in early 90’s to just 6 tons in 2004 and 2005 that is more than 10 times less. The stocking of pikeperch programme in the Daugava River and adjusted freshwater basins has been implemented since 1993. Annually 200-300 thousand one summer old specimens has been released near to Daugava River mouth (1993-1995) or in Bullupe and Kisezers (1996-2005). However the amounts of artificially reproduced and released juveniles as it can be seen are insufficient to maintain the stock in high fishing effort situation. Hence, strong additional measures are needed to ensure pikeperch stock recovery.

However it is essential to know the structure of the stock for elaboration of any effective stock management and recovery plan. Previous tagging experiments together with results of microchemical and genetical analyses provided by this GMM project will provide the necessary knowledge for elaboration of pikeperch stock protection measures.

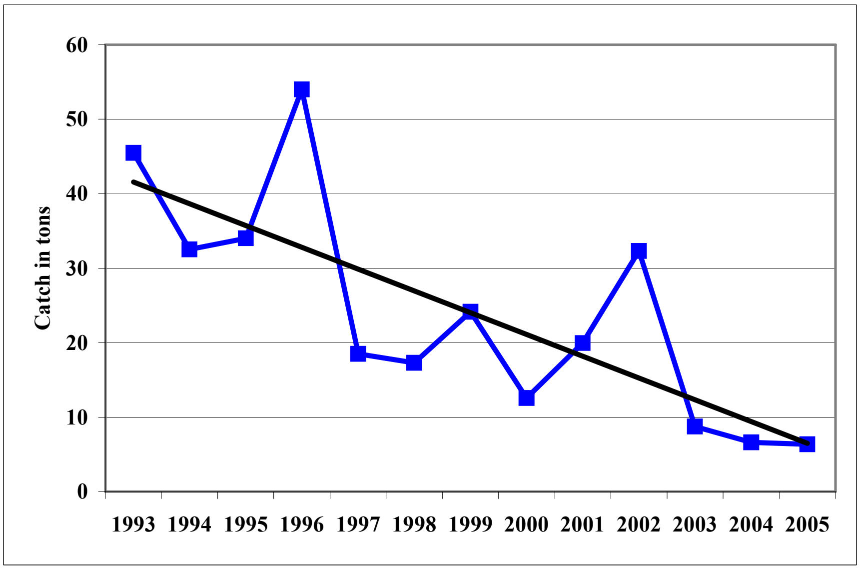


Fig. 3. Pikeperch catch in tones of in Latvian coastal waters of Riga Gulf in 1993-2005

In Latvia perch is one of the most abundant fish species in the coastal waters of Gulf of Riga and therefore has great importance also in coastal fisheries. Its market price is not as high as for eel and pikeperch but it has tendency to increase and presently is around 1USD per kg. The most part (over 80 %) of annual perch landings come from Riga Gulf, where it is fished mainly by fykenets. Annual perch landings in the Riga Gulf have been relatively stable since early 90's (Fig. 4) and are around 30-40 tons. The stock structure of perch in Riga Gulf is unknown. In previous years a tagging experiment was carried out which ended without any significant result due to extremely low number of recaptured fish. There still are several hypotheses about perch population structure and possible spawning in the coastal zone that cannot be proved or denied without genetical analyses and otolyth microchemistry analyses performed in this GMM project. Presently the fishing pressure on perch is relatively small and annual landing sizes depend more on perch yearclass strength. However in the situation when abundance of other commercially important fish decreases the importance and share of perch fisheries will increase. Therefore good knowledge about perch stock structure is vital for effective stock management.

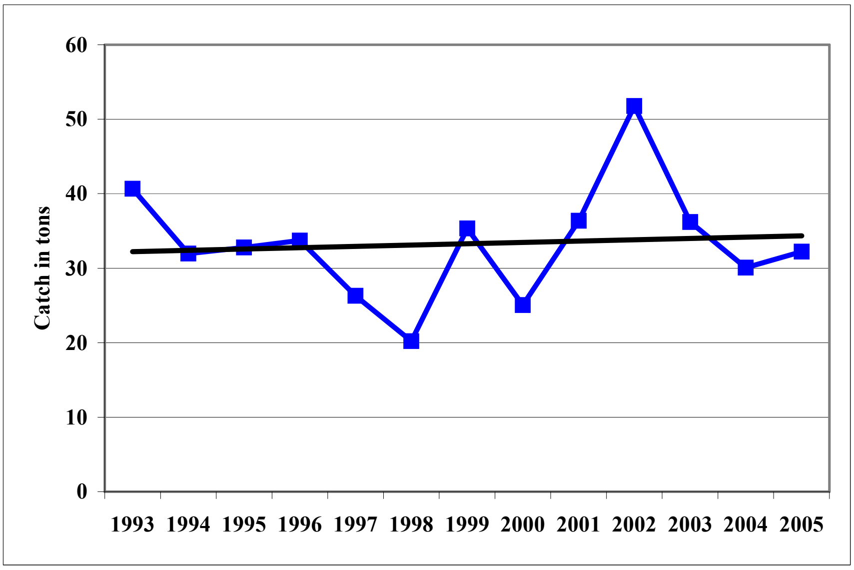
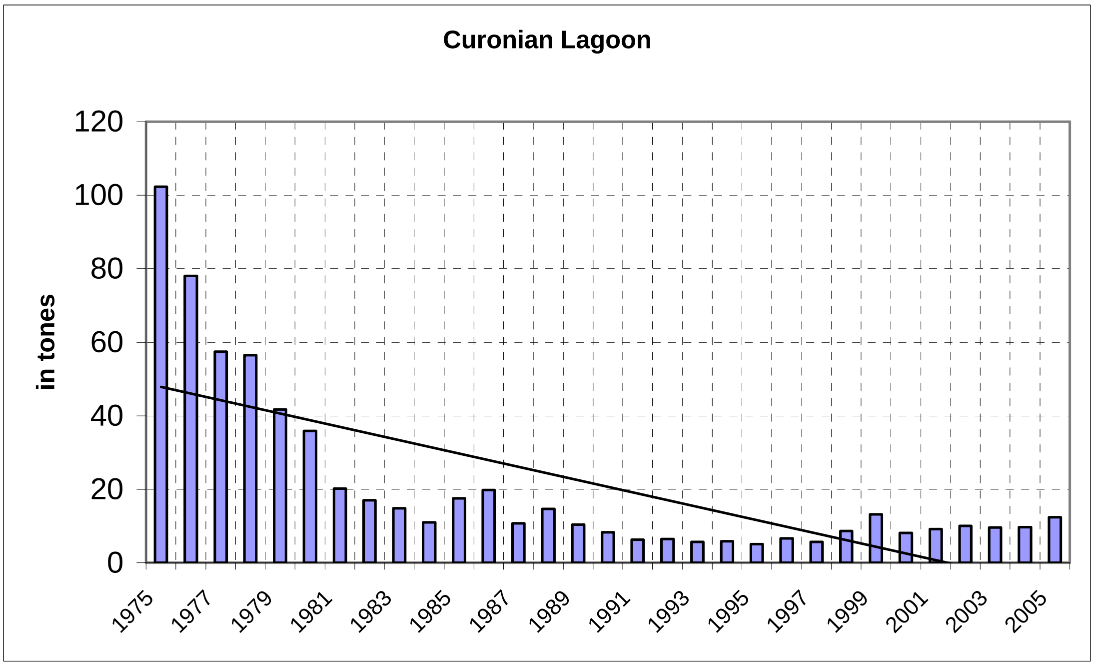
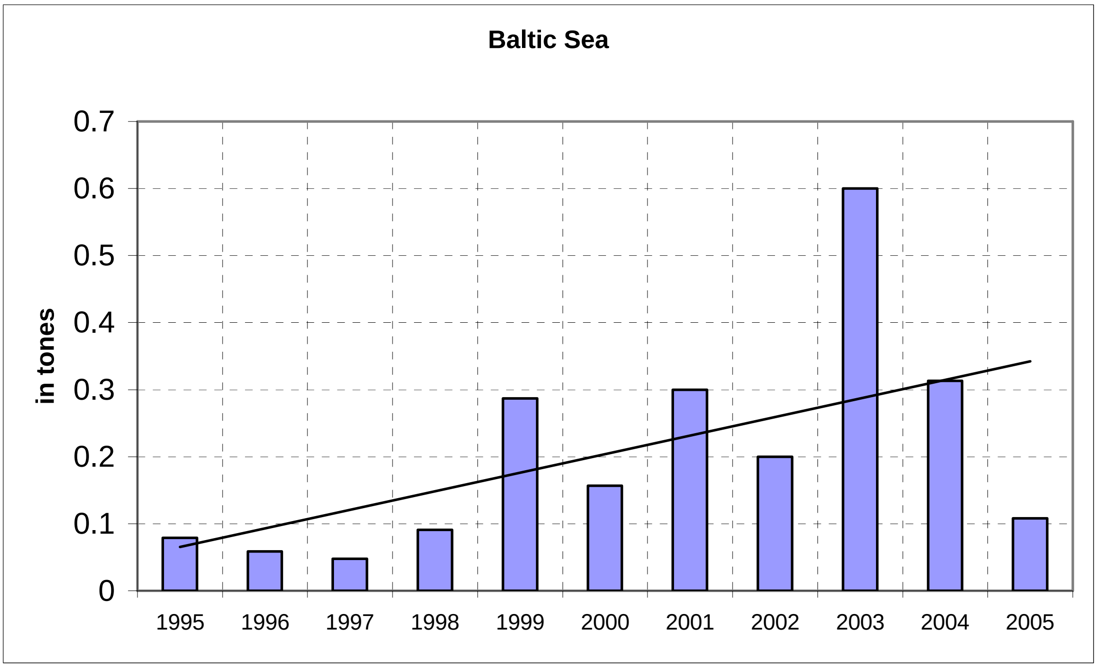


Fig. 4. Perch catch in tones in Latvian coastal waters of Riga Gulf in 1993-2005

Eels are exploited in most European countries, as well as in Lithuania and therefore are important not only as a natural asset but also as an economic resource for European fishermen. Eel landings in the Curonian Lagoon, the main are for eel fishery in Lithuania, demonstrates very negative decrease drends as overall in the rest Europe. Some positive landing trends during the last decade in the coastal waters is caused by increase in fishing efforts, but not by improving of stock status (Fig. 5).





5 Fig. Eel landings in the Curonian Lagoon (during 1975-2005) and Baltic Sea (during 1995-2005), Lithuania

Eel in Latvia is one of most expensive fishes in market today as the price per kg is above 17 USD for fresh eel and twice as high for smoked eel. This is why coastal fishermen are very interested in eel fishery. Despite that eel catches in Latvian coastal waters are small comparing with other Baltic Sea countries. Eel fishing is usually done with different fykenets. Longlines are used only in small scale fishery by some fishermen. Specialised eel fykenets are used only in limited numbers, because there is always significant amount of undersized fishes of different species bycatch in these fishing gears. Although eels are caught along all Latvian coast line, significant landings occur only in Riga Gulf and usually make more than 80% of total eel catches. Usually annual European eel landings are somewhere around 1.5 – 2 tons. The biggest landings were recorded in 2005 (Fig. 6) where they reached almost 3.5 tons in Riga Gulf. The reasons of such significant increase trend in the Gulf of Riga that it is opposite to general stock development trend of European eel are not clear. However it can be speculated that one reason of such increase is determined by improvement of landing reporting by fishermen.

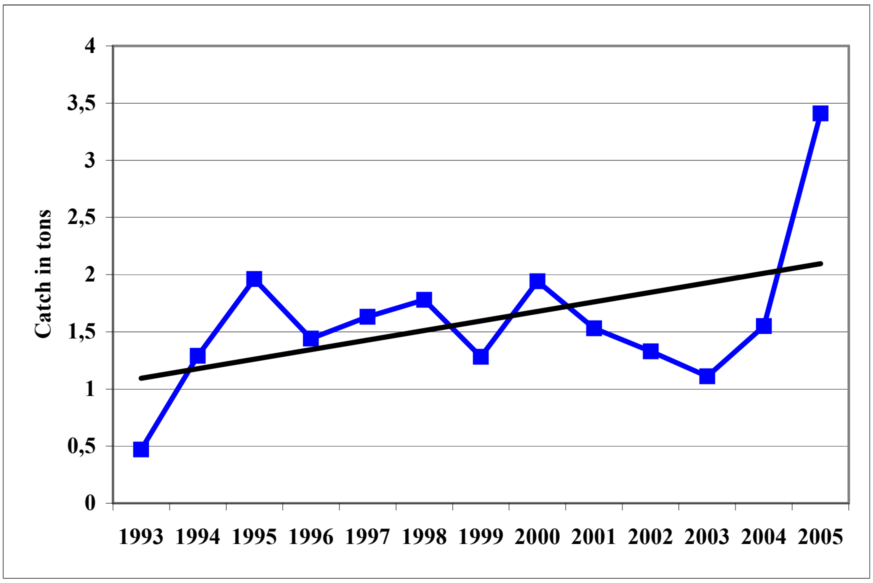


Fig. 6. Catch in tones of European eel in Latvian coastal waters
of Riga Gulf in 1993-2005

In Latvian inland waters the first official restocking of glass eel and young yellow ell were carried out in 1927 in the lake Odzes. More regularly eel restocking has been performed during 1960-1988 when were released 30.1 millions glass eels in 51 lakes (Annon, 2005). Since 1990 eel restocking is recorded only three times. Till 1990'ies the restocking was made by state but in recent decades - by private enterprises e.g. fishing rights/lakes leaseholder. In all cases stocking material was imported from the France.

Restocking was aimed to improve the fishing possibilities in given lake. Therefore all release was done in lakes which outlets were equipped with eel traps or so called "eel weir". The eel weirs covered practically all the outlet for all escapements during sea dwelling migrations. Although all lakes are situated in the midland of Latvia via rivers and its branches lakes are connected to the sea. However, downstream migration of eel additionally to eel weirs is limited by hydroelectric power station dams. Particularly since beginning of 1990'ies, when approximately 150 small hydroelectric power stations have been built on small rivers (12 of which are the new), it result that in some cases up to 7 dams can restrict eel downstream migrations. It should be mentioned that these stations are not equipped with eel ladders.

It is considered that eel catches in inland waters are mainly based on restocked eel because the upstream migrations are restricted also by above mentioned obstacles (Fig. 7).

Recently in the inland waters eel catches are reported in 16 lakes belonging to 3 river basins. Only 2 of these lakes are accessible for diadromous fish e.g. Liepajas and Kisezers (ICES, 2006). The last one is located near the Riga city and close to southern part of the Gulf of Riga. Historically there was no eel restocking in these lakes and it is assumed that catch in this lakes are based on wild stock. Although it is observed decrease of eel landings in the inland waters of Latvia in the Liepaja Lake and Kizezers the catches are almost stable (Table 1).

Table 1. Eel catch (kg) in selected lakes of Latvia with highest eel catch during 1996-2004

No	**Lake**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**
1	Raznas	13809	16744	13390	6841	5461	5915	2793	2956	3021
2	Usmas	2272	2615	3041	1643	2300	2810	2018	1639	2462
3	Sivera	23	327	713	500	254	467	371	450	884
4	Alauksta	1657	2137	826	447	1202	848	454	784	748
5	Saukas			680	706		332	483	161	483
6	Kisezers	142	148	96	240	294	387	415	363	357
7	Ludza	811	485	1191	735	828	1197	448	258	334
8	Cirma	1567	1716	880	1240	688	525	324	301	275
9	Aluksnes	2114	2202	2137	1467	1333	2934	518	473	252
10	Liepajas	273	247	62	165	180	291	381	448	218
The others		1772	1432	1366	788	712	859	580	634	255
Total		24440	28053	24382	14772	13252	16565	8785	8467	9289

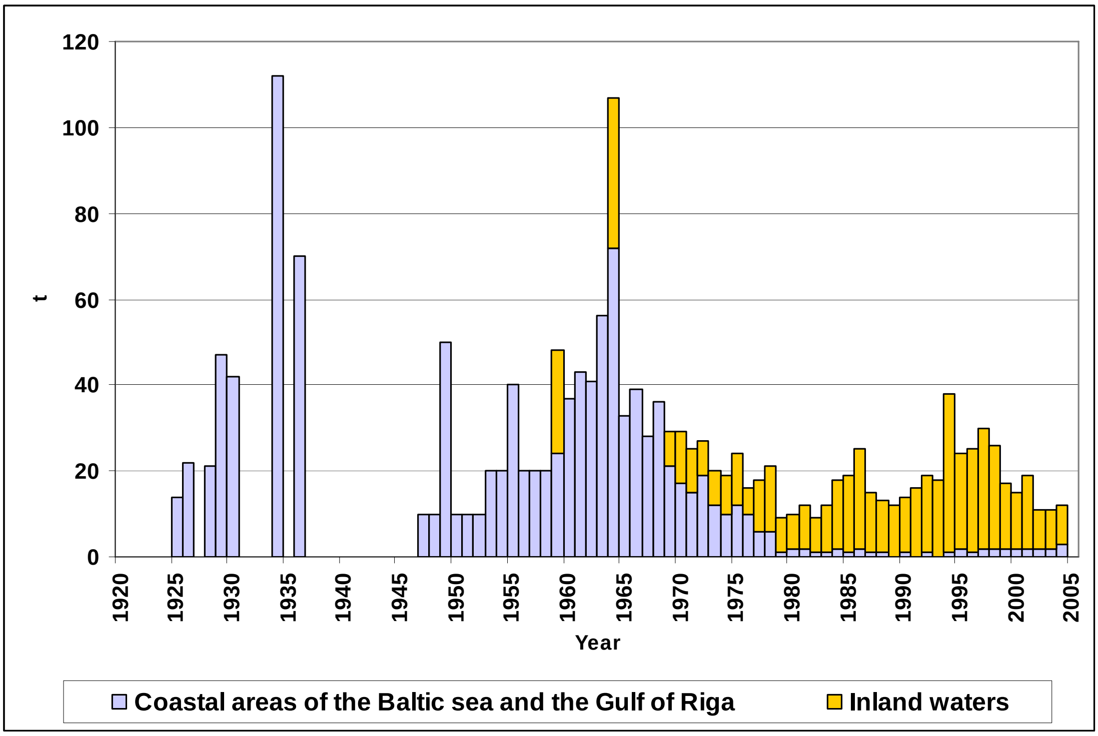


Fig. 7. Eel landings in Latvia (t) in inland waters and the Baltic Sea (landing statistics of eel in inland waters during 1920-1960 is incomplete and recreational fishing of eel is not taken into account)

Recruitment of the European eel has declined markedly in the past few decades as well as eel landings in almost all Europe. According to ICES (2001, *The International Council for the Exploration of the Sea*), a steep decline in recruitment has occurred over most of the continent since the early 1980s, to about 10 % of the former levels in 2001. In 2002 ICES/EIFAC Working Group on Eel (ICES C.M. 2003/ACFM:06; EIFAC - *European Inland Fisheries Advisory Commission*) considered that the stock remains outside safe biological limits. Recent recruitments of *A. anguilla* glass eel were estimated to be only 1% of the level before the 1980s (Dekker, 2004). At the end of 2003 the Commission of the European Communities taking into consideration a critical stock state declared in the Communication *"Development of a Community Action Plan for the Management of European Eel"* (COM(2003) 573) the first steps of the Development of the Community Action Plan for management of the European Eel. More recently, the Commission proposed "*Council regulation establishing measures for the recovery of the stock of European Eel*" (COM(2005) 472). Among other management instruments restocking glass eels from river estuaries or yellow eels from aquaculture production should be used. Incoming natural recruitment as well as silver eel migration for spawning should be one of the important research subjects and might contribute to the management of stock and fisheries. Re-stocking is traditional practice carried out in most Community Member States, in some cases dating back to the 19th century. Re-stocking is based on capture of eels from the wild for their release in other areas. Re-stocking in Lithuania has taken place since the first decades of the 20th century up to date. In the period after the early fifties Lithuania stocked to the fresh water bodies (inland lakes mainly) about 50 million young eels, while Latvia – 31 million. However, natural recruitment is unknown due to specific natural conditions in the main recruitment areas, i.e. Curonian Lagoon and coastal waters of the Baltic Sea in Lithuania and Gulf of Riga in Latvia due to limited possibilities for recruitment monitoring. Do to similar reasons little is known about migrating silver eel escapement in Lithuania and Latvia as well. Consequently, natural recruitment, stocking success, as well as the behavior (migration pattern) of stocked eels remain unexplored in Lithuania and Latvia waters. Moreover, natural recruitment remains unexplored not only in Lithuania and Latvia, but also in the overall South-Eastern Baltic Sea basin area. Knowledge of this kind is important from both fundamental and management points of view at all levels - local, national or international scale. Knowledge obtained within the GMM project on the genetic stock structure, the migrating behavior of stocked and naturally recruited eels, growth of naturally recruited and restocked eels as one of the best indicators for population strength and potential to contribute to stock reproduction cycle could contribute to the attempts made by the European Community to rebuilt European eel stocks.

1.4 Fish collection and sampling sites

Lithuania.

Perch and pikeperch were collected by gill netting in the Curonian Lagoon and Baltic Sea coastal waters (Fig. 8). During the spawning time and growing phase in mid-summer. Perch were collected during the spawning time close to the Vente cape, which is located in the North-eastern part of the freshwater Curonian Lagoon. Pikeperch were collected during the spawning time in the delta of Nemunas River, which flows to the Curonian Lagoon. Spawning of pikeperch in the research region (Lithuania) is identified and observed only in Nemunas river delta. Pikeperch were also sampled in spring time or early summer in the Northern part of the Curonian Lagoon just before emigration to the Baltic Sea. There was not observed pikeperch spawning in this area. In mid summer during the active growth phase perch and pikeperch were collected in the Curonian Lagoon close to Vente cape in the Curonian Lagoon and Lithuania coastal waters of the Baltic Sea.

The total length (L_T) and weight (W) of each perch and pikeperch was measured to the nearest 5.0 mm and 1.0 g. Sexes were determined macroscopically from the gross morphology of the gonads.

Silver and yellow-stage European eels *A. anguilla* were collected by fyke nets and long lining from Baltic coastal waters, the Curonian Lagoon in western Lithuania and the freshwater Lakes Baluošai and Dringis in eastern Lithuania The lake is about 300 km from the Curonian Lagoon and about 350 km from the Baltic Sea, to which it is connected via a system of small streams, lakes, the river Nemunas and the Curonian Lagoon. Natural recruitment to these lakes has never been reported and may not occur; however, the possibility cannot be excluded. Elvers have been regularly stocked since 1960 into the system of lakes in the Baluošai Lake region.

The total length (L_T) and weight (W) of each eel was measured to the nearest 1.0 mm and 1.0 g. Sexes were determined macroscopically from the gross morphology of the gonads, where eels with thin, regularly lobed organs (Syrski’s organ) were considered males, while individuals with more broad and folded curtain-like gonads were females (Tesch, 2003). The eels were classified as yellow and silver eels, by their external colour, fin shape and eye size.

The Baltic Sea is the largest brackish water body in the world, with an area of 412,000 km^2. Surface salinity varies from less then 1 psu in the upper Bothnian bay to more then 34 psu in the outer Skagerrak. Water salinity in the Gulf of Bothnia and Gulf of Finland varies between 2-6 psu; Northern Baltic proper (including the Archipelago Sea, the Åland Sea and the Gulf of Riga) between 6-8 psu; Southern Baltic (including the Arkona Basin, the Bornholm Basin and the Gdansk Bay) salinity ranges between 7-10 psu; Western Baltic (German coast, the Belt area) between 7-10 ; Southern Kattegat 12-34 psu (Bonsdorff & Pearson, 1999).

The salinity in the Lithuania coastal waters (depths to 20 m) of the Baltic Sea is influenced by inflow of fresh inland water from the Curonian Lagoon in some areas depending on wind direction and varies from about 1 psu in the area of inflow to 7 psu in some kilometers distance from the inflow area, which is typical salinity for Lithuania coastal and open sea waters (Vyšniauskas 2003). Water transparency (Secchi-disc depth) in the coastal waters varies from 1 m at the Klaipeda Strait where fresh inland water inflows to the sea to 14 m in open areas (Vyšniauskas & Lesys, 1998).

The Curonian Lagoon is 1584 km^2 in area, with a mean depth of 3.7 m and seasonal water transparency fluctuating in the range 0.35-2.0 m (I. Prochorova, pers. commun.); the Lagoon is separated by a narrow sand spit (0.5 – 4.0 km) from the Baltic Sea and is connected to it through the narrow Klaipėda Strait and is a fresh water basin, fluctuating from 0.03 psu, in the southern part of the Lagoon, up to 1.60 psu in the Klaipėda Strait. During stormy inflows of seawater the salinity may episodically increase up to 5-6 psu in the northern areas (Olenin 1996). The average water level in the Lagoon is 15 cm higher than sea level, thus the seawater penetration into the Lagoon is rare.

Lakes Dringis and Baluošai are located in the eastern part of Lithuania in about 300 km distance from the Curonian Lagoon and the Baltic Sea. Both lakes are of glacial origin. The area of Dringis Lake is 731 ha, with maximum depth 24 m, mean – 8.4 m, water transparency fluctuates from 1.8 to 6.0 m (Kavaliauskienė, 1996). Four streams inflows to the lake, while one flows out and connects the lake to some other lakes and Nemunas river basin which is the biggest Lithuania river and flows to the Curonian Lagoon.

The area of Baluošai Lake is 250 ha, maximum depth is 37.5 m, average – 12.5 m, typical water transparency in summer is 2.8 m. Two streams inflow to the lake, while one flows out to the nearby bigger lake Asveja through which connects to Nemunas river basin which connects the lake to the Curonian Lagoon and Baltic Sea.

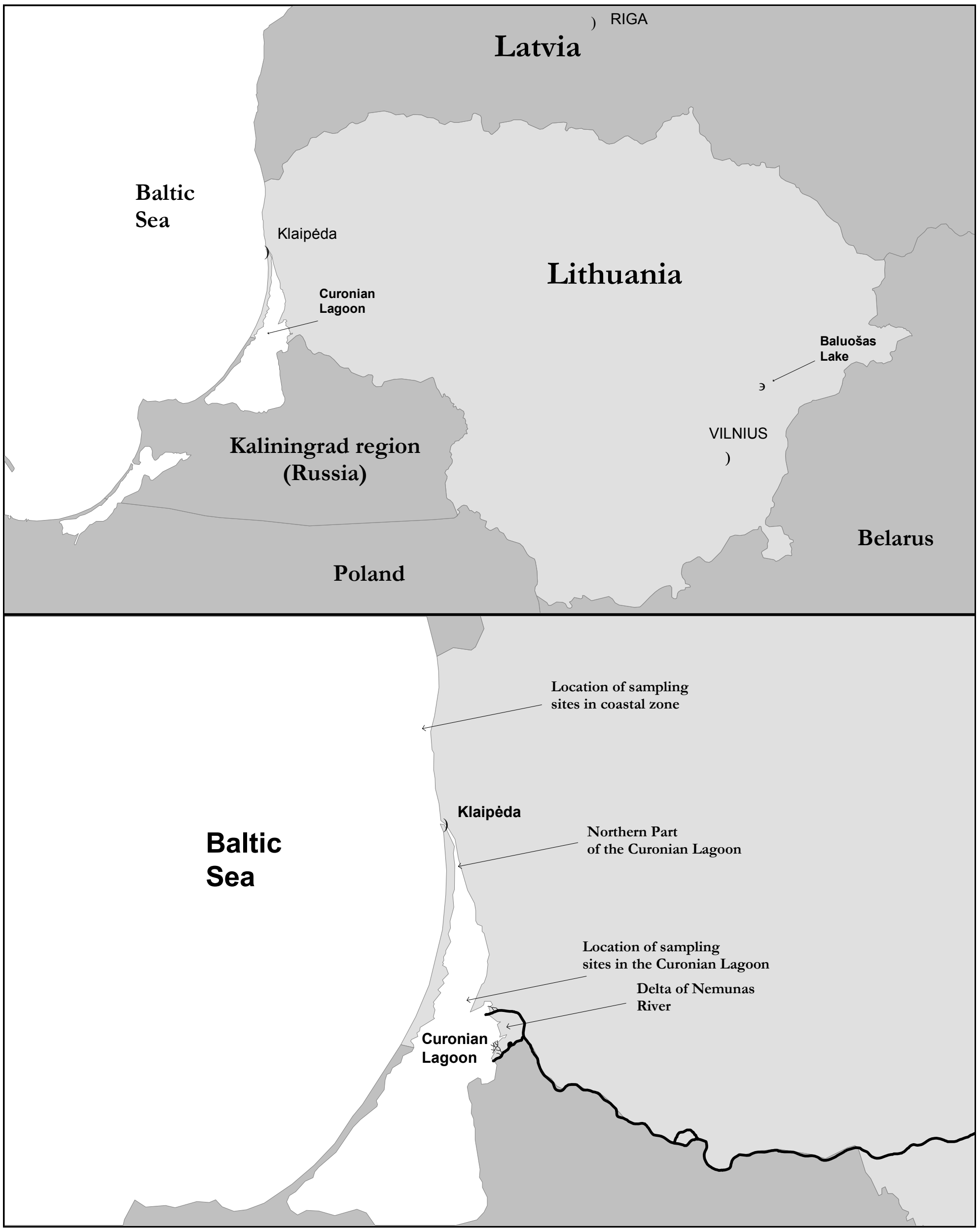


Fig. 8. Sampling locations in the Baltic Sea, Curonian Lagoon and Lake Baluošai (Lithuania).

Latvia.

Gulf of Riga is located in the eastern part of the Baltic. From the Baltic Proper it is separated Kurzemes peninsula and by Estonian islands: Saaremaa, Hiiumaa, Muhu and Vormsi. Irbe strait connects Gulf of Riga with Baltic proper in the west and Muhu basin - in north. Mean depth of the gulf is about 26m; maximum depth is in the central part and is about 60 m. Gulf of Riga is a brackish water body with low water salinity, only 2-7 ‰ depending on region and season and can be considered as highly eutrophic.
Length of the Latvian coastline of the Gulf of Riga is about 250 km. Average depth in the coastal (2 mile) zone is about 10 m. In the Eastern part the bottom is covered mainly by sandy gravel and sandy gravel with pebbles and boulders (Stiebrins and Väling 1996). In southern and western part of the coastal zone the bottom is covered mainly by sand and in some places also by sandy gravel with pebbles and boulders (Stiebrins and Väling 1996). Bottom vegetation in the coastal waters is unevenly distributed. Brown algae *Fucus vesiculosus* is the dominating species in the Latvian coastal zone. Less abundant are filamentous green algae *Cladophora spp.* and red algae *Ceramium spp.*, *Furcellaria lumbricalis* (A. Minde, unpub. data). Other species of different algae are present in very low densities.

Lake Ķīšezers is located close to the coast and is connected to the Baltic Sea through lower reach of river Daugava. It is freshwater lake but during strong Western winds brackish water from Gulf of Riga can enter the lake for short time period. The area of this lake is 1730 ha. It has average depth of 2,4 m and maximum depth 4,5 m, which is found only in one small area in the central part of it. Lake Ķīšezers is also characterised by brown colour of water and low transparency – in the summer only 0,5 m.

The river Daugava is largest river in Latvia with the largest river basin as well. There are 3 big hydroelectric plants constructed on Daugava, the last one located just 28 km from the river mouth and has not equipped with a fishpass. The lower reach flows through Riga city and therefore has diverse significant anthropogenic impact. The width of Daugava in the lower reach is around 500 m and the depth of 15 m.

According to the project work plan eels were sampled in Southern Gulf of Riga, near the Daugava river mouth (Fig. 9) in August – September 2005. In total 31 specimens were caught, 16 of them were yellow eels, 15 – have already reached silver eel stage. All fish have been measured to the nearest mm and weighted; also sex determination and eye measurements were made. From each specimen both *sagitta* otolyths were taken for microchemical analyses and age determination and blood and flesh samples were taken for genetical studies.

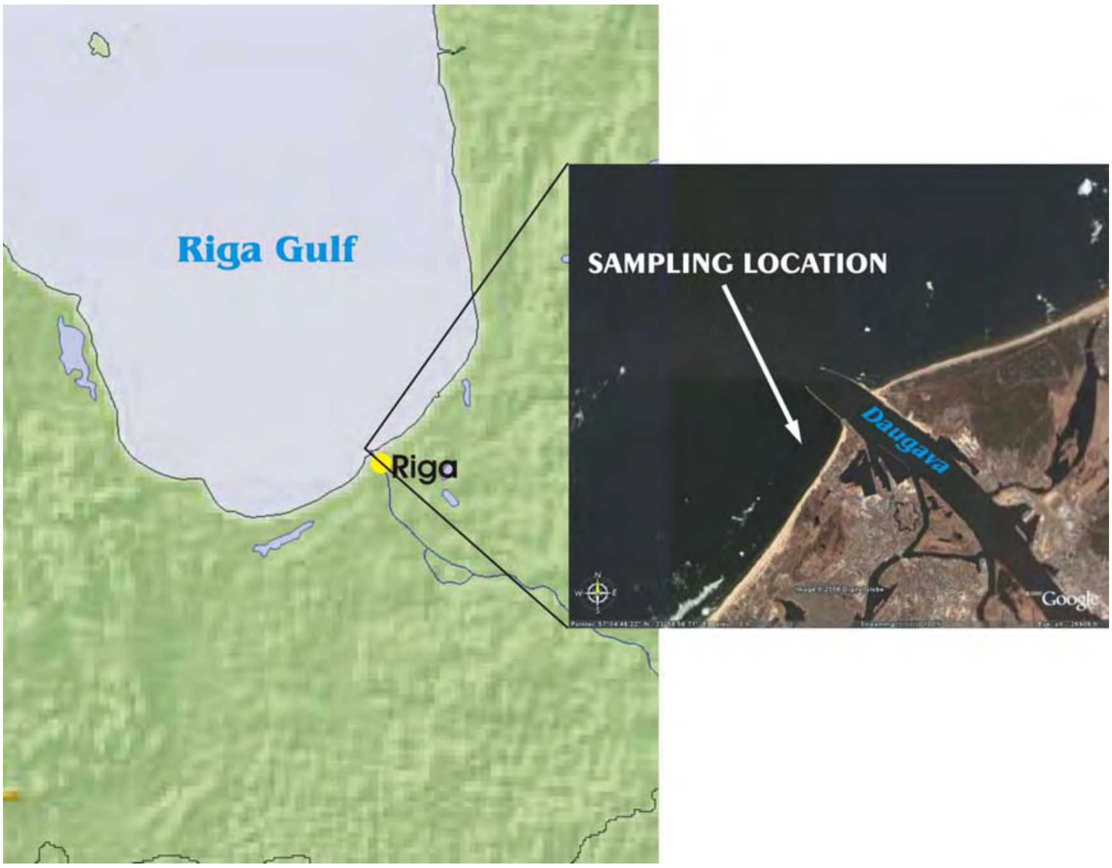


Fig. 9. Eel sampling location in Riga Gulf, summer 2005

Pikeperch were sampled during spawning time in river Daugava (Fig.10) in spring from May till end of June. According to project proposal and work plan in total 30 pikeperch were sampled. Each fish was measured to the nearest mm, weighted and also sex determination was made. *Sagitta* otolyths were extracted for microchemistry analyses and age determination and blood and tissue samples were taken for genetic studies.

Pikeperch sampling in coastal waters according to project proposal is planned in summer 2006 in the Eastern part of Riga Gulf.

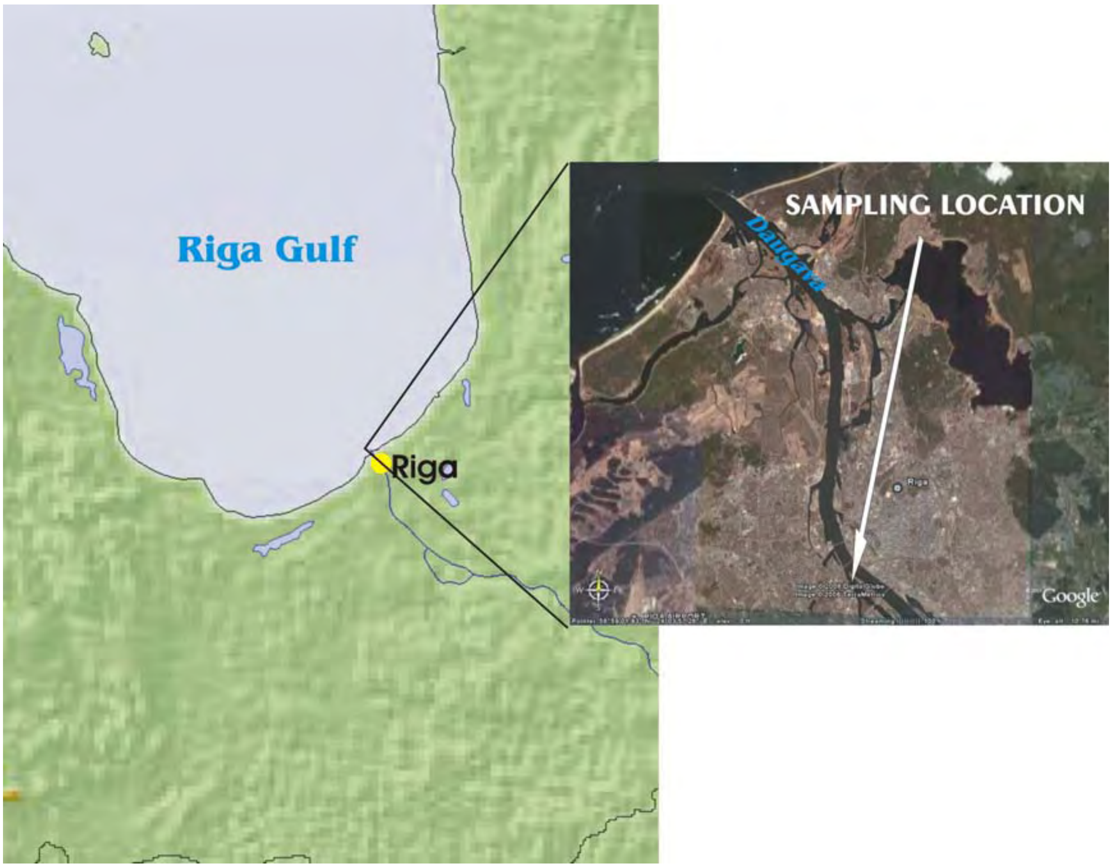


Figure 10. Pikeperch sampling location in Daugava river, spring 2005

Perch were sampled once during spawning time in lake Ķīšezers and twice - in spawning time and summer – in coastal zone near Daugava river mouth (Fig. 11). Sampling was carried out in spring from May till end of June and in summer from August till beginning of September. According to project proposal and work plan in total 100 perch were sampled. Each fish was measured to the nearest mm, weighted and also sex determination was made. *Sagitta* otolyths were extracted for microchemistry analyses and age determination and blood and tissue samples were taken for genetic studies.

Perch sampling in coastal waters according to project proposal is planned also in summer 2006 at the coast of Baltic Proper.

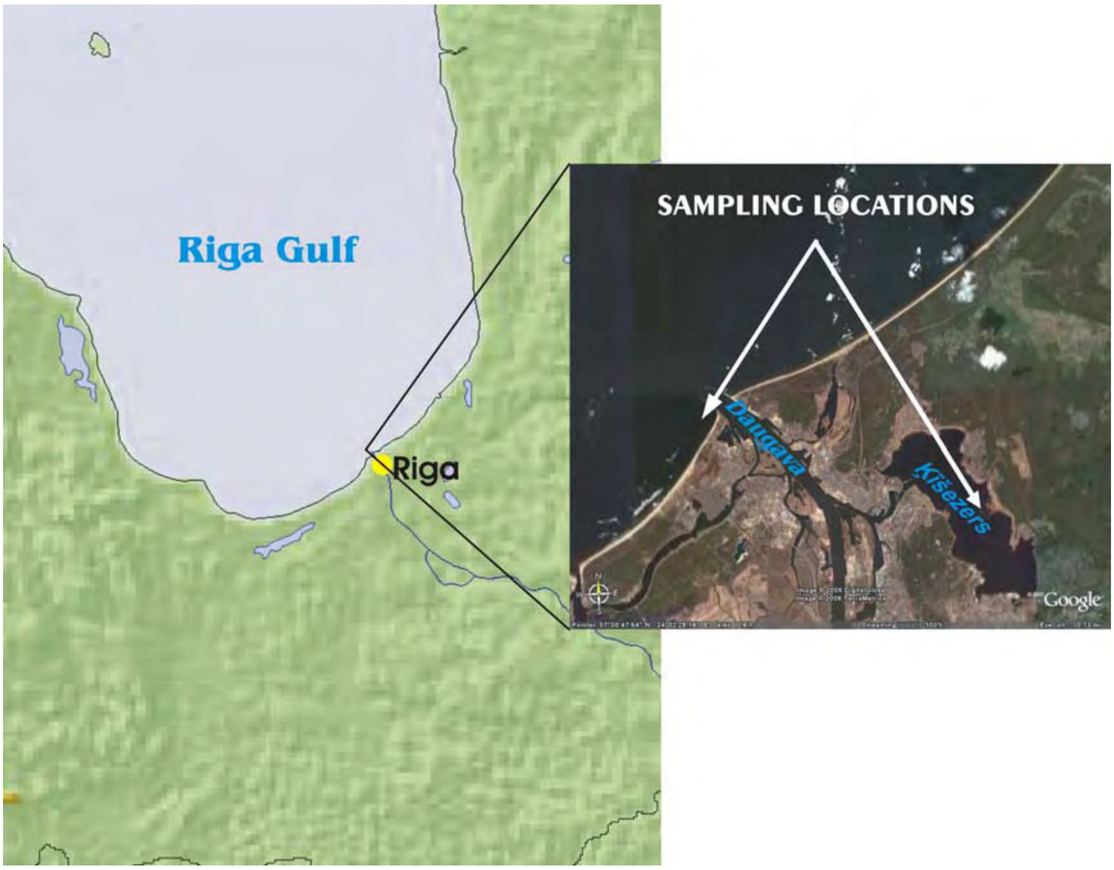


Fig. 11. Perch sampling locations in lake Ķīšezers and coastal zone in 2005

Sampling areas of eel in Latvia in 2006

According to the project work plan eels were sampled in lake Ķīšezers, which has close connection to Daugava river mouth and Riga Gulf (Fig. 12) and Mērsrags in Western part of Riga Gulf in August – September 2006. In total 46 specimens were caught, all of them were yellow eels. All fish have been measured to the nearest mm and weighted; also sex determination and eye measurements were made. From each specimen both *sagitta* otolyths were taken for microchemical analyses and age determination and blood and flesh samples were taken for genetical studies.

Figure 12. Eel sampling sites in 2006 – Mērsrags (blue dot) and Ķīšezers (yellow dot).

Perch were sampled in summer in coastal zone of Open Baltic Sea (Fig.13). Sampling was carried out in August. According to project proposal and work plan in total 30 perch were sampled. Each fish was measured to the nearest mm, weighted and also sex determination was made. *Sagitta* otolyths were extracted for microchemistry analyses and age determination and blood and tissue samples were taken for genetic studies.

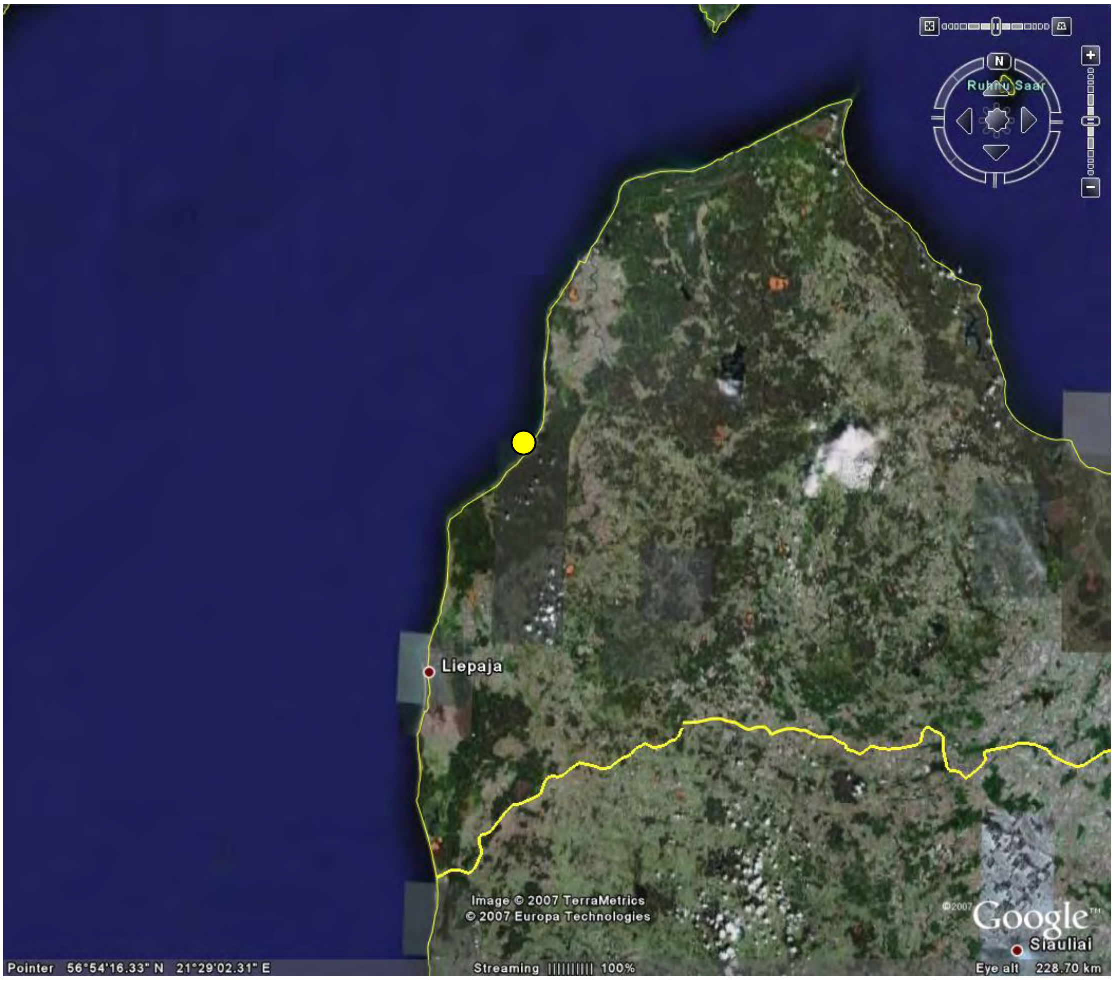


Figure 13. Location of perch sampling site in 2006.

II. DESCRIPTION OF THE RESULTS

2.1 MICROCHEMICAL ANALYSIS OF FISH OTOLITHS

Summary

- The first year report about the eel migration, movement and identification of stocked and naturally-recruited eels in Lithuania has been written into a scientific paper and has been accepted for publication by a prestigious journal, Journal of Fish Biology. This paper "Migratory patterns and contribution of stocking to the population of European eel in Lithuanian waters as indicated by otolith Sr:Ca ratios" was included in this report as an appendix I.

- Based on the outcomes of the first year, we further evaluate the effects of stocking, migratory histories and habitat on eel growth. The full paper is almost finished and will be submitted to a journal for publication soon. The draft "Effects of habitat and stocking programs on the growth rate of European eel *Anguilla anguilla* " is also included in the report as an appendix II.

- During the second year period of this project, we analyzed 33 eels and 31 eels from Lithuania and Latvia, respectively. We found higher proportion of stocked eels in Latvia than in Lithuania. Different migratory behaviors of the eels were also identified. Detailed data and results were discussed in the report.

- We also analyzed otolith Sr:Ca ratios from 22 perch and 53 pikeperch individuals collected in Lithuania. For the first time, both resident and migratory individuals were disclosed in this study.

Materials and Methods

Otolith preparation and Sr:Ca analysis

The largest pair of otoliths (sagittae) from eel, perch and pikeperch was removed, dried in air, embedded in Epofix resin, ground and polished until the core was exposed. For electron probe microanalysis, the polished otoliths were coated with carbon under a high-vacuum evaporator. Sr and Ca concentrations in the otolith were measured from the otolith core to the edge at 10 µm intervals for eel otolith and at 10 µm or 25 µm for perch and pikeperch otoliths. Quantitative analyses were conducted with an electron probe microanalyzer (JEOL JXA-8900R), using beam conditions of 15 kV for the acceleration voltage, 3 nA for the current, a 5 × 4 µm rectangular scanning beam with interval of the 10 µm for each analysis or 5 nA for the current, 10 µm beam size with interval of 25 µm for each analysis. The quantitative data were corrected by the PRZ (phi-rho-z) method to calculate oxide compositions (e.g., Goldstein *et al.*, 1984; Reed, 1993). The peak concentration of Sr Lα was counted for 80 s with background measurements for 20 s on each side. The peak concentration of Ca Kα was counted for 20 s and each background for 10 s. A synthesized aragonite ($CaCO_3$) and strontianite ($[Sr_{0.95}Ca_{0.05}]CO_3$; NMNH R10065) were used as calibration standards. Since aragonite-structure carbonates are similar to otoliths, the standards have smaller matrix corrections than other types of standards such as oxide or silicate (Jarosewich & White, 1987). The standards were mounted in epoxy resin and polished. The carbon coating for the standards and otoliths had the same thickness (25-35 nm). After microchemical analysis, the otolith was polished to remove the carbon layer, then etched with 5% EDTA for 1 to 2 min to reveal the annual rings for age determination. The duration of the fish in freshwaters and sea/brackish waters was estimated by relating the otolith Sr:Ca ratio profile to the otolith annuli. The criteria for the discrimination between freshwater- and sea/brackish water-residents are defined in the results.

Results

Otolith Sr:Ca ratios of the eels collected in the Baltic Sea (Lithuanian territorial waters)

According to the finding in the first year project (Shiao et al., 2006), 32 eels collected in the Baltic Sea of Lithuania territorial waters can be classified into 30 naturally-recruited eels and 2 stocked eels after the analysis of otolith Sr:Ca ratios (Table 2). The mean otolith Sr:Ca ratios of 30 naturally-recruited eels show (Fig. 12) a gradually decline from approximately 6×10^{-3} after the glass eel stage to approximately 3×10^{-3} at catch. This pattern is consistent to the decreasing profile of salinity from North Sea, Baltic Sea to the Lithuanian coasts. The 30 naturally-recruited eels may invade the freshwater once to several times in their life (so called inter-habitat shifter, $n = 15$) or merely reside in the brackish/sea waters (so called sea eels, $n = 15$). The diversified migratory patterns of the naturally-recruited eels were described in the first year project report and published in the Journal of Fish Biology (Shiao et al., 2006).

Stocked eels always show a period of lower otolith Sr:Ca ratios (so called freshwater signature) after the glass eels stage, as shown in Fig. 13-14. Stocked eels (No. 13 and 24) occupied approximately 6.25% of the 32 eels collected in the Baltic Sea of Lithuanian territorial waters. The proportion of stocked eel is higher than that (2%) collected in 2004. However, the proportion of stocked eel in 2005 might be over-estimated due to the smaller sample size examined. If the samples of 2004 and 2005 were pooled, the proportion of stocked eel in Baltic Sea of Lithuanian territorial waters was approximately 3.75%; that was 3 stocked eels found in total 80 eels. This indicated that the out-migrant stocked eel from the freshwater lagoon or rivers into the Baltic Sea was not predominant.

Table 2. Biological characteristics (mean ± SD) of the European eels collected in the Baltic Sea of the Lithuanian territorial waters. N: sample size.

Origin of the eels	Sampling period	Developmental stage	N	Mean (±SD) and range		
				Total length (cm)	Body weight (g)	Age (year)
Naturally-recruited eel	June-August	Yellow eel	31	61.8 ± 11.0 (36.5 - 79.0)	556.6 ± 348.9 (79.0 - 1207.0)	10.0 ± 2.2 (5 – 15)
Stocked eel	June-August	Yellow eel	2 (No. 13 and 24)	60.5 for No. 13 77.5 for No. 24	437 for No. 13 1169 for No. 24	10 for No. 13 9 for No. 24

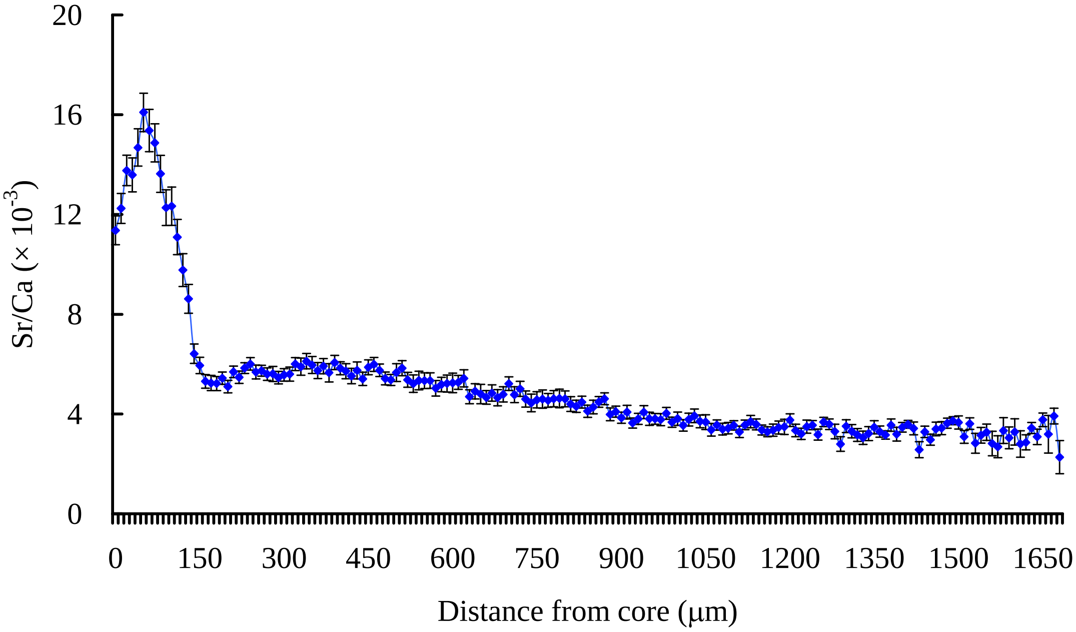


Fig. 12. Temporal changes of mean otolith Sr:Ca ratios from 30 **naturally-recruited** European eels (*Anguilla anguilla*) collected in the Baltic Sea of Lithuanian territorial waters. Y error bar represents the standard error of mean.

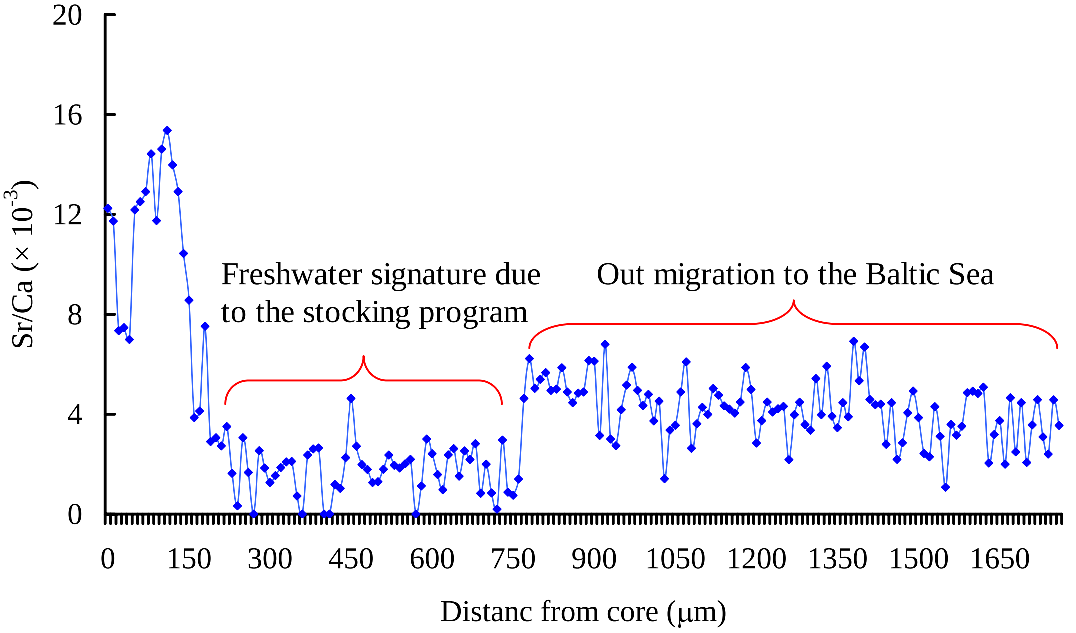


Fig. 13. Temporal changes of otolith Sr:Ca ratios from a **stocked** European eel (*Anguilla anguilla*) No. 13 collected in the Baltic Sea of Lithuanian territorial waters. Y error bar represents the standard error of mean.

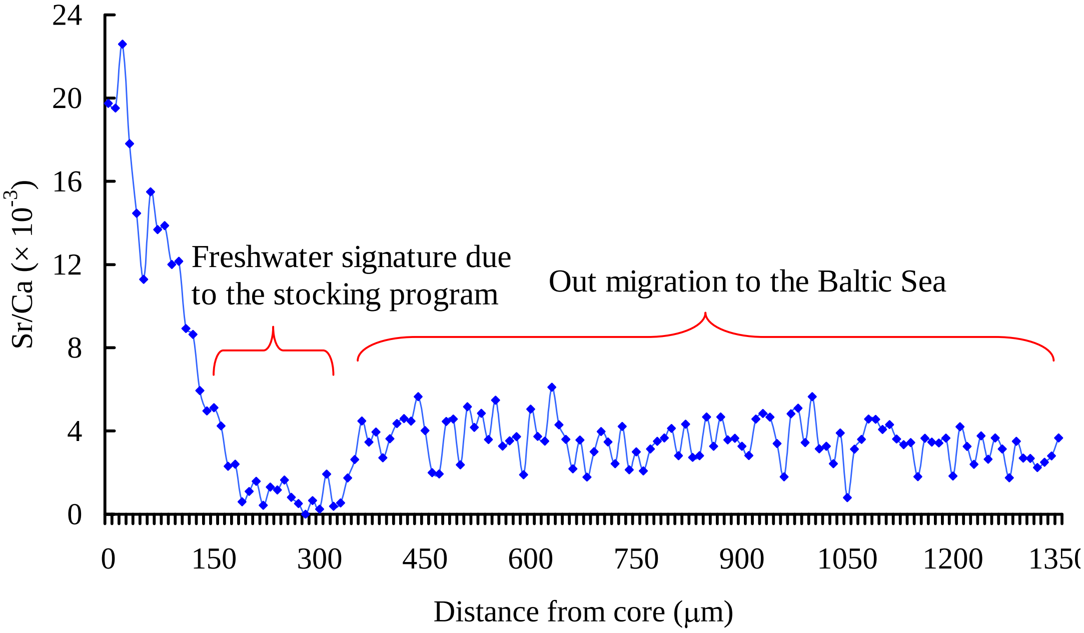


Fig. 14. Temporal changes of otolith Sr:Ca ratios from a stocked European eel (*Anguilla anguilla*) No. 24 collected in the Baltic Sea of Lithuanian territorial waters. Y error bar represents the standard error of mean.

Otolith Sr:Ca ratios of the eels collected in Latvian waters

Thirty-one eels collected in Latvian rivers were analyzed for otolyth Sr:Ca ratios. Among them 19 eels showed the pattern of naturally-recruited eel and 12 eels showed the pattern of stocked eels (Table 3). The mean otolyth Sr:Ca ratios of the naturally-recruited eels and stocked eels were shown in the Figs. 15 and 16, respectively. The mean otolyth Sr:Ca ratios of 19 naturally-recruited eels gradually decrease from approximately 6×10^{-3} (around 200μm from the otolyth core) after the glass eel stage to approximately 3×10^{-3} at catch. The profiles of otolyth Sr:Ca ratios of naturally-recruited eels collected in Latvia were very similar to that found in Lithuania since the naturally-recruited eels all experienced the same migrating routes from high saline North Sea, semi-saline Baltic Sea to very low saline Latvian coasts. The naturally-recruited eels collected in Latvia, as that collected in Lithuania, also showed diversified migratory patterns. All the 19 naturally-recruited eels except maybe only one specimen evidently invaded the freshwater once to several times in their life (so called inter-habitat shifter) and there be no sea eel observed. The most evident example of inter-habitat shifting can be observed for specimen No. 158 (Fig.18). In this example specimen has entered freshwater shortly after arriving in Baltic Sea, spent there around 3 years and then returned in the sea. After 3-4 years spent in the sea, this individual repeated similar habitat shift one more time.

The eels were collected very close to the river delta and thus had continuous opportunity to enter freshwater. It has to be mentioned also that great amount of freshwater continuously flows in the sampling area from the inlets of two biggest Latvian rivers Daugava and Lielupe periodically (depending on weather conditions) lowering water salinity. This factor can also lead to absence of truly “sea eel” pattern in the sample. Therefore, it was reasonable to see that no sea eel was found in the sampling area, however the existence of see eels in the are can not be fully excluded. The eel No.146 can arguably be see eel (Fig.19). Although in the previous study in Lithuania the Sr:Ca ratio for seawater residents was $>3.23 \times 10^{-3}$ in Riga Gulf salinity is lower so the ratio also could be lower. In this respect additional sampling is needed in area that is not so influenced by freshwater to reveal or deny existence of sea eels in Latvian coastal waters.

In addition, the 12 stocked eels all showed consistent low otolyth Sr:Ca ratio from the elver stage to yellow or silver eel stage (Fig. 16 and 17), which was different from that found in the eels collected in the Baltic Sea of Lithuanian territorial waters. The consistent low otolyth Sr:Ca ratios without any higher otolyth Sr:Ca ratios indicates that the stocked eels completely reside in the freshwater environments and no indication for the out-migration to the Baltic Sea. The stocked eels were released probably in some midland lakes, which are far from the sea coast and connected to sea with rivers. However, downstream migration from lakes where eels has been stocked is restricted by eel weirs or hydroelectric power station dams. Therefore, the high proportions of stocked eels (39%) observed in the southern part of the Gulf of Riga only can be explained by escapement of certain portion of yellow or silver eel from Daugava basin upstream lakes via eel weirs during non traditional sea dwelling migration timing or even possibly trough hydroelectric power station turbines. Although the last one possibility is considered to be dependent from turbine characteristics and the mode of operation of power plant as well as from size of eel (EPRI, 2001; Hadderingh & Baker, 1998) there is no investigations and evidences of such migration in Daugava River. Additionally the possibility of eel migration along the sea coastline from other river basins should be considered. The possible origins of the stocked eels could be revealed only after careful studies of restocking activities performed in Latvia during previous decades.

Table 3. Biological characteristics (mean ± SD) of the European eels collected in the area of Latvian river. N: sample size.

Origin of the eels	Sampling period	Developmental stage	N	Mean (±SD) and range		
				Total length (cm)	Body weight (g)	Age (year)
Naturally-recruited eel	August – September	Yellow eel	15	60.6 ± 10.7 (43.9 - 77.4)	670 ± 356.1 (179 – 1161)	12 ± 3.2 (5 – 19)
Naturally-recruited eel	August – September	Silver eel	5	61.3 ± 2.2 (58.9 – 63.6)	446 ± 79.6 (371 – 521)	12 ± 1.9 (10 – 14)
Stocked eel	August – September	Yellow eel	1	60.9 for No.142	499 for No. 142	16 for No. 142
Stocked eel	August – September	Silver eel	10	68.15 ± 10.0 (55.9 - 91.0)	866.5 ± 370.4 (368 – 1365)	21.5 ± 5.7 (13 – 30)

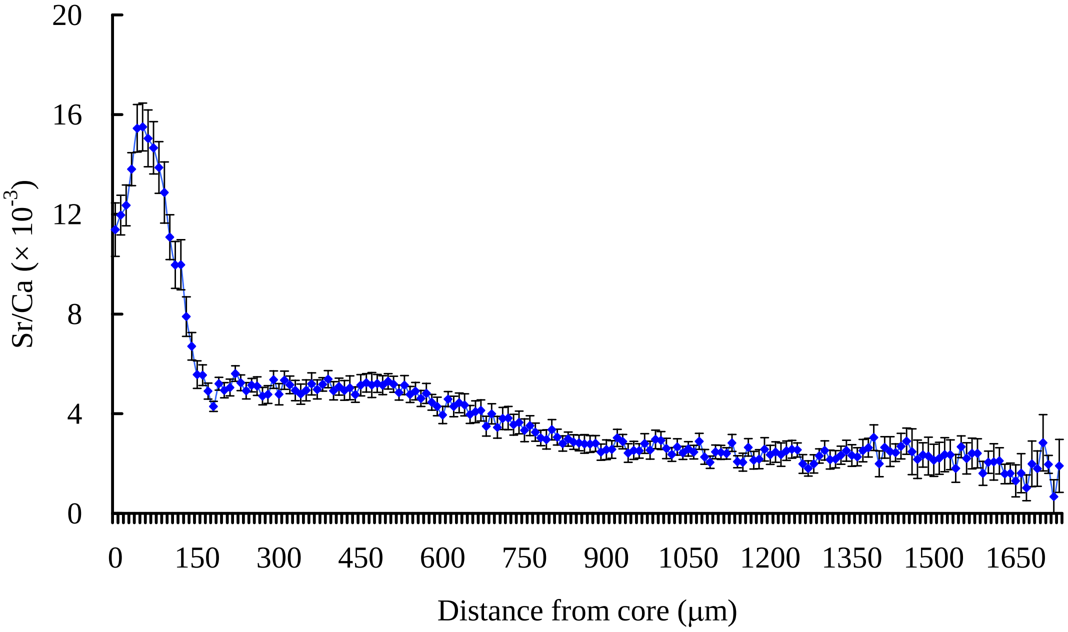


Fig. 15. Temporal changes of mean otolith Sr:Ca ratios from 19 **naturally-recruited** European eels (*Anguilla anguilla*) collected in the Latvian waters. Y error bar represents the standard error of mean.

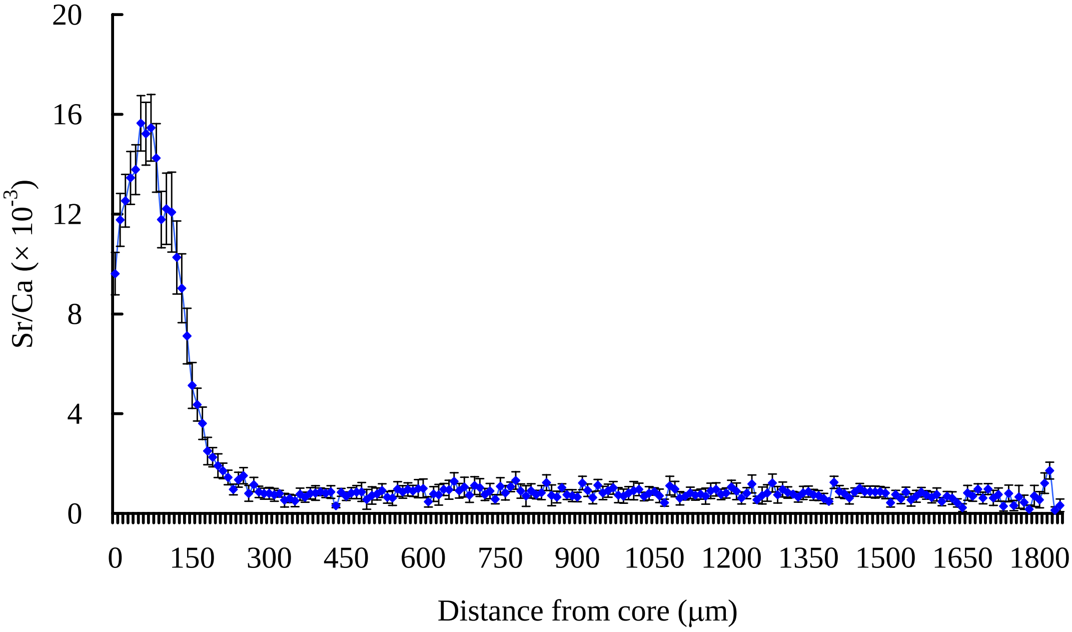


Fig. 16. Temporal changes of mean otolith Sr:Ca ratios from 12 **stocked** European eels (*Anguilla anguilla*) collected in the Latvian waters. Y error bar represents the standard error of mean.

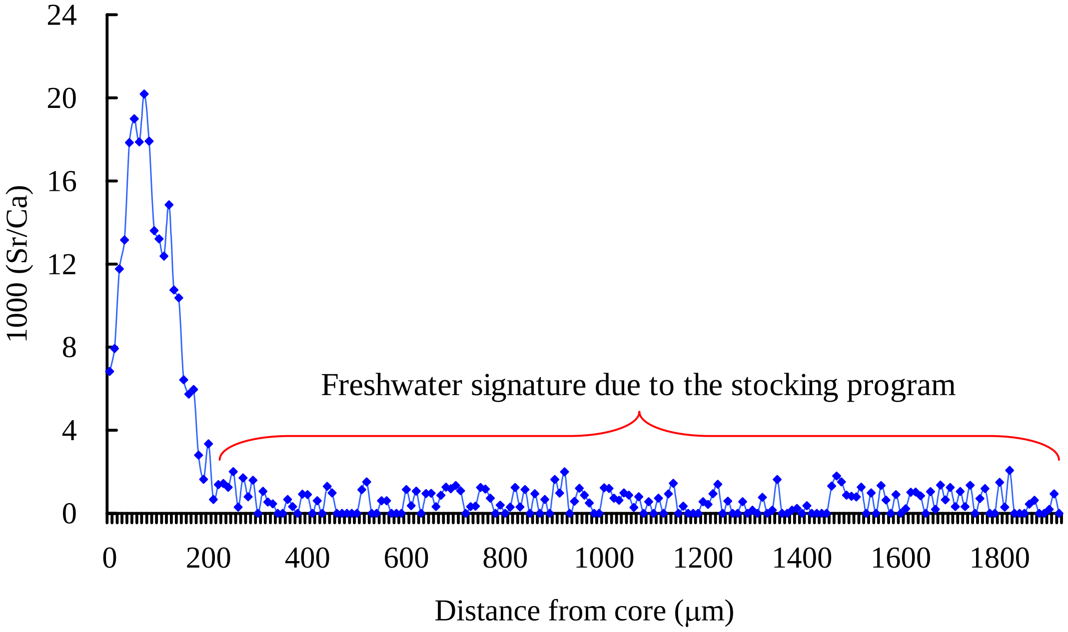
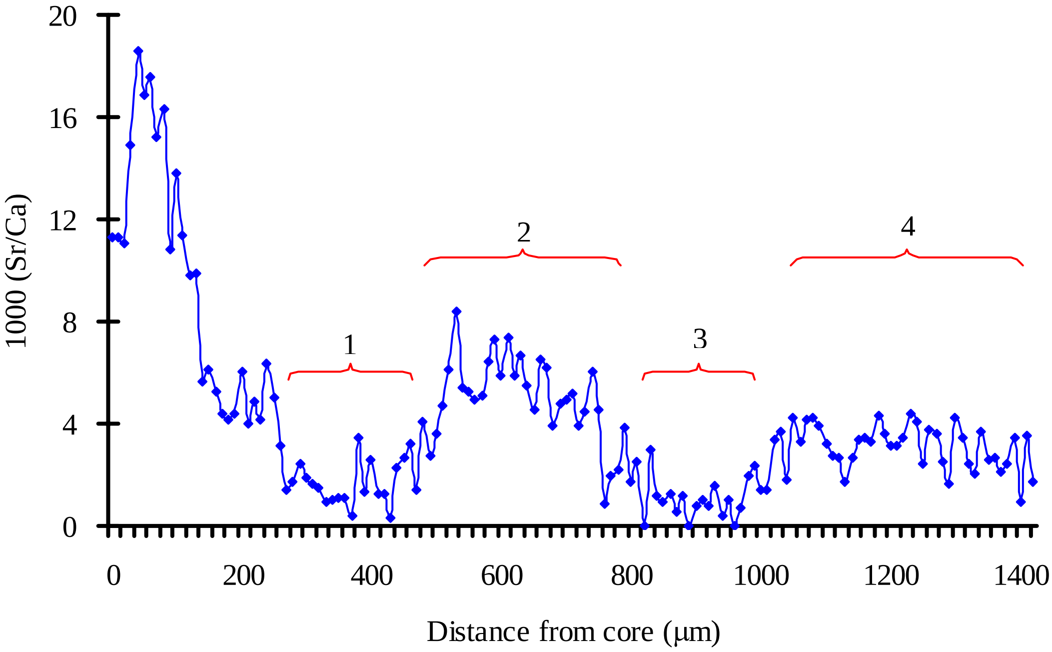


Fig. 17. Temporal changes of the otolith Sr:Ca ratios from a typical **stocked** European eels (*Anguilla anguilla*) (No. 156) collected in the Latvian waters. The consistent low otolith Sr:Ca ratios without relative high otolith Sr:Ca ratio indicated that the eel completely reside in the freshwater environment and did not out migrate to the Baltic Sea. Y error bar represents the standard error of mean.



Figure

Fig. 18. Temporal changes of Sr:Ca ratio for a typical inter-habitat shifter, 1 and 3 indicating freshwater residence, 2 and 4 – sea residence

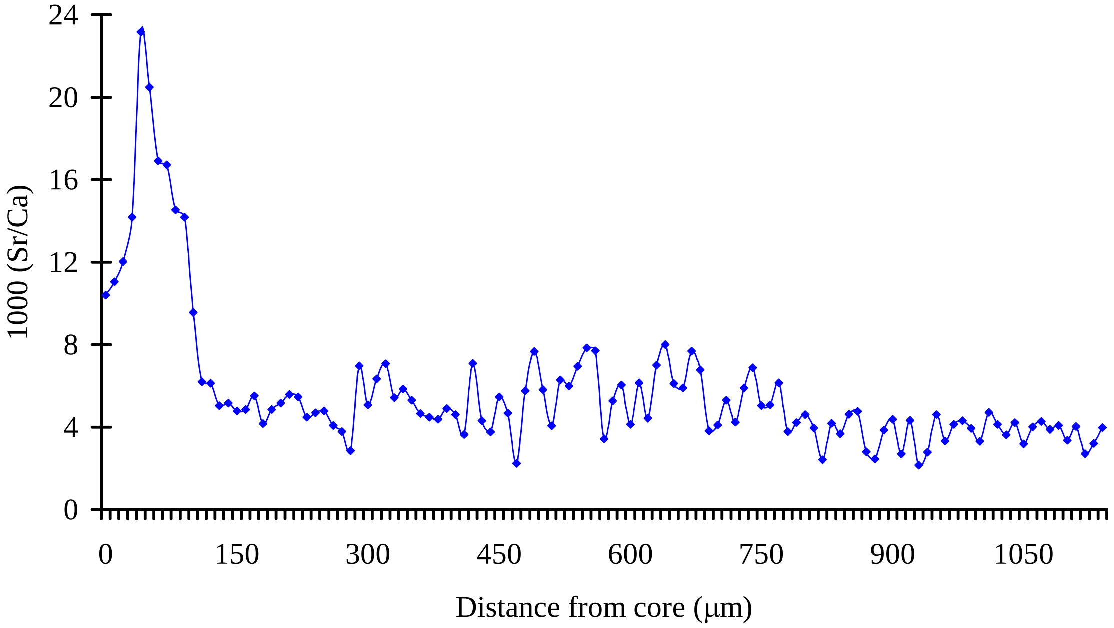


Fig. 19. Temporal changes of Sr:Ca ratio for possible sea eel, specimen from Latvian sample No.146

Otolith Sr:Ca ratios of the perch and pikeperch collected from the inland lake

Seven perch and ten pikeperch individuals were collected from inland freshwater lake. These fish never experience saline water in their life so that their otolith Sr:Ca ratios shall represent the freshwater signature of these two species. The otolith Sr:Ca ratios of these 17 fish were very small and range from 0 to 2.87 × 10^{-3} (Figs. 20-21). The mean values (± SD) were 0.58 ± 0.53 × 10^{-3} and 0.51 ± 0.51 × 10^{-3} for the perch and pikeperch, respectively. The frequent distribution of all otolith Sr:Ca ratios were shown in Fig 22-23. About 90% of the values are less than 2.0 × 10^{-3}. Therefore the value of 2.0 × 10^{-3} was used as a criterion to separate the freshwater residency from brackish/seawater residency. Furthermore, the values between 2.0 – 2.5 × 10^{-3} was regarded as the transition between freshwater and brackish/seawater environments in the follow sections.

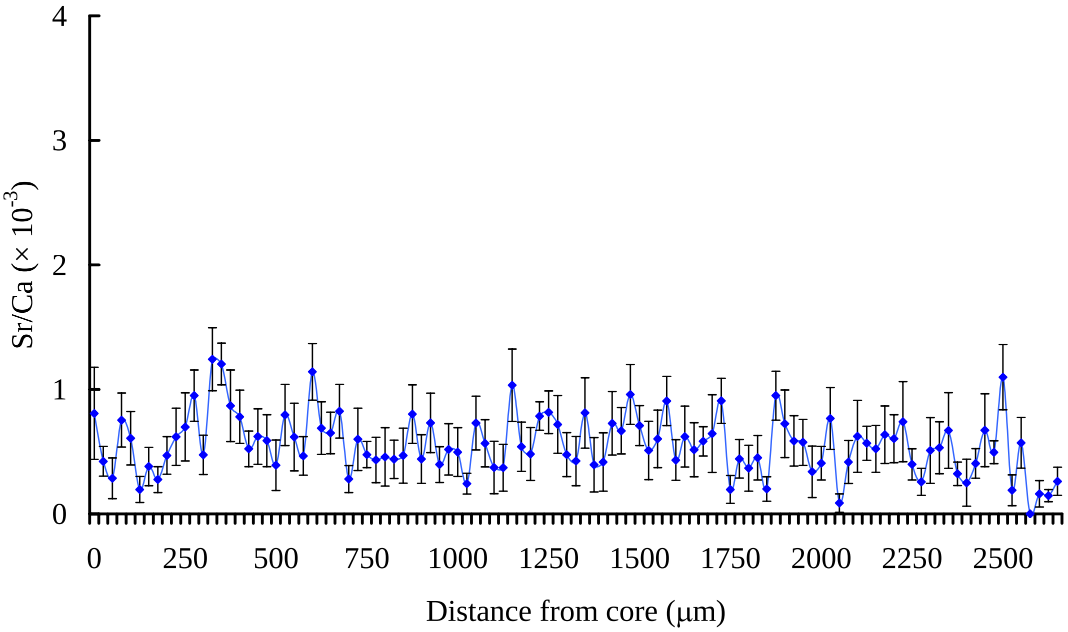


Fig. 20. Temporal changes of mean otolith Sr:Ca ratios from 7 perch collected in the lake. Y error bar represents the standard error of mean.

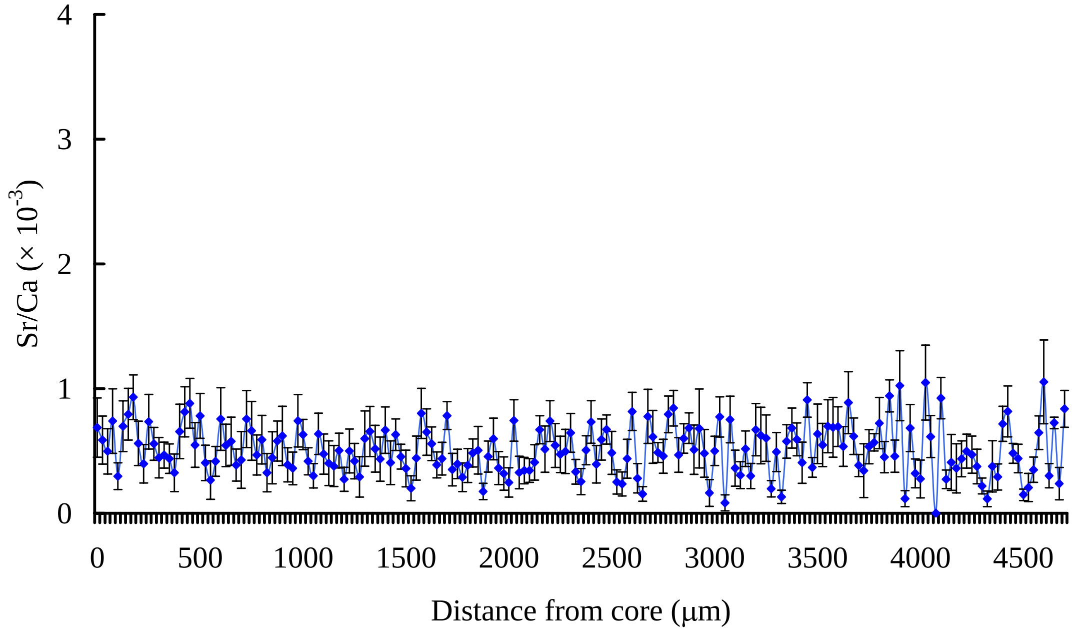


Fig. 21. Temporal changes of mean otolith Sr:Ca ratios from 10 pikeperch collected in the lake. Y error bar represents the standard error of mean.

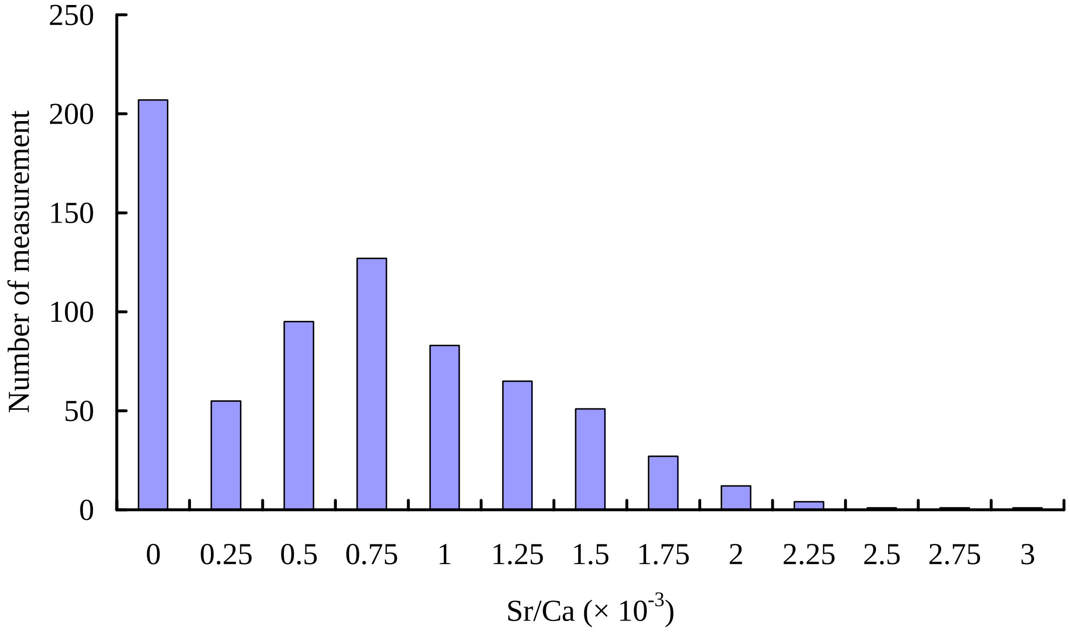


Fig. 22. Frequency distribution of otolith Sr:Ca ratios from 7 perch collected in the lake.

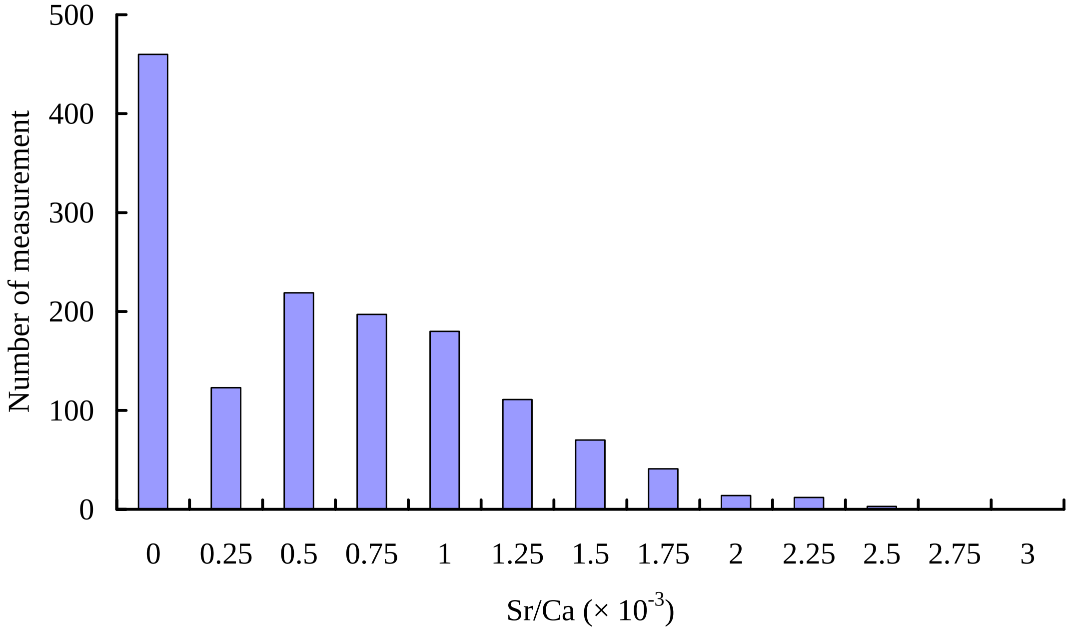


Fig. 23. Frequency distribution of otolith Sr:Ca ratios from 10 pikeperch collected in the lake.

Otolith Sr:Ca ratios of the pikeperch collected from the Curonian Lagoon

Sr:Ca ratio analysis were conducted on 24 pikeperch individuals collected in the Curonian Lagoon, 2004. Otolith Sr:Ca ratios were measured each 10 μm or 25 μm interval from the core. Both beam conditions indicated very low Sr:Ca ratios along the life history transect analysis (Fig. 24-25). The consistent low otolith Sr:Ca ratios indicated that these 24 pikeperches only resided in the freshwater Curonian Lagoon and did not move to the Baltic Sea during their life span. The otolith Sr:Ca ratios of these 24 pikeperches ranged from 0 to 3.66 × 10^{-3}, with a mean value of 0.69 ± 0.66 × 10^{-3}. Some fish show a single measurement larger than 3.0 × 10^{-3}, which is not regarded as a habitat transition from freshwater to seawater environment. These unusual signals were considered as the analytic artifacts due to the uneven otolith surface.

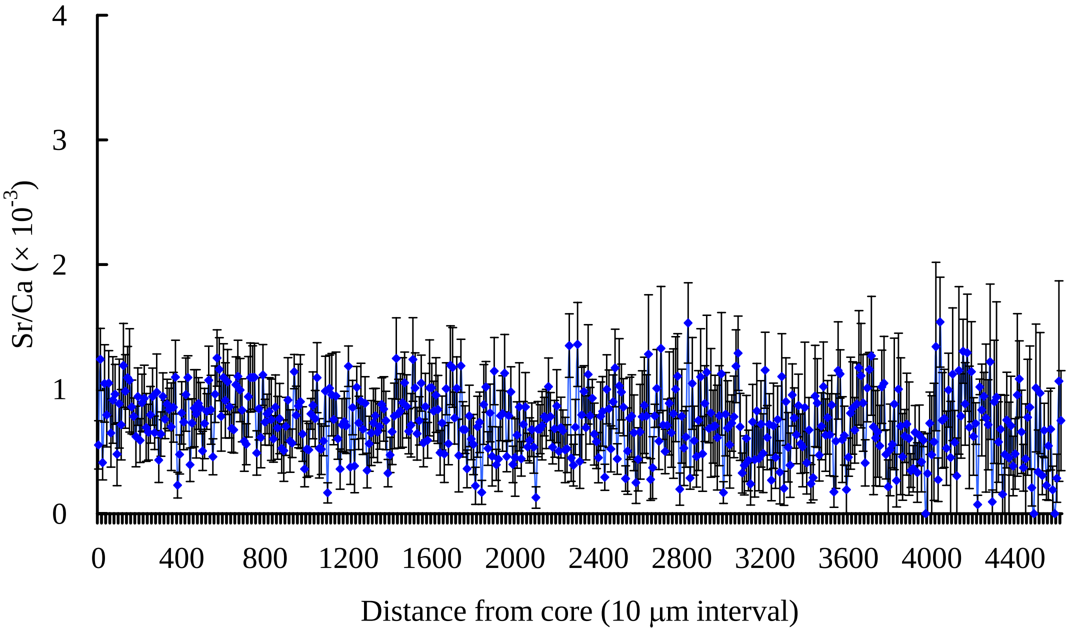


Fig. 24. Temporal changes of mean otolith Sr:Ca ratios from 9 pikeperch collected in the Curonian Lagoon in year 2004. Analysis was conducted each 10 μm from the core. Y error bar represents the standard error of mean.

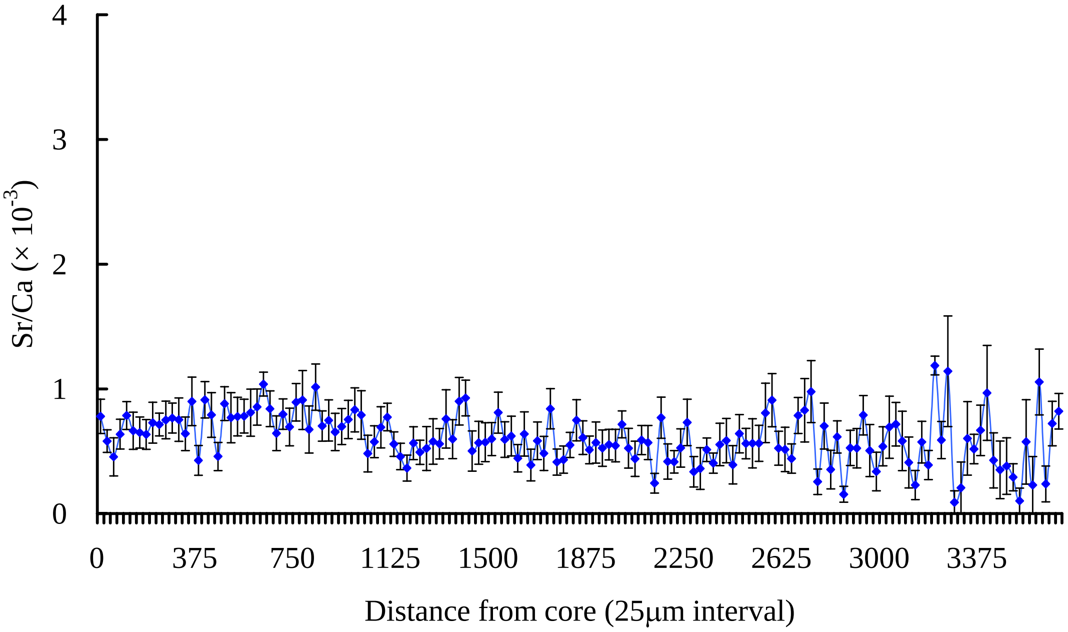


Fig. 25. Temporal changes of mean otolith Sr:Ca ratios from 15 pikeperch collected in the Curonian Lagoon in year 2004. Analysis was conducted each 25 µm from the core. Y error bar represents the standard error of mean.

Otolith Sr:Ca ratios of the pikeperch collected from the Baltic Sea

Otoliths from 19 pikeperch individuals collected in the Baltic Sea coastal waters were analyzed for Sr:Ca ratios. Otolith Sr:Ca ratios ranged from 0 to 4.76 × 10^{-3} with a mean value of 1.17 ± 0.81 × 10^{-3}. More than 20% of the measurements exceed 2.0 × 10^{-3} (Fig. 26). The mean values of the temporal changes of all samples were evidently larger than the counter parts collected from the inland freshwater lake (Fig. 27). According to the temporal changes of otolith Sr:Ca ratios, several migratory behaviors were identified. Most fish show freshwater to brackish/seawater movement after a period of freshwater residence. The movements between freshwater and brackish/seawater environments are seasonal or regularly (Fig. 28). There are three fish (No. 56, 57, 58) that show freshwater residence although they were collected from the Baltic Sea (Fig. 29). These three fish might reside in the freshwater environments (Curonian Lagoon?) and moved to the Baltic Sea ultimately. The residence in the Baltic Sea might be too short to imprint the brackish/seawater signature of higher Sr contents in the otoliths.

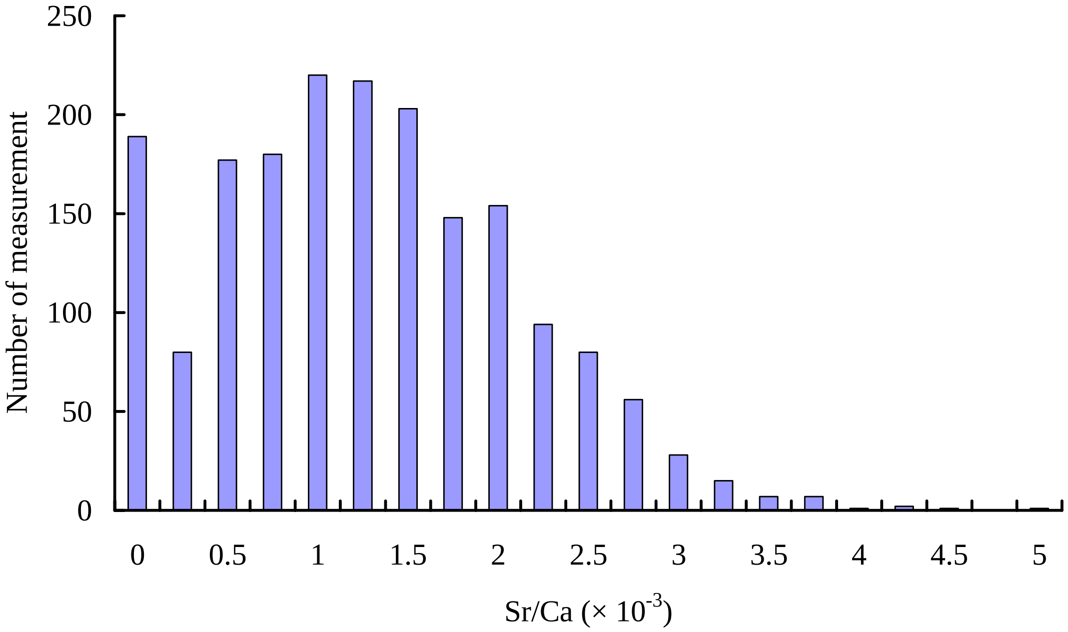


Fig. 26. Frequency distribution of otolith Sr:Ca ratios from 19 pikeperch collected in the Baltic Sea in the year 2005.

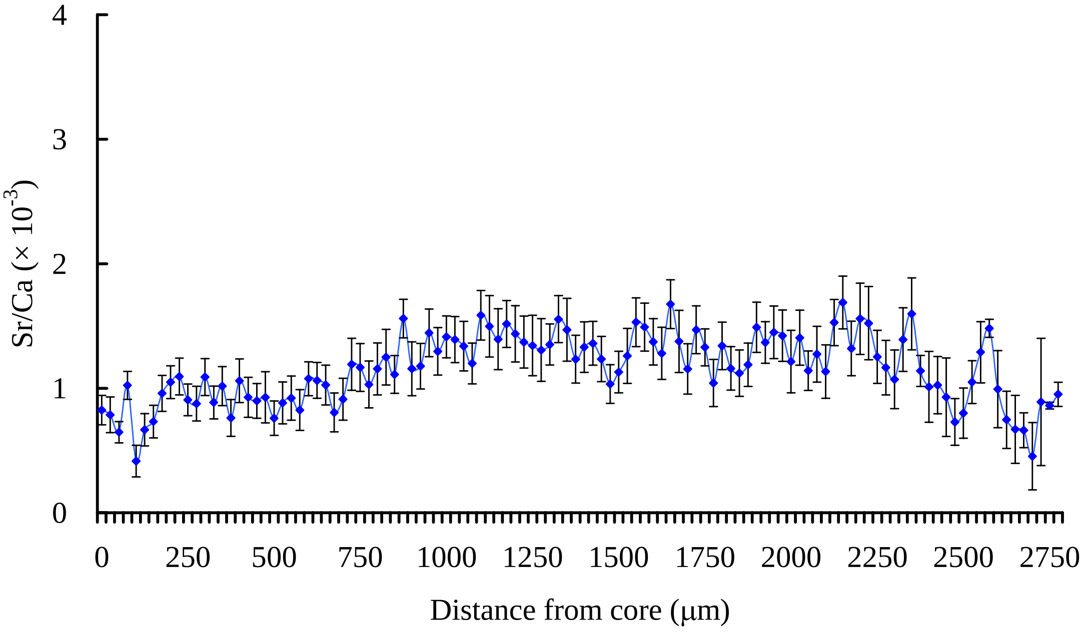


Fig. 27. Temporal changes of mean otolith Sr:Ca ratios from 19 pikeperch collected in the Baltic Sea in the year 2005. Y error bar represents the standard error of mean.

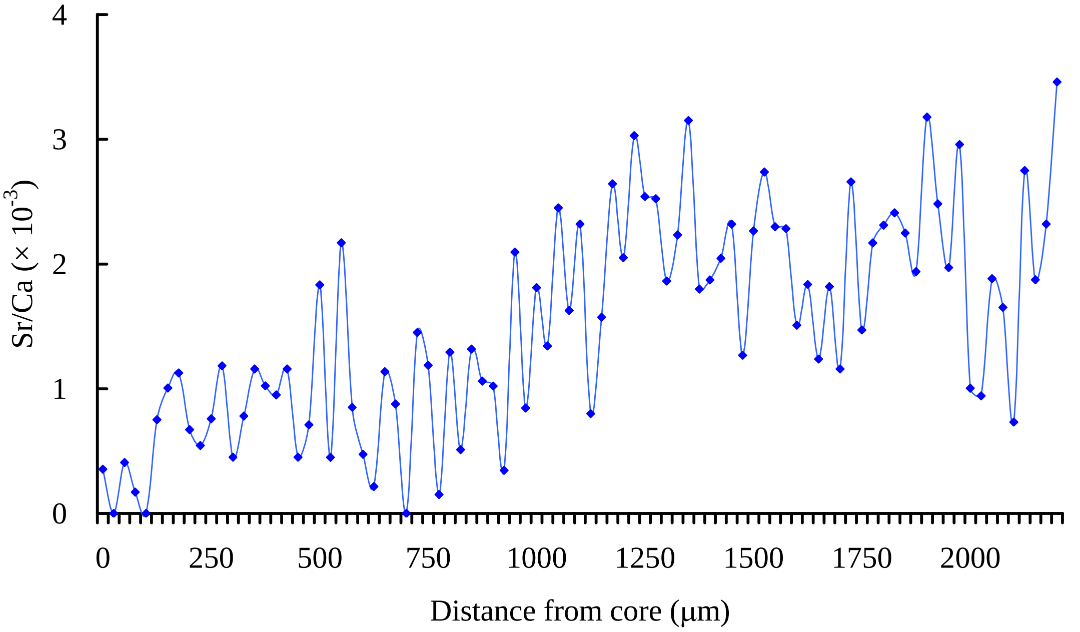


Fig. 28. Temporal changes of otolith Sr:Ca ratios of pikeperch no. 53 collected in the Baltic Sea in the year 2005. This fish reside in freshwater environment for a period of time (before 1000µm) then move to brackish Baltic Sea until being caught.

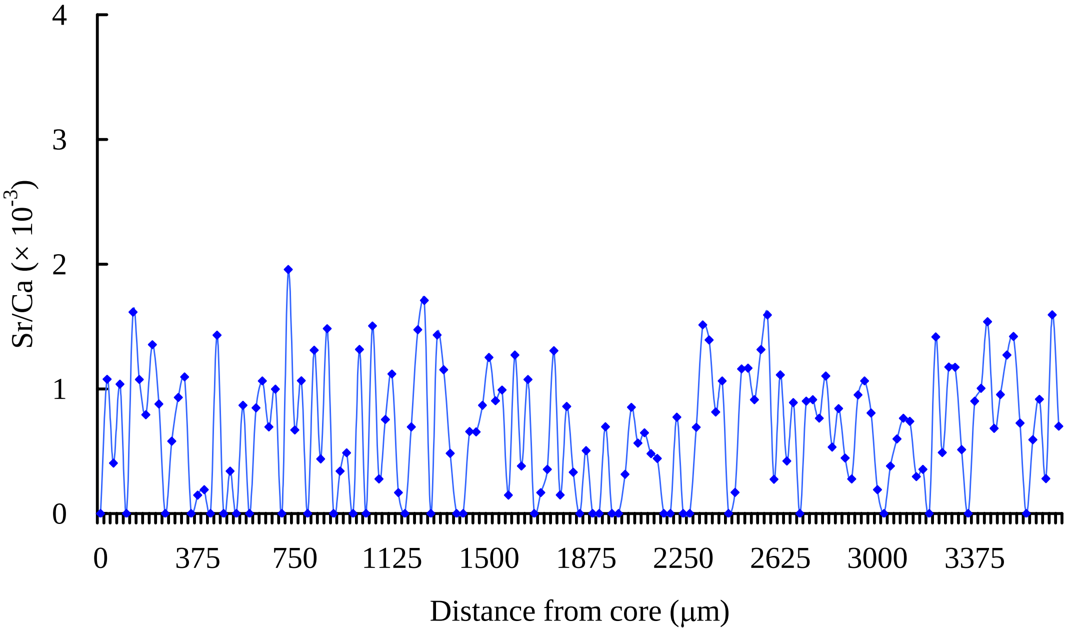


Fig. 29. Temporal changes of otolith Sr:Ca ratios of pikeperch no. 58 collected in the Baltic Sea in the year 2005. No evident movement between freshwater and brackish water was observed in this fish.

Otolith Sr:Ca ratios of the perch collected from the Baltic Sea in 2005

Otoliths from 15 perch collected in the Baltic Sea were analyzed for Sr:Ca ratios. Otolith Sr:Ca ratios ranged from 0 to 7.31×10^{-3} with a mean value of $1.91 \pm 1.37 \times 10^{-3}$. Approximately 50% of the measurements exceed 2.0×10^{-3} (Fig. 30). The mean values of the temporal changes of all samples show gradual increase from around 1.0×10^{-3} to around 3.0×10^{-3} (Fig. 31). According to the temporal changes of otolith Sr:Ca ratios, several migratory behaviors were also identified. Most fish show movement from freshwater to brackish water after an initial residency in freshwater environment. The movements between freshwater and brackish waters are seasonal or irregularly (Fig. 32-33). There is no freshwater-residence fish found in these samples. The perch evidently have larger otolith Sr:Ca ratios than that of pikerperch. This may indicate that perch resided in more saline water or the perch has more efficient Sr-uptake ability than the pikeperch.

Biological information of the perch and pikeperch collected for otolith Sr:Ca ratio analysis in Lithuania is presented in the Table 4.

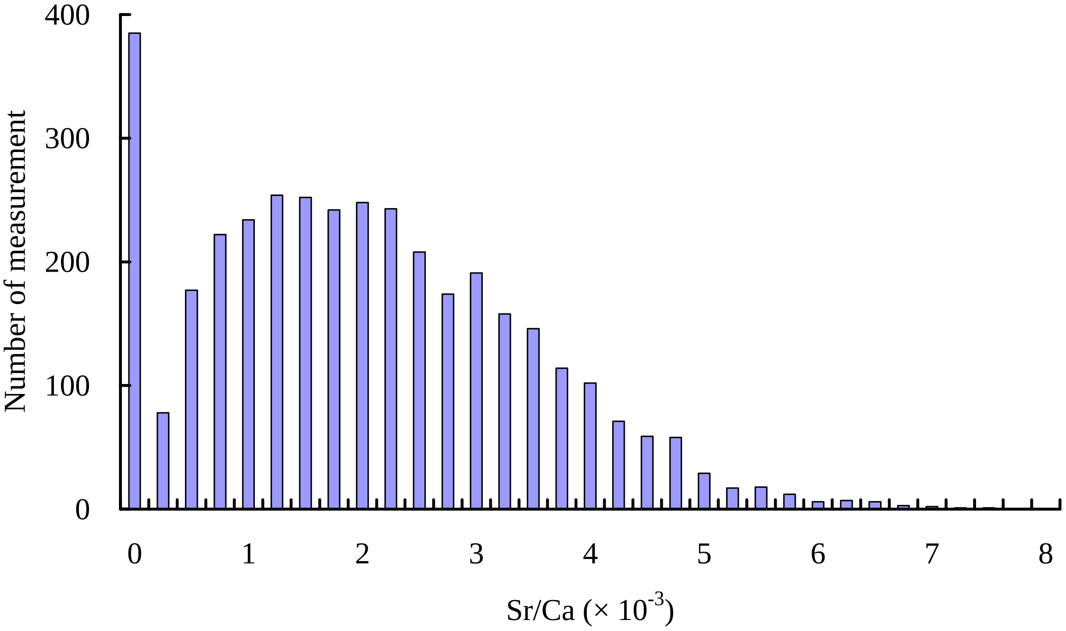


Fig. 30. Frequency distribution of otolith Sr:Ca ratios from 15 perch collected in the Baltic Sea in the year 2005.

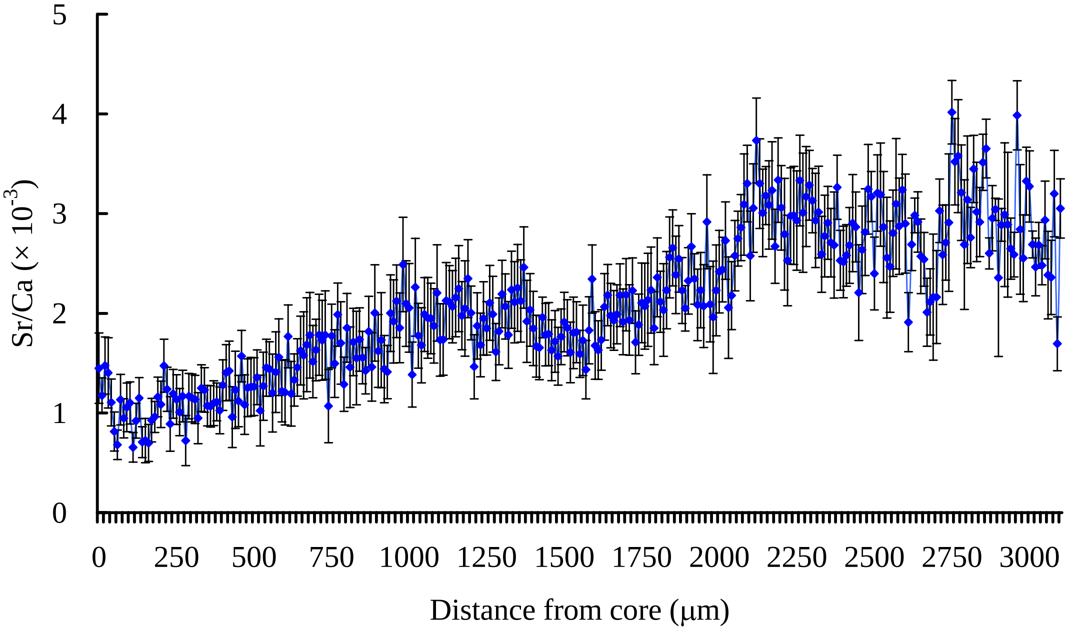


Fig. 31. Temporal changes of mean otolith Sr:Ca ratios from 15 perch collected in the Baltic Sea in the year 2005. Y error bar represents the standard error of mean.

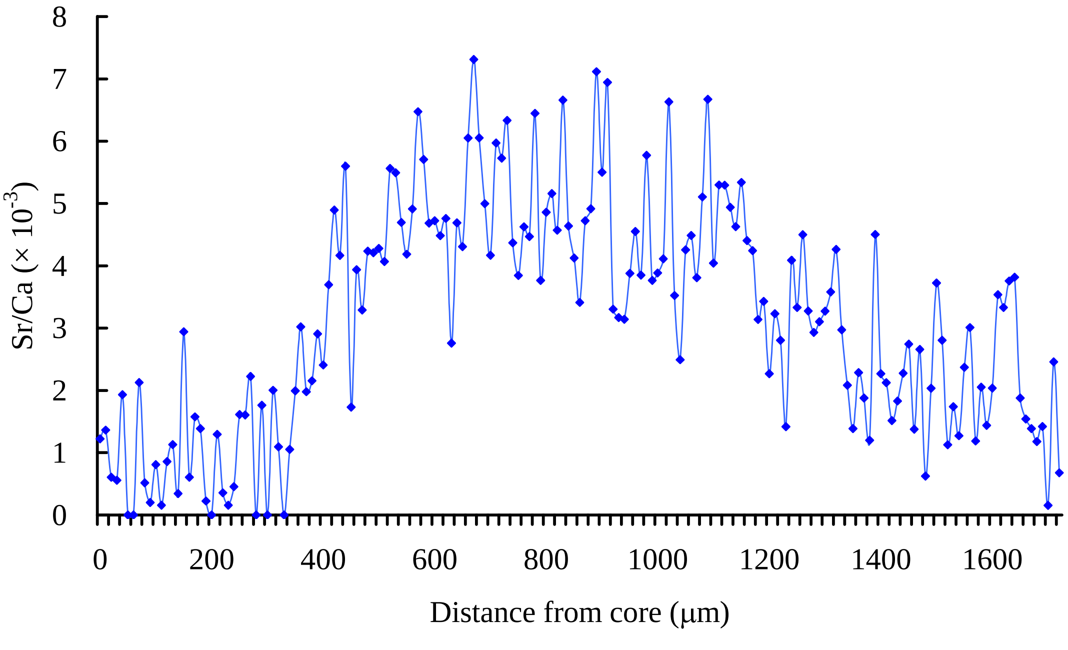


Fig. 32. Temporal changes of otolith Sr:Ca ratios of perch no. 10 collected in the Baltic Sea in the year 2005. This fish reside in freshwater environment for a period of time (before 400µm) then move to brackish Baltic Sea for another period of time and move to freshwater again.

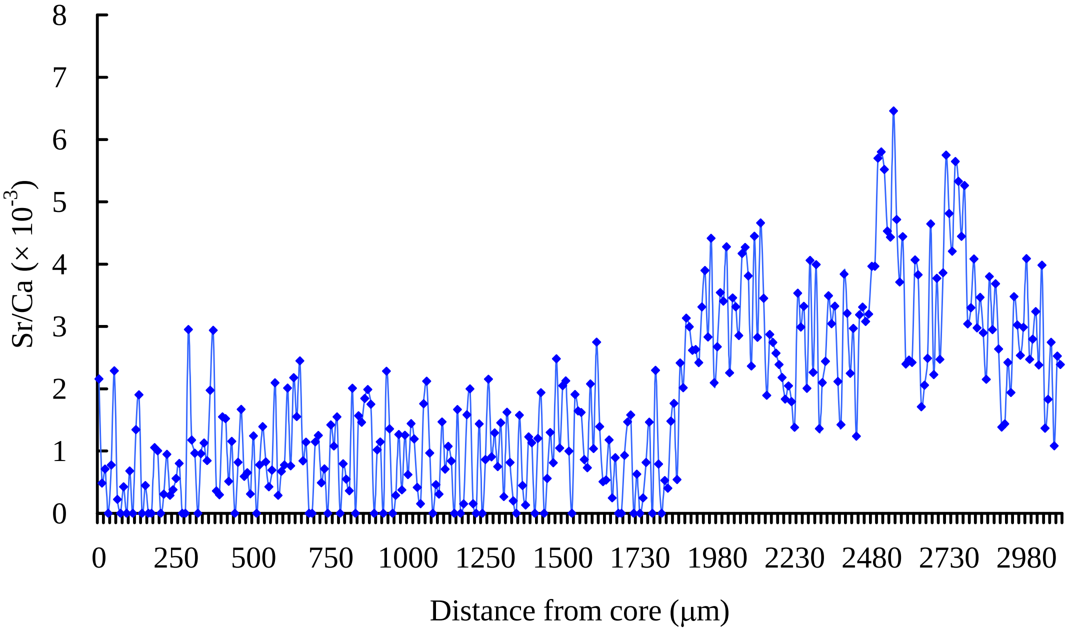


Fig. 33. Temporal changes of otolith Sr:Ca ratios of perch no. 12 collected in the Baltic Sea in the year 2005. This fish reside in freshwater environment for a period of time (before 1900μm) then move to brackish Baltic Sea until being caught.

Table 4. Biological information of the perch and pikeperch collected for otolith Sr:Ca ratio analysis.

Species	Location	Sampling date	Mean Sr:Ca ratios of freshwater contingent	Mean Sr:Ca ratios of diadromous contingent	Total length (cm)	Weight (g)
Pikeperch	Inland lake	2005	$0.51 \pm 0.51 \times 10^{-3}$ (n=10)	(n=0)	43.5±11.5	823.0±738.6
Pikeperch	Curonian Lagoon	2004	$0.69 \pm 0.66 \times 10^{-3}$ (n=24)	(n=0)	48.7±8.7	1208±737.6
Pikeperch	Baltic Sea	2005	$0.71 \pm 0.54 \times 10^{-3}$ (n=3)	$1.32 \pm 0.83 \times 10^{-3}$ (n=19)	42.0±6.7	746.5±493.1
Perch	Inland lake	2005	$0.58 \pm 0.53 \times 10^{-3}$ (n=7)	(n=0)	23.7±2.9	195.3±64.7
Perch	Baltic Sea	2005	(n=0)	$1.91 \pm 1.37 \times 10^{-3}$ (n=15)	25.3±2.0	244.9±54.7

Otolyth Sr:Ca ratios of the eels collected in Latvian waters in 2006.

In total 46 eels were sampled in August – September 2006. All eels were sampled with eel fykenet. All specimens were determined as females. Average total length and weight for caught eels differed by site. In lake Ķīšezers it was 81,3 cm (69,4-91,7 cm) and 979,8 g (521-1330 g), in Mērsrags – 77,9 cm (60,3-90,7 cm) and 1009,8 g (416-1560 g). All of them were yellow eels (Table _ and _). 40 of the sampled fish were naturally recruited, 6 were restocked eels that came from some freshwater bodies.

In summer 2006 30 perch were sampled in Jūrkalne on Open Baltic Sea coast. The average length and weight of the sampled fish was 27,5 ± 2,6 cm (24,4-34,3 cm) and 312,2 ± 96,1 g (198-567 g) (Table _). 19 perch in the sample were femailes, 11 were males making the sex ratio approximately 2:1.

Results

In total 46 eels collected in Latvian waters in 2006 were analyzed for otolyth Sr:Ca ratios. Among them 40 eels showed the pattern of naturally-recruited eel and 6 eels showed the pattern of stocked eels. The mean otolyth Sr:Ca ratios of the naturally-recruited eels and stocked eels were shown in the Fig. 1 and 2, respectively. The mean otolyth Sr:Ca ratios of 40 naturally-recruited eels gradually decrease from approximately 6×10^{-3} (around 200 m from the otolyth core) after the glass eel stage to approximately 3×10^{-3} at catch. The profiles of otolyth Sr:Ca ratios of naturally-recruited eels collected in Latvia were very similar to that found in Lithuania since the naturally-recruited eels all experienced the same migrating routes from high saline North Sea, semi-saline Baltic Sea to very low saline Latvian coasts. The naturally-recruited eels collected in Latvia, as that collected in Lithuania, also showed diversified migratory patterns. 30 naturally-recruited eels evidently invaded the freshwater once to several times in their life (so called inter-habitat shifters). Typical Sr:Ca ratio pattern of such specimens is shown in Figure 4. 15 eels were collected in lake Ķīšezers which is connected to river Daugava and thus has short connection with Riga Gulf. Therefore it is reasonable that no “sea eel” pattern was evident in the sample from this area. However 10 specimens from eels collected in Mērsrags could be considered as sea eels that have never entered freshwater at all. Typical Sr:Ca ratio for such specimens is shown in Fig.5. Although in the previous study in Lithuania the Sr:Ca ratio for seawater residents was $>3.23 \times 10^{-3}$ in Riga Gulf salinity is lower so the ratio also could be lower.

4 of the stocked eels, showed consistent low otolyth Sr:Ca ratio from the elver stage to yellow or silver eel stage (Fig. 2 and 3), which was different from that found in the eels collected in the Baltic Sea of Lithuanian territorial waters. The consistent low otolyth Sr:Ca ratios without any higher otolyth Sr:Ca ratios indicates that the stocked eels completely reside in the freshwater environments and there is no indication for the out-migration to the Baltic Sea. However 2 specimens according to their Sr:Ca ratio, have entered coastal waters after their release. One of them was collected in Mērsrags, second one – in lake Ķīšezers. All of the stocked eels were released probably in some midland lakes, which are far from the sea coast and connected to sea with rivers. However, downstream migration from lakes where eels has been stocked is restricted by eel weirs or hydroelectric power station dams. Therefore appearance of stocked eels observed in the sampling sites can be explained by escapement from Daugava basin upstream lakes via eel weirs during non traditional sea dwelling migration timing or even possibly trough hydroelectric power station turbines. Although the last one possibility is considered to be dependent from turbine characteristics and the mode of operation of power plant as well as from size of eel (EPRI, 2001; Hadderingh and Baker, 1998) there is no investigations and evidences of such migration in Daugava River. Additionally the possibility of eel migration along the sea coastline from other river basins

should be considered. The possible origins of the stocked eels could be revealed only after careful studies of restocking activities performed in Latvia during previous decades.

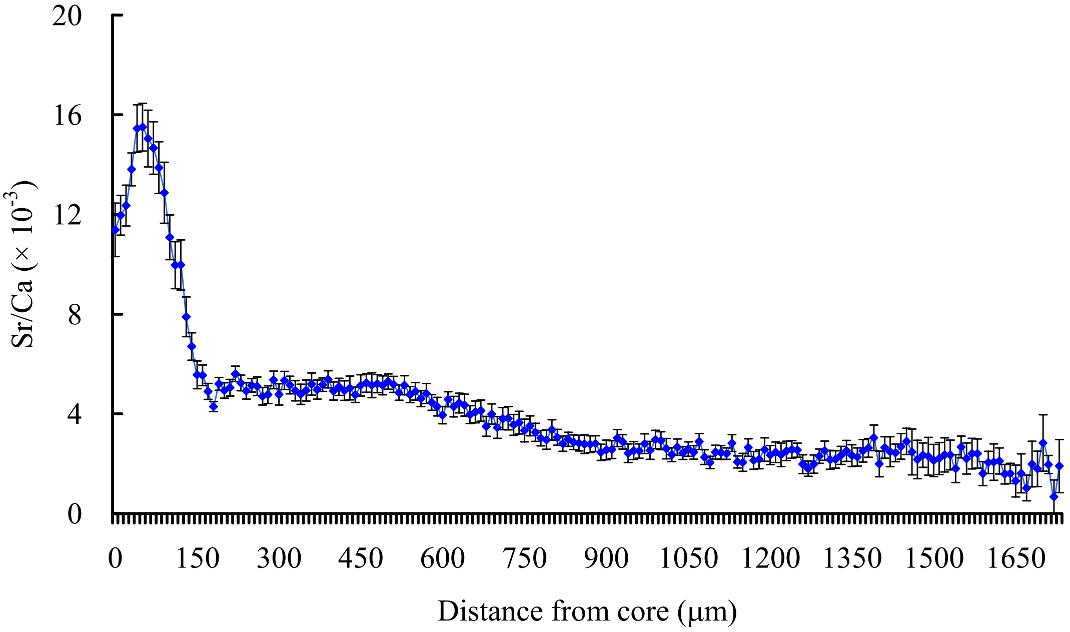


Figure 1. Temporal changes of mean otolith Sr:Ca ratios from 19 **naturally-recruited** European eels (*Anguilla anguilla*) collected in the Latvian waters. Y error bar represents the standard error of mean.

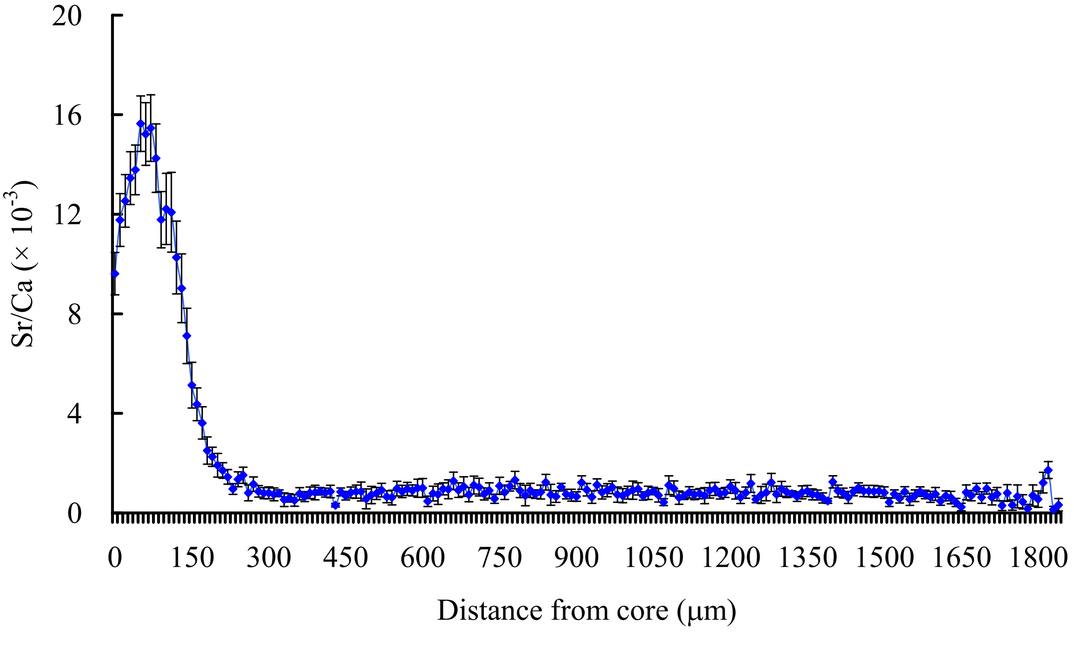


Figure 2. Temporal changes of mean otolith Sr:Ca ratios from 12 **stocked** European eels (*Anguilla anguilla*) collected in the Latvian waters. Y error bar represents the standard error of mean.

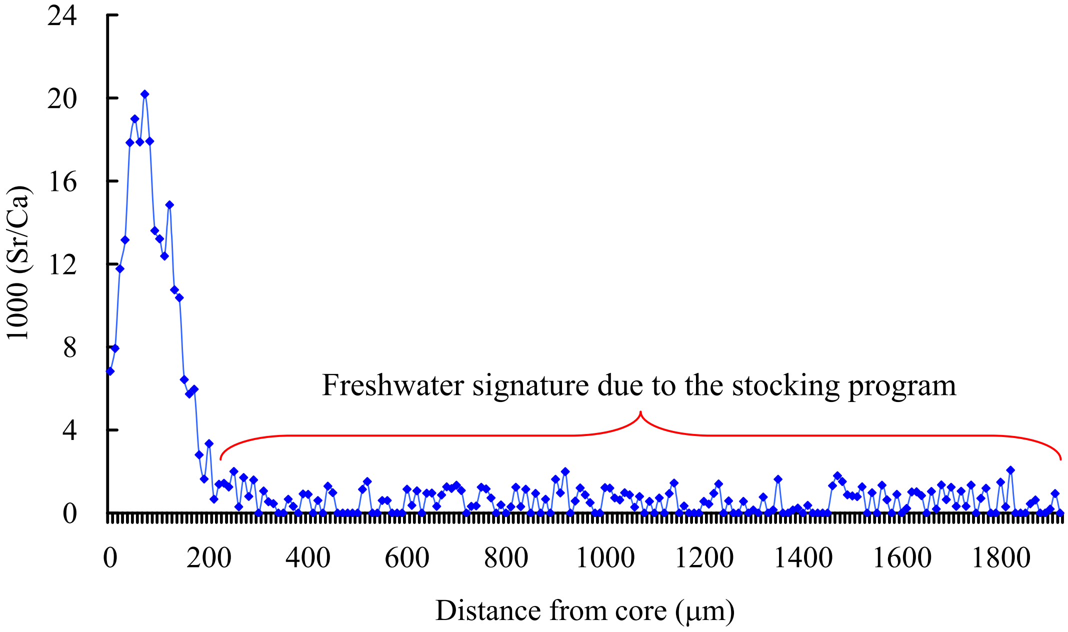


Figure 3. Temporal changes of the otolyth Sr:Ca ratios from a typical **stocked** European eels (*Anguilla anguilla*) (No. 156) collected in the Latvian waters. The consistent low otolyth Sr:Ca ratios without relative high otolyth Sr:Ca ratio indicated that the eel completely reside in the freshwater environment and did not out migrate to the Baltic Sea. Y error bar represents the standard error of mean.

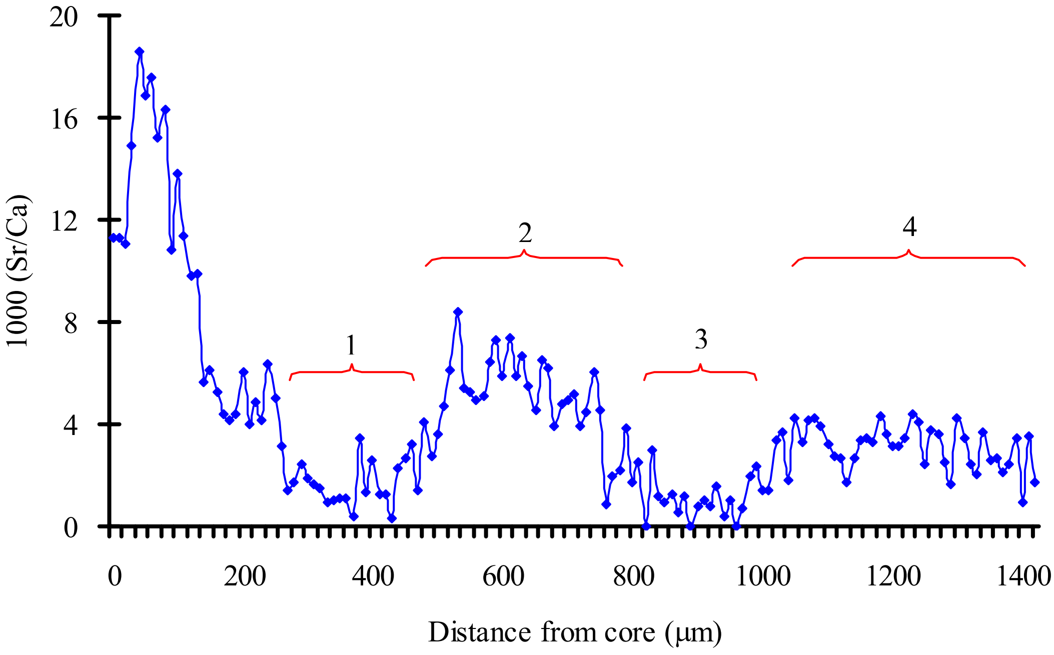


Figure 4. Temporal changes of Sr:Ca ratio for a typical inter-habitat shifter, 1 and 3 indicating freshwater residence, 2 and 4 – sea residence

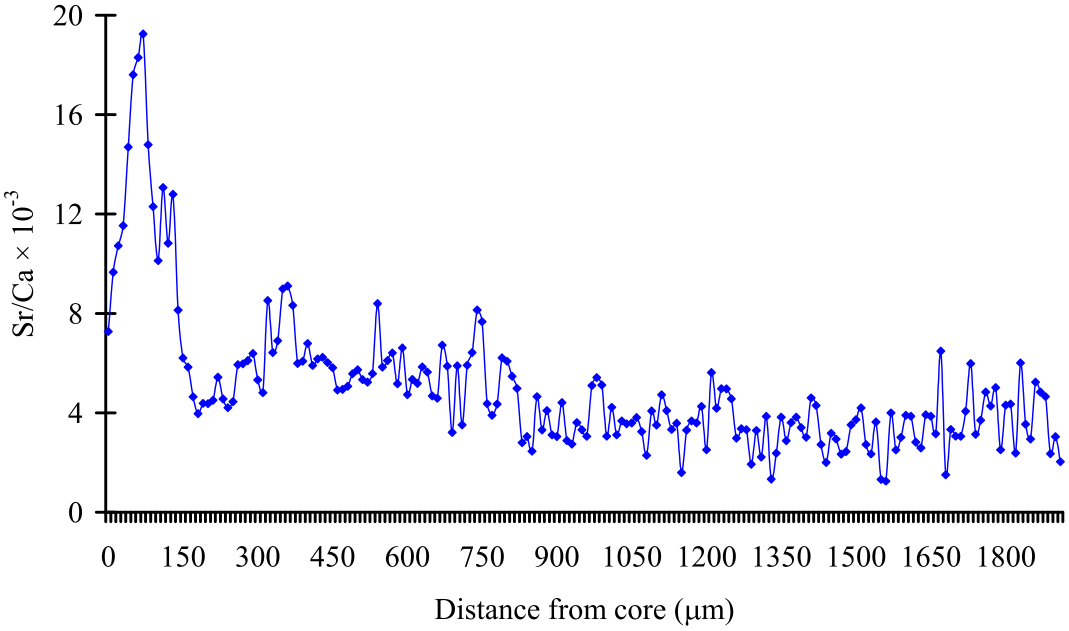


Figure 5. Temporal changes of Sr:Ca ratio for typical sea eel specimen from Latvian sample.

Table 5. Biological characteristics (mean ± SD) of the European eels collected in the lake Ķīšezers. N: sample size.

Origin of the eels	Sampling period	Developmental stage	N	Mean (±SD) and range		
				Total length (cm)	Body weight (g)	Age (year)
Naturally-recruited eel	September	Yellow eel	12	81.1 ± 7.3 (69.4 - 91.7)	980.8 ± 250.9 (521 – 1330)	12.8 ± 1.4 (11 – 15)
Stocked eel	September	Yellow eel	4	81.8 ± 6.7 (73.8 - 89.2)	976.8 ± 281.5 (712 – 1230)	14.3 ± 3.3 (11 – 18)

Table 6. Biological characteristics (mean ± SD) of the European eels collected in Mērsrags, Latvian coastal zone. N: sample size.

Origin of the eels	Sampling period	Developmental stage	N	Mean (±SD) and range		
				Total length (cm)	Body weight (g)	Age (year)
Naturally-recruited eel	August – September	Yellow eel	28	78.8 ± 6.2 (66.2 – 90.7)	1038.7 ± 258.3 (543 – 1560)	12.2 ± 1.8 (9 – 16)
Stocked eel	August – September	Yellow eel	2	No.210 60,3 No.224 65,7	No.210 416 No.224 650	15 -

Table 7. Biological characteristics (mean ± SD) of the perch collected in Jūrkalne, open Baltic Sea coast. N: sample size

Sampling site	Sampling period	N	Mean (±SD) and range	
			Total length (cm)	Body weight (g)
Jurkalne, Open Baltic Sea	August	30	27.5 ± 2.6 (24.4 – 34.3)	312.2 ± 96.1 (198 – 567)

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2.2 MICROCHEMICAL ANALYSIS OF FISH OTOLITHS. APPENDIXES.

Appendix I

Migratory patterns and contribution of stocking to the population of European eel in Lithuanian waters as indicated by otolith Sr:Ca ratios

Running headline: Stocking success of European eel in Lithuania

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Abstract

Anguilla anguilla (Linnaeus, 1758) elvers have been stocked in Lithuanian waters and mixed with naturally recruited eels for several decades. Otolith Sr:Ca ratios were examined to evaluate the contribution of the stocked eels to the native eel population. Stocked eels were identified by the freshwater signature (Sr:Ca ratios $< 2.24 \times 10^{-3}$) on the otolith after the glass eel stage. Naturally-recruited eels, that had migrated through the North and Baltic Seas, were characterized by an extended sea/brackish water signature (Sr:Ca ratios $> 3.23 \times 10^{-3}$) after the glass eel stage. Of 108 eels analyzed, 21 eels had otolith Sr:Ca ratio profiles consistent with stocking while 87 showed patterns of natural recruitment. The ages of naturally-recruited eels arriving in Lithuanian freshwaters varied from 1 – 10 years, with a mean age of 5.2 ± 2.1 years. Eels from the inland lake Baluošai were all freshwater residents of stocked origin. However, stocked eels accounted for only 20% of the eels from the Curonian Lagoon and 2% of eels sampled in Baltic coastal waters. This finding does not support the hypothesis that the eel fishery in the Curonian Lagoon mostly depends on stocking. This study demonstrates the application of otolith microchemistry in the discrimination of mixed populations and evaluation of the stocking effectiveness of diadromous fishes.

KEY WORDS: *Anguilla anguilla*; European eel; Migratory history; Otolith microchemistry; Stocking.

Introduction

Catadromous eels are commercially valuable species and support worldwide eel aquaculture and eel fisheries. The abundance of several species (i.e., *Anguilla anguilla* Linnaeus, 1758, *A. rostrata* Lesueur, 1817, and *A. japonica* Temminck & Schlegel, 1847) have declined throughout their distribution ranges due to over-fishing, anthropogenic activities and changes of oceanic currents induced by global weather anomalies (Castonguay *et al.*, 1994; Dekker, 2003; Tatsukawa, 2003). In addition, the eel's mysterious life cycle further compounds its fate and makes conservation and recovery more difficult.

A. anguilla inhabits coastal waters, estuaries, rivers and lakes in Europe and North Africa (Bertin, 1956; Tesch, 2003). Their leaf-like larvae, leptocephali, drift via the Gulf Stream and North Atlantic Current from oceanic spawning grounds in the Sargasso Sea to the continental shelves of Europe and North Africa, or enter the Mediterranean Sea. The larvae metamorphose into glass eels on the continental shelf. Glass eels become pigmented elvers when they enter estuaries. Some elvers stay in salt or brackish water along the coast while others migrate upriver. Major elver runs occur into the Atlantic-facing estuaries of France, Spain, Portugal and the Bristol Channel and Severn Estuary in the United Kingdom. Lesser migrations occur to other countries in the Baltic and Mediterranean Seas (Knights & White, 1998).

Declines in eel recruitment in Scandinavia have been noted since the 1940s (Moriarty, 1996), but the greatest decreases in the recruitment of eels throughout Europe have occurred since the early 1980s (Dekker, 2000). Recent recruitments of *A. anguilla* glass eel were estimated to be only 1% of the level before the 1980s (Dekker, 2004). Poor natural recruitment from the oceanic migration phase, exacerbated by habitat degradation, pollution, artificial physical barriers to migration and high fishing pressure on glass eels (Moriarty & Dekker, 1997) has led to the need for stocking to maintain, enhance, restore or establish stocks. Intensive stocking programmes have been undertaken in the Baltic Sea region over the past 50 years. The most intense stocking programmes have been implemented in the Baltic Sea drainage using eels originating from western Europe.

The first eel stockings in Lithuania occurred between 1928-1939 when 3.2 million elvers were released into lakes of the Vilnius region (~300 km from the Baltic coast). Since the mid 1960s, Lithuanian lakes have been stocked with about 50 million elvers or young yellow eels (Ložys, 2002) at an average stocking rate of 1.1 million eels yearly. However, studies on stocking effectiveness have not been carried out and the post-stocking movements of stocked eels remain largely unknown. Natural recruitment is unknown due to the lack of suitable locations to monitor recruitment in the main area of invasion - Klaipeda strait (Curonian Lagoon). Therefore, statements by fishers and fisheries managers that the Curonian Lagoon eel fishery depends on eel stocking are speculative. The degree to which stocked and naturally recruited eels contribute to the eel fishery and eventually to the spawning stock is unknown. In the absence of tagging, it is difficult to discriminate stocked eels from naturally recruited eels since they are morphologically similar. Fish tagging can help identify different stocks, but most conventional tagging is not feasibly applied to small glass eels. Internal marking of the otolith, such as by tetracycline or alizarin complexone, is feasible (Tsukamoto, 1988). However, most stocking programmes, as indicated by Cowx (1999), were carried out without evaluation of their potential success. Marks were not applied to the stocked eels before their release into lakes or the lagoon. The analysis of a natural tag, the otolith strontium (Sr): calcium (Ca) ratio, has been extensively used to study the migratory history of diadromous fishes and provides alternative resolution to discriminate stocked eel from naturally-recruited eel. (see Elsdon & Gillanders, 2003 for review).

The metabolically inert otolith records biological as well as environmental information throughout the fish's life. Fish can absorb Sr in the ambient water and substitute for Ca in the process of $CaCO_3$ deposition in the otolith. The positive relationship between salinity and otolith Sr:Ca ratios has been validated for different species including eels (e.g., Tzeng 1996; Secor *et al.*, 1998; Kraus & Secor, 2003). Accordingly, seawater-resident fish uptake and deposit more Sr in the otolith than do freshwater fish. Otolith Sr:Ca ratios in combination with age data have been used to elucidate the migratory environmental history of diadromous fishes, including anguiilid eels (Tsukamoto & Arai 2001; Jessop *et al.*, 2002; Shiao *et al.*, 2003).

Elvers purchased in the United Kingdom and France are directly released into Lithuanian freshwater lakes and the Curonian Lagoon. These stocked eels do not experience the long migratory journey through the North and Baltic Sea and thus they should show a freshwater signature of low otolith Sr:Ca ratios immediately after the elver stage. In contrast, eels naturally recruited to Lithuania must pass the North and Baltic Seas and should show an extended sea/brackish water signature of high (North Sea) and intermediate (Baltic Sea) otolith Sr:Ca ratios after the elver stage. Thus, a life history scan of otolith Sr:Ca ratios should be able to discriminate between both stocked and naturally recruited eels. Clarification of the eel's migratory history will also help to evaluate the contribution and interaction of the two possible eel origins (stocked or naturally recruited) to each population along the Baltic coasts and in the Curonian Lagoon and inland lakes.

Materials and Methods

Fish collection and sampling sites

Silver and yellow-stage European eels *A. anguilla* were collected by fyke nets and long lining from Baltic coastal waters, the Curonian Lagoon in western Lithuania and the freshwater Lake Baluošai in eastern Lithuania in 2003-2004 (Fig. 1). The lake is about 300 km from the Curonian Lagoon and about 350 km from the Baltic Sea, to which it is connected via a system of small streams, lakes, the river Nemunas and the Curonian Lagoon. Natural recruitment to these lakes has never been reported and may not occur; however, the possibility cannot be excluded. Elvers have been regularly stocked since 1960 into the system of lakes in the Baluošai Lake region.

The shallow Curonian Lagoon (mean depth - 3.7 m) is separated by a narrow sand spit (0.5 – 4.0 km wide) from the Baltic Sea and is connected to the Baltic Sea through the narrow (0.5 km wide) Klaipėda Strait. The salinity of the Baltic Sea adjacent to Lithuania varies from 4.9 to 6.8 (Dubra & Dubra, 1998). The Lagoon is 1584 km^2 in area and is a fresh water basin. Rivers supply the lagoon with about 3.6 times more freshwater than the water volume in the lagoon itself and the mean water level in the lagoon is 15 cm higher than sea level. Therefore, brackish water penetration into the Lagoon is rare. The salinity fluctuates from 0.03 in the southern part of the Lagoon up to 2.7 in the Klaipėda Strait. During stormy inflows of brackish water the salinity may episodically rise to 5-6 in the northern areas (Olenin, 1996). The Curonian Lagoon was stocked by young yellow eels during 1996-1997 (43000 eels), 2000-2003 (10000 eels) and by elvers in 1995 (150000 eels) and 2003 (60000 eels).

The total length (L_T) and weight (W) of each eel was measured to the nearest 1.0 mm and 1.0 g. Sexes were determined macroscopically from the gross morphology of the gonads, where eels with thin, regularly lobed organs (Syrski's organ) were considered males, while individuals with more broad and folded curtain-like gonads were females (Tesch, 2003). The eels were classified as yellow and silver eels, by their external colour, fin shape and eye size.

Water Sr and Ca concentrations around the eel sampling locations were determined by atomic absorption spectrophotometer (Hitachi Z-5000). Standard solutions from Merck (Darmstadt, Germany) were used to make the standard curve. Sr and Ca concentrations of the water collected from the Baltic coast (salinity 5.8) were approximately 1.67×10^{-5} and 2.60×10^{-3} M, respectively (6.44×10^{-3} for the Sr:Ca ratio). Water collected in the Curonian Lagoon (salinity 0) contained approximately 1.36×10^{-6} M of Sr and 1.51×10^{-3} M of Ca (0.90×10^{-3} for the Sr:Ca ratio). Water Sr and Ca concentrations in the Baltic coast and Curonian Lagoon were in the range of normal brackish and fresh water.

Fig. 1 A. Northern Europe, showing the North and Baltic Seas, and B. Sampling locations (dots) in the Baltic Sea, Curonian Lagoon and Lake Baluošai.

Otolith preparation and Sr:Ca analysis

The largest pair of eel otoliths (sagittae) was removed, dried in air, embedded in Epofix resin, ground and polished until the core was exposed. For electron probe microanalysis, the polished otoliths were coated with carbon under a high-vacuum evaporator. Sr and Ca concentrations in the otolith were measured from the otolith core to the edge at 10 μm intervals. Quantitative analyses were conducted with an electron probe microanalyzer (JEOL JXA-8900R), using beam conditions of 15 kV for the acceleration voltage, 3 nA for the current, and a 5 × 4 μm rectangular scanning beam. The quantitative data were corrected by the PRZ (phi-rho-z) method to calculate oxide compositions (e.g., Goldstein *et al*., 1984; Reed, 1993). The peak concentration of Sr Lα was counted for 80 s with background measurements for 20 s on each side. The peak concentration of Ca Kα was counted for 20 s and each background for 10 s. A synthesized aragonite ($CaCO_3$) and strontianite ($[Sr_{0.95}Ca_{0.05}]CO_3$; NMNH R10065) were used as calibration standards. Since aragonite-structure carbonates are similar to otoliths, the standards have smaller matrix corrections than other types of standards such as oxide or silicate (Jarosewich & White, 1987). The standards were mounted in epoxy resin and polished. The carbon coating for the standards and otoliths had the same thickness (25-35 nm). After microchemical analysis, the otolith was polished to remove the carbon layer, then etched with 5% EDTA for 1 to 2 min to reveal the annual rings for age determination (Fig. 2). The duration of the eel in freshwaters and sea/brackish waters was estimated by relating the otolith Sr:Ca ratio profile to the otolith annuli. The criteria for the discrimination between freshwater- and sea/brackish water-residents are defined in the results.

Data analysis

Data are expressed as means ± SD (n = number of fish). Statistical differences among groups (locations) were evaluated by one-way analysis of variance (ANOVA) or Mann-Whitney Rank Sum Test. Differences among groups were identified by Tukey's pairwise multiple comparison test. Significance was set at $p < 0.05$.

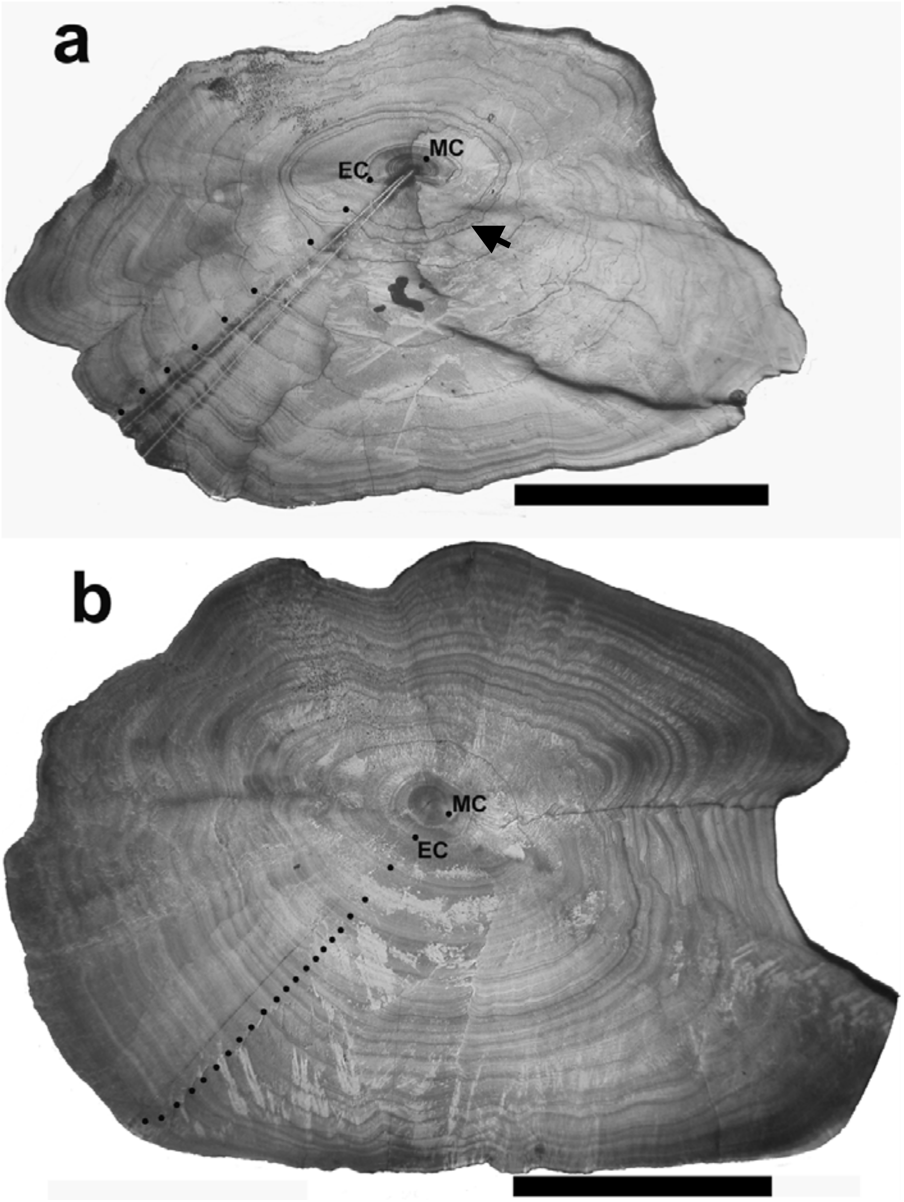


Fig. 2 *Anguilla anguilla*. Otoliths of European eels showing the metamorphosis check (MC), elver check (EC) and annuli (remaining dots). Panel a: restocked eel no. 3 (yellow eel, 63 cm TL) collected from Curonian Lagoon, showing rapid growth as inferred from wide otolith annuli. Panel b: restocked eel no. 105 (silver eel, 74 cm TL) collected from Lake Baluošai showing slow growth inferred from narrow annuli. A conspicuous check (indicated by the arrow) fused with the second annuli is regarded as a false annulus in eel no. 3. Scale bar = 1mm.

Results

Total length, body weight, ages and sexes of the eels among locations

All 48 eels collected in the coastal waters of the Baltic Sea were at the yellow eel stage as were the 49 eels collected at the Curonian Lagoon, with the exception of 1 silver eel (Table I). All 10 eels collected in the Lake Baluošai were migrating silver eels. All eels collected in the 3 sites were all females except one male in Lake Baluošai. There were no significant differences in mean length (F = 1.56, df = 2, p = 0.21) and weight (F = 1.54, df = 2, p = 0.22) among sampling locations. However, the mean ages of the eels from Lake Baluošai were significantly greater than those from the Curonian Lagoon and the Baltic coast (F = 84.8, df = 2, $p < 0.001$). This implies that the eel grows faster in the Baltic coasts and Curonian Lagoon than in Lake Baluošai.

Table 1. Biological characteristics (means ± SD) of the European eels collected from Lithuanian sites.

Sampling Location	Sampling period	Developmental stage	Sample size*	Mean (±SD) and range		
				Total length (cm)	Body weight (g)	Age (year)
Baltic coasts	June-September	Yellow eel	48	63.0 ± 7.3 (47.5 - 81.0)	582.4 ± 274.6 (180.0 - 1400.0)	11.0 ± 1.8 (8 - 16)
Curonian Lagoon	June-August	Yellow eel (except one silver eel)	50	66.3 ± 10.4 (49.0 - 92.0)	691.4 ± 441.7 (201.0 - 2126.0)	10.8 ± 1.7 (6 - 15)
Lake Baluošai	April	Silver eel	10	64.7 ± 11.0 (43.3 - 80.0)	519.9 ± 266.2 (127.0 - 930.0)	19.0 ± 3.0 (15 - 24)

* All female except one male in Lake Baluošai

Life history scan of otolith Sr:Ca ratios

Sr:Ca ratios in eel otoliths increased from approximately 8 - 10 × 10^{-3} in the core to a peak of approximately 18 - 24 × 10^{-3} about 60 - 100 μm from the core. Otolith Sr:Ca ratios then sharply decreased (Figs. 3 - 6), which corresponds to the metamorphosis from leptocephalus to glass eel (Arai *et al*., 1997). Otolith Sr:Ca ratios before the elver stage were similar among individuals since the eels have similar migratory histories at the leptocephalus and glass eel stages. The patterns of otolith Sr:Ca ratios beyond the elver stage were variable, indicating diverse migratory histories during the yellow eel to silver eel stages. The migratory patterns of the eels were classified as follows:

Freshwater pattern: There were 16 eels (10 from Lake Baluošai and 6 from the Curonian Lagoon) which showed consistently low otolith Sr:Ca ratios from the elver to silver eel stage (Fig. 3). No eels collected in the Baltic Sea had a pattern of consistently low otolith Sr:Ca ratios. The mean otolith Sr:Ca ratio of these 16 eels after the elver stage was 0.72 ± 0.76 × 10^{-3}, which is consistent with previous studies on European and American eels (Tzeng *et al*., 1997; Cairns *et al*., 2004). This pattern suggested that these eels resided in freshwater from the elver to the yellow or silver eel stage.

Table 2. Migratory patterns of European eels as inferred from otolith Sr:Ca ratios. Freshwater residents, Sr:Ca ratios consistently $<2.24 \times 10^{-3}$; Seawater residents, Sr:Ca ratios consistent $>3.23 \times 10^{-3}$; Inter habitat shifters, Sr:Ca ratios covering the ranges of freshwater and seawater values.

Habitat	Sample size	Migratory patterns of the eels (%)		
		Freshwater residents	Seawater residents	Interhabitat shifters
Baltic coast	48	-	48	52
Curonian Lagoon	50	12	-	88
Lake Baluošai	10	100	-	-
Total	108	14.8	21.3	63.9

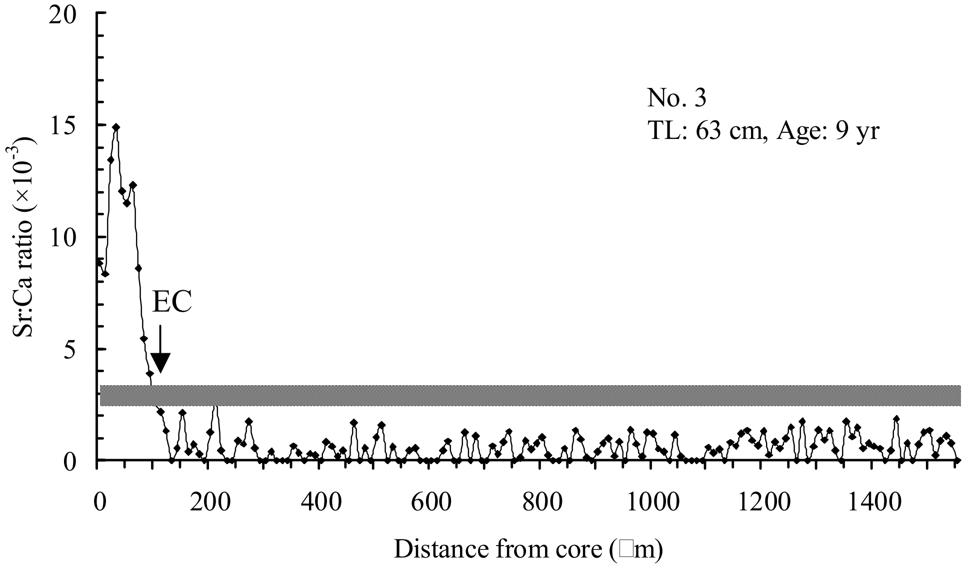


Fig. 3. Sr:Ca ratio transects from eel otolith core to the edge illustrating consistent freshwater residence after the glass eel stage. Eel was collected from the Curonian Lagoon.

Sea/brackish water pattern: Twenty-three eels (48%) collected in the Baltic coastal waters had otolith Sr:Ca ratios consistently above 3×10^{-3} between the elver check and the otolith edge (Fig. 4). The consistently high otolith Sr:Ca ratios suggested that these eels resided in brackish waters or seawaters without entering freshwater from the elver stage through the yellow eel stage. Eels with this kind of otolith Sr:Ca ratio pattern were considered to be sea/brackish water eels (Tsukamoto *et al.*, 1998; Tzeng *et al.*, 2000). The mean otolith Sr:Ca ratio from the elver check to the otolith edge of the sea/brackish water-resident eels was $4.84 \pm 1.61 \times 10^{-3}$, which was significantly higher than that of the freshwater-resident eels ($0.72 \pm 0.76 \times 10^{-3}$) (Fig.3). Therefore, the mean otolith Sr:Ca ratios of the eels collected in the freshwater Lake Baluošai and in the Baltic Sea were used as criteria to classify different migratory environmental histories of the eel. Eels with otolith Sr:Ca ratios lower than 2.24×10^{-3} (mean otolith Sr:Ca ratios of 16 freshwater eels + 2 SD) were considered as freshwater residents while eels with ratios larger than 3.23×10^{-3} (mean otolith Sr:Ca ratios of 23 sea/brackish water eels - 1 SD) were considered as sea/ brackish water residents. Intermediate values were regarded as transition between freshwater and sea waters. No eels collected in the Curonian Lagoon demonstrated the sea/brackish water pattern; all showed freshwater residency ($< 2.24 \times 10^{-3}$) for all or part of their life's span.

Some sea/brackish water-resident eels showed relatively high otolith Sr:Ca ratios between the elver and yellow eel stages and gradually decreased to lower otolith Sr:Ca ratios in the later part of the yellow stage (Fig. 4). For example, eel no. 34 had higher Sr:Ca ratios (5 - 12 × 10^{-3}) before 600 µm (age 5) and lower ratios (3 - 5 × 10^{-3}) between 700 and 1000 µm (age 6 - 10) (Fig. 4a). Eel no. 44 showed otolith Sr:Ca ratios that decreased from 6 - 10 × 10^{-3} at 350 µm (age 2) to 3 - 4 × 10^{-3} around 800 µm (age 8) (Fig. 4d). Decreasing trends of otolith Sr:Ca ratios were found in 20 sea/brackish water eels that indicated a habitat shift from high to low salinity by these eels (Figs. 4, 5). The pooled profile of these 20 sea/brackish water-resident eels showed a relatively large mean otolith Sr:Ca ratio (5.51 ± 1.57 × 10^{-3}, range 4 - 8 × 10^{-3}, n = 1240) between 160 - 770 µm from the core and a small otolith Sr:Ca ratio (3.64 ± 1.10 × 10^{-3}, range 3 - 5 × 10^{-3}, n = 1119) between 780 - 1500 µm (Fig. 7). The gradient of mean otolith Sr:Ca ratios in Fig. 7 may reflect the migratory history of the eel from the full-strength sea water in North Sea to the brackish waters in the southeastern Baltic coasts.

Table 3. Relative contribution of restocked and naturally recruited European eels in different habitats .

Habitat	Sample size	Origin of the eel (%)	
		Restocked	Naturally-recruited
Baltic coasts	48	2	98
Curonian Lagoon	50	20	80
Lake Baluošai	10	100	-
Total	108	19	81

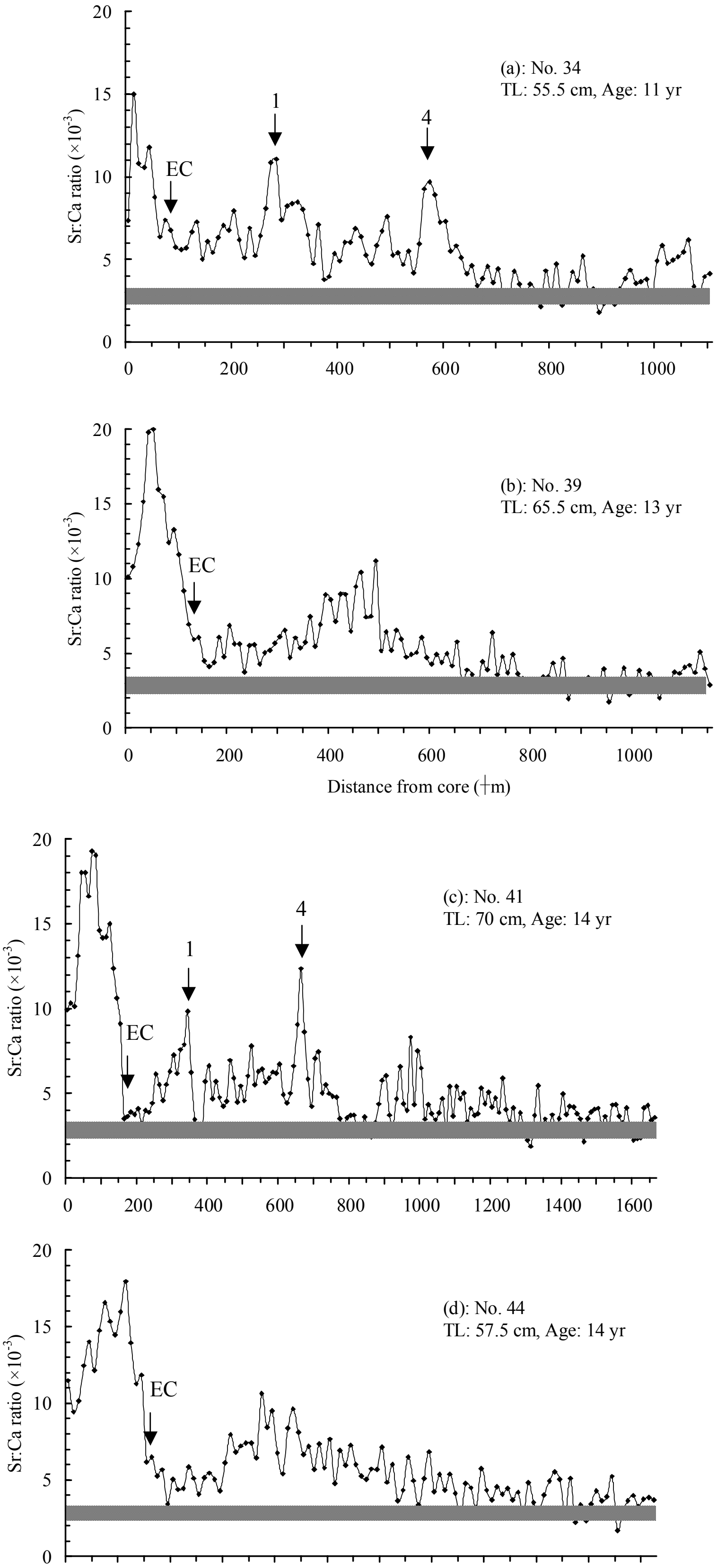
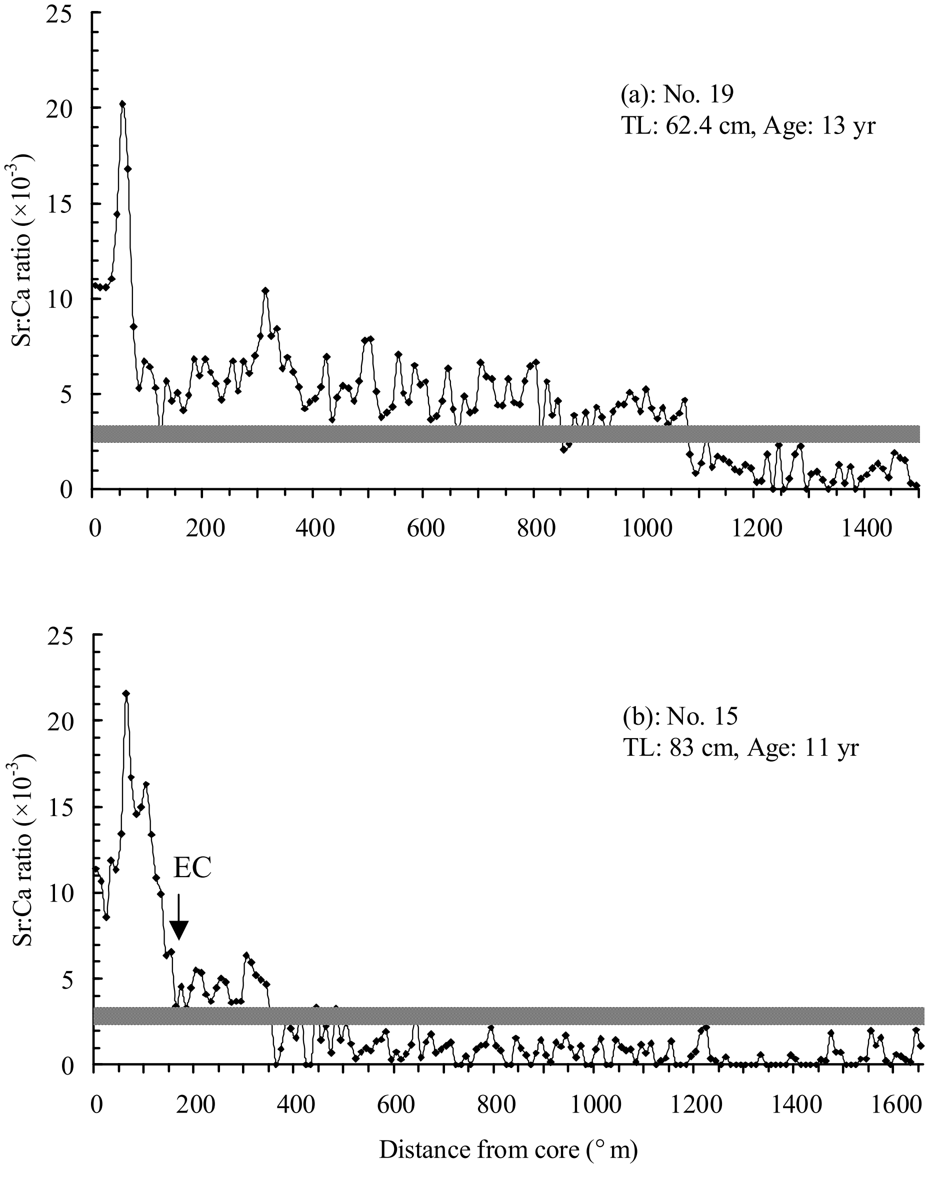


Fig. 4. Sr:Ca ratio transects from eel otolith cores to the edge illustrating consistent seawater residence after the glass eel stage. The arrows and numerals indicate the annuli corresponding to the peaks of Sr:Ca ratios. Eels were collected from the Baltic Sea.

Patterns of interhabitat shifters: Most eels caught in the Curonian Lagoon (88%) and half from the Baltic Sea (52%) migrated between habitats (Table II) i.e., their otolith Sr:Ca ratios fluctuated between freshwater and seawater levels. Most interhabitat shifters (eels migrating between fresh water and sea/brackish water) showed high otolith Sr:Ca ratios ($>3.23 \times 10^{-3}$) for several years after the elver stage that were then followed by relatively low ($< 2.24 \times 10^{-3}$) or fluctuating ratios between the freshwater and seawater levels (Fig. 5). Eel no. 1 resided in sea/brackish water for the first five years (otolith Sr:Ca ratios range: $4 - 12 \times 10^{-3}$) and invaded freshwater at age 5 (Fig. 5d). This eel showed seasonal peaks in otolith Sr:Ca ratios at each annulus, suggesting that it wintered in brackish water but spent the remainder of each year in fresh water (Tzeng *et al.*, 1997; 1999). The coincidence of otolith Sr:Ca ratio peak and annulus was also found in other eels e.g., no. 4, 34 and 41 (Figs. 4, 5). In light of the high plasticity of eel phenotypes, an explicit description or classification of the diversified migratory behaviors is not feasible and seems not necessary. Briefly, these varying patterns suggested that the eels recruited to freshwater and resided there until being caught or moved seasonally/irregularly between fresh water and sea water.



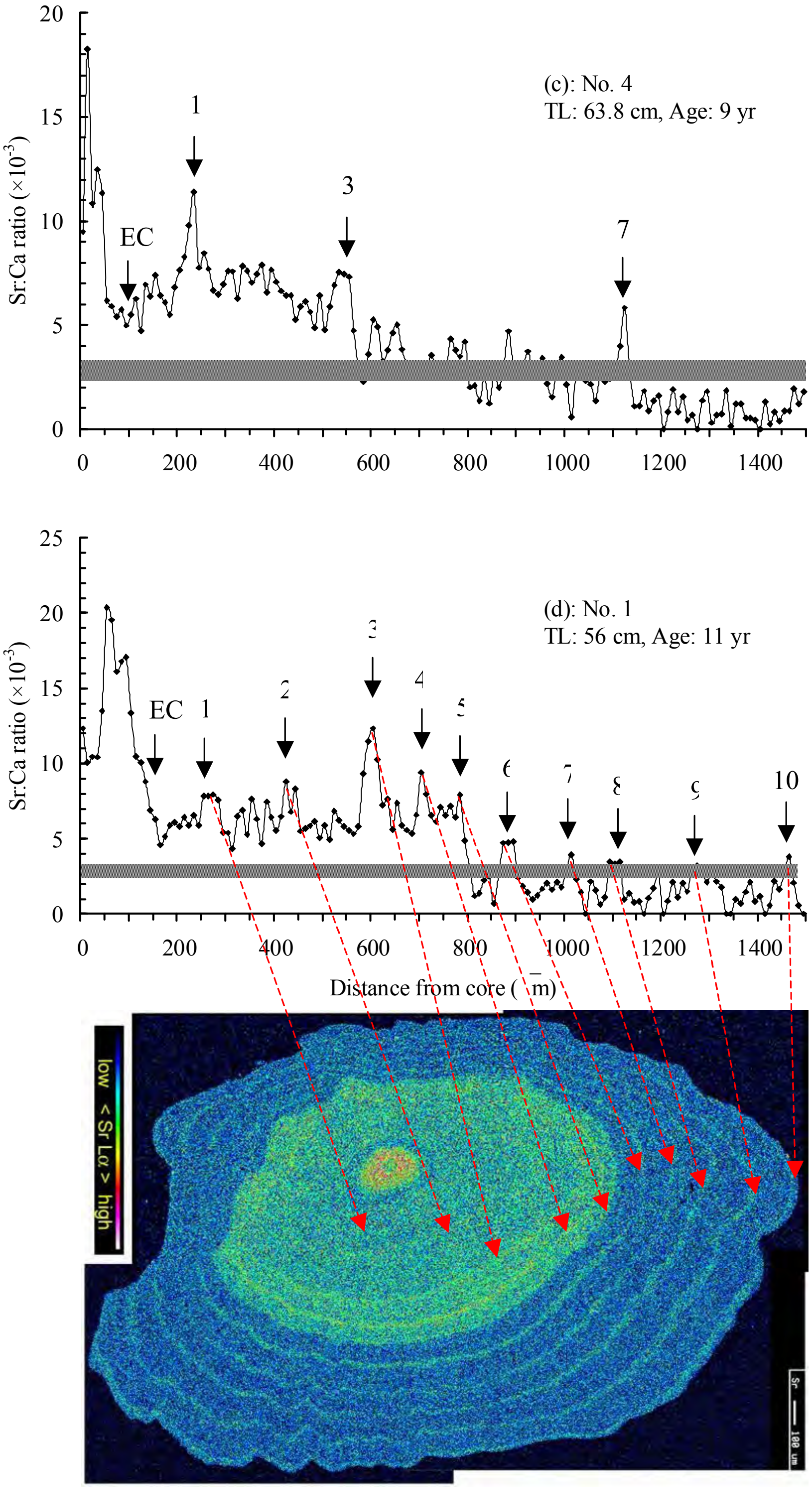


Fig. 5. Sr:Ca ratio transects from eel otolith cores to the edge illustrating eel movement into freshwaters after a period of seawater residence. Eel no. 4 demonstrate a gradual decline of Sr:Ca ratio profile corresponding to the movement from high salinity through low salinity to freshwater. Eel no. 1 shows seasonal migration between high salinity and low salinity or freshwaters. The arrows and numerals indicate the annuli corresponding to peaks in the Sr:Ca ratios. The X-ray intensity mapping display high Sr content from age 1- 5 and high Sr rings at annuli 6 to 10. Eels were collected from the Curonian lagoon.

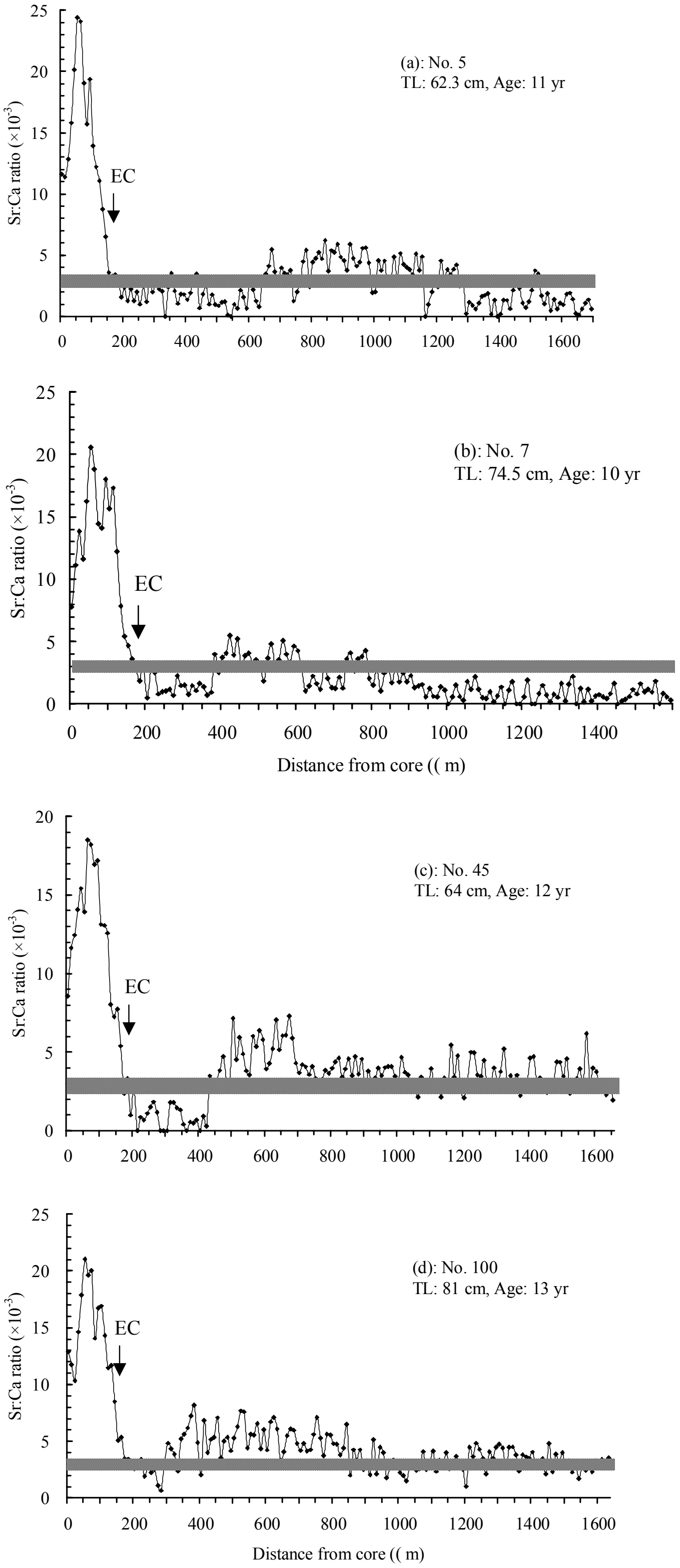


Fig. 6. Sr:Ca ratio transects from eel otolith cores to the edge illustrating rapid entry into fresh water at the elver stage then a return to seawater (eels no. 45 and 100) or movement between freshwater and seawater (eels no. 5 and 7). Eels no. 5 and 7 were collected from the Curonian Lagoon. Eels no. 45 and 100 were collected from the Baltic Sea.

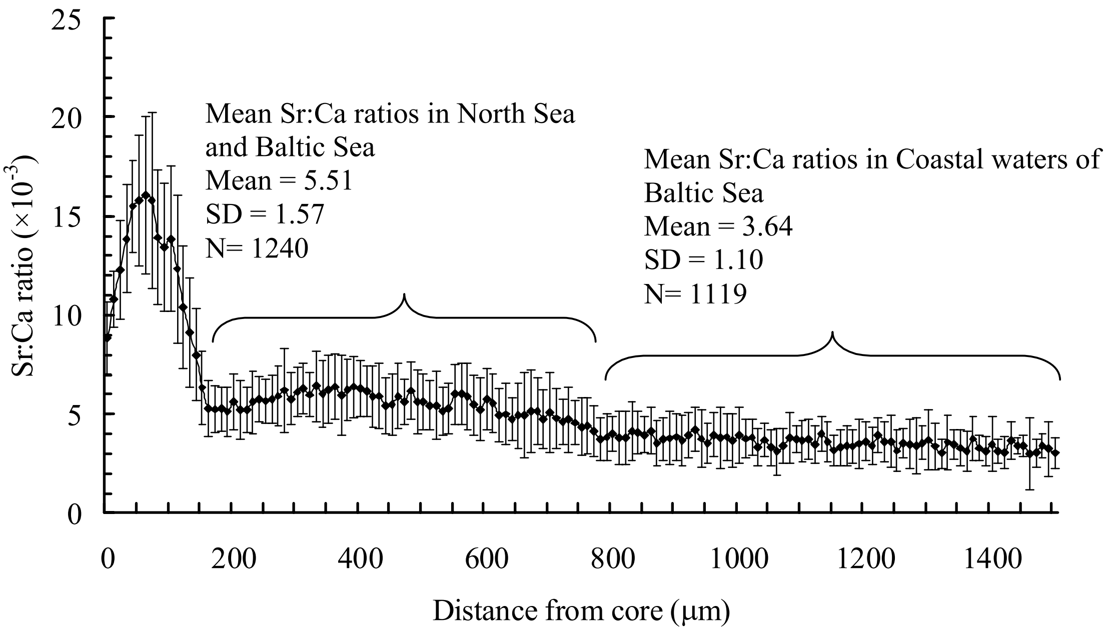


Fig. 7. The pooled otolith Sr:Ca ratio profile of 20 European eels that showed a gradual decline in Sr:Ca ratios during movement from the North Sea to the Baltic Sea. The 20 eels were collected in the coastal waters of the Baltic Sea.

Migratory patterns and origin of the eels among locations

The eels collected along the Baltic coast were either sea/brackish water-resident eels (48%) or interhabitat shifters (52%); none were freshwater eels (Table II). The eels collected from the Curonian Lagoon were primarily interhabitat shifters (88%) while freshwater-resident eels accounted for about 12% (Table II). Eels from the freshwater Lake Baluošai were all freshwater eels (Table II).

The presence or absence of an extended sea/brackish water signature (otolith Sr:Ca ratios $> 3.23 \times 10^{-3}$) after the glass eel stage was used to distinguish stocked eels from naturally recruited eels. The 6 eels from the Curonian Lagoon and 10 eels from Lake Baluošai showing a consistent freshwater signature throughout their life after the glass eel stage must be the stocked eels. Another 85 eels with extended large otolith Sr:Ca ratios after glass eel stage should be the naturally recruited eels if the stocked elvers have not migrated immediately to seawater.

In a few interhabitat shifters (N = 7), a period of low otolith Sr:Ca ratios ($< 2.24 \times 10^{-3}$) appeared before the extended high otolith Sr:Ca ratio ($>3.23 \times 10^{-3}$), making it difficult to tell whether these 7 eels were stocked eels or naturally recruited eels (Fig. 6). This may indicate that the eels had invaded freshwater at the elver stage for a period of time, then returned to sea/brackish water. Eels no. 5, 6 and 7 resided in fresh water for approximately 2 years, returned to sea/brackish waters for 1 - 4 years, and then moved back to freshwater (Fig. 6a, b). Eels no. 14, 45 and 100 resided in freshwater for approximately 1 - 4 years and subsequently returned to sea/brackish waters for their remaining life (Fig. 6c, d). Differences in their migratory histories were evident. Eels no. 45 and 100, collected on the Baltic coast, (Figs. 6c, d) showed relatively high otolith Sr:Ca ratios (400 - 700μm for eel no. 45 and 350 - 800μm for eel no. 100) at the early stage and a decreasing trend in otolith Sr:Ca ratio in the later stage, which is very similar to the pattern of naturally recruited eels. Therefore, eels no. 45 and 100 might be naturally recruited eels that have entered freshwater somewhere prior to entering Lithuanian waters. In contrast, eels no. 5, 6, 7, 14 collected in the Curonian Lagoon (Figs. 6a, b) and eel no. 60 from Baltic coast might be stocked eels that stayed in a freshwater for a few years then returned to the Baltic coast or migrated between both sites. This interpretation was based on otolith Sr:Ca ratios for the sea/brackish water signature that were smaller, intermittent or shorter than that for naturally recruited eels and that showed no decline in otolith Sr:Ca ratios. Overall, 87 individuals were naturally recruited eels while 21 individuals were stocked eels among the samples collected in the Curonian Lagoon (N = 10), Baltic coasts (N = 1) and Lake Baluošai (N = 10) (Table III). Stocked eels accounted for about 20% of the eels in the Curonian Lagoon and 2% on the Baltic coast; however, the eels in the Lake Baluošai were 100% of stocked origin (Table III).

The ages on arrival in Lithuanian waters

Sixty-three naturally recruited eels initially entered freshwater at ages of 1 to 10 yrs with a mean age of 5.2 ± 2.1 yrs (Fig. 8). This implies that after reaching the Baltic, the eels spent a number of years in marine/brackish waters before entering freshwater. Twenty-two of these 63 eels showed consistent low otolith Sr:Ca ratios after extended high Sr:Ca ratios, indicating that they continuously resided in fresh waters after freshwater entry (Figs. 5a, b). Mean age of these 22 eels at freshwater entry was 4.6 ± 2.2 yrs (Fig. 8b), not significantly different from the mean ages of the other 41 eels (5.6 ± 1.9) that entered fresh water but showed subsequent movements between freshwater and sea/brackish waters ($p = 0.16$, Mann-Whitney Rank Sum Test). This indicated these eels spent several years in the Baltic Sea then entered fresh water at the area of capture. After the initial freshwater entry, the eels spent 5 – 6 years (range: 1 - 10 years) in the Curonian Lagoon or Baltic coast before capture (Fig. 8).

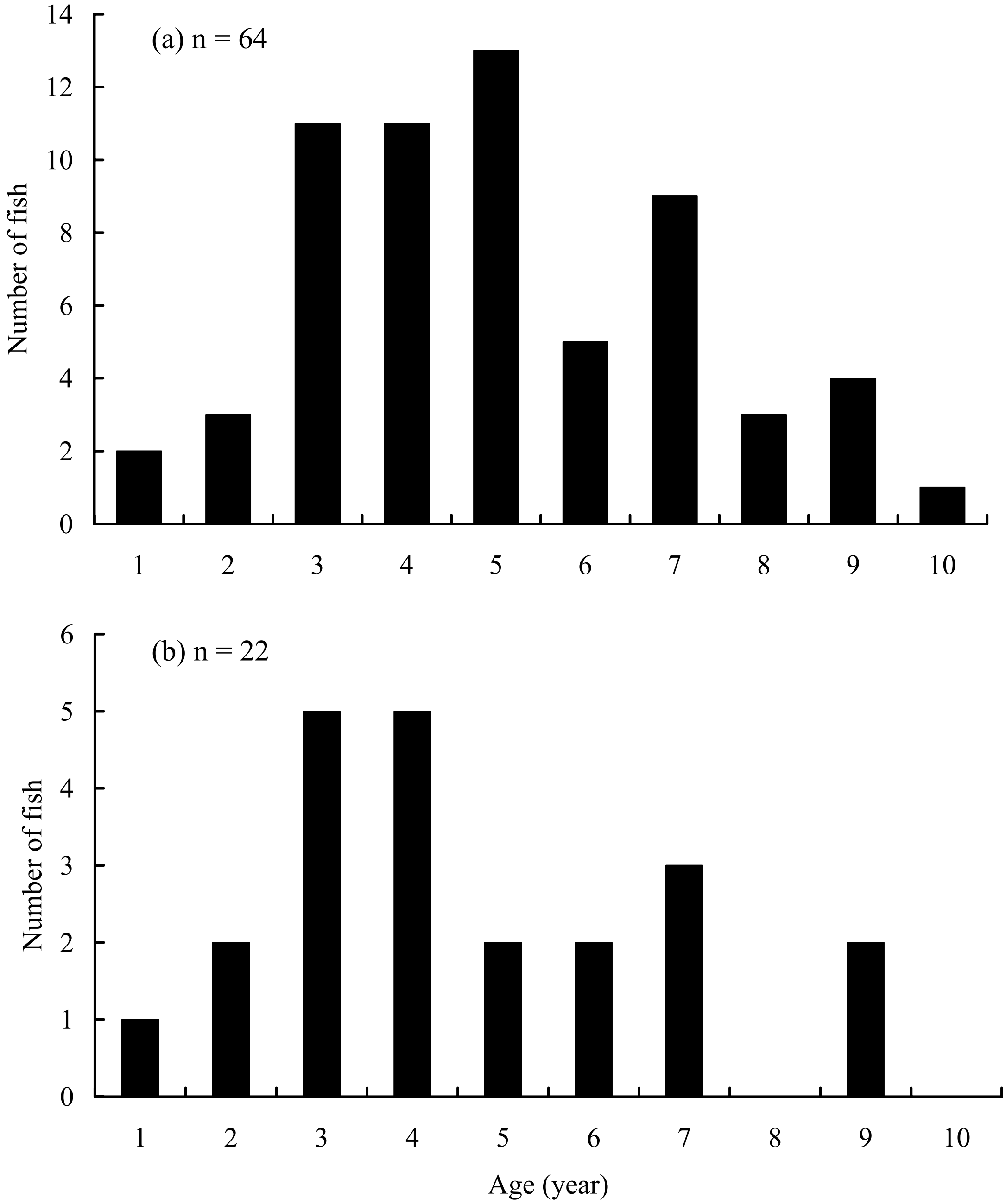


Fig. 8. Frequency distribution of ages of naturally recruited eels at initial entry into freshwater entry (panel a), Panel b, only naturally recruited eels continuously reside in Lithuanian freshwaters after initial entry.

Discussion

Interpretation of eel migration by otolith Sr:Ca ratios

Bath *et al.* (2000) and Kraus & Secor (2004) pointed out that it was Sr:Ca ratios in the water rather than salinity that primarily determined the incorporation of Sr into the otolith. This finding implied that otolith Sr:Ca ratios, as the proxy of salinity, should be interpreted based on knowledge of the ambient water chemistry. This is because fish living in Sr-rich fresh water may incorporate Sr at the same level or even higher than the fish living in normal sea/brackish water. However, Kraus & Secor (2004) also admitted that natural Sr-rich fresh water, if it can be found, is rarely seen. Generally, Sr is about 100-fold greater in seawater

(8.7×10^{-5} M) than in fresh water (9×10^{-7} M) (Campana, 1999). We measured more than 10-fold higher water Sr concentration or 7-fold higher water Sr:Ca ratios in the Baltic coast than in the Curonian Lagoon. Elsdon & Gillanders (2005) found significantly increased otolith Sr:Ca ratios by enhancing ambient water Sr:Ca ratios by 2 to 4 fold. Their experimental results justified the use of otolith Sr:Ca ratios in interpreting migratory history of the eels across different salinity environments.

Salinity decreases gradually from about 35 in the North Sea to about 15 in the southwestern Baltic Sea and to around 6 in the coastal waters of the southeastern Baltic Sea. The migration from a high salinity (North Sea) to low salinity environment (southeastern Baltic) was clearly imprinted in 20 naturally recruited eels with decreasing otolith Sr:Ca ratios (Fig. 7). Owing to the asymptotic relationship between otolith Sr:Ca ratios and salinity (Tzeng, 1996; Limburg *et al.*, 2003), there is limited ability to distinguish full-strength and half-strength sea water salinities by otolith Sr:Ca ratios. However, freshwater eels showed very low otolith Sr:Ca ratios, distinct from those in sea/brackish water eels. This result is consistent with the Zimmerman (2005) finding that salmonid otolith Sr:Ca ratios were linearly related to salinity but the sensitivity of otolith Sr:Ca ratios was only enough to discriminate between fresh water, brackish water and seawater.

Contribution of stocked and naturally recruited eels to the population

The composition of stocked and naturally recruited eels differed among locations. Based on the patterns of otolith Sr:Ca ratios, eels caught in Lake Baluošai are all of stocked origin, suggesting that few, if any naturally recruited eels reach this area and that eel fisheries in the inland lakes are all based on the stocked eels. Further studies with larger sample sizes are required to determine if there is any natural recruitment to the inland waters of eastern Lithuania. The proportions of stocked eels decreased to 20% and 2% in the Curonian Lagoon and Baltic coast, respectively (Table III). There was no evidence to suggest that stocked eels from inland lakes of eastern Lithuania will emigrate downriver and contribute to the eel stock in the Curonian Lagoon or the Baltic coast. It seems unlikely that stocked eels undergo the long migration, approximately 300 km, from Lake Baluošai or other lakes from the same water basin to the Curonian Lagoon during the yellow eel growth-phase. The eels caught in Lake Baluošai showed narrow otolith annuli (Fig. 2b), which is distinct from that of residents in the Curonian Lagoon and Baltic coast (Fig. 2a). The narrow annuli indicate a slow growth rate due to the limited prey available in Lake Baluošai, determined by higher eel population density based on catch-per–unit-effort in the stocked lakes of eastern Lithuania (L. Ložys, unpubl. data). The growth differences might also be determined by lower lake productivity and by feeding differences at the sampling sites. The Curonian Lagoon is eutrophic (Jašinskaitė, 1998) while Lake Baluošai is meso/oligothrophic (K. Arbačiauskas, pers. comm.). Eel dietary studies demonstrated that Lagoon and Baltic Sea eels eat a high proportion of fish while Lake Baluošai eels eat largely invertebrates (E. Bacevičius, pers. comm.). None of the 11 stocked eels found in the Curonian Lagoon and Baltic coast showed narrow otolith annuli, suggesting that few, if any, stocked eels migrate downriver to the Curonian Lagoon or Baltic coast waters until the spawning migration. A reasonable hypothesis is that the eel populations in inland lakes of eastern Lithuania and the Curonian Lagoon are independent during the growth phase. Energy costs, density-dependent migration and variable habitat quality could influence the geographic distribution as well as the migration of eels within the river (Feunteun *et al.*, 2003).

Some glass or small yellow eels have been released in Curonian Lagoon, but the stocking rate has been low: (only 1.7 eels ha^{-1} in 1995 - 2003). Only one stocked eel (no. 60) was found to have emigrated from the Curonian Lagoon to the nearby Baltic coast while 4 stocked eels (no. 5, 6, 7, 14) eventually returned to the Curonian Lagoon after short movement to the Baltic coast. This suggests that stocked eels prefer to settle in the location where they are released. Accordingly, 91% (n = 10) of the stocked eels (n = 11) remained in the Curonian Lagoon where they were released, assuming that no or very few eels had descended from lakes via the Nemunas River. In addition, about 9% (n = 1) of the stocked eels in the Curonian Lagoon emigrated to the Baltic coast and constituted about 2% of the local eel population.

Eels are important commercial and recreational species in central and eastern Europeand make important contributions to local and regional economies. Hence, the original aim of stocking programmes in the Lithuania and other Baltic countries was enhancement of inland fisheries. Intensive exploitation of the stocked eels presumably led to high fishing mortality and a low rate of escapement by silver eels. However, Limburg *et al*., (2003) found that 26.7% of the migrating silver eels at the Baltic Sea exit were of stocked origin, comparable to our finding that stocked eels account for 20% of the population in the Curonian Lagoon. Without related information on natural recruitment as well as the survival rate of the stocked eels, it is difficult for this first evaluation of the contribution of stocked eels to the naturally recruited population in the southeastern Baltic Sea to determine stocking effectiveness. However, it is possible that the stocked glass eels in Lithuania (e.g., stocked to the Curonian Lagoon) or other Baltic countries migrate to the Baltic Sea too soon after release to allow the freshwater signature to be recorded in the otolith. If so, the proportion of stocked eels in the Baltic Sea will be underestimated. Alternatively, most stocking programmes in the Baltic countries are focused on fisheries enhancement in inland lakes and quick migration over long distances to the Baltic Sea without creating a freshwater signature in the otoliths seems unlikely.

Sea/brackish water-resident eels accounted for only 23% of eels examined from Baltic coastal waters and the lagoon, while interhabitat shifters comprised approximately 70% of the eels. The proportion of naturally-recruited eels that have experienced fresh water is more than twice as high as found in the migrating silvers at the exit of the Baltic Sea (Limburg *et al*., 2003). If only the eels collected in the Lithuanian Baltic coast were considered, the proportion of the eels experiencing freshwater is still as high as 50%. The differences between these 2 independent studies may be due to the different geographical and habitat constraints or different behaviors of the eels. Long-distance migratory eels may be more active in exploring different habitats than their counterparts that settle down earlier. After entry into Baltic Sea, eels trapped in this closed system may explore optimal habitats at minimal energy cost to benefit maximal growth. Diversified habitats usually provide more foods and shelters than does a less diversified habitat, which presumably encourages euryhaline fish to explore different habitats. This may explain the flexible and complex migratory behavior of the eels, which reflects their environmental and evolutional adaptation.

Ages of eel on arrival in SE Baltic Sea

To our knowledge, this is the first study that estimates the ages of the eels arriving in the southeastern Baltic area. Most naturally-recruited eels showed an initial freshwater entry at age 1 - 10 years (5.2 ± 2.1 years). High variability in the age at initial freshwater entry indicates that some eels might migrate quickly through the Baltic Sea and into freshwater within one or a few years, while some eels showed very slow migration eastward. The broad ranges of age at initial freshwater entry also suggest a random distribution of the eels in the Baltic Sea rather than size or age dependent distribution. The eel density in the North Sea and Skagerrak/Kattegat Sounds may influence ages at arrival in the southeastern Baltic. Low eel density in the Baltic Sea due to low recruitment of young eels (Westin, 2003) may discourage eastward migration due to low intraspecific competition, so the eels arrive at older ages. It is known that populations in the lower reaches of rivers achieve high densities but as eels grow, relative biomass and hence competition for food and space increase. Agonistic encounters may then act as a stimulus for further upstream migration (Knights, 1987). Interestingly, on the Baltic coast of Denmark, at the monitoring site of eel recruitment at the Harte hydropower station, 50% of trapped eels were glass eels in the 1960s, while glass eels are rarely seen today. Thus, the mean size of recruiting eels has probably increased over the years due to the delayed recruiting process. In Vester Vedsted brook on the Danish North Sea coast, glass eels, elvers and yellow eels are found, however pigmented glass eels are most common at the lower part of the brook and are considered as new recruits (Pedersen, 2002). More to the south of the European coast, in the Netherlands, new recruits are partly but never fully pigmented glass eels (Dekker, 2002), while in coastal Germany at recruitment monitoring sites both true glass eel and fully pigmented elvers are found (Kuhlmann *et al*., 2002). In southwest Norway in the river Imsa, all sampled eels are fully pigmented elvers or young yellow eels that have stayed for one winter or more in marine or estuarine waters (Vøllestad, 2002). In Sweden, the catch in the River Viskan discharging to the North Sea consists mainly of elvers; however, at other rivers of the same coast age ranged from 0 to about 8 (Wickström, 2002). Overall, eels from rivers along the eastern coast of Sweden (Baltic Sea) are older than in rivers closer to the coast of Skagerrak/Kattegat sound (Wickström, 2002) i.e., the sound between the Baltic and North Seas. Hence, presumably eels to the southeastern Baltic should not be glass eels on arrival. Our observed arrival ages clearly support the hypothesis of eel recruitment to the southeastern Baltic at the yellow eel stage and explains why regional ichthyologists and managers were so uncertain about the contribution of natural recruitment, i.e., the absence of truly glass eels in the coastal waters led to hypotheses of natural recruitment weakness or even overall absence in the region.

Stocking programmes are a common and usually effective strategy to mitigate population decline, restore fisheries or to create new fisheries. The majority of stocked lakes in Latvia, Lithuania and Poland were almost devoid of eels before intensive stocking programmes began in the 1950s. The stocking programmes did create new eel fisheries in these inland lakes and also partially support the eel population in the Curonian Lagoon. However, many stocking programmes, including eel stocking, are carried out without evaluation of their effectiveness or actual success (Cowx, 1999). The use of otolith Sr:Ca ratios enabled this study to discriminate stocked eels from naturally recruited eels and to evaluate their contribution to the population. The understanding of population structure is fundamental for successful management and conservation.

Acknowledgements

We are grateful to M. Dragūnas, R. Rimkus and R. Repečka for assistance in sampling and measuring the eels and to B.M. Jessop and D. K. Cairns for constructive suggestions. Financial support was provided by the Ministry of Environment, Republic of Lithuania and the Lithuanian Fisheries Produces' Association for the field studies. The Lithuania-Latvia-Taiwan (Republic of China) Mutual Fund provided financial support for the otolith analysis (Contract No. NSC 93-2313-B-002-114).

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Appendix II

Effects of habitat and stocking programs on the growth rate of European eel *Anguilla anguilla*

Abstract

The European eel *Anguilla anguilla* is traditionally seen as a catadromous fish, growing up in freshwater, and spawning in the sea. Recently, this conventional view has been challenged by the finding of sea eels, inferred from otolith Sr:Ca ratios. These eels never invade freshwater, and complete their entire life history in the marine environment. The migration behavior of the eel is thus more complicated than originally thought. Meanwhile, the eel restocking program has been conducted in Lithuania in response to population declines occurred since 1980. To understand the effect of restocking and habitat on the growth of eel, otolith Sr:Ca ratios and ages were determined for 128 female eels collected from the Baltic area. Samples were from Baltic coastal waters (salinity 4.9 ~ 6.8 ppt, n = 47), an adjoining lagoon (salinity 0.03 ~ 1.6 ppt, n = 49) and two inland lake Baluošai Lake and Dringis Lake (salinity 0, n = 9 and 23) in Lithuania. The eels from coastal waters were all naturally recruited with different migratory histories; those in the lake had all been restocked, while those in lagoon comprised of naturally recruited and restocked ones. Length at age was back-calculated and compared among different habitats and between restocked and natural recruited eels. Mean age (± SD) of the eels was significantly greater in the lakes than in coastal waters and lagoon, (19 ± 3, 16 ± 3, 11 ± 2, 11 ± 2 years, respectively) ($p < 0.001$), but no significant differences in body length (64.7 ± 11.0, 62.6 ± 7.1, 66.5 ± 10.5 cm) and weight (519.9 ± 266.2, 571.8 ± 271.9, 693.4 ± 433.9 g) were found among samples in Baltic sea, lagoon and Baluošai Lake ($p > 0.05$) except significantly smaller individuals in Dringis Lake (52.6 ± 11.0 cm and 263.9 ± 112.9 g, $p < 0.05$). The mean growth rate, the slop of length-weight relationship back-calculated lengths were significantly different among habitats, whereas within habitats, these biological parameters were not significantly different among eels with different migratory environment histories ($p > 0.05$). Moreover for the eels collected from the Curonian Lagoon, the back-calculated lengths of the restocked eels grew faster than the naturally-recruited eels from age 5 ~ 8 ($p < 0.05$). Eels in the brackish Baltic Sea and the lagoon, mostly naturally recruited eels, were generally larger than the restocked eels from inland lakes, but the restocked eels in the lagoon were larger during a certain age range, which suggested that the growth rate of European eels in Lithuania was influenced both by habitats and by artificial transplantation.

Keywords: European eels, *Anguilla anguilla,* Sr:Ca ratios,Habitats, Growth.

Introduction:

Migration, either for spawning, feeding or over-wintering, is widely found in various fish species. Migratory fish usually benefit from many aspects such as escaping from predators and parasites in original habitat, benefiting by optimal physical conditions of new habitat, e.g. optimal temperature or water salinity and utilizing new resources in a newly exploited habitat with less competition. However, high energetic costs for migration may retard fish growth and the risks exposing to new predators during migration are the perceived drawbacks

(McKeown 1984, Gross 1987). For the diadromous fish, migration involves habitat-switches between fresh and saline water bodies. Energy requirement for environmental adaptation and osmo-regulation are also energetically highly demanding processes.

The facultatively catadromous European eel *Anguilla anguilla* spawn in the Sargasso Sea. After hatching, the leaf-like larvae migrate to the continental shelf, metamorphosing to glass eels, then either enter freshwater, reside at the coastal waters or migrate in between until sexually mature, then ends up its life after return to Sargasso Sea for spawning (Tsukamoto et al. 1998, Tzeng et al 1997, 2000, 2002, Tesch 2003).

The diversified habitat use of anguilid eels has received extensive attentions of ichthyologists and fisheries managers (ex: Shiao et al. 2003, Daverat et al. 2006). Most studies focused on how the environmental factors influence the eel growth and it is generally suggested that differences in environmental productivity and temperature might explain the growth differences of the eels inhabiting various habitats (ex. Jessop et al 2004). Acou et al. (2003) found eels (i.e. *A. Anguilla*) living in the brackish lagoon grew faster and matured earlier than those in the freshwater channel. They concluded that the growth differences were resulted by different growth potential provided in different environment, which was further supported by Daverat & Tomás (2006). For the American eel *A. rostrata*, eels with a history of primarily estuary residence have longer total length at migration and higher annual growth rates than primary freshwater residences (Jessop et al. 2004). The Japanese eel *A. japonica*, with residence in estuary also grew faster than in freshwater (Tzeng et al. 2003).

In addition, eel stocking programs have been extensively conducted in Europe for more than half century, but post-stocking concerns were mainly concentrated in stocking efficiency (ex: Feunteun 2002), dispersion of parasites (ex: Audenaert et al. 2003), bio-economical yield (Wickström et al. 1996), ecological consequences (Holmlund and Hammer 2004) or behavioral differences compared to naturally recruited eel (Westin 1998, 2003; Limburg et al. 2003). Little is known about the possible stocking effects on eel growth except Thorpe (2004) that indicated stocking program can retard or enhance fish growth. Intensive stocking programs have been undertaken in the Baltic Sea region over the past 50 years due to the poor natural recruitment over several decades (Svedäng 1996, ICES 2003). The majority of stocked lakes in Latvia, Lithuania and Poland were almost devoid of eels before intensive stocking programs began in the 1950s. Most intense stocking programs have been implemented in the area of the Baltic Sea drainage using eels originating from west Europe, in which eels were directly stocked to freshwater at the glass eel stage, but sometimes were on-grown in aquaculture for several months.

Shiao et al. (2006) found that naturally recruited European eels averagely spent 5 years (range 1 - 10 years) migrating from the eastern Atlantic coasts to the southeastern Baltic. However, the eels stocked in southeastern Baltic were artificially transported (mainly from France and United Kingdom) into waters where they were released and skipped the migration in the Baltic, a distance about at least about 1200 km (i.e. the distance between outer Skagerrak and Lithuanian coastal waters). For the naturally recruited eels, more energy allocation to migration will be expected since long-distance migration is energy consuming for the young eels passing through the Baltic while the restocked eels colonized in Baltic were without such energetic expenditure.

The objectives of this study are to evaluate (1) habitat effects on eel growth by comparing the eels inhabiting in different habitat with different productivity. (2) effects of migratory histories on eel growth by comparing different migrating contingents. In addition, based on the assumption that the stocked eels that save the energy in long-distance migration may benefit the growth, we further examined (3) stocking effects on eel growth by comparing the growth trajectory of naturally recruited and stocked eels.

Materials and Methods

Sampling

European eels *Anguilla anguilla* in yellow and silver-stage were collected in the Baltic Sea coastal waters, Curonian Lagoon, the inland Lake Baluošai and Lake Dringis in eastern Lithuania in 2003-2004 (Baltic Sea: June-September 2003; Curonian Lagoon: June-August 2003; Baluošai lake: April 2004; Dringis lake: 2004 August-September)(Fig. 1).The total length (TL) and weight (W) of each eel were measured to the nearest 1 mm and 1 g. Sexes of the eels were determined macroscopically from the gross morphology of the gonads, where eels with thin, regularly lobed organs (Syrski's organ) were considered males, while individuals with more broad and folded curtain-like gonads were females (Tesch 2003). The eels were classified as yellow and silver eels, by their external color, fin shape and eye size.

Fig. 1 Sampling locations (dots) in the Baltic Sea, Curonian Lagoon, Lake Baluošai and Lake Dringis.

Description of the sampling sites:

The Baltic Sea is the largest brackish water body in the world, with an area of 412,000 km^2. Surface salinity varies from less then 1 psu in the upper Bothnian bay to more then 34 psu in the outer Skagerrak. Water salinity in the Gulf of Bothnia and Gulf of Finland varies between 2-6 psu; Northern Baltic proper (including the Archipelago Sea, the Åland Sea and the Gulf of Riga) between 6-8 psu; Southern Baltic (including the Arkona Basin, the Bornholm Basin and the Gdansk Bay) salinity ranges between 7-10 psu; Western Baltic (German coast, the Belt area) between 7-10 ; Southern Kattegat 12-34 psu (Bonsdorff & Pearson 1999).

The salinity in the Lithuania coastal waters (depths to 20 m) of the Baltic Sea is influenced by inflow of fresh inland water from the Curonian Lagoon in some areas depending on wind direction and varies from about 1 psu in the area of inflow to 7 psu in some kilometers distance from the inflow area, which is typical salinity for Lithuania coastal and open sea waters (Vyšniauskas 2003). Water transparency (Secchi-disc depth) in the coastal waters varies from 1 m at the Klaipeda Strait where fresh inland water inflows to the sea to 14 m in open areas (Vyšniauskas & Lesys 1998).The Curonian Lagoon is 1584 km^2 in area, with a mean depth of 3.7 m and seasonal water transparency fluctuating in the range 0.35-2.0 m (I. Prochorova, pers. commun.); the Lagoon is separated by a narrow sand spit (0.5 – 4.0 km) from the Baltic Sea and is connected to it through the narrow Klaipėda Strait and is a fresh water basin, fluctuating from 0.03 psu, in the southern part of the Lagoon, up to 1.60 psu in the Klaipėda Strait. During stormy inflows of seawater the salinity may episodically increase up to 5-6 psu in the northern areas (Olenin 1996). The average water level in the Lagoon is 15 cm higher than sea level, thus the seawater penetration into the Lagoon is rare.

Lakes Dringis and Baluošai are located in the eastern part of Lithuania in about 300 km distance from the Curonian Lagoon and the Baltic Sea. Both lakes are of glacial origin. The area of Dringis Lake is 731 ha, with maximum depth 24 m, mean – 8.4 m, water transparency fluctuates from 1.8 to 6.0 m (Kavaliauskienė, 1996). Four streams inflows to the lake, while one flows out and connects the lake to some other lakes and Nemunas river basin which is the biggest Lithuania river and flows to the Curonian Lagoon.

The area of Baluošai Lakeis 250 ha, maximum depth is 37.5 m, average – 12.5 m, typical water transparency in summer is 2.8 m. Two streams inflow to the lake, while one flows out to the nearby bigger lake Asveja through which connects to Nemunas river basin which connects the lake to the Curonian Lagoon and Baltic Sea.

Differences in water temperature regimes at studied water bodies were evaluated comparing mean daily temperature measured in shore line three times per day in 2004. For estimation of inland lake temperature regime daily temperatures measured at Tauragnai lake were used. Hydrological features of Tauraganai Lake are similar to other lakes in the region. The Lake is in 10 km distance from Dringis lake, hence the water temperatures in shore line are very close to water temperatures in Dringis and Baluošai Lakes. Furthermore, it is known that stocked eels distribute through the stocked lake basin and eels caught in e.g. Baluošai Lake spent most their life indeed in another lake of the region.

The Curonian Lagoon was stocked by on-grown eels during 1996-1997 (43.000 individuals), 2000-2003 (10.000) and by glass eel in 1995 (150.000) and 2003 (60.000 individuals), and the Lake Baluošai and Dringis belong to the lake system of the Eastern Lithuania which have been regularly restocked by glass eels since 1960.. Even if only one lake in the region is stocked, eels spread to the nearby lakes through streams or small rivers. The natural recruitment to these lakes in eastern Lithuania is unknown, never observed or reported and may not occur, the rather that commercial landing reports did not mention eels before the lakes of the region were stocked with eels; however, the possibility could not be fully excluded.

Length-Weight relationship:

The length-weight relationship was represented by the formula:

$$BW = a \times BL^b$$

where BW denotes the body weight, and BL is the body length, *a* and *b* are constants which were derived by linear regression of the formula:

$$Log_e(BW) = Log_e\, a + b \times Log_e(BL)$$

a and *b* were then compared among habitats and between migratory histories to reveal the different environment or migratory effects acting on the eels. As suggested by Cone (1989, 1990), the condition factors were not calculated and used for violation of assumption for common slope of length-weight relationship.

Otolith preparation and Sr:Ca ratio measurement.

The largest (sagitta) otolith was removed, dried in the air, embedded in Epofix resin, ground and polished until the core was exposed. The polished otoliths were coated with carbon under a high-vacuum evaporator. Sr and Ca concentrations in the otolith were measured from the core to the edge of the otolith at intervals of 10 µm by Electron Probe Microanalyzer (EPMA, JEOL JXA-8900R). Quantitative analyses were conducted using beam conditions of 15 kV for the accelerating voltage, 3 nA for the beam current, and a 5 × 4 µm rectangular scanning beam. A synthesized aragonite ($CaCO_3$) and Strontianite ($[Sr_{0.95}Ca_{0.05}]CO_3$; NMNH R10065) were used as the standards to calibrate the concentration of Ca and Sr in the eel otolith. The Sr:Ca ratios were used for discrimination of the naturally-recruited and stocked eels (Shiao et al. 2006).

Classification of migratory types and restocked eels

The migratory patterns of the eels were classified according to Shiao et al (2006): (a) Seawater resident: otolith Sr:Ca ratios were consistently larger than 3×10^{-3} from the elver check to the otolith edge and (b) Freshwater resident type: otolith Sr:Ca ratios were consistently smaller than 2.24×10^{-3} from the elver check to the otolith edge. (c) Inter-habitat shifter: large otolith Sr:Ca ratios ($>3.23 \times 10^{-3}$) appeared for several years after the glass eel stage and followed by relatively small otolith Sr:Ca ratios ($< 2.24 \times 10^{-3}$) or the values fluctuating between freshwater and seawater range in the yellow eel stage. Moreover, the naturally-recruited and stocked eels were also distinguished by otolith Sr:Ca ratios profile according to Limburg (2003) and Shiao et al. (2006), that the Sr:Ca profiles of naturally recruited eels after elver stage were characterized by a period of salt-water experience followed by either freshwater entrance or remained in salt-water, while those of restocked ones were directly show freshwater signature (otolith Sr:Ca values $<2.24 \times 10^{-3}$) without saltwater experience after elver stage, which indicates the translocation from France to Lithuania.

Otolith measurement and back-calculation procedures.

After microchemical analysis, the otoliths were repolished to remove the carbon layer, then etched with 5% EDTA for 1 to 2 min to reveal the annual rings for age determination. The radius was measured by image software (SigmaScan Pro 5.0, SPSS Inc.) to nearest 0.001 mm along the longest line from the core to edge (Fig.2). The duration of the eel in freshwaters and seawaters was then estimated by relating the Sr:Ca ratios profile to the otolith annuli. The length at each age i (L_i) was estimated by Dahl-Lea method (Francis 1990):

$$L_i = L_c \times (R_i / R_c)$$

where L_c is the length at capture, R_i is the i^th otolith radius, and R_c is the otolith radius from core to the edge. The back-calculation was stared from the age 1 (the 2nd annuli), as suggested by Poole et al. (2004). The back-calculated lengths at each age i (L_i) would be compared between locations, between migratory groups, and between naturally-recruited and restocked eels

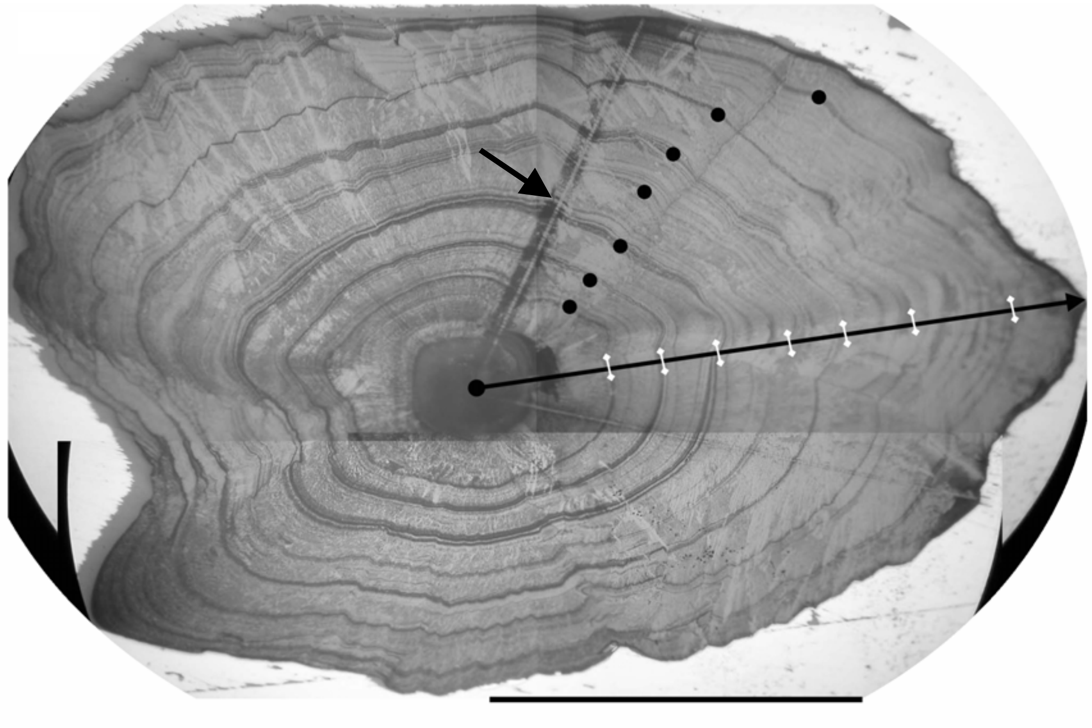

Fig. 2 Otolith of European eel *Anguilla anguilla* showing the annuli and measurement of the growth. The black arrow and black line indicate the tract where microchemical analysis and the radius measurement were conducted, respectively. White arrows on the black line denote where the annuli locate.

Data analysis

All numeral variables being compared were first tested for normal distribution and homogeneity of variances (Kolmogorov-Smirnov Test and Bartlett's Test, respectively). The variables did not meet the assumptions above would be log-transformed by $log_e(x+1)$ or otherwise be compared by non-parameter methods. The body length and weight were compared among locations by Analysis of Variance (ANOVA), and a multiple comparison (Student-Newman-Keul test, SNK test) was conducted to elucidate the differences among groups. The age, mean growth rate (MG), derived from body length divided by age, and back-calculated lengths at each age i (L_i) were compared by non-parametric method (Mann-Whitney Test and Kruskal-Wallis Test). The slope of the length-weight relationship was t-transformed by:

$$\frac{(\text{Slope of population1 - Slope of population 2})}{\text{Pooled standard error}}$$

and then compared by unpaired t test among habitats with a comparisonwise significant level $\alpha = 0.0167$. To evaluate the stocking effects, the back-calculated lengths of naturally-recruited eels between elver stage and initial freshwater entry were compared with the back-calculated length of the stocked eels at the same ages by Mann-Whitney U Test. All statistical procedures were conducted by SAS® (version 8.01), and significant level α was set at 0.05.

Results:

Comparisons of eel growth among habitats

131 eels were caught in Lithuania during 2003-2004 (Baltic Sea: June-September 2003; Curonian Lagoon: June-August 2003; Baluošai lake: April 2004; Dringis lake: 2004 August-

September), in which 48, 50, 10 and 23 eels were from Baltic coast, Curonian Lagoon, Lake Baluošai and Lake Dringis, respectively. Eels from Curonian Lagoon and Baltic coast were all yellow eels, while eels from Lake Baluošai were all silver eels. All eels collected were all females except one male caught in Lake Baluošai, thus the male was excluded from analyses and all comparison were done in females. The body length, weight, mean growth rate and age were significantly different (ANOVA and Kruskal-Wallis Test, $p < 0.01$) among locations, in which the eels from Lake Dringis were smaller with less weight and the eels from both inland lakes were older with slower growth rate averagely (Kruskal-Wallis Test, $p < 0.01$) (Table1).

The length-weight relationship was shown as Table1 and Fig.3. The slops of ln(BL) on ln(BW) are significantly different among locations, with highest value in eels from the Baltic Sea, middle value in the eels from the Curonian Lagoon and smallest values in the eels from both lakes (unpaired t test, $p < 0.0167$), which might suggest that the eels in the coasts obtain weight faster, than those in the lagoon and the eels from the lakes.

The growth patterns of eels among locations were revealed by plotting the back-calculated lengths to the age (Fig. 4a, b, c). It was shown the eels grew fast during age 2 to 10 and beyond that the growth rate slow down. Some individuals extend the fast growth for a longer period compared to others, resulting in the peak around age 13 - 22 (Fig. 4). This situation was found in the eels collected from almost all locations. Furthermore, the back-calculated lengths of the eels from Baltic coast and Curonian Lagoon were similar at all ages (Mann-Whitney Test, $p > 0.05$), but gradually exceed the length of the eels from both lakes, with significant differences between ages 7 - 13 (Kruskal-Wallis Test, $p < 0.05$). Moreover, the growth of the eels in both freshwater lakes were similar in first 4 years but eels from Lake Baluošai evidently grow fast than those from Lake Dringis between 4 - 13 years (Mann-Whitney U Test, $p < 0.05$).

Table1. Summary table of the European eels *Anguilla anguilla* collected from Lithuania (means ± SD). #: the number of eels. MG: mean growth rate in length (BL/age). Different letters indicated significant difference ($\alpha = 0.05$), and all comparisons were among female eels.

Sampling site	Baltic Sea	Curonian Lagoon	Lake Baluošai	Lake Dringis
Sample time	June-September	June-August	April	
#	48	50	10	23
Development stage	Yellow eel	Yellow eel except one silver eel	Silver eel	
Sex ratio (F:M)	48 : 0	50 : 0	9:1	
Total length (range, cm)	63.0 ± 7.3^{a} (47.5 ~ 81.0)	66.3 ± 10.4^{a} (49.0 ~ 92.0)	64.7 ± 11.0^{a} (43.3 ~ 80.0)	52.6 ± 11.0^{b} (38.7 ~ 69.5)
Total weight (range, g)	582.4 ± 274.6^{a} (180.0 - 1400.0)	691.4 ± 441.7^{a} (201.0 - 2126.0)	519.9 ± 266.2^{a} (127.0 - 930.0)	263.9 ± 112.9^{b} (78.0 - 560.0)
Age (range, year)	11.0 ± 1.8^{a} (8 ~ 16)	10.8 ± 1.7^{a} (6 ~ 15)	19.0 ± 3.0^{b} (15 ~ 24)	16.0 ± 2.6^{b} (12 ~ 22)
MG (cm/year)	5.9 ± 0.9^{a} (4.1 ~ 7.9)	6.3 ± 1.3^{a} (3.8 ~ 9.1)	3.6 ± 0.6^{b} (2.7 ~ 4.3)	3.3 ± 0.5^{b} (2.3 ~ 4.2)
Length-weight relationship	$BW = 2.1 \times 10^{-5} \times BL^{4.11}$ $R^{2} = 0.8951$	$BW = 1.3 \times 10^{-4} \times BL^{3.66}$ $R^{2} = 0.9581$	$BW = 4.6 \times 10^{-4} \times BL^{3.32}$ $R^{2} = 0.9538$	$BW = 4.5 \times 10^{-4} \times BL^{3.34}$ $R^{2} = 0.9469$
Power of Length-weight relationship	4.11^{a}	3.66^{b}	3.32^{c}	3.34^{c}
Migratory Patterns				
Migratory, # (%)	25 (52 %)	44 (88%)	0 (0 %)	0 (0 %)
Seawater, # (%)	23 (48 %)	0 (0 %)	0 (0 %)	0 (0 %)
Freshwater, # (%)	0 (0 %)	6 (12%)	10 (100%)	23 (100%)
Origin of eels				
Natural-recruited	47 (98 %)	40 (80 %)	0 (0 %)	0 (0%)
Restocked	1 (2 %)	10 (20 %)	10 (100%)	23 (100%)

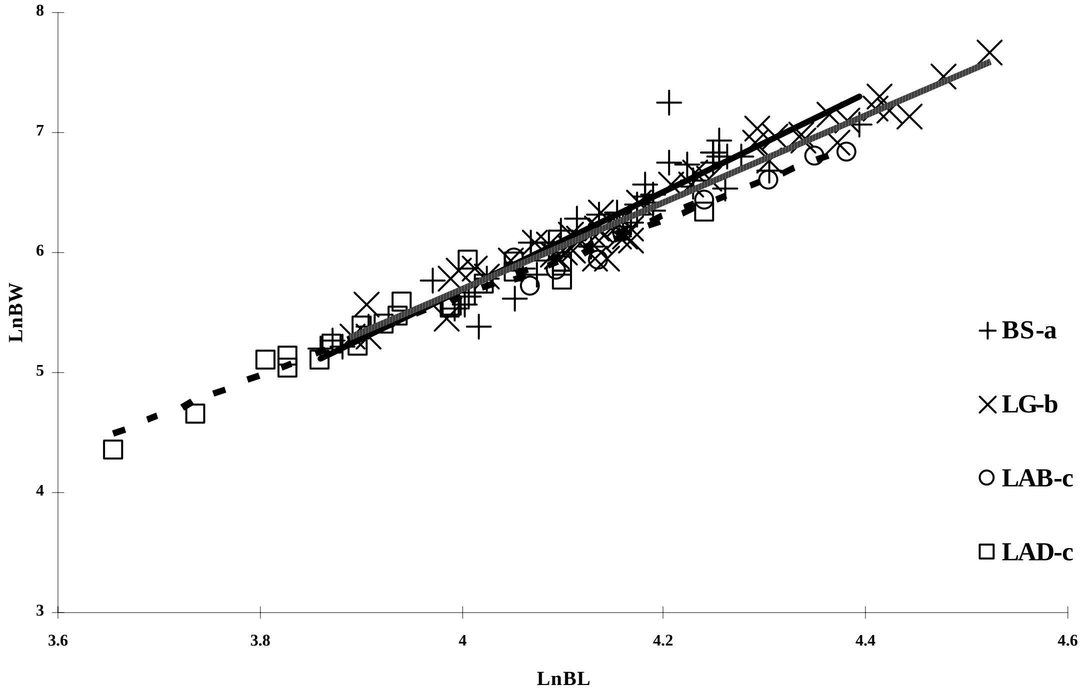

Fig. 3 Length-weight relationship of eels among the habitats. BS, LG, LAB and LAD represent eel caught from the Baltic Sea, Curonian Lagoon, Lake Baluošai and Lake Dringis, respectively. Different letters behind the abbreviation indicate significant difference in slope (unpaired t test, $p < 0.05$).

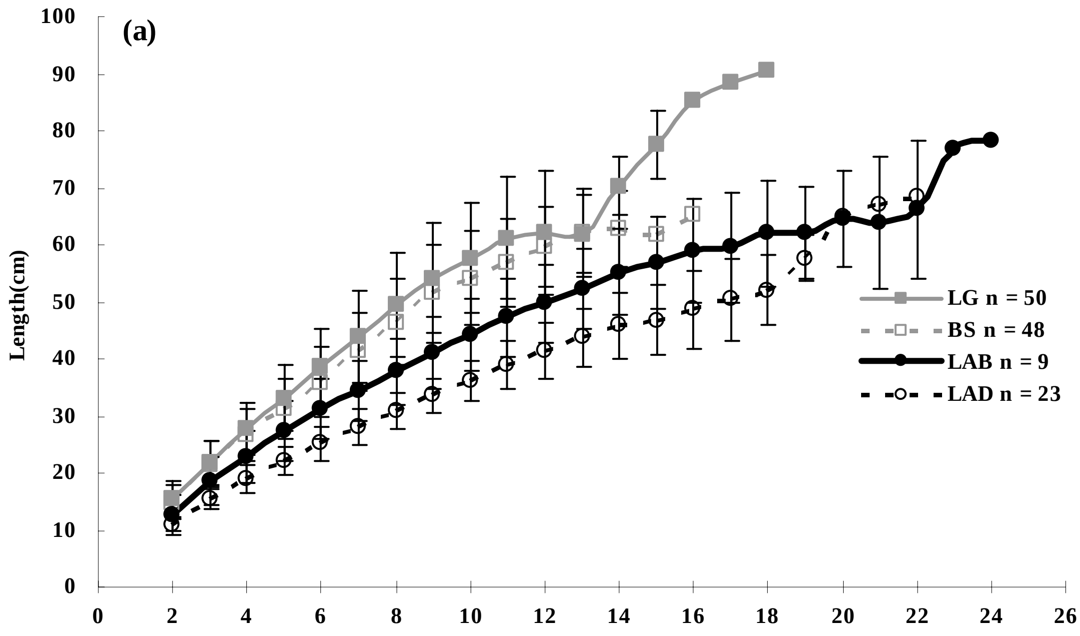

Fig. 4a

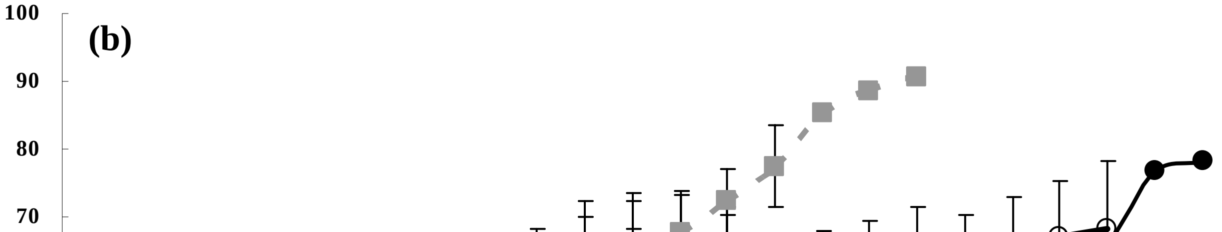

Fig. 4b

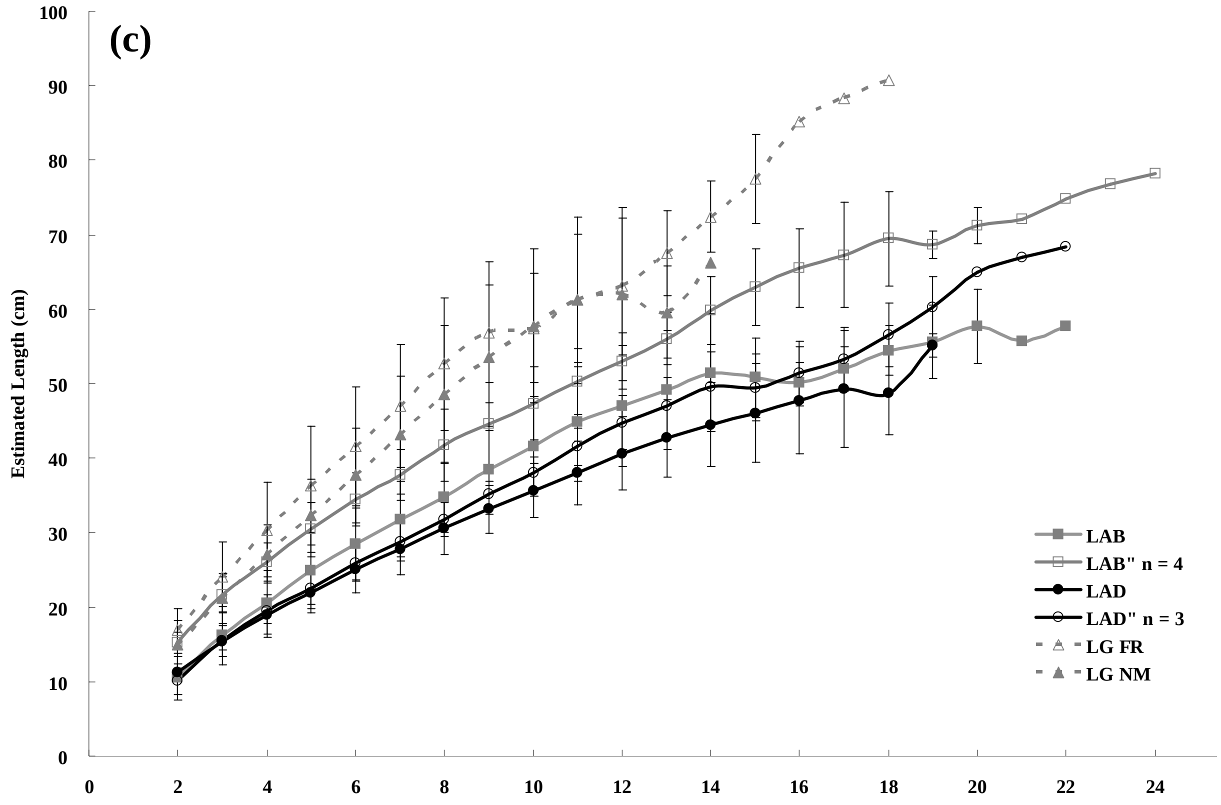
(c)
Estimated Length (cm)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
LAB
LAB" n = 4
LAD
LAD" n = 3
LG FR
LG NM

Fig. 4c

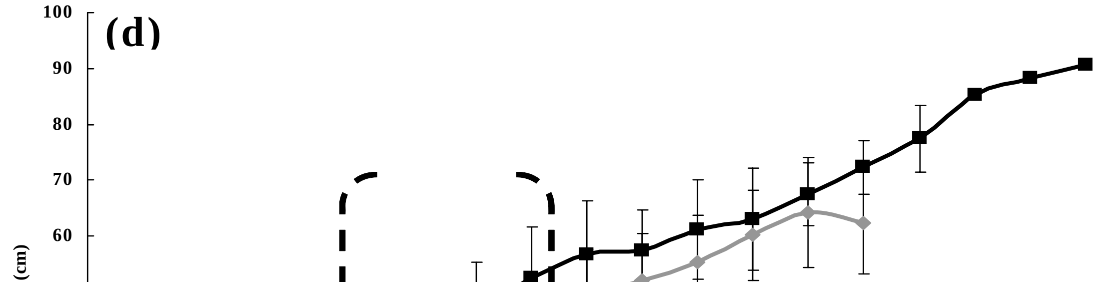
(d)
100
90
80
70
60
(cm)

Fig. 4 The back-calculated length-at-age at each age among (a) habitats (b) migratory types (c) Eels contributing the peaks in the length-at-age were separated and (d) between natural-recruited and restocked eels. The brackets include where the length-at-age were significantly different. BS, LG, LAB LA B", LA D and LAD" represent eel caught from the Baltic Sea, Curonian Lagoon, Lake Baluošai, exceptional large eels in Lake Baluošai, Lake Dringis and exceptional large eels in Lake Dringis, respectively.

Migratory type and restocked classification

It was found that eels collected along the Baltic coast were either Seawater residents (48%) or inter-habitat shifters (52%); none were freshwater eels. The eels from the Curonian Lagoon were mainly composed of inter-habitat shifters (88%) eels, while freshwater residents accounted for about 12%. The otolith Sr:Ca ratios in eels from the inland Lake Baluošai and Dringis all showed freshwater pattern. The eels in the Baltic Sea coasts and Curonian Lagoon were mostly naturally-recruited eels (98% and 80%, respectively), while the eels from both inland lakes were all stocked eels (100%) (Table 1).

Comparisons of eel growth based on the migratory history

The body length, weight, age, and mean growth rate (MG) were compared between different migratory groups of the eels from Baltic Sea coast and Curonian Lagoon, respectively. There were no significant differences found (ex.: length, weight, age, and mean growth rate) between different migratory groups (Mann-Whitney Test, $p > 0.05$, Table 2). Furthermore, the freshwater-residents in Curonian Lagoon seemed to be larger and had higher back-calculated length-at-ages than migratory- residents, but it was not significantly different at each age class between migratory groups, either in eels from Baltic Sea or Curonian Lagoon (Mann-Whitney Test, $p > 0.05$, Fig. 4b). It was found that the length-at-age peaked in their late life stage, in the lagoon, and both freshwater lakes (Fig. 4.a). Thus the eels contributed to these peaks were separated (Fig. 4c). For eels in the lagoon, the separated were all freshwater residents and with longer age, but no significant differences in the mean growth rate and back-calculated length-at-age in each age class (Mann-Whitney Test, $p > 0.05$). Similarly, the mean growth rates were not significantly different (Mann-Whitney Test, $p > 0.05$) between separated and remained eels, but the separated eels in the lake Baluošai were found with significantly larger length-at-age in the age range of 15 ~ 18 (Mann-Whitney Test, $p < 0.05$).

Table2. Comparisons (means ± SD) between different migratory groups of the European eels *A. anguilla* in different sites. MG: mean growth rate in length (BL/age). Eels from both lakes were all freshwater type so that they were dropped out. Comparison of variables between migratory group were all insignificant ($p > 0.05$).

Migratory group	Seawater type	Migratory type	Freshwater type
Baltic Sea			
Total length (range, cm)	62.7 ± 7.7 (48 ~ 81)	63.2 ± 7.5 (47.5 ~ 74.0)	-
Total weight (range, g)	533.3 ± 269.6 (195.0 ~ 1170.0)	632.6 ± 305.3 (180.0 ~ 1400.0)	-
Age (range, year)	10.9 ± 1.7 (8 - 14)	10.8 ± 1.8 (8 ~ 16)	-
MG (cm/year)	5.9 ± 0.9 (4.1 ~ 7.4)	6.0 ± 1.0 (4.1 ~ 7.9)	-
Curonian Lagoon			
Total length (range, cm)	-	69.4 ± 10.1 (60.5 ~ 92.0)	65.7 ± 10.6 (49.0 ~ 88.0)
Total weight (range, g)	-	804.5 ± 542.3 (382.0 ~ 2126.0)	655.6 ± 405.1 (201.0 ~ 1735.0)
Age (range, year)	-	10.9 ± 3.1 (9 ~ 18)	10.8 ± 1.6 (6 ~ 14)
MG (cm/year)	-	6.6 ± 1.1 (5.1 ~ 8.3)	6.2 ± 1.3 (3.8 ~ 9.1)

Table.3 Comparisons (means ± SD) between natural-recruited before entering to freshwater and restocked eels. FW: Freshwater. All were with no significant difference ($p > 0.05$).

	Natural-recruited eels	Restocked eels
Age before entering FW	5.2 ± 2.1 (1 ~ 10)	
Total length (range, cm)	64.2 ± 9.1 (47.5 ~ 88)	69.4 ± 10.1 (60.5 ~ 92.0)
Total weight (range, g)	621.4 ± 346.6 (180.0 ~ 1735.0)	804.5 ± 542.3 (382.0 ~ 2126.0)
Age (range, year)	10.8 ± 1.7 (6 ~ 16)	10.9 ± 3.1 (9 ~ 18)
MG (cm/year)	6.1 ±1.1 (3.8 ~ 9.1)	6.6 ± 1.1 (5.1 ~ 8.3)

Growth comparisons between naturally-recruited and restocked eels

Totally 90 naturally-recruited eels and 9 stocked eels were found in Baltic Sea and Curonian Lagoon. The body length, weight, age, and mean growth rate were not significantly different between naturally-recruited and stocked eels (Mann-Whitney U Test, $p > 0.05$, Table 3). However, the mean length-at-age of naturally-recruited eels before the first freshwater entry were significantly smaller than the stocked eels in Curonian Lagoon between the 5 - 8 year (Mann-Whitney Test, $p < 0.05$) (Fig. 4 d).

Discussion:

The identification of the migratory patterns, naturally-recruited eels and stocked eels were based on the study of Shiao et al. (2006). The growth difference of the eels from inland lake and the lagoon was also noticed in their paper, and thus this study further conduct explicit analysis on the growth of the eels either collected from different locations, habitats, or among different migratory histories and origins i.e., naturally-recruited and stocked eels:

Habitat effects

Either annual mean growth rate, the slope of length-weight relationship or back-calculated length-at-age all indicated that eels resided in the fresh water inland lake grow slower than the counter parts living in the Curonian Lagoon and Baltic coasts. This result supports the assumption of Shiao et al. (2006) that eutrophic water body and longer growing season in the Curonian Lagoon and the Baltic coasts will benefit the eel growth than that living in mesothrophic inland lake with shorter growing season. This observation is also consistent with previous studies in French waters e.g., Acou et al. (2003) and Daverat & Tomás (2006), that *A.anguilla* were found also with higher growth rate in estuary than freshwater. Morrison & Secor (2003) and Cairns et al. (2004) found that the growth rate of American eels *A. rostrata* caught in the brackish water was higher than that collected in upstream freshwater sites, and the estimated eel production was also higher in brackish habitats. On the other hand, *A. anguilla* glass eels live in saltwater were found to feed more intensely and grew faster than individuals live in freshwater (Edeline et al, 2005). This indicated a habitat-effect and locomotor activity on different behavior response and growth (Edeline & Elie 2004). The growth discrepancy between eels living in different habitats might reflect the difference in the individual behaviors, eel density, water temperature, food availability, changes of feeding preferences (Tesch 2003) and productivity of ambient environment (Gross 1987).

In the Curonian Lagoon, some freshwater eels (stocked eels) exhibited a faster growth rate after 12 years old. But, this was not observed in the naturally-recruited eels. The stocking activity may change the growth pattern of some eels. The growth curve peak among the lagoon eels was contributed by freshwater resident eels, which may be possibly due to the freshwater residents continue growing for a long period of time, while most naturally-recruited ones stopped growing after the 12th year. Besides, some individuals show a larger length-at-age throughout all the life span (Fig. 4c) and the peaks in the growth curve among the lake eels were contributed by some individuals that displaying larger length-at-age throughout all the life span (Fig. 4c). Lee (1982) indicated that some large eels experienced fast growth rate at younger stage could continue continental life for a longer period thereby attained a large size. The reasons of the occurrences of exceptional large eels were still unclear and perhaps were due to returning to sedentary stage, re-absorption of oocytes (Bezedenezhnykh 1973 in Moriarty & Hackett 1976) or some hierarchy effects found in eel aquaculture that some eels would display better growth condition irrespective similar food availability and outer environment compared to others.

Migratory history effects

At least three main migratory patterns i.e., seawater, freshwater residents and inter-habitat shifters were identified in the Curonian Lagoon and Baltic coasts. There is no growth differences among migratory groups were found although evident growth differences among different migratory eels were reported (ex. Jessop et al. 2002, 2004, Tzeng et al. 2003). This might suggest that the migratory history have very minor effects on the growth of the eels recruited to Lithuanian waters. For example, the salinity difference is much less between Baltic coast and Curonian Lagoon compared with the other studies in the river system connecting to the real ocean or the manipulating experiments in the lab (Edeline et al, 2005).

Stocking effects

The stocking programs seem to be beneficial for the growth of stocked eels. The length-at-age between 5 - 8 years were significantly larger in the stocked eels than the naturally-recruited eels although there is no difference found in body length, weight, age and mean growth rate. Some possible reasons are discussed as follows:

Environment factor: Temperature

Yearly water temperature in the inland freshwater lake (mean: 8.8 °C) was significantly lower than that in the Curonian Lagoon (mean: 9.6 °C) (student's t test, $p<0.05$), however did not differ ($p>0.05$) from that in the Baltic Sea (mean: 8.9°C) in 2004. Differences in water temperature between Curonian Lagoon and the Baltic Sea were significant ($p<0.05$). The physiological optimum for eel growth is found at 22 – 23 °C for yellow eels (Sadler, 1979) and glass eels (Elie & Daguzan 1976), however at temperatures < 10 °C eels become inactive (Tesch 2003) and as the result growth should stop. According to Elie & Daguzan (1976) from 10 °C and below, eel growth is inhibited, the individual activity and the alimentary taking are very low or non-existent; however, after temperature is elevated, from 11.5 °C, growth can start off again. In the Curonian Lagoon mean daily water temperatures > 10 °C were during 180 days (mean: 16.5 °C) in 2004, during 161 days in the coastal waters of the Baltic Sea (mean: 15.4 °C) and 157 days in the inland lake (mean: 16.2°C). The water temperature in periods >10 °C was significantly different ($p < 0.05$) in Curonian Lagoon than the Baltic Sea coast region, whereas that between the Baltic Sea and inland lake was not different ($p > 0.05$). By the viewpoint of water temperature, the Curonian Lagoon seems to be the best habitat for growth, followed by the inland lake, and the last one is the Baltic coastal region, which is contrary to observed growth phenomenon. Hence, it is indicated that the water temperature played a relatively minor role in the growth of the eels in Lithuania.

Stocking population density

Population density is one of factors influencing growth of all individuals in a population. Determination of growth rate of eels in a water body show that the slow growth of eels in natural waters results mainly from competition in a population that is too densely populated (Tesch 2003). Einsel (1961) found that eels stocked in the eel-free lake in Austria have reached longer body length in the 4th year compared to other natural populations, which might be due to less intra-species competition. But in Russian eels introduced in reservoirs with high density (200/ha) were smaller than in previous case in the 2nd year (Kokhnenko & Borovik 1958). The stocking rate is relatively lower: (only 1.7 ind.*ha^{-1} in 1995-2003) in the Curonian Lagoon, and this low population density may favor better growth condition for the eel growth. However, the stocked eels only displayed relatively higher growth rate at 5 - 8 year, suggesting other effects may also be involved

Environment factor: Salinity

Effect of water salinity on fishes is not well revealed. Some authors have demonstrated the positive influence of external salinity on growth capacities in other fish species (e.g. Tsintsadze 1991, Konstantinov & Martynova, 1993, Ložys 2004a). However, the effect of salinity on growth of freshwater species appears to vary among species. Specific growth rates for the channel catfish (*Ictalurus punctatus*) and goldfish (*Carassius auratus*) were highest in fresh water and decreased with increasing salinity, while within the same experiment three other species, i.e. rainbow trout (*Oncorhynchus mykiss*), striped bass (*Morone saxatilis*) and Gulf sturgeon (*Acipenser oxyrinchus desotoi*) grew faster in 3 and 9 psu than in lower salinities (Altinok & Grizzle, 2001). For eels, Tzeng et al. (2003) speculated that eels living in brackish waters may save more energy cost in osmo-regulation and resulting in better growth. In addition, Edeline et al (2005) also found for glass eels living in SW (salt water), the length started increasing earlier and they born higher growth rate than for those living in freshwater. Some studies (Pic, 1978; Hales et al., 1990; Mazik et al., 1991) suggest that isosmotic environment could be beneficial for both freshwater and marine fishes and fish growth should be improved in an isosmotic environment (Foss et al., 2001). However, recent ones indicate that the osmotic cost is not as high (roughly 10%) as this (Bœuf, 2001). Data are available in terms of food intake and food conversion stimulation, depending on the environmental salinity (e.g. Lambert et al., 1994; Conides et al., 1997; Altinok & Grizzle, 2001). Many hormones (e.g. growth hormone GH, insulin-like growth factor IGF1, Prolactin PRL, cortisol, thyroid hormones) are known to be active in both osmoregulation and growth regulation (e.g. Bern & Madsen, 1992; Bœuf, 1993; Sakamoto et al., 1993). It was found that GH cells were activated in eels acclimatized to brackish water (Olivereau & Ball, 1970). Pituitary gland explants from SW-adapted eels were shown to release less GH than those from FW-adapted specimens (Ball, 1981). However, the stocked eels living in the freshwater lagoon still grow fast, indicating that salinity does not overcome effect of other factors in this case. This is in contradiction with study done on another fish species *Perca fluviatilis* in freshwater Curonian Lagoon and brackish Baltic Sea coastal waters (Ložys 2004a). In this study low salt concentrations overcome other factors such as food and temperature and was crucial factor determining faster growth. Moreover, highly significant effect of brackish environment on perch growth was proven under experimental conditions. However, the fast growing eels in freshwater lagoon is in accordance with Tesch (2003), who refer to several studies when slower growth in coastal waters compare to freshwater was observed. (but the examples Tesch refered are old, about 1970~1980, and recently research ex Jessop 2004, Morrison and Secor 2002 has found that the eel in brackish water may have greater growth condition, thus here I may accept that the freshwater environment

Food availability and productivity of water sites

One of the parameters most well characterizing water body trophic status is the chlorophyll-a concentration in the phytoplankton, since it well reflects inflow of trophic elements into water body and is an indicator of primary production. According to data on chlorophyll-a concentrations in lake Dringis in 1988 provided by Kavaliauskienė (1996) and calculations of trophic index done as suggests Carlson (1977), trophic index of Dringis lake is 47, while trofic index of Baluošai lake in 2005 according to D. Kalytytė (pers. commun.) was calculated to be 39.8. According to Vaičiūtė (2004) trophic index of the Curonian Lagoon was on average 67 in 2004. The same index at the Baltic Sea according to the data on chlorophyll-a concentrations by Schrimpf et al. (2005) during the period 1998-2004 trophic index was rather stable, with values ranging from 38.7 to 39.6. However, Lithuania coastal waters as well as other coastal water areas of the Baltic Sea where eels spend most their lifes are under some influence of trophic elements inflow from inland waters and is higher than average trophic index values for the whole Baltic Sea. E.g. according to the chlophyll-a data provided by Marine Research Center, trophic index in the coastal waters was on average 47.5 in 2004. Hence, according to the Carlson (1977) clasification both lakes and Baltic Sea are mezotrophic water bodies, while Curonian Lagoon is hypertrophic.

Available information suggests that feeding conditions in the Curonian Lagoon may be better than those in the coastal waters. The abundance of fish juveniles in the lagoon is significantly higher (mean g per 100 m^2 ± SD: 4025 ± 572, dominant species: *Perca fluviatilis, Rutilus rutilus, Gobio gobio*, vs. 502 ±431, dominant species: *Osmerus eperlanus, Sprattus sprattus sprattus, Ammodytes tobianus*, $t = 11.8$, $p < 0.0001$; according to Repečka et al., 1996). Test fishing with 17-mm and 22-mm mesh size gill nets was conducted at both sites in 2000 and 2001. Significantly higher CPUE (catch per unit effort per 30 m of net per 8 h) was established in the Curonian Lagoon (CPUE: 5707 ± 2184 vs. 1123 ± 596, in g, $t = 7.01$, $p < 0.0001$; Ložys, unpubl. data). However, eels forage not only on small fish. Part or even all their diet can consist of different species of benthic invertebrates (Tesch, 2003). A zoobenthos study in the lagoon and the Lithuanian coastal zone down to the depth 40 m (Olenin, 1997), demonstrated that invertebrate biomass (molluscs excluded) was greater in the lagoon (Curonian Lagoon: 11.8 g m^{-2}; Baltic Sea: 5.5 g m $^{-2}$). Hence, the available information suggests that faster growth of eels in the Curonian Lagoon could be the resulted by higher food availability.

Genetic structure factors

The stocked eels were caught mainly from UK and French coasts then transported to Lithuanian. In one hand, Wirth & Bernatchez (2001) has demonstrated genetic differentiation occurred in European eels in different regions. In the other hand, Dannewitz et al. (2005) recently argued this approach and suggested that genetic variation in temporal samples European eel within sites clearly exceeds the geographical component, i.e. isolation-by-distance and supported the panmixia hypothesis. The question of the European eel genetic structure is still under the discussion and needs more studies to clarify this phenomenon. Consequently, there are no sufficient evidences to support the genetically driven growth difference in this study but the possibility can not be fully expelled.

Energy cost due to difference in migration distance

Comparing to naturally-recruited eels, the stocked eels colonized in the Curonian Lagoon without the extended migration from north Europe coast across the Baltic Sea. Bohlin et al (2001) has suggested energy cost of migration in trout could reduce fitness. According to evaluations done under experimental conditions, silver eel for migration use 10% of total energy allocations (fats) per 1000 km (Thillart et al. 2004). However, unlike migrating adults, eel juveniles or small yellow eels lack fat depots and other available stores of energy. Hence, during the migration, a regression in growth (Jellyman, 1977) and a reduction of caloric availability per eel (Tarr & Hill, 1978) occurs. Thus the longer migration of naturally-recruited eels have a negative effect on the growth due to the energy allocation to migration while stocked eels settling down at the elver stage have less energy spending in migration. Moreover, the mean age of naturally-recruited eels first entering to freshwater is 5.2 year (range: 1 – 10 yrs, Shiao et al. 2006), coincidental to the range where the significant differences in length-at-age were observed between naturally-recruited and stocked eels. The difference in energy cost due to migration may be accumulated from the elvers, and reached its maximum in about the 5th years when the eels arrived at Lithuania. The differences in length might be later replenished by compensation growth, as indicated by Graynoth & Taylor (2000), or also by the better energy transformation efficiency from feeding of naturally-recruited eels due to longer distant migration compared with stocked eels (Bernatchez & Dodson 1987).

Conclusion

The growth condition of European eels would be different among habitats, which eels caught in the inland freshwater lake grew slowly, and those caught in the lagoon and coast grew fast. Eels with different migratory histories were found no significant difference in growth, presumably due to connectivity, similarity and seasonal movement between the lagoon and the coast. Although in gross (??) the restocked were not different with naturally-recruited eels, but in the 5 - 8 year it was found the stocked eels were larger than the naturally-recruited eels. The difference during this period might result from different migration cost or different migration routes artificially induced due to stocking programs.

Acknowledgements

Temperature data for Curonian Lagoon and Baltic Sea were provided by Marine Research Center (Ministry of Environment); temperature records on Tauragnai Lake temperatures provided Lithuanian Hydrometeorological Service (Ministry of Environment). Financial support was provided by the Ministry of Environment, Republic of Lithuania and the Lithuanian Fisheries Produces’ Association and by the Lithuania-Latvia-Taiwan (Republic of China) Mutual Fund (Contract No. NSC 94-2313-B-002 -043) for the field studies and elemental analysis.

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2.3 DESCRIPTION OF GENETIC ANALYSIS RESULTS

2.3.1 Evaluation of genetic diversity of Perch (*Perca fluviatilis*) and Pikeperch (*Sander lucioperca*) populations inhabiting Lithuanian part of the Curonian Lagoon and costal zone of the Baltic Sea

Introduction

Many interpretations of phylogeographical and population genetic studies of fish are characterised by uncertainty arising from artificial stocking, intensive fishing or a lack of information about migrating behaviour of species of interest. Recent development of methods of investigation as the assay of microsatellites provides us with the opportunity to evaluate the intraspecific genetic variability in order to describe the genetic structure of population, establishment of existence of subpopulations reflecting the strategy of species adaptation that can be proved by the microsatellite analysis. Moreover, the new modern method of detection of the Sr/Ca ratio in the otoliths enable us to receive essential information about the seasonal habitat of commercially important fish species (Englbrecht et al. 2002, Swan et al. 2003, Campana and Thorrold 2001). Application of the above-mentioned modern methods provides researchers with tools for the evaluation of the condition of the populations of the endangered commercial fish species. Changes in the genetic structure of fish populations could be monitored on the basis of the data obtained by means of the genetic analysis.

The objective of this part of study is to carry out the investigation of the genetic variability of different populations of commercially important freshwater and diadromous-like behaving populations of pikeperch (*Sander lucioperca*) and perch (*Perca fluviatilis*) inhabiting Lithuanian part of the Curonian Lagoon on the basis of the data derived from the microsatellite assay.

The assay of microsatellite is carried out by the polymerase chain reaction (PCR) amplification of a specific microsatellite as defined by the unique primers. Usually microsatellite loci are detected by radiolabelling. The use of fluorescent detection may be either in a real time reading format as employed by systems such as the Li-Cor or PE Applied Biosystems fluorescent sequencer or delayed in which a conventional gel apparatus is used to separate PCR products and the gel is scanned following the completion of separation technology used by the Molecular Dynamics FluorImager. An alternative separation of amplified DNA fragments in a conventional electrophoresis apparatus followed by staining with etidium bromide and the evaluation of alleles by means of the computer program Total Lab v1.10 was applied in this study.

Material and methods

Blood and tissue samples of pikeperch and perch from different sites in the estuary of the Nemunas River, the Curonian Lagoon and inshore Lithuanian territorial water sites of the Baltic Sea were collected during the first stage of the study in June-August of 2004 (Fig. 1). Samples of DNA were extracted by using proteinase K and salt-extraction method (Aljanabi & Martinez, 1997).

Amplification of DNA fragments was carried out by means of microsatellite primers (Table 1.). The PCR has been performed in 25µl final volume containing the following: 100-200 ng of genomic DNA; 200 ng of a single primer; 0.75 unit of Taq DNA polymerase (MBI Fermentas, Lithuania) and 200µl each of dATP, dCTP, dGTP and dTTP. The reaction buffer contained 1.5 mM $MgCl_2$, 10 mM Tris-HCL pH 8.8 (at 20^0C), 50 mM KCL and 0.08% NP-40. Amplifications have been carried out in Ependorf Gradient Mastercycler (Eppendorf, Germany). Following the initial denaturation step at 95^0C for 2 min., the reaction was subject to 30 cycles of amplification denaturing at 94^0C (30 sec.), anealling at various temperatures depending on primer specificity (45 sec.), elongation at 72^0 C (45 sec.) and final elongation at 72 º C (5 min.).

Samples of the amplification products (15 µl) have been dissolved electrophoretically on 10% polyacrilamide gels in the TBE buffer for 4 hours. DNA fragments have been photographed, saved and analysed by means of MiniDoc^{TM} Documentation System (Herolab) and TotalLab V1.10 software.

Estimation of parameters of the genetic variability and differences between the populations were evaluated by standard measures with the help of computer programs TFPGA, FSTAT and GENEPOP.

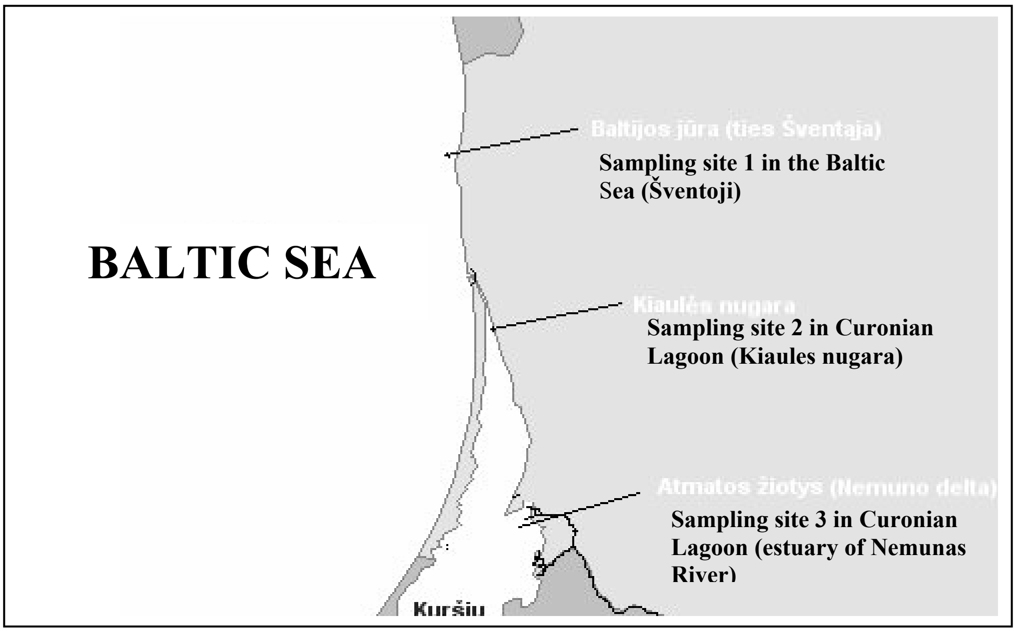

Figure 1. Sampling of material in estuary of Nemunas River, Curonian Lagoon and inshore Lithuanian territorial water sites of the Baltic Sea

Table 1. Specification of oligonucleotides used in the studies

Primer pairs	Sequences of oligonucleotides	Melting temperature
Svi-L2 (F) *Svi-L2 (R)*	5'–3' – TAAGACATAAACATACTCTG 5'–3' – TATACTGAGAGTGGAGACAT	51.8°C 56.4°C
Svi-L7(F) *Svi-L7 (R)*	5'–3' – GATGTGCATACATTTACTCC 5'–3' – GCTTTAATCTGCTGAGAAC	59.8°C 59.6°C
Svi-L8 (F) *Svi-L8 (R)*	5'–3' – GCTTATACGTCGTTCTTATG 5'–3' – ATGGAGAAGCAAGTTGAG	59.2°C 61.1°C
Svi-L10 (F) *Svi-L10 (R)*	5'–3' – GGTAATGTATTTTCAGTTATTGC 5'–3' – GCTGTTCTCCAAGTAAAGCC	60.5°C 65.6°C
Pfla-L1 (F) *Pfla-L1 (R)*	5'–3' – AAGCAGCCTGATTATATATC 5'–3' – CAGACAATTAAACATGCAAC	49.0°C 49.0°C
Pfla-L2 (F) *Pfla-L2 (R)*	5'–3' – GTAAAGGAGAAAGCCTTAAC 5'–3' – TAGCATGACTGGCAAATG	58.8°C 64.2°C
Pfla-L8 (F) *Pfla-L8 (R)*	5'–3' – GCCTTATTGTGTGACTTATCG 5'–3' – GGATCTTTCACTTTTTCTTTCAG	63.8°C 64.4°C
Pfla-L9 (F) *Pfla-L9 (R)*	5'–3' – GTTAGTGTGAAAGAAGCATCTGC 5'–3' – TGGGAAATGTGGTCAGCGGC	66.6°C 77.9°C

Results and discussion

Two sampling sites of perch (*Perca fluviatilis)* and two sampling sites of pikeperch (*Stizostedion lucioperca*) inhabiting different areas of the Curonian Lagoon and the Baltic Sea (sampling sites 1, 2 and 3, Figure 1.) were investigated during the second stage of the project.

Species specificity of the primers developed for taxonomically close fish species yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*) were investigated first of all (Table 2).

Table 2. Species specificity of primers set used in the study

Locus	Species used for primer development	Pikeperch-Curonian Lagoon (Kiaulės nugara)	Pikeperch-Curonian Lagoon (estuary of Nemunas river)	Perch (Baltic sea, Šventoji)	Perch (Curonian Lagoon, estuary of Nemunas river)
Pfla-L1	*Perca flavescens*	m	m	p	p
Pfla-L2	*Perca flavescens*	m	m	p	p
Pfla-L8	*Perca flavescens*	p	p	m	m
Pfla-L9	*Perca flavescens*	p	p	-	-
Svi-L2	*Stizostedion vitreum*	m	m	-	-
Svi-L7	*Stizostedion vitreum*	-	-	-	-
Svi-L8	*Stizostedion vitreum*	p	p	-	-
Svi-L10	*Stizostedion vitreum*	p	p	p	p

(m – monomorphic locus, p – polymorphic locus, - - no amplification).

Application of primer pair *Svi–L8* allowed amplification of polymorphic locus in all individuals of pikeperch (*Stizostedion lucioperca*) investigated in this study (Figure 2).

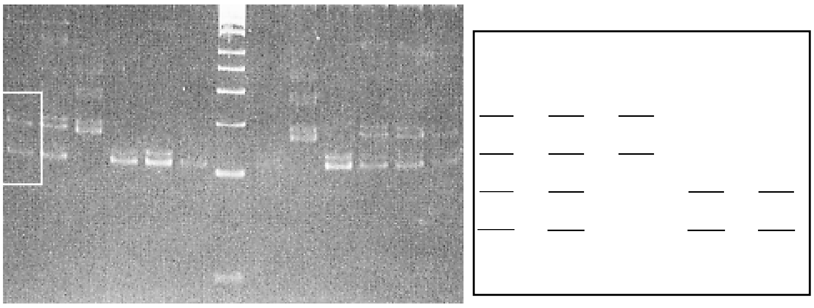

Figure 2. Schematically illustrated (right picture) fragments reflects spectrum of amplified DNA fragments (left picture, spectrum in white frame) by using pikeperch samples and primer pair *Svi – L8: 1 and 2 – heterozygous individuals, 3 –homozygous ind. (slow moving allele), 4 – 5 – homozygous ind. (fast moving allele), R –GeneRuler^{TM}50bp DNA Ladder, MBI Fermentas)*

Optimisation of PCR conditions for primer pairs Pfla-L8, Pfla-L9 and Svi-L10 also allowed us to detect separate polymorphic loci for pikeperch (Figure 3 and Figure 4). Number of alleles and allele sizes were identified by comparative mobility of amplified DNA fragments using TotalLab v1.10 software.

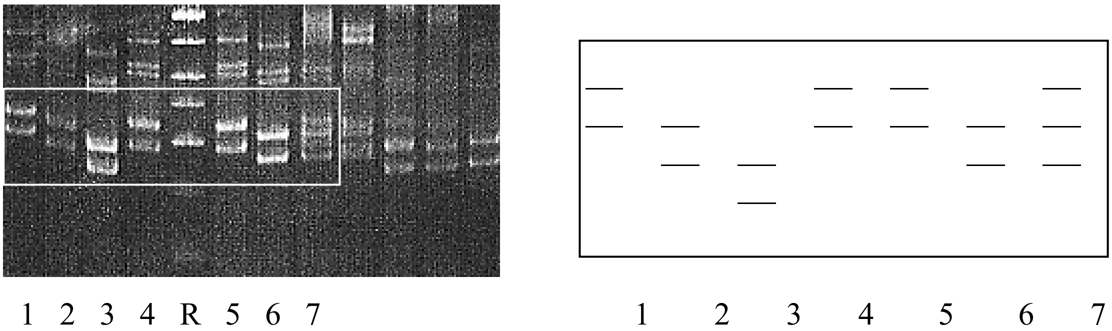

Figure 3. Schematically illustrated (right picture) fragments reflects spectrum of amplified DNA fragments (left picture, spectrum in white frame) by using pikeperch samples and primer pair Pfla–L8: lines *1,2,3,4,5 and 6 – homozygous individuals with alleles of three different length, 7 –heterozygous individual, R –GeneRuler^{TM}50bp DNA Ladder, MBI Fermentas)*

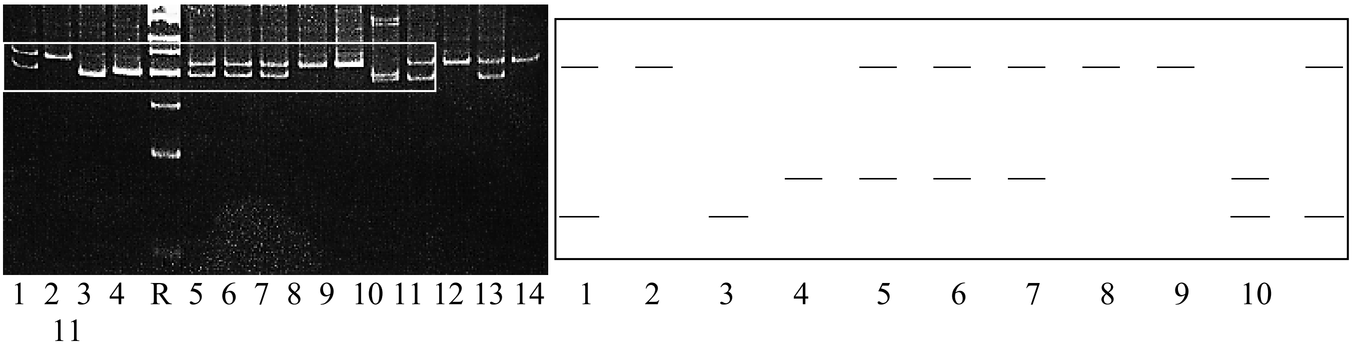

Figure 4. Schematically illustrated (right picture) fragments reflects spectrum of amplified DNA fragments (left picture, spectrum in white frame) by using pikeperch samples and primer pair Pfla–L9: *lines-1 and 11 – heterozygous individuals with slowest and most fast alleles;lines 2, 8 and 9 – homozygous individuals with slowest allele; line 3 - homozygous individual with most fast allele, line 4 - homozygous individual with intermediate allel; lines 5,6 and 7 – heterozygous individuals with intermediate and slowest alleles; R –GeneRuler^{TM}50bp DNA Ladder, MBI Fermentas)*

A total of four polymorphic loci with 2-4 alleles per locus and three monomorphic loci were amplified for all samples of pikeperch (Table 2). Following similar optimisation procedures the same primer set was screened for specificity of the perch DNA.

Contrary to pikeperch the same primer pair Pfla–L8 allowed us to amplify the monomorpic DNA fragment at locus *Pfla–L8* for all perch samples (Figure 5).

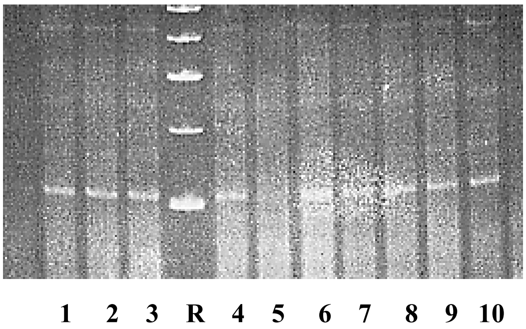

Figure 5. Spectrum of monomorphic DNA fragments amplified using perch samples and primer pair Pfla–L8: lines from *1 to 10 represent individuals from different sites, R – GeneRuler^{TM}50bp DNA Ladder, MBI Fermentas)*

Unlike the above-mentioned case, the primer pair Pfla-L2 allowed amplification of polymorphic loci using perch DNA samples (Figure 6) to be carried out when all individuals were monomorphic for pikeperch samples (data not shown).

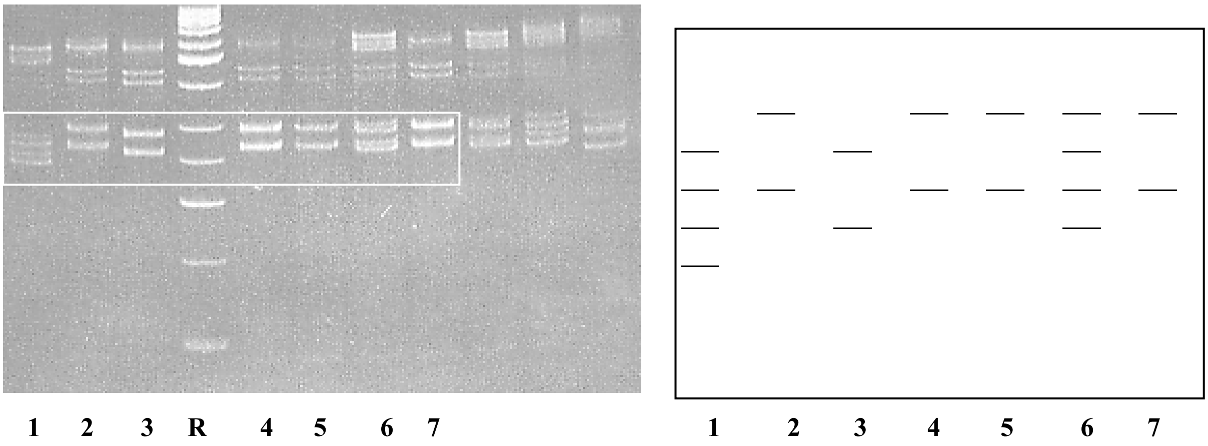

Figure 6. Schematically illustrated (right picture) fragments reflects spectrum of DNA fragments (left picture, spectrum in white frame) amplified using perch samples and primer pair Pfla–L2: *lines 1 and 6 – heterozygous individuals; lines 2, 4 5 and 7 – homozygous individuals with slowest allele; line 3 - homozygous individual with more fast moving allele,; R –GeneRuler^{TM}50bp DNA Ladder, MBI Fermentas)*

As it is common for microsatellites, we could confirm that in our case, on the basis of the amplified DNA spectrum analysis all polymorphic loci for fish species investigated are codominant features with both alleles being distinguishable in heterozygous individuals.

Four polymorphic loci were amplified for the pikeperch (*Pfla-L8, Pfla-L9, Svi-L8, Svi-L10*) and three for the perch (*Pfla-L1, Pfla-L2, Svi-L10*) out of 8 primer pairs investigated (Table 3). A further analysis was carried out using polymorphic loci only. A total of 66 individuals of perch and pikeperch from two different sites were genotyped after evaluating other comparative allele sizes. Allele frequencies were calculated using TFPGA software.

Table 3. Frequencies of alleles, expected (He) and observed (Ho) heterozygosity of perch and pikeperch samples

Locus	**Alleles (bp)**	Pikeperch Curonian Lagoon (Kiaulės nugara)	Curonian Lagoon (estuary of Nemunas river)	**Alleles (bp)**	Perch (Baltic sea)	(Curonian Lagoon)
Pfla-L1	**204**	1.000	1.000	**119**	0.5833	0.6000
				128	0.1667	0.4000
				143	0.2500	0.0000
Ho					0.8333	0.8000
He					0.6212	0.5333
Pfla-L2	**234**	1.000	1.000	**250**	0.0938	0.1000
				265	0.4688	0.5500
				271	0.4375	0.3500
Ho					0.3125	0.5500

He					0.5988	0.5650
Pfla-L8	**165**	0.1875	0.6875	**107**	m	m
	153	0.8125	0.3125			
Ho		0.3750	0.3750			
He		0.3250	0.4297			
Pfla-L9	**184**	-	-	-	-	-
	196					
	221					
Ho						
He						
Svi-L2	**238**	1.000	1.000	-	-	-
Svi-L8	**107**	0.6250	0.6071	-	-	-
	142	0.3750	0.3929			
Ho		0.5000	0.6429			
He		0.5000	0.4947			
Svi-L10	**230**	0.0625	0.0000	**172**	0.9286	0.9063
	236	0.5625	0.5714	**180**	0.0714	0.0938
	243	0.3750	0.4286			
Ho		0.3750	0.0000		0.1429	0.0625
He		0.5391	0.4898		0.1376	0.1699

- no amplification

Comparing products of amplification for the pikeperch and the perch differences in the allele sizes at the homologous loci between two fish species became evident (see Table 3).

The mean number of alleles per locus (A) was similar in both fish species - 2.66 and 2.33 in the perch and the pikeperch, respectively.

The genotype distribution corresponded to the Hardy-Weinberg equilibrium in the most loci except for *Pfla-L2* ir *Svi-L10* where a significant deviation ($p<0.05$) was detected in the perch population sampled in the Baltic Sea and in the pikeperch population sampled in the Curonian Lagoon, the estuary of the Nemunas River (Table 4), respectively. This deviation might be caused by insufficient sample size or the gene drift.

4. Lentelė. Significance values calculated for testing of deviation from Hardy-Weinberg equilibrium among fish samples investigated

	Significance values at different samples			
Loci	Pikeperch Curonian Lagoon (Kiaulės nugara)	Pikeperch Curonian Lagoon (estuary of Nemunas river)	Perch (Baltic Sea)	Perch (Curonian Lagoon)
Pfla-L1	-	-	0.6502	0.4270
Pfla-L2	-	-	**0.0262**	0.5317
Pfla-L8	1.0000	1.0000	**-**	-
Pfla-L9				
Svi-L2	-	-	-	-
Svi-L8	1.0000	0.3268	-	-
Svi-L10	0.4387	**0.0162**	1.0000	0.1009

A significant deficit or the excess of heterozygotes was not detected at any locus for both fish species investigated. The level of the genetic variability and observed heterozygosity calculated for both species were higher among the perch population as compared with the pikeperch population. Otherwise, in the pikeperch population observed heterozygosity was higher than expected contrary to the perch population (Table 5).

Table 5. Mean heterozygosity in pikeperch and perch populations

Species/Sampling sites	Mean heterozygosity	
	Observed	Expected
Pikeperch / Curonian Lagoon (Kiaulės nugara)	0.4167	0.4375
Pikeperch / Curonian Lagoon (estuary of Nemunas river)	0.3393	0.4655
Perch/(Baltic Sea)	0.4296	0.4274
Perch/(Curonian Lagoon)	0.4708	0.4294

On the basis of the data obtained (allele frequencies at microsatellite loci) the calculated Nei‘s (1972) genetic distance between two perch samples collected in the Curonial Lagoon and the Baltic Sea was 0.9623 and it was lower than the genetic distance (0.8461) between two pikeperch samples collected in different areas of the Curonian Lagoon.

Genetic differentiation between two samples of the same fish species was estimated by means of the Raimond and Rousset test (1995). Despite different salinity conditions typical of the sample collection sites both for the perch and the pikeperch significant genetic differentiation was not ascertained (p values of significance were 0.7614 and 0.1805 for the perch and the pikeperch, respectively). Thus investigated samples of the perch and the pikeperch could be considered as representatives of non-differentiated populations.

Insufficient differentiation of the perch and pikeperch samples was confirmed by theta P coefficient corresponding to the Wright‘s F_{ST} fixation index (coefficient of inbreeding). For the perch this coefficient was equal to 0.085 and for the pikeperch it was 0.0842. Using theta P values the number of migrants per generation (N_m = (1-theta P) / (4thetaP)) [DeSalle, Giribet, Wheeler, 2001] was calculated as the parameter, which could reflect a relatively high gene flow between the pikeperch populations (N_m = 2.69) and even a higher gene flow (N_m = 29.16) between the perch populations.

The Perch population of the Curonian Lagoon is characterized by higher heterozygosity as compared with the sample representing the Baltic Sea perch population. It might be caused by partial migration of individuals of a similar age composing a shoal of perch moving through the Klaipėda waterfront to more advantageous feeding areas spreading along the inshore. If this behavioural model is true then the perch population of the Curonian Lagoon should be characterized by higher heterozygosity as is confirmed by our calculations.

A further investigation of the genetic diversity of the perch and pikeperch populations involved additional sampling sites for both species making possible the comparison of samples representing a different year.

Application of the additional type of genetic markers as partial sequences of control region of mtDNA was successfully introduced in our laboratory. Now it became possible to compare two types of informative genetic markers – microsatellite and mtDNA analysis in attaining the objectives of the project.

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2.3.2 Investigation of Genetic Variability in the European Eel *Anguilla anguilla* (L.) in Lithuania Using Microsatellite DNA Markers

Introduction

The genus *Anguilla* comprises 15 species (Tzeng 2004; Watanabe *et al.* 2005) and belongs to the order *Anguilliformes* (Jing & LI 1999; Virbickas 2000). A complex life cycle is characteristic of *Anguilla sp.* (Ellerby *et al.* 2001b; Sasai *et al.* 2001; Tseng *et al.* 2003; Dannewitz *et al.* 2005), since these fish species have a few morphological forms, inhabit a different environment depending on their life stage or age (Poole & Reynolds 1998; Tzeng *et al.* 1999; Tsukamoto & Arai 2001; Powles & Warlen 2002;) and migrate over long distances (Virbickas 2000; Katoh & Kobayashi 2001; Okamura *et al.* 2002; Limburg *et al.* 2003; Bardonnet & Riera 2005) to their spawning grounds. No one knows exactly where the European eel's spawning ground is (Lokman & Young 2000; Ringuet *et al.* 2002). The smallest larvae found in the Sargasso Sea, south of Bermuda, suggest that spawning of the European eel as well as that of the American eel (*Anguilla rostrata*) occurs nearby (Wirth & Bernatchez 2001, 2003; Mank & Avise 2003; Versonnen *et al.* 2004; Dannewitz *et al.* 2005; Palstra *et al.* 2006). Meanwhile, the Japanese eel's spawning ground appears to be in the west of the Mariana Islands in the North Pacific (Katoh & Kobayashi 2001; Tsukamoto & Arai 2001; Ringuet *et al.* 2002; Tseng *et al.* 2003; Tsukamoto 2006, Nature). Species of the *Anguillidae* family, especially the European eel (Virbickas 2000; Hansen *et al.* 2001; Tzchori *et al.* 2004), the American eel and the Japanese eel (*Anguilla japonica*) (Katoh & Kobayashi 2001) are of great commercial interest (Jing & LI 1999; Tsukamoto & Arai 2001; Ringuet *et al.* 2002; Altun *et al.* 2005). Consequently, a decrease in European (Limburg *et al.* 2003; Dannewitz *et al.* 2005), American (Wirth & Bernatchez 2003) and Japanese eel (Tseng *et al.* 2003) catches during the past decades in Europe, America and Asia, respectively, has inspired many studies on physiological (Ellerby *et al.* 2001a, 2001b), ecological (Poole & Reynolds 1998; Tzeng *et al.* 1999; Okamura *et al.* 2002; Bardonnet & Riera 2005; Palstra *et al.* 2006) and genetic (Jing & LI 1999; Wirth & Bernatchez 2001, 2003; Mank & Avise 2003; Tseng *et al.* 2003; Dannewitz *et al.* 2005) aspects of eel biology and an increased concern over the eel restocking strategy (Ringuet *et al.* 2002). As an outcome of various studies, it has become clear that the decrease in stocks of all the three eel species during the past decades could have been caused by a number of factors: abiotic (Ringuet *et al.* 2002; Wirth & Bernatchez 2003), biotic (Kirk *et al.* 2002, Genç *et al.* 2005), anthropogenic, including intense stock exploitation, habitat loss (Katoh & Kobayashi 2001; Limburg *et al.* 2003; Palstra *et al.* 2006,), and maybe genetic factors (Wirth & Bernatchez 2001; Dannewitz *et al.* 2005; Laffaille *et al.* 2005). Despite various hypotheses the essential cause of eel stock decline remains indeterminate.

It is worth mentioning that up till now no *A. anguilla* studies seem to have been carried out to find out if naturally recruited European eels differ genetically from the introduced *A. anguilla* group within a particular region of the range. However, if true genetic differentiation in *A. anguilla* populations exists, eel restocking can have a negative effect on *A. anguilla* populations (Dannewitz *et al.* 2005). Therefore further investigations into *A. anguilla* population genetics are necessary.

The Baltic Sea and the Curonian Lagoon are the most important water bodies for commercial eel fishery in Lithuania. However, during the 1955-1975 period European eel landings in the Curonian Lagoon declined from 200-480 t per year to 27-87 t per year and did not increase during the past decades (Virbickas 2000). The decline in eel stocks and great commercial interest in Lithuania have boosted importation of glass eels from England and France (Shiao *et al.* 2006) and their release into Lithuanian water bodies, mostly lakes. Recent findings based on the European eel otolith microchemistry analysis suggest that eel fishery in the Curonian Lagoon mainly depends on naturally recruited eel catches (Shiao *et al.* 2006), since restocked eels accounted for only 20% of eels in the Curonian Lagoon and 2% in the Lithuanian coastal zone of the Baltic Sea. Therefore, Lithuanian fishery in the coastal waters and the Curonian Lagoon depends on natural recruitment primarily, while restocking is still not very essential. Furthermore, besides other eel restocking problems, such as spread of parasites or infections (Madsen *et al.* 2000; Hansen *et al.* 2001; Marco-Noales *et al.* 2001; Kirk *et al.* 2002; Shin 2004), eel restocking can modify genetic variability and even reduce reproductive success if genetic differentiation in *A. Anguilla* populations exists.

This study presents results of the initial investigation into the European eel population in Lithuania using microsatellite DNA markers. The study aimed at finding out if naturally recruited eels sampled in the Baltic Sea and the Curonian Lagoon differ from introduced eels sampled in Lake Dringis and Lake Siesartis genetically. Overall genetic differentiation among sampled eel populations from all the studied water bodies was evaluated too.

Material and Methods

Sampling

During the 2004-2006 period 60 European eels, of which 21 were silver eels and 39 yellow eels, were sampled from the Baltic Sea, the Curonian Lagoon, Lakes Dringis and Siesartis (Figure 1). 24, 8, 8 and 20 eels were taken from these locations, respectively. Samples from the Baltic Sea and the Curonian Lagoon predominantly represented the naturally recruited eel population, while fish from the lakes were eels introduced from Western Europe.

Figure 1. Sampling locations of European eel in Lithuania.

Genotyping and data analysis

Genetic variation was estimated at 5 microsatellite loci: *Aro121*, *Aro063*, *Ang101*, *Ang151* and *Ang114* corresponding to the same set of primers used by Wirth et al. (Wirth and Bernatchez 2001). For that purpose, oligonucleotides AF237898, AF237899, AF237900, AF237901 and AF237902 synthesized by biometers.net GmbH (Table 1) were used respectively.

Table 1. Charakteristics of primers (F-forward and R-reverse primers).

Primers	Primers pairs sequences (5‘-3‘)	T_m, °C	Species of origin	Loci

AF237898	F: TTGGGAAGGTCATGGACGTG	73.8	*Anguilla rostrata*	*Aro121*
	R: CTAATAAATGTCTGGGTAGGC	61.7		
AF237899	F: CCAGATACCTTGACAACGGC	69.7	*Anguilla rostrata*	*Aro063*
	R: TCAAGAGCTTCCTGACCCTC	69		
AF237900	F: GAAAACAATCGGGTACCACAG	68.1	*Anguilla anguilla*	*Ang101*
	R: ACAGTCAGTCACAATGAGCC	66.3		
AF237901	F: GATCTGTGGAGAGATGTTGG	65.5	*Anguilla anguilla*	*Ang151*
	R: AGTAGCATGCCTAGAACTGG	63.8		
AF237902	F: CCTGTGAATCCAACAGGTGG	71	*Anguilla anguilla*	*Ang114*
	R: GGATAATGCGGCAGAGTTCCC	73.5		

DNA was extracted from the frozen- or ethanol-preserved muscle tissue using universal and rapid salt-extraction of genomic DNA for PCR-based analysis (Aljanabi & Martinez 1997). PCR was performed using the Eppendorf Mastercycle gradient PCR machine. PCR volume was 25 µl and consisted of: 5 µl of genomic DNA, the concentration of which was 50 µg/µl, 0.75 µl 1 U Taq Polymerase (MBI Fermentas), 2.5 µl PCR buffer, 2.5 µl $MgCl_2$, the concentration of which was 2.5 mM, 2.5 µl 2mM dNTP, 0.1 µM forward and reverse primers and water. Since different pairs of primers required different annealing temperatures, four PCR programmes (Table 2) were used.

Table 2. PCR conditions for the microsatellite assays.

Loci	Initial denaturation		Cycle denaturation		1 cycle annealing		12 cycle annealing		30 cycle annealing		Cycle elongation		Final elongation	
	°C	**Min**	**°C**	**Min**	**°C**	**Min**	**°C**	**Min**	**°C**	**Min**	**°C**	**Min**	**°C**	**Min**
Aro121	94	3	94	1	53	1	64	1	65	1	72	1	72	5
Aro063	94	3	94	1	57	1	68	1	69	1	72	1	72	5
Ang101	94	3	94	1	67	1	67	1	67	1	72	1	72	5
Ang151	94	3	94	1	53	1	64	1	65	1	72	1	72	5
Ang114	94	3	94	1	60	1	71	1	72	1	72	1	72	5

Procedure for the amplification of the loci *Aro121* and *Ang151* was as follows: initial denaturation for 3 min at 94 °C, every cycle denaturation for 1 min at 94 °C, first cycle annealing for 1 min at 53 °C, every cycle annealing (until cycle 13) for 1 min adding +1 °C for the next cycle, from cycle 13 to 30 annealing for 1 min at 65 °C, every cycle elongation for 1 min at 72 °C, ending with a 5 min final elongation step at 72 °C. PCR procedures for the amplification of the loci Aro063, Ang101 and Ang114 were the same as those applied for the loci Aro121 and Ang151. However, the annealing temperature for the amplification of the locus Ang101 was stable, annealing temperatures for the amplification of other loci differing. After amplification each PCR product was mixed with 4 µl of loading dye and 2 µl of sacharose and then loaded in 12 % polyacrylamide gel. Electrophoresis was carried out in the MAXI vertical dual plate electrophoresis unit, 20x20cm, for 4-5 hours at 200 V following staining with ethidium bromide solution for 15 min. Then gel was examined using the Herolab firm MiniDoc^{TM} system. The results were registered and documented.

Relative allele sizes at all loci were determined using the TotalLab v1. 10 programme (Amersham Biosciences). Allele frequencies, heterozigosity, HWE, F statistics and genetic distance were calculated using the TFPGA v1.3 programme.

Results

This study examined five microsatellite loci and established that relative allele sizes varied from 109 to 228 bp among all loci (Table 3). In total, 35 alleles were scored at 5 microsatellite loci, of which 8 were recorded at *Aro121*, 9 at *Aro063*, 4 at *Ang101*, 5 at *Ang151* and 9 at *Ang114*. The largest and the smallest allele number was 31 and 22 in the Baltic Sea and Lake Dringis populations, respectively. Two specific alleles were detected in the Curonian Lagoon population and one in each of the remaining populations.

Table 3. Allele frequencies in microsatellite loci in different sampled European eel populations.

Locus	Alleles	Allele frequencies			
		Baltic sea	Curonian lagoon	Lake Dringis	Lake Siesartis
Aro121	1	0.0833	0.0000	0.0000	0.1250
	2	0.1250	0.0625	0.0625	0.2500
	3	0.1875	0.3125	0.1250	0.1000
	4	0.1250	0.1875	0.1875	0.1750
	5	0.1875	0.1250	0.2500	0.1000
	6	0.1875	0.0625	0.1875	0.1750
	7	0.0833	0.1875	0.1250	0.0750
	8	0.0208	0.0625	0.0625	0.0000
Aro063	1	0.0000	0.0625	0.0000	0.0000
	2	0.0208	0.0625	0.0000	0.0250
	3	0.0208	0.0625	0.0000	0.0250
	4	0.0000	0.0000	0.1875	0.0000
	5	0.1875	0.3125	0.3750	0.3500
	6	0.6042	0.3750	0.2500	0.4000
	7	0.1458	0.0000	0.1875	0.1750
	8	0.0208	0.0000	0.0000	0.0250
	9	0.0000	0.1250	0.0000	0.0000
Ang101	1	0.2500	0.3750	0.3125	0.1750
	2	0.4167	0.4375	0.2500	0.2250
	3	0.2917	0.1875	0.4375	0.4250
	4	0.0417	0.0000	0.0000	0.1750
Ang151	1	0.0208	0.0000	0.0000	0.0250
	2	0.1458	0.0625	0.1875	0.0000
	3	0.5208	0.3125	0.3750	0.2250
	4	0.2917	0.5625	0.3750	0.7000
	5	0.0208	0.0625	0.0625	0.0500
Ang114	1	0.0208	0.1250	0.1250	0.0000
	2	0.1250	0.0625	0.0625	0.0250
	3	0.0417	0.0625	0.1250	0.0250
	4	0.2917	0.1250	0.6875	0.3750
	5	0.1875	0.1875	0.0000	0.2000
	6	0.2500	0.2500	0.0000	0.2250
	7	0.0625	0.1875	0.0000	0.1250
	8	0.0000	0.0000	0.0000	0.0250
	9	0.0208	0.0000	0.0000	0.0000

The observed and expected mean heterozygositiy values calculated for all sampled populations are presented in Table 4.

Table 4. Observed and expected heterozygosity values in sampled European eel populations.

Loci	**H_E**				**H_O**			
	Populations				**Populations**			
	Baltic sea	Curonian lagoon	Lake Dringis	Lake Siesartis	Baltic sea	Curonian lagoon	Lake Dringis	Lake Siesartis
Aro121	0.8670	0.8583	0.8833	0.8564	0.6250	0.5000	0.5000	0.5500
Aro063	0.5895	0.7833	0.7750	0.7026	0.3333	0.6250	0.5000	0.4000
Ang101	0.6915	0.6750	0.6917	0.7256	0.5000	0.3750	0.2500	0.4000
Ang151	0.6348	0.6167	0.7250	0.4679	0.2917	0.5000	0.8750	0.4500
Ang114	0.8121	0.8833	0.5250	0.7705	0.7083	0.8750	0.5000	0.6000
Mean heterozyg	0.7190	0.7633	0.7200	0.7046	0.4917	0.5750	0.5250	0.4800

A significant deviation from HWE at two different loci was detected in each investigated population, except the Curonian Lagoon population. The latter had only one significant deviation from HWE at the locus *Aro121*. Such two loci with deviations from HWE were characteristic of the group of naturally recruited eels and three loci were detected in the group of introduced European eels.

On the basis of the F_{IS} value (0.3084), which reflects the degree of inbreeding in a population, we drew the conclusion that non-random mating is characteristic of the European eel population in Lithuania represented by all individuals sampled in the sea and lakes in the course of this investigation (Table 5). This F_{IS} value can also reflect *A. anguilla* restocking nuances. Glass eels migrate from spawning sites in the Sargasso Sea to Europe in shoals, consisting of presumably akin individuals, and accumulate in river entries. There they are caught by fishermen and are later translocated upstream, into other river basins or even other countries within great distances.

Table 5. Evaluation of inbreeding (F_{IS}) and genetic differentiationand (F_{ST}) within naturally recruited and introduced European eel populations using F statistics

Populations of European eel	F_{IS}	F_{ST}
Naturally recruited	0.3046	0.0000
Introduced	0.3128	0.0238
Overall	0.3084	0.0228

Overall genetic differentiation among the sampled eel populations from all the studied water bodies was evaluated too. Since the F_{ST} value at all loci was 0.0228 and the p value was 0.0281, it seems that there is small but significant genetic differentiation among the 4 examined populations (Table 5). Similarly, small but significant genetic differentiation was detected within the naturally recruited and introduced European eel groups. Since the F_{ST} value calculated for the naturally recruited and introduced European eel groups was 0.000 and 0.0238, respectively, no genetic differentiation is likely to exist in the group of naturally recruited European eels. However, there is small but significant genetic differentiation within introduced fish groups. The lack of genetic differentiation within the group of naturally recruited European eels suggests that the Baltic Sea and the Curonian Lagoon samples could belong to a genetically uniform population.

One of the reasons determining a rather high calculated value of the inbreeding coefficient F_{IS} (0.3084) in the Lithuanian eel population, which is represented by the individuals caught in the Baltic Sea, the Curonian Lagoon, as well as Lakes Siesartis and Dringis, can be a decline in heterozygosity. The latter phenomenon can be explained by mating specificity and a non-random distribution of individuals at spawning sites that have an effect on the formation of rather homogeneous shoals of glass eels, consisting of individuals of similar genotypes. It might be suggested that the majority of naturally recruited eels reaching the Lithuanian coastal waters yearly belong to such a genetically uniform shoal. This particular model of spatial distribution of this species might be one of the main reasons causing the high F_{IS} value. Significant differentiation between most pairs of these populations was confirmed using the Raimond and Rousset test. Samples from the Curonian Lagoon and the Baltic Sea formed the only significantly non-differentiated ($p=0.1120 > 0.05$) pair among the investigated samples (Table 6). This fact means that they represent a single population consisting of presumably naturally recruited eels.

Table 6. Significance of genetic differentiation between pairs of populations (Raimond & Rousset test)

Populations	Coronian Lagoon	Baltic Sea	Lake Dringis	Lake Siesartis
Coronian Lagoon	-			
Baltic Sea	0.1120	-		
Lake Dringis	0.0243*	0.0153*	-	
Lake Siesartis	0.0140*	0.0022*	0.0008*	-

* - significant differentiation

It is known that the introduction of glass eels into Lakes Dringis and Siesartis was performed repeatedly over several years. Presumably, they were introduced from different countries or at least from different locations within the country. As a result, eel populations are likely to have different genetic pools in the lakes under study (p value was 0.0008 < 0.05, Raimond and Rousset test).

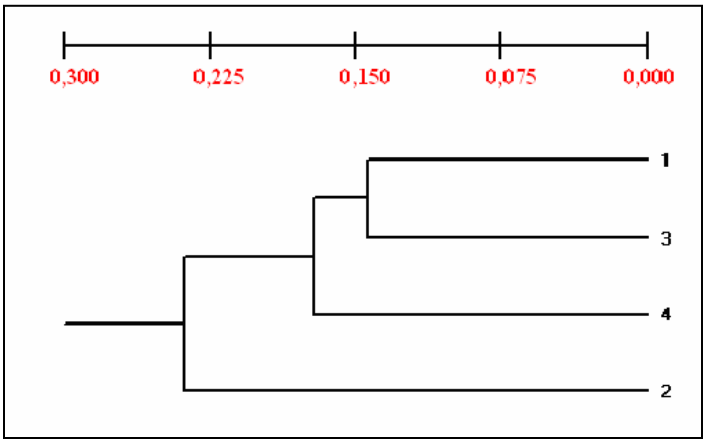

Figure 2. Dendrogram based on Nei (1972) genetic distances. Numbers 1, 2, 3 and 4 indicate Curonian lagoon, lake Dringis, Baltic sea and lake Siesartis populations, respectively.

The dendrogram based on standard Nei genetic distances (Figure 2) indicates that the Curonian Lagoon and the Baltic Sea samples form one cluster which also reflects the closest relations between these groups of European eels. What is more, these groups consisting of naturally recruited eels could be considered as subpopulations of a single population. The dendrogram, as well as the Raimond & Rousset test confirms our suggestion that glass eels introduced into Lake Dringis and Lake Siesartis were transported from different locations or countries in different time periods and, as a result, they exhibit higher genetic distances.

Discussion

During this study the genetic structure of the European eel populations sampled in the Baltic Sea, the Curonian Lagoon, Lake Dringis and Lake Siesartis was examined using microsatellite assay. The percentage of introduced eels among samples taken from the Baltic Sea and the Curonian Lagoon was determined using otolith microchemistry analysis by a group of Taiwanese and Lithuanian researchers (Shiao *et al.* 2006). It was ascertained that introduced eels constitute 2% and 20% of eel populations in the Baltic Sea and the Curonian Lagoon, respectively, whereas individuals collected from the lakes were introduced totally. Although there is no reliable information regarding the countries from which glass eels were introduced into Lake Dringis and Lake Siesartis, it is known that the restocking of Lithuanian inland waters with glass eels from England and France was performed on a regular basis over several decades (Shiao *et al.* 2006). Furthermore, there are reliable data indicating that all eels, which inhabit Lake Baluošai were introduced (Shiao *et al.* 2006). Eels inhabiting Lakes Dringis and Siesartis are likely to have been introduced in a similar way.

Results of the study suggest that sampled eels from two lakes might be considered as representatives of different populations. However, the Baltic Sea and the Curonian Lagoon samples could be ranged as subpopulations of a single population. As it was suggested earlier, the significant genetic differentiation between the populations of the two lakes might be caused by the following factors: different age of individuals (which means that glass eels were caught and translocated into two lakes in different years); different restocking time and different sites from which glass eels were collected. It was demonstrated that naturally recruited European eels differed genetically from the introduced ones. Thus, there is small but significant genetic differentiation in the *A. anguilla* population consisting of the groups of naturally recruited and introduced European eels. Due to some methodical and sampling differences it should not be stressed that the calculated F_{ST} values were higher than those of other studies (Wirth & Bernatchez 2001, Dannewitz *et al.* 2005). However, previous studies did not examine genetic differentiation between the groups of naturally recruited and introduced European eels. Hence, the comparison of the F_{ST} value with the results of alternative studies is complicated.

Wirth and Bernatchez (2001) found genetic differentiation within the European eel stock, which could be determined applying the isolation by distance (IBD) pattern in *A. anguilla*. However, some other studies proposed contradictory explanations, stating that there was no genetic differentiation determined by the IBD pattern between different European eel populations, whereas the presence of the temporal genetic structure in the European eel was determined (Dannewitz *et al.* 2005).

The purpose of the European eel restocking is to increase its resources in a particular country (Ringuet *et al.* 2002; Wickström 2005). However, some countries take a different approach to the European eel restocking strategy (Limburg *et al.* 2003; Sjöberg *et al.* 2005). For example, in Sweden eels are released not only into inland waters but even into the sea itself. Therefore, the purpose of fish restocking is not only to enhance local fishery but also to sustain *A. anguilla* population by means of stock protection (Sjöberg *et al.* 2005; Wickström 2005). As *A. anguilla* otolith microchemistry studies describing spawning migrations of the Silver eel are controversial (Limburg *et al.* 2003; Sjöberg *et al.* 2005; Wickström 2005), the question as to whether introduced European eels reach the Sargasso Sea or lose their way somewhere in the Baltic Sea still remains unanswered. Furthermore, the effect of crossbreeding of restocked and naturally recruited eels on this species in the Sargasso Sea could be examined too. If Wirth and Bernatchez's (2001) hypothesis concerning the different timing of reaching the Sargasso Sea by eels from different geographical regions of their distribution range is correct, it is plausible that the European eels introduced into a particular country could reach the Sargasso Sea at the same time as the naturally recruited individuals.

Our findings on the existence of genetic differentiation among eels could be in agreement with the conclusions drawn by Dannewitz *et al.* (2005) due to the existence of temporal genetic variation, which means that samples consisting of individuals of similar age have similar genetic structures. As there are different populations with different age structures in Lake Dringis and Lake Siesartis (Ložys, unpubl. data), it is plausible that our F_{ST} values reflect temporal genetic variation in the studied populations too.

To sum up, the initial investigations into genetic variability and genetic structure of the European eel population representing naturally recruited and introduced individuals do not support the hypothesis on the existence of complete panmixia in *A. anguilla* species. Consequently, we think that the restocking strategy of this fish should be viewed with precaution. More investigations must be carried out to find out whether introduced European eels are able to reach the Sargasso Sea and if there is genetic differentiation caused by the isolation by distance in this fish species.

Acknowledgements

Financial support was provided by the Ministries of Education and Science of Republic of Lithuania and Republic of Latvia and by the Mutual Fund of Lithuania-Latvia-Taiwan (Republic of China).

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2.3.3 VARIETY OF mtDNA HAPLOTYPES IN THE POPULATIONS OF THE EUROPEAN PERCH (*Perca fluviatilis*) OF THE CURONIAN LAGOON, THE COASTAL ZONE OF THE BALTIC SEA AND THE GULF OF RIGA

Summary

A total of 56 perches attributed to three different populations of the Baltic Sea (Lithuanian costal zone), the Curonian Lagoon (Lithuania) and the Gulf of Riga (Latvia) were investigated. The amplified mtDNA fragment consisting of 378 bases includes the *trnP* gene and the 5‘-gale fragment of the control region consisting of 260 bases. Ten variable positions were identified and 15 haplotypes of the European perch of mitochondrial DNA (mtDNA) D-loop were established. Three new haplotypes C4, F8 and G2 characteristic of the perch caught in Lithuanian territorial waters and two haplotypes L1 and L2 characteristic of the perch caught in the Gulf of Riga were determined. Five rarer haplotypes were found in the samples of the Lithuanian perch and four – in the samples of the Latvian perch, which characterises the scope of reproductive isolation among the populations of these perches. The similarity in the distribution of the most frequently found haplotypes A and F in the Lithuanian samples investigated illustrates closeness of the populations of the perch, substantiating the assumption about possible seasonal migration of the perch between the Curonian Lagoon and the coastal zone of the Baltic Sea.

Introduction

Considerable climate changes that have lasted for the recent 2.5 million years, the Baltic Sea and part of the European continent periodically becoming covered with ice, as well as other physical-geographical barriers, had an impact on the formation of the genetic structure of the species living here. The unequal frequency of a distribution of different alleles in the populations that were isolated for some time reflects this process (Nesbø et al. 1999). The said statements have been confirmed by the investigation into freshwater fishes carried out in North America, which revealed significant correlation between postglacial migration and the distribution of genetic lineages in the fishes under investigation (Bernatchez & Wilson, 1998). Since the analysis of the isoenzyme systems of such widespread species as the European perch (*Perca fluviatilis*) revealed especially low informativeness of genetic markers (Mardsen et al. 1995), the analysis of randomly amplified polymorphic DNA fragments and mtDNA markers was used to investigate the peculiarities of the genetic structure of the European population of this species (Nesbø et al, 1998b). It should be underlined that suitability of the perch to the investigations into the formation of the genetic structure of the populations is defined by such features as a universal distribution of the species in the European network of river basins, their adaptation to live under conditions of a varying water temperature and even salinity. Effectiveness of the use of DNA markers is illustrated by the investigation results when in analysing the variability of the sequence of mitrochondrial DNA (mtDNA) D-loop of the perch, phyllogenetic relationships of the samples of the perch caught in the territorial waters of Sweden in the Baltic Sea, were evaluated. In the samples of the perch, which represented the population noted for different migration behaviour a peculiar spectrum of haplotypes was discovered, and the reliability of phyllogenetic relationships was alternatively confirmed using the RAPD markers analysis (Nesbø et al. 1998b). The authors of another fundamental investigation during which 44 different samples of the perch from various river basins of the European continent were compared established the distribution frequency of mitochondrial DNA (mtDNA) D-loop and proved that there were several centres from which the perch could spread into northern Europe in the postglacial period (Nesbø et al. 1999). However, thus far there has been no data available about the investigations into the genetic diversity of the perch caught in the inland waters of Lithuania and Latvia or in the coastal waters of the Baltic Sea using DNA markers. The only survey known in the region for perch populations in Kaunas city reservoir, the Curonian Lagoon and the Baltic Sea coastal zone is the study, in which izoenzymes as biochemical markers were used (Paulauskas & Ložys, 2001). All this underlines the gap in the population-genetic investigations into this species in our region only. Therefore, seeking to reveal the genetic structure of the populations of this species of fish, which is significant from the point of view of fishery, and to deepen the theoretical basis on the grounds of which recommendations could be put forward to fishery manager concerning the optimisation of the perch stock management, the aim of the present work was to determine polymorphism of sequences of mitochondrial DNA (mtDNA) D-loop and to evaluate the genetic diversity of the populations of the perch in different areas, the individuals representing which were caught in the Curonian Lagoon, in the coastal wasters of the Baltic Sea near Šventoji (Lithuania) and in the Gulf of Riga (Latvia).

Material and Methods

56 European perches *(Perca fluviatilis)* caught in the year 2004 and 2005 were used for the investigation. Out of eleven individuals caught in the Baltic Sea, 20 individuals caught in the Curonian Lagoon in 2004 and 9 individuals caught in the Baltic Sea (near Monciškės), five individuals caught in the Baltic Sea (near Būtingė), 16 individuals caught in the Curonian Lagoon (near the location named *Kiaulės nugara*), 8 individuals caught in the Curonian Lagoon (near Ventės ragas) in 2005, as well as 23 European perches caught in the Gulf of Riga near the mouth of the River Daugava in the summer of 2006, genomic DNR was isolated from the samples of muscle tissues fixed in ethanol by means of the salt extraction method (Aljanabi & Martinez, 1997).

Amplification of mtDNA fragments was carried out using *Mastercycler Gradien* amplifier manufactured by the *Eppendorf* Company. For amplification of mitochondrial DNA sequences the following primers were used: HV2: TTCCCCGGTCTTGTAAACC; and CSB-D: GGAACCAAATGCCAGGAA.

PCR was carried out by denaturation at 96°C temperature for 5 minutes, then 30 cycles at 96°C temperature for one minute, at 54°C temperature (HV2 – CSB-D) for one minute, following elongation step at 72°C temperature for 2 minutes and finishing with final elongation step at the 72°C temperature for 5 minutes. The PGR product was checked in 1.5% agarosis gel and purified by CIAP and ExoI nucleases: for 1 5 minutes at 37°C and then for 15 minutes at 85°C.

Sequencing was carried out at the Sequencing Centre of the Institute of Biotechnology, DNA sequences were determined by the ABI Prism 377 automatic sequencer using the same primers as for PCR.

The sequencing results were compared with the PFY14724 sequence using the BLAST database. The computer program CLC Free Workbench version 0.91 was used for the sequence analysis and for construction of phylogenetic tree.

Results and Discussion

A total of 56 perches attributed to three different populations of the Baltic Sea, the Curonian Lagoon and the Gulf of Riga were investigated. The amplified mtDNA fragment consisting of 378 bases includes the *trnP* gene and the 5‘-gale fragment of the control region consisting of 260 bases. Ten variable positions were identified and 15 haplotypes of the European perch of mitochondrial DNA (mtDNA) D-loop were established. Three new haplotypes C4, F8 and G2 characteristic of the perch caught in Lithuanian territorial waters and two haplotypes L1 and L2 characteristic of the perch caught in the Gulf of Riga were determined.

Table 1. Haplotypes of mtDNR *trnP* gene and control region fragment determined in the European perch *Perca fluviatilis* caught in the territorial waters of Lithuania and Latvia (data are compared with the mtDNA sequence PFY14724, haplotype A presented in the Gene Bank)

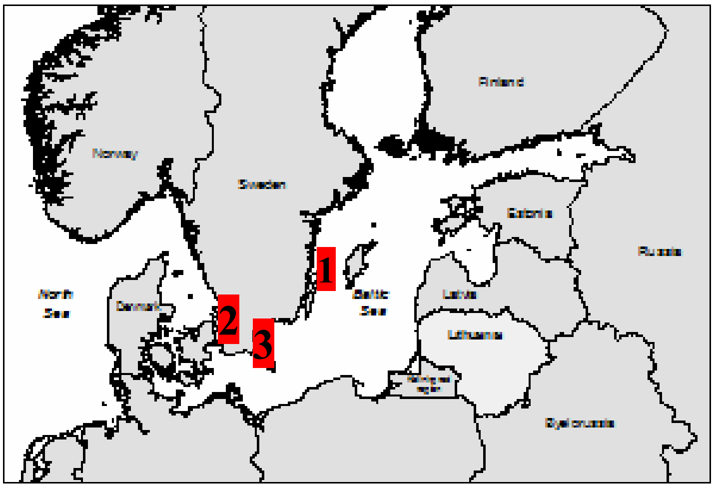
Haplotypes	98-99	103	129	130	135	136	137	139	264	299	**N**
A	**-**	**A**	**G**	**C**	**A**	**A**	**T**	**T**	**T**	**C**	***6***
A2	-			T							***1***
A7	-		T	T							***4***
C	-									T	***7***
C1	-		T	T						T	***4***
C4	-		T	T					A	T	***1***
E	-	T					-			T	***2***
F	-	T								T	***23***
F1	-	T	T	T						T	***1***
F2	-	T	T	T	G					T	***1***
F7	-	T	T							T	***2***
F8	-	T				-				T	***1***
G2	-							-		T	***1***
L1	Ins*	T		T						T	***1***
L2	Ins*			T							***1***
Total:											**56**

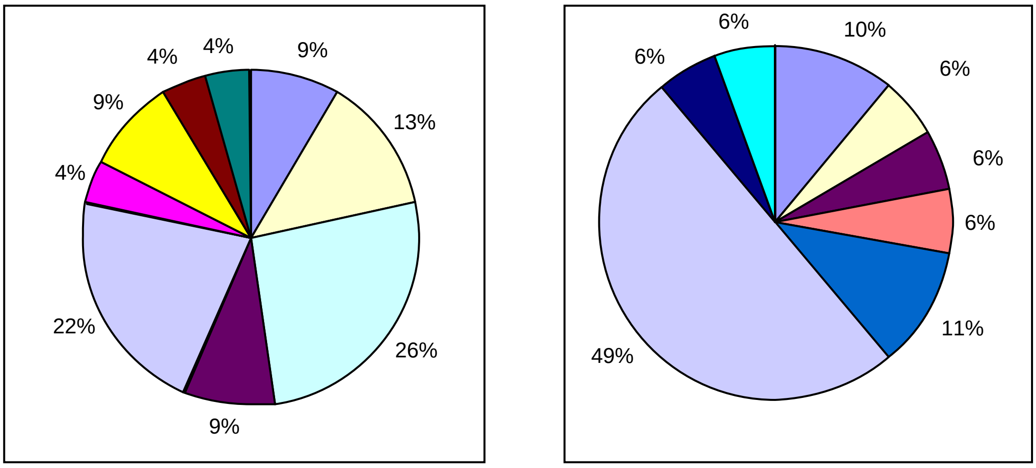
C4, G2, F8, L1 ir L2 – newly identified haplotypes
N – the number of individual studied
T, A and G – substitution of nucleotides
- – deletion of nucleotides
Ins* - insertion of ten nucleotides TTGCAAGCAC

The most frequent F haplotype was discovered in the samples of the perch caught both in the Curonian Lagoon and the Baltic Sea near Šventoji. It should be noted that this, as well as other haplotypes of group F (F1-F7) that are close to it by origin, were found most often after many other perch populations of Western Europe had been investigated. Refseth (1998) and Nesbø (1998b) determined a total of 35 haplotypes grouped into eight closely related groups by the nature of base substitutions. The scheme of frequency distribution of haplotypes of the perch presented in the publication based on the data of the said authors (Nesbø and others, 1999) several zones, segregated from the phylogenetic point of view, in which mtDNA variants attributed to different groups of haplotypes prevail, become clear. As has already been mentioned, in all perch populations of Western Europe haplotypes of group F prevailed, with the exception of the river basins stretching in the territory of Norway where haplotypes (A, A1-A9) of group A prevailed in the perch caught there, as well as the coastal zone of the Adrian Sea and river basins of the Balkan countries in which haplotypes (M, M1) of group M, which are oldest in term of origin, prevailed. Whereas in samples of the perch caught in the continental part of the mainland, in the basins of the Nemunas, Dnieper Rivers, as well as in the remote Baikal Lake, the Angara River basin, haplotypes (C, C1-C3) of group C prevailed, which testify to the attribution of the perch in the basins of the rivers stretching across this territory to a separate Euro-Asian phyllogeographical region.

According to our data, haplotype A is found to be second by frequency in the populations of the Lithuanian perch, whereas haplotypes C and E are most often found in the populations of the perch of the Gulf of Riga (Fig. 1).

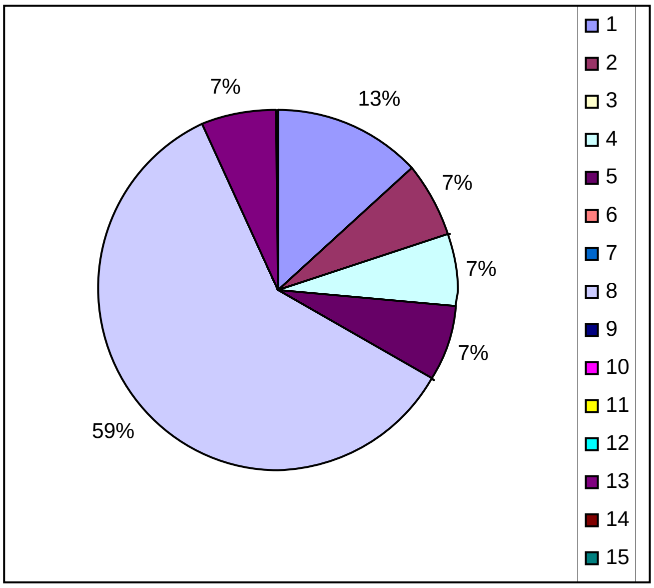
Haplotypes C1 and A7 were found in all the populations of the perch that we investigated. Five rarer haplotypes were found in the samples of the Lithuanian perch and four – in the samples of the Latvian perch, which characterises the scope of reproductive isolation among the populations of these perches. It should be noted that haplotype E was found with minimum frequency on the coastal zone of the Baltic Sea near Šventoji only in the samples of 2004 and 2005 and could be related to the anadromous behaviour, i.e., a periodical change in the environment when fish feeding in more saline waters of the coastal zone of the Baltic sea swim to spawn to the fresh waters of the river mouths. A repeated discovery of haplotype E in the samples of the perch caught in the coastal strip near Monciškės not far from the settlement of Šventoji in different years can be related to the fact that a larger part of the individuals keep near the spawning site located in the approaches of the mouth of the Šventoji River, and rarer variants of haplotypes (halpotype E), inherited with steady, though small frequency, reflect the fact that the population is in Hardy-Weinberg balance.





1) Gulf of Riga

2) Baltic Sea near Šventoji



Haplotypes
1 – A
2 – A2
3 – A7
4 – C
5 – C1
6 – C4
7 – E
8 – F
9 – F1
10 – F2
11 – F7
12 – F8
13 – G2
14 – L1
15 – L2

3) Curonian Lagoon

Fig. 1. Percentage distribution of all investigated haplotypes of mitrochondrial DNA (mtDNR) D-loop in the populations of the perch in the Gulf of Riga (1), the Baltic Sea near Šventoji (2) and the Curonian Lagoon (3).

Newly identified haplotypes C4 and F8 were found in the samples of the Baltic Sea, G2 – in the samples of the Curonian Lagoon are characteristic of the Lithuanian population of the perch and are related to the group of haplotypes E-F (Fig. 2). The presence of insertion of ten nucleotides characteristic of newly identified haplotypes L1 and L2 could be accounted for by a close phyllogenetic link of these haplotypes, however, on the basis of grouping of these samples in the phyllogenetic tree, according to the nature of single base substitutions haplotype L1 is closest to the group of haplotypes F1-F2, which is related to the group of haplotypes F fully dominating in Western Europe, and haplotype L2 is closest to the group of haplotypes A typical of the western and northern parts of Scandinavia (Norway).

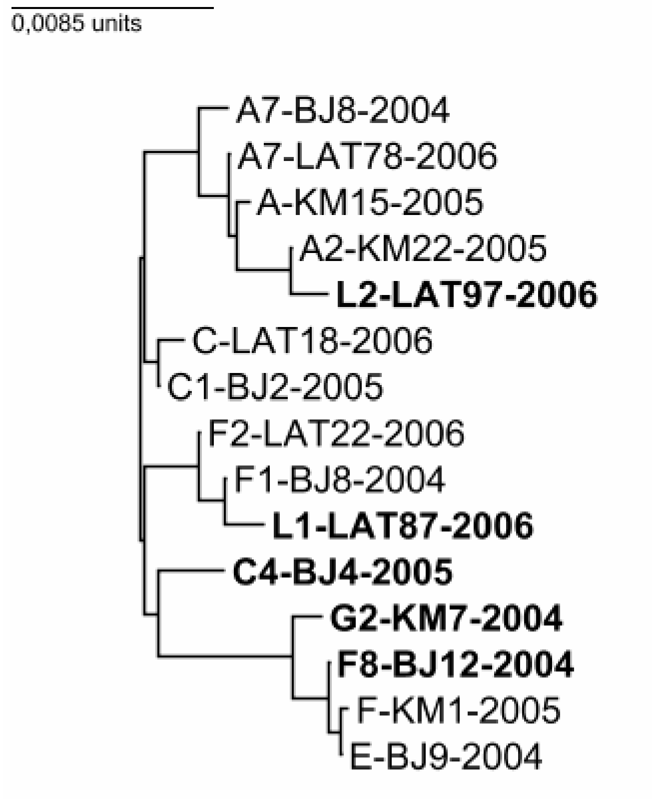


Fig. 2. The *Neighbour joining* phylogenetic tree representing the origin of haplotypes (the first letter of abbreviation stands for the haplotype, BJ-samples from the Baltic Sea, KM – samples from the Curonian Lagoon, LAT – samples of the perch caught in the Gulf of Riga)

By the way, a combination of these two groups of haplotypes A and F is characteristic of the samples of the perch named the Matsalu population investigated in the geographical range of the Baltic Sea (territorial waters of Estonia) (Table 2), which geographically is one of the closest to the sample of the Latvian perch investigated by us, however, which differs greatly from the Emjajogi and Peipsi populations (the Gulf of Finland, territorial waters of Estonia) in which haplotypes of groups A and C found testify to the influence of the Northern and Eastern phylo-geographical regions on the formation of the perch population of the Gulf of Finland.

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Table 2. Sample collection sites and distribution of haplotypes in the population of the European perch

Item No.	Population	Region	Number of individuals studied	Water basin	Haplotypes (N*)
1.	Curonian Lagoon	Lithuanian territorial waters	15	Baltic Sea, mouth of the Nemunas	A(2), A7(1), C(1), C1(1), F(9), G2(1)
2.	Baltic Sea	Lithuanian territorial waters	18	Baltic Sea near Šventoji	A(2), A2(1), C(1), C4(1), E(2) F(9), F1(1), F8(1)
3.	Gulf of Riga	Latvian territorial waters	23	Baltic Sea, the Gulf of Rig near the Daugava mouth	A(2), A7(3), C(6), C1(2), F(5), F2(1), F7(2), L1(1), L2(1)
4.	L.Krylovo**	Russia, inland waters	5	Basins of the Vilija/Nemunas Rivers	C(5)
5.	L.Hencza**	Inland waters of Poland	5	The Nemunas basin	C(4), C1(1)
6.	L.Dubrovskoje**	Inland waters of Russia	10	The Dnieper basin	C(10)
7.	Gulf of Matsalu**	Inland waters of Estonia	10	Baltic Sea	A8(3), F(4), F1(1), F7(1), E2(1)
8.	Emjajogi R.**	Inland waters of Estonia	5	Baltic Sea, the Gulf of Finland	A(1), A7(1), A8(1), C(2), J1(1)
9.	L.Peipsi	Inland waters of Estonia	9	Baltic Sea, the Gulf of Finland	A(1), A7(1), A8(1),C(2), J1(1)
10.	L.Vourasjarve	Inland waters of Norway	10	Karasjakka/Tana	A(4), A2(6)
8.	The Angara River**	Inland waters of Russia	10	Baikal Lake, the Angara basin	C(10)
9.	L.Rybinsk**	Inland waters of Russia	14	The Volga basin	A(1), C(7), C2(1), C3(2), G1(1), J(2)
10.	The gulf of Bothnia**	Territorial waters of Sweden	20	Baltic Sea	A7(2), C(14), F1(4)
11.	Anadromous A**	Territorial waters of Sweden	20	Baltic Sea	A(2), A7(4), C(8), F(3), F1(2), G(1)
12.	Anadromous B**	Territorial waters of Sweden	17	Baltic Sea	A(4), C(6), C1(1), F(2), F1(2), G(2)
13.	L.Ängersjön**	Territorial waters of Sweden	19	Lake near the Baltic Sea	A5(1), C(13), C1(4), F1(1)
14.	L.Race**	Inland waters of Slovenia	10	Drava/Danube	C(1), M1(9)

* - the equal number of haplotypes is given in brackets

** - data about the investigated perch populations published in Molecular Ecology, 8, 1387-1404 (Nesbø et al. 1999)

The phylogenetic link between the Estonian and Latvian perch populations is confirmed by the fact that, as in the above-mentioned Matsalu population, haplotypes of A and F groups are most frequently found in the perch population of the Gulf of Riga, however, the Latvian perch population is noted for a rather high frequency of haplotypes C, which, as has been mentioned above, is related to the Euro-Asian phyllo-geographical region extending in the East. This frequency of haplotypes of group C is three times lower in the populations of the Lithuanian perch in the samples of both the Curonian Lagoon and the coastal zone of the Baltic Sea, hence, the total spectrum of frequencies of haplotypes is closer to the Western European one, though the 'eastern' haplotypes of group C entirely dominate in the samples of the perch investigated in the basins of the Vilija and Nemunas Rivers in other than Lithuanian territory.

The smallest diversity of haplotypes (6) was discovered in the perch population of the Curonian Lagoon. The interesting thing is that these data coincide with the data of the investigations carried out into the perch populations of the Gulf of Bothnia in the territorial waters of Sweden, which are noted for a different migration behaviour, where it was determined that the samples of stable, that is, non-migrating perch populations in which 3 and 4 different haplotypes in each were found, respectively, were noted for the smallest diversity of haplotypes, whereas in two samples of the perch noted for an anadromous behaviour 6 different haplotypes in each were discovered (the size of samples fluctuated from 17 to 20 individuals). The data confirming this phenomenon were obtained during our investigation. When analysing the results obtained it was noticed that in brackish coastal waters of the Baltic Sea near Šventoji and in the Gulf of Riga where the perch noted for the anadromous behaviour usually live, a greater diversity of haplotypes, 8 and 9 respectively, was determined. The similarity in the distribution of the most frequently found haplotypes A and F in the samples investigated illustrates closeness of the populations of the Lithuanian perch, substantiating the assumption about possible seasonal migration of the perch between the Curonian Lagoon and the coastal zone of the Baltic Sea, where grown up individuals migrate for summer to the brackish Baltic Sea probably due to low water salinity beneficial effect on the perch growth, whereas in autumn they return to the freshwater Lagoon for winter and spawning the following spring (Ložys, 2004). In this case the smaller diversity of haplotypes reflecting the genetic diversity of the summer population of the perch of the Curonian Lagoon testifies to the assumption about seasonal migrations of part of the perch to the Baltic Sea.

Taking into consideration the results of this investigation and seeking to reveal such subtleties of the structure of populations as the tendency of some part of individuals to seasonally migrate to brackish waters, apart from using mtDNR genetic markers inherited through the maternal lineage, it is necessary to make use of micro-satellite markers, which are especially suitable to the analysis of the population structure (Brunner et al. 1998, Englbrecht et al. 2002), all the more so, that on the basis of the DNA investigations several specific micro-satellite primers have already been created for the species of the yellow perch, which is taxonomically close to the European perch (Leclerc et al., 2000), thus, sufficiently complicated and expensive overcoming of the stage of creating specific primers for the species raises the possibility for the investigators to make use of the available information in continuing investigations into the interspecific genetic diversity and population structure of the European perch.

Acknowledgements

We express our gratitude to the Mutual Funds for the Scientific Co-operation of the joint project of Taiwan, the Republics of Latvia and Lithuania "Application of genetic and micro-chemical markers as implements for diadromous and endangered commercial fish species populations management" for its financial assistance and the provided possibility to carry out this project.

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2.3.4 DNA SAMPLING AND PCR PERFORMING OF LATVIAN PERCH, PIKEPERCH AND EUROPEAN EEL

DNA from collected samples was extracted by standard procedures using “Fermentas” DNA extracting kit. Quality of extracted DNA was checked either by electrophoresis or by analysis by the spectrophotometer. Altogether 99 DNA samples were extracted, including 62 perch samples (Table 1), 15 pikeperch samples (Table 2) and 22 samples of European eel (Table 3).

Table 1. List of extracted perch DNA samples

Sample number	Sampling date	Sampling place	Sex
1	16.05.2005	Ķīšezers	M
2	16.05.2005	Ķīšezers	M
3	16.05.2005	Ķīšezers	M
6	16.05.2005	Ķīšezers	M
7	16.05.2005	Ķīšezers	M
8	16.05.2005	Ķīšezers	M
9	16.05.2005	Ķīšezers	M
10	16.05.2005	Ķīšezers	M
13	16.05.2005	Ķīšezers	M
14	16.05.2005	Ķīšezers	M
15	16.05.2005	Ķīšezers	M
16	19.05.2005	Ķīšezers	M
17	19.05.2005	Ķīšezers	M
18	19.05.2005	Ķīšezers	M
19	19.05.2005	Ķīšezers	M
20	19.05.2005	Ķīšezers	F
21	19.05.2005	Ķīšezers	M
22	19.05.2005	Ķīšezers	M
24	19.05.2005	Ķīšezers	F
25	19.05.2005	Ķīšezers	M
26	19.05.2005	Ķīšezers	F
29	19.05.2005	Ķīšezers	M
62	14.06.2005	Daugavgrīva	F
63	14.06.2005	Daugavgrīva	M
64	14.06.2005	Daugavgrīva	M
65	14.06.2005	Daugavgrīva	M
66	14.06.2005	Daugavgrīva	F
67	14.06.2005	Daugavgrīva	M
68	14.06.2005	Daugavgrīva	F
69	14.06.2005	Daugavgrīva	M
70	14.06.2005	Daugavgrīva	F
71	14.06.2005	Daugavgrīva	F
72	14.06.2005	Daugavgrīva	F
73	14.06.2005	Daugavgrīva	F
75	14.06.2005	Daugavgrīva	F

78	14.06.2005	Daugavgrīva	F
79	14.06.2005	Daugavgrīva	F
82	14.06.2005	Daugavgrīva	F
83	14.06.2005	Daugavgrīva	M
84	14.06.2005	Daugavgrīva	F
85	14.06.2005	Daugavgrīva	F
93	30.06.2005	Daugavgrīva	M
94	30.06.2005	Daugavgrīva	M
97	30.06.2005	Daugavgrīva	F
102	29.08.2005	Daugavgrīva	M
104	29.08.2005	Daugavgrīva	F
106	29.08.2005	Daugavgrīva	M
107	29.08.2005	Daugavgrīva	F
109	29.08.2005	Daugavgrīva	M
111	29.08.2005	Daugavgrīva	F
112	29.08.2005	Daugavgrīva	M
113	29.08.2005	Daugavgrīva	F
114	29.08.2005	Daugavgrīva	F
116	29.08.2005	Daugavgrīva	M
118	29.08.2005	Daugavgrīva	F
119	29.08.2005	Daugavgrīva	M
121	29.08.2005	Daugavgrīva	M
123	05.09.2005	Daugavgrīva	F
124	05.09.2005	Daugavgrīva	F
126	05.09.2005	Daugavgrīva	F
127	05.09.2005	Daugavgrīva	F
128	05.09.2005	Daugavgrīva	F

Table 2. List of extracted pikeperch DNA samples

Sample number	Collecting date	Collecting place	Sex
32	31.05.2005	Vecdaugava	F
33	31.05.2005	Vecdaugava	M
34	31.05.2005	Vecdaugava	M
35	31.05.2005	Vecdaugava	F
37	31.05.2005	Vecdaugava	M
38	31.05.2005	Vecdaugava	M
39	31.05.2005	Vecdaugava	F
41	13.06.2005	Vecdaugava	M
43	13.06.2005	Vecdaugava	M
44	13.06.2005	Vecdaugava	F
45	13.06.2005	Vecdaugava	M
46	13.06.2005	Vecdaugava	F
47	13.06.2005	Vecdaugava	M
50	13.06.2005	Vecdaugava	M
60	30.06.2005	Vecdaugava	M

Table 3. List of extracted European eel DNA samples

Sample number	**Collecting date**	**Collecting place**	**Sex**
131	29.08.2005	Daugavgrīva	F
133	29.08.2005	Daugavgrīva	F
134	29.08.2005	Daugavgrīva	F
135	29.08.2005	Daugavgrīva	F
136	29.08.2005	Daugavgrīva	F
137	29.08.2005	Daugavgrīva	F
138	29.08.2005	Daugavgrīva	F
139	29.08.2005	Daugavgrīva	M
142	29.08.2005	Daugavgrīva	F
143	29.08.2005	Daugavgrīva	F
144	29.08.2005	Daugavgrīva	F
145	05.09.2005	Daugavgrīva	F
146	05.09.2005	Daugavgrīva	F
147	05.09.2005	Daugavgrīva	F
149	05.09.2005	Daugavgrīva	F
150	05.09.2005	Daugavgrīva	F
151	05.09.2005	Daugavgrīva	F
152	05.09.2005	Daugavgrīva	F
156	05.09.2005	Daugavgrīva	F
157	05.09.2005	Daugavgrīva	F
160	05.09.2005	Daugavgrīva	F
161	05.09.2005	Daugavgrīva	F

Analysis of microsatellites

PCR was performed in a Gene Amp PCR System 9700 (Applied Biosystems) Using 20-30 ng DNA. The reaction mixture incleded 10.5 µl molecular water, 2 µl 10X PCR buffer (Fermentas), $MgCl_2$, (25mM) (Fermentas), 0.4 µl dNTP (10mM) (Fermentas), 2 µl F and 2 µl R primers and 0.1 µl Tag polymerase (Fermentas).

PCR reaction conditions:

The amplification started with 4 min at 96 ^{0}C followed by 30 cycles of 30 s at 96 ^{0}C, 1 min 53 ^{0}C and 1 min at 72 ^{0}C. This was followed by 7 min 72 ^{0}C.

PCR samples (5 µl) were electrophoresed on a 1.5 % agarose gel ((Carl Roth GmGh) using 1X TAE (tris-acetate-EDTA) electrophoresis buffer (Fermentas) at 79 V for 1 h, visualized by staining with ethidium bromide and photographed using UV light box (UVI tec) and program GeneSnap. A 100-bp DNA ladder (Fermentas) was used as the molecular weight standard.

PCR products for DNA samples oh the Latvian populations were detected for following primers (Tab. 4,5, 6 and 7).

Table 4.Primers with fluorescent markers used for PCR reaction of eel samples

Primers	**Sequences (5‘-3‘)**
AF237898 (F)	FAM- TTGGGAAGGTCATGGACGTG
AF237898 (R)	CTAATAAATGTCTGGGTAGGC
AF237899 (F)	HEX-CCAGATACCTTGACAACGGC
AF237899 (R)	TCAAGAGCTTCCTGACCCTC
AF237902 (F)	NED- CCTGTGAATCCAACAGGTGG
AF237902 (R)	GGATAATGCGGCAGAGTTCCC
AJMS-3 (F)	FAM- GGTATGAATGCAGGCGTTTATG
AJMS-3 (R)	GCAACCGATTTGATCTCCAG
AF237901 (F)	HEX- GATCTGTGGAGAGATGTTGG
AF237901 (R)	AGTAGCATGCCTAGAACTGG
AF237900 (F)	NED- GAAAACAATCGGGTACCACAG
AF237900 (R)	ACAGTCAGTCACAATGAGCC
AJMS-6 (F)	FAM- ACAGAGCCAGACAAACAGAC
AJMS-6 (R)	GGTCAGCAAGCAAAACGAAC
AJMS-10 (F)	HEX-TGTCTAACACTAAGAAAAGGAGAGG
AJMS-10 (R)	GGCTGCCAGTATCTTCTCAAAG
AJMS-2 (F)	NED- ATTTCACGTCATCGGACCTGC
AJMS-2 (R)	GCTGGGAGCGACGCTTTATC
AJMS-5 (F)	FAM- CCTTCAGATTGCTAGCAC
AJMS-5 (R)	CGGAGTCTAATTGTCTCCTC

Table 5. Primers with fluorescent markers used for PCR reaction of pikeperch and perch samples

Primers	**Sequences (5‘-3‘)**	**Species**
Svi-L11 (F) *Svi-L11 (R)*	HEX- AGGGTATGGCATGATAAG CTCTACATTTCATCAGACAG	pikeperch
Svi-L8 (F) *Svi-L8 (R)*	FAM- GCTTATACGTCGTTCTTATG ATGGAGAAGCAAGTTGAG	pikeperch
Pfla-L8(F) *Pfla-L8 (R)*	HEX- GCCTTATTGTGTGACTTATCG GGATCTTTCACTTTTTCTTTCAG	pikeperch
Svi-L2 (F) *Svi-L2 (R)*	HEX- TAAGACATAAACATACTCTG TATACTGAGAGTGGAGACAT	pikeperch
Svi-L6(F) *Svi-L6 (R)*	FAM- AGAGGAAGAAGAGGTATC TTAAAGGGTAAGTCCACTG	perch
Pfla-L6 (F) *Pfla-L6 (R)*	HEX- GCATACATATAAGTAGAGCC CAGGGTCTTCACTATACTGG	perch
Pfla-L1 (F) *Pfla-L1 (R)*	FAM- AAGCAGCCTGATTATATATC CAGACAATTAAACATGCAAC	pikeperch perch
Pfla-L5 (F) *Pfla-L5 (R)*	HEX- TGAGAGCCCATGAATTAC GCAAACACAGCCAATTTAG	perch
Pfla-L2 (F) *Pfla-L2 (R)*	NED- GTAAAGGAGAAAGCCTTAAC TAGCATGACTGGCAAATG	perch
Pfla-L4 (F) *Pfla-L4 (R)*	FAM- AAAGGGAAAAGGCTACGGTG ATCAGCAGTGCTTATGTTG	perch
Pfla-L10 (F) *Pfla-L10 (R)*	HEX- TCCACCCTTTGATAAGGGAC ACAAATCTCCTGTCAAACGC	perch
Pfla-L9 (F) *Pfla-L9 (R)*	NED-GTTAGTGTGAAAGAAGCATCTGC TGGGAAATGTGGTCAGCGGC	pikeperch perch
Svi-L1 (F) *Svi-L1 (R)*	FAM - CTACAGGCTACAAACAAG CACAAACATTTTGGGCAG	pike
Svi-L7(F) *Svi-L7 (R)*	HEX- GATGTGCATACATTTACTCC GCTTTAATCTGCTGAGAAC	pikeperch perch
Svi-L10 (F) *Svi-L10 (R)*	NED- GGTAATGTATTTTCAGTTATTGC GCTGTTCTCCAAGTAAAGCC	pikeperch perch

Table 6. Primers with fluorescent markers used for PCR reaction of eel samples

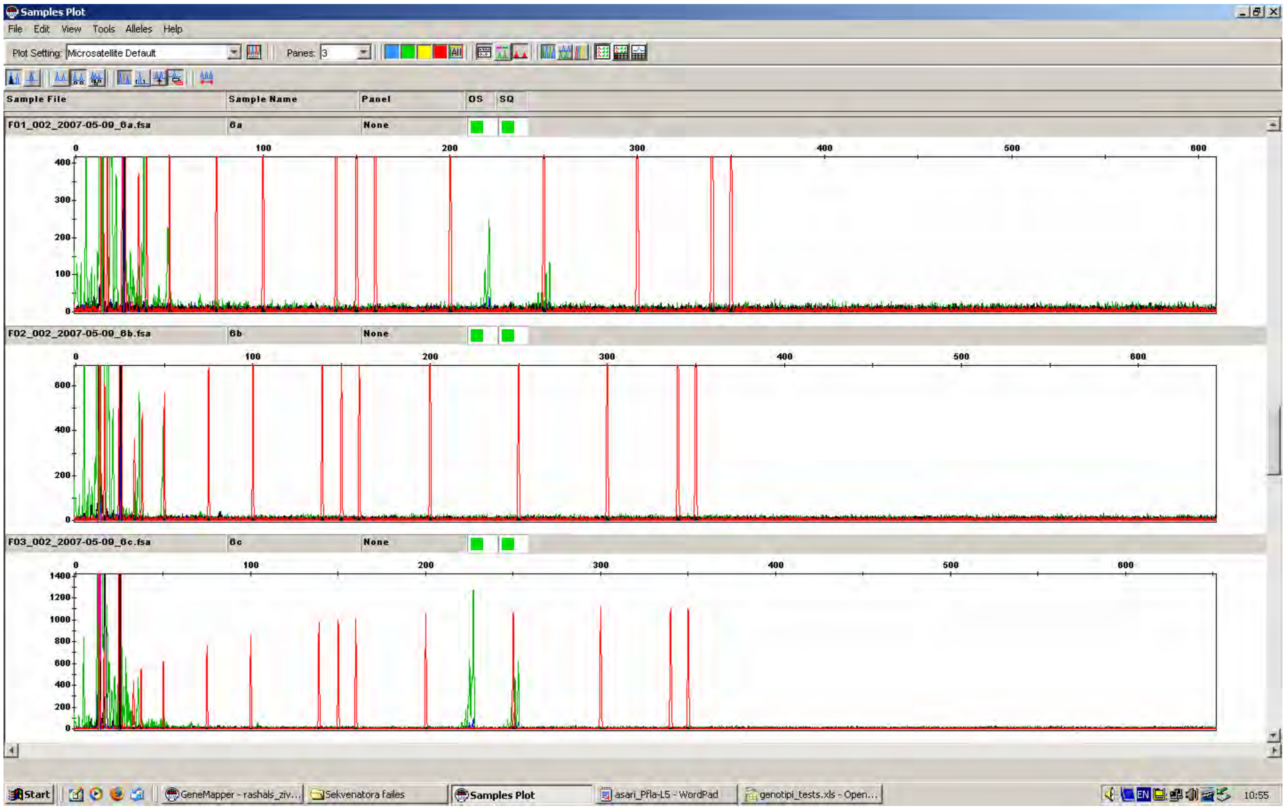
Primers	Sequences (5‘-3‘)
AF237898 (F) *AF237898 (R)*	FAM- TTGGGAAGGTCATGGACGTG CTAATAAATGTCTGGGTAGGC
AF237899 (F) *AF237899 (R)*	HEX-CCAGATACCTTGACAACGGC TCAAGAGCTTCCTGACCCTC
AF237902 (F) *AF237902 (R)*	NED- CCTGTGAATCCAACAGGTGG GGATAATGCGGCAGAGTTCCC
AJMS-3 (F) *AJMS-3 (R)*	FAM- GGTATGAATGCAGGCGTTTATG GCAACCGATTTGATCTCCAG
AF237901 (F) *AF237901 (R)*	HEX- GATCTGTGGAGAGATGTTGG AGTAGCATGCCTAGAACTGG
AF237900 (F) *AF237900 (R)*	NED- GAAAACAATCGGGTACCACAG ACAGTCAGTCACAATGAGCC
AJMS-10 (F) *AJMS-10 (R)*	HEX-TGTCTAACACTAAGAAAAGGAGAGG GGCTGCCAGTATCTTCTCAAAG

Table 7. Primers with fluorescent markers used for PCR reaction of pikeperch and perch samples

Primers	Sequences (5‘-3‘)	Species
Svi-L11 (F) *Svi-L11 (R)*	HEX- AGGGTATGGCATGATAAG CTCTACATTTCATCAGACAG	pikeperch
Svi-L8 (F) *Svi-L8 (R)*	FAM- GCTTATACGTCGTTCTTATG ATGGAGAAGCAAGTTGAG	pikeperch
Pfla-L8(F) *Pfla-L8 (R)*	HEX- GCCTTATTGTGTGACTTATCG GGATCTTTCACTTTTTCTTTCAG	pikeperch
Svi-L2 (F) *Svi-L2 (R)*	HEX- TAAGACATAAACATACTCTG TATACTGAGAGTGGAGACAT	pikeperch
Pfla-L6 (F) *Pfla-L6 (R)*	HEX- GCATACATATAAGTAGAGCC CAGGGTCTTCACTATACTGG	perch
Pfla-L1 (F) *Pfla-L1 (R)*	FAM- AAGCAGCCTGATTATATATC CAGACAATTAAACATGCAAC	pikeperch perch
Pfla-L5 (F) *Pfla-L5 (R)*	HEX- TGAGAGCCCATGAATTAC GCAAACACAGCCAATTTAG	perch
Pfla-L2 (F) *Pfla-L2 (R)*	NED- GTAAAGGAGAAAGCCTTAAC TAGCATGACTGGCAAATG	perch
Pfla-L4 (F) *Pfla-L4 (R)*	FAM- AAAGGGAAAAGGCTACGGTG ATCAGCAGTGCTTATGTTG	perch
Pfla-L10 (F) *Pfla-L10 (R)*	HEX- TCCACCCTTTGATAAGGGAC ACAAATCTCCTGTCAAACGC	perch
Pfla-L9 (F) *Pfla-L9 (R)*	NED-GTTAGTGTGAAAGAAGCATCTGC TGGGAAATGTGGTCAGCGGC	pikeperch perch
Svi-L1 (F) *Svi-L1 (R)*	FAM - CTACAGGCTACAAACAAG CACAAACATTTTGGGCAG	pike
Svi-L7(F) *Svi-L7 (R)*	HEX- GATGTGCATACATTTACTCC GCTTTAATCTGCTGAGAAC	pikeperch perch
Svi-L10 (F) *Svi-L10 (R)*	NED- GGTAATGTATTTTCAGTTATTGC GCTGTTCTCCAAGTAAAGCC	pikeperch perch

Microsatellite alleles

Microsatellite analysis was done by ABI 3100 capillary sequencer (Fig. 1).



Plot Setting: Microsatellite Default
Panes: 3

Sample File	Sample Name	Panel	OS	SQ
F01_002_2007-05-09_6a.fsa	6a	None	🟩	🟩
F02_002_2007-05-09_6b.fsa	6b	None	🟩	🟩
F03_002_2007-05-09_6c.fsa	6c	None	🟩	🟩

Fig. 1. Fragment analysis by the ABI 3100 capillary sequencer of three perch individuals DNA samples for microsatellite Pfla-L10 loci detection.

Several individuals of each species were analysed to check usefulness of selected primers. Results are presented in tables 8, 9-and 10.

Table 8.Microsatellite alleles detected in Latvian perch samples

Microsatellite	Alleles				
Pfla-L1	124	132			
Pfla-L6	131	132			
Pfla-L2	245	250	263	273	
Pfla-L4	128	130	132	138	140
Pfla-L5	147	149	154		
Pfla-L10	221	227	253		
Pfla-L9	no product				
Svi-L7	199	221	230	243	
Svi-L1	393	395	396	400	401
Svi-L10	no product				

Table 9. Microsatellite alleles detected in Latvian pikeperch samples

Microsatellite	Alleles						
Pfla-L1	no product						
Pfla-L9	182	190	213				
Pfla-L8	164	183					
Svi-L7	199	220	221	222	230	235	243
Svi-L10	232	240					
Svi-L11	114						
Svi-L8	104	133	135	137	144		
Svi-L2	no product						

Table 10. Microsatellite alleles detected in Latvian eel samples

Microsatellite	Alleles					
AJMS-3	82	84				
AJMS-10	173	177	211			
AF237901	171	172	180	182		
AF237900	163	171	172	177	180	182
AF237899	180	181	183	184	187	200
AF237902	192	219	222	225	229	231
AF237898	103	105	126	132	145	

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2.4 GENERAL CONCLUSIONS AND RECCOMENDATIONS FOR STUDIED FISH SPECIES MANAGEMENT

CONCLUSIONS:

- European eels recruited in natural way predominate in Lithuania and Latvia coastal waters;
- Some eels in coastal waters are of the restocked origin. Restocked eels in silver eel stage reach the Baltic Sea and migrate together with naturally recruited individuals for spawning to Sargasso Sea along Latvian coast in Riga Gulf; eel of restocked origin numbers in silver eel stage increase and predominate in coastal waters during migration phase in Riga Gulf;
- Inland eel fishery in both countries depends on artificial eel restocking. No evidences of natural recruitment to inland lakes were observed during the study;
- Naturally recruited eels spend from 1 to 10 years (mean 5 years) for migration to reach the area of study; this suggests that ell recruitment at the glass eel stage (which is typical recruitment stage in other sites of the range of European eel distribution) should not be expected in the studied area, i.e. eels reach Lithuania and Latvia in yellow eel stage;
- The growth of naturally recruited eels did not differ significantly between the brackish Baltic coasts and the freshwater Curonian lagoon (opposite some other studies);
- Three migratory patterns, seawater-residents, freshwater-residents and inter-habitat shifters, were identified from the Curonian Lagoon and Baltic coasts; no significant growth differences among migratory groups were found although such growth differences occur in other anguillid eels;
- Significantly larger the lengths-at-age between 4-7 years for stocked eels leads to hypothesis that the artificial restocking allows the energy savings due to non-migration, while naturally recruited eels have to allocate energy for long distance migration that stocked eels do not. After the arrival of naturally recruited eels in the Baltic Sea, any differences in length might be reduced by compensatory growth;
- It was found that the growth of the eels might be more enhanced in estuary and coastal waters than in freshwater lakes. In this study, it was further suggested that the productivity and longevity of the growing season, rather than the salinity, might play a more important role in growth of the eels because that of the eels in the nearly freshwater lagoon was higher than in freshwater lakes;
- The analysis of eel otolith samples provides the first confirmation that vaterite zones can exist in the aragonite otoliths of the European eel. The prevalence of otoliths with mosaic vaterite was found in nearly half of the eels examined. If mosaic vaterite is not distinguished from aragonite, the fish migratory history may be misidentified when otolith elemental signatures are used as biological tracers;
- Naturally recruited and restocked eels belong to different populations and differ genetically from each other; eel populations from different lakes can differ as well hypothetically depending on the stocked eel originating area (the area from which eels in glass eel stage were translocated; the distance between restocking and glass eel catch site are thousands kilometers usually);
- The study indicates that the Percid fish migration to brackish water is rather irregular than regular. Percids perform migrations to the Baltic Sea in later age, while juveniles stay in fresh water. However, to reveal the migratory history of Percids completely, further complete analysis of the study results is needed;

- The results of the genetic studies on Percid fish populations is in accordance with the observed irregular Percid fish migrations revealed by microchemical analysis. The data of genetic studies do not support the hypothesis of two “migrating” and “non-migrating” subpopulations existence in the Curonian Lagoon: according to the study results, Percid fish population in the Curonian Lagoon should be considered as a “single population”;
- The hypothesis of “migrating pikeperch” spawning grounds in the Northern part of the Lagoon was denied during the study. Field observations enable to conclude that pikeperch in the northern part of the Lagoon concentrate before their migration to the Sea awaiting favorable temperature conditions in the Baltic Sea;
- Percid fish populations in Lithuania and Latvia should be considered as different subpopulations due to observed genetic.

RECCOMENDATIONS FOR STUDIED FISH SPECIES MANAGEMENT:

- Eel stocks in both Lithuania and Latvia should be considered and accordingly managed as consisting of two differently originated populations: naturally recruited, which predominate in the coastal waters and restocked, which predominated in the inland lakes;
- Eel stock managers in Lithuania and Latvia should use precautionary approach and avoid mixing of natural stocks with restocked, i.e. should not stock water sites where natural recruits prevail using transported glass eels from other areas of the distribution range to avoid possible genetic over mixing of “local populations” with restocked eels;
- The abilities of restocked eels to contribute to reproduction (to reach spawning grounds and spawn successfully) is disputable; therefore stock protection should focus on naturally recruited eels primarily;
- Data obtained during the study concerning eel stock structure, recruitment timing, growth rate, maturation, abiotic and biotic effects on eel growth should be used preparing European eel management plans in Lithuania and Latvia; both countries are obligated by European Union to complete plans until the end of 2008;
- Perch and pikeperch stocks should be considered as one single stock and managed accordingly; the study failed to find two different “migrating” and “non-migrating” subpopulations;
- The study denied hypothesis about “migrating” pikeperch spawning grounds in the northern part of the Curonian Lagoon and this area should be managed as pikeperch concentration area but not spawning ground; spawning grounds in the area of the Curonian Lagoon where found in Nemunas river delta only.
- Perch and pikeperch stocks between Lithuania and Latvia are isolated by means of distance and should be managed as separate stocks.

The collaborative research study between Lithuania, Latvia and Taiwan (ROC) contributed to integration and promotion of cooperation between the regions and scientists involved into the study and will result continuous cooperation, generating new research ideas and series of new scientific publications based on GMM project results. The knowledge obtained within GMM *project provided the European eel and Percid fish management by knowledge of primary importance.*

Acknowledgement

We are grateful to Lithuania-Latvia-Taiwan (Republic of China) Mutual Fund for the financial support of the collaborative research project between the countries.

計畫成果自評：本三年期計畫的成果符合當初的構想，對於目標魚種的人工放流，洄游生活史以及族群結構等相關問題提出充分的解釋，目前為止共有4篇報告發表在SCI的期刊上，另外尚有兩篇報告正在準備中，預計總共最少會有6篇報告發表在SCI的期刊。
以下為已經發表的報告：

1. 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. Journal of Fish Biology 69: 749–769.

2. Sruoga A, Rashal I, Butkauskas D, Lozys L (2007) Variety of mtDNA haplotypes in the populations of the european perch (*Perca fluviatilis*) of the curonian lagoon, the coastal zone of the Baltic Sea and the Gulf of Riga. PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B, Vol. 61 (2007), No. 3/4 (650/651), 91–95.

3. Lin YJ, Lozys L, Shiao JC, Iizuka Y, Tzeng WN (2006) Growth differences between naturally recruited and stocked European eel *Anguilla anguilla* from different habitats in Lithuania. Journal of Fish Biology 71: In press.

4. 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. In press.

Journal of Fish Biology (2006) **69,** 749–769
doi:10.1111/j.1095-8649.2006.01147.x, available online at http://www.blackwell-synergy.com

Migratory patterns and contribution of stocking to the population of European eel in Lithuanian waters as indicated by otolith Sr:Ca ratios

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(Received 24 October 2005, Accepted 22 March 2006)

Otolith Sr:Ca ratios were examined to evaluate the contribution of the stocked eel *Anguilla anguilla* elvers, which have been stocked in Lithuanian waters and mixed with naturally recruited eels for several decades, to the native eel population. Stocked eels were identified by the freshwater signature (Sr:Ca ratios $<2{\cdot}24 \times 10^{-3}$) on the otolith after the glass eel stage. Naturally recruited eels, that had migrated through the North and Baltic Seas, were characterized by an extended seawater and brackish-water signature (Sr:Ca ratios $>3{\cdot}23 \times 10^{-3}$) after the glass eel stage. Of 108 eels analysed, 21 eels had otolith Sr:Ca ratio profiles consistent with stocking while 87 showed patterns of natural recruitment. The ages of naturally recruited eels arriving in Lithuanian fresh waters varied from 1 to 10 years, with a mean ± S.D. age of 5·2 ± 2·1 years. Eels from the inland Lake Baluošai were all freshwater residents of stocked origin. Stocked eels, however, accounted for only 20% of the eels from the Curonian Lagoon and 2% of eels sampled in Baltic coastal waters. This finding does not support the hypothesis that the eel fishery in the Curonian Lagoon depends mostly on stocking.

Key words: *Anguilla anguilla*; European eel; migratory history; otolith microchemistry; stocking.

INTRODUCTION

Catadromous eels are commercially valuable species and support worldwide eel aquaculture and eel fisheries. The abundance of several species, *e.g. Anguilla anguilla* (L.), *Anguilla rostrata* (Lesueur) and *Anguilla japonica* Temminck & Schlegel, have declined throughout their distribution ranges due to overfishing, anthropogenic activities and changes of oceanic currents induced by global weather anomalies (Castonguay *et al.*, 1994; Dekker, 2003; Tatsukawa, 2003).

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In addition, the eel's mysterious life cycle further compounds its fate and makes conservation and recovery more difficult.

Anguilla anguilla inhabits coastal waters, estuaries, rivers and lakes in Europe and North Africa (Bertin, 1956; Tesch, 2003). Their leaf-like larvae, leptocephali, drift *via* the Gulf Stream and North Atlantic Current from oceanic spawning grounds in the Sargasso Sea to the continental shelves of Europe and North Africa or enter the Mediterranean Sea. The larvae metamorphose into glass eels on the continental shelf. Glass eels become pigmented elvers when they enter estuaries. Some elvers stay in salt or brackish water along the coast while others migrate upriver. Major elver runs occur into the Atlantic-facing estuaries of France, Spain, Portugal and the Bristol Channel and River Severn estuary in the U. K. Lesser migrations occur to other countries in the Baltic and Mediterranean Seas (Knights & White, 1998).

Declines in eel recruitment in Scandinavia have been noted since the 1940s (Moriarty, 1996), but the greatest decreases in the recruitment of eels throughout Europe have occurred since the early 1980s (Dekker, 2000). Recent recruitment of *A. anguilla* glass eel was estimated to be only 1% of the level before the 1980s (Dekker, 2004). Poor natural recruitment from the oceanic migration phase, exacerbated by habitat degradation, pollution, artificial physical barriers to migration and high fishing pressure on glass eels (Moriarty & Dekker, 1997) has led to the need for stocking to maintain, enhance, restore or establish stocks. Intensive stocking programmes have been undertaken in the Baltic Sea region over the past 50 years. The most intense stocking programmes have been implemented in the Baltic Sea drainage using eels originating from western Europe.

The first eel stockings in Lithuania occurred between 1928 and 1939 when 3·2 million elvers were released into lakes of the Vilnius region (*c.* 300 km from the Baltic coast). Since the mid 1960s, Lithuanian lakes have been stocked with *c.* 50 million elvers or young yellow eels at an average stocking rate of 1·1 million eels yearly (Ložys, 2002). Studies on stocking effectiveness, however, have not been carried out and the post-stocking movements of stocked eels remain largely unknown. Natural recruitment is unknown due to the lack of suitable locations to monitor recruitment in the main area of invasion, the Klaipėda strait (Curonian Lagoon). Therefore, statements by fishers and fisheries managers that the Curonian Lagoon eel fishery depends on eel stocking are speculative. The degree to which stocked and naturally recruited eels contribute to the eel fishery and eventually to the spawning stock is unknown. In the absence of tagging, it is difficult to discriminate stocked eels from naturally recruited eels since they are morphologically similar. Fish tagging can help identify different stocks, but most conventional tagging is not feasibly applied to small glass eels. Internal marking of the otolith, such as by tetracycline or alizarin complexone, is feasible (Tsukamoto, 1988). Most stocking programmes, as indicated by Cowx (1999), however, were carried out without evaluation of their potential success. Marks were not applied to the stocked eels before their release into lakes or the lagoon. The analysis of a natural tag, the otolith strontium (Sr):calcium (Ca) ratio, has been extensively used to study the migratory history of diadromous fishes and provides alternative resolution to discriminate stocked eel from naturally recruited eel (Elsdon & Gillanders, 2003).

The metabolically inert otolith records biological as well as environmental information throughout the fish's life. Fishes can absorb Sr in the ambient water and substitute for Ca in the process of $CaCO_3$ deposition in the otolith. The positive relationship between salinity and otolith Sr:Ca ratios has been validated for different species including eels (Tzeng, 1996; Secor *et al.*, 1998; Kraus & Secor, 2003). Accordingly, seawater-resident fish uptake and deposit more Sr in the otolith than do freshwater fishes. Otolith Sr:Ca ratios in combination with age data have been used to elucidate the migratory environmental history of diadromous fishes, including anguillids (Tsukamoto & Arai, 2001; Jessop *et al.*, 2002; Shiao *et al.*, 2003).

Elvers purchased in the U. K. and France are directly released into Lithuanian freshwater lakes and the Curonian Lagoon. These stocked eels do not experience the long migratory journey through the North and Baltic Seas and thus they should show a freshwater signature of low otolith Sr:Ca ratios immediately after the elver stage. In contrast, eels naturally recruited to Lithuania must pass the North and Baltic Seas and should show an extended seawater and brackish-water signature of high (North Sea) and intermediate (Baltic Sea) otolith Sr:Ca ratios after the elver stage. Thus, a life-history scan of otolith Sr:Ca ratios should be able to discriminate between both stocked and naturally recruited eels. Clarification of the eel's migratory history will also help to evaluate the contribution and interaction of the two possible eel origins (stocked or naturally recruited) to each population along the Baltic coasts and in the Curonian Lagoon and inland lakes.

MATERIALS AND METHODS

FISH COLLECTION AND SAMPLING SITES

Silver and yellow-stage *A. anguilla* were collected by fyke nets and long lining from Baltic coastal waters, the Curonian Lagoon in western Lithuania and the freshwater Lake Baluošai in eastern Lithuania in 2003–2004 (Fig. 1). The lake is *c.* 300 km from the Curonian Lagoon and *c.* 350 km from the Baltic Sea to which it is connected *via* a system of small streams, lakes, the River Nemunas and the Curonian Lagoon. Natural recruitment to these lakes has never been reported and may not occur; however, the possibility cannot be excluded. Elvers have been regularly stocked since 1960 into the system of lakes in the Baluošai Lake region.

The shallow Curonian Lagoon (mean depth 3·7 m) is separated by a narrow sand spit (0·5–4·0 km wide) from the Baltic Sea and is connected to the Baltic Sea through the narrow (0·5 km wide) Klaipėda Strait. The salinity of the Baltic Sea adjacent to Lithuania varies from 4·9 to 6·8 (Dubra & Dubra, 1998). The lagoon is 1584 km^2 in area and is a freshwater basin. Rivers supply the lagoon with *c.* 3·6 times more fresh water than the water volume in the lagoon itself and the mean water level in the lagoon is 15 cm higher than sea level. Therefore, brackish water penetration into the lagoon is rare. The salinity fluctuates from 0·03 in the southern part of the lagoon up to 2·7 in the Klaipėda Strait. During stormy inflows of brackish water, the salinity may episodically rise to 5–6 in the northern areas (Olenin, 1996). The Curonian Lagoon was stocked by young yellow eels during 1996–1997 (43 000), 2000–2003 (10 000) and by elvers in 1995 (150 000) and 2003 (60 000).

The total length (L_T) and mass (M) of each eel was measured to the nearest 1·0 mm and 1·0 g. Sexes were determined macroscopically from the gross morphology of the gonads, where eels with thin, regularly lobed organs (Syrski's organ) were considered

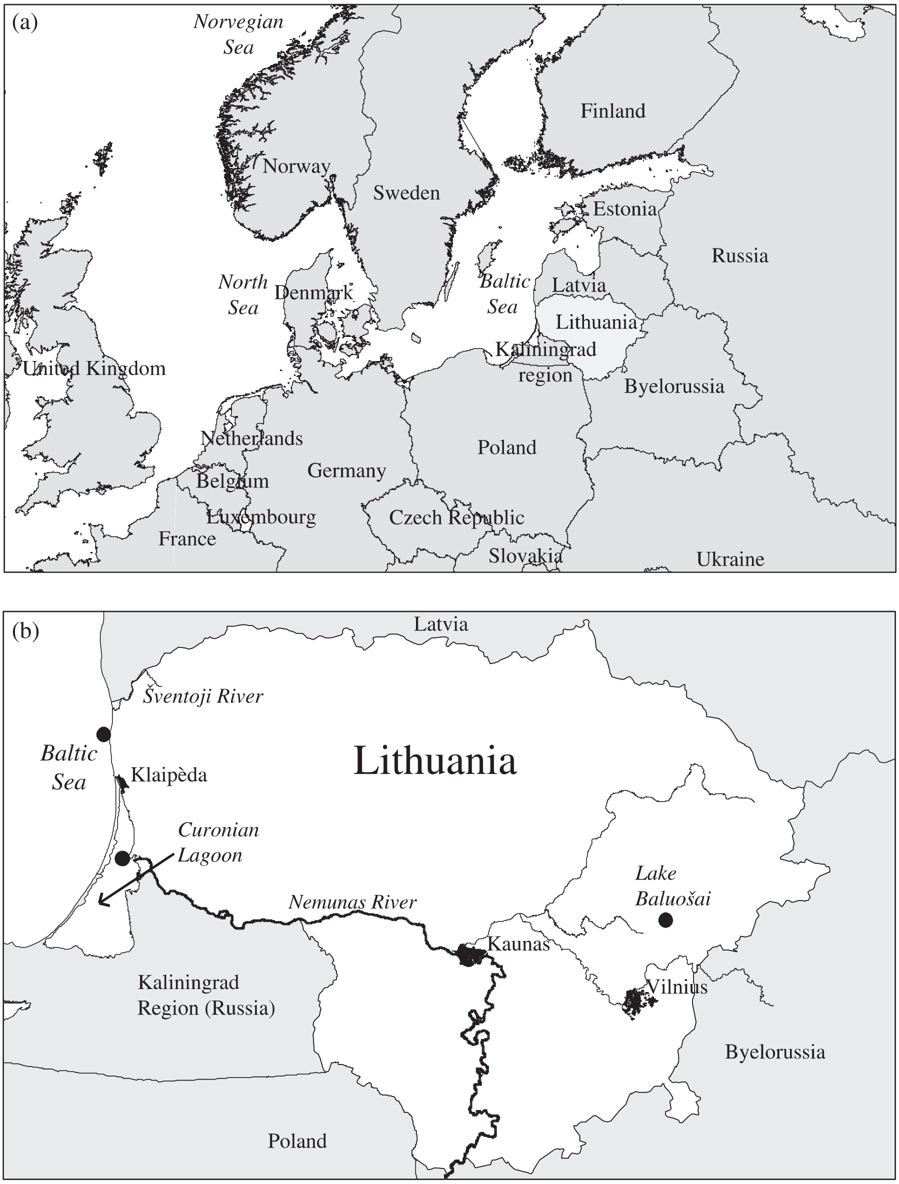

FIG. 1. Sampling locations: (a) northern Europe, showing the North and Baltic Seas and (b) sampling locations (●) in the Baltic Sea, Curonian Lagoon and Lake Baluošai.

males, while individuals with more broad and folded curtain-like gonads were females (Tesch, 2003). The eels were classified as yellow and silver eels, by their external colour, fin shape and eye size.

Water Sr and Ca concentrations around the eel sampling locations were determined by atomic absorption spectrophotometer (Hitachi Z-5000). Standard solutions (Merck, Darmstadt, Germany) were used to make the standard curve. Sr and Ca concentrations of the water collected from the Baltic coast (salinity 5·8) were *c.* $1{\cdot}67 \times 10^{-5}$ and $2{\cdot}60 \times 10^{-3}$ M, respectively ($6{\cdot}44 \times 10^{-3}$ for the Sr:Ca ratio). Water collected in the Curonian Lagoon (salinity 0) contained *c.* $1{\cdot}36 \times 10^{-6}$ M of Sr and $1{\cdot}51 \times 10^{-3}$ M of Ca ($0{\cdot}90 \times 10^{-3}$ for the Sr:Ca ratio). Water Sr and Ca concentrations in the Baltic coast and Curonian Lagoon were in the range of normal brackish and fresh water.

OTOLITH PREPARATION AND SR:CA ANALYSIS

The largest pair of eel otoliths (sagittae) was removed, dried in air, embedded in Epofix resin, ground and polished until the core was exposed. For electron probe microanalysis, the polished otoliths were coated with carbon under a high-vacuum evaporator. Sr and Ca concentrations in the otolith were measured from the otolith core to the edge at 10 μm intervals. Quantitative analyses were conducted with an electron probe microanalyzer (JEOL JXA-8900R), using beam conditions of 15 kV for the acceleration voltage, 3 nA for the current, and a 5 × 4 μm rectangular scanning beam. The quantitative data were corrected by the PRZ (phi-rho-z) method to calculate oxide compositions (Goldstein *et al.*, 1984; Reed, 1993). The peak concentration of Sr Lα was counted for 80 s with background measurements for 20 s on each side. The peak concentration of Ca Kα was counted for 20 s and each background for 10 s. A synthesized aragonite ($CaCO_3$) and strontianite [$(Sr_{0{\cdot}95}Ca_{0{\cdot}05})\ CO_3$; NMNH R10065] were used as calibration standards. Since aragonite-structure carbonates are similar to otoliths, the standards have smaller matrix corrections than other types of standards such as oxide or silicate (Jarosewich & White, 1987). The standards were mounted in epoxy resin and polished. The carbon coating for the standards and otoliths had the same thickness (25–35 nm). After microchemical analysis, the otolith was polished to remove the carbon layer, then etched with 5% EDTA for 1–2 min to reveal the annual rings for age determination (Fig. 2). The duration of the eel in fresh waters and sea and brackish waters was estimated by relating the otolith Sr:Ca ratio profile to the otolith annuli.

DATA ANALYSIS

Data are expressed as means ± S.D. (n = number of fish). Statistical differences among groups (locations) were evaluated by one-way ANOVA or Mann–Whitney rank sum test. Differences among groups were identified by Tukey's pair-wise multiple comparison test. Significance was set at $P < 0{\cdot}05$.

RESULTS

TOTAL LENGTH, BODY MASS, AGES AND SEXES OF THE EELS AMONG LOCATIONS

All 48 eels collected in the coastal waters of the Baltic Sea were at the yellow eel stage as were the 49 eels collected at the Curonian Lagoon, with the exception of one silver eel (Table I). All 10 eels collected in the Lake Baluošai were migrating silver eels. All eels collected in the three sites were all females except one male in Lake Baluošai. There were no significant differences in mean L_F ($F_{2,105}$, $P > 0{\cdot}05$) and M ($F_{2,105}$, $P > 0{\cdot}05$) among sampling locations. The mean ages of the eels from Lake Baluošai, however, were significantly greater than those from the Curonian Lagoon and the Baltic coast ($F_{2,105}$, $P < 0{\cdot}001$). This implied that the eel grew faster in the Baltic coasts and Curonian Lagoon than in Lake Baluošai.

LIFE-HISTORY SCAN OF OTOLITH SR:CA RATIOS

Sr:Ca ratios in eel otoliths increased from *c.* 8–10 × 10^{-3} in the core to a peak of *c.* 18–24 × 10^{-3} *c.* 60–100 μm from the core. Otolith Sr:Ca ratios

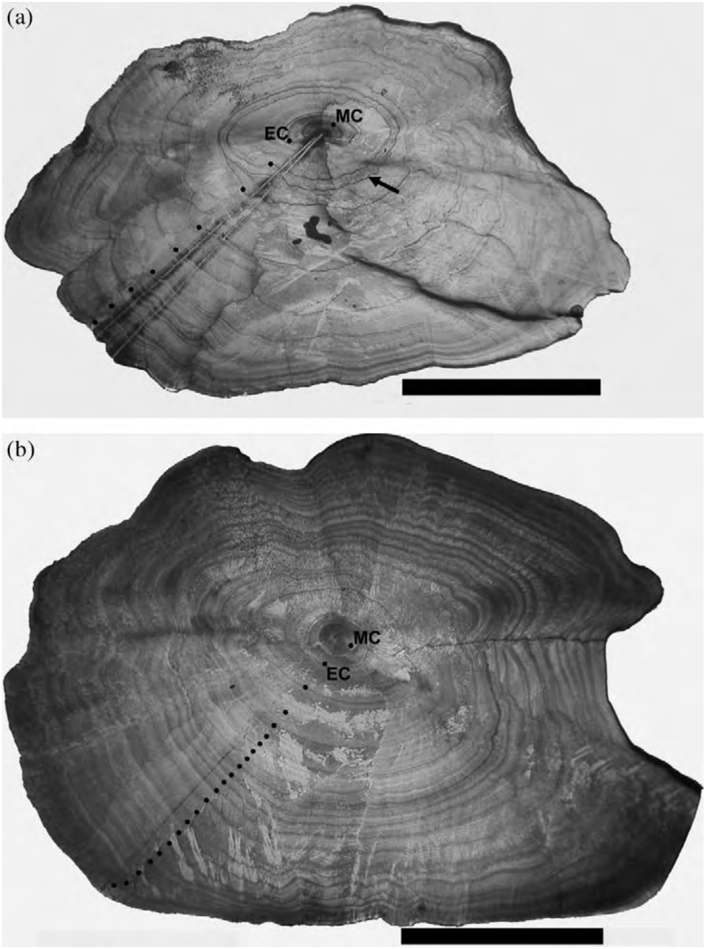

FIG. 2. Otoliths of *Anguilla anguilla* showing the metamorphosis check (MC), elver check (EC) and annuli (•••). (a) Stocked eel number 3 (yellow eel, 63 cm total length, L_T) collected from Curonian Lagoon, showing rapid growth as inferred from wide otolith annuli. A conspicuous check (→) fused with the second annuli was regarded as a false annulus. (b) Stocked eel number 105 (silver eel, 74 cm L_T) collected from Lake Baluošai showing slow growth inferred from narrow annuli. Scale bar = 1 mm.

then sharply decreased (Figs 3–6), which corresponded to the metamorphosis from leptocephalus to glass eel (Arai *et al.*, 1997). Otolith Sr:Ca ratios before the elver stage were similar among individuals since the eels have similar migratory histories at the leptocephalus and glass eel stages. The patterns of otolith Sr:Ca ratios beyond the elver stage were variable, indicating diverse migratory histories during the yellow eel to silver eel stages. The migratory patterns of the eels were classified as follows.

Freshwater pattern

There were 16 eels (10 from Lake Baluošai and six from the Curonian Lagoon), which showed consistently low otolith Sr:Ca ratios from the elver

TABLE I. Biological characteristics (mean ± s.d.) of *Anguilla anguilla* collected from Lithuanian sites

Sampling location	Sampling period	Developmental stage	n*	Mean ± s.d. and range		
				L_T (cm)	Body mass (g)	Age (years)
Baltic coasts	June to September	Yellow eel	48	63·0 ± 7·3 (47·5–81·0)	582·4 ± 274·6 (180·0–1400·0)	11·0 ± 1·8 (8–16)
Curonian Lagoon	June to August	Yellow eel except one silver eel	50	66·3 ± 10·4 (49·0–92·0)	691·4 ± 441·7 (201·0–2126·0)	10·8 ± 1·7 (6–15)
Lake Baluošai	April	Silver eel	10	64·7 ± 11·0 (43·3–80·0)	519·9 ± 266·2 (127·0–930·0)	19·0 ± 3·0 (15–24)

*All female except one male in Lake Baluošai.
n, sample size; L_T, total length.

and through their life span (Fig. 3). No eels collected in the Baltic Sea had a pattern of consistently low otolith Sr:Ca ratios. The mean otolith Sr:Ca ratio of these 16 eels after the elver stage was $0{\cdot}72 \pm 0{\cdot}76 \times 10^{-3}$, which is consistent with previous studies on European and American eels (Tzeng *et al.*, 1997; Cairns *et al.*, 2004). This pattern suggested that these eels resided in fresh water from the elver to the yellow or silver eel stage.

Seawater and brackish-water pattern

Twenty-three eels (48%) collected in the Baltic coastal waters had otolith Sr:Ca ratios consistently $>3 \times 10^{-3}$ between the elver check and the otolith edge (Fig. 4). The consistently high otolith Sr:Ca ratios suggested that these

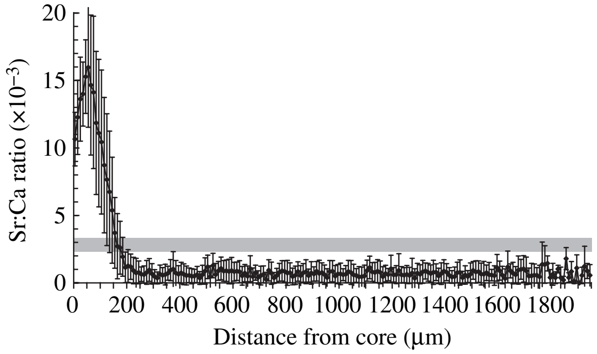

FIG. 3. Transects of mean ± s.d. otolith Sr:Ca ratios ($0{\cdot}72 \pm 0{\cdot}76 \times 10^{-3}$) from 16 freshwater-resident eels, illustrating consistent low values after the glass eel stage. Eels were collected from the Curonian Lagoon ($n = 6$) and Lake Baluošai ($n = 10$). The transition of freshwater and sea and brackish residence (▬) is indicated.

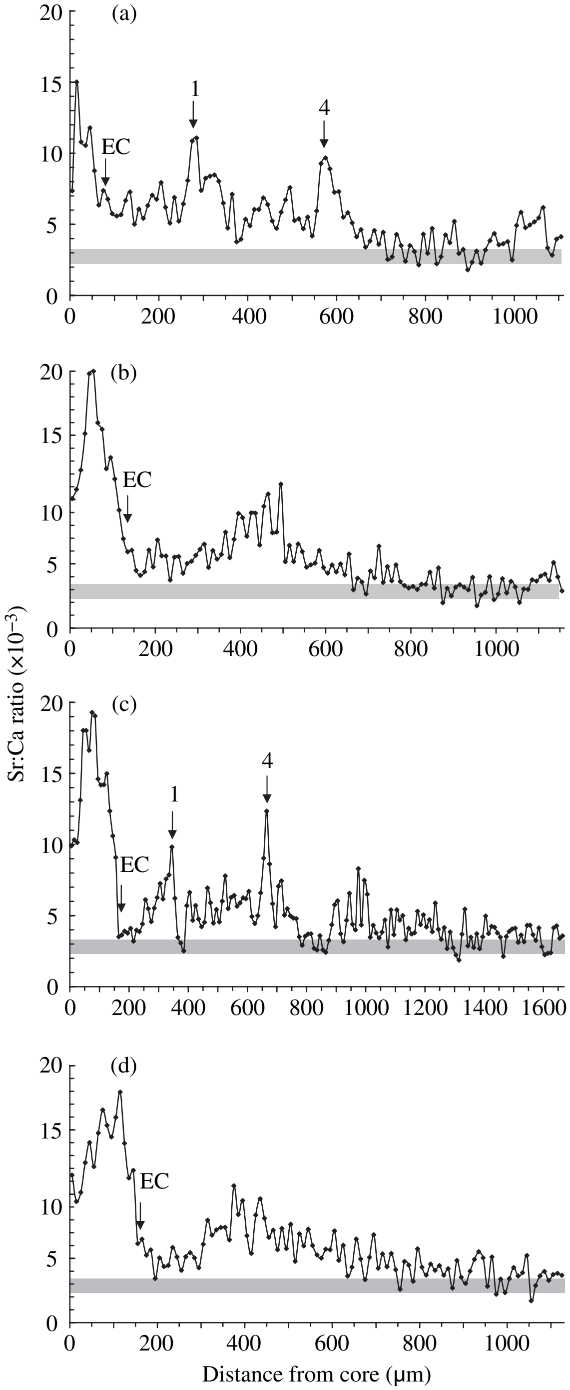

FIG. 4. Otolith Sr:Ca ratio transects illustrating some eels with seawater and brackish-water residence after the glass eel stage. ↓ and 1 and 4, the annuli corresponding to the peaks of Sr:Ca ratios; EC, elver check. Eels were collected from the Baltic Sea: (a) number 34, 55·5 cm total length, L_T, age

eels resided in brackish or sea waters without entering fresh water from the elver stage through to the yellow eel stage. Eels with this kind of otolith Sr:Ca ratio pattern were considered to be seawater and brackish-water eels (Tsukamoto *et al.*, 1998; Tzeng *et al.*, 2000). The mean otolith Sr:Ca ratio from the elver check to the otolith edge of the seawater–and brackish water–resident eels was $4·84 \pm 1·61 \times 10^{-3}$, which was significantly higher than that of the freshwater-resident eels ($0·72 \pm 0·76 \times 10^{-3}$) (Fig. 3). Therefore, the mean otolith Sr:Ca ratios of the eels collected in the freshwater Lake Baluošai and in the Baltic Sea were used as criteria to classify different migratory environmental histories of the eel. Eels with otolith Sr:Ca ratios $<2·24 \times 10^{-3}$ (mean otolith Sr:Ca ratios of 16 freshwater eels + 2 S.D.) were considered as freshwater residents while eels with ratios $>3·23 \times 10^{-3}$ (mean otolith Sr:Ca ratios of 23 seawater and brackish-water eels − 1 S.D.) were considered as seawater and brackish-water residents. Intermediate values were regarded as a transition between fresh and sea water. No eels collected in the Curonian Lagoon demonstrated the seawater and brackish-water pattern; all showed freshwater residency ($<2·24 \times 10^{-3}$) for all or part of their life span.

Some seawater–and brackish water–resident eels showed relatively high otolith Sr:Ca ratios between the elver and the yellow eel stages and gradually decreased to lower otolith Sr:Ca ratios in the later part of the yellow stage (Fig. 4). For example, eel number 34 had higher Sr:Ca ratios ($5–12 \times 10^{-3}$) before 600 μm (age 5 years) and lower ratios ($3–5 \times 10^{-3}$) between 700 and 1000 μm (age 6–10 years) [Fig. 4(a)]. Eel number 44 showed otolith Sr:Ca ratios that decreased from $6–10 \times 10^{-3}$ at 350 μm (age 2 years) to $3–4 \times 10^{-3}$ *c.* 800 μm (age 8 years) [Fig. 4(d)]. Decreasing trends of otolith Sr:Ca ratios were found in 20 seawater and brackish-water eels that indicated a habitat shift from high to low salinity by these eels (Figs 4 and 5). The pooled profile of these 20 seawater–and brackish water–resident eels showed a relatively large mean otolith Sr:Ca ratio ($5·51 \pm 1·57 \times 10^{-3}$, range $4–8 \times 10^{-3}$, $n = 1240$) between 160 and 770 μm from the core and a small otolith Sr:Ca ratio ($3·64 \pm 1·10 \times 10^{-3}$, range $3–5 \times 10^{-3}$, $n = 1119$) between 780 and 1500 μm (Fig. 7). The gradient of mean otolith Sr:Ca ratios (Fig. 7) may reflect the migratory history of the eel from the full-strength sea water in North Sea to the brackish waters in the south-eastern Baltic coasts.

Patterns of interhabitat shifters

Most eels caught in the Curonian Lagoon (88%) and half from the Baltic Sea (52%) migrated between habitats (Table II), *i.e.* their otolith Sr:Ca ratios fluctuated between freshwater and seawater levels. Most interhabitat shifters (eels migrating between fresh water and sea and brackish water) showed high otolith Sr:Ca ratios ($>3·23 \times 10^{-3}$) for several years after the elver stage that

FIG. 4. (Continued) 11 years, (b) number 39, 65·5 cm L_T, age 13 years, (c) number 41, 70 cm L_T, age 14 years and (d) number 44, 57·5 cm L_T, age 14 years. The transition of freshwater and sea and brackish residence (▬) is indicated.

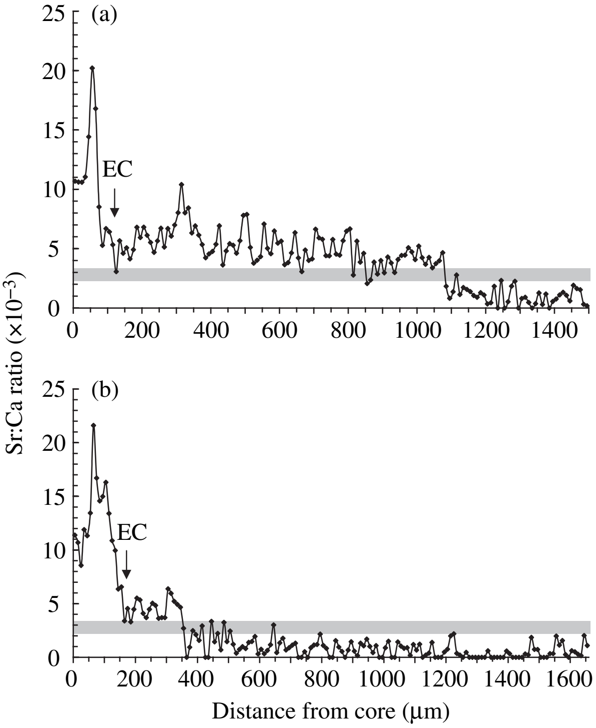

FIG. 5. Otolith Sr:Ca ratio transects illustrating eel movement into fresh water after a period of seawater and brackish-water residence: (a) eel number 19, 62·4 cm total length, L_T, age 13 years and (b) number 4, 63·8 cm L_T, age 9 years. (c) Eel number 15, 83 cm L_T, age 11 years demonstrates a gradual decline of the otolith Sr:Ca ratio profile corresponding to the movement from high salinity through low salinity to fresh water. (d) Eel number 1, 56 cm L_T, age 11 years, shows seasonal migration between high salinity and low salinity or fresh waters. ↓ and 1–10, the annuli corresponding to peaks in the otolith Sr:Ca ratios; EC, elver check. (e) The X-ray intensity mapping displays high Sr content from age 1 to 5 years and high Sr rings at annuli 6–10 in eel number 1. All eels were collected from the Curonian Lagoon. The transition of freshwater and sea and brackish residence (▬) is indicated.

were then followed by relatively low ($<2{\cdot}24 \times 10^{-3}$) or fluctuating ratios between the freshwater and the seawater levels (Fig. 5). Eel number 1 resided in sea and brackish water for the first 5 years (otolith Sr:Ca ratios range: 4–12×10^{-3}) and invaded fresh water at age 5 years [Fig. 5(d)]. This eel showed seasonal peaks in otolith Sr:Ca ratios at each annulus, suggesting that it wintered in brackish water but spent the remainder of each year in fresh water (Tzeng *et al.*, 1997, 1999). The coincidence of otolith Sr:Ca ratio peak and annulus was also found in other eels, *e.g.* number 4, 34 and 41 (Figs 4 and 5). In light of the high plasticity of eel phenotypes, an explicit description or classification of the diversified migratory behaviours is not feasible and seems not necessary. Briefly, these varying patterns suggested that the eels recruited to fresh water and resided there until being caught or moved seasonally and irregularly between fresh and sea water.

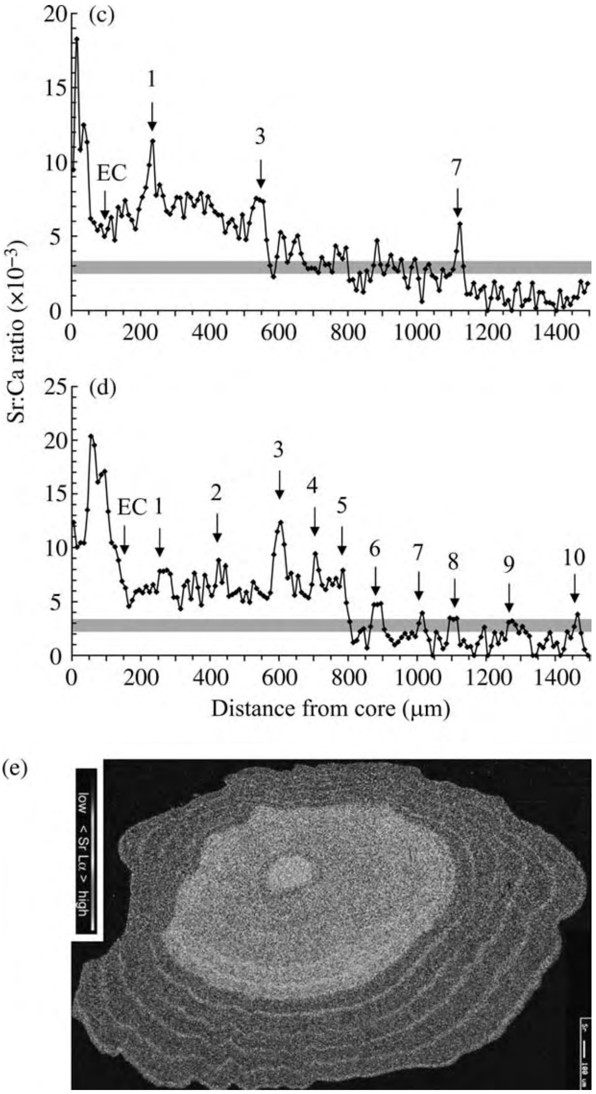

FIG. 5. Continued.

MIGRATORY PATTERNS AND ORIGIN OF THE EELS AMONG LOCATIONS

The eels collected along the Baltic coast were either seawater–and brackish water–resident eels (48%) or interhabitat shifters (52%); none were freshwater eels (Table II). The eels collected from the Curonian Lagoon were primarily interhabitat shifters (88%) while freshwater-resident eels accounted for *c.* 12% (Table II). Eels from the freshwater Lake Baluošai were all freshwater eels (Table II).

The presence or absence of an extended seawater and brackish-water signature (otolith Sr:Ca ratios $>3{\cdot}23 \times 10^{-3}$) after the glass eel stage was used to

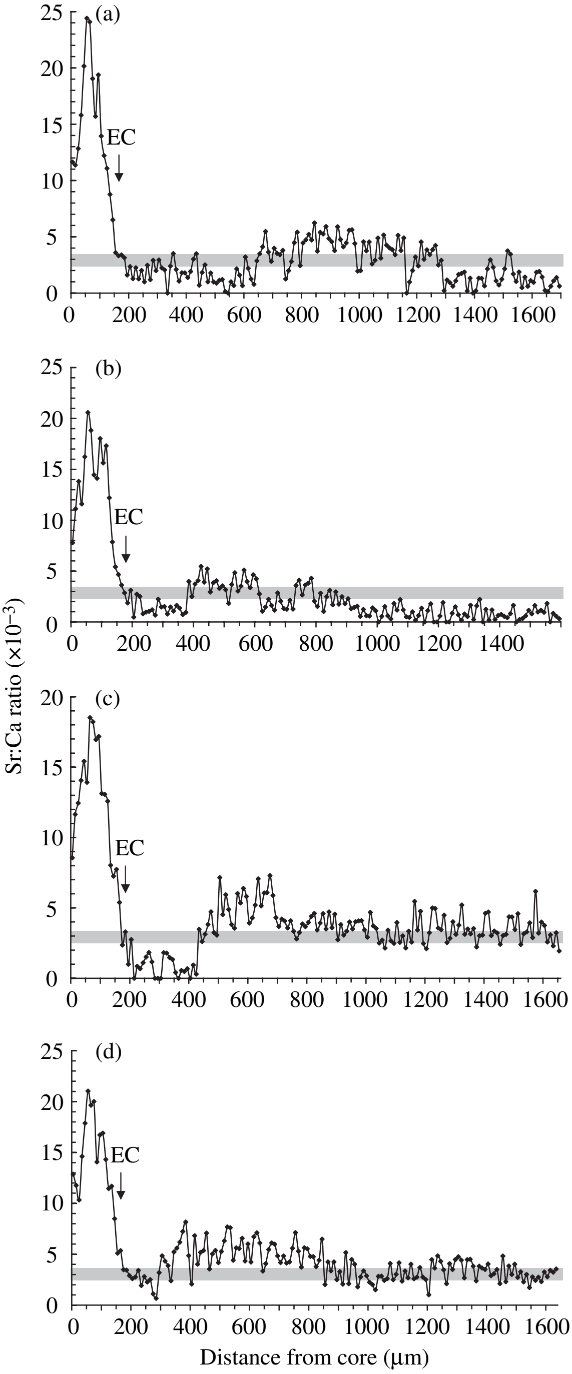

FIG. 6. Otolith Sr:Ca ratio profiles of interhabitat shifters. Otolith Sr:Ca ratio transects illustrating movement between fresh water and sea and brackish water: (a) eel number 5, 62·3 cm total length, L_T, age 11 years and (b) number 7, 74·5 cm L_T, age 10 years (eels number 5 and 7 were collected from

distinguish stocked eels from naturally recruited eels. The six eels from the Curonian Lagoon and 10 eels from Lake Baluošai showing a consistent freshwater signature throughout their life after the glass eel stage must have been the stocked eels. Another 85 eels with extended large otolith Sr:Ca ratios after glass eel stage should have been the naturally recruited eels if the stocked elvers had not migrated immediately to sea water.

In a few interhabitat shifters ($n = 7$), a period of low otolith Sr:Ca ratios ($<2{\cdot}24 \times 10^{-3}$) appeared before the extended high otolith Sr:Ca ratio ($>3{\cdot}23 \times 10^{-3}$) making it difficult to tell whether these seven eels were stocked eels or naturally recruited eels (Fig. 6). This may indicate that the eels had invaded fresh water at the elver stage for a period of time, then returned to sea and brackish water. Eels number 5, 6 and 7 resided in fresh water for *c.* 2 years, returned to sea and brackish waters for 1–4 years, and then moved back to fresh water [Fig. 6(a), (b)]. Eels number 14, 45 and 100 resided in fresh water for *c.* 1–4 years and subsequently returned to sea and brackish waters for their remaining life [Fig. 6(c), (d)]. Differences in their migratory histories were evident. Eels number 45 and 100, collected on the Baltic coast, [Fig. 6(c), (d)] showed relatively high otolith Sr:Ca ratios (400–700 μm for eel number 45 and 350–800 μm for eel number 100) at the early stage and a decreasing trend in otolith Sr:Ca ratio in the later stage, which is very similar to the pattern of naturally recruited eels. Therefore, eels number 45 and 100 might be naturally recruited eels that have entered fresh water somewhere prior to entering Lithuanian waters. In contrast, eels number 5, 6, 7 and 14 collected in the Curonian Lagoon [Fig. 6(a), (b)] and eel number 60 from Baltic coast might be stocked eels that stayed in a fresh water for a few years then returned to the Baltic coast or migrated between both sites. This interpretation was based on otolith Sr:Ca ratios for the seawater and brackish-water signature that were smaller, intermittent or shorter than that for naturally recruited eels and that showed no decline in otolith Sr:Ca ratios. Overall, 87 individuals were naturally recruited eels while 21 individuals were stocked eels among the samples collected in the Curonian Lagoon ($n = 10$), Baltic coasts ($n = 1$) and Lake Baluošai ($n = 10$) (Table III). Stocked eels accounted for *c.* 20% of the eels in the Curonian Lagoon and 2% on the Baltic coast; however, the eels in the Lake Baluošai were 100% of stocked origin (Table III).

THE AGES ON ARRIVAL IN LITHUANIAN WATERS

Sixty-three naturally recruited eels initially entered fresh water at ages of 1–10 years with a mean age of 5·2 ± 2·1 years (Fig. 8). This implies that after reaching the Baltic, the eels spent a number of years in marine and brackish waters before entering fresh water. Twenty-two of these 63 eels showed consistent low otolith Sr:Ca ratios after extended high Sr:Ca ratios, indicating that they

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FIG. 6. (Continued) the Curonian Lagoon) and rapid entry into freshwater at the elver stage (EC, elver check) then a return to sea and brackish water; (c) eel number 45, 64 cm L_{T}, age 12 years and (d) eel number 100, 81 cm L_{T}, age 12 years (eels number 45 and 100 were collected from the Baltic Sea). The transition of freshwater and sea and brackish residence (▬) is indicated.

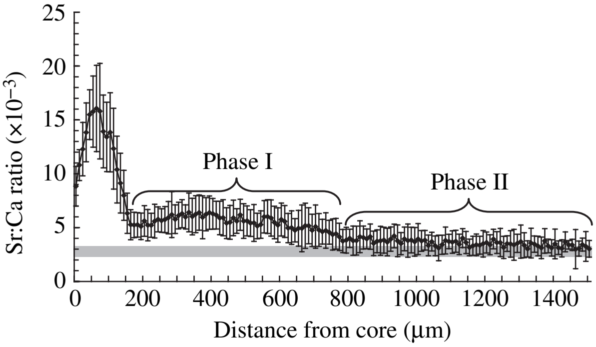

FIG. 7. The mean ± S.D. otolith Sr:Ca ratio profile of 20 European eels that showed a gradual decline pattern, indicating the movement from the full strength of salinity in the North Sea into brackish water of the Baltic Sea (phase I: mean Sr:Ca ratio = 5·51 ± 1·57 × 10^{-3}, $n = 1240$) and ultimately arrived at the coastal waters of Lithuania (phase II: mean Sr:Ca ratio = 3·64 ± 1·10 × 10^{-3}, $n = 1119$). The transition of freshwater and sea and brackish residence (▬) is indicated.

continuously resided in fresh water after freshwater entry [Fig. 5(a), (b)]. Mean age of these 22 eels at freshwater entry was 4·6 ± 2·2 years [Fig. 8(b)], not significantly different from the mean ± S.D. ages of the other 41 eels (5·6 ± 1·9 years) that entered fresh water but showed subsequent movements between fresh and sea and brackish waters ($P = 0·16$, Mann–Whitney rank sum test). This indicated these eels spent several years in the Baltic Sea then entered fresh water at the area of capture. After the initial freshwater entry, the eels spent 5–6 years (range: 1–10 years) in the Curonian Lagoon or Baltic coast before capture (Fig. 8).

DISCUSSION

INTERPRETATION OF EEL MIGRATION BY OTOLITH SR:CA RATIOS

Bath *et al.* (2000) and Kraus & Secor (2004) pointed out that it was Sr:Ca ratios in the water rather than salinity that primarily determined the incorporation

TABLE II. Migratory patterns of *Anguilla anguilla* as inferred from otolith Sr:Ca ratios. Freshwater residents, Sr:Ca ratios consistently <2·24 × 10^{-3}; seawater and brackish-water residents, Sr:Ca ratios consistently >3·23 × 10^{-3}; interhabitat shifters, otolith Sr:Ca ratios covering the ranges of freshwater and seawater values

		Migratory patterns of the eels (%)		
Habitat	Sample size	Freshwater residents	Seawater and brackish-water residents	Interhabitat shifters
Baltic coast	48	—	48	52
Curonian Lagoon	50	12	—	88
Lake Baluošai	10	100	—	—
Total	108	14·8	21·3	63·9

TABLE III. Relative contribution of stocked and naturally recruited *Anguilla anguilla* in different habitats

		Origin of the eel (%)	
Habitat	Sample size	Stocked	Naturally recruited
Baltic coasts	48	2	98
Curonian Lagoon	50	20	80
Lake Baluošai	10	100	—
Total	108	19	81

of Sr into the otolith. This finding implied that otolith Sr:Ca ratios, as the proxy of salinity, should be interpreted based on knowledge of the ambient water chemistry. This is because fishes living in Sr-rich fresh water may incorporate Sr at the same level or even higher than the fishes living in normal sea and brackish water. Kraus & Secor (2004), however, also admitted that natural Sr-rich fresh water, if it can be found, is rarely seen. Generally, Sr is *c.* 100-fold greater in sea water ($8{\cdot}7 \times 10^{-5}$ M) than in fresh water (9×10^{-7} M) (Campana,

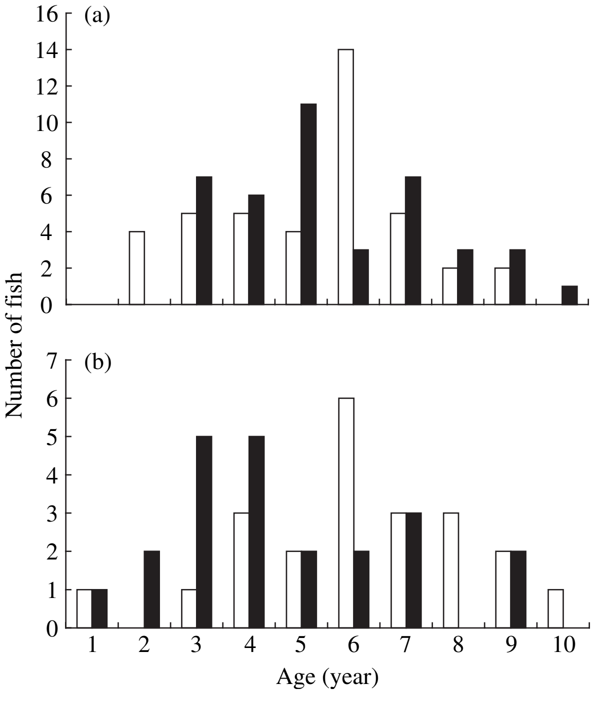

FIG. 8. The naturally recruited eels showed a variable range of the ages at first freshwater entry (■) as well as the duration between first freshwater entry and capture (□). (a) The eels showed more than one freshwater entry. The mean ± S.D. age at the first freshwater entry was 5·6 ± 1·9, years ($n = 41$) (■) and the age between the first freshwater entry and the capture was 5·3 ± 1·9, years ($n = 41$) (□). (b) The eels only showed one freshwater entry. The mean age at first freshwater entry was 4·6 ± 2·2, years ($n = 22$) (■) and the age between the first freshwater entry and the capture was 6·1 ± 2·1, years ($n = 22$) (□).

1999). More than 10-fold higher water Sr concentration or seven-fold higher water Sr:Ca ratios were measured in the Baltic coast than in the Curonian Lagoon. Elsdon & Gillanders (2005) found significantly increased otolith Sr:Ca ratios by enhancing ambient water Sr:Ca ratios by two- to four-fold. Their experimental results justified the use of otolith Sr:Ca ratios in interpreting migratory history of the eels across different salinity environments.

Salinity decreases gradually from *c.* 35 in the North Sea to *c.* 15 in the south-western Baltic Sea and to *c.* 6 in the coastal waters of the south-eastern Baltic Sea. The migration from a high salinity (North Sea) to low salinity environment (south-eastern Baltic) was clearly imprinted in 20 naturally recruited eels with decreasing otolith Sr:Ca ratios (Fig. 7). Owing to the asymptotic relationship between otolith Sr:Ca ratios and salinity (Tzeng, 1996; Limburg *et al.*, 2003), there is limited ability to distinguish full-strength and half-strength seawater salinities by otolith Sr:Ca ratios. Freshwater eels, however, showed very low otolith Sr:Ca ratios, distinct from those in seawater and brackish-water eels. This result is consistent with the finding of Zimmerman (2005) that salmonid otolith Sr:Ca ratios were linearly related to salinity, but the sensitivity of otolith Sr:Ca ratios was only enough to discriminate between fresh, brackish and sea water.

CONTRIBUTION OF STOCKED AND NATURALLY RECRUITED EELS TO THE POPULATION

The composition of stocked and naturally recruited eels differed among locations. Based on the patterns of otolith Sr:Ca ratios, eels caught in Lake Baluošai are all of stocked origin, suggesting that few, if any naturally recruited eels reach this area and that eel fisheries in the inland lakes are all based on the stocked eels. Further studies with larger sample sizes are required to determine if there is any natural recruitment to the inland waters of eastern Lithuania. The proportions of stocked eels decreased to 20 and 2% in the Curonian Lagoon and Baltic coast, respectively (Table III). There was no evidence to suggest that stocked eels from inland lakes of eastern Lithuania will emigrate downriver and contribute to the eel stock in the Curonian Lagoon or the Baltic coast. It seems unlikely that stocked eels undergo the long migration, *c.* 300 km, from Lake Baluošai or other lakes from the same water basin to the Curonian Lagoon during the yellow eel growth-phase. The eels caught in Lake Baluošai showed narrow otolith annuli [Fig. 2(b)], which is distinct from that of residents in the Curonian Lagoon and Baltic coast [Fig. 2(a)]. The narrow annuli indicate a slow growth rate due to the limited prey available in Lake Baluošai, determined by higher eel population density based on catch-per-unit effort in the stocked lakes of eastern Lithuania. The growth differences might also be determined by lower lake productivity and by feeding differences at the sampling sites. The Curonian Lagoon is eutrophic (Jašinskaitė, 1998) while Lake Baluošai is mesotrophic to oligotrophic (K. Arbačiauskas, pers. comm.). Eel dietary studies demonstrated that the lagoon and Baltic Sea eels eat a high proportion of fishes while Lake Baluošai eels eat largely invertebrates (E. Bacevičius, pers. comm.). None of the 11 stocked eels found in the Curonian Lagoon and Baltic coast showed narrow otolith annuli, suggesting that few, if any, stocked eels migrate downriver to the Curonian Lagoon or Baltic coast waters until the spawning migration. A reasonable hypothesis is that the eel populations in inland lakes of eastern Lithuania and the Curonian Lagoon are independent during the growth phase. Energy costs, density-dependent migration and variable habitat quality could influence the geographic distribution as well as the migration of eels within the river (Feunteun *et al.*, 2003).

Some glass or small yellow eels have been released in Curonian Lagoon, but the stocking rate has been low: (only 1·7 eels ha^{-1} in 1995–2003). Only one stocked eel (number 60) was found to have emigrated from the Curonian Lagoon to the nearby Baltic coast while four stocked eels (number 5, 6, 7 and 14) eventually returned to the Curonian Lagoon after short movements to the Baltic coast. This suggests that stocked eels prefer to settle in the location where they are released. Accordingly, 91% ($n = 10$) of the stocked eels ($n = 11$) remained in the Curonian Lagoon where they were released, assuming that no or very few eels had descended from lakes *via* the Nemunas River. In addition, *c.* 9% ($n = 1$) of the stocked eels in the Curonian Lagoon emigrated to the Baltic coast and constituted *c.* 2% of the local eel population.

Eels are important commercial and recreational species in central and eastern Europe and make important contributions to local and regional economies. Hence, the original aim of stocking programmes in the Lithuania and other Baltic countries was enhancement of inland fisheries. Intensive exploitation of the stocked eels presumably led to high fishing mortality and a low rate of escapement by silver eels. Limburg *et al.* (2003), however, found that 26·7% of the migrating silver eels in the area connecting the Baltic Sea with the North Sea were of stocked origin, comparable to the present finding that stocked eels account for 20% of the population in the Curonian Lagoon. Without related information on natural recruitment as well as the survival rate of the stocked eels, it is difficult for this first evaluation of the contribution of stocked eels to the naturally recruited population in the south-eastern Baltic Sea to determine stocking effectiveness. It is possible, however, that the stocked glass eels in Lithuania (*e.g.* stocked to the Curonian Lagoon) or other Baltic countries migrate to the Baltic Sea too soon after release to allow the freshwater signature to be recorded in the otolith. If so, the proportion of stocked eels in the Baltic Sea will be underestimated. Alternatively, most stocking programmes in the Baltic countries are focused on fisheries enhancement in inland lakes and quick migration over long distances to the Baltic Sea without creating a freshwater signature in the otoliths seems unlikely.

Seawater–and brackish water–resident eels accounted for only 23% of eels examined from Baltic coastal waters and the lagoon, while interhabitat shifters comprised *c.* 70% of the eels. The proportion of naturally recruited eels that have experienced fresh water is more than twice as high as found in the migrating silvers in the area connecting the Baltic Sea to the North Sea (Limburg *et al.*, 2003). If only the eels collected in the Lithuanian Baltic coast were considered, the proportion of the eels experiencing fresh water is still as high as 50%. The differences between these two independent studies may be due to the different geographical and habitat constraints or different behaviours of the eels. Long-distance migratory eels may be more active in exploring different habitats than their counterparts that settle down earlier. After entry into Baltic Sea, eels trapped in this closed system may explore optimal habitats at minimal energy cost to benefit maximal growth. Diversified habitats usually provide more food and shelter than does a less diversified habitat, which presumably encourages euryhaline fishes to explore different habitats. This may explain the flexible and complex migratory behaviour of the eels, which reflects their environmental and evolutional adaptation.

AGES OF EEL ON ARRIVAL IN SOUTH-EAST BALTIC SEA

As for as is known, this is the first study that estimates the ages of the eels arriving in the south-eastern Baltic area. Most naturally recruited eels showed an initial freshwater entry at age 1–10 years (mean ± S.D. 5·2 ± 2·1 years). High variability in the age at initial freshwater entry indicates that some eels might migrate quickly through the Baltic Sea and into fresh water within 1 or a few years, while some eels showed very slow migration eastward. The broad ranges of age at initial freshwater entry also suggest a random distribution of the eels in the Baltic Sea rather than size- or age-dependent distribution. The eel density in the North Sea and Skagerrak and Kattegat Sounds may influence ages at arrival in the south-eastern Baltic. Low eel density in the Baltic Sea due to low recruitment of young eels (Westin, 2003) may discourage eastward migration due to low intraspecific competition, so the eels arrive at older ages. It is known that populations in the lower reaches of rivers achieve high densities, but as eels grow, relative biomass and hence competition for food and space increase. Agonistic encounters may then act as a stimulus for further upstream migration (Knights, 1987). Interestingly, on the Baltic coast of Denmark, at the monitoring site of eel recruitment at the Harte hydropower station, 50% of trapped eels were glass eels in the 1960s, while glass eels are rarely seen today. Thus, the mean size of recruiting eels has probably increased over the years due to the delayed recruiting process. In Vester Vedsted brook on the Danish North Sea coast, glass eels, elvers and yellow eels are found, however, pigmented glass eels are most common at the lower part of the brook and are considered as new recruits (Pedersen, 2002). More to the south of the European coast, in the Netherlands, new recruits are partly but never fully pigmented glass eels (Dekker, 2002), while in coastal Germany at recruitment monitoring sites both true glass eel and fully pigmented elvers are found (Kuhlmann *et al.*, 2002). In south-west Norway in the River Imsa, all sampled eels are fully pigmented elvers or young yellow eels that have stayed for one winter or more in marine or estuarine waters (Vøllestad, 2002). In Sweden, the catch in the River Viskan discharging to the North Sea consists mainly of elvers; however, at other rivers of the same coast age ranged from 0 to *c.* 8 years (Wickström, 2002). Overall, eels from rivers along the eastern coast of Sweden (Baltic Sea) are older than in rivers closer to the coast of the Skagerrak and Kattegat Sound (Wickström, 2002), *i.e.* the sound between the Baltic and the North Seas. Hence, presumably eels to the south-eastern Baltic should not be glass eels on arrival. The present observed arrival ages clearly support the hypothesis of eel recruitment to the south-eastern Baltic at the yellow eel stage, and explains why regional ichthyologists and managers were so uncertain about the contribution of natural recruitment, *i.e.* the absence of truly glass eels in the coastal waters led to hypotheses of natural recruitment weakness or even overall absence in the region.

Stocking programmes are a common and usually effective strategy to mitigate population decline, restore fisheries or to create new fisheries. The majority of stocked lakes in Latvia, Lithuania and Poland were almost devoid of eels before intensive stocking programmes began in the 1950s. The stocking programmes did create new eel fisheries in these inland lakes and also partially support the eel population in the Curonian Lagoon. Many stocking programmes, including eel stocking, however, are carried out without evaluation of their effectiveness or actual success (Cowx, 1999). The use of otolith Sr:Ca ratios enabled this study to discriminate stocked eels from naturally recruited eels and to evaluate their contribution to the population.

We are grateful to M. Dragūnas, R. Rimkus and R. Repečka for assistance in sampling and measuring the eels and to B. M. Jessop and D. K. Cairns for constructive suggestions. Financial support was provided by the Ministry of Environment, Republic of Lithuania and the Lithuanian Fisheries Produces' Association for the field studies. The Lithuania-Latvia-Taiwan (Republic of China) Mutual Fund provided financial support for the otolith analysis (Contract No. NSC 93-2313-B-002-114). The first two authors contributed equally to this work.

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PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B, Vol. 61 (2007), No. 3/4 (650/651), pp. 91–95.

VARIETY OF mtDNA HAPLOTYPES IN THE POPULATIONS OF THE EUROPEAN PERCH (*Perca fluviatilis*) OF THE CURONIAN LAGOON, THE COASTAL ZONE OF THE BALTIC SEA AND THE GULF OF RĪGA

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Contributed by Isaak Rashal

A total of 56 perch individuals attributed to three different populations—the Baltic Sea (Lithuanian costal zone), the Curonian Lagoon (Lithuania) and the Gulf of Rīga (Latvia)—were investigated. The amplified mtDNA fragment consisting of 378 bases includes the trnT *gene and the 5‘-fragment of the control region consisting of 260 bases. Ten variable positions were identified and 15 haplotypes of the European perch of mitochondrial DNA (mtDNA) D-loop were established. Three new haplotypes C4, F8 and G2 characteristic of the perch caught in Lithuanian territorial waters and two haplotypes L1 and L2 characteristic of the perch from the Gulf of Rīga were determined. Five rarer haplotypes were found in the samples of the Lithuanian perch and four in the samples of the Latvian perch, which characterise the scope of reproductive isolation among the populations. The similarity in the distribution of the most frequently found haplotypes A and F in the Lithuanian samples investigated illustrate the similarity of the perch populations and substantiate the assumption of possible seasonal migration of the perch between the Curonian Lagoon and the coastal zone of the Baltic Sea.*

Key words: *perch, mtDNA, haplotypes.*

INTRODUCTION

Considerable climatic changes during the last 2.5 million years resulted in the fact that the Baltic Sea and part of the European continent periodically became covered with ice. This, together with other physically geographical barriers, had an impact on the formation of the genetic structure of the species living there. The unequal frequency of a distribution of different alleles in the populations reflects this process (Nesbø *et al.* 1999). This has been supported by the investigation of freshwater fishes carried out in North America, which revealed significant correlation between postglacial migration and the distribution of genetic lineages (Bernatchez, 1998).

Since the analysis of the isoenzyme systems of widespread species, such as the European perch (*Perca fluviatilis*), revealed their low informativeness as genetic markers (Mardsen *et al*., 1995), DNA markers, including randomly amplified polymorphic DNA fragments and mtDNA markers have been used to investigate the features of the genetic structure of the European population of this species (*Nesbø et al*., 1998). Suitability of perch in investigations of the formation of the genetic structure of populations conferred by its universal distribution in the European network of river basins, and their adaptation to the life under conditions of varying water temperature and even salinity. Effectiveness of the use of DNA markers has been illustrated by the analysis of the variability of the sequence of mitochondrial DNA (mtDNA) D-loop of perch and the phylogenetic relationships of perch samples from Swedish territorial waters in the Baltic Sea. In samples of perch representing populations with different migration behaviour, a distinct spectrum of haplotypes was discovered, which was confirmed by construction of phylogenetic relationships from the analysis of RAPD markers (Nesbø *et al.,* 1998). In another investigation of 44 samples of perch from river basins of the European continent the distribution frequency of mtDNA D-loop was established and proved that there were several centres from which the perch could spread into Northern Europe in the postglacial period (Nesbø *et al*., 1999).

However, till now there has been no DNA marker data available concerning the genetic diversity of the perch in the inland waters of Lithuania and Latvia or in the coastal waters of the Baltic Sea. The only survey known in the region is the analysis of perch populations in Kaunas city reservoir, in the Curonian Lagoon and in the Baltic Sea coastal zone by means of isoenzymes as biochemical markers (Paulauskas and Ložys, 2001). The aim of the present work was to determine polymorphism of sequences of mtDNA D-loop and to evaluate the genetic diversity of the populations of perch representing different areas of the Baltic Sea.

MATERIAL AND METHODS

A total of 56 European perches *(Perca fluviatilis)* caught in the years 2004–2006 were used for the investigation. Altogether 15 individuals were caught in the Baltic Sea, 18 individuals in the Curonian Lagoon and 23 in the Gulf of Rīga. Among them, in 2004, nine individuals were caught in the Baltic Sea near Monciškės and six individuals caught in the Baltic Sea near Būtingė, both Lithuania. In 2005, ten individuals were caught in the Curonian Lagoon (Lithuania) near Kiaulės nugara and eight individuals in the same lagoon near Ventės ragas, and in 2006, 23 European perches in the Gulf of Rīga near the mouth of the River Daugava (Latvia).

Genomic DNA was isolated from the samples of muscle tissues fixed in ethanol by means of the salt extraction method (Aljanabi and Martinez, 1997).

Amplification of mtDNA fragments was carried out using *Mastercycler Gradien* amplifier manufactured by the *Eppendorf* Company. For amplification of mitochondrial DNA sequences the following primers were used: HV2: TTCCCCGGTCTTGTAAACC; and CSB-D: GGAACCAAATGCCAGGAA.

PCR was carried out by denaturation at 96 ^{o}C temperature for 5 minutes, then 30 cycles at 96 ^{o}C for one minute, at 54 ^{o}C (HV2 – CSB-D) for one minute, followed by an elongation step at 72 ^{o}C for 2 minutes and finishing with a final elongation step at the 72 ^{o}C for 5 minutes. The PGR product was checked in 1.5% agarose gel and purified by CIAP and ExoI nucleases: for 15 minutes at 37 ^{o}C and then for 15 minutes at 85 ^{o}C.

Sequencing was carried out at the Sequencing Centre of the Institute of Biotechnology (Vilnius, Lithuania). DNA sequences were determined by an ABI Prism 377 automatic sequencer. Sequencing results were compared with the PFY14724 sequence using the BLAST database. The computer programme CLC Free Workbench version 0.91 was used for the sequence analysis and for the construction of phylogenetic tree.

RESULTS

A total of 56 perches attributed to three different populations of the Baltic Sea, the Curonian Lagoon and the Gulf of Rīga were investigated. The amplified mtDNA fragment consisting of 378 bases includes the *trnT* gene and the 5‘-fragment of the control region consisting of 260 bases. Ten variable positions were identified and 15 haplotypes of the European perch of mtDNA D-loop were established (Table 1). Three new haplotypes, we designated as C4, F8, and G2, characteristic of the perch caught in Lithuanian territorial waters and two haplotypes, designated as L1 and L2, characteristic of the Latvian perch were determined (Table 1).

The distribution of haplotypes in the studied populations is presented on Figure 1. The most frequent F haplotype was discovered in samples of perch caught both in the Curonian Lagoon and the Baltic Sea near Šventoji. Haplotype A was found to be the second by frequency in the populations of the Lithuanian perch, and haplotypes C and E are most often found in the populations of perch of the Gulf of Rīga. Haplotypes C1 and A7 were found in all investigated populations. Five rarer haplotypes were found in the samples of the Lithuanian perch and four in the samples of the Latvian perch, which characterises the scope of reproductive isolation among the populations of these perches. Haplotype E was found with minimum frequency on the coastal zone of the Baltic Sea near Šventoji only in the samples of 2004 and 2005.

Table 1

HAPLOTYPES OF mtDNR *trnT* gene AND CONTROL REGION FRAGMENT DETERMINED IN EUROPEAN PERCH *Perca fluviatilis* SAMPLES CAUGHT IN THE TERRITORIAL WATERS OF LITHUANIA AND LATVIA (data are compared with the mtDNA sequence PFY14724, haplotype A presented in the Gene Bank)

Haplotypes	Position										N
	98–99	103	129	130	135	136	137	139	264	299	
A	-	A	G	C	A	A	T	T	T	C	6
A2	-			T							1
A7	-		T	T							4
C	-									T	7
C1	-		T	T						T	4
C4	-		T	T					A	T	1
E	-	T					-			T	2
F	-	T								T	23
F1	-	T	T	T						T	1
F2	-	T	T	T	G					T	1
F7	-	T	T							T	2
F8	-	T				-				T	1
G2	-							-		T	1
L1	Ins*	T		T						T	1
L2	Ins*			T							1
Total:											56

C4, G2, F8, L1 and L2 – newly identified haplotypes.

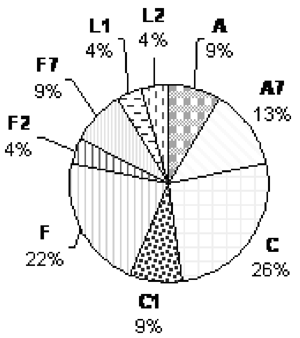
N, number of individuals.

T, A, and G, substitution of nucleotides.

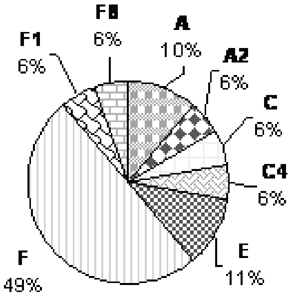
- – deletion of nucleotides.

Ins*, insertion of ten nucleotides (TTGCAAGCAC).

(a) Gulf of Riga



(b) Baltic Sea near Šventoji



(c) Curonian Lagoon

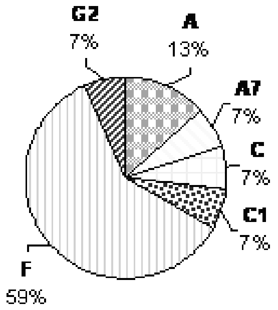


Fig. 1. Frequencies of mtDNR haplotypes in the investigated populations of European perch in the Gulf of Rīga (a), the Baltic Sea near Šventoji (b) and the Curonian Lagoon (c)

DISCUSSION

Nesbø *et al.* (1998) and Refseth *et al.* (1998) determined in total 35 haplotypes grouped into eight closely related groups by the nature of base substitutions. Based on this data, several zones, separated from the phylogenetic point of view, were postulated, with prevailing different groups of mtDNA haplotypes (Nesbø *et al.*, 1999). In all perch populations of Western Europe, haplotypes of group F dominated, with the exception of those in river basins stretching in the territory of Norway where haplotypes of group A (A, A1–A9) prevailed, as well as the coastal zone of the Adriatic Sea and river basins of the Balkan countries (oldest in the term of origin), in which haplotypes of group M (M, M1) prevailed. In samples of perch of the continental part of the mainland, in the basins of the Nemunas, Dnieper rivers, as well as in the remote Lake Baikal, the Angara River basin, haplotypes of group C (C, C1–C3) prevailed. This indicated separation of perch in the basins of the rivers stretching across this territory to a separate Euro-Asian phylo- geographical region.

Haplotype E was found with minimum frequency on the coastal zone of the Baltic Sea near Šventoji only in the samples of 2004 and 2005 which could be related to the anadromous behaviour of those perch, i.e., a periodical change in the environment when fish feeding in more saline waters of the coastal zone of the Baltic Sea swim to spawn to the fresh waters of the river mouths. A repeated discovery of haplotype E in the samples of the perch caught in the coastal strip near Monciškės not far from the settlement of Šventoji in different years can be related to a larger part of the individuals keeping near the spawning site located in the approaches of the mouth of the Šventoji River, and rarer variants of haplotypes (haplotype E), inherited with steady, though small frequency, reflect the Hardy-Weinberg balance of the population.

Newly identified haplotypes C4 and F8 were found in the samples of the Baltic Sea. Also, haplotype G2 found in the samples from the Curonian Lagoon are characteristic of the Lithuanian population of the perch and are related to the group of haplotypes E–F (Fig. 2). The presence of an insertion of ten nucleotides characteristic of the newly identified haplotypes L1 and L2 could be accounted for by a close phylogenetic link of these haplotypes. However, on the basis of grouping of these samples in the phylogenetic tree, according to the nature of single base substitutions, haplo-

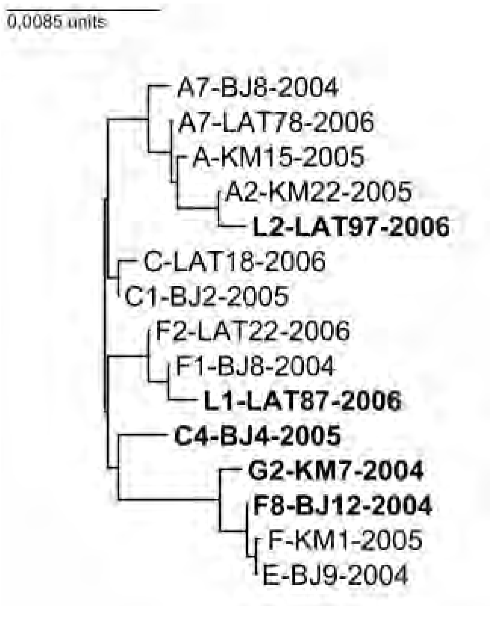


Fig. 2. The *Neighbour joining* phylogenetic tree representing the origin of haplotypes.

The first letter of abbreviation stands for the haplotype, BJ-samples from the Baltic Sea, KM—samples from the Curonian Lagoon, LAT—samples of perch caught in the Gulf of Rīga.

Table 2

SAMPLE COLLECTION SITES AND DISTRIBUTION OF HAPLOTYPES IN POPULATIONS OF THE EUROPEAN PERCH

Population	Region	Number of individuals studied	Water basin	Haplotypes (N*)
Curonian Lagoon**	Lithuanian territorial waters	15	Baltic Sea, mouth of the Nemunas	A(2), A7(1), C(1), C1(1), F(9), G2(1)
Baltic Sea**	Lithuanian territorial waters	18	Baltic Sea near Šventoji	A(2), A2(1), C(1), C4(1), E(2) F(9), F1(1), F8(1)
Gulf of Rīga**	Latvian territorial waters	23	Baltic Sea, the Gulf of Rīga near the Daugava mouth	A(2), A7(3), C(6), C1(2), F(5), F2(1), F7(2), L1(1), L2(1)
L. Krylovo***	Russia, inland waters	5	Basins of the Vilija/Nemunas Rivers	C(5)
L. Hencza***	Inland waters of Poland	5	The Nemunas basin	C(4), C1(1)
L. Dubrovskoje***	Inland waters of Russia	10	The Dnieper basin	C(10)
Gulf of Matsalu***	Inland waters of Estonia	10	Baltic Sea	A8(3), F(4), F1(1), F7(1), E2(1)
Emjajogi R.** *	Inland waters of Estonia	5	Baltic Sea, the Gulf of Finland	A(1), A7(1), A8(1), C(2), J1(1)
L. Peipsi ***	Inland waters of Estonia	9	Baltic Sea, the Gulf of Finland	A(1), A7(1), A8(1),C(2), J1(1)
L. Vourasjarve ***	Inland waters of Norway	10	Karasjakka/Tana	A(4), A2(6)
The Angara River***	Inland waters of Russia	10	Baikal Lake, the Angara basin	C(10)
L. Rybinsk***	Inland waters of Russia	14	The Volga basin	A(1), C(7), C2(1), C3(2), G1(1), J(2)
The gulf of Bothnia***	Territorial waters of Sweden	20	Baltic Sea	A7(2), C(14), F1(4)
Anadromous A***	Territorial waters of Sweden	20	Baltic Sea	A(2), A7(4), C(8), F(3), F1(2), G(1)
Anadromous B***	Territorial waters of Sweden	17	Baltic Sea	A(4), C(6), C1(1), F(2), F1(2), G(2)
L. Ängersjön***	Territorial waters of Sweden	19	Lake near the Baltic Sea	A5(1), C(13), C1(4), F1(1)
L. Race***	Inland waters of Slovenia	10	Drava/Danube	C(1), M1(9)

* in brackets the number of individuals of the particular haplotype is given

** data from this research

*** data from Nesbø *et al*., 1999

type L1 is closest to the group of haplotypes F1 and F2, which is related to the group of haplotypes F fully dominating in Western Europe, and haplotype L2 is closest to the group of haplotypes A typical of the western and northern parts of Scandinavia (Norway).

A combination of haplotypes A and F is characteristic of the samples of the Matsalu perch population investigated in the geographical range of the Baltic Sea (territorial waters of Estonia) (Table 2), which geographically is one of the closest to the sample of the Latvian perch investigated by us but, however, which differs greatly from the Emjajogi and Peipsi populations (the Gulf of Finland, territorial waters of Estonia) in which the haplotypes of groups A and C suggest influence of the Northern and Eastern phylo-geographical regions on the formation of the perch population of the Gulf of Finland.

The phylogenetic link between the Estonian and Latvian perch populations is confirmed by the most frequently presence of haplotypes of groups A and F in the Matsalu population of the Gulf of Rīga, but the Latvian perch population is noted for a rather high frequency of haplotypes C, which is related to the Euro-Asian phylo-geographical region extending in the East. The frequency of haplotypes of group C is three times lower in the populations of the Lithuanian perch in the samples of both the Curonian Lagoon and the coastal zone of the Baltic Sea, hence, the total spectrum of frequencies of haplotypes is closer to that of the Western Europe, although the “eastern” haplotypes of group C entirely dominate in the perch in the basins of the Vilija and Nemunas rivers in other than Lithuanian territory.

The lowest diversity of haplotypes (six) was observed in the perch population of the Curonian Lagoon. Similarly, perch populations of the Gulf of Bothnia in the territorial waters of Sweden, non-migrating perch populations had three or four different haplotypes, the lowest diversity of haplotypes, while perch with an anadromous behaviour had six different haplotypes (the size of samples fluctuated from 17 to 20 individuals). In brackish coastal waters of the Baltic Sea near Šventoji and in the Gulf of Rīga, where the perch generally have anadromous behaviour, a greater diversity of haplotypes—eight or nine—was found. The similarity in the distribution of the most frequently found haplotypes A and F in the samples investigated illustrates the similarity of the populations of the Lithuanian perch, supporting the idea of possible seasonal migration of the perch between the Curonian Lagoon and the coastal zone of the Baltic Sea: adult individuals migrate in summer to the brackish Baltic Sea probably due to the beneficial effect of water salinity on the perch growth, whereas in autumn they return to the freshwater Lagoon, stay there in winter and spawn the following spring (Ložys, 2004). In this case, the lower diversity of haplotypes in summer population of the perch of the Curonian Lagoon suggests seasonal migrations of part of the perch population to the Baltic Sea.

Further work on the structure of populations in relation to seasonal migration to brackish waters requires the use of microsatellite markers (Brunner *et al*., 1998; Englbrecht *et al*., 2002). Several specific microsatellite primers have already been developed for species of yellow perch, which is taxonomically close to the European perch (Leclerc *et al*., 2000), thereby aiding to overcome the stage of creating specific primers for the species, and allowing investigators to make use of the available information in continuing investigations into the interspecific genetic diversity and population structure of the European perch.

ACKNOWLEDGEMENTS

We express our gratitude to the Mutual Funds for the Scientific Co-operation of the joint project of Taiwan, the Republics of Latvia and Lithuania "Application of genetic and micro-chemical markers as implements for diadromous and endangered commercial fish species populations management" for its financial assistance and the provided possibility to carry out this project.

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Received 20 November 2006

mtDNS HAPLOTIPU DAŽĀDĪBA KURŠU LĪČA, BALTIJAS JŪRAS PIEKRASTES ZONAS UN RĪGAS LĪČA ASARU (*Perca fluviatilis*) POPULĀCIJĀS

Īpatņiem no trim asaru populācijām tika pētīts mtDNS haplotipu sadalījums. Trīs jauni haplotipi atrasti starp Lietuvas paraugiem, divi – starp Latvijas paraugiem. Atrasti vairāki reti haplotipi, kas raksturo Latvijas un Lietuvas populāciju īpatnības, kuru cēlonis ir reproduktīvā izolācija. Savukārt A un F grupu haplotipu līdzīgais sadalījums Kuršu līča un Lietuvas Baltijas jūras piekrastes populācijās liecina par iespējamo sezonālo migrāciju starp šiem ūdens baseiniem.

國際合作計畫赴國外研究心得報告

計畫編號	NSC 95-2313-B-002 -016 -
計畫名稱	Application of Genetic and Microchemical Markers as Implements for Diadromous and Endangered Commercial Fish Species Populations Management
出國人員姓名 服務機關及職稱	曾萬年教授（國立臺灣大學） 蕭仁傑助理教授(國立臺灣大學） Yoshiyuki Iizuka (中央研究院研究助師）
出國時間及地點	2007 年 9 月 3-7 日， Riga, Latvia
合作研究機構	1. Institute of Ecology of Vilnius University 2. Institute of Biology, University of Latvia 3Latvian Fisheries Research Institute
合作計畫名稱	Application of Genetic and Microchemical Markers as Implements for Diadromous and Endangered Commercial Fish Species Populations Management
合作計畫主持人	曾萬年教授
出國事由	The third year Lithuania-Latvia-Taiwan GMM project

一、內容及成果：

本次會議為此三年期的國際合作計畫中的最後一次，主要的任務為討論第三年的研究結果並且檢討這三年來的所有成果，以及將來的合作機會。我方在會議中提出三篇報告，首先由 Yoshiyuki Iizuka 就耳石 Sr/Ca ratio 的分析方法，進行技術層面的報告，其題目為：EPMA as a tool in studying the migratory environmental history of Diadromous fishes:Application and limitation。之後由曾萬年教授報告三年來關於歐洲鰻魚的人工放流的成效與洄游行為等相關成果，其題目為：Migratory environmental history and habitat use of the European eels in Lithuanian and Latvian waters as revealed by otolith Sr/Ca ratios。最後由蕭仁傑助理教授報告三年來關於 Perch 與 Pikerperch 的河海洄游生活史之成果，其題目為：Comparison of migratory environmental history and habitat use between perch and pikeperch in Lithuanian and Latvian waters as revealed by otolith Sr/Ca ratios。

Lithuania 則由 Dalius Butkauskas 與 Linas Lozys 提出關於 Perch 與 Pikerperch 的族群結構之相關成果，其題目為：Evaluation of genetic diversity of Perch (*Perca fluviatilis*) and Pikeperch (*Sander lucioperca*) populations inhabiting Lithuanian water areas of the Curonian Lagoon and the Baltic Sea。

Latvia 則由 Isaak Rashal, Maris Plikshs, Atis Minde 提出關於鰻魚的族群遺傳結構的結果，其題

目為：Investigation of the genetic diversity of naturally recruited and introduced European eels in Lithuania and Latvia。會議中則有 Latvian Fish Resources Agency 的所長簡報該研究單位的編制，任務與研究工作，其報告題目為：State Agency “Latvian Fish Resources Agency”。

研究進行中雖然三方人員經常以電子郵件保持聯絡與討論，但是此次的會議讓所有與會人員能夠完全空下三天時間，專注地針對相關議題與結果數據，進行討論與審視，對於釐清數據所代表的意義有非常大的助益。由於討論相當熱烈，原本有另外一位 Latvian Fish Resources Agency 的研究人員 Janis Birzaks 要報告 Latvia 的鰻魚資源與研究概況，由於時間不足，只好取消，但是 Janis Birzaks 研究員慷慨留下其報告內容的 powerpoint 檔案”Status of eel in LV”，以供與會人員參考，這份報告與所有的會議簡報內容皆一併附加在此心得報告之後，以供參考。

會議的第二天與第三天則由 Isaak Rashal, Maris Pliksh s, Atis Minde 帶領與會人員前往研究魚類對象的採集地點，實地瞭解該地的水域環境與採樣方法，並且於現場討論於分析中所發現的魚類行為，洄游與生活史等相關的問題，使得我們對於實驗室中所獲得的資料，更能詮釋其生物意義，但是還是有些問題無法在會議與實地參訪中獲得肯定的答案，這些問題則留待往後的研究再行設計實驗以驗證。其次也順便參觀一些漁業活動，例如七鰓鰻的漁撈作業。雖然匆忙的拜訪行程無法清楚瞭解該國的實際漁業狀況，但是對於該國以核發漁業捕撈權的方式控管漁撈的捕獲量，對於有效管理漁業資源是非常有效的方式，該國不僅重視基礎研究工作，更以務實的方式落實到有效的管理運作層面。沿途順便參觀 Latvia 的重要歷史景點，此次會議不僅對研究魚種有深刻與具體的瞭解與掌握，對 Latvia 的歷史與文化也留下十分深刻的印象。

三年的研究工作所獲得的資料與數據，目前發表了 4 篇 SCI 的報告，回答了許多關於鰻魚與河鱸的洄游生活史相關問題，卻也延伸出許多新的問題，冀望往後能有經費持續支助本項研究計畫。

二、建議事項

三、攜回資料

攜回資料附加在心得報告之後

State Agency “Latvian Fish Resources Agency”

www.latzra.lv

Status

- State management institution under the supervision of Ministry of Agriculture

- Established in November 2004, merging Latvian Fisheries Research Institute and 7 state fish hatcheries

- In 2006 included in the Register of Latvian Research Institutions

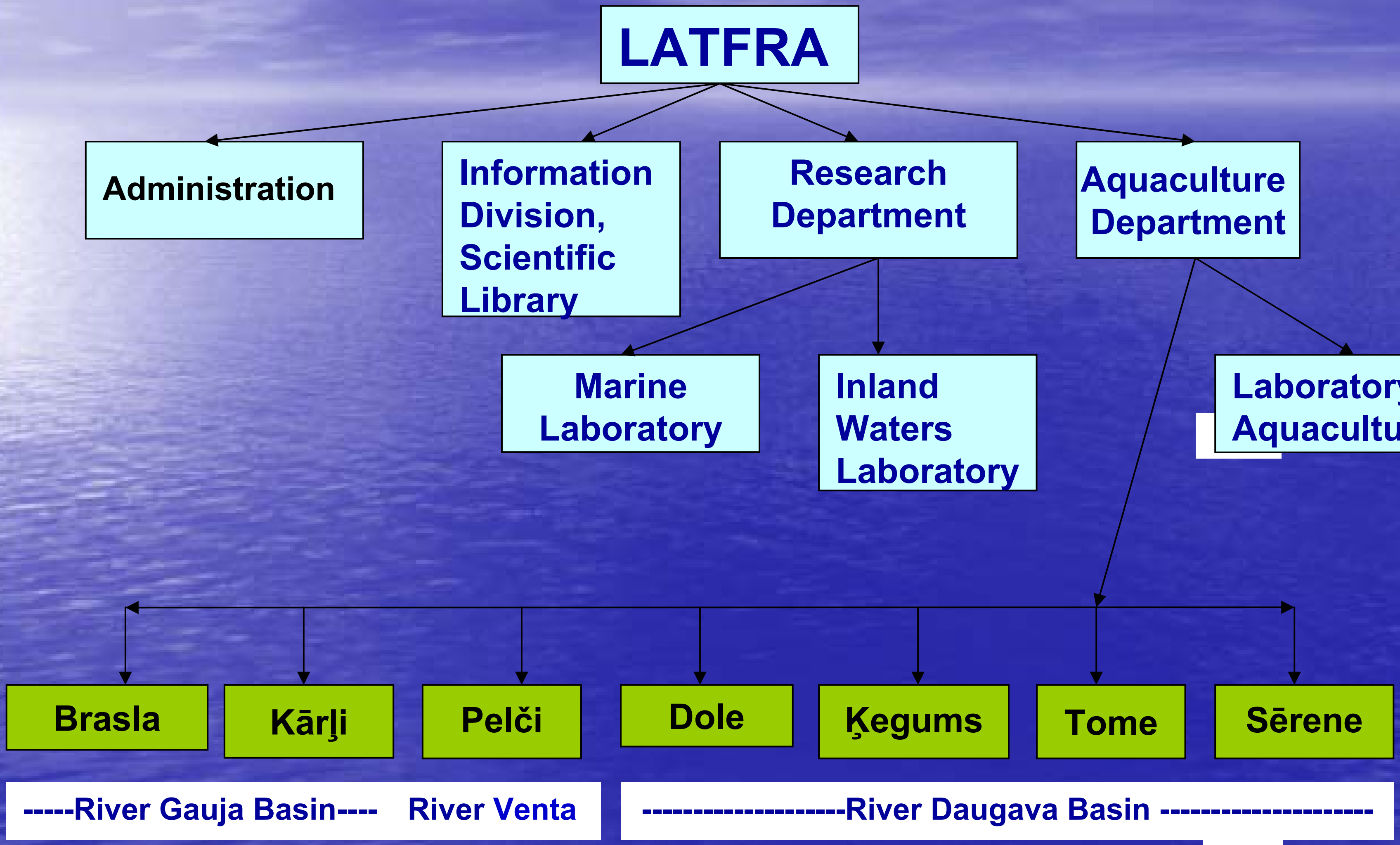
Mission

- Promote sustainable commercial and recreational fishery in the Baltic Sea, Gulf of Riga and inland waters based on stable fish resouces and favourable environment
- Implement state policy in fisheries sector

Tasks

- Implement fisheries and aquaculture research, provide scientific advice on management of fish resources and protection of biodiversity in marine and freshwaters
- Ensure implementation of state policy of restocking of fish resources
- Provide public services in fisheries and aquaculture research, restocking, fisheries expertises
- Provide public information

Capacity



LATFRA

Administration

Information Division, Scientific Library

Research Department

Aquaculture Department

Marine Laboratory

Inland Waters Laboratory

Laboratory of Aquaculture

Brasla

Kārļi

Pelči

Dole

Ķegums

Tome

Sērene

-----River Gauja Basin---- River Venta

--------------------River Daugava Basin ---------------------

🟦 Central Office in Riga

🟩 Affiliations – fish hatcheries

Capacity

- Number of employees – 208

  incl.: research - 51

  hatcheries – 143

  administration – 14
- Funding from State Budget (mill. EUR)

  total – 3.29

  incl.: research – 1.7

  restocking - 1.59

Programmes, projects

- Latvian National Fisheries Data Collection Programme
- Baltic Sea Regional Project (BSRP), 2004-2007
- European Network of Exelence for Ocean Ecosystems Analysis (EUR-OCEANS), 2004-2007
- Understanding the Mechanism of Stock Recovery (UNCOVER), 2006-2009
- MPAs for Ecosystem Conservation and fisheries management (PROTECT), 2005-2008
- Improved Methodology for Baltic Cod Age Estimation (DECODE), 2007-2008
- Application of Genetic and Microchemical Markers as Implements for Diadromous and Endangered Commercial Fish Species Populations Management (GMM), 2006-2007

- State Programme on Restocking of Fish Resources, 2001-2010
- Impact of Climat Changes on Latvian Waters Environment, State Research Programme, 2006-2009

Cooperation

- EC STECF, sub-groups
- RACs, Baltic and NAFO RCMs
- ICES committees, working, study groups
- NAFO Scientific Council
- EFARO
- EAS (European Aquaculture Society)
- BSRP
- Bilateral cooperation with research institutes

Thanks !

Status of eel in LV

By Janis Birzaks
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Eel distribution in Latvia

- Along the coastline~ 500 km;
- 17 lakes/lakes systems:

-2 with free access for eel~8000 ha;

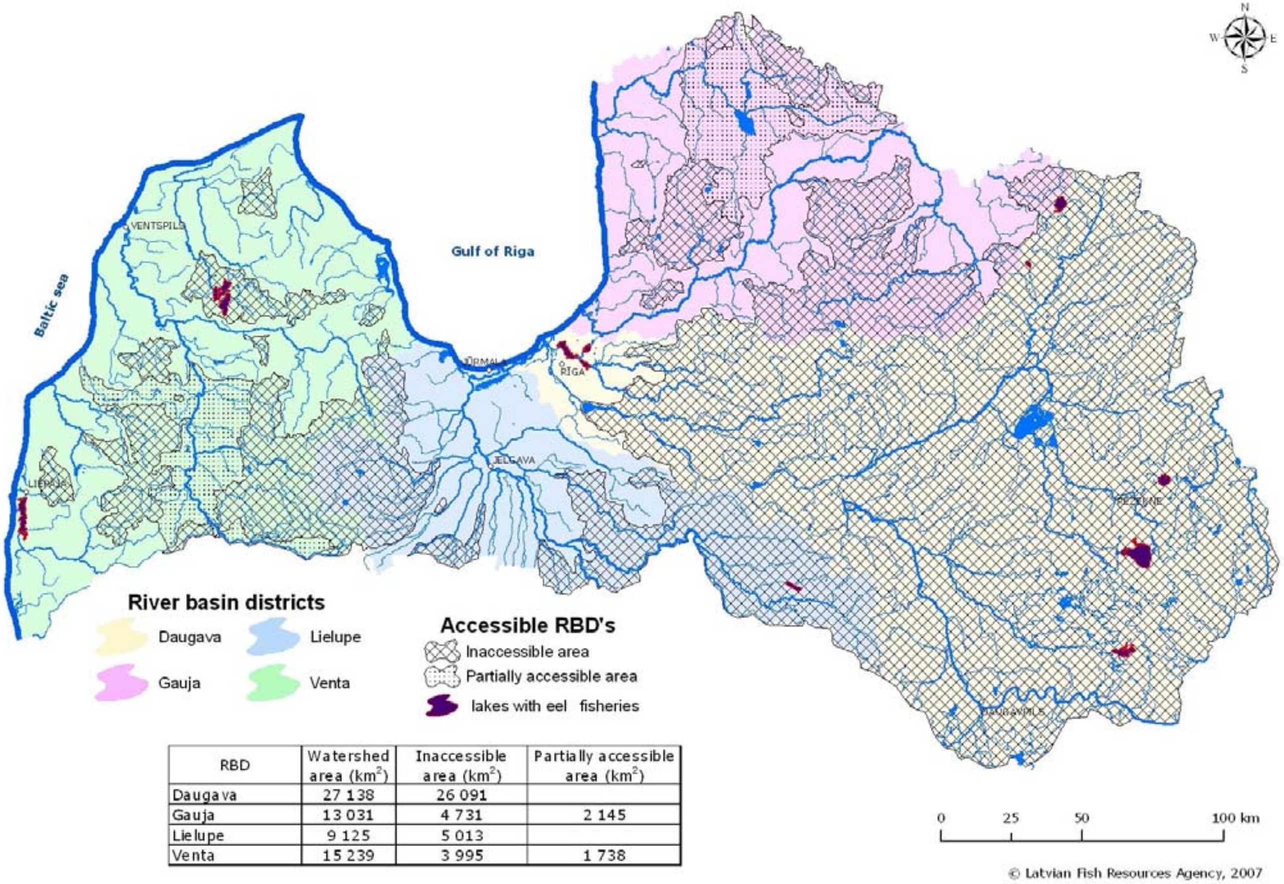
-15 with restocked eel (migration obstacles)~22400 ha

- Separate rivers

without HPS dams;

In total more than 60% of country territory is inaccessible for migratory fish species

Accessibility of the river basin districts (RBD), Latvia



RBD	Watershed area (km²)	Inaccessible area (km²)	Partially accessible area (km²)
Daugava	27 138	26 091	
Gauja	13 031	4 731	2 145
Lielupe	9 125	5 013	
Venta	15 239	3 995	1 738

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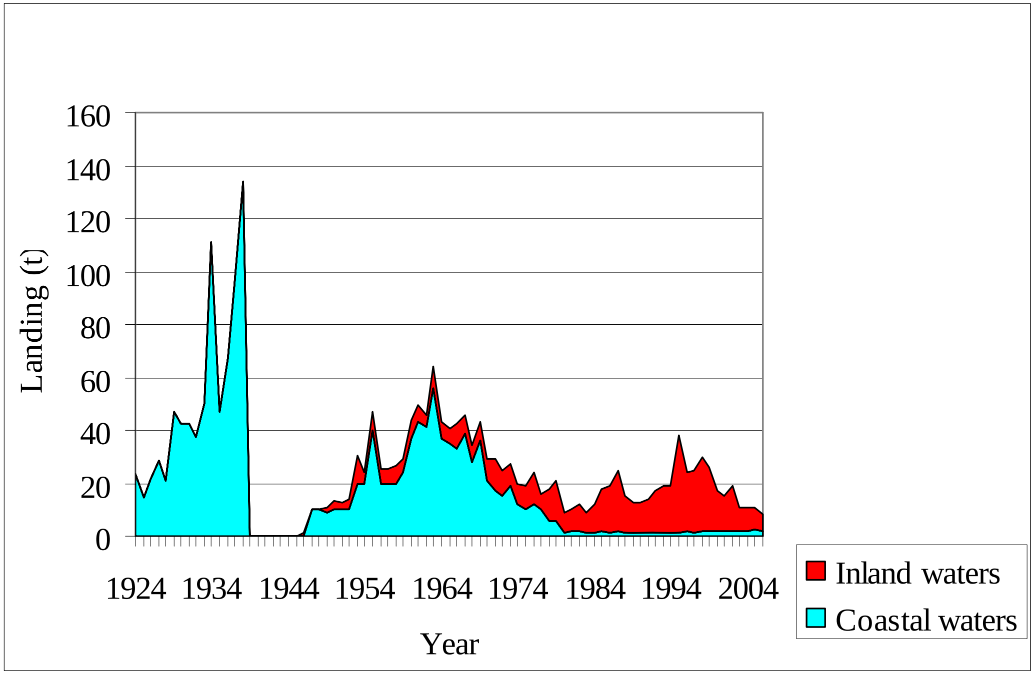
Eel fisheries

- Only stationary gears are used:

In 30- 50ies anchored long lines in coastal waters and fyke-nets in lakes fisheries;

Currently- fyke and trapnets, in some lakes- eel

- The eel landings in LV coastal and inland waters continue decreasing, in fact it reaches historically lowest level.



Fishing capacity:

-in 2006 66 full and part time fishermens reported eel landing in coastal waters;

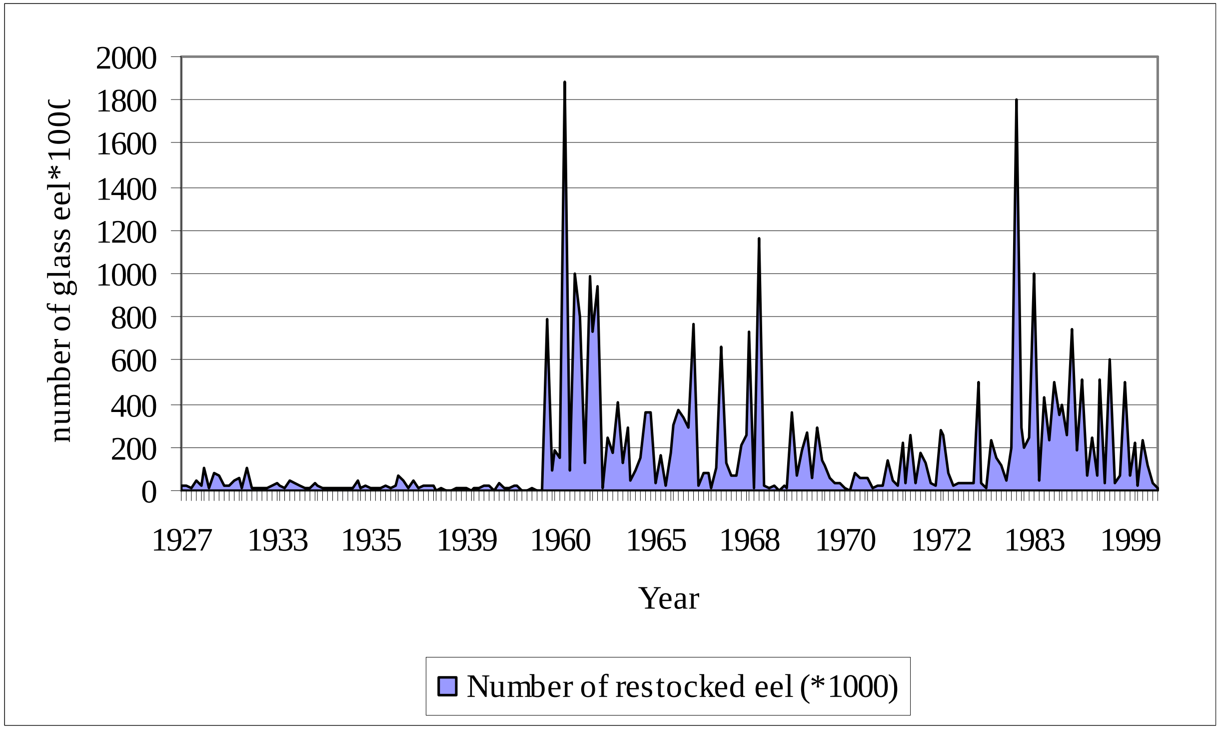
-38 small enterprises reported the eel landing in inland waters

Eel restocking

First glass eel restockings carried out in 1927

The number of restocked eel increase in soviet period, when releasing was organized as state supported program

In total eel was introduced in 80 lakes



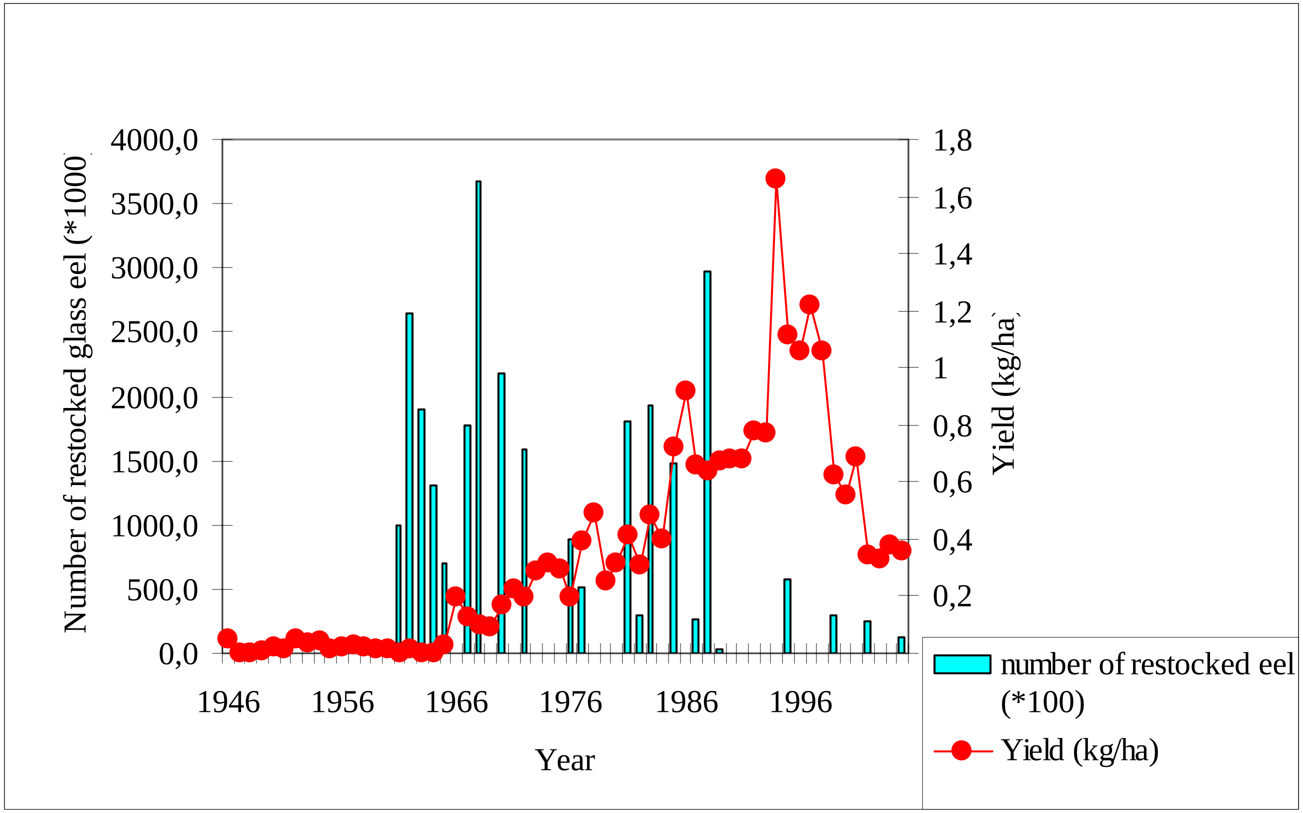
Eel restocking peaked in 60- 70s and 80- 90

Currently restocking carried out by private companies- fishing rights leasholders

Effectiveness of eel restocking, yield

In ecological conditions in East Baltic eel restocking efectiveness in average not exceed 2 kg/ha.

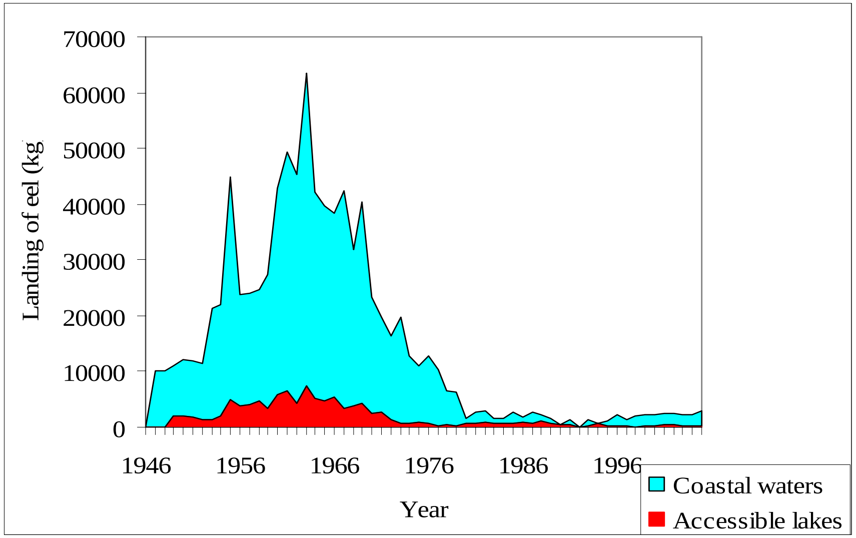
In some more productive lakes these productiviti increase up to 4-5 kg/ha



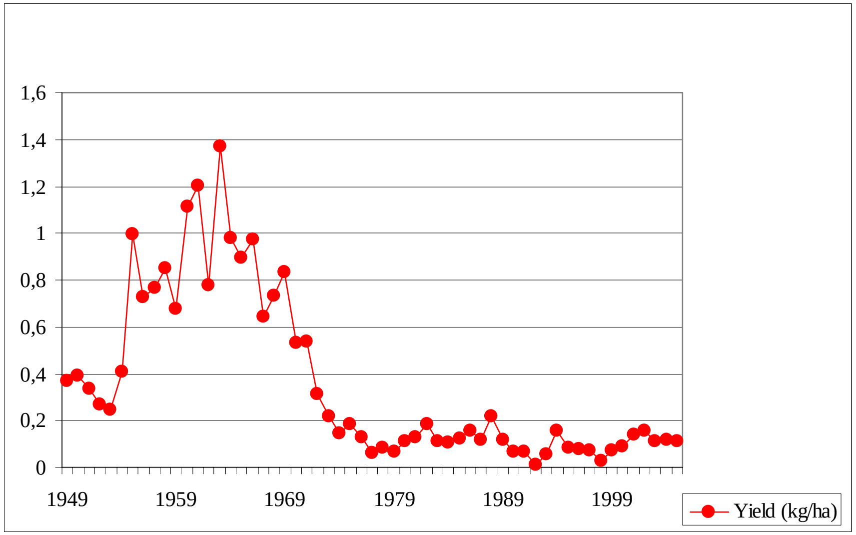
First results of restocking of glass eel for fisheries become relevant only after 8- 10 years later. Effect would be sustainable at least 20 years

Natural production of eel

The landings of natural eel decreased in period from 1970- 1980 in coastal waters and in lakes accessible for eel migration;



Natural eel production in lakes and lakes systems accessible for migratory fish demonstarted the same picture- fast decreasing of yield in 1970s



Samplin and research

Sampling oragnized in cooperation with two small fisherie's enterpises: in Gulf of Riga near the river Daugava outlet and lake Kisezers;

Currently special studies not planned

However, to contribute the DCR requirements biological data- lenght, weight, otholits etc. collected in 2006/07: 200 specimens per year

Perspectives for cooperation

- As partner for cooperation LFRA would provide the sampling, including tissues or organs
- LFRA as partner for cooperation are interested for eel originality and age determination. This data would be important for Eel management plan developing what will be obligation for every EU member contry

PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B, Vol. 61 (2007), No. 3/4 (650/651), pp. 91–95.

VARIETY OF mtDNA HAPLOTYPES IN THE POPULATIONS OF THE EUROPEAN PERCH (*Perca fluviatilis*) OF THE CURONIAN LAGOON, THE COASTAL ZONE OF THE BALTIC SEA AND THE GULF OF RĪGA

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Contributed by Isaak Rashal

A total of 56 perch individuals attributed to three different populations—the Baltic Sea (Lithuanian costal zone), the Curonian Lagoon (Lithuania) and the Gulf of Rīga (Latvia)—were investigated. The amplified mtDNA fragment consisting of 378 bases includes the trnT *gene and the 5'-fragment of the control region consisting of 260 bases. Ten variable positions were identified and 15 haplotypes of the European perch of mitochondrial DNA (mtDNA) D-loop were established. Three new haplotypes C4, F8 and G2 characteristic of the perch caught in Lithuanian territorial waters and two haplotypes L1 and L2 characteristic of the perch from the Gulf of Rīga were determined. Five rarer haplotypes were found in the samples of the Lithuanian perch and four in the samples of the Latvian perch, which characterise the scope of reproductive isolation among the populations. The similarity in the distribution of the most frequently found haplotypes A and F in the Lithuanian samples investigated illustrate the similarity of the perch populations and substantiate the assumption of possible seasonal migration of the perch between the Curonian Lagoon and the coastal zone of the Baltic Sea.*

Key words: *perch, mtDNA, haplotypes.*

INTRODUCTION

Considerable climatic changes during the last 2.5 million years resulted in the fact that the Baltic Sea and part of the European continent periodically became covered with ice. This, together with other physically geographical barriers, had an impact on the formation of the genetic structure of the species living there. The unequal frequency of a distribution of different alleles in the populations reflects this process (Nesbø *et al.* 1999). This has been supported by the investigation of freshwater fishes carried out in North America, which revealed significant correlation between postglacial migration and the distribution of genetic lineages (Bernatchez, 1998).

Since the analysis of the isoenzyme systems of widespread species, such as the European perch (*Perca fluviatilis*), revealed their low informativeness as genetic markers (Mardsen *et al*., 1995), DNA markers, including randomly amplified polymorphic DNA fragments and mtDNA markers have been used to investigate the features of the genetic structure of the European population of this species (*Nesbø et al*., 1998). Suitability of perch in investigations of the formation of the genetic structure of populations conferred by its universal distribution in the European network of river basins, and their adaptation to the life under conditions of varying water temperature and even salinity. Effectiveness of the use of DNA markers has been illustrated by the analysis of the variability of the sequence of mitochondrial DNA (mtDNA) D-loop of perch and the phylogenetic relationships of perch samples from Swedish territorial waters in the Baltic Sea. In samples of perch representing populations with different migration behaviour, a distinct spectrum of haplotypes was discovered, which was confirmed by construction of phylogenetic relationships from the analysis of RAPD markers (Nesbø *et al.,* 1998). In another investigation of 44 samples of perch from river basins of the European continent the distribution frequency of mtDNA D-loop was established and proved that there were several centres from which the perch could spread into Northern Europe in the postglacial period (Nesbø *et al*., 1999).

However, till now there has been no DNA marker data available concerning the genetic diversity of the perch in the inland waters of Lithuania and Latvia or in the coastal waters of the Baltic Sea. The only survey known in the region is the analysis of perch populations in Kaunas city reservoir, in the Curonian Lagoon and in the Baltic Sea coastal zone by means of isoenzymes as biochemical markers (Paulauskas and Ložys, 2001). The aim of the present work was to determine polymorphism of sequences of mtDNA D-loop and to evaluate the genetic diversity of the populations of perch representing different areas of the Baltic Sea.

MATERIAL AND METHODS

A total of 56 European perches *(Perca fluviatilis)* caught in the years 2004–2006 were used for the investigation. Altogether 15 individuals were caught in the Baltic Sea, 18 individuals in the Curonian Lagoon and 23 in the Gulf of Rīga. Among them, in 2004, nine individuals were caught in the Baltic Sea near Monciškės and six individuals caught in the Baltic Sea near Būtingė, both Lithuania. In 2005, ten individuals were caught in the Curonian Lagoon (Lithuania) near Kiaulės nugara and eight individuals in the same lagoon near Ventės ragas, and in 2006, 23 European perches in the Gulf of Rīga near the mouth of the River Daugava (Latvia).

Genomic DNA was isolated from the samples of muscle tissues fixed in ethanol by means of the salt extraction method (Aljanabi and Martinez, 1997).

Amplification of mtDNA fragments was carried out using *Mastercycler Gradien* amplifier manufactured by the *Eppendorf* Company. For amplification of mitochondrial DNA sequences the following primers were used: HV2: TTCCCCGGTCTTGTAAACC; and CSB-D: GGAACCAAATGCCAGGAA.

PCR was carried out by denaturation at 96 ^{o}C temperature for 5 minutes, then 30 cycles at 96 ^{o}C for one minute, at 54 ^{o}C (HV2 – CSB-D) for one minute, followed by an elongation step at 72 ^{o}C for 2 minutes and finishing with a final elongation step at the 72 ^{o}C for 5 minutes. The PGR product was checked in 1.5% agarose gel and purified by CIAP and ExoI nucleases: for 15 minutes at 37 ^{o}C and then for 15 minutes at 85 ^{o}C.

Sequencing was carried out at the Sequencing Centre of the Institute of Biotechnology (Vilnius, Lithuania). DNA sequences were determined by an ABI Prism 377 automatic sequencer. Sequencing results were compared with the PFY14724 sequence using the BLAST database. The computer programme CLC Free Workbench version 0.91 was used for the sequence analysis and for the construction of phylogenetic tree.

RESULTS

A total of 56 perches attributed to three different populations of the Baltic Sea, the Curonian Lagoon and the Gulf of Rīga were investigated. The amplified mtDNA fragment consisting of 378 bases includes the *trnT* gene and the 5‘-fragment of the control region consisting of 260 bases. Ten variable positions were identified and 15 haplotypes of the European perch of mtDNA D-loop were established (Table 1). Three new haplotypes, we designated as C4, F8, and G2, characteristic of the perch caught in Lithuanian territorial waters and two haplotypes, designated as L1 and L2, characteristic of the Latvian perch were determined (Table 1).

The distribution of haplotypes in the studied populations is presented on Figure 1. The most frequent F haplotype was discovered in samples of perch caught both in the Curonian Lagoon and the Baltic Sea near Šventoji. Haplotype A was found to be the second by frequency in the populations of the Lithuanian perch, and haplotypes C and E are most often found in the populations of perch of the Gulf of Rīga. Haplotypes C1 and A7 were found in all investigated populations. Five rarer haplotypes were found in the samples of the Lithuanian perch and four in the samples of the Latvian perch, which characterises the scope of reproductive isolation among the populations of these perches. Haplotype E was found with minimum frequency on the coastal zone of the Baltic Sea near Šventoji only in the samples of 2004 and 2005.

Table 1

HAPLOTYPES OF mtDNR *trnT* gene AND CONTROL REGION FRAGMENT DETERMINED IN EUROPEAN PERCH *Perca fluviatilis* SAMPLES CAUGHT IN THE TERRITORIAL WATERS OF LITHUANIA AND LATVIA (data are compared with the mtDNA sequence PFY14724, haplotype A presented in the Gene Bank)

Haplotypes	Position										N
	98–99	103	129	130	135	136	137	139	264	299	
A	-	A	G	C	A	A	T	T	T	C	6
A2	-			T							1
A7	-		T	T							4
C	-									T	7
C1	-		T	T						T	4
C4	-		T	T					A	T	1
E	-	T					-			T	2
F	-	T								T	23
F1	-	T	T	T						T	1
F2	-	T	T	T	G					T	1
F7	-	T	T							T	2
F8	-	T				-				T	1
G2	-							-		T	1
L1	Ins*	T		T						T	1
L2	Ins*			T							1
Total:											56

C4, G2, F8, L1 and L2 – newly identified haplotypes.

N, number of individuals.

T, A, and G, substitution of nucleotides.

- – deletion of nucleotides.

Ins*, insertion of ten nucleotides (TTGCAAGCAC).

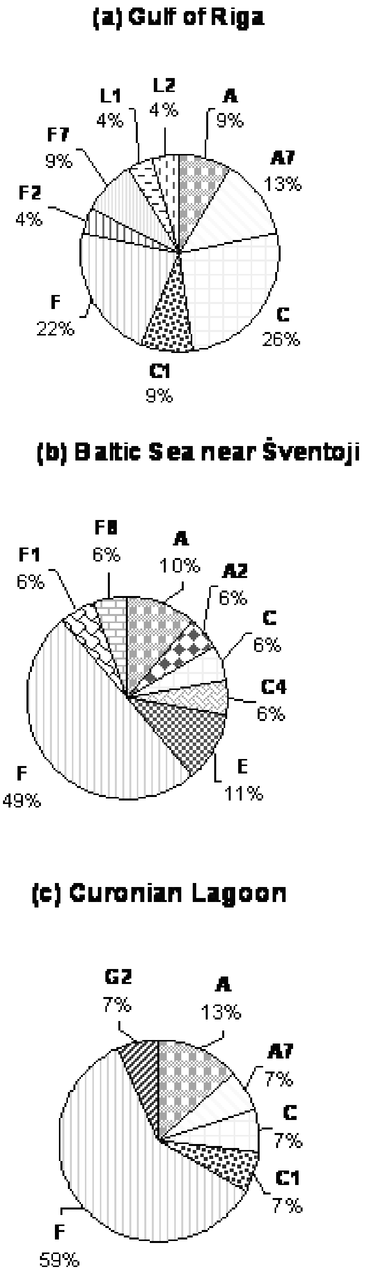

Fig. 1. Frequencies of mtDNR haplotypes in the investigated populations of European perch in the Gulf of Rīga (a), the Baltic Sea near Šventoji (b) and the Curonian Lagoon (c)

DISCUSSION

Nesbø *et al.* (1998) and Refseth *et al.* (1998) determined in total 35 haplotypes grouped into eight closely related groups by the nature of base substitutions. Based on this data, several zones, separated from the phylogenetic point of view, were postulated, with prevailing different groups of mtDNA haplotypes (Nesbø *et al.*, 1999). In all perch populations of Western Europe, haplotypes of group F dominated, with the exception of those in river basins stretching in the territory of Norway where haplotypes of group A (A, A1–A9) prevailed, as well as the coastal zone of the Adriatic Sea and river basins of the Balkan countries (oldest in the term of origin), in which haplotypes of group M (M, M1) prevailed. In samples of perch of the continental part of the mainland, in the basins of the Nemunas, Dnieper rivers, as well as in the remote Lake Baikal, the Angara River basin, haplotypes of group C (C, C1–C3) prevailed. This indicated separation of perch in the basins of the rivers stretching across this territory to a separate Euro-Asian phylo- geographical region.

Haplotype E was found with minimum frequency on the coastal zone of the Baltic Sea near Šventoji only in the samples of 2004 and 2005 which could be related to the anadromous behaviour of those perch, i.e., a periodical change in the environment when fish feeding in more saline waters of the coastal zone of the Baltic Sea swim to spawn to the fresh waters of the river mouths. A repeated discovery of haplotype E in the samples of the perch caught in the coastal strip near Monciškės not far from the settlement of Šventoji in different years can be related to a larger part of the individuals keeping near the spawning site located in the approaches of the mouth of the Šventoji River, and rarer variants of haplotypes (haplotype E), inherited with steady, though small frequency, reflect the Hardy-Weinberg balance of the population.

Newly identified haplotypes C4 and F8 were found in the samples of the Baltic Sea. Also, haplotype G2 found in the samples from the Curonian Lagoon are characteristic of the Lithuanian population of the perch and are related to the group of haplotypes E–F (Fig. 2). The presence of an insertion of ten nucleotides characteristic of the newly identified haplotypes L1 and L2 could be accounted for by a close phylogenetic link of these haplotypes. However, on the basis of grouping of these samples in the phylogenetic tree, according to the nature of single base substitutions, haplo-

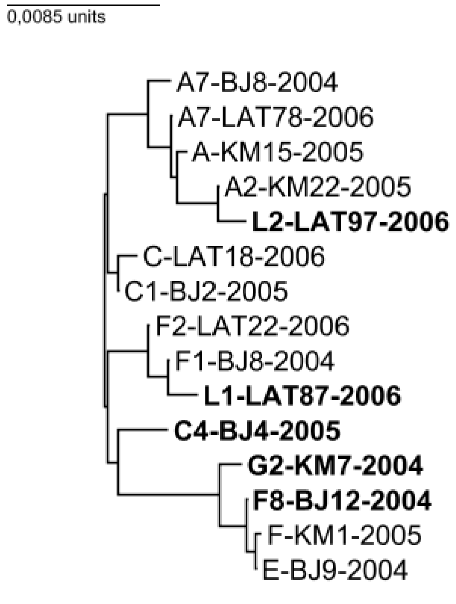

Fig. 2. The *Neighbour joining* phylogenetic tree representing the origin of haplotypes.

The first letter of abbreviation stands for the haplotype, BJ-samples from the Baltic Sea, KM—samples from the Curonian Lagoon, LAT—samples of perch caught in the Gulf of Rīga.

Table 2

SAMPLE COLLECTION SITES AND DISTRIBUTION OF HAPLOTYPES IN POPULATIONS OF THE EUROPEAN PERCH

Population	Region	Number of individuals studied	Water basin	Haplotypes (N*)
Curonian Lagoon**	Lithuanian territorial waters	15	Baltic Sea, mouth of the Nemunas	A(2), A7(1), C(1), C1(1), F(9), G2(1)
Baltic Sea**	Lithuanian territorial waters	18	Baltic Sea near Šventoji	A(2), A2(1), C(1), C4(1), E(2) F(9), F1(1), F8(1)
Gulf of Rīga**	Latvian territorial waters	23	Baltic Sea, the Gulf of Rīga near the Daugava mouth	A(2), A7(3), C(6), C1(2), F(5), F2(1), F7(2), L1(1), L2(1)
L. Krylovo***	Russia, inland waters	5	Basins of the Vilija/Nemunas Rivers	C(5)
L. Hencza***	Inland waters of Poland	5	The Nemunas basin	C(4), C1(1)
L. Dubrovskoje***	Inland waters of Russia	10	The Dnieper basin	C(10)
Gulf of Matsalu***	Inland waters of Estonia	10	Baltic Sea	A8(3), F(4), F1(1), F7(1), E2(1)
Emjajogi R.** *	Inland waters of Estonia	5	Baltic Sea, the Gulf of Finland	A(1), A7(1), A8(1), C(2), J1(1)
L. Peipsi ***	Inland waters of Estonia	9	Baltic Sea, the Gulf of Finland	A(1), A7(1), A8(1),C(2), J1(1)
L. Vourasjarve ***	Inland waters of Norway	10	Karasjakka/Tana	A(4), A2(6)
The Angara River***	Inland waters of Russia	10	Baikal Lake, the Angara basin	C(10)
L. Rybinsk***	Inland waters of Russia	14	The Volga basin	A(1), C(7), C2(1), C3(2), G1(1), J(2)
The gulf of Bothnia***	Territorial waters of Sweden	20	Baltic Sea	A7(2), C(14), F1(4)
Anadromous A***	Territorial waters of Sweden	20	Baltic Sea	A(2), A7(4), C(8), F(3), F1(2), G(1)
Anadromous B***	Territorial waters of Sweden	17	Baltic Sea	A(4), C(6), C1(1), F(2), F1(2), G(2)
L. Ängersjön***	Territorial waters of Sweden	19	Lake near the Baltic Sea	A5(1), C(13), C1(4), F1(1)
L. Race***	Inland waters of Slovenia	10	Drava/Danube	C(1), M1(9)

* in brackets the number of individuals of the particular haplotype is given

** data from this research

*** data from Nesbø *et al.*, 1999

type L1 is closest to the group of haplotypes F1 and F2, which is related to the group of haplotypes F fully dominating in Western Europe, and haplotype L2 is closest to the group of haplotypes A typical of the western and northern parts of Scandinavia (Norway).

A combination of haplotypes A and F is characteristic of the samples of the Matsalu perch population investigated in the geographical range of the Baltic Sea (territorial waters of Estonia) (Table 2), which geographically is one of the closest to the sample of the Latvian perch investigated by us but, however, which differs greatly from the Emjajogi and Peipsi populations (the Gulf of Finland, territorial waters of Estonia) in which the haplotypes of groups A and C suggest influence of the Northern and Eastern phylo-geographical regions on the formation of the perch population of the Gulf of Finland.

The phylogenetic link between the Estonian and Latvian perch populations is confirmed by the most frequently presence of haplotypes of groups A and F in the Matsalu population of the Gulf of Rīga, but the Latvian perch population is noted for a rather high frequency of haplotypes C, which is related to the Euro-Asian phylo-geographical region extending in the East. The frequency of haplotypes of group C is three times lower in the populations of the Lithuanian perch in the samples of both the Curonian Lagoon and the coastal zone of the Baltic Sea, hence, the total spectrum of frequencies of haplotypes is closer to that of the Western Europe, although the “eastern” haplotypes of group C entirely dominate in the perch in the basins of the Vilija and Nemunas rivers in other than Lithuanian territory.

The lowest diversity of haplotypes (six) was observed in the perch population of the Curonian Lagoon. Similarly, perch populations of the Gulf of Bothnia in the territorial waters of Sweden, non-migrating perch populations had three or four different haplotypes, the lowest diversity of haplotypes, while perch with an anadromous behaviour had six different haplotypes (the size of samples fluctuated from 17 to 20 individuals). In brackish coastal waters of the Baltic Sea near Šventoji and in the Gulf of Rīga, where the perch generally have anadromous behaviour, a greater diversity of haplotypes—eight or nine—was found. The similarity in the distribution of the most frequently found haplotypes A and F in the samples investigated illustrates the similarity of the populations of the Lithuanian perch, supporting the idea of possible seasonal migration of the perch between the Curonian Lagoon and the coastal zone of the Baltic Sea: adult individuals migrate in summer to the brackish Baltic Sea probably due to the beneficial effect of water salinity on the perch growth, whereas in autumn they return to the freshwater Lagoon, stay there in winter and spawn the following spring (Ložys, 2004). In this case, the lower diversity of haplotypes in summer population of the perch of the Curonian Lagoon suggests seasonal migrations of part of the perch population to the Baltic Sea.

Further work on the structure of populations in relation to seasonal migration to brackish waters requires the use of microsatellite markers (Brunner *et al*., 1998; Englbrecht *et al*., 2002). Several specific microsatellite primers have already been developed for species of yellow perch, which is taxonomically close to the European perch (Leclerc *et al*., 2000), thereby aiding to overcome the stage of creating specific primers for the species, and allowing investigators to make use of the available information in continuing investigations into the interspecific genetic diversity and population structure of the European perch.

ACKNOWLEDGEMENTS

We express our gratitude to the Mutual Funds for the Scientific Co-operation of the joint project of Taiwan, the Republics of Latvia and Lithuania "Application of genetic and micro-chemical markers as implements for diadromous and endangered commercial fish species populations management" for its financial assistance and the provided possibility to carry out this project.

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Received 20 November 2006

mtDNS HAPLOTIPU DAŽĀDĪBA KURŠU LĪČA, BALTIJAS JŪRAS PIEKRASTES ZONAS UN RĪGAS LĪČA ASARU (*Perca fluviatilis*) POPULĀCIJĀS

Īpatņiem no trim asaru populācijām tika pētīts mtDNS haplotipu sadalījums. Trīs jauni haplotipi atrasti starp Lietuvas paraugiem, divi – starp Latvijas paraugiem. Atrasti vairāki reti haplotipi, kas raksturo Latvijas un Lietuvas populāciju īpatnības, kuru cēlonis ir reproduktīvā izolācija. Savukārt A un F grupu haplotipu līdzīgais sadalījums Kuršu līča un Lietuvas Baltijas jūras piekrastes populācijās liecina par iespējamo sezonālo migrāciju starp šiem ūdens baseiniem.