Jyrki Torniainen

Migratory Connectivity amongst Baltic Sea Salmon

$\delta^{13}C_{DIC}$ $\delta^{18}O$

20:5ω-3

$\delta^{13}C_{diet}$

$\delta^{15}N$

22:6ω-3

JYVÄSKYLÄN YLIOPISTO

Jyrki Torniainen

Migratory Connectivity
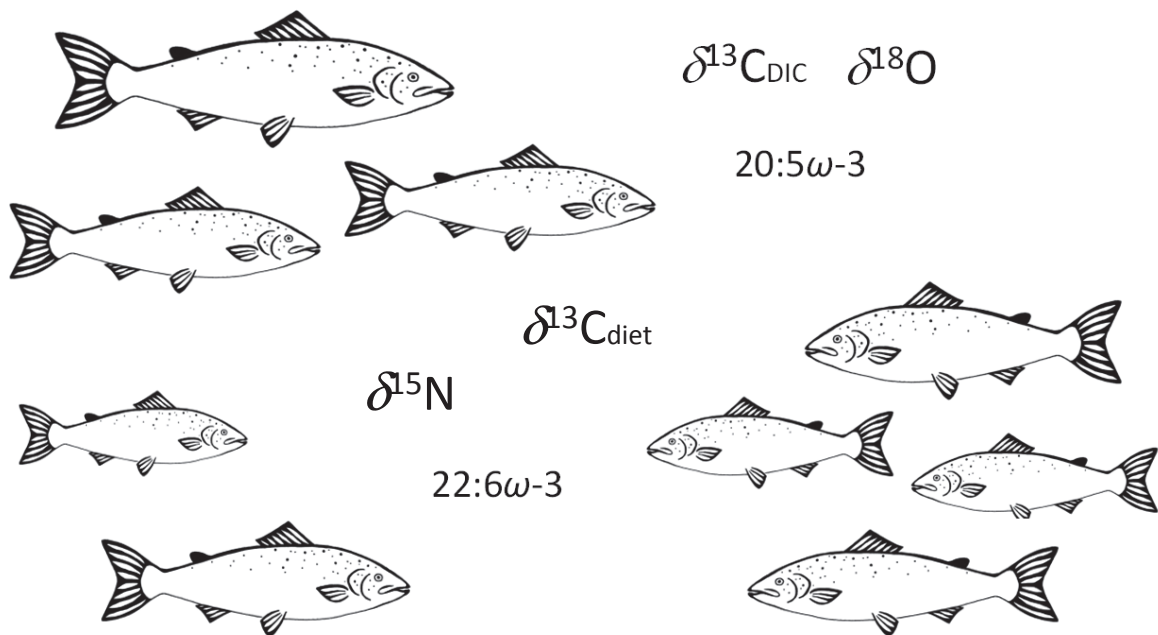# amongst Baltic Sea Salmon

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2014

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Cover drawing by Kaisa Raatikainen.

Dedicated to my grandparents

ABSTRACT

Migratory connectivity refers to the extent to which migratory individuals areas of a species or population occupy the same breeding and non-breeding areas. A population showing strong migratory connectivity could be threatened if the single non-breeding area habitat is degraded. A population showing weak migratory connectivity might be safer, if one of the non-breeding areas is threatened but the rest are secure. Although the situation is improving, preservation of the last remaining wild Baltic Sea salmon populations is still crucial and better understanding of their migration patterns is needed. Previous tag-recovery methods in salmon migration studies have relied on commercial fishing in the sea areas and are thus ineffective for revealing any consequences of migration patterns on individual characteristics. Stable isotope analysis of salmon scales and otoliths was used in this study to follow movements of Baltic Sea salmon individuals retrospectively in more detail, and even through the whole lifetime of a salmon by transect analysis of its otoliths. In addition, analyses of salmon surviving to ascend the spawning rivers better represent the actual breeding population than fish caught at sea. River Simojoki salmon mainly feed in the Baltic Proper, and River Kymijoki salmon in the Gulf of Finland. Other sea areas provide feeding areas for smaller but appreciable parts of both populations. Migratory connectivity was weak for both populations, but remained rather fixed through the studied timespan. Otolith analysis revealed striking movements of individual salmon between summer and winter. Swimming movements were almost entirely in a northern direction apparently due to the onset of the spawning migration. Fatty acid composition of muscle and eggs was higher in quality for those ascending salmon assigned as having been feeding in the Baltic Proper, indicating the Baltic Proper to be a higher-quality feeding area than the other areas. However, wild and hatchery-reared salmon had similar migratory patterns in the sea, which complicates possible strategies for conservation of wild salmon in the sea areas.

Keywords: Fatty acids; feeding area quality; isoscape; migratory connectivity; M74 syndrome; otolith micromilling; stable isotope analysis.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-IV.

I wrote the papers where I am first author and made a significant contribution to writing of III. I had an important role in planning the papers with the assistance of co-authors. I sampled, prepared and analysed the material and data for papers I and IV and made a major contribution to II and III. Mikko Kiljunen carried out the otolith micromilling for II and III. All papers were finalised with the co-authors.

I   Torniainen J., Vuorinen P.J., Jones R.I., Keinänen M., Palm S., Vuori K.A.M. & Kiljunen M. 2014. Migratory connectivity of two Baltic Sea salmon populations: retrospective analysis using stable isotopes of scales. *ICES Journal of Marine Science* 71: 336–344.

II   Torniainen J., Lensu A., Vuorinen P.J., Sonninen E., Keinänen M., Jones R.I., Patterson W.P. & Kiljunen M. 2014. Oxygen and carbon isoscapes for the Baltic Sea and an example of their potential for revealing probable fish locations. Submitted manuscript.

III Kiljunen M., Torniainen J., Lensu A., Keinänen M., Patterson W.P., Sonninen E., Vuorinen P.J. & Jones R.I. 2014. Seasonal movements of individual salmon in the Baltic Sea revealed by otolith oxygen stable isotopes. Manuscript.

IV   Torniainen J., Kainz M.J., Vuorinen P.J., Keinänen M., Jones R.I. & Kiljunen M. 2014. The final nonbreeding area quality of Atlantic salmon (*Salmo salar*) estimated by fatty acid and stable isotope composition. Submitted manuscript.

ABBREVIATIONS

ARA	arachidonic acid, 20:4n-6
DA	discriminant analysis
DIVA	Data Interpolating Variational Analysis
DHA	docosahexaenoic acid, 22:6n-3
EPA	eicosapentaenoic acid, 20:5n-3
FA	fatty acid
FGFRI	Finnish Game and Fisheries Research Institute
ICES	International Council for the Exploration of the Sea
ODV	Ocean Data View
PL	phospholipids
SIA	stable isotope analysis
TAG	triacylglycerols
$\delta^{13}C_{DIC}$	carbon isotope value of dissolved inorganic carbon in water
$\delta^{18}O_{H2O}$	oxygen isotope value of water

1 INTRODUCTION

1.1 Migration

The term migration can be considered to refer to a type of directional movement in an ecological context, either one-way or to-and-fro. It is a common and widely observed ecological phenomenon among almost all taxa (e.g. Baker 1978, Dingle and Drake 2007). Probably the most famous migrations are undertaken by birds, which have also been the most inspirational target group for migration studies (Hobson and Wassenaar 2008). Studies of insects, mammals and fish have all also contributed to understanding this ecological phenomenon (e.g. Hobson and Wassenaar 2008). Migration as a term is somewhat subjective and a thorough definition accepted by everyone does not exist (Dingle 2006). However, it is generally agreed that migration evolves ubiquitously in situations with considerable variation in resources and seasonality (Cresswell *et al.* 2011), or threat (e.g. McKinnon *et al.* 2010). In other words, migration occurs as a response to reasonably predictable cyclic events and to habitat heterogeneity (Milner-Gulland *et al.* 2011).

Migration is a movement of an individual, and can be scaled up to populations, species, and eventually to the landscape level (Milner-Gulland *et al.* 2011). Dingle and Drake (2007) have listed four concepts in which use of the term migration is appropriate: "*1) a type of locomotory activity that is notably persistent, undistracted and straightened out; 2) a relocation of the animal that is on a much greater scale and involves movements of much longer duration, than those arising in its normal daily activities; 3) a seasonal to-and-fro movement of populations between regions where conditions are alternatively favourable or unfavourable (including one region in which breeding occurs); and 4) movements leading to redistribution within a spatially extended population*". The first two are individual concept descriptions and the last two apply at a wider level. Within previous definitions there are at least the following types of different migratory behaviours: to-and-fro, round-trip, one-way, altitudinal and nomadic (Dingle and Drake 2007).

Countless factors relating to the evolution of migration can be listed. Fryxell *et al.* (2011) mentioned several: "*to take advantage of spatially distributed*

resources; to evade predators; to gain access to rich but temporary resources; to find suitable habitats for different life stages with markedly different physical needs". These attributes are widely found in anadromous salmonid life-cycles involving migration between freshwater breeding areas and marine feeding areas. In addition, instead of being obligate, migration within a population can be also plastic or facultative. It is not always necessary that all individuals of the migratory species migrate. Several bird species are partially migratory and the evolution of partial migration can be rather fast (Bearhop *et al.* 2005). This flexibility in migratory behaviour is also found in salmonid fish species (Dodson *et al.* 2013). For example, brown trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.) display migratory and non-migratory (or delayed migration) behaviour within the same populations (Klemetsen *et al.* 2003, Syrjänen and Valkeajärvi 2010).

During the last decades there have been numerous observations of shortening and even loss of migration in terrestrial (Wilcove 2008, Harris *et al.* 2009) and aquatic animals (e.g. Auer 1996, Mills 2003, Hanrahan *et al.* 2004). Observations are linked to human activities like climate change and building barriers like damning (Wilcove and Wikelski 2008) and overexploitation via unsustainable fishing policy (Syrjänen and Valkeajärvi 2010). Conservation of threatened migratory species differs from that of non-migratory species due to the migratory species lifecycle complexity (Wilcove 2008). Considering the classic to-and-fro migration as an example, with seasonal movement between the breeding and the non-breeding areas, in addition to understanding the species requirements (physiological, behavioural etc.) conservation planning needs understanding of the characteristics and conditions in both areas (Wilcove 2008, Shuter *et al.* 2011). It may be useless to conduct serious conservative actions in the breeding area, if the ultimate reasons for population decline are at the opposite end of the migratory species seasonal journey.

Migratory species have a cyclic impact on the habitat functioning in the areas where they perform their life-cycle of appearance and disappearance. On the one hand they offer resources when they arrive, but then they also share the same resources as resident species. Migratory species connect the opposite ends of their life-cycle habitats, and also the possible stopover areas to each other.

1.1.1 Migratory connectivity

Migratory connectivity refers to the extent to which individuals in the same population behave in unison with respect to migration (Webster *et al.* 2002, Webster and Marra 2004). Migratory connectivity can be considered strong when all individuals of a single population breed in one place and migrate to the same non-breeding area. In contrast, when all individuals disperse evenly to separate non-breeding areas migratory connectivity can be considered weak (Webster *et al.* 2002) (Fig. 1).

Migratory connectivity is a useful concept, which clarifies the connections between breeding and non-breeding areas of a species or population. For example, a population showing strong migratory connectivity could be

threatened or even face extinction if the single non-breeding area habitat is degraded. In contrast, a population showing weak (or weaker) migratory connectivity might be safer, if one of the non-breeding areas is experiencing habitat degradation but the rest are secure. On the other hand, a population showing strong migratory connectivity could respond better to conservation activities, whereas a population showing weak connectivity might be more demanding for conservation efforts (Webster *et al.* 2002, Webster and Marra 2004).
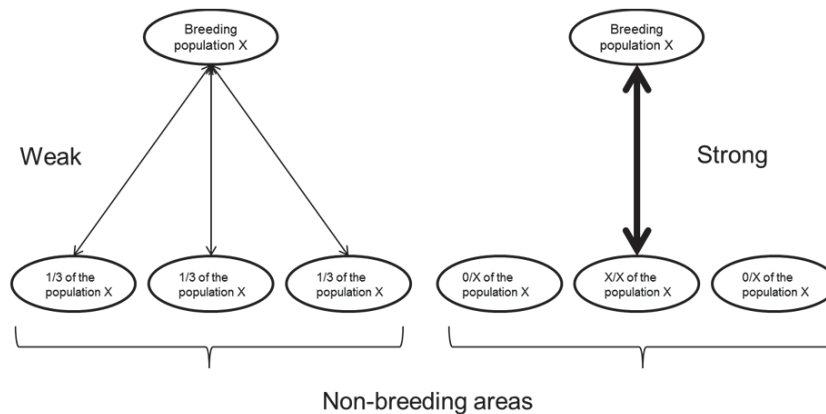
FIGURE 1   Schematic illustration of two extremes of migratory connectivity.

1.2   Baltic salmon

The well-known Atlantic salmon (hereafter Baltic salmon) life-cycle (e.g. Thorstad *et al.* 2011) begins as an egg buried by a female salmon in the gravel of a river bottom. A yolk-sac fry hatches in spring, but remains hidden in the gravel utilizing the yolk reserves. When a young salmon emerges from gravel, it starts a 2–4 year riverine phase as a parr. The following smolt phase begins when a parr becomes a silvery colour and several physiological changes prepare the young salmon for the saline sea environment. During the smolt phase, the salmon migrates downstream towards the river estuary, and eventually to the sea. The next phase is a post-smolt migration towards the sea final feeding areas. Adult salmon in the Baltic Sea grow fast by foraging mainly on sprat (*Sprattus sprattus* (L.)) and herring (*Clupea harengus membras* L.) (Hansson *et al.* 2001). Usually after 1-3 years, a salmon starts its spawning migration back to the natal river (e.g. Mills 2003, Ikonen 2006, Thorstad *et al.* 2011).

Salmon from rivers draining into the Bothnian Bay mainly utilize the southern areas of the Baltic Sea as a foraging area, although a minor proportion forage in the Bothnian Sea (e.g. Salminen *et al.* 1994, Ikonen 2006, Jutila 2008)

(Fig. 2). The population of the River Kymijoki (drains into the Gulf of Finland) has been shown to exhibit more limited migration and to forage mainly in the Gulf of Finland, although appreciable numbers migrate further to the Baltic Proper (Kallio-Nyberg and Ikonen 1992). According to these observations migratory connectivity is rather weak for both populations, but R. Kymijoki salmon perhaps show stronger connectivity due to the apparent irrelevance of the Bothnian Sea as a feeding area.

Spawning migration of salmon foraging in the Baltic Proper starts in March–April (Christensen and Larsson 1979). Migrating salmon are caught by fishermen in the Bothnian Sea already in May (Ikonen 2006) and river ascendants are observed in June (Siira *et al.* 2009). Comprehensive studies concerning the spawning migration routes throughout the Baltic Sea are absent. However, Aro (1989) and Ikonen (2006) have reported that two main types of routes have been observed: Direct northward migration from the Baltic Proper to their northern natal rivers occurs via the coastlines of Finland and Sweden. On the other hand, some salmon swim appreciable distances into the Gulf of Finland before moving to the Bothnian Sea on their way to the northern natal rivers. In addition, Siira *et al.* (2009) showed rather detailed salmon migratory behaviour in the northernmost area of the Bothnian Bay right before ascending to rivers.

1.2.1 Present threats to salmon

For centuries people have been fishing salmon throughout their distribution. Initially fishing of salmon was focused on rivers by catching salmon during their upstream spawning migration. With technical development of fishing gear, commercial sea fishing has become more important and fishing in the rivers has become more recreational. Although salmon is economically (and ecologically) valuable, the interaction between humans and salmon has become imbalanced (Kulmala *et al.* 2012), and salmon have faced several environmental impacts (Mills 2003). Damming of many spawning rivers for hydropower ended salmon migration in those rivers and several populations were lost. There are 83 potential salmon rivers in the Baltic, of which only 38 still have wild salmon to some extent. In Finland only the River Simojoki still produces wild salmon, together with the River Tornionjoki along the border between Finland and Sweden (Anon. 1999).

In many situations power industry is forced to compensate the loss of wild salmon via hatchery-reared salmon stockings. However, it has been observed that reared salmon might be less viable than wild (Siira *et al.* 2006), and there are some indications that wild and reared salmon do not necessarily display similar migratory behaviour (Salminen *et al.* 1994, Kallio-Nyberg *et al.* 2006).

Hatched fry of female salmon suffering from yolk sac fry mortality disorder (M74 syndrome) die due to thiamine (vitamin B$_1$) deficiency (Bengtsson *et al.* 1999). Mikkonen *et al.* (2011) showed that main causes lie in the Baltic Proper area, especially during those years when large cohorts of young sprat are dominant in the area and are thus eaten extensively by salmon. Young

sprat are a high-fat diet, but also have relatively small amount of thiamine in their tissues. Salmon foraging on young sprat gain weight fast, are larger and have higher condition factor feeding than salmon feeding on other prey items, but have lower thiamine concentration (Keinänen *et al.* 2012).

M74 syndrome has had a severe impact on salmon reproduction, since during the worst years, in the 1990s, nearly all fry died because of this syndrome (Karlsson and Karlström 1994, Keinänen *et al.* 2008). A similar disorder has been observed in the Great Lakes of North America, where early mortality syndrome (EMS) was reported, and has been considered to be a result of similar factors as M74 (e.g. Fitzsimons *et al.* 1999).

1.3 Methods for tracking animal movements

Several marking approaches have been employed to address questions in migration ecology. Conventionally, the markers are different kinds of attached indicators, which allow tracking of an individual between the time of marking and the time of recapture or recovery. Ideal markers should also fulfil several requirements for avoidance of unbiased data (Nielsen 1992, Lucas and Baras 2000). Here it is focused on two categories, which include commonly used methods in fish migration studies.

1.3.1 Extrinsic markers

Extrinsic markers are here categorised as added markers, tags or imprints placed by humans. Many marking applications and variations exist (Lucas and Baras 2000) from nearly harmless ink tattoos to commonly used Carlin tags, which are individually-identified metal or plastic flags secured to the fish body through the skin with narrow wires. In common to all these is that tagged individuals have to be recaptured to acquire spatial information. The spatial data are usually only one-time spatial coordinates. One can only guess if the caught fish is a local inhabitant or on its way to another area. All these methods also require large numbers of markings, with relatively few recoveries. However, tag-recovery methods have their merits and have revealed areas where fish forage, crude pathways of migration and growth rates, and can provide estimates of fishing mortality and population size (Lucas and Baras 2000, Ikonen 2006).

Telemetry is a method which does not require recapture of the marked fish. However, a tagged fish with the transmitter has to be located with a receiver as many times as is needed for sufficient data to answer the study questions (Lucas and Baras 2000, Keskinen *et al.* 2005). More advanced satellite telemetry for teleost fish is limited due to requirements of the location data transfer through air. However, recent satellite geolocators have been applied to track fish movements. For example pop-up archival satellite tags (PSATs) (e.g. Lutcavage *et al.* 1999) have been used in salmon migration studies in the

northern Atlantic (Chittenden *et al.* 2013). These transmitters can offer valuable data about an individual's movements and its ambient environment, but are still expensive and therefore application for studies at batch or population level are often limited. In addition, these geolocators are quite large and thus possible only for larger fish, although some development in size reduction has occurred (Aarestrup *et al.* 2009). Due to the size of the equipment, the effect on the swimming ability of fish is also questionable. Apart from telemetry, all the mentioned methods rely on the hope of recapture.

1.3.2 Intrinsic markers

In contrast to extrinsic markers, all animals are biochemically (intrinsically) marked by the environment they live in and this is independent of recapture. In the laboratory such markers can be analysed from sample tissue. Biochemical components that have been applied as biomarkers in migration studies include DNA-analysis, contaminants, fatty acids, stable isotopes and trace elements (Secor *et al.* 1995, Rafseth *et al.* 1998, Hobson 1999, Braune and Simon 2003, MacKenzie *et al.* 2011a). The measured biomarker value from a caught individual is compared to reference values. The best comparable reference is a sample of the same tissue grown in the habitats assessed to answer the study questions. Regardless of reference, the assignment of an individual to a certain area works via classification with received probabilities of between-values correspondence (Wunder 2010).

For stable isotopes, there are global gridded data sets which may serve as a reference (West *et al.* 2010). Recent applications in migration studies using stable isotopes incorporate these isotopic landscapes (isoscapes: Hobson and Wassenaar 2008, West *et al.* 2010). Isoscapes are based on global survey stations or compiled measurements (e.g. Global Network of Isotopes in Precipitation, GNIP; Global Seawater Oxygen-18 Database). Collected information is interpolated to create a global gridded data set (Bowen *et al.* 2005). The isotopic composition of sampled study tissue reflects environmental values. Using the relationship between the tissue and the grid values (transfer functions) it is possible to receive a probability estimate of where this particular tissue has been formed (Patterson *et al.* 1993, Hobson and Wassenaar 1997).

For elemental applications, the most suitable samples for migration studies are tissues which are inert (chemical composition is fixed) after formation. Such tissues in fish are scales and otoliths (ear stones) that retain chemical information about the location and diet where they were formed (Patterson *et al.* 1993, Hutchinson and Trueman 2006, MacKenzie *et al.* 2011a, 2011b) and can be analysed afterwards. These markers even allow tracking fish movements from the time of hatching and back to the natal origin as an adult. A great advantage in using biochemical markers is that they can be linked to those individuals that actually survived the migration to their breeding habitats (Lucas and Baras 2000), and which therefore better represent the population. These conditions are rarely achieved using conventional tag-recovery methods. Some biomarkers have additional applications. For example, fatty acids can be

used as a tool for spatial assignment, but they have also qualitative capabilities to indicate condition of individual animal (Jobling 1994, Bell 1998, Sargent *et al.* 2002).

1.4 Aims of the study

The overall aim of this study was to reveal salmon movements at individual level in the Baltic Sea using new methodological approaches, and hence to evaluate salmon migratory connectivity.

The study had following specific objectives:
  i) to use a combination of modern methods to clarify salmon feeding migration characteristics in the Baltic (I, II, III);
  ii) to investigate possible seasonal and annual variability in feeding area selection and migratory connectivity within and between different salmon populations (I, III);
  iii) to compare migratory behaviour between hatchery-reared salmon and salmon of wild origin; (I)
  iv) to investigate how final sea feeding area may affect salmon fatty acid composition and hence reproductive fitness (IV).

These objectives were tested by analysis of salmon scale and otolith stable isotope values of oxygen (O), carbon (C) and nitrogen (N) together with the Baltic Sea water stable isotope values of O and dissolved inorganic C (DIC) (I, II, III). The studied salmon were from the River Simojoki, the River Kymijoki and three main salmon feeding areas of the Baltic Sea. The R. Simojoki is one of the last remaining undammed rivers in Finland, which still produce wild salmon, whereas the R. Kymijoki salmon are practically all of hatchery-reared origin. Almost 1900 salmon scale samples were obtained to evaluate the isotopic composition of salmon scales from different feeding areas and for the two salmon populations (I). One main purpose was to determine sea water stable isotope values of O and C to create the Baltic Sea water isoscapes (II). Using otolith micromilling to obtain a chronosequence of samples analysed for O and C stable isotopes, and comparing these values to the Baltic Sea isoscapes, the aim was to reveal detailed movements and habitats of individual salmon (II, III). Fatty acid compositions were analysed from female salmon muscle tissue and eggs to evaluate how the fitness of salmon ascending the rivers might depend on the likely final sea feeding area (IV). Using the intrinsic marker methods described in this thesis, it was possible to study movements of large numbers of salmon individuals and in more detail (even over the entire lifetime) during their sea feeding phase, and to focus on representatives of R. Simojoki salmon population that had actually survived to the stage of ascending the river for spawn.

2 MATERIALS AND METHODS

2.1 Study site - the Baltic Sea

The Baltic Sea in northern Europe (Fig. 2) is one of the most studied brackish water basins in the world (Lass and Matthäus 2008, Ojaveer *et al.* 2010). It is a shallow sea with several special characteristics. One striking characteristic is the progressive north to south increase in marine influence (Kullenberg 1981), which also leads to divergence in stable isotopic composition (e.g. Rolff and Elmgren 2000, Kiljunen *et al.* 2008). The development of a summertime thermocline, and the year-round halocline and deep water hypoxia reflect water column stratification. Occasional oxygen-rich salt water pulses through the Danish straits periodically refresh the deep hypoxic areas (Dickson 1973, Matthäus and Lass 1995). The northern part of the sea is ice-covered in the winter, but southern areas remain open. Taxa inhabiting the Baltic have clear marine characteristics in the south but fresh water characteristics in the far ends of the Gulf of Bothnia and the Gulf of Finland (Feistel *et al.* 2008).

2.2 Scale SIA method for salmon spatial assignment (I, IV)

Salmon scale samples were acquired from the scale archives of the FGFRI, the University of Turku and the Swedish University of Agricultural Sciences from the years 1988–2011 (except 1999–2004). Those reference scales from salmon in the sea feeding phase were from ICES subdivisions of the Baltic (BSDs). BSDs 25, 26, 28, 30 and 32 were included. BSDs 25, 26 and 28 represent the Baltic Proper area, whereas 30 and 32 represent the Bothnian Sea and the Gulf of Finland, respectively (Fig. 2).
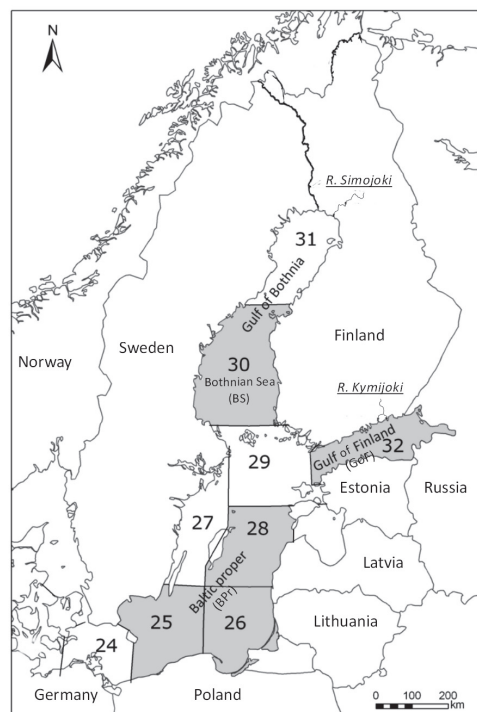
FIGURE 2   The Baltic Sea, showing the ICES subdivisions (sampled subdivisions are indicated in grey) and the location of the River Simojoki and River Kymijoki whose salmon populations were studied in this thesis.

Prior to SIA all scales were soaked in deionised water and wiped clean with non-lint tissue to remove adherent material (mucus, pigment etc.) possibly affecting SIA. The outermost scale growth region, which represents the SI composition of the salmon final sea feeding season, was cut on a glass plate under a microscope using a scalpel. The outermost scale parts were acidified (1.2 N HCl) for 2 min to remove exogenous carbonates, rinsed five times (for details see Perga and Gerdeaux 2003) and dried overnight (60 °C). At least 0.2 mg of scale sample was weighed into tin cups to give sufficient sample material for accurate SIA. The origin of the salmon (wild or hatchery-reared) and age were determined from the scale growth pattern (details: Hiilivirta *et al.* 1998).

Predatory fish show a positive relationship between size and trophic level (Romanuk *et al.* 2011). Therefore larger salmon tend to show higher $\delta^{15}N$ values (Satterfield and Finney 2002, MacKenzie *et al.* 2011a, 2011b, Trueman *et al.* 2012), which could lead to incorrect assignment to those sea areas showing higher $\delta^{15}N$ values. This is especially the case when river-ascending salmon tend to be on average larger than salmon in the sea feeding phase. In contrast, $\delta^{13}C$ values in fish tissues do not show this bias (e.g. Vander Zanden and Rasmussen 1999). To adjust for size effects, $\delta^{15}N$ values were therefore standardized to salmon

lengths for each annual salmon dataset when linear regression between length and δ^{15}N value was statistically significant.

SIA was performed at the University of Jyväskylä using a FlashEA 1112 Elementar Analyzer connected to a Thermo Finnigan DELTA^{plus}Advantage mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA). Pike (*Esox lucius* L.) white muscle tissue was used as an internal working standard. The reference materials used were International Atomic Energy Agency (IAEA) standards of known relation to the international standards of Vienna Pee Dee Belemnite (for C) and atmospheric N_2 (for N). Precision for each run was better than 0.35 ‰ for C and 0.20 ‰ for N based on the standard deviation of replicates of the internal working standards. Sample analysis also yielded percentage of C (%C) and N content (%N) of samples from which C:N ratios (by weight) were derived. These additional data were included in statistical analyses as they improved the precision of assignment to sea feeding areas.

DA was applied to estimate the most likely final sea feeding area for each salmon caught ascending the R. Simojoki and the R. Kymijoki. For each year the ascending salmon SI values were compared (via DA) to previous wintertime reference salmon scale SI values to ensure correct assignment to sea feeding area. A stepwise method was used for DA with variables δ^{13}C, δ^{15}N, %C, %N and C:N. The criterion for including a variable was Wilks' λ ($F \geq 3.84$). The stepwise approach minimizes the multicollinearity occurrence and between-variable correlations; tolerances of the variables entered at each step were checked (tolerance > 0.4) for confirmation.

2.3 Baltic Sea isoscapes (II, III)

Water samples were collected during two cruises in summer 2010 and one in the following winter 2011 by the R/V Aranda of the Finnish Environment Institute. To receive an overview of δ^{13}C$_{DIC}$ and δ^{18}O$_{H2O}$ values and to evaluate possible seasonal impact on those values, two summer sets and one winter set of samples were collected. The first cruise in summer covered the Baltic Sea excluding the Gulf of Finland, which was the only area sampled during the second cruise in summer. The third cruise, in winter, covered the Baltic except the areas south from Gotland.

Sea water was sampled at a depth of 10 m from every sampling station, assuming this to represent an average depth where salmon spend their time in the Atlantic (Hansen *et al.* 2003). The same average depth was assumed to apply in the Baltic. From some stations, water was also sampled vertically from the sea surface to the bottom (metric intervals: 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, and at further 50 m intervals to the bottom) to obtain an overview of the vertical distribution pattern of both isotopes (II). Water samples were taken using a CTD/Rosette sampler (Rosette 1015, Seabird, SBE 911/General Oceanics, SIS: Plus 500).

Samples collected for $\delta^{13}C_{DIC}$ analysis, were placed in 12 ml borosilicate vials prepared in the laboratory, where 0.2 ml of 85 % orthophosphoric acid (H_3PO_4) was added into each vial, which was then sealed with a cap (containing a rubber septum) and flushed and filled with a helium atmosphere. In the field 2–4 ml of sea water from each station was injected through the rubber septum into the vial. The syringe and needle were changed for each sample.

Samples collected for $\delta^{18}O_{H2O}$ analysis were placed in 20 ml glass scintillation vials. All samples were stored in a refrigerator pending laboratory analysis.

In the laboratory, the same kind of vials used for $\delta^{13}C_{DIC}$ samples were filled with 0.5 ml sea water and equilibrated with CO_2 for at least 24 h at 25 ºC. All water samples were analysed for both $\delta^{13}C_{DIC}$ and $\delta^{18}O_{H2O}$ at the Laboratory of Chronology, Finnish Museum of Natural History, University of Helsinki, using a GasBench II and Delta^PLUS^XL (Thermo Fisher Scientific, Bremen, Germany). In addition to sampled water $\delta^{18}O_{H2O}$ values, salinity from the Baltic Marine Environment Protection Commission (HELCOM) data archives was used to model $\delta^{18}O_{H2O}$ values for the Baltic Sea in 2008–2011 (III). The model was created from analysed samples for the Baltic Sea areas used in this study. Modelled and analysed $\delta^{18}O_{H2O}$ values were pooled (III).

Isoscapes for Baltic Sea water $\delta^{13}C_{DIC}$ and $\delta^{18}O_{H2O}$ samples were constructed using ODV (Schlitzer 2002, 2011). Map data for horizontal maps were obtained from the database of the Leibniz Institute for Baltic Sea Research Warnemünde (Seifert *et al.* 2001) and for both horizontal maps and vertical profiles from the General Bathymetric Chart of the Oceans. This grid had to be converted into NetCDF format compatible with ODV with R Statistics software v 3.0.1 (The R Development Core Team 2013) using the package RNetCDF (Michna 2012). Interpolated maps were produced using DIVA gridding software (Troupin *et al.* 2012) included in ODV.

2.4 Salmon otolith micromilling and SIA (II, III)

Both sagittal otoliths were removed from the heads of two sea winter salmon initially caught for the FGFRI M74 monitoring program (Keinänen *et al.* 2012). Otoliths were cleaned of adherent organic tissue and dried overnight (60 °C). Prior to micromilling and SIA of O and C, each otolith was attached to a microscope slide using two-component adhesive, and then carefully sanded and polished flat in the sagittal plane until the otolith nucleus was reached. Otoliths were photographed using a stereomicroscope for determination of growth rings in order to characterize suitable paths for milling. Micromilling was conducted using a custom-built three-dimensional micromilling system in the Saskatchewan Isotope Laboratory facilities at the University of Saskatchewan (details: Wurster *et al.* 1999). This system allowed 31–32 sampling paths to be followed in parallel with the growth banding (II). Eventually, only

6–9 sampling paths were needed per otolith to estimate the final summer and final winter growth spots. In the final assignment only the lowest (summer) and highest (winter) SI values were used (III). The required sample size for isotope analysis (~10–15 µg) was obtained from all sampling paths. Isotope ratios of samples were determined at the University of Saskatchewan using a Finnigan MAT 253 directly coupled to a Kiel-IV automated carbonate preparation device (Thermo–Fisher Scientific, Waltham, MA, U.S.A.).

2.4.1 Temperature data

Baltic Sea surface water temperatures are sometimes over 20 °C, but the temperature drops rapidly to below 10 °C in the thermocline zone between 10–20 m (Matthäus *et al.* 2008). Because salmon prefer cool water, the thermocline probably has a restrictive effect on their occupation of the upper and warmer water column. Handeland *et al.* (2008) and Forseth *et al.* (2011) reported that a water temperature of ~14 °C temperature is the most preferred by post-smolt salmon. The temperature data received from HELCOM indicates high temperatures at 10 m depth (mean ~17 °C) in summer. It is very unlikely that salmon would spend significant time at those temperatures. In addition, due to the thermocline in the Baltic Sea, salmon have the possibility to occupy water at the most preferred temperature during the summer. Based on these considerations, a summer water temperature of 14 °C was applied to the whole Baltic. For winter isotope corrections we used temperature data from HELCOM covering the whole Baltic Sea.

2.4.2 Spatial assignment

Understanding the dependence of otolith $\delta^{18}O$ on ambient water temperature and its $\delta^{18}O$ values is required to obtain comparable values of otolith $\delta^{18}O$ and $\delta^{18}O_{H2O}$ for spatial assignment (e.g. Patterson *et al.* 1993). Moreover, otolith $\delta^{13}C$ value is a mix of bicarbonate $\delta^{13}C_{DIC}$ from ambient water and metabolically-derived diet bicarbonate $\delta^{13}C$ (Wurster and Patterson 2003, Solomon *et al.* 2006). Therefore $\delta^{18}O_{H2O}$ and $\delta^{13}C_{DIC}$ values were corrected using transfer functions, after which the corrected values could be considered as "otolith isoscapes" of both elements. This then permits a direct comparison of otolith and Baltic Sea water isotopes, thereby providing a probabilistic spatial assignment of salmon during their sea-feeding. Temperature-related fractionation causes seasonal fluctuations in otolith $\delta^{18}O$ values (Wurster and Patterson 2003). During the sea migration phase of Baltic salmon the otolith carbonate with the highest otolith $\delta^{18}O$ values are accreted during winter, while the lowest $\delta^{18}O$ values are generated during summer. Therefore 3 measured points, namely 1st sea winter, the following summer, and the 2nd sea winter from the otolith data of each salmon were used to assign the salmon to Baltic Sea areas for each period (II) (in III only summer and the following winter were used).

Probability density surfaces were calculated by using a deterministic grid covering the Baltic Sea following the approach presented by Wunder (2010)

with R Statistics software v 3.0.1 (Anon. 2013a). All DIVA interpolation results (water and diet-based isotopes, temperatures) from ODV first had to be re-interpolated into a deterministic grid to enable the transfer functions for all grid locations to be calculated. This re-interpolation was performed with local inverse-distance weighting interpolation available in the R package gstat (Pebesma 2004). The interpolated isotope values were used as the mean parameter, and standard deviations of $\delta^{18}O$ and $\delta^{13}C$ values were estimated based on observed variations of $\delta^{18}O$ ($\varepsilon = 0.019$) and $\delta^{13}C$ ($\varepsilon = 0.216$) values obtained from the same location and depth, and the variation of observed $\delta^{18}O$ values in the otoliths of several fish individuals ($\varepsilon = 0.207$ estimated from Godiksen et $al.$ 2010) (II).

Only $\delta^{18}O$ values of otoliths and water were used for estimating the seasonal movements and distances of individual salmon (III). Calculation of probability surfaces resulted in several high probability spots for each salmon individual in the sea. It is nearly equally probable for a salmon to be located in any of these high probability locations. Therefore the 3 highest probability locations for every salmon were chosen in both seasons with distance of 2° coordinate radius from the highest probability at minimum. All combinations of directions of 3 × 3 locations were added to the circular diagram of migratory directions (Rose Diagram in R) to visualize the salmon movements. For evaluating swimming distance between seasons and final ascending to the R. Simojoki, the optimised migratory paths were based on the migration routes reported by Ikonen (2006) (IV) and were conducted using ArcGIS Advanced v. 10.2 (ESRI, Redlands, CA) with Network Analyst Extension (ESRI, Redlands, CA).

The coastlines in the maps are based on the Global Self-consistent Hierarchical High-resolution Shorelines database v 2.1 (Wessel and Smith 1996).

2.5 Pre-spawning salmon muscle and egg FA analysis (IV)

Female salmon ascendants (N = 49) were caught from the R. Simojoki in years 2008–2011. All salmon individuals had spent 2 yr in the sea before ascending to their natal river. Salmon were kept in glass fibre basins with through-flowing river water at the Keminmaa Hatchery (FGFRI) for about 4 mo before stripping of eggs and sampling of muscle tissue in October. It was hypothesised that due to the effect of fasting on muscle diet-related FA composition, muscle TAG FA will not show areal differences, but differences are retained in PL FA and eggs (Fig. 3). Muscle samples were collected from dorsal muscle tissues (from below the dorsal fin to the lateral line) sets of samples were stored at –80 °C. Salmon were assigned to likely feeding areas based on scale SIA (I) to evaluate the possible effect of different areas on FA composition. Only salmon with areal assignment probability of ≥ 0.5 were included in FA analysis. Freeze-dried salmon muscle tissues and eggs were analysed for total lipids and fatty acids according to Heissenberger et $al.$ (2010). TAG were separated from PL by 1-

dimensional thin layer chromatography (TLC). Salmon tissue fatty acids were analysed in WasserCluster Lunz, Austria.
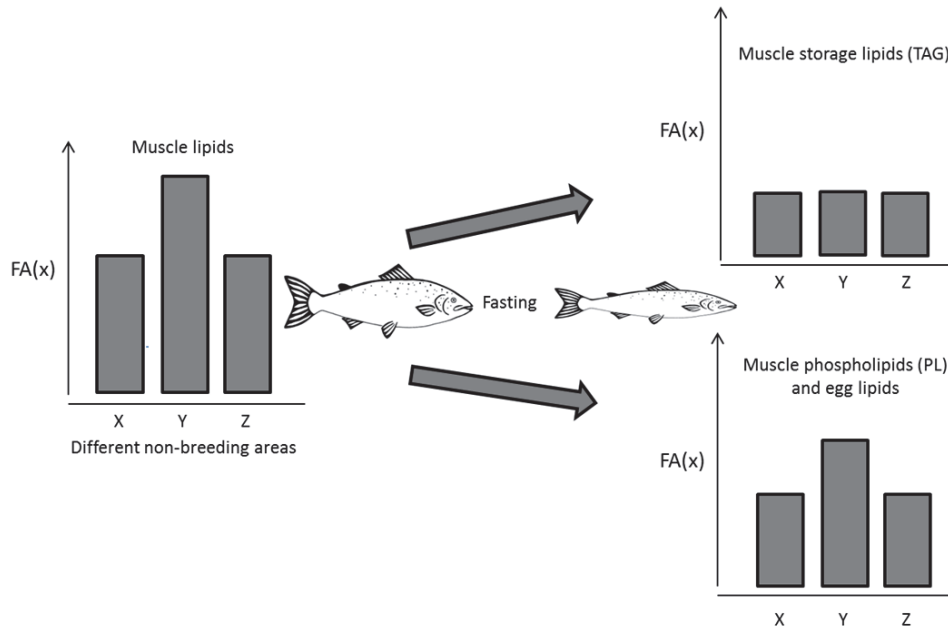
FIGURE 3   Schematic illustration of the effect of pre-spawning fasting on female salmon diet-related fatty acids. Salmon were drawn by Kaisa Raatikainen.

2.6   Quantifying the migratory connectivity

Simpson's index of diversity D (Simpson 1949) was used to provide a simple indication of the relative strength of migratory connectivity of R. Simojoki salmon and R. Kymijoki salmon:

$$D = 1 - \sum_{i=1}^{s} (p_i)^2$$

where p_i is the proportion of individuals of salmon in feeding area i based on DA analysis yearly probabilities. This D, hereafter "*migratory connectivity index (MCI)*", gives values from 0 (strong connectivity) to $1 - 1 / S$ (weak connectivity), where S is the number of salmon feeding areas. In our case with 3 feeding area system the maximum value would thus be 2/3, however, for convenience the values were scaled to vary from 0 (strong connectivity) to 1 (weak connectivity). These scaled values are used to compare different sets of migrations.

2.7 Statistics

Discriminant analysis (DA) was used for estimating salmon final sea feeding areas using salmon scale SI values. Linear regression was used to test change during study time-span in salmon final feeding areas and for standardising salmon length and δ^{15}N. DIVA interpolation was used to create C and O isoscapes from the Baltic Sea. Paired samples *t*-test was used to test seasonal differences in Baltic Sea water isotopes and to test differences in salmon mean weight between arrival at the river and spawning. Arcsine-sqrt-transformation for FA proportions was conducted to satisfy assumptions for analysis of variance (ANOVA). If significant differences in FA mean values were found, Tukey's HSD and LSD test were performed for post-hoc comparisons at $P \leq$ 0.05. If required conditions for equality of variances were not met, the non-parametric Kruskal-Wallis test was used with Bonferroni-corrected Mann-Whitney's test for post-hoc comparisons at $P \leq 0.05$. Spearman's correlation was used to test associations between the salmon arrival to the R. Simojoki and swimming distance. Statistical analyses were performed using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA), R Statistics software v 3.0.1, ODV v 4.5.5 and 4.6 and ArcGIS Advanced v. 10.2 (ESRI, Redlands, CA).

3 RESULTS & DISCUSSION

3.1 Variability in salmon isotope values

Values of both salmon scale isotopes exhibited marked variation, both within a year and in mean annual values over the 22-year timespan (I). This variation is caused by abiotic and biotic factors, such as annual variation in primary producer isotopic values transferred through the foodweb (DeNiro and Epstein 1978, DeNiro and Epstein 1981, Vander Zanden and Rasmussen 1999, McCutchan *et al.* 2003) and factors related to seawater temperature changes (MacKenzie *et al.* 2011a). The decrease in $\delta^{13}C$ of atmospheric CO_2 over recent decades due to global use of fossil fuel and deforestation (the Suess effect; Verburg 2007) has also affected the CO_2-$\delta^{13}C$ value in aquatic environments (Schloesser *et al.* 2009) and might have an influence in the Baltic. At least salmon scale $\delta^{13}C$ values exhibited rather constant and clear decrease over the timespan.

During the 22-year study period (I), the Baltic Sea pelagic foodweb has undergone a well-documented regime shift (e.g. Möllmann *et al.* 2009), whereby the system has changed from a food-web dominated by cod (*Gadus morhua*) to one more dominated by sprat in the 1990s (Möllmann *et al.* 2009). During this period there has been a shift in stock size of the two main prey species of salmon, from herring, which was previously more abundant, to sprat (Möllmann *et al.* 2004, Mikkonen *et al.* 2011) especially in the Baltic Proper. Of these two prey species, sprat appears to hold a lower trophic position i.e. lower $\delta^{15}N$ values (Kiljunen *et al.* 2008). Consumers are fundamentally influenced by the isotopic composition of organisms at the bottom of the foodweb (e.g. Vander Zanden and Rasmussen 1999, Satterfield and Finney 2002). Hence the changes in stock biomass over time of the two most important prey species for salmon may be the main driver of the declining trend in salmon scale $\delta^{13}C$ and fluctuation in $\delta^{15}N$ (I), rather than changes in salmon migratory behaviour.

Scale isotope values represent an average of the final season isotope values of consumed prey and of environment. This averaging is due to analysing the

whole final growth region of the scale (Hutchinson and Trueman 2006). Detailed information of variation in assimilated environmental and prey isotopic composition are hidden and averaged in the scale. In principle, salmon could have migrated long distances between several feeding areas with sufficient variation in areal isotope values, and based on otolith analysis swimming distances could actually be substantial (III). However, this cannot be detected from the analysed scale isotope values, thus preventing the use of scale isotope values for investigating the whole lifetime migration of salmon.

In contrast, changes in otolith isotope values are due to different factors. Micromilling revealed the isotopic profiles of salmon otoliths from the nucleus to the edge (II). It was clear that the change in values represents the salmon whole life cycle and it would be ideal to reconstruct the whole migration route of the salmon. The analysed micromilled sample from the certain path of the otolith reflects very accurately the present conditions in the environment at that particular moment (i.e. temperature and water isotope values) (e.g. Patterson *et al.* 1993). Especially, temperature has a significant effect on otolith isotope values. This is clearly seen in the change of isotope values micromilled from the otolith nucleus to the edge. The highest values of $\delta^{18}O$ and also of $\delta^{13}C$ are formed during the winter and the "pit" between the winter values is formed during summer. This is due to temperature-related fractionation of heavier and lighter isotopes from water to otolith carbonates (aragonite) (Patterson *et al.* 1993).

In conclusion, the variations and changes in scale and otolith values depend on different factors. The changes in otolith values between seasons can provide additional insights into what lies behind the isotope values of scales, in which finer-scale temporal details are obscured by the integration that occurs in scale analysis.

3.2 Salmon migratory connectivity

Based on DA results of SIA of salmon scales, the migratory connectivity of the R. Simojoki and R. Kymijoki salmon populations differed from each other. Majority of R. Simojoki salmon were likely feeding in the Baltic Proper (mean occupancy ~60 %). The clearest distinction is between the proportions of sub-populations of both river salmon with likely feeding in the Bothnian Sea and in the Gulf of Finland. For R. Kymijoki salmon the Bothnian Sea was virtually irrelevant, whereas ~20 % of R. Simojoki salmon was likely feeding in that area. In addition, a similar ~20 % proportion of R. Simojoki salmon fed in the Gulf of Finland as in the Bothnian Sea, but R. Kymijoki salmon mostly occupied the Gulf of Finland with ~70 % of ascendants coming from that area (Fig. 4) (I). However, it should be noted that ascendants analysed from the R. Simojoki had been caught over 18 yr in contrast to only 2 yr for R. Kymijoki salmon. This difference could affect the direct comparison. There was no significant change

in the final feeding area probabilities of ascending R. Simojoki salmon through the studied time-span, but the variation between years was considerable.

The results from scale-based SI method are rather consistent with previous tag-recovery data (Cristensen and Larson 1979, Salminen *et al.* 1994, Ikonen 2006). However, the SI method appears to indicate more salmon feeding in the Gulf of Finland than the tag-recovery method (I). This observation is probably due to salmon migratory behaviour. Salmon have been observed to migrate via the Gulf of Finland to their northern spawning rivers (Aro 1989, Ikonen 2006), or they may have fed in the nearby areas thus reflecting the SI values of the Gulf of Finland. At least salmon feeding in the northern areas of the Baltic Proper could show values reflecting more the Gulf of Finland values than the Baltic Proper values. The overlap in SI values from those 2 areas might introduce false DA assignment. On the other hand, otolith analysis partly supports the idea of migration via the Gulf of Finland, since some salmon were assigned to the Gulf of Finland before the spawning migration (III). Commercial fishing has also been small-scale recently in the Gulf of Finland and thus the recoveries are sparse (Anon. 2013b).
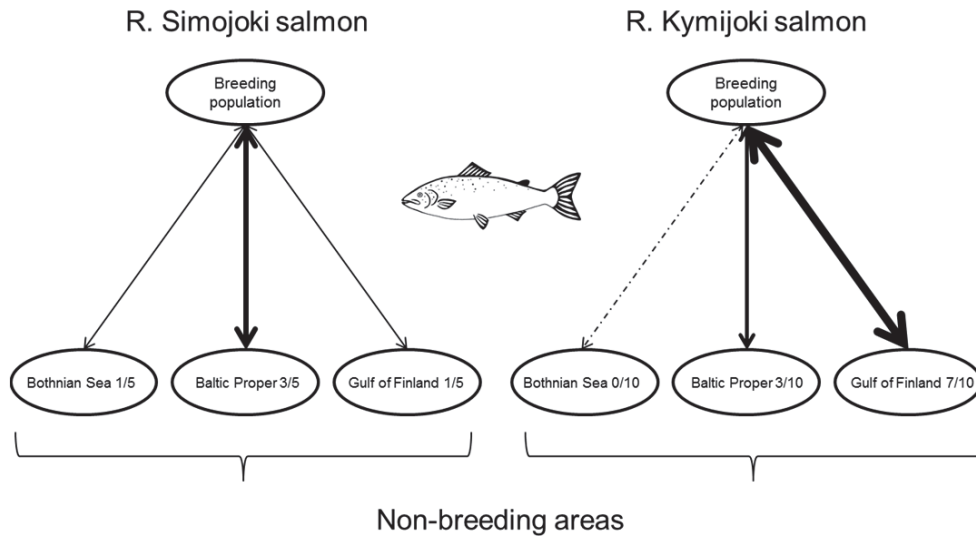
FIGURE 4   Migratory connectivity of two Baltic salmon populations. The thickness of the arrow indicates the proportion of the breeding population in the feeding area. Salmon was drawn by Kaisa Raatikainen.

There were no significant differences between wild and hatchery-reared R. Simojoki salmon migratory connectivity (I). That result is consistent with the report by Palm *et al.* (2008) that adult salmon shoals are a mixture of individuals of different origin and relation. On the other hand, Salminen *et al.* (1994) and Kallio-Nyberg *et al.* (1999) have shown that stocked immature salmon show more restricted migration than wild salmon. This behaviour has been attributed

to the decreased migration activity (Jutila *et al.* 2003) and the larger size of stocked smolts. Wild post-smolts are smaller and mainly feeding on invertebrates at the moment they are in the Bothnian Sea (Salminen *et al.* 1994), but larger stocked post-smolts have better ability to feed on the prey fish available in the Bothnian Sea (Kallio-Nyberg *et al.* 2011). Salmon feeding on invertebrates continue their migration, whereas some proportion of salmon already using a fish diet stay in the Bothnian Sea. In contrast, in this SI study salmon were adult fish and included only those individuals that had survived to ascend their natal river. Perhaps the capacity of the Bothnian Sea as a feeding area for salmon is rather limited. Large numbers of post-smolts in the Bothnian Sea might be competing for resources and may not survive as well as in the other areas. At least the prey fish numbers are far lower in the Bothnian Sea than in the Baltic Proper (Anon. 2012).

To my knowledge there are only few attempts which have been trying to quantify the migratory connectivity. Problem has been the more or less subjective or arbitrary determination of the boundaries of breeding and non-breeding areas. However, Ambrosini *et al.* (2009) have conducted an objective method, which seems to successfully quantify connectivity of such breeding and non-breeding areas of which boundaries are not geographically determined. However, in this study there are 3 fixed salmon feeding areas, which have clear limits (i.e. the BSDs) and thus the investigation of the migratory connectivity using MCI is done. The simplified index of migratory connectivity MCI enables the estimation and clarification of different Baltic Sea salmon population migratory behaviour and dependence on 3 feeding areas. Lower MCI indicates higher dependence on a single feeding area, and thus higher vulnerability to possible threats in that area. Based on the presented MCI, the migratory connectivity is rather weak for both salmon populations and the annual variation in MCI for R. Simojoki salmon is considerable, although remaining in the weaker side of the proposed qualification except in 2 years (Fig. 5). On average R. Kymijoki salmon (average MCI = 0.62, range: 0.61–0.63, N = 2) show stronger and more stable connectivity than does R. Simojoki salmon (MCI = 0.78 range: 0.25–0.99, N = 18), although the R. Kymijoki average values are from only 2 yr which clearly makes them unreliable. Thus, in principle the R. Kymijoki salmon population might be considered less secure than the R. Simojoki salmon population. R. Kymijoki salmon might face serious problems if the Gulf of Finland is degraded. However, based on otolith analysis, R. Simojoki salmon could then also face problems at some level, since part of the population migrates via the Gulf of Finland to their natal river to spawn. In addition, these 2 studied salmon populations would suffer little in a scenario of future degradation of the Bothnian Sea as that area seems to have the least importance as a feeding area. In 1996 migratory connectivity of R. Simojoki salmon appeared very strong, but this probably reflects the low number of analysed ascendants, which are almost all assigned to the same feeding area. The MCI produce a clear index for quantifying each studied salmon population, and populations can be compared against each other in terms of migratory connectivity. However, MCI is incapable to indicate which

of the areas is the most used if the area identification is not available. Imagine, e. g., ~70 % of R. Kymijoki salmon feed in the Gulf of Finland and the rest ~30 % in the Baltic Proper now (MCI = 0.62), but if the situation turns upside down the MCI would still be the same. In addition, the usage of MCI is restricted to only those beforehand determined areas, which might be something else than the actual salmon feeding areas.

Unlike birds, which mainly perform classical annual to-and-fro migrations, salmon spend several years in their feeding areas (i.e. non-breeding areas). This prolonged feeding period offer a possibility for salmon to move to a feeding area with better resources. Salmon otolith analyses indicate movements of an individual salmon (II, III), which could be expressed as a seasonal change in migratory behaviour of individuals i.e. *variable migratory connectivity*. This cannot be detected from scale SI analysis and makes the interpretation of the migratory connectivity more complicated. Salmon might change their feeding areas slightly between seasons (II) or perhaps salmon spread to the Baltic more widely in summer than in winter (III). For example, in summer 2010 the majority of salmon were close to the Danish straits, but with an appreciable number of salmon located in the central Baltic Proper (III). During winter most salmon were rather tightly located in the Bothnian Sea and just a few fish were located in the Gulf of Finland, indicating stronger connectivity in winter. Although we are dealing with mature salmon at the beginning of their spawning migration, this is an issue that has not previously been addressed in migration studies.

Studied salmon populations in the Baltic Sea prefer different feeding areas. Similar observations of different feeding area occupancy between salmon populations have been shown also in Atlantic (Mackenzie *et al.* 2011a). These observations are logical at least the way that salmon from different population are not competing from the same resources. Different feeding areas probably have different habitat characteristics and require adaptations. It might be that each salmon population is therefore genetically adapted to those specific feeding area conditions and has a unique genetic signature and that value should be acknowledged. Environmental influence has been shown in genomic divergence of Atlantic salmon throughout its distribution by Bourret *et al.* (2013). Divergence of choosing different feeding areas is not only a characteristic of salmon or salmonid fish. There are studies that indicate segregation of feeding area migratory behaviour in other fish species. Alarcos and Timi (2013) showed different populations of flounders (*Xystreurys rasile*) habiting at least 2 different feeding areas in Argentine Sea and Aranda *et al.* (2013) showed that Atlantic Bluefin tuna (*Thunnus thynnus*) mainly feed in the North Atlantic but minor proportion of the population feed in the Mediterranean.
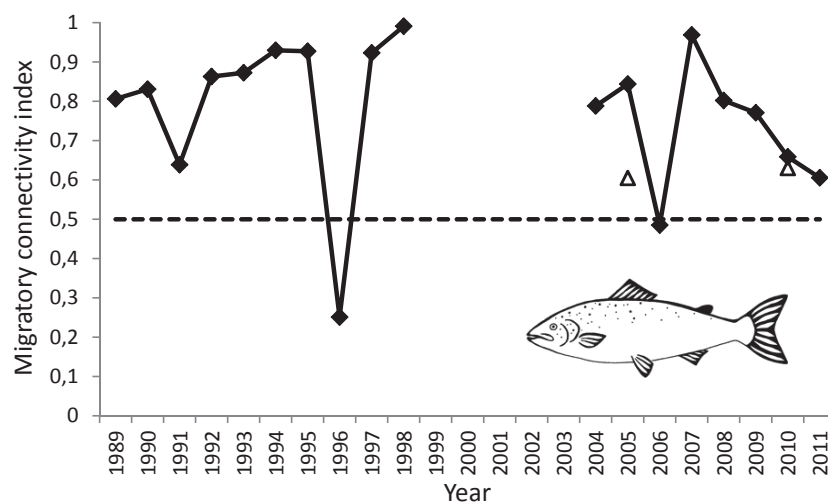
FIGURE 5 Annual migratory connectivity index of R. Simojoki (filled diamonds) and R. Kymijoki (open triangles) salmon. The measure is derived from Simpson's index of diversity D (see text for details). Weak connectivity (value of 1) indicates salmon being distributed uniformly across all three feeding areas before ascending the river to spawn; strong connectivity (value of 0) indicates all individuals occupying only one feeding area. Dashed line represents the arbitrary transition from strong to weak migratory connectivity (value of 0.5). Salmon was drawn by Kaisa Raatikainen.

3.3 Salmon locations via otolith analysis and isoscapes

Reported salmon movements in oceans are based on a few satellite telemetry studies (e.g. Chittenden *et al.* 2013). Based on otolith and water isotopes it is possible to obtain rather accurate information about salmon (and other fish) movements. Even entire lifetime movements of salmon can be traced. In this study mainly 2 time points at sea, the final summer and the following winter, were investigated. Based on tag-recoveries it is easy to show the distance from the tagging place to the recovery spot; the salmon is known to have travelled that far, but nothing else can be inferred. In contrast, the otolith method offers a new approach which allows identification of multiple sequential locations of the same individual.

Direct comparison of otolith values with the water values is not possible, because fractionation between otolith and water $\delta^{18}O$ values depends on temperature. There are experimentally-derived temperature fractionation functions (i.e. transfer functions) between otolith and water O isotopes (e.g. Patterson *et al.* 1993, Storm-Suke *et al.* 2007, Godiksen *et al.* 2010) for other salmonids, but the only available parameters specifically for Atlantic salmon were from Hanson *et al.* (2013). These parameters were initially applied in this study, but they gave questionable salmon assignments especially in summer

(II). Further consideration of the above-mentioned transfer function parameters showed distinct differences in salmon assignments, and a decision regarding the final parameters to be used was difficult. Finally, a compromise was adopted whereby average values from all available parameters seemed to fit plausibly to the salmon migration study between seasons (III).

O and C isoscapes show clear differences in isotope composition between different areas of the Baltic Sea (II). Especially the $\delta^{18}O$ values separate the areas well, because of the seasonal stability and areal distinction. Exceptions are the values of the Bothnian Sea and the Gulf of Finland, where the $\delta^{18}O$ values are very similar and there is thus an apparent problem in the separation between those 2 areas in terms of salmon locations. In contrast, $\delta^{13}C$ values change greatly between seasons and the areal segregation is vague. On the other hand, it seems that, irrespective of season, $\delta^{13}C$ values of the Bothnian Sea and the Gulf of Finland remain different, offering robust areal isotopic segregation. Therefore, by combining the $\delta^{18}O$ and $\delta^{13}C$ isoscapes it was possible to allocate those salmon which could not be separated between those 2 areas using $\delta^{18}O$ or $\delta^{13}C$ alone (II).

By combining the 2 isoscapes, the areal segregation of the Bothnian Sea and the Gulf of Finland was achieved and salmon were clearly assigned to the Bothnian Sea instead of the Gulf of Finland. The highest probabilities of the 2 example salmon were in the Baltic Proper (II). Estimated salmon assignments to the Baltic Proper and the Bothnian Sea instead of the Gulf of Finland are consistent with previous tag-recovery studies (Cristensen and Larson 1979, Salminen et al 1994, Ikonen 2006). However, the salmon assignment to the Gulf of Riga in summer was questionable. That area is shallow, very warm in summer and suitable prey are sparse (Lass and Matthäus 2008, Hammer *et al.* 2008, Anon. 2012), making it implausible that salmon would spend time there. Thus summer assignment may actually be more reliable from $\delta^{18}O$ alone without $\delta^{13}C$.

Based on salmon otolith and Baltic Sea water isotope analysis, most of the salmon received highest assignment probabilities to the south-western areas of the Baltic Sea in all study years (2008–2011) (II). Only a few salmon were located outside the main basin (III). Locations assigned in this study appear rather consistent with earlier findings of salmon distribution (e.g. Salminen et al 1994, Ikonen 2006), although the majority of the estimated salmon locations using the SI method are very close to the Danish straits. Tagging recoveries are obtained from slightly more north-eastern areas (Kallio-Nyberg and Ikonen 1992).

Salmon locations in the following winter before ascending the natal R. Simojoki are far more north. Most salmon are located in the Bothnian Sea and only a minor part is in the Gulf of Finland (III). Again, these results appear consistent with earlier findings, since some of the salmon migrate north via the Gulf of Finland (Aro 1989). As mentioned earlier, salmon in the Bothnian Sea in the last winter seem to be very close to each other regardless of the previous summer location. A similar rather tight gathering is observed for those salmon located in the Gulf of Finland, although the numbers are lower. Most of the salmon in the Bothnian Sea are located close to Swedish shore. Ikonen (2006)

showed that salmon migrate via shorelines following water currents. It seems that salmon are migrating to the same area and perhaps even using the same routes regardless of their location in summer. However, the salmon location in March in those latitudes may be unreliable. Christensen and Larson (1979) showed that the spawning migration begins just at that time from the Baltic Proper, but from the way the micromilled otolith isotope values decline rapidly at the end of the otolith, the spawning migration seems to be very fast and direct (II).

There wasn´t any association between the salmon individual travelling distance and arrival date to the river. Therefore, it could be interpreted that salmon with longer to travel need to swim faster or start migrating earlier to reach the river at the same time as those with a shorter distance to travel. The arrival date of salmon to the river changed to only slightly later during the studied years (III).

Vertical distribution patterns of the Baltic Sea water $\delta^{18}O$ and $\delta^{13}C$ values were similar for both elements. The $\delta^{18}O$ values remain fixed between seasons, but $\delta^{13}C$ values change. Photosynthesis preferentially incorporates ^{12}C (e.g. Fry 2006) as observed in the clear seasonal changes in the surface water values of $\delta^{13}C$. In vertical profiles of both values, the change in values is clear from a depth of 50 m. The halocline is at that depth and keeps the upper more fresh water and the lower more saline water separated. The only factor which could change the situation is a sufficient pulse of oxygen-rich saline water via the Danish straits (e.g. Matthäus and Lass 1995), which would push the older hypoxic saline water towards the Gulf of Finland. The observed change in water isotope values at 50 m could potentially have an effect on salmon isotope values and hence lead to false assignments. However, in practice this is not very likely, as especially in the Baltic Proper but also in the Gulf of Finland, water below that depth is usually very hypoxic or even anoxic (Lass and Matthäus 2008) and therefore unsuitable as a habitat for salmon.

3.4   Advantages of SIA over traditional methods

There are several advantages to the use of SI-based methods in migration studies compared with conventional tagging methods.

i) Conventional methods can be strongly susceptible to the bias that samples are obtained from a commercial fishery from the sea. They therefore do not represent the whole feeding salmon population, but are heavily dependent on the spatial distribution of fishing effort. The use of salmon scale SI based methods will not entirely overcome this problem, but because scale SI values of ascending salmon represent seasonal averages of the feeding area (Hutchinson and Trueman 2006), areal reference scale SI values should closely represent the actual feeding area irrespective of the fishing location.

ii) In SI-based methods all the specimens are naturally "tagged" and results do not rely on recoveries. In addition, the SI method offers a possibility to follow salmon (fish) movements through the whole life.

iii) Tag-recaptures are ineffective for studying the consequence of migration patterns (e.g. final feeding area) on individual characteristics which are not manifested in tagged fish but appear later in the life cycle. For example, M74 mortality of yolk-sac fry, which affects Baltic Sea salmon (Ikonen 2006, Keinänen *et al.* 2012), is related to the diet of salmon and therefore also to the feeding areas but only becomes evident at spawning or in offspring. SIA provides better information on individual dietary history than does tagging.

iv) Analysis of salmon that have survived to ascending the spawning river better represents the migratory connectivity of the actual breeding population than a tagged subsample of the population caught from the sea.

v) The scale SI method is non-lethal, which is very important when studying endangered species or populations.

3.5   Fatty acids in evaluating the feeding area quality

As expected, the salmon muscle total FA compositions were largely similar between salmon from different final feeding areas due to the apparent effect of the pre-spawning fasting period. However, the lowest proportion of oleic acid (18:1n-9) in muscle was in salmon assigned to the Baltic Proper. Interpretation of this result is difficult because oleic acid is characteristic for sprat (Røjbek *et al.* 2014) and sprat is the principal prey fish of salmon in the Baltic Proper (Hansson *et al.* 2001, Mikkonen *et al.* 2012, Vuorinen *et al.* 2014). The reason for this discrepancy may be the overlap of the SI values of salmon from the Baltic Proper with those from the two other areas, which is apparent in the reference values (Fig. 6). Oleic acid is an important component of cell membrane lipids and in build-up of oocytes during exogenous vitellogenesis; it is also easily used as fuel and is thus one of the preferred FAs for fuelling muscles in swimming (Sargent *et al.* 2002).

TLC-derived fatty acids of TAG (i.e. storage lipids; e.g. Jobling 1994) in salmon muscle tissues revealed no differences between salmon from different likely feeding areas. Instead, FA composition of muscle PL, which are integral parts of cell membranes (e.g. Jobling 1994), showed differences between salmon from different likely feeding areas (IV).

Several studies have shown that, in addition to the importance of particular single FA, the balanced ratios of ARA, EPA and DHA are also important. This is especially true for egg quality and early embryonic development, because of the high n-3 and n-6 PUFA requirements in fish (Sargent 1995, Bell 1998, Arts and Kohler 2009).

3.5.1 Muscle PL FA

A fasting phase of salmon occurs naturally for several months as salmon do not forage during their spawning migration (Ikonen 2006, Vuorinen *et al.* 2014). During the fasting phase salmon metabolise lipid resources for survival, as was clearly seen in the weight loss between arrival at the river and spawning, similarities in muscle TAG FA, and differences in total lipids of spawning salmon muscle tissue compared to the feeding phase salmon (Kiljunen *et al.* 2008, Vuorinen *et al.* 2012).

DHA and EPA are the 2 most important dietary FA for salmon (Arts and Kohler 2009) and therefore linked to environmental conditions. DHA is especially abundant in the brain and is particularly important in controlling functions of cell membranes (Bell *et al.* 1993). Large amounts of DHA in salmon tissue are also a reflection of a high-fat fish-based diet (Alvarez *et al.* 1998, Hemre and Sandnes 1999). In addition, EPA in salmon increases the anti-inflammatory response and decrease cardiac lesions in salmon (Bell *et al.* 1993). Higher EPA content in muscle PL was found in salmon which had likely been feeding in the Baltic Proper (IV). The ratios of EPA:ARA and DHA:EPA were better balanced in salmon assigned to the Baltic Proper. The results show that PL of cell membranes partly reflect dietary lipid supply and are thus not entirely intrinsically regulated by fish, as was reported from controlled feeding studies (e.g. Jobling and Bendiksen 2003).

3.5.2 Eggs

The EPA:ARA ratio in eggs of salmon that had been feeding in the Bothnian Sea was close to 5, whereas it was ≥ 6 in salmon from the Baltic Proper and the Gulf of Finland. Sargent (1995) suggested that the optimum in embryonic development should be between 5 and 10. Therefore all study areas offer suitable feeding areas for salmon, but the Baltic Proper seem to have the best diet in terms of FA.

Egg yolk should be rich in DHA for normal embryo development (Bell 1998). Eggs from salmon that had likely been feeding in the Baltic Proper showed the highest DHA:ARA ratio and the salmon from the Bothnian Sea the lowest (IV). This indicates that the diet in the Baltic Proper had contained more fish-based lipids, i.e foraging on sprat which is the main prey fish in the Baltic Proper, whereas in the Bothnian Sea the diet was principally herring (Hansson *et al.* 2001, Mikkonen *et al.* 2011). Although DHA is an important FA in eggs, it has been observed to act like a double-edged-sword. For example, Czesny *et al.* (2009) suspected that lake trout (*Salvelinus namaycush* Walbaum) foraging on alewife with high DHA content gave rise to the FA composition found in eggs and the incidence of the thiamine deficiency syndrome EMS. High DHA has also been linked to the occurrence of M74 syndrome via abundant sprat which are eaten by salmon (Keinänen *et al.* 2012).

The FA results from salmon muscle and eggs together could indicate higher diet quality in the Baltic Proper. The Bothnian Sea and the Gulf of

Finland are both suitable feeding areas with important resources for a minor but appreciable proportion of salmon in the Baltic Sea. In addition to physiologically important FA contents in salmon, the Baltic Proper also appears to be a high-quality feeding area due to the important prey fish in the Baltic Proper compared to other areas. Especially sprat, but also herring and some other species are abundant (Mikkonen *et al.* 2011). On the other hand, occasional occurrence of severe M74 syndrome originating from the Baltic Proper area causes high mortality of yolk sac fry of those female salmons that have been feeding extensively on sprat in that area (Keinänen *et al.* 2012).

3.6 Limitation of the study and future research

The scale SI method is hindered by the reference isotope values overlapping (Fig. 6). DA validates the analysis (i.e. reference overlapping) by cross-validation (leave-one-out method in this study). In this study the range of accuracy of the cross-validation was 74–97 % (average 85 %). This means that an average incorrect assignment of ~15 %. One way to improve the results is to choose those ascending salmon individuals which have a high assignment probability to a certain area. This is especially important when making interpretations about the feeding area effect on certain characteristics appearing in ascending salmon. This was done in the evaluation of salmon FA composition related to the final feeding area (only those salmon were included with an assignment probability ≥ 0.5) (IV). In addition, if an observation falls outside any reference value scatter, hence representing something other than all sampled references, it will anyway be assigned to the most closely corresponding reference area. However, in this study the scatter plots were checked to identify and exclude those outliers. In any case, the results were very similar to observations derived from tag-recovery, which suggests the SI method was giving robust results.

The accuracy of the otolith SI method is highly dependent on knowledge of the salmon temperature-related location in the sea. In this study water temperatures were approximations. There is a lack of information about the ambient temperatures in waters occupied by salmon in the Baltic Sea. In addition, it has to be recognized that there are always uncertainties when model estimates are used (II, III), while missing values of prey species $\delta^{13}C$ covering the whole Baltic Sea area might also lead to questionable salmon assignments (II). These unknown aspects offer pointers for future research. Revealing the salmon temperature preference in the Baltic (for example using PSAT) and conducting experimental studies to reveal the temperature-related fractionation for salmon would be extremely important.

The scale SI analysis integrates the final growing season isotopic composition, and hence is an average representation of all the areas where the scale was laid down. In contrast, otolith SI values are more dependent on the micromilling accuracy. Although the otolith SI method has clear advantages

(possibility to reconstruct the whole salmon life cycle, annual reference sampling unnecessary), it is laborious and rather expensive. In contrast, the scale SI method is more cost-effective compared, but does require annual reference values.

The analysis of salmon muscle FA during the feeding phase of salmon from the 3 different sea areas or immediate biopsy when ascending to the river would provide a better comparison among the pre-spawning salmon.

Overall, I want to emphasize the fact that the study salmon are those, which have actually survived from the sea area feeding phase. Because tag-recoveries rely on commercial fishing and fishing is not uniformly distributed to the sea it is also the main restrictive factor in interpreting the tag-recovery results. Recoveries are received only from areas where fishing if conducted. SI study salmon might equally originate from sea areas where fishing is sparse or absent. However, it is obvious that fishermen seek salmon instead of fishing randomly in every achievable sea area. But as a whole, it is uncertain how comparable the results are between salmon used in the SI method with those in tag-recovery studies.
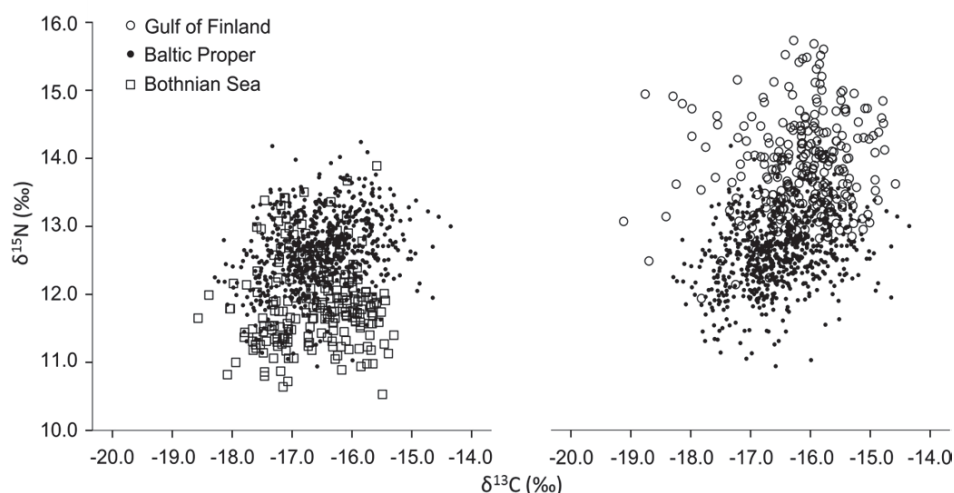
FIGURE 6   Reference salmon scale carbon (C) and nitrogen (N) isotope values from three main feeding areas of salmon in the Baltic Sea. Left figure shows values from the Baltic Proper and the Bothnian Sea, whereas right figure shows values from the Baltic Proper and The Gulf of Finland.

From the point of view of conservation of wild salmon, the sea area actions are demanding. Origins of salmon cannot be separated during sea area fishing. Recent news has indicated that reared salmon adipose fin should be cut for distinction of the origins and that wild salmon should then be identified and released. However, possible future directions in targeting sea fishing against reared individuals is challenging due to the similarity in migratory behaviour

of wild and reared adult salmon. For R. Kymijoki salmon, lower MCI indicates higher dependence on the Gulf of Finland, and thus higher vulnerability to possible threats in that area (e.g. fishing). On the other hand, salmon in that feeding area (and in the R. Kymijoki) are mostly of reared origin and hence fishing in the Gulf of Finland could be more beneficial for wild salmon. Seasonal change, or the term introduced in this study, the variable migratory connectivity, where salmon are moving from area to another, makes conservation even more demanding. Perhaps, reconsideration of overall fishing quota of salmon in the sea areas could be a workable solution.

Whatever the truth, future salmon migration studies should focus on all salmon populations in the Baltic Sea to obtain the most complete overall view of the Baltic Sea salmon migratory behaviour.

4 CONCLUSIONS

The stable isotope analysis of salmon scales and otoliths combined with the Baltic Sea water isotopic composition provided a powerful alternative tool, alongside the conventional tag-recovery method, to track salmon migrations from the time of hatching of young salmon to the feeding areas in the sea and all the way back to the natal river as an adult fish. In addition, this is the first time that seasonal movements of individual salmon have been studied using isotope markers.

Based on the SI method the migratory connectivity of salmon populations does differ, although both populations studied in this thesis showed rather weak migratory connectivity. A larger proportion of River Simojoki salmon (~60 %) were most likely feeding in the Baltic Proper, ~20 % were feeding in the Bothnian Sea and ~20 % in the Gulf of Finland. In contrast, ~70 % of River Kymijoki salmon were most likely feeding in the Gulf of Finland and ~30 % in the Baltic Proper, while the Bothnian Sea was irrelevant to R. Kymijoki salmon as a feeding area. R. Kymijoki salmon therefore had a lower average migratory connectivity index (MCI) indicating slightly stronger connectivity than R. Simojoki salmon. This demonstrates that different salmon populations can have different migratory behaviours. R. Simojoki salmon might be more flexible in their migratory behaviour than R. Kymijoki salmon, which might thus be more threatened during their sea migrations. In contrast to some previous reports, in this study wild and reared salmon from the same river population showed similar migratory behaviours.

Salmon perform impressive movements within a short time during their spawning migration. The salmon otolith SI method showed considerable between-area movements of different salmon individuals between seasons. This could also be seen as variability in the migratory connectivity. Most individuals were assigned to the south-west Baltic Proper during the summer and only few individuals were in the other areas. During the following winter most salmon had already migrated to the Bothnian Sea and some individuals were in the Gulf of Finland on their way to the natal River Simojoki to spawn. Due to the methodological uncertainty (selection of approximate temperatures and

transfer function parameters) these substantial movements might be overestimated.

However, the SI approach requires understanding of the sample structure. Otolith SI values are compared to water SI values, and it is crucial to understand the temperature-related fractionation (i.e. transfer function) between the SI values of the otolith and those of the ambient water. Without resolving that function, salmon spatial assignments can easily be incorrect. In the best case, otolith analysis provides totally new information about individual salmon movements in the sea.

Spatial assignments using otolith or scale SI rely on largely similar basic principles. In the otolith SI method, reference values are the sea water SI values, while ascended salmon scale SI values require salmon reference scale SI values from the sea areas. The latter requires a salmon catch from the sea and an adequate discrimination between the areal reference scale SI values. Heavy overlap between the SI values of feeding areas leads to a weak assignment probability of salmon to the sea areas.

In addition, FA proved a useful potential biomarker to evaluate the final feeding area quality even when salmon subsequently fast for several months. During the fasting period salmon muscle storage lipids are metabolised and differences in diet-related fatty acid composition between feeding areas disappear. In contrast, analysing phospholipid fraction of salmon muscle revealed persisting differences between feeding areas in the diet-based FA retention of structural lipids in cell membranes. Salmon feeding in the Baltic Proper showed the most balanced FA composition, indicating this area to be the most suitable feeding area in terms of FA. FA analysis of salmon eggs supported the interpretation of the Baltic Proper as a higher quality feeding area for salmon than the other areas. Moreover, the amount of prey is highest in that area, providing a resource-rich habitat that can support large numbers of feeding salmon. The Bothnian Sea and the Gulf of Finland offer suitable feeding areas for smaller but still appreciable numbers of salmon. However, the quality of the Baltic Proper as a feeding area is weakened by the occasional occurrence of yolk-sac fry mortality (M74 syndrome) in eggs from female salmon that have been feeding in that area.

Wild salmon populations are endangered in the Baltic Sea. Although the situation is improving, conservation of the remaining wild populations is still crucial. Baltic salmon occupy a very variable habitat and future research should focus on revealing migratory connectivity of all different Baltic salmon populations to acquire an improved understanding of the migratory behaviour of this special species in the Baltic Sea.

Acknowledgements

I am most grateful to my supervisors Dr. Mikko, Prof. Roger and Dr. Pekka. I cannot imagine any better supervisors. The eventual chance to fulfil my dream to do research work is mainly Mikko's fault, absolutely a good fault. Mikko's never ending enthusiasm and meticulousness in research work is enviable. I have to acknowledge the importance of Roger in stable isotope studies. The opportunity for me to have adopted and learned to apply this handy elemental tool is fundamentally Roger´s credit. Roger has an encouraging way as a supervisor to watch from behind-the-scenes (i.e. let the student work as he/she finds best), but you are there when a broader professional expertise and perspective is needed. I truly appreciate it. Pekka has a deep expertise in animal physiology and long-term experience in Baltic salmon studies. Although, you were located some distance from Jyväskylä (Helsinki) I always received the help, ideas and expertise whatever was necessary.

I am also appreciative of all co-authors. Especially Anssi's astonishing ability of helping with interpolation methods, creating the isoscapes and maps is inconceivable. Without your help this work would be still continuing, or would be at least far lower in quality. Eloni's shining and unreserved being with the couple of hundred sea water samples offered by me is unforgettable. Marja and Martin guided me to the demanding world of fatty acids. Martin also showed me the beautiful scenery of Lunz am See region. I would have been lost without your expertise. I also had a lottery chance to work in R/V Aranda through the Baltic Sea a couple of times. I want to thank the crew and staff of that "jollyboat" for being so friendly and collaborative with this research embryo. Professors Mönkkönen and Erkinaro, members in my thesis support group, offered valuable comments and support.

All researchers, fellows etc. in our department who have helped me with this work are thanked. Especially Hannu, Heikki, Jari, Timo (both) and Tuula and all the rest. You sure know who you are. I have to bring up my grand old roommates Antti, Fabio and Sanni (and recently Pauliina). Time working with you guys has been a pleasure. Those nearly insane conversations about any subject ever have been a true balancing force against thesis work! There is life beyond salmon migrations. Birding, fishing or just spending time and hanging together with Artsi, Bystööl, Havu-Meister, Heikki, Hurmeet, Häggson, Teppo etc. has been something unforgettable and has offered mental health(?). All kinds of gatherings and activities with spectacular persons of MUsTikka ry expanded my thoughts. Hopefully all this continues.

I have had support to this work from my family in many ways. Parents, and sister and bro together with your families – thank you.

Finally, Kaisa, my life companion, you if anyone has had the chance to enlarge, test and fulfil your sink with complaining, whining and nuisance during these years. I have to admit that those adjectives have been in a major role and for example compliments and joy have had far smaller role. Tremendous thank for your endless encouragement and depth of that sink!

YHTEENVETO (RÉSUMÉ IN FINNISH)

Itämeren lohen vaelluskäyttäytymisen alueellinen yhtenäisyys

Ihmisen aiheuttamista elinympäristömuutoksista johtuen luonnonvaraista alkuperää olevan Itämeren lohen (*Salmo salar* L.) tulevaisuus on vaarantunut. Suurin osa Suomen lohijokien luonnonvaraisista populaatioista on jo tuhoutunut jokiympäristön muututtua. Suomen lohijoista vain Tornion- ja Simojoessa tapahtuu enää luonnonvaraisen lohen lisääntymistä merkittävissä määrin. Jokaisen lohijoen populaatio on evoluution myötä sopeutunut ominaisuuksiltaan juuri kyseiseen jokeen. Kerran menetettyä alkuperäistä joen populaatiota ei saada enää takaisin, ja jonkin muun joen lohen kotiuttaminen tuhoutuneen tilalle voi olla vaikeaa. Itämeren lohikantaa on pyritty ylläpitämään istutuksin, mutta istutustulokset ovat huonontuneet viime aikoina. Onkin havaittu, että kasvatettu lohi ei ole yhtä elinvoimainen kuin luonnonvarainen lajitoverinsa, ja saadut viitteet syönnösvaelluskäyttäytymisen eroista merellä tukevat tätä havaintoa. Tämänhetkisessä merialueella tapahtuvassa sekakantakalastuksessa ei voi erottaa, onko lohi kasvatettua vai luonnonvaraista alkuperää. Luonnonvaraisen lohen tilanne on kuitenkin kohentunut huonoimmista vuosista parantuneen kalastuksensäätelyn myötä, mutta luonnonvarainen lohi on edelleen uhanalainen ja vaatii suojelutoimenpiteitä. Olisi tärkeää selvittää jäljellä olevien eri lohipopulaatioiden sekä luonnonvaraisen ja kasvatetun lohen syönnösvaelluskäyttäytymispiirteet Itämeren merialueilla.

Kalojen liikkeitä on perinteisesti tutkittu merkintämenetelmin, joissa kalaan usein kiinnitetään ulkoinen keinotekoinen merkki. Yleensä merkissä on yksilökohtainen kirjain–numerotunnus, jolla yksittäisen kalan liikkeittä voidaan arvioida merkin palauduttua takaisin tutkijalle. Ulkoiset merkit ovatkin käytännössä täysin riippuvaisia kalastuksesta. Sieltä missä ei kalasteta, ei myöskään tule merkkipalautuksia, vaikka kalat siellä oleskelisivatkin. Tämä voi johtaa vääristyneeseen tulkintaan kalojen liikkeistä. Yleensä joudutaan myös merkitsemään suuria määriä kaloja, jotta aikanaan saadaan edes yksi merkkipalautus.

Kalojen liikkeitä on seurattu myös telemetrian avulla. Laajoilla merialueilla menetelmän käyttö on kuitenkin miltei mahdotonta. Satelliittipaikannus on vesiympäristössä vaikeaa ja laitteet suurikokoisia haitaten mahdollisesti kalan luontaista liikkumista. Joka tapauksessa tietoa saataisiin vain yhden tai muutaman kalayksilön liikkeistä ja tulosten yleistäminen esimerkiksi populaatiotasolle on kyseenalaista.

Ravinnosta jää eläimen kudoksiin kullekin elinalueelle ominainen kemiallinen leima. Eläin on siis ikään kuin jo merkattu eikä varsinaista merkintätoimenpidettä enää tarvita. Kyseiset merkkiaineet voidaan analysoida jälkikäteen ja verrata arvoja oletettujen elinalueiden vastaaviin. Näin saadaan paikkatietoon perustuva arvio siitä, millä todennäköisyydellä eläinyksilö on ollut kullakin oletetulla elinalueella. Erityisen hyviä kudoksia ovat sellaiset, joiden kemi-

allinen koostumus ei enää kudoksen muodostumisen jälkeen muutu. Tällaisia ovat muun muassa lintujen sulat ja kalojen suomut.

Tässä tutkimuksessa analysoitiin lohen suomujen ja kuuloluiden (otoliitti) hapen, hiilen ja typen isotooppisuhteita, jotta voitiin arvioida eri lohipopulaatioiden sekä luonnonvaraisen ja kasvatetun lohen merivaelluseroja. Suomujen viimeisen kasvuvyöhykkeen hiilen ja typen isotooppiarvoja verrattiin syönnösalueilta pyydettyjen lohien suomuarvoihin. Lisäksi Itämeren vedestä mitattiin hapen ja liuenneen epäorgaanisen hiilen isotooppiarvot. Mitatuista arvoista luotiin koko Itämeren kattavat interpoloidut isotooppikartat kesältä ja talvelta. Kuuloluiden mikroporauksen avulla saatujen näytteiden isotooppiarvoja ja veden isotooppiarvoja vertailemalla voitiin määrittää ensimmäistä kertaa lohiyksilön koko elämänkaaren pituinen vaellusreitti vaelluspoikasesta syönnösalueille ja paluu aikuisena takaisin kutujokeen. Lisäksi jokeen nousseen lohen lihaskudoksesta analysoitiin rasvahappokoostumus, jolla voitiin arvioida sen syönnösalueen laatua, jossa lohi oli viimeisen syönnöskautensa viettänyt.

Suomujen ja kuuloluiden isotooppiarvoista havaittiin, että valtaosa Simojoen lohista vaeltaa Itämeren pääaltaalle syönnökselle. Noin 40 % viettää syönnöskauttaan kuitenkin Suomenlahdella ja Selkämerellä määrän jakautuessa hyvin tasaisesti molemmille alueille. Sen sijaan pääosa Kymijoen lohista oli syönnöksellä Suomenlahdella. Noin 33 % käytti pääallasta syönnösalueenaan, mutta selkämeri oli syönnösalueena lähes merkityksetön. Kymijoen lohella näyttää olevan suppeampi merivaelluskäyttäytyminen verrattuna syönnösalueita laajemmin hyödyntävään Simojoen loheen. Tuloksista voidaankin päätellä, että jos elinympäristömuutokset merialueilla koskevat Suomenlahtea, Kymijoen lohi voi kohdata suurempia selviytymisvaikeuksia verrattuna Simojoen loheen. Simojoen lohen 22 vuoden mittaisessa aikasarjassa ei havaittu pitkän aikavälin systemaattista muutosta merivaelluskäyttäytymisessä. Luonnonvaraisen ja kasvatetun lohen vaelluskäyttäytymisessä ei havaittu eroja. Lohen kuuloluiden ja Itämeren veden isotooppiarvojen samankaltaisuuksiin perustuvan paikantamismenetelmän perusteella havaittiin kaksivuotiaiden Simojoen lohien oleskelevan kesällä aivan Itämeren lounaisimmassa osassa. Seuravana talvena, jolloin kutuvaellus käynnistyi, pääosa niistä oli siirtynyt jo Selkämerelle muutamien yksilöiden ollessa Suomenlahdella. Siirtyminen oli hämmästyttävän nopeaa, mutta tuloksia selittänee menetelmän riippuvuus tutkimukseen valituista veden lämpötila-arvioista sekä käytetyistä muuttujien arvoista veden ja kuuloluun välisessä lämpötilariippuvassa korjauksessa. Isotooppimäärityksiin perustuvassa menetelmässä on myös varmistettava, että vertailuaineiston arvoissa eri syönnösalueilla on riittävästi eroja. Erojen puuttuessa tai ollessa pieniä lohien paikantaminen jollekin alueelle on epätarkkaa.

Rasvahappoanalyysillä havaittiin, että kutua edeltävä paasto kuluttaa lohien rasvavarastoja ja siten hävittää eri merialueilla mahdollisesti syntyneiden rasvahappokoostumusten väliset eroavaisuudet lohen rasvakudoksesta. Eri merialueilla syötyjen erilaisten ravintokohteiden vaikutus lohen rasvahappokoostumukseen oli kuitenkin havaittavissa solukalvolipideissä sekä mätimunissa. Näyttää siltä, että Itämeren pääallas on rasvahappokoostumuksen perusteella

laadultaan korkein ja Selkämeri ja Suomenlahti heikompia, mutta nekin tarjoavat silti merkittävälle joukolle lohia hyvät syönnösalueolot. Pääaltaalla on myös eniten silakkaa ja kilohailia, jotka ovat Itämeren lohen yleisimmät ravintokohteet. Toisaalta vahvan kilohailivuosiluokan takia ruskuaispussipoikaskuolleisuus (M74 oireyhtymä) voi aiheuttaa lohinaaraan koko jälkeläistuotannon menehtymisen. Niinpä pääallas voi toisinaan olla syönnösalueena laadukkain mutta toisinaan epäedullisin.

Molemmat isotooppimenetelmät siis näyttävät tuovan lisätietoa lohien liikkeistä Itämeressä ja rasvahappoanalyysi lisätietoa eri syönnösalueiden laadusta lohen lisääntymisen kannalta. Tulokset kuvaavat myös paremmin tietystä joesta peräisin olevien kutulohien vaelluskäyttäytymistä kuin merkintäaineisto, koska analysoidut lohiyksilöt ovat selvinneet jokeen asti kudulle. Lisäksi on syytä mainita, että suomunäytteiden keruu isotooppianalyysin ei ole tappava, mikä on ensiarvoisen tärkeää tutkittaessa uhanalaisia kalalajeja ja -populaatioita. Tulevaisuudessa olisi hyvä pyrkiä selvittämään kaikkien Itämeren lohipopulaatioiden syönnösvaelluskäyttäytyminen. Näin saataisiin selkeämpi kokonaiskuva koko Itämeren lohen syönnösalueista ja sen vaihtelusta sekä populaatioiden mahdollisista vuorovaikutuksista.

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ORIGINAL PAPERS

I

MIGRATORY CONNECTIVITY OF TWO BALTIC SEA SALMON POPULATIONS: RETROSPECTIVE ANALYSIS USING STABLE ISOTOPES OF SCALES

by

Jyrki Torniainen, Pekka J. Vuorinen, Roger I. Jones, Marja Keinänen, Stefan Palm, Kristiina A.M. Vuori & Mikko Kiljunen 2014

II

OXYGEN AND CARBON ISOSCAPES FOR THE BALTIC SEA AND AN EXAMPLE OF THEIR POTENTIAL FOR REVEALING PROBABLE FISH LOCATIONS

by

Jyrki Torniainen, Anssi Lensu, Pekka J. Vuorinen, Eloni Sonninen, Marja Keinänen, Roger I. Jones, William P. Patterson & Mikko Kiljunen 2014

Submitted manuscript

III

SEASONAL MOVEMENTS OF INDIVIDUAL SALMON IN THE BALTIC SEA REVEALED BY OTOLITH OXYGEN STABLE ISOTOPES

by

Mikko Kiljunen, Jyrki Torniainen, Anssi Lensu, Marja Keinänen, William P. Patterson, Eloni Sonninen, Pekka J. Vuorinen & Roger I. Jones 2014

Manuscript

IV

THE FINAL NONBREEDING AREA QUALITY OF ATLANTIC SALMON (SALMO SALAR) ESTIMATED BY FATTY ACID AND STABLE ISOTOPE COMPOSITION

by

Jyrki Torniainen, Martin J. Kainz, Pekka J. Vuorinen, Marja Keinänen, Roger I. Jones & Mikko Kiljunen 2014

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