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Developing novel means for unravelling population structure, provenance and migration patterns of European whitefish *Coregonus lavaretus* s.l. in the Baltic Sea

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ABSTRACT

We sought to investigate catch composition, provenance and migration patterns of an economically important coregonid, the European whitefish (EW, *Coregonus lavaretus* s.l.). EW displays complex life history and is heavily stocked in the Baltic Sea. By combining otolith geochemistry (Sr:Ca, Ba:Ca, ^{87}Sr:^{86}Sr) and gill raker counts we developed an assignment framework for determining the general ecological form (anadromous or sea-spawning) and provenance (wild or stocked) of EW adults caught in the mixed stock fishery. Among the adults sampled from the Estonian coastal sea ($N = 82$) 50% were stocked as fingerlings in Finland (1% were stocked as fingerlings in Estonia), and 35 and 14% were of wild anadromous and sea-spawning origin, respectively. Young-of-year individuals ($N = 55$) sampled from four geographically separated rearing facilities in Finland displayed highly distinct otolith natal fingerprints, therefore demonstrating great potential for pin-pointing the provenance of adult individuals that were stocked as fingerlings. Among the adults that were stocked as fingerlings 13 different otolith natal fingerprint groups were identified, however 81% of them originated from seven distinct sources. This study underscores the prevalence of stocked individuals in the EW mixed-stock fishery, and quantitatively demonstrates the low share of wild individuals. It is suggested that current stocking practices may lack the desired effect on wild populations, and they only serve to supplement the catches. The presented results will allow to monitor the structure of EW stocks in both local and regional scale.

1. Introduction

Understanding population structure and connectivity in economically valuable migratory fish species that can transcend country borders is paramount in their management and conservation. Such species are often anadromous and therefore depend on the accessibility and quality of freshwater spawning areas which are often negatively influenced by different anthropogenic factors such as damming and pollution (Limburg and Waldman, 2009; Cooke et al., 2012). To compensate for the effects of anthropogenic factors supplementary stocking of juveniles is a common practice (ICES, 2014; Jokikokko and Huhmarniemi, 2014). This will, however, add an extra dimension to the species' population structure and render stock management more difficult. New methods should be applied to resolve the population structure and/or catch composition in species with complex life-histories and extensive stocking practices.

European whitefish (EW, *Coregonous lavaretus,* s.l.) inhabiting the brackish Baltic Sea and its freshwater tributaries is an ecologically and economically important coregonid displaying anadromous, sea-spawning and freshwater-resident forms (Svärdson, 1979; Lehtonen, 1981; Sõrmus and Turovski, 2003). Anadromous individuals spawn in rivers with various discharge rate (Larsson et al., 2013), while the spawning areas of the sea-spawning form are mostly located in shallow bays with sandy, stony and/or gravely bottom (Leskelä et al., 1991; Sõrmus and Turovski, 2003). Majority of the progeny of anadromous individuals drift to the sea within 2–3 weeks post-hatch, however this can vary from one week to four months being subjected to inter-individual and –river variability (Leskelä et al., 1991; Lehtonen et al., 1992; Jokikokko et al., 2012). Feeding and spawning migrations of anadromous EW can be extensive reaching up to 700 km in specimens originating from rivers running to Bothnian Bay and feeding in the Archipelago Sea (Lehtonen and Himberg, 1992)

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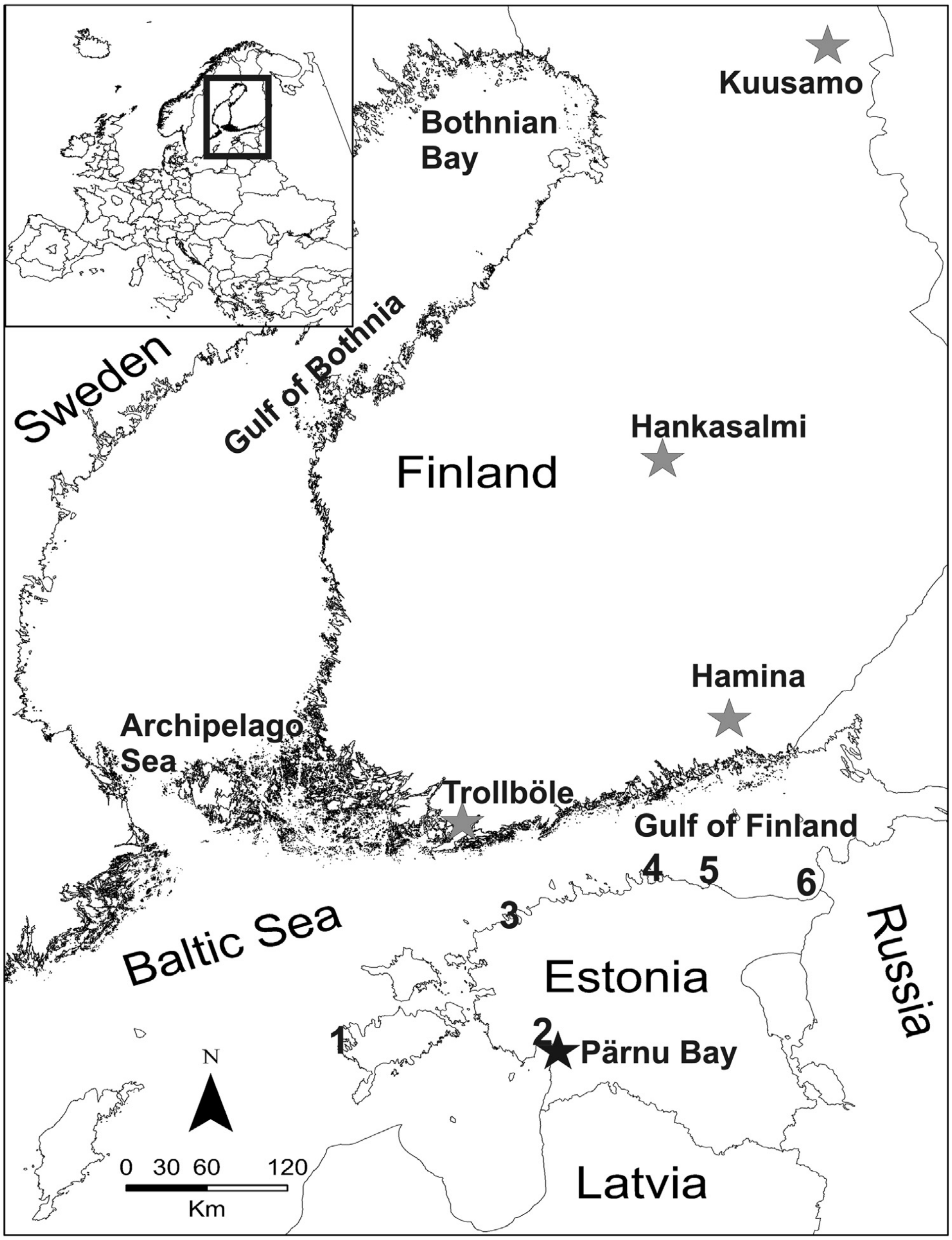

Fig. 1. Map of the study region indicating sampling sites of hatchery (grey stars) and wild origin (black star) young-of-year and adult (1–6) European whitefish. Refer to Table 1 for further details on sampling sites and collected samples.

(Fig. 1). The sea-spawning form is usually more stationary and rarely migrates >100–200 km (Lehtonen and Himberg, 1992). Natal homing is well developed in both forms as tagged fish are only occasionally caught in non-natal spawning areas (Ikonen, 1982; Lehtonen and Himberg, 1992).

To compensate for different anthropogenic factors negatively influencing EW, vast stocking programmes have been implemented throughout the Baltic Sea for decades (e.g. Salojärvi et al., 1985; Jokikokko and Huhmarniemi, 2014). These programmes mainly concentrate on stocking the fry and/or fingerlings of anadromous form, while the progeny of sea-spawning form are stocked in small numbers only in the Archipelago Sea and northern part of the Gulf of Finland (GoF) (Lari Veneranta, personal communication). Amongst the Baltic Sea countries, Finland is currently by far the largest stocker of EW due to large economic interest, but also because many anadromous EW spawning rivers are inaccessible to fish and/or with poor water quality (Leskelä et al., 2004). Other countries stock EW rather occasionally or/and in relatively small numbers (see methods for additional information on stocking practices). Despite the large-scale stocking events conducted in the Baltic Sea the abundance of EW is still in the lows (Leskelä et al., 2004; Sõrmus and Turovski, 2003; HELCOM, 2013). EW is currently enlisted among endangered species in the Baltic Sea (HELCOM, 2013).

Discrimination between wild anadromous and sea-spawning form has been traditionally based on morphological characteristics (e.g. gill raker counts; Lehtonen, 1981). However, as a result of large-scale stocking programmes genetic (and therefore morphological) integrity of distinct forms and spawning stocks has been obscured (Ozerov et al., 2015), making distinctions based on gill raker counts partly ambiguous but still useful (Verliin et al., 2013; Himberg et al., 2015). The identification of stocked (artificially unmarked) individuals remains to be an unsolved issue (Himberg et al., 2015), although one preliminary study based on scale chemistry is available (Schwartzberg et al., 1995). Therefore researchers have started to look for other possibilities such as methods based on naturally occurring trace elements in fish otoliths. Although such studies have so far been limited to solution-based whole otolith analyses and analyses with poor spatio-temporal resolution, useful results have already emerged (Lill et al., 2015; Himberg et al., 2015; Hägerstrand et al, in press). However, to advance our understanding on EW population structure in the Baltic Sea by utilizing methods based on natural chemical constituents of otoliths, further research using improved spatio-temporal resolution (e.g. line-scans) and additional chemical markers is warranted.

Otolith chemistry may have good potential in studies aiming to resolve the population structure and/or catch composition of species composed of individuals with distinct life-history strategies or origin, but which tend to display little or no genetic differentiation. This is because otolith chemistry relies on environmental not genetical signals, and it enables better resolution in terms of physical origin of individual fish. Different otolith chemistry based methods rely on naturally occurring trace elements and stable isotopes that are incorporated to otoliths in proportion with ambient environment (Campana, 1999; Walther and Limburg, 2012). Therefore, variable ambient water chemistry is generally a prerequisite for identifying distinct life-history strategies or pin-pointing provenance. However, this information can be extracted in several ways starting from bulk analyses to fine-scale femto-second laser ablations coupled with high resolution mass-spectrometers (Campana et al., 1997; Walther and Limburg, 2012; Tanner et al., 2016). Therefore, selection of the methodological approach ultimately determines which questions can be asked and how precise the answers can be.

The main aim of the present study was to provide a solution to the above described problem of determining the population structure and/or catch composition of Baltic Sea EW. To achieve this we applied fine-scale laser ablation line-scan techniques to quantify Sr:Ca, Ba:Ca and ^{87}Sr:^{86}Sr profiles in the otoliths of EW collected from various coastal sites in Estonia and four Finnish rearing facilities. Specifically, the aims were to combine otolith geochemistry and gill raker counts to: (i) investigate the possibility of differentiating among naturally reproducing forms and stocked individuals and (ii) estimate the proportion of stocked individuals among the adult EW inhabiting the coastal waters of Estonia. Additional aim was to use otolith geochemistry to investigate the possibility of differentiating among young-of-year (YOY) EW collected from four Finnish rearing facilities and one naturally reproducing anadromous population from Estonia.

2. Materials and methods

2.1. Study system and stocking programme

The Baltic Sea is a semi-enclosed brackish waterbody where salinities are mostly <8‰. Low salinities result from limited water exchange with the Atlantic Ocean and hundreds of rivers that drain into the Sea. These rivers sustain both freshwater resident and diadromous fish species, however, many of such rivers are dammed or suffer under poor water quality caused by anthropogenic pollution. To compensate for these anthropogenic effects large stocking programmes are undertaken for economically important migratory species like the EW. For example, during the last decade Finland has annually stocked 40–90 million EW fry and 4–8 million fingerlings to Gulf of Bothnia (GoB), and 1.3 million fry and 1.2 million fingerlings to GoF (Leskelä, 2006; Lari Veneranta, personal communication). These fish are usually stocked to rivers or estuaries, and occasionally directly to the sea. There has been a tendency of stocking progressively more fingerlings than fry, and as a consequence no or limited amount of fry has been stocked to the GoF during recent years (Lari Veneranta, personal communication). In Sweden stockings currently occur only in River Indalsälven (one million fry and 100 000 fingerlings annually) and River Ångermanälven (3–4 million fry annually), both located in the GoB (Ann-Britt Florin, personal communication). In Latvia, EW is stocked annually, but inconsistently to one or two of the following rivers: Daugava, Gauja, and Venta (16 000–47 000 fingerlings per year) (Jānis Birzaks, personal communication). In Poland, a total of ≈ 900 000 fry (0+ to 1 +) and 1.75 million larvae have been stocked to Puck Bay, Reda River and Szczecin Lagoon in the period of 2004–2013 (Bartel and Kardela, 2015). Russia stocks annually ≈ 150 000 EW fingerlings to Curonian Lagoon, but no stocking is known to occur in the GoF. No stocking of sea-dwelling EW currently occur in Lithuania. In Estonia the last two stocking events took place in 2006 and 2016 when 45 000 and 34 000 fingerlings, respectively, were released to River Pärnu. Germany also stocks EW, but this stocking source can be considered as too distant for our study.

2.2. Fish sampling

Hatchery-origin EW fingerlings were collected in autumn of 2013 from four different rearing facilities (ponds or indoor tanks) located in Finland (Fig. 1; Table 1). The fingerlings originating from Hamina and Kuusamo are the progeny of wild anadromous individuals; they are reared on natural food in outdoor freshwater ponds until autumn and subsequently stocked to the GoF. The fingerlings originating from Trollböle are the progeny of sea-spawning broodstock kept in fresh water; they are reared on commercial fish feed in outdoor freshwater ponds until autumn and subsequently stocked to the GoB and the GoF (sea area from Pori to Porvoo). The fingerlings originating from Hankasalmi are the progeny of anadromous broodstock kept in fresh water; they are reared on commercial fish feed in indoor freshwater tanks, but currently not stocked to the GoF. Wild EW YOY were collected on 6th of June 2013 from Pärnu Bay, close to the mouth of River Pärnu. These individuals were the progeny of wild anadromous River Pärnu stock, which is currently the only viable anadromous EW stock in Estonia. Adult individuals were sampled in 2012–2013 from six sites located in the coastal waters of Estonia (Fig. 1; Table 1). These samples were collected by commercial fishers or provided by the national fish monitoring programme. In most cases the heads of adult fish were frozen for later otolith removal and gill raker counting. Adult fish total length (mm) and sex were also recorded before the beheading and freezing process.

2.3. Otolith preparation and micro-chemical analysis

In the laboratory, the frozen heads of adult fish were thawed. Otoliths were removed, cleaned and stored dry in all sampled YOY and adult specimen. Gill rakers were enumerated only in adults by one experienced reader. Subsequently, one randomly chosen otolith (sagitta) per individual was glued sulcus side up onto a coverslip that was itself partially glued onto a standard glass slide. All otoliths were then manually grinded on silicon carbide grinding paper (P1200) until the core was visible. Final polish was given with size P4000 grinding paper. Individual sagittal thin-sections were finally glued onto standard glass slides and stored in clean plastic bags for later analysis. Before the chemical analyses, all slides containing the otolith thin-sections were ultrasonically cleaned for 15 min in NANOPure® water (Barnstead International, USA) and subsequently dried in laminar flow hood. All the chemical analyses were carried out at the Oregon State University's WM Keck Collaboratory for Plasma Spectrometry. Cleaned otoliths were first analyzed for ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{66}Zn, ^{86}Sr and ^{137}Ba with laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). A VG PQ ExCell ICPMS (Thermo

Table 1
Collected biological data ordered by sampling site. Mean total length (TL) ± SD is given in mm. Gill raker counts are expressed as mean ± SD and minimum and maximum values. YOY denotes young-of-year.

Sample site	Sampling date	*N*	TL	Gill raker count
YOY (reared)				
Hamina	Oct. 2013	11	78 ± 13	–
Kuusamo	Sept. 2013	11	102 ± 5	–
Trollböle	Aug. 2013	11	126 ± 28	–
Hankasalmi	Aug. 2013	11	111 ± 6	–
YOY (wild)				
Pärnu Bay	Jun. 2013	11	39 ± 1	–
Adults				
1– Vilsandi Island	Jun.-Nov. 2012–2013	19	455 ± 42	25.2 ± 5.1 (17–32)
2 – Pärnu Bay	Oct. 2012–2013	20	444 ± 51	23.5 ± 3.1 (18–28)
3 – Paldiski Bay	Aug.-Nov. 2012	21	384 ± 22	28.9 ± 2.6 (24–33)
4 – Käsmu Bay	Aug. 2010	1	591	29
5 – Kunda Bay	Apr.-Jul. 2012–2013	20	387 ± 47	28.4 ± 4.3 (21–35)
6 – Narva Bay	Apr. 2012	1	588	29

Scientific, USA) with a New Wave DUV193 excimer laser (New Wave Research, USA) was used. The laser was set at 7 Hz with a 40-μm ablation spot size and a scan speed of 5 μm·s^{-1} (YOY) or 10 μm·s^{-1} (adults). For the YOY (*N* = 55), a ≈ 500 μm transect was traced from the core towards the pararostrum. For the adults (*N* = 82), a continuous line scan was traced from the core to the edge of the pararostrum. Helium was used as a carrier gas. A glass reference material (NIST 612) and calcium carbonate standard (MACS-3) was analyzed before and after every 10 otoliths. Data reduction to element:Ca in mmol·mol^{-1} was achieved by following the methods of Miller (2007) as described in Rohtla et al. (2014a). A nine-point running mean followed by nine-point running median was used to smooth the data for visualization. The average precisions (%RSD) of quantifying ^{43}Ca, ^{86}Sr and ^{137}Ba for NIST 612 glass across runs during three days were 14%, 12% and 13%, respectively. Absolute accuracy was estimated from MACS-3, and the mean values for Sr:Ca and Ba:Ca were 17% and 53% higher than reported for the standard. Differences from standards were corrected for in data treatment. As Mg:Ca, Mn:Ca and Zn:Ca ratios displayed rather erratic and inconsistent profiles among the sampled individuals, they were not used in any further data interpretation or analysis. Mg:Ca ratio was, however, used for detecting vateritic inclusions. Zn:Ca profiles displayed some evidence on annual oscillations (sensu Limburg and Elfman, 2010), but not in all individuals.

After elemental analysis, the same otoliths were used for ^{87}Sr:^{86}Sr ratio quantification using a Nu-Plasma multi-collector inductively coupled plasma mass spectrometer (MC-ICPMS, Nu Instruments, UK) and a New Wave DUV193 excimer laser (New Wave Research, USA). Only otoliths with an indication of potential Sr:Ca freshwater signal were analyzed, except for YOY where all the individuals were analyzed. For the YOY (*N* = 55), a ≈ 500 μm transect (parallel to the transect traced during elemental analysis) was traced from the core area towards the pararostrum. For the adults (*N* = 61), a ≈ 500 μm transect was traced from the core area towards dorsal side of the otolith. The laser was set to a pulse rate of 10 Hz, with a 65 μm spot size that travelled at 5 μm·s^{-1}. The general method of Woodhead et al. (2005) as described in Miller and Kent (2009) was followed to correct for potential Kr and Rb interferences and monitor for Ca argide/dimer formation. To monitor instrument accuracy and precision, the ^{87}Sr:^{86}Sr of a marine gastropod (0.70918) was used as an in-house marine carbonate standard (Miller and Kent, 2009) and analyzed before and after every two otoliths. A mean value (± 2 SE) of 0.709272 ± 0.000032 (*N* = 89) was consistently obtained. All otolith ^{87}Sr:^{86}Sr values were corrected for this difference using the closest measured gastropod value.

2.4. Assignment framework development

To create a framework for determining the general ecological form (anadromous or sea-spawning), general provenance (wild or stocked) and the specific stocking practice (1–4; Table 2) of EW inhabiting the Baltic Sea, YOY and adult otolith Sr:Ca, Ba:Ca and ^{87}Sr:^{86}Sr profiles (examined in the present study) in conjunction with the known records of adult gill raker counts and published geochemical literature were examined. Both quantitative (i.e. chemical marker ratios from the natal area) and qualitative traits (e.g. shape) of the chemical profiles were used for the framework development (Table 2). However, only qualitative traits of the profiles were later used for quantitative assignment of adults in our case (see next paragraph). Published data on region specific gill raker counts were used if available. In Estonia, sea-spawning and anadromous EW generally have ≤24 and ≥25 gill rakers, respectively (Sõrmus and Turovski, 2003). In the GoB the respective values are ≤28 and ≥29 (Himberg et al., 2015). However, as large variation in gill raker counts can occur within one spawning stock and overlaps among different spawning stocks are common (Verliin et al., 2003), estimations on ecological form based solely on gill raker counts are often inconclusive and ambiguous. This necessitates additional methods, such as otolith geochemistry, for determining the ecological form of EW inhabiting the Baltic Sea. Therefore, published and unpublished geochemical background data for the Baltic Sea was also used to develop the assignment framework. For example, it is known that Sr:Ca ratios are high in the Baltic Sea, but moderate (Proterozoic-Archean intrusives) and low (Mesozoic-Paleozoic sediments) in its freshwater drainage areas (Löfvendahl et al., 1990). Also, ^{87}Sr:^{86}Sr ratio in the Baltic Sea is slightly higher than in the ocean (0.70918), but still fixed around 0.7093 and 0.7095 (Åberg and Wickman, 1987). ^{87}Sr:^{86}Sr ratios in the freshwaters, however, are in most cases significantly higher and display a general south to north gradient due to varying bedrock (Löfvendahl et al., 1990). This results in ^{87}Sr:^{86}Sr ratios of 0.711–0.718 and >0.720 in Estonian and Finnish freshwaters, respectively (Löfvendahl et al., 1990; Rohtla et al., 2014b). Based on the above, assignment rules were established. Estimations based solely on otolith chemistry vs. gill raker counts were compared. Specifically, it is common practice in Estonia that individuals possessing ≥25 gill rakers are generally regarded as having foreign (mostly Finnish) origin. Individuals possessing ≤24 gill rakers are generally regarded as having local origin. This common practice was put to a test by confronting the results of qualitative gill raker based assignment with the results of quantitative otolith chemistry assignments. Although the development of the assignment frame-

Table 2
Established assignment rules for determining general ecological forms and stocking practices in European whitefish mixed-stock fishery in the Baltic Sea. Rules are based on Sr:Ca, Ba:Ca and $^{87}Sr{:}^{86}Sr$ ratios quantified from the natal area just outside the maternally influenced area, qualitative traits of Sr:Ca and Ba:Ca profiles, and gill raker counts. FW – fresh water; SW – sea water; FWR – freshwater residency; GRC – gill raker count; GoB – Gulf of Bothnia; GoF – Gulf of Finland. Words italicized in the first column indicate possible confusion with other ecological form or stocking practice. ≈ – mean value.

Ecological form or stocking practice	Sr:Ca ($mmol{\cdot}mol^{-1}$)	Ba:Ca ($mmol{\cdot}mol^{-1}$)	$^{87}Sr{:}^{86}Sr$	Gill raker count (rule of thumb)	Qualitative "presence-absence" traits of the chemical profiles
Wild-origin					
Sea-spawning *(Practice 3)*	>2.0	<0.01	0.7093-0.7095	≤24 in EST, LV, PL, Gotland ≤28 in GoB ≈29 in GoF FIN	Exclusively high Sr:Ca and low Ba:Ca values. No Sr:Ca nadir and Ba:Ca peak next to the otolith core (Fig. 2g). GRC should help to distinguish from (rare) Practice 3.
Anadromous* *(Practice 2)*	0.1–2.0	>0.01	0.7093-0.7095 or <0.711	≥25 ≥29 in GoB	Sr:Ca nadir and Ba:Ca peak next to the otolith core, usually short FWR that does not allow the true natal signature to stabilize (Fig. 2f). Longer stays in FW will result in stabilized natal signatures, but this is rare.
Hatchery-origin					
Practice 1 *(Practice 4)*	0.1–2.0	0.003–0.06	>0.711	≥25 ≥29 in GoB	Long FWR (Fig. 2h, i), labile chemical profiles and high Sr:Ca and low Ba:Ca values indicate commercial (marine-origin) fish feed. GRC should help to distinguish from Practice 4.
Practice 2* *(wild anadromous)*	0.1–2.0	>0.01	0.7093-0.7095 or <0.711	≥25 ≥29 in GoB	Sr:Ca nadir and Ba:Ca peak next to the otolith core, usually short FWR that does not allow the true natal signature to stabilize (Fig. 2f). Longer stays in FW will result in stabilized natal signatures, but this is rare.
Practice 3 *(wild sea-spawning)*	>2.0	<0.01	0.7093-0.7095	≥25 ≥29 in GoB	Exclusively high Sr:Ca and low Ba:Ca values. No Sr:Ca nadir and Ba:Ca peak next to the otolith core (Fig. 2g). GRC should help to distinguish from (rare) Practice 3.
Practice 4 *(Practice 1)*	0.1–2.0	0.003–0.06	>0.711	≈29 in GoF FIN ≤28 in GoB	Long FWR (Fig. 2h, i), labile chemical profiles and high Sr:Ca and low Ba:Ca values indicate commercial (marine-origin) fish feed. GRC should help to distinguish from Practice 1.

Note: Practice 1–parents wild anadromous or anadromous origin broodstock, progeny are reared in FW and stocked to FW or SW (rare) as fingerlings; Practice 2 – parents wild anadromous or anadromous origin broodstock, progeny hatch in FW and are stocked to FW as fry; Practice 3 (rare) – parents wild anadromous or anadromous origin broodstock, progeny hatch in FW and are stocked directly to SW as fry; Practice 4 (rare) – parents wild sea-spawning or sea-spawning origin broodstock, fish are reared in FW and stocked to SW as fingerlings (conducted only in Åland and Trollböle).
*Differentiation between wild anadromous and Practice 2 individuals remains to be an unresolved issue and should be addressed in the future. However, in the context of the assignments made in the present study this is irrelevant because no or only limited amount of fry are currently stocked to the GoF or to any other region within reasonable proximity to the adult sampling sites in the present study. Also, stocking small quantities of fry have no or limited influence on catch numbers in the GoF (Salojärvi et al., 1985). Nor is the Practice 4 relevant for the present study as sea-spawning European whitefish rarely migrate >100 km (Lehtonen and Himberg, 1992).

work is partly based on otolith chemical profiles of adults that were collected from the Estonian coastal sea, it is reasonable to assume that (qualitatively) same otolith chemical profiles are present in other regions of the Baltic Sea (e.g. the GoB and the southern regions of the Baltic Sea). Quantitative values of the profiles can be region specific (especially the natal freshwater values) and are therefore denoted as range of values in Table 2.

2.5. Data analysis

Multinomial logistic regression was used to assign adult individuals with unknown ecological form ($N = 72$) as "wild sea-spawning", "wild anadromous", or "anadromous stocked as fingerling". Training data set contained known-origin YOY anadromous individuals from Pärnu Bay ($N = 11$), to-be-stocked fingerlings from four Finnish rearing facilities ($N = 44$) and spawning individuals from Vilsandi Island ($N = 9$). Binomial presence (1) or absence (0) values were assigned to three otolith chemical profile parameters: 1) Sr:Ca nadir next to the otolith core (= anadromous if present; = sea-spawning if absent), 2) presence of Ba:Ca peak next to the otolith core (= anadromous if present; = sea-spawning if absent) and 3) long freshwater period next to the core as evidenced by low Sr:Ca values (= stocked as a fingerling when present). Although Table 2 encompasses three other types of stocking practices, these can be omitted in the present situation because such stocking practices are not utilized in our region or relevant in our case (see Table 2 for further explanations). However we are confident that these general classification rules (and applied quantitative approach) for identifying anadromous, sea-spawning, and stocked as fingerlings individuals can be used throughout the Baltic Sea. Qualitative assignment of adults with unknown ecological form was also performed (based on the same data) and the results were compared with the results from quantitative multinomial logistic regression to evaluate the agreement between two approaches. The next step would be to determine the exact origin of individuals that were stocked as fingerlings, but this will require sampling all the potential sources of fingerlings (not attempted in the present study). This will also require the use of other quantitative approaches (e.g. decision trees, maximum likelihood estimations, discriminant analysis) as continuous variables must be used in the model. Multinomial logistic regression used in the present study can here be used to screen out the individuals which were stocked as a fingerlings.

To characterize spatial variation in elemental and isotopic signatures across freshwater rearing habitats, mean otolith Sr:Ca, Ba:Ca and ^{87}Sr:^{86}Sr ratios were calculated from the otolith region accreted during residency in rearing facilities (hatchery YOY and adults) and rivers (wild YOY and adults). This would potentially allow to pin-point the most successful recruits and the hatcheries/rivers that provide them. However, as this study was not designed to incorporate all potential natal sources of freshwater-origin EW, individual back-assignment of adults to the specific rearing/spawning water bodies was statistically not attempted. ANOVA was used to examine spatial differences in YOY otolith Sr:Ca, Ba:Ca and ^{87}Sr:^{86}Sr ratios. As dependent variables (i.e. chemical markers) were not linearly correlated to each other, separate ANOVAs were used for each dependent variable instead of a MANOVA. Upon identifying significant differences among locations Tukey's HSD test was employed to identify pairwise differences. Canonical analysis of principal coordinates (CAP test using PRIMER PERMANOVA+; Andersson et al., 2008) was used to visualize the variation in multi-elemental signatures among sampled locations for YOY and adults. Data was square-root transformed and normalized before the analysis. Canonical discriminant analysis (CDA) was used to measure the relative importance of each variable. Finally, a quadratic discriminant function analysis (QDFA) using log-transformed values and accompanied with jackknife cross-validation procedure was performed to determine the accuracy of individual YOY EW assignment to their natal rearing locations.

3. Results

3.1. Ecological form and stocking practice assignment

By sampling the YOY fish from Pärnu Bay it was possible to determine representative otolith chemical profiles for the anadromous form. All sampled individuals displayed a moderate Sr:Ca nadir and a prominent Ba:Ca peak in the beginning of their profiles (Fig. 2a). This suggests that these fish descended to the sea within two weeks post-hatch and resided in a high Ba environment, which is usually found at river estuaries where ambient Ba maxima occurs due to mixing processes (Coffey et al., 1997; Rohtla et al., 2014a). Therefore high Ba:Ca near the otolith core indicate that the particular fish has resided in an estuary and therefore is anadromous (Fig. 2f). By sampling the spawning fish near the Vilsandi Island (which usually have ≤24 gill rakers; Sõrmus and Turovski, 2003) it was possible to determine representative otolith chemical profiles for the sea-spawning form. All nine fish with gill raker count 17–24 had stable and high Sr:Ca profiles with no prominent Ba:Ca peaks near the core (Fig. 2g). Therefore lack of Sr:Ca nadir and Ba:Ca peak near the otolith core indicate that a particular fish is a sea-spawner. By sampling YOY fish from the Finnish rearing facilities it was possible to determine representative otolith chemical profiles for the stocked fingerlings. These fish are kept in fresh water throughout the summer and stocked in autumn (Fig. 2b–e). As a result of such stocking practice, the chemical profiles of those fish are markedly different from the wild anadromous individuals which descend to the sea mostly within few weeks after hatching. Therefore long-term freshwater residency indicates that a particular fish was stocked as a fingerling (Fig. 2h, i) or even as an older individual.

Based on the established assignment framework (Table 2) the following results emerged. The multinomial logistic regression model performed with 100% accuracy (κ=1, $CI_{95\%}$ = 0.8235; 1). The agreement between qualitative and quantitative assignment was 97.3% ($CI_{95\%}$ = 0.926; 0.9997). Amongst the adults sampled near Vilsandi Island (N = 19), 47% were local sea-spawners, 42% were stocked as fingerlings in Finland, and 11% were of wild anadromous origin. Amongst the adults sampled in Pärnu Bay (N = 20), 95% were of wild anadromous origin (River Pärnu stock), and only one fish was stocked as a fingerling in Estonia (from the stocking event in 2006; Fig. 2i). Amongst the adults sampled in Paldiski Bay (N = 21), 90% were stocked as fingerlings in Finland, 5% were of wild anadromous origin and 5% were local sea-spawners. Amongst the adults sampled in Kunda Bay (N = 20), 60% were stocked as fingerlings in Finland, 30% were of wild anadromous origin, and 10% were local sea-spawners. Both of the individuals sampled in Käsmu and Narva Bay were stocked as fingerlings in Finland.

Sixty-three per cent (N = 52) of the sampled adults were estimated to be of Finnish origin based solely on gill raker counts (≥25; without information on maturity or location and date of capture). Otolith chemistry confirmed it (i.e. stocked as fingerlings that were reared in non-Estonian location) in 77% of the cases (N = 40). One individual that was stocked as a fingerling was actually reared in an Estonian hatchery. In other cases it could not be unambiguously confirmed whether a specific individual was of local or foreign origin. This applies especially for minority of individuals with intermediate gill raker count (i.e. 25–26) in which case both local and foreign origin are possible (A. Verliin, unpublished data). In such cases otolith chemistry solved the issue if a specific individual was stocked as fingerling, but if not, then exact provenance could not be unambiguously determined. Individuals that were stocked as fingerlings based on otolith chemistry had 25–33 gill rakers. Individuals with low gill raker count (18–24) never possessed a natal fingerprint representative to a stocked fingerling. The latter fish always had an otolith chemical profile representative to a (local) sea-spawning or (local) anadromous individual. Vateritic inclusions were observed only in one individual; sharp declines in both Sr:Ca and Ba:Ca were accompanied by corresponding increases in Mg:Ca.

3.2. Otolith fingerprint variation in freshwater rearing sites

Elemental profiles of all wild anadromous individuals sampled in the present study were characterized by limited freshwater residency before the onset of seaward migration as demonstrated by erratic Sr:Ca values near the core (Fig. 2a, f); only one individual displayed more or less stabilized natal values. This means that the observed natal fingerprint in wild anadromous individuals is a mix of fresh- and seawater endmembers, and therefore renders it unusable as a true natal fingerprint. Such fingerprint can only be used to categorize a specific individual as anadromous, and cannot be regarded as representative to the river in which a specific fish hatched. For this reason elemental profiles of wild anadromous individuals were only considered with YOY (for comparative purposes with to-be-stocked YOY) and not for adults.

Univariate analyses (ANOVA) indicated significant differences among YOY sampling sites in otolith Sr:Ca ($F_{4,50}$ = 38, P < 0.001), Ba:Ca ($F_{4,50}$ = 19, P < 0.001) and ^{87}Sr:^{86}Sr ratios ($F_{4,50}$ = 2779, P < 0.001) (Fig. 3). Relatively labile Sr:Ca and ^{87}Sr:^{86}Sr ratios and Ba:Ca ratios <0.01 mmol·mol^{-1} were observed in Trollböle and Hankasalmi where fish are reared on commercial (marine-origin) fish feed. Relatively stable Sr:Ca and ^{87}Sr:^{86}Sr ratios and Ba:Ca ratios >0.01 mmol·mol^{-1} were observed in Kuusamo and Hamina where fish are reared on natural food. CAP demonstrated strong geographical separation among YOY based on otolith elemental fingerprints (Fig. 4a). CDA showed that the first two canonical variates explained >99% of variation within the data. Canonical structure coefficients indicated that ^{87}Sr:^{86}Sr ratio was by far the most influential variable in the first canonical variate, while Sr:Ca ratio dominated in the second canonical variate with smaller contribution from Ba:Ca ratio (Table 3). Jackknifed reclassification accuracy from the QDFA was 100% for all five YOY sampling sites.

Among the adults that were stocked as fingerlings (N = 42) several groups encompassing similar individual fingerprints, but different sampling locations, emerged (Fig. 4b). However, not any of those individual fingerprints completely matched with the fin-

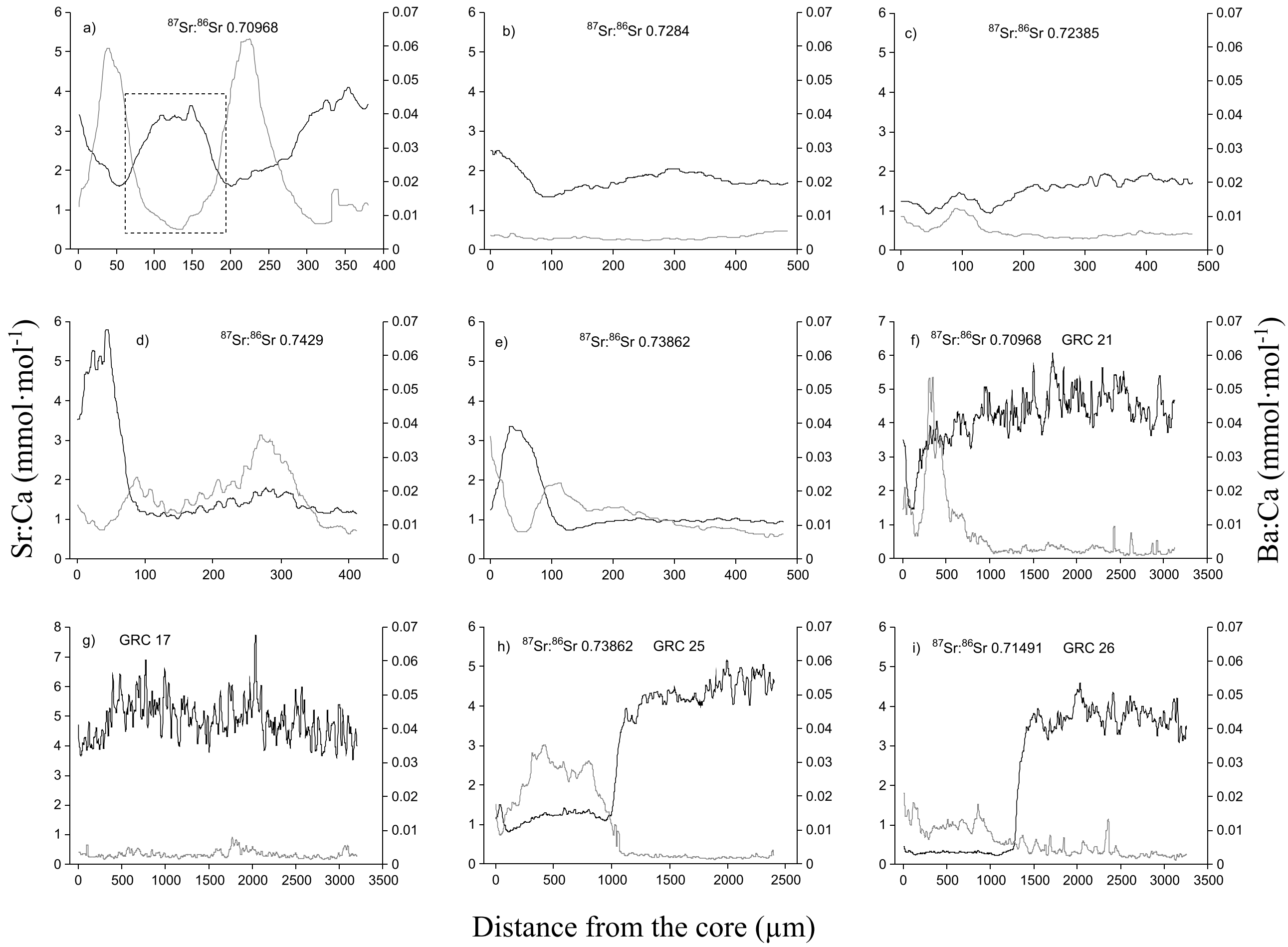

Fig. 2. Representative otolith Sr:Ca (▬) and Ba:Ca (▬) profiles of young-of-year and adult European whitefish collected from four Finnish rearing facilities and various coastal sites in Estonia. (a) wild anadromous young-of-year from Pärnu Bay; (b) fingerling from Hankasalmi; (c) to-be-stocked fingerling from Trollböle; (d) to-be-stocked fingerling from Hamina; (e) to-be-stocked fingerling from Kuusamo; (f) wild anadromous adult from Pärnu Bay; (g) wild sea-spawning adult from Vilsandi Island; (h) Finnish origin stocked adult from Vilsandi Island; (i) Estonian origin stocked adult from Pärnu Bay. Otolith natal ^{87}Sr:^{86}Sr values and gill raker counts (GRC) are also provided where available. In most cases core-to-edge elemental transects are provided with the exception of (a) where almost an edge-to-edge profile is provided for explanatory purposes. Area within the dashed rectangle represents the maternally influenced area (sensu Kalish, 1990).

Table 3
Total canonical structure coefficients for canonical discriminant analysis performed on Sr:Ca, Ba:Ca and ^{87}Sr:^{86}Sr ratios in otolith natal areas of anadromous young-of-year European whitefish collected from four Finnish rearing facilities and one coastal site in Estonia.

Marker	CV1	CV2
Sr:Ca	−0.156	0.812
Ba:Ca	−0.158	−0.547
^{87}Sr:^{86}Sr	0.999	−0.053

gerprints that were quantified in YOY sampled from four Finnish rearing facilities. All adults which were stocked as fingerlings had Ba:Ca natal values >0.01 mmol mol^{-1}. CAP identified 13 distinct otolith fingerprint groups (D = 1) (Fig. 4b).

4. Discussion

4.1. Ecological form and stocking practice assignment

This study provided the first comprehensive assignment framework for resolving the population structure and/or catch composition of EW inhabiting the Baltic Sea. By combining the chemical information stored in otoliths and the number of gill rakers, it was possible to determine the general ecological form (anadromous or sea-spawning) and general provenance (wild or stocked) of adult EW caught in the coastal waters of Estonia. Morphological parameters such as gill raker counts that are traditionally used for differentiating between forms and stocks display increasingly overlapping patterns, and as such cannot be utilized unambiguously. This study demonstrated that otolith geochemistry should be the preferred method when more confident estimations on population structure or catch composition are desired. However, gill raker counts provide useful additional information, especially in situations where distinct spawning stocks still exist.

Fifty per cent of the adults sampled in the coast of Estonia were stocked as fingerlings in Finland. As expected, the share of Finnish origin hatchery fish was higher in the GoF averaging at 77%. From a study on River Kymijoki (GoF) origin EW it is known that <1% of the tagged individuals were recaptured on the Estonian coast (Ikonen, 1982). This has lead researchers to conclude that most of the EW stocked to Finnish rivers draining to the GoF are actually caught by Finnish fishers (Salojärvi et al., 1985). Our results, however, demonstrate that Finnish origin stocked individuals are abundant in the Estonian coast. The reason why past tagging-studies have not documented higher recapture rates from the Estonian coast may lay on the fact that Estonia was part of the Soviet Union, and foreign correspondence was not allowed. However, more recent data also demonstrate EW recapture rates of ≈1% on the Estonian coast (Kunnar Klaas, unpublished data). Estonian commercial fish-

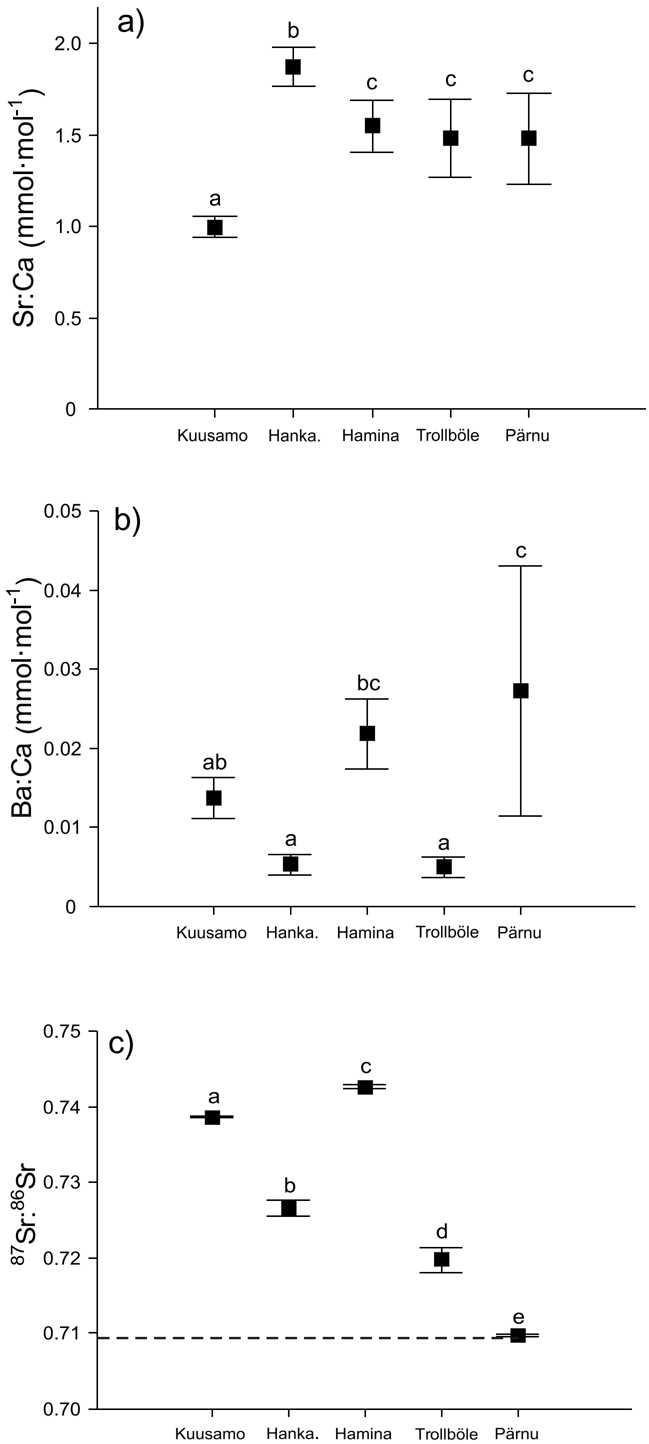

Fig. 3. Mean ± SD (a) Sr:Ca, (b) Ba:Ca, and (c) $^{87}Sr:^{86}Sr$ ratios in the otolith natal areas of young-of-year European whitefish collected from four Finnish rearing facilities and one wild anadromous population from Estonia. Abbreviation is used for one site: Hankasalmi (Hanka.). Different letters indicate pair-wise differences within the respective one-way model (Tukey HSD test $P < 0.05$). Dashed line indicates $^{87}Sr:^{86}Sr$ ratio in the Baltic Sea (≈0.7095; Åberg and Wickman, 1987).

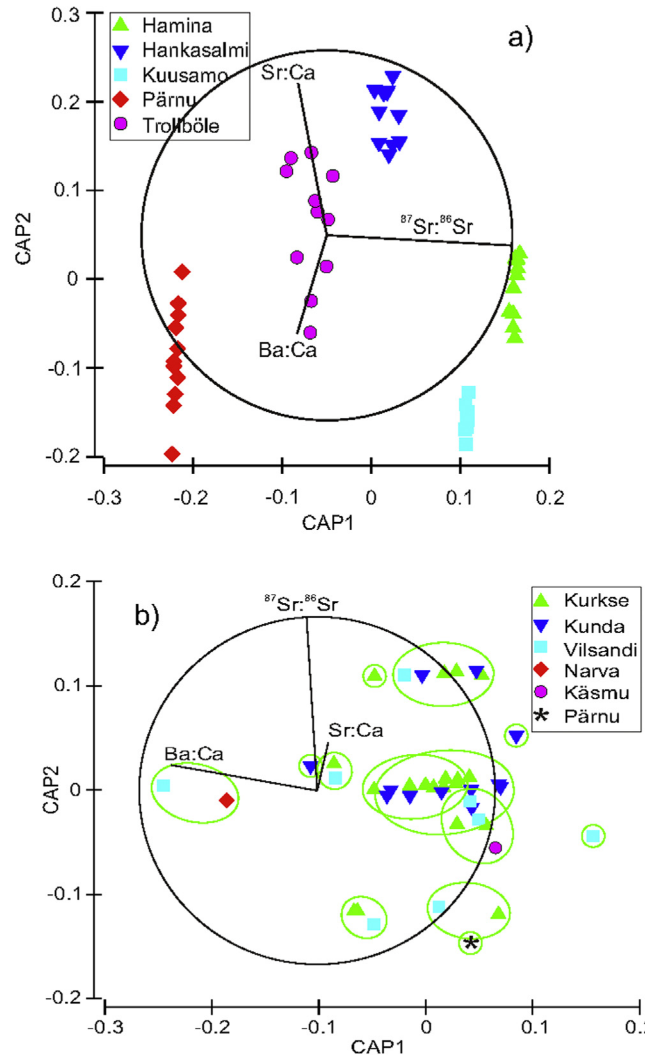

Fig. 4. Canonical analysis of principal coordinates of European whitefish otolith natal fingerprints from (a) young-of-year collected from four Finnish rearing facilities and one coastal site in Estonia, and (b) adults that were stocked as fingerlings and sampled in coastal areas of Estonia.

ers have landed ca 22–25 t of EW per year during the last decade from the GoF (www.agri.ee), and as no EW spawning currently exist in the rivers located in Estonian side of the GoF, the low recapture rates reported from the Estonian coast by different tagging studies should be considered as inconclusive. In fact, we argue that trans-GoF migrations of the Finnish origin EW are common, and Estonian fishers take a large toll on mostly artificially sustained GoF EW stocks.

One may also argue that the sampled stocked fish originate from countries other than Finland. Currently only Finland and Sweden have significant and regular EW stocking programmes around the Baltic Sea. This is supported by otolith geochemistry results obtained in the present study as all adult specimen that were stocked as fingerlings possessed high $^{87}Sr:^{86}Sr$ and/or Sr:Ca ratios that are representative to the older bedrock present in Finland and most of Sweden. In Sweden, stocking of EW fingerlings is currently conducted only in River Indalsälven (Bothnian Sea) from which EW is known to migrate only 50–100 km southward (Lindroth, 1957). Therefore, it is highly unlikely that EW originating from River Indalsälven reach Estonian coast. In fact, it is known that limited mixing occurs between EW from the GoB and other basins such as the GoF (Lehtonen and Himberg, 1992). Based on the reasoning presented above, Finland is the only source from where the sampled stocked adults can originate.

The proportion of wild anadromous and sea-spawning individuals among the adults sampled in Estonia was 35 and 14%, respectively. However, these numbers cannot be regarded as entirely representative because in Vilsandi and Pärnu most of the individuals were sampled just before or during the spawning period, so the local fish were expected to be over-represented in these sites. As these sites represent the few remaining known spawning areas of sea-spawning and anadromous EW in Estonia, sampling was concentrated to the spawning period to obtain representative otolith chemical profiles for each form. Therefore, the total share of wild individuals in our total sample in most likely overestimated. This is also exemplified by the other more representative sampling sites (Paldiski, Kunda, Käsmu and Narva Bay; $N = 43$) where the total share of wild anadromous and sea-spawning individuals was 18% and 5%, respectively. At the moment it is not possible to determine the exact provenance (i.e. country of origin or natal river for anadromous fish) of wild individuals with otolith chemistry as no known-origin foreign wild EW were available for the present study. It is likely that the peculiarities of anadromous EW life-history (i.e. immediate descent to the sea) and chemical homogeny of brackish water will not allow to create distinctive otolith natal chemistry fingerprints in wild anadromous and sea-spawning individuals. Although EW with gill raker count $\leq$24 are generally regarded as local in Estonia, estimations based solely on gill raker counts can be ambiguous due to overlaps between different origin spawning stocks.

Three decades ago Salojärvi et al. (1985) claimed that the sea-spawning form dominates in the GoF EW catches. Currently, we have demonstrated that hatchery-origin anadromous EW dominate in the Estonian coast of the GoF. This conclusion is strongly supported by long-term gill raker counting data obtained from various locations in the Estonian coastal sea (A. Verliin, unpublished data). Therefore, it seems that fingerling stocking that was promoted by Salojärvi et al. (1985) has paid off in that sense. Unfortunately, it seems that in the meantime, the stocks of wild sea-spawning EW have plummeted. Degradation of wild sea-spawning EW stocks has already been documented in the GoB and the Estonian coastal sea (e.g. Verliin et al., 2003; Veneranta et al., 2013).

Otolith Ba:Ca values helped to identify freshwater recruits. As it is known that the larvae and/or fry of most wild anadromous EW stocks soon end up in the estuary or the sea (Leskelä et al., 1991; Lehtonen et al., 1992; Lari Veneranta, personal communication), traces of pure freshwater signature are lost. Instead, a mixture of fresh and brackish water signature is incorporated to the otolith. Usually this is identifiable as a moderate Sr:Ca nadir in the beginning of the core-to-edge transect. However, present study showed that additional confirmation can be gained by quantifying Ba:Ca profiles. This is needed when otolith Sr:Ca profiles are not conclusive as was demonstrated with several fish in the present study (i.e. emigration to the sea occurred within few days). All Pärnu Bay collected individuals, which originated from River Pärnu displayed Ba:Ca peak(s) of > 0.01 mmol·mol^{-1} in the beginning of their profiles. These peaks are most likely derived from ambient Ba maxima located in the mixing zones of estuaries or river mouths, where fresh water meets with brackish water, and as a result Ba is released from riverine clay-borne particulates and becomes dissolved in the water (Coffey et al., 1997; Stecher and Kogut, 1999). On the other hand, the spawning (and hence local) fish collected near Vilsandi Island all displayed Ba:Ca values < 0.01 mmol·mol^{-1}. Therefore, it is argued that these observations indirectly verify the utility of otolith Ba:Ca values as a tool for identifying riverine recruits that are swept to the brackish water soon after hatching or stocking. Only some anadromous EW populations from large undammed rivers are known to display a component of prolonged freshwater residency during early ontogeny (i.e. up to four months; Jokikokko et al., 2012), but those individuals should be identifiable based on the duration of low Sr:Ca phase. Furthermore, the contribution of such individuals to the fishery is likely low.

4.2. Otolith fingerprint variation in freshwater rearing sites

Hatchery-origin fingerlings from geographically separated rearing facilities can be readily distinguished with otolith geochemistry. Ideally, this would allow to determine the rearing facilities which produce the most successful recruits in terms of post-stocking mortality. It would be also possible to investigate dispersal and/or return rates of fingerlings which originate from a certain rearing facility and are stocked to a certain river or area in the sea – information that is often crucial for local stakeholders that finance the stocking. However, to be successful in the aforementioned tasks, all relevant sources of fingerlings should be accounted for. For determining the most successful rearing facilities this means that all sources of hatchery-origin fingerlings that can potentially give recruits to a specific area under study must be accounted for. To investigate dispersal and/or return rates of fingerlings originating from a certain hatchery, it is only important to assure that no other identical fingerprints exist in the study area of interest. Although present study demonstrated strong differences in otolith fingerprints among four distinct rearing facilities, it must be stressed that similar fingerprints can emerge if additional sources of fingerlings are added. This is most plausible in the GoB, where EW fingerlings from numerous sources are stocked to the gulf. However, the results from the present study and also historical geochemical data (Åberg and Wickman, 1987; Löfvendahl et al., 1990) suggest high geochemical variability in the freshwater basins of the Baltic Sea, meaning that there is great potential in characterizing all or at least most sources of hatchery-origin EW fingerlings.

4.3. Provenance of adults stocked as fingerlings

Thirteen distinct otolith elemental fingerprint groups were identified among the adults that were stocked as fingerlings. However, 81% of the stocked adults originated from seven distinct sources. A bit surprisingly, none of the stocked adult possessed a natal fingerprint that completely matched with YOY fingerprints from the sampled rearing facilities. There are several known and hypothetical reasons responsible for such discrepancy. First of all, the aim of the present study was not to include all possible sources (i.e. ponds) of to-be-stocked EW fingerlings, and therefore most of them were not sampled for baseline characterization. Second, fingerlings from Hankasalmi are currently not stocked to the GoF, and fingerlings from Trollböle are all progeny of sea-spawning form which are known to be rather stationary and do not perform long migrations (Lehtonen and Himberg, 1992). Therefore, fingerlings from Kuusamo and Hamina are the only potential sources of stocked fingerlings relevant to the present issue. However, as it turned out later, fish obtained from a rearing pond near Hamina actually form a minor proportion of the EW fingerlings stocked to the GoF by that specific organization. Among the hypothetical reasons, inter-annual variability in natal fingerprint values could complicate the back-assignment, but other studies conducted elsewhere have demonstrated that at least ^{87}Sr:^{86}Sr values generally remain relatively stable across years (e.g. Barnett-Johnson et al., 2008). Still, inter-annual variability in otolith chemical fingerprints of to-be-stocked fingerlings should be definitely investigated in the future. Furthermore, fingerlings that are fed with commercial fish feed demonstrate labile Sr:Ca and ^{87}Sr:^{86}Sr profiles. For example, individual ^{87}Sr:^{86}Sr values from YOY from Trollböle varied between 0.718 and 0.722, which can be considered a large variation for one site. So, a rearing facility that is maintained on commercial feed can produce a number of different otolith fingerprint groups. However, rearing to-be-stocked EW fingerlings on commercial feed is rare around the Baltic Sea, and Trollböle is the only known exception.

Among the adults that were stocked as fingerlings several groups encompassing similar individual fingerprints, but different sampling locations, were identified. This implies that EW originating from a certain rearing facility can disperse to various locations. Lehtonen (1981) also suggested that after spawning in rivers EW will descend to the sea and disperse. Feeding and spawning migrations of anadromous EW can be relatively extensive, reaching up to 700 km in EW originating from rivers running to Bothnian Bay and feeding in the Archipelago Sea (Lehtonen and Himberg, 1992). However, in the Bothnian Sea and the GoF these migrations are generally <80–100 km with maximum reported distances of ≈ 340 km (Lindroth, 1957; Ikonen, 1982). However, the stocked fish ($N = 8$) from Vilsandi must have travelled at least ≈ 200 km to reach their sampling site. Feeding migrations of such distance inevitably raise a question whether such individuals return to spawn in the river they were originally stocked. Most wild anadromous EW drift to the sea soon after hatching (Lehtonen et al., 1992; Jokikokko et al., 2012), and imprinting to the natal river must occur during early ontogeny. To-be-stocked fingerlings, however, rear in freshwater ponds/tanks over summer and are stocked to rivers, estuaries, and even sea in autumn. Will these fish imprint as successfully as wild conspecifics? While the fish stocked directly to the sea will most likely become "fish with no home to return to", those stocked to the rivers will at least have a chance to imprint if physiologically possible. It remains to be sought out whether stocked fingerlings actually contribute to the spawning stock they were intended to supplement (if this is indeed the agenda), or whether they just become vagrants that ultimately contribute to the "illusion of healthy whitefish fishery" (sensu Veneranta et al., 2013) in the Baltic Sea. Judging from the decreasing catches that occur in the GoB despite tremendous stocking efforts (HELCOM, 2013), it can be suggested that the current stocking policy lacks the desired effect on wild populations, and the success of different stocking practices should be evaluated for better comprehension on developing the one stocking practice that has the greatest positive effect on wild populations.

4.4. New approaches and future prospects

In addition to the proposed assignment framework and new biological data, present study provided evidence that the spatio-temporal resolution in otolith analyses is paramount in determining the general ecological form and/or provenance of EW. Previous studies have utilized solution-based analyses (Himberg et al., 2015; Hägerstrand et al., in press) or spot-analyses with poor spatio-temporal resolution (Lill et al., 2015), which most likely fail to identify individuals with limited natal freshwater residency (e.g. wild anadromous or stocked fry) and can therefore misclassify them as sea-spawning. So, continuous line-scans or dense array of high resolution 3–5 μm spots coupled with Sr:Ca and Ba:Ca quantification are required to confidently determine the ecological form and provenance of EW inhabiting the Baltic Sea. Quantification of natal $^{87}Sr:^{86}Sr$ ratio in adults who were stocked as fingerlings or in wild anadromous adults with prolonged freshwater residency, will serve as an additional powerful marker for pin-pointing provenance to a specific rearing pond or a river. As Baltic Sea EW display a complex and dynamic stock structure, the provided assignment framework should be considered as a first attempt to organize this variability for enhanced stock management. Future case studies with larger sample sizes are needed to further elucidate the catch composition and population structure of EW inhabiting the Baltic Sea. Additional studies are required to find methods for discriminating between wild anadromous individuals and fry stocked to the rivers. This is especially relevant for the GoB where all possible stocking practices are utilized. Unravelling the detailed population structure of EW inhabiting the GoB may require artificial marking of stocked individuals coupled with coordinated and well instructed stocking programmes. Although artificial chemical marking of EW has been successfully applied in some studies in the GoB (Lehtonen et al., 1992; Leskelä et al., 2004), innovative and more cost-effective methods such as transgenerational and immersion batch marking should be put to in use (Munro et al., 2009; Woodcock et al., 2011; Warren-Myers et al., 2015).

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