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Temporally stable, weak genetic structuring in brackish water northern pike (*Esox lucius*) in the Baltic Sea indicates a contrasting divergence pattern relative to freshwater populations

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1 **Temporally stable, weak genetic structuring in brackish water northern**
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3 **pattern relative to freshwater populations**

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20 Right running head: Weak genetic structure of Baltic pike

21 Left running head: Wennerström et al.

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Abstract

Understanding spatio-temporal population genetic patterns is important for conservation management of ecologically and socio-economically important species. This is particularly so in species poor environments such as the brackish Baltic Sea. We examined over 600 northern pike (*Esox lucius*), a coastal predator and treasured sport fish, collected over major parts of the Baltic Sea coastline. We found low genetic divergence among populations indicating a contrasting genetic structure of brackish water coastal spawners compared to previous reports on anadromous Baltic pike migrating up freshwater streams for spawning. A pattern of genetic isolation by distance either over shortest waterway, or primarily along the mainland coast with islands as stepping stones, suggested that gene flow is primarily taking place among neighboring populations, possibly with some migration over open water. Temporal data showed a stable genetic structure over a decade. Within a single sampling year, however, spatial divergence was larger during spawning than feeding season, indicating increased mixing of populations during the feeding season. Management should assure connectivity among brackish spawning grounds and large population sizes at identified core areas.

41

Key words

Conservation genetics, Fisheries management, Genetic monitoring, Microsatellites,
Population structure

45 **Introduction**

46 Understanding patterns of spatial and temporal population genetic structure is important in
47 evolutionary and conservation biology (Allendorf et al. 2008). Research over the last decade
48 has shown that genetic diversity can affect species productivity, viability, resilience, and
49 adaptive potential (Frankham 2005; Reusch et al. 2005; McGinnity et al. 2009; Hellmair and
50 Kinziger 2014). Insights from these and earlier studies are reflected in international
51 conservation policies such as the Convention on Biological Diversity (CBD), which
52 specifically calls for conservation and sustainable management of genetic diversity, within
53 and between populations (www.cbd.int). The particular importance of maintaining genetic
54 diversity of species of socio-economic value is also highlighted in the Aichi Target 13 of the
55 CBD Strategic Plan for 2011-2020 (UNEP/CBD/COP/DEC/X/2; www.cbd.int/sp/targets).

56
57 In species poor environments, such as the brackish Baltic Sea, genetic diversity has been
58 suggested to be of particular importance (Laikre et al. 2008; Johannesson et al. 2011) as it can
59 have similar effects on ecosystem functioning as species diversity (Hughes et al. 2008;
60 Schindler et al. 2010; Cook-Patton et al. 2011; Yang et al. 2015). Moreover, species-specific
61 patterns of genetic variation and divergence within the Baltic Sea apparently reflect a variety
62 of evolutionary histories and patterns of genetic drift and gene flow among species
63 (Wennerström et al. 2013).

64
65 The northern pike (hereafter pike) is a Baltic Sea species to which the CBD Aichi Target 13
66 typically applies, and for which assessment of genetic diversity is particularly warranted
67 (Ljunggren et al. 2010; Karlsson et al. 2014). It is one of few predatory fish species in Baltic
68 Sea coastal areas and is hence ecologically important. Declines of pike populations have been
69 related to trophic cascades yielding mesopredator fish release and eutrophication symptoms

70 such as blooms of ephemeral filamentous algae (Eriksson et al. 2009; 2011). In addition, pike
71 is a much appreciated target for primarily recreational but also small-scaled coastal
72 commercial fisheries, and thus of obvious socio-economical value (Karlsson et al. 2014).
73 Severe local declines of Baltic pike have been reported (Andersson et al. 2000; Westin and
74 Limburg 2002; Lehtonen et al. 2009; Rohtla et al. 2012), and although pike is not regarded as
75 threatened in the Baltic Sea there is still reason for conservation concern (Ljunggren et al.
76 2010).

77

78 Several recent studies focusing on anadromous Baltic Sea pike populations (pike feeding in
79 the Baltic Sea but migrating up freshwater streams and tributaries connected to the Baltic Sea
80 for spawning) have found fine-scaled genetic sub-structuring, small effective population sizes,
81 and local adaptations among spawning populations (Bekkevold et al. 2015; Larsson et al.
82 2015; Tibblin et al. 2015). A proportion of the feeding population of pike in the Baltic Sea
83 spawn in brackish coastal areas (Müller and Berg 1982; Westin and Limburg 2002; Engstedt
84 et al. 2010) where fewer barriers to dispersal and gene flow appear to occur. Earlier tagging
85 studies indicate a high degree of philopatry in pike both in freshwater habitats (Miller et al.
86 2001) and in the Baltic Sea (Karås and Lehtonen 1993). Results from the first population
87 genetic screening of Baltic pike, focusing on samples from the Swedish coastline, were in line
88 with these observations indicating gene flow along the coast that resulted in an isolation by
89 distance-pattern of divergence (Laikre et al. 2005). Similarly, a later screening over a larger
90 part of the Baltic coastline showed weak genetic structuring and isolation by distance
91 (Wennerström et al. 2013). In both these studies samples were, however, largely collected
92 outside spawning time, potentially leading to biased low estimates of genetic differentiation
93 due to mixing of different spawning populations during feeding season (Laikre et al. 2005;
94 Bekkevold et al. 2015). In addition, no information is available on the stability of the genetic

differentiation is over time. Temporally separated samples can add important information on the stability of populations over generations, as well as over different parts of the life cycle (Laikre et al. 2005). To best conserve genetic resources information on spatial and temporal population genetic patterns should form the basis for management and conservation (Dannewitz et al. 2005; Schwartz et al. 2007; Palm et al. 2009).

In the present study we use the currently largest sample of pike from the brackish Baltic Sea of 662 specimens collected both during and after spawning time over large parts of the entire Baltic coastline in order to further explore if brackish spawning Baltic pike show contrasting patterns of genetic structure as compared to freshwater spawners. We also include samples separated in time (ten years vs. within a single year) to address the degree of temporal stability of divergence patterns of brackish Baltic pike.

Material and Methods

We sampled and analyzed a total of 662 georeferenced pike individuals from 16 areas (3 - 147 per area) covering major parts of the Baltic Sea (Table 1; Fig. 1, Supplemental Table S1). One sampling area (no 3, Kokkola) only included three individuals and was excluded from all analyses on the area level thus resulting in 15 areas with 10-147 individuals per area for those analyses (Table 1). The three individuals from Kokkola were, however, included in individual-based analyses. The main part of the sampling was performed during spawning season (April-June) in 2010 (Supplemental Table S1). For long term temporal comparisons genotypic information for an additional 129 pikes collected in 2001 (Laikre et al. 2005) were included (see below; Supplemental Table S2)

Genotyping

Fin clips were stored in 95% ethanol. DNA was extracted using a silica-based purification method modified from Elphinstone et al. (2003). All samples were genotyped for 11 microsatellite markers, 10 developed for northern pike and one for the closely related muskellunge (*Esox masquinongy*): *Elu 87*, *Elu 51*, *Elu 76*, *Elu 19*, *Elu 276*, *Elu 64* (Miller and Kapuscinski 1996), *EluB e INRA*, *EluB 38 INRA* (Launey et al. 2003), *Elu 10*, *Elu 12* (Hansen et al. 1999), and *EMA D4* (Sloss et al. 2008). Standard protocols and annealing temperatures between 58 and 60 °C were used (exact protocols are available on request). The genotypes were scored using *Genemarker 1.96* (SoftGenetics) and following visual inspection, exported to a spreadsheet program for downstream analyses. Genotyping errors were assessed by re-extracting DNA of 10 randomly picked individuals, and analyzing them blindly in comparisons to original genotypes. Using this procedure no differences were detected. All laboratory work was carried out at the Center of Evolutionary Applications, University of Turku, Finland.

Genetic variation, *F*-statistics

We used traditional population genetic approaches with a priori grouping of the material into the 15 sampling areas (excluding the sample from site 3 Kokkola with only three individuals, cf. Fig. 1). *F*-statistics were applied to quantify spatial heterogeneity and deviations from Hardy-Weinberg (HW) assumptions, including significance testing, using 10,000 MCMC repeats in *Genepop 4.2* (Raymond and Rousset 1995). Significance testing of F_{ST} -values were performed with χ^2 – tests in the software *Chifish* (Ryman 2006). Genetic variation in terms of observed and expected heterozygosity and mean allelic richness, rarefied to the smallest sample size with complete genotypes for all 11 loci, $n = 5$, were assessed in *Fstat 2.9.3*

(Goudet 1995). Potential correlations between genetic variation and latitude and longitude were assessed using *R* 3.2.1 (R Core Team 2014).

Genetic clustering

Genetic clustering and the most likely number of genetic clusters (K) were estimated for the total material ($n = 662$) using the Bayesian approach implemented in *Structure* 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). The posterior probability of clustering of data $\ln P(K)$ was estimated for different number of potential genetic clusters ($K = 1-20$) using an admixture model with correlated allele frequencies with 500,000 Markov Chain Monte Carlo (MCMC) iterations following a burn-in of 200,000 iterations, and results were averaged over 10 runs with the software *Clumpp* (Jakobsson and Rosenberg 2007). Two separate runs were performed, one including no prior information on sampling location and one where sample location was given as an additional prior. The most likely number of clusters was assessed using the second order rate of change of log probabilities (ΔK) following Evanno et al. (2005), and by inspection of $\ln P(K)$ values. An additional structure analysis using only spring samples (April-June) in order to reflect sampling close to spawning time, was performed with the same settings as above and with sampling location as a prior.

Isolation by distance

The relationship between genetic distance ($F_{ST}/(1-F_{ST})$) and geographic distance was tested with Mantel tests with 9,999 permutations in the R-package *Ade4* 1.6-2 (Dray and Dufour 2007). Geographic distance was measured in three ways: *i*) shortest waterway distance among sampling areas was used to reflect potential gene flow in any direction, and *ii*) shortest coastal distance was used to reflect gene flow only along the mainland coastline (island samples Gotland, Åland, and Estonia from which majority samples were collected on the islands

Saaremaa and Hiiumaa in western Estonia were excluded here) *iii*) shortest coastal distance with the Island of Åland as a stepping stone, while allowing for migration between Gotland and Estonia and their nearest mainland sample. Absolute geographic distances were used for all analyses of isolation by distance. Additional Mantel tests on log transformed distances were performed for the analyses over shortest waterway distance, because gene flow in that model is expected to occur in two dimensions (Rousset 1997). To find out if the correlation between geographic and genetic distance differs among the three models confidence intervals of correlation coefficients (r) were estimated after using Fisher's r to z' transformation on these coefficients (because correlation coefficients are typically not normally distributed). Also for each pair of the migration models pairwise significance tests were performed by calculating the normal deviate (z) between the z' transformed correlation coefficients (Cohen and Cohen 1983; equation 2.8.5).

Temporal variation

We studied the long- and short-term temporal stability of genetic patterns of divergence among sampling localities in three areas for which temporal samples were available: Småland-Östergötland, Stockholm Archipelago, and Åland (Fig. 1). Long-term temporal variation was studied using three points in time; 2001, 2005, and 2010 (total $n = 454$; Supplemental Table S2). Short-term temporal patterns were studied between spawning and feeding seasons within one year in the same three localities (2010; total $n = 270$; Supplemental Table S1). Changes in F_{ST} between seasons were tested statistically with paired t-tests.

For long-term temporal comparisons 140 individuals sampled in 2001 and previously presented in Laikre et al. (2005) were added to the samples collected for this study. These

individuals from 2001 were genotyped at six of the eleven microsatellite markers used in the current study (*Elu87*, *Elu51*, *Elu76*, *Elu19*, *Elu12*, *Elu276*). Allele sizes for the added samples were calibrated for consistency with newly scored individuals by regenotyping a subset of individuals sampled in 2001. The regenotyped individuals were chosen to represent all alleles reported in Laikre et al. (2005). All previous alleles were identified and could be calibrated to the 2010 sample. Thus, long-term comparisons (2001-2010) were based on six loci whereas temporal changes within one single sampling year (2010) used 11 loci.

Poptree2 (Takezaki et al. 2010) was used to construct neighbor joining trees based on Nei's standard genetic distance (D_{ST} ; Nei 1972) to visualize long-term changes within the three areas Småland-Östergötland, Stockholm Archipelago, and Åland where long-term data were available (2001-2010; six loci). Genetic differentiation (F_{ST}) was estimated among sampling years, within localities. Short-term temporal variation was measured among the 2010 samples from Småland-Östergötland, Stockholm Archipelago, and Åland (11 loci) by comparing divergence (F_{ST}) during spawning season (April-June) vs. feeding periods (July-November).

Results

Genotyping success was high; 98% of the individuals were successfully genotyped for at least 8 loci and 91% of the individuals were genotyped for 10 or 11 loci. A total of 127 alleles were observed across the 11 loci with an average of 11.6 alleles per locus. Most alleles were shared among areas, but 26 alleles (20%) were private, i.e., only found in a single sample. The majority of private alleles were found in the Stockholm Archipelago sample, which was also the largest one (Table 1).

Allelic richness (AR) and heterozygosity (H_e) among samples were correlated ($P < 0.001$, $F_{1,14} = 26.33$, $r = 0.82$), and subsequent tests were performed using only AR . AR increased both eastwards and northwards, being highest in the samples from the Bothnian Bay and the Inner Gulf of Finland (multiple regression $P = 0.010$; $F_{2,13} = 6.52$, $r = 0.73$) when the outlier population Swedish Quark, which had much lower levels of genetic variation than any other sample, was excluded. When included, the correlation between AR and latitude was non-significant.

Deviations from HW-proportions, manifested as statistically significant positive F_{IS} -values were detected in two samples (Finnish Quark and Estonia; Table 1). The F_{IS} -values in Estonia remained significant when the sample was divided into two smaller samples according to geographic proximity (data not shown).

The overall F_{ST} was 0.033 and highly significant ($P < 0.001$; Table 1), and pairwise F_{ST} -values among all pairs of areas ranged in the interval 0 – 0.14 (Supplemental Table 3). The majority of these pairwise values were significant, except between Gävle and the samples from Småland-Östergötland and Åland, and between Uppland and the samples from Åland, Gävle, Gotland, Stockholm Archipelago, and Archipelago Sea (Supplemental Table S3). The sample from the Swedish Quark was highly divergent from all other samples (Table 1).

Genetic clustering

Results of genetic clustering were very similar between *Structure* runs using only spring samples (i.e. sampling around the spawning season) and runs using the total material of 662 individuals. Runs with and without location as a prior information gave similar results, but runs without such information gave a less clear spatial separation of clusters, and lower

assignment probabilities (data not shown). Below we report results from runs using sampling location as a prior and the total material ($n = 662$).

The largest second order rate of change of log probabilities for different K (ΔK) occurred at $K = 2$. However, the division of the total material into these two suggested main clusters made little biological sense (Fig. 2). The clusters did not group geographically, and individual assignment probabilities (Q) to either of the clusters were to a large extent intermediate.

The second highest value of ΔK occurred at $K = 6$ (Supplemental Table S4) and this number of clusters made more biological sense than $K = 2$ with respect to the geographic distribution of clusters. Assignment probabilities of individual pike were typically low to all clusters for $K = 6$, but the clusters were reasonably geographically separated (Fig. 2). Two of these six *Structure* clusters corresponded completely to samples from each of two separate sampling areas, i.e. Swedish Quark (number 5, yellow color in Fig. 2) and Estonia (16, red) formed clearly distinct groups. A third cluster (orange) included pike predominantly from Stockholm Archipelago (12) and Småland-Östergötland (13). A fourth and northern cluster (purple) dominated the samples from Swedish and Finnish Bothnian Bay (1 and 2, respectively) and Finnish Quark (4). The three individuals from Kokkola (3) were assigned to a fifth cluster (green) along with pike distributed irregularly over most of the sampled area. Finally, a sixth cluster (blue) was geographically widespread and included individuals from Blekinge (15), Gotland (14), and Inner Gulf of Finland (10). Fish from the central part for the Baltic Sea (i.e. Gävle (6), Uppland (7), Åland (11), Archipelago Sea (8), and Outer Gulf of Finland (9)) exhibited highly admixed genomes suggesting considerable contribution from several clusters, or intermediate genotypes in an isolation by distance scenario.

When only considering individuals with high assignment probabilities ($Q > 0.8$), only 166 pike were assigned to a cluster, and the "widespread" Blekinge/Gotland/Inner Gulf of Finland (blue) cluster came out empty because no pike had a $Q > 0.8$ to this cluster. Similarly, only a single fish was assigned to the green cluster characterized by a poorly defined geographic distribution.

Isolation by distance

There was a clear pattern of isolation by distance when geographic distance was measured as shortest waterway distance ($P = 0.020$, $r = 0.409$; Fig. 3) as well as when measured as coastal distance, including Åland as a stepping stone and allowing for migration between Gotland and Estonia and their nearest mainland sample ($P = 0.004$, $r = 0.467$). There was no significant isolation by distance, however, when geographic distance was measured along the mainland coast only and excluding all three island samples (i.e. excluding Åland, Gotland, Estonia; $P = 0.790$, $r = 0.129$). Additional tests on log transformed geographic distances over shortest waterway distance resulted in minor differences in correlation coefficients, and no difference in statistical significance (data not shown).

The correlation coefficients were significantly higher when the Swedish Quark sample was excluded from the isolation by distance analyses both over shortest waterway distance (Table 2; Supplemental Table S5; migration model 1 vs. 2; $z = 2.33$, $P = 0.020$) and stepping stone coastal distance (migration model 7 vs. 8; $z = 2.27$, $P = 0.023$). Excluding also island samples (Gotland, Åland, and Estonia) did not significantly change the correlation coefficients.

The correlation coefficients were consistently higher when geographic distance was measured along the coast and including Åland as a stepping stone, than when geographic distance was

measured as shortest waterway distance, but these differences were not significant as seen by the highly overlapping confidence intervals (Table 2) and statistical tests for differences in correlation coefficients (Supplemental Table S5). Thus, it was not possible to differentiate between migration models measuring geographic distance as shortest waterway distance and migration models measuring geographic distance along the mainland coast, including Åland as a stepping stone and allowing migration from Gotland and Estonia to nearest mainland.

Temporal patterns

There was no indication of genetic heterogeneity between the 2001 and 2010 samples from Stockholm Archipelago and Åland; F_{ST} -values were small and non-significant. In Småland-Östergötland there was a significant difference among sampling years, due to the sample from 2005 being significantly different from the others, which could be due to the 2005 sample being from a very restricted location. The samples from 2001 and 2010 did not differ significantly from each other (Table 3; Supplemental Table S6). Similarly, a dendrogram separated samples according to location rather than time of collection (Fig. 4). Levels of genetic variation were similar in temporally separated samples from the same sampling areas (Supplemental Table S2).

With respect to short-term temporal variation (within 2010), the degree of genetic differentiation among sampling areas changed somewhat over the year. Pairwise F_{ST} -values between sampling areas were highest in the spring around spawning time (Apr-Jun); they decreased and approached zero later in the year suggesting increased mixing during feeding seasons (Fig. 4). The reduction in F_{ST} from spawning to feeding season was significant in the Åland and Stockholm Archipelago comparison (paired t -test, $t_{10} = 2.46$, $P = 0.033$). In the Småland-Östergötland and Stockholm Archipelago comparison the reduction in F_{ST} was close

to statistically significant (paired t -test, $t_{10} = 2.04$, $P = 0.068$), while in the Småland-Östergötland and Åland comparison the reduction was non-significant (paired t -test, $t_{10} = 0.66$, $P = 0.523$). All of the pairwise F_{ST} -values were significant between sampling areas, regardless of season (Supplemental Table S7). Within sampling areas two out of three of the F_{ST} -values were non-significant among sampling seasons in Åland and Stockholm Archipelago, but highly significant in Småland-Östergötland (Supplemental Table S7), indicating some changes in the genetic composition between spawning and feeding seasons.

Discussion

The present study of brackish water pike in the Baltic Sea showed relatively weak but temporally stable population genetic structure. The system is characterized by genetic isolation by distance, following either a migration model over shortest waterway distance, or along the coast, including the islands of Åland as a stepping stone and allowing for migration between the island samples Gotland and Estonia and their nearest mainland sample. We also found indications of a more pronounced genetic structure during spawning time than feeding season. However, the overall degree of spatial differentiation over the Baltic Sea is very much in line with previous and less extensive studies that did not focus on pike collected during spawning season (Laikre et al. 2005; Wennerström et al. 2013). Our data indicate that brackish water spawning Baltic pike display much weaker spatial subdivision than that observed for Baltic freshwater spawning pike (Larsson et al. 2015; Tibblin et al. 2015). Overall F_{ST} for Baltic brackish water spawners was 0.033 (Table 1) whereas a similar estimate for Baltic freshwater spawners separated by short distances (< 100 km) has been reported to be 0.069 (Tibblin et al. 2015).

An important contribution of the present study compared to earlier ones is that the current samples mainly were collected close to spawning time. Previously reported levels of genetic divergence in brackish water Baltic pike have been regarded as surprisingly low considering the philopatry indicated from tagging studies (Saulamo and Neuman 2002). Mixing of spawning populations has been proposed as a possible reason when sampling has been performed outside spawning time (Laikre et al. 2005a; Bekkevold et al. 2015). Our results suggest that such mixing occurs, but it appears not to have a major impact on the overall pattern of genetic structure displayed in brackish water spawning pike.

The low levels of divergence (average $F_{ST} = 0.03$) observed in our study are in stark contrast to what has been observed for anadromous populations of Baltic pike that migrate to freshwater for spawning (Bekkevold et al. 2015; Tibblin et al. 2015). Those studies and our used microsatellite markers, many of them overlapping between studies and with similar levels of variation. Thus, marker choice is probably not the reason for the different levels of divergence across studies. Rather, the differences likely reflect true dissimilarities in gene flow between the two types of pike. Anadromous populations of Baltic pike have been demonstrated to exhibit homing behavior to a limited number of creeks and outlets for spawning, creating isolated populations similar to those of salmonids and coregonids (Engstedt et al. 2010; Dierking et al. 2014). As a contrast, gene flow in brackish spawning pike might be higher due to a more continuous dispersal of suitable spawning grounds in coastal waters without obvious geographic barriers. This, in turn, might facilitate some degree of migration between spawning grounds. Still, the levels of divergence in brackish water Baltic pike are much higher than what is typically seen in marine species in the Baltic Sea, e.g. herring (e.g. Larsson et al. 2010; Barrio et al. 2016). In contrast to many truly marine

species, pike eggs are attached to a substrate and larvae and juveniles are stationary (Craig 1996). Thus, gene flow is probably maintained mainly through active adult dispersal.

A recent study of pike in another large open water basin of similar size, Lake Ontario, has reported differentiation among spawning populations at low levels similar to those of our study (Ouellet-Cauchon et al. 2014). In large water systems without barriers to migration the potential for gene flow among multiple populations is higher than in small and restricted freshwater systems. Large water bodies can also sustain larger population sizes, and thus the rate of genetic drift is expected to be smaller. These features could be an explanation for the relatively low levels of population genetic divergence found in both the Baltic Sea and Lake Ontario. A major difference between these two systems however, is that Lake Ontario is a freshwater lake, but the Baltic Sea harbors both freshwater and brackish water spawning populations of pike. Further studies in the Baltic should focus on the detailed genetic relationship between fresh- and brackish water pike, including potential adaptations to the different habitats and the extent of gene flow between the two types of pike.

We found a spatial pattern with weak indications of northern, central, and south-eastern groupings, along with isolation by distance over shortest waterway, or along the coast, with the islands of Åland as a stepping stone. The weak genetic clusters could to some degree be an artifact of an overall isolation by distance pattern, and should thus be interpreted with some caution. There is a lack of isolation by distance when geographic distance is measured solely along the mainland coast, which in part is due to genetic similarities between populations in southern Sweden (Blekinge, Småland-Östergötland, Gotland) and in the Gulf of Finland and the Archipelago Sea (cf. Figs 2 and 3). This suggests that gene flow is, or has been, occurring across the Baltic Sea, e.g. between Sweden and Finland. Correlation coefficients were

consistently higher for the coastal stepping stone models, than for the shortest waterway models, hence suggesting that gene flow may primarily take place along the coast. However, these differences were not significant and thus we cannot differentiate between a migration model including gene flow over open water and a migration model including gene flow primarily along the coast, but with possible gene flow over the islands of Åland. The latter model is biologically likely, because pike is mostly restricted to coastal areas and tagging studies indicate short dispersal distances along the coast (Saulamo and Neuman 2002). There is only about 40 km of open water between the Swedish coast and Åland, which is within recorded migration distances of individual pike (Karås and Lehtonen 1993). Between Åland and the Finnish coast there is an almost unbroken archipelago. An interesting parallel has been observed in terrestrial systems, where the relationship between geographic and genetic distance is stronger when geographic distances are measured along likely dispersal routes than as simple Euclidian distances (Coulon et al. 2004; Broquet et al. 2006).

The suggested genetic relationship across the Baltic may hence be explained by *i*) natural gene flow occurring to some degree over open waters, *ii*) man-mediated gene flow as a result of stocking of fish transferred across the Baltic, or *iii*) post-glacial colonization patterns that still remain. Although not documented in the scientific literature, there is anecdotal evidence for large sized pike following and feeding on herring schools in the open waters of the Baltic Sea. The concept of these pelagic so called “herring pikes” is well known among sport fishermen (Engström 1972), potentially suggesting the existence of dispersal and resulting gene flow across open waters.

Stocking of fish might have large effects on population genetic patterns and stocking of non-native populations might disrupt local adaptations (Allendorf et al. 2008; Dierking et al.

2014). Human translocation of populations might lead to homogenization of populations as has been documented e.g. for pike in France (Launey et al. 2006). Further, release of non-native stocks and secondary dispersal, so called “stepping stone and diffusion” might have genetic effects over large geographic areas (Gozlan et al. 2010). Stocking of pike has occurred in both Sweden and Finland, including the islands of Åland (Lars Lundahl and Antti Lappalainen, personal communication), but documentation of the origin of released fish is generally poor (Laikre et al. 2006). Thus, some of the genetic patterns we observe in this study might have been affected by stocking. For example, the apparent high levels of admixture in the *Structure* analysis in the sample from e.g. the Archipelago Sea (8) might indicate homogenization of populations due to stocking. The genetic similarity of pike from the southern Swedish coast and Gulf of Finland could also be explained by human translocation of pike between the Swedish and Finnish coast. However, these patterns could also be explained by natural gene flow mediated over the islands of Åland.

Little is known with respect to post-glacial colonization of Baltic pike. Northern pike probably colonized Scandinavia from the south-west after the last glaciation (Skog et al. 2014). Contrary to what would be expected if this colonization was the major force shaping current genetic patterns (Hewitt 1996; Widmer and Lexer 2001), we observe statistically significant increased levels of genetic variation towards the north and the east, further away from a possible core area or a glacial refugium. Thus, post-glacial colonization does not appear to have a major effect on current patterns in levels of genetic variation of brackish water pike in the Baltic Sea.

Although we cannot rule out the effects of stocking in creating the genetic patterns observed, we find evidence for gene-flow to some degree occurring across open waters. This hypothesis

is further supported by the results from *Structure* in suggesting a genetic connectivity between Sweden and Finland via the Island of Åland.

Conservation and management implications

The spatial genetic pattern of the coastal spawning Baltic pike indicates a high degree of genetic connectivity compared to freshwater spawning pike. Thus, a combination of metapopulation and local management appears warranted for this species in the Baltic. For freshwater spawning pike local management is needed, whereas a metapopulation approach assuring connectivity of coastal spawning grounds over large areas seems important from a conservation perspective for the brackish spawning pike. The high degree of genetic connectivity among brackish spawning populations suggests that actions in one area can affect pike in other areas of the Baltic also. This implies that e.g., population reductions in one area can lead to increased isolation and genetic drift not only in that area, but also in those previously connected to the site of population reduction. Similarly, release of pike of non-native origin may have genetic effects not only locally in the area of release, but may result in the spread of non-native genes over large areas (Gozlan et al. 2010). Thus, we recommend that stocking should generally be avoided (cf. Laikre et al. 2010), and if releases are carried out for conservation purposes to re-establish or support of weak populations, closely located Baltic coastal spawners should be used. Geographic areas where we find less genetically admixed individuals include the Swedish Quark, Estonia (western archipelago), and the Stockholm Archipelago with Småland-Östergötland (Fig. 2), potentially indicating core areas where it might be particularly important to ensure maintenance of pike. However, caution is needed here, because the *Structure* analysis can be affected by an overall isolation by distance, which makes identifications of separate genetic clusters less certain. Highest levels of genetic variation are found in the Bothnian Bay and in the Inner Gulf of Finland.

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469 The ten-year monitoring data show a relatively stable genetic structure with no indications of
470 reduced genetic variation at our presumably neutral microsatellite loci over this time period.
471 This suggests that there is no immediate risk for changes in the genetic composition in this
472 species, and that genetic monitoring more frequently than over ten year periods might not be
473 necessary for securing gene level biodiversity. The samples used in this study and the
474 previous ones of brackish water Baltic pike (Laikre et al. 2005, Wennerström et al. 2013), are
475 stored in a frozen tissue bank at Stockholm University allowing future genetic monitoring
476 options. In addition, the short-term temporal patterns showed more pronounced genetic
477 structuring with higher pairwise F_{ST} values around spawning time than during feeding time.
478 This in turn indicates increased mixing outside spawning time, and that fishing of pike outside
479 the spawning time to some degree might target mixtures of fish from multiple spawning
480 populations with inherent risks to stock components with lower productivity (Hilborn 1985).

481

482

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Draft

747 **Table 1.** Mean allelic richness per locus (AR ; rarefied to sample size of $n = 5$, the smallest
748 sample with complete genotypes at all loci), expected and observed heterozygosities (H_e and
749 H_o), and summary F-statistics for 15 areas around the Baltic Sea ($n = 659$; excluding sampling
750 location Kokkola with $n = 3$; cf. Fig. 1) based on genotyping of 11 microsatellite loci.
751 Numbers in parentheses refer to area numbers as in Fig. 1. Asterisks denote significant
752 deviations from HW-proportions (significant F_{IS} values, * $P < 0.05$, *** $P < 0.001$). F_{ST} is
753 calculated as the average of all the pairwise F_{ST} -values between each particular area and all
754 other areas. For separate pairwise F_{ST} -values and significance testing see text and
755 Supplemental Table S3.
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Area	<i>n</i>	<i>AR</i>	Private alleles	<i>H</i> _o	<i>H</i> _e	<i>F</i> _{IS}	<i>F</i> _{ST}
Swedish Bothnian Bay (1)	12	3.52	1	0.602	0.628	0.054	0.065
Finnish Bothnian Bay (2)	15	3.77	1	0.592	0.661	0.113	0.073
Finnish Quark (4)	57	3.44	1	0.583	0.601	0.031*	0.035
Swedish Quark (5)	11	3.08	0	0.492	0.577	0.153	0.112
Gävle (6)	10	3.20	0	0.581	0.571	-0.023	0.043
Uppland (7)	14	3.37	1	0.610	0.607	-0.001	0.021
Archipelago Sea (8)	39	3.52	0	0.592	0.596	0.009	0.028
Outer Gulf of Finland (9)	18	3.31	0	0.523	0.546	0.044	0.042
Inner Gulf of Finland (10)	49	3.52	3	0.622	0.618	-0.006	0.031
Åland (11)	58	3.55	1	0.619	0.611	-0.013	0.030
Stockholm Archipelago (12)	147	3.38	8	0.573	0.594	0.037	0.041
Småland-Östergötland (13)	120	3.36	2	0.573	0.595	0.038	0.065
Gotland (14)	20	3.39	2	0.570	0.600	0.050	0.048
Blekinge (15)	20	3.22	3	0.547	0.551	0.005	0.048
Estonia (16)	69	3.37	3	0.526	0.566	0.071***	0.054
Average/Total	659	3.57		0.574	0.595	0.033***	0.033

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Table 2. Models for testing isolation by distance in pike in the Baltic Sea. Geographic distances were measured as shortest waterway distance (Model 1-4), coastal distance along the mainland coast (Model 5-6), and shortest coastal distance, including the Island of Åland as a stepping stone (Model 7-9; Fig. 1) and allowing migration from island samples Gotland and Estonia to location of nearest mainland sample (Models 7-8). r is the correlation coefficient with confidence intervals in parenthesis. The exclusion of the outlier population Swedish Quark (5 in Fig. 1) significantly improved the models over shortest waterway distance and coastal stepping stone compared to models including this population.

Distance measured	Migration model	Populations excluded	r	P -value
Shortest waterway	1	-	0.409 (0.235-0.557)	0.020
	2	Swe Qvark	0.649 (0.551-0.754)	<0.001
	3	Swe Qvark, Got, Est	0.686 (0.558-0.740)	<0.001
	4	Swe Qvark, Got, Est, Åland	0.649 (0.525-0.810)	<0.001
Coastal distance	5	Got, Est, Åland	-0.129 (-0.117-0.360)	0.790
	6	Got, Est, Åland, Swe Quark	0.013 (-0.253-0.277)	0.495
Coastal distance, Åland as stepping stone	7	-	0.467 (0.302-0.604)	0.004
	8	Swe Qvark	0.684 (0.556-0.780)	<0.001
	9	Swe Quark, Got, Est	0.723 (0.582-0.821)	<0.001

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Table 3. Results from tests of genetic differentiation among regions within years, and among years within regions. Numbers in parentheses refer to area numbers as in Fig. 1.

Sample		<i>n</i> (tot)	<i>F</i> _{ST}	<i>P</i> -value
2001	Småland-Östergötland (13), Stockholm Archipelago (12), Åland (11)	140	0.012	<0.001
2010	Småland-Östergötland (13), Stockholm Archipelago (12), Åland (11)	270	0.021	<0.001
Åland (11)	2001, 2010	73	0.002	0.445
Stockholm Archipelago (12)	2001, 2010	205	0.002	0.192
Småland-Östergötland (13)	2001, 2005, 2010	176	0.004	<0.001
	2001, 2010	132	0.004	0.089

Fig. 1. Sampling locations of the 662 northern pike included in this study. Arrows indicate how geographic distance was measured along the coast, and how the Island of Åland was included as a stepping stone while allowing for migration between the islands of Gotland and Estonia and their nearest mainland sample. Only three individuals were sampled in Kokkola, and this location was excluded from all analyses on location level (cf. Table 1).

Fig. 2. Upper: assignment probabilities (Q) from *Structure* results for $K = 2$ and 6 with sampling location as a prior for 662 northern pike individuals. Each bar denotes one individual, and the black lines separate the sampling areas. The sampling areas are in the order: 1. Swedish Bothnian Bay, 2. Finnish Bothnian Bay, 3. Kokkola, 4. Finnish Quark, 5. Swedish Quark, 6. Gävle, 7. Uppland, 8. Archipelago Sea, 9. Gulf of Finland Outer, 10. Gulf of Finland Inner, 11. Åland, 12. Stockholm Archipelago, 13. Småland-Östergötland, 14. Gotland, 15. Blekinge, 16. Estonia. Below: Geographic location of samples for $K = 6$ using the same color coding as in the upper plate. In the lower panes the sampling location for each individual is shown. For visualization, the samples are scattered within 0.25 degrees along longitude and 0.1 degrees along latitude. In the lower left plate each individual is assigned to the cluster to which it has the highest assignment probability. To the lower right only individuals with an assignment probability >0.8 are colored (others are white).

Fig. 3. Isolation by distance over shortest waterway distance (a), along the coast (b), and along the coast with Åland as a stepping stone (c). Each point represents a pairwise comparison between two samples. All circles and dotted lines represent pairwise comparison among all samples, filled circles and full lines represent pairwise comparisons, excluding the samples from the Swedish Quark. There is a significant isolation by distance when geographic distance is measured as shortest waterway distance ($P = 0.020$, $r = 0.409$) and when

geographic distance is measured along the mainland coast and including Åland as a stepping stone ($P = 0.004$, $r = 0.467$), but not when geographic distance is measured only along the mainland coast ($P = 0.790$, $r = -0.129$).

Fig. 4. Long-and short term temporal patterns. a) Long-term (2001-2010): Neighbor joining tree based on Nei's standard genetic distance (D_{ST}). Bootstrap-values based on 10 000 iterations. b) Short-term (within 2010): Pairwise F_{ST} -values between sampling areas in spawning (Apr-Jun) vs. feeding (Jul-Nov) seasons. All pairwise F_{ST} -values between areas are significant (Supplemental Table S7).

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Fig. 2. Upper: assignment probabilities (Q) from Structure results for $K = 2$ and 6 with sampling location as a prior for 662 northern pike individuals. Each bar denotes one individual, and the black lines separate the sampling areas. The sampling areas are in the order: 1. Swedish Bothnian Bay, 2. Finnish Bothnian Bay, 3. Kokkola, 4. Finnish Quark, 5. Swedish Quark, 6. Gävle, 7. Uppland, 8. Archipelago Sea, 9. Gulf of Finland Outer, 10. Gulf of Finland Inner, 11. Åland, 12. Stockholm Archipelago, 13. Småland-Östergötland, 14. Gotland, 15. Blekinge, 16. Estonia. Below: Geographic location of samples for $K = 6$ using the same color coding as in the upper plate. In the lower panes the sampling location for each individual is shown. For visualization, the samples are scattered within 0.25 degrees along longitude and 0.1 degrees along latitude.

In the lower left plate each individual is assigned to the cluster to which it has the highest assignment probability. To the lower right only individuals with an assignment probability >0.8 are colored (others are white).

Fig. 3. Isolation by distance over shortest waterway distance (a), along the coast (b), and along the coast with Åland as a stepping stone (c). Each point represents a pairwise comparison between two samples. All circles and dotted lines represent pairwise comparison among all samples, filled circles and full lines represent pairwise comparisons, excluding the samples from the Swedish Quark. There is a significant isolation by distance when geographic distance is measured as shortest waterway distance ($P = 0.020$, $r = 0.409$) and when geographic distance is measured along the mainland coast and including Åland as a stepping stone ($P = 0.004$, $r = 0.467$), but not when geographic distance is measured only along the mainland coast ($P = 0.790$, $r = -0.129$).

Fig. 4. Long-and short term temporal patterns. a) Long-term (2001-2010): Neighbor joining tree based on Nei's standard genetic distance (DST). Bootstrap-values based on 10 000 iterations. b) Short-term (within 2010): Pairwise F_{ST} -values between sampling areas in spawning (Apr-Jun) vs. feeding (Jul-Nov) seasons. All pairwise F_{ST} -values between areas are significant (Supplemental Table S7).

Supplemental material

Temporally stable, weak genetic structuring in brackish water northern pike (*Esox lucius*) in the Baltic Sea indicates a contrasting divergence pattern relative to freshwater populations

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Draft

Table S1. Sampling area and sampling time for pike genotyped for 11 microsatellite loci.

	April	May	June	July	August	September	October	November	December	Total
2001										
Gävle (6)							3	7		10
Uppland (7)						1	2	1		4
Åland (11)							3		2	5
Stockholm Archipelago (12)							2	1		3
Småland-Östergötland (13)							1	2		3
Gotland (14)							2	1		3
Blekinge (15)							5	15		20
Tot 2001						1	18	27	2	48
2005										
Småland-Östergötland (13)	44									44
Tot 2005	44									44
2010										
Swedish Bothnian Bay (1)		10	2							12
Finnish Bothnian Bay (2)		5	5	3			2			15
Kokkola (3)		3								3
Finnish Quark (4)		57								57
Swedish Quark (5)		11								11
Uppland (7)			6	4						10
Archipelago Sea (8)		3	18	9			9			39
Outer Gulf of Finland (9)		12				6				18
Inner Gulf of Finland (10)			3	17		8	14	7		49
Åland (11)		23	21	5	4					53
Stockholm Archipelago (12)	13	25	77	8	6	13	2			144
Småland-Östergötland (13)	9	16	23	12	8	1	3	1		73
Gotland (14)		13	4							17
Estonia (16)				61	4	2	2			69
Tot 2010	22	178	159	119	22	30	32	8		570
Tot 2001-2010	66	178	159	119	22	31	50	35	2	662

Table S2. Samples used for long term temporal studies and genotyped for six microsatellite loci (*Elu87*, *Elu51*, *Elu76*, *Elu19*, *Elu12*, *Elu276*). Samples from 2001 are from Laikre et al. (2005a). *n* denotes the number of individuals, *Ho* and *He* is observed and expected homozygosity, respectively. *AR* is allelic richness, interpolated from a smallest sample size of 20 individuals.

Sample	<i>n</i>	<i>Ho</i>	<i>He</i>	<i>AR</i>
Åland 2001	20	0.550	0.502	5.167
Åland 2010	53	0.526	0.546	5.235
Småland-Östergötland 2001	59	0.504	0.544	4.510
Småland-Östergötland 2005	44	0.536	0.572	4.501
Småland-Östergötland 2010	73	0.536	0.577	4.866
Stockholm archipelago 2001	61	0.494	0.547	4.919
Stockholm archipelago 2010	144	0.508	0.540	4.972

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Table S3. Pairwise F_{ST} values between 15 areas in the Baltic Sea (Kokkola excluded from the total material; $n=659$) based on 11 microsatellite loci estimated in GenePop (Raymond and Rousset 1995) and using 10,000 MCMC repeat. Numbers in parentheses refer to area numbers in Fig. 2. F_{ST} estimates below diagonal, significances indicated above diagonal. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = non significant.

	Åland	Blekinge	Estonia	FinBott	FinQuark	Gävle	GoF Inner	GoF Outer	Gotland	Småland-Ö	Stockholm A	Swe Bott	Swe Quark	Archipelago S	Uppland
Åland (11)		***	***	***	***	ns	***	***	***	***	***	***	***	**	ns
Blekinge (15)	0.037		***	***	***	***	***	***	***	***	***	***	***	***	***
Estonia (16)	0.041	0.035		***	***	***	***	***	***	***	***	***	***	***	***
FinBott (2)	0.060	0.075	0.110		***	***	***	***	***	***	***	*	***	***	***
FinQuark (4)	0.017	0.021	0.041	0.057		***	***	***	***	***	***	***	***	***	***
Gävle (6)	0.013	0.075	0.086	0.036	0.035		*	*	***	*	**	**	***	ns	ns
GoFInner (10)	0.018	0.020	0.031	0.061	0.014	0.031		***	***	***	***	***	***	***	*
GoFOuter (9)	0.026	0.027	0.030	0.106	0.030	0.047	0.013		***	***	***	***	***	**	ns
Gotland (14)	0.040	0.032	0.039	0.082	0.040	0.069	0.015	0.030		***	***	***	***	***	ns
Småland-Ö (13)	0.018	0.037	0.032	0.072	0.032	0.021	0.017	0.033	0.028		***	***	***	***	***
Stockholm A (12)	0.015	0.046	0.044	0.087	0.041	0.023	0.026	0.032	0.040	0.014		***	***	***	**
SweBott (1)	0.052	0.090	0.075	0.024	0.058	0.033	0.055	0.080	0.082	0.071	0.068		***	***	***
SweKQuark (5)	0.081	0.139	0.134	0.140	0.091	0.095	0.115	0.114	0.132	0.112	0.114	0.128		***	***
Archipelago S (8)	0.008	0.018	0.026	0.056	0.004	0.030	0.010	0.017	0.028	0.016	0.024	0.055	0.100		ns
Uppland (7)	-0.003	0.029	0.026	0.057	0.011	0.010	0.003	0.010	0.011	0.001	-0.002	0.041	0.096	-0.001	

Table S4. Results from Structure. Mean $\text{LnP}(K)$ and standard deviation averaged over 5 runs for K 1-20, and the second order rate of change (ΔK) for each K .

K	Mean $\text{LnP}(K)$	StDev $\text{LnP}(K)$	ΔK
1	-18456	0.10	-
2	-18086	22.06	11.48
3	-17969	30.21	2.22
4	-17918	111.18	0.47
5	-17815	63.10	0.33
6	-17732	33.39	7.15
7	-17888	232.69	1.38
8	-17722	83.43	1.91
9	-17715	119.64	1.42
10	-17879	73.41	1.84
11	-17908	362.80	0.40
12	-18082	632.03	0.17
13	-18151	373.65	0.82
14	-17912	189.82	2.67
15	-18179	253.78	0.04
16	-18456	354.14	0.07
17	-18706	1566.38	0.10
18	-18801	836.59	0.11
19	-18804	598.27	0.41
20	-18561	419.83	-

Table S5. Statistical testing of correlation coefficients (*r*) for different migration models (Table 2, main text). Differences are tested by calculating the normal curve deviate between *z*'-transformed correlation coefficients (Cohen and Cohen 1983, equation 2.8.5).

Migration model A	<i>r</i> (A)	Migration model B	<i>r</i> (B)	<i>z</i>	<i>P</i> -value
1	0.409	2	0.649	2.331	0.020
1	0.409	3	0.686	2.533	0.011
1	0.409	4	0.649	1.990	0.047
1	0.409	5	0.129	1.901	0.057
1	0.409	6	0.013	2.473	0.013
1	0.409	7	0.467	0.513	0.608
1	0.409	8	0.684	2.764	0.006
1	0.409	9	0.723	2.992	0.003
2	0.649	3	0.686	0.405	0.686
2	0.649	4	0.649	0.000	1.000
2	0.649	5	0.129	3.901	0.000
2	0.649	6	0.013	4.348	0.000
2	0.649	7	0.467	1.838	0.066
2	0.649	8	0.684	0.418	0.676
2	0.649	9	0.723	0.850	0.395
3	0.686	4	0.649	0.356	0.721
3	0.686	5	0.129	3.988	0.000
3	0.686	6	0.013	4.416	0.000
3	0.686	7	0.467	2.085	0.037
3	0.686	8	0.684	0.023	0.982
3	0.686	9	0.723	0.413	0.680
4	0.649	5	0.129	3.436	0.001
4	0.649	6	0.013	3.878	0.000
4	0.649	7	0.467	1.569	0.117
4	0.649	8	0.648	0.010	0.992
4	0.649	9	0.723	0.749	0.454
5	0.129	6	0.013	0.623	0.533
5	0.129	7	0.467	2.350	0.019
5	0.129	8	0.684	4.283	0.000
5	0.129	9	0.723	4.401	0.000
6	0.013	7	0.467	2.895	0.004
6	0.013	8	0.684	4.709	0.000
6	0.013	9	0.723	4.808	0.000
7	0.467	8	0.684	2.271	0.023
7	0.467	9	0.723	2.544	0.011
8	0.684	9	0.723	0.468	0.639

Table S6. Pairwise F_{ST} -values between temporally separated samples below diagonal. F_{ST} -values are averaged over 6 microsatellite loci (*Elu87*, *Elu51*, *Elu76*, *Elu19*, *Elu12*, *Elu276*). Statistical significance from *ChiFish* above diagonal. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non significant. Shaded areas represent temporal comparisons within sampling area.

	Åland 2001	Åland 2010	Småland- Östergötland 2001	Småland- Östergötland 2005	Småland- Östergötland 2010	Stockholm archipelago 2001	Stockholm archipelago 2010
Åland 2001		ns	***	***	***	***	***
Åland 2010	0.0018		***	***	***	***	***
Småland-Östergötland 2001	0.0268	0.0188		***	ns	ns	ns
Småland-Östergötland 2005	0.0276	0.0179	0.0048		*	***	***
Småland-Östergötland 2010	0.0305	0.0264	0.0042	0.0043		***	***
Stockholm Archipelago 2001	0.0218	0.0102	0.0019	0.0108	0.0175		ns
Stockholm Archipelago 2010	0.0255	0.0225	0.0027	0.0184	0.0184	0.0024	

Table S7. Pairwise F_{ST} - values between temporally separated samples within one year (2010) comparing genetic divergence at spawning (April-June) vs. feeding (July-November) season. F_{ST} -values averaged over 11 microsatellite loci below diagonal. Statistical significance from *Chifish* (Ryman 2006) above diagonal. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = non significant. Shaded areas represent temporal comparisons within sampling area.

	Åland Apr-Jun	Åland Jul-Nov	Småland-Östergötland Apr-Jun	Småland-Östergötland Jul-Nov	Stockholm Archipelago Apr-Jun	Stockholm Archipelago Jul-Nov
Åland Apr-Jun		ns	***	***	***	***
Åland Jul-Nov	0.0012		***	***	**	*
Småland-Östergötland Apr-Jun	0.0192	0.0173		***	***	***
Småland-Östergötland Jul-Nov	0.0101	0.0136	0.0013		***	*
Stockholm Archipelago Apr-Jun	0.0165	0.0118	0.0173	0.0079		ns
Stockholm Archipelago Jul-Nov	0.0149	0.0037	0.0124	0.0029	0.0000	