Combining biotelemetry and genetics provides complementary insights relevant to the management and conservation of a freshwater predator (Esox lucius) living in brackish lagoons.

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Supplementary material

Supplementary methods

Study Area

Table S1: Annual mean hydrochemical conditions from 2010 to 2020 in different lagoons (data source: LUNG, reproduced from (Arlinghaus et al. 2023b).

Parameter	BAT	WRB	NRBC	S	GB	PS
Chlorophyll a (mg/m^3)	27.7 ± 19.6	7.9 ± 6.9	16.8 ± 12	15 ± 9.1	14.6 ± 13.6	63.6 ± 48.1
Total phosphorus (µmol/l)	1.8 ± 0.7	1.3 ± 0.6	1.8 ± 1.1	1.6 ± 0.6	1.5 ± 0.7	3.2 ± 1.9
Salinity (PSU)	8.3 ± 1.6	8.7 ± 1.1	8.3 ± 1	7.8 ± 1.1	7.2 ± 0.9	3.2 ± 2.1
Secchi depth (m)	1 ± 0.8,	1.9 ± 0.8	1.4 ± 0.7	1.4 ± 0.6	1.7 ± 0.8	0.7 ± 0.5
Water (°C)	11.5 ± 6.6	11.6 ± 6.7	12.5 ± 6.5	11.9 ± 7	11.7 ± 6.8	11.9 ± 6.7
Mean depth (m)	2	1.8	3.5	3.9	5.8	2.6
Max depth (m)	16.5	7.6	10.3	16	13.5	16
Catchment area (km^2)	1.578	NA	312	238	665	5.772
Area (km^2)	59.8	231	132.9	47.6	540.1	181.9
Macrophyte coverage	Low-Medium	Medium	Medium-High	Low-Medium	Low-Medium	Low

BAT – Barther Bodden; WRB – Western Rügen Bodden; NRBC – Northern Rügen Bodden chain; S – Strelasund; GB – Greifswalder Bodden; PS – Peenestrom.

Values after ± represent standard deviations.

The macrophyte coverage represents expert judgement at the time of study as there is no objective data.

Acoustic telemetry

Different tag types did not affect the probability of detection by receivers. For each transmitter, we calculated the average number of detections per day at liberty and performed a t-test comparing both transmitter types and found no significant differences:

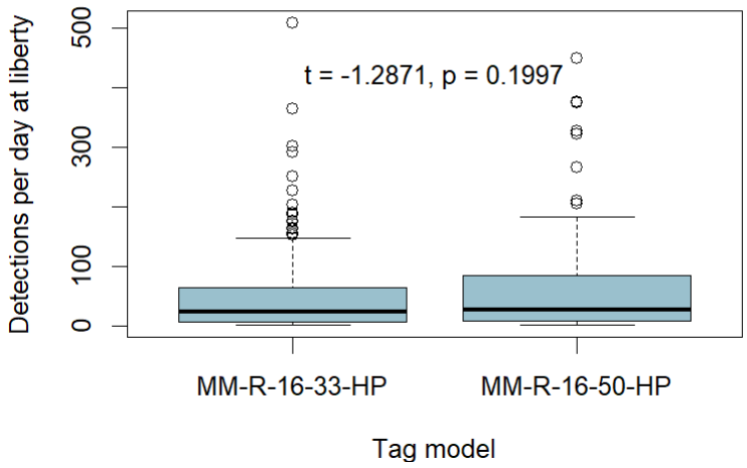

Fig. S1. T-test comparing detectability of the two transmitter tags used in the study.

Acoustic data processing

When a tagged fish occurred within the detection range of a receiver, its ID and the date and time were recorded. Data was stored on the receivers and downloaded annually in winter, and processed in Fathom (Innovasea Systems Inc., Massachusetts, U.S.A.) to correct for clock drift (Dhellemmes et al., 2023). The data was then filtered using *ATfiltR* (Dhellemmes et al. 2023a). We first trimmed the detections to keep only detections that were collected after the deployment and before the retrieval of receivers (i.e., removing detections potentially obtained when a receiver was being serviced on a boat or on land), and of transmitters (i.e., removing data from transmitters that aren't implanted in a fish). We then removed detections from tags that were detected only once on a given receiver in a 48h window. Then, to filter out impossible movements between our study areas, we identified any group of five or less detections that occurred alone in a given lagoon, and filtered them out as ghost detections. Finally, since the average pulse rate of the transmitters used in this study was 120 seconds, any detection of a given ID that occurred within 120 seconds of the previous one was regarded as a duplicate and removed. We assumed that this would reduce the number of detections in locations with a high receiver density and would not have a significant effect on connectivity between the areas considered for the analysis described below. Throughout our filtering protocol, we visually checked the data by comparing individual detection networks created in R package *igraph* (Csárdi and Nepusz 2006) before and after each filtering step.

Null model

To test whether the observed patterns of movement differed from random, the observed networks were compared to those generated from null models, i.e., randomised movement networks. Random networks were constructed as random walks according to the method developed by (Lea et al. 2016). To create such random walks, the first detection at the first receiver was retained from the observed track of each individual. Then a movement distance was calculated based on the time elapsed before each next detection and the predetermined swimming speed of the animal. The swimming speed of each pike was calculated as 0.019 x Body-Length^{0,75} in accordance with Wolter and Arlinghaus (2003). For each step of the random walk, receivers were selected at random until two were found within range of the swim distance and the closest of them was then chosen as the next position in the random track. If no receiver met the criteria after 100 random selections, then no movement was deemed to occur, and the current station was assigned. This procedure was carried on for the whole duration of the track, generating a random walk through the receiver array, constrained by the observed detection intervals and individual animal characteristics. This was repeated 100 times for each individual, thus producing 100 random tracks per fish. The random walks were created in *Python 3.8.10* via the *Anaconda 3 distribution* (2020) in the *Spyder* environment (Raybaut 2009) using the *pandas* (McKinney 2010) and *NumPy* (Harris et al. 2020) software libraries. To test whether empirical individual networks were different from random, *edge density* (proportion of edges present in a network, out of the total number of edges possible in it (Jacoby et al. 2012)) was derived from observed network and tested against the same metric of random networks for each individual with Wilcoxon one-sample signed rank test (Lea et al. 2016).

Population genetics and its link to ecological connectivity, geographical distance, and salinity gradients

Roser et al. (2023) assessed the genetic population structuring of pike using a pool-sequencing approach in 11 lagoon and river locations around the island of Rügen, Germany. Tissue samples were collected from 45-50 individuals from each of 11 locations (535 pike in total; see locations on Figure S2), and DNA was extracted following a standard phenol-chloroform protocol before being pooled and sequenced using Illumina technology. The genomic differentiation among

populations was assessed through F_{ST} estimation and visualized using a Neighbor-Joining distance tree as described in Roser et al. (2023):

> "[] allele frequencies and F_{ST}s for every single nucleotide polymorphisms (SNP) were estimated in popoolation2, using the sliding-windows option with a window size of one in order to take pool sizes into account. Average genomic differentiation, measured as F_{ST}, was calculated for all pairwise comparisons using a custom perl script. To visualize genetic sub-structuring, we built a Neighbor-Joining tree from the pairwise F_{ST}s using the PHYLIP/NEIGHBOR v. 3.695 (Felsenstein, 2005) and FIGTREE v.1.4.4 (Rambaut, 2011) software. Finally, we used popoolation v.1.2.2 (Kofler et al., 2011) to calculate genome-wide estimates of nucleotide diversity (pi, Nei and Li, 1979) for each chromosome separately, using window sizes corresponding to chromosome sizes and averaging chromosomal pi values at the end."

Pairwise F_{ST} values ranged from 0.0128 (Greifswalder Bodden vs. Kubitzer/Schaproder Bodden) to 0.0547 (Greifswalder Bodden vs. Stettiner Haff) and were generally highest in lagoon locations (Bodden) vs. Peene River and Stettiner Haff (an oligohaline estuary of the river Oder) comparisons (range: 0.036–0.0547). Neighbor-Joining distance tree (Figure S2) shows that one cluster is formed of brackish-water pike populations in Barther Bodden (BAT), Schaproder/Kubitzer Bodden (SB/KB), Großer Jasmunder Bodden (GJB) and Greifswalder Bodden (GB), which are all mesohaline lagoons. Freshwater populations from rivers Barthe (west of Rügen) and Peene (southeast of Rügen) and the oligohaline lagoons Peenestrom (P), and Stettiner Haff in the estuary of river Oder form another cluster with a notably higher among-population divergence than the mesohaline brackish water populations (BAT, GJB, SB/KB, GB). Putative anadromous populations are given by the smaller rivers/streams/ditches Sehrowbach, Neuendorfer Hechtgraben, and Ziese River. We call them putative anadromous because in all cases we observed migrations into the rivers during spawning time and very limited to no resident freshwater pike (in contrast to the larger rivers Barthe and Peene, where freshwater residents occur year-round). River Ziese, which connects the GB and P, was sampled close to the inflow into P and is part of the cluster including freshwater populations and oligohaline lagoons (SH, P) of the river Oder estuary. Importantly, Neuendorfer Hechtgraben (draining into BAT) and Sehrowbach (draining into SB/KB) show less divergence from the brackish-water populations than the putatively freshwater populations in the largest rivers that we sampled (rivers Barthe and Peene). Note also that the rivers Barthe and Peene are

geographically on opposite sides of the lagoon network and not connected through the same lagoon. It is possible that the samples of pike from the rivers Barthe and Peene encompassed both freshwater residents and anadromous pike, which the pooled sequencing cannot resolve. Taken together, the tree demonstrates divergence of putative anadromous population samples in smaller rivers at a level that is comparable with what is observed among putative freshwater populations and populations in oligohaline lagoons (SH, P) and suggests genetic divergence of anadromous pike from brackish water pike captured in mesohaline lagoons as well as from populations from different freshwater sites. The tree also suggests genetic structure by geography and salinity gradients, whose individual contributions are resolved in the main text.

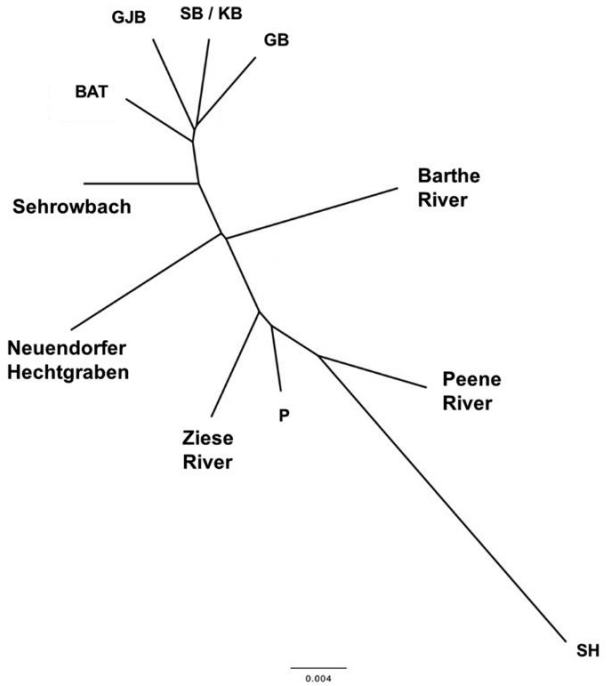

Fig. S2. Neighbor-Joining distance tree based on 1.190.970 SNPs from whole-genome sequences of pooled individuals from different study sites. Full names of the areas: BAT – Barther Bodden; GB – Greifswalder Bodden; GJB – Großer Jasmunder Bodden; SB/KB – Schaproder and Kubitzer Bodden; P – Peenestrom; Sehrowbach – Sehrowbach stream; SH – Stettiner Haff. Figure from Roser et al. (2023).

Table S2. Pairwise F_{ST} values.

	Barthe river	Peene river	SB/KB	GJB	GB	Sehrowbach	BAT	Ziese river	Neuendorfer Hechtgraben	P	SH
Barthe river		0.029	0.029	0.029	0.029	0.024	0.026	0.029	0.026	0.025	0.048
Peene river	0.029		0.036	0.036	0.036	0.030	0.034	0.022	0.033	0.016	0.036
SB/KB	0.029	0.036		0.014	0.013	0.019	0.013	0.029	0.028	0.028	0.053
GJB	0.029	0.036	0.014		0.014	0.019	0.014	0.029	0.028	0.027	0.053
GB	0.029	0.036	0.012	0.014		0.019	0.013	0.029	0.027	0.027	0.055
Sehrowbach	0.024	0.030	0.019	0.019	0.019		0.018	0.027	0.026	0.024	0.049
BAT	0.026	0.034	0.013	0.014	0.013	0.018		0.027	0.024	0.025	0.052
Ziese river	0.029	0.022	0.029	0.029	0.029	0.027	0.027		0.027	0.013	0.044
Neuendorfer Hechtgraben	0.026	0.033	0.028	0.028	0.027	0.026	0.024	0.027		0.026	0.054
P	0.025	0.016	0.028	0.027	0.027	0.024	0.025	0.013	0.026		0.037
SH	0.048	0.036	0.053	0.053	0.055	0.049	0.052	0.044	0.054	0.037	

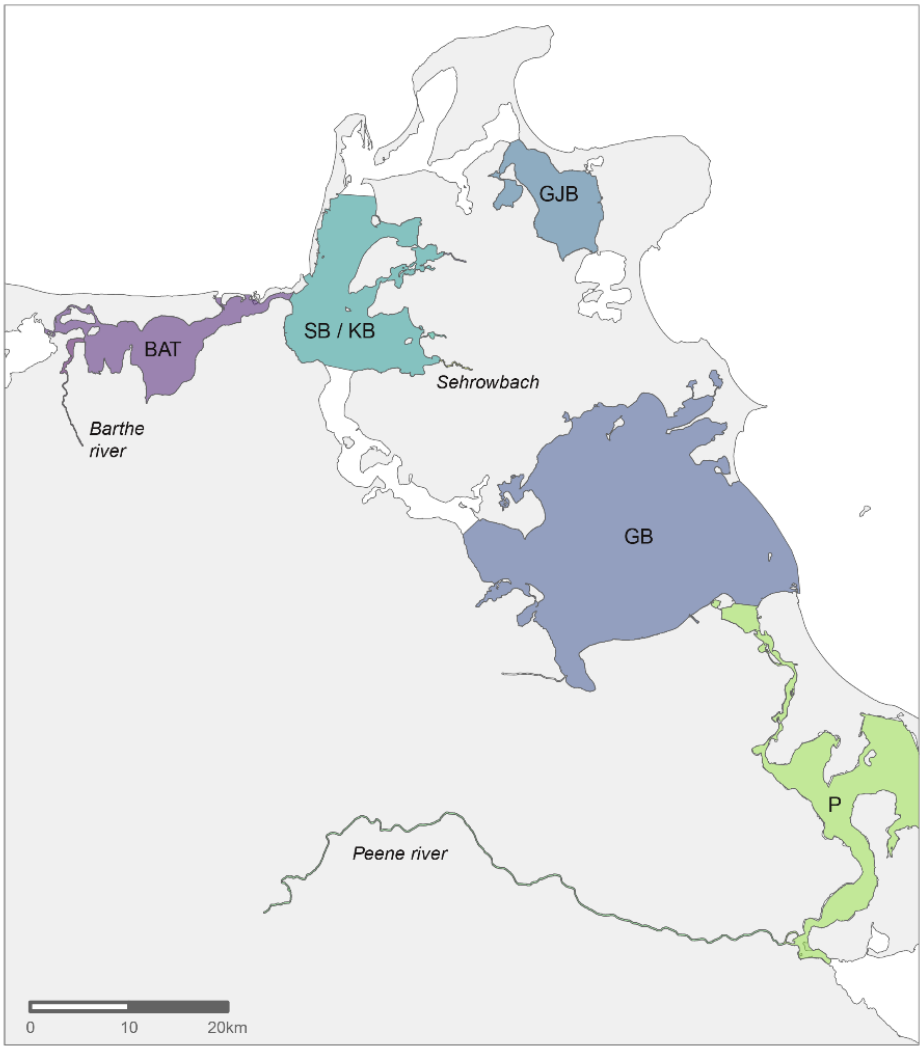

Fig. S3. Map displaying section of the study area where both genetic and telemetry data were available and which, therefore, were used for models assessing the links between population genetics and ecological connectivity, geographical distance, and salinity gradients. The locations included mesohaline brackish-water (Barther Bodden (BAT), Kubitzer/Schaproder Bodden (KB / SB), Großer Jasmunder Bodden (GJB), Greifswalder Bodden (GB)), possibly resident freshwater (Barthe and Peene river), oligohaline brackish (Peenestrom (P)) and a putative anadromous population (Sehrowbach).

Supplementary results

Seasonal movement networks

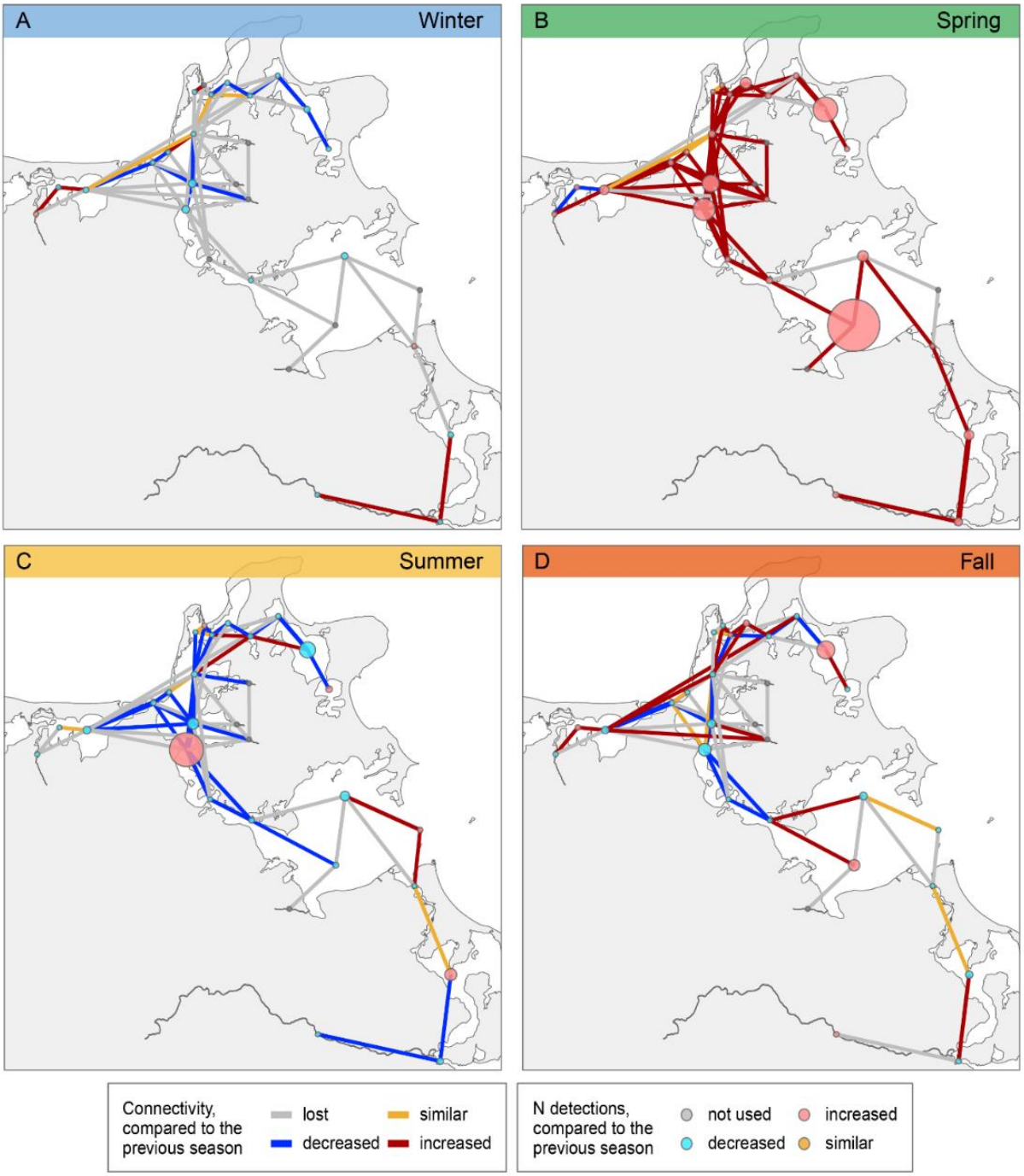

Fig. S4. Seasonal changes in pike movements across the lagoons, freshwater tributaries, and "gates". (A-D) Seasonal movement networks. Colours show the differences in metrics compared to the previous season (e.g., winter to fall), where: node colours reflect changes in the total number of daily detections in each area (node strength) and colours of the edges reflect the change in connectivity between areas (frequency of internodal movements; edge weight). The size of nodes and the edge line thickness correspond to the absolute values of respective metrics

Population genetics and its link to ecological connectivity, geographical distance, and salinity gradients

GLM model

```
m0<-glmmTMB(data = data, formula = linFST~scaledEdgeWeight+scaledMeanDist+scaledSalinityDiff+Freshwater+(1|from), family = glmmTMB::beta_family())

stepAIC(m0, trace = TRUE, direction= "both")
```

```
## Start:  AIC=-449.44
## linFST ~ scaledEdgeWeight + scaledMeanDist + scaledSalinityDiff +
##     Freshwater
##
##                   Df     AIC
## - scaledEdgeWeight    2 -452.84
## <none>                -449.44
## - Freshwater          3 -435.81
## - scaledMeanDist      2 -421.61
## - scaledSalinityDiff  2 -418.58
##
## Step:  AIC=-452.84
## linFST ~ scaledMeanDist + scaledSalinityDiff + Freshwater
##
##                   Df     AIC
## <none>                -452.84
## + scaledEdgeWeight    1 -451.43
## - Freshwater          2 -437.45
## - scaledSalinityDiff  1 -420.23
## - scaledMeanDist      1 -417.52

## Formula:         linFST ~ scaledMeanDist + scaledSalinityDiff + Freshwater
## Data: data
##       AIC       BIC    logLik  df.resid
## -452.8400 -440.6879  232.4200        50
##
## Number of obs: 56
##
## Dispersion parameter for beta family (): 1.54e+03
##
## Fixed Effects:
##
## Conditional model:
##       (Intercept)     scaledMeanDist  scaledSalinityDiff       Freshwaterone
##          -3.73642            0.16127            0.27324           -0.06829
##     Freshwatertwo
##           0.42581
```

It seems that connectivity did not significantly improve the model fit.

Fit the best model:

```
m1<-glmmTMB(data = data, formula = linFST~scaledMeanDist + scaledSalinityDiff + Freshwater + (1|from), family = glmmTMB::beta_family())

DHARMa::plotQQunif(DHARMa::simulateResiduals(m1))
```

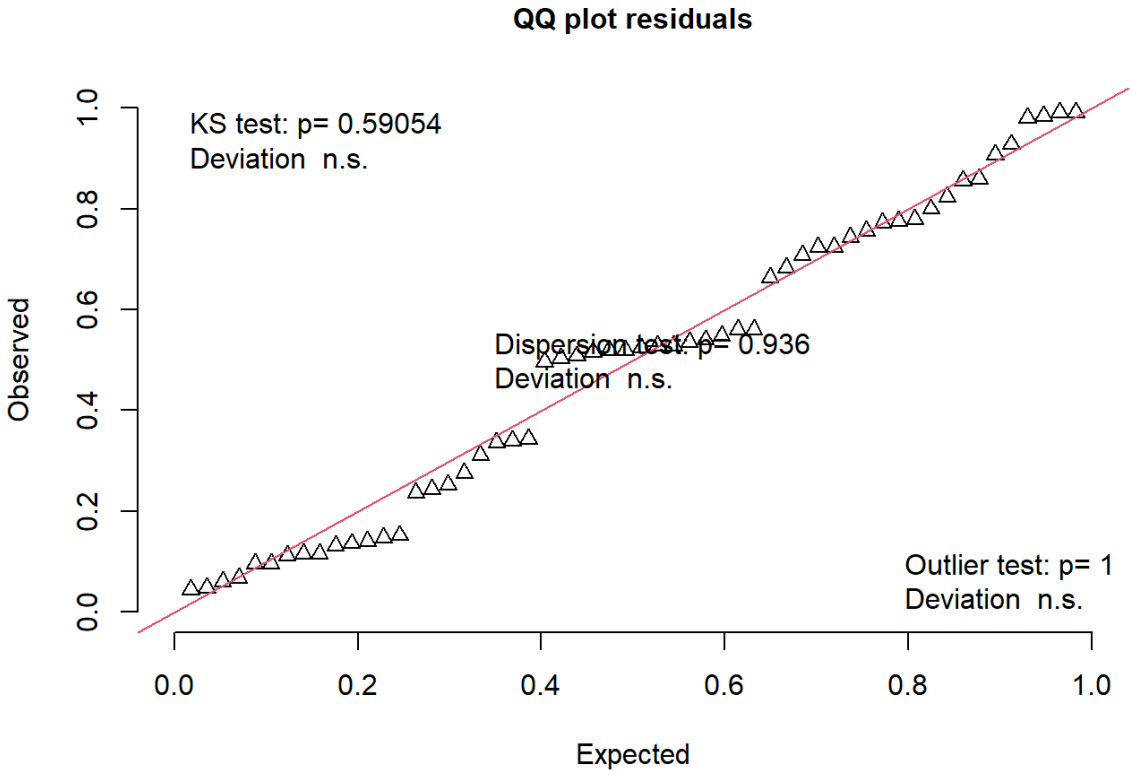

Fig. S5. Assessment of the fit of the obtained most parsimonious model.

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