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Eutrophication causes speciation reversal in whitefish adaptive radiations

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Species diversity can be lost through two different but potentially interacting extinction processes: demographic decline and speciation reversal through introgressive hybridization. To investigate the relative contribution of these processes, we analysed historical and contemporary data of replicate whitefish radiations from 17 pre-alpine European lakes and reconstructed changes in genetic species differentiation through time using historical samples. Here we provide evidence that species diversity evolved in response to ecological opportunity, and that eutrophication, by diminishing this opportunity, has driven extinctions through speciation reversal and demographic decline. Across the radiations, the magnitude of eutrophication explains the pattern of species loss and levels of genetic and functional distinctiveness among remaining species. We argue that extinction by speciation reversal may be more widespread than currently appreciated. Preventing such extinctions will require that conservation efforts not only target existing species but identify and protect the ecological and evolutionary processes that generate and maintain species.

Effectively counteracting the biodiversity crisis requires identifying and protecting the ecological and evolutionary processes that generate and maintain diversity[1,2]. Species can go extinct through two distinct but potentially interacting processes. In the first, demographic decline results in population extirpation and eventually the total extinction of the species. In the second, introgressive hybridization erodes differentiation until species collapse into a hybrid swarm[3]. A special case of introgressive hybridization is speciation reversal[4], in which changes in selection regimes increase gene flow between sympatric species, thus eroding genetic and ecological differences. Speciation reversal may be particularly important in adaptive radiations with recently diverged sympatric species that lack strong intrinsic post-zygotic isolation[5–8].

Adaptive radiation is the evolution of ecological diversity in rapidly speciating lineages[9]. It is often characterized by 'ecological speciation', in which traits that are under divergent natural selection, or those genetically correlated with them, contribute to reproductive isolation[9–13]. When reproductive isolation between ecologically differentiated populations is maintained by the temporal and spatial clustering of breeding aggregations, adaptive radiation occurs through the correlated partitioning of ecological and reproductive niche spaces. Because intrinsic post-zygotic isolation is typically weak during adaptive radiation[12], environmental changes that reduce niche space and relax the selective forces maintaining reproductive isolation[14,15] can lead to extinction by speciation reversal[4–8].

Fish of post-glacial lakes are model systems for studying adaptive radiation owing to their recent origins and repeated patterns of diversification in independent lineages[16–18]. These radiations are characterized by the correlated partitioning of ecological and reproductive niche spaces[16,19,20]. In the European Alps, at least 25 lakes harbour 1 to 5 whitefish species (*Coregonus* spp.)[18,21] (Fig. 1a and Supplementary Table 1). For 17 of these lakes, 13 of which contain multiple sympatric species, the whitefish diversity was described by Steinmann 60 years ago[22]. This diversity has arisen since deglaciation within nine hydrologically independent lake systems[18].

Reproductive isolation in central European whitefish radiations is maintained mainly by pre-zygotic mechanisms (divergence in spawning depth[23], time, possibly mate choice (B. Lundsgaard-Hansen et al. unpublished data) and extrinsic rather than intrinsic post-zygotic mechanisms[24]. Generally, large-bodied species with few, widely spaced gill-rakers (benthic invertebrate feeders), spawn in winter in shallow littoral habitats, whereas small-bodied species with many densely spaced rakers (zooplankton feeders), spawn in deeper water in winter or summer. Exceptions to this rule are profundal summer-spawning species with very low numbers of gill-rakers that exist in Lake Thun and existed in Lake Constance[22]. Summer-spawning species choose cold and well-oxygenated spawning habitats below the thermocline (>20 m in depth). Eggs settle onto the lake-floor sediment and require an oxygenated water–sediment interface to develop and hatch[25]. Because whitefish use most of the lacustrine habitats, and because of their large biomass and ecological diversity, they are keystone species in the ecosystems of pre-alpine lakes, which are commonly referred to as whitefish lakes.

Although eutrophication threatens lake ecosystems worldwide[26,27], the manner and mechanisms by which it has affected adaptive radiations, and whitefish in particular, remain unclear[22,23]. Many Swiss whitefish lakes lie in densely populated areas and were subjected to high nutrient inputs in the twentieth century, a fact that led Steinmann to suggest in 1950 that eutrophication was the cause of the extinction of eight whitefish populations[22]. By the 1970s, eutrophication had increased primary production in all Swiss lakes (Fig. 2d and Supplementary Fig. 1). The associated increase in microbial decomposition rates resulted in oxygen depletion at the water–sediment interface, especially below the thermocline, leading to reduction or complete failure of whitefish recruitment[25]. Eutrophication also affected the biomass and diversity of zooplankton (Supplementary Fig. 2) and

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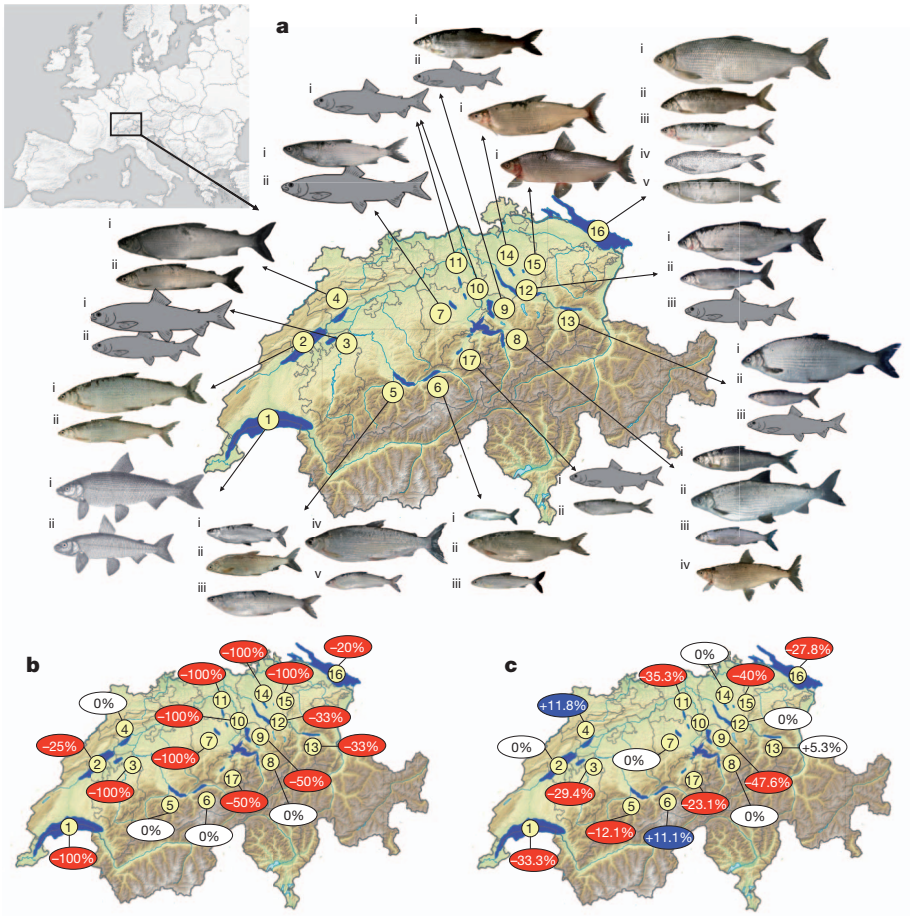

Figure 1 | Distribution of historical whitefish diversity and recent diversity loss. **a**, Whitefish species diversity in Swiss lakes (numbered as in Table 1, fish are named in Supplementary Information; for details of taxonomy see quantification of whitefish diversities in Supplementary Information). **b**, Species richness change in 17 lakes. **c**, Functional diversity change in 16 lakes. Red ellipses, more than 10% diversity loss; white ellipses, little or no change; blue ellipses, increase in diversity of more than 10%. The observed functional diversity increase in Lake Brienz is due to the presence of one species (*C.* sp. '*Balchen*') that Steinmann was unaware of[22].

probably of benthic invertebrates[28–30], thus altering the ecological and reproductive niche spaces that were associated with whitefish radiations. Improved sewage treatment and phosphorus management have allowed some lakes to return to near their natural trophic state (Fig. 2d). However, in other lakes, the sediment–water interface remains anoxic and zooplankton biomass is higher than before eutrophication[28].

We suspected that loss of deep spawning habitat weakened reproductive isolation, and that at the same time, increased productivity led to an increase of zooplankton density at the expense of zooplankton diversity (Supplementary Fig. 2), whereas the associated hypoxia probably led to loss of zoobenthos density in the profundal zone[31]. By disproportionately affecting the availability of one type of prey more than the other along the principal axis of whitefish feeding divergence, eutrophication probably changed the shape of the adaptive landscape from multimodal towards unimodal or flat, thus relaxing divergent selection. We therefore proposed that eutrophication caused speciation reversal in addition to demographic decline. We show that the speciation reversal hypothesis is supported by historical and contemporary patterns of diversity across lakes and by changes through time in genetic and phenotypic distinctiveness of sympatric species.

Diversity loss in polluted lakes

Most whitefish assemblages have lower species and functional diversity today than historically (Fig. 1, Table 1, Supplementary Table 1 and quantification of whitefish diversity in Supplementary Information). On average, species richness has decreased by 38% (Wilcoxon test $N = 17$, $V = 91$, $P < 0.001$), functional diversity (range in gill-raker numbers) by 14% ($N = 16$, $V = 60$, $P = 0.018$) and the difference between sympatric species in gill-raker mean counts by 28% (Welch's t-test $N = 8$, $t = 7.79$, d.f. $= 7$, $P < 0.001$). Declines in species richness were explained by eutrophication level (linear regression $N = 17$, $R^2 = 0.50$, $P < 0.001$; Fig. 2a and Supplementary Table 2). Reductions in gill-raker count range were poorly predicted by eutrophication, probably because some variation is retained in hybrid swarms and stocking programmes have maintained some diversity even in the most polluted lakes[25] (Table 1 and Supplementary Table 1). Eutrophication reduced the oxygenated depth (depth range with $O_2 > 2.5$ mg l^{-1}; see Supplementary Information) across 16 lakes (Supplementary Fig. 3). Egg survival was measured in a subset of those lakes and was found to decrease with nutrient load ($N = 12$, $R^2 = 0.45$, $P = 0.010$; Fig. 3f) and was close to zero once the maximum phosphorus exceeded 150 μg l^{-1}.

Predicting the origin and loss of diversity

Because available depth affects the diversity of spawning and feeding habitats[22,23], and because all lakes were oxygenated to the greatest depths before eutrophication, we expected maximum lake depth (D_{max}) to predict pre-eutrophication diversity. By contrast, we expected maximum phosphorus concentration (P_{max}) and minimum oxygenated depth ($D_{O,min}$) during eutrophication to predict patterns of contemporary diversity (Table 1).

Maximum lake depth does indeed predict historical species richness ($N = 17$, $R^2 = 0.48$, $P = 0.001$, Fig. 3a) and the use of vulnerable reproductive niches. This pattern held when tested with evolutionarily independent lineages from hydrologically isolated lake

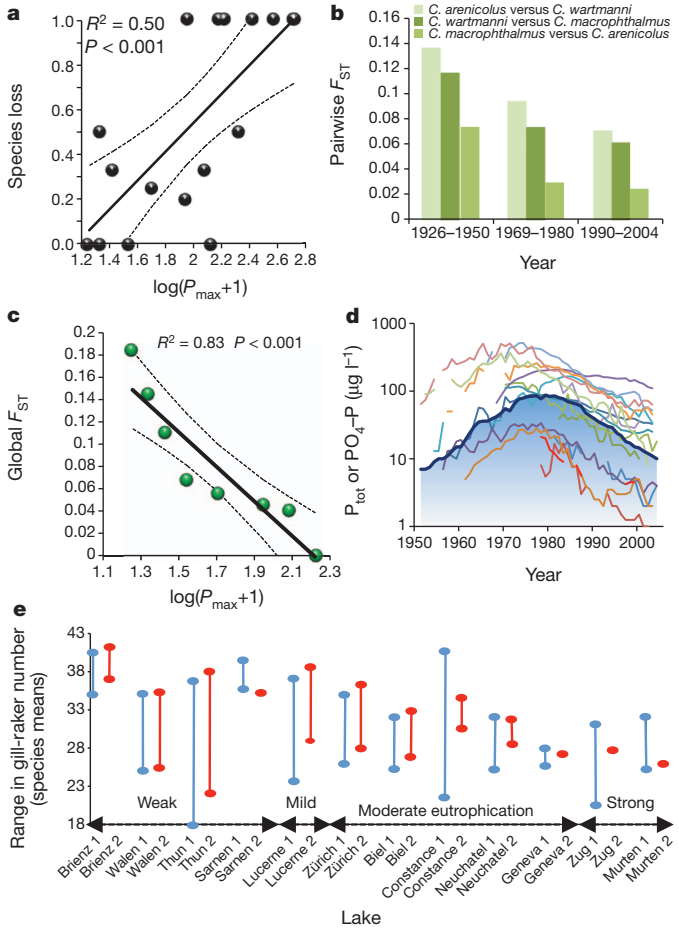

Figure 2 | Diversity loss through speciation reversal. **a**, Species diversity loss regressed against the maximum phosphorus concentration, P_{max} (µg l^{-1}). **b**, The pairwise F_{ST} values among three *Coregonus* species from Lake Constance observed through time. **c**, The global genetic differentiation among species within each lake plotted against the maximum phosphorus concentration. **d**, Fifty-year trends in phosphorus concentration from our study lakes are included. Lake Constance is highlighted as a blue gradient surface. Lake details are given in Supplementary Fig. 1. **e**, Ranges of species means in gill-raker counts for each lake, prior to (historical; 1, shown in blue) and after (contemporary; 2, shown in red) pollution. Lakes are arranged from weakly to strongly polluted. For panels **a** and **c** the dashed lines represent the 95% confidence intervals for the regression line.

groups[18,22] as the unit of observation ($N = 9$, $R^2 = 0.51$, $P = 0.019$). Historical functional diversity also increased with maximum lake depth ($N = 17$, $R^2 = 0.30$, $P = 0.013$; Fig. 3b). Lakes that historically harboured summer- and deep-spawning species are significantly deeper than lakes that did not ($N_{Summer} = 8$, $N_{NoSummer} = 9$: $t = 3.25$, d.f. = 12.97, $P = 0.006$; $N_{Deep} = 11$, $N_{NoDeep} = 6$: $t = 5.05$, d.f. = 14.36, $P < 0.001$).

Oxygenated depth during eutrophication predicts contemporary species richness and functional diversity slightly better than does maximum lake depth, although the difference is not significant (difference in Akaike's corrected information criterion (ΔAICc) = 1.96 and 1.91; see regression model selection in Supplementary Information) (species richness: $N = 17$, $R^2 = 0.55$, $P < 0.001$, Fig. 3c, versus $R^2 = 0.49$, $P < 0.001$; functional diversity: $N = 16$, $R^2 = 0.40$, $P = 0.005$, Fig. 3d, versus $R^2 = 0.32$, $P = 0.013$). This was also true for historical species richness and functional diversity, but oxygenated depth explained slightly more of the variance in contemporary than in historical diversity (Supplementary Table 2). Moreover, lakes that lost summer- or deep-spawning species were more eutrophied than those that retained these species ($N_{SummerLoss} = 3$, $N_{SummerNoLoss} = 5$: $t = 3.04$, d.f. = 5.99, $P = 0.023$; $N_{DeepLoss} = 3$, $N_{DeepNoLoss} = 8$: $t = 2.98$, d$f = 7.13$, $P = 0.020$), whereas maximum depth was not different between these lakes ($N_{SummerLoss} = 3$, $N_{SummerNoLoss} = 5$: $t = 0.44$, d.f. = 5.90, $P = 0.675$; $N_{DeepLoss} = 3$, $N_{DeepNoLoss} = 8$: $t = -0.301$, d.f. = 2.27, $P = 0.783$).

Among lakes, the contemporary number of genetically differentiated species (see Methods) is best predicted by maximum depth ($N = 8$, $R^2 = 0.50$, $P = 0.031$; Fig. 3e). The level of genetic differentiation among species, on the other hand, is predicted by the severity of eutrophication, to which it is strongly negatively correlated ($N = 8$, $R^2 = 0.83$, $P < 0.001$; Fig. 2c). In combination with the previous results, these data suggest that the depth-mediated legacy of adaptive radiation has been modified by speciation reversal driven by eutrophication.

Species loss through speciation reversal

If extinction resulted from demographic decline, pairwise genetic differentiation among contemporary species at neutral markers (measured using the fixation index (F_{ST})) would remain unchanged or increase owing to genetic drift as effective population sizes declined[32]. By contrast, extinction by speciation reversal should involve declines in pairwise F_{ST} values among extant species[6]. Lake Constance suffered eutrophication, but phosphorus concentrations never exceeded 150 µg l^{-1} at which egg development fails even in shallow waters (Table 1). We extracted DNA from samples of all species collected before (1926–50, $P_{max} < 10$ µg l^{-1}), during (1969–80, $P_{max} = 87$ µg l^{-1}) and after (1990–2004, $P_{max} = 39$ µg l^{-1}) peak eutrophication. Genetic cluster analysis identified four species, with all four being well represented in pre-eutrophication scale samples. Out of all of the post-eutrophication samples, only five individuals were assigned to the now extinct summer- and deep-spawning *Coregonus gutturosus* (Supplementary Table 3). However, the morphological (gill-raker counts) and reproductive (winter instead of summer spawning) traits of these individuals did not match those of historical *C. gutturosus*. We therefore calculated pairwise F_{ST} with and without these genetically assigned *C. gutturosus*-like individuals. Pairwise genetic differentiation among the three extant species has dropped dramatically through time (Fig. 2b) and global F_{ST} has decreased over twofold (0.108/0.165 to 0.046/0.047, without/with *C. gutturosus*, respectively).

Speciation reversal should also increase genetic variation within extant species. Consistent with this prediction, allelic richness has increased through time in *Coregonus wartmanni* ($N = 10$, d.f. = 8, $t = 3.38$, $P = 0.009$) and a similar trend is seen in *Coregonus macrophthalmus* ($N = 10$, d.f. = 8, $t = 2.17$, $P = 0.062$; Supplementary Table 4). Out of 11 alleles found only in *C. gutturosus* among the pre-eutrophication samples (private alleles), 5 were found in contemporary *Coregonus* species of Lake Constance (Supplementary Table 5). The probability of finding at least one of these alleles in pre-eutrophication samples of the other species, assuming similar frequencies, is 98% and suggests that the extinction of *C. gutturosus* involved hybridization with other species.

Data from Lake Brienz, the lake that is least polluted and that has no loss in species or functional diversity (Table 1), contrast and complement those from Lake Constance. For the three endemic species, global genetic differentiation (global F_{ST}) was historically (1952–70) identical to that in Lake Constance (0.166) but has not declined until the present (0.183). Moreover, no significant increase in allelic richness was observed in any of the three species. Nevertheless, out of 12 historically private alleles of the summer- and deep-spawning *Coregonus albellus*, 7 were also found in contemporary samples of other species, suggesting that gene flow between species has also occurred in this lake (see also ref. 33).

Additional support for speciation reversal comes from Lakes Zürich and Walen, which share a single-origin species pair, a small, deep-spawning species (*Coregonus heglingus*) and a large, shallow-spawning species (*Coregonus duplex*[18]; Supplementary Fig. 5). Despite a common evolutionary history, pairwise F_{ST} between the species

Table 1 | Whitefish species and functional diversity in 17 pre-alpine lakes.

Lake (no.)	Species diversity and genetic differentiation					Functional ecological diversity					Mean egg survival (%)	Oxygenated lake depth (m)	P_{max} (μg l^{-1})	Maximum lake depth (m)
	Historical species	Contemporary species	Species loss (%)	Genetic species	Change in genetic differentiation (%)	Historical gill-raker range	Contemporary gill-raker range	Functional loss (%)	*N* Historical gill-raker range	*N* Contemporary gill-raker range				
Lake Geneva (1)	2	0 (1‡)	−100			15	10*	−33.30	61	24*	84.4	254.17	90	309
Lake Neuchatel (2)	2	1.5	−25	1.5		17	17	0.00	?	341	43.8	153.00	50	152
Lake Murten (3)	2	0	−100			17	12*	−29.40	?	30*		8.93	150	45.5
Lake Biel (4)	2	2	0			17	19	11.80	?	49	17.9	27.50	132	74
Lake Thun (5)	5	5	0	5		33	29	−12.10	471	331	67.2	214.00	21	217
Lake Brienz (6)	3	3	0	3	10	18	20	11.10	>123	100		243.97	17	261
Lake Sempach (7)	2	0	−100	1		13	13*	0.00	>12	76*	0.7	8.26	165	87
Lake Lucerne (8)	4	4	0	4		23	23	0.00	180	730	42.0	203.49	34	214
Lake Zug (9)	2	1	−50			21	11*	−47.62	?	20*	0.3	8.50	208	198
Lake Baldegg (10)	1	0	−100			17			?			4.34	517	66
Lake Hallwil (11)	1	0	−100			17	11*	−35.30	?	20*	0.9	6.69	260	47
Lake Zürich (12)	3	2	−33	2		18	18	0.00	76	66	35.3	9.72	119	136
Lake Walen (13)	3	2	−33	2		19	20	5.30	?	236	37.8	144.00	26	145
Lake Greifen (14)	1	0	−100			11	11*	0.00	?	50*		0.00	507	32.3
Lake Pfäffiker (15)	1	0	−100			15	9*	−40.00	?	19*		0.00	367	35
Lake Constance (16)	5	4	−20	3	−57 (−71.5†)	36	26*	−27.80	694	79*	31.4	248.91	87	254
Lake Sarnen (17)	2	1	−50			13	10*	−23.10	?	20*	59.4	47.33	21	52
Total/average	41	25.5	−38		−24 (−31†)			−13.78		2,191				

The number of historically observed and presently observed phenotypically distinct, naturally recruiting whitefish species (Historical species[22] and Contemporary species, respectively. ‡The present wild population observed in Lake Geneva does not correspond to either of the two described species); the percentage loss in species numbers (Species loss); the number of genetically distinct species observed today (Genetic species), in which 1.5 represents a species cline observed in Lake Neuchâtel[23]; the percentage reduction in global genetic differentiation; the historically and currently observed gill-raker count range (Historical gill-raker range and Contemporary gill-raker range, respectively); the functional diversity (Functional loss); the sample sizes for historical data (Historical gill-raker range, *N*) and contemporary gill-raker analysis (*N* Contemporary gill-raker range), the mean egg survival (Mean egg survival), the biologically available depth during eutrophication with more than 2.5 mg l^{-1} dissolved oxygen (Oxygenated lake depth); the maximum total phosphorus concentration observed during the eutrophic period (P_{max}); and the maximum lake depth (Maximum lake depth).

* Gill-raker ranges adjusted for unequal sample sizes (see Methods, Supplementary Information, Supplementary Table 6 and Supplementary Fig. 4).

† Number in brackets corresponds to the loss if the phenotypically extinct *C. gutturosus* is included in the analysis (see Supplementary Table 3).

today in eutrophic Lake Zürich (0.041) is less than half of that in oligotrophic Lake Walen (0.110).

Phenotypic signs of speciation reversal

Speciation reversal is expected to erode interspecific phenotypic distinctiveness[4,6]. Gill-raker counts provide a measure of heritable phenotypic trophic adaptation[19], and the contemporary range in gill-raker number and total body shape disparity of individuals in a lake are correlated ($N = 15$, slope $= 0.49$; $R^2 = 0.36$, $P < 0.011$; Supplementary Fig. 6). Across lakes, the distances of species means from the historical midpoint of species means in a lake have become significantly smaller over time ($N = 19$, $t = 2.56$, d.f. $= 18$, $P = 0.020$). Extant species have converged in moderately and strongly polluted lakes ($N = 10$, $t = 2.43$, d.f. $= 9$, $P = 0.038$, Fig. 2e) but not in weakly and mildly polluted lakes ($N = 9$, $t = 1.06$, d.f. $= 8$, $P = 0.319$). Relative contemporary disparity (see phenotypic tests of speciation reversal in Supplementary Information) was significantly lower in moderately and strongly polluted lakes than in weakly and mildly polluted lakes ($N = 5$ (moderately and strongly polluted lakes), $N = 6$ (weakly and mildly polluted lakes), $t = 2.48$, d.f. $= 9$, $P = 0.035$; Fig. 2e). The best general linear model contained maximum phosphorus concentration, maximum lake depth and oxygenated depth, with phosphorus having the largest and most significant effect ($N = 10$ lakes, $R^2 = 0.85$, $P < 0.001$, ΔAICc $= 7.95$; regression coefficient for phosphorus -0.79, $P = 0.002$).

In all but two radiations, species with few gill-rakers spawn in shallow water, whereas species with many gill-rakers spawn deeper[22,23]. Speciation reversal predicts that the range in gill-raker number should contract from both ends of the distribution, whereas extinction through demographic decline of deep spawners predicts a contraction at the high end of the distribution. Consistent with speciation reversal, diversity has been lost from both ends of the distribution in each lake that experienced a range reduction (Table 1), independent of whether the two deep-spawning species with a low gill-raker count were included or not (mean loss at lower end is -3.4 or -2.7 gill rakers, respectively; Wilcoxon test: $Z = -2.54$, $N = 8$, d.f. $= 7$, $P = 0.011$ for both cases; mean loss at upper end is -2.75, $Z = -2.54$, $N = 8$, d.f. $= 7$, $P = 0.011$ for both cases). This result was robust to the removal of Lakes Murten, Hallwil and Pfaeffiker where natural recruitment had ceased and stocks are maintained by stocking from hatcheries (mean loss at lower end is -3.4 or -2.4 gill rakers, respectively; Wilcoxon test, $Z = -2.03$, $N = 5$, d.f. $= 4$, $P = 0.042$ for both cases; mean loss at upper end is -3; $Z = -2.03$, $N = 5$, d.f. $= 4$, $P = 0.010$ for both cases).

Thus, in cases in which several species persisted in sympatry after eutrophication, their phenotypes converged, and the extinction of species was associated with evolution to intermediate phenotypes in the remaining species. This is consistent with partial and complete speciation reversal, respectively.

Discussion

Our evidence suggests anthropogenic eutrophication has led to speciation reversal in whitefish radiations by increasing gene flow between previously ecologically differentiated species. Although divergent natural selection could in principle maintain species differences in the face of increased gene flow, eutrophication seems to have altered reproductive and ecological niche spaces to the degree that selection cannot counteract the homogenizing effects of gene flow. It is possible that accidental hybridization in hatcheries has contributed to interspecific gene flow. However, while reductions in genetic differentiation were related to eutrophication, hatcheries operate on all lakes. Thus, this alone cannot explain observed patterns of diversity loss.

The study lakes have lost 38% of species diversity, 14% of functional diversity and 28% of functional disparity among species. At least eight endemic species and seven distinct populations of extant species have become extinct (Table 1 and Supplementary Table 1). Only 4 of 17 lakes suffered no species loss. Among remaining species, genetic differentiation is reduced. This loss of species richness, phenotypic diversity and genetic differentiation occurred mainly unnoticed despite the commercial importance of whitefish. Similar large losses of whitefish diversity may have occurred in other lakes outside Switzerland (Supplementary Table 1) and the extinction of endemic char species pairs in some of the same lakes could have involved similar mechanisms[21]. Finally, we also note that similar patterns of diversity loss have been observed in several other taxa[4–6,15,34,35].

Loss of biodiversity through speciation reversal may be underappreciated for two reasons. First, the process can be difficult to detect because it does not require changes in distribution or abundance but can manifest through subtle changes in patterns of variation within

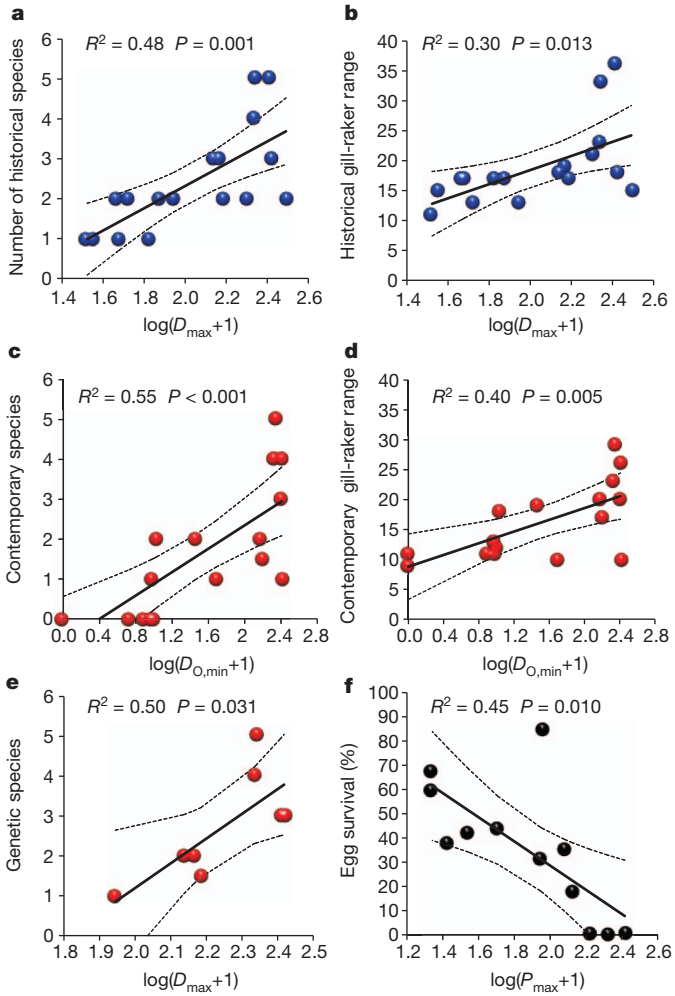

Figure 3 | Whitefish diversity explained by environmental variables. **a**, **b**, Historical whitefish diversity as species numbers (**a**) and the range in gill-raker numbers (**b**), plotted against maximum lake depth (D_{max}). **c**, **d**, Contemporary diversity, measured as species numbers (**c**) and the range in gill-raker numbers (**d**), plotted against oxygenated depth ($D_{O,min}$). **e**, The number of contemporary genetically differentiated species plotted against maximum lake depth. **f**, The relationship between the maximum phosphorus concentration (P_{max}) and the viability of the whitefish eggs in 12 lakes. The dashed lines represent the 95% confidence intervals for the regression line.

multi-species assemblages[4]. Second, speciation reversal is a potentially rapid process, by which species can collapse in just a few generations[5,6,14]. Compelling tests of speciation reversal will often require historic samples with DNA of sufficient quality. Our results add to a growing body of evidence suggesting that freshwater fish radiations, but also terrestrial radiations[14,15], are threatened by anthropogenic activities that disrupt the ecological conditions and evolutionary processes that promote adaptive radiation[4–6,35]. There is evidence from lake ecosystems that eutrophication-mediated speciation reversal may threaten diversity simultaneously at interacting trophic levels[36], and the effects on food webs require investigation. If the loss of ecologically dominant species, such as planktivorous fish, affects other ecosystem components, the impacts of speciation reversal may extend beyond the simple loss of species[37,38]. Regardless of the mechanistic details, preserving ecosystem services requires maintaining functional ecosystems, which in turn requires protecting the ecological conditions and evolutionary mechanisms that generate and maintain species diversity[2,37,39,40].

METHODS SUMMARY

Between 2004 and 2010 we collected 2,449 whitefish from 16 lakes. Muscle tissue was preserved in 100% ethanol for genetic analyses. The first gill arch was removed from 2,191 individuals for gill-raker counts (Table 1). Scale samples were used to analyse historical trends in genetic differentiation of species in Lakes Constance (1926–50: $N = 133$; 1969–80: $N = 92$) and Brienz (1952–70: $N = 66$). We collected data on historical species richness in 17 lakes and on contemporary richness in 16 lakes. We determined three different metrics of historical diversity and four metrics of contemporary diversity for each lake assemblage: first, species richness, identified using morphology, spawning ecology and taxonomic literature; second, the observed range in gill-raker number, a measure of heritable functional diversity; third, genetic species differentiation, using genotypes based on ten microsatellite loci (for methods see ref. 23); fourth, phenotypic distinctiveness of species using gill-raker mean counts. When possible, individual assignments to species was based on a Bayesian population inference algorithm (STRUCTURE version 2.3.3[41]; 30,000 burn in and 300,000 Markov chain Monte Carlo steps). Environmental variables for each lake were obtained from the literature[42] and government databases. Maximum phosphorus concentration corresponds to the highest value observed between 1951 and 2004. Oxygenated depth was the minimum depth range observed during the eutrophic phase with the water containing at least 2.5 mg l^{-1} dissolved oxygen (see environmental variables in Supplementary Information). Whitefish eggs were collected from the lake bottom in twelve lakes on several samplings between 1968 and 2008, and the percentage of normally developing eggs was calculated[43].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions P.V. contributed to conception and design of the study, collected fish, generated gill-raker and contemporary genetic data, and carried out most of the statistical analyses. D.B. collected fish, generated gill-raker, historical and contemporary genetic data. A.G.H. collected fish and generated gill-raker and geometric morphometric data. K.A.Y. participated in designing the study and writing the manuscript. R. M. collected and analysed egg data and contributed to fish collection. B.L.-H. contributed to fish, gill-raker, and genetic data collection. D.R. contributed to analyses and writing. S.D.P. contributed to collection of historical genetic data. C.R.L. supervised parts of sampling, gill-raker counting and contemporary genetic data collection. O.S. conceived and designed the project, supervised the project, and contributed to data analyses. P.V. and O.S. wrote the paper.

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METHODS

Sampling. Between 2004 and 2010 we collected 2,449 whitefish from 16 lakes. We collected at least 20 individuals of each known species from 16 lakes, except *C. heglingus* in Lake Zürich (17 individuals), and *C.* sp. *'Felchen'* in Lake Thun, which could not be obtained. In most lakes, we collected fish directly on the spawning grounds. In six lakes (Lakes Sempach, Walen, Lucerne, Thun, Brienz and Neuchâtel), fish were collected several times from many different spawning sites to distinguish intraspecific genetic population structure and species structure. No geographical or temporal differences within species could be observed (ref. 23 and B.L.H., personal communication). We sampled systematically along water-depth gradients during the spawning period in Lakes Neuchâtel[23] and Lucerne (B.L.-H., P.V., A.G.H., K. Lucek and O.S., unpublished data). The length, weight and sex of every fish was recorded. Muscle tissue was removed and preserved in 100% ethanol for DNA analysis. The first gill arch was removed from 2,191 individuals for gill-raker counting (Table 1). Scale samples were used for molecular genetic analyses of historical trends in species differentiation in Lake Constance (1926–50: $N = 133$; 1969–80: $N = 92$) and Lake Brienz (1952–70: $N = 66$).

Historical and contemporary diversity. We collected data on historical and contemporary diversity in 17 and 16 Swiss lakes, respectively. We determined three different metrics of historical and four of contemporary diversity (details in Supplementary Information). First, contemporary species richness was determined using the same traits and procedures as Steinmann in 1950, who determined historical species richness using morphological and meristic traits, and information on spawning ecology[22]; Second, contemporary ranges in gill-raker numbers were collected from our recent samples (see above) and historical gill-raker data were taken from Steinmann[22]. In whitefish, gill-raker number is related to feeding ecology[44] and is highly heritable (0.79)[19], and thus provides an ecologically meaningful and taxonomically independent (Supplementary Fig. 7) estimate of heritable functional diversity. To enable comparisons between historical and contemporary data when sample sizes were unequal or (for historical data) unknown, we used available data for each species to create normal distributions from which 100 virtual individuals were then randomly sampled. Third, genetic species differentiation was determined by genotyping historical and contemporary samples at 10 microsatellite loci. Details of laboratory methods that were used for contemporary samples are given in ref. 23. Whenever possible, the identification of sympatric genetically differentiated species and individual assignment were performed using the Bayesian population inference algorithm in STRUCTURE version 2.3.3 (ref. 41) (30,000 burn in and 300,000 MCMC steps). However, STRUCTURE is typically inefficient when $F_{ST} < 0.05$ (unless many loci are sampled)[45]. This was found to be the case between some species in Lake Lucerne and in Lake Zürich. A combination of morphology and spawning ecology was used to identify species in these lakes that was confirmed a posteriori by significant F_{ST} values observed between species sampled in sympatry. We calculated the extent of contemporary genetic differentiation among species in eight lakes, and the historical differentiation in two lakes; one that was moderately impacted (Lake Constance) and the other little impacted (Lake Brienz) by eutrophication. Fourth, phenotypic distinctiveness of species was determined using gill-raker mean counts for each species in each lake.

DNA extraction and PCR amplification of DNA from historical material. Total DNA was extracted from historical dried scales using a modified standard phenol-chloroform-ethanol extraction method[46]. The DNA quantity was measured using a Nanodrop ND-1000 (Nanodrop technologies) spectrophotometer and all samples containing less then 20 ng μl^{-1} DNA were excluded from further investigations. Polymerase chain reaction (PCR) was performed according to the QIAGEN Multiplex standard protocol with an annealing temperature (T_{AN}) of 57 °C and 35 cycles (Sets 1 and 3) or 45 cycles (Sets 2 and 4). Denatured fragments were resolved on an automated DNA sequencer (ABI 3100). Genotypes were determined with the GENEMAPPER 4.0 (ABI) software and checked visually. Each sample was amplified twice in a separate PCR. When both genotypes were identical we used these genotypes for further analysis (81.5% of all genotypes). When both genotypes were missing, no further attempt was taken to genotype a sample at that locus (8.9% of all genotypes). Finally, when only one of the two genotypes could be determined (9.6%), a third—or when needed, a fourth—separate PCR was performed to confirm genotypes. To estimate reproducibility, 28 samples were independently extracted and the procedure that is described above was repeated. 240 genotypes were compared and 8 mismatches were found (reproducibility, 96.7%.). Only individuals with a minimum of six successfully genotyped loci were considered for population genetic data analysis. The level of missing data in loci with large fragment lengths was considerable in historical populations (40.5% for locus CoCl-61, 26.4% for locus CoCl-10 and 14.5% for locus CoCl-45). Separate analyses excluding these loci yielded very similar results (data not shown). Therefore, all analyses were performed including all loci.

Environmental variables. Lake depths (m) and maximum phosphorus content (P_{tot} (mg m^3)) data were obtained from ref. 42. O_2 depth profiles (mg l^{-1}) (Supplementary Table 7) were obtained from the Federal Office for the Environment (FOEN), Swiss Federal Institute of Aquatic Science and Technology (EAWAG) and the Internationale Gewässerschutzkomission für den Bodensee (IGKB). For maximum phosphorus concentration, we took the highest value that was observed in time series covering the period from 1951 to 2004, which includes the onset and peak of the eutrophic phase and the re-oligotrophication that began in the 1980s. The maximum depth of a lake was the depth measured from the lake surface to the deepest point of the lake. Oxygenated depth was calculated as the minimum water depth range observed during the eutrophic phase with the water containing at least 2.5 mg l^{-1} dissolved oxygen. The limit of 2.5 mg l^{-1} was chosen to correspond to the critical oxygen level at which embryo development is negatively affected[47].

Egg survival data. Whitefish eggs were collected from the lake bottom in 12 lakes on several occasions between 1968 and 2008. Sampling was done in each lake between early January and early March, before the anticipated beginning of mass hatching of the corresponding whitefish species. As a comparative measure of egg viability, we calculated the percentage of eggs that developed normally. Details of the sampling methods can be found in ref. 43 and in Supplementary Information.

Statistical analyses. We used least squares regressions and an information theoretic approach to select the models that best explain the relationship between predictor and response variables (Supplementary Information). Before comparing data, we tested for significant deviations from normal distributions using a Shapiro Wilks test. For data that did not significantly deviate from normality, a standard or paired Welch's *t*-test was used. When the data significantly deviated from normality, a Wilcoxon signed rank or a Mann–Whitney U test was used. All tests were two-tailed.

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SUPPLEMENTARY INFORMATION

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Eutrophication causes speciation reversal in whitefish adaptive radiations

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Comparative quantification of whitefish diversity

Identification of historical species diversity

Whitefish species diversity in the European Alps is characterized by parallel adaptive radiations that occurred in all larger lakes[1-5]. In 1950, Steinmann published a detailed and data rich monograph on taxonomy, ecology and evolution of whitefish in these lakes[6]. Based on his own data and a detailed literature review, he used morphological and meristic traits (relative head size, mouth positioning, relative eye size, growth rates, number of scales along the lateral line, gill-raker counts), and reproductive ecology (spawning time, spawning depth and habitat) to identify and characterize the different species present in each lake. Even though he described them as biological species, he applied an antiquated taxonomical nomenclature where all species were named as intraspecific rankings within *Coregonus lavaretus*. Early molecular investigations confirmed for many lakes that these sympatric forms were distinct species[2, 3]. More recent taxonomical treatments[7, 8] resurrected binary species names for most of Steinmann's taxa. Recent genetic and morphological work confirmed and sometimes refined these classifications[1, 4, 9] (Table S1). We use the term "distinct populations" for populations from different lakes that are phenotypically similar and belong to the same genetic cluster[1], yet cannot have exchanged genes regularly and may have significant F_{ST} between them.

Identification of contemporary species diversity

In the first step, the contemporary presence or absence of historically described species was investigated for all the 16 lakes that we sampled. All whitefish sampled from spawning locations were aged using scale rings and from this data, growth rates were determined[10], and their gill-rakers were counted. The resulting data were compared to the descriptions of Steinmann[6] and Kottelat & Freyhof[8]. The data were further confirmed using the knowledge of local fisheries authorities and professional fishermen. A species was considered extinct when no recent observations, neither in our data nor by local authorities or professional fishermen, existed. Extinction included cases where subsequent to natural extinction whitefish have been maintained in hatcheries or introduced from hatchery stocks of mixed origins[1] (Tables 1 + S1). Such introductions explain why a "range in gill-raker numbers" can presently be observed in lakes whose endemic species are considered extinct. The complete list of known, extinct and extant species is shown in Table S1. Additionally, we summarized our data from Lake Bourget (France) and reviewed the literature regarding Bavarian and Austrian lakes and added to Table S1 a section on pre-alpine lakes outside of Switzerland.

For Figure 1 in the main text, we use the taxonomy of Kottelat & Freyhof[8] and local common names for as yet undescribed species, or species whose assignment to a described taxon is not clear († marks extinct species). (1) Lake Geneva: (i) *C. fera* † (ii) *C. hiemalis* † (from Jurine 1825); (2) Lake Neuchâtel: (i) *C. palea* (ii) *C. candidus*; (3) Lake Murten: (i) *C. palea* † (ii) *C. confusus* †; (4) Lake Biel: (i) *C. palea* (ii) *C. confusus*; (5) Lake Thun: (i) *C. albellus* (ii) *C. alpinus* (iii) *C. fatioi* (iv) *C. sp.* "Balchen" (v) *C. sp.* "Felchen"; (6) Lake Brienz: (i) *C. albellus* (ii) *C. sp.* "Balchen" (iii) *C. sp.* "Felchen"; (7) Lake Sempach: (i) *C. suidteri* † (ii) not described †; (8) Lake Lucerne: (i) *C. nobilis* (ii) *C. sp.* "Bodenbalchen" (iii) *C. zugensis* (iv) *C. sp.* "Schwebbalchen"; (9) Lake Zug: (i) *C. sp.* "Zugerbalchen" (ii) *C. sp.* "Zugeralbeli" †; (10) Lake Baldegg (i) *C. cf. suidteri* †; (11) Lake Hallwil (i) *C. cf. suidteri* †; (12) Lake Zuerich: (i) *C. duplex* (ii) *C. heglingus* (iii) *C. zuerichensis* †; (13) Lake Walen: (i) *C. duplex* (ii) *C. heglingus* (iii) *C. zuerichensis* †; (14) Lake Greifen: (i) *C. cf. duplex*; (15) Lake Pfaeffiker: (i) *C. cf. zuerichensis*; (16) Lake Constance: (i) *C. arenicolus* (ii) *C. macrophthalmus* (iii) *C. wartmanni* (iv) *C. gutturosus* † (from Steinmann 1950) (v) *C. sp.* "Weissfelchen"; (17) Lake Sarnen: (i) *C. sp.* "Sarnerfelchen" (ii) *C. sp.* "Sarnerbalchen" †.

Functional diversity as a taxonomy-independent measure of diversity

Steinmann provided data on the range of gill-raker counts for most Swiss whitefish species prior to 1950, before the most severe anthropogenic impacts had occurred[6]. The number of gill-rakers on the first gill arch varies

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considerably among species, is highly heritable[11] and is expected to correlate with feeding ecology[12]. As such, the observed diversity in gill-raker counts is a reasonable measure of genetically inherited functional diversity. The advantage of this diversity measure is that it is not influenced by taxonomic interpretations of species richness as it is based on individual fish independently of their taxonomic assignment. Unequal sample sizes can however affect the observed range in gill-raker numbers. For some species we could not recover information on Steinmann's sample sizes, whereas means and ranges were available. For others his sample sizes are published. Assuming that the unknown sample sizes of Steinmann were no smaller on average than those known, our contemporary sample sizes from lakes Geneva, Murten, Sempach, Zug, Hallwil, Greifen, Pfaeffiker, Constance and Sarnen were likely smaller than the historical sample sizes (Table 1). We therefore estimated gill-raker count normal distributions for each species in each of these lakes from the mean and variance in contemporary data (Table S6), and subsequently virtually sampled 100 individuals from these distributions for each species in each of these eight lakes. Because the accuracy of the estimate of standard deviation is affected by sample size, we estimated mean gill-raker counts and standard deviations from real data (N = 100 individuals, Species: *C. zugensis* from Lake Lucerne) for sample sizes of N = 1 to 100. The random sub-sampling was repeated 100 times to estimate 95 % confidence intervals. The results show that a sample size of $N > 20$ fishes yields relatively reliable estimates of means and standard deviations (Fig. S4). Least squares regressions and an information theoretic model selection approach (see below) were then used to identify the model that best explained the relationship between the range in gill-raker number and the number of observed species. It revealed that the pre-eutrophication range in gill-raker numbers was strongly positively correlated with the pre-eutrophication estimate of species richness (second order polynomial regression , N = 17 lakes, R^2 = 0.86, $P < 0.001$; Fig. S7a). Contemporary range in gill-raker numbers is similarly strongly correlated with the observed numbers of species present today (linear regression, N = 10 lakes, R^2 = 0.87, $P < 0.001$; Fig. S7b; 6 lakes were excluded because the contemporary whitefish population is artificially maintained in hatchery schemes), and also with molecular marker-based estimates of current minimum numbers of genetically differentiated species (linear regression, N = 8 lakes, R^2 = 0.79, P = 0.002; Fig. S7c). Therefore, the range in gill-raker numbers observed in a lake is a good surrogate for species richness, independent of taxonomic considerations.

Population genetic data analyses

For each species, expected (H_E) and observed (H_O) heterozygosity was calculated using Arlequin 3.1[13]. Deviations from Hardy-Weinberg equilibrium (HWE) were tested with Fisher's exact tests[14] for each locus and each species using GENEPOP 3.4[15] (1,000,000 steps in the Markov chain and 5,000 dememorization steps). Allelic richness (A_R) was calculated in FSTAT version 2.9.3[16] except for historical samples with a larger number of missing data for some loci (Table S3). Inbreeding coefficients (F_{IS}[17]) were calculated across all loci for all species and tested for significant deviations from zero using FSTAT. P-values for deviations from HWE and for F_{IS} are given in Table S3 and were corrected for multiple comparisons using the sequential Bonferroni method[18]. Deviations from linkage equilibrium between all pairs of loci for each species were tested using ARLEQUIN 3.1[13] with a significance level of $P < 0.01$. The global genetic differentiation (Global F_{ST}) of species within each lake was calculated in a hierarchical analysis of molecular variance[19] using ARLEQUIN 3.1[13].

Significant deviations from HWE and significant F_{IS} values after Bonferroni correction were observed only for two loci in the historical sample of *C. gutturosus* in Lake Constance. These loci (COCL-Lav61, COCL-Lav45) are characterized by longer DNA fragments (230-260 BP), suggesting an effect of DNA quality. Several deviations from HWE and several F_{IS} values were significant before correcting for multiple testing, the most significant ones were mostly found in historical samples. These deviations were likely due to non-amplifying alleles instead of population substructure. 35 out of 1395 pairwise linkage tests (2.51 %) were significant at a significance level of 0.01. Because they consisted of different pairs of loci in different populations and because at least six of the ten loci used in this study are known to be unlinked in North American whitefish (*C. clupeaformis*)[20], we conclude that they were the result of a type one error instead of physical linkage between loci. The global F_{ST} within lakes that currently contain more than one species ranged from 0.041 (Lake Zuerich) to 0.183 (Lake Brienz). Mean allelic richness within species ranged from 2.99 (*C. alpinus*, Lake Thun) to 4.70 (*C. Sp. "Schwebbalchen"* Lake Lucerne). Population genetic summary statistics are provided in Table S3.

Finally, we analysed the frequency of five alleles that were historically found only in the extinct summer, deep-spawning *C. gutturosus* of Lake Constance (Table S4). In our contemporary samples, four of these occur in other species. Because their frequencies in contemporary samples of other species were low (0.012 - 0.038), we checked how likely their absence from our historical samples of the other species was due to chance. We calculated the probability (L_{Hist}) to observe each (or at least one) putatively (historically) private *gutturosus* allele in a binomial distribution where the rare allele was expected to have a frequency equal to its frequency in contemporary species (Table S4) and N was set to the sample size of our historical (1926 - 1950) samples. The results are shown in Table S4. Our estimate is conservative because for the calculation of current frequencies in other species of originally private *gutturosus* alleles, we excluded the *gutturosus* alleles found in 4 contemporary individuals that were genetically assigned to *C. gutturosus*, with moderate probability, but did not belong to that species. We did the same analysis for the summer, deep-spawning species *C. albellus* of little-polluted Lake Brienz. These results are also presented in Table S4.

Note also that our test of speciation reversal, *i.e.* testing the prediction of eroding F_{ST} between sympatric species may be conservative. This is because speciation reversal may be undetectable in F_{ST} of a multi-species assemblage in the special situation where one species in a collapsed species pair was genetically more distinct than the average species in the assemblage, and hybridisation was contained within this pair. In this case, speciation reversal could even be accompanied by an increase in pairwise F_{ST} between the surviving species.

Environmental variables

There are many data for biotic and abiotic variables available for all the large pre-alpine lakes of Europe. Because they affect the availability and diversity of spawning and feeding habitat[4, 21], we predicted that lake depth, nutrient load and oxygenation of the water column would influence whitefish species richness (see main text). Lake depths and maximum phosphorus content (Ptot-P [mg/m^3]) data were obtained from[22]. We used 6499 O_2 depth profiles (mg/l) collected between 1921 and 2011 (Table S7) to calculate the maximum depth at which the water contained at least 2.5 mg/l dissolved oxygen, corresponding to the critical oxygen level at which embryo development is negatively impacted[23]. It thus corresponds to the biologically available depth for successful whitefish recruitment. The minimum of these available depth values observed in the time series for each lake was taken for further analyses (Oxygenated depth).

Regression model selection

We used Least Squares Regressions and an information theoretic approach to select the models that best explain the relationship between predictor (environmental) and response (whitefish diversity) variables (Table S2). For each model, we estimated Akaike's information criterion[24], corrected for sample size and model complexity[25], as an estimation of model fit (AICc). In the model selection procedure, we tested all variables independently with increasing model complexity (i.e. from a linear model (y = a + bx) to a polynomial model of second order (higher order polynomials were explored, but the results yielded little or no decrease in AICc)). This was repeated until AICc reached minimal values. A model was considered more likely when ΔAICc $\geq$4[25]. When two models had similar values (ΔAICc<4), the model with lower complexity (i.e. lower model d*f*) was selected. If two models had similar AICc values and identical model complexity, the model with the highest R^2 value was selected. All analyses are summarized in Table S2. The polynomial regression results are only shown when the model was more likely than the linear model. We also tested whether including multiple predictor variables in the model (stepwise forward selection) considerably increased ΔAICc (i.e. ΔAICc $\geq$4). This was however never the case (results not shown). To test if an environmental variable still predicted whitefish diversity in the original pre-pollution radiation when the historical and phylogenetic non-independence was taken into account, we calculated a linear model by which lakes were nested in independent watersheds as follows: (1) Lake Geneva; (2) Lakes Murten, Neuchâtel, Biel; (3) Lakes Thun, Brienz; (4) Lake Sempach; (5) Lakes Sarnen, Lucerne, Zug; (6) Lakes Hallwil, Baldegg; (7) Lakes Zuerich, Walen; (8) Lakes Pfaeffiker, Greifen; (9) Lake Constance.

Egg survival data

Different species of whitefish spawn either directly over the lake bottom or in the water column. The eggs of all species settle to the sediment surface, where their development requires a well-oxygenated sediment surface[6]. To test if egg development was constrained by eutrophication-induced oxygen depletion, whitefish eggs were collected from the lake bottom in 12 lakes at multiple times between 1968 and 2008. The sample sizes (number of eggs analysed/number of sampling years) were as follows: Lake Constance (7169/15), Lake Sempach (5106/16), Lake Hallwil (4513/13), Lake Thun (2223/3), Lake Zug (1852/6), Lake Biel (1404/3), Lake Geneva (1400/2), Lake Sarnen (675/6), Lake Zuerich (649/2), Lake Lucerne (399/3), Lake Walen (90/1), Lake Neuchâtel (32/1). All eggs were taken to the laboratory, sorted and grouped into six classes: (1) Developing normally [D_N]; (2) developing but embryo deformed [D_D]; (3) unfertilized [U]; (4) dead/undeterminable [T]; (5) empty with a small hole [E] (a sign of invertebrate predation); (6) empty/split open (hatched) [H]. As a comparative measure of egg viability, we calculated the percentage of eggs that developed normally (containing almost hatched embryos of developmental stage 12-13[26]) plus hatched egg shells among all possibly fertilized eggs (Sum of D_N, D_D, T and H) using the following equation:

$$\frac{D_N + H}{D_N + D_D + T + H} \times 100$$

Phenotypic tests of speciation reversal

To the extent that speciation reversal is driven by the convergence of two or more previously distinct fitness peaks in eco-morphological phenotype space, the hypothesis of speciation reversal makes two predictions of relative disparity loss: i) where speciation reversal is complete, i.e. one hybrid population is now found in place of two species, the remaining population is phenotypically intermediate to the original pair of species. ii) where speciation reversal is incomplete, phenotypes would converge on the historical midpoint of species means without reaching it. We tested these predictions using historical and contemporary data on gill-raker counts (Table S1). We calculated for each species its historical mean gill-raker count, and found for each lake assemblage the range of historical species means (a measure of assemblage disparity) and its midpoint. We then compared the distance of historical and contemporary species means from the midpoint of the historical range, using *t* tests. When two or more species had survived in a lake that used to have more species, we took the historical range midpoint of only those species still present today. When more than two species still existed in a lake, we used only

the phenotypically extreme species among those remaining to calculate distances from historical midpoints. These procedures are conservative with regard to our test. As predicted by speciation reversal, the species distances from the historical midpoint have significantly decreased between the first half of the 20th century and now (N = 19, t = 2.56, df = 18, P = 0.020), and this trend was much stronger in the more strongly polluted lakes (N = 10, t = 2.43, df = 9, P = 0.038) than in the mildly and weakly polluted lakes (N = 9, t = 1.06, df = 8, P= 0.319). Finally, we calculated the relative contemporary disparity for each lake as the ratio of [contemporary distance of a species' mean from the historical range midpoint]/[historical distance of that same species' mean from the historical range midpoint], averaged over the two phenotypically most extreme species still present today in each lake. This relative contemporary disparity was significantly more strongly reduced in more strongly polluted (> 50 ug P/l) than in mildly and weakly polluted lakes (N_1 = 5, N_2 = 6, t = 2.48, df = 9, P = 0.035). Relative contemporary disparity was 28% lower than historical disparity on average.

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Table S1. Complete compilation of the known whitefish diversity in pre-alpine lakes with reference to recent taxonomic treatments.

Lake	Nr.	Steinmann (1950)	Kottelat & Freyhof (2007)	Present	ecotype	historical GR mean	contemp. GR mean	Growth/Adult Size	Spawning season	Spawning depth	extinction
Geneva*	1 (i)	*rhodanensis*, ecot. *primigenius*	*C. fera*	*C. fera*	LGR	26.4	-	fast/large	W	Deep	extinct
Geneva*	1 (ii)	*rhodanensis*, ecot. *nanus*	*C. hiemalis*	*C. hiemalis*	LGR	28.5	-	intermediate	W	Shallow	extinct
Geneva*		not known to author	*C. lavaretus*	*C. lavaretus or C. palaea*	LGR	-	27.7	fast/large	W	Shallow	introduced?
Neuchâtel*	2 (i)	*jurassica*, ecot. *primigenius*	*C. palaea*	*C. palaea*	LGR	26	29.1	fast/large	W	Shallow	
Neuchâtel*	2 (ii)	*jurassica*, ecot. *nanus*	*C. candidus*	*C. candidus*	MGR	32.5	32.2	slow/small	W	Deep	
Murten*	3 (i)	*jurassica*, ecot. *primigenius*	*C. palea*	*C. palea*	LGR	26	26.6	fast/large	W	Shallow	extinct
Murten*	3 (ii)	*jurassica*, ecot. *nanus*	*C. confusus*	*C. confusus*	MGR	32.5	-	slow/small	W	Deep	extinct
Biel*	4 (i)	*jurassica*, ecot. *primigenius*	*C. palaea*	*C. palaea*	LGR	26	27.6	fast/large	W	Shallow	
Biel*	4 (ii)	*jurassica*, ecot. *nanus*	*C. confusus*	*C. confusus*	MGR	32.5	33.2	slow/small	W	Deep	
Biel*		not known to author	not known to authors	*C. cf. albellus**	HGR	-	-	slow/small	S	Deep	
Thun	5 (ii)	*arurensis*, ecot. *profundus*	*C. alpinus*	*C. alpinus*	VLGR	(13-25)	22.8	slow/small	S	Deep	
Thun	5 (iv)	*arurensis*, ecot. *litoralis*	not known to authors	*C. sp.* "Balchen" *sp.nov.*	LGR	(20-37)	30.6	fast/large	W	Shallow	
Thun	5 (v)	not known to author	not known to authors	*C. sp.* "Felchen" *sp.nov.*	MGR	-	34.6	intermediate	W	Deep	
Thun	5 (iii)	*arurensis*, ecot. *primigenius*	*C. fatioi*	*C. fatioi*	MGR	(22-36)	34.7	intermediate	W	Intermediate	
Thun	5 (i)	*arurensis*, ecot. *nanus*	*C. albellus*	*C. albellus*	HGR	(32-42)	38.1	slow/small	S	Deep	
Brienz	6 (ii)	not known to author	not known to authors	*C. sp.* "Balchen" *sp.nov.*	LGR	-	28.8	fast/large	W	Shallow	
Brienz	6 (iii)	*arurensis*, ecot. *primigenius*	*C. fatioi*	*C. sp.* "Felchen" *sp.nov.*	MGR	35.3	37.3	intermediate	W	Intermediate	
Brienz	6 (i)	*arurensis*, ecot. *nanus*	*C. albellus*	*C. albellus*	HGR	40.6	41.2	slow/small	S/W	Deep	
Sempach	7 (ii)	"kleiner Balchen des Sempachersees"	not considered	*not found*	LGR	-	-	slow/small	W	?	extinct
Sempach	7 (i)	*intermedia*, ecot. *Primigenius*	*C. suidteri*	*C. suidteri*	MGR	33.5	31.9	fast/large	W	Shallow	*?*
Lucerne	8 (ii)	*riusensis*, ecot. *primigenius*	*C. suidteri*	*C. sp.* "Bodenbalchen" *nov.sp*	LGR	24.4	29.4	fast/large	W	Shallow	
Lucerne	8 (iv)	not known to author	not known to authors	*C. sp.* "Schwebbalchen" *sp.nov.*	MGR	-	33.2	intermediate	W	Intermediate	
Lucerne	8 (i)	*riusensis*, ecot. *pelagicus*	*C. nobilis*	*C. nobilis*	HGR	36	37.3	intermediate	S	Deep	
Lucerne	8 (iii)	*riusensis*, ecot. *nanus*	*C. zugensis*	*C. zugensis*	HGR	37.4	38.8	slow/small	S/W	Deep	
Zug	9 (i)	*riusensis*, ecot. *primigenius*	*C. suidteri ?*	*C. sp.* "Zugerbalchen" *sp.nov.*	LGR	(19-24)	28.3	fast/large	W	Shallow	*?*
Zug	9 (ii)	*riusensis*, ecot. *nanus*	*C. zugensis*	*C. sp.* "Zugeralbeli"	HGR	(24-39)	-	slow/small	S/W	Deep	extinct
Baldegg	10 (i)	*intermedia*, ecot. *primigenius*	*C. suidteri*	*C. cf. suidteri*	MGR	32	-	fast/large	W	Shallow	extinct
Hallwil	11 (i)	*intermedia*, ecot. *primigenius*	*C. suidteri*	*C. cf. suidteri*	MGR	32	31.9	fast/large	W	Shallow	extinct
Zuerich	12 (i)	*lindimacensis*, ecot. *litoralis*	*C. duplex*	*C. duplex*	LGR	26.7	28.6	fast/large	W	Shallow	
Zuerich	12 (iii)	not considered by author	*C. zuerichensis*	*C. zuerichensis*	MGR	-	-	intermediate	W	Pelagic	extinct
Zuerich	12 (ii)	*lindimacensis*, ecot. *nanus*	*C. heglingus*	*C. heglingus*	HGR	35.2	36.5	slow/small	S/W	Deep	S extinct****
Walen	13 (i)	*lindimacensis*, ecot. *litoralis*	*C. duplex*	*C. duplex*	LGR	25.8	26.1	fast/large	W	Shallow	
Walen	13 (iii)	*lindimacensis*, ecot. *pelagicus*	*C. zuerichensis*	*C. zuerichensis*	MGR	34.1	-	intermediate	W	Pelagic	extinct
Walen	13 (ii)	*lindimacensis*, ecot. *nanus*	*C. heglingus*	*C. heglingus*	HGR	35.4	35.6	slow/small	S/W	Deep	
Greiffen	14 (i)	*intermedia*, ecot. *primigenius*	*C. zuerichensis*	*C. cf. duplex*	LGR	29.1	29.7	fast/large	W	Shallow	extinct
Pfaeffiker	15 (i)	*intermedia*, ecot. *primigenius*	*C. zuerichensis*	*C. cf. zuerichensis*	MGR	29.8	30.8	fast/large	W	Shallow	extinct
Constance	16 (iv)	*bodanensis*, ecot. *profundus*	*C. gutturosus*	*C. gutturosus*	VLGR	19.97	-	slow/small	S	Deep	extinct
Constance	16 (i)	*bodanensis*, ecot. *litorlis*	*C. arenicolus*	*C. arenicolus*	LGR	22.35	31.1	fast/large	W	Shallow	
Constance	16 (v)	*bodanensis*, ecot. *primigenius*	*C. arenicolus*?	*C. sp. "Weissfelchen" sp.nov.*	MGR	33.35	-	intermediate-fast/large	W	Int.	
Constance	16 (iii)	*bodanensis*, ecot. *pelagicus*	*C. wartmanni*	*C. wartmanni*	HGR	36.15	34.5	intermediate	W	Pelagic	
Constance	16 (ii)	*bodanensis*, ecot. *nanus*	*C. macrophthalmus*	*C. macrophthalmus*	HGR	40.65	34.8	intermediate	W	Int.	
Sarnen	17 (ii)	*riusensis*, ecot. *Primigenius*	not considered	*C. sp.* "Sarnerbalchen"	LGR	36	-	fast/large	W	Shallow	extinct
Sarnen	17 (i)	*riusensis*, ecot. *pelagicus-nanus*	*C. zugensis*?	*C. sp.* "Sarnerfelchen" *sp.nov.?*	HGR	38-41	35.5	slow/small	W	Deep	
Pre-alpine lakes in France, Bavaria and Austria not studied by us (except Lake Bourget, France) (historical data for Bavaria and Austria from Wagler 1937)											
Bourget			*C. lavaretus*	*C. lavaretus*	HGR	(33-42)	(26-38)	fast/large	W	Shallow	
Bourget			*C. bezola*	*C. bezola*	LGR	(26-33)	-	intermediate	W	Deep	extinct
Atter		"Blaufelchen"	*C. atterensis*	not assessed by us	LGR	(24-34)		intermediate	W	Pelagic	
Atter		"Gangfisch"	*C. austriacus*	not assessed by us	MGR	(30-39)	-	slow/small	W	Deep	extinct?
Ammer		"Gangfisch"	*C. renke*	not assessed by us	HGR	(31-40)	(31-35?)	intermediate	W	Pelagic	
Ammer		"Kilch"	*C. bavaricus*	not assessed by us	VLGR	(18-21)	(19-30)	slow/small	S	Deep	almost extinct
Ammer		"Sandfelchen"		not assessed by us	LGR		-		W	Shallow	extinct
Traun			*C. renke*	not assessed by us	M or HGR	(30-40)		intermediate	W	?	
Traun			*C. danneri*	not assessed by us	HGR	(30-42)		slow/small	?	?	
Starnberger			*C. renke*	not assessed by us	MGR	(30-33)	(31-35?)	intermediate	W	Shallow	
Starnberger			*C. sp.*	not assessed by us	VHGR	(37-44)	-	slow/small	W	Pelagic	extinct
Chiem		"Blaufelchen"		not assessed by us	HGR	(23-34)	-	intermediate		Pelagic	extinct?
Chiem		"Gangfisch"	*C. sp.*	not assessed by us	MGR	(20-30)		slow/small		Int.	
Chiem		"Sandfelchen"	*C. hoferi*	not assessed by us	LGR	(24-32)	-			Shallow	probably extinct

Classifications of Steinmann[6] (all species were considered subspecies and ecotypes of *Coregonus lavaretus*), Kottelat & Freyhof[8] and our own work[1, 4, 9, 27]. Ecological and eco-morphological characteristics: Gill-raker counts (VLGR=Very low (15-25) gill-raker count, LGR=Low gill-raker count (20 - 32), MGR=Medium gill-raker count (27 - 37), HGR=High gill-raker count (35 - 47)); Growth rate and adult size; Spawning time (W=Winter (Nov-Feb), S=Summer (May - Oct), S/W = with summer and winter spawning populations (May-Feb)); Spawning depth (Deep: > 30m, Intermediate: > 10m & < 30m, Shallow: < 10m); and extinction status.

* Fatio in 1890[5] suggested that 3 species were present in each of these lakes. Steinmann[6] could distinguish just 2 species but did not examine Lake Murten fish. Finally, Kottelat & Freyhof[8] applied the Fatio taxonomy to Lake Murten, whereas they applied the Steinmann taxonomy to Lake Biel and express uncertainty in Lake Neuchâtel.

** Kottelat & Freyhof applied the Dottrens[28] taxonomy in Lake Geneva and described three species. Fatio and Steinmann recognized just two species, both of which are extinct. Our genetic data [1] suggest the extant third species was introduced, consistent with Steinmann.

*** This third species stems from a recent colonization by a species from Lake Thun (*C. cf. albellus;* Bittner et al., in preparation). The colonisation was made possible by a deviation of the Aare river (outflow of Lake Thun), that today drains into Lake Biel, which was historically not the case. Here we consider that two species were historically present in Lake Biel.

**** The summer spawning population in Lake Zürich is extinct.

Table S2. Summary of model selection

Response variable [y]	Predictor variable [x]	Model	Direction	*N*	k	Df	AICc	Adj. R2	*P-value*
Diversity metrics:									
Historical GR count range	N Hist. spec.	y=a+bx	positive	17	2	15	**44.60**	0.73	<0.001
Historical GR count range	**N Hist. Spec,**	**$y=a+bx+cx^2$**	positive	**17**	**3**	**14**	**34.63**	**0.86**	**<0.001**
Contemp. GR count range	**N Pres. spec.**	**y=a+bx**	**positive**	**11**	**2**	**9**	**20.56**	**0.87**	**<0.001**
Contemp. GR count range	N Pres. spec.	$y=a+bx+cx^2$	positive	11	3	8	20.50	0.90	<0.001
Contemp. GR count range	**N Pres. gen. spec.**	**y=a+bx**	**positive**	**8**	**2**	**6**	**16.95**	**0.79**	**0.002**
Contemp. GR count range	N Pres. gen. spec.	$y=a+bx+cx^2$	positive	8	3	5	20.58	0.77	0.011
Explaining historical diversity:									
N historical species	**Log(Oxygenated depth+1)**	**y=a+bx**	**positive**	**17**	**2**	**15**	-1.76	**0.51**	**<0.001**
N historical species	Log(Maximum depth+1)	y=a+bx	positive	17	2	15	-0.18	0.48	0.001
N historical species	Log(Maximum phosphor+1)	y=a+bx	negative	17	2	15	1.92	0.41	0.003
Historical GR count range	Log(Oxygenated depth+1)	y=a+bx	positive	17	2	15	62.38	0.24	0.026
Historical GR count range	**Log(Maximum depth+1)**	**y=a+bx**	**positive**	**17**	**2**	**15**	**60.65**	**0.30**	**0.013**
Historical GR count range	Log(Maximum phosphor+1)	y=a+bx	-	17	2	15	65.53	0.07	0.162
Explaining historical diversity: controlling for evolutionary nonindependence									
N historical species	**Log(Oxygenated depth+1)**	**y=a+bx**	**positive**	**9**	**2**	**7**	**1.50**	**0.53**	**0.016**
N historical species	Log(Maximum depth+1)	y=a+bx	positive	9	2	7	1.92	0.51	0.019
N historical species	Log(Maximum phosphor+1)	y=a+bx	negative	9	2	7	2.51	0.47	0.024
Historical GR count range	**Log(Oxygenated depth+1)**	**y=a+bx**	**-**	**9**	**2**	**7**	35.55	**0.32**	**0.065**
Historical GR count range	Log(Maximum depth+1)	y=a+bx	-	9	2	7	36.50	0.24	0.100
Historical GR count range	Log(Maximum phosphor+1)	y=a+bx	-	9	2	7	37.95	0.11	0.199
Explaining contemporary diversity:									
N contemp. species	**Log(Oxygenated depth+1)**	**y=a+bx**	**positive**	**17**	**2**	**15**	5.44	**0.55**	**<0.001**
N contemp. species	Log(Maximum depth+1)	y=a+bx	positive	17	2	15	7.40	0.49	<0.001
N contemp. species	Log(Maximum phosphor+1)	y=a+bx	negative	17	2	15	8.24	0.47	0.001
Contemp. GR count range	**Log(Oxygenated depth+1)**	**y=a+bx**	**positive**	**16**	**2**	**14**	53.22	**0.40**	**0.005**
Contemp. GR count range	Log(Maximum depth+1)	y=a+bx	positive	16	2	14	55.13	0.32	0.013
Contemp. GR count range	Log(Maximum phosphor+1)	y=a+bx	negative	16	2	14	55.50	0.30	0.016
N "genetic species"	Log(Oxygenated depth+1)	y=a+bx	-	8	2	6	5.43	0.28	0.103
N "genetic species"	**Log(Maximum depth+1)**	**y=a+bx**	**positive**	**8**	**2**	**6**	**2.53**	**0.50**	**0.031**
N "genetic species"	Log(Maximum phosphor+1)	y=a+bx	-	8	2	6	5.45	0.28	0.104
Contemporary genetic differentiation:									
Global F_{ST}	Log(Oxygenated depth+1)	y=a+bx	-	8	2	6	-44.64	0.32	0.085
Global F_{ST}	Log(Maximum depth+1)	y=a+bx	-	8	2	6	-44.75	0.33	0.081
Global F_{ST}	**Log(Maximum phosphor+1)**	**y=a+bx**	**negative**	**8**	**2**	**6**	**-55.93**	**0.83**	**<0.001**
Diversity loss:									
GR count range loss	Relative depth loss	y=a+bx	-	16	2	14	-55.06	-0.01	0.358
GR count range loss	Log(Maximum phosphor+1)	y=a+bx	-	16	2	14	-51.04	0.09	0.132
Species loss	Relative depth loss	y=a+bx	positive	17	2	15	-34.80	0.40	0.004
Species loss	**Log(Maximum phosphor+1)**	**y=a+bx**	**positive**	**17**	**2**	**15**	**-38.09**	**0.50**	**<0.001**
Effect of pollution on oxygenated lake depth:									
Log(Maximum phosphor+1)	**Log(Oxygenated depth+1)**	**y=a+b x**	**negative**	**17**	**2**	**15**	**-24.08**	**0.70**	**<0.001**
Log(Maximum phosphor+1)	Log(Oxygenated depth+1)	$y=a+bx+cx^2$	positive	17	3	14	-25.32	0.7498	<0.001

Given are the predictor and the response variables included in each model, the model equations (Model), the direction of the relationship among the variables (Direction), sample size (*N*), number of parameters included in each model (k), degrees of freedom (d*f*), Akaike's information criterion as an estimation of model fit including a second order correction for small sample sizes (AICc), the R^2 adjusted for model complexity for each model and the corresponding *P*-value. The best fitting models for each analysis are highlighted in bold characters. GR count = gill-raker count. *N* "genetic species" refers to the minimum number of genetically differentiated sympatric species recognized by STRUCTURE using 10 microsatellite loci.

Table S3. Summary of population genetic analyses

Lake	Species	*N*	Global F_{ST}	H_O	H_E	*P*-Value	A_R	F_{IS}	*P*-Value	N_{LD} $P < 0.01$
Lake Sempach	*C. suidteri*	60	-	0.50	0.53	0.530	4.24	0.043	0.068	0
Lake Neuchâtel	*C. palea*	193	0.056	0.55	0.54	0.569	3.94	-0.032	0.029°	0
	C. candidus	226		0.57	0.55	0.130	4.29	-0.025	0.044°	0
Lake Walen	*C. duplex*	186	0.110	0.45	0.48	0.045°	3.46	0.052	0.004°°	1
	C. heglingus	338		0.47	0.47	0.986	3.45	0.006	0.337	1
Lake Lucerne*	*C. sp.* "Bodenbalchen"	137	0.068	0.57	0.59	0.667	4.32	0.020	0.125	0
	C. sp. "Schwebbalchen"	118		0.57	0.59	0.016°	4.70	0.027	0.419	4
	C. zugensis	276		0.48	0.49	0.226	4.29	0.024	0.033°	0
	C. nobilis	83		0.48	0.48	0.777	3.68	-0.006	0.419	2
Lake Thun	*C. sp.* "Balchen"	60	0.144	0.49	0.49	0.977	3.31	-0.013	0.354	2
	C. fatioi	47		0.58	0.55	0.542	3.99	-0.061	0.031°	1
	C. albellus	56		0.46	0.46	0.463	3.67	-0.016	0.330	0
	C. alpinus	89		0.48	0.47	0.880	2.99	-0.037	0.099	2
Lake Zürich*	*C. duplex*	45	0.041	0.51	0.54	0.244	3.67	0.015	0.116	1
	C. heglingus	17		0.54	0.54	0.147	4.32	0.041	0.429	0
Lake Brienz 1952-1970)	*C. albellus*	26	0.166	0.39	0.40	0.062	3.49	0.025	0.305	3
	C. sp. "Balchen"	17		0.40	0.45	0.161	***	0.119	0.042°	1
	C. sp. "Felchen"	23		0.45	0.52	0.030	3.48	0.124	0.012°	2
Lake Brienz (2003-2006)	*C. albellus*	102	0.183	0.42	0.42	0.477	3.32	-0.002	0.489	0
	C. sp. "Balchen"	67		0.41	0.43	0.625	3.09	0.054	0.051	0
	C. sp. "Felchen"	89		0.48	0.49	0.207	3.40	0.026	0.172	1
Lake Constance (1926-1950)	*C. arenicolus*	24	0.108 (0.165)	0.38	0.45	0.143	***	0.176	0.003°°	2
	C. macrophthalmus	22		0.48	0.51	0.170	***	0.061	0.127	2
	C. wartmanni	22		0.43	0.51	0.017°	***	0.177	0.007°°	0
	C. gutturosus	65		0.40	0.45	0.001°°°	3.29	0.104	0.001°°°	3
Lake Constance (1969-1980)	*C. arenicolus*	18	0.072 (0.076)	0.46	0.47	0.613	***	-0.005	0.463	1
	C. macrophthalmus	25		0.60	0.59	0.198	***	-0.022	0.327	2
	C. wartmanni	48		0.51	0.49	0.278	3.99	-0.040	0.127	1
	C. gutturosus **	1		-	-	-	-	-	-	-
Lake Constance (1990-2004)	*C. arenicolus*	50	0.046 (0.047)	0.52	0.53	0.947	3.56	0.019	0.280	2
	C. macrophthalmus	46		0.59	0.56	0.429	4.01	-0.055	0.050	0
	C. wartmanni	25		0.56	0.56	0.165	4.01	-0.003	0.483	1
	C. gutturosus **	4		-	-	-	-	-	-	-
Total/overall		2605		0.49	0.50		3.76			35 (2.51%)

° $P < 0.05$; °°$P < 0.01$; °°° Significant after Bonferroni correction.

* STRUCTURE failed to detect genetic clustering among samples from Lakes Lucerne and Zürich. However, all pairwise F_{ST} were significant (all $p < 0.05$) and $F_{ST} > 0.015$. Ecological and morphological information (spawning time, spawning depth, growth rates, gill-raker counts) was used to assign fish to species.

** Some fish where assigned to *C. gutturosus* by STRUCTURE. The morphology (gill-rakers) and the spawning ecology of those fish (winter-spawning versus summer-spawning for *C. gutturosus*) suggests that these individuals are introgressed individuals that carry a higher frequency of *C. gutturosus* microsatellite alleles without bearing phenotypic resemblance to that species.

*** There was too much missing data for some loci to calculate an accurate A_R estimate

Population genetic summary statistics for contemporary samples of 20 species from eight lakes, and historical samples of seven species from Lakes Brienz and Constance. Given are the sample sizes for each species and sampling period (*N*), the global F_{ST} value calculated over all species within each lake and sampling period (Global F_{ST}). The Global F_{ST} for all three Lake Constance sampling periods (1926-1950, i.e. before the onset of eutrophication; 1969-1980, during the peak of the eutrophication; 1990-2004, after the peak eutrophication) were calculated twice, once excluding *C. gutturosus* and once including it (in brackets) this species, which was phenotypically extinct after 1969. Observed (H_O) and expected heterozygosity (H_E) with corresponding *P*-value of Fishers exact test of deviation from HWE across all loci, the inbreeding coefficient (F_{IS}) with corresponding *P*-value and the number of deviations from linkage equilibrium (N_{LD}) out of a total of 45 pairs of loci observed at a significance level of $P < 0.01$.

Table S4. Analyses of historical versus contemporary allellic richness.

Locus	Lake Constance						Lake Brienz					
	C. wartmanni		*C. macrophthalmus*		*C. arenicolus*		*C. sp.* "Balchen"		*C. albellus*		*C. sp.* "Felchen"	
	1926-1950	1990-2004	1926-1948	1990-2004	1935-1948	1990-2004	1952-1970	2003-2006	1952-1970	2003-2006	1952-1970	2003-2006
	N = 22	*N* = 25	*N* = 22	*N* = 46	*N* = 24	*N* = 50	*N* = 17	*N* = 67	*N* = 26	*N* = 102	*N* = 23	*N* = 89
BWF2	2.11	2.07	3.12	3.93	3.76	4.11	4.12	2.85	4.17	5.25	4.79	3.95
C2-157	2.66	2.53	2.66	2.40	1.62	1.75	4.00	2.99	1.92	1.94	2.53	2.41
COCL-Lav10	2.91	3.46	2.70	3.22	2.53	3.16	2.00	2.28	3.77	3.00	2.68	2.79
COCL-Lav18	2.49	3.22	2.57	2.90	2.68	2.89	2.68	2.02	3.32	3.13	3.79	2.96
COCL-Lav4	2.32	2.66	2.17	2.22	2.27	2.22	3.00	3.26	2.00	1.99	2.00	2.52
COCL-Lav45	1.94	2.00	1.68	1.65	1.62	1.67	2.68	2.88	2.00	2.27	2.12	2.28
COCL-Lav49	3.56	4.12	2.93	3.36	3.64	3.12	2.94	2.98	3.61	3.54	3.64	3.65
COCL-Lav6	1.98	2.66	3.08	3.34	2.86	2.90	6.43	4.92	8.61	9.64	6.58	8.21
COCL-Lav61	1.00	2.15	1.90	2.04	1.95	2.09	4.85	4.19	5.42	5.42	5.73	5.27
COCL-Lav68	3.12	3.21	3.09	3.22	2.03	2.76	2.00	2.00	2.00	2.00	2.00	2.00
Mean	2.41	2.81	2.59	2.83	2.49	2.67	3.47	3.04	3.68	3.82	3.59	3.60

Allelic richness observed at ten microsatellite loci in historical and contemporary samples of the same three whitefish species from Lake Constance and three species from Lake Brienz. Allelic richness has been calculated for each lake independently. Because allelic richness is corrected for effects of sample size, estimates differ from the values given in Table S3 where the calculation was done across all species from all lakes.

Table S5. Private allele analyses.

Sample	*N* samples	*N* samples	Frequency of private alleles historically found in *C. gutturosus*												L_{Hist}
	1926-1950	1990-2004	1	2	3	4	5	6	7	8	9	10	11		
C. gutturosus 1926-1950	65	-	0.008	0.015	0.031	0.009	0.008	0.015	0.023	0.354	0.008	0.021	0.008		
*C. arenicolus*1990-2004	24	50		0.038											0.844
C. wartmanni 1990-2004	22	25				0.021									0.607
C. macrophthalmus 1990-2004	22	46						0.012		0.012					0.657
C. gutturosus-like 1990-2004	-	4											0.125		
Total															0.979

Sample	*N* samples	*N* samples	Frequency of private alleles historically found in *C. albellus*												L_{Hist}
	1952-70	2003-05	1	2	3	4	5	6	7	8	9	10	11	12	
C. albellus	26	102	0.019	0.019	0.038	0.019	0.019	0.019	0.058	0.019	0.019	0.019	0.019	0.019	
C. sp. "Balchen"	17	67						0.03				0.007			0.720
C. sp. "Felchen"	23	89					0.006	0.011	0.039	0.006	0.006	0.011	0.006		0.981
Total															0.995

The frequency of private alleles found in historical samples of *C. gutturosus* of Lake Constance or *C. albellus* of Lake Brienz that can now be found in contemporary samples of sympatric species (*C. wartmanni*, C. *macrophthalmus* and *C. arenicolus* in Lake Constance; *C. sp.* "Balchen" and *C. sp.* "Felchen" in Lake Brienz). Given are the historical and contemporary sample sizes (*N* samples), the frequency of private alleles in the historical samples, their frequencies in contemporary samples of other sympatric species, and the probability with which we would have seen an allele in our historical samples had it been present (L_{HIST}). Total is the probability with which we would have seen at least one of the historical private alleles in our historical samples of the other species where those alleles present. *C. gutturosus-like* are four contemporary samples that were assigned genetically with largest probability to the extinct species, *C. gutturosus*, but clearly do not belong to that species as judged by phenotype and ecology (see text). The alleles in these individuals were conservatively not counted for the calculation of the contemporary frequencies in other species of alleles that were historically private to *gutturosus*.

Table S6. Gill-raker distribution normality analyses.

Lake	Species	*N*	*W*	*P-value*	*P* < 0.05	*P* < 0.01	Estimation
Geneva	*C. lavaretus or C. palaea*	24	0.964	0.5147	n.s.	n.s.	Yes
Neuchâtel	*C. palaea*	29	0.969	0.5290	n.s.	n.s.	No
Neuchâtel	*C. candidus*	49	0.962	0.1136	n.s.	n.s.	No
Biel	*C. palaea*	29	0.971	0.5772	n.s.	n.s.	No
Biel	*C. confusus*	20	0.892	0.0290	Sig.	n.s.	No
Murten	*C. palea*	29	0.912	0.019	Sig.	n.s.	Yes
Thun	*C. albellus*	49	0.898	0.0005	Sig.	Sig.	No
Thun	*C. fatioi*	46	0.969	0.2574	n.s.	n.s.	No
Thun	*C. sp.* "Balchen" *sp.nov.*	56	0.978	0.4101	n.s.	n.s.	No
Thun	*C. alpinus*	77	0.755	0.0000	Sig.	Sig.	No
Brienz	*C. albellus*	21	0.779	0.0003	Sig.	Sig.	No
Brienz	*C. sp.* "Balchen" *sp.nov.*	59	0.973	0.2223	n.s.	n.s.	No
Brienz	*C. sp.* "Felchen″ *sp.nov.*	20	0.963	0.6040	n.s.	n.s.	No
Sempach	*C. suidteri*	33	0.967	0.4003	n.s.	n.s.	Yes
Lucerne	*C. sp.* "Bodenbalchen" *sp.nov.*	126	0.969	0.0049	Sig.	Sig.	No
Lucerne	*C. sp.* "Schwebbalchen" *sp.nov.*	24	0.927	0.0856	n.s.	n.s.	No
Lucerne	*C. nobilis*	49	0.931	0.0065	Sig.	Sig.	No
Lucerne	*C. zugensis*	96	0.956	0.0025	Sig.	Sig.	No
Zug	*C. sp.* "Zugerbalchen" *sp.nov.*	20	0.960	0.5425	n.s.	n.s.	Yes
Hallwil	*C. cf. suidteri*	19	0.885	0.0267	Sig.	n.s.	Yes
Zürich	*C. duplex*	20	0.938	0.2238	n.s.	n.s.	No
Zürich	*C. heglingus*	19	0.965	0.6665	n.s.	n.s.	No
Walen	*C. duplex*	50	0.968	0.1987	n.s.	n.s.	No
Walen	*C. zuerichensis*	31	0.961	0.3038	n.s.	n.s.	No
Greifen	*C. cf. duplex*	39	0.947	0.0673	n.s.	n.s.	Yes
Pfaeffiker	*C. cf. zuerichensis*	19	0.950	0.3991	n.s.	n.s.	Yes
Constance	*C. arenicolus*	29	0.976	0.7232	n.s.	n.s.	Yes
Constance	*C. macrophthalmus*	26	0.960	0.3947	n.s.	n.s.	Yes
Constance	*C. wartmanni*	17	0.909	0.0955	n.s.	n.s.	Yes
Sarnen	*C. sp.* "Sarnerfelchen" *sp.nov.?*	20	0.913	0.0742	n.s.	n.s.	Yes

We tested whether within species gill-raker distributions deviate significantly from normality using a Shapiro-Wilks test. Given are for each lake and species the sample size (*N*), the Shapiro-Wilks test statistic (W), the *P*-value, significance or lack thereof at $P<0.05$ and $P<0.01$, and whether the data was used in the estimation of the range in gill-raker numbers. None of the range in gill-raker numbers were estimated assuming a normal distribution when $p<0.01$.

Table S7. Oxygen depth profile data

Lake	*N* profiles analysed	Time Period
Geneva	621	1957-2010
Neuchâtel	475	1962-2009
Biel	234	1967-2010
Murten	120	1934-2008
Thun	185	1994-2011
Brienz	191	1994-2010
Sempach	423	1938-2006
Lucerne	125	1964-2010
Zug	355	1950-2002
Baldegg	395	1921-2006
Hallwil	392	1969-2010
Zürich	839	1936-2008
Walen	371	1972-2007
Greifen	621	1942-2006
Pfaeffiker	560	1941-2006
Constance	568	1963-2010
Sarnen	24	1972-2007
Total	6499	1921-2011

Summary of the available oxygen measures as depth profiles for all lakes: the Number of profiles analysed (*N* profiles analysed) and the period in which these profiles were taken (Time Period). All measures were obtained from FOEN (Swiss federal office for environment) and EAWAG except for Lake Constance for which the data was obtained from the Internationale Gewässerschutzkomission für den Bodensee (IGKB).

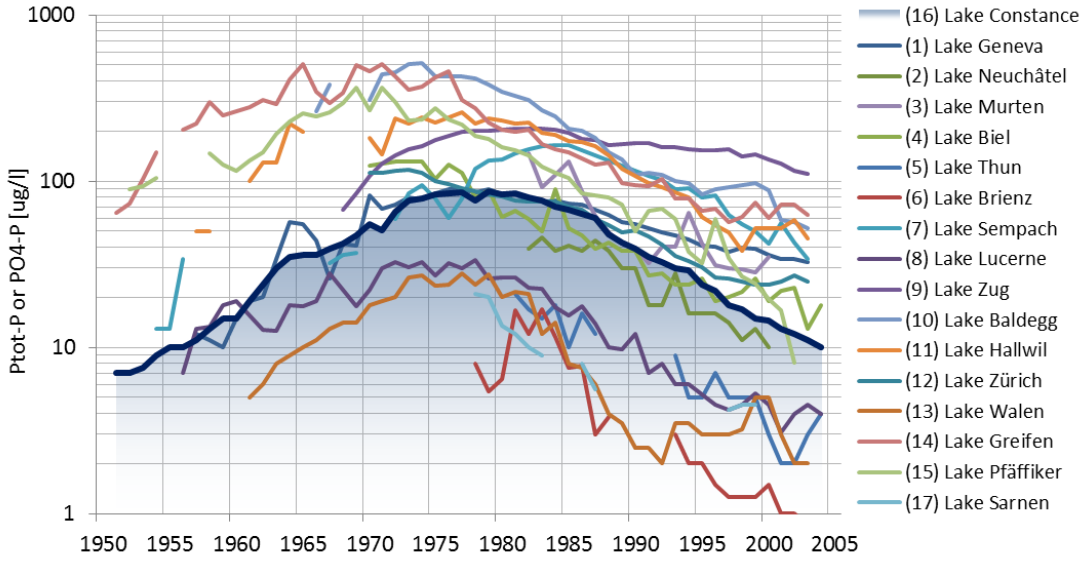

Figure S1 | Change in phosphorus concentrations through time. 50 year trends in phosphorus concentration [µg/l] during lake overturning from 17 lakes included in this study. Lake numbering corresponds to the numbering of Fig. 1 in the main paper.

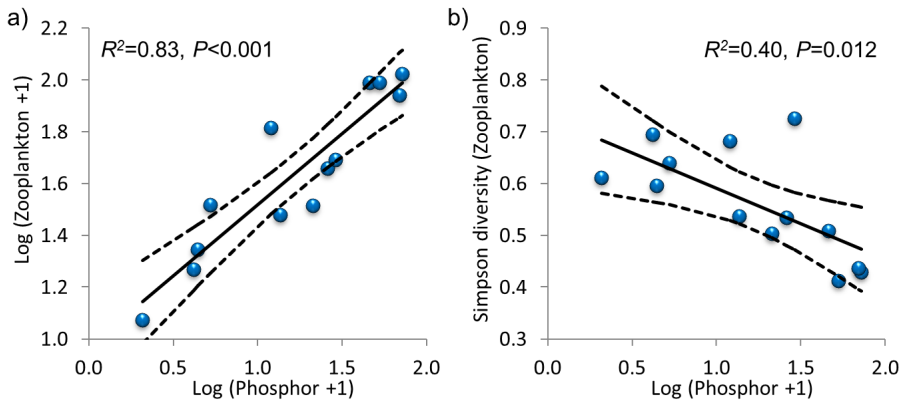

Figure S2 | Total phosphorus concentration in lakes predicts zooplankton biomass and diversity. **a,** Relationship between the average zooplankton biomass [g/m^2; measured between 1999 and 2006] and the average total phosphorous concentration (Ptot-P [µg/l]) measured in 13 alpine lakes. **b,** Relationship between the Simpson diversity index for zooplankton (calculated from zooplankton biomass [g/m^2] of five major functional groups: herbivorous Cladocera, carnivorous Cladocera, herbivorous Copepoda, carnivorous Copepoda, omnivorous Copepoda) and the average total phosphorous concentration (Ptot-P [µg/l]) for the same lakes.

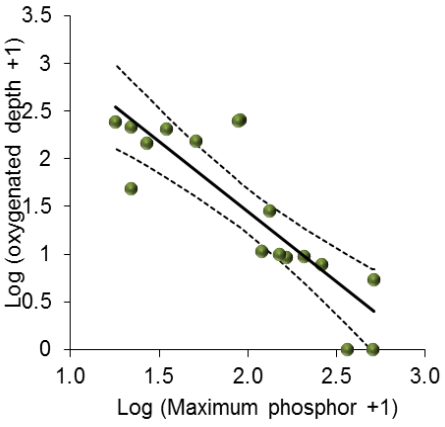

Figure S3 | Relationship between pollution and oxygenated lake depth. The correlation between the maximum phosphorus measured in each lake against the maximum depth with at least 2.5 mg/l dissolved oxygen.

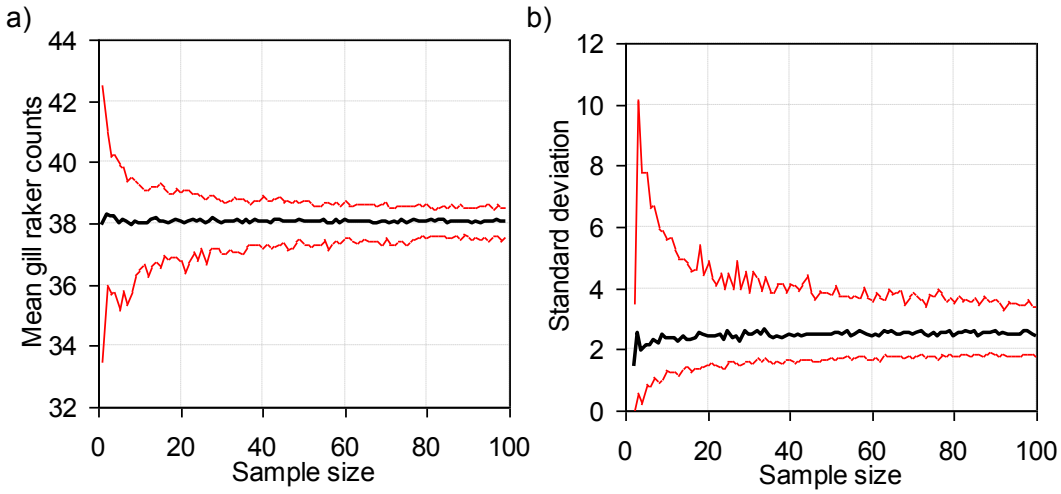

Figure S4 | Simulation of the mean gill-raker count and corresponding standard deviation based on real gill-raker count data. Gill-raker counts from 100 Lake Lucerne *C. zugensis* were used to randomly sample with replacement from 1 to 100 individuals. For each *N*, 100 replications were performed and 95 % confidence intervals calculated: **a,** We show the mean of the observed means (black line) with 95 % confidence intervals (red lines); **b,** the mean standard deviations observed (black lines) with 95 % confidence intervals.

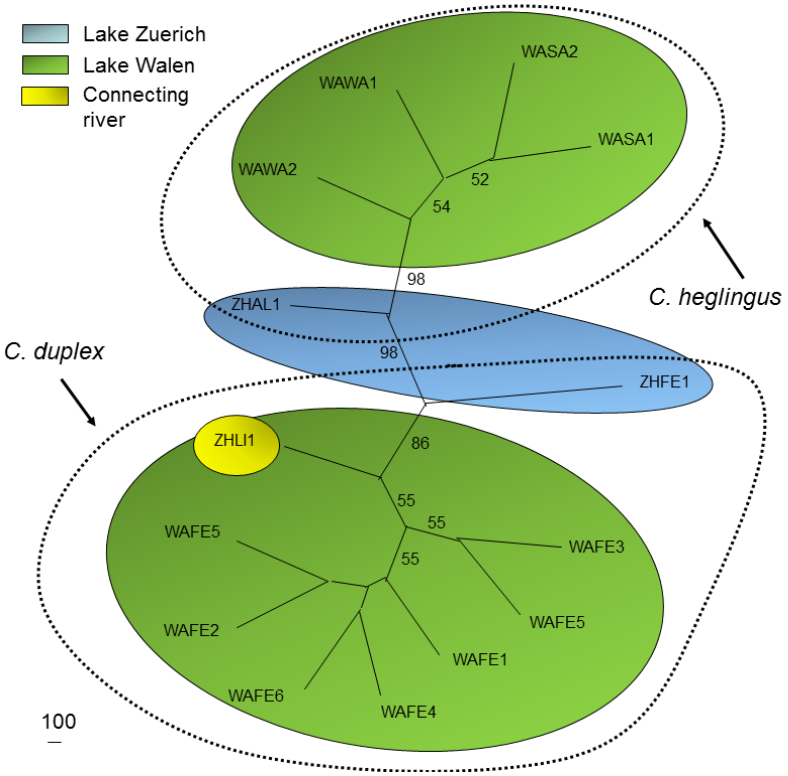

Figure S5 | Phylogeographic signature of introgression in eutrophic Lake Zürich. Lake Walen and Lake Zürich were basins of a single larger lake until well into the Holocene and contain the same pair of whitefish species endemic to this system. *C. duplex* is a large-growing and shallow-spawning, *C. heglingus* a small and deep-spawning whitefish species. A neighbour joining consensus tree based on Cavallis-Sforza chord distance (D_C) with 1000 bootstrap replicates[29] reveals that while the two species are still reciprocally monophyletic, the sympatric populations of these species in recently eutrophic Lake Zuerich (blue background) are genetically much more closely related to each other than the populations in never-eutrophied Lake Walen (green background). The F_{ST} between the species is 0.110 in Lake Walen but only 0.041 in Lake Zürich. Also included are whitefish spawning in the river "Linthkanal" that connects the two lakes (yellow background).

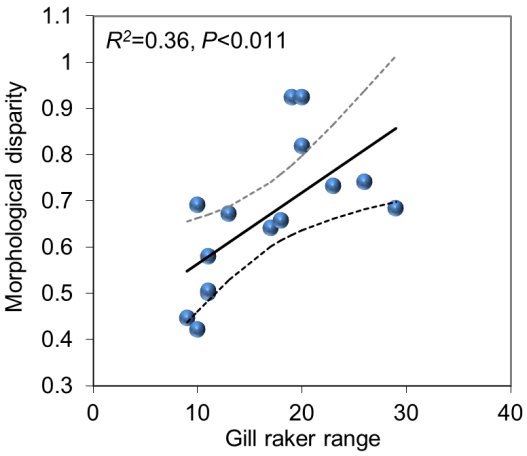

Figure S6 | range in gill-raker numbers **predicts morphological disparity in body shape.** Morphological disparity[30] in body shape regressed against range in gill-raker numbers observed in each lake. Morphological disparity was quantified using a landmark-based analysis of body shape. Thirteen homologous landmarks[4] were placed on a total of 1257 digital images that included 24 species from 15 lakes using TPSDIG2 (http://life.bio. sunysb.edu/morph/). Raw landmarks configurations were imported into MORPHOJ 1.02d[31] and subjected to Generalized least-squared Procrustes super-imposition. Generated Procrustes coordinates were corrected for allometry using pooled lake and species-specific regressions against size. Corrected Procrustes coordinates were then split by lake and used as input into the program DispartyBox7 (http:// www3.canisius.edu/ ~sheets/imp7.htm), which estimates the general spread of individuals in multidimensional morphospace.

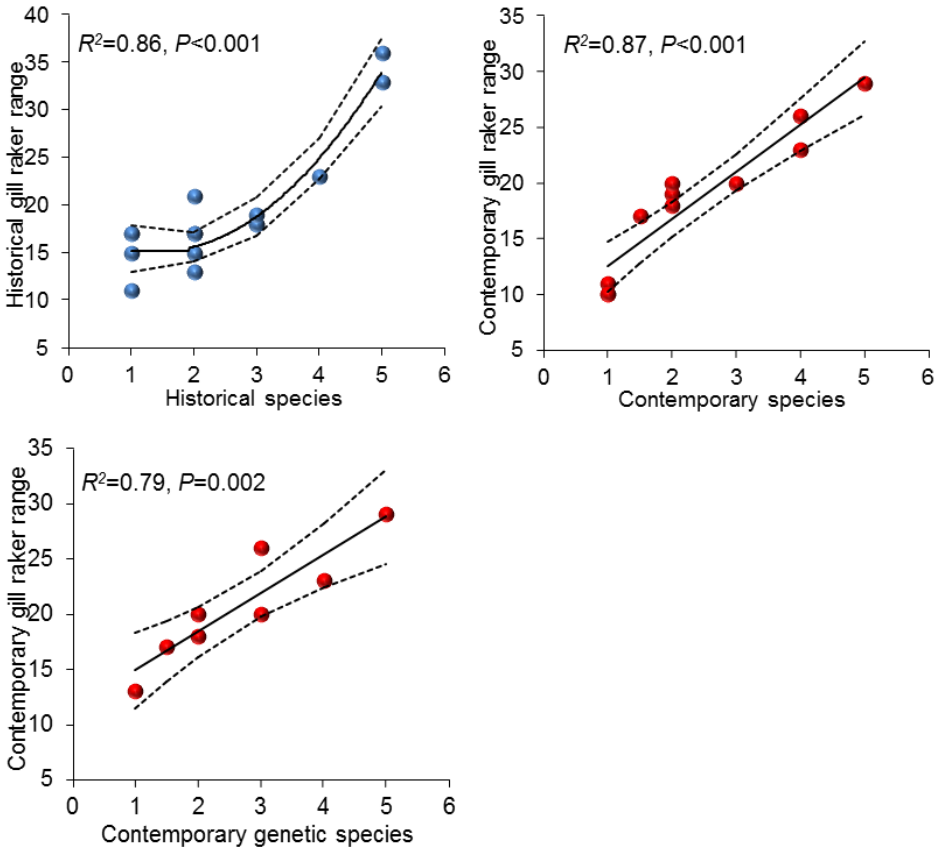

Figure S7 | Range in gill-raker numbers **in a lake predicts species numbers. a,** The correlation between the historically recognized number of whitefish species[6] and historical range in gill-raker numbers for 17 Swiss lakes. **b,** The correlation between the number of contemporarily species we recognized and the contemporary range in gill-raker numbers observed in 12 lakes. **c,** The correlation between the minimum number of genetically differentiated sympatric species and the contemporary range in gill-raker numbers observed in eight lakes.