

Allochrony as a potential driver for reproductive isolation in adaptive radiations of European whitefish ecomorphs

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Abstract

In northern Fennoscandian lakes, monophylogenetic lineages of postglacial fishes are radiating into several adaptive forms, but the speciation process is still at an incipient stage. The speciation process has received increased attention over the years, but the underlying mechanisms and drivers are still debated and poorly understood. European whitefish (*Coregonus lavaretus* [L.]) is the most abundant fish species in these lakes and has evolved into several ecomorphs adapted to different trophic niches and habitats. Genetic divergence has been observed among these ecomorphs, but the mechanism(s) responsible for the ongoing build-up of reproductive isolation has still to be revealed. As these systems are young in evolutionary time (<10 kyr), pre- and post-zygotic extrinsic isolation mechanisms are thought to be more likely to contribute to the reproductive isolation than intrinsic isolation mechanisms. We determined the gonadosomatic index (GSI) of three ecomorphs in two replicated lake systems and used GSI as a proxy to investigate the pre-zygotic isolation mechanism, allochrony, as a driving factor of divergence in this adaptive radiation of whitefish. We found that the three ecomorphs differed in GSI-values within and between lakes, suggesting different spawning times of the ecomorphs. We also show that males of one ecomorph had equal onset of maturity as another ecomorph, giving novel insights into the ongoing gene flow observed between ecomorphs. The result supports allochrony as a driver for the divergence process of whitefish ecomorphs, but more evidence is still needed to rule out that the three ecomorphs make use of different spawning grounds.

Keywords:

Coregonus lavaretus, whitefish, gonadosomatic index, habitat preference, spawning time, allochrony, ecological speciation

Introduction

Adaptive radiation is the rapid evolutionary divergence of individuals from a common ancestor into a variety of adaptive forms (Futuyma 1998) that exploit different ecological niches (Grant & Grant 2008). Understanding what processes drive and maintain an adaptive radiation is a central question in evolutionary ecology. The availability of various ecological opportunities in an ecosystem allows for different niches that may favour different behavioural and morphological adaptations. The association between a particular morphology and a specific niche is recognized as an important factor in adaptive radiation (Schluter 2000), and in fish, this association is often related to foraging traits, e.g. head shape and trophic niche (Schluter 1996). These associations can lead to genetic divergence and reproductive isolation of different adaptive forms (Rundle & Nosil 2005, Schluter 2000). Pre-zygotic and post-zygotic isolation are different isolation mechanisms that can result in reproductive isolation. Pre-zygotic isolation involves spatial and temporal isolation (e.g., different spawning sites and time) and sexual selection (Ritchie 2007, Taylor & Friesen 2017), while post-zygotic isolation includes ecological inviability (extrinsic), hybrid inviability, and sterility (intrinsic) (Coyne & Orr 2004). Despite extensive efforts in understanding why and how reproductive isolation accumulates in adaptive radiations, the exact drivers still remain to be identified for many species.

Polymorphic populations are commonly found in several freshwater fish species in postglacial lakes of the northern hemisphere. The fish species inhabiting these lakes have shown rapid (<10 kyr), convergent phenotypic divergence and adaptive radiation into multiple ecomorphs (Häkli et al. 2018, Østbye et al. 2005a, Østbye et al. 2006, Schluter 2000, Taylor 1999). European whitefish (*Coregonus lavaretus* [L.], referred to as whitefish further on) is a widely distributed fish species in Europe and is highly abundant in northern Fennoscandia. Here it has diverged from a monophyletic

lineage (Østbye et al. 2005a) into distinct ecological morphs through adaptive radiation (Svärdson 1979, Østbye et al. 2006). Three distinct ecomorphs of whitefish have been described (Kahilainen & Østbye 2006, Siwertsson et al. 2010), which show clear differences in niche utilization and trophic morphology, e.g. head shape and gill rakers (Amundsen et al. 2004, Harrod et al. 2010, Kahilainen & Østbye 2006), and have also been found to differentiate genetically (Præbel et al. 2013a, Siwertsson et al. 2013). However, the reproductive isolation among the ecomorphs is not complete, evidenced by the frequent occurrence of hybrids between the ecomorphs (Bhat et al. 2014).

The three whitefish ecomorphs are associated to the feeding niches found in three main habitats of the lakes; the littoral, pelagic and profundal zones (Kahilainen et al. 2003, Østbye et al. 2006). The ecomorphs are named according to morphology of gill rakers (Kahilainen & Østbye 2006). The densely rakered whitefish ecomorph (hereafter DR whitefish) resides mainly in the pelagic habitat and is a zooplanktivorous specialist, the large sparsely rakered whitefish (LSR whitefish) mainly feeds on benthic macroinvertebrates in the littoral habitat, and the small sparsely rakered whitefish (SSR whitefish) is mainly found in the profundal habitat feeding on benthic invertebrates (Harrod et al. 2010). In a recent study it was found that initial divergence of the ecomorphs into different habitats was a result of the presence of pike (*Esox lucius*) in the lakes (Öhlund et al. unpublished). The presence of pike very likely has caused the whitefish to either refuge into new, but less suitable habitats, or maximize growth to reach a safe size. Apart from variation in food resources and basal sources of energy in the three principal habitats (Harrod et al. 2010), they also differ with regard to their thermal conditions (Hayden et al. 2013; Evans et al. 2014). Thermal stratification of the water column in postglacial lakes means that littoral and pelagic habitats undergo large seasonal changes in water temperature from 10-20 °C in the warm summer

months and to 0-2 °C in the cold winter months when covered with ice (Hayden et al. 2013), whereas the profundal habitat display a cold, but stable, temperature of 2-8 °C, throughout the year.

The isolation mechanism driving the ongoing divergence in adaptive radiation of whitefish still has to be identified in these northern systems. This is important in understanding the biological, ecological, and genetic mechanisms involved in their adaptive radiation. Studies on whitefish in more southern Swedish lakes (Öhlund et al. unpublished, Svärdson 1979) suggested spatial and temporal differences in spawning time, but little is known about the exact spawning times and places for the different ecomorphs in northern Fennoscandian lakes. Based on field observations in Lake Paadar of spawning shoals in shallow water, there were indications of the DR whitefish being the first ecomorph to spawn, followed by the LSR whitefish and finally SSR whitefish (K. Kahilainen pers. obs. in Kahilainen et al. 2014). A fourth ecomorph, LDR, is also present in lake Padaar but it occurs in low abundance and is only present in the Pasvik watercourse, not the Alta watercourse where this study was performed. In coregonids, such interspecific variation in spawning time are important factors driving reproductive isolation (Bernatchez et al. 2010, Hudson et al. 2007, Svärdson 1979). The late spawning time of SSR whitefish may reflect the dark and stable cold temperatures in profundal habitats which slows down metabolic processes (Ohlberger et al. 2008). Physiological adaptation, such as metabolic and maturation processes, to a particular light and thermal regime should differ substantially among the ecomorphs, but so far only niche driven and respiration adaptations have been suggested as drivers of phenotypic divergence and reproductive isolation in northern post-glacial lakes (Evans et al. 2014, Harrod et al. 2010, Keller & Seehausen 2012, Østbye et al. 2006).

In this study, we investigated the pre-zygotic isolation mechanism(s) among the three whitefish ecomorphs. The challenge of revealing reproduction in these northern systems, compared to more southern lakes e.g. in Sweden, Denmark, and the European Alps, is that the spawning takes place in late October to December, when the lake ice is too thin to work on, but too thick for boating. Based on our own field observations, information from locals, and the presence of hybrids between the ecomorphs (Bhat et al. 2014), it seems that all three ecomorphs mainly share the same spawning ground(s) in the littoral zone, and with the knowledge of whitefish ecomorphs' thermal niches, we hypothesized that differences in spawning time (allochrony) acts as the main driver of reproductive isolation. This differs from the spatial divergence in spawning grounds observed in other more southern whitefish systems (Østbye et al. 2005b; Vonlanthen et al. 2009). We sampled European whitefish ecomorphs in two replicated northern lakes, as close to their natural spawning time as possible, and calculated a gonadosomatic index (GSI) as a measurement of sexual maturity. GSI has previously been used to determine sexual maturity, seasonal changes, and reproduction timing in fish (e.g. Flores et al. 2015, McQuinn 1989, Valdés et al. 2004). Seasonal changes in GSI of female whitefish (of a morph equivalent to the DR morph in this study) in lake Constance, Germany, showed that the GSI increased steadily over the summer and autumn until it reached a maximum, just before spawning in the winter (Rösch 2000). To our knowledge, no previous study has used GSI to investigate allochrony in a polymorphic species, and by comparing sexual maturation of the three whitefish ecomorphs, our goal was to infer whether the whitefish ecomorphs display differences in spawning time.

Materials and Methods

Sample collection

In October 2016, over a period of seven days, two lakes located in the northern Fennoscandia were sampled for European whitefish ecomorphs, Lake Stuorajávri (69°06'N, 22°49'E) and Lake Suohpatjávri (68°56'N, 23°05'E). Stuorajávri covers an area of 24 km² with a maximum depth of 30 m, and Suohpatjávri covers 2 km² with a maximum depth of 25 m. The two lakes are oligotrophic, harbour six fish species in addition to the whitefish, and have relatively equal distribution of shallow and deep areas. Both lakes have three principal habitats: the littoral habitat (shore water, close proximity to the bottom, <10 m depth; >1% of light at surface), the profundal habitat (close proximity to the bottom, >10 m depth; <1% of light at surface), and the pelagic habitat (open water, 0–6 m depths). Three different ecomorphs of whitefish are found in both of these lakes: DR, LSR and SSR whitefish (Siwertsson et al. 2013) (Figure 1). Fish sampling was performed with standardized gillnets of different mesh sizes, 10, 12.5, 15, 18.5, 22, 26, 35, and 45 mm. Benthic gillnets (1.5 m high) were used in the littoral and profundal zones, whereas the pelagic habitat was sampled using 6-m-high floating nets. A total of 364 whitefish were collected (Table 1). For each individual fish, total body weight and gonad weight were determined in the field laboratory using a Sartorius BP 310s scale and subsequently used to calculate the gonadosomatic index ($GSI = [\text{testis or ovary weight} / \text{body weight}] * 100$) per individual (Table 1). We further recorded the sex (female/male), sexual maturity (immature/mature), and total length for all individuals. Figure 1 shows a subsample of gonads for each whitefish ecomorph documented using a Nikon D610 camera body mounted with a Nikon 16.0-35.0 mm f/4.0ED lens. A fin tissue sample was also collected and stored in 96 % ethanol at -20°C for later genetic analysis.

[Insert Figure 1 near here]

[Insert Table 1 near here]

Ecomorph assignment and discrimination

A phenotypic analysis of the fish was performed in the field, where each individual was classified as DR, LSR or SSR whitefish according to appearance, head and body shape, and a visual evaluation of the gill raker morphology (Amundsen et al. 2004, Kahilainen & Østbye 2006). Ecomorph assignment was further verified in the laboratory by assessing the first left branchial arch under a microscope and counting the number of gill rakers (Table 1). We did not distinguish between male and female when counting of gill rakers.

To assess genetic divergence between the ecomorphs we followed the protocol of Præbel et al. (2013b) using a panel of 22 microsatellite markers. The data was screened for scoring errors, allele dropouts and null alleles using Micro-Checker 2.2.3 (van Oosterhout et al. 2004). Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were tested using exact tests (Guo & Thompson 1992) as implemented in GenePop 4.0 (Rousset 2007). Sequential Bonferroni corrections (BFC) was used to correct pairwise comparisons for multiple comparisons following Rice (1989). Genetic divergence among morphs was estimated using the pairwise.fst function in the Adegenet R-package (Jombart 2008), which computes Nei's estimator of pairwise F_{ST} , and tested for significance using 1000 permutations. For the genetic divergence analysis, we used both immature and mature individuals.

Preliminary analyses and quality control of GSI-maturity data

For each lake, fish were divided into groups based on ecomorph, sex and sexual maturity (immature and mature). Division of sex was necessary because the weight of the gonads differs greatly between males and females, and the assessment of sexual maturity was necessary because we only were interested in using mature individuals for this study. Individuals that did not fulfil these criteria were excluded from the analyses, leaving 168 females and 148 males for the statistical

analyses. Gonadosomatic index was calculated and used as a measurement for sexual maturity, i.e. the advancement of sexual maturity towards spawning, because all individuals were mature. This GSI-maturity functioned as our proxy for differences in spawning time between whitefish ecomorphs. This means that the GSI-values are under the assumption that greater GSI-values equals a more sexually mature fish, and consequently, has an earlier spawning time, than a fish with a lower GSI-value. The GSI-values were transformed using the logarithmic function to more closely meet the assumption of linear models and because our dependent variable is a proportion (Baum 2008). An overview of the number of mature individuals, percentage of maturity and the average GSI for each ecomorph in the two lakes is found in Table 1.

Determination of variation in GSI-maturity among whitefish ecomorphs

For the statistical data analyses of GSI-maturity among ecomorphs we carried out a two-way analysis of variance (ANOVA) using R v.1.0.44 (R Core Team 2016). The first step was model selection using the *MuMin* package in R (Bartoń 2017) to find the linear model that best explained the differences in maturity of whitefish ($\log_{10}(\text{GSI})$). Using a factorial design of relevant variables, we tested 16 different linear models. The additive model of two factors (or independent variables), population (ecomorphs) and lake, was the most parsimonious fit according to Akaike's Information Criteria (AIC) statistics for model selection. This model had, for both sexes, the lowest AIC-value with the lowest degrees of freedom and most weight to it. Next step was a control of the model to check if the data fitted a normal distribution by plotting a Q-Q plot. To detect the presence of outliers in our dataset we used the Interquartile Range Rule (IQR). For the males only, the data contained nine outliers outside $1,5 \times \text{IQR}$ that skewed the distribution and thus needed to be removed from the dataset. This left us with 139 males for further analysis. The nine outliers removed from the male dataset consisted of 8 individuals with much lower GSI-values, indicating

they were wrongly assigned as sexually mature in the field when in fact they were immature. One individual had a much higher GSI-value than average, which may be the result of a typo-error during the field work. The gonad weight of this particular individual is >50% more than average. After the preliminary steps, the ANOVA statistics were performed and finally, post hoc analyses using pairwise comparisons of least-squares means for the specified factors in the linear model using the *estimability* and *lsmeans* packages in R (Lenth 2016a, 2016b).

Additionally, we caught an unusually low number of SSR whitefish in Lake Suohpatjávri, only one mature male in total. This was clearly not enough to do proper statistical analysis, but we included the individual anyway because this individual was in fact not an outlier in the model control step allowing us to obtain some insight into the GSI-values of male SSR whitefish.

[Insert Table 2 near here]

Results

Genetic differentiation among whitefish morphs

The number of alleles at each microsatellite locus ranged from 2 to 21 across populations (Table S1). Micro-Checker detected homozygote excess due to possible null alleles in four of 22 tested loci: Cocl_lav27 (Stuorajávri, DR whitefish), and BWF1, ClaTet06 and Cocl_lav10 (Stuorajávri, SSR whitefish) (Table S1). Although none of the possible null alleles were found in more than one population at a time, those four loci were excluded from further analysis. Deviations from HWE were indicated in 1 out of 108 tests (0.9%) after sequential Bonferroni corrections, which is less than expected by chance (5%). Out of 918 tests, significant linkage disequilibrium was found in three (0.3%) loci comparisons after Bonferroni correction. Pairwise F_{ST} values between populations

were small yet statistically significant, ranging from 0.011 to 0.059, where the smallest genetic difference was found between LSR whitefish in different lakes (Table 2).

Field-determined maturity of whitefish morphs

The abundance of mature individuals differed both between the ecomorphs and lakes (Table 1). When individuals of both sexes were combined, we found a pattern that most often the DR whitefish had more mature individuals than the LSR whitefish, which again had more mature individuals than the SSR whitefish. The only exception being females in Stuorajávri, where 85.2% of DR whitefish were mature versus the 94.7% of LSR whitefish (Table 1). Between lakes, there was a higher proportion of mature whitefish caught in Stuorajávri than in Suohpatjávri, especially for the LSR and SSR whitefish. The DR whitefish appeared to have an equal proportion of mature individuals between the lakes.

Variation in GSI-maturity of whitefish ecomorphs

The two-way ANOVA showed that there are significant differences of the advancement of sexual maturity ($\log_{10}GSI$) between the three distinguished whitefish ecomorphs for both sexes (females: $F_{2,165}=17.359$, $P<0.001$; males: $F_{2,135}=9.097$, $P<0.001$). There were also significant differences in GSI-maturity for both sexes between the two lakes (females: $F_{1,165}=15.293$, $P<0.001$; males: $F_{1,135}=46.838$, $P<0.001$). For the females, the DR whitefish was significantly more mature than the LSR whitefish ($P<0.001$, $R_2=0.2186$) and the SSR whitefish ($P<0.001$, $R_2=0.2186$), but the LSR whitefish was not significantly more mature than the SSR whitefish ($P=0.597$, $R_2=0.2485$). For the males, there was no difference in maturity between DR whitefish and LSR whitefish ($P=0.752$, $R_2=0.310$), but both the DR whitefish ($P<0.001$, $R_2=0.310$) and LSR whitefish ($P<0.001$, $R_2=0.2615$) were significantly more mature than the SSR whitefish.

For the post hoc analyses, we did pairwise comparisons using least-square means of $\log_{10}(\text{GSI})$ on the specified factors in the linear model (Figure 2a and 2b). These analyses contrasted the advancement of sexual maturity of the whitefish ecomorphs individually from each lake. For the female whitefish, the whitefish from Stuurajávri were significantly more mature compared with whitefish in Suohpatjávri. Also, for females, the DR whitefish in both lakes were more mature compared to the LSR whitefish and SSR whitefish, which in turn were equally mature. For the male whitefish, as with the females, the whitefish in Stuurajávri were more mature compared with whitefish in Suohpatjávri. Furthermore, the male DR whitefish and LSR whitefish were equally mature, whereas they were significantly more mature than SSR whitefish in both lakes.

[Insert Figure 2a and 2b near here]

Discussion

We found statistically significant differences in the advancement of sexual maturity, based on GSI-values, between the three genetically and morphologically divergent ecomorphs of whitefish. We demonstrated that the pelagic DR whitefish were generally further advanced in sexual maturity than the littoral LSR whitefish, which in turn were more mature than the profundal SSR whitefish at the moment of capture. This pattern was prevalent in both of the sampled post-glacial lakes and for both sexes. The pattern was also evident from the morphological appearance of the gonads of the ecomorphs, where DR whitefish had far more developed oocytes than the other ecomorphs at the moment of capture (Figure 1). However, our results do not permit inference about the temporal pattern of gonad development, nor do they provide information about ecological factors that drive the maturation. Rösch (2000) investigated, over a five-year period, seasonal changes in GSI of the

Blaufelchen whitefish (morph equivalent to the DR morph in this study) in Lake Constance-Obersee and found that GSI increased from low values in summer to about 24% of the body weight immediately before spawning in December. A similar result was obtained for Pollan whitefish in Lough Neagh, Ireland (Wilson & Pitcher 1983), suggesting that GSI is a useful measure of sexual maturity in *Coregonus*. The whitefish radiations in Northern Fennoscandia have been shown to be driven by ecological opportunity (Siwertsson et al. 2010), where each of the ecomorphs have adopted a life-history and specialization to each of the principal lake niches (littoral, pelagic, profundal). The DR whitefish feed on pelagic prey, which leads to the smallest body size and earliest sexual maturation, the LSR whitefish feed on littoral benthic macroinvertebrates, which leads to large size and late sexual maturation, and the SSR whitefish feed on profundal benthic prey and grow to intermediate body size and late sexual maturation (Harrod et al. 2010; Hayden et al., 2013; Kahilainen et al. 2003, 2005; Østbye et al. 2006). Our results of GSI-estimated maturity extend the understanding of the general life history characteristics of the ecomorphs by adding a likely spawning chronology, similar to what has been observed in the field on a single occasion (K. Kahilainen pers. obs. in Kahilainen et al. 2014).

Estimating the advancement of sexual maturity with GSI was based on the assumption that GSI-values increase with increasing sexual maturity and earlier spawning time. According to Ohlberger's theory (2013), the temporal spawning segregation (allochrony) develops because individuals living in different habitats experience different water temperatures and therefore mature at different rates. For whitefish, a discussed mechanism to drive differences in timing of spawning activities, and thus differences in sexual maturity between diverging ecomorphs, is the exposure to different environmental conditions, e.g. light and temperature regimes (Evans et al. 2014, Kahilainen et al. 2014). Adaptation to divergent thermal niches is also likely to be of crucial

importance to the relative capacity to assimilate the energy required to reach sexual maturity and the development of gonads (Kahilainen et al. 2014). These habitat specific differences reflect what the three whitefish ecomorphs in northern postglacial lakes experience, where the DR, LSR, and SSR whitefish segregate in the pelagic, littoral and profundal habitats, respectively (Østbye et al. 2006, Præbel et al. 2013a, Siwertsson et al. 2013, Svärdson 1979). The relative contribution of ecological and physical factors on timing of sexual maturity for these whitefish ecomorphs remains to be investigated. But, all together, our hypothesis is supported in that the accumulation of genetic differences observed between the three whitefish ecomorphs (Østbye et al. 2006, Præbel et al. 2013a, Siwertsson et al. 2013), is driven by a pre-zygotic extrinsic isolation mechanism, and is best explained by differences in spawning time, allochrony.

The lakes, as local entries, also appear to have an influence on sexual maturity as we found statistically significant difference of the advancement of sexual maturity between the two lakes. The whitefish from Stuorajávri were significantly more advanced in sexual maturity than the whitefish from Suohpatjávri. Stuorajávri is larger (24 km²) and a little deeper (max depth of 30 m) compared to Suohpatjávri (2.0 km², 25 m) and may therefore retain an overall warmer annual water temperature. Increased environmental temperatures are known to accelerate the physiological rate of animals and may induce earlier maturation of the Stuorajávri whitefish. Locals and scientists have also observed Stuorajávri whitefish to spawn in October-December (Pers. Com. Rune Knudsen) and in Suohpatjávri in November-January (Pers. Com. Eleonor Beck). However, further long-term studies are needed to elucidate the relative contribution of light and temperature to the extrinsic postzygotic isolation mechanisms in the northern Fennoscandian whitefish radiations to confirm these observations.

Where, when, and how the whitefish spawn in nature is evidently important for understanding the evolution of these radiations. Earlier maturation of male whitefish and their presence on the spawning grounds before and after the arrival and spawning of females, have been observed for Pollan whitefish in Lough Neagh, Ireland (Dabrowski 1981). We also found, in both lakes, that male and female DR and male LSR whitefish displayed a similar state of maturity, while the female LSR and male and female SSR whitefish were less mature. This suggests that in these lakes, the temporal chronology of reproduction is: DR whitefish, LSR whitefish, and SSR whitefish. Notably, we show that the male LSR whitefish is mature at the same time as DR whitefish, suggesting a sex-specific route for the ongoing gene flow observed between the whitefish ecomorphs in their adaptive radiation (Bhat et al. 2014, Häkli et al. 2018, Præbel et al. 2013a, Siwertsson et al. 2013). Hybrids between whitefish ecomorphs frequently occur in all northern Fennoscandian lakes, but genetic studies have only observed hybridisation between DR-LSR whitefish (Bhat et al. 2014) and between LSR-SSR whitefish (Præbel et al. unpublished). A study, using genome-wide coverage of SNPs to infer genomic consequences of speciation reversal in whitefish ecomorphs, showed that hybridisation between female DR and male LSR is the most likely route of gene flow between the whitefish ecomorphs (Bhat 2016). The exact mechanism of this hybridisation is not known and a potential explanation could be sneaking behaviour of LSR males, which has been shown in other salmonids (e.g., Baxter et al. 1997, Garcia-Vazquez et al. 2001, Redenbach & Taylor 2003). However, this behaviour has thus far only been described for small males and we therefore find it most likely to be caused by an overlap in spawning. Collectively, the ongoing gene flow between whitefish ecomorphs may be explained by sexually mature male LSR whitefish that spawn with mature DR and we propose that male SSR x Female LSR is a possible sex-specific route for gene flow between the SSR and LSR whitefish ecomorphs (Figure 2a and 2b). These results also support

our hypothesis that the ecomorphs share spawning ground(s), but are reproductively isolated in time.

The most pronounced difference in terms of trophic ecology, morphology, and reproductive isolation has been found between the SSR and DR whitefish (Harrod et al. 2010, Kahilainen & Østbye 2006, Præbel et al. 2013a, Siwertsson et al. 2013). Our results also support these observations in both lakes and for both sexes. There was no overlap of GSI-values between the DR and SSR ecomorphs indicating that extrinsic pre-zygotic isolation mechanisms are in play, quite possibly allochrony, and thus drive the diverging populations. Temporal separation can be important in achieving reproductive isolation in incipient ecological speciation (Schluter 2001), where intrinsic post-zygotic isolation mechanisms, such as genomic incompatibility, has yet to be accumulated between the diverging ecomorphs. A recent study by Johannsson et al. (2017) established that spawning window and population age were positively correlated in all ecomorphs of a lake. Hence, the older the lake is, the more prevalent divergence is for the whitefish ecomorphs. Overall spawning time is therefore increased as an intrinsic isolation mechanism to reduce contact and avoid interbreeding. This supports allochrony as a potential main driver for reproductive isolation between whitefish ecomorphs.

In conclusion, we found statistically significant differences in maturity between whitefish ecomorphs. Overall, the pelagic, zooplanktivorous DR whitefish displayed greater sexual advancement than the littoral, benthic feeding LSR whitefish, which again showed greater maturity than the profundal, benthic feeding SSR whitefish. This was the general pattern for both lakes, despite Stuurajávri showing greater proportion of mature individuals than Suohpatjávri. The morphological divergence between the ecomorphs, their adaptation to different thermal niches, and

sex-specific routes for gene flow between the ecomorphs, all suggest allochrony as a pre-zygotic isolation mechanism that contributes to the adaptive radiation in European whitefish.

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Data availability

The data is available from UiT Open Research Data, DOI: xxxx.

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Conceived and designed the investigation: KP, JBT

Performed field and/or laboratory work: JBT, KH, SB, KP

Analyzed the data: JBT, KH, KP, SB

Contributed materials, reagents and/or analysis tools: JBT, KH, KP

Wrote paper: JBT, KP, KH, SB

Table 1

Overview of the whitefish dataset including average number of gill rakers $\pm SD$; number of individuals used in F_{ST} -calculations; number of mature individuals; average length, weight and gonad weight $\pm SD$; percentage of maturity; and average GSI for the three ecomorphs in Stuorajávri and Suohpatjávri, divided by sex.

FEMALES									
Ecomorph	Lake	Gill rakers (N)	N(F_{ST}) §	N(mature) ¶	Length (mm)	Weight (g)	Gonad weight (g)	%Maturity	AverageGSI(g)
DR	Stuorajávri	34.8 ± 2.1	16	23	310 ± 22.75	236 ± 32.48	22.77 ± 5.37	85.2%	9.59 ± 1.34
	Suohpatjávri	39.0 ± 2.5	51	79	237 ± 23.52	89 ± 22.89	5.54 ± 2.53	97.6%	6.35 ± 3.05
LSR	Stuorajávri	24.4 ± 1.6	19	18	342 ± 48.67	317 ± 152.6	31.58 ± 34.51	94.7%	8.05 ± 8.09
	Suohpatjávri	26.5 ± 2.4	27	21	313 ± 40.49	245 ± 117.4	8.83 ± 14.04	63.6%	3.66 ± 4.85
SSR	Stuorajávri	22.0 ± 1.6	26	22	267 ± 42.37	154 ± 101.8	10.28 ± 17.31	75.9%	5.14 ± 4.38
	Suohpatjávri	23.6 ± 3.3	10	5	269 ± 7.92	134 ± 23.93	0.94 ± 0.52	50%	0.71 ± 0.34
MALES									
Ecomorph	Lake	Gill rakers (N)	N(F_{ST}) §	N(mature) ¶	Length (mm)	Weight (g)	Gonad weight (g)	%Maturity	AverageGSI(g)
DR	Stuorajávri	34.8 ± 2.1	31	50	317 ± 13.26	241 ± 30.76	3.35 ± 0.90	100%	1.39 ± 0.33
	Suohpatjávri	39.0 ± 2.5	18	27	232 ± 24.65	85.5 ± 27.21	0.83 ± 0.34	100%	0.96 ± 0.15
LSR	Stuorajávri	24.4 ± 1.6	26	26	348 ± 28.46	331 ± 99.81	4.55 ± 2.40	93.1%	1.32 ± 0.29
	Suohpatjávri	26.5 ± 2.4	24	14	296 ± 42.35	207 ± 122	2.47 ± 2.14	67.9%	1.12 ± 0.31
SSR	Stuorajávri	22.0 ± 1.6	25	21	259 ± 32.79	131 ± 53.30	1.32 ± 0.71	85.2%	1.00 ± 0.22
	Suohpatjávri	23.6 ± 3.3	2	1 †	356 †	422 †	3.12 †	50% †	0.74 †

0 Abbreviations: DR = densely rakered whitefish, LSR: large sparsely rakered whitefish, SSR = small sparsely rakered whitefish.

1 §Both mature and immature individuals were genotyped, but not all individuals. Therefore, the number of individuals can vary from the
2 number of mature individuals used for the GSI-calculations.

3 ¶Outlier males were removed from the dataset and is therefore not included here. A total of 139 mature males.

4 †Only one mature male SSR, therefore averages and percentages calculated for this ecomorph is not correct. We kept the values to display
5 the one male we did include in the study.

6 **Figure 1**

7 Drawings of the three ecomorphs of European whitefish (*Coregonus lavaretus* L.) from northern
 8 Norway and their respective gill rakers: (A) DR, densely rakered, (B) LSR, large sparsely rakered,
 9 and (C) SSR, small sparsely rakered ecomorph (line drawings modified from Harrod et al. 2010).
 10 The photos show three examples of female gonads for each ecomorph at the exact same time of
 11 year in Suohpatjávri: DR (mature, mature, immature) LSR (mature, immature, immature) SSR
 12 (mature, immature, immature). The GSI-index values calculated as a measure of sexual maturity,
 13 which account for any size differences between fish, are provided next to the corresponding gonad.

14

15

16 **Table 2**

17 F_{ST} -Table of all genotyped individuals. Below diagonal are pairwise F_{ST} -values, above diagonal are
 18 P-values.

	StD	StL	StS	SuD	SuL	SuS
StD		0.001	0.001	0.014	0.001	0.001
StL	0.049		0.005	0.001	0.030	0.001
StS	0.035	0.016		0.001	0.005	0.008
SuD	0.012	0.059	0.042		0.001	0.001
SuL	0.037	0.011	0.017	0.047		0.008
SuS	0.038	0.020	0.016	0.032	0.014	

19 Abbreviation of populations are: St (Stuorajávri), Su (Suohpatjávri), D (DR whitefish), L (LSR
 20 whitefish), S (SSR whitefish). Code is a combination of lake and morph name.

21 **Figure 2a and 2b:**

22 Plots showing the differences in maturity of the whitefish ecomorphs, for both sexes and across two
23 lakes. This was done using least-square means for the specified factors in the linear model and *p*-
24 values were adjusted for multiple comparisons by Tukey's HSD. Boxes indicate the least-square
25 mean of $\log_{10}(\text{GSI})$ and error bars indicate the 95% confidence interval of the least-square mean.
26 Means sharing a letter have no statistically significant difference at the *alpha* level 0.05.

27

28

29 **Appendix**

30 **Table S1**

NUMBER OF ALLELES PER POPULATION							
Locus ID	Total	StD	StL	StS	SuD	SuL	SuS
BWF2 _a	5	3	3	3	4	3	3
BFRO-018 _b	3	1	2	1	2	3	2
ClaTet01 _c	11	7	6	6	5	9	5
ClaTet03 _c	9	4	6	5	8	6	3
ClaTet09 _c	13	8	10	9	7	12	6
ClaTet10 _c	21	11	12	12	16	17	8
ClaTet05 _c	11	5	7	6	3	9	3
ClaTet12 _c	17	10	11	9	10	13	6
ClaTet13 _c	7	6	6	7	6	6	5
ClaTet15 _c	5	4	4	5	4	4	5
ClaTet17 _c	15	8	9	7	10	10	4
ClaTet18 _c	4	4	2	3	4	2	2
Coel-Lav4 _c	2	2	1	1	1	2	1
Coel-Lav6 _c	4	3	3	2	2	2	2
Coel-Lav18 _a	2	2	2	2	2	2	1
Coel_Lav49 _a	7	2	4	5	6	3	2
Coel-Lav52 _a	13	8	9	9	9	8	7
C2-157 _c	5	2	1	2	1	4	1

31 Details of microsatellite loci used for estimating genetic differentiation among whitefish
 32 ecomorphs. Total number of alleles in each locus and number of alleles in each locus per
 33 population. Abbreviation of populations are: St (Stuorajávri), Su (Suohpatjávri), D (DR whitefish),
 34 L (LSR whitefish), S (SSR whitefish). Code is a combination of lake and morph name. _a(Patton et
 35 al., 1997); _b(Susnik et al., 1999); _c(Winkler & Weiss, 2008); _d(Rogers et al., 2004); _e(Turgeon et al.,
 36 1999).