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# Ecological and evolutionary effects of anthropogenic change in freshwater fish communities

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### Abstract

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Environmental change is ongoing, driven by human activities that have profoundly modified the modern world. Natural ecosystems are directly affected by these anthropogenic activities. This thesis explores the effects of anthropogenic change on natural ecosystems. Being this an extremely broad topic, we narrow down our research to some key areas of investigation, without aiming to be exhaustive. We also explore related themes. In **Paper I**, we review the effects of anthropogenic change on the process of speciation. We show that human intervention can alter geography or selective regimes. In both cases, this can either lead to an increase or decrease in reproductive isolation between species. In the following papers, we focus on the study case of two sister species with similar morphology and ecological niches, the common bream (*Abramis brama*) and the white bream (*Blicca bjoerkna*). In **Paper II**, we demonstrate that changes in abiotic and biotic conditions in lakes can disrupt the delicate co-occurrence and abundance patterns between the two species. In particular, increases in turbidity, predation and competition pose a threat to the local survival of white bream. In **Paper III**, we examine the effects of abiotic and biotic conditions on the phenotype of our species of interest. For this purpose, we focus on the average individual size and its relative difference between the species as a phenotypic proxy. We show that the relative difference in size is quite insensitive to external perturbations. However, we find that the presence of pike in the lake increases the similarity in the size between the study species. This can potentially threaten niche specialisation and species cohesion. Throughout **Paper II** and **Paper III**, we investigate the temporal trends of environmental and biotic factors over the last few decades, finding an increase in temperature, turbidity and the proportion of perch, along with a decrease in the proportions of pike and roach. In **Paper IV**, we conduct a genomic study based on whole-genome resequencing, which reveals that the morphological approach is inadequate for the taxonomical identification of our study species. Genomic data also show that hybridisation occurs, but it does not pose an immediate threat to species cohesion in the study area. Intra-specific genetic variation is low, and it suggests a pattern of isolation-by-distance. Overall, this thesis highlights that an analysis of anthropogenic influences on natural systems should be approached from different angles, to provide a comprehensive overview of such a complex phenomenon.

*Keywords:* ecology, co-occurrence, hybridisation, genetic variation, selection, adaptation, phenotypic change, freshwater environments, fish, common bream, white bream

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# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. Ålund, M., Cenzer, M., Bierne, N., Boughman, J. W., Cerca, J., Comerford, M. S., **Culicchi, A.**, Langerhans, B., McFarlane, S. E., Möst, M. H., North, H., Qvarnström, A., Ravinet, M., Svanbäck, R., Taylor, S. A. (2023). Anthropogenic change and the process of speciation. *Cold Spring Harbor Perspectives in Biology*, 15(12): a041455. <https://doi.org/10.1101/cshperspect.a041455>.
- II. **Culicchi, A.**, Svanbäck, R., Brännström Å. Environmental and biotic stressors impact co-occurrence of closely-related species: a case study in freshwater fish. *Manuscript*.
- III. **Culicchi, A.**, Brännström, Å., Svanbäck R. Potential exception to character displacement: predator-induced character convergence in a freshwater species pair. *Manuscript*.
- IV. **Culicchi, A.**, Svanbäck R., Brännström Å., Backström N. A genomic approach for accurate species identification and characterisation of genetic variation in hybridising freshwater fish species. *Manuscript*.

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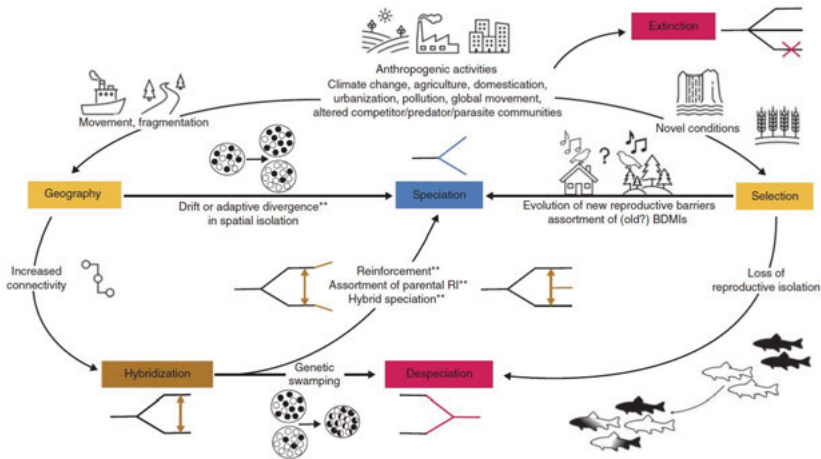
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# Introduction

## A changing world: human activities and speciation

The world has always undergone changes throughout its existence. However, over the last 200 years, the rate of change has significantly accelerated, primarily due to increasing anthropogenic impacts on the environment, biological species, ecosystems and communities (Ellis and Ramankutty, 2008; Lewis and Maslin, 2015). One aspect that has been traditionally overlooked is the impact of human activities on the speciation process (Figure 1). Human activities vary in nature and can either enhance or reduce reproductive isolation between species (Coyne and Orr, 2004). While a reduction of reproductive isolation can lead to reverse speciation, it is unlikely that its increase as a result of anthropogenic activities will lead to complete speciation (Servedio and Hermisson, 2020). The main mechanisms through which humans disrupt the speciation process involve altering the geographical arrangement of organisms and changing the selective regimes to which organisms are exposed. Both kinds of activities can either result in an increase or a decrease of reproductive isolation (Coyne and Orr, 2004).



**FIGURE 1** Schematic describing the general ways anthropogenic activities can influence the formation and loss of species by changing the geography, selection, and rates of hybridization between lineages. \*\* Indicate indirect changes in selection due to changed geography or via hybridization. (RI) Reproductive isolation, (BDMIs) Bateson–Dobzhansky–Muller incompatibilities.

## Reduction in reproductive isolation

Human activities may lead to a reduction in reproductive isolation between species through various mechanisms. Alterations of geography include transport of species, such as through horticulture, agriculture, aquaculture, shipping, pet trade and others, as well as the creation of corridors like channels, bridges and maritime traffic (Crispo et al., 2011; Miles et al., 2018, 2019). This can result in the loss of intra-specific genetic variation and in the secondary contact between already divergent species. Increased gene flow and hybridisation can in turn potentially lead to reverse speciation (Thompson et al., 2023). An example of this phenomenon is the transport of *Ciona* tunicate species from the Pacific Ocean to the Atlantic Ocean, which caused gene introgression into some of the Atlantic species (Le Moan et al., 2021). Human-induced environmental change (e.g. climate change) can also facilitate secondary contact between species by means of range expansion, potentially involving the breakdown of both geographical and ecological barriers (Taylor et al., 2014). A specific case is the southern flying squirrel (*Glaucomys volans*), whose range expanded into the range of the northern flying squirrel (*Glaucomys sabrinus*), provoking hybridisation (Garroway et al., 2010, 2011), competitive exclusion (Wood et al., 2016) and spread of parasites (Krichbaum et al., 2010).

However, the reduction of reproductive isolation can also be caused by alterations in selective regimes. This can happen, for instance, when humans



contribute to the homogenisation of the environment. An example of this is represented by eutrophication in Lake Victoria, where reduced visibility levels led to the reverse speciation of several species of cichlid fishes (Seehausen et al., 1997). Additionally, the reduction of reproductive isolation can result from the creation of novel, artificial, selective scenarios, such as the development of pollutant or pesticide resistance (Le Corre et al., 2020; Valencia-Montoya et al., 2020). A special case is the feralisation of domesticated species, where humans are responsible for both the domestication and subsequent secondary contact with a non-domesticated lineage, as in the case of the wolf and the dog (Pilot et al., 2018).

## Increase in reproductive isolation

Reproductive isolation may grow as a result of human activities. In this scenario as well, alterations in both geography and selective regimes can be the primary factors that contribute to such increase. The genetic mechanisms that underlie this phenomenon include drift, similar selection and divergent selection (Nosil and Flaxman, 2011; Nosil, 2012; Langerhans and Riesch, 2013). Elements such as environmental fragmentation and geographical barriers are straightforward examples that can drive a reduction in connectivity between different populations. These elements include infrastructures like roads, agricultural fields, power lines, dams and cities (Trombulak and Frissell, 2000). The Great Wall of China, for instance, significantly reduced gene flow between plant populations, by limiting both wind- and insect-mediated pollination (Su et al., 2003). Fragmentation can accompany changes in the ecology of previously homogeneous areas, speeding up the process of divergence. This is what happens with alterations to flow regimes caused by the construction of dams in river ecosystems (Franssen et al., 2013). The active or passive transport of species can expedite the build-up of reproductive isolation, especially when introduced to a novel environment with different selective pressures. For example, populations of common reed (*Phragmites australis*) introduced to North America diverged from native European populations over the last 150 years (Guo et al., 2018).

Human activities can impact mechanisms of selection, influencing a number of phenotypic traits, such as the timing and duration of reproduction (Sirkiä et al., 2018), song frequency, timing and composition (Reichard et al., 2020), mate-choice modality and male colour signals (Giery et al., 2015; de Jong et al., 2018; Koneru and Caro, 2022). A clear example of how human alteration of selective regimes was pivotal to the build-up of reproductive isolation is the introduction of domesticated apples in North America, which led to the origin of a derived ecotype of the hawthorn fruit fly, the apple maggot fly (Walsh BJ, 1867; Bush, 1992). Altered selection and geographical barriers can act together within the same scenario. The case of the legume *Trifolium*

*repens* is quite illustrative: urban populations of this species share more similarities with each other than with surrounding populations in natural areas (Santangelo et al., 2022). Similar selection, as well, can drive divergence between lineages, via the evolution of different traits in response to similar selection regimes, provided that gene flow remains sufficiently low. However, this scenario requires further study and investigation.

In addition to the aforementioned scenarios, in which the build-up of reproductive isolation is mediated by changes in geography or selection, secondary contact between divergent species can further strengthen reproductive barriers under specific circumstances, by means of reinforcement or the formation of a hybrid *taxon*. The Italian sparrow (*Passer italiae*), for example, is a hybrid species, which originated when populations of the house sparrow (*Passer domesticus*) were introduced into the distribution range of the Spanish sparrow (*Passer hispaniolensis*) (Elgvin et al., 2017; Runemark et al., 2018).

## Altered speciation dynamics and long-term insights into speciation

One of the most striking differences between human-induced reduction and increase in reproductive isolation is that the former may involve loss of species cohesion, leading in some cases to reverse speciation, with a consequent decline in biodiversity. With a few exceptions (e.g. the Italian sparrow, as described above), instead, it is very uncommon to observe complete speciation, in the short term, as a result of anthropogenic change. Instead, we may detect the initial development of reproductive isolation, which may be lost if the conditions that facilitated it cease. This is due to the fact that anthropogenic effects are new in the history of life, where the time required for speciation is much longer than the time required for despeciation. If the conditions promoting divergence do not persist during this period, the incipient speciation process may reverse and be lost (Coyne and Orr, 2004). Consequently, this represents an opportunity to investigate the early stages of speciation. Indeed, anthropogenic hybrid zones serve as natural laboratories, being replicated in space (Simon et al., 2020; Westram et al., 2021), precisely dated in written records (Calfee et al., 2020; McFarlane et al., 2020) and potentially offering precise demographic estimates (Grabenstein and Taylor, 2018).

Having some speculations on the predicted change in the number of species, in the short-term we may expect a general loss of biodiversity, given the different rate at which anthropogenic extinction and anthropogenic speciation occur. A deeper look into the future, however, reveals insights gained from previous mass extinctions, which show that they are usually followed by eras with accelerated speciation rates, after ecological communities have recovered (Harries et al., 1996; Hallam A and Wignall P B, 1997; Foote, 2023). For

instance, peri-alpine whitefish species (*Coregonus* sp.) underwent a severe decline in biodiversity, due to eutrophication in their natural environment (Vonlanthen et al., 2012). Following environmental restoration, genetic and functional diversity was quickly recovered in some of the *taxa* (Smith and Schindler, 2009; Jacobs et al., 2019). This suggests that conservation strategies should focus on preserving (re-)diversification rather than solely protecting individual species.

## Effect of a changing environment on co-occurrence, abundance and phenotype

Species patterns of co-occurrence and abundance have been extensively studied in a variety of organisms (Wisz et al., 2013). Traditionally, three kinds of filters have been described as key drivers of species distributions in different habitats: capability of dispersal, environment and biotic interactions (Hardy et al., 2012). Several abiotic and biotic parameters affecting the distribution of microbial, fungal, plant and animal species have been identified (Karst et al., 2005; Fierer and Jackson, 2006; Kraan et al., 2020). However, disentangling the effects of different elements is not an easy task. The use of appropriate statistical tools is crucial for understanding the effect of different factors on species presence and abundance. This allows us to predict shifts in ecological communities based on the changes of monitored parameters (Guisan and Thuiller, 2005; Ferrier and Guisan, 2006). Substantial effort has been put in assessing the relative contribution of environmental parameters with respect to species interactions in shaping the ecological community composition (Blois et al., 2014; D’Amen et al., 2018). Patterns of co-occurrence have been specifically studied (Heino and Grönroos, 2013; Larsen and Ormerod, 2014), with habitat size often emerging as a significant variable affecting species co-occurrence probability (Azeria et al., 2012; Levine J.M. and Hart S.P., 2020). In lakes as well, the effects of several variables on ecological communities have been studied, with size and depth playing a pivotal role (Griffen and Drake, 2008; Alahuhta et al., 2018; Cordero and Jackson, 2021; Mehner et al., 2021). However, more research is needed to develop a comprehensive view of these effects (Jackson et al., 2001).

Notably, environmental factors and species interactions have an important effect on species phenotype as well (Kaul and Thornton, 2014; Trubenová et al., 2019), playing an important role in adaptation to the ecosystem (Rellstab et al., 2020; Limberger and Fussmann, 2021). For example, it has been found that the presence of pike in lakes favours niche specialisation and phenotypic divergence between populations of European whitefish, *Coregonus lavaretus*. This process is particularly interesting, as it can be interpreted as an incipient step towards speciation (Öhlund et al., 2020).

## Effect of co-occurrence on phenotype (character displacement)

Character displacement is the phenomenon according to which closely-related species that occupy similar ecological niches have their characters driven apart when they co-occur, with respect to instances in which they are not in sympatry. This mechanism helps the two species to fully exploit the ecological niches they have adapted to, minimising the risks for overlap, and leading to character differentiation (Brown and Wilson, 1956). As a result of this process, character displacement can also promote speciation (Losos, 2000).

In many organisms, body size is an important character that affects their ecology and their fitness (Damuth, 1991; Rudolf et al., 2014). It is particularly prone to be displaced when two species occur in sympatry. Where they do occur, populations of closely-related species will show more divergent size with respect to places where they do not co-occur (Douglas, 1987; Kawano, 2002; Bothwell et al., 2015). Character displacement usually involves differentiation of one or more characters in co-occurrence, but occasionally such characters may actually converge in sympatry, possibly to enhance competitive ability (Grant, 1972; Dunham et al., 1979).

## Genetics as a tool to identify signatures of human-induced environmental change

We refer to the genetic diversity among individuals of the same species as intra-specific genetic diversity, whereas we define inter-specific genetic diversity the one that exists between different species. In particular, intra-specific genetic diversity is pivotal to adaptation to the environment, as it provides the variation which evolutionary forces can act on (Fisher 1930). Inter-specific genetic variation, instead, results from divergence between lineages and speciation, and can reveal information on evolutionary relationships (Coyne and Orr 2004). Selection favours genetic traits that are best adapted to the current environment (Endler 1986). Since many phenotypic traits are underlain by genotype, it appears clear how adaptation and evolution are influenced by selection on certain genes. The study of selection can shed light on the mechanism by which individuals and species respond to it, and on the process of adaptation with changing environmental conditions (Barrett and Hoekstra 2011).

## The common bream and the white bream in their ecological context

Human modifications of natural environments have severely affected northern latitude lakes. In particular, a sensible increase in temperature and a decrease in visibility has been reported (Whitehead et al. 2009; Dokulil 2014), mainly due to eutrophication (Larsen et al. 2011; Solomon et al. 2015). These changes may have a wide array of impacts on several biological aspects of aquatic ecosystems and on their communities. For the purpose of this thesis, we focus on the two species common bream, *Abramis brama*, and white bream, *Blicca bjoerkna*. They are widespread all over Western Asia and most of Europe (Backiel and Zawisza 1968; Bogutskaya and Naseka 2004). Their distribution ranges overlap to a great extent, including in southern Sweden. The common bream, however, reaches more northern latitudes. The common bream and the white bream are sister species, and they occupy similar ecological niches. As juveniles, their main food source is represented by phytoplankton, whereas as adults they gradually shift to feeding on benthic invertebrates (Billard 1997; Kottelat and Freyhof 2007). Despite being very similar to each other, the common bream and the white bream exhibit some differences as well. For instance, while the common bream is a pelagic species, the white bream occupies a habitat at the margin of the pelagic and the littoral zones (Lammens et al. 1992). On average, populations of white bream have fewer individuals than populations of common bream (Tátrai et al. 2008). Common bream can also reach larger size than white bream at maturity (Kottelat and Freyhof 2007).

In the context of Swedish freshwater bodies, the common bream and the white bream engage in competitive relationships mainly with the European perch, *Perca fluviatilis* (from now on, perch) and with the common roach, *Rutilus rutilus* (from now on, roach). The perch, like the common bream and the white bream, mainly feeds on plankton in the juvenile stage, and gradually shifts to feeding on benthic invertebrates, once it reaches the adult stage (Hjelm 2000; Svanbäck and Eklöv 2002). However, other fish, including common bream and white bream, constitute a part of perch diet as well (Yazıcıoğlu et al. 2016; Yazıcıoğlu 2019). The roach is an omnivorous species (Kottelat and Freyhof 2007). The main predator of the common bream and the white bream is represented by the northern pike, *Esox lucius* (from now on, pike), which is mainly piscivorous (Craig 1996).

The common bream and the white bream exhibit phenotypic responses to external factors. Specifically, temperature is positively correlated to fish size at maturity (Domagała et al. 2015). However, not enough studies are available on co-occurrence patterns in response to external abiotic and biotic conditions and on the extent of phenotypic response to change of external conditions. Therefore, more investigation is needed in this field.

Many cases of hybridisation have been reported in fish. In our two species of interest, it is known that the common bream can hybridise with the roach (Pitts et al. 1997) and with the common bleak, *Alburnus alburnus* (Blachuta and Witkowski 1984). Interestingly, however, the common bream and the white bream can also give birth to fertile hybrids when they reproduce with each other. This is easily understandable in the light of the recent divergence time between the two species, their similar morphology and the similarity in the ecological niches they are adapted to (Swinney and Coles 1982; Demandt and Bergek 2009). Therefore, hybridisation between these two species can raise a potential flag for the risk of loss of species cohesion, which may happen either at a local or at a global scale.

The morphological and ecological similarity and the close-relatedness of the common bream and the white bream is very important for our purposes, as it makes these two species ideal candidates to study patterns of co-occurrence and abundance, reciprocal morphological change and hybridisation. The importance of these two species will be entirely clear to the reader once we outline the papers that constitute this thesis and we deal with its aims, in the next paragraphs.

## Overview of the papers

**Paper I:** The first chapter of the thesis is a scientific review, which addresses the influence of human activities on the process of speciation.

**Paper II:** The second chapter of the thesis makes use of a large database with extensive information about environmental parameters and fish species and abundance in more than 3,000 lakes all over Sweden, to investigate the effects of such abiotic and biotic variables on patterns of co-occurrence and abundance between the common bream and the white bream. The temporal trends of the variables included in the database are also investigated.

**Paper III:** The third chapter of the thesis makes use of the same database used in Paper II to investigate the effects of abiotic and biotic variables on average size per individual, a proxy for phenotype, in the common bream and in the white bream. Reciprocal change in size between the two species is investigated as well. Particular emphasis is put on testing the theory of character displacement.

**Paper IV:** The fourth chapter of the thesis is a population genomics study of common bream, white bream and hybrids between the two species, in five Swedish lakes, along a gradient of distance and eutrophication. In this chapter, a genomic approach for the identification of the two species and the hybrids is proposed, as traditional morphological criteria are proven to be insufficient. We analyse several genetic parameters and explore species cohesion in the study system, detecting the contribution of parental species to the hybrids, together with intra- and inter-specific genetic diversity.

## Aims of the thesis

The present thesis mainly deals with anthropogenic environmental change, and tries to highlight some of its consequences and potential risks for biodiversity. Being an extremely broad topic, our goal is not to be exhaustive, but we focus on some specific areas of investigation. After an initial chapter in which we summarise existing literature on the effects of human-induced change on the process of speciation, the following chapters work on the case study represented by the common bream and the white bream to investigate ecological equilibria (co-occurrence and abundance), phenotype (body size), genetic structure and hybridisation, in the context of their response to a changing environment. Our work also aims at testing whether environmental change, in its abiotic and biotic components, is actually ongoing in Swedish lakes. In dealing with such issues, we also touch upon the validation of morphological taxonomic classification with a genomic approach, as well as the exploration of hybridisation and inter- and intra-specific genetic variation in our study species. The questions presented below will help to focus our research interests.

### Q1. What is the effect of human activities on speciation?

Nowadays we live in a constantly changing world, where human presence is ubiquitous, and where the effect of humans on the environment and on living species are of outstanding clearness. It is foreseeable that such interaction between man and the environment is bound to get even stronger with time. Species and ecosystems are now being exposed to human-induced change at a pace that had never been observed before the appearance of man on Earth. Therefore, one of the goals of the thesis is to explore patterns of anthropogenic influence on the process of speciation. In the context of this thesis, **Paper I** is the main chapter that deals with this question. In this scientific review, we recap the main literature that describes effects of human activities on speciation. We are interested in mechanisms that may promote divergence between species, accelerating the accumulation of reproductive isolation, but also in mechanisms that may hamper the process of speciation itself, potentially leading to reverse speciation. To present an overview of the topic, we conveniently address separately altered geography and altered selection. The former represents scenarios in which humans directly or indirectly modify the environment, by means of the creation, alteration or removal of geographical barriers between species or populations. The latter represents direct or indirect instances of human-induced changes in species selective regimes. In the following sections of the paper, we discuss how altered geography and altered selection can affect hybridisation between closely-related species, and how human activities considerably alter species turnover rates.

We indirectly address the effects of human activities on speciation also in our study system, which comprises the common bream and the white bream, in **Paper III**. Here, we aim at understanding how proportional change in phenotype, specifically size, might accelerate or hamper the process of hybridisation, given that the two species may be more or less morphologically similar based on co-occurrence or other on external parameters. Increased or decreased morphological similarity may, in turn, impact species recognition and hybridisation rate.

Q2. Do stress factors and deteriorated environmental conditions reduce chances of co-occurrence between closely-related species?

The general deterioration of environmental conditions that mankind is responsible for raises more than just concern for the conservation of delicate ecological equilibria in ecosystems. In particular, closely-related species tend to occupy similar ecological niches. Changes in their environment may alter these equilibria, and separate niches may not be available anymore. With this idea in our mind, in **Paper II** we decide to explore the effects of the change in abiotic and biotic factors on patterns of co-occurrence and abundance in two closely-related freshwater fish species. The investigated species, the common bream and the white bream, represent an ideal study system to address this question, since they are sister species, they are morphologically very similar and they occupy similar niches in lentic environments.

Q3. Do stress factors, deteriorated environmental conditions and co-occurrence affect phenotype and its divergence in closely-related species?

Ongoing environmental change that is object of study in this thesis may have manifold effects. Not only may co-occurrence be affected (see previous question), but there may be complex effects on species phenotype, as a result of either genetic adaptation, phenotypic plasticity or both. In **Paper III**, we analyse the effects of the change in abiotic and biotic factors on size and size divergence in two closely-related freshwater fish species. The chosen species, the common bream and the white bream, are sister species and are again an ideal study system. Indeed, their morphological and ecological features are similar. Due to this similarity, it is possible, with some approximation, to select body size as a proxy for phenotype. Factors that affect divergence in phenotype may drive a different degree of similarity between the species. Considering the role of visual cues in reproduction, this might in turn affect species discrimination and hybridisation, potentially impacting species cohesion.



Q4. Have environmental conditions in Swedish lakes been deteriorating over the last few decades? What have the most important changes been?

The speed at which environmental change occurs has been increasing alarmingly all over the world. In **Paper II** and **Paper III** we want to test whether change is also ongoing in Swedish lakes. We make use of statistical models to test whether water temperature has been increasing and visibility has been decreasing over the last few decades, as expected from literature. Together with environmental variables, also the change in abundance of common bream, white bream and other species that significantly interact with them is studied. Testing whether change in the environment and in its species concerns Nordic freshwater ecosystems can highlight potential threats, which could trigger the mechanisms that are the object of study of the other questions. Identifying such risks could suggest in what areas work may be needed to come up with prevention and reparation measures.

Q5. How accurate is morphological taxonomic identification in our study system? Is morphology a reliable classification tool for closely-related species?

The common bream and the white bream exhibit similar morphologies. Body size is their primary distinguishing feature. The potential presence of hybrids complicates the morphological identification of these species. Hence, in **Paper IV**, we aim at evaluating the accuracy of morphological assessment in these species, by comparing it with results from genome resequencing. This approach allows us to test the reliability of species identification conducted during test-fishing, which relies solely on traditional morphological criteria, for species with very similar morphological features.

Q6. Does hybridisation in common bream and white bream hamper species cohesion?

In **Paper I**, we extensively discuss the decrease in reproductive isolation, and how this can lead to hybridisation and potential loss of species cohesion. In **Paper IV**, we investigate whether this is the case for the common bream and the white bream. We collect individuals that are morphologically assessed as belonging to the two species, as well as hybrids. Using this data, we implement a genomic approach to estimate the contribution of each parental lineage to the hybrids and to assess the degree of separation between the two species. This, in turn, can shed light on the risk of species cohesion loss in our study system in the study area.

Q7. What are the patterns of genetic variation in common bream and white bream populations? Are there any genetic signatures of anthropogenic change or isolation-by-distance?

Not only does anthropogenic change affect ecological equilibria of species, but it also has an effect on the adaptation of species to the new conditions. While a sign of this adaptation can be highlighted by phenotype, it is only at genotypic level that we can observe a fine-scale signature of this process. In **Paper IV**, we conduct a preliminary investigation into the genomic signatures of anthropogenic change in our study system. To do so, we select lakes along a gradient of distance and eutrophication, to represent different degrees of anthropogenic pressure. We scan genomes and we investigate population structures of common bream and white bream, to determine whether patterns of differentiation align better with an isolation-by-distance scenario or with an adaptation-to-eutrophication scenario.

# Methods

## NORS database

For analyses on co-occurrence, abundance and size of common bream and white bream and on temporal trends of environmental and biotic variables, we made use of the Swedish Database for Survey test-fishing (NORS), which is managed by the Swedish University of Agricultural Sciences (National Register of Survey test-fishing - NORS. Swedish University of Agricultural Sciences 2021) (**Papers II-III**). This database contains information on environmental parameters and species abundance for more than 3,000 lakes all over Sweden, surveyed over the last few decades. The database was updated to September 2021. The included environmental variables were: temperature, Secchi depth, average depth, area, altitude, latitude and EQR8, a measure of the ecological status (Naturvårdsverket 2007; Havs- och vattenmyndigheten 2013). As a preliminary step, all the measurements that detected a Secchi depth higher than 90% with respect to the average depth of the lake were removed from the data. This measure was taken to ensure that, for each observation, water was not so clear that the bottom of the lake could be seen and recorded as Secchi depth. Subsequently, all the measurements that were not taken in the months of June, July, August and September were also excluded, to minimise seasonal influence on the observations. With the exclusion of the analyses on temporal trends, all the observations of each lake were aggregated together, and an average was calculated for each variable. This helped avoiding problems of pseudoreplications. We only focused on lakes in which at least common bream or white bream had been detected. Our criteria restricted the total amount of considered lakes to 697. All the analyses were performed with R version 4.2.1 (R Core Team 2021).

## Statistical analyses on the NORS database

Giving an exhaustive account of the statistical analyses performed on the NORS database is a challenging effort. Several statistical tests were performed throughout **Paper II** and **Paper III**, where they are described in detail. The reader will forgive us if, in this section, we do not follow the logical concatenation of questions and tools for investigation, as in good scientific practice. Indeed, doing so would require describing the same methods multiple times

in these following lines. For such dissertation, we redirect the reader to the relevant papers. Here, instead, we will give an overview of the main methods used, tentatively in order of complexity, and refer to the corresponding analyses, providing only a few details about them.

Two-tailed *t*-tests were used whenever it was necessary to compare the means of two normally distributed groups of values (**Paper III**). This kind of test was implemented with the package WEIGHTS version 1.0.4 (Pasek et al. 2021) for the following analyses: effect of the presence of pike on the absolute difference of weight per individual between the common bream and the white bream; effect of the presence of pike on the relative difference of weight per individual between the common bream and the white bream; effect of the co-occurrence between the studied species on the weight per individual in the white bream; effect of the co-occurrence between the studied species on their absolute difference in weight per individual; effect of the co-occurrence between the studied species on their relative difference in weight per individual.

The Wilcoxon rank-sum test was used to assess the difference in weight per individual in common bream, between those populations found to be in co-occurrence with white bream and those found not to be in co-occurrence (**Paper III**). This test is non-parametric, and it was necessary, as an alternative to a *t*-test, given the non-normal distribution of the sample values.

Comparing environmental and biotic variables across more than two categories was accomplished using univariate analyses of variance (ANOVAs), in the case in which the variable was normally distributed, or Kruskal-Wallis rank-sum tests, whenever the variable distribution was not normal. These kinds of analyses were implemented when it was necessary to compare variables values between lakes in which only common bream was present, lakes in which only white bream was present, and lakes in which both species were present (**Paper II**). These statistical tests were followed by post-hoc analyses, aimed at conducting pairwise comparisons between categories. Specifically, ANOVAs were followed by Tukey's range tests, whereas Kruskal-Wallis rank-sum tests were followed by the corresponding non-parametric analyses, Wilcoxon signed-rank tests. For Tukey's range tests, we used the package RSTATIX version 0.7.0 (Kassambara A 2021) (**Paper II**).

Resampling-based multivariate analyses of variance (MANOVAs) allowed us to test the overall difference of sets of abiotic and biotic variables between the categories of lakes that were defined for the previous analyses (only common bream, only white bream or both species) (**Paper II**). These analyses were run with the package MANOVA.RM version 0.5.3 (Friedrich et al. 2022).

Throughout **Papers II-III**, extensive use was made of general linear models (GLMs), general linear mixed models (GLMMs), generalised linear models (GLIMs) and generalised linear mixed models (GLIMMs). While for the first kind of models, no extra packages were utilised, we made use of the package LME4 version 1.1.30 (Bates et al. 2015) for the other three types of

models. The kind of selected model depended on the data at our disposal and on the analyses that we wanted to perform. Whenever the residuals of the response variable followed a normal distribution, GLMs and GLMMs were implemented. In the other cases, we run GLIMs and GLIMMs. What follows is an account of the analyses that it was possible to conduct with each category of models. With GLMs the following analyses were conducted: effect of abiotic and biotic factors on the reciprocal proportion of common bream and white bream, whenever the two species co-occurred in the same lake (**Paper II**); effect of abiotic and biotic factors on weight per individual in white bream (**Paper III**); effect of the proportion of pike on the absolute difference in weight between common bream and white bream (**Paper III**); effect of abiotic and biotic factors on the absolute difference in weight per individual between common bream and white bream (**Paper III**); effect of the proportion of pike on the relative difference in weight per individual between common bream and white bream (**Paper III**); effect of abiotic and biotic factors on the relative difference in weight per individual between common bream and white bream (**Paper III**); effect of co-occurrence of common bream and white bream on the weight per individual in white bream (**Paper III**). With GLMMs the following analyses were conducted: temporal trends of environmental variables and of some of the biotic variables (**Papers II-III**). With GLIMs the following analyses were conducted: effect of abiotic and biotic factors on the assignation of the observation to different categories (only common bream, only white bream or both species) (**Paper II**); effect of abiotic and biotic factors on weight per individual in common bream (**Paper III**). With GLIMMs the following analyses were conducted: temporal trends of some of the biotic variables (**Paper II**).

## Sampling

Samples of putative common bream, white bream and hybrids between the two species were collected from five Swedish lakes, along a distance and eutrophication gradient, in the Summer of 2021 (**Paper IV**). One of the five lakes is Vänern, located in the west of the country. It is the fourth biggest lake in Europe and the biggest in Sweden. The other four lakes are smaller and located around 250 km to the east of lake Vänern. The distance among the smaller lakes is around 10-15 km. Eutrophication in each lake was estimated based on information on Secchi depth contained in the NORS database. The identity of the species was assessed based on morphological criteria.

## Laboratory work and data processing

DNA extraction was performed on each sample, followed by library preparation and short-read shotgun sequencing. Sequencing was executed on a single Illumina NovaSeq6000 S4-300 lane, with 2x150bp reads. The read depth was of around 12X per individual (**Paper IV**).

The common bream genome assembly was downloaded from the National Center for Biotechnology Information, and a sequence dictionary was created for it. Adapters were trimmed away. Bases with a base call accuracy < 99%, reads shorter than 100 bp and reads with > 5 undetermined bases were removed. The reads were aligned to the reference genome, compressed and sorted. PCR duplicates were marked, and variant calling was performed. The files that were obtained were merged according to the scaffold of the reference genome. Genotyping was then carried out. Another filtering step was implemented, in which the following variants were filtered out: variants with a quality by depth < 2.0, with a Fisher strand bias score > 60.0, with a mapping quality < 30, with a mapping quality rank sum < -12.5 and with a read position rank sum < -8.0. We proceeded with removing indels, along with sites that were neither monoallelic nor biallelic. A third filtering step was necessary to remove variants with a proportion of missing genotypes > 5% and with an allele count < 5. Finally, we concatenated files belonging to different scaffolds into a unique file (**Paper IV**).

## Molecular-based species identification and genetic variation

Our dataset was pruned for SNPs in linkage disequilibrium, with the following settings: window size = 50 sites; step size = 10 sites; threshold for  $r^2 = 0.1$ . A Principal Component Analysis (PCA) was conducted a first time with all samples. The species of each sample was reassessed based on visual inspection of the PCA. Three additional PCAs were conducted, based on the reassessment: on common bream, on white bream with potential backcrosses and on white bream without potential backcrosses (**Paper IV**).

A maximum likelihood estimation of individual ancestries (admixture analysis) was conducted on the pruned dataset with all samples, with their species assessed on morphological criteria. The species of each sample was reassessed based on visual inspection of the admixture analysis. This reassessment was in line with the one conducted through the PCA. Three additional admixture analyses were executed, based on the reassessment: on common bream, on white bream with potential backcrosses and on white bream without potential backcrosses. All the admixture analyses were conducted with a number of clusters  $K$  varying between 1 and 10 (**Paper IV**).

## Genome scans

We calculated LD decay all over the genome. After visually inspecting the LD decay patterns, we decided to use sliding windows of 80 kb, with a minimum of 10 kb sites covered, for the successive analyses (**Paper IV**).

Genome scan analyses were performed to calculate the following statistics: genetic differentiation between groups ( $F_{ST}$ ), absolute genetic divergence between groups ( $D_{XY}$ ) and genetic diversity within each group ( $\pi$ ). The genome scans were conducted at species level and at population level. For  $F_{ST}$  and  $D_{XY}$ , the analyses were conducted in the form of pairwise comparisons of different groups (species and populations). For  $\pi$ , the analyses were conducted within groups (species and populations). The grouping into species was based on the genomic reassessment. The grouping into populations, within each species, was based on the lake where the sample came from. We excluded from these analyses F1 hybrids and potential backcrosses. It is worth noting that the genomic reassessment revealed the absence of common bream samples from lake Erken. Moreover, only a single white bream individual was retrieved from lakes Syningen and Lommaren. For this reason, since it was not possible to obtain reliable estimates of genetic diversity for these populations, these white bream samples were included only in analyses at species level (**Paper IV**).

# Results and discussion

## **Paper II: Impact of environmental and biotic factors on co-occurrence between the common bream and the white bream**

In the present section, for reasons of conciseness and clarity, whenever pairwise comparisons between different categories of lakes based on species presence (“Common bream”, “Both” and “White bream”) are conducted, we report the results for the comparison “Common bream” – “Both”, with biotic independent variables considered as proportions in terms of weight.

At first, we tested whether lakes belonging to different categories in terms of species co-occurrence are overall different, across the selected environmental (temperature, Secchi depth, average depth, area, altitude, latitude, EQR8) and biotic (proportion of perch, proportion of pike, proportion of roach) variables, using MANOVAs. This test reveals a significant difference between lakes that host only common bream and lakes that host both species. The subsequent ANOVAs and Kruskal-Wallis rank-sum tests were aimed at assessing whether the different lake categories differ for single variables. Post-hoc analyses were used to conduct pairwise comparisons, revealing that altitude, EQR8, proportion of perch and proportion of pike are significantly higher in the category “Common bream”, whereas area and latitude are significantly higher in the category “Both”.

The effect of each variable to determine the assignation of the observation to different categories of lakes was investigated by GLIMs in pairwise comparisons. The comparison between lakes belonging to the category “Common bream” and lakes belonging to the category “Both” shows that higher average depth, altitude, EQR8 and proportion of perch, pike and roach disfavour the co-occurrence of white bream, whereas higher values of Secchi depth and area favour their presence along common bream.

In cases of co-occurrence between the two species, we tested with GLMs the effect of the selected variables on their reciprocal abundance, finding out that the proportion of roach favours common bream over white bream (Figure 2; Table 1).

Finally, temporal trends of selected variables analysed by means of GLMMs and GLIMMs show that Secchi depth, EQR8, proportion of pike, proportion of roach and proportion of common bream have been decreasing



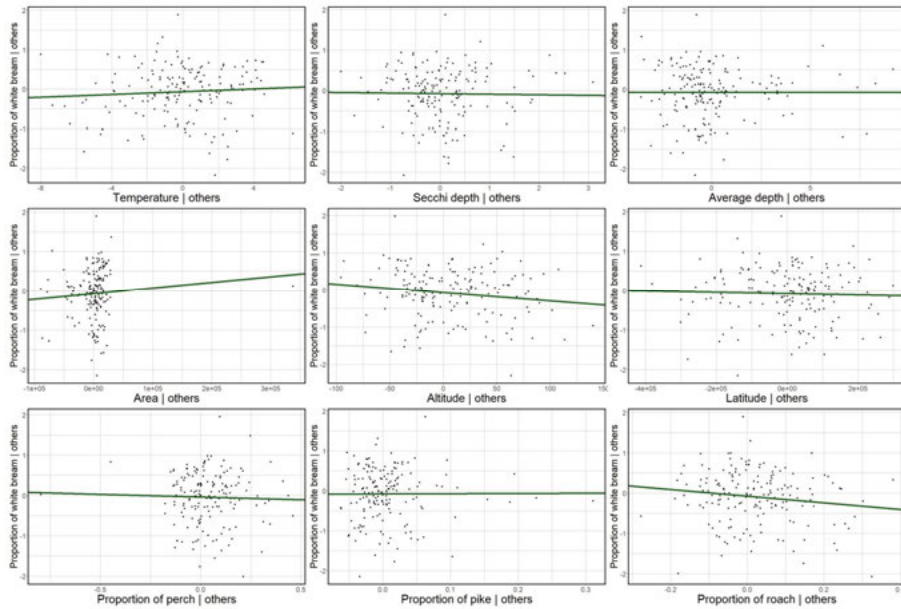
significantly. On the other hand, temperature, proportion of perch and relative proportion of white bream out of the sum of common bream and white bream have been increasing significantly.

Most of the previous studies have made use of null model analyses of species co-occurrence patterns to investigate the connection between environmental parameters and the composition of the community (Azeria et al. 2012; Heino and Grönroos 2013; Blois et al. 2014; Haynes et al. 2014; D'Amen et al. 2018). Our approach, instead, aims at testing for the effects of different explanatory variables on co-occurrence, allowing the identification of specific variables that might be responsible for the detected patterns.

The presented results show an ongoing environmental deterioration in Swedish lakes, particularly evident in the increase in temperature and the reduction of water clarity over the last few decades. Environmental fluctuations and change, in turn, play a very important role in shaping the ecological community, and this study highlights their effects on a pair of sister species, the common bream and the white bream, whose patterns of co-occurrence and abundance are very sensitive to external disturbance, given the similarities in their morphology and in the ecological niches that they occupy. In particular, it is remarkable that increased turbidity seems to decrease the chances of co-occurrence of white bream with common bream, which can be due to the fact that environmental deterioration may affect more severely the white bream, which is the species with the smaller population size. The local presence and abundance of white bream seem to be also negatively affected by the proportion of perch, of pike and of roach. Since the proportion of perch and of roach were used as a proxy for competitive pressure, and the presence of pike were used as a proxy for predatory pressure, these results suggest that the white bream may be the most vulnerable species to increased levels of competition and predation. This is probably due to the lower numerosity of white bream with respect to common bream populations.

With regard to co-occurrence between the studied species, the vast majority of the lakes that were taken into account in the present study either host solely common bream or both species. Out of a total of 697 lakes, only 13 of them host white bream populations, with no common bream present either. Although these data may reflect the rarity of such category of lakes, increasing their sample size would be highly beneficial in increasing the power of our statistical analyses. Moreover, we need to be aware that only a limited number of variables has been included in our models, due to the fact that we were restricted to the information contained in the NORS database, but also due to the need to avoid excessive collinearity among explanatory variables. Finally, to keep understanding the effects of the environment and interactions with other species on co-occurrence patterns, we believe that this kind of studies should be extended to other species pairs too, particularly to the ones that occupy similar ecological niches.

### Multiple regression – Biotic variables as proportions in terms of weight



**FIGURE 2** Added-variable plots of the multiple regression which shows the correlation between selected variables (temperature, Secchi depth, average depth, area, altitude, latitude, proportion of perch, proportion of pike and proportion of roach) and the proportion of white bream out of the sum of common bream and white bream. Biotic variables are expressed as proportions in terms of number of individuals. The effect of each independent variable has been corrected for the other variables in the model. When building the model, the response variable  $y$  has been transformed according to the formula  $\log_{10}(y/(1-y))$ , in order to meet the assumptions of linear models. The model has been weighted based on the sum of common bream and white bream.

**TABLE 1** Results of general linear models showing the effects of selected predictors (temperature, Secchi depth, average depth, area, altitude, latitude, proportion of perch, proportion of pike and proportion of roach) on the proportion of white bream out of the sum of common bream and white bream, given that both species are present. Independent variables have been standardised. The response variables  $y$  have been transformed according to the formula  $\log_{10}(y/(1-y))$ , in order to meet the assumptions for linear models. Observations have been weighted according to the sum of common bream and white bream. Biotic variables are analysed as proportions in terms of overall weight of the catch.

Variable	Estimate	Std. error	<i>T</i> value	<i>P</i>
Temperature	-0.013	0.049	-0.264	0.792
Secchi depth	-0.169	0.097	-1.746	0.083 .
Average depth	-0.038	0.104	-0.368	0.713
Area	0.090	0.069	1.307	0.193
Altitude	-0.114	0.059	-1.940	0.054 .
Latitude	-0.017	0.052	-0.326	0.745
Proportion of perch	0.104	0.059	1.777	0.077 .
Proportion of pike	-0.024	0.062	-0.386	0.700
Proportion of roach	-0.108	0.053	-2.017	0.045 *

### Paper III: Impact of environmental and biotic factors on phenotype in the common bream and the white bream

In the present section, for the sake of conciseness and clarity, we only report analyses in which biotic independent variables are considered as proportions in terms of weight.

First of all, we wanted to analyse the effect of selected environmental (temperature, Secchi depth, average depth, area, altitude, latitude) and biotic (proportion of perch, proportion of pike, proportion of roach) variables on the average weight per individual, separately for the common bream and the white bream, by means of a GLM and a GLIM. These analyses show that Secchi depth and altitude have a positive significant effect on the response variable in the common bream (Figure 3). Instead, for the white bream, a negative significant effect is detected for Secchi depth and latitude, and a positive significant effect is detected for average depth and altitude (Figure 4).

It was investigated what the effect of the selected variables is on the average absolute difference in weight between the two species. The GLM implemented for this analysis does not reveal any significant effects. It was then tested whether the presence of pike, analysed as a categorical variable, has any effect on the response, using a *t*-test. It was found out that, in lakes where pike are present, common bream and white bream exhibit a significantly more similar size, with respect to lakes where pike are not present. However, this effect becomes non-significant if considered in a GLM with the other environmental and biotic variables utilised before (Table 2).

We then moved to testing the effects of the selected variables on the relative difference in average weight per individual between the common bream and the white bream. The GLM that we used does not manage to highlight any significant effects of any of the predictors. We conducted a *t*-test to check whether the presence of pike, analysed as a categorical variable, has any effects on the response variable. It turns out that, in lakes where pike are present, common bream and white bream are significantly relatively more similar, with respect to lakes without pike. This result is robust, and is confirmed when the effect of the presence of pike is analysed in a multiple regression with the other predictors, in a GLM (Table 3).

Finally, we came to investigate the effect of co-occurrence between the two species on their average weight per individual. The Wilcoxon rank-sum test, for the common bream, and the *t*-test, for the white bream, reveal that both species are significantly smaller when they co-occur. However, this effect becomes non-significant in both species when it is analysed in a multiple regression with the other explanatory variables. A *t*-test reveals that the absolute difference in weight per individual is significantly lower in case of co-occurrence (Table 4). There is no effect of co-occurrence on the relative difference in weight per individual, though.

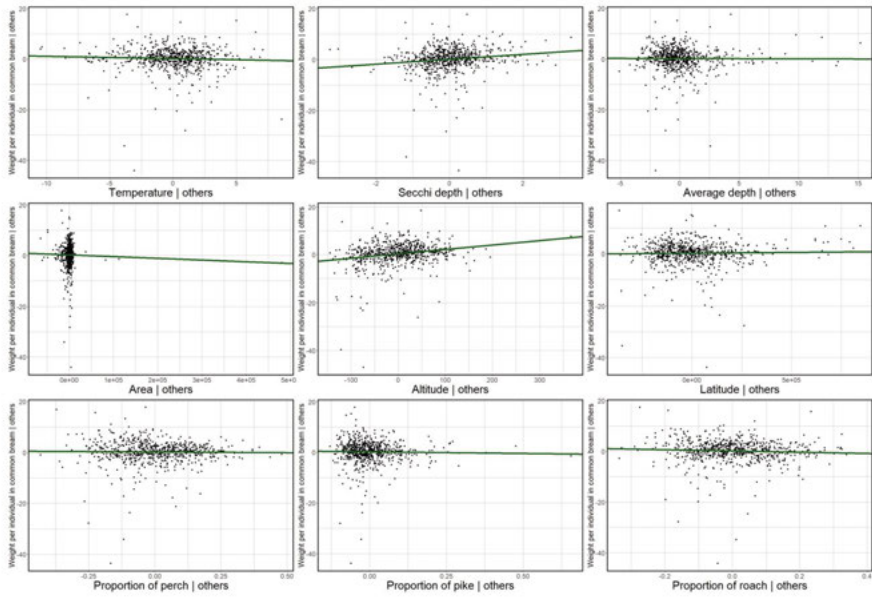
In this study, we aim at analysing the effects of the environment and of biological interactions on a phenotypic proxy, the average weight per individual, in a pair of sister species, the common bream and the white bream. In doing this, we analyse both the effect of selected variables on the two species independently and in relation to each other. The independent approach helps to clarify what factors drive the two species bigger or smaller. On the other hand, the analyses conducted in relation to each other help to understand what conditions favour an increased similarity between the two species. They also help to test the theory of character displacement. In turn, this may be a hint on the degree on niche overlapping and on hybridisation risk, following the change in the monitored parameters.

When analysed separately for each species, weight per individual is significantly affected by a number of factors. Among them, it is interesting to note the opposite significant effect of Secchi depth in the two species. With an increase in water turbidity, common bream become smaller, whereas white bream are driven bigger. This potentially hints at a role of visual cues in niche specialisation. However, neither the absolute nor the relative difference in size between the two species are significantly affected by turbidity. Co-occurrence only affects the absolute difference in weight between the species, but not their relative difference. This shows that no detectable mechanism of character displacement on size is established between the two species. The only explanatory variable that affects the relative difference in weight between the two species is the presence of pike. When pike are present, an increased similarity in weight between the studied species is detected.

It has been shown earlier that the presence of a predator, such as the pike, was associated with an increased phenotypic divergence in Swedish lakes between populations of European whitefish (Öhlund et al. 2020). Therefore, we expected to reveal a similar pattern of divergence between the common bream and the white bream. This would promote the consolidation of the process of speciation between them. Despite our expectations, we detected the opposite effect of the presence of pike on the relative size of our studied species. Although the causes of this phenomenon go beyond the scope of this study, we hypothesise that being more similar in size may be helpful in decreasing predation risk.

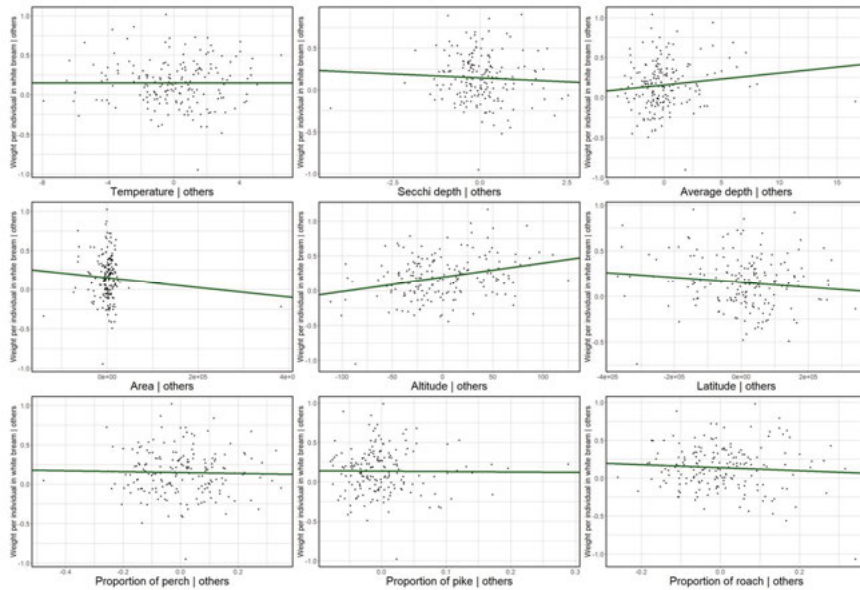
Our study makes a wide use of multiple regressions. This instrument strongly increases the reliability of our results, controlling for the effect of several variables at a time. Although this approach can pinpoint a number of significant correlations between environmental and biotic variables and size in our studied species, it will be necessary to conduct further experimental studies, in order to explore the causality of these relationships. Overall, we demonstrated that changes in the environment affect species phenotype, potentially leading to significant eco-evolutionary outcomes. This should raise concern about ongoing environmental changes, since they can jeopardise species adaptation and survival.

### Multiple regression – Biotic variables as proportions in terms of weight



**FIGURE 3** Multiple regression showing the correlation between selected variables (temperature, Secchi depth, average depth, area, altitude, latitude, proportion of perch, proportion of pike and proportion of roach) and the weight per individual in common bream. Independent biotic variables are expressed as proportions in terms of overall weight of the catch. The effect of each predictor has been corrected for the other variables. A generalised linear model has been run, with a Gamma family assigned to it. The response variable  $y$  has been transformed according to the formula  $\log_e(y)$ . The model has been statistically weighted based on the total number of common bream.

### Multiple regression – Biotic variables as proportions in terms of weight



**FIGURE 4** Multiple regression showing the correlation between selected variables (temperature, Secchi depth, average depth, area, altitude, latitude, proportion of perch, proportion of pike and proportion of roach) and the weight per individual in white bream. Independent biotic variables are expressed as proportions in terms of overall weight of the catch. The effect of each predictor has been corrected for the other variables. A linear model has been run, and the response variable  $y$  has been transformed according to the formula  $\log_{10}(y)$ , in order to meet the assumptions for linear models. The model has been statistically weighted based on the total number of white bream.

**TABLE 2** Results of general linear models showing the effects of selected predictors (temperature, Secchi depth, average depth, area, altitude, latitude, proportion of perch, presence of pike and proportion of roach) on the difference in weight per individual between common bream and white bream in each lake. Continuous independent variables have been standardised, whereas the variable “presence of pike” is binary (categorical). The response variable  $y$  has been transformed according to the formula  $\log_{10}(y + 287)$ . The addition of a positive value (287) to the variable  $y$  is motivated by the need to only have positive values, in order to apply a logarithmic transformation. Observations have been statistically weighted according to the sum of the number of common bream and white bream. The amounts of perch and roach are analysed as proportions in terms of overall weight of the catch.

Variable	Estimate	Std. error	T value	P
Temperature	-0.006	0.011	-0.521	0.603
Secchi depth	0.010	0.022	0.449	0.654
Average depth	-0.004	0.026	-0.141	0.888
Area	-0.002	0.017	-0.097	0.923
Altitude	-0.001	0.015	-0.060	0.952
Latitude	-0.002	0.013	-0.181	0.857
Proportion of perch	-0.003	0.014	-0.229	0.819
Presence of pike	-0.035	0.041	-0.866	0.388
Proportion of roach	-0.001	0.014	-0.087	0.931

**TABLE 3** Results of general linear models showing the effects of selected predictors (temperature, Secchi depth, average depth, area, altitude, latitude, proportion of perch, presence of pike and proportion of roach) on the proportion of weight per individual for white bream respect to common bream in each lake where both species are present. Continuous independent variables have been standardised, whereas the variable “presence of pike” is binary (categorical). The response variable  $y$  has been transformed according to the formula  $\log_{10}(y)$ . Observations have been statistically weighted according to the sum of the number of common bream and white bream. The amounts of perch and roach are analysed as proportions in terms of overall weight of the catch.

Variable	Estimate	Std. error	T value	P
Temperature	0.003	0.030	0.115	0.909
Secchi depth	-0.065	0.057	-1.137	0.257
Average depth	0.048	0.067	0.721	0.472
Area	-0.003	0.043	-0.079	0.937
Altitude	0.014	0.039	0.368	0.714
Latitude	-0.027	0.033	-0.832	0.407
Proportion of perch	-0.010	0.038	-0.262	0.794
Presence of pike	0.231	0.106	2.173	0.031 *
Proportion of roach	-0.002	0.035	-0.053	0.958



**TABLE 4** Results of a *t*-test aimed at comparing the differences between the average weight per individual in co-occurrence in each lake and the average value calculated for the average weight per individual in a situation of non-co-occurrence in each lake, for common bream and white bream. The values *y* of the two vectors used for the *t*-tests have been transformed according to the formula  $\log_{10}(y + 246)$ , to meet the criteria for a parametric test. The addition of a positive value (246) to the variable *y* is motivated by the need to only have positive values, in order to apply a logarithmic transformation. Observations have been weighted according, respectively, to the amount of common bream and of white bream in co-occurrence.

Two tailed <i>t</i> -test	df	Std. error	<i>T</i> value	<i>P</i>
Co-occurrence	173.604	0.047	-11.024	< 0.001 ***

## Paper IV: Species identification and genetic variation in common bream and white bream populations from central Sweden

The PCA performed on all samples, assessed on a morphological basis, allows us to reassess the individuals as belonging to three taxonomic groups: common bream, white bream and F1 hybrids. Some of the white bream individuals show signs of potential introgression from the common bream (Figure 5). Results from the admixture analysis align with these findings. The successive PCAs conducted on common bream and on white bream without potential backcrosses, after the species reassessment, highlight low inter-population differentiation and low intra-population genetic variation. Samples from lake Vänern are an exception for both species, since they are, at the same time, more spread along the first two principal components and more separated from the other lakes (Figure 6; Figure 7). The PCA conducted on white bream with potential backcrosses confirms that potential backcrosses are separated from the rest of the white bream. Admixture analyses at the specific level do not reveal any intra-specific structure.

The genome scan on  $F_{ST}$  between the two species shows an average value of 0.468, whereas the average values in comparisons between populations are slightly below 0, in both species. The average  $D_{XY}$  value detected by the genome scan between the two species is 0.022, whereas comparisons between populations always highlight average values < 0.001, in both species. The genome scans on  $\pi$  at species level reveal an average value of 0.008 in common bream and of 0.010 in white bream. Values of  $\pi$  at the population level are always very similar to the  $\pi$  value of the species the population belongs to (Table 5).

This genomic study is based on whole-genome resequencing of the common bream and the white bream, from samples coming from central Sweden. First, we demonstrate that morphological assessment in these two species and in their hybrids is inaccurate, and needs to be complemented with molecular

information. Second, we demonstrate the presence of F1 hybrids and possibly of common bream introgression into white bream populations. Finally, we estimate intra- and inter-specific genetic variation by means of genome scans on  $F_{ST}$ ,  $D_{XY}$  and  $\pi$ .

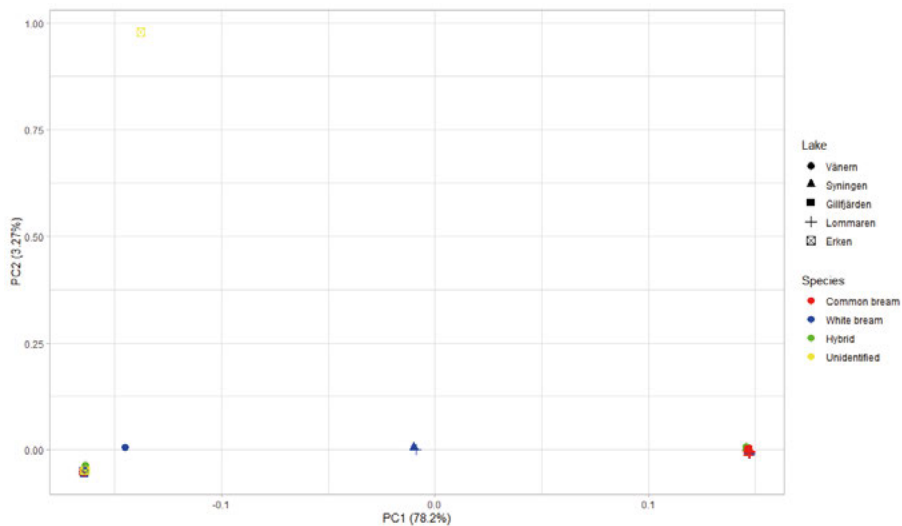
Species identification on a genomic basis was conducted through a PCA and an admixture analysis. These methods have been useful to identify grouping patterns in our samples. From these analyses, the presence of two groups, corresponding to the two species, is clear. The group that mostly comprises individuals morphologically assessed as common bream is assumed to be made up entirely of common bream individuals, and vice versa for white bream. A third group is found to be intermediate between the other two. In the PCA, this third group is located halfway between common bream and white bream, along the first principal component. In the admixture analysis, with  $K = 2$ , the individuals of this group have approximately 50% contribution from each species. We believe that these individuals are F1 hybrids between the two species. Among the white bream, some individuals are clearly separated from the rest in the PCAs, and they show signs of introgression from common bream in the admixture analysis conducted on all samples. Hence, we believe these may be backcrosses. Interestingly, the correspondence between morphological and genomic identification is rather weak. For instance, none of the specimens that were morphologically identified as hybrids had their identity confirmed by genomic data. This study confirms that hybridisation between the common bream and the white bream occurs and is probably not uncommon. Morphological identification criteria for the two species are insufficient, and should be aided by genomic information to correctly determine the taxonomic identity of specimens, especially in the light of hybridisation.

The two studied species appear to be well separated in all the analyses conducted. This suggests the absence of any risks for loss of species cohesion in the near future in the study area. Intra-specific genetic variation is very low in both species. The only exception comes from lake Vänern, whose common bream and white bream individuals are separated from the individuals of the other lakes and show higher intra-population diversity. This pattern overall indicates that isolation by distance can have a key role in the process of genetic differentiation of these species. Instead, differential selective regimes, in the form of different visibility levels, do not seem to have left any identifiable genomic footprints.

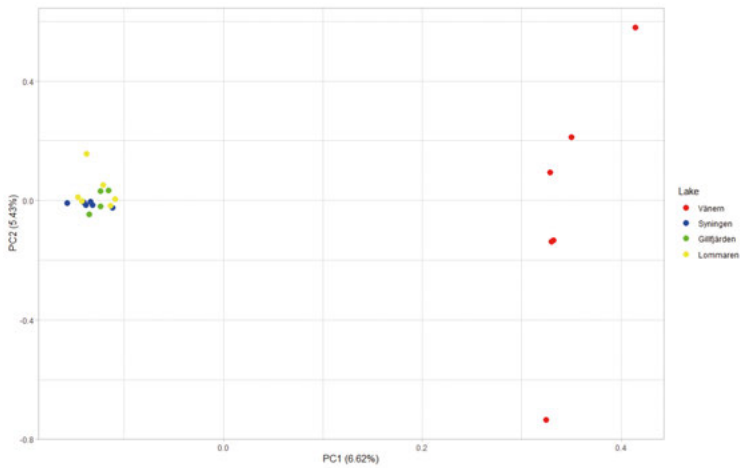
The limitations of traditional morphological criteria in identifying the common bream, the white bream and their hybrids had already been recognised through the investigation of geometric morphometry and genetic markers (Demandt and Bergek 2009). Previous research highlights uneven hybridisation, with most backcrosses occurring with common bream (Demandt and Bergek 2009). However, our study is the first to apply a genomic approach to these species. Contrary to what previously reported, the only potential backcrosses that we identified are with white bream.

In this study, not only does the use of genome scans allow us to calculate average values of  $F_{ST}$ ,  $D_{XY}$  and  $\pi$  across the genome, but also to identify specific regions subjected to particularly high levels of recombination, gene flow or selection. The lack of genome annotation at the time the study was conducted, though, does not allow us to identify the functional role of such regions. Moreover, it is important to note that all our results have to be interpreted taking the reduced sample size into account.

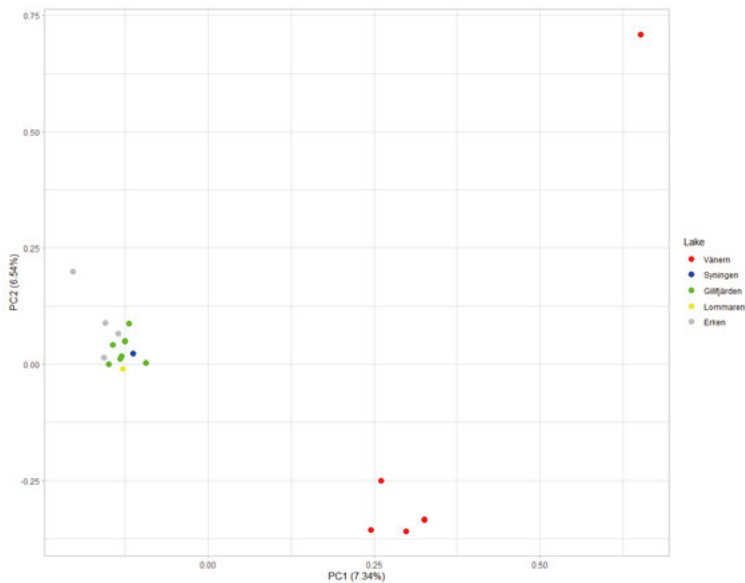
To sum up, here we demonstrate the clear separation of the common bream from the white bream. We also demonstrate their hybridisation, which, however, does not pose a risk to species cohesion. We prove that the identification of the studied species should be aided by molecular tools, and we call for these tools to be implemented in survey test-fishing. We encourage expanding the sampling effort to other areas of the distribution range of the studied species. Finally, identifying the genes that have altered levels of  $F_{ST}$ ,  $D_{XY}$  or  $\pi$  may shed light on the process of adaptation to different environments in freshwater fish.



**FIGURE 5** First two axes of a PCA showing all the samples object of this study. Samples are grouped by colour, according to the species of the sample, and by shape, according to the lake where the sample comes from. The species of the samples in this PCA has been assessed morphologically. The grouping pattern of this PCA has been used to reassess the sample species for the PCAs in Figure 6 and Figure 7.



**FIGURE 6** First two axes of a PCA showing all the common bream samples object of this study. Samples are grouped by colour, according to the lake where the sample comes from. The species of the samples in this PCA has been reassessed based on the PCA shown in Figure 5.



**FIGURE 7** First two axes of a PCA showing all the white bream samples object of this study, excluding the two samples that show potential signs of introgression. Samples are grouped by colour, according to the lake where the sample comes from. The species of the samples in this PCA has been reassessed based on the PCA shown in Figure 5.

**TABLE 5 (a)** Average  $F_{ST}$  values in comparisons between species and between populations within each species. **(b)** Average  $D_{XY}$  values in comparisons between species and between populations within each species. **(c)** Average  $\pi$  values in each species and in each population within each species.

(a)  $F_{ST}$

Comparison	Average $F_{ST}$ value
Common bream / White bream (overall populations)	0.468
Common bream – Vänern / Syningen	-0.0145
Common bream – Vänern / Gillfjärden	-0.0195
Common bream – Vänern / Lommaren	-0.0138
Common bream – Syningen / Gillfjärden	-0.0254
Common bream – Syningen / Lommaren	-0.0204
Common bream – Gillfjärden / Lommaren	-0.0254
White bream – Vänern / Gillfjärden	-0.00758
White bream – Vänern / Erken	-0.0113
White bream – Gillfjärden / Erken	-0.0158

(b)  $D_{XY}$

Comparison	Average $D_{XY}$ value
Common bream / White bream (overall populations)	0.0215
Common bream – Vänern / Syningen	0.00756
Common bream – Vänern / Gillfjärden	0.00757
Common bream – Vänern / Lommaren	0.00756
Common bream – Syningen / Gillfjärden	0.00750
Common bream – Syningen / Lommaren	0.00749
Common bream – Gillfjärden / Lommaren	0.00750
White bream – Vänern / Gillfjärden	0.00969
White bream – Vänern / Erken	0.00975
White bream – Gillfjärden / Erken	0.00956

(c)  $\pi$

Species or population	Average $\pi$ value
Common bream (overall populations)	0.00750
White bream (overall populations)	0.00954
Common bream – Vänern	0.00776
Common bream – Syningen	0.00780
Common bream – Gillfjärden	0.00799
Common bream – Lommaren	0.00779
White bream – Vänern	0.00998
White bream – Gillfjärden	0.00976
White bream – Erken	0.0100

# Conclusions

In this thesis, we mainly explore the impact of environmental changes of anthropogenic origin on living organisms. To do so, we select some topics of interest, which can serve as an example of such a multifaceted phenomenon. In **Paper I**, we start with a general review of anthropogenic effects on the process of speciation. In doing this, we touch upon hybridisation and despeciation as well. In the following papers, as a study case, we focus on a pair of freshwater fish sister species that inhabit European and Swedish lakes, the common bream and the white bream. In **Paper II**, we investigate how external disturbances, potentially of anthropogenic origin, affects the patterns of co-occurrence and abundance in our study system. In this paper, we also try to identify changes in environmental and biotic conditions that have been occurring over the last few decades. In **Paper III**, we analyse how changes in abiotic and biotic conditions affect phenotype in the study species. Mechanisms of character displacement for size are also taken into account. Finally, **Paper IV** is a genomic study that broadens the scope of this thesis, by aiming at verifying traditional morphological criteria for taxonomic identification of common bream and white bream. In this paper, we also examine intra- and inter-specific genetic variation. In line with the main topic of this thesis, we also try to detect genomic signatures of adaptation to a changing environment. Now, we come back to the questions that we asked ourselves in the “Aims of the thesis”, and we try to reflect on them again, in the light of the results of the present thesis.

Q1. What is the effect of human activities on speciation?

As shown in **Paper I**, it is not possible to give a short reply to this question, as the effects are manifold. For a comprehensive review of such effects, we redirect the reader to the paper, or to the “Introduction” section of this thesis. However, we also feel compelled to summarise the answer in a few words. Human activities that may have an impact on the speciation process can be divided into two main categories: alteration of geographical features and alteration of selective regimes. For both categories, we can identify two main outcomes: increase in reproductive isolation and decrease in reproductive isolation. We show that the latter outcome is probably more common than the

former. Loss of reproductive isolation usually involves hybridisation, and can lead to reverse speciation.

The contribution that **Paper III** gives to this question is mostly speculative. However, this paper helps to understand the potential consequences of human activities on species cohesion in a specific study case. In the paper, we show that the presence of pike is the only factor, among the considered ones, to drive the size of the two species relatively more similar to each other. This may hamper, in principle, species recognition, and lead to an increase in the hybridisation rate.

Q2. Do stress factors and deteriorated environmental conditions reduce chances of co-occurrence between closely related species?

The study system that we work on seems to be particularly sensitive to changes in external conditions. Among the most interesting findings of **Paper II**, we find that an increase in water turbidity reduces the chances of co-occurrence between the common bream and the white bream. The species that is more likely to disappear is the white bream. A similar effect is detected with an increase in competition and predation coming from other species. These results seem to suggest that environmental disturbances may strike more easily the species with a smaller population size. Such species may be more susceptible to local extinction, with respect to the other closely related species with a higher numerosity.

Q3. Do stress factors, deteriorated environmental conditions and co-occurrence affect phenotype and its divergence in closely related species?

In **Paper III**, we show that a number of environmental and biotic factors significantly affect the average size per individual, both in common bream and in white bream. However, the relative difference in size between the two species seems to be rather stable, indicating that common bream and white bream exhibit similar responses to environmental changes. The only exception to this pattern of stability of the relative size difference to external factors is represented by the effect of the presence of pike in the lake. When pike is present, the two species are significantly more similar in size, in relative terms. This may be a mechanism that two closely-related species put into place to decrease chances of predation. Finally, we do not detect any character displacement on size, when the two species co-occur.

Q4. Have environmental conditions in Swedish lakes been deteriorating over the last few decades? What have the most important changes been?

In **Paper II** and **Paper III**, we test whether there were any changes in the considered environmental and biotic variables over the last five decades.

Indeed, many of the monitored variables have been changing. Temperature, for instance, has increased significantly, as has turbidity, probably as a result of eutrophication. The proportion of perch has also significantly increased, whereas the proportions of pike and roach have significantly decreased. These changes may, in turn, have important impacts on the patterns of co-occurrence of common bream and white bream, and potentially on the local survival of white bream itself. This should raise concern, and further effort should be put into mitigating the effects of human activities on environmental changes.

Q5. How accurate is morphological taxonomic identification in our study system? Is morphology a reliable classification tool for closely-related species?

Surprisingly, in **Paper IV**, it turns out that the correspondence between morphological and genomic species identification in common bream and white bream is rather low. Out of a total of 41 samples that were morphologically assessed as either being common bream, white bream or hybrids, 14 of them were classified in a different taxonomic group with genomic data. Notably, in the case of hybrids, there is no correspondence whatsoever between the two classification systems. This shows that molecular aid to species identification during survey test-fishing may be crucial, especially for populations with a low numerosity or with a high degree of hybridisation. These considerations may be extended to other hybridising species pairs.

Q6. Does hybridisation in common bream and white bream hamper species cohesion?

The data collect in **Paper IV** suggest that there is no immediate risk of species cohesion loss for the common bream and the white bream in our study area. Indeed, the two species are well separated. Among the 44 sampled individuals, we find 2 first-generation hybrids and 2 white bream individuals with minimal genomic contributions from common bream. However, we need to consider that our sample size is limited and that our sampling is not random, but specifically aimed at collecting enough specimens from each taxonomic group.

Q7. What are the patterns of genetic variation in common bream and white bream populations? Are there any genetic signatures of anthropogenic change or isolation-by-distance?

Part of the effort of **Paper IV** is put into studying genetic diversity in the common bream and the white bream to investigate, among others, the relative importance of differential selective pressure with respect to geographical distance in determining population differentiation. In our case, the difference in selective regimes is represented by different visibility levels across the



sampled lakes. We partially succeeded in achieving this objective. For both species, genetic differentiation seems to be unrelated to the levels of visibility, but we observe that, with an increase of distance, genetic differentiation increases as well. However, these results are just preliminary, as are drawn by the mere visual inspection of PCAs, without any statistical support. We approach the matter also making use of genome scans. Although areas with exceptionally high parameter values pinpoint genomic regions that may be subjected to selection, the lack of genome annotation makes it impossible to investigate the function of such regions. Nonetheless, we show that the overall amount of intra-specific genetic variation is low in both species.

Overall, this thesis sheds light on the diverse effects of anthropogenic change on biological species, but it expands in other directions as well. We demonstrate that alterations in geography and selective regimes can influence the degree of reproductive isolation between species. By studying two sister species, we show that environmental and biotic changes can drive local extinction in species pairs with similar ecological niches, along with changes in important phenotypic features, which can, in turn, affect niche specialisation. Finally, we conduct a genomic project on our species of interest, showing that molecular tools are an important complement to morphology for species identification. We also show that hybridisation occurs, but does not threaten species cohesion. Genomic data may highlight some effects of adaptation to environments subject to anthropogenic change, but further studies are necessary to confirm this hypothesis. These findings underscore the importance of integrating multiple approaches to fully understand the impacts of human activities on biodiversity.

## Abstract in Swedish / Svensk sammanfattning

Miljöförändringarna pågår ständigt och drivs på av mänskliga aktiviteter som på ett genomgripande sätt har förändrat den moderna världen. Förändringstakten har ökat dramatiskt under de senaste decennierna. Naturliga ekosystem påverkas direkt av dessa antropogena aktiviteter, och det finns en oro för bevarandet av arter, ekosystem och ekologiska jämvikter. I denna avhandling undersöks effekterna av antropogena förändringar på naturliga ekosystem. Med tanke på ämnets breda omfattning begränsar vi vår forskning till några viktiga undersökningsområden, utan att sträva efter att vara uttömmande. I den sista delen av avhandlingen undersöker vi också relaterade teman, vilket ger ett bredare perspektiv. I **Papper I** undersöker vi effekterna av antropogena förändringar på utbildningsprocessen. Vi visar att mänsklig påverkan kan ske i form av förändrad geografi och förändrad ekologi. Det senare har konsekvenser för selektiva regimer. I båda fallen kan detta antingen leda till en ökning eller minskning av den reproduktiva isoleringen mellan arter. I hela rapporten ger vi en lång rad exempel. Särskild uppmärksamhet ägnas åt hybridisering, som diskuteras både i samband med förstärkning och risk för förlust av artsammanhållning. I de följande artiklarna fokuserar vi på två systerarter, braxen (*Abramis brama*) och björkna (*Blicca bjoerkna*). Dessa två arter har liknande morfologi, där storleken är den viktigaste särskiljande egenskapen. I **Papper II** utnyttjar vi det faktum att braxen och björknan upptar liknande ekologiska nischer. Detta gör dem till utmärkta fallstudier för att undersöka hur känsliga deras förekomster är för yttre störningar. I den här artikeln visar vi att förändringar i abiotiska och biotiska förhållanden i sjöar kan störa de känsliga mönstren för samexistens mellan och förekomst av de två arterna. Framför allt utgör ökad grumlighet, predation och konkurrens ett hot mot björknans lokala överlevnad. Detta kan bero på att björkna har en lägre populationsstorlek jämfört med braxen. Björkna är därför den art som är mest känslig för stressfaktorer i form av förändrade miljöförhållanden eller interaktioner med andra arter. I **Papper III** undersöker vi effekterna av abiotiska och biotiska förhållanden i sjöar på fenotypen hos brax och björkna. För detta ändamål fokuserar vi på den genomsnittliga individstorleken och dess relativa skillnad mellan arterna. Med tanke på likheten i morfologi mellan braxen och björkna kan storlek vara en bra fenotypisk proxy. Även om storleken i sig påverkas av ett antal yttre faktorer hos båda arterna, visar vi att den relativa skillnaden är ganska okänslig för yttre störningar. Inte ens samexistens mellan

de två arterna verkar utlösa några mekanismer för karaktärsförskjutning. Vi finner dock att närvaron av gädda i sjön ökar likheten i storlek mellan de studerade arterna. Detta resultat är överraskande, eftersom det inte överensstämmer med liknande studier som genomförts på andra sötvattensfiskarter. Detta kan dock tolkas som en försvarsmekanism för att minska risken för predation, eftersom gäddan är en viktig predator på braxen och björkna. En minskad storleksskillnad mellan de två arterna kan i sin tur försvåra både artkänning och nischspecialisering och potentiellt hota artens sammanhållning. I **Papper II** och **Papper III** undersöker vi de tidsmässiga trenderna för miljöförhållanden och förekomsten av några viktiga arter i svenska sjöar under de senaste decennierna, och finner en ökning av temperatur, grumlighet och andelen abborre, tillsammans med en minskning av andelen gädda och mört. Dessa resultat är intressanta, eftersom vissa av de övervakade parametrarna utgör ett hot mot den lokala överlevnaden av björkna. Detta väcker frågor om bevarandet av sårbara arter. Slutligen, i **Papper IV**, genomför vi en genomisk studie baserad på sekvensering av hela genomet. Huvudresultatet av denna studie är att det morfologiska tillvägagångssättet är otillräckligt för taxonomisk identifiering av våra studiearter. Dessa överväganden kan utvidgas till hybridisering av närbesläktade arter, vilket tyder på att molekylära verktyg bör vara ett viktigt komplement till morfologiska kriterier, särskilt när en exakt redogörelse för antalet individer i populationer behövs, som vid provfiske undersökningar. Genomiska data bekräftar att hybridisering förekommer, eftersom vi lyckas identifiera både F1-hybrider och återkorsningar med björkna. Det utgör dock inte ett omedelbart hot mot artsammanhållningen i studieområdet, eftersom de två arterna ser väl separerade ut. Den intraspecifika genetiska variationen är låg hos båda arterna. De mest differentierade populationerna är dock de som kommer från den mest avlägsna sjön. Även om detta bara är ett preliminärt resultat tyder det på att genomisk differentiering följer ett mönster av isolering genom avstånd, snarare än att vara resultatet av anpassning till olika miljöförhållanden. Sammantaget visar denna avhandling att en analys av antropogena influenser på naturliga system bör angripas från olika vinklar för att ge en heltäckande bild av ett så komplext fenomen.

## Abstract in Italian / Abstract in italiano

I cambiamenti ambientali sono ubiquitari. Le attività umane hanno fortemente contribuito alla loro velocizzazione. Il ritmo dei mutamenti è aumentato drasticamente negli ultimi decenni. Gli ecosistemi naturali sono direttamente influenzati da queste attività antropiche. Ciò desta preoccupazione per la conservazione delle specie viventi, degli ecosistemi e degli equilibri ecologici. Questa tesi esplora gli effetti dei cambiamenti di natura antropica sugli ecosistemi naturali. Trattandosi di un argomento di così ampia portata, la nostra ricerca si restringe ad alcune aree chiave, senza la pretesa di effettuare una trattazione esaustiva della materia. Nella parte finale della tesi, esploriamo anche temi correlati, fornendo una prospettiva di più ampio respiro sulla biologia della conservazione. Nell'**Articolo I**, esaminiamo gli effetti dei mutamenti di natura antropica sul processo di speciazione. Dimostriamo che l'intervento umano può avvenire sotto forma di alterazione delle barriere geografiche o di alterazione degli equilibri ecologici. Quest'ultima forma di intervento porta a sua volta a conseguenze sull'azione della selezione naturale. Sia che siano la geografia o l'ecologia ad essere mutate, tali cambiamenti possono portare a un rafforzamento o a un indebolimento delle barriere riproduttive tra le specie. In questo articolo, viene fornita un'ampia gamma di esempi di questi meccanismi. Particolare attenzione è dedicata al processo di ibridazione, che viene discusso sia nel contesto di un incremento dell'isolamento riproduttivo che alla luce del rischio di perdita di biodiversità dovuto alla formazione di uno sciame ibrido. Negli articoli successivi al primo, ci concentriamo sul caso di studio di due specie sorelle, l'abramide comune (*Abramis brama*) e la blicca (*Blicca bjoerkna*). Queste due specie hanno una morfologia simile, con le loro dimensioni come principale caratteristica distintiva. Nell'**Articolo II**, ci basiamo sul fatto che l'abramide comune e la blicca occupano nicchie ecologiche simili. Questo li rende un caso di studio eccellente per indagare quanto la loro coesistenza sia sensibile ai disturbi esterni. In questo lavoro, dimostriamo che i mutamenti nelle condizioni abiotiche e biotiche dei laghi possono alterare i delicati schemi di coesistenza e abbondanza tra le due specie. In particolare, l'aumento della torbidità, della predazione e della competizione rappresentano una minaccia per la sopravvivenza locale della blicca. Ciò può essere dovuto al fatto che le popolazioni di blicca hanno solitamente un numero inferiore di individui, rispetto a quelle di abramide comune. Pertanto, la blicca sarebbe la specie più

suscettibile ai fattori di stress, rappresentati dai cambiamenti delle condizioni ambientali e delle interazioni con altre specie. Nell'**Articolo III**, esaminiamo gli effetti delle condizioni abiotiche e biotiche dei laghi sul fenotipo delle nostre specie di interesse. A questo scopo, ci concentriamo sul peso medio per individuo e sulla sua differenza relativa tra le specie. Data la somiglianza morfologica tra l'abramide comune e la blicca, il peso può essere un buon indicatore fenotipico. Sebbene il peso medio per individuo sia influenzato da una serie di fattori esterni in entrambe le specie, in questo articolo dimostriamo che la sua differenza in termini relativi è piuttosto insensibile alle perturbazioni esterne. Anche la coesistenza tra le due specie non sembra innescare alcun meccanismo di spostamento dei caratteri. Tuttavia, in risposta alla presenza del luccio nel lago, il nostro studio rivela un aumento della somiglianza relativa in peso tra le specie oggetto di studio. Tale risultato è sorprendente, poiché non si allinea con studi simili condotti su altre specie di pesci d'acqua dolce. Questo potrebbe essere interpretato come un meccanismo difensivo per ridurre le possibilità di predazione, dal momento che il luccio è un importante predatore dell'abramide comune e della blicca. Una maggiore somiglianza fenotipica tra le due specie può, a sua volta, ostacolare il loro riconoscimento reciproco e la specializzazione nelle rispettive nicchie ecologiche, potenzialmente portando ad un incremento del processo di ibridazione. Nell'**Articolo II** e nell'**Articolo III**, analizziamo l'evoluzione temporale delle condizioni ambientali e dell'abbondanza di alcune specie ittiche di interesse nei laghi svedesi negli ultimi decenni, riscontrando un aumento della temperatura, della torbidità e dell'abbondanza del pesce persico, oltre a una diminuzione dell'abbondanza del luccio e del rutilo. Questi risultati sono interessanti, poiché alcuni dei parametri monitorati possono rappresentare una minaccia per la sopravvivenza locale della blicca. Ciò sottolinea la responsabilità dei gestori degli ecosistemi naturali, e della società in generale, nella conservazione delle specie, specialmente delle più vulnerabili. Infine, nell'**Articolo IV**, presentiamo uno studio genomico basato sul risequenziamento dell'intero genoma di individui di abramide comune e di blicca. Il risultato principale di questo studio è che l'approccio morfologico è inadeguato per l'identificazione tassonomica delle nostre specie di studio. Questa considerazione può essere estesa a specie sorelle o strettamente correlate che producono ibridi, suggerendo che gli strumenti molecolari dovrebbero essere un importante complemento ai criteri morfologici, soprattutto quando è necessario un resoconto preciso della numerosità delle popolazioni, come nei monitoraggi dell'ittiofauna. I dati genomici confermano che le due specie producono ibridi fertili, in quanto siamo riusciti ad identificare sia ibridi di prima generazione che ibridi con la blicca di generazioni successive. Tuttavia, tale processo di ibridazione non rappresenta una minaccia immediata per la sopravvivenza dell'abramide comune e della blicca nell'area di studio, poiché le due specie appaiono ben separate a livello genomico. La diversità genetica intraspecifica è bassa in entrambe le specie.

Le popolazioni più differenziate, tuttavia, sono quelle provenienti dal lago più remoto all'interno dell'area di studio. Sebbene questo sia solo un risultato preliminare, esso suggerisce che la differenziazione genomica segue un modello di isolamento per distanza, piuttosto che essere il risultato di un adattamento a condizioni ambientali differenti. Nel complesso, questa tesi evidenzia come l'analisi delle influenze antropiche sui sistemi naturali debba essere affrontata da diverse angolazioni, per fornire una panoramica completa di un fenomeno così complesso.

## Abstract in Russian / Аннотация на русском

Изменения окружающей среды происходят постоянно, они обусловлены деятельностью человека, которая коренным образом поменяла современный мир. За последние несколько десятилетий темпы изменений резко возросли. Природные экосистемы подверглись непосредственному воздействию антропогенной деятельности, что вызвало обеспокоенность в связи с сохранением видов, экосистем и экологического равновесия. В данной диссертации мы исследуем влияние антропогенных изменений на природные экосистемы. Учитывая широту охвата данной темы, мы сузили круг исследований до некоторых ключевых областей. В заключительной части диссертации мы также исследуем смежные темы, обозначаем более широкую перспективу. В **разделе I** мы рассматриваем влияние антропогенных изменений на процесс видообразования. Мы показываем, что вмешательство человека может выражаться в изменении географии и экологии. Последнее имеет последствия для селекционных режимов. В обоих случаях это может привести либо к увеличению, либо к уменьшению репродуктивных изоляций каждого отдельного вида. На протяжении всего раздела мы приводим множество примеров. Особое внимание уделено гибридизации, которая обсуждается как в контексте усиления, так и в контексте риска потери сплочённости вида. В следующих разделах мы сосредоточимся на изучении двух сестринских видов: леща (*Abramis brama*) и густеры (*Blicca bjoerkna*). Эти два вида имеют схожую морфологию, но главным отличительным признаком является размер. В **разделе II** мы рассмотрели тот фактор, что лещ и густера занимают схожие экологические ниши. Это делает их отличным примером для изучения их совместного обитания и взаимодействия с внешними факторами. В данной работе мы продемонстрировали, что изменения абиотических и биотических условий в озёрах могут нарушить тонкие закономерности совместного обитания и численности этих двух видов. В частности: повышение мутности воды, хищничество и конкуренция представляют угрозу для выживания густеры. Это может быть связано с меньшей популяцией густеры по сравнению с лещом. Следовательно, густера является видом, наиболее восприимчивым к стрессовым факторам, представленным изменениям в условиях окружающей среды, а также при взаимодействии с другими видами. В

**разделе III** мы исследуем влияние абиотических и биотических условий в озерах на фенотип интересующих нас видов. Для этого мы сосредоточимся на среднем размере особей и его относительных различиях между видами. Учитывая сходство в морфологии леща и густеры, размер может быть хорошим фенотипическим показателем. Хотя размер, сам по себе, подвержен влиянию ряда внешних факторов у обоих видов, мы показываем, что его относительная разница довольно нечувствительна к внешним колебаниям. Даже совместное обитание двух видов, похоже, не запускает механизмов смешения признаков. Однако, мы обнаружили, что присутствие щуки в озере увеличивает сходство в размерах между исследуемыми видами. Этот результат удивляет, поскольку не согласуется с аналогичными исследованиями, которые провели на других видах пресноводных рыб. Это можно интерпретировать как защитный механизм, снижающий вероятность хищничества, поскольку щука является важным хищником среди леща и густеры. Повышенное сходство в размерах между этими двумя видами может, в свою очередь, препятствовать как распознаванию видов, так и специализации ниш, и потенциально угрожать сплочённости вида. В **разделах II и III** мы исследуем временные тенденции, изменения экологических условий и численности некоторых видов, представленных в шведских озёрах за последние несколько десятилетий. Мы обнаружили повышение температуры, мутность воды, увеличении популяции речного окуня, но снижение популяции щуки и плотвы обыкновенной. Эти результаты интересны, поскольку некоторые из отслеживаемых параметров представляют угрозу для местного выживания густеры. Это заставляет задуматься о сохранении уязвимых видов рыб. Наконец, в **разделе IV** мы проводим геномное исследование, основанное на полногеномном секвенировании. Основной вывод этого исследования заключается в том, что морфологический подход не совсем точен для таксономической идентификации изучаемых нами видов. Эти соображения можно распространить на гибридизацию близкородственных видов, предполагая, что молекулярные инструменты должны стать важным дополнением к морфологическим критериям, особенно, когда необходим точный учёт численности популяций, как при контрольном отлове. Геномные данные подтверждают, что гибридизация имеет место, поскольку нам удалось выявить гибриды первого поколения и обратное скрещивание с густерой. Однако это не представляет непосредственной угрозы для сплочённости видов в месте исследования, поскольку эти два вида хорошо разделены. Внутривидовая наследственная изменчивость у обоих видов низкая. Наиболее дифференцированными являются популяции, происходящие из самого отдалённого озера. Хотя это лишь предварительный результат, он позволяет предположить, что геномная дифференциация следует схеме пространственной изоляции, а не



является результатом адаптации к различным условиям окружающей среды. В целом, данная диссертация подчеркивает, что к анализу антропогенного влияния на природные системы необходимо подходить с разных сторон, чтобы дать всестороннее представление о таком сложном явлении.

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I have finally come to write the acknowledgements section of this doctoral thesis. The attentive reader will have already noticed the shift of persona in my writing. I have indeed abandoned the “we” that has characterised the previous sections of this scientific enterprise, to embrace the “I” that will guide the reader throughout these acknowledgements, to express my gratitude to the people that I have met along this journey. Such a careful reader shall forgive this inconsistency, because this change is the only way I can properly thank my supervisors, as well as everybody else.

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