


# Hybridization in the Anthropocene – how pollution and climate change disrupt mate selection in freshwater fish

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## ABSTRACT

Chemical pollutants and/or climate change have the potential to break down reproductive barriers between species and facilitate hybridization. Hybrid zones may arise in response to environmental gradients and secondary contact between formerly allopatric populations, or due to the introduction of non-native species. In freshwater ecosystems, field observations indicate that changes in water quality and chemistry, due to pollution and climate change, are correlated with an increased frequency of hybridization. Physical and chemical disturbances of water quality can alter the sensory environment, thereby affecting chemical and visual communication among fish. Moreover, multiple chemical compounds (e.g. pharmaceuticals, metals, pesticides, and industrial contaminants) may impair fish physiology, potentially affecting phenotypic traits relevant for mate selection (e.g. pheromone production, courtship, and coloration). Although warming waters have led to documented range shifts, and chemical pollution is ubiquitous in freshwater ecosystems, few studies have tested hypotheses about how these stressors may facilitate hybridization and what this means for biodiversity and species conservation. Through a systematic literature review across disciplines (i.e. ecotoxicology and evolutionary biology), we evaluate the biological interactions, toxic mechanisms, and roles of physical and chemical environmental stressors (i.e. chemical pollution and climate change) in disrupting mate preferences and inducing interspecific hybridization in freshwater fish. Our study indicates that climate change-driven changes in water quality and chemical pollution may impact visual and chemical communication crucial for mate choice and thus could facilitate hybridization among fishes in freshwater ecosystems. To inform future studies and conservation management, we emphasize the importance of further research to identify the chemical and physical stressors affecting mate choice, understand the mechanisms behind these interactions, determine the concentrations at which they occur, and assess their impact on individuals, populations, species, and biological diversity in the Anthropocene.

*Key words:* reproductive barriers, hybrids, mate choice, speciation, water quality, contaminants, environmental stressors.

## CONTENTS

I. Introduction	2
II. How anthropogenic stressors may affect the integrity of reproductive barriers	3

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(1) Changing home range and spawning times	4
(2) Disruption of sexual cue production (chemical and visual)	5
(a) Expression of chemical cues	5
(b) Expression of visual cues	6
(3) Disruption of sexual cue transmission (chemical and visual)	7
(a) Transmission of chemical cues	7
(b) Transmission of visual cues	7
(4) Disruption of sexual cue reception (chemical)	8
(5) Decreased encounter rate among conspecifics	8
III. Evidence of differences in tolerance or susceptibility to environmental stressors between hybrids and parental fish species	9
IV. Relevance of anthropogenic-induced hybridization to biodiversity and conservation	10
V. Assessing key knowledge gaps that inform future research needs	10
VI. Conclusions	12
VII. Acknowledgements	12
VIII. Author contributions	12
IX. Data availability statement	12
X. References	12
XI. Supporting information	15

## I. INTRODUCTION

Speciation (see Table 1 for key definitions), the process of divergence from single populations into multiple species, is the foundation of biodiversity. This process requires the build up of reproductive isolation and consequently the reduction of gene flow between diverging populations. Mating between two evolutionarily distinct lineages (i.e. hybridization, see Table 1) is limited by reproductive barriers (see Table 1) between species, which can reduce the production, viability, or fertility of hybrid offspring (see online Supporting Information, Appendix S1 for further information about reproductive barriers). Barriers that prevent interspecific mating (i.e. pre-copulatory barriers) are often effective in limiting hybridization (Coyne & Orr, 2004) and may include the use of chemical, visual, acoustic, tactile, and electrical signals used in mate choice (see Table 1) decisions, as well as morphological differences in reproductive organs (Rometsch, Torres-Dowdall & Meyer, 2020). However, these barriers are often fragile, and their disruption can lead to hybridization (Rosenthal, 2013).

The potential for changing environments to disrupt pre-copulatory barriers is especially relevant in freshwater ecosystems, which humans have dramatically altered. Physical alterations, like changing river courses and water flow, shifting phenological patterns due to climate change, and translocating and stocking fishes have shifted species distributions and interactions (Crispo *et al.*, 2011; Meraner *et al.*, 2013; Reid *et al.*, 2019; Fennell *et al.*, 2023). Freshwater ecosystems are particularly vulnerable to anthropogenic stressors due to the influences of air temperature on water quality and quantity (e.g. warm temperatures decrease dissolved oxygen levels, changes in flow regimes are linked to changes in physicochemical processes), less mitigation *via* dilution than in marine ecosystems, and their proximity to human populations (Capon, Stewart-Koster & Bunn, 2021). Moreover, water

quality has degraded in the last two centuries due to inputs of nutrients and chemical contaminants from anthropogenic activities (Reid *et al.*, 2019). From first principles, physical and chemical changes in water quality (e.g. increased turbidity, eutrophication, and exposure to pollutants) may affect courtship behaviour, expression of secondary sexual traits (see Table 1), and communication *via* visual or chemical cues, which could all impact mate selection (Candolin & Wong, 2019). Changes in physical parameters (e.g. temperature) may induce range shifts, leading to secondary contact, which can indirectly facilitate hybridization (Heath, Bettles & Roff, 2010; Hudson *et al.*, 2013; Muhlfeld *et al.*, 2017). These changes have fundamentally altered the environmental landscape for freshwater fishes, which can affect species interactions, including hybridization (Heath *et al.*, 2010; Hudson *et al.*, 2013; Monette *et al.*, 2020; Fennell *et al.*, 2023; Banerjee *et al.*, 2023).

Some studies have reported correlations between hybridization of fishes in nature and anthropogenic disturbances in aquatic ecosystems. For example, changes in water temperature associated with global warming have increased rates of sympatry and overlap of spawning times between closely related species, facilitating hybridization (Monette *et al.*, 2020; Fennell *et al.*, 2023). Logging (associated with increased sediment load and turbidity) and the development of urban infrastructure (associated with contaminant runoff) were positively correlated with hybridization in trout populations (Heath *et al.*, 2010). In addition, eutrophication was suggested as the driver of hybridization and loss of biodiversity in Alpine whitefish in Europe (Hudson *et al.*, 2013) and in cichlid species in Lake Victoria (Seehausen & Alphen, 1998). More recently, we reported ongoing hybridization between *Xiphophorus variatus* and pre-existing *X. malinche* × *X. birchmanni* hybrids in a section of a river impacted by treated and untreated sewage (Banerjee *et al.*, 2023). Although studies on these topics are accumulating, the literature currently lacks a synthesis and detailed

Table 1. Glossary of terms.

Concept	Definition
Acetylcholinesterase	An enzyme primarily found at postsynaptic neuromuscular junctions, especially in muscles and nerves, that breaks down the neurotransmitter acetylcholine and terminates neuronal transmission (Boison, 2007)
Adverse outcome pathway	A model that identifies the sequence of molecular and cellular events required to produce a toxic effect when an organism is exposed to a substance, as defined by the National Toxicology Program, U.S. Department of Health and Human Services (NTP, 2024)
Con-/heterospecific	Belonging to the same (con-) or different (hetero-) species
Cyclooxygenase	An enzyme involved in the biosynthesis of prostaglandins (e.g. prostaglandin F <sub>2</sub> α), thromboxane and levuloglandins, mediators of inflammatory processes, and the pharmacological target of non-steroidal anti-inflammatory drugs (NSAIDs; Fitzpatrick, 2004)
Genetic incompatibilities	Alleles unique to each parent species that interact dysfunctionally in hybrids, reducing viability and/or fertility
Genetic swamping	The disappearance of alleles and phenotypes unique to a rare species due to overwhelming rates of gene flow from a more abundant species
Gynogenetic species	A species with an asexual mode of reproduction that requires the presence of sperm for egg activation without the actual contribution of paternal DNA
Hormonal pheromones	A subset of reproductive pheromones (i.e. hormones or synthetically related compounds) that act as potent exogenous signals that synchronize reproductive functions among conspecific individuals (Stacey & Sorensen, 2009)
Hybridization	Mating and reproduction between species which have otherwise been reproductively isolated across substantial areas and/or timespans
Hypothalamus–pituitary–gonadal axis	The combined system of the hypothalamus, pituitary gland, and gonadal glands which regulates development and reproduction
Mate choice	Any aspect of an animal's phenotype (neural, chemical, or morphological) that leads to their being more likely to mate with certain individuals than with others
Olfactory sensory neurons	Cells in the olfactory epithelium which detect odorant molecules and transmit information to the central nervous system
Prostaglandin F <sub>2</sub> α	A non-steroidal hormonal pheromone known to stimulate ovulation and sexual receptivity in teleosts
Reproductive barriers	Mechanisms that prevent different species from successfully interbreeding and producing viable offspring
Reproductive endocrine-disrupting compounds	A highly heterogenous group of molecules (e.g. industrial chemicals and byproducts, pesticides, pharmaceuticals, plasticizers) which interfere with the functioning of the reproductive endocrine system by affecting the synthesis and fate of hormones, or simulating, potentiating, or antagonizing their actions
Secondary sexual traits	Aspects of phenotype which are sex specific but not part of the reproductive system
Speciation	An evolutionary process by which a single biological population diverges into two or more genetically distinct populations
Vitellogenin	An egg yolk precursor protein which is only expressed in vertebrate females, and is thus a biomarker for exposure to feminizing compounds

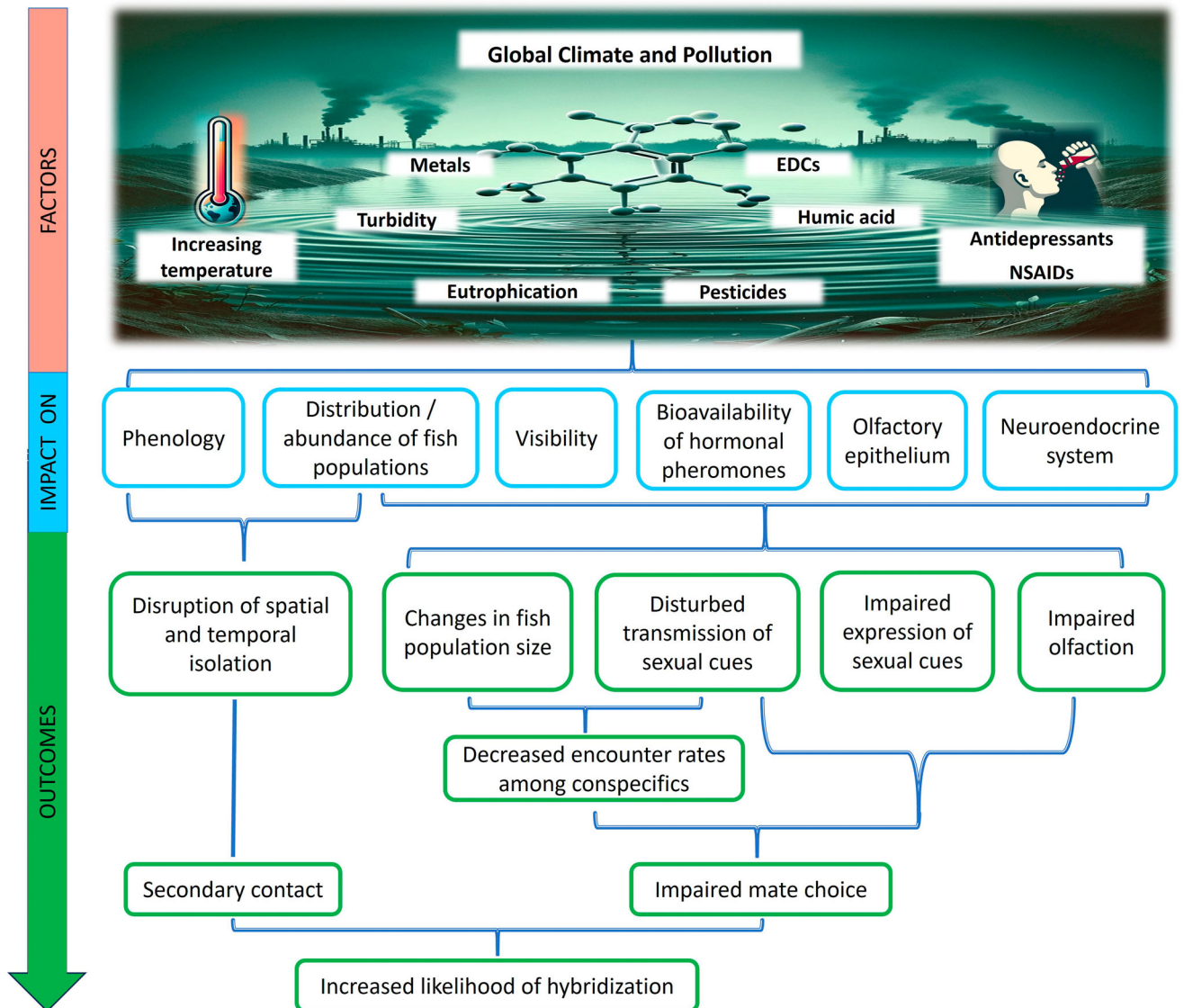
exploration of how these freshwater stressors may facilitate hybridization, and what this means for biodiversity and species conservation.

Here, we conduct a systematic review of an interdisciplinary body of literature to increase our understanding of the drivers of freshwater fish hybridization in the Anthropocene. Details of the methodology we followed for this systematic literature review (Appendix S2; Fig. S1; Table S1), the list of studies analysed at the full-text level (Appendix S3), and the set of studies that fulfilled our eligibility criteria (Table S2) are provided as online supporting information. We synthesize current knowledge about how climate change and chemical pollution facilitate hybridization in freshwater fishes (Section II), their effects on hybrid individuals (Section III), and their relevance to biodiversity and conservation (Section IV). We also identify key gaps in the current

understanding of these processes, highlighting where further research is needed (Section V). Overall, this synthesis underscores important links between anthropogenic disturbance and hybridization. Understanding these links will be critical for preserving biological diversity in the Anthropocene.

## II. HOW ANTHROPOGENIC STRESSORS MAY AFFECT THE INTEGRITY OF REPRODUCTIVE BARRIERS

In this section, we synthesize available studies (29 in total) relevant to the mechanisms by which anthropogenic changes in physical and chemical water quality may affect hybridization in fishes (Fig. 1). These include disruption of spatial and



**Fig. 1.** Mechanisms by which global warming and pollution-driven changes in water quality could facilitate hybridization between freshwater fish species. EDCs, endocrine-disrupting compounds; NSAIDs, non-steroidal anti-inflammatory drugs.

temporal isolation (i.e. home range shifts and overlapping spawning times), behavioural isolation (caused by alterations in the expression/production and transmission of visual and non-visual cues among fish species), disruption of olfactory morphology and physiology (crucial for perception of chemical cues such as hormonal pheromones), and changes in fish population size [affecting encounter rates between conspecifics and heterospecifics (see Table 1)].

### (1) Changing home range and spawning times

Changes in water quality (e.g. temperature, pH, and salinity) and other factors associated with climate change (e.g. precipitation patterns and stream water level) can affect the distribution of fish populations (Muhlfeld *et al.*, 2009b; Culumber

*et al.*, 2012; Splendiani *et al.*, 2016) and spawning times (Araujo *et al.*, 2021; Fennell *et al.*, 2023). We found 23 studies evaluating the correlation between these types of environmental factors and a higher incidence of hybridization (Table S2). Global warming has changed the temperature gradients of rivers and precipitation patterns in relatively rapid time periods (Capon *et al.*, 2021). This has induced species range shifts [upstream and/or latitudinal displacement (Monette *et al.*, 2020; Muhlfeld *et al.*, 2017; Young *et al.*, 2016)], altered spawning times (Fennell *et al.*, 2023), and/or reduced habitat availability (Araujo *et al.*, 2021). These phenological and habitat changes can lead to the spread of non-native species, create secondary contact between formerly allopatric species (Muhlfeld *et al.*, 2009b; Culumber *et al.*, 2012; Splendiani *et al.*, 2016) and drive spatial and temporal overlap of spawning grounds

(Araujo *et al.*, 2021; Fennell *et al.*, 2023). We summarize relevant examples of these phenomena below.

In the USA and Canada, warmer temperatures have facilitated a range shift for introduced rainbow trout (*Oncorhynchus mykiss*) throughout the Rocky Mountains, increasing sympatry with the native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and promoting hybridization (Young *et al.*, 2016; Muhlfeld *et al.*, 2017). Increasing water temperature also led to overlapping spawning times among Yellowstone cutthroat trout (*O. clarkii bouvieri*) and introduced rainbow trout (Fennell *et al.*, 2023). Moreover, low water levels (associated with increasing air temperatures and water diversion) have reduced habitat availability and as a result, increased the frequency of co-spawning between chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) (Araujo *et al.*, 2021). Other changes in water quality (i.e. turbidity, conductivity/salinity, and dissolved oxygen) have been correlated with changes in the distribution of populations of other fish species and the spread of hybridization (Seehausen *et al.*, 2008; Walters *et al.*, 2008; Rudolfson *et al.*, 2019).

These studies support the idea that the emergence of spatiotemporal overlap among previously isolated species is the first step to facilitating hybridization, a broadly supported result (Grabenstein & Taylor, 2018). Species that have not historically been in sympatry may not have evolved pre-mating reproductive barriers that reduce hybridization (Brennan *et al.*, 2014). However, even in species that do have strong pre-mating barriers, these barriers can be broken down in certain environmental conditions.

## (2) Disruption of sexual cue production (chemical and visual)

Conspecific mate preferences are widespread across animal species and are a crucial mechanism in reducing hybridization (Crispo *et al.*, 2011). Mate selection in fish, as with other complex behaviours, relies on the correct integration and functioning of sensory, hormonal, and neuromuscular systems properly to generate, receive, and process sexual cues (Passos *et al.*, 2015). These cues can be visual (e.g. coloration or courtship display), chemical (e.g. hormonal pheromones) or involve other sensory modalities (e.g. electrical, tactile, auditory or vibratory; not discussed here) and different species rely on different cues or combinations of cues for species discrimination and mate choice (Rometsch *et al.*, 2020). Given that we found only one study directly connecting chemical disturbance, cue production, and hybridization, we expand our discussion here to include studies that demonstrate how chemical pollutants may affect secondary sexual characteristics and/or courtship behaviours, but do not directly test hypotheses about mate choice and/or hybridization.

### (a) Expression of chemical cues

We found no studies directly testing how chemical pollution affects mating between conspecifics. Many industrial,

agricultural, and pharmaceutical chemicals are detected in aquatic ecosystems (Naidu *et al.*, 2021), and some have been shown to interfere with the expression of chemical cues in fish. Chemical substances that affect the physiology of the hypothalamus–pituitary–gonadal (HPG; see Table 1) axis or activity of cyclooxygenase (COX; see Table 1) may affect the synthesis of hormonal pheromones and impair mate choice (see Appendix S4 for further information about the physiological control of the synthesis of hormonal pheromones in fish). Numerous and diverse chemical compounds have been shown to affect the functioning of the reproductive endocrine system with subsequent changes in levels of circulating reproductive hormones and in reproductive behaviours (Scott & Sloman, 2004). The release of hormonal pheromones is closely related to levels of circulating hormones, so disturbances in their synthesis may have consequences for mate preferences and mating behaviour. For instance, male guppies (*Poecilia reticulata*) showed a preference for chemical cue-containing water from conspecific females exposed to 17 $\alpha$ -ethinylestradiol (a synthetic oestrogen used in oral contraceptive pills) compared to water from unexposed conspecific females (Saaristo *et al.*, 2019), suggesting that females increased their production of pheromones when exposed to this synthetic oestrogen. Anthropogenic chemicals interfering with chemical cues used in mating systems may be increasingly relevant as the number and amount of chemicals introduced into aquatic ecosystems increases with growing human populations. Below, we provide context that may motivate future research on this topic.

Antidepressants are commonly found in aquatic ecosystems (Chen *et al.*, 2022). They are designed to alter concentrations of monoamine neurotransmitters like dopamine, serotonin, and noradrenaline in the brain (Thompson & Vijayan, 2022). These neurotransmitters, in turn, modulate levels of gonadotropin-releasing hormone (GnRH), gonadotropins, oestrogen, and maturation-inducing steroids in the gonads (Thompson & Vijayan, 2022). The antidepressant fluoxetine (brand name Prozac) is known from laboratory-controlled studies to be a neuroendocrine disruptor in fish, reducing concentrations of serotonin in the brain of hybrid striped bass (*Morone saxatilis*  $\times$  *M. chrysops*) (Bisesi Jr *et al.*, 2016), and causing changes in levels of 17 $\beta$ -oestradiol and decreased expression of oestrogen receptors in female goldfish (*Carassius auratus*) (Mennigen *et al.*, 2008). A mix of antidepressants (fluoxetine and venlafaxine) caused additive effects on brain serotonin levels in hybrid striped bass (Bisesi Jr *et al.*, 2016).

Non-steroidal anti-inflammatory drugs (NSAIDs, e.g. ibuprofen, naproxen, and diclofenac) constitute the pharmaceutical group most commonly found in aquatic environments and can affect the production of prostaglandin hormonal pheromones by inhibiting the enzyme COX (Świacka *et al.*, 2021). Exposure of goldfish to the NSAID indomethacin decreased serum prostaglandin F $2\alpha$  (PGF $2\alpha$ ; see Table 1) concentrations and blocked female sexual behaviour (Sorensen *et al.*, 2018). Besides affecting the synthesis of prostaglandins, exposure to environmentally

relevant concentrations of NSAIDs modulated the synthesis of the steroid hormones 17 $\beta$ -oestradiol and testosterone, and increased expression of vitellogenin (a biomarker of exposure to oestrogenic compounds; see Table 1) in Japanese medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*) (Hong *et al.*, 2007; Ji *et al.*, 2013).

Several pesticides also cause endocrine-disrupting effects downstream of the GnRH pathway (León-Olea *et al.*, 2014; Giroux, Gan & Schlenk, 2019) that disturb the reproductive endocrine system in salmonids (*Oncorhynchus* spp.; Crago & Schlenk, 2015; Giroux *et al.*, 2019) and zebrafish (Hu *et al.*, 2022; Kung *et al.*, 2015). These endocrine effects of exposure to pesticides at early developmental stages persisted through development in zebrafish (Kung *et al.*, 2015).

Finally, some metals (e.g. copper, lead, and cadmium) are known to affect neurotransmitter systems and the HPG axis in the brain of fish. At environmentally relevant concentrations, copper causes species-specific changes in brain serotonin and dopamine levels (De Boeck *et al.*, 1995; De León *et al.*, 2020), lead decreases brain dopamine and blood luteinizing hormone levels after waterborne and dietary exposures (Sionkowski *et al.*, 2017; De León *et al.*, 2020), and cadmium caused a decrease in brain dopamine and serotonin after multi-generational exposure in zebrafish (Li & Li, 2020).

As these studies show, multiple chemical contaminants common in freshwater ecosystems are neuroactive compounds that can alter neurotransmitter systems in the brain and neuroendocrine signalling along the HPG axis. However, how such disruption of the HPG axis is impacting the generation of chemical cues for communication and mate choice, relevant to hybridization, has not been explored.

#### (b) Expression of visual cues

Visual cues important for mate choice in fish include secondary sexual characteristics (e.g. size, coloration, and fin elaborations) as well as courtship displays, and are often species specific. Like chemical cues, they can be important in generating conspecific mate preferences. Attenuation of the conspicuousness of secondary sexual traits or disruption of proper courtship displays [e.g. by neuroactive substances and reproductive endocrine-disrupting compounds (EDCs; see Table 1)] may affect mate choice, even in species that rely on a combination of visual and chemical cues (Hankison & Morris, 2003).

We found only one study (Ward & Blum, 2012) evaluating the influence of chemical or physical water quality parameters on visual cues and its impact on mate choice. Bisphenol A, a weak xenoestrogen commonly used in plastic manufacturing and found in aquatic environments, decreased the intensity of body and fin coloration in male red shiner (*Cyprinella lutrensis*) and reduced female and male preference for conspecifics (Ward & Blum, 2012). However, changes in female discrimination were not tightly linked to male phenotypic traits, indicating that bisphenol A could have also directly affected female responsiveness to chemical cues or behaviour, or that bisphenol A caused changes in the

male phenotype other than skin discoloration (Ward & Blum, 2012). Secondary sexual characteristics, which are typically developed and maintained under the influence of sex steroid hormones, are potential targets of reproductive EDCs (Gore, Holley & Crews, 2018). Reproductive EDCs, like bisphenol A, interfere in the functioning of the reproductive endocrine system and affect either synthesis, release, transport, or elimination of hormones, or mimic, potentiate or block their actions (Gore *et al.*, 2018).

Some studies demonstrated how chemical pollutants may affect secondary sexual characteristics and/or courtship behaviours, but did not directly test hypotheses about mate choice and/or hybridization. There are several examples of contaminants affecting the expression of phenotypic traits, which have been reviewed elsewhere (Gore *et al.*, 2018). Given that courtship behaviour is strongly dependent on circulating gonadal hormones (Gore *et al.*, 2018), EDCs could also impair courtship displays. In many fish species, females prefer males that exhibit more extensive courtship or specific courtship behaviours (Wootton & Smith, 2014). As a result, exposure to EDCs may affect behaviours important for conspecific mate choice. We found no studies evaluating pollutant-induced changes in courtship behaviour associated with impaired mate choice. However, several substances have been shown to reduce male interest in courtship and mating at environmentally relevant concentrations (e.g. the antibacterial triclosan; Wang *et al.*, 2018), whereas others may increase coercive mating behaviour (e.g. the organophosphate chlorpyrifos; De Silva & Samayawardhena, 2005). Increased coercive copulatory behaviour in males may lead to increased chances of mating with both conspecific and heterospecific individuals (Fujimoto *et al.*, 2021). Pollution-induced changes in courtship behaviour could be relevant in species pairs where one of the species is more sensitive to xenobiotics, whether that species is native or invasive. Invasive species are often more tolerant to a broader range of biotic and abiotic conditions (Sax & Brown, 2000) and, in some cases, have been shown to be more resistant to pollution than native species (Karatayev *et al.*, 2009; Kim, Atique & An, 2021). Courtship behaviour could also be affected by substances that impair neurotransmitter systems. Changes in serotonin and dopamine metabolism (like those caused by antidepressants) were shown to disrupt the neural circuitry involved in mate choice in other taxa (Fisher, Aron & Brown, 2006a). Organophosphate (OP) and carbamate pesticides, commonly used in urban and agricultural settings, can disturb locomotion by inhibiting the enzyme acetylcholinesterase (see Table 1; Wijngaarden, Brock & Brink, 2005), and potentially affect complex behaviours that require the integration of the neuromuscular system (Renick *et al.*, 2016), such as courtship.

Given the abundance of evidence for behavioural effects of endocrine-disrupting and neuroactive compounds in fish, chemical contaminants that impair the expression of phenotypic sexual traits and courtship behaviour could be playing a substantial role in the reported higher incidence of hybridization in areas impacted by urban development, agricultural

activities, and chemical disturbance (Walters *et al.*, 2008; Heath *et al.*, 2010; Banerjee *et al.*, 2023).

### (3) Disruption of sexual cue transmission (chemical and visual)

Beyond the initial production of cues, successful communication relies on the transmission of that cue from producer to receiver. Transmission of sexual cues through water is affected by both its physical and chemical characteristics (Rosenthal & Stuart-Fox, 2012), and thus can be impacted by changes therein. In this section, we review the literature – only seven studies in total – that assessed how changes in water quality (physical and chemical) may interfere with mate selection by affecting the expression and/or transmission of chemical and visual cues.

#### (a) Transmission of chemical cues

Once hormonal pheromones are secreted into water, they need to interact with the olfactory receptors of other individuals to induce a physiological response. In theory, the chemical properties of water may interfere with the biological function of hormonal pheromones by affecting their bioavailability, persistence, or chemical integrity. We found only two studies evaluating the impact of changes in water quality (increased concentrations of humic acid) on the transmission of chemical cues related to mate choice in fish (Table S2).

Humic acid, a product of the degradation of organic matter, was shown to interfere with the detection of hormonal pheromones and the discrimination of chemical cues from conspecific and heterospecific mates (Hubbard, Barata & Canario, 2002; Fisher, Wong & Rosenthal, 2006b; Fabian *et al.*, 2007). In goldfish, humic acid at 1 mg/l reduced the electro-olfactogram response of the olfactory epithelium to the steroid pheromone 17 $\alpha$ ,20 $\beta$ -dihydroxy-4-pregnen-3-one (17,20-P), and at higher concentrations ( $\geq 100$  mg/l) affected the response to PGF2 $\alpha$  (Hubbard *et al.*, 2002). The preference for conspecific mates was affected by humic acid at 20 mg/l in female *X. birchmanni* (Fisher *et al.*, 2006b), and in zebrafish in a single test of exposure to humic acid at 200 mg/l (Fabian *et al.*, 2007). Humic acid seems to affect mate choice by binding hormonal pheromones (with a much higher affinity for 17,20-P than for PGF2 $\alpha$ ), which reduces their availability to olfactory receptors (Mesquita, Canário & Melo, 2003), and can interfere with the proper timing of chemical communication. Given the differences in the affinity of humic acid for different pheromones, its disruptive potential may depend on the particular pheromones a species employs. Humic acid from different sources will differ in composition (Huljev, 1986); however, studies conducted with humic acid have not measured the chemical composition of water, so potential effects of other substances present (e.g. metals) on the reported dysfunction of the olfactory epithelium cannot be disregarded. Moreover, changes in other physical and chemical properties of water quality (e.g. pH) may also affect the bioavailability of hormonal pheromones

(Mesquita *et al.*, 2003). However, as discussed in Section II.2, few studies have explored how changes in water quality may interfere with chemical signalling through water and mate choice to facilitate hybridization.

#### (b) Transmission of visual cues

Visual cues are important mating signals in diverse fish species (Wootton & Smith, 2014). The transmission of visual cues can be impacted by the turbidity and light conditions of fresh water. Decreased visibility can result from changes in water quality (e.g. increased turbidity caused by increased nutrient input and subsequent eutrophication) or a shift in habitat to a location with different light conditions (e.g. a population moving deeper in the water column in response to increasing surface water temperature) (Caves & Johnsen, 2021). Decreased visibility masks coloration (Seehausen *et al.*, 2008) and interferes with the ability of fishes to see courtship displays (Engström-Öst & Candolin, 2007). In low-visibility water, species that rely on visual cues may also become less choosy due to a low frequency of encounters with conspecific mates (Berglund, 1995) and more likely to hybridize with heterospecific mates. The importance of assessing visual cues on mate selection was shown in *Xiphophorus pygmaeus*, where in the absence of visual cues (i.e. only chemical cues were presented) preference for conspecific mates was suppressed (Hankison & Morris, 2003). Our systematic review identified three laboratory-controlled and two field-relevant studies (Table S2).

Turbidity-driven disturbance of mate choice was shown in three laboratory-controlled studies. Females of haplochromine cichlids from Lake Victoria preferred conspecific over heterospecific mates under white light, but not under monochromatic light (a light condition that masks interspecific colour differences and simulates increased turbidity) (Seehausen & Alphen, 1998). Additionally, both conspecific and heterospecific behavioural interactions (number of approaches and association time) between invasive red shiner and native blacktail shiner (*Cyprinella venusta*) increased under greater turbidity, which could result in a higher incidence of hybridization (Glotzbecker *et al.*, 2015). Notably, native blacktail shiner females showed a weaker preference for conspecific males in turbid conditions (Glotzbecker *et al.*, 2015). Moreover, increased turbidity with the addition of argillaceous earth powder [Edasil<sup>®</sup> bentonite; turbidity = 25 Nephelometric Turbidity units (NTU)] decreased the association time of *Poecilia latipinna* males with conspecific and heterospecific [the gynogenetic (see Table 1) sexual parasite *Poecilia formosa*] females (Heubel & Schlupp, 2006). Heubel & Schlupp (2006) proposed that mate choice under turbid conditions could be so time-consuming that males respond by decreasing their choosiness.

Deleterious effects of turbidity on mate choice have also been demonstrated in field studies. Anthropogenic eutrophication of pre-alpine European lakes led to hybridization in whitefish (*Coregonus* spp.) radiations (Vonlanthen *et al.*, 2012; Hudson *et al.*, 2013). In cichlids, turbidity was shown to affect

the perception and distribution of some coloration phenotypes more than others based on the amount of light penetrating the water column and the light-scattering properties of particulate and dissolved matter (Seehausen *et al.*, 2008). In Lake Victoria, more diverse intra- and interspecific colour morphs were found in clearer waters, and less diverse colour phenotypes were found in turbid water (Seehausen *et al.*, 2008; Witte *et al.*, 2013), suggesting that these phenotypic classes have collapsed due to a breakdown in visual discrimination. In freshwater ecosystems, short wavelengths are absorbed more strongly (Caves & Johnsen, 2021), hence visual cues in fish species with blue and green coloration patterns are most affected by increased turbidity. Turbidity effects are likely to increase with global warming and continued anthropogenic disturbance. Global warming leads to changes in rainfall patterns and intensity (IPCC, 2021), associated increased runoff, soil erosion, and nutrient and sediment deposition (Sahoo & Anandhi, 2023).

#### (4) Disruption of sexual cue reception (chemical)

Olfaction and olfaction-mediated behaviours can be disrupted by impairing the morphological and/or physiological integrity of the olfactory epithelium (Tierney *et al.*, 2010). The olfactory epithelium in fish is composed of olfactory sensory neurons (OSNs; see Table 1), among other cell types (see Appendix S5 for a description of the olfactory epithelium in teleost fish), which have specialized receptors for different types of chemical cues (e.g. hormonal pheromones, alarm substance, and food cues). Hence, the olfactory epithelium is involved in several complex behaviours, including mating behaviour. The olfactory epithelium is in direct contact with ambient water, which directly exposes it to waterborne chemical pollutants. However, we found no studies investigating the effects of impairment of the olfactory epithelium on fish mate preferences (Table S2). Below, we discuss evidence from the ecotoxicological literature demonstrating how pollutants can impair olfaction as a proxy for how we may expect pollutants to impact mate preferences. Toxicants impacting the olfactory epithelium were reviewed by Tierney *et al.* (2010).

Metals can cause sublethal lesions and death of OSNs, diminish olfactory acuity, and impair olfaction-dependent behaviours (Tierney *et al.*, 2010). For example, copper exposure (20 µg/l) resulted in the downregulation of Na<sup>+</sup>/K<sup>+</sup>-ATPase genes in olfactory rosettes in wild yellow perch (*Perca flavescens*), which could impair the ability of the OSNs to restore electrochemical gradients and result in decreased olfactory acuity (Azizishirazi *et al.*, 2015). In addition, copper can increase the paracellular permeability of the olfactory epithelium (Razmara & Pyle, 2021) and cause a loss of ciliated OSNs (cOSNs; Beyers & Farmer, 2001). At a physiological level, copper can decrease olfactory acuity to chemical cues (Morris *et al.*, 2019) and decrease attraction towards conspecific pheromones (Saucier & Astic, 1995), which could potentially affect mate choice. Other metals, such as cadmium, were shown to suppress antipredator and feeding

behaviours (Scott *et al.*, 2003), as well as responses to adult migratory pheromones (Baker & Montgomery, 2001). Interestingly, different types of OSNs showed different susceptibility to contaminants. cOSNs were more sensitive to metals (Cu, Zn, and Ni) than microvillous OSNs (mOSNs) (Hentig & Byrd-Jacobs, 2016; Lazzari *et al.*, 2017, 2019). Given that cOSNs are known to mediate social behaviours and mOSNs to mediate feeding behaviour, complex behaviours such as identification of conspecifics and pheromone-mediated mate choice are likely to be more sensitive to contaminants than feeding behaviours.

Empirical evidence suggests that other chemicals could affect the ability of the olfactory epithelium to respond to hormonal pheromones. Humic acid may affect the olfactory epithelium, since a reduced preference for conspecific male chemical cues persisted for several days in female *X. birchmanni* after exposure (Fisher *et al.*, 2006b). However, there is currently no direct evidence that humic acid damages the olfactory epithelium. Similarly, mating responses to hormonal pheromones could be impaired by neuroactive pharmaceuticals. Exposure of male goldfish to the antidepressant fluoxetine suppressed the increase in milt production in response to the female primer pheromone 17,20-P or the releaser pheromone, PGF2α (Mennigen *et al.*, 2010). Whether antidepressants affect responsiveness of male olfactory epithelium to pheromones or act by other mechanisms is not clear.

Given the range of ecological interactions facilitated by olfaction (i.e. mating, recognition of conspecific or related individuals, predator avoidance, feeding, and migration), even pollutant-induced olfactory deficits that are reversible (Saucier & Astic, 1995) can have important outcomes at both the individual (e.g. wasting mating effort on heterospecific individuals) and population levels (e.g. sporadic hybridization events).

#### (5) Decreased encounter rate among conspecifics

Hybridization may also occur simply because of a scarcity of conspecific mates and a high abundance of heterospecific individuals, as reported in several animals, including fishes (Mayr, 1963). As encounter rates with conspecifics decrease, choosiness often declines, leading to an increased acceptance of heterospecifics (Willis, Ryan & Rosenthal, 2011). Although we did not discover any studies specifically relevant to this within our systematic review, we discuss how chemical pollution and climate change can drive changes in population sizes and sex ratios, and could result in low encounter rates with conspecifics that trigger hybridization. First, differences in sensitivity to pollutants among fish species (van Treeck, Van Wichelen & Wolter, 2020) may lead to a decline in the frequency of the most sensitive taxon in a given community, promoting a higher encounter rate with heterospecifics. Pollutants may also cause skews in the sex ratio if males and females differ in their sensitivity to pollutants (Guillante *et al.*, 2023). Alternately, feminizing or masculinizing effects of specific pollutants, as is the case for hormones and EDCs (Dang & Kienzler, 2019), may directly impact the ratio of



reproductively active males and females in the population. Second, in species with environmentally sensitive sex-determination systems, the sex ratio can be skewed by changes in temperature or pH (Ospina-Álvarez & Piferrer, 2008; Reddon & Hurd, 2013). Third, as visibility becomes limited by increasing turbidity, mate encounter rates may decrease. As a consequence, fish may become less choosy and more likely to hybridize with heterospecific mates. Fourth, invasive species, by displacing native ones, lead to lower encounter rates among conspecific native fish, facilitating hybridization with the most abundant invasive species. Notably, invasive species also tend to be more tolerant to a broader range of biotic and abiotic conditions (Sax & Brown, 2000), and thus may be more likely to thrive in chemically disturbed environments. Therefore, in situations where encounters among conspecific mates are low, fish may expend more time and energy looking for mates (Heubel & Schlupp, 2006), decrease choosiness for conspecifics, and/or use non-specific phenotypic and behavioural traits such as male size and display rates to choose mates, which may lead to increased mating with heterospecific individuals (Seehausen & Alphen, 1998; Willis *et al.*, 2011).

### III. EVIDENCE OF DIFFERENCES IN TOLERANCE OR SUSCEPTIBILITY TO ENVIRONMENTAL STRESSORS BETWEEN HYBRIDS AND PARENTAL FISH SPECIES

In addition to facilitating hybridization by affecting reproductive barriers, anthropogenic stressors have the potential to affect the survival of parental and hybrid individuals directly. Vulnerability and/or tolerance to anthropogenic stressors can ultimately affect the stability of both hybrid and parental populations. For example, interspecific hybridization is a common practice in aquaculture, used as a strategy to increase growth rate and tolerance to some environmental conditions, presumably as a result of hybrid vigour in first-generation hybrids (Bartley, Rana & Immink, 2000). However, anthropogenic stressors can have either negative or positive effects on naturally occurring interspecific hybrid individuals and populations. In natural populations, interspecific hybrids may also benefit from hybrid vigour or combinations of traits that allow them to colonize novel environments (Todesco *et al.*, 2016; Brauer *et al.*, 2023). More generally, later-generation hybrids can also allow for the transfer of beneficial alleles between species through a process known as 'adaptive introgression' (Edelman & Mallet, 2021). Alternatively, hybridization may lead to decreased fitness through a range of mechanisms, which compromises the viability or fertility of hybrids (Todesco *et al.*, 2016; Moran *et al.*, 2021). Because of the intricate relationship between environmental factors and natural selection, and the variation in hybrid viability and fertility among hybrid crosses, the net negative or positive effects of interspecific hybridization and introgression on fish

populations are difficult to predict. As a result, anthropogenic stressors could reduce or increase the potential for hybrid individuals to persist, depending on the tolerance of hybrids for those particular stressors compared to parental species. In our systematic review, we found 11 studies comparing fitness among naturally occurring hybrids and parental fish species focusing on a range of traits (Table S2).

One of the underlying factors impacting whether hybrid individuals persist in a given environment is their ability to occupy niches not used by parental species. Through recombination and/or epistatic interactions between the parental genomes, hybridization may facilitate the ability of individuals to live under different environmental parameters than the parental species (Seehausen, 2004). This is the case for hybrids between rainbow trout and native westslope cutthroat trout in the Rocky Mountains, USA and Canada (Rasmussen, Robinson & Heath, 2010; Yau & Taylor, 2013). In this case, hybrids have remained restricted to intermediate elevations, where hybrids may have an advantage relative to their progenitor species, which are found in either warmer, lower reaches or colder headwaters. Another example comes from hybrids among sympatric populations of roach (*Rutilus rutilus*) and bream (*Abramis brama*) in lacustrine habitats. Roach × bream hybrids displayed an intermediate position between parental species regarding diet, niche breadth, and trophic position, and a greater flexibility in diet that has probably facilitated their spread and abundance in Irish lakes (Hayden *et al.*, 2011). In the long term, the increasing temperature associated with global warming may push thermally separated, closely related fish species populations into greater spatial overlap and induce the formation of new hybrid swarms.

By contrast, some studies report a lack of hybrid vigour, or even a reduced tolerance to anthropogenic stress. For example, swordtail fish (*Xiphophorus* spp.) hybrids showed severe viability deficits due to genetic incompatibilities (see Table 1; Powell *et al.*, 2020; Moran *et al.*, 2024). Likewise, hybrids between invasive rainbow trout and native westslope cutthroat trout had a lower probability of survival and showed evidence of selection against alleles derived from the invasive rainbow trout (Muhlfeld *et al.*, 2009a; Rasmussen *et al.*, 2010). This result was observed even in relatively warm environments where rainbow trout are predicted to have a selective advantage *a priori* (Kovach *et al.*, 2016). Rainbow × cutthroat trout hybrids, with more than 20% rainbow trout ancestry, showed a roughly 50% reduction in reproductive success (Muhlfeld *et al.*, 2009a), decreased fecundity, and different migratory behaviour and growth rate compared to westslope cutthroat trout (Corsi, Eby & Barfoot, 2013). Moreover, there was a tendency towards decreased survival of hybrids as the proportion of rainbow trout alleles increased (Rasmussen *et al.*, 2010). Another example is the *Phoxinus* species complex, which includes an asexual hybrid distributed in Canada and the USA, which coexists with either or both of their progenitor species, finescale dace (*Phoxinus neogaeus*) and northern redbelly dace (*Phoxinus eos*). The lack of hybrid vigour, and possibly the existence of hybrid disadvantage,

in the *Phoxinus* species complex (Mee & Rowe, 2006; Mee, Brauner & Taylor, 2011), could facilitate the coexistence of asexual and sexual dace in this system (Mee *et al.*, 2011) and more stable parental populations. Finally, hybrids between pumpkinseed (*Lepomis gibbosus*) and bluegill (*Lepomis macrochirus*) sunfish showed mostly intermediate tolerance to hypoxia and intermediate physiological measures of O<sub>2</sub> transport and metabolism (Borowiec *et al.*, 2016), as well as a higher production of reactive oxygen species (ROS) than both parental species (Du *et al.*, 2017). Because the strength and direction of selection varies across environmental clines (Savolainen, Lascoux & Merilä, 2013) and distinct populations (Siepielski *et al.*, 2013), fitness outcomes expected for naturally occurring hybrids are equally variable.

The literature evaluating the impact of naturally occurring hybridization on fish fitness, performance, and tolerance to environmental factors is limited to a few hybrid species complexes, which highlight a diversity of possible outcomes – from outperforming their progenitor species in novel environments to reduced hybrid fitness. It is noteworthy that none of the above studies focused on tolerance relevant to chemical pollution.

#### IV. RELEVANCE OF ANTHROPOGENIC-INDUCED HYBRIDIZATION TO BIODIVERSITY AND CONSERVATION

Although hybridization could increase genetic variation in species with low genetic diversity, it can also pose a threat to species survival (Todesco *et al.*, 2016; Brauer *et al.*, 2023). Hybridization could decrease genetic variation and limit plasticity against further habitat disturbances. For fish and other aquatic species, habitat disturbance has significantly increased during the Anthropocene (Reid *et al.*, 2019). These changes are projected to worsen in the foreseeable future, given current estimates of increasing temperature (IPCC, 2021) and human populations (United Nations, Department of Economic and Social Affairs, Population Divisions, 2017). Human population growth directly leads to an increase in the production and release of household, industrial, and agricultural wastewater (Dudgeon, 2019). In other words, human population density correlates with aquatic pollution. As discussed above, the breakdown of reproductive barriers due to changes in water quality associated with climate change and pollution, as well as potential differences in tolerance to stressors between parentals and hybrids, may facilitate the formation and persistence of new hybrid populations. For endemic species that are not widely distributed, this could lead to their extinction by genetic swamping (see Table 1).

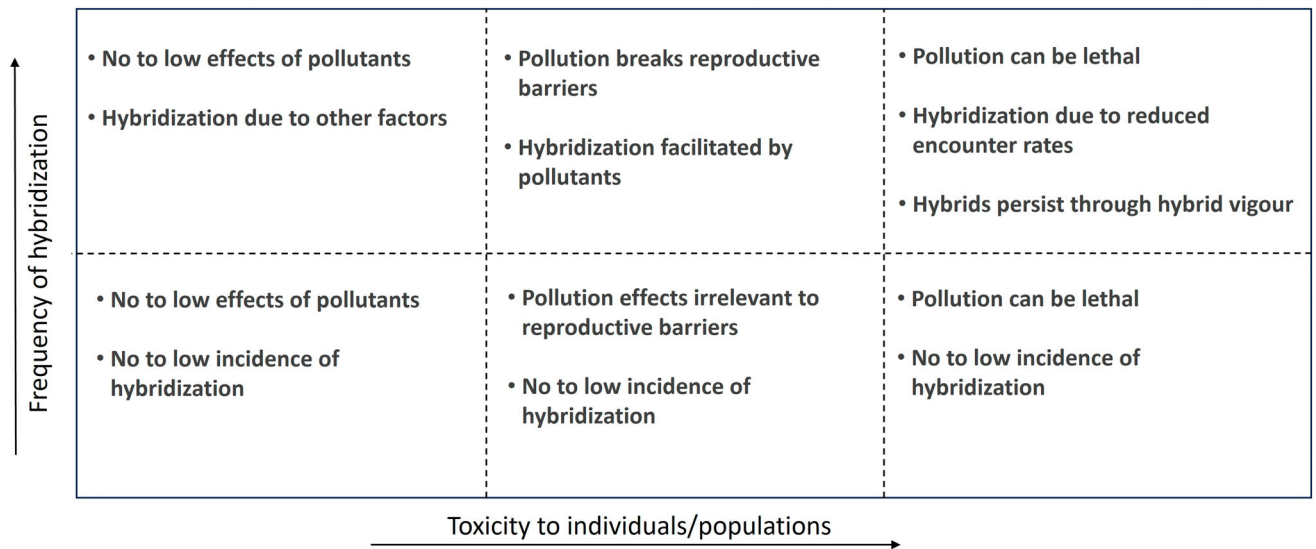
Before the advent of whole-genome sequencing, interspecific hybridization was estimated to occur in about 10% of animal species (Mallet, 2005), but the true frequency is likely much higher (Taylor & Larson, 2019). In freshwater fishes, hybridization was first reported in the mid-20th century

(Hubbs, 1955), and it seems to be occurring more frequently in recent decades due to rapid environmental changes imposed by human activities and climate change (Heath *et al.*, 2010; Hudson *et al.*, 2013; Monette *et al.*, 2020; Fennell *et al.*, 2023; Banerjee *et al.*, 2023). Climate change is affecting the distribution of fish populations, hence promoting secondary contact and hybridization between formerly allopatric species as well as between invasive and native ones. The occurrence of secondary contact caused, for example, by warming temperatures may more likely affect closely related species with less well-developed reproductive barriers. On the other hand, chemical disturbance of freshwater ecosystems and increased turbidity may facilitate hybridization and introgression among more distant species by impairing visual and chemical communication. Moreover, under environmental conditions in which hybrid individuals and populations have a fitness advantage relative to parental species (Rasmussen *et al.*, 2010; Hayden *et al.*, 2011; Yau & Taylor, 2013), the number of hybrid individuals may become more prevalent, assuming that other factors (e.g. genetic incompatibilities) do not prevent their survival or reproduction. A high frequency of hybrid individuals could result in a decreased encounter rate for parentals – leading to increased mating among hybrids and further stabilization of hybrid populations (Rosenthal, 2013).

Our systematic review reveals that current research does not focus sufficiently on how hybridization due to anthropogenic stressors is affecting species, and thereby affecting biodiversity in aquatic ecosystems. This seems to be especially the case for chemical pollution, which may interact with fish hybridization and hybrid populations in several ways (Fig. 2). In part, this may be attributable to the emphasis placed by regulators on studying lethal outcomes, whereas sublethal outcomes may be at least as common and capable of causing drastic changes in ecosystems. Given that hybridization and gene flow are significant evolutionary factors influencing biodiversity (Crispo *et al.*, 2011), further research is warranted to assess the role that changes in water quality, due to climate change and pollution, are playing in fish population decline and biodiversity loss in the sixth mass extinction, and to prioritize conservation management strategies better. Moreover, such research would inform chemical manufacturers, users, risk assessors, and policy-makers about chemical contaminant-driven processes affecting fish olfaction, mate selection, and, therefore, biodiversity.

#### V. ASSESSING KEY KNOWLEDGE GAPS THAT INFORM FUTURE RESEARCH NEEDS

Our systematic review of the literature generated only 38 studies related to changes in fish mate choice and hybridization associated with climate change- and/or pollution-driven changes in water quality. Most of the studies (23 papers) evaluated hybridization associated with changes in the distribution of fish populations and spawning times,



**Fig. 2.** Diagram describing the potential interactions between chemical pollution and hybridization. At non-toxic levels, occurrence of hybridization is caused by other factors (bottom and top left squares). As toxicity increases, sublethal effects of chemical pollutants can be inconsequential to hybridization (centre bottom square) or break reproductive barriers, which facilitates hybridization (centre top square). At lethal concentrations, chemical pollutants can either affect all populations and be inconsequential to hybridization (right bottom square), or selectively affect some populations and facilitate hybridization due to low encounter rates among conspecifics (right top square).

driven by changes in temperature and/or hydrology (i.e. relevant to climate change). Only eight studies evaluated impacts related to pollution-related changes in water quality (i.e. humic acid, turbidity, and bisphenol A) affecting the expression and transmission of chemical and visual cues. In addition, we found 11 studies that evaluated differences in fitness (i.e. temperature ranges, tolerance to hypoxia, and diet flexibility) between naturally occurring hybrids and parental fish species (Table S2).

Although we only found three studies assessing how chemical pollution affects mate choice, there is evidence for molecular initiating events and consequent key events (e.g. changes in gene expression, protein production and tissue function) induced by several chemical groups (e.g. EDCs, NSAIDs, antidepressants, neuroactive pesticides, and metals) that could potentially be linked with altered mate choice and hybridization. However, no adverse outcomes associated with hybridization have been reported, except for a few exceptional cases. Research on how these chemical groups impact the synthesis and bioavailability of hormonal pheromones, skin coloration, courtship behaviour, the functioning of sensory organs (i.e. olfactory epithelium), and mate choice is sorely needed to understand better the sequence of molecular and cellular events required for the breakdown of pre-mating reproductive barriers that facilitates hybridization [i.e. the adverse outcome pathways (see Table 1) leading to hybridization]. Additionally, it remains necessary to investigate at what concentrations chemical pollutants can break down reproductive barriers. Moreover, there is a need for further research regarding the influence of substances that affect turbidity (e.g. humic acid) because of their potential

to limit the bioavailability of hormonal pheromones, in addition to limiting visual communication. Finally, further research is needed to identify factors that impact differences in tolerance to pollution between hybrids and parentals to inform whether hybrid individuals may have a fitness advantage in some contaminated environments.

Future work in this field should be of interest to ecotoxicologists and risk assessors. If research demonstrates that pollution plays an important role in hybridization, we recommend including tests relevant to how chemicals impact sensory cues relevant for mating within risk assessment frameworks for newly marketed chemicals. Given the involvement of fish olfaction in population- and species-level processes (e.g. mate choice, prey avoidance, feeding, homing, and migration), it is striking that very little attention has been given to including questions and tests relevant to mating behaviour and the functioning of physiological structures (e.g. olfactory epithelium) by regulatory agencies [e.g. U.S. Environmental Protection Agency (EPA) and The Organisation for Economic Co-operation and Development (OECD)] for the marketing of new chemical products.

In summary, we identified the following key research questions to inform future work on how pollution affects hybridization: (i) how prevalent is an overlap of polluted areas with hybrid zones, and how much hybridization that we observe in nature is fully or partially caused by pollution? (ii) What chemical or physical parameters of water quality interfere with the stability or bioavailability of hormonal pheromones? (iii) How does chemical pollution affect hybridization, speciation, and biodiversity regionally and globally? (iv) Which pollutants are most likely to impact mate choice and facilitate

hybridization, and what are the mechanisms (e.g. disruption of visual and/or chemical cues, impacts on the structure and function of olfactory epithelium)? (v) What is the combined effect of chemical pollutants and other anthropogenic stressors (e.g. climate change and invasive species) on hybridization? (vi) Which species are more susceptible to the breakdown of reproductive barriers and, therefore, to interspecific hybridization?

## VI. CONCLUSIONS

- (1) Our systematic literature review indicates that climate change-driven changes in water quality and chemical pollution may affect visual and chemical communication processes relevant for mate choice and thus could facilitate hybridization of fishes in freshwater ecosystems.
- (2) Very few studies have tested hypotheses about how these anthropogenic stressors, especially chemical pollution, could disrupt mating barriers.
- (3) We can no longer ignore the potential for chemicals to interact with mating systems and processes in freshwater organisms. We need to identify the chemical and physical stressors that affect mate choice, the mechanisms involved, the concentrations at which this occurs, and the extent of its impact on individuals, populations, species, and biodiversity in nature.
- (4) An increased understanding of the link between anthropogenic stressors and hybridization is urgently needed and will be vital for conservation efforts as well as for both applied and basic research.

## VII. ACKNOWLEDGEMENTS

This work was supported by a Human Frontier Science Program grant (Grant RGY0081/2020) to M. S., V. C. S., C. B. and C. M. R. We are grateful to the editor and anonymous reviewers for their insightful and valuable comments. The authors declare no competing interests. ChatGPT-4 with DALL-E were used to create Fig. 1.

## VIII. AUTHOR CONTRIBUTIONS

W. F. R.-D. and C. M. R. co-lead the writing. W. F. R.-D. performed the literature search. W. F. R.-D. and B. M. M. screened the literature. All authors contributed to reviewing and editing.

## IX. DATA AVAILABILITY STATEMENT

The methodology followed to prepare this systematic literature review and the list of articles that were reviewed at the

full-text level with reasons for exclusion are available as online supporting information.

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## XI. SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1.** Reproductive barriers in fish.

**Appendix S2.** Methods and materials.

**Appendix S3.** List of studies analysed at the full-text level.

**Appendix S4.** Synthesis of hormonal pheromones.

**Appendix S5.** Olfactory epithelium in fish.

**Fig. S1.** Systematic literature review development steps.

**Table S1.** Search queries used to locate peer-reviewed, primary research articles.

**Table S2.** Original research articles evaluating the influence of physical and chemical parameters of water quality on different processes associated with mate choice and hybridization in fish.

(Received 8 April 2024; revised 17 July 2024; accepted 22 July 2024)