

## **Effects of global warming on sex ratios in fishes**

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### **Abstract**

In fishes, sex is either determined by genetics, the environment, or an interaction of both. Temperature is among the most important environmental factors that can affect sex determination. As a consequence, changes in temperature at critical developmental stages can induce biases in primary sex ratios in some species. However, early sex ratios can also be biased by sex-specific tolerances to environmental stresses that may, in some cases, be amplified by changes in water temperature. Sex-specific reactions to environmental stress have been observed at early larval stages before gonad formation starts. It is therefore necessary to distinguish between temperature effects on sex determination, generally acting through the stress axis or epigenetic mechanisms, and temperature effects on sex-specific mortality. Both are likely to affect sex ratios and hence population dynamics. Moreover, in cases where temperature effects on sex determination lead to genotype-phenotype mismatches, long-term effects on population dynamics are possible. For example, temperature-induced masculinization potentially leading to the loss of Y chromosomes, or feminization to male-biased operational sex ratios in future generations. To date, most studies under controlled conditions conclude that if temperature affects sex ratios, elevated temperatures mostly lead to a male bias. The few studies that have been performed on wild populations seem to confirm this general trend. Recent findings suggest that transgenerational plasticity could potentially mitigate the effects of warming on sex ratios in some populations.

Keywords: Climate change, sex determination, environmental sex reversal, cortisol, methylation, sex-specific mortality

## 1 Introduction

Sex determination in fishes is probably best seen as a threshold trait, i.e. processes that occur early in development can act as a switch that determines the cascade of processes of gonad development (Beukeboom & Perrin, 2014). Regardless of whether these early processes have a genetic basis, they are often modifiable by external factors (Devlin & Nagahama, 2002). Temperature is among the most important external factors that can determine or interfere with these early steps of sex differentiation (Ospina-Álvarez & Piferrer, 2008; Baroiller & D’Cotta, 2016).

Temperature-dependent sex determination (TSD) in fishes was probably first described in the Atlantic silverside *Menidia menidia* (L. 1766) where more males are produced under relatively high temperature regimes in the wild (Conover & Kynard, 1981). Since then, many studies report an effect of temperature on sex determination in various fish families, with different levels of domestication (Devlin & Nagahama, 2002; Ospina-Álvarez & Piferrer, 2008). This include species domesticated for many generations, like zebrafish *Danio rerio* (Hamilton 1822) (Uchida *et al.*, 2004), olive flounder *Paralichthys olivaceus* (Schmidt 1904) (Tabata, 1995), European sea bass *Dicentrarchus labrax* (L. 1756) (Piferrer *et al.*, 2005), goldfish *Carassius auratus* (L. 1758) (Goto-Kazeto *et al.*, 2006), Nile tilapia *Oreochromis niloticus* (Trewavas 1983) (Baroiller *et al.*, 1995), pike silverside *Chirostoma estor* (de Buen 1940) (Corona-Herrera *et al.*, 2016) and African spiny catfish *Clarias gariepinus* (Burchell 1822) (Santi *et al.*, 2016). But similar results were obtained for the F1 progeny of wild populations of pejerrey *Odontesthes bonariensis* (Valenciennes 1935) (Strüssmann *et al.*, 1996), Nile tilapia (Bezault *et al.*, 2007), spiny chromis damselfish *Acanthochromis polyacanthus* (Bleeker 1855) (Donelson & Munday, 2015), *Poecilia melanogaster* (Günther 1866), 35 species of the genus *Apistogramma* (Römer & Beisenherz, 1996), and even the F2 of wild atipa *Hoplosternum littorale* (Hancock 1828) (Hostache *et al.*, 1995) and spiny chromis damselfish (Donelson & Munday, 2015). Overall, this strongly suggests that thermosensitive sex determination has not evolved with domestication but is also common in the wild, perhaps with the exception of the zebrafish (Wilson *et al.*, 2014).

Species with genetic sex determination (GSD) can have their phenotypic sex modulated by temperature as, for example, medaka *Oryzias latipes* (Temminck & Schlegel 1846) (Kitano *et al.*, 2012), sockeye salmon *Oncorhynchus nerka* (Walbaum 1792) (Craig *et al.*, 1996; Azuma *et al.*, 2004), rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Valdivia *et al.*, 2014) and sablefish *Anoplopoma fimbria* (Pallas 1814) (Huynh *et al.*, 2019). An interesting observation in this context is that juveniles of Nile tilapia or African catfish (both species with GSD) sometimes seem actively to seek warmer temperatures around the time of sex differentiation in order to develop into males (Santi *et al.*, 2017; Nivelles *et al.*, 2019). Such temperature-induced genotype-phenotype mismatches can produce interesting long-term effects on demography, as discussed below.

In nearly all cases, high temperatures lead to a higher ratio of males in species with TSD, or induce masculinization in species where GSD can be overruled by temperature. It therefore seems that male bias at high temperature is a rule in fishes. This suggests that producing males may often be adaptive in harsh conditions (Geffroy & Douhard, 2019). Different hypotheses have been proposed in this context, including higher migration propensity and quicker maturation, leading to a greater relative fitness for males than females in unpredictable environments (Geffroy & Douhard, 2019). However, female bias at high temperatures are common in other taxa (e.g. Janzen 1994), and there are at least two examples of temperature-related female bias in fishes, in sockeye salmon (Craig *et al.*, 1996) and channel catfish *Ictalurus punctatus* (Rafinesque 1818) (Patiño *et al.*, 1996). Patiño *et al.* (1996) could not exclude sex-specific mortality as confounding factor in their study but they argued that such selective mortality was unlikely to have produced the observed effects

because there were no sex-ratio biases in low-temperature treatment groups, despite significant mortality.

Temperature effects on sex ratios can be a threat to natural populations. Here we discuss how and when changed temperature regimes affect primary and operational sex ratio in fishes, and we outline possible short-term and long-term consequences of temperature-induced sex ratio biases.

## 2 Types of temperature effects on sex determination

So far, most studies investigating temperature effects on sex ratios have concentrated on species within the Perciformes, a comparatively young order of fishes. The phylogenetic tree of teleost fishes started, however, about 300 to 360 million years ago in the Carboniferous. Many orders have been evolutionarily separated by hundreds of millions of years, which may explain why fishes exhibit such great diversity in sex determination and sex differentiation (Wang *et al.*, 2018). The Cypriniformes, Salmoniformes, and Perciformes, for example, have been separated for over 200 million years each (Near *et al.*, 2012). Much research has been done on these orders because they are well represented in aquaculture (Baroiller & D’Cotta, 2001). Each of these orders includes species where temperature effects on sex determination have been described, and species where no such temperature effects could be found (Figure 1).

Figure 2 illustrates the range of possible temperature effects on sex ratios. Masculinization at high temperature (scenarios B2 and B3 in Figure 2) is the commonest response observed so far, but there are potential examples of other responses (Figure 2), and further patterns cannot currently be excluded. The patterns are often not very strong (see, for example, the Supplementary Table 1 of Ospina-Alvarez and Pifferer (2008). Moreover, there is often significant variation among families, as in zebrafish (Ribas *et al.*, 2017), African catfish (Santi *et al.*, 2016), and Nile tilapia (Baroiller & D’Cotta, 2001; Nivelles *et al.*, 2019), and there can be significant variation among experimental runs, as observed in European sea bass (Vandeputte & Pifferer, 2018). Some of this variation could, however, be due to sex-specific mortality at very early stages (see discussion below).

Most studies on temperature effects on sex ratio have been performed under laboratory conditions. A recent study on the southern flounder *Paralichthys lethostigma* (Jordan & Gilbert 1884) combined laboratory experiments with field observations (Honeycutt *et al.*, 2019). This is a species where temperature extremes (warm and cold) had previously been reported to induce masculinization (Luckenbach *et al.*, 2003) (C2 in Figure 2). Honeycutt *et al.* (Honeycutt *et al.*, 2019) used gonadal expression of sex-specific markers to determine the phenotypic sex of juveniles caught at different locations along the east coast of North Carolina (USA) and observed increasingly male-biased sex-ratios with increasing water temperatures. These temperature effects were then largely confirmed under controlled conditions in the laboratory (Honeycutt *et al.*, 2019). In another study system, however, laboratory experiments by Pompini *et al.* (2013) did not confirm the link between water temperatures and sex ratios that Wedekind *et al.* (2013) had found in a wild population of grayling *Thymallus thymallus* (L. 1758). The authors concluded that the link between sex ratio and temperature in the wild is more likely caused by sex-specific mortality (see below) than by temperature effects on sex determination, a conclusion that was further supported by the first description of the sex determining locus in this species (Yano *et al.*, 2013).

It is important to note that climate change does not necessarily lead to increased temperatures during the critical stages of sex differentiation. Elevated summer temperatures can, for example, delay spawning of brook trout *Salvelinus fontinalis* (Mitchill 1814) in autumn or winter (Warren *et al.*, 2012), and elevated winter temperatures can advance spawning in spring for grayling (Wedekind & K ung, 2010). In the latter case, 40 years of

continuous temperature measurements revealed a somewhat counterintuitive effect, namely that the observed shift of spawning season (by over 3 weeks over a few decades) caused increasingly colder temperature environments for larvae around hatching, i.e. around a time that has been found to be critical for sex determination in other species (Devlin & Nagahama, 2002). This is because climate change seemed to cause temperatures to rise increasingly more slowly in early than in late spring in the habitat of the study population.

In conclusion, even if global change typically leads to warmer temperatures and even if most examples in fishes indicate that masculinization is more likely than feminization at higher temperatures, global change could, in some cases, also induce feminization (e.g. by exposure to colder temperatures at critical developmental stages because of temporal shifts in important life history events). Therefore, the likely effects of both, masculinization and feminization, need to be considered.

### **3 Physiology of temperature effects on sex determination**

#### **3.1 Primary sex ratios in lower vertebrates**

The term “primary sex ratio” is usually defined as sex ratio at the time of conception, but this definition excludes many lower vertebrates because it implies that sex is only genetically determined. In fishes with environmental influence on sex determination, i.e. with either environmental sex determination or environmental sex reversal (environmental factors overruling genetic factors), the primary sex ratio can only be determined at a time when the decisive processes that occur early in development have set the path to the development of testes or ovaries. It is not surprising that at this time, typically during embryogenesis and/or early larval stages (Devlin & Nagahama, 2002), the sexes show sex-specific gene expression (Geffroy *et al.*, 2016; Maitre *et al.*, 2017). At that time, they also show sex-specific reactions to environmental stress such as, for example, exposure to exogenous sex hormones at ecologically relevant concentrations (Selmoni *et al.*, 2019).

#### **3.2 Cortisol**

The link between environmental variables (particularly high temperature and fish density) and the stress axis (through the production of cortisol) has been established as an important factor influencing sex determination in various fish species, typically resulting in masculinization (Geffroy & Douhard, 2019). Incubating rainbow trout eggs in cortisol leads to the development of testes (van den Hurk & van Oordt, 1985), and cortisol administration induces masculinization in three-spotted wrasse *Halichoeres trimaculatus* (Quoy & Gaimard 1834) (Nozu & Nakamura, 2015) and orange-spotted grouper *Epinephelus coioides* (Hamilton 1822) (Chen *et al.*, 2020). Cortisol is also expected to play a crucial role in sex-change of various sequential hermaphrodite species (Goikoetxea *et al.*, 2017; Olivotto & Geffroy, 2017). A brief exposure (minutes to hours) to a temperature that is outside the thermal preference of a fish can elicit an increase in cortisol production (e.g. Cockrem *et al.*, 2019). Chronic exposure to high temperatures can also lead to higher basal concentrations of cortisol (Madaro *et al.*, 2018) although it is not yet established whether this represents a state of physiological stress (Schreck & Tort, 2016) with higher routine metabolic rate and enzyme activities (see review of Alfonso *et al.* 2020 in this special issue).

If it is indeed mainly stress that affects sex differentiation, temperature would play an indirect role in some fishes. That is, stressful temperatures would induce the production of cortisol and then typically induce male development, as exemplified in the pejerrey (Hattori *et al.*, 2009). To date, most of our knowledge on the underlying mechanisms has been gathered from three species: the medaka, the pejerrey, and the olive flounder. Four distinct and direct mechanisms linking temperature-induced stress and sex have been demonstrated. Two involved a down-regulation of feminizing genes (*cyp19a1a* and *fshr*) while the two others

involved an up-regulation of masculinization genes (*dmrt1a* and *hsd11b2*) (Figure 3). For the former, the complex formed by cortisol and its receptor the glucocorticoid receptor (CORT/GR), bound to a specific promoter zone (glucocorticoid response element, GRE) of the gene encoding gonadal aromatase (*cyp19a1a*), the enzyme that converts androgens into estrogens (Guiguen *et al.*, 2010), as demonstrated in the olive flounder (Yamaguchi *et al.*, 2010). This results in down-regulation of *cyp19a1a*, which in turn impedes estrogen production and leads to an accumulation of testosterone in the undifferentiated gonad. Another mechanism involves the follicle stimulating hormone receptor *fshr*. In medaka, the CORT/GR can bind to a GRE in the promoter region of *fshr* (Hayashi *et al.*, 2010), which may lead to an imbalance between androgens and estrogens.

More recently, *in vitro* experiments showed that CORT/GR stimulates expression of the autosomal *dmrt1a* (a duplicate of *dmrt1bY*, the master sex-determining gene) in XX medaka, potentially explaining masculinisation of these genetic females (Adolfi *et al.*, 2019). Interestingly, disrupting the cortisol synthesis pathway using CRISPR/Cas9 technology targeting corticotropin-releasing factor *crf* (released by the hypothalamus at the beginning of the cortisol synthesis cascade) inhibited sex reversal in XX medaka reared at high temperature (Cortés *et al.*, 2019).

It should be emphasized that the synthesis of 11-oxygenated androgens is related to that of cortisol because their synthesis and inactivation involves the same steroidogenic machinery. The last step of cortisol synthesis is performed by 11- $\beta$ -hydroxylase (CYP11B) but this enzyme is also responsible for the 11-hydroxylation of testosterone into 11- $\beta$ -OH-testosterone (Borg, 1994). The catabolization of cortisol into cortisone (its biologically inactive metabolite) is performed by 11 $\beta$ -hydroxysteroid dehydrogenase (11- $\beta$ -HSD) that is also responsible for conversion of 11- $\beta$ -OH-testosterone into 11-ketotestosterone (11-KT). This has led some authors to propose that the production of biologically active androgens (*e.g.* 11-KT) is only a collateral outcome of high production of 11- $\beta$ -HSD, to deal with the elevated production of cortisol at warm temperatures (Fernandino *et al.*, 2013). Indeed, *in vitro* treatment of testes of pejerrey with cortisol caused an up-regulation of 11-KT synthesis, through activation of 11- $\beta$ -HSD (Fernandino *et al.*, 2012). Overall, although our understanding of the mechanisms involved in temperature-stress-sex regulation is increasing, it is not yet clear why binding of CORT/GR to GREs can elicit either an up- or a down-regulation of glucocorticoid-responsive genes.

### 3.3 Epigenetics

Epigenetics can be defined as "... the study of phenomena and mechanisms that cause chromosome-bound, heritable changes to gene expression that are not dependent on changes in DNA sequence" (Deans & Maggert, 2015). With regard to temperature-induced masculinization of gonochoristic species, most of our knowledge of epigenetics is from studies analyzing methylation of promoter regions of various key sex differentiation genes (see Piferrer *et al.*, 2019; Ortega-Recalde *et al.*, 2020 for review). A common mechanism appears to be conserved in many species that are sensitive to temperature. That is, exposure to warmer temperature consistently increases DNA methylation of the *cyp19a1a* promoter, resulting in decreasing expression of *cyp19a1a* in phenotypic (and possibly sex-reversed) males of European sea bass (Navarro-Martín *et al.*, 2011), in ZW pseudomales of the tongue sole *Cynoglossus semilaevis* (Liu *et al.*, 2019), in XX pseudomales of the Nile tilapia (Wang *et al.*, 2019) and in XX pseudomales of the olive flounder (Wen *et al.*, 2014). Many other sex-specific genes, particularly *dmrt1* (Wen *et al.*, 2014), appear to be differentially methylated after exposure to high temperatures, which warrants further investigation (Piferrer *et al.*, 2019). While various potential processes have been revealed for cortisol-induced masculinization, we do not yet know the mechanisms by which the temperature induces

specific methylation of target genes (Piferrer *et al.*, 2019). In reptiles with TSD, a calcium and redox (CaRe)-mediated signal has recently been proposed as a “cellular sensor” that links environment factors (e.g. temperature) to epigenetic processes (Castelli *et al.* 2020), which is an interesting avenue of investigation for fishes.

Epigenetic modifications can be responsible for transgenerational plasticity that allows fishes to rapidly modulate sex-determination in response to prevailing thermal conditions (Grossen *et al.* 2011). For instance, exposing spiny chromis damselfish to elevated temperatures during development causes male-biased sex ratios, but when both parents and F1 are reared at those elevated temperatures, no such bias is observed (Donelson & Munday, 2015). In a domesticated strain of zebrafish where sex determination is under genetic and environmental influence, parents exposed to warm temperatures produced more male offspring in the unexposed F1 but these temperature effects varied among families, and the sex ratio of the unexposed F2 was not affected by the warm exposure of their grandparents (Valdivieso *et al.*, 2020).

#### **4 Sex-specific mortality**

Population sex ratios are a product of primary sex ratios, sex-specific life histories, and sex-specific mortality (Figure 4). The latter can, in principle, occur at any life stage. If it happens during early developmental stages, it may sometimes be difficult to distinguish from environmental effects on sex determination. As a consequence, any kind of mortality should be seen as a potentially confounding factor in studies of environmental effects on sex determination.

The development of sex-linked genetic markers (Yano *et al.*, 2013) has permitted investigation of sex-linked mortality at very early developmental stages within various species. Moran *et al.* (2016), for example, used such markers in brown trout *Salmo trutta* (L. 1758) families, to gain evidence of sex-specific mortality during embryo and early larval stages in some of families but not others. Such mortality may typically be induced by symbiotic microbial communities (Wilkins *et al.*, 2015a) whose composition varies with temperature (Wilkins *et al.*, 2015c) but also with embryo age (Wilkins *et al.*, 2015b), embryo genetics (Wilkins *et al.*, 2016), and maternal environmental effects, e.g. egg carotenoids content (Wilkins *et al.*, 2017). Such family-specific stress environments are likely to cause family- and sex-specific reactions that, on a population level, may cancel each other out. If so, sex-specific mortalities would easily be missed if studied in populations rather than families. In Nile tilapia (with a XX/XY system), a role for sex-specific mortality was ruled out by i) raising XX progenies at high temperature, which resulted in the production of males (no female mortality, but rather a sex-reversal) and ii) identifying XX males in temperature treated groups in many different experiments (Baroiller *et al.*, 2009).

Not much is known about the physiology of sex-specific mortality at very early stages. However, the sexes often differ significantly in their life history at later developmental stages and such sex-specific strategies are likely to induce sex-specific sensitivities to certain stress factors at certain times. Sex differences in life history and behavior are often most profound shortly before and during spawning season, and the factors that induce sex-specific mortality can be diverse, including predation and pathogens. The relevance of these factors often depends on water temperature, e.g. the virulence of *Tetracapsuloides bryosalmonae* that causes proliferative kidney disease in many salmonids is temperature dependent (Bettge *et al.*, 2009). As a consequence, changes in thermal regimes can influence sex ratios around spawning time.

Sex differences in life history are easily overlooked if they are expressed early in life, e.g. before or around gonad formation. Such sex differences can, however, lead to sex-specific larval or juvenile mortality in response to environmental stressors, many of which

may interact with temperature. Wedekind *et al.* (2013) compared water temperatures with adult sex ratios of wild grayling at their spawning sites in a pre-Alpine region, over a period of about 40 years, and found a significant male-biased sex ratio since the early 1990s. This may be a contributing factor to declines in these populations (Wedekind & Küng, 2010). The bias started five years after a significant shift in average water temperature, which coincided with a temperature regime shift in Europe and elsewhere (Reid *et al.*, 2016). Interestingly, this five-year gap between temperature change and change in sex ratio corresponds well with the average age of the fish that were sampled, namely 5 years. Pompini *et al.* (2013) therefore tested experimentally whether the temperature change could have influenced sex determination in the population. They found no evidence for such temperature effects and therefore concluded that sex-specific mortality during the first year of life is the most parsimonious explanation for the shifted sex ratio. Maitre *et al.* (2017) then used sex-linked molecular markers to study gonad development of genetic males and females, to find marked differences in the timing of gonad development: females start during their first summer (i.e. at a time when the natural population is exposed to increasingly higher water temperatures) whereas males grow faster during these months and only start developing testes in autumn, after the warm period. Such different life histories could potentially cause female-biased juvenile mortality linked to climate change.

Sex differences in the timing of gonad development have also been found in channel catfish (Patiño *et al.*, 1996): when reared at 27 °C, females and males started sex differentiation around 20 and 100 days after fertilization, respectively, and sex ratio was essentially balanced. When reared at 34°C, however, a significant female-bias was observed at 102 days after fertilization. Patino *et al.* (1996) could not resolve whether the female bias at warmer temperature was due to feminization of genetic males, sex-biased mortality, or a combination of both. If sex-biased mortality played a role, males would be more susceptible to warmer temperature in this species, early gonad formation would not necessarily make one sex more susceptible to higher temperature than the other.

## **5 Sex-specific fertility**

Changes in temperature regimes may not only affect life histories and mortality but also fertility, by affecting gamete number and viability, as demonstrated in various taxa including some fishes (Walsh *et al.*, 2019). Temperature effects on fertility are likely to be sex-specific, especially in ectotherms (Iossa, 2019). Such sex-specific effects may be critical for effects of climate change on population viability. Information about population sex ratios may then be inadequate and operational sex ratios, i.e. the ratio of males and females that are ready to mate, could instead be used to evaluate the consequences of global warming for the two sexes and overall population dynamics.

Iossa (2019) argues that male fertility is typically less resilient to temperature-induced stress than female fertility, but stresses that sex differences may well be taxon-specific. The physiology of temperature effects on fertility of the two sexes therefore needs to be studied within various taxa. Heritability of susceptibility to such temperature effects may induce rapid evolution and hence local adaptation (Grossen *et al.*, 2011).

## **6 Consequences for population dynamics**

Temperature effects on sex ratios can potentially induce both immediate and long-term effects on population dynamics. One potential immediate effect is a change in recruitment rates. Obviously, if egg numbers limit population growth, male-biased sex ratios can cause or reveal a reduction of absolute female numbers that may cause a population decline, as has been proposed for temperate eels (Geffroy & Bardounet, 2016). The male bias in the grayling population studied by Wedekind *et al.* (2013) seems to provide a good example. The

population is fully protected, water quality in their habitat is high and no environmental factor can, so far, be linked to the observed population decline except the significant increase in water temperature over recent decades. In other species like some cichlids (e.g. *Sarotherodon melanotheron*) (Kishida & Specker, 2000) or seahorses (Stölting & Wilson, 2007), immediate negative effects on recruitment rates might also result from male parental care and protective incubation, which limits population growth so that a female-biased sex ratio could have drastic consequences for the population.

There are two main forms of potential long-term consequences of biased sex ratios for population dynamics. One could result from temperature-induced sex reversal (Figure 4). Such early modifications of sex determination do usually not create significant long-lasting effects on growth and survival (Senior *et al.*, 2012, 2016) but they can create genotype-phenotype mismatches with impacts on population demography. For example, if sex determination is male dominant (XX/XY), continuous masculinization can drive Y chromosomes to extinction and hence change the sex determination system of a population from GSD to ESD (Kanaiwa & Harada, 2008; Grossen *et al.*, 2011). The maintenance of the two sexes would then depend upon environmental masculinization. Analogous dynamics are possible if sex determination is female dominant (ZZ/ZW) (Schwanz *et al.*, 2020).

Populations can then rapidly go extinct if the environmental pressure that causes the sex reversal ceases (Cotton & Wedekind, 2009). On the other hand, if climate change leads to a female-biased sex ratio, either because of direct effects of high temperature on physiology or because of changed environments due to shifts in the timing of spawning or other life-history events (as discussed above), the resulting XY females are expected to produce male-biased families when reproducing with wild-type XY males: 25% of the F1 will be XX, 50% XY, and 25% YY. The latter genotypes are usually viable in fishes (Senior *et al.*, 2012). Without further feminization, the XY and YY genotypes will be males, so that the family sex ratio of such sex-reversed females will be strongly male-biased. Moreover, a third of these males (i.e. the YY males) would only produce XY offspring in the F2 generation. Environmental feminization would therefore be expected to first create female-biased sex ratios but then male-biased sex ratios in subsequent generations. Continuous feminization over several generations could amplify the sex biases and hence threaten population viability, especially if it involves feminization of YY individuals (Bókony *et al.*, 2017; Wedekind, 2017).

The other form of possible long-term consequences for population dynamics of biased sex ratios derives from the effects that unequal sex ratios have on effective population size ( $N_e$ ). This effect can be estimated as

$$N_e = 4N_m N_f / (N_m + N_f) \quad (1)$$

based on male and female numbers ( $N_m$  and  $N_f$ , respectively) (Hartl, 1988). Figure 5 illustrates how biased sex ratios affect  $N_e$ . A declining  $N_e$  signifies a loss of genetic diversity within a population and hence a reduction in the potential to adapt to changing environments. Since human activities are causing various types of rapid environmental change, a loss of evolutionary potential could be a major threat to population resilience (Hendry *et al.*, 2017). Another potential threat that is linked to a reduction of genetic diversity within populations is co-evolving pathogens, whose virulence can increase in response to low genetic diversity in their hosts (e.g. Kubinak *et al.*, 2012). Lehnert *et al.*, (2019) reported a strong association between the decline of more than half of 172 Atlantic salmon *Salmo salar* (L. 1758) populations over recent decades and the decline of their respective  $N_e$ . A strong correlate of these declines seemed to be increased winter temperatures and their effects on development of



early developmental stages, but it remains unclear whether and how these temperature changes affected sex ratios.

## 7 Conclusion

Based upon many laboratory studies and a few investigations in the field, the general patterns that emerge are that, if climate change affects population sex ratios in fish, (i) male-biased population sex ratios seem generally more likely than female-biased; (ii) sex ratios can differ from generation to generation, due to factors such as transgenerational plasticity or the demographic effects of phenotype-genotype mismatches that sex reversal can create; (iii) there can be significant family effects on sex ratios, which could mask environmental effects on sex determination at a population level, and (iv) epigenetic and stress-related mechanisms probably play a role in responses to warming. Another general trend is that, if sex ratios are affected by temperature, the reactions seem similar in wild and domesticated species. Given the limited studies on wild populations, however, it is difficult to predict how global warming will affect the sex ratio of a given population. There are various factors that determine whether climate change causes either increased or decreased water temperatures at developmental stages that are critical for sex determination in a given fish population and species. Changed temperature regimes may affect the timing of spawning and, hence, the temperature regime during earlier developmental stages. Coupled with associated changes in day length (due to a shift in the timing of events) or changes in other environmental factors (e.g. pH), this could influence embryo and larval development at critical stages. Another important challenge for future field studies will be to separate environmental effects on sex determination from environmental effects on sex-specific mortality.

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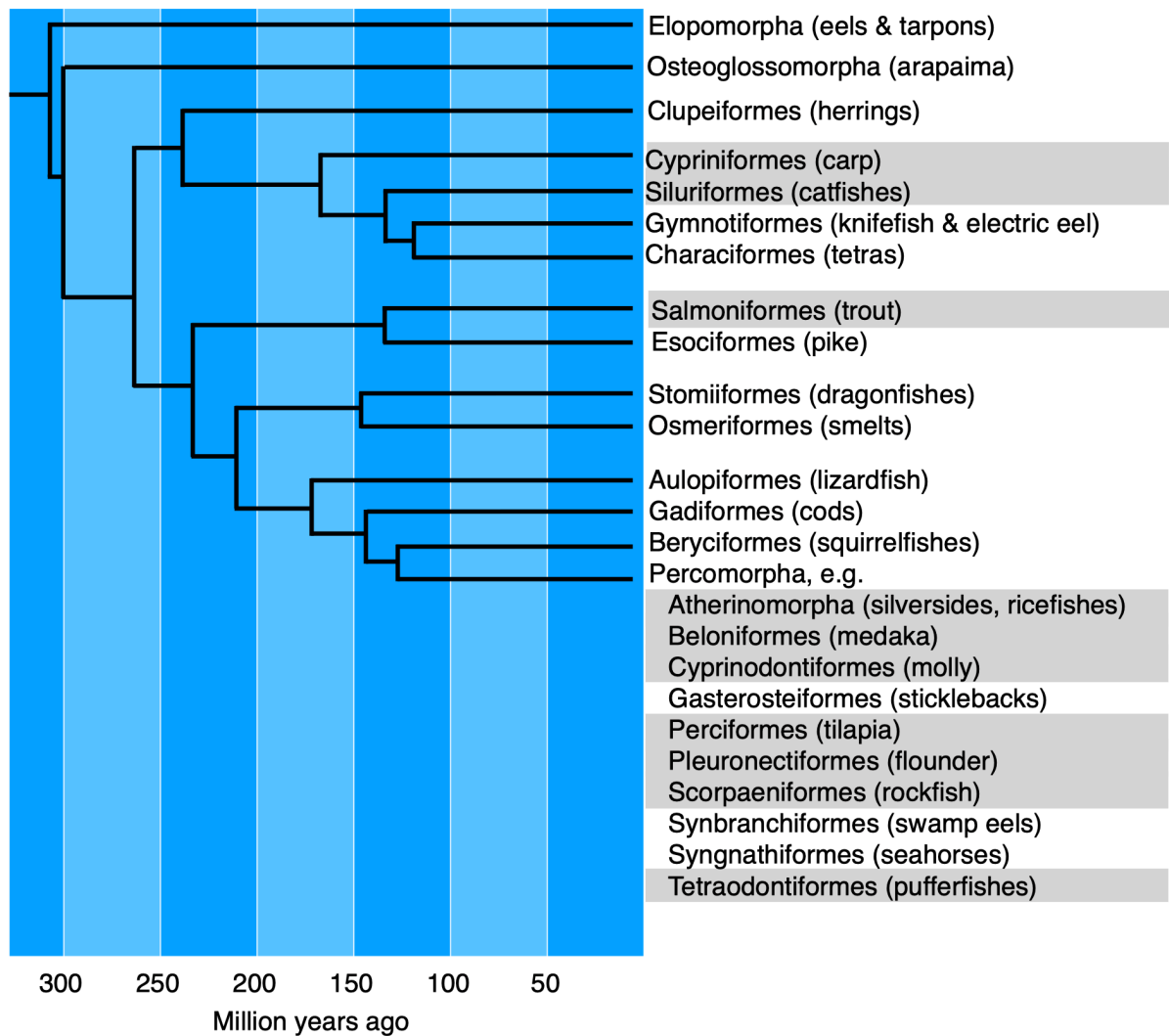


Figure 1. Phylogeny of major orders or superorders of teleost fishes, time-calibrated by Near et al. (2012). Orders with examples of temperature effects on sex determination are highlighted, the parentheses give examples.

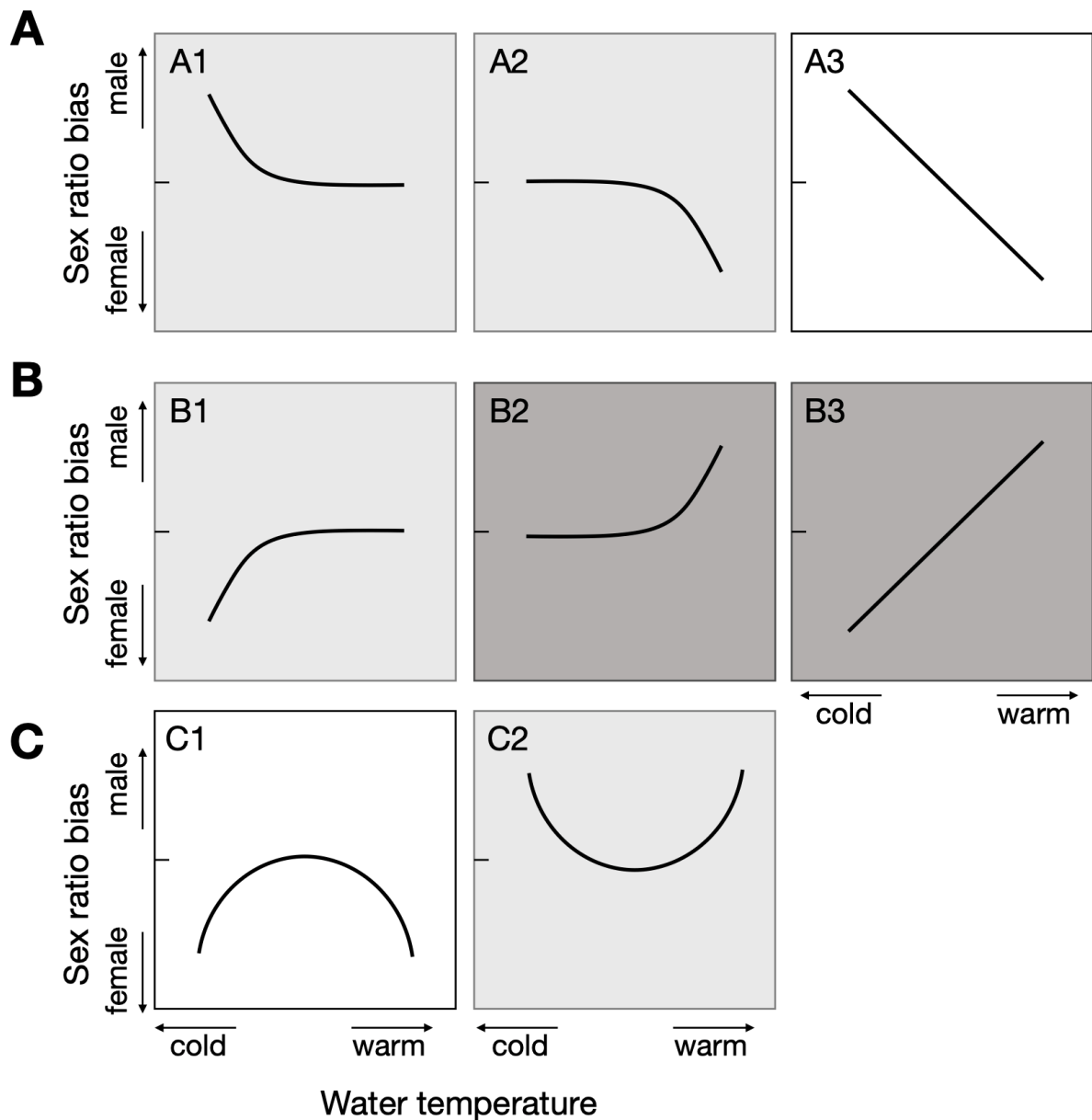


Figure 2. The types of potential temperature effects on shifts in population sex ratio: Decrease (A) or increase (B) in proportion of males with increasing temperature; decrease or increase in proportion of males at extreme temperatures (C). Most observations so far show male biases at high temperature (emphasized in dark grey, examples mentioned in Introduction), but other sex ratio biases have been reported (emphasized in light grey), for example A1: tiger puffer *Takifugu rubripes* (Zhou *et al.*, 2019), A2: channel catfish *Ictalurus punctatus* (Patiño *et al.* 1996), B1 : tidewater silverside *Menidia peninsulae* (Middaugh & Hemmer, 1987), C2: southern flounder *Paralichthys lethostigma* (Luckenbach *et al.*, 2003).

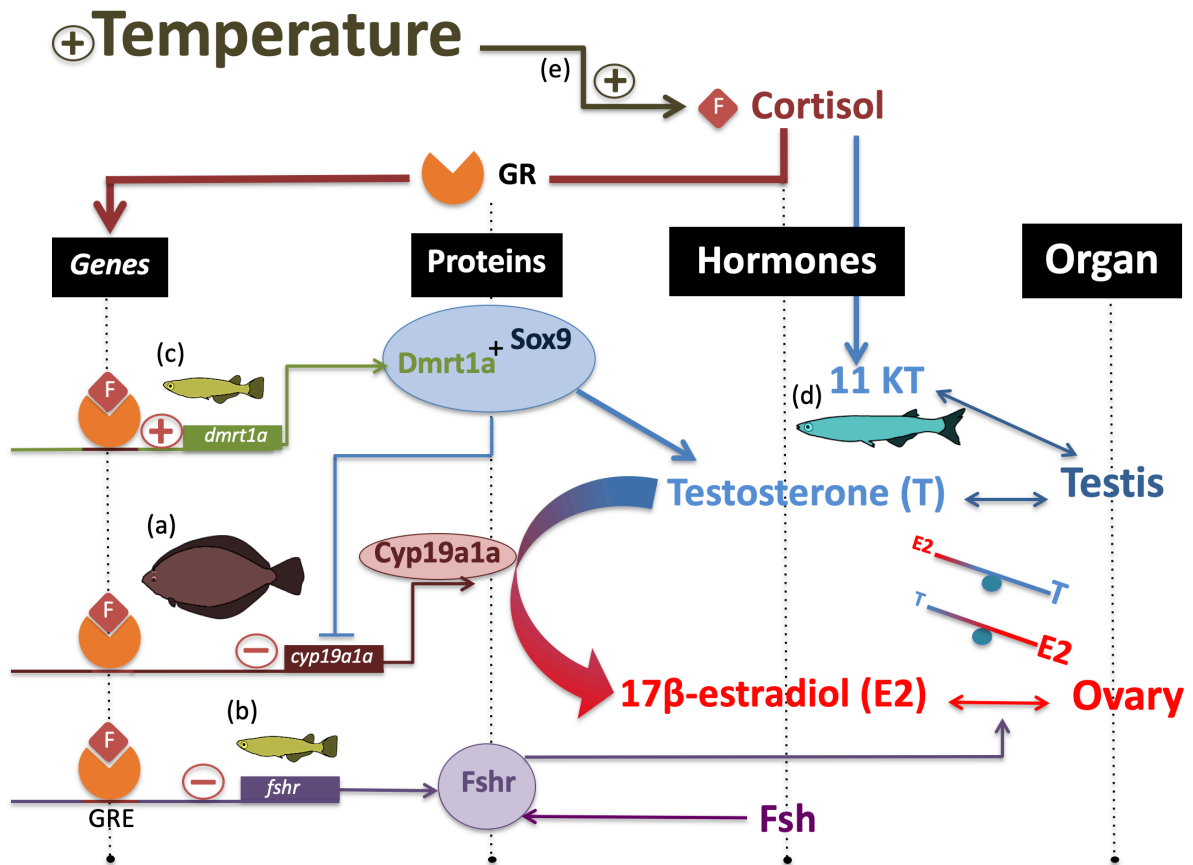


Figure 3. A scheme of how temperature affects sex through cortisol increases in different fish species. a) Yamaguchi et al., 2010 b) Hayashi *et al.*, 2010 c) Adolfi *et al.*, 2019 d) Fernandino *et al.*, 2013 e) Alfonso et al. 2020 (this issue). These various studies indicate that the glucocorticoid receptors (GR)/cortisol association binds to DNA regions known as glucocorticoid response element (GRE) that ARE located in the promoter sequence of different genes: *dmrt1a* or *fshr* in the medaka and *cyp19a1a* in the olive flounder. This triggers either an up- or down-regulation of downstream genes, which results in a differential translation of proteins involved in sex determination/differentiation or of the related to sex steroid balance. Finally, the undifferentiated gonads exposed to different levels of sex steroids develop into testis or ovary. More details of the process are provided in the main text. ‘Proteins’ refers to sex-related proteins. GR: (in fishes the mineralocorticoid receptor is also a cortisol receptor); Fsh: follicle stimulating hormone. Full arrows represent direct link.



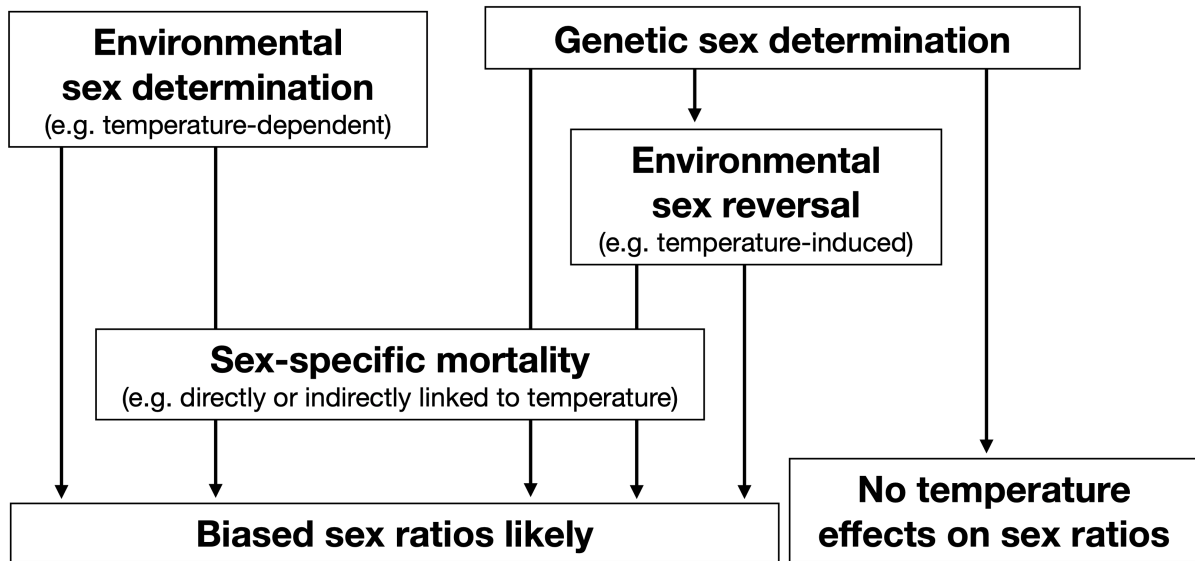


Figure 4. Temperature effects on sex determination and on sex-specific mortality can cause biases in population sex ratios. These temperature effects can vary among families (and potentially cancel each other out at the population level). Further factors that can cause biased population sex ratios or operational sex ratios are temperature-induced and sex-specific changes in, for example, the timing of maturation, migration, spawning behavior, and fertility.

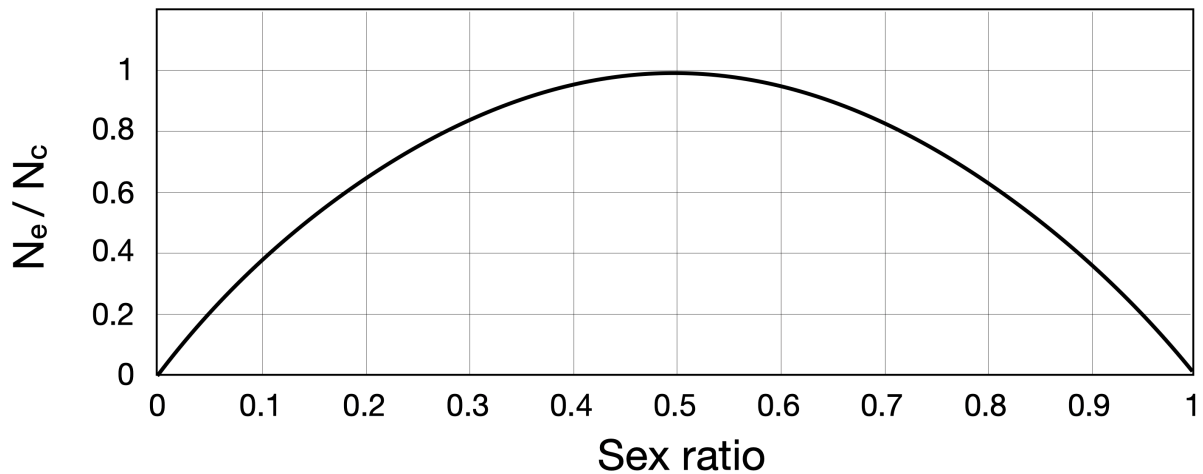


Figure 5. Effect of biased sex ratios, for example due to temperature effects on sex determination, sex-specific mortality, or sex-specific fertility, on genetically effective population size ( $N_e$ ) relative to the census size ( $N_c$ ).