Poeciliid Research

Endocrine and environmental drivers of female masculinization in *Xiphophorus hellerii*: integrating species-specific evidence with general Teleost mechanisms

[1,2,3,4]I. Valentin Petrescu-Mag, [1]Cristian Iederan, [1]Petru Burduhos, [5]Florin D. Bora

[1] Department of Environmental Engineering and Protection, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania; [2] Bioflux SRL, Cluj-Napoca, Romania; [3] University of Oradea, Oradea, Romania; [4] WABBA International Bodybuilding and Fitness LTD, 8 Gainsborough Road, London, United Kingdom; [5] Viticulture and Oenology Department, Advanced Horticultural Research Institute of Transylvania, Faculty of Horticulture and Business for Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania. Corresponding author: F. D. Bora, florindumitru.bora@usamvcluj.ro

Abstract. Masculinization of genetic females represents a form of sexual plasticity documented in many teleost fishes, yet it remains poorly investigated in the swordtail *Xiphophorus hellerii*. This mini review synthesizes the limited direct evidence available for this species alongside broader mechanistic insights from teleost models to identify likely pathways underlying female masculinization. Experimental exposure of adult female *X. hellerii* to hydrocortisone, particularly in interaction with temperature, induces masculinization of secondary sexual traits, notably the formation of a caudal "sword", without complete gonadal sex reversal. Histological alterations include ovarian degeneration and impaired embryonic viability rather than functional testicular development. These findings indicate that glucocorticoid signaling can modulate phenotypic sex traits independently of full gonadal transformation. Comparative evidence from other teleosts demonstrates that environmental stressors such as temperature, oxidative stress, and endocrine disruption frequently promote masculinization through activation of the hypothalamic–pituitary–interrenal axis, suppression of aromatase expression, reduced estrogen synthesis, and upregulation of male pathway genes such as dmrt1, amh, and sox9. Epigenetic mechanisms, particularly DNA methylation of the aromatase gene promoter, play a key role in stabilizing these shifts. In *X. hellerii*, masculinization appears primarily to affect secondary sexual traits and reproductive physiology rather than overriding the genetically determined sex pathway under natural conditions. These findings place female masculinization in swordtails at the intersection of conserved endocrine plasticity and endocrine disruption, highlighting the need for future molecular and epigenetic studies to clarify the mechanistic basis and ecological implications of this phenomenon.

Key Words: aromatase, cortisol, endocrine disruption, environmental sex differentiation, glucocorticoids, hydrocortisone, masculinization, secondary sexual traits, sex differentiation, sexual plasticity, teleost fish, *Xiphophorus hellerii*.

Introduction. We did not find any research that specifically analyzes factors influencing the masculinization of female *Xiphophorus hellerii* beyond one experimental study on hydrocortisone and temperature (Al-ali & Ibrahim 2020). The other available papers deal either with general mechanisms of environmental or endocrine sex differentiation in teleosts, with sex determination and male differentiation in *Xiphophorus* at a broad, evolutionary level, or with experimental sex reversal in other fish species (Vallowe 1957; Yamaguchi et al 2010; Navarro-Martín et al 2011; Sharma et al 2016; Díaz & Piferrer 2017; Ribas et al 2017; Liu et al 2020; Rajendiran et al 2021; Xu et al 2021; Yu et al 2021; Mukai et al 2022; Tran et al 2022; Fedder 2023; Yamaguchi & Kitano 2023; Yu et al 2023). The review below therefore combines what is known directly for *X. hellerii* with general teleost mechanisms that are likely to underlie or parallel masculinization in this species; where extrapolation is made, this is indicated.

Sexual differentiation and male traits in *Xiphophorus helleri*. Swordtail fishes (genus *Xiphophorus*) are viviparous teleosts characterized by marked sexual dimorphism in body shape and secondary sexual traits, especially the caudal sword of males (Mag-Mureșan & Pop 2004; Petrescu-Mag & Popa 2018; Fedder 2023). In *X. hellerii*, sex determination follows predominantly genetic systems, but the genus shows a remarkable diversity of sex-determining mechanisms, including male and female heterogamety and more complex multi-chromosomal systems (Lindholm & Breden 2002; Volff & Schartl 2002; Petrescu-Mag 2007; Oroian et al 2019; Fedder 2023). Classical histological work showed that gonads of both sexes are initially indifferent; ovarian and testicular pathways diverge shortly after birth, and no spontaneous gonadal sex reversal was detected under normal conditions (Vallowe 1957; Sun et al 2013) (Figure 1). Nonetheless, experimental endocrine manipulation of juveniles with testosterone or estradiol can induce spermatogenesis in ovaries and oogenesis in testes, respectively, indicating that the gonad retains substantial sexual plasticity during early differentiation (Vallowe 1957).

Male differentiation in swordtails encompasses both gonadal sex and the development of male-specific secondary traits such as the sword and gonopodium. Evolutionary analyses highlight that the sword itself has repeatedly been lost in the genus, whereas female preference for sworded males persists, illustrating a tension between sexual and natural selection and suggesting that male differentiation pathways and female perception have been under strong, sometimes divergent selection pressures (Fedder 2023). At the neuroendocrine level, variation in melanocortin 4 receptor (Mc4r) signaling has been implicated in controlling puberty onset and male body size in *Xiphophorus* species, including *X. hellerii*, with higher mc4r expression in large males and colocalization of mc4r and mrap2 in hypothalamic and preoptic regions (Liu et al 2020). Although not a masculinization factor in females per se, these findings show that hypothalamic neuroendocrine axes finely tune male phenotype expression and could, in principle, modulate the expression of male traits in genetic females if upstream sex-determining signals are perturbed.

Experimental masculinization of *X. hellerii* females by hydrocortisone and temperature. The only study directly examining masculinization of female *X. hellerii* used chronic exposure of adults to hydrocortisone at different ambient temperatures (Al-ali & Ibrahim 2020). Mature females treated for one month with hydrocortisone sodium succinate at 2.5 mg L^{-1}, both in summer (28-34°C) and winter (16-21°C), developed progressive elongation of the ventral portion of the caudal fin, forming a "sword" morphologically similar to that of males. This change in a quintessential male secondary sexual character occurred without complete gonadal sex reversal: histological analysis of treated females revealed ovaries containing atretic oocytes, degenerating embryos and malformed or dead embryos, rather than testes or ovotestes (Al-ali & Ibrahim 2020). Thus, hydrocortisone, in interaction with temperature, promoted phenotypic masculinization confined to external morphology and was associated with disrupted ovarian physiology and embryonic viability rather than a full transformation into functional males.

From a physiological standpoint, hydrocortisone is a glucocorticoid analog, and its masculinizing effect on the sword suggests crosstalk between stress axes and androgen-dependent pathways controlling fin outgrowth. In many teleosts, glucocorticoid elevation, as part of the generalized stress response, can masculinize developing gonads by suppressing aromatase expression and ovarian estrogen synthesis, thereby shifting the steroid milieu toward androgens (Yamaguchi et al 2010; Mukai et al 2022). Although *X. hellerii* hydrocortisone exposure was applied to adults after gonadal differentiation, similar mechanisms may promote local androgenic signaling in caudal fin tissues, driving male-like fin growth while compromising ovarian function. The observation that hydrocortisone "acts as a promoter of sexual transformation" at the level of external traits, but not of complete gonadal sex reversal, underscores that different components of sexual phenotype (gonads versus secondary characters) have distinct sensitivity windows and thresholds to endocrine disruption (Al-ali & Ibrahim 2020).

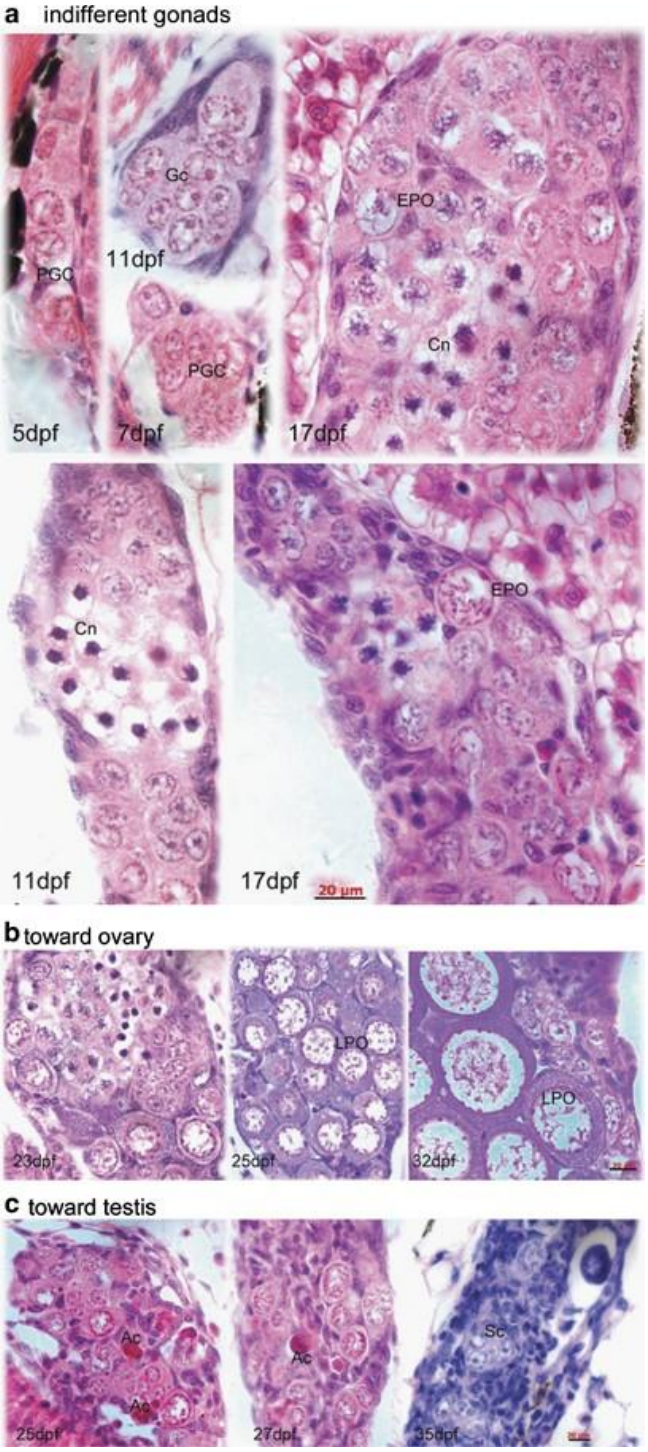

Figure 1. Gonadal development and differentiation in zebrafish. Indifferent gonad (a), only primordial germ cells (PGCs) are found in the gonad at 5 d.p.f. PGCs contain a large nucleus and possess a distinct nuclear membrane, several big nucleoli, and a network of fine chromatin filaments distributed throughout the karyoplasm. Along the inner side of the nuclear membrane, deeply staining granulosa material is seen. The weakly stained cytoplasm accounts for only a small fraction of the cell volume. At 7 d.p.f., PGCs differentiate into gonocytes (Gc). At 11 d.p.f., more Gcs and meiotic gonocytes with condensed chromatin (Cn) are seen, in addition to PGCs. At 17 d.p.f., early perinucleolar oocytes (EPOs) appear. During juvenile gonadal differentiation toward the ovary (b), more meiotic germ cells are seen (23 d.p.f.), showing densely packed oocytes (27 d.p.f.) and tight connections between late perinucleolar oocytes (LPOs) at 35 d.p.f. In the presumptive testis (c), stromal cells increase and oocyte degenerations appear as acid staining cells (Ac). Sc, spermatogonia cyst. Scale bar=20 µm. Reproduced from Sun et al (2013), Cell Death & Disease 4:e930. Licensed under CC BY 4.0.

Temperature in this experiment did not by itself induce swords in control females, but hydrocortisone plus temperature altered ovarian histology more severely at both high and low ranges, suggesting that thermal stress may exacerbate glucocorticoid-mediated effects on reproduction (Al-ali & Ibrahim 2020). This is consistent with broader evidence that temperature and cortisol often act in concert during environmental sex differentiation in fish (Yamaguchi et al 2010; Yu et al 2023).

Environmental and endocrine factors driving masculinization in teleosts: relevance to *X. helleri*. Beyond this specific experiment, a large body of work in teleosts documents that a wide range of environmental stressors, including elevated temperature, hypoxia, crowding and contaminants, consistently bias sex differentiation toward masculinization, often through shared endocrine and epigenetic pathways (Yamaguchi et al 2010; Sharma et al 2016; Ribas et al 2017; Rajendiran et al 2021; Xu et al 2021; Yu et al 2021; Mukai et al 2022; Petrescu-Mag & Proorocu 2022; Yu et al 2023). Systematic review of more than 100 species shows that virtually all classes of environmental stress can shift sex ratios toward males or induce female-to-male sex reversal, a process termed environmental sex differentiation (ESDi) (Yu et al 2023). At the physiological level, stressors frequently activate the hypothalamic–pituitary–interrenal (HPI) axis, elevating cortisol, which has been causally implicated in temperature-dependent masculinization in species such as Japanese flounder and yellow catfish (Yamaguchi et al 2010; Yu et al 2021). In these fishes, high temperature raises cortisol during the critical window of gonadal differentiation, and exogenous cortisol alone can induce XX female-to-male sex reversal, whereas inhibition of cortisol synthesis blocks thermal masculinization (Yamaguchi et al 2010; Yu et al 2021).

At the molecular level, cortisol and other stressors often converge on the aromatase gene cyp19a1a in the gonad. Increased DNA methylation of the cyp19a1 promoter and reduced transcription lead to lower estrogen synthesis, allowing testicular pathways (e.g. amh, dmrt1, sox9) to dominate, thereby masculinizing the gonad (Navarro-Martín et al 2011; Yamaguchi & Kitano 2023). This epigenetic suppression of cyp19a1a has been demonstrated in temperature-induced masculinization of European sea bass and is emerging as a common mechanism connecting environmental stress with male-biased sex ratios in teleosts (Navarro-Martín et al 2011; Yamaguchi & Kitano 2023; Yu et al 2023). Oxidative stress can also induce masculinization without elevating cortisol, as shown in medaka, where hydrogen peroxide treatment masculinized XX fish via pathways requiring gsdf and pparaa and upregulating male pathway genes (Mukai et al 2022). Thyroid hormone is another endocrine modulator that can masculinize genetic females by increasing expression of male-biased genes (amh, ar) while repressing female-biased genes (cyp19a1a, estrogen receptors) during testicular differentiation in zebrafish (Sharma et al 2016). Finally, direct androgen exposure, or indirect androgenization via aromatase inhibitors, can induce robust gonadal masculinization in numerous species, including rainbow trout and mosquitofish, supporting a general model in which reduction of estrogen signaling or enhancement of androgen signaling shifts gonadal fate toward maleness (Xu et al 2021; Tran et al 2022).

Although these mechanisms have not been directly dissected in *X. hellerii*, the hydrocortisone-induced masculinization of the sword strongly suggests that stress hormone pathways can access and modulate the sexual differentiation networks governing secondary sexual traits in this species as well. Given the conservation of key sex-differentiating genes (dmrt1, amh, cyp19a1a) and of cortisol-dependent pathways in teleosts (Ribas et al 2017; Rajendiran et al 2021; Fedder 2023; Yamaguchi & Kitano 2023), it is plausible that chronic elevation of glucocorticoids, elevated temperature or endocrine-disrupting pollutants in natural or captive environments could, under appropriate developmental timing, masculinize both gonads and secondary traits in genetic females of *X. hellerii* by suppressing ovarian aromatase, shifting steroid balance toward androgens, and possibly altering DNA methylation of sex-related genes.

Evolutionary and adaptive perspectives on female masculinization in swordtails. From an evolutionary standpoint, masculinization of genetic females is a form of sexual plasticity that can influence population sex ratios and the expression of sexually dimorphic traits. Across teleosts, environmental masculinization is thought to represent an adaptive response to moderate environmental stress, because populations composed of more males may better withstand resource limitation or density stress, given lower direct reproductive costs of males (Yu et al 2021, 2023). However, strong and persistent male bias can reduce effective population size and elevate extinction risk in small or inbred populations (Ribas et al 2017; Yu et al 2021).

In *Xiphophorus*, the evolutionary context is further shaped by elaborate sexual selection on male swords and body size, and by diverse sex-determining systems (Liu et al 2020; Fedder 2023). Female masculinization at the level of secondary sexual traits, such as the hydrocortisone-induced sword, could transiently increase the frequency of sworded individuals, potentially altering the dynamics of female mate choice and intrasexual competition. Yet, because hydrocortisone-treated females showed impaired ovarian function and nonviable or malformed embryos, such partial masculinization is likely maladaptive for individual fitness and would not contribute functionally male gametes to the next generation (Al-ali & Ibrahim 2020). This underscores a common pattern in anthropogenically induced masculinization: endocrine disruption may co-opt evolutionary conserved plasticity mechanisms but in ways that are decoupled from, and often detrimental to, reproductive success and long-term population adaptation (Yu et al 2021; Fedder 2023; Yu et al 2023).

In contrast, controlled, heritable shifts in the balance between male and female pathways, mediated by selection on sex-determining loci and downstream regulators such as mc4r, have allowed swordtails to evolve multiple male phenotypes and divergent sex-determining systems while retaining the capacity for endocrine responsiveness (Liu et al 2020; Fedder 2023). Endocrine- or environment-induced masculinization of females therefore sits at the interface between adaptive plasticity and pathological disruption. In *X. hellerii*, the available evidence suggests that experimentally induced masculinization of females is primarily an endocrine stress response affecting secondary sexual traits and ovarian function, while the underlying genetic sex-determining system and core gonadal pathway remain robust under natural conditions. Further work linking hydrocortisone and other stressors to transcriptional and epigenetic changes in cyp19a1a, amh, dmrt1 and related genes in *X. hellerii* would be necessary to fully elucidate the mechanistic parallels with better-studied teleost models of environmental masculinization.

Conclusions. Available evidence indicates that masculinization of female *Xiphophorus hellerii* is primarily mediated through endocrine pathways associated with stress physiology rather than through spontaneous or naturally occurring gonadal sex reversal. Hydrocortisone exposure demonstrates that glucocorticoids can induce the development of male-specific secondary sexual traits, particularly the caudal sword, while simultaneously impairing ovarian structure and reproductive viability. This dissociation between external masculinization and gonadal identity highlights the differential sensitivity of sexual phenotype components to endocrine modulation and suggests that secondary sexual traits remain responsive to hormonal signals even after gonadal differentiation is complete.

Comparative data from teleost fishes strongly support a conserved mechanistic framework in which environmental stressors activate glucocorticoid signaling, suppress aromatase expression, reduce estrogen synthesis, and shift the endocrine balance toward androgen-dependent pathways. These processes may involve epigenetic regulation of key sex-determining genes, thereby stabilizing masculinized phenotypes. In *X. hellerii*, such mechanisms likely operate at least at the level of secondary sexual trait expression, although the genetic sex determination system appears robust under natural conditions.

From an evolutionary and ecological perspective, experimentally induced masculinization in this species appears maladaptive, as it compromises reproductive function without producing functional males. This pattern is consistent with endocrine disruption rather than adaptive sexual plasticity. Future research integrating transcriptomic, endocrine, and epigenetic analyses will be essential to determine whether environmental stressors can induce permanent gonadal masculinization in *X. hellerii*, to identify critical developmental windows of susceptibility, and to assess the implications of endocrine-mediated sexual plasticity for population dynamics and environmental risk assessment.

Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Ioan Valentin Petrescu-Mag, Department of Environmental Engineering and Protection, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372 Cluj-Napoca, Romania, e-mail: zoobiomag2004@yahoo.com
Cristian Iederan, Department of Environmental Engineering and Protection, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372 Cluj-Napoca, Romania, e-mail: cristi.iederan@usamvcluj.ro
Petru Burduhos, Department of Environmental Engineering and Protection, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372 Cluj-Napoca, Romania, e-mail: petru.burduhos@usamvcluj.ro
Florin Dumitru Bora, Viticulture and Oenology Department, Advanced Horticultural Research Institute of Transylvania, Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372 Cluj-Napoca, Romania, e-mail: florindumitru.bora@usamvcluj.ro

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