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Beyond the Binary: Controlling Natural Sex-Change in Hermaphroditic Fishes

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ABSTRACT

Sex-change in hermaphroditic fishes is a complex but natural biological phenomenon that has significant implications for aquaculture fingerling production, selective breeding, and grow-out. Understanding the physiological, endocrinological, and molecular pathways underlying hermaphroditism in fishes offers unique opportunities to manipulate male-to-female and female-to-male transitions and maintain desired sex-ratios in hatcheries. Control over natural sex-change in hermaphroditic fishes can be achieved in vivo through socio-environmental manipulation, neuro-endocrine regulation, exogenous steroid treatment, or inhibition of aromatase activity with aromatase inhibitors (AIs). This review synthesises the current body of literature and knowledge of the tools associated with controlling sex-change, specifically in hermaphroditic fishes. Importantly, the recent advances in applying steroidal and non-steroidal hormones/compounds to control sex-change are described, as well as the effect of these approaches on gamete quality, fertility, and reproductive success of fishes in this unique group. Through investigating current practices and potential side-effects of controlling natural sex-change, we aim to guide the development of more effective and viable methods for maintaining desired sex-ratios in aquaculture selective breeding. This review highlights the need for further research to optimise control strategies, minimise unintended impacts, and promote best practices in controlling sex-change in hermaphroditic fishes.

1 | Introduction

Hermaphroditism is a reproductive process where an individual can reproduce as both a male and a female at some stage in its life history [1, 2]. Hermaphroditism is a unique sexual development strategy, present in less than 1% of vertebrate species and mostly observed in teleosts and some anurans [3–6]. In this rare reproductive development strategy, both male and female reproductive functions can exist either simultaneously (synchronous) or sequentially within a single individual [5]. In teleosts, individuals with simultaneous hermaphroditism concurrently possess both fully functional ovarian and testicular tissue and produce both types of gametes (e.g., barred hamlet, *Hypoplectrus puella*), whereas individuals with sequential hermaphroditism first sexually mature with either functional testicular tissue and then functional ovarian tissue (termed protandry; e.g., barramundi, *Lates calcarifer*), or vice versa (termed protogyny; e.g., protogynous red-spotted grouper, *Epinephelus akaara*) [2, 3, 7, 8]. Recent studies have reported hermaphroditism in about 1.5% of teleost species, with

sequential hermaphroditism being the most common reproductive strategy present in >88% of hermaphroditic species [4, 9–11]. Of the sequential hermaphrodite fishes, 72% are protogynous, while 13% are protandrous; the remaining 15% of sequential hermaphroditic fishes can change sex in both directions (bi-directional, e.g., gobiid fish *Trimma okinawae*) [12]. Sex-change in sequential hermaphrodites, therefore, is a programmed event where during their development that changes in transcriptional profile, radical restructuring of the gonadal morphology, and behaviour changes take place [8, 13–16]. Several studies have extensively reviewed the incidence, distribution, phylogeny, and mating system of sex-changing simultaneous and sequential hermaphroditic fishes [4, 5, 17]. However, they lack information on how natural sex-change of these unique fish groups is controlled, particularly concerning the role of internal and external cues. A considerable number of studies have investigated sex-change in hermaphroditic fishes, and the underlying genetic, environmental, and hormonal drivers that underpin natural sex-change in several species are increasingly well documented (reviewed by Todd et al. [16]). Moreover, several studies have demonstrated that a range of intervention strategies (e.g., social, environmental, neuroendocrine, gene editing, epigenetics, transgenesis, and hormonal/chemical administration) have become successful in inducing male-to-female (MTF) or female-to-male (FTM) sex-change in hermaphroditic species (see reviews [12, 16, 18–22]). However, these studies often focus on individual strategies relevant to the species and conditions under which it is being investigated, lacking comprehensive comparisons and synthesis of their effectiveness under varying conditions or replicability of outcomes that are likely across fish species. A critical gap, therefore, exists in the comparative evaluation of the efficacy of various sex-change manipulation strategies. This lack of comparative data and synthesis hinders our understanding of the optimal timing, dosage, duration, and route of administration for each strategy in different species and life stages. Moreover, how different sex-change control strategies, either through transient or persistent changes in gonadal morphology, affect gamete quality and the spawning success of sex-reversed individuals are often overlooked.

This review synthesises research on different sex-change control strategies (with special focus on hormonal and chemical control) as applied to hermaphroditic fishes, highlighting their efficacy in various species and life stages depending on the dosage/exposure, treatment duration, and route of administration. The review also highlights findings reported on different sex-change manipulation strategies and their impact on broodstock gamete quality, fertility, and reproductive success. We also explore the need for developing alternative methods for effective and predictable sex-change control programmes in aquaculture breeding and responsible use of techniques for regulating the sex of hermaphroditic fishes.

2 | Reproductive and Sex-Change Strategies in Hermaphroditic Fishes

Unlike in gonochoristic fishes where sexual identity is primarily genetically determined at fertilisation and remains fixed throughout development, hermaphroditic fishes exhibit profound sexual plasticity [23, 24]. Natural sex-change in hermaphroditic fishes often involves interactions between physiological, neuroendocrinological, hormonal, and environmentally mediated axes [16, 23]. Internal cues (e.g., age, size, and growth rate) and external factors (e.g., social dynamics, temperature) are hypothesised to trigger the initiation of sex-change primarily at the neuroendocrine level [12, 19]. This process is mediated by the interaction between the hypothalamic–pituitary–gonadal (HPG) and the hypothalamic–pituitary–interrenal (HPI) axes, leading to a rapid cascade of physiological responses [2, 13, 16, 20, 21, 25]. These responses include altered brain chemistry, shifts in plasma sex steroid levels, reprogramming of gene activity within the gonad, and subsequent structural and functional changes in the reproductive organs and associated behaviours, ultimately resulting in the transition between sexes [2, 13, 16, 20, 21, 23, 24, 26, 27]. In combination, these mechanisms are enabled and executed in varied ways to achieve the different forms of hermaphroditism observed in fishes, including protogyny, protandry, bidirectional, and simultaneous hermaphroditism (see review [16]) (Figure 1). In protogynous fishes, the FTM transformation involves the replacement of ovarian tissue with testicular tissue, leaving only remnants of the original female structure [37]. During the onset of protogynous sex-change, ovarian follicles (including granulosa and theca cells) and oocytes undergo degenerative processes [16]. The degenerated gonads are subsequently replaced through the progressive proliferation of Sertoli cells, Leydig cells, and spermatogonia within the peripheral ovarian lamellae, after which spermatogenesis continues to progress and fully functional testes are developed (see review [16]) (Figure 1A).

In contrast, during the onset of MTF sex-change in protandrous fishes, testicular tissue is replaced by the initial development of ovarian tissues at the ventral periphery of the gonad, followed by progressive inward migration of ovarian tissue (Figure 1B). In some species (e.g., anemonefish, *Amphiprion clarkii* and barramundi), non-functional previtellogenic oocytes may also be dispersed in small numbers throughout an adult's testicular tissue prior to the onset of sex-change [38] (Figure 1Bii,iii). Sex-change in bidirectional fishes proceeds from a bisexual gonad or ovotestis where ovarian and testicular portions are either contained simultaneously (syngonic; e.g., divine dwarfgoby, *Eviota epiphanes*) or separated by connective tissue (digonic; e.g., species under the genera *Lythrypnus*, *Trimma*, *Gobiodon*, and *Paragobiodon* spp.). In bidirectional fishes, either the male or female portion of the gonad has mature gametes (with full reproductive function) at one time and determines the fish's current functional sexual phenotype [39–41]. In simultaneous hermaphroditism, individuals contain both mature testis and ovary and reproduce either by outcrossing or self-crossing [3]. Since there is significant variation in the onset of sex-change among species of hermaphroditic fishes, the precise timing and triggers of sex-change are not fully understood [42]. Consequently, this lack of knowledge presents a significant challenge to effective management of these sex-changing species in aquaculture settings. Achieving control over the natural sex-change process is essential for optimising breeding programmes, maximising production efficiency, and leveraging the advantages of fish sexual plasticity for the aquaculture industry [18].

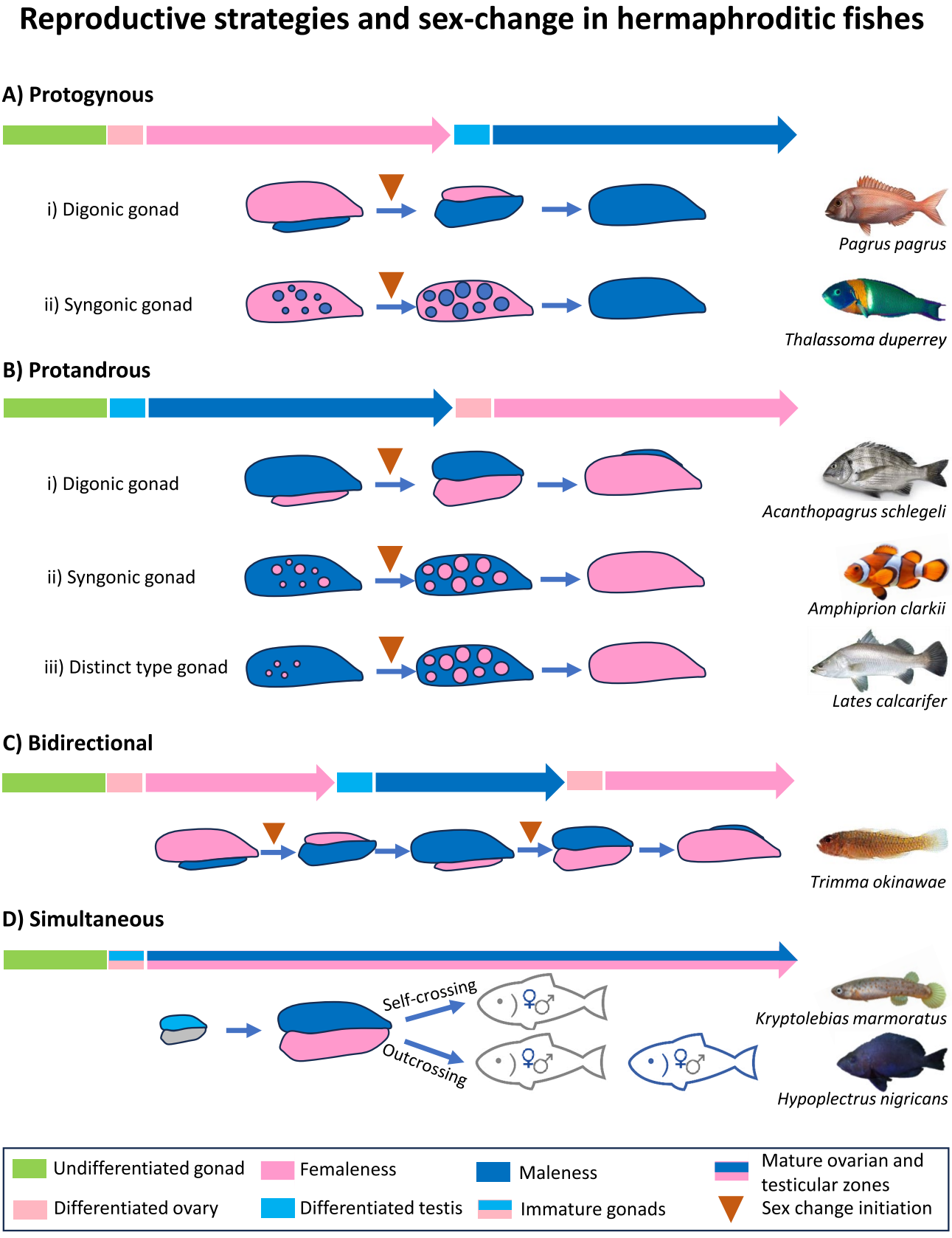

FIGURE 1 | Legend on next page.

FIGURE 1 | Schematic representation of reproductive strategies and sex-change in hermaphroditic fishes. A(i) Protogynous fish (e.g., red porgy, *Pagrus pagrus*) gonads that contain a dorsal ovarian and a ventral testicular area are separated by connective tissue (digonic). In the first sex phase (female), the ovarian part is dominant (pink). As sex-change progresses towards male, the mature ovarian cells decrease in size, while the testicular cells grow, multiply and become the dominant part (blue) [28]. A(ii) In the female sex phase (e.g., the saddleback wrasse, *Thalassoma duperrey*), the immature testicular cells are scattered within the ovary (syngonic). During the onset of sex-change, the scattered cells grow and finally occupy the gonad as testis (second sex phase as male) [29]. B(i) Protandrous fish (e.g., black porgy, *Acanthopagrus schlegeli*) gonad with initial male sex phase where a dominant testis and immature ovary are separated by connective tissue (digonic). As the sex-change proceeds, the ovarian cells grow and become dominant and functional (female sex phase) [30]. B(ii) Ovotestis gonad where the testicular tissue develops, dominates and functions within the immature ovaries without a distinct connective tissue boundary, syngonic (male sex phase). In the female sex phase, scattered ovarian cells grow and mature and become functional part of the gonad (anemonefish, *Amphiprion clarkii*) [31]. B(iii) In this protandrous type (e.g., barramundi, *Lates calcarifer*), gonads filled with testicular tissue (often have low numbers of non-functional previtellogenic oocytes) during the male phase and packed with ovarian tissue during female phase [32]. (C) In this hermaphroditism (e.g., gobiid fish *Trimma okinawae*), the testes remain fully mature and functional during the male phase, while the ovaries remain in an immature state. Conversely, during the female phase, the ovaries are mature and functional, while the testes remain immature. However, the immature gonadal tissue in either male or female phase does not undergo degeneration, but persists within the gonad [33]. (D) During simultaneous hermaphroditism, individuals possess both functional testes and ovaries. This adaptation allows them to release sperm and eggs at any time after maturation. During spawning, individuals either reproduce by self-crossing (e.g., mangrove killifish, *Kryptolebias marmoratus*) [34] or alternate roles: One acts as a male, releasing sperm, while the other acts as a female, releasing eggs (outcrossing, e.g., black hamlet, *Hypoplectrus nigricans*) [35]. In the subsequent spawning event, the roles are reversed, meaning the previous male acts as a female and vice versa. Figure revised and adapted from [36].

3 | Why Is Control Over Natural Sex-Change Necessary?

Unlike gonochoristic fishes where sex is determined either as male or female and remains fixed throughout life, the sexual phenotype of hermaphroditic fishes is highly plastic [43, 44]. The diverse patterns of sex-change in hermaphroditic fishes, with different species evolving distinct mechanisms and associated life-history strategies, often makes their reproductive management difficult in an aquaculture setting [16, 23, 43, 45] (Figure 2). While several hermaphroditic fishes (e.g., protandrous barramundi; several protogynous groupers, *Epinephelus* sp.) have become major seafood species consumed globally, the lack of control over their natural sex-change limits the efficient propagation of these species due to difficulties in hatchery breeding [18, 46–48]. However, significant plasticity of the phenotypic sex in hermaphroditic fishes provides a unique opportunity to develop a better understanding of the underlying mechanisms of gonadal differentiation and sex-change of these fishes. Furthermore, if well understood, it is possible to develop methods to achieve complete control of the sex-change process and improve breeding outcomes significantly [18, 49]. Complete control over sex-change can allow breeders and hatchery managers to achieve desired sex-ratios and increase the rate of genetic gain obtained in selective breeding programmes [50]. In many protogynous groupers (e.g., white grouper, *Epinephelus aeneus*; red-spotted grouper), for example, natural female-to-male sex-change appears at the age of 5–6 years, or even later, and therefore artificial breeding is mostly reliant on repeated capture of mature wild-caught males [51–53]. However, there is a paucity in the supply of wild-sourced mature males and waiting for fish to naturally change sex in captivity is time-consuming and costly, leading to significant constraints in the selective breeding of the species [51–53]. In this scenario, producing precocious males (i.e., earlier than naturally occurring) is a potential solution, and sex-change control strategies could support further growth of grouper aquaculture throughout Asia [50, 52–58]. The necessity of improved sex control has also been demonstrated in the protandrous Australian farmed barramundi (*Lates calcarifer*), where in the wild males do not usually undertake MTF transition till they are ~4–6 years of age [59, 60]. This age-related sex-change hinders same-age and generation mating among males and females, increases the generational breeding interval, and reduces the rate of genetic progress that can be realised over time [61, 62]. Conversely, highlighting the flexibility in the MTF transition process, even within the same species, precocious MTF sex-change at <2 years of age has been reported in Singaporean farmed barramundi [63]. This results in high female-skewed populations and a scarcity of mature males for breeding [63]. Moreover, the use of immature males may lead to suboptimal breeding and reduced spawning success. In both scenarios, full control over the sex-change process in barramundi is crucial for broodstock management and breeding. Similar issues occur with other protandrous species (e.g., gilthead seabream, *Sparus aurata*; sharpsnout seabream, *Diplodus puntazzo*), where if the broodstock sex-ratios of these species are not managed for several years, the breeding populations will become highly female skewed with no mature males left in the breeding population [64–66]. In addition, dynamic environmental conditions in aquaculture settings can also alter the timing and direction of sex-change and cause distorted sex-ratios due to the inherent sexual plasticity of hermaphroditic fishes [21, 67]. This unpredictability poses significant challenges for aquaculture management, as it can lead to undesired or skewed sex-ratios within populations, complicating breeding programmes, impacting reproductive success, and hindering efficient production planning [12, 20, 21, 68]. Moreover, sex-change can lead to significant alterations in the possible genetic combinations within a breeding population [69] complicating selective breeding programme operation and making it difficult to achieve specific breeding goals. Sexual dimorphism in growth traits, including growth rate, body size, shape, colour, and precocious maturation, is also another important reason for controlling sex-reversal and for producing mono-sex populations when one sex is preferred over the other [70, 71]. In gonochoristic species, sex control is often employed to produce mono-sex populations, focusing

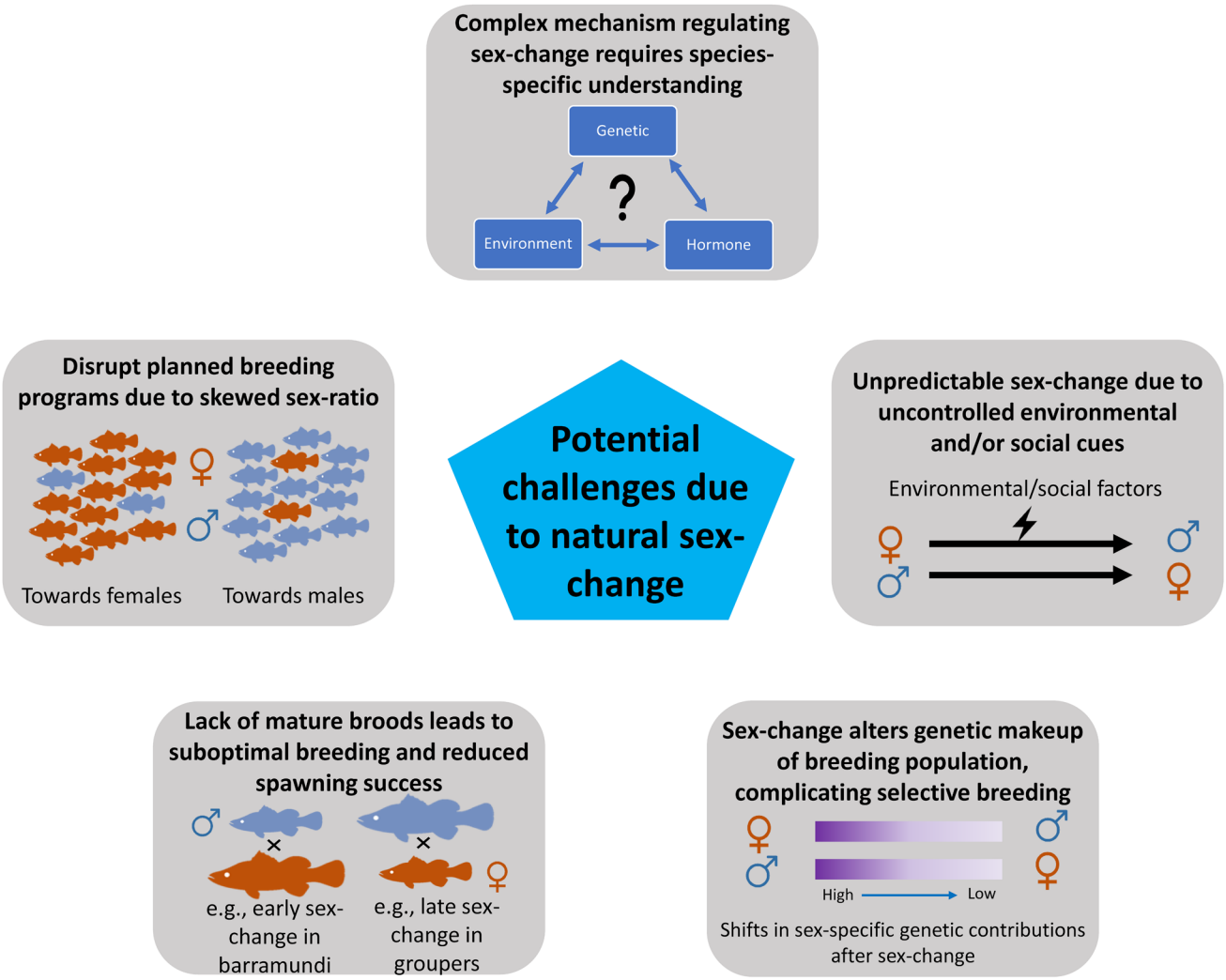

FIGURE 2 | Conceptual diagram illustrating the challenges that can occur because of the natural sex-change in hermaphroditic fishes in the context of aquaculture breeding.

on the production of a single sex that shows improved growth performance [18, 70–74]. There is little information on the effect of sex on the growth rate of hermaphroditic fishes and as such the potential of using sex control for these purposes remains under explored. Considering all these standpoints, achieving full control over sex-change processes presents the potential to maximise the benefits associated with specific sexes in selective breeding programmes.

4 | Sex-Change Control Strategies in Hermaphrodite Fishes

This section provides a brief overview of the various strategies, including socio-environmental, neuroendocrinological, hormonal and chemical, and genetic and epigenetic manipulations, utilised for controlling sex-change in hermaphroditic fishes. Considering the widespread adoption of hormonal, especially sex steroids, and chemical regulation of sex-change in aquaculture and the prevailing knowledge gap on comprehensive comparative assessment of the efficacy across diverse hermaphroditic fish species, the next section (Section 5) will provide a more detailed description of these topics.

4.1 | Socio-Environment-Mediated Sex-Control

Social and environmental (e.g., temperature, photoperiod, pH, salinity, density, and hypoxia) manipulations have been reported to effectively induce sex-change to maintain desired sex-ratios in a number of teleosts [75–80]. In socially controlled hermaphroditic fishes (e.g., bluehead wrasses, *Thalassoma bifasciatum*; parrotfish, *Sparisoma radians*; and cleaner fish, *Labroides dimidiatus*), the introduction and removal of a dominant male has been demonstrated to be effective for preventing or inducing protogynous sex-change in subordinate and larger females, respectively [13, 81–84]. Conversely, the removal of a large dominant female from a social group prompts the protandrous sex-change of the male of a monogamous anemonefish mating pair (*Amphiprion* and *Premnas* spp.) [85, 86]. In a commercially important protogynous red-spotted grouper, rearing of a larger dominant and a smaller subordinate fish of the same sex in the same tank for 1 year triggers sex-change in both directions (MTF and FTM) [87]. This result suggests that manipulating social conditions holds the potential for controlling sex-change in a number of marine hermaphroditic fish families, including Labridae, Scaridae, Pomacentridae, and Gobiidae [13, 83, 88].

Environmental manipulation studies have received less scientific attention in hermaphroditic fishes, with most studies instead focusing on gonochoristic fishes [49, 80, 83, 88–99]. Many environmental manipulation studies of gonochoristic fishes have been found effective in inducing sex-change, overriding the otherwise determined sex. The few studies that have investigated the influence of environmental factors in hermaphroditic fishes have shown the promising potential of temperature (e.g., for delaying sex-change in barramundi) and pH (e.g., for controlling sex-ratio in swordtail, *Xiphophorus helleri*) [80, 93, 96, 100, 101]. However, further studies are needed, particularly in the context of hermaphroditic aquaculture species, to elucidate the full potential of manipulating the environment for control over natural sex-change.

4.2 | Neuroendocrine Regulation for Controlling Sex-Change

To date, there have only been a limited number of studies on the use of neuroendocrine manipulations (exogenous administration of neuroendocrine substances, including the gonadotropin releasing hormone, GnRH; monoamine neurotransmitters; and neuropeptides) in orchestrating sex-change in hermaphroditic fishes (see review [19]). However, these studies have highlighted their potential as a tool for sex control. For example, in the protogynous rice-field eel (*Monopterus albus*) and bluehead wrasse, GnRH analogue is reported to influence FTM sex-reversal by increasing the number of GnRH-immunoreactive neurons in the preoptic area (POA) of the hypothalamus [102–106]. The quantitative changes in GnRH-immunoreactive neurons presumably stimulate the secretion of gonadotropic hormones, induce the development of testicular lobules and Leydig cells, and promote male development [106]. Conversely, several researchers argued that the increase in GnRH-immunoreactive cell numbers does not reliably induce sex-change, and is instead correlated with male reproductive function, as evidenced in ballan wrasse, *Labrus berggylta* [107, 108]. A similar observation was also reported by Larson et al. [109, 110], who claimed that GnRH can induce sex-change when it is administered in combination with other chemicals (i.e., dopamine antagonists). Studies have also suggested that monoamine neurotransmitters (e.g., dopamine, norepinephrine, serotonin, and catecholamine) can play regulatory roles in modulating sex in gonochoristic fishes, but their roles in controlling sex-change in hermaphroditic fishes remain largely unexplored [111–117]. A small number of studies have reported the role of dopamine, norepinephrine, and serotonin in regulating sex-change in protogynous saddleback wrasse and protandrous dusky anemonefish (*Amphiprion melanopus*) [109, 110, 118]; however, there is also evidence of incomplete sex-reversal in some cases [113]. Neuropeptides (e.g., arginine vasotocin, isotocin, and neuropeptide Y) have also been reported to influence sex-change by regulating hormone secretion and gonad development in fish [25, 114, 116], but their direct role in controlling sex-ratio is yet to be explored. Other studies claimed that the administration of neuropeptides (i.e., arginine vasotocin) enhanced testosterone production and induced the spawning response in some gonochoristic fishes, including rainbow trout (*Oncorhynchus mykiss*) and killifish (*Fundulus heteroclitus*) [119, 120]. In contrast, the findings from a study indicate that neuropeptide Y (NPY) can induce sex-reversal in the protogynous bluehead wrasse [121]. The dichotomous and contrasting findings suggest the need for further studies to understand the complex network of neuropeptides and neurotransmitter mechanisms underlying sex-change, with special focus on hermaphroditic fishes.

4.3 | Use of Hormones/Chemicals for Sex-Control

In hermaphroditic fishes, sex-change is orchestrated by a delicate balance of estrogens and androgens, primarily 17ß-estradiol (E_2) and 11-ketotestosterone (11-KT) [122]. These sex-steroid hormones have critical roles within the differentiation and development of male and female gonadal tissues (e.g., determining fate of germ-cell differentiation into oogonia or spermatogonia), along with other secondary sexual characteristics in protogynous, protandrous, bidirectional and simultaneous hermaphrodites (see reviews [12, 16, 25, 123]) (Figure 3). Therefore, to obtain better control over sex-change in hermaphroditic fishes, the administration of exogenous sex-steroids, hormones and chemicals that either promote or inhibit male and female hormonal pathways has seen significant research focus [22, 42, 50, 52, 54, 106, 123, 126–145]. Application of natural androgen, such as 11-KT, has been reported to play a key role in controlling natural sex-change in many hermaphroditic fish species, particularly in protogynous species [126–131]. In addition, exogenous androgenic enhancers, including 17α-methyltestosterone (MT), 11β-hydroxyandrostenedione (OHA), and 17α-methyldihydrotestosterone (MDHT), have demonstrated masculinisation potency and have been extensively used to manipulate sex-change in these species [26, 29, 52, 128, 129, 132]. Similarly, the use of estrogenic agents, such as natural E_2 and synthetic 17α-ethynylestradiol (EE_2), to induce phenotypic feminisation of genetically determined gonochoristic species or precocious feminisation of protandrous species is well documented [133–138]. Apart from sex-steroid hormones, exposure to cortisol, either by dietary administration or intraperitoneal injection, has been observed to promote masculinisation in some protogynous and bidirectional sex-changing species [139–142]. In addition, in sex-changing fish, aromatase inhibitors (AIs), including letrozole (LET) and fadrozole (FAD), block the conversion of androgens to estrogens by impeding aromatase activity. These act to promote sex-change in protogynous species and prevent or delay sex-change in protandrous species [129, 143–145]. While hormonal/AIs treatments have been found effective for controlling sex-change in some fishes, the treatment efficacy is not consistent, varying with dosage, treatment duration, and route of administration employed, along with different species and hermaphroditic strategy [146–149]. Furthermore, no studies of the long-term maintenance of the induced sex phenotype have been undertaken, and the evaluation of the reversibility of these changes remains limited [146, 147, 150]. Due to the importance of these issues in determining the utility of these approaches for industry, further research is required to develop this understanding.

4.4 | Gene Editing and Epigenetic Modifications: Tools for Controlling Sex-Change

During the sex-change process, the production of androgens and estrogens is widely suggested to be regulated by a network of key

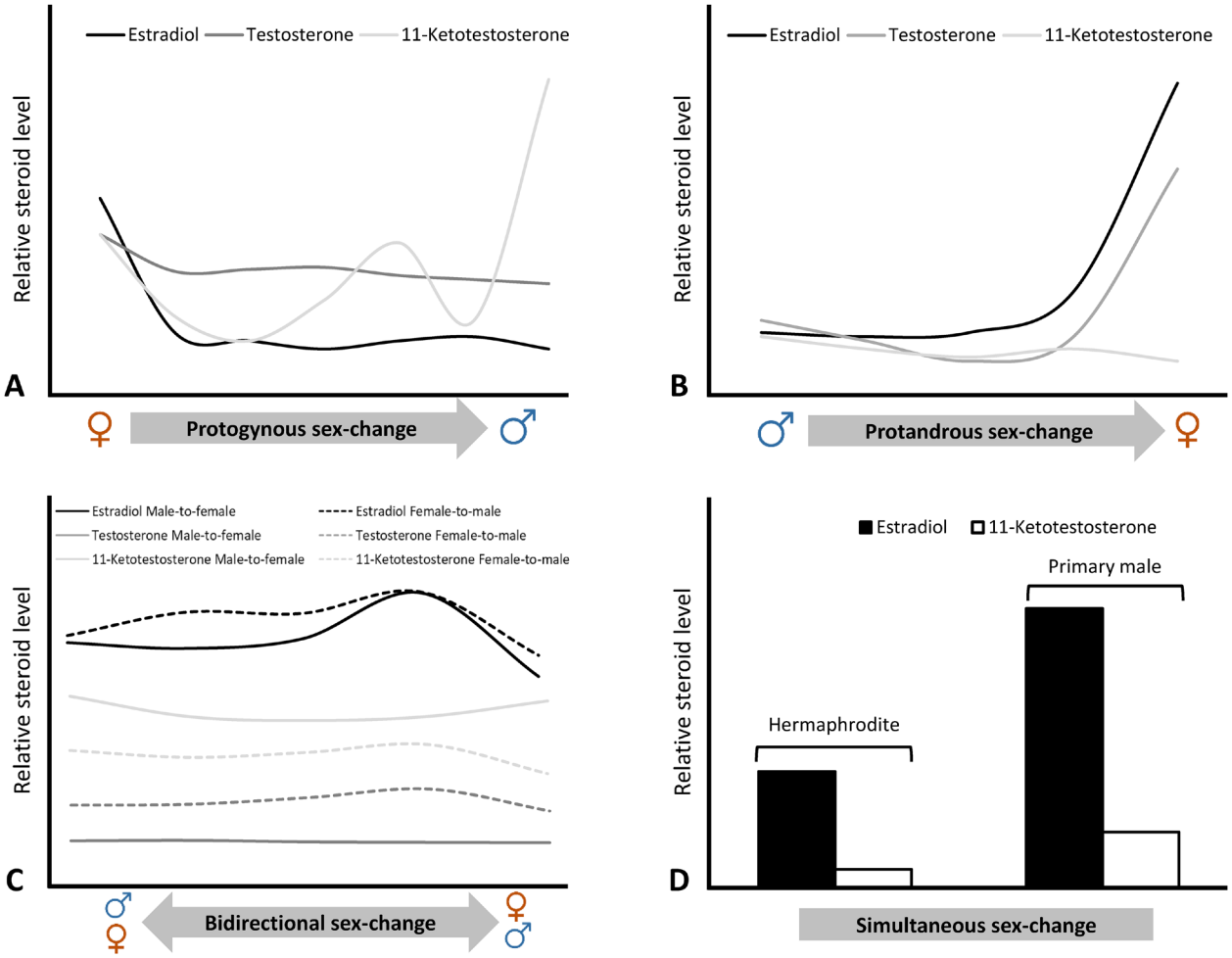

FIGURE 3 | Schematic representation of hormonal shifts during sex-change in different hermaphroditic fishes. (A) Protogynous sex-change in the saddleback wrasse (*Thalassoma duperrey*) involves a shift from estrogen-dominant to androgen-dominant hormonal profiles [29]. (B) Protandrous sex-change in anemonefish (*Amphiprion melanopus*) involves the opposite shift, with a transition from androgen-dominant to estrogen-dominant hormonal profiles [124]. The persistent presence of testosterone in protandrous females is suggested to occur due to being the precursor for estrogen biosynthesis. (C) Bidirectional sex-change involves steroidal shifts between male-to-female and female-to-male directions of gorgeous goby, *Lythrypnus pulchellus* [82]. Male and female pairs of similar size. (D) Simultaneous sex-change involves hormonal shifts between hermaphroditic and primary male of the mangrove killifish (*Kryptolebias marmoratus*) [125].

genes involved in testis development (e.g., double sex- and mab-3-related transcription factor-1, *dmrt1*; anti-Müllerian hormone, *amh*; SRY-box transcription factor 9, *sox9*; and gonadal soma-derived factor, *gsdf*) and ovary formation (e.g., cytochrome P450 family 19 subfamily A polypeptide 1a, *cyp19a1a*; and fork head box L2, *foxl2*) [151] (Figure 4). The association between steroidogenic and genetic pathways is now well documented and comprehensively reviewed by Zhou et al. [155]. Studies have demonstrated that gene-editing tools (e.g., zinc finger nucleases, ZFNs; transcription activator-like effector nucleases, TALENs; and clustered regularly interspaced short palindromic repeats, CRISPR) can regulate the expression of specific sex determining genes by inhibiting mRNA translation and are also effective in controlling sex-change (see reviews [155–157] for protocols and application, and Section 7.2.1 for more information) (Table 1).

In addition to gene editing, epigenetic modifications (e.g., DNA methylation, histone modifications, and non-coding RNAs (for details see review [21])) also play a significant role in sex differentiation and sex-change [169–171]. Research in protogynous fishes (e.g., rice-field eel) shows that the hypermethylation of the *cyp19a1a* promoter correlates with the downregulation of *cyp19a1a* expression and orchestration of ovarian tissue to testicular tissue [172]. Conversely, in the protandrous fishes (e.g., black porgy), demethylation of the *cyp19a1a* promoter led to increased *cyp19a1a* expression and MTF sex-change [173]. In protandrous barramundi, higher methylation of the *dmrt1* gene was observed in females than in males, while in protogynous species this gene was reported to be demethylated through FTM sex-change [152, 171]. In addition to DNA methylation, some hermaphrodite species showed that histone modifications are involved in maintaining the differentiated gonad [172, 174]. These findings suggest that epigenetic modifications play a crucial role in fish in regulating gene expression and thereby influencing phenotypic plasticity. As such, the manipulation of epigenetic modifications is also an active area of research for achieving sex control and studying sex-change, demonstrating promise in a range of hermaphroditic and gonochoristic fishes [172, 175–177]. For instance, in the rice-field eel, the natural FTM sex-change involves DNA methylation of the *cyp19a1a* promoter and reduced

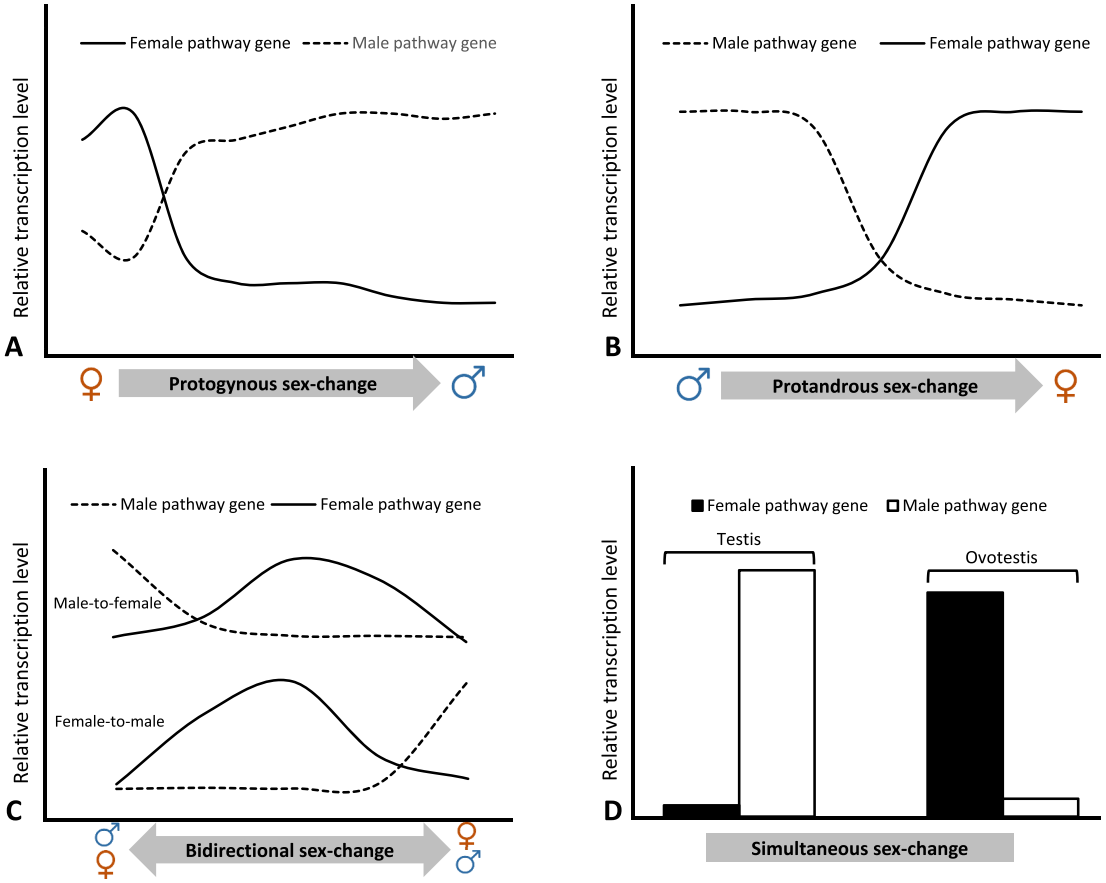

FIGURE 4 | Schematic representation of relative transcription levels of male and female sex-determining genes during sex-change in different hermaphroditic fishes. (A) Protogynous sex-change in bluehead wrasse is accompanied by downregulation of female-specific genes and upregulation of male-specific genes [152], (B) Protandrous sex-change in black porgy involves the opposite shift, with an increasing trend in female gene expression pathways following steady decrease in male-biased gene expression [16, 153], (C) Bidirectional sex-change involves changes in genetic pathways between female-to-male and reversible male-to-female directions of orange-spotted grouper, *Epinephelus coioides* [150]). Male and female pairs of similar size. (D) Gonadal gene expression levels between simultaneous sex-changing hermaphroditic and primary male of mangrove killifish (*Kryptolebias marmoratus*) [154].

transcription of *cyp19a1a* [172]. Intraperitoneal implantation (1 and 5 mg kg^{-1} body weight, BW) of 5-aza-2′-deoxycytidine (5-aza-dC, a DNA methyltransferase inhibitor that blocks DNA methylation when incorporated in the DNA as a cytidine nucleoside analogue) resulted in decreased methylation of the *cyp19a1a* promoter and increased gonadal *cyp19a1a* expression, leading to the reversal of natural sex-change of rice-field eels [172]. The use of 5-aza-dC has also been demonstrated to successfully induce sex-reversal in Nile tilapia and zebrafish [175–177]. While the techniques for manipulating epigenetic modifications are still evolving, these studies highlight intriguing potential for manipulating sexual fate in fishes, particularly in highly sexually plastic hermaphroditic species. However, the use of demethylating agents, such as 5-aza-dC, should be considered carefully, as these compounds can cause genome-wide demethylation and indiscriminately affect epigenetic modification [178].

5 | Steroidal and Chemical-Based Sex-Change Control Strategies: Recent Advances

Among vertebrates, fish sex is highly plastic and shows a greater response to external cues [23]. Since the sex differentiation of fish is ultimately influenced by sex steroids, manipulating gonadal sex through hormone administration is considered the simplest and most convenient way to modulate sex-change, and has been well established in many gonochoristic and hermaphroditic fish species [29, 38, 124, 179–181]. This section provides a detailed comparative discussion on the most commonly used sex-steroidal (estrogens and androgens) and chemical (estrogen blockers and aromatase inhibitors) sex-control techniques applied to hermaphroditic fishes, examining their effectiveness in sex-reversal and reproductive performance, which varies with species, life stage, dosage, treatment duration, and administration route.

5.1 | Estrogenic Agents and Estrogen Blockers

5.1.1 | Estrogenic Enhancers: Tools for Shaping Female-Biased Sex-Ratios

Being the most potent and prevalent natural estrogen, E_2 has a key role in sexual differentiation and sex-change through stimulating the development of ovarian tissue [182]. For inducing MTF sex-change and producing precocious females, the

TABLE 1 | Key sex-related genes that have been targeted via genome editing technologies to control sex-reversal.

Gene abbreviations	Full name	Primary role in sex determination	Purpose of targeted mutation[a]	Gene-editing tool(s) used[b]	Studied species	References
cyp19a1a	Cytochrome P450, Family 19, Subfamily A, Polypeptide 1a (Aromatase)	Involved in converting androgens to estrogens	Knock out to disrupt estrogen synthesis and promote male development	TALENs CRISPR	*Monopterus albus*	[158, 159]
foxl2	Forkhead Box L2	Ovarian determining/ maintaining factor	Knock out to promote male development or FTM sex-reversal	TALENs	*Monopterus albus*	[158, 159]
amhy	Y-specific duplicate of the *anti-Müllerian hormone*	Plays a role in inhibiting ovarian development and essential for male sex determination	Knock out to induce female development and MTF sex-reversal	CRISPR/Cas9	*Oreochromis niloticus*	[160]
dmy	DM-related gene on the Y chromosome	A master male-determining gene in certain species, such as medaka	Knock out to MTF sex-reversal	TALENs	*Oryzias latipes*	[161]
dmrt1	Doublesex and Mab-3 Related Transcription Factor 1	A key factor in testis differentiation and maintenance across many vertebrates	Knock out to cause MTF sex-reversal	TALENs CRISPR	*Cynoglossus semilaevis*, *Oreochromis niloticus*, *Danio rerio*	[162–165]
gsdf	Gonadal Somatic Cell Derived Factor	Crucial for normal gonadal somatic cell development and male differentiation in many fish species	Knock out to induce MTF sex-reversal	ZFNs CRISPR	*Oreochromis niloticus*, *Oryzias latipes*	[166, 167]
sdY	Sexually Dimorphic on the Y chromosome	Master male sex-determining gene in rainbow trout	Knock out to inactivate male sex-determination and to induce MTF sex-reversal	ZFNs	*Oncorhynchus mykiss*	[168]

[a]Purpose of targeted mutation: FTM and MTF indicate female-to-male and male-to-female, respectively.
[b]Gene-editing tool(s) used: TALENs (transcription activator-like effector nucleases), CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9), and ZFNs (zinc finger nucleases).

administration of exogenous E_2 has been demonstrated to be potent in a number of hermaphroditic fish families, including Centropomidae, Gobiidae, Labridae, Latidae, Pomacentridae, and Sparidae [123, 183] (Table 2). However, the efficacy is significantly influenced by the age and developmental stage of the fish, dosage, treatment duration, and methods of administration [183].

Studies in protandrous fishes (e.g., barramundi; black porgy) demonstrate that the efficacy of E_2 in inducing sex-change is significantly influenced by the developmental stage of the fish [133–136, 146, 190]. Individuals, particularly those in early developmental stages, or with undifferentiated gonads, appear more responsive to E_2 treatment, often requiring lower treatment duration to achieve complete sex-reversal compared to adult fish or fish with differentiated gonads [135, 146, 190]. However, the dosage of E_2 required for controlling sex-change is species-specific. Studies have highlighted that high dosages of E_2 (such as 4 mg kg^{-1} of feed for adult black porgy, 8 mg kg^{-1} of body weight (BW) for adult barramundi, and 20 mg kg^{-1} of feed for juvenile undifferentiated barramundi) can successfully induce complete feminisation [133, 134, 136]. In adult barramundi, implants with lower dosages (4 mg kg^{-1} of BW) induced a lower percentage of precocious females compared to the higher dosage (8 mg kg^{-1} BW) but resulted in complete suppression of testicular development [134]. However, the same dosages administered via implants (4 and 8 mg kg^{-1} BW of E_2 implants) caused mortality in common snook (*Centropomus undecimalis*) [186]. In contrast, dietary E_2 treatment with dosage 60 mg kg^{-1} feed induced 100% feminisation without affecting the survival of Mexican snook (*Centropomu poeyi*) [195]. These results indicate the species-specific and delivery method-specific tolerance of E_2. Therefore, the difference in E_2 efficacy in feminisation could also be due to variation in application methods [123, 133, 134, 179, 185, 188, 194, 195, 197]. Studies have shown that E_2 administration using implants requires lower amounts of hormones compared to dietary administration, even when administered to adults with differentiated gonads [200]. For example, in the common snook, juveniles provided with 50 mg E_2 kg^{-1} feed for 21 to 42 days (followed by 204 days of rearing), and adults implanted with 0.5 mg E_2 kg^{-1} BW for 120 days, both resulted in similar levels of feminisation (93 and 100% feminisation, respectively) [186, 194]. These findings in protandrous barramundi, common snook, gilthead seabream and bidirectional coral goby (*Gobiodon erythrospilus*) also support the conclusion that controlled release implants require comparatively lower amounts of E_2 to induce sex-change [42, 133, 134, 184, 187, 189, 201]. While low E_2 levels were shown effective for MTF sex-reversal in some hermaphrodites (e.g., the common snook, at 0.5 mg kg^{-1} BW), the low levels can also support testicular development and milt production, as observed in protandrous black porgy (at dosages under 1 mg kg^{-1} of feed) [136, 186]. This finding suggests that E_2 can play a dichotomous role and highlights the necessity of species-specific investigations to determine appropriate dosage for successful sex-reversal. Although E_2 acts as a key hormonal regulator during sex-change by promoting the development of female characteristics, and suppressing male characteristics, its administration often includes some critical drawbacks, including reversible sex-change [146]. For instance, transient sex-reversal and reversible sex-change were observed in 2-month-old black porgy 2–3 months after ending E_2 treatment, leading to the re-development of testicular tissue with spermatocytes, spermatids, and spermatozoa [146]. In contrast to this outcome, permanent sex-change has been reported in some protandrous species (e.g., common snook, Mexican snook, and barramundi) where functional feminisation was seen even after cessation of E_2 treatment for a prolonged period (6 months to 3 years) [138, 194, 195]. Given the variability in efficacy in controlling sex-change observed among species, species-specific studies to elucidate the stability of E_2 effects are necessary.

Studies in some gonochoristic fishes have demonstrated that 17α-ethynylestradiol (EE_2) has stronger feminising effects than E_2 [183, 202–204]. Both immersion and dietary administration of EE_2 have been shown to be effective at inducing feminisation in gonochoristic fish species when administered at their early developmental stage (e.g., chinook salmon, *Oncorhynchus tshawytscha*; rainbow trout; blue tilapia, *Oreochromis aureus*), but some variations in feminising effects are seen by species, dosage, and treatment duration [203, 205]. For instance, in rainbow trout and chinook salmon larvae, a 2-h immersion (400 μg EE_2 L^{-1}) treatment induced 94.5 to 100% feminisation, where 4- and 8-h immersions resulted in decreased survival and reduced feminisation rates [203, 206]. In rainbow trout, dietary administration required comparatively higher dosage (20 mg kg^{-1} feed) and longer treatment duration (30 days) than immersion, even when applied at the same developmental stage [206]. Although EE_2 induction demonstrates feminisation success in some gonochoristic fishes, studies on its use in hermaphroditic species have been limited [133, 199] (Table 2). Intriguingly, existing studies did not provide conclusive evidence that EE_2 successfully induces sex-change in hermaphroditic fishes. For example, unlike gonochoristic fishes, comparatively lower dosages of EE_2 in protandrous barramundi (5 to 10 mg kg^{-1} food) and in simultaneous mangrove rivulus (*Kryptelobias marmaratus*) (0.1 to 1.0 ppm in immersion) did not result in feminisation but instead resulted in phenotypically detrimental effects [133, 198]. This might be due to EE_2 acting paradoxically to downregulate expression of the gene *cyp19a1a* (through a negative feedback loop) resulting in decreased native E_2 plasma levels, or that the dosage and treatment duration of EE_2 were not appropriate relative to the species' developmental stage to induce sex-reversal [133, 198]. However, a recent study showed that short-term (28 days) and low-dosage (2.5 to 5 μg g^{-1} feed) treatment of EE_2 in protandrous gilthead seabream during its reproductive cycle prevented natural MTF sex-change but resulted in long-lasting disruptive effects [199]. Similarly, findings from another study demonstrated that dietary treatment of EE_2 (5 μg g^{-1} feed) during the spermatogenesis stage of gilthead seabream caused a reduction in sperm concentration [207]. Similar to E_2, secondary sex-reversal has also been observed in gonochoristic species (e.g., zebra fish, *Danio rerio*) after EE_2 treatment ceased, suggesting that EE_2 sex-changed fish may be able to revert back to their original sex [208]. This outcome contrasts with the findings observed in gilthead seabream, where short-term EE_2 exposure induced persistent alterations in gonads, even after long (146 days) recovery periods [199]. The differences in treatment effects and associated gaps in our understanding of EE_2 use highlight the importance of further studies in hermaphroditic species to determine the optimal balance between dosage and treatment duration for successful control over natural sex-change processes. Therefore, it is crucial to fully understand the underlying mechanism

TABLE 2 | Estrogenic agents used in hermaphroditic fishes for purposes of controlled sex-change (from 1994 to 2024).

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Females[f]	References
Protogynous bidirectional	*Gobiodon erythrospilus*	Coral goby	Adult male	E_2	Imp	1.4 mg fish^{-1}	NM	6 W	Induced male-to-female sex-change	[184]
			Adult female						Prevented female-to-male sex-change	
Protogynous	*Halichoeres poecilopterus*	Multicolour fin rainbowfish	Primary male	E_2	Imp	2.5 mg kg^{-1} BW	NM	51–63 D	66.67 (3)	[185]
						10 mg kg^{-1} BW			50 (4)	
Protogynous	*Halichoeres tenuispinis*	Wrasse	Primary male	E_2	Imp	2.5 mg kg^{-1} BW	NM	51–63 D	62.5 (8)	[185]
						10 mg kg^{-1} BW			80 (5)	
Protandrous	*Centropomus undecimalis*	Common snook	Adult male	E_2	Imp	0.5, 1.0, 4.0 and 8.0 mg kg^{-1} BW	3 YO	120 D	100 (7)	[186]
Protandrous	*Lates calcarifer*	Barramundi	Adult male	E_2	Imp	4 mg kg^{-1} BW	15 MO	9 W	44 (12)	[134]
						8 mg kg^{-1} BW			78 (12)	
Protandrous	*Centropomus undecimalis*	Common snook	Adult male	E_2	Imp	2 mg kg^{-1} BW	3 YO	90 D	100 (20)	[42]
Protandrous	*Centropomus undecimalis*	Common snook	Adult male	E_2	Imp	2 mg kg^{-1} BW	5.5 YO	90 D	100 (20)	[187]
Protandrous	*Centropomus viridis*	White snook	Adult male	E_2	Imp	0.5 mg kg^{-1} BW	512 DPH	120 D (implanted at day 0, 30, 60 and 90) + 150 D rest period	29 (14)	[188]
						1.5 mg kg^{-1} BW			87 (15)	
Protandrous	*Lates calcarifer*	Barramundi	Adult male	E_2	Imp	0.5 mg kg^{-1} BW	20 MO	400 D (implanted at day 0, 28 and 52)	80 (20)	[189]
						1.0 mg kg^{-1} BW			85 (20)	
						1.5 mg kg^{-1} BW			75 (20)	
Protandrous	*Acanthopagrus schlegeli*	Black porgy	Juvenile	E_2	D	4 mg kg^{-1} feed	1 YO	5 M	100 (15)	[190]

(Continues)

TABLE 2 | (Continued)

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Females[f]	References
Protandrous	*Acanthopagrus schlegeli*	Black porgy	Juvenile	E_2	D	4 mg kg^{-1} feed	1 YO	5 M	100 (8–10)	[136]
						1 mg kg^{-1} feed		5 M	Delayed spermatogenesis, increased spermiation	
						0.25 mg kg^{-1} feed		7 M	Delayed spermatogenesis, increased spermiation	
Protandrous	*Acanthopagrus schlegeli*	Black porgy	Adult male	E_2	D	4 mg kg^{-1} feed	2 YO	5 M	100 (8–10)	[135]
Protandrous	*Acanthopagrus schlegeli*	Black porgy	Adult male	E_2	D	6 mg kg^{-1} feed	2 YO	7 M	100 (8–16)	[191]
Protandrous	*Sparus aurata*	Sea bream	Juvenile	E_2	D	2 mg kg^{-1} feed	~1 YO	14 W	90 (4)	[192]
						15 mg kg^{-1} feed			20 (4)	
Protandrous	*Acanthopagrus schlegeli*	Black porgy	Fingerling	E_2	D	6 mg kg^{-1} feed	2 MO	5 M	100 (10)	[146]
Protandrous	*Acanthopagrus schlegeli*	Black porgy	Differentiated males	E_2	D	0.25 mg kg^{-1} feed	7 MO to 1 YO	6 M	Induced testicular development	[148]
						6 mg kg^{-1} feed			100 (20)	
Protandrous	*Centropomus undecimalis*	Common snook	Juvenile	E_2	D	50 mg kg^{-1} feed	NM	45 D	68.4 (10) with 5.26% intersex	[193]
						100 mg kg^{-1} feed			90 (10)	
Protandrous	*Centropomus undecimalis*	Common snook	Juvenile	E_2	D	50 mg kg^{-1} feed	NM	21, 28, 35 and 42 D + 204 D rearing	93–100 (20)	[194]
Protandrous	*Centropomus poeyi*	Mexican snook	Juvenile	E_2	D	50 and 60 mg kg^{-1} feed	340 DPH	60 D	100 (10)	[195]
						40 mg kg^{-1} feed	340 DPH		77.8 (10)	
						30 mg kg^{-1} feed	227 DPH		63 (20)	
						20 mg kg^{-1} feed	227 DPH		59 (20)	
						10 mg kg^{-1} feed	227 DPH		37 (20)	

(Continues)

TABLE 2 | (Continued)

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Females[f]	References
Protandrous	*Lates calcarifer*	Barramundi	Juvenile	E_2	D	10 mg kg^{-1} feed	30 DPH	130 D	33 (36)	[133]
						20 mg kg^{-1} feed			0 (36) Suppressed testicular development	
Protandrous	*Amphiprion biaculeatus*	Maroon clownfish	Non-breeder male	E_2	Inj	1.5 μg g^{-1} BW	60 DPH	90 D (injected at 1st, 3rd & 5th week of rearing)	100 (3)	[196]
Simultaneous	*Kryptelobias marmaratus*	Mangrove rivulus	Adult	E_2	Inj	1 & 100 μg g^{-1} BW	6 MO to 1 YO	30 D (injected once)	Inhibit ovarian development (24)	[197]
Simultaneous	*Kryptelobias marmaratus*	Mangrove rivulus	Fry	EE_2	Imm	0.1, 0.5, or 1.0 ng L^{-1}	Newly hatched	4 W	Impaired reproductive health	[198]
Protandrous	*Sparus aurata*	Gilthead seabream	Mature	EE_2	D	2.5 and 5 μg g^{-1} diet	2nd reproductive cycle	28 D	Prevent MTF sex-reversal	[199]
Protandrous	*Lates calcarifer*	Barramundi	Juvenile	EE_2	D	5 mg kg^{-1} feed	30 DPH	130 D	0 (36) Gonadal fibriosis	[133]
						10 mg kg^{-1} feed			0 (36) Gonadal fibriosis	

[a]Estrogenic agents used: E_2, 17β-estradiol; EE_2, 17α-ethynylestradiol.
[b]Route of administration: D, orally through diet; Imm, by immersion; Imp, via implant; Inj, via injection.
[c]Dosage: Expressed as mg kg^{-1} or μg g^{-1} feed when an estrogenic agent is administered through the diet; ng L^{-1} water during immersion; mg kg^{-1} BW via implantation; and μg g^{-1} BW via injection.
[d]Timing: Commencement of treatment is given as age in days post hatch (DPH), months old (MO), and years old (YO) when provided by authors. Otherwise, as specified by the authors, or not mentioned (NM) if not found in the study.
[e]Duration: Treatment period is given in days (D), weeks (W) or months (M).
[f]Percent: Percentage of resulting sex-changed females, otherwise as specified by the authors. Values in parentheses indicate the total number of treated individuals.

and long-term effects to achieve complete control over the sex-change process, particularly in hermaphroditic fishes.

5.1.2 | Estrogen Blockers: Shifting Gonadal Development Towards Males

Tamoxifen, an anti-estrogenic chemical agent, acts primarily by blocking estrogen receptor binding sites, thereby suppressing estrogen synthesis and disrupting normal female development [209–212]. Studies in some gonochoristic fishes (Nile tilapia, *Oreochromis niloticus*; bagrid catfish, *Pseudobagrus fulvidraco*; Japanese flounder, *Paralichthys olivaceus*; Japanese medaka, *Oryzias latipes*; and common carp, *Cyprinus carpio*) demonstrated a dosage-dependent masculinising effect of tamoxifen, where higher dosages during their labile period led to a greater percentage of males [212–215]. However, inconsistent results were also observed, with higher dosages resulting in the induction of intersex individuals and reduced survival in Nile tilapia, and a decrease in the fecundity and fertility of Japanese medaka [212, 216]. Moreover, tamoxifen demonstrated no masculinisation effect in all-female juvenile populations of rainbow trout (after 2 months of treatment with 2, 20 and 100 mg kg^{-1} feed) [217]. As a key consideration, the all-female populations used in this study were likely generated by mating neomales (XX males) with normal females, which may have influenced the effectiveness of treatment. However, the authors proposed that the physiological differences associated with being a cold-water species could be the potential reason for no sex-reversal effectiveness of tamoxifen in rainbow trout compared to other species [217]. The differences in effectiveness in sex-reversal could be due to species-specific characteristics that require further investigations. Although tamoxifen has proved its suitability in inducing masculinisation using immersion, dietary, and injection methods in several gonochoristic fishes, there have been no studies to date specifically conducted for hermaphrodite fishes with the purpose of controlling sex-change [211, 212, 217]. In adult protandrous gilthead seabream, however, García-Hernández et al. [207] conducted trials on the effects of dietary administration of tamoxifen on male reproductive function, finding that tamoxifen enhanced androgen production, proliferated testicular cells, and increased sperm quality and concentration, while downregulating *cyp19a1a* expression. Intriguingly, the estrogenic effects (e.g., the up-regulation of hepatic vitellogenin, estrogen receptor α, and G protein-coupled estrogen receptor genes, along with increased E_2 serum levels) exerted by tamoxifen in gilthead seabream suggest dichotomous roles in steroidogenic pathways [207]. While this study was not specifically trying to control the sex-change process in gilthead seabream, the contradictory results raise intriguing possibilities for use as a possible chemical compound to manipulate sex in this species. Further research should be undertaken to understand the utility of tamoxifen in sex-reversal programmes for hermaphroditic fishes, as observations in seabream may not be universally applicable to other hermaphroditic fish species.

5.2 | Testosterone Enhancers/Androgenic Agents

The roles of androgens in morphological differentiation of testis, spermatogenesis, and maintenance of male sex characteristics have been studied in a number of fish species [218–221] (Table 3). Application of androgens, particularly 17α-methyl testosterone (MT) and 11-ketotestosterone (11-KT), has been reported to be effective in producing male-dominated fish populations [132]. However, different androgens have distinct biological effects, and hence varying levels of effectiveness in promoting masculinisation and sex-reversal in fish. Understanding these differences is crucial for optimising hormone manipulation in hermaphroditic fish species.

5.2.1 | Aromatisable Androgens

Testosterone is a primary, natural, steroidal, and aromatisable male androgen that acts directly by binding to cell androgen receptors, or indirectly becomes converted into 5α-dihydrotestosterone (DHT) by 5α-reductase and/or estradiol by aromatase [252]. There have been a considerable number of studies exploring the use of testosterone for inducing sex-change in gonochoristic fishes using immersion, implantation, and injection treatments; however, there have been very few successful reports of achieving complete control over the sex-change process using this approach [253–255]. It has been suggested that paradoxical feminisation (i.e., feminisation instead of masculinisation after androgen treatment), where high-dosage or extended use of testosterone converts to E_2, shifts the sexatio in favour of females and reduces its efficacy in controlling sex-change [255, 256]. In contrast, in protogynous hermaphrodite fishes, a small number of early studies reported that testosterone can effectively induce sex-change (e.g., as in Mediterranean rainbow wrasse, *Coris julis*) [257, 258]. This effect has not been observed consistently, with different outcomes seen between species studied to date [223, 224, 259–262]. In some species, testosterone induced gonadal sex-reversal but did not induce the development of secondary sexual characters (e.g., slippery dick wrasse, *Halichoeres bivittatus* and Caribbean wrasse, *H. garnoti*), while in others, secondary sexual characteristics (e.g., colouration) develop without sex-change occurring [259–261]. Moreover, the administration of testosterone, either in the specific form of injections or pellet implants, did not induce protogynous sex-change in bluehead wrasse or rice-field eel [102, 223, 224, 262] (Table 3). Likewise, testosterone implants in two-year-old protogynous black porgy did not prevent natural FTM sex-change [30]. Despite observing that testosterone prolongs the duration of spermiation and increases milt volume in some gonochoristic and hermaphrodite fishes, its direct application for sex-reversal in fish in recent years is limited [222, 263]. Use of testosterone is often avoided due to the risk of paradoxical feminisation and the development of other alternate androgens that are more effective and have higher potency.

17α-methyltestosterone (MT) is a synthetic aromatisable androgen and acts to activate androgen receptors and male-biased gene pathways [264, 265]. Upon activation, it suppresses the expression of the female-promoting aromatase gene, inhibits ovarian function and endometrial growth, and stimulates male sexual development [264–266]. Since the late 1950s, MT has been used to successfully induce sex-change in a number of gonochoristic fishes and control sex-change in hermaphroditic species, with notable success in protogynous hermaphrodites, particularly within the genus *Epinephelus* [50, 52, 56, 131, 145, 226, 237, 267–277] (Table 3). The efficacy

TABLE 3 | Androgenic hormones (testosterone enhancers) used in hermaphroditic fishes for purposes of controlled sex-change (from 1974 to 2025).

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Aromatisable androgens										
Protandrous	*Acanthopagrus schlegeli*	Black porgy	Juvenile male	T	D	0.5 & 4 mg kg^{-1} feed	8 MO	7 M	Increased testicular function	[222]
Protogynous	*Monopterus albus*	Rice-field eel	Adult female	T	Inj	8 μg g^{-1} fish $injection^{-1}$	Breeding season	8 W (injected on alternate days)	No sex-reversal	[223]
Protogynous	*Thalassoma bifasciatum*	Bluehead wrasse	Adults mostly with ovarian tissue	T	Imp	Pellet weight: 7.2 ± 0.6 mg Release rate: 28.0 μg $24\,h^{-1}$	Yellow colouration phase	21 D 40 D	Blue coloration phase and regressed oocytes	[224]
Protogynous	*Mycteroperca microlepis*	Gag grouper	Adult	17α-MT	D	1 mg kg^{-1} BW	NM	150 D	Transitional ovotestis/early male phase	[225]
Protogynous	*Epinephelus fario*	Blue-spotted grouper	Juvenile	17α-MT	D	0.5 mg kg^{-1} feed 1 mg kg^{-1} feed	2 YO	5 M	100 (18) 100 (18)	[226]
Protogynous	*Epinephelus marginatus*	Dusky grouper	Young and female	17α-MT	D	5 mg kg^{-1} feed	1+ to 4+ YO	2.5 M	100 (6)	[227]
Protogynous	*Epinephelus coioides*	Orange-Spotted Grouper	Juvenile	17α-MT	D	50 mg kg^{-1} feed	2 YO	60 D	100 (20)	[228]
Protogynous	*Epinephelus marginatus*	Dusky grouper	Juvenile	17α-MT	D Inj	1 mg kg^{-1} BW (daily 5 days a week) 5 mg kg^{-1} BW (weekly in a single dose)	NM	180 D	100 (9) 77.8 (9)	[229]
Protogynous	*Epinephelus malabaricus*	Malabar Grouper	Immature	17α-MT	D	50 μg g^{-1} feed	120 DPH	7 M	80 (10)	[147]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Juvenile	17α-MT	D	10 mg kg^{-1} feed	1.5 YO	96 D	100 (30)	[230]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Fry	17α-MT	D	10 mg kg^{-1} feed	90 DPH	96 D	100 (5)	[231]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Fry	17α-MT	D	5 mg kg^{-1} feed	90 DPH	90 D	Induced testicular differentiation	[232]

(Continues)

TABLE 3 | (Continued)

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Juvenile and mature	17α-MT	D	10 mg kg^{-1} feed	6 MO to 3 YO	150 D	100 (30)	[233]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Juvenile	17α-MT	D	10 mg kg^{-1} feed	90 DPH	90 D	100 (25)	[233]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Juvenile	17α-MT	D	30 mg kg^{-1} feed	7 MO	3 M	100 (50)	[234]
			Immature		Imp	20 mg kg^{-1} BW	2–3 YO	4 W	100 (10)	
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Juvenile	17α-MT	D	30 mg kg^{-1} feed	<2.5 YO	3 M	100 (4)	[235]
Protogynous	*Epinephelus coioides*	Orange-Spotted Grouper	Immature	17α-MT	D	50 mg kg^{-1} feed	4 MO	3 M	100 (with immature spermatogenesis)	[236]
Protogynous	*Epinephelus suillus*	Orange-spotted rock cod	Juvenile	17α-MT	Inj	0.5 mg kg^{-1} BW	2 YO	6 M (injected every 15 days)	Spermiation	[237]
						1 mg kg^{-1} BW			Spermiation	
						5 mg kg^{-1} BW			Testis with active spermatogenesis but no spermiation	
Protogynous	*Epinephelus bruneus*	Longtooth grouper	Juvenile	17α-MT	Inj	5 mg kg^{-1} BW	NM	21 W	Induced spermatogenesis	[238]
Protogynous	*Epinephelus marginatus*	Dusky grouper	Juvenile	17α-MT	Inj	15 mg kg^{-1} BW	NM	90 D (injected at 0, 30 and 60 days)	100 (12)	[239]
Protogynous	*Epinephelus akaara*	Red Spotted Grouper	Juvenile	17α-MT	Imm	1 mg L^{-1} (Once weekly)	70 DPH	4 W	0	[240]
								8 W	0	
Protogynous	*Epinephelus akaara*	Red Spotted Grouper	Juvenile	17α-MT	Imm	5 mg L^{-1}	70 DPH	4 W	0	[240]
								8 W	100	
Simultaneous	*Kryptolebias marmoratus*	Mangrove killifish	Embryo	17α-MT	Imm	0.025 μg ml^{-1}	12 DPF	10 D	97 (63)	[154]
Protogynous	*Epinephelus aeneus*	White grouper	Adult	17α-MT	Imp	23 mg crystalline 17α-MT/pellet	NM	5–17 M	100 (3) but one turned back into female	[51]

(Continues)

TABLE 3 | (Continued)

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Protogynous	*Epinephelus coioides*	Grouper	Adult	17α-MT	Imp	1.0 mg kg^{-1} BW	Post-spawning season	90 D	85.7 (7)	[131]
				Testosterone mixture (T + MT + TP)		1.0 mg kg^{-1} BW		90 D	85.7 (7)	
Protogynous	*Epinephelus coioides*	Grouper	Maturing	Testosterone mixture (T + MT + TP)	Imp	1.0 mg kg^{-1} BW	2 YO	90 D	85.7 (7)	[50]
						10.0 mg kg^{-1} BW		90 D	100 (7)	
Protogynous	*Epinephelus akaara*	Red-spotted grouper	Adult	17α-MT	Imp	10.0 mg kg^{-1} BW	NM	30 D	100 (5)	[52]
Protogynous	*Epinephelus marginatus*	Dusky grouper	Juvenile	17α-MT	Imp	~11 mg kg^{-1} BW (once a month)	21 MO	12 W	100 (5)	[241]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Adult	17α-MT	Imp	NM	3 YO	4 W	100 (20)	[242]
Protogynous	*Epinephelus coioides*	Orange-Spotted Grouper	NM	17α-MT	Imp	10 mg kg^{-1} BW	3 YO	4 W	100 (5)	[243]
Protogynous	*Epinephelus coioides*	Orange-Spotted Grouper	Juvenile	17α-MT	Imp	10 mg kg^{-1} BW	2 YO	6 W	100 (8)	[244]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Adult	17α-MT	Imp	10 mg kg^{-1} BW	3 YO	4 W	100 (5)	[245]
Protogynous	*Epinephelus bruneus*	Longtooth grouper	Juvenile	17α-MT	Imp	2 mg kg^{-1} BW	NM	14 W	64 (14)	[246]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Juvenile	17α-MT	Imp	10 mg kg^{-1} BW	NM	6 W	100 (40)	[247]
Protogynous	*Epinephelus coioides*	Orange-Spotted Grouper	Juvenile	17α-MT	Imp	20 mg kg^{-1} BW	7 MO	30 D	100 (6)	[149]
Protogynous	*Epinephelus coioides*	Orange-Spotted Grouper	Juvenile	17α-MT	Imp	20 mg kg^{-1} BW	> 2.5 YO	4 W	100 (18)	[150]
Protogynous	*Epinephelus aeneus*	White grouper	Juvenile	17α-MT	Imp	10 mg kg^{-1} BW	NM	4 M (monthly)	100 (6)	[54]
Protogynous	*Epinephelus coioides*	Orange-Spotted Grouper	Juvenile	17α-MT	Imp	10 mg kg^{-1} BW	NM	3 W	100 (5)	[248]
Protogynous	*Epinephelus bruneus*	Longtooth grouper	Juvenile	17α-MT	Imp	2 mg kg^{-1} BW	2 YO	80 D	100 (16)	[249]
						5 mg kg^{-1} BW			100 (19)	

(Continues)

TABLE 3 | (Continued)

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Protogynous	*Epinephelus bruneus*	Longtooth grouper	Juvenile	17α-MT	Imp	2 mg kg^{-1} BW	1 YO	1–2 M	100 (16)	[250]
						10 mg kg^{-1} BW			100 (15)	
Protogynous	*Epinephelus bruneus*	Longtooth grouper	Juvenile	17α-MT	Imp	2 mg kg^{-1} BW	12 MO	2 M	100 (16)	[250]
						10 mg kg^{-1} BW			100 (15)	
Protogynous	*Plectropomus leopardus*	Leopard coral grouper	Adult	17α-MT	Imp	1 mg kg^{-1} BW	4 YO	3 M	62.5 transitional gonad (8)	[251]
						5 mg kg^{-1} BW			87.5 transitional gonad (8)	
Non-Aromatisable androgens										
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	11-KT	D	150 mg kg^{-1} feed	NM	6 W	100 (9)	[128]
Protogynous	*Sparisoma viride*	Stoplight Parrotfish	Adult	11-KT	Inj	5 μg g^{-1} BW	NM	16 D (one-time injection)	60 (5)	[127]
Protogynous	*Coryphopterus nicholsii*	Blackeye Goby	Adult	11-KT	Imp	0.26 mg fish^{-1}	Non-breeding season	43 D	100 (4)	[129]
				11-KA		0.3 mg fish^{-1}		42 D	90 (10)	
Protogynous	*Epinephelus coioides*	Grouper	Adult	11-KT	Imp	1.0 mg kg^{-1} BW	Post-spawning season	90 D	0 (7)	[131]
Protogynous	*Epinephelus merra*	Honeycomb Grouper	Adult	11-KT	Imp	10 mg kg^{-1} BW	Pre-spawning season	75 D	100 (12)	[126]
Protogynous	*Pseudolabrus sieboldi*	Wrasse	Adult	11-KT	Imp	500 nmol (per capsule)	Breeding season Non-breeding season	40 D	Secondary testes or inter-sexual gonads (8)	[130]
Protogynous	*Epinephelus akaara*	Red-spotted grouper	Adult	17α-MDHT	Imp	10.0 mg kg^{-1} BW	NM	30 D	100 (5)	[52]

[a]Testosterone enhancers used: 11-KT, 11-Ketotestosterone; 11-KA, 11-ketoadrenosterone; 17α-MT, 17α-Methyltestosterone; 17α-MDHT, 17α-Methyldihydrotestosterone; T + MT + TP, testosterone (T) + 17α-methyltestosterone (MT) + testosterone propionate (TP).
[b]Route of administration: D, orally through diet; Imp, via implantation; Inj, via injection; Imm, through immersion.
[c]Dosage: Expressed as mg kg^{-1} or μg g^{-1} feed when administered through the diet; mg fish^{-1}, mg kg^{-1} BW or dosage (nmol) per capsule via implantation; μg g^{-1} fish or mg kg^{-1} BW via injection; and μg ml^{-1} or mg ml^{-1} in water during immersion.
[d]Timing: Commencement of treatment is expressed as age in days post hatch (DPH) or days post fertilization (DPF), months old (MO), and years old (YO). Seasonal timing (e.g., pre-spawning, post-spawning, breeding and non-breeding season) and colouration phase (e.g., yellow colouration phase) are provided when specified by the authors. Otherwise, it is indicated as 'Not Mentioned' (NM).
[e]Duration: Treatment duration is given as in days (D), weeks (W), or months (M).
[f]Percent: Percentage of resulting sex-changed males, otherwise as specified by the authors. Values in parentheses indicate the total number of treated individuals.

of MT treatment varies significantly depending on factors such as dosage, duration, and the developmental stage of the fish. While MT can effectively masculinise mature female grouper, inducing sex-reversal in immature individuals has been more challenging due to variation in treatment efficacy, requiring a longer treatment window [131, 150, 230]. In dusky grouper (*Epinephelus marginatus*) and red-spotted grouper, MT-induced sex-change was found to be permanent when MT is applied to juveniles, with sex-changed individuals completing spermatogenesis, and sustaining reproductive function and spawning capability [227, 240, 241]. However, in some cases, short-term MT treatments in juveniles or immature female groupers (e.g., orange-spotted grouper; malabar grouper, *Epinephelus malabaricus*) only induced temporary sex-change, with individuals reverting again to female phenotypes shortly after treatment ceased [147, 150]. Furthermore, complete testicular development and functional male characteristics were not achieved in all individuals of juvenile blue-spotted groupers, *Epinephelus fario* and longtooth groupers, *Epinephelus bruneus* treated with MT [226, 250]. These findings highlight the importance of considering longer treatment duration for these species if precocious sex-change is necessary during their early (juvenile or immature) stages of development. Likewise, MT treatment efficacy also varies depending on the method of administration. While both implantation and dietary administration of MT have been proven to be successful, dietary administration may require longer treatment durations (e.g., 8–28 weeks) to achieve results comparable to implantation (e.g., 3 weeks to 12 weeks), as observed in studies on orange-spotted grouper [50, 131, 147, 150, 226, 228–230, 233, 234, 241–243, 248]. Moreover, in several grouper species, MT implantation can induce masculinisation at a rate similar to that for dietary treatment, irrespective of developmental stages. Importantly, implantation achieves these comparable outcomes with comparatively low dosages [147, 228, 234, 235, 243–247, 249, 250]. Apart from implantation and dietary administration, dose-specific sex-reversal efficacy was also reported when fishes were treated with intramuscular injections. In juvenile mud grouper, *Epinephelus suillus* (Valenciennes), although lower injection dosage (0.5–1.0 mg kg^{-1} BW) induced sex-change, spermatogenesis was accelerated and maintained when MT was administered at higher dosage (5 mg kg^{-1} BW) with prolonged period [237]. Likewise, high MT injection dosages (5–15 mg kg^{-1} BW) with prolonged treatment duration accelerated spermatogenesis in several grouper species, demonstrating the importance of considering both fish developmental stage and treatment parameters (dosage and duration) for successful sex-reversal [237–239]. While high dosages and prolonged treatment durations may be necessary to induce complete and stable sex-reversal in some cases [226, 237, 241], this approach can also have unintended consequences, including malformation or agenesis of the sperm duct system, and inhibition of spermiation [253]. Moreover, like testosterone, MT can be converted by aromatase to E_2 resulting in paradoxical feminisation, thereby reducing its utility in an aquaculture setting [91, 147, 150, 256, 278].

5.2.2 | Non-Aromatisable Androgens

In recent years, non-aromatisable synthetic androgens have gained prominence as preferred chemical compounds for ensuring a consistent and predictable masculinising effect in sex control of fishes [279]. 11-ketotestosterone (11-KT) is a potent naturally occurring non-aromatisable androgen that, unlike testosterone and MT, cannot be converted by aromatase to estrogen [280, 281]. 11-KT treatment has been found to successfully induce sex-change in various teleost fish species, particularly in protogynous hermaphrodites, including adult female three-spot wrasse (*Halichoeres trimaculatus*), blackeye goby (*Coryphopterus nicholsii*), and honeycomb grouper (*Epinephelus merra*) using dietary and implantation approaches [126, 128–130] (Table 3). However, the masculinisation efficacy of 11KT was found to vary with treatment dosage, treatment duration, species, and developmental stage [126, 128, 129]. For instance, use of a high dose and short treatment duration (150 mg kg^{-1} dietary 11-KT for 6 weeks) successfully induced sex-reversal in adult female three-spot wrasse, but use of a lower dose implant with comparatively longer treatment duration (1 mg kg^{-1} BW 11-KT for 90 days) failed to induce sex-change in mature female orange-spotted grouper during the post-spawning stage [128, 131]. Likewise, in adult female wrasse, studies have found dietary administration of 11KT required comparatively high dosage and duration of treatment compared to use of implants [128, 130]. These findings highlight the potential of 11-KT as a valuable tool for sex control in aquaculture; however, treatment conditions need to be optimised carefully for the target species. Furthermore, while 11-KT application induced sex-reversal in many protogynous species, there have been no long-term studies on the stability of 11-KT induced sex-change. Notably, in various protogynous species, 11-KT treatment (by either implantation or injection) also induced rapid changes in androgen-dependent secondary sexual characteristics, such as coloration and genital papilla development [127, 282, 283]. It is, therefore, necessary to gain further understanding of the effect of 11-KT on other key male characteristics such as sperm quality and spawning performance within sex-reversed fish to confirm their utility in commercial breeding programmes.

17α-methyldihydrotestosterone (MDHT) is a synthetic, methylated form of 5α-dihydrotestosterone (DHT) that, due to its 5α-reduction, cannot be converted to E_2 via aromatase [52]. Due to this, MDHT has commonly been found to induce a higher proportion of sex-reversed males than treatment with MT or aromatase inhibitors (e.g., fadrozole, FAD) in a number of gonochoristic and hermaphroditic fish species including Atlantic halibut (*Hippoglossus hippoglossus*), rainbow trout, brook trout (*Salvelinus fontinalis*), Nile tilapia, red-spotted grouper, and European sea bass (*Dicentrarchus labrax*) [52, 146, 279, 284–290]. Studies in some gonochoristic fishes (e.g., European sea bass) have shown that lower-dosage MDHT treatment can achieve a complete masculinisation effect compared to MT or aromatase inhibitors like FAD [74, 287]. In contrast, findings in other fishes (e.g., Atlantic cod, *Gadus morhua*) have demonstrated that MDHT has dosage-dependent effects, with higher concentrations resulting in a greater proportion of males, suggesting its species-specific effect [291]. To date, while studies on MDHT-induced sex-change are scarce in hermaphrodite fishes (Table 3), the results demonstrated promising levels of success, highlighting strong potential for increased control over the sex-change process [52]. For instance, in a study of protogynous red-spotted grouper, mature females implanted with MDHT

exhibited a higher percentage late-phase males than MT and MT-FAD treatments [52]. This suggests that MDHT is capable of controlling the sex-ratio in hermaphroditic fishes even when individuals have fully differentiated gonadal phenotypes. While existing studies in gonochoristic fishes showed that individuals with immature or undifferentiated gonads, either immersed in or fed with MDHT, result in permanent masculinisation, studies into MDHT effects on immature gonads in hermaphrodite fishes deserve greater attention because this area remains largely unexplored [292]. Although MDHT is a non-aromatisable synthetic androgen, paradoxical feminisation has unexpectedly also been reported in the channel catfish (*Ictalurus punctatus*) following MDHT exposure [293]. Although the authors suggested that androgen aromatisation may not be necessary for paradoxical feminisation in channel catfish, this would mean that the paradoxical mechanism in this species does not involve the androgen aromatisation to estrogen [293]. The underlying mechanism of this counterintuitive result remains unclear and has not been reported to date in other species [74, 287, 291–294]. Given the significant success of MDHT in inducing sex-change in gonochoristic species, robust research is needed to explore its potential for controlling sex-change in hermaphroditic fishes, particularly those species that are economically important [292].

5.3 | Aromatase Inhibitors (AIs)

Aromatase inhibitors (AIs) inhibit estrogen (mainly E_2) biosynthesis by binding reversibly or irreversibly to the aromatase enzyme, leading to reduced estrogen production and masculinisation in many teleosts [295, 296] (Table 4). Although AIs are classified into their generations (i.e., first, second and third generation), they can also be categorised as Type I and Type II based on their structures [309]. Type I have a steroidal structure, similar to androgens, and permanently inactivate aromatase by irreversibly blocking the substrate-binding site where the conversion of androgens to estrogens normally occurs (e.g., formestane, and exemestane) [309–311]. Type II are non-steroidal and are reversible, temporarily blocking the conversion of androgens to estrogens, allowing for finer scale control over the duration of estrogen suppression (e.g., FAD, anastrozole, and letrozole (LET)) [309, 310] (Figure 5). For sex manipulation programmes, the development of non-steroidal methods is of strategic value to the aquaculture industry, as there is low social acceptance of the use of steroid hormones in animal production [312]. Therefore, the use of non-steroidal AIs in particular is recognised as a viable alternate approach to obtain sex control. However, in this review, a brief overview of both steroidal and non-steroidal AIs is provided.

5.3.1 | Irreversible Steroidal AIs

Formestane (also known as 4-androsten-4-ol-3, 17-dione, 4-hydroxyandrostenedione or 4-OHA) is a second-generation steroidal and irreversible aromatase inhibitor, known to significantly reduce plasma levels of estrogen in humans [313, 314]. While formestane has demonstrated its capability in promoting male sexual characters in the female dogwhelk snail (*Nucella lapillus*) and in inducing sex-change in ZW/ZZ-type Japanese wrinkled frogs (*Rana rugosa*), there has been no study on its application to fishes to date [315–317]. This may be because formestane must be administered through deep intramuscular injection, which has been linked to increased injection-site reactions, making it less practical than other aromatase inhibitors [318, 319].

Exemestane, a third-generation AI, also functions as an irreversible steroidal inhibitor (or inactivator), binding to the substrate-binding pocket of the aromatase enzyme, leading to its degradation [320]. Exemestane has shown efficacy in masculinising both gonochoristic and protogynous fish species at a number of different life stages. Complete masculinisation, for example, in Nile tilapia, was achieved with exemestane administered during the labile period (the critical period for sex differentiation) [316], while in adult female black sea bass (*Centropristis striata*) and three-spot wrasse, short-term dietary exposure to exemestane ($1\,mg\,g^{-1}$ diet for 2–4 weeks) successfully induced sex-change [143, 144, 298, 300] (Table 4). Intriguingly, Chen et al. [139] reported an anti-masculinisation effect of exemestane in adult female zebrafish, but not in juveniles, suggesting that the species has lower sex plasticity in adults than juveniles. Conversely, Rahaman et al. [321] claimed that sex plasticity remains in the mature ovaries of zebrafish, whereby intraperitoneal injection of exemestane induced sex-reversed male individuals within 3 months that were able to spawn effectively to produce viable offspring. This result suggests that a comparatively longer treatment duration is required for adult gonochoristic fishes rather than for juveniles to achieve complete sex-control. However, exemestane may also have a negative effect upon male reproductive function, as observed in three-spot wrasse, where both low and high exemestane dosages (0.002 and $0.2\,mg\,g^{-1}$ diet) negatively impacted spermatogenesis [297, 299]. While, like other AIs, both dosages of exemestane used resulted in increased 11-KT and decreased E_2 production, the findings also indicated that 11-KT is not the only hormone for spermatogenesis in three-spot wrasse [297, 299]. The findings indicate that exemestane-induced suppression of estrogen synthesis may have negatively impacted spermatogonial proliferation in adult wrasse, with low levels of estrogen needed for the activation of spermatogenesis and renewal of spermatogonia [297, 322]. These findings suggest that male reproductive function for some species may be more sensitive to exemestane use and that its application requires careful consideration while inducing bidirectional sex-change [143, 298, 316]. Although a dosage-dependent effect of exemestane is not generally reported [139, 143, 298, 316, 321], López et al. [323] found a dosage-dependent effect, whereby higher dietary dosage ($100\,mg\,kg^{-1}$) led to a higher proportion of males in red tilapia (*Oreochromis* spp.) larvae than the low dosage ($25\,mg\,kg^{-1}$). While exemestane's efficacy in sex-change control is evident in gonochoristic fishes through direct inhibition of aromatisation, its application in hermaphroditic species presents a more complex scenario that requires further studies, particularly to fully elucidate its role in maintaining functional masculinity.

5.3.2 | Reversible Non-Steroidal AIs

Aminoglutethimide (AGT, 3-[p-aminophenyl]-3-ethyl-piperidine-2,6-dione), a first-generation non-steroidal AI, was used to treat estrogen-dependent breast cancer five decades ago

TABLE 4 | Aromatase inhibitors (AIs) used in hermaphroditic fishes for purposes of controlled sex-change (from 2000 to 2025).

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Irreversible steroidal AIs										
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	Exemestane	D	2 μg g^{-1} feed 200 μg g^{-1} feed	Initial phase males	10 W	Decreased spermatogonial proliferation	[297]
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	Exemestane	D	1 mg g^{-1} feed	NM	30 D	Sex-changed in most individuals involving different stages	[298]
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	Exemestane	D	2 μg g^{-1} feed 200 μg g^{-1} feed	Initial phase males	10 W	Decrease in spermatogonia and spermatocytes	[299]
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	Exemestane	D	1000 μg g^{-1} feed	NM	2 W	Different stages of male development (64)	[300]
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	Exemestane	D	1000 μg g^{-1} feed	NM	30 D (replicated twice)	87.5 (40) 93.33 (30)	[144]
Protogynous	*Centropristis striata*	Black sea bass	Adult	Exemestane	D	1 mg g^{-1} feed	~1–2 YO	30 D	Early sex-change	[143]
Reversible non-steroidal AIs										
Protandrous	*Acanthopagrus schlegeli*	Black porgy	Adult	FAD and 1,4,6-androstatriene-3,17-dione	D	Each 10 mg kg^{-1} feed	2 YO	8.5 M	100 (21)	[145]
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	FAD	D	100 mg kg^{-1} feed	NM	6 W	100 (6)	[128]
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	FAD	D	500 μg g^{-1} feed	NM	3 D 5 D 10 D	0 (6) 100 (8) 100 (5) Data after 30 D of treatment starts	[301]
Protogynous	*Halichoeres trimaculatus*	Three-spot wrasse	Adult	FAD	D	200 μg g^{-1} feed	NM	2 W	100 (28)	[302]
Protogynous	*Epinephelus bruneus*	Longtooth grouper	Juvenile	FAD	Inj	3 mg kg^{-1} BW 5 mg kg^{-1} BW	NM	21 W (one-time injection)	Gonads contained spermatogonia and spermatocytes (20) Induced advanced stages of spermatogenesis (20)	[238]

(Continues)

TABLE 4 | (Continued)

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Protogynous	*Coryphopterus nicholsii*	Blackeye Goby	Adult	FAD	Imp	0.1 mg fish^{-1}	Non-breeding season	43 D	14.28 (7)	[129]
						1 mg fish^{-1}		42 D	42.85 (7)	
						5 mg fish^{-1}		43 D	83.33 (8)	
Protogynous	*Epinephelus merra*	Honeycomb Grouper	Maturing	FAD	Imp	1 mg kg^{-1} BW	Pre-spawning season	2.5 M	100 (3)	[303]
						10 mg kg^{-1} BW			100 (15)	
Protogynous	*Epinephelus merra*	Honeycomb Grouper	Immature female	FAD	Imp	100 μg fish^{-1}	Non-breeding season	3 M	Transitional with intersex gonad (8)	[216]
						500 μg fish^{-1}				
						1000 μg fish^{-1}			Transitional and male phases (8)	
Protogynous	*Epinephelus merra*	Honeycomb Grouper	Adult	FAD	Imp	10 mg kg^{-1} BW	Pre-spawning season	75 D	100 (15)	[304]
Protogynous	*Epinephelus merra*	Honeycomb Grouper	Adult	FAD	Imp	1 mg fish^{-1}	Breeding season	3 W	60 (10)	[305]
Protogynous	*Epinephelus aeneus*	White grouper	Juvenile	FAD	Imp	1 mg kg^{-1} BW	NM	4 M (monthly)	Transitional phase	[54]
						3 mg kg^{-1} BW			Testicular differentiation	
Protandrous	*Lates calcarifer*	Barramundi	Adult	FAD	Imp	8 mg kg^{-1} BW	15 MO	9 W	100 (12)	[134]
Protogynous	*Epinephelus coioides*	Orange-spotted grouper	Fry	LET	D	5 mg kg^{-1} feed	70 DPH	110 D	Gonad development seized at the primitive gonad stage	[232]
						100 mg kg^{-1} feed			Female-to-male sex-reversal	
Protogynous	*Monopterus albus*	Rice-field eel	Juvenile	LET	D	300 mg kg^{-1} feed	2 MO	4 M	100 (5)	[306]
Protogynous	*Epinephelus marginatus*	Dusky grouper	Adult	LET	Inj	100 mg kg^{-1} BW	Breeding season (spring)	9 W (injected every 4 weeks until semen detection)	Induced spermiating individuals able to fertilise oocytes	[307]
							End of breeding season (summer)		Incomplete sex-change with intersex individuals	

(Continues)

TABLE 4 | (Continued)

Hermaphrodite type	Species	Common name	Life stage	Hormone name[a]	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Protogynous	*Epinephelus marginatus*	Dusky grouper	Juvenile	LET	Inj	100 mg kg^{-1} BW	NM	90 D (injected at 0, 30 and 60 days)	100 (12)	[239]
Protogynous	*Epinephelus akaara*	Red spotted grouper	Adult	LET	Imp	5 mg kg^{-1} BW	24 MO	8 W	100 (30)	[308]
Protogynous	*Plectropomus leopardus*	Leopard coral grouper	Adult	LET	Imp	1 mg kg^{-1} BW $month^{-1}$	4 YO	3 M	100 (7)	[251]
						5 mg kg^{-1} BW $month^{-1}$			100 (8)	

[a]Aromatase inhibitors used: Exemestane; FAD, fadrozole; and LET, letrozole.
[b]Route of administration: D, orally through diet; Imp, via implantation; and Inj, via injection.
[c]Dosage: Expressed as μg g^{-1}, mg kg^{-1} feed when administered through the diet; μg $fish^{-1}$, mg $fish.^{-1}$ or mg kg^{-1} BW via implantation; and mg kg^{-1} BW via injection.
[d]Timing: Commencement of treatment is given as: age in days post hatch (dph), months old (MO), and years old (YO). Seasonal timing (e.g., pre-spawning, breeding, or non-breeding season) and phases of development (e.g., initial male phase) are provided when specified by the authors. Otherwise as not mentioned (NM) if not found in the study.
[e]Duration: Period of AI treatment is given in days (D), weeks (W) or months (M).
[f]Percent: Percentage of resulting sex-changed males, otherwise as specified by the authors. Values in parentheses indicate the total number of treated individuals.

[324, 325]. AGT has had only limited application in fish sex-reversal due to its relatively low potency [326]. Studies have demonstrated either minimal or no inhibitory effects of AGT on ovarian aromatase activity in fishes (e.g., goldfish, *Carrasius auratus*; rainbow trout; and Atlantic salmon, *Salmo salar*) and amphibians (e.g., urodele newt, *Pleurodeles waltl*), indicating its lower efficacy compared to more potent aromatase inhibitors currently employed in sex-reversal programmes [312, 327–329].

Fadrozole (FAD) is a potent, non-steroidal, and selective second-generation AI that reversibly binds to aromatase's iron-porphyrin nucleus, inhibiting oxygen binding and reversibly inactivating the enzyme [296, 330]. FAD has shown high potency, causing masculinisation in many gonochoristic fishes, including peacock blenny (*Salaria pavo*), Nile tilapia, zebrafish, golden rabbitfish (*Siganus guttatus*), common carp, and Japanese flounder [210, 272, 331–335]. In addition to gonochoristic fishes, FAD has demonstrated efficacy in controlling male-biased sex-ratios in several protogynous hermaphrodites through dietary administration, implantation, or injection, despite having some dosing related variation in efficacy among administration routes [54, 128, 238, 301–305] (Table 4). Studies have shown that dietary FAD administration typically requires higher dosages (10–500 g/kg feed) than implantation or injection methods (0.1–10 mg kg^{-1} BW) [54, 128, 129, 216, 238, 301, 303–305]. While effective masculinisation or preventing sex-change through dietary and implantation methods often requires treatment durations ranging from 1 to 12 weeks, injection treatments may require longer treatment periods (e.g., 21 weeks) [128, 129, 216, 238, 301–304]. In addition to inducing FTM sex-change in protogynous fishes, recent studies have utilised FAD to maintain testicular development in protandrous fishes (e.g., barramundi, black porgy) and prevent MTF sex-change from occurring [32, 145]. In protogynous fishes, dosage-dependent effects of FAD on masculinisation across different species and developmental stages have been demonstrated [129, 216, 238]. For example, in immature honeycomb grouper, higher FAD dosages were necessary to achieve complete masculinisation, while lower dosages resulted in the development of intersex individuals with early transitional gonads with atretic perinucleolar oocytes and sparse peripheral spermatogonial proliferation [216]. Similarly, in juvenile protogynous longtooth grouper and gonochoristic golden rabbitfish larvae, increased doses of FAD led to more advanced stages of spermatogenesis, while lower dosages resulted in incomplete masculinisation [238, 334]. In addition to early developmental stages, dosage optimisation is also crucial in mature individuals. Kroon & Liley [129] reported dosage-dependent masculinisation success in mature blackeye goby, where the effect of FAD increased with increasing concentration (from 0.1 to 5 mg $fish^{-1}$), with complete sex-change in some individuals at the highest dosage. Intriguingly, in mature red-spotted grouper, higher implantation dosages (1.0 and 10 mg kg^{-1} BW) significantly increased the proportion of males compared to a lower concentration (0.1 mg kg^{-1} BW), but the highest dosage (10 mg kg^{-1} BW) resulted in some individuals remaining female [53]. These findings highlight the importance of carefully adjusting FAD dosage based on species, age, and desired outcome to maximise masculinisation success, while minimising potential side effects and ensuring optimal reproductive function in the resulting males. Apart from the dosage-dependent effects, FAD's effect on masculinisation often relates to treatment time, as observed in

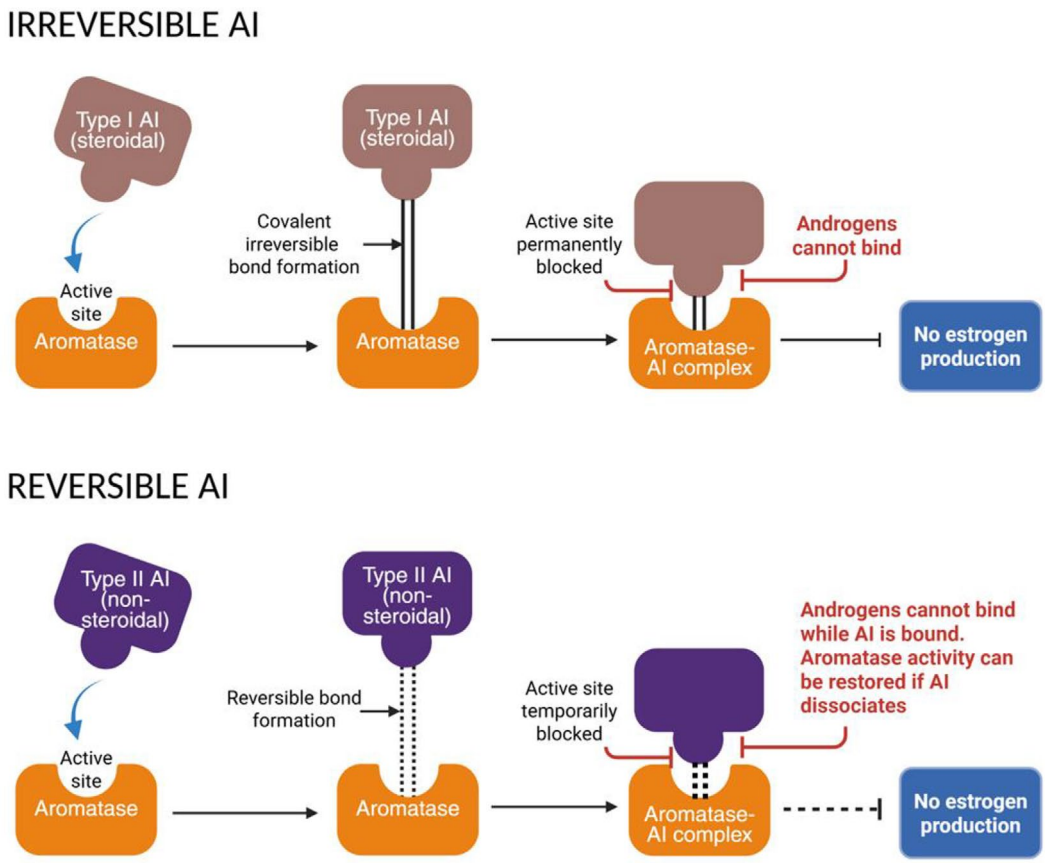

FIGURE 5 | Schematic pathways of irreversible and reversible aromatase inhibitors inhibiting estrogen synthesis. Solid lines represent strong binding between AI and aromatase whereas dotted lines depict a transient bond. A solid inhibitory line represents an irreversible blocked pathway, whereas a dotted inhibitory line indicates a reversible blocked pathway. Figure based on Mokbel [309].

protogynous adult female three-spot wrasse [301]. In this species, a shorter treatment duration (3-day) induced the onset of sex-change, while a comparatively longer treatment duration (10-day) induced complete sex-change [301]. This suggests that treatment duration plays a crucial role in the efficacy and extent of FAD-induced sex-reversal. Although in some cases, FAD-induced sex-reversed males have been observed to successfully spawn and produce viable offspring, there are very few studies examining the gamete quality and spawning performance of FAD-induced sex-reversed males [304]. Moreover, long-term studies investigating prolonged stability of FAD-induced sex-reversal and potential for reversibility in different hermaphrodite fish species have yet to be explored and warrant further research.

Anastrozole is a non-steroidal third-generation AI that selectively targets the aromatase enzyme complex [336]. Although anastrozole treatment is commonly used in humans and other vertebrates, its use to control sex in fish has not been widely explored [337–339]. The few studies that have looked at this chemical treatment are restricted to gonochoristic species, including dwarf gourami (*Trichogaster lalius*), common carp, and zebrafish [335, 340, 341]. In these studies, anastrozole treatment was found to be effective in inducing masculinisation using both dietary administration and immersion methods [335, 340, 341]. There are no studies to date of the administration of anastrozole in hermaphroditic fishes, emphasising that more research is needed to understand its application in other species.

In recent years, the use of letrozole (LET), a third-generation reversible aromatase inhibitor, has emerged as a powerful tool for maintaining male-biased sex-ratio in several gonochoristic and hermaphroditic fishes, suggesting its broad-spectrum potency in inducing masculinisation by inhibiting the aromatase pathway [215, 251, 270, 306, 308, 342–345]. LET can be administered to fish via immersion, incorporation into feed, or use of implants, with variation of dosage and treatment duration among the respective methods of administration [251, 308, 342–344] (Table 4). Studies have shown that dietary administration of LET requires comparatively high dosages and treatment windows than do implantation and immersion techniques, as observed in different gonochoristic and hermaphroditic fishes [215, 251, 270, 306–308, 343, 346]. While the existing studies in both gonochoristic and hermaphroditic fishes suggest that dietary treatment of LET can successfully induce masculinisation in both early and adult developmental stages, immersion and implantation methods are particularly focused on young and adult developmental stages, respectively [251, 306–308, 343, 346]. For small-sized fish, studies suggest that immersion treatment of LET can be more effective than dietary treatments, whilst implantation can be more effective in mature individuals, as evidenced in gonochoristic Siamese fighting fish (*Betta splendens*) and several protogynous grouper species [251, 307, 308, 343]. Although LET can induce functional masculinisation irrespective of administration methods, studies of spawning performance have been restricted to dietary and implantation methods [306, 307]. The evaluation of spawning performance revealed that LET-induced

sex-reversal can be stable and that sex-reversed males can spawn effectively, achieving fertilisation rates that are comparable to those of natural males, as observed in protogynous dusky grouper and rice-field eel [306, 307]. While the use of LET has shown promising results in the masculinisation of fish, the optimal dosage and treatment duration of LET can vary significantly between fish species. For instance, successful masculinisation can be achieved in red-spotted grouper with relatively low treatment duration and very low dosages compared to dusky grouper [307, 308] (Table 4). Although a dosage-dependent effect of LET has been observed in several gonochoristic fishes (e.g., Japanese medaka and Siamese fighting fish), where higher dosages result in reduced oocyte growth and increased masculinisation, further study is required to investigate any unintended impacts on reproductive development and performance [343, 347]. The current body of research indicates that the appropriate dosage and treatment duration of LET can be varied among species and it is necessary to conduct species-specific studies based on age and maturity to optimise treatment protocols and determine the most appropriate dosage of LET for species of interest.

6 | Impacts of Sex-Reversal on Gamete Quality, Fertility and Reproductive Success

Gametes must be of sufficient quality to fertilise eggs in the case of spermatozoa, or to be fertilised to generate normal embryos in the case of oocytes [348]. Spawning success can be strongly influenced by gamete quality, where poor-quality gametes may reduce reproductive success [349]. A number of factors may affect gamete quality, and thereby, spawning success, including genetic background, nutritional availability, hormones, or environmental conditions [348] (Figure 6). While a number of techniques have been used to successfully control sex-ratios in various fish species, their potential impacts on gamete quality and subsequent reproductive success remain a critical area of investigation. Changes in hormone regulation caused by induced sex-change may influence the quality and quantity of gametes produced compared to non-induced individuals, potentially affecting fertilisation rates, embryo development, and overall offspring quality [64, 351]. However, studies often overlook the assessment of the long-term effects of hormonal manipulation on gamete quality and larval survival, particularly in hermaphroditic fishes [352]. Therefore, it is important to understand whether there are effects of endocrine manipulation on gamete quality, fertility, and reproductive success, along with the potential for using sperm/eggs from sex-controlled fish in industrial breeding programmes.

6.1 | Impacts of Feminisation on Oocyte Quality

The quality of eggs produced in aquaculture hatcheries has a critical influence on commercial production and profitability [350]. Several findings have raised concern that MTF sex-changed fish may have reduced oocyte quality, or their oocytes may not have developed through vitellogenesis. For instance, in adult protandrous white snook (*Centropomus viridis*), E_2 implanted with silastic tube at a dose of 1.5 mg kg^{-1} BW resulted in 87% feminisation with the development of primary oocytes, but these oocytes did not reach the vitellogenesis stage even after 1 year of maturation [188]. While the presence of previtellogenic oocytes has been reported in E_2-mediated sex-changed protandrous common snook, the spawning success of feminised fish remains untested [193]. In contrast, E_2 treatment of protandrous juvenile Mexican snook was confirmed to produce fertile precocious females that were able to undergo spawning to generate fertilised eggs and viable larvae [195]. Likewise, in a recent study of barramundi that were feminised using cholesterol-based E_2 implants, precocious females completed vitellogenesis after 8 months of maturation, at which point spawning was successfully induced [138]. Importantly, the quantity and quality of larvae were comparable to commercial spawns involving natural females [138]. Both the egg size (751.4–795.0 μm) and oil droplet size (227.3 to 276.0 μm) of precocious female barramundi were within the normal range (egg size 700 to 850 μm and droplet size 200 to 270 μm) [138, 353–357]. In another study involving 20-month-old male barramundi, E_2 administrated using ethylene-vinyl-acetate (EVAc) implants induced precocious females that also successfully spawned and produced viable larvae [189], although a much lower fertilisation success (25%–35%) was reported compared to previously reported by Guppy et al. [138]. While different implant methods were used between the two studies, the difference in fertilisation rates is likely to be due to differences in size (~4.2 kg, Guppy et al. [138] vs. ~2.6 kg, Fine-Idan et al. [189]) and functional maturity of the precocious females used in each study. Higher fertilisation rates were observed in E_2-EVAc females as female broodstock weight increased to ~4.0 kg in later trials [189], aligning more closely with those reported earlier [138]. This strongly indicates that the physiological maturity of hormonally induced females, often reflected by their body weight, is a critical factor for optimal fertilisation success. Given the potentials of using E_2-induced sex-changed individuals for the operation of selective breeding, further investigations on optimising maturation and spawning protocols are necessary.

Studies of the reproductive competency of EE_2-induced females have been limited to protandrous hermaphroditic fishes, and their effects on reproductive viability remain largely unexplored [133]. EE_2 has been administered to simultaneous hermaphrodite mangrove rivulus, and attempts have been made to evaluate reproductive success; however, treated fish demonstrated impaired reproductive health that included decreased fertility, delayed reproductive maturity, and increased sterility [198]. Similarly, the findings of EE_2-induced reproductive success in gonochoristic fishes do not provide enough confidence for meeting commercial breeding standards. For instance, in EE_2-treated female chinook salmon (*Oncorhynchus tshawytscha*), sex-reversed fish had smaller ovaries and reduced oocyte numbers than untreated females, representing the lower reproductive potential of EE_2 females [203]. Since fish oocytes cannot be cryopreserved and loss of quality can have a large effect on the success of a breeding programme, the selection of estrogenic agents along with their dosages and treatment windows for sex manipulation should be strategically determined to produce the required numbers of high-quality eggs “on demand”.

6.2 | Impacts of Masculinisation on Gamete Quality

Motility and fertilisation success are important metrics of sperm quality [348]. In FTM sex-reversal programmes,

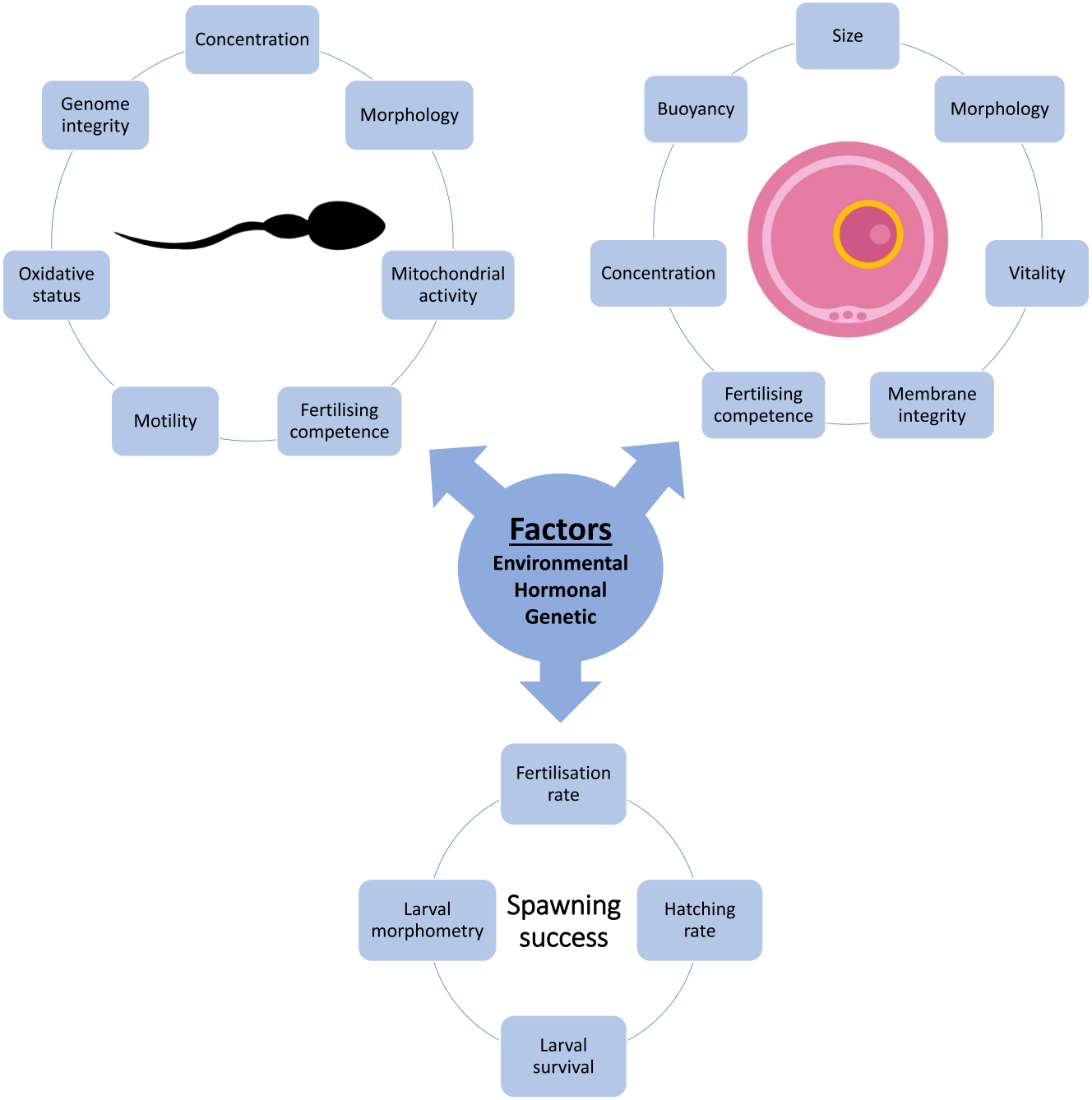

FIGURE 6 | Multifaceted factors influencing gamete quality and spawning success in fishes. Sperm/egg quality is dependent on various intrinsic characteristics, such as size, morphology, membrane and genome integrity, motility, and fertilising competence. These are mainly influenced by three primary factors: Environmental, hormonal, and genetic. Environmental factors include temperature, pH, salinity, and endocrine disruptor pollutants. Hormonal factors encompass the endogenous/exogenous endocrine influence on sperm/egg development and function. Genetic factors encompass inbreeding, genetic variability and inherited traits that contribute to sperm/egg quality. The interplay of these factors ultimately affects spawning success, including fertilisation rate, hatch rate, larval development, and survival. Figure derived from studies [348–350].

suboptimal hormonal/chemical regulations can disrupt spermatogenesis by directly impacting germ-cell development, leading to reduced sperm concentration, decreased motility, and abnormal sperm morphology [49, 183, 358]. Moreover, structural and functional abnormalities of testicular development can occur if the hormone dosages are inappropriate or given at high concentration or for prolonged periods [359]. To date, the majority of studies on the effects of sex-reversal on sperm quality, fertility, and reproductive success have been undertaken in gonochoristic salmonids because of the advantages to produce sperm from sex-reversed females (XX-males) to produce all-female populations and undertake cryopreservation [352, 360–363]. Studies have demonstrated that the sperm quality (e.g., sperm motility and viability), fertilisation rates, and larval survival of offspring of sex-reversed female salmonids are comparable to those of normal males

[352, 360–363]. Therefore, at present, the use of sperm from MT/MDHT/OHA-induced sex-reversed females to produce all-female populations is very common in salmon breeding programmes (see review [364]). Moreover, the cryopreserved semen of sex-reversed rainbow trout demonstrated high fertilisation rates (83.0% ± 5.5%–90.3% ± 3.5%), suggesting the possibility of incorporating cryopreserved semen of sex-reversed fish into breeding programmes [365]. Many commercial facilities, including Benchmark (based in Chile, Iceland and Norway) and AquaGen (based in Norway), are now utilising cryopreserved semen from sex-reversed female salmon in their breeding programmes to produce "all-female" products to export to land-based farms [366]. The existing literature shows that studies on sperm quality, fertility, and reproductive competency of FTM sex-controlled hermaphroditic fishes are limited to only a few species (e.g., honeycomb grouper, dusky grouper, and rice-field eel), but demonstrate promising results when using androgen enhancers and aromatase inhibitors [56, 126, 227, 229, 304–307, 367–369]. For instance, while studies have demonstrated varying sperm volume from sex-controlled males depending upon the compound used and treatment duration, these are often comparable to those of natural males [227, 229, 307, 367]. In protogynous dusky grouper, higher sperm volume (360 ± 26 μL) was observed when fishes were treated with dietary MT for 180 days compared to the MT implantation for 10 weeks (5–400 μL) and LET implantation for 9 weeks (118.20 ± 24.83 μL) [229, 307, 367]. However, no variation in sperm density was observed in this species using MT and LET [229, 307, 367, 369]. The sperm motility characteristics of both MT- and LET-induced males were comparable to natural males of dusky grouper [307, 367, 370]. Intriguingly, other studies have demonstrated that androgen (e.g., 11-KT) and AI-treated (e.g., FAD) sex-changed males of honeycomb grouper can successfully fertilise eggs collected from natural females, and the resulting larvae exhibit no morphological deformities [126, 304, 305]. Even after cryopreservation, the post-thawed sperm of both MT-nd LET-induced sex-controlled males has been successfully used to fertilise eggs collected from natural dusky grouper females, achieving fertilisation rates (69.5% ± 17.7%–73.2% ± 10.4%) that are acceptable for a commercial breeding programme [307, 367]. Conversely, Marino et al. [368] and Glamuzina et al. [227] argued that the fertility rate of sperm collected from MT-induced sex-reversed juvenile dusky grouper can be lower than in natural males, although successful egg fertilisation was obtained using milt from MT-induced males. Successful egg fertilisation and hatching can be achieved using sperm from LET-treated male rice-field eel, even 5 months after ceasing the treatment, with results that are comparable to natural males [306]. These findings suggest that AI treatment can effectively induce functional males with good sperm quality, highlighting their potential as a method for sex control in aquaculture. The utility of sex-reversal of females to males in many programmes is to enable the storage of individuals' sperm in gene banks. In reality, a male fish provides far more sperm than required during artificial insemination protocols, and as such, a slight loss of quality is easier to accommodate than for females. Therefore, further studies are warranted across a number of commercially important hermaphroditic fishes to explore the technical viability of incorporating sex-changed male individuals into breeding programmes.

7 | Further Research for Effective Sex-Change Control

7.1 | Identification of Suitable Combinations of Hormones for Sex-Control Strategies

For a successful sex-control strategy, determining an appropriate hormone or combination of hormones to obtain functional, fertile sex-reversed individuals is crucial. Although the application of a single hormone (or a chemical compound) has proven successful in controlling sex-change in a number of gonochoristic and hermaphroditic fishes, combined treatments of multiple hormones or chemical compounds may be a promising approach to achieve complete sex-reversal and full reproductive function. For example, in the gonochoristic spotted scat (*Scatophagus argus*), sole treatment by E_2 (300 μg g^{-1} feed) for 3 months resulted in partially sex-reversed individuals with malformed and sterile gonads, whereas combined dietary treatments of E_2 (300 μg g^{-1}) and an androgenic inhibitor, trilostane (TR) (300 μg g^{-1}) for 90 days resulted in completely sex-reversed individuals with no abnormalities in their ovaries [371, 372]. Similarly, in hermaphroditic fishes, Yeh et al. [131] found the highest masculinisation rates in mature female protogynous orange-spotted grouper while using a combination of androgens (testosterone, methyltestosterone, and testosterone propionate) implanted intramuscularly compared to their sole administration. Consequently, this multi-hormone approach effectively induced sex-reversal in all treated females, resulting in functional males (M2 stage with spermatozoa) that were capable of spawning successfully with natural females [131]. Additionally, the combined use of aromatase inhibitors (e.g., LET) and androgens (e.g., 17α-MT) has recently been reported as effective in shifting the sex-ratio towards male [251, 373]. For example, in the protogynous adult greasy grouper (*Epinephelus tauvina*) and leopard coral grouper (*Plectropomus leopardus*), the combined treatment with LET and MT resulted in higher masculinisation rates compared to the sole treatment by MT [251, 373]. Likewise, MT and LET co-administration induces hormone-implanted orange-spotted grouper females to become ripe males within a short period of time [57]. The findings suggest that the introduction of androgens with AIs could prove more effective for inducing masculinisation since combined treatment would decrease estrogen levels by blocking the transformation of androgens to estrogens, thereby increasing the androgen/oestrogen ratio and reducing the paradoxical feminisation effect. Although the findings highlight the potential benefits of utilising a combination of hormones or chemical treatments rather than a single agent for increasing the efficacy of sex-reversal, long-term studies to elucidate its stability, effects on gamete quality, and spawning performance of sex-reversed fish are needed.

7.2 | Genome Manipulation Technologies

Genomic engineering technologies, including gene editing, RNA interference-induced gene silencing, and transgenesis, are powerful tools for studying the function of genes within reproduction, and hold promise for advancing selective breeding programmes [155–157, 374–376]. Although this is an emerging field, the development of novel genome-editing technologies in recent years has allowed for targeting and editing genes with great

efficiency in a range of teleost species for different aquaculture applications [377–380]. In this section, the potential application of genomic manipulation techniques for controlling sex-change will be discussed with a focus upon gonochoristic fishes. Given the limited research on genomic sex-control strategies in hermaphroditic fishes, examples from studies on hermaphroditic species will also be incorporated where available.

7.2.1 | Gene Editing

In teleosts, newly emerged gene-editing tools hold promise for controlling sex-change by knocking out targeted key sex-determination and sex-differentiation-related genes [378, 381]. Gene knockout with gene-editing tools relies on creating targeted double-strand breaks in the genes using engineered nucleases, which are inaccurately repaired via non-homologous end joining, leading to inactivating mutations (insertions, deletions, or frameshifts) of genes [377–379, 382]. ZFNs (zinc finger nucleases) are artificial restriction enzymes (first developed in 1996) that combine specific DNA-recognition domains (to target specific regions of DNA sequences) with the non-specific type IIS restriction enzyme endonuclease (to perform cleavage) [382–384]. ZFN-based gene-editing has been successfully used to induce MTF sex-reversal in rainbow trout and medaka by knocking out the *sdy* (sexually dimorphic on the Y chromosome) gene and the *gsdf* (gonadal somatic cell derived factor) gene, respectively [167, 168]. Since the development of TALENs (transcription activator-like effector nucleases) and CRISPR (clustered regularly interspaced palindromic repeats) in the early 2010s, these approaches have now largely replaced the use of ZFN for gene-editing because of their higher gene editing specificity and efficiency, among other advantages [385, 386]. TALENs provide greater target accuracy as they are sensitive to single base pair mismatches in the target whereas ZFNs utilise a three-base pair binding mechanism that results in less precise sequence specificity [387, 388]. CRISPR utilises CRISPR-associated protein 9 (Cas9) to cleave DNA, with the target specified by a single-guide RNA (sgRNA) that is complementary to the target DNA [389, 390]. The sgRNA-Cas9 complex binds to the target DNA, where the Cas9 protein creates a double-strand break; the cell's subsequent error-prone non-homologous end joining repair then induces inactivating mutations and functionally knocks out the target gene [378, 382]. The knock-out of the key enzyme involved in estrogen biosynthesis, the ovarian aromatase (*cyp19a1a*) and its transcription factor *foxl2* has been achieved in both gonochoristic and hermaphroditic fishes (e.g., rice-field eel). The use of CRISPR and TALENs both led to decreased E_2 production and increased 11-KT production in these studies and eventually induced FTM sex-reversal [158, 159, 163, 165, 166, 391–394] (Table 5). Conversely, gene-editing of male-biased genes, including *dmy*, *dmrt1*, *amhy*, and growth factor *gsdf* with CRISPR and TALENs resulted in increased E_2 production and decreased 11-KT production and led to MTF sex-change [160, 166, 394, 397] (Table 5).

Even though gene-editing tools have potential as powerful tools for genetic manipulation within oocytes and early embryos, the strong regulatory framework, ethical concerns, and public scepticism to date have confined their application mostly to studying gene function and understanding genetic pathways [376, 381]. Moreover, sterile or intersex individuals that possess compromised testis (regressed or ovotestis) or have disrupted spermatogenesis are seen among gene-edited individuals [162, 164]. The use of gene-editing tools for controlling sex-change needs to be further investigated to fully understand its potential to reliably induce sex-changed individuals while ensuring functional gametes are produced. With the technologies becoming increasingly mature in various fish models (including hermaphrodites), it is anticipated that gene editing will significantly contribute to the field of aquaculture selective breeding in the future.

7.2.2 | Gene Silencing or Knockout of Sex Determining Gene Using RNA-Interference

RNA interference (RNAi) is a gene silencing/knockdown technique that uses double-stranded RNA (dsRNA) to degrade target mRNA and prevent its translation, leading to the silencing and knockdown of target gene expression [398]. Gene silencing or knockdown has proved invaluable in understanding gene function and pathways in developmental processes [399]. In our context, the use of RNAi has been demonstrated to be successful at inducing sex-reversal (see reviews [374, 376] for more comprehensive discussion on its application in aquaculture) in a range of vertebrates (e.g., in avians and teleosts) and invertebrates (e.g., arthropods) [375, 400, 401]. In chicken (*Gallus gallus*) and gonochoristic medaka, the knockdown of male promoting genes (e.g., *dmy*) with RNAi caused upregulation of female promoting genes and functional feminisation of genetic males [375, 400]. Similarly, in the giant freshwater prawn, *Macrobrachium rosenbergii*, the knockdown of insulin-like receptor (MrIR) using RNAi microinjection efficiently stopped spermatogenesis and yielded neofemales with similar reproductive structures (e.g., brood chambers, ovipositing setae and ovigerous setae) to natural females [401]. Although the findings demonstrate the potential of RNAi as a tool for controlling sexual fate in gonochoristic fishes, its application in hermaphroditic fishes remains limited. In undifferentiated protandrous black porgy, the mutation of *dmrt1* using virus-based RNAi resulted in increased expression of the ovarian marker gene, *figla* (folliculogenesis-specific basic helix–loop–helix transcription factor), leading to the disruption of testicular differentiation and stimulation of ovarian development [395] (Table 5). However, when the *dmrt1* gene was knocked down in differentiated black porgy, the effects of the treatment varied [395, 396]. Some knocked-down males had regressed testis and developed primary oocytes in their gonads [395], while others did not undergo MTF sex-reversal [396] (Table 5). The lack of MTF sex-reversal in differentiated individuals may be because the loss of *dmrt1* expression occurred after Sertoli cells were already established in the testis. *Dmrt1* plays a key role in the differentiation of germ cells into Sertoli cells, but once present, ongoing production of androgens would be expected to prevent feminisation of germ cells. However, these outcomes require further investigation to develop a better understanding of underlying mechanisms. Despite the potential of RNAi-based gene silencing for altering sex-ratios, it is likely these methods will predominantly be used to study reproductive pathways of different species, rather than within commercial production due to regulatory constraints (see Section 8.2). Moreover, practical constraints related to delivery difficulties (e.g., efficiency, protection and stability of the RNAi molecules, and route of administration) and cost both limit the widespread use of RNAi for sex control [374].

TABLE 5 | Genomic manipulation techniques used in hermaphroditic fish species.

Tools used	Reproductive process	Species	Mutated gene[a]	Mutation efficiency (%)[b]	Mutation effect	Phenotypic effect[c]	Generation[d]	References
Virus-based RNAi	Protandrous	Black porgy *(Acanthopagrus schlegeli)* (undifferentiated)	*dmrt1*	ND	Increased expression of ovarian marker gene, *figla* (folliculogenesis-specific basic helix–loop–helix)	Disrupted testicular differentiation and stimulated ovarian development	P_0	[395]
Virus-based RNAi	Protandrous	Black porgy *(Acanthopagrus schlegeli)* (differentiated)	*dmrt1*	ND	Decreased expression of *amh* (anti-Müllerian hormone)	Regressed testis and presence of primary oocytes	P_0	[395]
Virus-based RNAi	Protandrous	Black porgy *(Acanthopagrus schlegeli)* (differentiated)	*dmrt1*	ND	*Cyp19a1a*-expressing cells in the degenerated testis	Significant decrease in germ cells number; no male-to-female sex-reversal	P_0	[396]
TALEN	Protogynous hermaphrodite	Rice-field eel (*Monopterus albus*)	*cyp19a1a*	87.5	*Cyp19a1a* and *foxl2* expression significantly downregulated; decreased serum E_2 levels	Ovarian differentiation	P_0	[158]
TALEN	Protogynous hermaphrodite	Rice-field eel (*Monopterus albus*)	*foxl2*	58.3	Decreased serum E_2 levels, increased *dmrt1* expression significantly	Undifferentiated gonad?	P_0	[158]
TALEN	Protogynous hermaphrodite	Rice-field eel (*Monopterus albus*)	*dmrt1*	75	Decreased *sox9a1* expression significantly	Undifferentiated gonad?	P_0	[158]
CRISPR/Cas9	Protogynous hermaphrodite	Rice-field eel (*Monopterus albus*)	*cyp19a1a*	100	Decrease in serum E_2 levels; blocking of ovarian development	12-month-old fish with undifferentiated gonads containing germ stem cells or oogonia but no oocytes	P_0	[159]
CRISPR/Cas9	Protogynous hermaphrodite	Rice-field eel (*Monopterus albus*)	*foxl2*	100	Minimal decrease in serum E_2 levels; increase in *foxl2-l*, *foxl3*, and *dmrt1* transcription	12-month-old fish differentiated into female	P_0	[159]

[a]Mutated gene: *dmrt1* (double-sex and Mab-3 related transcription factor 1), *cyp19a1a* (cytochrome P450 family 19 subfamily A member 1a), and foxl2 (forkhead box protein L2).
[b]Mutation efficiency in percentage: ND, not determined if not specified by authors.
[c]Phenotypic effect: question mark (?) represents no clear information on the phenotypic effect in the studied literature.
[d]Generation: P_0 refers to the parental generation zero. These individuals were directly subjected to the genome manipulation technique.

7.2.3 | DNA Vector-Based Transgenic Approaches

Gene transfer is the process of introducing a foreign genetic material (a recombinant DNA construct) into cells of an animal to drive expression of a gene of interest [382, 402]. This is often achieved by delivering specific DNA sequences via pronuclear microinjection, retroviral or non-viral vectors, or DNA transposons, allowing the recipient cell to express the introduced gene [382, 402]. Introducing synthetic gene variants or genes from other species can disrupt or override endogenous sex-determination pathways and can induce targeted sex-reversal, as evidenced in mammals, avians, and teleosts [375, 403–407]. For example, in gonochoristic teleosts (such as yellow catfish, *Pelteobagrus fulvidraco*; medaka), a DNA vector-based transgenic approach has been used to control the sexual fate (either male or female) during embryonic stages [375, 403]. However, there are limitations in breeding and sex-change control due to the random location of transgene integration into the host genome, leading to unpredictable expression of the desired phenotype, disruption of normal reproductive processes, and decreased popularity compared to other genomic technologies [408]. Moreover, its application in commercially produced animals remains largely constrained by stringent regulatory frameworks [382, 409]. To date, only a handful of transgenic animals, most notably the AquAdvantage growth hormone-transgenic Atlantic salmon, have successfully navigated the complex approval processes to reach market production [382, 410, 411]. However, none of these products are under widespread production by conventional farmers, as the regulatory frameworks, which involve comprehensive assessments of environmental impact, food safety, and animal welfare, severely restrict their commercial release [382, 409, 412].

7.3 | Bioactive Compounds to Induce Sex-Change

In recent years, the use of bioactive compounds extracted from medicinal plants has received increasing attention as a tool for sex-reversal and fertility enhancement in fishes [413]. Many phytochemicals or plant-derived bioactive compounds, including flavonoids, isoflavonoids, saponins, stigmasterol, lupeol, quercetin, lignans, and stilbenes, have estrogenic or androgenic activity and can be used as an alternative to steroids during induced sex-change of different fish species [72, 413–422] (Table 6). Studies in gonochoristic Nile tilapia and convict cichlid (*Cichlasoma nigrofasciatum*) have demonstrated that puncture vine (*Tribulus terrestris*) seed extract, often attributed to its steroidal saponin (protodioscin) content, demonstrates varying degrees of masculinisation (e.g., 55%–92% depending on dosage in oral administration) across different dosages and life stages [414, 418, 425, 430, 431]. In comparison, treatment of Nile tilapia with *Basella alba* leaf extracts that contain a broad spectrum of bioactive compounds demonstrated moderate masculinisation, achieving typically below 85% sex-change [416, 418, 425, 426]. The Javanese chili (*Piper retrofractum* Vahl.), administered via injection or diet to adult rice-field eels and maturing striped catfish (*Pangasianodon hypopthalmus*) respectively, also induced masculinisation, albeit with intersex individuals observed in the eels [423, 427]. To date, the masculinisation efficacy of plant extracts has been variable. Outcomes have depended on the bioactive compounds in each specific extract and their mechanism of action, as well as the dosage, administration method, and fish species assessed. Although the mechanism of action is highly variable depending on the presence of specific bioactive compounds, it is hypothesised that these bioactive compounds modulate both genetic and endocrine systems to induce sex-change in fish [434, 435]. Phytochemicals, including phytoandrogens (e.g., flavonoids, saponins, and steroids) are hypothesised to inhibit aromatase (*cyp19a1a*), resulting in reduced estrogen synthesis and increased testosterone levels to favour male characteristics, while phytoestrogens (e.g., isoflavonoids, lignans, and stilbenes) exert estrogen-like biological actions that promote ovarian development [415, 435–440].

The majority of existing plant-based sex-reversal studies in gonochoristic fishes focus on early life stages, specifically fry and larvae, targeting early development (the labile period) as a critical window for controlling sex [73, 414, 416, 418, 425–433]. However, the studies on gonochoristic striped catfish and hermaphroditic rice-field eels demonstrate that plant-based sex-reversal is also possible in the adult stage [423, 427].

Dosage-dependent effects have been observed with several plant extracts (e.g., *Aloe vera*, *Tribulus terrestris*) in gonochoristic Nile tilapia and convict cichlids, where higher dosages generally correlated with increased masculinisation rates [414, 429]. Conversely, an inverse relationship between dosage and masculinisation has also been reported where the highest dosages of some plant extracts (e.g., *Basella alba*, *Tribulus terrestris*) led to a reduction in masculinisation rates [73, 416, 425, 428]. The inconsistency in masculinisation rates may be due to variation in bioactive compounds depending on using different extraction methods that can interact with each other and lead to synergistic or antagonistic effects on sex differentiation and sex-change (see review [441]). Moreover, high dosages of some plant extracts may have toxic effects and can disrupt biological pathways, leading to the intersex development and reduction in masculinisation rate [423]. However, the complex interplay between these factors remains largely unexplored. Therefore, careful selection of plant extract dosage for sex-change control is imperative, requiring species-specific studies. Studies have demonstrated that treatment duration ranges from a few days to several weeks depending upon the administration methods [72, 73, 414, 416, 418, 425–433]. Furthermore, the use of combined plant extracts, such as *Tribulus terrestris* and *Mucuna pruriens*, and simultaneous treatment with androgen appear promising for enhanced masculinisation [428, 431], warranting further investigation into synergistic effects. Although some findings have demonstrated both the negative and positive effects of some plant extracts (e.g., pawpaw (*Carica papaya*) seed extract, neem (*Azadirachta indica*) leaves powder) on sperm quality of sex-changed individuals [432], comprehensive studies are necessary to fully elucidate their effects on reproductive capacity and spawning performance. Moreover, the need for further studies on finding potential bioactive compounds to feminise fish and to control sex-change in hermaphroditic fishes is crucial.

TABLE 6 | Plant extracts used in hermaphroditic and gonochoristic fishes for purposes of controlled sex differentiation and sex-change.

Reproductive process	Common name	Scientific name	Life stage	Plant name and part used[a]	Bioactive agent	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Protogynous Hermaphrodite	Rice-field eel	*Monopterus albus*	Adult	Javanese chili extract (*Piper retrofractum* Vahl)	Piperine and sitisterol	Inj	187.5 g kg^{-1} fish	NM	6 W	75 (rest intersex)	[423]
							375 g kg^{-1} fish (5 times with an interval of 7 days)			16.67 (rest intersex)	
Gonochoristic	Convict Cichlid	*Cichlasoma nigrofasciatum*	Newly hatched larvae	*Tribulus terrestris* extract	Steroidal saponins (Protodioscin)	Imm	0.10 g L^{-1}	0 D	60 D (once in a week)	79 (93)	[414]
							0.20 g L^{-1}			85 (94)	
							0.30 g L^{-1}			87 (95)	
Gonochoristic	White molly	*Poecilia latipinna*	Hatchlings	*Tribulus terrestris* extract	Steroidal saponins (Protodioscin)	Imm	100 mg L^{-1} 150 mg L^{-1} 200 mg L^{-1} 250 mg L^{-1} 300 mg L^{-1}	0 D	60 D	Masculinisation with advanced testis development	[424]
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Basella alba* leave extract	Tannins, saponins, steroids and alkaloids	Imm	0.05 g L^{-1}	3 D	4 W	59.7 (240)	[416]
							0.10 g L^{-1}			70.3 (240)	
							0.15 g L^{-1}			55.35 (240)	
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Basella alba* leaves extract	Tannins, saponins, steroids and alkaloids	Imm	0.05 g L^{-1}	3 D	30 D	61.1 (40)	[425]
							0.10 g L^{-1}	3 D	30 D	71.9 (40)	
							0.15 g L^{-1}			56.7 (40)	
				Tribulus terrestris seed extract	Steroidal saponin (protodioscin)		0.05 g L^{-1}			71.5 (40)	
							0.10 g L^{-1}			72.0 (40)	
							0.15 g L^{-1}			81.4 (40)	
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Egg	*Basella alba* leaves	Flavonoids, tannins, steroids, and saponin	Imm	0.12 g L^{-1}	Yellow eggs	96 h	80.2 (10)	[426]
			Fry				0.12 g L^{-1} for 2 h	5 D	28 D (weekly)	75 (10)	
Gonochoristic	Striped catfish	*Pangasianodon hypopthalmus*	Maturing males	*Piper retrofractum* Vahl. extract	Piperine and sitisterol	D	187.5 g kg^{-1} BW	NM	8 W	75	[427]
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	MT + *Tribulus terrestris* extracts	Steroidal saponin (protodioscin)	D	40 mg kg^{-1} feed	NM	66 D	85.4 (2000)	[428]
							50 mg kg^{-1} feed			100 (2000)	
							60 mg kg^{-1} feed			97.50 (2000)	

(Continues)

TABLE 6 | (Continued)

Reproductive process	Common name	Scientific name	Life stage	Plant name and part used[a]	Bioactive agent	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Basella alba* leaves	Tannins, saponins, steroids and alkaloids	D	5 g kg^{-1} feed	3 D	30 D	61.1 (40)	[425]
							10 g kg^{-1} feed			70.3 (40)	
							15 g kg^{-1} feed			53.3 (40)	
				Tribulus terrestris seeds	Steroidal saponin (protodioscin)		5 g kg^{-1} feed			55.8 (40)	
							10 g kg^{-1} feed			64.1 (40)	
							15 g kg^{-1} feed			76.6 (40)	
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Tribulus terrestris* seed extract	Steroidal saponin (protodioscin)	D	2 g kg^{-1} feed	3 D	30 D	91.5 (50)	[73]
							2.5 g kg^{-1} feed			59.7 (50)	
							3 g kg^{-1} feed			57.2 (50)	
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Basella alba* leaves extract	Flavonoids, tannins, steroids, and saponin	D	Extract from 250 g leaves kg^{-1} feed	Fry with absorbed yolks	28 D	64.9 (10)	[426]
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Aloe vera* powder	Saponin	D	0.5% kg^{-1} feed	First-feeding stage	30 D	54.7 (210)	[429]
							1.0% kg^{-1} feed			58.5 (210)	
							2.0% kg^{-1} feed			60.9 (210)	
							4.0% kg^{-1} feed			67.6 (210)	
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Tribulus terrestris* seed extract	Steroids, flavonoids, saponins, resins, & alkaloids	D	2 g kg^{-1} feed	NM	28 D + 72 D normal diet	85.5 (500)	[430]
				Tribulus terrestris seed powder			200 g kg^{-1} feed			83 (500)	
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Tribulus terrestris* (TT) powder	Steroids, flavonoids, saponins, resins, & alkaloids	D	2 g kg^{-1} feed	Newly hatched	30 D	91.5 (200)	[431]
				Mucuna pruriens (MP) seed extract			2 g kg^{-1} feed			92.7 (200)	
				TT + MP			1 g TT + 1 g MP kg^{-1} feed			89.8 (200)	

(Continues)

TABLE 6 | (Continued)

Reproductive process	Common name	Scientific name	Life stage	Plant name and part used[a]	Bioactive agent	Route[b]	Dosage[c]	Timing[d]	Duration[e]	%Males[f]	References
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	Pawpaw *(Carica papaya)* seeds Powder	Saponin (oleanolic acid 3-glucoside)	D	6 g kg^{-1} feed	1 D	45 D	68 (300)	[432]
				Neem *(Azadirachta indica)* leaves powder	Saponins and flavonoids		1 g kg^{-1} feed		60 D	50 (300)	
Gonochoristic	Rainbow trout	*Oncorhynchus mykiss*	Larvae	*Asparagus racemosus* root + *Tribulus terrestris* seed extracts (combinedly used in equal proportion)	Steroidal saponins	D	200 mg kg^{-1} feed	15 D	120 D	70.1 (250)	[433]
					Steroidal saponins (Protodioscin)		400 mg kg^{-1} feed			84.6 (250)	
Gonochoristic	Nile tilapia	*Oreochromis niloticus*	Fry	*Basella alba* leaf ethanol extract	Flavonoids, tannins, steroids, and saponin	D	1.0 g kg^{-1} feed	3 D	30 D + 90 D normal diet	83.7	[418]
				Tribulus terrestris seed ethanol extract	Steroidal saponins (Protodioscin)		2.0 g kg^{-1} feed			91.8	
				Mucuna pruriens seed methanol extract	Resins & alkaloids		0.2 g kg^{-1} feed			92.7	
				Asparagus racemosus root methanol extract	Steroidal saponins		0.2 g kg^{-1} feed			55.2	

[a]Plant name and part used: Details the plant's botanical name and the form (e.g., extract, powder) or plant parts (e.g., leaves, seed, root) investigated.
[b]Route of administration: D, orally through diet; Imm, by immersion; Inj, through injection.
[c]Dosage: Expressed as mg kg^{-1}, g kg^{-1} or % kg^{-1} feed when plant extract is administered through the diet; in g L^{-1} or mg L^{-1} water when administered by immersion, and in g kg^{-1} fish when administered through injection.
[d]Timing: Commencement of treatment is given as age in days (D). Developmental timing (e.g., newly hatched, yellow eggs, absorbed yolks, feeding stage etc.) is provided when specified by authors other than specific age. Otherwise as not mentioned (NM) if not found in the study.
[e]Duration: Period of treatment is given in hours (hrs), days (D) or weeks (W).
[f]Percent: Percentage of resulting sex-changed males, otherwise as specified by the authors. Values in parentheses indicate the total number of treated individuals.

8 | Responsible Application of Sex-Change Control Strategies and the Importance of Good Practice

8.1 | Endocrine Disruptors

The responsible use of endocrine disruptors in fish sex-reversal programmes is a complex issue that requires careful consideration of environmental, ethical, and economic factors. While both steroidal and non-steroidal hormones and chemical compounds are widely being used in aquaculture and breeding, animal husbandry, and medicine, concerns have arisen regarding potential environmental hazards and human health risks associated with both parent compounds and their metabolites [442]. In grow-out farming systems, there have been consumer health concerns where hormones are directly applied to non-brood fish destined for harvest and consumption [18]. Some natural steroids (e.g., testosterone, E_2, and progesterone) and chemical compounds (e.g., trenbolone acetate and zeranol) are allowed in countries such as Argentina, Australia, Canada, New Zealand, and the United States in food-producing animals, including aquaculture species [443, 444]. In those countries, the residues of synthetic compounds with maximum residual levels are controlled [443]. However, there are some countries where certain hormones are banned for use in aquaculture operations. For example, MDHT is banned in the European Union, and both MT and MDHT are currently restricted substances controlled by the Drug Enforcement Administration (DEA) of the United States [290, 445]. Moreover, Brazil has banned all forms of natural and synthetic compounds for animal production, and only certain substances (e.g., testosterone, progesterone, E_2, zeranol, trenbolone acetate) are allowed for therapeutic uses in livestock [443]. In fish hatcheries, for sex-reversal programmes, overdoses and long exposure to hormones can lead to fish health problems, including organ deformities, sterility, or altered sexual behaviour [70,74]. However, the use of hormones for controlling sex-change is not regulated by the legislative framework of any country, nor any regional (European Commission) or international (Codex Alimentarius Commission, the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives) bodies that deal with food safety [446]. Some studies have postulated that MT treatment does not adversely affect tissues of tilapia fry after treatment ceases, implying that consumption of sex-reversed fish does not present a human health risk because some steroids (e.g., estradiol and MT) can easily be metabolised after the hormonal treatment withdrawal period [447, 448]. However, the indiscriminate use of steroidal hormones or synthetic compounds, or non-compliant withdrawal periods could lead to high residual levels that could pose a hazard to human health [444]. Sex-control programmes that exclusively target the production broodstock animals alleviate these concerns as these animals will not be consumed. Regardless of the programme's goals, the potential occupational risks to workers involved in manipulating the sex of fishes must be carefully considered as improper handling of hormones and chemical compounds could lead to unintended health effects in workers. Therefore, it is important to consider the potential effects of these hormones on fish health, consumer safety, and environmental safety [449, 450]. By adopting best practices and responsible use, such as minimising hormone dosage, optimising treatment timing, monitoring hormone residues, and safe disposal of treatment water, it is possible to mitigate risks and ensure the responsible production of farmed fish. For example, precise dosing and careful monitoring can help to ensure that the hormones have not bioaccumulated in the fish to avoid adverse effects on human consumers [18] and are not released into the surrounding environment so as to minimise environmental impacts (see review [451] for removal techniques of some endocrine disruptors). Conducting thorough risk assessments and implementing appropriate mitigation measures are necessary to ensure that the benefits of hormone use outweigh any potential risks. Furthermore, continued research and development of alternative methods, such as bioactive compounds and gene editing, are essential for exploring an additional set of approaches to sex control in aquaculture. However, the safety, efficacy, and potential impact of all such methods must be rigorously and comprehensively assessed before their widespread application.

8.2 | Genome-Manipulation Technologies

While the emergence of genome-manipulation technologies holds great promise in advancing fish selective breeding, it also presents technical and regulatory challenges that must be navigated in a responsible manner (see reviews [382, 452]). Studies have shown that off-target events and mosaicism are primary obstacles to the application of genome manipulation in commercial aquaculture [453, 454]. In both RNAi and genome-editing experiments, off-target events can occur when partially homologous genes are unintentionally knocked out or silenced other than the intended targets [454–457]. Similarly, mosaicism occurs when introduced genetic material or edits are not fully or uniformly incorporated during early development [453, 458]. Since genetic drift persists in a population, possible off-target effects can be transmitted each generation, and the effects of mutations may increase as generations progress [459, 460]. In field applications, these alterations can lead to diseases or other side effects in animals, possess unintended variability of experimental outcomes, and affect treatment efficacy [461–463]. Such a situation may adversely affect animal welfare and ethics, leading to questions regarding the responsible use of these technologies [460]. Moreover, some studies have raised concerns of escaping genetically modified fish into the wild that can pose genetic risks and deleterious effects upon ecosystem dynamics since transgenic animals can interbreed with wild populations [464, 465]. However, regulatory frameworks that control release and commercialisation of genetically modified fishes are not consistent from one country, region, or governing body to another, creating challenges for researchers, industries, and other stakeholders to deal with an inconsistent set of regulations [466, 467]. Additionally, consumer acceptance over genetically engineered fish remains questionable because of the safety concerns and risks [468]. However, in recent years, consumer acceptance has appeared to favour genome editing rather than transgenesis due to perceived similarity between genome editing and natural mutations [466]. Responsible and wise use along with stringent guidelines and increased public awareness on the science, benefits, and risks of these technologies

are required to dispel misconceptions, build trust, and facilitate acceptance [382, 467].

9 | Conclusion

The phenomenon of sex-change in hermaphroditic fishes is evidence of their remarkable biological plasticity and has been a subject of interest and scientific inquiry for decades [43, 49, 469]. This review synthesises information on recent advancements in different sex-change control strategies and their potential effects on sex-ratio and reproductive success of hermaphroditic fishes, (with illustrative effects in gonochoristic species), examining the effect of administration methods, dosages, treatment durations, and the developmental stage chosen for induction. Since environmental cues and physiological processes play pivotal roles in shaping the expressed sexual phenotype, hormonal manipulation techniques require careful consideration in terms of chosen dosage and treatment duration to achieve the desired sex-ratio and optimise gamete quality and subsequent reproductive performance. Moreover, despite the potential, there are only a few studies focussed on non-aromatisable androgens (e.g., MDHT) and non-hormonal AIs (e.g., LET) in hermaphroditic fishes. Therefore, a deeper investigation of their use is essential to explore how these hormones/compounds trigger sex-change based on varied developmental stages. Moreover, the potential use of combined androgen and AI treatments to minimise the paradoxical feminisation effect needs to be validated by functional experiments. Most of the sex-reversal studies are focussed upon the effect of hormone/compounds on sex-ratio and on genetic and hormonal pathways. Long-term studies focussing on maturation, oocyte and sperm quality, and spawning ability and reproductive success (e.g., fertility, hatching rate, and larval survivability) of sex-reversed fish are essential to incorporate such protocols into commercial breeding programmes. Current knowledge on alternative approaches, including gene editing and bioactive compounds, is based on the study of gonochoristic fishes, emphasising the need to extend the scope to hermaphroditic fishes to elucidate the potentials for effective control over their sex-change process. Compliance with the regulations of hormonal and chemical use is essential for responsible use and good practice; carefully considering the ethical implications is necessary to address public concerns and maintain social licence.

Author Contributions

Roni Chandra Mondal: writing – original draft, conceptualisation, data curation, visualisation, validation. **Jarrod L. Guppy:** writing – review and editing, conceptualisation, validation, supervision, project administration. **Maria A. Villacis-Escobar:** writing – review and editing, validation. **Dean R. Jerry:** writing – review and editing, conceptualisation, validation, supervision, project administration. All authors read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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