The lasting impact of Toki-o Yamamoto's pioneering chapter on fish sex determination and differentiation: A retrospective analysis of its contributions to reproductive biology and influences on aquaculture and fisheries sciences

J. Adam Luckenbach[1,2,*], Kiyoshi Kikuchi[3], Takashi Iwamatsu[4], Yoshitaka Nagahama[5,6], and Robert H. Devlin[7]

[1]Environmental Physiology Program, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd E, Seattle, WA 98112, USA
[2]Center for Reproductive Biology, Washington State University, Pullman, WA 99164, USA
[3]Fisheries Laboratory, University of Tokyo, Hamamatsu, Shizuoka 431-0214, Japan
[4]Aichi University of Education, Igaya-cho, Kariya, Aichi 448-0001, Japan
[5]National Institute for Basic Biology, Okazaki 444-8585, Japan
[6]College of Science and Engineering, Kanazawa University, Kanazawa 920-1192, Japan
[7]Fisheries and Oceans Canada, 4160 Marine Drive, West Vancouver, BC, Canada V7V 1N6
[*]Corresponding author: adam.luckenbach@noaa.gov

Keywords (5–10 terms): sex determination; gonadal sex differentiation; reproduction; gonochorism; hermaphroditism; sex steroids; hormonal sex reversal; monosex; aquaculture; fishes

Manuscript word count: ~5,000 words

Abstract

In 1969, Professor Toki-o Yamamoto published a landmark review on sex differentiation in fishes, which provided the first comprehensive synthesis of the various influences on sex phenotype, clarified the nomenclature, and sought to define the mechanisms that determine sex. Yamamoto's review highlighted the genetic basis of sex determination and the influence of sex steroids on gonadal sex differentiation. He argued that genetic sex determination was influenced by the net effect of sex chromosomes, when present, and contributions of female- and male-influencing alleles. Yamamoto also demonstrated that sex steroid treatments could override underlying genetic controls, resulting in sex reversal (e.g., XX-genotype males or XY-genotype females) in gonochoristic species. His comprehensive review has since stimulated a wide range of fields including aquaculture and fisheries sciences, genetics, and aquatic toxicology. This retrospective discusses Yamamoto's scientific legacy and remarkable life, which was devoted to studying the reproductive biology of fishes.

The process of sexual differentiation has fascinated scientists and scholars for centuries. From Aristotle to modern-day geneticists, physiologists, and environmental biologists, researchers have sought to understand the intricate factors that determine sex and drive the differentiation of testes or ovaries in different species. In 1969, Professor Toki-o Yamamoto published a landmark paper that provided the first comprehensive synthesis of the various influences involved in fish sex differentiation. Prior to Yamamoto's work, the field was complicated by conflicting hypotheses and a lack of consensus. Yamamoto drew on a wide range of research and presented a clear assessment of the validity of the existing theories. The paper established a firm foundation for subsequent researchers to build upon and remains a seminal work.

1. A brief overview of Yamamoto (1969)

Yamamoto (1969) defined the sex of an individual based on the obvious production of eggs or sperm, and further described the major sexual systems in fishes: gonochorism, which is reflected by two distinct sexes, female and male (the case with most fish species), or hermaphroditism, which has synchronous or consecutive forms (Fig. 1). Synchronous (also known as simultaneous) hermaphroditic species can produce eggs and sperm simultaneously, which, remarkably, allows for self-fertilization in some species (Tatarenkov et al. 2009). Consecutive (or sequential) hermaphroditic species can be further divided into protandrous hermaphrodites that mature first as males then change to females, or protogynous hermaphrodites that mature first as females then change to males (Fig. 1). Yamamoto noted that both protogynous and protandrous species start gonadal development containing testis and ovarian rudiments, suggesting that individuals do not switch sex, but rather emphasize the development and maturation of eggs or sperm at different phases of their reproductive life history. He prophetically hypothesized (see more below in present section) that the endogenous production of male vs. female sex hormones plays a role in controlling whether male or female gonadal development ensues.

During gonadal development in some gonochoristic fishes and other vertebrates, the gonads first develop as “undifferentiated” forms, which resemble ovaries, then secondarily the gonads in half of the individuals become testes. In differentiated forms, the gonads directly develop into either testes or ovaries. Yamamoto (1969) noted at that time that there were few known documented differentiated gonochorists (e.g., medaka (*Oryzias latipes*) and platyfish (*Xiphophorus maculatus*)), whereas today both undifferentiated and differentiated gonochorists are prevalent. Interestingly, undifferentiated forms are considered more labile and subject to hermaphroditism.

Yamamoto (1969) described clearly and at length the curious case of all-female (monosex) species, where mothers produce only daughters. Some females of the genus *Poecilia* are able to modify meiosis to generate ova (all of female genotype) and utilize sperm from other related species to activate their ova while, remarkably, preventing retention of the paternal genome. Similarly generated all-female progeny are also found in goldfish (genus *Carassius*). In another monosex female species group, *Poeciliopsis*, females can produce diploid ova (likely through a failure of polar body extrusion) which can be fertilized by a male from a related species. In this case, the male genome is retained in the resulting triploid progeny but excluded during meiosis to yield all-female ova in the next generation. Yamamoto's inclusion of this peculiar mode of reproduction provides the reader with an enhanced appreciation of the breadth of mechanisms influencing reproductive development among fishes.

Understanding the heritable basis of sex has been a major objective of geneticists for more than a century, with fishes being significant contributors to that understanding. Yamamoto (1969) presented previous experimental data from his own lab as well as that from others. At that time, sex chromosomes had been identified in many species and two main genetic sex determination (GSD) systems had been described, XX-XY and WZ-ZZ (Fig. 1), which Yamamoto took great care to explain, especially the nuance of GSD systems for some unusual but well-studied species, such as swordtails (genus *Xiphophorus*). Depending on the population, sex chromosomes in different genetic backgrounds could act as either male or female determinants. It is important to note that the human gene encoding the *sex determining region Y protein* (*SRY*) was not discovered until 1990 (Sinclair et al., 1990) and the first sex-determining gene in fish was not discovered until 2002 (Matsuda et al., 2002; Nanda et al., 2002; see below). Still, Yamamoto (1969) emphasized that "sex genes" existed and likely caused a "chain of events" that lead to the sexual phenotype. This quite accurately describes the processes of sex determination and differentiation in gonochoristic species as we know it today (Luckenbach and Yamamoto, 2018; Nagahama et al., 2021).

It is also noteworthy that Yamamoto (1969) included a section on polygenic sex determination (PSD), which is influenced by epistatic sex genes on sex chromosomes and/or autosomes. Yamamoto felt that sex is determined by the sum of genetic tendencies to induce each sex phenotype, with sex chromosomes (e.g., Y or W) possessing the strongest influences relative to those from autosomes or the second allosome. This polygenic view, also presented by Winge (1934), explained examples of apparent sex reversal simply as occurrences of genetic deviations from the normal balance of male or female genetic determinants. Yamamoto (1969) provided experimental data with medaka showing that sex-determining factors can be subject to selection such that males can develop in an otherwise XX genetic background, consistent with Aida's (1936) earlier findings. More recently, PSD has been found to be more widespread, including in zebrafish which possess family effects with multiple loci involved, and cichlids in which multiple sex chromosomes and sex determination systems co-exist (Bachtrog et al., 2014; Nagahama et al., 2021). Changes in genetic systems arising from captive breeding are also thought to play an important role in sex-determination systems in zebrafish (Wilson et al., 2014).

Yamamoto (1969) highlighted a number of examples of species that are "characterized by diverse sex ratios." Although environmental sex determination (ESD; Fig. 1) was not characterized in fishes until more than a decade later (Conover and Kynard, 1981), Yamamoto's appreciation of anomalous sex ratios provided the basis for subsequent research investigating the high degree of sexual plasticity observed in some fishes. We of course now know that ESD, and temperature-dependent sex determination (TSD) in particular, is widespread (50+ fish species; Luckenbach and Yamamoto, 2018), especially when reared in captivity under unnatural conditions which can trigger sex reversal and give rise to skewed sex ratios. The stress endocrine axis and epigenetic modifications have more recently been demonstrated to play important roles in mediating the ESD response (Yamamoto and Luckenbach, accepted).

It is clear when reading Yamamoto (1969) that sex control research (manipulation of sex with exogenous treatments) was one of his greatest scientific passions; note that the thought-provoking quote at the outset of the *Control of Sex Differentiation* section was one of his own: "Science unfolds and controls nature." Arguably, one of Professor Yamamoto's most impactful areas of research was on hormonal sex control using medaka as a model species (Fig. 2). He discovered that any steroid downstream of androstenedione can induce sex reversal. Through sex reversal experiments with medaka, he was able to generate XX-genotype male and YY-genotype female medaka and explore outcomes from a series of breeding crosses demonstrating that sex steroid treatment can override the underlying GSD mechanisms. His own research summarized in this chapter was noteworthy for a number of reasons, including demonstrating that 1) exogenous androgen treatment can drive testicular differentiation in genetically-female fish, 2) exogenous estrogen treatment can drive ovarian differentiation in genetically-male fish, 3) sex reversal can be induced with relatively low steroid dosages, and 4) monosex populations can be generated indirectly through sex reversal in the parental line followed by targeted breeding crosses (see section 3.2). Yamamoto thus posited that natural sex steroids play a critical role in sex differentiation. These were important conclusions that are supported by today's science and have been a topic in numerous subsequent reviews (e.g., Pandian and Sheela 1995; Donaldson, 1996; Piferrer, 2001; Devlin and Nagahama, 2002; Piferrer and Guiguen, 2008; Luckenbach and Yamamoto, 2018; Nagahama et al., 2021). He also proposed that the balance between androgens and estrogens is important in determining whether male or female differentiation occurs. As discussed further below, we now know that estrogens play a pivotal role in fish ovarian differentiation, development, and maintenance (Guiguen et al., 2010; Nakamoto et al., 2018; Nagahama et al., 2021; Zhou et al., 2021). However, the role of androgens in testicular differentiation is less clear, with some species potentially dependent on androgen production and signaling, and others not (Piferrer and Guiguen, 2008).

Yamamoto (1969) emphasized the criticality of the timing and dosage of steroid treatment application for successful induction of "complete" sex reversal. He noted that both sexes are responsive to steroid treatment and could result in at least partial transformation (e.g., induction of ovo-testis and testis-ova, referred to as "intersex") in fish treated at later stages of development. Treatment of undifferentiated gonochorists with androgens or estrogens during early development can induce sex reversal. However, differentiated species such as medaka may not be capable of complete sex reversal if treatments are applied after the fry stage and gonadal differentiation has begun. To overcome this difficulty, ovoviviparous adult females could be treated with sex steroids at a time when embryos they carry still possessed undifferentiated gonads, resulting in fully sex-reversed progeny. The key point was that steroid administration must be initiated “at the stage of the indifferent gonad and continue through the stage of gonadal sex differentiation” (Yamamoto, 1969).

The nature of “sex inducers” was discussed by Yamamoto (1969) where he presented how this may function in controlling sex differentiation. Prior work by other authors suggested that non-sex steroids were capable of modulating phenotypic sex, and that some androgens in high doses can cause feminization. These works were consistent with a non-sex steroidal mechanism of control. In contrast, working with medaka, Yamamoto noted that androgens and estrogens were highly specific in their effects on sex differentiation (e.g., no paradoxical feminization) and, as mentioned above, they required very low doses to induce sex reversal. Based on these observations, and that of Hishida (1962) who localized labeled sex steroids in juvenile gonads, Yamamoto (1969) concluded that a sex steroid-based control of sex differentiation was likely operating in fishes, but also noted that research on the specific steroidal pathways utilized was at a very early stage of what should be “promising areas of research.” As you will see in Section 3.2 below, his prediction was correct.

Although the main focus of Yamamoto (1969) was determination and differentiation of the fish gonad (“primary sex differentiation”), his chapter closes with an overview of the differentiation of secondary sex characters with an emphasis on influences of gonadectomy or sex steroid treatment. Male-specific secondary characteristics (most common) can typically be induced by androgen treatment and suppressed by castration, and female-specific characteristics can be stimulated by estrogen treatment and suppressed by ovariectomy. However, in fewer cases, removal of the ovaries can stimulate the appearance of male-specific characteristics in females, consistent with an inhibitory control of the trait arising from the ovaries.

Perhaps one of the most important take-away messages from Yamamoto (1969) is his demonstration that sex determination and differentiation are highly complex and diverse processes among fishes. Indeed, this conclusion has been found to be true based on subsequent research (see recent reviews, Luckenbach and Yamamoto, 2018; Nagahama et al., 2021; Yamamoto and Luckenbach, accepted).

2. *The life and career of Toki-o Yamamoto*

Toki-o Yamamoto (Fig. 3) was born February 12, 1906 in a small village in the Akita prefecture, the northern part of Japan’s main island of Honshu. He grew up surrounded by nature, which developed his keen interest in insects and fishes, and motivated him to begin studying zoology in 1926 at the University of Tokyo, Department of Zoology. He received his Ph.D. in 1936 and became an Assistant Professor immediately in the same department. During his term in Tokyo, he carried out numerous physiological studies on fertilization and activation of ova using several fishes, most notably medaka. Later, Yamamoto postulated that an invisible “fertilization wave” was propagated ahead of the wave of the breakdown of cortical alveoli within the ova (Yamamoto, 1944, 1961).

In 1942, the school of science was established in Nagoya Imperial University (later, Nagoya University) where Yamamoto was promoted to Associate Professor and subsequently to

Professor in 1943. Yamamoto's laboratory was reduced to ashes during the Second World War and he lost almost all of his research data and materials. However, Yamamoto's strong passion for research and observational skills allowed him to rebuild and initiate new research projects, including his important work on fish sex differentiation (reviewed in Yamamoto, 1969). Using a new d-rR strain of medaka that he developed (Fig. 2), Yamamoto successfully induced artificial sex reversal for the first time ever in medaka (Yamamoto, 1953).

During his 27-year term at Nagoya University, Yamamoto expertly instructed many students and researchers. While his love of nature was especially noteworthy, he was also passionate about drinking sake (rice wine), collecting stones and seashells, and listening to classical music. He frequented a classical music café near the university where he wrote and revised manuscripts with his students, including Takashi Iwamatsu, Yamamoto's last direct successor and co-author of the present article. When Yamamoto socialized at banquets, he often sang the self-composed tune, "The Song of Medaka."

After his retirement at the age of 63 in 1969, he continued to serve as a Professor at the Faculty of Agriculture, Meijo University, Nagoya. Despite being diagnosed with cancer in April of that year, he continued to conduct research for the next eight years while enduring the illness. Yamamoto died at the age of 71 in Nagoya on April 5, 1977. During his illustrious career, he received several honors including, the 1950 Zoological Society of Japan Award, 1957 Genetics Society of Japan Prize, 1976 Imperial Prize of the Japan Academy, 1976 the Order of the Rising Sun, Gold Rays with Neck Ribbon, and 1977 Junior Third Rank.

3. The legacy of Yamamoto (1969)

Yamamoto's (1969) chapter in the enduring book series "Fish Physiology" has been cited over 800 times and has influenced a variety of scientific fields including reproductive biology, aquaculture, fisheries science, genetics, aquatic toxicology, and behavioral biology (Fig. 4). Importantly, Yamamoto's chapter provided the first comprehensive overview of the research questions, hypotheses, and discoveries from the previous 100 years, many of which were complicated by conflicting hypotheses and a lack of consensus. It must be emphasized that this was nothing like writing a review article today – running literature searches and perusing downloaded PDF files on one's desktop computer. A significant amount of the prior literature was published in other languages, such as German and French, thus it was surely painstaking work to obtain the reference articles/books and translate the text. However, through his effort, Yamamoto provided a review that has stood the test of time and greatly benefitted the subsequent basic and applied fish sex determination and differentiation work described below. We were limited to mostly citing major review articles but encourage readers to further explore the primary literature on basic and applied aspects of fish sex determination and differentiation influenced by Yamamoto's chapter.

3.1. Basic discoveries stemming from Yamamoto (1969)

A recurrent theme in Yamamoto (1969) is that sex determination is largely genetically controlled in gonochoristic species. This conclusion was based on segregation patterns of sex-linked phenotypes, as well as sex ratios of progeny derived from hormonally-treated, sex-reversed parents, which confirmed that a chromosomal basis of sex determination is operating in many species. With the advent of molecular biology and genomics, Y-chromosome-linked sequences were identified in fishes, and subsequently specific genes were located in the sex-determining region of sex chromosomes. The first master sex-determining gene in a fish, identified in medaka (*DMY*, Matsuda et al., 2002; *dmrt1by* Nanda et al., 2002), was found to have high homology to *dmrt1*, a transcription factor involved in testicular differentiation in vertebrate and invertebrate species. While gain- and loss-of-function experiments clearly demonstrated the masculinizing abilities of *DMY/dmrt1by* in medaka, its specific role in inducing male development remains unknown. It is also interesting that *DMY/dmrt1by* is not the master sex-determining gene in some other members of the genus *Oryzias*, but rather, other sex-determining genes (i.e., *gsdf* and *sox3*) are in control (Nagahama et al., 2021). This lack of a conserved master sex-determining gene among medaka species is a revealing example of how modification of sex-associated genes can direct initiation of sex determination. In another case where *dmrt1* is essential to male sex determination, in tongue sole, *Cynoglossus semilaevis*, testicular differentiation is induced through dosage effects of the Z-chromosome-linked *dmrt1* gene present in two copies in ZZ males versus one copy in WZ females (Cui et al., 2017).

To date, ten master sex-determining genes have been identified in fishes (Nagahama et al., 2021; Yamamoto and Luckenbach, accepted). The mechanisms by which these genes regulate sex differentiation are not conserved, and include neomorphic and hypermorphic transcription factors, dosage effects, and cell signaling. For the latter, altered cell signaling (TGF-β superfamily) has been associated with sex determination via *amhy* and *amhr2* genes (e.g., Hattori et al., 2012; Kamiya et al., 2012; Kikuchi and Hamaguchi, 2013). In contrast, a male determining variant of *gsdf*^{Y} was found to possess altered regulatory sequences which caused ectopic expression of *gsdf*^{Y} and induction of male differentiation in multiple species (e.g., *O. luzonensis* (Myosho et al., 2012); sablefish *Anoplopoma fimbria* (Herpin et al., 2021)). The gene *sox3* in *O. dancena* has also been found to possess modified regulatory sequences that increase *sox3*^{Y} expression, which in turn elevates *gsdf* and drives testicular differentiation (Takehana et al., 2014). Uniquely, sex is determined in salmonids by *sdY*, a gene that originated not by modification of other sex-determining genes, but rather by modification of a completely novel gene (a truncated immune-related gene) not previously associated with sex determination/differentiation pathways (Bertho et al., 2018).

The above studies have also shown the clear interplay among known sex-determining genes as well as their roles in controlling sex differentiation pathways (e.g., *foxl2*, *cyp19a1a*, and *hsd17b1* in females, and *dmrt1*, *sox*, *gsdf*, and *amh* in males, among others; Nagahama et al., 2021). The genes *gsdf* and *DMY/dmrt1by* are co-expressed at the same developmental stage, consistent with *DMY/dmrt1by* being able to bind to the *gsdf* promoter to increase its expression. These analyses, as well as other studies, have clearly shown the direct control of the male path of sex differentiation by *DMY/dmrt1by* directed by *gsdf*. Similarly, functional studies with *amh* in other species have found this gene to be necessary and sufficient to stimulate testicular differentiation (Nagahama et al., 2021). In females, *cyp19a1/cyp19a1a* is essential for gonadal estradiol-17β synthesis and is one of the earliest markers of ovarian differentiation (Piferrer and Guiguen, 2008). Similarly, *foxl2* seems to play a supportive role in estrogen production. Germ cells are also critical for ovarian development, as sterile fish, lacking germ cells, exhibit elevated androgens and decreased estrogen (Tanaka, 2019).

As mentioned earlier, Yamamoto (1969) hypothesized that sex steroids were endogenous sex inducers in fish embryos, which significantly catalyzed research on their involvement in the early stages of gonadal development. This has led to an accumulation of knowledge on the timing of gene expression and enzyme activity related to steroid production (e.g., *cyp19a1* and *cyp11c*) in fish gonads (Piferrer and Guiguen, 2008). Furthermore, evidence derived from functional analyses using steroid inhibitors and steroid-deficient fish largely corroborates the model that estrogens play indispensable roles in initiating and maintaining the process of ovarian differentiation in many fishes (Guiguen et al., 2010; Zhou et al., 2021). Interestingly, depletion of estrogens can also induce functional female-to-male sex reversal in sexually-mature female gonochoristic species, including medaka, demonstrating that gonochorists maintain their sexual plasticity until adulthood and estrogens play a critical role in maintaining the female phenotype (Nagahama et al., 2021).

In conjunction with identification of the diverse master sex-determining genes, it can reasonably be surmised that estrogen synthesis is situated downstream of these master genes (Nagahama et al., 2021). However, a direct link between the master genes and estrogens had not been elucidated until recently. The first link was reported in rainbow trout (*Oncorhynchus mykiss*); its sex-determining protein, SdY, directly suppresses *cyp19a1* expression in cooperation with cofactors (Foxl2 and Nr5a1 proteins), resulting in depletion of estrogens in the male genotype (XY) (Bertho et al., 2018). A more direct link was reported in amberjack fish including *Seriola dumerili* whose sex-determining locus encodes Hsd17b1 (Koyama et al., 2019). In these fish, a Z-linked variant of *hsd17b1* has been found to have a reduced ability to produce estradiol-17β and testosterone compared to the W-linked allele, which supports high levels of steroid production in the female genotype (ZW) (Koyama et al., 2019). These studies clearly support the steroidal basis of sex differentiation in fishes as advanced by Yamamoto (1969).

It is worth noting that the role of estrogens in the onset of ovarian differentiation in medaka remains controversial; the manifestation of apparent sex-reversal/intersex characteristics in the female genotype (XX) occurs only during the later stages of development subsequent to pharmacological and genetic suppression of estrogen synthesis (e.g., Nakamoto et al., 2018). Dr. Takashi Iwamatsu is continuing research on this issue.

In protandrous and protogynous fishes (Fig. 1), the shift in dominance of testicular vs. ovarian tissues is correlated with expression of masculinizing and feminizing genes (see above, and Avise and Mank, 2009; Nagahama et al., 2021). This is not simply a consequence of natural shifts in ovarian and testicular tissue, but rather is supported by functional studies diminishing masculinizing influences (e.g., via *dmrt1* knockout strains that lack male germ cells, resulting in promotion of ovarian differentiation; Wu et al., 2012). Masculinization of the gonad is possible since undifferentiated germ cells reside in the ovary that can be recruited to begin testis development during sex change in protogynous fishes. Indeed, even some gonochorists have been found to possess undifferentiated germ cells in ovaries. For simultaneous hermaphrodites, the role of the brain and pituitary has been found key, mediated via expression of gonadotropins (Lh and Fsh).

As noted earlier, ESD has now also been described in many fishes. Most often, elevated temperature induces testicular differentiation, but multiple additional factors can also affect sex differentiation, including growth rate, photoperiod, exposure to particular wavelengths of light, salinity, pH, and breeding behavior. ESD-based masculinization is associated with downregulation of female pathway genes such as *cyp19a1* and *foxl2*, and upregulation of male pathway genes like *dmrt1* and *amh* (Yamamoto and Luckenbach, accepted). Environmentally-induced masculinization has been associated with increased epigenetic modification (methylation) of the *cyp19a1* promoter in some species (Navarro-Martin et al., 2011; Piferrer et al., 2021). These observations reveal how non-genetic influences are linked and can play important roles in sex determination.

3.2. Practical applications stemming from Yamamoto (1969)

The case can certainly be made that the field of hormonal sex control in fishes was spawned from Yamamoto (1969). Although his primary goal was to examine effects of steroids on gonadal sex differentiation to unveil the underlying genetics and potential role of sex steroids as "natural inducers," Yamamoto's work, and that summarized in his chapter, was foundational to a number of applied sex control technologies that have greatly influenced aquaculture and environmental sciences (Fig. 4).

One of the most important practical sex control techniques used in aquaculture and fisheries science, foreshadowed by Yamamoto (1969), is that of genetic sex identification (Devlin et al., 1991; Piferrer, 2001; Luckenbach and Guzmán, 2022). Being able to modify the phenotypic potential of an individual, independent of its genotypic sex, allows development of populations of fish that possess aquaculture production benefits relative to normal mixed-sex populations (Martínez et al., 2014). Yamamoto showed that medaka can be sex reversed (via appropriate steroid treatment timing and dosage) to generate XX males that can be mated with regular XX females to sire all-female populations (referred to nowadays as "indirect feminization;" Fig. 5). Thus, for some aquacultured species where performance differs between the sexes (e.g., sablefish, salmon, carp, tilapia), restricting production to the better performing sex using "monosex technology" can be highly advantageous (Piferrer, 2001; Martínez et al., 2014; Luckenbach et al., 2017). For this scheme, the genetic sex of XX and XY males can be distinguished by using test crossing (two generations) or molecular genetic assays (one generation). Depending on the degree to which sex is determined by sex chromosomes versus polygenic or environmental influences, monosex strains can be very stable.

Monosex approaches also have benefits in reducing impacts arising from introduced species by combining monosex and chromosome-set manipulation methods. For example, in rainbow trout, triploid females fail to produce functional ovaries, whereas triploid males develop testes and produce functional, albeit aneuploid sperm that is capable of fertilization but produces nonviable embryos (Lincoln and Scott, 1984). To enhance numbers of fish for commercial and sportfishing harvest, introduction of triploid fish has been used to reduce genetic introgression into wild populations by preventing interbreeding between introduced and wild fish (Piferrer et al., 2012). Releasing triploid males has the potential to modify the genetic architecture of the population, for example by competition with wild males (via mate selection) and females (affecting numbers of viable offspring and their genotypes). Thus, fisheries managers can opt to introduce all-female triploids that can grow and survive in nature, but do not contribute to breeding and thereby maintain the genetic integrity of the wild population. Sex control methods have also been developed to control non-indigenous invasive species using "Trojan" sex chromosome technology, which uses repeated introductions of YY females to reduce the number of regular females in a population (Cotton and Wedekind, 2007).

Yamamoto's lucid description of sex differentiation processes has provided background for numerous additional discoveries based on an elevated understanding of the sensitivity of fish sex differentiation to disruption by exposure to sex steroids or other compounds. For example, during the decades since the Yamamoto (1969) review, it has been discovered that many chemicals (steroidal and non-steroidal) in nature are capable of disrupting gonadal development (Endocrine Disrupting Chemicals; EDCs), in particular environmental estrogens released to nature by human activity (Sumpter and Johnson, 2008). These EDCs or "gender benders" are now routinely monitored in human wastewater in an effort to mitigate potential impacts on aquatic ecosystems and human health. Furthermore, in fisheries science, skewed sex ratios in natural populations can be analyzed with sex-specific DNA markers to determine whether such deviations arose from sex reversal or by natural demographic fluctuations. Other examples of fish DNA sex technology now in use include determination of the sex of prey in scat samples (e.g., preferential selection of Chinook salmon by Orca whales), eDNA estimates of sex ratios, and determination of sex of salmon in indigenous People's ancient middens (Ford and Ellis, 2006; Royle et al., 2018).

At the end of the 1960s, when Yamamoto published his review, little was known regarding the physiological and genetic pathways responsible for fish sex determination and differentiation. However, he was clearly aware of the importance of this area as he noted to the reader that "Research along these lines should be fruitful." Indeed, Yamamoto was correct in that prophecy, such that today, in the era of molecular biology and genomics, an abundance of genes involved in fish sex determination/differentiation are now known (Nagahama et al., 2021). In addition to providing a deep understanding of how these genes function to influence sex, they have also provided opportunities in applied science to control the reproductive or survival capabilities of fishes. Similar to the above objectives for sex control, transgenic and CRISPR-Cas9 based approaches have been developed to conditionally control gene constructs capable of supporting reproduction or inducing sterility (Xu et al., 2023). A variety of genetic or physiological pathways are being explored including the *dead end* gene (affecting migration and identity of PGCs), thiaminase (inducing vitamin dependency), Lh (control of gonadal maturation), and Zp3:caspase constructs affecting egg development. In addition, efforts are underway to generate CRISPR-Cas9 gene-drive systems (e.g., daughterless based) that could be used to control invasive/unwanted populations in nature (e.g., lamprey; Ferreira-Martins et al., 2021). None of these approaches are yet deployed in fishes in nature or aquaculture, and, while significant debate surrounds their use, they do hold significant promise to control unwanted reproduction.

4. In closing

In the closing section of his chapter, Yamamoto (1969) states that “sex is a phenotypic expression” that of course results from diverse and complex mechanisms. Although the genetic sex for most fishes is established at the time of fertilization, a high degree of plasticity remains whereby minor genetic, epigenetic, and/or environmental factors, as well as exogenous factors (e.g., EDCs, water temperature) may influence the ultimate sexual phenotype of the individual. Yamamoto again concludes that endogenous sex steroids likely play a key role in the natural process of gonadal sex differentiation, but also notes that “artificial control of sex differentiation may be one of the key projects in biology,” thus showing his appreciation for how the ability to control sex might influence aquaculture, genetics, and fisheries science into the future. As discussed above, these were profound conclusions from his own life’s work and effort towards developing Yamamoto (1969).

We can all learn from Professor Yamamoto’s enduring nature and passion for research. After his aquatic facilities and laboratory were destroyed in World War II, despite the significant losses, he rebuilt and carried on with his work. The same occurred after he was first diagnosed with cancer; he was unstoppable until the end. In his quest to review the body of literature on fish sex differentiation, Yamamoto fearlessly challenged prior findings and ideas (e.g., see pg. 129 Oka) and clearly sought to identify common, unifying threads in the research up to 1969, identify spurious published results or conclusions, and establish consistent nomenclature for the field. He minced no words in his chapter and was willing to hold colleagues to task in order to find the truth regarding the mechanisms of sex differentiation. Overall, Yamamoto’s contributions greatly advanced our understanding of sex differentiation and serve as a model for scientific rigor in research.

Acknowledgements

Each of the authors of this retrospective review benefited from Yamamoto-sensei’s pioneering empirical and theoretical works on sex determination and differentiation, for which we are most grateful. The authors thank Su Kim (NWFSC), Visual Information Specialist, for her suggestions on graphic design of the figures. The diagram presented in Figure 5 was modified from that previously published in Aquaculture, Volume 479, J.A. Luckenbach, W.T. Fairgrieve, and E.S. Hayman, Establishment of monosex female production of sablefish (*Anoplopoma fimbria*) through direct and indirect sex control, pages 285–296, Copyright Elsevier (2017). The authors also sincerely appreciate comments by Yuzo Yanagitsuru (NWFSC) on an earlier draft of the manuscript.

References

Aida, T., 1936. Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation. Genetics 21, 136-153. doi: 10.1093/genetics/21.2.136.

Avise, J.C., Mank, J.E., 2009. Evolutionary perspectives on hermaphroditism in fishes. Sex. Dev. 3, 152–163. doi:10.1159/000223079.

Bachtrog, D., Mank, J.E., Peichel,C.L., Kirkpatrick, M., Otto, S.P., Ashman, T.L., Hahn, M.W., Kitano, J., Mayrose, I., Ming, R., Perrin, N., Ross, L., Valenzuela, N., Vamosi, J.C., 2014. Tree of Sex Consortium. Sex determination: why so many ways of doing it? PLoS Biol. 12, e1001899, doi:10.1371/journal.pbio.1001899.

Bertho, S., Herpin, A., Branthonne, A., Jouanno, E., Yano, A., Nicolb, B., Mullerc, T., Pannetierd, M., Pailhouxd, E., Miwae, M., Yoshizaki, G., Schartl, M., Guiguen, Y., 2018. The unusual rainbow trout sex determination gene hijacked the canonical vertebrate gonadal differentiation pathway. Proc. Nat. Acad. Sci. USA 115, 12781-12786. doi.org/10.1073/pnas.1803826115.

Conover, D.O., Kynard, B.E., 1981. Environmental sex determination – interaction of temperature and genotype in a fish. Science 213, 577-579. doi: 10.1126/science.213.4507.57.

Cotton, S., Wedekind, C., 2006. Control of introduced species using Trojan sex chromosomes. Trends in Ecol. Evol. 22, 441-443. doi:https://doi.org/10.1016/j.tree.2007.06.0

Cui, Z., Liu, Y., Wang, W., Wang, Q., Zhang, N., Lin, F., Wang, N., Shao, C., Dong, Z., Li, Y., Yang, Y., Hu, M., Li, H., Gao, F., Wei, Z., Meng, L., Liu, Y., Wei, M., Zhu, Y., Guo, H., Cheng, C.H., Schartl, M., Chen, S., 2017. Genome editing reveals *dmrt1* as an essential male sex-determining gene in Chinese tongue sole (*Cynoglossus semilaevis*). Sci. Rep. 7, doi: 10.1038/srep42213.

Devlin, R.H., McNeil, B.K., Solar, I.I., Donalson, E.M., 1991. Isolation of a Y-chromosomal DNA probe capable of determining genetic sex in Chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 48, 1606-1612. doi.org/10.1139/f91-190.

Devlin, R.H., Nagahama, Y., 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208, 191-364. doi.org/10.1016/S0044-8486(02)00057-1.

Donaldson, E.M., 1996. Manipulation of reproduction in farmed fish. Anim. Reprod. Sci. 42, 381–392. https://doi.org/10.1016/0378-4320(96)01555-2.

Ferreira-Martins, D., Champer, J., McCauley, D.W., Zhang, Z., Docker, M.F., 2021. Genetic control of invasive sea lamprey in the Great Lakes. J. Great Lakes Res. Supplement 1, 47, S764-S775. https://doi.org/10.1016/j.jglr.2021.10.018

Ford, J.K.B., Ellis, G.M., 2006. Selective foraging by fish-eating killer whales *Orcinus orca* in British Columbia. Mar. Ecol. Prog. Ser. 316, 185–199. doi:10.3354/meps316185

Guiguen, Y., Fostier, A., Piferrer, F., Chang, C.F., 2010. Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. Gen. Comp. Endocrinol. 165, 352–366. doi: 10.1016/j.ygcen.2009.03.002.

Hattori, R.S., Murai, Y., Oura, M., Masuda, S., Majhi, S.K., Sakamoto, T., Fernandino, J.I., Somoza, G.M., Yokota, M., Strüssmann, C.A., 2012. A Y-linked anti-Müllerian hormone duplication takes over a critical role in sex determination. Proc. Natl. Acad. Sci. USA 109, 2955-2959. doi.org/10.1073/pnas.1018392109.

Herpin, A., Schartl, M., Depincé, A., Guiguen, Y., Bobe, J., Hua-Van, A., Hayman, E.S., Octavera, A., Yoshizaki, G., Nichols, K.M., Goetz, G.W., Luckenbach, J.A., 2021. Allelic diversification after transposable element exaptation promoted Gsdf as the master sex determining gene of sablefish. Genome Res. 31, 1366-1380. www.genome.org/cgi/doi/10.1101/gr.274266.120.

Hishida, T., 1962. Accumulation of testosterone-4-C^{14} propionate in larval gonad of the medaka, *Oryzias latipes*. Embryologia Nagoya 7, 56-67. doi.org/10.1111/j.1440-169X.1962.tb00159.x.

Kamiya, T., Kai, W., Tasumi, S., Oka, A., Matsunaga, T., Mizuno, N., Fujita, M., Suetake, H., Suzuki, S., Hosoya, S., Tohari, S., Brenner, S., Miyadai, T., Venkatesh, B., Suzuki, Y., Kikuchi, K., 2012. A trans-species missense SNP in Amhr2 is associated with sex determination in the tiger pufferfish, *Takifugu rubripes* (fugu). PLoS Genet. 8(7), e1002798. doi.org/10.1371/journal.pgen.1002798.

Kikuchi, K., Hamaguchi, S., 2013. Novel sex-determining genes in fish and sex chromosome evolution. Dev. Dynam. 242, 339-353. doi.org/10.1002/dvdy.23927.

Koyama, T., Nakamoto, M., Morishima, K., Yamashita, R., Yamashita, T., Sasaki, K., Kuruma, Y., Mizuno, N., Suzuki, M., Okada, Y., Ieda, R., Uchino, T., Tasumi, S., Hosoya, S., Uno, S., Koyama, J., Toyoda, A., Kikuchi, K., Sakamoto, T., 2019. A SNP in a steroidogenic enzyme is associated with phenotypic sex in *Seriola* fishes. Curr. Biol. 29, 1901–1909. doi.org/10.1016/j.cub.2019.04.069.

Lincoln, R.F., Scott, A.P. 1984., Sexual maturation in triploid rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 25, 385-392. https://doi.org/10.1111/j.1095-8649.1984.tb04886.x).

Luckenbach, J.A., Guzmán, J.M., 2022. Reproduction. *In* Midway, S.R., Hasler, C.T., Chakrabarty, P., eds. *Methods for Fish Biology*, 2nd edition. Chapter 12. American Fisheries Society, Bethesda, Maryland. doi: https://doi.org/10.47886/9781934874615

Luckenbach, J.A., Yamamoto, Y., 2018. Genetic & environmental sex determination in cold-blooded vertebrates: fishes, amphibians, and reptiles. *In*: Encyclopedia of Reproduction, 2nd edition (Skinner, M.K., ed.), Volume 6, Chapter 29. Elsevier Publishing. https://doi.org/10.1016/B978-0-12-809633-8.20553-0

Luckenbach, J.A., Fairgrieve, W.T., Hayman, E.S., 2017. Establishment of monosex female production of sablefish (*Anoplopoma fimbria*) through direct and indirect sex control. Aquaculture 479, 285-296. doi.org/10.1016/j.aquaculture.2017.05.037.

Martínez, P., Viñas, L., Sánchez, A. M., Díaz, L., Ribas, N., Piferrer, F., 2014. Genetic architecture of sex determination in fish: applications to sex ratio control in aquaculture. Front. Genet. 5, 340. doi.org/10.3389/fgene.2014.00340.

Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C.E., Shibata,, N., Asakawa,, S., Shimizu, N., Hori, H., Hamaguchi, S., Sakaizumi, M., 2002. *DMY* is a Y-specific DM-domain gene required for male development in the medaka fish. Nature 417, 559-563. doi:10.1038/nature751.

Myosho, T., Otake, H., Masuyama, H., Matsuda, M., Kuroki, Y., Fujiyama, A., Naruse, K., Hamaguchi, S., Sakaizumi, M., 2012. Tracing the emergence of a novel sex-determining gene in medaka, *Oryzias luzonensis*. Genetics 191, 163-170. doi:10.1534/genetics.111.137497

Nagahama, Y., Chakraborty, T., Paul-Prasanth, B., Ohta, K., Nakamura, M., 2021. Sex determination, gonadal sex differentiation, and plasticity in vertebrate species. Physiol. Rev. 101(3), 1237-1308. doi.org/10.1152/physrev.00044.2019.

Nakamoto, M., Shibata, Y., Ohno, K., Usami, T., Kamei, Y., Taniguchi, Y., Todo, T., Sakamoto, T., Young, G., Swanson, P., Naruse, K., Nagahama, Y., 2018. Ovarian aromatase loss-of-function mutant medaka undergo ovary degeneration and partial female-to-male sex reversal after puberty. Mol. Cell. Endocrinol. 460, 104-122. doi.org/10.1016/j.mce.2017.07.013.

Nanda, I., Kondo, M., Hornung, U., Asakawa, S., Winkler, C., Shimizu, A., Shan, Z., Haaf, T., Shimizu, N., Shima, A., Schmid, M., Schartl, M., 2002. A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*. Proc. Natl. Acad. Sci. USA 99, 11778–11783. doi.org/10.1073/pnas.182314699.

Navarro-Martin, L., Vinas, J., Ribas, L., Diaz, N., Gutierrez, A., Di Croce, L., Piferrer, F., 2011. DNA methylation of the gonadal aromatase (*cyp19a*) promoter is involved in temperature-dependent sex ratio shifts in the European sea bass. PLoS Genet 7, 12, e1002447, 2011. doi:10.1371/

Pandian, T.J., Sheela, S.G., 1995. Hormonal induction of sex reversal in fish, Aquaculture 138, 1-22. doi.org/10.1016/0044-8486(95)01075-0.

Piferrer, F., 2001. Endocrine sex control strategies for the feminization of teleost fish. Aquaculture 197, 229–281. doi.org/10.1016/S0044-8486(01)00589-0.

Piferrer, F., Beaumont, A., Falguiré, J.C., Flajšhans, M., Haffray, P., Colombo, L., 2009. Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293, 125-156. doi:10.1016/j.aquaculture.2009.04.036

Piferrer, F., Guiguen, Y., 2008. Fish gonadogenesis. Part II: Molecular biology and genomics of sex differentiation. Rev. Fish. Sci. 16, 35–55. doi.org/10.1080/10641260802324644.

Piferrer, F., 2021. Epigenetic mechanisms in sex determination and in the evolutionary transitions between sexual systems. Philos. Trans. R. Soc. Lond. B Biol. Sci. 376(1832). doi.org/10.1098/rstb.2020.0110.

Royle, T.C.A., Sakhrani, D., Speller, C.F., Butler, V.L., Devlin, R.H., Cannon, A., Yang, D.Y., 2018. An efficient and reliable DNA-based sex identification method for archaeological Pacific salmonid (*Oncorhynchus* spp.) remains. PLoS ONE 13(3), e0193212. doi.org/10.1371/journal.pone.0193212

Sinclair, A.H., Berta, P., Palmer, M.S., Hawkins, J.R., Griffiths, B.L., Smith, M.J., Foster, J.W., Frischauf, A.M., Lovell-Badge, R., Goodfellow, P.N., 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346, 240–244. doi.org/10.1038/346240a0.

Sumpter, J.P., Johnson, C., 2008. 10th Anniversary Perspective: Reflections on endocrine disruption in the aquatic environment: from known knowns to unknown unknowns (and many things in between). J. Environ. Monit. 10, 1476-1485.

Takehana, Y., Matsuda, M., Myosho, T., Suster, M.L., Kawakami, K., Shin-I, T., Kohara, Y., Kuroki, Y., Toyoda, A., Fujiyama, A., Hamaguchi, S., Sakaizumi, M., Naruse, K., 2014. Co-option of Sox3 as the male-determining factor on the Y chromosome in the fish *Oryzias dancena*. Nat. Commun. 5, 4157. doi.org/10.1038/ncomms5157

Tanaka, M., 2019. Regulation of germ cell sex identity in medaka. Curr. Top. Dev. Biol. 134, 151-165. doi:10.1016/bs.ctdb.2019.01.010.

Tatarenkov, A., Lima, S.M., Taylor, D.S., Avise, J.C., 2009. Long-term retention of self-fertilization in a fish clade. Proc. Natl. Acad. Sci. 106, 14456-14459. doi.org/10.1073/pnas.0907852106

Wilson, C.A., High, S.K., McCluskey, B. M., Amores, A., Yan, Y., Titus, T.A., Anderson, J.L., Batzel, P., Carvan, M., Schartl. M., Postlethwait, J.H., 2014. Wild sex in zebrafish: loss of the natural sex determinant in domesticated strains. Genetics 198, 1291–1308. https://doi.org/10.1534/genetics.114.16928

Winge, O., 1934. The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by *Lebistes*. Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol. 21, 1-49.

Wu, G.C., Chiu, P.C., Lin, C.J., Lyu, Y.S., Lan, D.S., Chang, C.F., 2012. Testicular dmrt1 is involved in the sexual fate of the ovotestis in the protandrous black porgy. Biol. Reprod. 86, 1–11. doi:10.1095/biolreprod.111.095695.

Xu, L., Mingli, R., Hyung, J., Hayman, E.S., Fairgrieve, W.T., Zohar, Y., Luckenbach, J.A., Wong, T.T., 2023. Reproductive sterility in aquaculture: A review of induction methods and an emerging approach with application to Pacific Northwest finfish species. Rev. Aquac. 15, 220-241. doi.org/10.1111/raq.12712.

Yamamoto, T., 1944. Physiological studies on fertilization and activation of fish eggs. II. The conduction of the “fertilization wave” in the eggs of *Oryzias latipes*. Anno. Zool. Jap. 22 (3), 126-136.

Yamamoto, T., 1953. Artificially induced sex-reversal in genotypic males of the medaka (*Oryzias latipes*). J. Exp. Zool. 123 (3), 571-594. doi.org/10.1002/jez.1401230309.

Yamamoto, T., 1961. Physiology of fertilization in fish eggs. Int. Rev. Cytol. 12, 361-405. doi: 10.1016/s0074-7696(08)60545-8.

Yamamoto, T., 1969. Sex differentiation. *In:* Hoar, W.S., Randall, D.J. Eds., Fish Physiology, vol. III, Reproduction, Academic Press, New York, pp. 117–175. doi.org/10.1016/S1546-5098(08)60113-2.

Yamamoto, Y., Luckenbach, J.A., accepted. Sex determination and gonadal sex differentiation. *In* Alderman, S., Gillis, T., eds. *Encyclopedia of Fish Physiology*, 2nd edition. Chapter 30001. Elsevier.

Zhou, L., Li, M., Wang, D., 2021. Role of sex steroids in fish sex determination and differentiation as revealed by gene editing. Gen. Comp. Endocrinol. 313, Article number 113893. doi.org/10.1016/j.ygcen.2021.113893.

Figure Legends

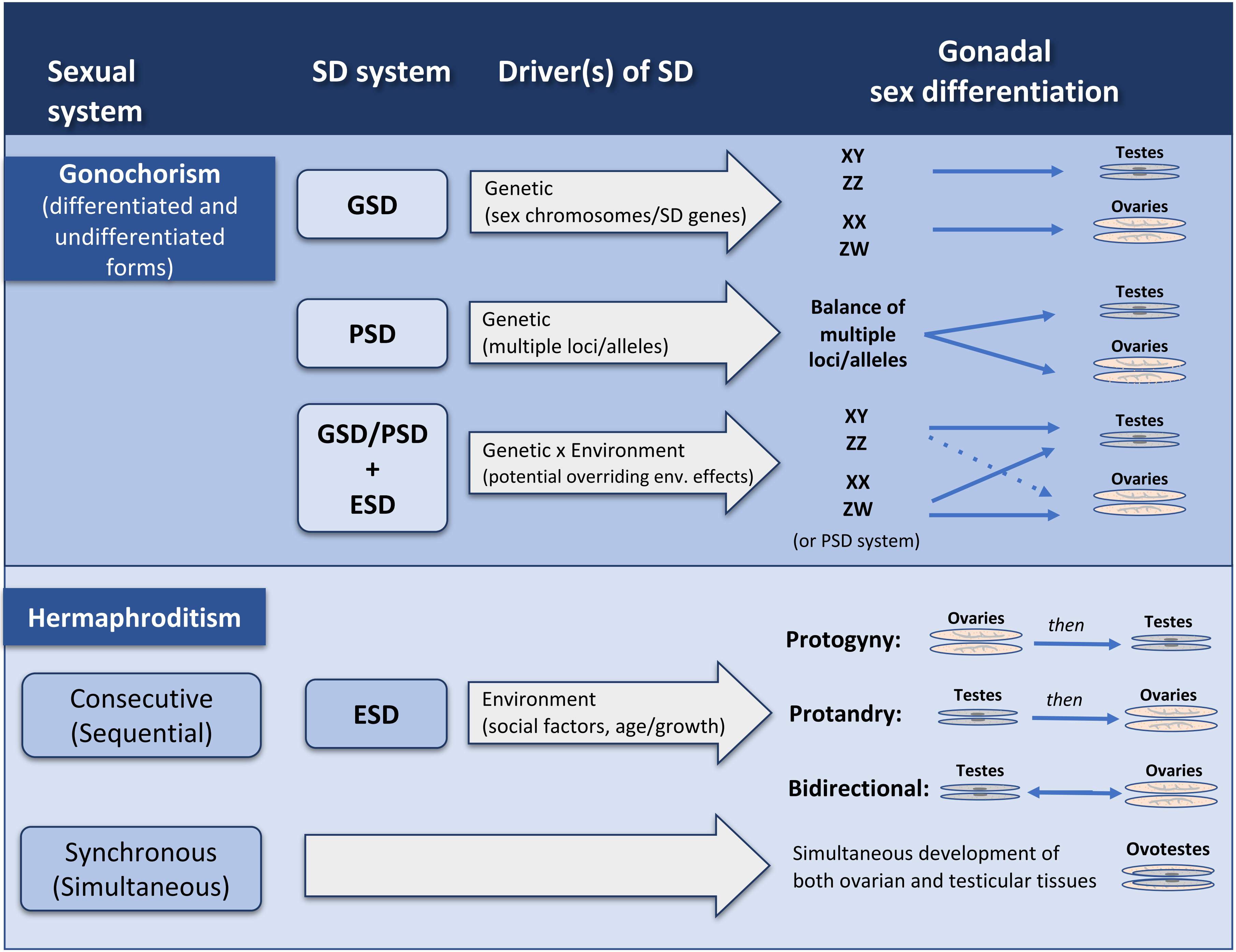
Fig. 1. Schematic diagram of the diverse sexual systems and mechanisms/drivers of sex determination (SD) and gonadal sex differentiation in fishes. Sex determination systems shown are genetic sex determination (GSD), polygenic sex determination (PSD), environmental sex determination (ESD), and combinations thereof. In gonochoristic species, naturally occurring instances of masculinization of genotypic females is much more common (arrow with solid line) than feminization of genotypic males (arrow with dashed line). Drawing based on Yamamoto and Luckenbach (accepted).

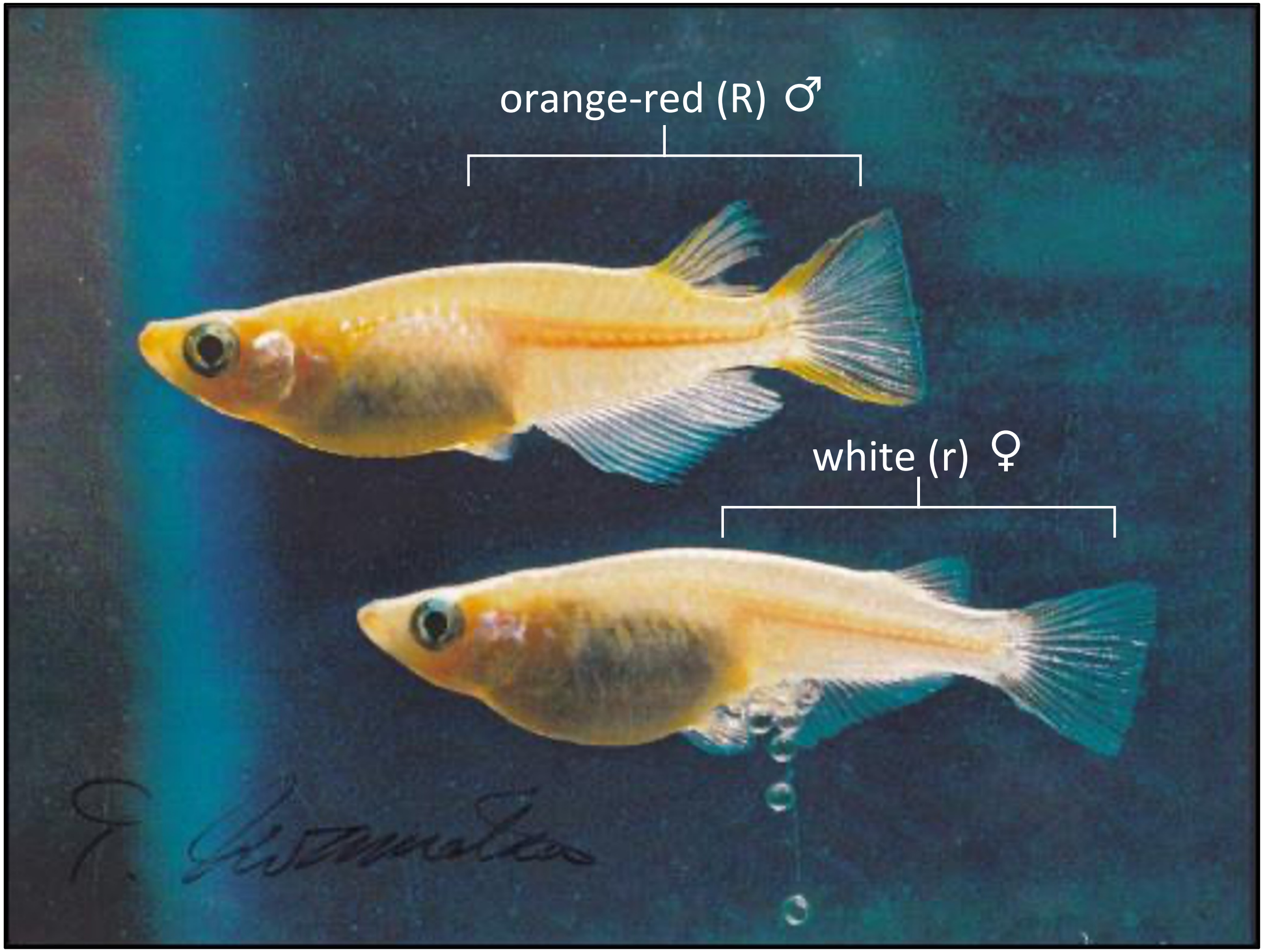
Fig. 2. Medaka (*Oryzias latipes*) d-rR strain developed and used for research by Professor Toki-o Yamamoto. Top: Orange-Red strain (male shown; Bottom: White Strain (female shown). Note the color difference along the dorsal surface between morphs. Photo credit: Takashi Iwamatsu.

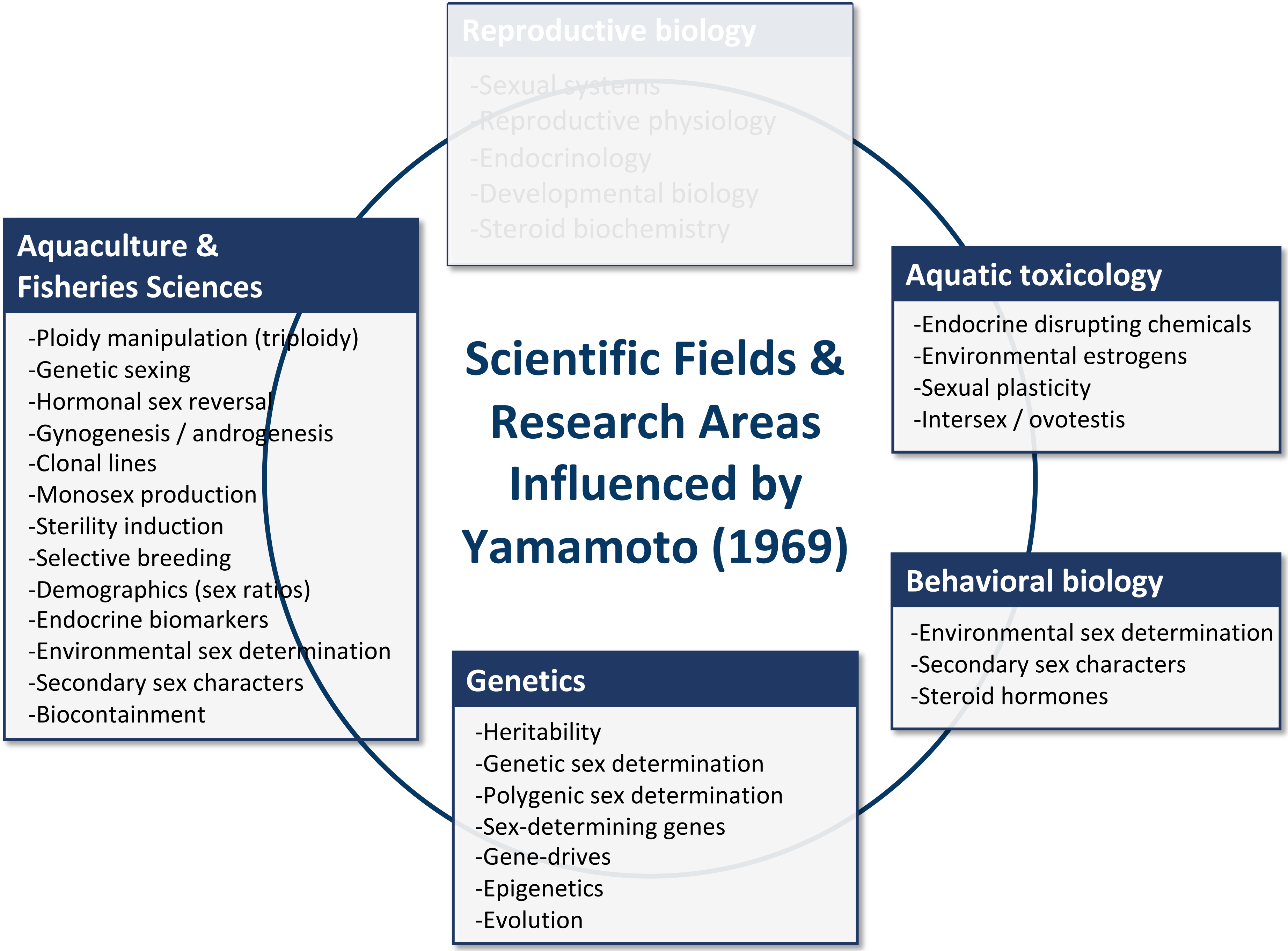
Fig. 3. Professor Toki-o Yamamoto in his aquarium facility at the Biological Institute, Nagoya University, Nagoya, Japan (1962). He stands among tanks used to hold medaka for his research. Photo credit: Takashi Iwamatsu.

Fig. 4. Some of the scientific fields and areas of research influenced by Yamamoto (1969) over the past 50+ years.

Fig. 5. Direct and indirect feminization approaches successfully used to produce monosex female populations of sablefish (*Anoplopoma fimbria*) for aquaculture. Direct feminization may be effective regardless of the sex determination system utilized by a species, whereas indirect feminization is streamlined in XX/XY species, such as sablefish, and can produce monosex female populations in the F1 generation. Genetic sexing methods can greatly facilitate sex control work by allowing researchers to determine the underlying genetic sex for individual fish with only DNA isolated from a fin clip. Schematic diagram reproduced from Luckenbach et al. (2017).


Sexual system
SD system
Driver(s) of SD
Gonadal sex differentiation
Gonochorism (differentiated and undifferentiated forms)
GSD
Genetic (sex chromosomes/SD genes)
XY
ZZ
XX
ZW
Testes
Ovaries
PSD
Genetic (multiple loci/alleles)
Balance of multiple loci/alleles
Testes
Ovaries
GSD/PSD + ESD
Genetic x Environment (potential overriding env. effects)
XY
ZZ
XX
ZW
(or PSD system)
Testes
Ovaries
Hermaphroditism
Consecutive (Sequential)
ESD
Environment (social factors, age/growth)
Protogyny:
Ovaries
then
Testes
Protandry:
Testes
then
Ovaries
Bidirectional:
Testes
Ovaries
Synchronous (Simultaneous)
Simultaneous development of both ovarian and testicular tissues
Ovotestes


orange-red (R) ♂
white (r) ♀



Scientific Fields & Research Areas Influenced by Yamamoto (1969)

Reproductive biology

- -Sexual systems
- -Reproductive physiology
- -Endocrinology
- -Developmental biology
- -Steroid biochemistry

Aquaculture & Fisheries Sciences

- -Ploidy manipulation (triploidy)
- -Genetic sexing
- -Hormonal sex reversal
- -Gynogenesis / androgenesis
- -Clonal lines
- -Monosex production
- -Sterility induction
- -Selective breeding
- -Demographics (sex ratios)
- -Endocrine biomarkers
- -Environmental sex determination
- -Secondary sex characters
- -Biocontainment

Aquatic toxicology

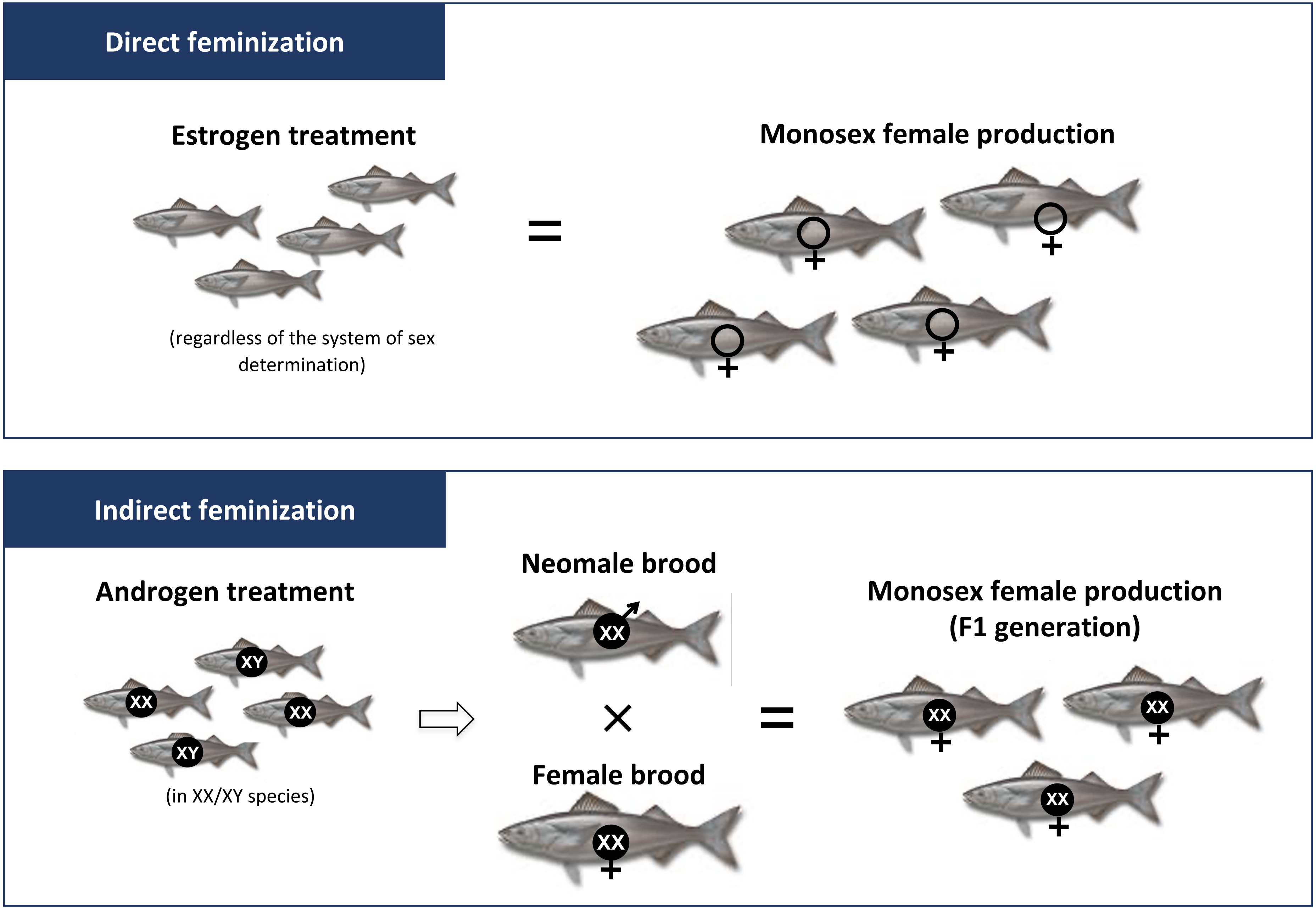
- -Endocrine disrupting chemicals
- -Environmental estrogens
- -Sexual plasticity
- -Intersex / ovotestis

Behavioral biology

- -Environmental sex determination
- -Secondary sex characters
- -Steroid hormones

Genetics

- -Heritability
- -Genetic sex determination
- -Polygenic sex determination
- -Sex-determining genes
- -Gene-drives
- -Epigenetics
- -Evolution


Direct feminization
Estrogen treatment
(regardless of the system of sex determination)
=
Monosex female production
Indirect feminization
Androgen treatment
XY
XX
XX
XY
(in XX/XY species)
Neomale brood
XX
×
Female brood
XX
=
Monosex female production (F1 generation)
XX
XX
XX