水研機構研報, 第 53 号, 79 – 88, 令和 8 年
Bull. Jap. Fish. Edu. Agen. No. 53, 79-88, 2026

Monosex breeding technology for sablefish aquaculture: a mini-review

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Abstract: The process of sexual differentiation (i.e. development of ovaries or testes) in fishes is much more plastic than for humans or other mammals. Even in fish species for which genetic sex determination systems have been well described, exogenous factors such as high temperature or steroid exposure may override those genetic controls and induce sex reversal—a mismatch between genetic and phenotypic sex. Sablefish *Anoplopoma fimbria*, also known as black cod (or "gindara" in Japan), is an important United States fishery and emerging marine aquaculture species, and a focal species for intensive aquaculture research and development at the Northwest Fisheries Science Center (NWFSC, Seattle, Washington, USA).

Upon initiating our work with sablefish, we recognized that it exhibits sexually dimorphic growth, with females growing significantly faster and attaining a larger body size than males, a phenomenon seen in many other species as well. This led to a major goal to develop a non-GMO method to produce monosex female (100% female) sablefish stocks for aquaculture to capitalize on their faster growth. A series of studies was conducted to gain an understanding of the basic processes of sex determination and differentiation (e.g., When do the gonads differentiate? What molecular and morphological changes signal the occurrence of gonadal sex differentiation?) and to develop an effective method for feminization of sablefish populations. The labile period of sex differentiation was successfully characterized and indirect feminization was achieved. First, sex differentiation of XX-genotype fish was redirected toward testicular development instead of ovarian development using dietary 17 alpha-methyltestosterone (MT) treatment during the labile period. Second, putative neomale (i.e., XX-genotype male) broodstock were obtained and ultimately bred with normal female broodstock. Offspring resulting from neomale × female crosses were 100% female, while those from control male × female crosses were ~50% female. Sperm from neomale sablefish broodstock could then be routinely utilized to generate all-female populations without the use of hormones, allowing for semi-commercial evaluations of their aquaculture performance.

Economic analyses were conducted using data obtained from the wild fishery, industry, and our own grow-out trials. This highlighted the importance of this monosex breeding technology on the economics of sablefish aquaculture production, including the reduction of time to harvest. Current research seeks to address common reproductive problems observed in neomale sablefish broodstocks through the use of alternatives to MT treatment (aromatase inhibitors) that allow for more 'natural' testicular differentiation to occur in neomales.

In summary, the advancement of monosex breeding technology for sablefish not only enhances the economic viability of sablefish aquaculture but also sets a precedent for sustainable practices in the industry. The successful development of hormone-free methods to produce all-female populations represents a significant step

Accepted on Feb 26, 2026
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toward optimizing growth performance while minimizing potential environmental impacts. This research contributes valuable insights that can be applied to other aquaculture species, supporting the industry's shift toward more sustainable and efficient production practices.

Key words: aquaculture; reproductive physiology; sex differentiation; economics

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Background on Sablefish Aquaculture Development

Over half the world's seafood now comes from aquaculture (FAO 2024). The United States is a leading consumer of seafood; however, it recently ranked 17th in aquaculture production worldwide and continues to import much of its farmed faish. To ameliorate this issue, the United States Department of Agriculture (USDA), National Oceanic and Atmospheric Administration (NOAA), and other agencies and research institutes have begun to identify focal species for aquaculture development in the USA to bolster its aquaculture production (e.g., the "Status of Marine Finfish for USA Aquaculture" project and associated species-status publications in Journal of the World Aquaculture Society in 2021).

One such focal species is sablefish (*Anoplopoma fimbria*), often marketed in the USA as "black cod" and in Japan as "gindara." Sablefish is a long-lived, deepwater fish native to the North Pacific Ocean. It is a relatively hardy species that exhibits fast growth in the aquaculture environment (Goetz *et al*. 2021). In addition, there are strong, established markets for sablefish in Japan and other parts of Asia, as well as growing markets in the USA, Europe, and Canada (Hartley *et al*. 2020). At NOAA's Northwest Fisheries Science Center (NWFSC; Seattle, Washington, USA), a multidisciplinary approach to the development of sablefish aquaculture was taken over the past decade. It was seen as critical to first gain a basic understanding of aspects of sablefish biology that are important to aquaculture, such as reproduction, growth, nutrition, and immunology. This involved NWFSC researchers with expertise in various scientific disciplines working together to study sablefish in captivity. Specific research projects focused on improving or refining methods for reproduction of captive broodstocks, larval rearing, and grow-out. One focal area for our own laboratory has been the development of methods for all-female (monosex) production of sablefish, which is the focus of this mini-review.

Capitalizing on Sexually Dimorphic Growth

When we began working with sablefish in captivity, one aspect of its biology we recognized was a distinct sex-associated growth pattern. Our observations of captive fish supported earlier reports in the fisheries literature demonstrating that female sablefish achieve larger sizes than males (Mason *et al*. 1983; Echave *et al*. 2012), a phenomenon known as sexual growth dimorphism or sexually dimorphic growth, which is also observed in many other fishes (Martínez *et al*. 2014). Studies were conducted in our laboratory with juvenile sablefish to determine whether sexually dimorphic growth occurs in cultured fish prior to them reaching the typical harvest size of ~2.5 kg. If so, its impacts could be significant, whereas, if it occurs after harvest size it might have little or no impact on sablefish aquaculture. We found that sexually dimorphic growth indeed occurs in the aquaculture setting and females and males diverge in body weight and length prior to reaching the target harvest size (Luckenbach *et al*. 2017; Hartley *et al*. 2020).

This finding led to a major objective to develop a non-GMO approach to produce all-female (monosex) sablefish populations for aquaculture to capitalize on their faster growth relative to males. Monosex female stocks were anticipated to grow more rapidly than mixed-sex (i.e., mixed male and female) stocks, thereby reducing the duration of grow-out prior to harvest and labor costs for industry operations. If such stocks could be obtained, they would provide significant economic benefits to growers and bolster marine aquaculture production, potentially offsetting the domestic trade imbalance for seafood noted above.

Understanding Sex Determination and Differentiation in Sablefish

Fish exhibit a high degree of sexual plasticity relative to mammals (Nagahama *et al*. 2021; Yamamoto and Luckenbach 2024). Species may exhibit environmental sex determination, whereby abiotic factors (e.g., water temperature) or social/behavioral cues influence their sex; genetic sex determination, whereby underlying genetically driven signaling determines their sex; or a combination of genetic and environmental sex determination. Regardless of what mechanisms are at play, and even in species with strictly genetically controlled systems, the sexual phenotype of fish may be readily influenced by exposure to hormones or other factors during the labile or critical sensitive period of sex differentiation (Luckenbach *et al*. 2023; Yamamoto and Luckenbach 2024). This period typically corresponds with fish embryonic or larval development when the gonads are sexually undifferentiated and thus sensitive to exogenous perturbations.

To identify the labile period of sexual differentiation and develop research 'tools' to understand and monitor this process in sablefish, we conducted a series of studies in our laboratory. One study sought to characterize the morphological changes sablefish gonads undergo during sex differentiation using histology. An ontogenetic series of gonad tissue samples was collected to track the development of sexually undifferentiated gonads into either testes or ovaries ("morphological sex differentiation;" Luckenbach and Fairgrieve 2016). We found that morphological sex differentiation was well correlated with fish length, first apparent in fish ~80 mm long, and was completed in all individuals by 150 mm. Thus, we concluded that ~70-150 mm is the period of morphological sex differentiation for sablefish (Fig.1).

Other studies focused on characterizing earlier events associated with sex determination and differentiation (Rondeau *et al*. 2013; Smith *et al*. 2013; Hayman *et al*. 2021; Herpin *et al*. 2021). Advanced molecular biology approaches such as next-generation sequencing and quantitative RT-PCR were used to identify sexually dimorphic genes expressed in the gonads that may regulate the development of either testes or ovaries (Smith *et al*. 2013; Hayman *et al*. 2021). This research provided insights into the sequence of molecular events associated with gonadal sex differentiation (i.e., "molecular sex differentiation;" Fig.1), which precedes morphological sex differentiation, and established robust molecular markers to distinguish each gonadal phenotype (Smith *et al.* 2013; Hayman *et al.* 2021). Molecular markers of sablefish sex differentiation include genes encoding transcription factors, steroidogenic enzymes, gonadotropin and steroid receptors, and transforming growth factors.

Complementary work conducted in collaboration with researchers at the National Research Institute for Agriculture, Food and Environment (INRAE; Rennes, France) and academic partners led to the identification of the sablefish male sex-determining gene, *gonadal soma-derived factor on the Y chromosome* (*gsdfY*), and revealed the ontogeny of its expression and potential mechanisms of action (Herpin *et al.* 2021). The *gsdfY* gene was the ontogenetically earliest sexually dimorphic gene that we identified and showed a male-specific expression pattern that began shortly after embryos had hatched (Herpin *et al.* 2021). Results of this research revealed that sablefish *gsdfY* had evolved through gene duplication and subfunctionalization to become the master sex-determining gene.

Altogether, these basic studies of early reproductive development provided a blueprint of the normal process of gonadal sex differentiation in sablefish (Fig.1) that, as described below, informed our targeted manipulation of the process to create all-female populations via indirect feminization. This approach did not involve the use of genetic modification techniques and instead relied on the inherent plasticity of gonadal sex differentiation in fishes.

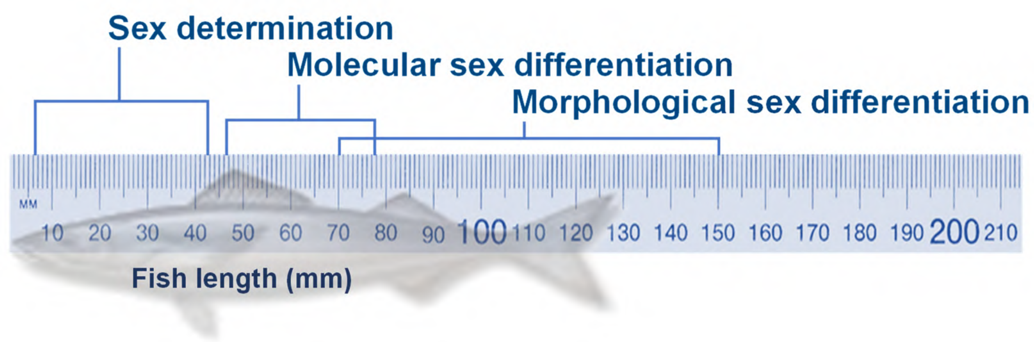


Fig.1 Schematic depicting the developmental timing of sablefish (*Anoplopoma fimbria*) sex determination and gonadal sex differentiation, which were well correlated with and predictable based on fish body length

Data to inform each period were generated through a series of experiments tracking ontogenetic development of sablefish produced and reared in captivity (Smith *et al*. 2013; Luckenbach and Fairgrieve 2016; Hayman *et al*. 2021; Herpin *et al*. 2021).

An Indirect Feminization Strategy

As we set out to devise a non-GMO strategy for monosex female production of sablefish, several potential approaches were considered. One such approach was 'direct feminization' whereby young, sexually undifferentiated fish can be exposed via the diet to low levels of estrogen to promote ovarian differentiation in 100 % of the treated fish (Piferrer 2001; Luckenbach *et al.* 2017). The caveat with this approach, however, is that the food fish (i.e. those intended for human consumption) would be directly treated with exogenous hormones. An alternative and preferred approach was 'indirect feminization,' which often entails treating sexually undifferentiated fish with an androgen, most commonly 17 alpha-methyltestosterone (MT), to induce masculinization of the genetically female proportion of the fish (e.g. the XX proportion in species with an XX/XY sex determination system) (Fig. 2). If successful, these will develop as "neomales"—genotypic female individuals with functional testes. The neomales can be grown over several years post-treatment until they reach sexual maturity; they are then crossed with female broodstock to potentially generate all-female offspring in the filial-one (F1) generation (Fig.2). Conceptually, the resulting progeny will be 100% genotypically female and should develop normal ovaries if no unintended environmental effects on sex determination occur. Given the indirect nature of this approach, the food fish are never treated with exogenous hormones, which is preferred for safety and environmental sustainability.

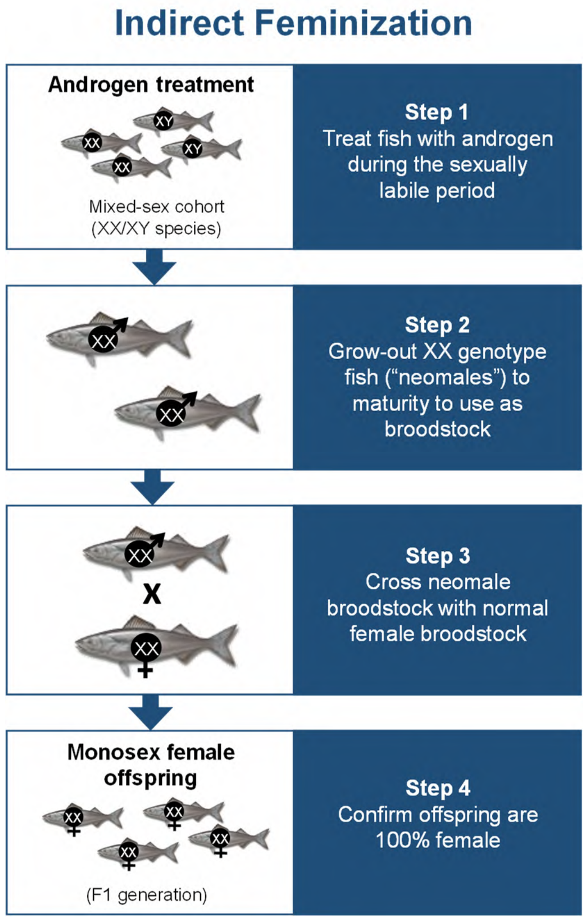


Fig.2 Schematic depicting the indirect feminization approach used to produce monosex female stocks of sablefish via dietary 17 alpha-methyltestosterone (MT) treatment

Modified from Luckenbach *et al.* (2017).

As a first step in the process of indirect feminization (Fig.2), dietary treatment of juveniles with MT was applied so that the treatment spanned the entire period of sex differentiation in an attempt to generate neomale sablefish. The intention was that female genotype fish in the treated population would be redirected toward testicular instead of ovarian development (see Luckenbach *et al.* 2017 for details). After a treatment duration of 2-4 months the fish were provided a standard, untreated diet. After approximately two years of grow-out, the fish were subsampled to evaluate their genotypic and phenotypic sex and maturity status. Gonadal histology and genotypic sexing using the sex marker *gsdf* demonstrated that some genotypic females treated with MT had testes with tubules full of spermatozoa, a promising result (Luckenbach *et al.* 2017).

The remaining fish were reared for an additional year (until reaching 3 years old) and checked for maturation in winter. At that time, some of the neomales and normal males (controls) were producing milt and were used in targeted breeding crosses with normal female broodstock (Fig.2). The results of those crosses clearly demonstrated the success of indirect feminization. As shown in Table 1, eight control male × female crosses yielded an average of 55 % female offspring (not significantly different from a 1 female:1 male sex ratio), while 10 neomale × female crosses yielded 100 % female offspring (Luckenbach *et al.* 2017). Results were consistent between genotypic sex ratios for sampled embryos (determined by the *gsdf* sex marker; Rondeau *et al.* 2013), and later evaluations of the same populations for phenotypic sex (i.e., genotypic females all developed normal ovaries). This study also established that sablefish exhibit an XX/XY-type sex determination whereby the presence or absence of a 'Y chromosome' determines the sex of resulting offspring (Luckenbach *et al.* 2017). In the years that have followed this breakthrough, no phenotypic males have ever been observed

Table 1 Genotypic sex ratios for F1 embryos resulting from control male × female crosses and neomale × female crosses. Summarized from Luckenbach *et al*. (2017)

Control Crosses	Genotypic Sex		Female (%)	Neomale Crosses	Genotypic Sex		Female (%)
	Male (n)	Female (n)			Male (n)	Female (n)	
1	15	26	63.4	1	0	42	100
2	16	15	48.4	2	0	30	100
3	12	23	65.7	3	0	31	100
4	12	19	61.3	4	0	31	100
5	15	16	51.6	5	0	35	100
6	14	18	56.3	6	0	33	100
7	19	12	38.7	7	0	31	100
8	15	16	51.6	8	0	31	100
				9	0	30	100
				10	0	31	100
		Average % female	54.6 ± 8.9			Average % female	100 ± 0.0

in the populations resulting from neomale × female crosses. Milt from neomale broodstock can now be routinely collected from existing broodstock and used to generate all-female populations without the use of hormones.

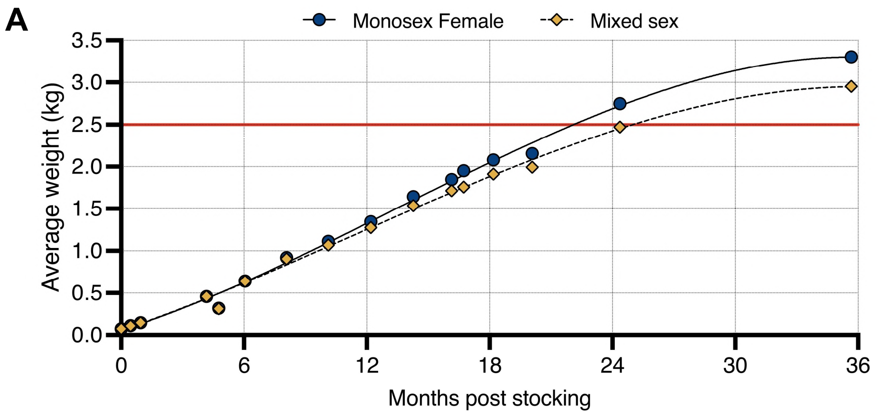
Of note, follow-up work in our lab has demonstrated that low proportions of neomales can also be generated by rearing genotypic female sablefish at high water temperatures throughout the period of sex differentiation (Huynh *et al*. 2019). However, this approach requires further investigation to evaluate its potential for aquaculture.

Economic Benefits of Monosex Female Production

After successfully producing monosex female stocks of sablefish, there was interest in knowing whether the growth rate advantages of using monosex stocks in place of mixed-sex would be a game changer for commercial-scale aquaculture of sablefish. The performance of monosex and mixed-sex stocks was evaluated under research conditions in trials conducted at the NWFSC Manchester Research Station (Port Orchard, Washington, USA). We found that mixed-sex and monosex fish grew equally well through the first winter and spring post-stocking, but that thereafter, the weight gain of male fish slowed dramatically compared with females. Net productivity of the mixed-sex stock was reduced by an increased time to harvest, greater losses due to higher total mortality, and a higher proportion of undersized, mostly male fish in the mixed-sex population. Based on this information, a semi-commercial grow-out trial was conducted with monosex female sablefish in marine net pens. The monosex sablefish grew rapidly and were ready for harvest at an average weight of 2.48 kg, almost 3 months earlier than a typical net pen stocked with mixed-sex sablefish (Hartley *et al*. 2020; Goetz *et al*. 2021).

A comprehensive econometric analysis was conducted using data obtained from the wild fishery, the small Canadian sablefish aquaculture industry, and our own grow-out trials (Hartley *et al*. 2020; Goetz *et al*. 2021). Cash-flow simulation models (Monte Carlo simulations) were developed to estimate the bottom-line impact of using monosex females instead of mixed-sex sablefish in net-pen aquaculture were developed. Two different simulations were run using actual weight-at-age data from our tank- and net-pen-based studies to make predictions (Fig.3A). In the first simulation, the grower harvests all of their fish at once, while in the second simulation, the grower selectively size-grades and harvests fish as they reach a market size of ~2.5 kg (Hartley *et al*. 2020). The scenario shown in Fig.3A reflects the former, where all fish are harvested after a fixed 24-month production cycle. In the simulation, both monosex and mixed-sex stocks were grown in commercial pens and harvested after 24 months, allowing for five complete production cycles to take place over a 10-year simulation period. We assumed fixed operational costs equivalent to commercial salmon net-pen farms that were operating in Puget Sound, Washington (USA), at that time. We also assigned size-based premium prices to the farmed fish that were equivalent to those for wild-caught sablefish as larger sablefish command a higher price, particularly in the Japanese market.

Results of the cash-flow simulations clearly demonstrated the advantages of farming monosex female stocks relative to mixed-sex stocks (Fig.3B; Hartley *et al*. 2020). Monosex



B

	Mixed sex	Monosex female	Difference
Average fish weight (kg)	2.4 kg	2.7 kg	+12.50%
Sized-based price ($/kg)	$9.42	$9.96	+5.70%
Operational costs ($/kg)	$5.82	$5.51	-5.30%
Gross margin ($/kg)	$3.60	$4.41	+22.50%

Fig.3 Growth rate data used for cash-flow simulations of monosex female versus mixed-sablefish stocks during the grow-out phase of aquaculture (A) and key information derived from the cash-flow simulation of a 2-year production cycle (B)

See Hartley *et al*. (2020) for details.

female sablefish had a higher average weight and received a better price. Because of the greater biomass produced, operational expenses consisting of feed, labor, purchase of fingerlings, and harvesting costs were reduced, leaving a higher gross margin. Overall, the cash-flow simulations predicted that using monosex female stocks would add 6-15% to the Internal Rate of Return (IRR) for growers compared to mixed-sex stocks (Hartley *et al*. 2020). In practical terms, a higher IRR translates to higher profits for growers who switch from farming mixed-sex to monosex stocks.

Conclusions, Challenges, and Future Directions

In summary, a multidisciplinary effort within NOAA has led to several key advances in sablefish aquaculture. Research on sexually dimorphic growth in this species paved the way for targeted research to capitalize on the superior growth of females relative to males through the development of methods for monosex female production. Basic studies were conducted using captively reared sablefish to first develop an understanding of sex determination and differentiation toward ultimately controlling those processes to generate monosex stocks to enhance sablefish aquaculture. Through the use of dietary MT treatment, indirect feminization of sablefish was achieved whereby monosex female offspring could be produced *en masse* via neomale broodstock without the use of genetic modification or exogenous hormone treatment.

Econometric analyses showed clear benefits of this monosex female breeding technology, including higher profits to growers when compared to previous mixed-sex farming practices. Most importantly, this technology has been adopted by industry: monosex sablefish are now being farmed in the United States and Canada. This line of research also generated immense biological and genetic information about sablefish, one of the highest-value fishery species in the United States on a per-weight basis (Hartley *et al*. 2020). Hence, information obtained should continue to be of value to sablefish aquaculture and fishery management well into the future.

The advancement of monosex breeding technology for sablefish not only enhances the economic viability of sablefish aquaculture but also sets a precedent for sustainable practices in industry. The successful development of indirect methods to produce all-female populations represents a significant step toward optimizing growth performance while minimizing potential environmental impacts. This research contributes valuable insights that can be applied to other aquaculture species, supporting the industry's shift toward more sustainable and efficient production practices.

Despite all of these successes, several challenges have been encountered with neomale sablefish broodstock that affect their utility for commercial-scale production of monosex offspring. The problems observed that may negatively affect neomale broodstock performance or having sufficient gametes for spawning include anomalous testis morphology, inability to manually express and collect milt for spawning, and/or low sperm motility. For example, a high proportion of neomales possess testes that have anomalous morphology and intersex characteristics (Fig.4). Intersex gonads are not encountered in wild-sourced or untreated, captively reared sablefish, and therefore likely reflect incomplete masculinization by MT. It is therefore critical to refine the current MT treatment protocol to generate fully masculinized neomale broodstock for commercial-scale aquaculture.

Current research in our lab seeks to address the reproductive problems observed in neomale broodstock through the use of an alternative to MT treatment. Our approach was informed by examining the natural process of ovarian versus testicular differentiation in sablefish and emerging evidence in the field suggesting a lack of estrogens, and not necessarily the presence of androgens, is critical to testicular differentiation in fishes (Fig.5; Zhou *et al*. 2021). Since MT is an androgen, and neomales (genotypic females) likely have competing endogenous estrogen signaling, MT treatment does not mimic the natural process of testicular

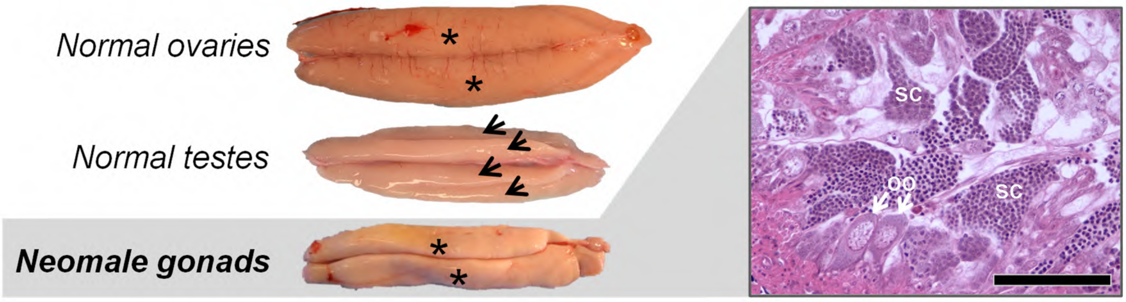


Fig.4 Typical gonadal morphology of female and male sablefish compared to that of a neomale sablefish generated by methyltestosterone treatment

Normal ovaries are cylindrical in shape and single-lobed (i.e., lacking folds) as indicated by asterisks, whereas normal testes are dual-lobed as indicated by arrows. Neomale gonads are single-lobed like ovaries and may contain both male and female germ cells (sc, spermatocytes; oo, oocytes), which are indicative of an intersex phenotype due to incomplete sex reversal. Scale bar = 100 μm. Images of normal gonads from Guzmán *et al*. (2017).

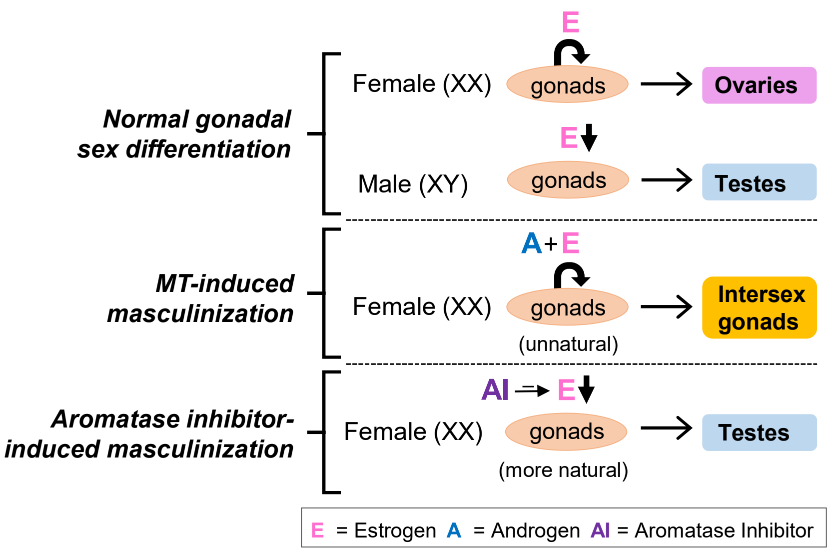


Fig.5 Conceptual diagram of the normal gonadal sex differentiation process; methyltestosterone (MT)-induced testicular differentiation, which overrides endogenous E2 effects; and aromatase inhibitor (AI)-induced testicular differentiation, which blocks E2 production and is viewed as a more ‘natural’ induction method

Curved arrows indicate the positive feedback of estrogen (E) production on the gonads.

differentiation. We hypothesized that a better approach would be to block estrogen production via treatment with an aromatase inhibitor (AI). AIs are widely used in human medicine and have also been vetted in fish to block estrogen synthesis by inhibiting aromatase/Cyp19a1, the rate-limiting enzyme responsible for the conversion of testosterone to estrogen (Piferrer 2001; Guiguen *et al*. 2010; Luckenbach *et al*. 2023). Thus, AI treatment may allow more 'natural' testicular differentiation to occur in neomales as opposed to the unnatural actions of MT (Fig.5). We are excited about this ongoing research using AIs to overcome prevalent issues with sablefish neomale broodstock—as well as those of other aquaculture species—and the future expansion of monosex breeding technologies to improve the economic and environmental sustainability of aquaculture.

Acknowledgments

This research was supported by a variety of funding sources including the NOAA Office of Aquaculture via Internal Competitive Aquaculture Funds (ICAF) and the NWFSC Internal Grants Program. The authors are grateful to S. Kim and A. Brown (NWFSC NOAA) for their helpful suggestions on an earlier version of the manuscript.

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Annotated Bibliography of Key References

(1) Martínez P, Vinas AM, Sanchez L, Diaz N, Ribas L, Piferrer F (2014) Genetic architecture of sex determination in fish: applications to sex ratio control in aquaculture. *Front. Genet*., **5**, 340. https://doi: 10.3389/fgene.2014.00340

This article reviews the state-of-the-art for fish sex determination and approaches to control sex ratios in aquaculture in order to capitalize on enhanced performance characteristics of either females or males. The primary characteristic of interest in aquaculture is growth, which may differ between sexes. This phenomenon, known as sexually dimorphic growth or sexual growth dimorphism, is common in fishes and may greatly influence the time to harvest, uniformity of harvested fish, and other aspects of aquaculture. The authors begin with an introduction on the main features of early gonadal development, including the processes of sex determination and gonadal sex differentiation (i.e., differentiation of the gonads into either ovaries or testes), as well as gonadal gene expression and morphological changes observed in most fish species. They also highlight the complex interplay between environmental and genetic factors that influence sex determination, with particular focus on how manipulating environmental conditions (e.g., temperature, hormones) or employing genetic breeding schemes can be used to control sex ratios. These approaches are of particular interest for improving productivity in aquaculture settings, where the selective production of a single sex can lead to faster growth rates or other desirable traits. A number of examples of sex-control strategies for various species, such as turbot, European sea bass, and tilapia are provided. Finally, the authors discuss the potential ethical and ecological implications of these methods, offering a comprehensive view of both the benefits and challenges of sex ratio manipulations in aquaculture.

(2) Luckenbach JA, Kikuchi K, Iwamatsu T, Nagahama Y, Devlin RH (2023) Chapter 11-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. *Fish Physiol*., **40**, 401-419. https://doi.org/10.1016/bs.fp.2023.08.003

This chapter, developed in collaboration with Japanese and Canadian colleagues, provides a retrospective analysis of Dr. Toki-o Yamamoto's foundational work on fish sex determination and its continuing influence on modern aquaculture practices. While honoring Yamamoto's 1969 chapter in the famous *Fish Physiology* book series edited by Hoar and Randall, the authors place significant emphasis on how Yamamoto's research has informed current strategies for sex control and genetic selection in aquaculture. The text outlines key advancements in understanding the genetic and environmental mechanisms governing fish sex differentiation, offering insights into the development of techniques such as hormonal sex reversal and breeding of monosex populations. These methods, derived from Yamamoto's early findings, are critical for enhancing fish production in aquaculture by enabling the manipulation of sex ratios to produce all-male or all-female stocks, depending on the desired traits (e.g., faster growth rates in one sex). The chapter also highlights the application of these approaches in various species and the potential for integrating genetic selection techniques to improve growth performance and disease resistance. Overall, the analysis underscores the enduring relevance of Dr. Yamamoto's work for optimizing genetic outcomes in aquaculture, making it a good resource for researchers and practitioners focused on sustainable fish production.

(3) Luckenbach JA, Fairgrieve WT, Hayman ES (2017) Establishment of monosex female production of sablefish (*Anoplopoma fimbria*) through direct and indirect sex control. *Aquaculture*, **479**, 285-296. https://www.sciencedirect.com/science/article/abs/pii/S0044848617306361

This paper focuses on the development of methods for both direct and indirect feminization of sablefish populations for aquaculture and establishes for the first time that sablefish have an XX/XY system of sex determination. For direct feminization, the authors administered estradiol-17 beta (E2) via the diet starting just after weaning for two or four months. They found that E2 treatment successfully feminized genetic males, as fish sampled at both time points all developed ovaries. For indirect feminization, 17 alpha-methyltestosterone (MT) was used, with the intention to masculinize genetic females. After four months of MT treatment, fish had all developed testes, while a two-month treatment resulted in intersex gonads, indicating incomplete sex reversal. Once sexually mature, the "neomales" (genetic females with masculinized traits) were crossed with females and their offspring were all female, confirming that sablefish have an XX/XY sex determination system. The study demonstrates that indirect feminization using neomales can yield all-female populations of sablefish for aquaculture without the need for hormone treatment of food fish. This approach, which avoids direct hormone treatment of market fish, offers significant advantages to the aquaculture industry by improving production and ensuring consumer safety.

(4) Hartley ML, Schug DM, Wellman KF, Lane B, Fairgrieve WT, Luckenbach JA (2020) Sablefish aquaculture: An assessment of recent developments and their potential for enhancing profitability. NOAA Technical Memorandum NMFS-NWFSC-159, Seattle, Washington, 89 p. https://doi.org/10.25923/cb0y-n468

This article provides a comprehensive assessment of the history of sablefish aquaculture and recent advancements in research, and evaluates their potential to improve the profitability of the industry. The authors, some of whom are economists, summarize technological innovations and biological research aimed at overcoming key production challenges, such as feed efficiency, broodstock management, and disease resistance. In particular, they emphasize developments in monosex female sablefish production and the use of novel feeds, both of which have the potential to reduce production costs and enhance growth rates. The authors report findings from the first economic simulation of monosex (all female) compared to mixed-sex (both females and males) grow-out for sablefish, which demonstrated a significant increase in the internal rate of return to growers. Additionally, the article explores market factors, such as consumer demand and pricing trends, offering insights into how improved production practices could meet market needs and increase profitability. This resource is important to stakeholders seeking to enhance the sustainability and economic viability of sablefish farming. Many of the principles therein may apply broadly to aquaculture of other fish species.