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REVIEW ARTICLE

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Status of sablefish, *Anoplopoma fimbria*, aquaculture

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Abstract

Sablefish, *Anoplopoma fimbria* (also called black cod), is a long-lived marine species with wide distribution extending from Baja California to Alaska, the Bering Sea, and through to the eastern coast of Japan. The landed weight of sablefish in the U.S. commercial fisheries is not large compared with other species; however, the exceptional value of sablefish has ranked it high compared with other species such as pollock, sockeye salmon, and Pacific cod. Sablefish are high in omega-3 fatty acids and have white firm flesh with superior quality and taste. Current population levels are lower

relative to historic ones and harvests have decreased within the last decade. The exceptional value of sablefish and decreases in wild populations have stimulated the development of methods to commercially aquaculture this species. Over the last 20 years, significant progress has been made in addressing the production of sablefish, and while there is still research that needs to be completed, sablefish have been commercially aquacultured by a small number of Canadian companies. In the Pacific Northwest, it is relatively easy to collect sablefish broodstocks from the wild and to transition them to land-based rearing facilities. However, they must be maintained at cold temperatures to successfully reproduce. Captive broodstocks for genetic selection are not commercially available, though producers have begun their own development. Incubation conditions for yolk-sac larvae have been developed and currently require long incubation periods at low temperatures, elevated salinity, and light exclusion. Although incubation times are long, they do not require very much attention during this phase. Exogenously feeding larvae currently require a regimen of rotifers and *Artemia* prior to dry feed habituation. However, tank characteristics, water turbidity, temperature, and illumination, as well as live feed enrichments have been studied. With the research that has been accomplished so far, survival rates of 10–40% have been routinely obtained at the larval stage. Despite a scarcity of species-specific nutritional studies, researchers have shown that sablefish can be successfully cultured from the juvenile to the adult stage on commercial salmon feeds. Off-the-shelf salmon feeds have been used successfully in net-pen grow-out trials and are used by commercial producers. In addition, sablefish have proven to be a good cold-water marine model for alternative feeds research. Still, research is needed to optimize nutritional requirements for all life stages of sablefish, develop practical feeds with these nutrient profiles, optimize feeding schedules, and produce life-stage specific diets since the growth of sablefish differs according to size—most likely reflective of their complex life history. Sexually dimorphic growth in sablefish occurs during the typical grow-out period, affecting time to harvest, the proportion of undersized (male) fish, and thus overall economic return to the

producer. Production of all-female monosex offspring at semi-commercial scale using F-1 progeny of neomales (XX males) generated through dietary treatment with 17α-methyltestosterone is now possible. Results of long-term feeding trials suggest that time to harvest at 2.5 kg from stocking at 75 g may be reduced by almost 3 months when monosex stocks are used. Econometric models reveal that internal rates of return are 11–15% higher for monosex relative to mix-sex stocks over a 10-year period under typical cage culture conditions. Sablefish are susceptible to diseases (furunculosis and vibriosis) brought on by atypical *Aeromonas salmonicida* and *Vibrio anguillarum*. Vaccination of sablefish using commercial vaccines to *A. salmonicida* (typical and atypical) has demonstrated that fish can be protected against a subsequent challenge by *A. salmonicida*, but this has only been effective by injection of the vaccine (not immersion) and how long the protection lasts has not been studied. More research is required to develop more effective vaccines, methods for vaccine delivery, and to understand conditions (ontogenetic and environmental) that may promote or enhance pathogenesis.

KEYWORDS

broodstocks, disease, growth, neomales, nutrition, pathogens, sablefish, black cod, yolk-sac fry, larval development, sexually dimorphic growth

1 | INTRODUCTION

1.1 | Sablefish life history

Sablefish (also called black cod) is a long-lived marine species with wide distribution extending from Baja California to Alaska, the Bering Sea, and through to the eastern coast of Japan. Even though their range is extensive, no distinct genetic population structure is evident (Jasonowicz, Goetz, Goetz, & Nichols, 2017). This may be a result of the potential to move great distances as adults (Hanselman, Heifetz, Echave, & Dressel, 2015) as well as movements that are part of the life history of younger sablefish (Maloney & Sigler, 2008). Adults are considered deep-water inhabitants (Sasaki, 1985), and there appears to be a relationship between depth and size with larger fish living at greater depths (Afanasyev, Orlov, & Novikov, 2014; Laidig, Adams, & Samiere, 1997; Sogard & Berkeley, 2017). There also appears to be seasonal movement of adults to deeper water in the winter and shallower in the summer (Karinen, Barnett, & Masuda, 2010). From several studies using popup satellite archival tags, it is evident that adult sablefish undergo diel vertical migrations at certain times of the year that are extensive, spanning ≥250 m daily (Goetz, Jasonowicz, & Roberts, 2018; Sigler & Echave, 2019). This diel vertical migratory activity has been hypothesized to be involved in foraging (Goetz et al., 2018). Spawning sites are unknown for sablefish but are proposed to be deep (Mason, Beamish, & Mcfarlane, 1983) with developing larvae ascending gradually to the surface (Alderdice, Jensen, & Velsen, 1988a; Mcfarlane & Beamish, 1992). There is a pelagic larval phase; and in southeast Alaska, juveniles have

been shown to inhabit inshore areas (Rutecki & Varosi, 1997). The varied life history of sablefish, from larvae to adults, figures prominently in how they are reared in aquaculture operations as discussed in this review.

1.2 | History of sablefish aquaculture

Sablefish is a commercially important species throughout its North American range. The landed weight of sablefish in the U.S. commercial fisheries is not large compared with other species; however, the exceptional value of sablefish has ranked it high compared with other species such as pollock, sockeye salmon, and Pacific cod (Hartley et al., 2020). Sablefish are high in omega-3 fatty acids and have white firm flesh with superior quality and taste, particularly favored in Asia where it is eaten as sushi, sashimi, or in various marinated forms (e.g., gindara misozuke). Current population levels are lower relative to historic ones and harvests have decreased within the last decade. The exceptional value of sablefish, fast growth, and decreases in wild populations have stimulated the development of methods to commercially aquaculture this species.

Aquaculture of sablefish actually began early in the 1970s in the Pacific Northwest with the grow out of juveniles captured from the wild (Kennedy, 1972, 1974). When it was found that significant numbers of juveniles could not be obtained to support an industry, there was a hiatus in their culture until techniques to obtain eggs from wild broodstocks could be developed (Solar, Baker, & Donaldson, 1987). In the 1980s, a sablefish mariculture program was developed at the Pacific Biological Station (PBS; Department of Fisheries and Oceans, Canada) at Nanaimo, British Columbia (Canada), that covered all culture aspects including the collection of adult broodstocks from the wild, spawning induction, egg and larval incubation, and larval and juvenile growth (reviewed: McFarlane & Nagata, 1988). An important part of this program was the contributions of Alderdice et al. (1988a) who developed the techniques to incubate sablefish eggs and defined the timing and stages of sablefish embryonic development (Alderdice, Jensen, & Velsen, 1988b). The contribution of these studies to the culture of sablefish cannot be overstated. At the time of that program, aquaculture of sablefish was projected to be economically feasible given fishing quotas and wild population sizes (McFarlane & Nagata, 1988). There was another hiatus in the development of sablefish culture due to a lack of funding but resumed again in the mid-1990s as a collaboration between PBS and industry. By 1999, significant numbers of juveniles were produced at PBS and at a company called Island Scallops Ltd. (Canada) that enabled grow-out trials to be undertaken at commercial farms (Minkoff & Clarke, 2003). Since the initial development of sablefish aquaculture, nearly all of the commercial production has been in Canada. Although a number of Canadian farm sites have had licenses to produce sablefish, only a small number have actually produced and sold them. Island Scallop Ltd. appears to have been the first company to rear sablefish, but this was followed by Sablefish Canada, Sablefin, and then Golden Eagle Sable Fish Inc. (Sablefin Hatcheries merged in 2008 with Sablefish Canada, which was acquired by Golden Eagle Sable Fish Inc. in 2014: Hartley et al., 2020). Other farms or entities in Canada that produce or have produced sablefish are Totem Sea Farm (now closed) and, more recently, Hub City Fisheries.

In the United States, Troutlodge Marine produced sablefish fingerlings for several years at a hatchery in Brinnon, WA, but there was low demand for them and no serious commercial grow out of sablefish in the continental United States. In 2007, Troutlodge Marine obtained a facilities lease at the Natural Energy Laboratory of Hawaii at Kona, HI, where landside rearing facilities were located. This site had been used previously by a Canadian company, Unlimited Aquaculture Corporation, to raise sablefish in land-based ponds, but they could not raise enough to be commercially viable (Hartley et al., 2020). The Kona site was used by Troutlodge Marine to explore production of market-sized sablefish and to obtain grow-out information. Troutlodge was successful in establishing a sablefish market in Hawaii; however, the cost and logistics of producing fry and transporting them from their Brinnon facility to Hawaii were significant and use of the Hawaii facility for rearing sablefish was discontinued. Currently, there is interest by a business venture composed of Cooke Aquaculture Pacific and the Jamestown S'Klallam Tribe (Sequim, WA) to develop net-pen grow out of sablefish and other native fish species in Puget Sound, WA.

2 | BROODSTOCKS

2.1 | Acquisition

Although captive broodstocks would be the ultimate goal for obtaining gametes for commercial production, sablefish broodstocks have historically been acquired from the wild off the Canadian (McFarlane & Nagata, 1988) and Washington coasts (Cook et al., 2015). In part, this relates to the long time to reach sexual maturity for adults in captivity, and the difficulty in holding large sablefish for many years at conditions conducive to reproductive maturation (see later). Even so, captive broodstocks have been developed by some companies including Golden Eagle Sable Fish Inc. and are supplemented with wild broodstocks during the reproductive season (Hartley et al., 2020). Since sablefish live in deepwater, acquiring broodstocks from the wild has involved commercial fishermen using longlines or pots. Given the depths (≥300 m) at which these fish are caught, it would seem that many problems would arise from the pressure differential between the depths at capture and the surface. However, sablefish do not have swimbladders (Rummer, Roshan-Moniri, Balfry, & Brauner, 2010) or problems with the equilibration of gases in the circulation that are generally observed in deep sea fishes brought to the surface. Further, sablefish are robust and very little mortality is observed when bringing wild sablefish into culture facilities. Thus, acquiring wild broodstocks is generally not a problem. The temperature of the water at which sablefish are captured is 4–5°C (Goetz et al., 2018), so broodstocks are maintained at similar temperatures in aquaculture facilities and this usually requires chilling throughout much of the year. Wild sablefish reproduce in the winter and reproductive life history studies on populations off the Washington State coast indicate that their reproduction is fairly synchronized during January and February (Guzman et al., 2017), though others have suggested that reproduction can extend to March (Bell & Gharrett, 1945; Fujiwara & Hankin, 1988). The onset of ovarian development in populations off the Washington coast begins as early as April with vitellogenesis starting in May and essentially complete by October (Guzman et al., 2017). In males, development begins in April and spermiating individuals are observed in November (Guzman et al., 2017). If broodstock collections are conducted in October off the Washington State coast, then essentially both males and females are very close to reproducing and very little further gonadal development needs to occur in captivity. However, if fish are collected in the spring or summer, then further development must occur (Guzman et al., 2017). At the NOAA Manchester Research Station (NMRS) (Port Orchard, WA), fish are routinely collected in October with great reproductive success from January to March. However, they have also been collected in the spring and held until the following winter. The issue is maintaining them in healthy conditions for over 6 months so that reproduction is not inhibited. Out-of-cycle gonadal development and spawning has been achieved at the NMRS through photoperiod manipulation and is currently used by Canadian producers (Hartley et al., 2020), but the methods have not been published.

2.2 | Spawning induction

Female sablefish do not appear to spawn (release eggs) in tanks in captivity; they need to be hormonally induced to undergo the final stages of ovarian development referred to as oocyte maturation and ovulation (Berlinsky, Kenter, Reading, & Goetz, 2020; Goetz, 1983). Both partially purified salmon gonadotropin (SG-G100) and gonadotropin releasing hormone (GnRH) were first reported to stimulate ovulation of sablefish held in captivity (Solar et al., 1987), but details about spawning and the ability of the eggs to undergo fertilization and subsequent development were not provided. Sablefish are believed to be batch spawners, releasing multiple clutches of eggs during the reproductive season (Hunter, Macewicz, & Kimbrell, 1989). At the NMRS, pelleted salmon GnRH (Ovaplant-Syndell) at a dose of $\sim$50 μg kg^{-1} body weight has been used to initiate the spawning cycle of sablefish (Cook et al., 2015). This generally results in females undergoing oocyte maturation and ovulation within 10–14 days following implantation and females will continue to ovulate multiple batches of eggs at a frequency of 24–48 hr depending on the individual.

The progression of these ovulatory events is followed noninvasively using ultrasonography and when eggs are clearly ovulated and channelized in the ovary, then stripping of the female can occur (Berlinsky et al., 2020; Cook et al., 2015). Not all fish treated in this manner ovulate eggs that have good rates of fertilization and cell division symmetry perhaps indicating different sensitivities of females to the hormone. We have investigated the use of lower pellet doses (25 μg kg^{-1} body weight) of GnRH as well as pre-injections of 10-fold lower doses of GnRH a week prior to the primary pellet implant. Although all three methods induce ovulation, there were not any significant differences in the parameters of spawning such as number of spawns/female or % fertilization or cell division symmetry (Table 1). However, what seems to be different is that pre-injection tightens the time when fish are ovulating eggs and, therefore, can be stripped. When fish are only given implants, some individuals appear to ovulate early, whereas others ovulate later. However, a lower pre-injection appears to synchronize females (Figure 1). The effect of this pre-injection is likely to induce "meiotic" or "ovulatory competence" required for fully responding to the higher implant level (Berlinsky et al., 2020).

2.3 | Fertilization and cryopreservation

Fertilization protocols, as well as methods to cryopreserve sperm, have been developed for sablefish (Immerman & Goetz, 2014; Sanchez-Serrano, Paniagua-Chavez, Segovia, & Weirich, 2014). Sablefish sperms are activated by increased osmolality that is independent of the solute source since even solutions of urea or glucose will activate sperm if high enough in concentration (Immerman & Goetz, 2014). Sperm can be maintained in solutions of sea water diluted to 300 mOsm (Sanchez-Serrano et al., 2014) as well as physiological salines such as modified Cortland's (Immerman & Goetz, 2014). To distribute sperm evenly over the eggs for fertilization, sperm can be diluted in modified Cortland's (Immerman & Goetz, 2014) at pH 7.9 and 5°C, and then distributed over the eggs to which 100% seawater is immediately added at a dilution of 3:1 (volume of seawater:volume of eggs). Males produce copious amounts of sperm that is collected by stripping and can be stored at 5°C under oxygen for up to a week, or in seawater or physiological salines adjusted to 300 mOsm (Sanchez-Serrano et al., 2014). Thus, the amount of sperm used for fertilization is not limiting and generally 1–2 mL/500 mL of eggs is used. However, from our experience using cryopreserved milt, as little as 250 μl of sperm can be used to fertilize 500 ml of eggs (Goetz, 1983). Although males can produce mature sperm when held in culture tanks, they can also be induced with GnRH if necessary.

Cryopreservation protocols for sablefish sperm have been investigated several times with different results. Immerman and Goetz (2014) found that dimethyl sulfoxide (DMSO) provided the best sperm motility after freezing

TABLE 1 Effects of a pre-injection of GnRH at 5 μg kg^{-1} on sablefish spawning induced by a 50 μg kg^{-1} implant and effects of 50 and 25 μg kg^{-1} implants alone

	50 μg kg^{-1} implant	25 μg kg^{-1} implant	Pre-injection[a]
Egg volume (per spawn)	514.3	419.4	483.3
Egg and fluid volume (per spawn)	541.4	448.3	505.0
Fert% (weighted by volume of eggs)	62.2	75.7	66.7
Symmetry (weighted by volume of eggs)	52.7	62.7	77.4
Fert % (unweighted)	63.5	75.8	64.8
Symmetry (unweighted)	52.4	62.9	75.8
Mean days until first spawn	12.6	12.8	13.9
Mean # of spawns >100 ml	2.8	3.5	2.5
Number of fish (*n*)	5.0	10.0	8.0

[a]Pre-injection followed by 50 μg kg^{-1} implant 1 week later.

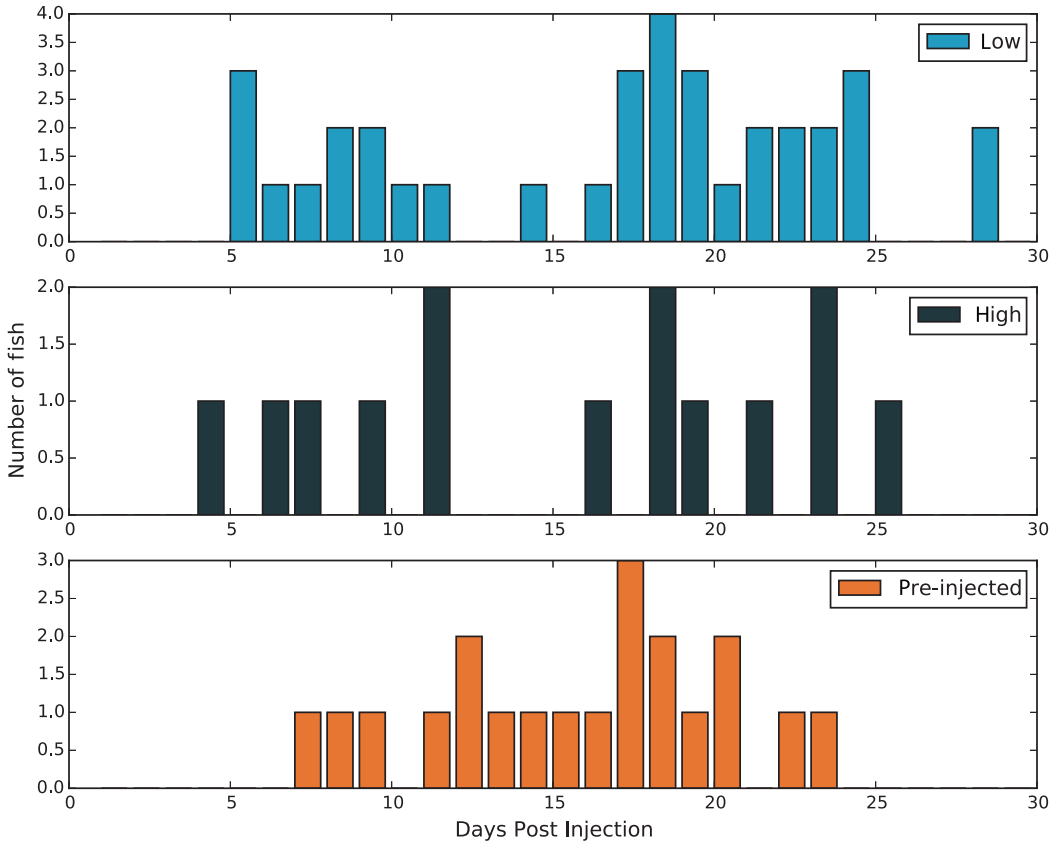

FIGURE 1 Number of fish spawning/day following an ovaplant implant alone 50 μg kg^{-1} (high), 25 μg kg^{-1} (low), and a pre-injection of GnRH (5 μg kg^{-1}) followed by a 50 μg kg^{-1} ovaplant implant

at several rates as compared with propylene glycol or glycerin. In contrast, Sanchez-Serrano et al. (2014) found glycerol to be better than DMSO or methanol as a cryoprotectant. However, the two studies used different physiological extenders and freezing rates so the results cannot be directly compared. At the NMRS, we routinely cryopreserve milt using DMSO diluted to 10% in Cortlands.

3 | EGG AND YOLK-SAC LARVAE INCUBATION

The methods and conditions for incubating sablefish embryos and endogenous feeding "yolk-sac" larvae are based on conditions where they are found in the wild. Sablefish spawning is believed to occur along the continental slope and at depths exceeding 300 m (Mason et al., 1983). The eggs and newly hatched yolk-sac larvae remain at depths greater than 400 m and the larvae eventually come into the surface waters as their yolk reserves are resorbed (Mason et al., 1983). Sablefish eggs are spherical, pelagic, and transparent and average 2 mm in diameter (Alderdice et al., 1988a; Kendall & Matarese, 1987; Mason et al., 1983). Initial efforts to incubate sablefish eggs revealed that they are very fragile, stenohaline, and stenothermal and susceptible to mechanical shock (Alderdice et al., 1988a; Alderdice et al., 1988b). Thus, incubation of eggs and yolk-sac larvae in the hatchery requires precise control of temperature and salinity. Also, since the eggs and larvae are found at depths that exceed the euphotic zone, white light is excluded during the incubation process and only red headlamps are used by staff while tending incubators.

The current hatchery system at the NMRS utilizes recirculated seawater, chilled to 5°C, salinity controlled, filtered to 1 μm, and UV sterilized. The neutral buoyancy of sablefish eggs ranges from 32.6 ppt at fertilization to 34 ppt during incubation and increases just before hatch (Alderdice et al., 1988a). Therefore, salinity in the hatchery is maintained at 33–33.5 ppt in which the eggs are slightly negatively buoyant (Cook et al., 2015). The lower salinity and the addition of air (one bubble/s) allow for better dispersal of eggs within the incubation tanks. After fertilization, the eggs are rinsed and a subsample is removed and placed in a 4°C incubator for approximately 16 hr to determine percent fertilization and cell division symmetry at the 8–16 cell stage (Alderdice et al., 1988a; Cook et al., 2015). Sablefish egg quality is highly variable, and evaluation of fertilization percentage and cell division symmetry is needed to identify groups of eggs for continued incubation. The fertilized eggs are placed into the incubation tank and, if evaluated to be of acceptable quality, are incubated for 10–12 days prior to moving them into yolk-sac tanks (see below).

Conical upwelling tanks with low water exchange rates (one exchange every 2 hr) are typically used for egg incubation of sablefish (Cook et al., 2015; Jensen, Clarke, Whyte, & Damen, 1992). Atlantic halibut hatcheries also use conical upwelling tanks for egg incubation and control the same water quality parameters (Shields, Gara, & Gillespie, 1999) as the two species have similar incubation requirements. During the incubation period, dead eggs are removed using a salt plug procedure (Jelmert & Rabben, 1987), which involves adding approximately 4 L of high saline (50 ppt) water to the bottom of the incubator. Dead eggs have a greater density and sink while the live or viable eggs float above the high saline layer. This procedure helps to maintain water quality during the incubation period. At 2 days prior to hatch, the eggs are floated to the surface using high saline water and collected using a fine mesh net. At this point, the number (by volume) and viability of the eggs are assessed and disinfection with peracetic acid is administered for 60 s at 200 ppm (Cook et al., 2015). The eggs are transferred prior to hatching because newly hatched larvae are extremely fragile and would not survive the transfer procedure.

The eggs are then transferred into the yolk-sac tanks and hatch approximately 2 days later. The yolk-sac tanks are upwelling, cylindroconical tanks with volumes ranging from 600 to 850 L. These tanks are deeper and allow for vertical movement of the larvae during incubation. Water exchange rates in these tanks are further reduced to about one exchange every 6–7 hr due to the fragility of the larvae. Gentle aeration introduced at the bottom (one bubble/s) and at the surface screen helps keep the fragile larvae suspended and from being impinged on the exit screen. Egg shells are removed by surface skimming and is aided by surface aeration. The yolk-sac larval incubation period is long, lasting 35 days after hatch (d.a.h.) at 5°C (Cook et al., 2015). Development rates for eggs and yolk-sac larvae at different incubation temperatures are described by Alderdice et al. (1988a) and Jensen and Damon (2002) and are very useful to identify critical points during the incubation process. During incubation of yolk-sac larvae, salt plugs are done only in the first week post hatch. As the larvae develop the salt plugs are less effective and the larvae are able to cross the salinity gradient. During the remainder of endogenous feeding only minor adjustments to aeration and water flow are made. Siphoning to remove dead eggs and larvae is done as needed. At approximately Day 49 post-fertilization (34 d.a.h.), the hatchery system water is switched to ambient flow-through water to begin the acclimation to the larval live feed tanks. This water source varies in temperature but matches the larval live feed tanks. On approximately Day 50 post-fertilization (245–250°C days), light is introduced to the top of the silo. The larvae swim toward the light and are collected and transferred to the larval live feed tanks. At this point, the yolk-sac has been completely resorbed and the larvae are ready to begin exogenous feeding.

4 | LARVAL REARING

4.1 | Introduction

First-feeding sablefish larvae range from 6 to 9 mm long, weigh ~25 μg/larvae (dry weight), are almost clear and have poorly developed eyesight, digestion, fins, and skeletal muscular system (Cook, Lee, Massee, Wade, & Goetz, 2018; Deary,

Porter, Dougherty, & Duffy-Anderson, 2019). Thus, larvae are initially poor swimmers and hunters and require a specific tank design, tank environment, high densities of nutritious live feeds, and specialized husbandry (Cook et al., 2015). Efforts made from 1970 to 1990 to culture larvae from first feeding to metamorphosis increased the understanding of early sablefish development (Alderdice et al., 1988a; Alderdice et al., 1988b; Clarke, Jensen, Klimek, & Pakula, 1999; Jensen et al., 1992; Kennedy, 1972, 1974); however, they did not result in established larval rearing protocols.

Sablefish larvae have weakly ossified jaw structures and jaw muscle attachments and are, therefore, prone to jaw and cranial malformations (Deary et al., 2019). We experience high rates of these malformations at the NMRS (15–90%—M.A. Cook, personal communication, July 1, 2019) depending on the year. Sablefish larvae develop quickly but still require high feed rates and specialized care until shortly after weaning. A larva is considered weaned and metamorphosed when it can digest and utilize dry feeds, has all its fins, and can feed and swim without added turbidity. Sablefish larvae are considered subjuveniles at this point. The following larval fish section provides an overview of general husbandry much of which has been previously reported (Cook et al., 2015; Lee, Britt, Cook, Wade, Berejikian, & Goetz, 2017; Lee, Cook, Luckenbach, et al., 2017).

4.2 | Physical rearing conditions: tanks

Many experiments at the NMRS are conducted in 500 L cylindrical fiberglass tanks with a dimensional volume of ~740 L (102 cm diameter × 91 cm deep). They have smaller volumes than production tanks (below), but their depth to surface area ratios were determined to work well in a prior tank design study (Cook et al., 2015). Interior tank bottoms are flat and painted with white epoxy paint. Water exits the tank from the bottom center through a 4″ internal center screen that changes in mesh size as the larvae get larger. Production tanks with a diameter of 2.4 m are fiberglass and cylindrical with a dimensional volume of ~5,700 L (244 cm diameter × 122 cm deep) and painted black on the sides with a white bottom. The bottom edge of the tank where the wall connects to the bottom is concave. Survival to weaning in these eight-foot diameter tanks is often better than in the 500 L experimental tanks, and consistently ranges from 15 to 40%.

4.3 | Physical rearing conditions: water turbidity

In contrast to the incubation of yolk-sac larvae, the water supply to externally feeding larvae is flow-through but is still temperature controlled, UV irradiated, and filtered to 1 μm. Like the larvae of many marine fish species, larval sablefish require turbidity from first feeding to weaning onto dry feeds (M.A. Cook, personal communication, July 1, 2019). Turbidity improves the ability of larvae to see their prey and properly orient themselves in rearing tanks (Boehlert & Morgan, 1985; Cobcroft, Shu-Chien, Kuah, Jaya-Ram, & Battaglene, 2012). Without sufficient turbidity, sablefish larvae "wall-nose", feed poorly, and do not survive. For example, rates of larval sablefish wall-nosing were more than triple in water with 2.4 NTU (nephelometric turbidity units) compared to water with 13.9 NTU (Lee, Cook, Luckenbach, et al., 2017). Algae (greenwater) can be used to generate turbidity through the entire larval period, but better larval growth is achieved when algae is replaced with clay (claywater) at the beginning of the second week of the larval period (Lee, Cook, Luckenbach, et al., 2017). Clay is also significantly cheaper than algae (<2% the cost to produce turbid seawater), does not contribute organic matter that will decay in tanks, and has been associated with reduced relative abundance of *vibrio* in larval sablefish as well as other species (Pierce, Lee, Dodd, & Poretsky, 2019; Stuart, Rotman, & Drawbridge, 2016). However, clay should not be used during the first week of sablefish feeding because high mortality rates will result (Lee, Cook, Luckenbach, et al., 2017). Adding algae to clay during that first week will lead to higher survival than clay alone, suggesting that mixtures of algae and clay may be possible during that first week, and that in the first week of the larval period, algae has important beneficial effects beyond turbidity (Lee, Cook, Luckenbach, et al., 2017). For example, some species of algae release dimethylsulfoniopropionate (DMSP), which is a chemical cue

that can stimulate feeding behavior or improve feeding or survival in some planktivorous fishes and birds, including sablefish (Lee et al., 2016).

The algae that has been used to green tanks at the NMRS is a 4:1 ratio of Instant Algae® Nanno 3600, (*Nannochloropsis* paste) and food-safe green dye (Liquid Color Green Shade, ESCO Foods). The clay that has been used is Kentucky Ball Clay OM4 (Kentucky-Tennessee Clay Company, Roswell, GA), both with and without green dye. The greenwater mixture or clay can be pumped directly into the tank and the water inlet system at a rate dependent on the incoming water flow rate. The mixture can also be pumped into the incoming seawater where it mixes prior to reaching the tank, for example, with an in-line static mixer (Cook et al., 2015).

4.4 | Physical rearing conditions: temperature and illumination

Hatchery water temperatures and light intensity are increased through larval ontogeny, mimicking temperature, and light changes in nature. In nature, eggs are fertilized in cold and dark waters at depths greater than 200 m, and slowly rise to the surface during development (Kendall & Matarese, 1987). In the hatchery, fertilized eggs are held in dark chilled water (5°C) through hatching and until yolk-sacs are depleted (Cook et al., 2015). Red headlamps are used during maintenance. Yolk-sac larvae appear to react to red light, but to a much lesser degree than to white light (K.C. Massee, personal communication, February 1, 2013). At yolk-sac depletion, silos are transitioned to ambient water (9–11°C in Puget Sound, WA) over 24 hr and stocked into rearing tanks. Upon yolk depletion, the larvae exhibit positive phototaxis and can be concentrated at the surface for removal by positioning a white light above the water surface.

Rearing tanks are filled with ambient water for the transfer from silos. Temperatures are then gradually increased by one degree per day, until the temperature reaches 14–15°C. During the first week after first feeding, light intensity is maintained at 10–40 lx, measured at the water surface, which appears to aid growth and survival (Lee, Britt, Cook, et al., 2017). This contrasts with the larvae of many other marine fish species, which tend to prefer brighter light intensities. Light intensity is increased to 80–100 lx about 1.5 weeks after stocking. Tank characteristics likely also affect optimal light intensities (Lee, Britt, Cook, et al., 2017). For example, deeper tanks might need brighter light intensities to properly illuminate lower areas of the tanks. Tanks with higher turbidity should also attenuate light more strongly than tanks with lower turbidity. Tanks have been illuminated with LED or fluorescent tubes hung 140 cm above the tank surface and changes in light intensity during rearing have been accomplished using sheets of landscape cloth placed inside the light fixture (Cook et al., 2015).

In the rearing tanks, 15°C is superior to 12 and 18°C (Cook et al., 2018; Lee, Cook, Berejikian, & Goetz, 2017). Compared to rearing at 12°C, higher rearing temperatures up to and including 18°C led to increased short-term growth and shortened the duration of the expensive larval period (Cook et al., 2018). However, the growth advantage in the 18°C treatment was only temporary, and also came with higher mortality. Nine months after temperature exposures, the growth advantage of 18°C fish had reversed itself, and fish from the 15°C treatment had the highest body weight of the three treatments (Lee, Cook, Berejikian, et al., 2017). Eight months after temperature exposures, deformity frequencies varied with larval temperature exposure treatment. Each temperature treatment was associated with higher rates of different deformities—spinal deformities, maxilla deformities, and pelvic fin deformities were highest in 12°C, 15°C, and 18°C treatments, respectively (Lee, Cook, Berejikian, et al., 2017). Nine months after temperature exposures, fish from the 15°C treatment had lower flesh firmness than fish from the 18°C treatment, but the difference was slight and likely undetectable by consumers (Lee, Cook, Berejikian, et al., 2017). Overall, rearing at 15°C leads to better results than 12 and 18°C.

4.5 | Live feeds, feed rates, and timing: rotifer production

Rotifers for sablefish larvae are produced in modified intensive machines originally purchased from Reed Mariculture. The machines consist of a 750 L culture tank and a 500 L biofilter tank. Water in them is partially recirculated, UV-

irradiated, oxygenated, and foam fractionated. Rotifers are cultured in two 1,350 L high-density recirculation systems at 26–27°C and a salinity of 28–29 ppt. Rotifers are cultured with Instant Algae Nanno 3600 (Reed Mariculture). The two systems can produce 1 billion rotifers per day during the intensive rotifer feeding period. Rotifers are harvested each morning from the system based on need and transferred to three, 368 L oxygenated enrichment silos; one silo for each of the three daily feedings. Rotifers are enriched with Ori-Green (Skretting) for the two daily feedings and Algamac 3050 (Aquafauna Bio-Marine, Inc.) for rotifers fed overnight. Enriched rotifers are fed three times daily (~9:00 a.m., 4:00 p.m., and 11:00 p.m.) from 1 to 16 days post first-feeding (dpff). The morning and afternoon feedings are performed by hand. The 11:00 p.m. feeding is delivered to the tanks by a pump on a timer. Experimental and production tanks are fed at a rate of 10–12 rotifers/ml for the two daytime feedings and 12–15 rotifers/ml for the overnight feeding from 1 to 16 days dpff. For production tanks, the rotifer feed rate is sometimes increased to 12–15 rotifers/ml for all three feeding for all 16 days depending on rotifer availability.

At the NMRS, we have compared different rotifer enrichments including Algamac 3050, Ori-green and Spresso (INVE Aquaculture Inc.). In trials using the three enrichments, we found no significant differences in final weights, lengths, and survival between the three (M.A. Cook, personal communication, May 1, 2020). Ori-green, however, was cleaner, easier to use, and enriching with Ori-green could be done in one third of the space and one sixth of the time compared to Spresso and Algamac-3050. As a result, rotifers for morning and afternoon feedings are now enriched with Ori-green and rotifers for the overnight feeding are enriched with Algamac 3050. We have also been testing a prototype automated rotifer machine (Industrial Plankton, Victoria, BC, Canada), which shows promise for producing large quantities of rotifers while minimizing both labor and supply expenses.

4.6 | Live feeds, feed rates, and timing: *Artemia* nauplii production

SEPart *Artemia* cysts (Aquaculture International LLC) are hatched in 650 L heavily aerated cone-bottomed cylinders at 25.6°C for 24 hr. Oxygen is turned on and the *Artemia* are held in the same tank for another 24 hr after hatching. On the morning of the third day, the nauplii and cyst waste are harvested and collected in a screened bucket and the nauplii are enriched for 12 hr at 22°C with Algamac 3050. Enriched *Artemia* nauplii are fed three times daily by hand (09:00 a.m., 4:00 p.m., and 11:00 p.m.) from 16 to ~32 dpff at 15°C. All tanks are fed at a rate of 1.0 nauplii/ml for the two daily feedings and 1.5 nauplii/ml for the night feeding from 16 to 26 dpff. The rate is increased to 1.5 nauplii/ml for all three feedings from 27 to 41 dpff. As with rotifers, different enrichments for *Artemia* have been investigated at the NMRS comparing Algamac 3050, Ori-green and Selco (INVE Aquaculture Inc.). However, no significant differences have been observed on larval growth between them (Cook, personal communication, May 1, 2020).

4.7 | Weaning diets

Sablefish larvae readily wean to dry artificial diets when co-fed during the *Artemia* feeding stage. Otohime™, a krill-based diet, is used at the NMRS as a weaning diet. At 15°C, we generally introduce dry diet at Day 24 and depending on development, larvae are weaned between Days 28 and 32. Production tanks are offered dry feed 24 hr per day via a belt feeder. Experimental tanks are fed during the day by hand or by shaker feeders on a timer. Sablefish larvae do not appear to need 24 hr dry diet feeding to transition off of live feeds. Once transitioned to a dry diet, larvae are kept another 1 or 2 weeks indoors before being graded, counted and moved outside where the feed rate and flows can be increased. Grading at this point is necessary to reduce cannibalism. Once in outdoor tanks, sub-juveniles are fed a mixture of Otohime™ C1 or C2 and BioVita 0.6 mm pellet (BioVita, Bio-Oregon) before being completely transitioned to BioVita. At the NMRS, we have yet to raise sablefish from first-feeding solely on microparticulate diets, by-passing all live feeds. However, replacing the *Artemia* nauplii period with a dry diet suitable for sablefish larvae would reduce costs and labor and potentially improve larval growth and survival. Trials have been conducted in which sablefish larvae were fed rotifers for

21 days, then either Otohime, Gemma Wean Diamond (Skretting) or *Artemia* nauplii (control) until weaning (Day 44). Although weaned by Day 22, larvae in the Gemma treatment did not survive beyond Day 35. Control larvae were significantly heavier than those weaned early to Otohime, but survival at Day 44 was not significantly different (Table 2). Deformities were significantly higher in the early weaning treatment (Table 2). The increased rate of deformities in the Otohime treatment may have been due to reduced feed consumption rather than nutritional differences; however, this was not studied specifically. This study showed that sablefish larvae could be weaned at Day 22 and grown without *Artemia*. Still, more studies are required to find or develop a diet appropriate for the earlier stages of development given the smaller size and higher deformities observed in early weaned fish.

4.8 | Microbial communities

The microbiome can have important effects on the health, growth, and survival of larval fish (Egerton, Culloty, Whooley, Stanton, & Ross, 2018). Two recent studies have shed light on how microbial communities can be affected by interactions among time, the surface on which the community exists, and the use of algae and clay as turbidity agents (Dodd, Pierce, Lee, & Poretsky, 2020; Pierce et al., 2019).

Larval sablefish microbial communities shift over time and development. For example, yolk-sac larvae in silos have different microbial communities than larvae in rearing tanks, a few days after first feeding on live prey (Pierce et al., 2019). Yolk-derived microbes likely influence the microbiomes of these young sablefish. Furthermore, while the environment can influence the sablefish microbiomes, larval skin microbes may in return also influence the environment. The skin of larvae-maintained microbiomes similar to (potentially influenced by) the silos they were hatched in, and after transfer out of silos and into rearing tanks, seawater microbial communities in the rearing tanks became more similar over time to microbiomes on larval skin (Dodd et al., 2020).

Microbial communities are also affected by interactions among the use of algae versus clay as turbidity agents. Seawater microbial communities shift closely with transitions between turbidity agents. For example, the use of algae in the first week of first feeding will lead to a greenwater-typical seawater microbial community, and a switch to clay in the second week will cause a shift to a claywater-typical seawater microbial community (Pierce et al., 2019). However, microbial communities inside sablefish larvae or on larval skin surfaces do not exhibit such a clear shift. Larvae reared with algae or clay in the first week after first feeding will have distinct microbial communities in the two different environments, but a shift from algae to clay for the second week after first feeding will not induce a shift to a clay-typical sablefish microbiome (Dodd et al., 2020; Pierce et al., 2019). Thus, the fish microbiome is more resistant to water additive induced change in the second week after first feeding, when compared to the first week and when compared to seawater microbial communities. Furthermore, sablefish larvae maintained distinct microbial communities from their surrounding seawater environment, although overlap between the seawater and larvae was observed. Another water additive, taurine, was not shown to significantly impact the sablefish microbiome (M.L. Pierce, personal communication, September 23, 2020), providing additional evidence that environment may not have as much of an influence as intrinsic factors.

TABLE 2 Final comparison of sablefish larvae fed *Artemia* nauplii prior to weaning according to a standard protocol versus weaned with Otohime dry diet only

	Control	Otohime
Survival (%)	3.6 ± 3.1	2.3 ± 0.6
Deformities (%)	25.00 ± 8.66a	53.33 ± 3.33b
Wet weight (mg final)	32.60 ± 4.90a	22.75 ± 1.60b
Length (mm final)	26.85 ± 1.39a	22.68 ± 0.72b
SGR	13.53 ± 0.98	11.81 ± 0.09

Note: Different letters in a row indicate significant difference ($p < .05$).
Abbreviation: SGR, specific growth rate.

In addition to the above-described differences, sablefish larvae reared with clay during the first week show greater inter-individual variation in microbial communities than algae-reared larvae, as well as high mortality rates (Pierce et al., 2019). This increased variation may reflect a dysbiosis that stems from the stressful/inferior environment (Pierce et al., 2019).

5 | SABLEFISH FEEDS AND NUTRITION

5.1 | Feeding wild caught sablefish

Early in the investigative stages of sablefish culture, it was realized that sablefish were opportunistic predators and were able to thrive on a variety of fresh diets. In a grow-out study with wild captured, Year-2 sablefish (~300 g starting weight), Kennedy (1972) observed fish grew equally well on herring, dogfish, and mixed diets containing herring and dogfish or chicken offal. Diets containing dogfish had the highest feed efficiency (FE) and fish in most treatments approached or exceeded 3 kg in their second year of captivity, with some reaching the target market size of 4 kg. A formulated salmon feed, the Oregon moist pellet, was also included in the mixed herring and chicken offal treatment at low amounts. Survival was high during the study, but approximately 20% of fish became blind in at least one eye suggestive of a nutrient deficiency in the fresh diets. Gores and Prentice (1984) conducted a similar study with wild captured Year-2 sablefish (mean starting weight 228 g) reared in salmon style net-pens after a 6-month conditioning period in land-based tanks. Fish were fed either herring, juvenile Pacific salmon, or a mix of the two diets for another 2 years in the net-pens situated at the NMRS. Growth was highest among fish that had received the herring diet, followed by the mix diet, and then the salmon diet; however, growth was good in all treatments and mean fish weight exceed 3 kg in all treatments. Again, blindness was observed in a large percentage (38%) of fish by the end of the study. Later, in an energetics study with wild caught adult sablefish (~2 kg starting weight), Sullivan and Smith (1982) observed good growth in the laboratory from a diet of ground mackerel, although growth was improved with the addition of squid to the diet. Adult sablefish fed a large ration (14% wet body weight) every 7–10 days for 8 months showed growth rates two to three times higher than known growth rates for wild fish. In addition, patterns of nitrogen excretion suggested it took adult sablefish up to 5 days to enter a post-absorptive state after a large meal.

Interest in sablefish culture grew as researchers began to observe the rapid growth capability of juvenile sablefish in captivity. In a series of feeding studies with wild caught Year-1 sablefish (starting weights 0.3–16.5 g), Shenker and Olla (1986) observed growth rates exceeding 2 mm per day when fish were fed an ad libitum diet of either brine shrimp, *Artemia salina* or mysid shrimp, *Archaeomysis grebnitzkii*. These high growth rates are rarely observed among marine fish and were the result of large daily rations, in excess of 30% body weight on a wet weight basis, and good feed efficiencies. Similar rapid growth was later observed by Sogard and Olla (2001) among wild caught Year-1 sablefish receiving a formulated salmon feed. Fish were fed either ad libitum or a low (3% body weight per day) ration for 3 weeks at eight different rearing temperatures, spanning 6–24°C. Mean daily growth rates exceeding 2 mm per day were observed in Week 3 of the experiment among fish fed ad libitum at rearing temperatures between 14 and 22°C. Again, fish fed an ad libitum diet were observed to consume large daily rations, which increased with rearing temperature up to a maximum of 40% body weight observed among 16 and 20°C fish. The feed used in these trials was BioDiet (BioOregon, Warrenton, OR), a semi-moist pellet developed for salmon culture containing 45% protein, 15% lipid, and 21% moisture. Feed efficiency (FE) was moderate among fish fed either ad libitum or the low ration between 10 and 20°C. Fish fed ad libitum at 20°C had the highest FE; however, the authors concluded that the high growth rates exhibited by sablefish in this experiment were not driven by particularly high FE, but rather high feed consumption rates.

5.2 | Cultured sablefish and formulated feeds

Early research on sablefish maturation, egg incubation (Alderdice et al., 1988b), and larval rearing (Whyte, Clarke, Ginther, Jensen, & Townsend, 1994) at PBS in the 1980s and then again later in the 1990s (Clarke et al., 1999) led to a supply of

cultured juvenile sablefish that were available for feeds, nutrition, and pilot grow-out studies. McFarlane and Nagata (1988) reported on two early feed studies conducted with formulated dry feeds. The first study evaluated fish growth from a practical dry diet, a purified diet, and a mixed fresh fish diet (60% herring, 25% pollock, and 15% shrimp). Both the practical and purified diets were formulated to 57% protein and 17% lipid. In a replicated study, juvenile fish (mean starting weight ~1 kg) were fed one of the three diets to apparent satiation every other day for 4 months. Fish growth in the mixed fish control tanks was good and approached a mean final weight of 2 kg, while growth of fish receiving the other two diets was similar, but less with mean final weights around 1.6 kg. The second study evaluated the potential of two practical dry diets made from pollock silage (45%), herring meal, and plant proteins against a mixed fish (75% herring, 25% squid) control diet. The three diets were each fed to a tank of juvenile fish (mean starting weight 1.1 kg) to apparent satiation every other day for 106 days. Again, growth of fish fed the control diet was good and approached a mean final weight of 2 kg. Growth of fish fed either silage diet was similar, but less, with mean final weights just under 1.6 kg. The silage diets in the second study, however, showed potential for sablefish culture as the reduced growth observed was associated with an initial reduction in feeding, followed by increased acceptance of the feeds. After 4 weeks, the fish became accustomed to the silage diets and growth rates were then similar to that of fish receiving the control feed.

5.3 | Protein and lipid requirements

Research studies determining nutrient requirements of sablefish are few and life stage specific diets have yet to be developed. Commercial salmon feeds are well accepted by sablefish and support adequate growth in culture (Luckenbach & Fairgrieve, 2016; Luckenbach, Fairgrieve, & Hayman, 2017; Minkoff & Clarke, 2003; Sogard & Olla, 2001). Protein and lipid content of these commercial feeds varied from an advertised composition of 45% protein, 15% lipid to 42% protein, and 33% lipid. A few studies have studied the nutrient requirements of juvenile sablefish (Fairgrieve, Shearer, Kettunen, & Johnson, 2012; Forster, Campbell, Morton, Hicks, & Rowshandeli, 2017; Johnson et al., 2015; Johnson, Fairgrieve, & Freitas, 2013), with the most prominent being the research of Forster et al. (2017), which used a mixture model approach to simultaneously optimize fishmeal, fish oil, and wheat ingredients in experimental sablefish feeds with the goal of predicting ideal relationships of dietary protein, lipid, and carbohydrates for juvenile sablefish. The experimental design, based on preliminary research by Fairgrieve et al. (2012), employed a total of 11 experimental diets that contained different levels of the three test ingredients. The sum of the three test ingredients accounted for 90% of the formulation of the experimental diets, with the remaining 10% consisting of an attractant, binders, vitamins, and minerals. Protein levels ranged from 31% to 43% and lipid levels ranged from 16% to 32%. Juvenile sablefish (mean starting weight 11 g) were fed to apparent satiation twice a day for 11 weeks. Fish growth was excellent during the study, with percent weight gains over 1,000% observed for all 11 diets and fish survival over 99%. Optimal fish growth and FE were associated with the feed containing the highest levels of both fishmeal and fish oil and the lowest level of wheat flour. This optimized feed had a calculated nutrient composition of 40% protein and 34% lipid, suggesting juvenile sablefish are able to utilize far more lipid than what is typical of commercial salmon feeds for this size of fish. Forster et al. (2017) additionally comment that as the optimal diet in this study also contained the highest protein and lipid contents examined, and that additional benefits may be realized with even higher levels of these nutrients. These findings complement those of Fairgrieve et al. (2012), and an earlier energy allocation study by Sogard and Spencer (2004), who concluded that lipostatic regulation of appetite was unlikely in juvenile sablefish and commented that when resources are unlimited, sablefish appear to adopt a maximizing strategy for both somatic growth and lipid accumulation.

5.4 | Alternative feed studies: fishmeal replacement

As sablefish have a natural propensity to consume a variety of diets, the species has proven useful in the evaluation of alternatives to fishmeal and fish oil in marine fish feeds. Global pelagic fisheries are currently fished at or near

maximum sustainable yield and alternatives to fishmeal and fish oil feed ingredients are needed if further development of the aquaculture industry is to be sustained. In addition to accepting a variety of feeds, juvenile sablefish grow extremely well in captivity with weight gains of 300% or higher typical of 8- to 10-week feeding studies. There are few cold-water marine species that grow as fast as sablefish, and differences in fish growth and FE attributable to an alternative ingredient are often easily detected. Early studies performed by Kennedy (1972) and McFarlane and Nagata (1988) showed chicken offal and fish silage, respectively, were readily accepted by sablefish and show potential as alternative ingredients for marine fish feeds. Later, a study by Nicklason, Barnett, Johnson, Tagal, and Pfutzenreuter (2003) demonstrated sablefish juveniles (~100 g starting weight) readily accept and grow well on feeds containing almost exclusively (over 97%) fish silage produced via a modified silage process (MSP) from Pacific sardines, sablefish, Pacific whiting, or spawned chinook salmon carcasses. The MSP, developed at the Northwest Fisheries Science Center (NWFSC), Seattle, WA, employs lower temperatures and higher pHs than that typically used for the production of acidic fish silage. Feed conversion ratios (FCRs) were especially good for the feeds containing sablefish (0.90) and salmon silage (0.93).

In a later study by Nicklason, Xu, Johnson, Sommers, and Armbruster (2016), a new piece of processing equipment was developed that would enable small scale fish producers to co-process fish trim with terrestrial plant proteins into sustainable, alternative feeds for sablefish. A heated ball mill was designed that would grind, pasteurize, and dry fish trim and plant feed ingredients in one step. The technology is scalable and designed for fish producers of more than one species who process their fish on site and wish to render fish processing trim from one species into a feed ingredient for a second species. In a short 4-week feeding study, juvenile sablefish (mean starting weight 58 g) were fed a plant protein diet containing either fishmeal (FM), salmon trim (ST), or a low molecular weight hydrolysate from Pacific whiting trim (LMWH) to apparent satiation every other day. Feed intake and fish growth among fish that received either trim diet was higher than of fish that received the fishmeal diet, with ST fish having the best growth. The ST feed, but not the LMWH feed, had a significantly lower FCR than the FM feed. The ST feed also increased lipid retention and lipid content in whole body tissue. Results from this study demonstrated heated ball mill processing of salmon fish trim waste or enzyme hydrolyzed whiting trim can increase the performance of alternative plant-based feeds for sablefish.

As mentioned previously, feeds containing high levels of marine proteins and oils are well utilized by sablefish, but are expensive, prone to spoilage, and represent potential barriers to the continued expansion of sablefish aquaculture. Through a series of alternative feeds studies at our laboratory, we have found sablefish readily accept feeds where soybean and corn protein concentrates replace the majority of fishmeal in the formulation. These plant-based feeds are naturally low in taurine and were useful in determining the nutrient requirement of taurine for juvenile sablefish (Johnson et al., 2015). Juvenile sablefish (mean starting weight of 52 g) were fed seven experimental feeds with taurine levels ranging from 0.1% to 5.8% taurine to apparent satiation every other day for 8 weeks. Fish grew well during the study with survival at 100%. The addition of taurine to the feeds significantly increased both weight gain and fish length. Feed efficiency and protein retention also significantly improved with the addition of taurine to the feeds. However, benefits to growth and FE were reduced at the highest dietary taurine concentrations examined. Peak weight gain and FE were estimated at 1.5% and 1.1% dietary taurine, respectively. Tissue taurine concentrations increased asymptotically with increasing dietary taurine supplementation and sablefish muscle became saturated at 0.34% taurine. In addition to improving nutrient utilization and protein retention in alternative plant-based feeds, follow-up electrophysiological studies by Sommers and Johnson (2016) suggest taurine may be physiologically essential for proper olfaction in sablefish.

5.5 | Alternative feed studies: Fish oil replacement

Alternative lipid studies with sablefish have evaluated the potential of replacing a portion of the added fish oil in sablefish feeds with plant oils, poultry fat, and blends of plant oils and novel algal and fungal oils containing long

TABLE 3 Experimental plant-based feeds fed to juvenile sablefish in alternative lipid studies (Johnson et al., 2013; Rhodes, Johnson, & Myers, 2016)

	Plant protein feeds				
	Corn oil	Flaxseed oil	Fish oil	Corn + DHA/ ARA oils	Flaxseed + DHA/ ARA oils
Ingredient (g kg^{-1})					
Fishmeal	90	90	90	90	90
Soy protein isolate	300	300	300	300	300
Corn gluten	260	260	260	260	260
Wheat flour	134	134	134	134	134
Fish gelatin	20	20	20	20	20
Corn oil	127	–	–	88	–
Flaxseed oil	–	127	–	–	88
Fish oil	–	–	127	–	–
DHA algal oil	–	–	–	35	35
ARA fungal oil	–	–	–	4	4
Choline	5.0	5.0	5.0	5.0	5.0
Betaine	2.5	2.5	2.5	2.5	2.5
L-Methionine	1.6	1.6	1.6	1.6	1.6
L-Lysine	2.0	2.0	2.0	2.0	2.0
Taurine	0.6	0.6	0.6	0.6	0.6
Vitamin pre-mix[a]	15.0	15.0	15.0	15.0	15.0
Mineral pre-mix[b]	1.0	1.0	1.0	1.0	1.0
Stabilized vitamin C[c]	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	20.0	20.0	20.0	20.0	20.0
Lignin sulfonate	20.0	20.0	20.0	20.0	20.0
Proximate analysis (%)					
Lipid	16.7	15.2	17.1	16.2	15.5
Protein	51.7	50.2	51.4	51.0	50.1
Ash	3.7	5.6	4.6	4.7	4.9
Moisture	11.7	11.8	11.7	12.0	12.0

Note: ARA, arachidonic acid; DHA: docosahexaenoic acid.
[a]USDA-ARS Vitamin Premix #702. Contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K_3 1.1 gm: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5 mg; cyanocobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5 mg; and inositol 600 mg.
[b]USFWS Mineral Premix #3. Contributed, per kg diet; zinc 75 mg, manganese 20 mg, copper 1.5 mg, and iodine 10 mg.
[c]L-Ascorbyl-2-polyphosphate, 35% ascorbic acid activity.

chain polyunsaturated fatty acids (LC-PUFAs, Friesen, Balfry, Skura, Ikonomou, & Higgs, 2013a, 2013b, Johnson et al., 2013, Rhodes et al., 2016). In an effort to reduce cost and contaminant levels in sablefish feeds, Friesen et al., 2013a, investigated the potential of using cold pressed flaxseed oil to replace up to 75% of the added fish oil in sablefish feeds. Juvenile sablefish (mean starting weight 154 g) were fed one of four experimental feeds containing anchovy meal and a blend of anchovy and flaxseed oils to apparent satiation twice a day for 15 weeks. The feeds were balanced for protein and lipid content at 46% protein and 20% lipid. The added anchovy and flaxseed oils

accounted for a total of 13% of the formulations. As flaxseed oil increased in the formulation, levels of persistent organic contaminants (polychlorinated biphenyls, PCBs, and polychlorinated dibenzo-*p*-dioxin/dibenzofurans, PCDD/Fs) in the feeds were reduced. Fish survival was 100% during the study and fish growth was moderate, approaching 300% weight gain in all treatments. Fish growth and FE were similar across treatments and levels of PCBs and PCDDs in the edible flesh were significantly reduced with the addition of flaxseed oil to the feeds. In addition, as flaxseed oil was added to the diets, levels of the long-chain polyunsaturated n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the edible flesh were reduced with concomitant increases in levels of the medium chain n-3 fatty acid, alpha linoleic acid (ALA). In conclusion, the authors (Friesen et al., 2013a) found flaxseed oil to be a suitable replacement for fish oil in sablefish feed up to 75% replacement. Such replacement results in a seafood product with slightly elevated levels of total n-3 fatty acids and reduced levels of persistent organic contaminants.

In a follow-up study, Friesen et al., 2013b further investigated the potential of replacing up to 75% of the added fish oil in sablefish feeds with poultry fat or blends of poultry fat and cold-pressed flaxseed oil. Larger juvenile sablefish (mean starting weight 422 g) were fed one of four experimental feeds to apparent satiation for 15 weeks. Similar to their previous study, feeds were formulated to 46% protein and 19% lipid and differed only in the type of added oil. Poultry fat was selected for this study due to its lower cost than plant oils. The added oils in the four diets were 100% anchovy oil; 25% anchovy oil with 25% poultry fat and 50% flaxseed oil; 25% anchovy oil with 50% poultry fat and 25% flaxseed oil; and 25% anchovy oil with 75% poultry fat. Fish survival was excellent, with over 98% survival in all treatments and fish growth was fair. The authors commented that unlike their previous study, feed intake with larger fish was inconsistent and appeared to be on a 2-day cycle, with fish consuming over 2% of their body weight on the first day and then less than 1% of their body weight on the second day. In studies at the NWFSC, we have similarly observed feed intake to be the most consistent when larger fish are fed every other day. Again, fish growth and FE were similar between treatments. There were noticeable differences in the fatty acid profiles of the edible flesh with the ratio of n-3 to n-6 fatty acids decreasing as poultry fat was added to the formulation, from a high of 3.88 for fish fed the 100% anchovy oil diet to 1.32 for fish fed the 75% poultry fat diet. The authors concluded that either poultry fat or blends of poultry fat and flaxseed oil were suitable replacements for fish oil in sablefish feeds and that further research is needed to optimize feeding schedules and feed efficiencies with larger fish.

In an effort to concomitantly reduce the levels of fishmeal and fish oil in sablefish feeds and increase the overall sustainability of sablefish culture, a study was conducted at the NWFSC investigating the effects of replacing the added fish oil in a plant-based sablefish feed with either corn or flaxseed oil, both alone or supplemented with terrestrially produced, specialty oils containing the LC-PUFAs, DHA, and arachidonic acid (ARA). The DHA oil was obtained from microalgae (DHASCO, Martek Biosciences, Columbia MD), whereas the ARA oil was obtained from fungi (VEVODAR, DSM Nutrition Products, Basel, Switzerland). As with the abovementioned studies by Friesen et al. (2013a; 2013b), the five experimental feeds had identical formulations with the exception of the added oil component and were identified as corn oil (C), corn oil plus LC-PUFA oils (C+), flaxseed oil (F), flaxseed oil plus LC-PUFA oils (F+), and fish oil (FO) feeds (Table 3). Feeds were formulated to contain less than 1% residual fish oil from 9% fishmeal in the formulation and contained 51% protein and 16% lipid. Juvenile fish (mean starting weight 38 g) were fed 3 days a week at an average ration of 1.125% body weight per day for 12 weeks. Feeding at this reduced rate ensured all feed was readily consumed by the fish. Fish were weighed every 2 weeks and the feed ration adjusted, accordingly. Fish grew at a moderate rate during the 12-week growth trial and survival was high with only three mortalities across all treatments. Growth was significantly influenced by treatment (Table 4). Fish fed plant oil feeds without the LC-PUFA supplements gained less weight than fish that had received either the supplemented feeds or the feed containing fish oil. There was no difference in weight gain between C+, F+, or FO fish. Corn, C, fish weight gain was significantly less than that of F fish at the end of the experiment, although growth between the two treatments was similar through Week 10 (Figure 2). Feed conversion ratio was inversely related to fish growth and ranged from 1.08 for F+ fish to 1.42 for C fish with significant difference between treatments (Table 4). There were also

TABLE 4 Fish growth, feed performance, and whole body proximate composition of juvenile sablefish fed plant-based feeds with alternative lipid ingredients (Johnson et al., 2013)

	Final fish, Week 12				
	Plant protein feeds, *N*=4				
	Corn oil	Flaxseed oil	Fish oil	Corn + DHA/ARA oils	Flaxseed + DHA/ARA oils
Initial weight (g)	37.9 ± 1.0	38.7 ± 0.8	37.9 ± 0.5	37.9 ± 1.5	38.1 ± 0.6
Final weight (g)	75.8 ± 1.8a	82.3 ± 2.8b	86.6 ± 1.0c	86.6 ± 1.6c	87.7 ± 0.6c
Weight gain (g)	37.9 ± 2.5a	43.6 ± 2.7b	48.7 ± 1.4c	48.7 ± 1.2c	49.6 ± 0.9c
CF	1.03 ± 0.03b	1.01 ± 0.02b	0.93 ± 0.01a	0.96 ± 0.02a	0.94 ± 0.01a
TGC	0.83 ± 0.05a	0.92 ± 0.05b	1.01 ± 0.03c	1.01 ± 0.03c	1.02 ± 0.02c
FCR	1.42 ± 0.09c	1.24 ± 0.08b	1.10 ± 0.03a	1.10 ± 0.03a	1.08 ± 0.02a
Proximate composition analysis (g kg^{-1})					
Lipid	83 ± 3a	103 ± 5b	100 ± 4b	96 ± 3b	97 ± 4b
Protein	127 ± 3a	135 ± 3b	143 ± 1c	139 ± 1bc	138 ± 5bc
Ash	19 ± 1	20 ± 1	21 ± 1	21 ± 1	20 ± 1
Moisture	770 ± 6b	740 ± 10a	736 ± 9a	745 ± 3a	744 ± 7a

Note: Within a row, different letters denote results that are significantly different between feed treatments ($p < .05$).
Abbreviations: ARA, arachidonic acid; CF, condition factor; DHA, docosahexaenoic acid; FCR, feed conversion ratio; TGC, thermal growth coefficient.

differences observed in fish condition factor between treatments. Condition factor was the highest among fish that had received the nonsupplemented plant oil feeds. The addition of DHA/ARA supplements to the plant oil feeds resulted in a significant decrease in condition factor. There was no difference in condition factor between fish that had received either of the supplemented feeds or the feeds containing fish oil.

Whole body lipid, protein, and moisture content were influenced by treatment (Table 4). The lipid content of C fish was less than that of F, FO, C+, and F+ fish, which were similar. Moisture content showed a inverse trend to lipid content and was greatest among C fish. Like whole body lipid, the protein content of C fish was less than all other treatments. The protein content of F fish was less than that of FO fish, but similar to that of C+ and F+ fish. Although we did not establish a nutrient requirement for LC-PUFAs for sablefish in this study, the restoration of growth and FCR by adding DHA and ARA to the flax and corn oil feeds suggest a dietary requirement for one or both of these long-chain fatty acids for sablefish. The study also demonstrated the potential to formulate marine fish feeds exclusively with terrestrially produced oils.

A follow-up study by Rhodes et al., 2016 detected adverse histomorphological changes in sablefish liver and intestinal tissue as well as changes in the gastrointestinal microbiome associated with sablefish fed the abovementioned alternative plant-based feeds that were deficient in LC-PUFAs and taurine. Juvenile sablefish (mean starting weight 36 g) were fed either the corn or flaxseed feeds listed in Table 3 or a salmon reference feed (BioBrood, Bio-Oregon, Longview, WA) for 8 weeks. The top three ingredients listed for the salmon reference feed were fishmeal, poultry meal, and marine fish oil. Fish growth was good among all treatments and fish survival was 100% during the study. Final fish weight and length were higher among reference fish and while not significant, condition factor showed an opposite trend and was lowest among reference fish. Intestinal mucosae were significantly less vacuolated among fish receiving the plant oil feeds and the frequency of intestinal mucous cells was reduced. Severe bile duct hyperplasia and hepatocellular lesions were present only in corn and flaxseed fish, indicating these feeds may be deficient in some nutrient or possibly harmful to sablefish. Significant differences in gastrointestinal microbiomes were present between all three treatments, with corn fish showing much less diversity than the other diets. Results demonstrated that diet-induced shifts in microbiome can occur quickly in sablefish but may not be quick enough to overcome nutritional deficiencies. Further studies are needed to identify whether restoring fish growth in these alternative feeds through the use of LC-PUFA supplements can reduce the observed adverse liver pathologies and changes in gut microbiome.

5.6 | Current alternative feeds research with sablefish

Recent alternative feeds studies at the NWFSC have explored the potential of full fat soybeans (Nicklason, Johnson, & Marancik, 2020), macroalgae (Johnson et al., 2020), and insect meal (Anulacion -personal communication) in alternative feeds for sablefish. Using heated ball mill processing, Nicklason et al. (2020) found sablefish readily accept and grow well on a diet containing approximately 20% full fat soybeans after processing with wet heat. Three alternative feeds were prepared for sablefish with Pacific whiting processing trim and either heated soybeans (HSB), minimally processed soybeans (SB), or a soybean protein concentrate (SPC). Juvenile fish (mean starting weight 120 g) where fed one of the three feeds to apparent satiation every other day for 8 weeks. Feed consumption and fish growth was good among HSB and SPC fish and higher than that observed for SB fish. At the conclusion of the study, histomorphologic evaluations of the distal intestines of final fish were conducted. Varying levels of inflammation were measured for all three treatments. Fish receiving the HSB feed had similar intestinal inflammation to that of SPC fish and significantly lower inflammation than that of SB fish. Results indicate that properly processed full fat soybeans perform as well as more expensive soybean protein concentrates for this marine species and that sablefish may be tolerant to some anti-nutritional biomolecules (e.g., oligosaccharides and saponins) found in full fat soybeans that reduce feed performance in other cultured species, such as Atlantic salmon. An economic analysis of feed costs revealed coprocessing of fish trim with full

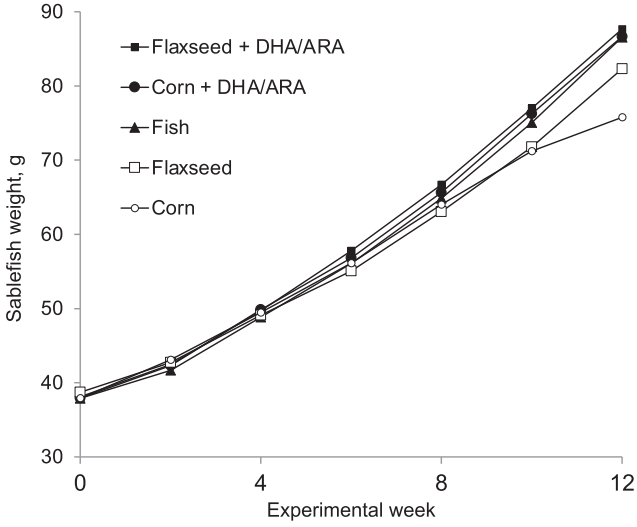

FIGURE 2 Growth of juvenile sablefish fed experimental plant-based feeds containing different added oils. Fish were fed 3 days a week at an average ration of 1.125% body weight per day for 12 weeks. Fish were weighed every 2 weeks and feed rations were adjusted, accordingly

fat soybeans shows great potential as an affordable process for rural or small-scale feed production for sablefish (Nicklason et al., 2020).

We are currently evaluating the potential of locally sourced green, red, and brown macroalgae species in plant-based feeds for sablefish at the NWFSC. In contrast to terrestrially produced plants, macroalgae needs no fresh water for growth and its culture is more environmentally sustainable for some regions. The most promising species investigated to date is the red macroalage Turkish Towel, *Chondracanthus exasperates*, which is a good source of dietary taurine (0.8%) with moderate (24%) protein (Johnson et al., 2020). In a 3×2 experimental design, Turkish Towel and taurine were added to plant-based feeds for juvenile sablefish to evaluate the potential of these ingredients, alone or combined, to increase fish growth and feed performance. Juvenile sablefish (mean starting weight 49 g) were fed one of six experimental feeds containing Turkish Towel at 0, 5, or 10% inclusion, with or without taurine supplementation (1%) to apparent satiation, every other day for 8 weeks. Fish grew well during the study with weight gain approaching 300% for the fastest growing treatments. Feed intake and fish growth increased with the addition of taurine and, to a lesser extent, Turkish Towel to the experimental feeds. Liver histomorphology of final fish was generally normal; however, evidence of cellular alteration was present in some fish by the end of the experiment. The number of fish affected was lower among fish receiving feeds containing Turkish Towel. In particular, fish with hepatocellular nuclear pleomorphism and clear cell foci pathologies were significantly fewer among Turkish Towel fish and there was a trend towards lower amounts of hepatocellular karyomegaly, although it did not reach statistical significance. The addition of taurine to experimental feeds had no effect on liver histomorphology. Overall, results from this study reaffirm taurine supplementation to be beneficial to sablefish receiving plant-based feeds and indicate Turkish Towel may improve fish health and be a promising functional feed ingredient for cold water marine fish.

6 | GROWTH

In the aquaculture environment, 5–6 years of growth in wild sablefish (Echave, Hanselman, Adkison, & Sigler, 2012) can be compressed into an approximately 2-year grow-out period leading up to harvest of fish at ~2.5 kg. This section focuses on methodologies and technological advancements related to sablefish growth during the early juvenile and grow-out phases, the latter of which is typically conducted in net-pens or large land-based tanks.

6.1 | Early juvenile rearing

Early juveniles (also known as "post larvae") are typically ready for transfer to outdoor tanks during late spring (~100 days post fertilization) when they are fully weaned to formulated feed and average 0.3–0.5 g. At this size, sablefish fry are robust, mortality rates low, and their gonads sexually undifferentiated (Luckenbach & Fairgrieve, 2016). Fish are distributed (N = 750–1,000) to 1.5 m diameter (1,550 L) tanks located outdoors under a shade structure at the NMRS. Each tank is continuously supplied with ambient temperature (10–14°C), filtered seawater at a rate of 15 L/min for the duration of the tank-rearing phase.

Cannibalism is a problem until the fish reach an average weight of 20–30 g and controlled by two methods. First, they are manually sorted to separate large from small individuals starting at weaning, and periodically thereafter based on feeding behavior and agonistic behavior among fish in each tank. Sablefish do not establish hierarchies or become territorial and can be reared at high stocking densities, provided that size variance is low. At the NMRS, densities of 16–20 kg/m^3 for 40 g fish are typical. Second, feed availability is managed to maintain near satiety of the fish at all times. To accomplish this, we use a temperature-dependent feeding model based on the delta-L method (Buterbaugh & Willoughby, 1967) to calculate a minimum daily ration which is continuously delivered to the fish via clockwork, belt feeders (Pentair Aquatic Eco-systems) 24 hr/day, 7 days per week. Additional feed is presented to the fish by hand several times during working hours so the operator can evaluate appetite and directly observe the fish for signs of agonistic behavior. Feeding schedules are adjusted according to actual growth data collected during biweekly sampling. There is no specialized feed for sablefish on the market today, but dry commercial salmon fry feeds are well accepted by the fish and support rapid growth (see 5. Sablefish Feeds and Nutrition).

At the NMRS, juvenile sablefish grow rapidly, averaging about 40 g after 70 days (Figure 3). They are then manually sorted and excessively small (underperforming) fish or those having head, jaw, and/or spinal deformities that may inhibit feeding, growth, or marketability are often culled. Typically, this may reach 15–20% of the total number of fish at this stage. The fish are vaccinated and held in larger tanks (4.4 × 1.2 m tanks filled to 14 m^3 with inflow of ~115 L/min) until they average 75–150 g and then transferred to net-pens (12 × 12 × 6 m deep, enclosed within a predator/escapement-prevention cage) for grow out. Stocking for grow out usually takes place in late fall.

6.2 | Sexually dimorphic growth

Early in our development of sablefish aquaculture, it was recognized that this species exhibits what is known as sexually dimorphic growth or sexual growth dimorphism. Sexually dimorphic growth is common in fishes, with some species exhibiting higher growth rates in females and others in males (Martinez et al., 2014; Mei & Gui, 2015). Sablefish do not have external, secondary sex characters that allow sex to be visually distinguished; however, fisheries data for wild sablefish clearly show that females outgrow males (Echave et al., 2012; Fujiwara & Hankin, 1988; Mason et al., 1983). In accordance, several studies conducted in captivity at the NWFSC have demonstrated that female

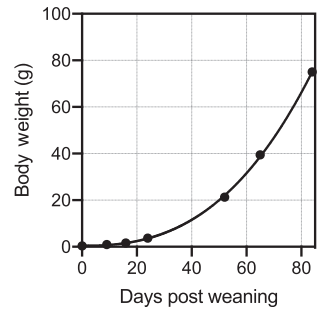

FIGURE 3 Typical growth pattern of early juvenile sablefish after weaning to formulated feed. Fish average 0.3–0.5 g when stocked into outdoor tanks and are provided dry salmon fry feeds on a daily basis until they are 60–80 g, when they are sorted and vaccinated before stocking into net pens or larger tanks for grow out

sablefish indeed grow faster than males and that sexually dimorphic growth occurs during the typical grow-out period (Figure 4; Luckenbach et al., 2017 and unpublished data), thereby influencing the economics and potential profitability of sablefish aquaculture (Luckenbach et al., 2017; Hartley et al., 2020; see Section 8). The desire to produce greater numbers of faster growing females, and avoid slow growing males, was the basis of the pursuit of methods for all-female (monosex) production of sablefish.

6.2.1 | Monosex female production

Monosex female stocks of fish may be obtained by either a direct or indirect strategy (Donaldson, 1996; Piferrer, 2001). Direct feminization typically entails treating fish directly with sex steroids either by immersion of the fish in water containing sex steroids or feeding them a diet supplemented with steroids to steer differentiation of the gonads toward the female path. Treatment with natural or synthetic estrogens has been widely used for this purpose (Devlin & Nagahama, 2002; Pandian & Sheela, 1995).

Indirect feminization, on the other hand, avoids direct exposure of the farmed fish to exogenous treatments and instead operates through the broodstock line (Figure 5; Luckenbach et al., 2017). A key step in this process is the production of what are known as "neomales." These are genetically female fish (i.e., XX genotype, for species that have an XX/XY-type system of sex determination) that are induced to develop as males through application of a masculinizing treatment during early development (Devlin & Nagahama, 2002; Piferrer, 2001). Sex phenotype is much more plastic in fishes than in mammals, for example, and there is typically a period of sexual lability from approximately first feeding of larvae to the initiation of morphological sex differentiation of the gonads (i.e., before ovaries and testes can be distinguished via histology). Treatment with masculinizing factors such as androgens, aromatase inhibitors, or even high temperature during this period can redirect sex differentiation and induce testicular development in genetic females (Baroiller, D'Cotta, & Saillant, 2009; Luckenbach & Yamamoto, 2018). Once neomales ultimately reach sexual maturity, often years later, they can then be used as broodstock and crossed with normal female broodstock to, in principle, produce monosex female offspring. In long-lived species like sablefish, neomale broodstock can be used year-after-year for spawning trials or their sperm may be cryopreserved for later use (see Section 2.3). Although methods have been developed for both direct and indirect feminization of sablefish (Luckenbach et al., 2017), due to regulatory restrictions, indirect feminization is thought to hold the most promise for commercial use by the U.S. aquaculture industry.

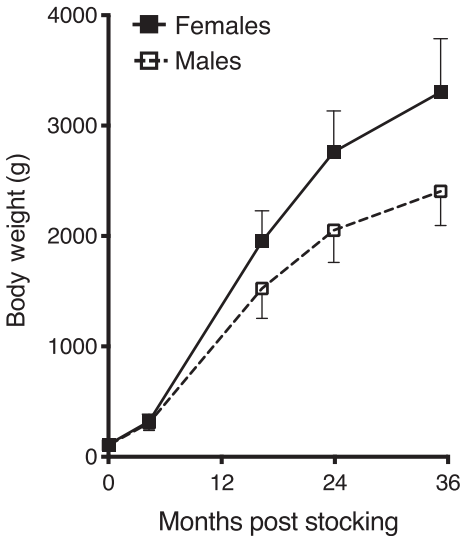

FIGURE 4 Sexually dimorphic growth pattern of female and male sablefish reared in a net-pen system at the Northwest Fisheries Science Center's Manchester Research Station (Port Orchard, WA). Data plotted are mean ± *SD*. Sex was determined using a PCR-based sex marker and confirmed with gonadal histology. See Luckenbach et al. (2017) for details

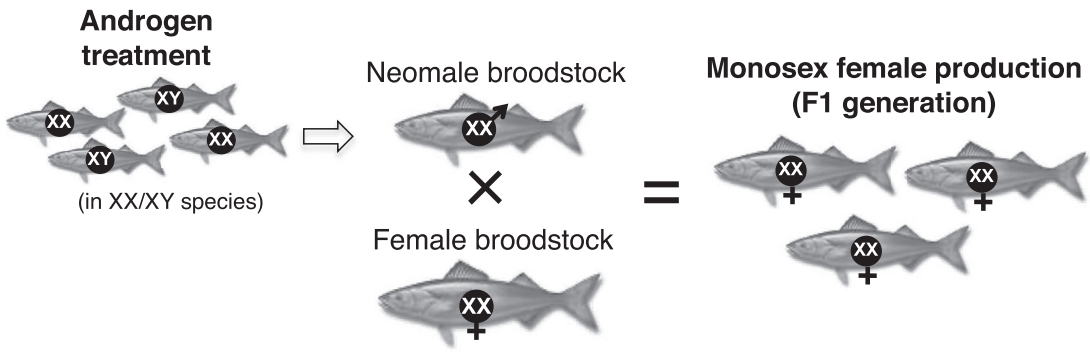

FIGURE 5 Schematic diagram of the process of indirect feminization used for monosex female production of sablefish for aquaculture. Genetic female (XX-genotype) fish are sex reversed to phenotypic males, known as neomales, by dietary androgen (methyltestosterone) treatment. Milt from these fish can then be used to fertilize eggs from female broodstock and produce all-female offspring in the F1 generation (see Luckenbach et al., 2017 for details)

Neomale broodstocks have been successfully generated at the NWFSC using dietary treatment with the synthetic androgen, 17α-methyltestosterone (MT; Luckenbach et al., 2017). The developmental timing of MT treatment in sablefish was critical to attain complete masculinization. When the initiation of treatment was too late in development, or duration of exposure too brief, the gonads of MT-treated genetic females developed ovaries or residual ovarian characteristics (Luckenbach et al., 2017; Luckenbach & Fairgrieve, 2016). Furthermore, an effective amount (dosage) of MT must be applied to induce complete masculinization while also avoiding sterilization, which can occur with excessive MT exposure (Luckenbach & Fairgrieve, 2016; reviewed by Pandian & Sheela, 1995). The current working protocol for sablefish neomale production is 5 mg MT/kg of feed for a period of 4 months, beginning shortly after weaning and continuing until they reach a size of ~250 mm (Luckenbach et al., 2017).

Importantly, 100% female offspring were obtained from neomale × female crosses in sablefish (Figure 5; Luckenbach et al., 2017 and subsequent demonstration trials). This indicated that sablefish possess an XX/XY system of sex determination in which the male is heterogametic (XY). In addition, the female phenotype of offspring obtained from neomale × female crosses was confirmed to be maintained throughout grow out, as only females were observed at harvest.

The process of indirect feminization of sablefish began with MT treatment of a mixed genotypic sex (XX and XY) stock of fish; however, another viable approach that has not been investigated in sablefish is induction of diploid gynogenesis to generate all-XX genotype larvae prior to dietary administration of MT (reviewed by Felip, Zanuy, Carrillo, & Piferrer, 2001; Devlin & Nagahama, 2002). This would ensure that all MT-treated fish were genetically female and thus had the potential to develop as neomales if masculinization were successful. This would preclude the need for genetic sexing of putative neomales (Luckenbach & Fairgrieve, 2016) and ultimate culling or repurposing of MT-treated, XY-genotype individuals.

Low proportions of neomales have also been produced by exposure of post-weaned, XX-genotype sablefish larvae to high water temperatures (~22°C; Huynh, Fairgrieve, Hayman, Lee, & Luckenbach, 2019). High temperature has been demonstrated to have masculinizing effects in numerous fishes (Baroiller et al., 2009; Luckenbach & Yamamoto, 2018; Ospina-Alvarez & Piferrer, 2008) and could potentially circumvent the use of MT for sablefish neomale production. However, further investigation is needed to determine whether this approach could be optimized to produce higher proportions of neomales and avoid increased rates of morphological deformities associated with high temperature exposure (Huynh et al., 2019).

In addition to achieving monosex female production for sablefish aquaculture, the sex control and genetics research described earlier, and conducted by other labs, has generated a tremendous amount of biological information and tools

related to sablefish reproductive and growth physiology and genetics. For example, a diploid number of 48 chromosomes and an XX/XY system of sex determination was established (Luckenbach et al., 2017; Phillips, Faber-Hammond, & Luckenbach, 2013), the processes of ovarian and testicular differentiation were characterized at both the molecular and morphological levels (Hayman, Fairgrieve, & Luckenbach, 2021; Luckenbach & Fairgrieve, 2016), and reliable genotypic and phenotypic sex markers were identified (Fairgrieve, Shibata, Smith, Hayman, & Luckenbach, 2016; Hayman et al., 2021; Rondeau et al., 2013; Smith, Guzman, & Luckenbach, 2013). This R&D is valuable to ongoing research to refine methods for neomale sablefish production and induction of maturation (see Section 8).

6.3 | Grow out in net-pens

A typical pattern of growth for mixed-sex (both males and females) and monosex (female only) stocks of sablefish reared under research conditions at the NMRS is depicted in Figure 6. Mixed-sex and monosex fish stocked in the late fall (Day 0) grow equally well through the first winter (~Day 75) and spring (~Day 170). Subsequently, the weight gain in male fish slows dramatically compared with females. Net productivity of a mixed-sex stock is reduced both by an increased time (~3 months) to reach an average harvest size of 2.5 kg and associated losses due to higher total mortality and a greater proportion of undersized, mostly male fish in the mixed-sex population (Hartley et al., 2020).

Based on this simulation, we conducted a grow-out trial with monosex female sablefish, produced via neomale × female crosses, under semi-commercial conditions. The goal was to evaluate the growth rate, survival to harvest, and dress-out yield of a monosex female stock. In mid-December, fish averaging about 135 g were stocked into net pens at an initial density of 6.1 fish/m^3 for rearing to a desired average weight of 2.5 kg at harvest. Optimum stocking densities have not been determined for sablefish, so pens were stocked so that the final density would not exceed 15 kg/m^3, which is typical for Atlantic salmon in the Pacific Northwest. Fish were fed by hand to apparent satiation by trained operators on a daily basis. Given that there are no performance optimized diets for sablefish grow out available on the market, commercial, high-energy diets (ranging from 46% protein with 27% lipid to 42% protein with 33% lipid, depending on pellet size), formulated for Atlantic salmon grow out and containing low levels of fish meal and oil were used for this trial. The fish were periodically sampled for growth and pellet size adjusted according to the feed manufacturer's recommendations for salmon.

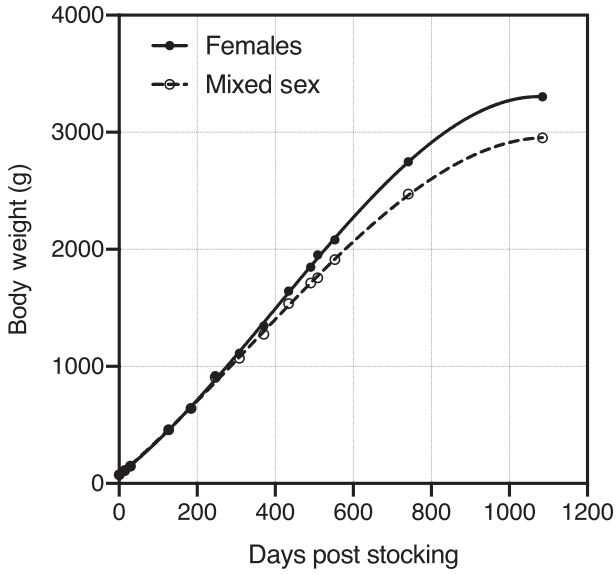

FIGURE 6 Growth curves for farm-raised sablefish modeled using data for males and females in mixed-sex populations across several trials. The average size at stocking was 75 g. Based on these models, the average individual in the monosex female stock will reach 2.5 kg in 667 days, while the average individual in the mixed-sex stock will attain that same weight in 760 days. This represents a 12.2% reduction in required grow-out days to attain an average market weight of 2.5 kg

The sablefish grew rapidly and attained an average weight of 2.48 kg in May, about 500 days post stocking (Figure 7; Weidenhoft, 2017). Chronic, low-level mortality was observed from the start of the trial, with most moribund or dead fish exhibiting external lesions characteristic of furunculosis, caused by infection with atypical *Aeromonas salmonicida* (see Section 7). An increase in mortality was observed during the summer months as water temperature increased to a seasonal maximum of 14–15°C and continued, albeit at a lower rate, into the fall and winter months. During the course of the trial, the fish were given medicated feed three times (TM 200F; 3.75 g/100 lb of fish biomass for 10 days). Seventy-eight percent of the fish stocked survived to harvest and a total of about 20.2 m.t. of marketable sablefish was obtained.

The influence of water temperature and stocking density on the occurrence of clinical disease in this trial is not known. In the wild, juvenile sablefish typically inhabit surface waters with temperatures as high as 18°C, although they can tolerate temperatures as high as 22°C (Huynh et al., 2019; Sogard & Olla, 2001). Older sablefish inhabit deeper offshore waters where temperatures are much colder (<6°C; Mason et al., 1983). Adult sablefish undergoing diel vertical migrations, presumably related to prey movement and feeding, generally ascended into water of 7–8°C, but temperatures of 9–11°C have been recorded (Goetz et al., 2018). Observations of sablefish held in commercial net pens located in areas with strong temperature stratification indicate that small fish (10–750 g) thrive in warmer surface waters (7–18°C), whereas larger fish avoid temperatures higher than 12°C (Golden Eagle Sable Fish Inc.; T. Brooks, personal communication, August 24, 2020). Tank-based studies with adult sablefish at our facility also link reduced feeding activity, growth depression, and elevated mortality with high water temperature, suggesting that siting plans should include size- and age-based criteria for seasonal water temperature maxima.

Optimal density criteria for sablefish reared in net-pens have not been determined. In our trial, density at harvest was 11.9 kg/m^3 or approximately 4.8 fish/m^3 of pen volume. Reid et al. (2017) reported densities of 3.95 kg/m^3 (2.9 fish/m^3 of pen volume) for sablefish averaging 1.37 kg at harvest, equating to 7.22 kg/m^3 for fish of 2.5 kg average weight. Density-related stress is well known to foster clinical disease epizootics in farmed fishes and was likely implicated in the poor survival of fish in our trial. In the future, we plan to adhere to the guideline of 10 kg/m^3, used by the Canadian producer of "Gindara Sablefish," Golden Eagle Sable Fish Inc. (Anonymous, 2020).

There are very few published studies on sablefish nutrition and optimal feeding strategies and diet formulations, especially for large fish in the final stages of grow out, have not been determined (see Section 5). In our trial, monosex sablefish fed daily to apparent satiation with high energy, nutrient dense commercial salmon diets (see earlier)

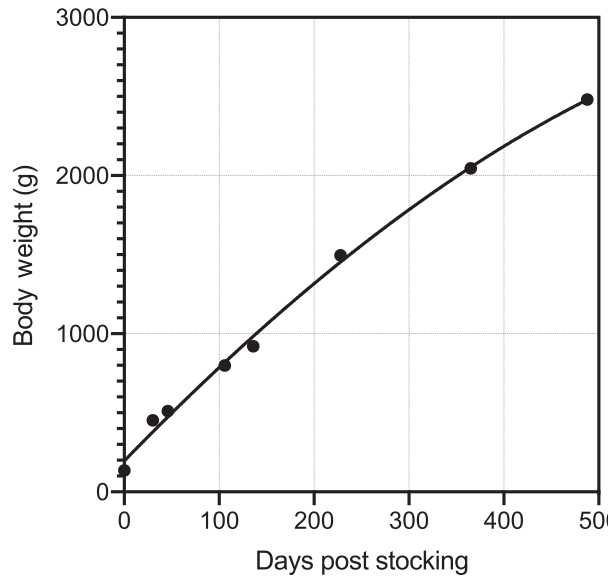

FIGURE 7 Growth of farm-raised, monosex female sablefish. The fish were stocked into net pens in December when they weighed 135 g and harvested about 500 days later at an average weight of 2.48 kg

grew from about 450 to 1,500 g in 7 months (Figure 7). In comparison, Reid et al. (2017) reported that sablefish fed daily until they were about 735 g with a diet containing about 37% protein and 17% lipid, and then on alternate days until harvest with the same diet, required about 10 months over a similar size range.

The satiation feeding strategy used in our monosex grow-out trial likely resulted in feed wastage from overfeeding. When productivity losses due to disease epizootics were also considered, we could not accurately determine how well feed was converted to body weight. Reid et al. (2017), however, reported that FCR increased with size from an average of 1.34 for 60–920 g fish to 1.62 for fish >920 g. Global Blue Technologies, Perciformes Group reported an FCR of 1.1 for sablefish reared to 500 g in tanks (Perciformes Group, 2019). These results underscore the importance of developing feeding strategies and performance optimized diets to take advantage of the full growth potential of sablefish and support efficient feed conversion, thereby reducing time to market and providing an overall cost savings to producers.

Because diets with imbalances in protein and lipid levels may reduce dressed yield of farmed fish due to contribution of the accumulation of visceral fat, we routinely monitor dressed yield of harvested fish. Sablefish are often prepared for market using the so-called Eastern cut, in which the pectoral girdle is removed along with the head. Eastern cut fish, which have historically been the primary wholesale product form for commercial fisheries, are also referred to as collar-off or Japanese-cut (J-cut) fish. In our evaluation of fish harvested from a mixed-sex stock fed the high energy diet, J-cut yields for fish weighing 2,000–2,400 g was ~70%. This compares very favorably to commercially caught wild sablefish, which average 62% (range 60–67%) from whole (round) weight (Crapo, Paust, & Babbit, 2004).

7 | DISEASE SUSCEPTIBILITY AND PREVENTION IN SABLEFISH AQUACULTURE

The significance of disease and host–pathogen interactions in sablefish culture is a relatively new area of study. Disease outbreaks have cost the aquaculture industry tens of billions of dollars over the last two decades (FAO, 2016). On a global scale, mortalities of 5% due to disease results in an estimated loss of $1 billion annually to the aquaculture industry (Dixon, 2012). A dramatic example is the outbreak of infectious salmon anemia in Chile's Atlantic salmon *Salmo salar* farms, which resulted in reduced smolt production and economic losses estimated at more than $2 billion (Asche, Hansen, Tveteras, & Tveteras, 2009). Disease outbreaks in sablefish aquaculture may result in high mortality during grow out and poor flesh quality in the survivors. Accordingly, disease can cause significant financial hardship for sablefish aquaculture operations.

7.1 | Disease research and pathogens of concern

The occurrence and virulence of most pathogens in sablefish have not yet been examined due to the novelty of sablefish as an aquaculture species. A number of potential pathogenic bacteria have been isolated from moribund sablefish and include *Vibrio logei* (Schulze, Alabi, Tattersall-Sheldrake, & Miller, 2006), *Vibrio splendidus* (Schulze et al., 2006), and *A. salmonicida* (Evelyn, 1971; NMFS, L. Rhodes, Personal Communication, January 15, 2015). Bacteria that have been demonstrated to cause disease in sablefish are as follows: *Vibrio anguillarum* (previously referred to as *Listonella anguillarum*) (Arkoosh & Dietrich, 2015); *Renibacterium salmoninarum* (Bell, Hoffmann, & Brown, 1990); and *A. salmonicida* (Arkoosh et al., 2018). In some of these instances, sablefish appear to be susceptible to common salmonid pathogens. Consequently, a number of pathogens that are of concern to salmon aquaculture also have the potential to affect sablefish aquaculture, including *Anisakis*, *Flavobacterium branchiophila*, epitheliocystis, leeches, papillomatosis, *Pseudomonas* sp., *Dactylogyrus* sp., *Diplostomum* sp., *Trichoina* sp., *V. anguillarum*, *R. salmoninarum*, and *A. salmonicida* (Sumaila, Volpe, & Liu, 2005).

The first study to demonstrate the susceptibility of sablefish to a pathogen (*R. salmoninarum*) in a controlled experiment was completed by Bell et al. (1990). In a study by Arkoosh and Dietrich (2015), the susceptibility of juvenile sablefish to three bacterial pathogens from the family *Vibrionaceae*, *V. anguillarum*, *Vibrio ordalii*, and *V. splendidus*, was examined. Most recently, sablefish susceptibilities to both typical and atypical *A. salmonicida* have also been characterized (Arkoosh et al., 2018; Vasquez et al., 2020).

7.1.1 | *Renibacterium salmoninarum*

R. salmoninarum is the etiological agent of Bacterial Kidney Disease (BKD). BKD is a significant disease in salmon that can result in mortality or chronic and systemic infection of the kidney. The study by Bell et al. (1990) documented 25% mortality in 20 sablefish injected with *R. salmoninarum* (Table 5). Twenty fish of 1,100 g size were injected with 1 ml of a 4.2×10^9 colony-forming units (cfu)/ml dose of *R. salmoninarum*, or alternatively saline (sham). Five of the 20 died between Days 50 and 71 post-infection of *R. salmoninarum* infection confirmed by culture and microscopy, as opposed to no mortalities among the sham group ($n = 5$). To our knowledge, no further research on sablefish has been conducted with this pathogen. Bell et al. (1990) also noted that no wild or farmed sablefish had been diagnosed with BKD at the time of that study.

7.1.2 | *Vibrio* spp.

Vibrio species are bacteria commonly found in the aquatic environment, most notably on plankton (Turner, Good, Cole, & Lipp, 2009). The prevalence of *Vibrio* tends to increase with elevated water temperatures (Powell & Loutit, 1994; Vezzulli, Colwell, & Pruzzo, 2013). *V. anguillarum* (Toranzo, Magarinos, & Romalde, 2005) and *V. ordalii* (Schiewe, Trust, & Crosa, 1981) are often described as salmonid pathogens, while *V. splendidus* is known as a shellfish pathogen (Lacoste et al., 2001), but has also been found to be pathogenic in marine fish, including turbot, *Scophthalmus maximus* (Gatesoupe, Lambert, & Nicolas, 1999) and gilthead sea bream, *Sparus aurata* L. (Zorrilla et al., 2003).

Arkoosh and Dietrich (2015) determined the susceptibility of sub-yearling sablefish to these three members of the family *Vibrionaceae* during controlled immersion exposures (Table 5). Groups of juvenile sablefish underwent pathogen challenges when they had reached 3.3–4.5 g and were exposed to five concentrations of each of the pathogens. Sablefish were susceptible to *V. anguillarum* at exposure concentrations $\geq 8.8 \times 10^4$ cfu/ml. Cumulative sablefish mortality increased with exposure to increasing *V. anguillarum* concentration. The greatest *V. anguillarum* concentration examined (8.8×10^6 cfu/ml) resulted in 24% mortality in juvenile sablefish. By contrast, sub-yearling sablefish were resistant to *V. ordalii* and *V. splendidus* at all exposure concentrations (up to 1.37×10^6 and 3.57×10^6 cfu/ml, respectively).

The authors also performed multiple logistic regression to determine the significance of association between weight, fork length, and bacterial concentrations on the probability of survival in sablefish (Arkoosh & Dietrich, 2015). The regression analysis indicated that sablefish survival to *V. anguillarum* exposure was significantly affected by their mass, with larger fish having a greater probability of survival. The mass of a sablefish may reflect the maturation or development (ontogeny) of the juvenile sablefish's immune system. In a study by Harrahy, Schreck, and Maule (2001), smaller Chinook salmon, *Oncorhynchus tshawytscha*, generated fewer antibody producing cells against a specific antigen than larger fish of the same age. Likewise, Johnson, Flynn, and Amend (1982) determined that protection from an immersion vaccine against vibriosis was greater in the larger salmonids from a same-age cohort, such that fish larger than 1.0 g were protected more than those ranging from 0.83 to 0.94 g. Therefore, size may play a role in the ability of sablefish to respond to bacterial infections, as well as influence the success of vaccines.

TABLE 5 Sablefish susceptibility to pathogens in controlled experiments

Reference	Fish size (g)	Pathogen	Challenge method	Dose	Survival (%)
Bell et al. (1990)	1400	*Renibacterium salmoninarum*, #384	Intraperitoneal injection	1 ml of 4.2×10^9 cells/ml	75
Arkoosh and Dietrich (2015)	4.5	*Vibrio ordalii* [ATCC 33509]	Immersion	1.37×10^2 cfu/ml	99[a]
				1.37×10^3 cfu/ml	98[a]
				1.37×10^4 cfu/ml	98[a]
				1.37×10^5 cfu/ml	99[a]
				1.37×10^6 cfu/ml	99[a]
	3.3	*Vibrio splendidus* [ATCC 33125]	Immersion	3.57×10^2 cfu/ml	99[a]
				3.57×10^3 cfu/ml	98[a]
				3.57×10^4 cfu/ml	99[a]
				3.57×10^5 cfu/ml	99[a]
				3.57×10^6 cfu/ml	100[a]
	3.3	*Vibrio anguillarum* [ATCC 68554]	Immersion	8.83×10^2 cfu/ml	100[a]
				8.83×10^3 cfu/ml	99[a]
				8.83×10^4 cfu/ml	96
				8.83×10^5 cfu/ml	91
				8.83×10^6 cfu/ml	76
Arkoosh et al. (2018)	8	*Aeromonas salmonicida*, atypical; T30	Immersion	1.54×10^5 cfu/ml	76
				1.54×10^6 cfu/ml	46
				1.54×10^7 cfu/ml	25
	8	*A. salmonicida*, typical; Banner#51	Immersion	9.33×10^4 cfu/ml	86
				9.33×10^5 cfu/ml	71
				9.33×10^6 cfu/ml	55
	15	*A. salmonicida*, typical; banner#51	Immersion[b]	4.9×10^6 cfu/ml	65
	15	*A. salmonicida*, atypical; T30	Immersion[b]	7.6×10^4 cfu/ml	48
				7.6×10^5 cfu/ml	16
	30	*A. salmonicida*, typical; banner#51	Immersion[b]	8.4×10^6 cfu/ml	48
	30	*A. salmonicida*, atypical; T30	Immersion[b]	9.7×10^5 cfu/ml	36
				9.7×10^6 cfu/ml	4
	100	*A. salmonicida*, typical; banner#51	Immersion[b]	2.4×10^6 cfu/ml	81
	100	*A. salmonicida*, atypical; T30	Immersion[b]	8.4×10^5 cfu/ml	43
	87	*A. salmonicida*, atypical; T30	Immersion[b]	8.4×10^5 cfu/ml	26
	88	*A. salmonicida*, atypical; T30	Immersion[b]	8.4×10^5 cfu/ml	45
	216			10^4 cfu/dose	93

TABLE 5 (Continued)

Reference	Fish size (g)	Pathogen	Challenge method	Dose	Survival (%)
Vasquez et al. (2020)		*A. salmonicida*, atypical; J410	Intraperitoneal injection		
				10^6 cfu/dose	7
				10^7 cfu/dose	3
	200	*A. salmonicida*, atypical; J410	Intraperitoneal injection[b]	10^7 cfu/dose	23

[a]Not significantly different from the no-pathogen, control, and treatment groups.
[b]Used as a sham treatment in a vaccine trial.

7.1.3 | *Aeromonas salmonicida*

A. salmonicida is able to affect a diversity of marine and freshwater fish, and its widespread distribution means it has potential to be devastating to fish culture. The five subspecies of *A. salmonicida* that have been identified (i.e., *salmonicida*, *achromogenes*, *masoucida*, *smithia*, and *pectinolytica*) can each cause the disease furunculosis (Han et al., 2011; Midtlyng, 2014). Furunculosis is used to describe a number of ulcer disease presentations caused by *A. salmonicida*. A further distinction is made between typical (classical) furunculosis and atypical furunculosis. Typical furunculosis is generally associated with an infection by *A. salmonicida* subsp. *salmonicida*. By contrast, atypical furunculosis arises from infection with the other *A. salmonicida* subspecies (Midtlyng, 2014). Atypical furunculosis occurs in over 20 species of both cultured and wild fish and is considered an "emerging disease" in Atlantic cod, *Gadus morhua*; Atlantic halibut, *Hippoglossus hippoglossus*; spotted wolfish, *Anarhichas minor*; common wolfish, *Anarhichas lupus*; and turbot, *S. maximus*, and some ornamental fish species (Gudmundsdottir & Bjornsdottir, 2007). In the case of sablefish, Evelyn (1971) first reported isolating a strain of *A. salmonicida* from a dead sablefish with a hemorrhagic lesion on its caudal peduncle characteristic of furunculosis. More recently, atypical *A. salmonicida* ("T30" isolate) was isolated and identified in diseased sablefish reared in net-pen culture at the NMRS during mortality events over a multiyear period by L. Rhodes (NWFSC, NOAA; Arkoosh et al., 2018). The subspecies of this atypical *A. salmonicida* has not been determined or reported.

Juvenile sub-yearling sablefish (50 ± 5.5 g) were found to be less susceptible to an immersion exposure of typical *A. salmonicida* relative to atypical *A. salmonicida* (Arkoosh et al., 2018) (Table 5). The typical isolate of *A. salmonicida* ("#51" isolate) was provided by Craig Banner (Oregon Department of Fish and Wildlife) originally collected from an infected Chinook salmon, and the atypical isolate of *A. salmonicida* was the T30 isolate provided by L. Rhodes (NWFSC, NOAA). Three dilutions of T30 atypical *A. salmonicida* (1.54×10^5, 10^6, and 10^7 cfu/ml) resulted in 75, 54, and 24% cumulative mortality, respectively, in sablefish 21 days post exposure, while three dilutions of #51 typical *A. salmonicida* of (9.33×10^4, 10^5, and 10^6 cfu/ml) produced 45, 29, and 14% cumulative mortality, respectively, in sablefish 23 days post exposure.

Sablefish susceptibility to atypical *A. salmonicida* strain J410, a strain isolated from infected cultured sablefish, was also demonstrated in a study by Vasquez et al. (2020) (Table 5). The authors developed a model of infection kinetics, finding that atypical *A. salmonicida* strain J410 has lower virulence in sablefish than some *A. salmonicida* strains infecting trout, but still exhibits high morbidity. The kinetics of infection were examined in 120 sablefish infected with one of three different doses of the bacteria to detect bacterial colonization in fish tissues 5 and 10 days post infection. The authors administered the pathogen by an intraperitoneal (IP) injection in juvenile sablefish (ca. 200 g). After 30 days, 97, 94, or 7% of the fish were infected when injected at 10^7, 10^6, or 10^4 cfu/dose, respectively. Although atypical *A. salmonicida* strain J410 did not display 100% mortality, it presented as chronic infection that persisted in fish tissues. Researchers then determined a median lethal dose (LD_{50}) for atypical *A.*

salmonicida J410 in sablefish of $\sim 3 \times 10^5$ cfu/dose (Vasquez et al., 2020). The authors of the study reported that this dose corresponds closely with the approximately 50% mortality observed for atypical *A. salmonicida* by Arkoosh et al. (2018). However, the two studies varied in sablefish age, size, as well as the mechanism of pathogen exposure that likely affected sablefish susceptibility. Additional research on this pathogen is discussed in greater detail later in terms of vaccine development.

7.2 | Prevention and treatment of sablefish diseases

Disease prevention and treatment in finfish aquaculture primarily involve the use of vaccines and antibiotics. No published data are available to support the use of antibiotics in sablefish. However, at the NMRS, diets containing oxytetracycline are effective in treating fish that are diagnosed with furunculosis (Goetz, personal communication). Some disadvantages have been established with the use of antibiotics in other aquaculture programs. Antibiotic presence in the environment may contribute to antibiotic resistance in the target (Ringo, Olsen, Jensen, Romero, & Lauzon, 2014), and nontarget bacteria (Cabello, 2006), as well as affect other nontarget organisms (Naylor & Burke, 2005). The use of antibiotics can also reduce the quantity of the nonpathogenic normal gut flora in fish (Ringo et al., 2014). By contrast, an effective vaccine strategy can be an economically, environmentally, and ethically appropriate method for controlling fish disease (Brudeseth et al., 2013). For example, vaccinating against *V. anguillarum* has been found to be highly effective in salmonids (Smith, 1988) and vaccines have been found to reduce epizootics due to *A. salmonicida* in salmon farms, in addition to reducing antibiotic expenses (Krkosek, 2010). Given the advantages of vaccines over antibiotics, initial studies have been carried out in order to test vaccines for use in sablefish aquaculture.

Vaccines are administered to fish through three routes, that is, injection, oral, and immersion, with each route having advantages and disadvantages (reviewed in Sudheesh & Cain, 2017, Dadar et al., 2017). The delivery route that is most effective for protecting fish depends on variables, such as fish size, the pathogen and its route of infection, water temperature, safety, and ontological development of the host (Dadar et al., 2017; Sudheesh & Cain, 2017). Thus far, injectable and immersion vaccines have been tested for use in sablefish with the pathogen *A. salmonicida* (Arkoosh et al., 2018; Vasquez et al., 2020).

7.2.1 | Vaccine efficacy in sablefish

Vaccine efficacy is determined by administering a potential vaccine to test groups of fish, where a control or sham group of fish is handled in exactly the same manner as fish administered the vaccine (i.e., equivalent immersion or injection handling), but the components of the vaccine are withheld. These fish are then subjected to a pathogen challenge protocol, to determine if any protection was conferred by the vaccine. In the vaccine studies reviewed herein, vaccine potency was determined by relative percent survival (RPS, Amend, 1980):

$$\mathrm{RPS} = [1 - (M_V/M_S)] \times 100\%, \quad (1)$$

where M_V is the percent mortality observed in the vaccinated treatment and M_S is the percent mortality observed in the sham treatment. Higher RPS values indicate that a vaccine successfully initiates an immune response in sablefish upon encounter with the pathogen after vaccination, where 100% would indicate complete immunity.

In the most recent study on *A. salmonicida* vaccines for sablefish, Vasquez et al. (2020) compared the efficacy of two commercial vaccines and one custom vaccine developed for use in salmon against *A. salmonicida* (Table 6). Commercial vaccines Alpha Ject Micro 4® (Pharmaq, Norway) consisting of formalin-killed strains of *A. salmonicida*, *V. anguillarum*, and *V. salmonicida*, and Forte Micro® (Elanco, Canada) consisting of formalin-killed strains of *A. salmonicida*, *V. anguillarum* serotypes I and II, *V. ordalii*, and *V. salmonicida* serotype I and II were compared with a

TABLE 6 Sablefish vaccine trials and subsequent challenges with *Aeromonas salmonicida*

Reference	Vaccine formulation	Route	Dose	Fish size[a] (g)	Challenge method	Pathogen isolate (cfu/ml)	Survival (RPS) (%)
Arkoosh et al. (2018)	AquaTactics, multivalent with oil-based emulsion	Injection	150 μl	50	Static immersion @ 5 weeks post vaccination	*A. salmonicida*, typical; Banner#51 (2.4×10^6)	99.3 (94.3)
						A. salmonicida, atypical; T30 (8.4×10^5)	90.0 (81.7)
		Immersion	2 rounds of 10% dilution for 1 min, 1 week apart	1.5	Static immersion @ 5 weeks post vaccination	*A. salmonicida*, typical; Banner#51 (4.9×10^6)	67.2 (7.3)
						A. salmonicida, atypical; T30 (7.6×10^5)	7.9 (−9.1)
						(7.6×10^4)	46.3 (−3.9)
					Static immersion @ 13 weeks post vaccination	*A. salmonicida*, atypical; T30 (8.4×10^5)	32.5 (8.5)
		Immersion	2 rounds of 10% dilution for 1 min, 1 week apart	4.5	Static immersion @ 5 weeks post vaccination	*A. salmonicida*, typical; Banner#51 (8.4×10^6)	49.9 (4.4)
						A. salmonicida, atypical; T30 (9.7×10^6)	7.8 (4.0)
						(9.7×10^5)	31.9 (−6.2)
					Static immersion @ 11 weeks post vaccination	*A. salmonicida*, atypical; T30 (8.4×10^5)	36.2 (−15.1)
Vasquez et al. (2020)	Bacterin mix from two isolates of atypical *A. salmonicida* in PBS	Injection	100 μl (10^9 cfu)	144	Injection @ 8/10[b] weeks post vaccination	*A. salmonicida*, atypical; J410 (10^7 cfu/dose)	73.3 (65.2)
	Alpha Ject Micro 4® Pharmaq; multivalent, oil-based	Injection	100 μl	135	Injection @ 10 weeks post vaccination	*A. salmonicida*, atypical; J410 (10^7 cfu/dose)	46.7 (30.4)
	Forte Micro® Elanco; multivalent, oil-based	Injection	100 μl	134	Injection @ 10 weeks post vaccination	*A. salmonicida*, atypical; J410 (10^7 cfu/dose)	66.7 (56.5)

[a]Fish size at vaccination.
[b]Authors report 8 weeks in the Discussion section and 10 weeks in the Methods section (Vasquez et al., 2020).
Abbreviation: RPS, relative percent survival.

custom autogenous bacterin vaccine consisting of formalin-killed strains *A. salmonicida* J409 (Accession number: CP047374-75), J410 (Accession number: CP047376-77), and J411 (Accession number: SUB6785506). The vaccines were IP injected in a single dose (100 μL for 10^9 cfu/dose for autogenous vaccine; according to manufacturer's instructions for commercial vaccines). Eight weeks after the injection, 140 immature juvenile sablefish (215 g) were challenged by IP injection with 100 times the LD_{50} dose (based on the LD_{50} determination described earlier). The challenge resulted in 53.5, 33.3, and 26.6% mortality in the vaccinated (Alpha Ject Micro 4®, Forte Micro®, and bacterin vaccines, respectively), and 76.67% mortality in the sham treatment. Consequently, RPS values ranged from 65.2% to 30.4% with the autogenous bacterin vaccine conferring the greatest protection and significantly higher IgM titers post-infection.

An efficacious vaccine that can be administered to smaller, sub-yearling fish is desirable in sablefish aquaculture. Arkoosh et al. (2018) published the first study to demonstrate that vaccination can protect juvenile sablefish against losses due to furunculosis (Table 6). Arkoosh et al. (2018) determined the efficacies of different vaccine administration methods against atypical and typical *A. salmonicida* for sablefish using a whole cell proprietary multivalent vaccine (AquaTactics; Kirkland, WA) that contained two isolates of formalin killed *A. salmonicida* (typical Hagerman and atypical T30 isolates) and three formalin killed *Vibrio* species (*V. anguillarum*, *V. ordalii*, and *V. salmonicida*). The vaccine was administered to groups of fish by either IP injection or immersion. The injection treatment consisted of one dose of 150 μL of the vaccine preparation in an oil-based emulsion administered to 250 fish at 50 g. The immersion treatment consisted of two 1-min immersions in a solution of one part vaccine to nine parts seawater that were separated by 1 week. Two separate groups of 500 fish were administered the immersion treatment when their mean mass was either 1.5 or 4.5 g.

Pathogen challenges with typical and atypical *A. salmonicida* were performed at approximately 5 weeks post-final vaccination for all three vaccine treatments (Table 6). In addition, a subset of fish from each immersion treatment was pathogen challenged alongside the injection-vaccinated fish, which corresponded to 11 and 13 weeks after their vaccination. The injection-vaccinated sablefish had 90% survival when challenged with atypical *A. salmonicida*, while the unvaccinated sham fish had 45% survival. The RPS of the injected vaccine was 81.7%. The injection-vaccinated sablefish had 99.3% survival when challenged with typical *A. salmonicida*, while sham-treated fish had 87.8% survival resulting in an RPS of 94.3%. By contrast, the immersion vaccine was not protective for sablefish against either atypical or typical *A. salmonicida* during any of the pathogen challenges.

At present, only IP-injected vaccines have shown any efficacy against *A. salmonicida* in juvenile sablefish. Multivalent vaccine formulations containing both *Aeromonas* and *Vibrio* species were used in the Arkoosh et al. (2018) and Vasquez et al. (2020) studies. Some multivalent vaccines have been more efficacious in salmon than monovalent vaccines against furunculosis (Austin, 2012; Hoel, Reitan, & Lillehaug, 1998). For example, increased resistance in Atlantic salmon was developed against *A. salmonicida* with a multivalent injectable vaccine containing *A. salmonicida*, *V. salmonicida*, and *V. anguillarum* (Midtlying, Reitan, & Speilberg, 1996). The enhanced protection was determined to be due to antibodies generated against *V. salmonicida* that were able to cross react with *A. salmonicida* whole cells and LPS (Hoel, Salonius, & Lillehaug, 1997).

Vaccine components and administration variables, for example, vaccine contact time, concentration, and incubation temperature, may affect the ability of immersion vaccines to confer protection (Du, Tang, Sheng, Xing, & Zhan, 2015). The use of carrier agents and mucosal adjuvants may also make immersion vaccination more effective with sablefish (Soto, Griffin, & Tobar, 2015). In addition, carrier molecules, such as liposomes, in immersion vaccine formulations have enhanced the uptake and effectiveness of an immersion vaccine against *A. salmonicida* in rainbow trout (Rodgers, 1990). Finally, enhanced immersion methods such as hyperosmotic infiltration, low-frequency sonophoresis, and dermal puncture may increase the vaccine uptake relative to direct immersion (reviewed in: Soto et al., 2015, Rombout & Kiron, 2014, Plant & Lapatra, 2011, Sudheesh & Cain, 2017).

7.3 | Understanding sablefish health and immune system

Sablefish disease susceptibility and vaccine trials have also advanced our understanding of the sablefish immune system and host-pathogen interactions. Specifically, researchers have started to characterize: the timing of immune

system development in sablefish; molecular protocols for assessing the stimulation of immune-relevant genes; histopathology and antibody response. Preliminary findings by Olson, Goetz, and Young (2016) showed that sablefish larvae expressed measurable levels of immune genes involved in the innate immune response (i.e., IL-1β, IL-10, MHC II, and Mx) and adaptive immune response (i.e., IgM and IgD antibodies) by Day 67 post-hatch, which corresponds with their transition from live to artificial diets. Presumably at this point, the larvae have a functional immune system and could respond if administered a vaccine. Histology also revealed that by 60 d.a.h., head kidney development was occurring and by 67 d.a.h., early development of the thymus was evident behind the operculum. Sablefish at this stage were about 35 mg and 1.2–1.5 cm length. This information suggests that vaccines could be administered to sablefish at a very early life stage, thereby maximizing protection during the most critical time points in the life stage of the fish and allowing farmers to move juveniles to net-pens as soon as possible.

In the study by Vasquez et al. (2020), specific IgM antibodies to *A. salmonicida* could not be determined due to the nonspecific binding of secondary antibodies to the A-layer of *A. salmonicida* used in the enzyme-linked immunoassay (ELISA). Therefore, the researchers determined total IgM and found that total IgM titers correlated to vaccine efficacy in sablefish. In addition, IgM titers were measured incrementally in post-vaccinated sablefish and were found to peak at 6 weeks post-immunization. This initial characterization of the antibody response may be useful in future studies, aiming to improve available options for vaccinating against this important disease.

8 | CONCLUSIONS AND FUTURE DIRECTIONS

Compared with salmonids, the development of sablefish aquaculture is nascent, spanning only a few decades. However, many of the bottlenecks for rearing sablefish for commercial aquaculture have been addressed and at least one company has successfully produced sablefish with net-pen grow out for several years. A number of the processes involved in production can certainly be made more efficient, efficacious, and cost-effective as noted below, so there is still research to be done on this species. While there is a significant commercial fishery for sablefish, a recent economic assessment indicates that there has been an expansion in the global market for sablefish that could dampen supply effects on pricing in the future (Hartley et al., 2020), providing a scenario where both aquaculture and the commercial fisheries coexist. This coexistence could be further enabled by the development of specific niche markets for an aquaculture product.

8.1 | Broodstocks and embryonic rearing

In the Pacific Northwest, it is relatively easy to collect sablefish broodstocks from the wild and to transition them to land-based rearing facilities. If these fish are maintained properly (e.g., temperature and diet), they will continue to reproduce, though not necessarily each year. However, to perform selection for traits of interest such as enhanced growth and disease resistance, genetically defined, captive broodstocks should be produced in the future and breeding schemes established. This could occur for both female and neomale lines, but the process will take time and resources given the age at maturity in this species and the requirement to rear broodstocks continually at low temperatures.

Sablefish eggs and yolk-sac larvae are fragile and require precise control of temperature, salinity, water flow, and light exclusion. Future research on early embryonic incubation and endogenous feeding larvae should focus on water temperature manipulation and its effects. Increasing temperature during incubation could reduce the duration of the hatchery phase and temperature manipulation for individual incubators could help synchronize larvae for stocking at the beginning of exogenous feeding.

8.2 | Larval rearing

Since 2007 when NMRS failed to produce any weaned juveniles, larval rearing protocols have been continually improved, leading to current survival rates that range from 15 to 40% and consistent production of greater than 20,000 weaned juveniles per year. Studies that focused on various aspects of larval rearing, including tank dimensions, water turbidity, temperature and illumination, live and dry feeds and enrichments, and microbial communities, have enabled improved larval production with greater cost efficiencies. Although survival rates during the larval stage have improved dramatically, within the current range (15–40%) it is still unpredictable, and larval rearing is still expensive. Future work will continue to optimize rearing parameters and should focus on reducing deformities, balancing optimal feed rates with live feed production costs, improving the transition from endogenous to exogenous feeding, and continued refinement of turbidity agents. This future work should help achieve consistently high production numbers, with minimized costs and improved product quality.

8.3 | Nutrition

Despite a scarcity of species-specific nutritional studies, researchers have shown that sablefish can be successfully cultured from juvenile through adult stages on commercial salmon feeds. In addition, sablefish have proven to be a good cold-water marine species model for alternative feeds research. Future research is needed that is directed towards optimizing nutritional requirements for all life stages of sablefish, developing practical feeds with these nutrient profiles, optimizing feeding schedules and producing life-stage specific diets.

8.4 | Growth

Sexually dimorphic growth in sablefish occurs during the typical grow-out period, affecting time to harvest, the proportion of undersized (male) fish, and thus overall economic return to the producer. Production of all-female monosex female offspring at semi-commercial scale using F-1 progeny of neomales generated through dietary treatment with 17α-methyltestosterone is now possible. Results of long-term feeding trials suggest that time to harvest at 2.5 kg from stocking at 75 g may be reduced by almost 3 months when monosex stocks are used. Econometric models reveal that internal rates of return are 11–15% higher for monosex relative to mix-sex stocks over a 10-year period under typical cage culture conditions (Hartley et al., 2020).

Despite these advances, challenges remain. Neomale sablefish generated via dietary treatment with MT may exhibit reproductive anomalies such as ovarian characteristics, reduced sperm production, and/or the inability to sexually mature in captivity—all factors that reduce their utility as male broodstock. To address these issues, research to optimize the timing, dosage and type of steroid used for neomales production is needed. Research should also include methods to reduce or eliminate use of exogenous steroid treatments, such as treatment with aromatase inhibitors or other nonsteroidal compounds.

8.5 | Pathogens and disease

Significant advances have been made in addressing disease prevention for sablefish aquaculture. Identifying the susceptibility and resistance of sablefish to pathogens and the progression of disease are critical to determining successful disease prevention strategies. In addition, further research should be conducted on immune system development, gene expression, antibody production, and pathology to provide researchers and practitioners with tools needed to assess sablefish health.

Immersion and oral vaccines should continue to be a focal point of disease prevention research in sablefish, given that sablefish are susceptible to diseases brought on by pathogens such as *A. salmonicida* and *V. anguillarum* when they are too small to be mass vaccinated through injection. Vaccine efficacy research should be fine-tuned using our growing understanding of the sablefish immune system as well as striving to improve methods that link immune responses to vaccination attempts. Disease prevention can also be enhanced in sablefish aquaculture by selecting for fish stocks that are healthy and robust and able to respond to low levels of environmental and opportunistic pathogens. Successful strategies could include minimizing stress, for example, from handling and overcrowding; maintaining optimal temperature regimens, and sablefish diets that incorporate immunostimulants, or therapies including probiotics or phages.

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