

REVIEW ARTICLE

Diet influences survival and growth of intensively reared larval saugeye (*Sander vitreus* × *Sander canadensis*)

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Funding information

University of Wisconsin Sea Grant Institute under grants from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and from the State of Wisconsin, Grant/Award Number: Federal grant number NA18OAR4170097

Abstract

We evaluated the performance of five commercial microstarter diets (Otohime, Gemma Micro, Gemma Wean, Optimal Starter, and Omega One Fry) fed to first feeding larval saugeye *Sander vitreus* × *Sander canadensis*. Triplicate tanks of 2500 larvae (initial weight: 2.6 ± 0.6 mg) were stocked at 4 days post-hatch (DPH) and fed treatment diets for 4 weeks. Fish were randomly sampled each week to monitor growth and development. Superior growth performance was observed in fish fed Otohime and Gemma Micro and was statistically significant as early as 13 DPH. At the conclusion of the trial, fish fed Otohime and Gemma Micro were approximately 3.6 times heavier than fish fed Gemma Wean and Optimal Starter. Survival was highest in fish fed Otohime ($39.7\% \pm 7.1\%$), with Gemma Micro performing second best ($14.1\% \pm 3.3\%$). Survival of fish fed Omega One Fry was too low for analysis (0.70 ± 1.1). Incidence of deformity among the dietary treatments ranged from 0% to 10.7%, primarily manifesting as a malformation of the jaw. Our results highlight the importance of diet for successfully raising saugeye through the larval/juvenile stage, and provide key information for this critical bottleneck in percid culture.

KEYWORDS

aquaculture, early development, feed evaluation, larvae diets, larviculture

1 | INTRODUCTION

Saugeye, a naturally occurring hybrid of walleye (*Sander vitreus*) and sauger (*Sander canadense*) (Billington et al., 1997), are a popular North American sportfish, and a promising candidate for intensive aquaculture. Currently, saugeye culture primarily focuses on pond rearing to fingerling size for stocking natural waters as a supplement to wild walleye populations (Jacob & Culver, 2010; Quist et al., 2010), but saugeye

are also well suited for intensive aquaculture for commercial food fish production. Saugeye grow faster than other percids in intensive culture, display less aggressive behaviour, and are not as influenced by routine disturbance and handling (Fischer et al., 2011; Garcia-Abiado et al., 2004; Malison et al., 1990; Siegwarth & Summerfelt, 1993). Successful procedures have been developed and employed for intensive saugeye production in- and out-of-season (Blawut et al., 2018; Garcia-Abiado et al., 2004), larval rearing (Fischer et al., 2011; Garcia-Abiado

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et al., 2004; Malison et al., 1990), and grow-out to market size (Siegarth & Summerfelt, 1993). However, a limited number of bottlenecks still exist for saugeye production on a commercial scale.

One such bottleneck is larval/juvenile survival and growth. Survival during intensive rearing of larval saugeye is generally low (12%–19%) and often coupled with variable growth rates (Czesny, 2000; Garcia-Abiado et al., 2004). This low survival and variable growth are often attributed to poor feed quality and palatability that leads to increased incidence of cannibalism and starvation (Garcia-Abiado et al., 2004). Commercial microstarter diets are the preferred feed for intensive culture of larval percids as it is less expensive and requires less labour than raising live feeds. Additionally, larval walleye have high acceptance of dry feeds without previous exposure to live feed (Rust et al., 2000), and have highest survival when dry feeds are introduced as early as possible (Aneshansley et al., 2001). Larval survival is also identical for pike perch (*Sander lucioperca*) fed commercial dry diets and live brine shrimp (*Artemia nauplii*) (Ostaszewska et al., 2005). Therefore, it is now common practice to begin feeding a 100% commercial dry diet at first feeding for walleye, sauger, pike perch, and saugeye (Johnson et al., 2021; Moodie, 1996; Moore, 1996; Summerfelt, 2004; Summerfelt et al., 2011). However, the nutritional requirements for saugeye have not been completely established, and no commercial diet has been specifically formulated for this species. As a result, the low larval survival bottleneck may be addressed by identifying a commercial feed that optimises larval saugeye survival and growth.

Feeds used as first food during larval development must be fine-grained, palatable, digestible, and meet the nutritional requirements of fast-growing fry (Hamre et al., 2013). A variety of these microstarter feeds developed for larval fish are available commercially. Otohime (Marubeni Nisshin Feed Co., Tokyo, Japan) is currently the most commonly used diet for intensively reared walleye and other percids. Other commercial microstarter feeds include Gemma Wean, Gemma Micro, and Nutra XP (Skretting USA, Tooele, UT, USA), Optimal Starter (Optimal Fishfood, Brookings, SD, USA), and Omega One Fry (Omegasea LLC., Painesville, OH, USA). While these feeds are all specifically formulated for intensively rearing larval/juvenile fish, it is unclear which are best suited for saugeye culture.

The objectives of this study are to evaluate the role of diet on survival and growth of intensively reared larval/juvenile saugeye, and identify the relative success of available commercial microstarter diets. Currently, there is little information available to inform best practices for this critical stage of intensive saugeye culture. Obtaining this information is crucial for determining how important diet is for larval survival and growth and will inform approaches to overcoming this bottleneck going forward.

2 | MATERIALS

2.1 | Fish

Walleye eggs and sauger milt were harvested in late April from fish collected in the Mississippi river (four female walleye and three male

sauger) and pooled. Milt was mixed into the eggs before the addition of water, following the dry method (Malison & Held, 1996; Piper et al., 1982), and fine clay was added to fertilised eggs to prevent adhesion followed by water hardening and disinfection with 100 mg/L iodine for 10 min. Fertilized eggs were then transported to the rearing facility, enumerated, and incubated in flow-through McDonald jars at 9°C. Incubation temperatures were gradually increased to a maximum of 12°C at hatch to maintain optimum incubation temperatures (Koenst & Smith, 2011). Fertilization success was 84.5% and hatching occurred 15 days post-fertilization.

2.2 | Larval rearing system and sampling procedure

We followed established procedures developed to provide optimal environmental conditions for larval walleye (Summerfelt & Johnson, 2015). Larvae were stocked 4 days post-hatch (DPH) into fifteen 240-L (220-L actual volume) round fiberglass rearing tanks with sides painted flat black and the bottom painted grey. The tanks were provided with aerated single-pass (flow-through) 20°C water, with clay (KT OM-4, L&R Specialties, MO) added to increase turbidity and to reduce clinging behaviour (Attramadal et al., 2012; Clayton et al., 2011; Rieger & Summerfelt, 1997). The clay suspension was maintained in a heavily aerated 500-L tank and delivered to a head tank via a peristaltic metering pump (Model A1N30F-7T, Blue-White Industries, Huntington Beach, CA). Water was delivered to each tank at an initial flow of 2 L/min (Flowatch flowmeter, JDC Electronic, Yverdon-les-Bains, Switzerland) in a manner to provide constant directional current, aligning the fry in the current to minimise cohort aggression and cannibalism. Water temperature, dissolved oxygen (ProDO, YSI Inc., Yellow Springs, OH), pH (PINPOINT pH Monitor, American Marine Inc., CT), and turbidity (2100Q Portable Turbidimeter, Hach Co., Loveland, CO) were measured daily and were not significantly different between tanks. Values averaged as follows (mean \pm SD): temperature, 20.3 \pm 0.5°C; oxygen, 8.9 \pm 0.3 mg/L; pH, 7.9 \pm 0.1; and turbidity, 45.5 \pm 6.5 nephelometric turbidity units; and these values meet optimal criteria for intensive culture of walleye and sauger (Bruner & DeBruyne, 2021). Total ammonia was measured in each fish culture tank weekly using procedures for the appropriate Hach TNT kit and DR/3900 spectrophotometer (Hach Co., Loveland, CO) and maintained below 0.05 mg/L in all tanks. Low lighting (<100 lux) was maintained 24 h a day, and a constant surface spray using horticultural hanging basket flex-misters (Hummert International, MO) was used to facilitate gas bladder inflation (Clayton et al., 2011).

Each larval rearing tank was stocked with 2500 (7.5 \pm 0.5 mm, 2.6 \pm 0.6 mg [mean \pm SD]) fry (11.4 fry/L), enumerated with a Jensorter fry counter (Model FCM, Jensorter, LLC, Hillsboro, OR), and randomly assigned one of five commercial microstarter diets (Figure 1). As the maximum granule sizes available for Gemma Micro and Wean are smaller than the other diets, an equivalent larger fry diet from the same manufacturer, Nutra XP, was utilised for fish that outgrew them (Figure 1). Samples of each diet were submitted for proximal analysis (SGS North America, Inc., Brookings, SD) (Table 1). Diets were fed

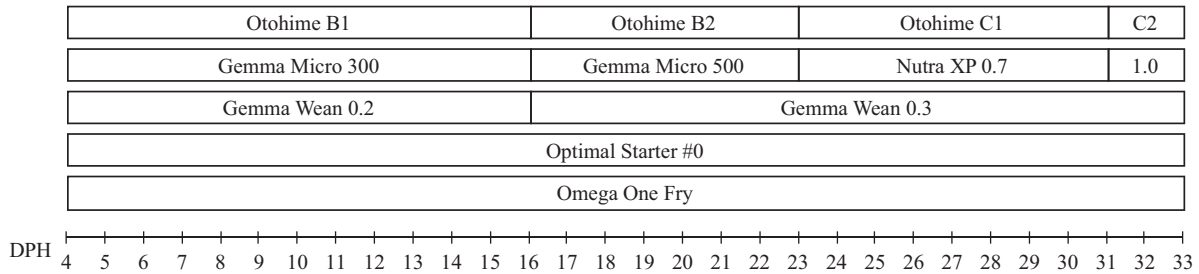


FIGURE 1 Feeding regimens of diets fed during the 4-week feeding trial. Marks separating the diets represent the mid-point of a 4-day transition between feeds where the new diet replaced the old in 25% increments in response to fish growth.

TABLE 1 Proximal composition and granule size of experimental diets fed to first-feeding saugeye

	Otohime B	Otohime C	Gemma Micro	Gemma Wean	Nutra XP	Optimal Starter	Omega One
Protein (%)	61.9 ± 2.7	62.7 ± 0.0	63.9 ± 1.1	67.7 ± 1.2	61.6 ± 0.2	53.5 ± 0.9	46.1 ± 1.4
Lipid (%)	16.0 ± 1.9	14.0 ± 1.1	19.8 ± 2.5	15.3 ± 0.6	17.4 ± 1.3	18.2 ± 1.8	11.0 ± 0.1
Fibre (%)	0.70 ± 0.4	0.45 ± 0.4	0.35 ± 0.2	0.55 ± 0.1	ND	2.00 ± 0.1	1.30 ± 0.3
Ash (%)	13.1 ± 0.2	14.3 ± 0.6	13.0 ± 0.5	9.60 ± 0.6	10.8 ± 0.1	11.3 ± 0.1	6.27 ± 0.3
Calcium (%)	1.89 ± 0.05	2.24 ± 0.18	1.51 ± 0.08	1.40 ± 0.21	2.02 ± 0.01	2.02 ± 0.21	0.86 ± 0.13
Phosphorus (%)	2.40 ± 0.01	2.48 ± 0.04	1.78 ± 0.08	1.42 ± 0.14	1.73 ± 0.01	1.59 ± 0.10	0.91 ± 0.05
Potassium (%)	1.05 ± 0.01	1.08 ± 0.05	0.98 ± 0.02	0.92 ± 0.02	0.81 ± 0.05	0.84 ± 0.21	0.78 ± 0.12
Magnesium (%)	0.33 ± 0.01	0.32 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.16 ± 0.00	0.20 ± 0.00	0.18 ± 0.03
Sodium (%)	1.03 ± 0.01	1.00 ± 0.01	0.92 ± 0.01	0.73 ± 0.09	0.58 ± 0.00	0.65 ± 0.15	0.34 ± 0.03
Sulphur (%)	0.84 ± 0.04	0.78 ± 0.01	1.38 ± 0.03	0.75 ± 0.02	0.68 ± 0.01	0.75 ± 0.00	0.47 ± 0.01
Copper (mg/L)	42.4 ± 7.28	32.2 ± 4.55	17.9 ± 0.47	20.0 ± 4.26	11.4 ± 0.61	76.6 ± 13.0	13.3 ± 0.22
Manganese (mg/L)	52.4 ± 1.07	51.6 ± 0.73	52.2 ± 2.91	56.5 ± 5.08	42.6 ± 5.09	85.0 ± 5.39	46.2 ± 10.1
Iron (mg/L)	595.9 ± 13.3	784.1 ± 199.0	272.2 ± 11.2	364.4 ± 117.4	208.8 ± 2.0	532.4 ± 67.3	120.9 ± 8.0
Zinc (mg/L)	216.4 ± 5.0	201.2 ± 0.2	158.8 ± 9.6	175.4 ± 7.6	259.7 ± 0.4	340.5 ± 4.1	60.1 ± 3.0
Granule size (µM)	B1 (250–360) B2 (360–620)	C1 (580–840) C2 (840–1410)	300 (200–500) 500 (400–700)	0.2 (250–400) 0.3 (350–500)	0.7 (700) 1.0 (1000)	#0 (250–500) #1 (800) #2 (1200)	Fry (250–500) MM ^a 1 (500) MM2 (1000)

Note: Data are presented as mean ± SD of duplicate samples. Figure 1 shows when each granule size from the different diets was provided during the feeding trial

^aMarine mini.

continuously over a 24-h period using custom made rotary automatic aquarium feeders, at an initial rate of 4 g per 1000 larvae per day. Feed rates were adjusted as needed to maintain a small amount of uneaten food each day and account for mortalities, ensuring that fish were not underfed. The tanks were inspected and cleaned daily by siphoning, with mortalities removed and enumerated. A random sample of 10 fish per tank was taken weekly to monitor growth and gut content, and this information was used to determine when to increase feed granule size and feed rate. Feed size was increased gradually over 4 days by increasing the ratio of new feed to old feed in 25% increments (Figure 1).

At the end of the trial (33 DPH), fish from each tank were enumerated and a random sample of 50 fish from each tank was collected for measurement of total length, body wet weight (BW), and presence of external deformities (fin erosion, head deformities,

and incomplete gill plate formation). Condition factor was calculated using the formula: $\frac{BW}{Length^3} \times 10,000$. Specific growth rate (SGR) expressed as percentage body weight increase per day was calculated as follows: $100 \times \frac{(\ln BW_{final} - \ln BW_{initial})}{Days elapsed}$. Cannibalism (unobserved mortality) was calculated as a percentage of the initial number of larvae that could not be accounted for (mortalities collected and enumerated during trial duration) and calculated using the formula: $\left(\frac{P_{initial} - P_{final} - P_{sampled} - Observed mortalities}{P_{initial}} \right) \times 100$, where P is the tank population. Jaw deformity and gas bladder inflation rates was calculated as a percent of the final sample. Feed presence in the gut was calculated as a percent of fish sampled at 13 DPH.

All data were analysed using R version 4.0.2 (R Core Team, 2013). Performance data (final length, final weight, SGR, survival, cannibalism, and jaw deformity) were analysed by one-way analysis of variance (ANOVA) with Tukey's post hoc test after testing for normality

TABLE 2 Comparative growth of saugeye measured regularly while on treatment diets and overall specific growth rate, survival, estimated prevalence of cannibalism, and prevalence of deformity

DPH		Otohime	Gemma Micro	Gemma Wean	Optimal Starter	Omega One	p
13	Length (mm)	13.87 ± 0.21 ^a	13.49 ± 0.35 ^a	11.00 ± 0.13 ^b	10.48 ± 0.16 ^b	10.74 ± 0.09 ^b	<0.0001
	Weight (mg)	17.80 ± 0.35 ^a	16.76 ± 1.39 ^a	8.00 ± 0.16 ^b	7.41 ± 0.40 ^b	7.86 ± 0.14 ^b	<0.0001
19	Condition factor	65.25 ± 1.20	66.74 ± 0.29	59.36 ± 2.25	63.74 ± 1.49	62.54 ± 1.89	0.065
	Length (mm)	20.59 ± 0.49 ^a	20.39 ± 0.15 ^a	13.31 ± 0.31 ^b	12.85 ± 0.16 ^b	12.67 ± 0.27 ^b	<0.0001
	Weight (mg)	71.20 ± 6.65 ^a	71.46 ± 1.64 ^a	13.07 ± 1.14 ^b	12.56 ± 0.57 ^b	11.51 ± 0.66 ^b	<0.0001
26	Condition factor	79.92 ± 2.17 ^a	82.53 ± 0.67 ^a	52.82 ± 0.43 ^b	56.44 ± 0.68 ^b	55.60 ± 0.82 ^b	<0.0001
	Length (mm)	28.91 ± 0.49 ^a	31.72 ± 0.73 ^b	15.15 ± 0.23 ^c	16.46 ± 0.34 ^c	14.68 ± 0.24 ^c	<0.0001
	Weight (mg)	198.7 ± 11.1 ^a	254.9 ± 17.2 ^b	18.44 ± 0.89 ^c	28.99 ± 1.49 ^c	17.58 ± 0.94 ^c	<0.0001
33	Condition factor	81.15 ± 1.10 ^a	79.36 ± 0.32 ^a	50.73 ± 0.16 ^b	60.73 ± 2.54 ^c	52.51 ± 0.53 ^b	<0.0001
	Length (mm)	39.89 ± 1.08 ^a	40.62 ± 0.18 ^a	27.09 ± 0.71 ^b	26.32 ± 1.33 ^b	–	<0.0001
	Weight (mg)	501.1 ± 49.6 ^a	507.6 ± 6.50 ^a	156.9 ± 15.4 ^b	135.3 ± 21.7 ^b	–	<0.0001
	Condition factor	77.87 ± 1.40	75.01 ± 1.03	74.34 ± 2.03	70.99 ± 1.22	–	0.062
	Specific growth rate	18.2 ± 0.3 ^a	18.2 ± 0.1 ^a	14.2 ± 0.3 ^b	13.6 ± 0.6 ^b	–	<0.0001
	Survival (%)	39.7 ± 7.1 ^a	14.1 ± 3.3 ^b	6.50 ± 0.8 ^c	5.10 ± 0.1 ^c	–	<0.0001
	Cannibalism (%)	35.0 ± 8.8 ^a	42.3 ± 5.1 ^{ab}	65.0 ± 2.2 ^b	60.6 ± 4.4 ^b	–	0.0219
Jaw deformity (%)	2.7 ± 0.7 ^a	0.0 ± 0.0 ^b	10.7 ± 2.7 ^c	7.3 ± 3.7 ^{ac}	–	<0.001	

Note: Data are presented as mean ± SE, with significant differences between treatments indicated by superscript letters. Ten fish per tank were sampled at 13, 19, and 26 days post-hatch (DPH) and 50 fish per tank were sampled at 33 DPH. The Omega One treatment was omitted from analysis at 33 DPH due to low survival.

and homoscedasticity, with proportional data being logit transformed before analysis. Jaw deformity rate was found to be heteroscedastic and was analysed using Welch's ANOVA with the Games-Howell post hoc test.

3 | RESULTS

3.1 | Survival and deformities

Survival of larval/juvenile saugeye was influenced by diet. Survival was highest in fish fed Otohime, intermediate in fish fed Gemma Micro, and lowest in fish fed Gemma Wean and Optimal Starter ($p < 0.0001$; Table 2). Daily mortality peaked from 6 to 8 DPH, levelling off by 12–16 DPH for most treatments with the exception of the Omega One treatment, which continued to experience some mortality not related to cannibalism throughout the trial (Figure 2). Estimated incidence of cannibalism was only significantly different between Otohime and the two lower performing diets, Gemma Wean and Optimal Starter ($p = 0.0219$; Table 2). The only deformity we observed was jaw malformation (Figure 3a), for which the incidence in fish fed Gemma Micro was significantly lower than the other diets (Table 2; $p < 0.001$), with Otohime being intermediate and the highest incidence in fish fed Optimal Starter, with Gemma Wean being not being significantly different from either Otohime or Optimal Starter. Incidence of failure to inflate the gas bladder (Figure 3b) was less than 2% in all experimental groups, with no significant differences between them ($p > 0.05$).

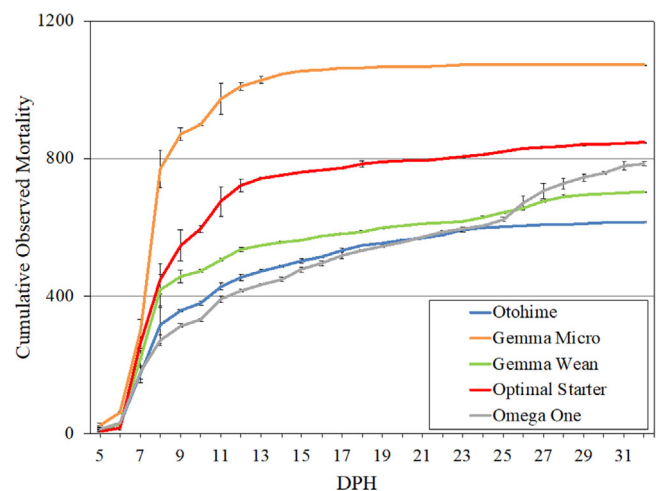


FIGURE 2 Cumulative observed mortality (unobserved mortality is included in Table 2) of each treatment represented by mean of observed mortality from daily collection of deceased fish from each tank. Error bars represent treatment standard error on each individual day.

3.2 | Growth

Fish fed Optimal Starter and Omega One Fry were not transitioned off initial feed sizes due to poor growth and lack of feed acceptance (Figure 1). Results of the Omega One Fry diet were not included in any statistical analyses at 33 DPH due to extremely low survival at the end of the trial ($0.70\% \pm 1.1\%$, mean ± SD), with two of the tanks

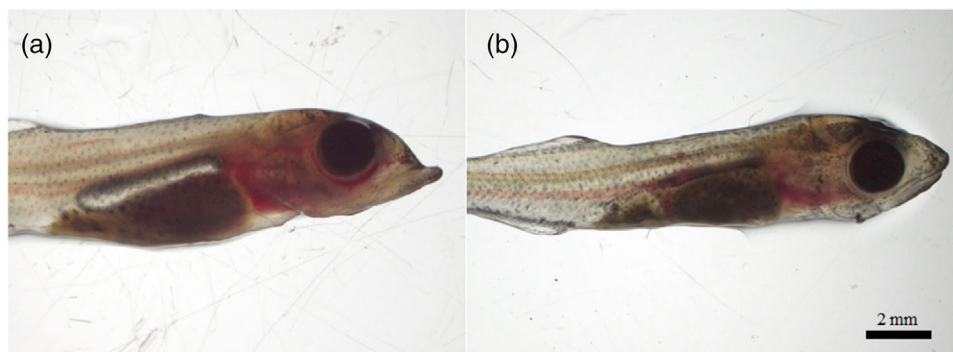


FIGURE 3 Jaw deformity (a) and non-inflated gas bladder (b) observed in juvenile saugeye (33 DPH) at the end of the feeding experiment

only having one or two surviving fish (Table 2). At 13 DPH, the Optimal Starter treatment had only 63.33% of fish with feed present in their gut. Omega One Fry (70%), Gemma Wean (70%), and Gemma Micro (73.33%) all had slightly higher presence of feed in the gut but were well below Otohime at 96.67%.

Otohime and Gemma Micro fed fish significantly outperformed the other treatments by 13 DPH with regard to total length and body-weight ($p < 0.0001$; Table 2) but not condition factor ($p = 0.065$). At 19 DPH, the Otohime and Gemma Micro regimens continued to outperform the other treatments, including condition factor ($p < 0.0001$; Table 2). At 26 DPH, fish on the Gemma Micro regimen were longer and heavier than fish on the Otohime regimen, and both continued to outperform the other treatments ($p < 0.0001$; Table 2). At trial completion (33 DPH), there were no differences in length, weight, or condition factor between fish fed the Otohime and Gemma Micro regimens, and they continued to outperform the other treatments in length, weight, and specific growth rate ($p < 0.0001$; Table 2), but not condition factor ($p = 0.062$).

4 | DISCUSSION

Our objectives were to evaluate the role of diet on survival and growth of intensively reared larval/juvenile saugeye and identify the relative success of available commercial microstarter diets. This stage is a critical bottleneck for successful culture of saugeye, and previous work suggests feed may be the most important factor at this stage. We found that diet has a considerable influence on larval/juvenile survival and growth for intensively reared saugeye. The Otohime diet resulted in the highest survival, highest condition factor, and highest specific growth rate at 33 DPH. The Gemma Micro diet resulted in similar growth performance but had lower survival than the Otohime diet. Gemma Wean, Optimal Starter, and Omega One all had poor growth performance, and consequently low survival. This information is necessary to inform best practices for this critical stage of intensive saugeye culture and highlights the importance of diet for minimising mortality and maximising growth.

Similar studies performed for walleye and closely related pikeperch (*S. lucioperca*) have also found that diet heavily influences survival and growth during the larval/juvenile stage (Hamza et al., 2008; Johnson et al., 2011; Kestemont et al., 2007). Johnson et al. (2011) compared

performance of INVE and Gemma Micro to a commonly used (but facing an import ban) Fry Feed Kyowa (FFK), finding that Gemma produced equivalent performance to Fry Feed Kyowa. They also observed the presence of several types of deformity, with the development of cataracts (opaque lens) being limited to fish fed the INVE diet. Jaw deformities similar to our observations (Table 2) were also recorded. While the nutrient requirements for walleye or saugeye have not been completely established, the mineral content of the diets we tested meets the minimum known requirements for most fish species (National Research Council, 2011). However, the significant differences we observed in prevalence of jaw deformities indicate the potential for lingering nutritional deficiencies that require further evaluation (Table 2). Similar jaw deformities have been reported in pikeperch (*S. lucioperca*), but diets supplemented with vitamin C and highly unsaturated fatty acids reduced the incidence of deformities (Kestemont et al., 2007). The higher rates of jaw deformity observed in the Gemma Wean and Optimal Starter diets suggest that these diets may not be meeting some critical nutritional requirement. Unfortunately, mechanistically linking the composition of our provided diets (Table 1) directly to observed deformity rates is beyond the scope of this study, but it would be an important avenue of research going forward.

Our data show that Gemma Micro produced similar growth performance to Otohime, albeit with reduced survival (14.1% vs. 39.7%; Table 2). However, the lower mortality of the Gemma Micro treatment is partly due to it experiencing a higher level of early mortality in the first week of the trial (Figure 2). While we cannot say with certainty why this treatment experienced greater early mortality, it could be related to palatability and related challenges with feed acceptance. This appears likely given our observed presence of feed in the gut for the Gemma Micro diet and Otohime diet at 13 DPH (73.33% vs. 96.67%, respectively), suggesting that Otohime was more readily accepted. Johnson et al. (2008) reported survival of walleye larvae fed Gemma Micro to 28 DPH at two separate sites of 56.7% and 11.7%, while Garcia-Abiade et al. (2004) reported survival of 19% for saugeye reared in-season on FFK. Johnson et al. (2011) initiated walleye larval rearing at 10–14°C, increasing to 18–20°C by 30 DPH, while larvae in our study were reared at approximately 20°C for the entire duration of the trial, which we have observed improves feed acceptance for saugeye fry. There was a moderately strong relationship between survival and the estimated degree of cannibalism (logistic regression, pseudo- $R^2 = 0.74$), which is expected. Once-a-day collection of

mortalities may be insufficient to accurately estimate the degree of cannibalism, especially for extremely small fish that may break down in the intervening 24 h between collections as postulated by Dabrowski et al. (2000). For future studies, both survival and accuracy of estimates of cannibalism could be improved by regular grading, removal, and enumeration of cannibalistic fish.

The significantly reduced growth performance and survival of the Omega One, Gemma Wean, and Optimal Starter feeding regimens were observable by 13 DPH. Concurrently, feed presence in the gut was 70% or lower for saugeye in each of these treatments (low relative to the 96.67% observed in the Otohime treatment). The significant reduction in condition factor for these treatments at 19 and 26 DPH strongly indicates their failure to stimulate feeding compared to the other two treatments. Subsequently, the improvement of condition factor at 33 DPH in these treatments is likely attributable to cannibalism coupled with improved feed acceptance. Failure of larvae to accept prepared diets is a major cause of mortality, due to both starvation and cannibalism (Kestemont et al., 2003; Naumowicz et al., 2017). Therefore, feed attractants are often an indispensable ingredient in larval diets (Hamre et al., 2013). Poor palatability can worsen cannibalism, as larvae that adapt to feed early grow large enough to consume others. Looking at the cumulative mortality curve for the Omega One diet, it is apparent that this treatment experienced continued losses due to starve-outs, which when combined with excessive cannibalism resulted in the extremely low survival observed (Table 2; Figure 2). The largest fish collected from this study were the two remaining fish in one Omega One fed tank and the sole surviving fish in another.

Besides cost and performance, another important consideration for larval diets is availability. We were unable to evaluate several well-regarded diets used in Canada due to their unobtainability in the United States, including Aglonorse (Trofi Aquaculture, Tromsø, Norway) and Larviva (BioMar Group, Aarhus, Denmark). Furthermore, Otohime is currently unavailable in Canada due to an importation ban by the Canadian Food Inspection Agency. FFK is similarly banned in North America due to concerns regarding bovine spongiform encephalopathy (Johnson et al., 2011). It is for this reason that it is important to continue evaluating commercial diets so that alternatives are available in the event the current industry standard diet becomes unavailable. Of the diets we evaluated, Gemma Micro remains the most suitable alternative to Otohime available in the United States, with Gemma Wean and Optimal Starter performing at a reduced level, and the Omega One diet being unsuitable for saugeye larviculture. However, these alternatives are currently not ideal replacements for Otohime, and further work identifying the specific dietary needs for saugeye (and other percids) and producing diets to meet those needs is required.

AUTHOR CONTRIBUTIONS

Gregory J. Fischer supervised the original investigation, acquired funding, collected the data, and wrote the manuscript. Tyler Firkus and Patrick Blauffess analysed the data, performed visualisation/data pre-

sentation, and reviewed and edited the manuscript. Kendall Holmes contributed in original investigation, collected the data, and reviewed and edited the manuscript. Jon Amberg prepared specific diets, analysed the data, and reviewed the manuscript. Christopher Hartleb acquired funding, analysed the data, and reviewed and edited the manuscript. All authors listed have contributed to this manuscript and have approved the final submission to the Journal of Aquaculture, Fish and Fisheries.

ACKNOWLEDGEMENTS

We acknowledge the Genoa USFWS Fish Hatchery for assisting with gamete collection and UWSP NADF staff for data collection and fish culture. Funding for this work was provided by the University of Wisconsin Sea Grant Institute under grants from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and from the State of Wisconsin (Federal grant number: NA18OAR4170097; project numbers R/SFA-16 and R/SFA-23).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be made available on the project page (<https://www3.uwsp.edu/cols-ap/nadf/Pages/Current-Projects-At-The-Facility.aspx>) repository upon acceptance.

ETHICS STATEMENT

All animal capture, handling, sampling, and sacrificial procedures were approved by the University of Wisconsin Stevens Point Institutional Animal Care and Use Committee (IACUC, Protocol Number: 2017.10.05).

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PEER REVIEW

The peer review history for this article is available at: <https://publons.com/publon/10.1002/aff2.86>.

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How to cite this article: Fischer, G.J., Firkus, T.J., Holmes, K., Blaufuss, P.C., Amberg, J.J., & Hartleb, C.F. (2022) Diet influences survival and growth of intensively reared larval saugeye (*Sander vitreus* × *Sander canadensis*). *Aquaculture, Fish and Fisheries*, 2, 450–457. <https://doi.org/10.1002/aff2.86>