

2025 NCRAC Termination Report

Instructions: NCRAC has typically funded 2-year projects, but many have continued for a longer duration. A termination report for the entire project or individual components (objectives) that have ended must be provided electronically to the Director of NCRAC within sixty (60) days of the termination date. To accomplish this, all investigators will prepare and provide project results to the Work Group chair who will then compile a project or project component termination report to submit to the Director. This report will be more comprehensive and detailed than annual reports.

Q1 Project Title: Asian Carp as an Initial Dietary Protein Source and Palatability Enhancer for Successful Production of Yellow Perch and Walleye Fingerlings

Q2 Key Word(s): Aquaculture Drugs, Aquaponics, Baitfish, Conferences/Workshops, Crayfish, Economics/Marketing, Extension, Hybrid Striped Bass, Largemouth Bass, National Coordinator for Aquaculture, **Nutrition/Diets**, Other, Salmonids, Sunfish, Tilapia, Viral Hemorrhagic Septicemia, **Walleye**, Wastes/Effluents, White Papers, **Yellow Perch**.

Q3 Dates of Work: (i.e. - 9/1/14-8/31/25)

August 2021 – August 2023 (extended through August 2025)

Q4 NCRAC Funding Level: (Total NCRAC-USDA funding)

\$198,614

Q5 Participants: (Funded cooperating personnel and institutions, agencies, and business entities including Industry Advisory Council liaison(s), extension liaison(s), and non-funded collaborators).

Karolina Kwasek, Michal Wojno – Southern Illinois University-Carbondale/University of New Hampshire
Tyler Firkus (as a replacement of Greg Fischer) – University of Wisconsin - Stevens Point

Q6 Extension Liaison: (name, organization, state)

Stuart Carlton, Illinois-Indiana Sea Grant | Purdue University
Amy Shambach, Illinois-Indiana Sea Grant | Purdue University
Emma Hauser - University of Wisconsin - Stevens Point

Q7 Industry Liaison: (name, organization, state)

Clarence Bischoff, CEO, Blue Water Farms

Q8 Reason for Termination: (Indicate objective(s) completed, funds terminated, or other specific reason for project termination.)

Project completion.

Q9 Project Objectives: (list)

1. To develop the optimal in vitro methodology for Asian carp muscle digestion using digestive enzymes obtained from adult yellow perch *Perca flavescens* and walleye *Sander vitreus* that can be used as a protein source and attractant in dietary formulations for larval and juvenile yellow perch and walleye.
 2. To evaluate the effect of Asian carp muscle protein hydrolysate obtained using methodology in Objective 1 as protein source in diets for yellow perch and walleye when used as first feed.
 3. To evaluate the effect of Asian carp muscle protein hydrolysate obtained using methodology in Objective 1 as an additive/palatability enhancer in diets for yellow perch and walleye on successful weaning to formulated feeds.
 4. To evaluate the effect of Asian carp muscle protein hydrolysate combined with soybean meal hydrolysate - both obtained using methodology in Objective 1, as additives in diets for yellow perch and walleye for successful weaning to formulated feeds and easier transition to plant-based feeds.
 5. To provide the aquaculture community within the North Central Region (NCR) with guidelines on successful larval rearing protocols for both yellow perch and walleye in indoor systems.
 6. To provide the feed/additive manufacturing industry with the knowledge and the tools required for production of high-quality well-digested dietary protein hydrolysate as a cost-effective source of protein and attractant for young fish feeds.
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Q10 Project Summary: (Text limited to 1,250 characters (approximately half a page) that describes the project in everyday language without the use of scientific or technical jargon. State the problem, challenge or issue your project addressed. Include dollar estimates if it's an economic issue (e.g., a potential decrease in feed costs). Briefly, tell how this project addressed or solved the problem or challenge. Answer the "Who cares?" or "So what?" question: Why is this worth the attention of people? How does this impact the lives of real people? What difference will it make, and to whom? What is the benefit or potential benefit of this project?)

Limited knowledge of larval/juvenile nutritional requirements, the reliance on live food, poor weaning success to formulated diets, and inefficient utilization of soybean meal-based feeds have all limited expansion of Percid fingerling production. We propose an innovative dietary protein source and dietary attractant that will precisely

match Percid larvae and juvenile requirements and induce high feed intake and positive growth responses when used as first feed and/or during weaning. This innovative dietary protein source will provide more control in production of Percid fingerlings by increasing dry diet acceptance and exposure to plant-based formulation at the earliest possible age. This innovative dietary ingredient and knowledge derived from the study will provide the aquaculture industry particularly in the NCR with the new approach for the development of high-quality starter feeds that will support sustainable expansion of the hatchery sector and consequently contribute to the development of competitive aquaculture market within the NCR.

Q11 Technical Summary and Analysis: (Text limited to 9,000 characters [approximately 3 pages]) (Describe the work undertaken and results obtained for each objective. Major results should be presented in detail, including graphs, figures, and/or tables. Methodology should be briefly described and statistical analyses included where appropriate. This section of the report should be written in a style similar to a professional publication. Manuscripts previously or currently prepared and/or accepted for publication may be submitted as part, or all, of this section. Keep in mind that once the final NCRAC report is published, all information will become public knowledge.)

Study 1 – Yellow Perch

This study aimed to deliver an innovative dietary methodology for reducing habituation time from live food and improving growth and survival during the challenging-vulnerable larval stage of YP. Specifically, this study intended to evaluate the effects of SC muscle hydrolysate obtained using YP digestive tracts as an enzyme source; and provided as a dietary protein source for first feeding YP. We hypothesized that, providing well-utilized diets based on the proper molecular weight and optimal amino acid composition from the start of feeding could enhance dietary amino acid assimilation and utilization for tissue protein synthesis—during a period when the larval YP retain an increased capacity for intracellular digestion.

The study was conducted in the Center for Fisheries, Aquaculture, and Aquatic Sciences at Southern Illinois University—Carbondale (SIUC). All experiments were carried out in accordance with institutional animal care guidelines and under the approval of the SIUC Institutional Animal Care and Use Committee (Protocol#19-033). During digestive enzyme collection a lethal recommended dose of tricaine methosulfate ($\geq 300\text{mg/L}$; pH 7.2) was used on the adult YP; adults were soaked in the solution until cessation of opercular movement was apparent. Ice slurry was used for larval sample collection/euthanasia because MS-222 acts by blocking gill ventilation and many larvae species are generally more resilient to common lethal MS-222 doses due to their ability to utilize cutaneous gas exchange which can be sufficient for weeks after hatch (Rombough 2007). All efforts were made to minimize pain, stress, and discomfort. The fish were under the care of researchers trained in accordance with SIUC Institutional Animal Care and Use requirement and monitored daily for signs of distress.

Silver Carp Muscle Harvest

Silver carp were sourced from the Illinois river (Marseilles, IL), and transported on ice. Dorsal carp muscle was harvested from whole frozen carp using a rotary meat saw (Industrial Tools, O'Fallon, IL), and skin removed using a common filet knife (Rapala, Helsinki, Finland), dorsal filets were fed through a meat grinder (Cabela's, Sydney, NE) 2-3 times using a ½ inch dye size.

In-vitro Digestion

The methodology for obtaining carp muscle hydrolysate followed Kwasek et al. (2021) with some modification. Briefly, adult YP were maintained at 21 degrees C and fed at 3% rate (based on fish biomass). On the day of

hydrolysis, the fish were fed twice, one hour apart, then harvested one hour later (one meal is approximately 1% of fish biomass). A total of 1.5 kg of digestive tracts (stomach and intestine) were collected from adult YP after a proper lethal dose of MS-222 was administered. Stomach and intestines were divided upon the harvesting of the adult YP and were subsequently ground. The collected stomach and hindgut portions were then placed in a centrifuge at 4000rpm for 10 minutes, and strained of any solids, these enzyme slurries were kept on ice. Skinless-frozen-carp fillets were ground, then homogenized with distilled water at a 1:1 ratio using a PowerGen1000 (Fisher Scientific, Waltham, MA). Kwasek et al. (2022) previously used a muscle-water dilution of 1:3; the equal-part ratio used here was anticipated to decrease the subsequent freeze-drying time, leading to improved feed production efficiency. The homogenized carp was mixed at high-speed using a continuous stirrer (VWS OS 16) ensuring even mixing throughout the entire hydrolysis process. The stomach enzyme slurry was added to the homogenized carp muscle once pH reached 4 and temperature was maintained at 20.5°C. The mixture was stirred continuously and incubated for 2 hours, using 3M hydrochloric acid to maintain a pH of between 3-4, mimicking stomach digestion. The inclusion ratio of total digestive tracts weight to carp muscle weight was half that of Kwasek et al. (2022), initial concentration of digestive tracts was 60-70g/1kg carp muscle. Due to the reduced digestive tracts-muscle ratio, the hydrolysis time was doubled to ensure effective enzymatic digestion. After 2 hours, the pH of the carp mixture was brought to 8.0 (± 1) using 2.5M sodium hydroxide. The intestinal enzyme slurry was then added to the carp mixture and incubated for 4 hours to simulate intestinal digestion. Kwasek et al. (2022) initially included complete digestive tracts for the entirety of the hydrolysis. Independent addition of the intestinal enzyme slurry minimizes risk of enzyme loss during the initial 2-hour acidic digestion, likely enhancing the hydrolysis process. The carp mixture was placed into a double boiler and heated, using a propane banjo burner (Flame King, Pico Rivera, CA), to 90°C to halt enzymatic processes. The mixture was then poured into 1-gallon bags (Ziploc, Racine, WI) and kept ridged by a 12L bin. The bags were sealed and placed in a freezer at -20 degrees C for at least 24 hours, or until needed.

Intact Muscle (IC)

The intact muscle was processed exactly according to the in-vitro digestion procedure, with the following difference. The collected stomach and intestines were combined and added to the homogenized carp mixture. The carp mixture was not incubated to mimic digestion, but the mixture was immediately brought to 90 degrees C to deactivate any enzyme activity. Both muscle sources were freeze dried (Labconco FreeZone 6, Kansas City MO) for subsequent analysis and inclusion in the feed. The Control feed (IC) was based solely on protein derived from the intact (non-hydrolyzed) carp muscle (Table 1).

Hydrolysate Diet Preparation (SCH)

Two experimental diets were made: 1) Silver Carp Hydrolysate (SCH) based diet obtained from carp muscle incubated with adult YP digestive enzymes, and 2) an “intact” muscle-based (IC) diet obtained from carp muscle mixed with adult YP digestive enzymes, but not incubated.

The SCH diet was based on 50% of protein derived from SC muscle hydrolysate and 50% of protein derived from intact SC muscle, a 1:1 ratio (Kwasek et al. 2021) (Table 1). The hydrolysate inclusion rates were determined by previous studies which indicate that, having not yet undergone enzymatic maturation, larval fish can better utilize small protein fractions due to increased cytosolic activity and high peptidase activity (Hevroy et al. 2005). All diets were formulated to be isonitrogenous and isolipidic and the essential amino acids included in levels required by YP or closely related species if the requirement data for some nutrients were not available (NRC 2011). Diets were formulated according to the preliminary Largemouth bass study (Kwasek et al. 2021) with some modification to meet both macro- and micronutrient requirements of YP (NRC 2011) (Table 2).

Table 1: Diet formulation for Silver Carp Hydrolysate (SCH) and Intact Control Diet (IC).

Ingredients	Silver Carp Hydrolysate Diet (%)	Intact Control Diet (%)
Silver Carp Meal	28.6	57.3
Silver Carp Hydrolysates	32.1	0
CPSP 90	5.9	5.9
Krill	5.1	5.1
Fish Oil	5.7	5.7
Lecithin	3.9	3.9
Dextrin	10.7	14.0
Mineral Mix	3.0	3.0
Vitamin Mix	3.0	3.0
CaHPO ₄	0.97	0.97

Table 2: Proximate composition of the three compound diets (Silver Carp Hydrolysate, Intact Control, Commercial Reference Diet).

Dry Matter (g/100g)	SCH Diet	IC Diet	CD Diet
Crude Protein	60.17 (±0.02)	60.46 (±0.02)	60.14 (±0.05)
Moisture	4.48 (±0.02)	4.44 (±0.02)	6.23 (±0.04)
Crude Fat	14.49 (±0.12)	14.35 (±0.15)	14.89 (±0.06)
Crude Fiber	0.20 (±0.02)	0.33 (±0.01)	2.45 (±0.05)
Ash	10.83 (±0.10)	10.31 (±0.08)	13.79 (±0.05)
Taurine	1.19 (±0.00)	1.36 (±0.00)	0.57 (±0.00)
Hydroxyproline	0.51 (±0.01)	0.56 (±0.01)	0.37 (±0.00)
Aspartic Acid	5.38 (±0.03)	5.55 (±0.01)	5.53 (±0.01)
Threonine	2.31 (±0.01)	2.39 (±0.00)	2.40 (±0.00)
Serine	2.09 (±0.01)	2.16 (±0.01)	2.13 (±0.00)
Glutamic Acid	8.09 (±0.02)	8.33 (±0.06)	7.52 (±0.01)
Proline	2.32 (±0.01)	2.43 (±0.02)	2.62 (±0.01)
Glycine	3.33 (±0.02)	3.48 (±0.02)	3.24 (±0.02)
Alanine	3.29 (±0.01)	3.41 (±0.00)	3.31 (±0.01)
Cysteine	0.53 (±0.00)	0.56 (±0.00)	0.61 (±0.00)
Valine	2.76 (±0.00)	2.83 (±0.02)	2.90 (±0.00)
Methionine	1.53 (±0.01)	1.61 (±0.00)	1.60 (±0.01)
Isoleucine	2.62 (±0.00)	2.68 (±0.03)	2.83 (±0.00)
Leucine	4.26 (±0.00)	4.39 (±0.02)	4.37 (±0.00)
Tyrosine	1.84 (±0.01)	1.91 (±0.01)	2.34 (±0.00)
Phenylalanine	2.24 (±0.00)	2.31 (±0.01)	2.54 (±0.00)
Lysine	4.97 (±0.00)	5.12 (±0.02)	4.44 (±0.01)
Histidine	1.42 (±0.00)	1.45 (±0.00)	1.45 (±0.00)
Arginine	3.27 (±0.01)	3.43 (±0.00)	3.63 (±0.01)
Tryptophan	0.63 (±0.02)	0.67 (±0.04)	0.66 (±0.00)

Diet preparation started with each dry ingredient being ground to 500um with a centrifugal mill (Retsch Haan, Germany). All dry ingredients were then manually sieved through a 250um screen to ensure all particles were of the appropriate and uniform size. All dry ingredients (excluding lecithin and choline chloride) were mixed for 15 minutes, followed by the addition of lecithin dissolved in the fish oil. The mixture was then stirred for an additional 15 minutes. Lastly, water (10-15% of total feed mass) with dissolved choline chloride was mixed until the desired firmness was reached. Final water additions to both the Hydro diet and Control diet may reach 50% of the total feed mass. Feeds were then slowly processed using an extruder (Caleva Extruder 20, Sturminster Newton Dorset, England) to obtain proper extrudate size and firmness. Extrudates were processed using a spheronizer (Caleva, Sturminster Newton Dorset, England) at 600rpm for 3 minutes, 1800rpm for 30 seconds, and then 600rpm for two to five minutes to finish the process. Finally, the spheres were freeze dried (Labconco, Kansas City, MO) and sieved by hand to their appropriate sizes. It should be noted that a large portion of the spheronized feed may be greater than 750um. When needed, sizes larger than 500um were ground and sieved down, by hand, to smaller desired sizes of 150um-350um. All finished feeds were stored in sealed bags at -20°C.

Larval Rearing

Fourteen YP egg ribbons were collected from a farm (Millcreek Perch Farm, Marysville, OH) and transported immediately in aerated, pond water-filled coolers to SIUC. Each egg ribbon was placed into a McDonald-style hatching jar within a UV-treated recirculated water system chilled (Aqualogic San Diego, CA) steadily at 12.1°C ($\pm 0.4^\circ\text{C}$). After 8 days, once a small proportion of newly hatched YP exhibited the swim up stage, the egg system was gradually raised to 15 degrees C and YP were transferred to the larval rearing system following proper acclimation.

The semi-recirculating larval rearing system used two mechanical (sand) filters (Pentair, Minneapolis, Minnesota) and inflow was intensely aerated using trickling tower biofilters. Municipal water was treated and replaced at 3 L/min, or around 4% of total system flow. Thirty black, 280L tanks were used. An inline chiller (Aqualogic, San Diego, CA) was used to maintain a temperature of 20°C ($\pm 2^\circ\text{C}$). During live feed stages salinity was kept at 1.49 (± 0.55) ppt to prolong the viability of the live food (Dabrowski and Miller 2018). A pH of 7.2 (± 0.2) was maintained throughout the study.

To minimize cannibalism, multiple precautionary measures were taken. Bristow and Summerfelt (1994) demonstrated the use of Clay (up to 50 NTU) as an effective method for reducing cannibalism and creating a contrast in color to aid larvae in foraging. Using clay to keep turbidity at levels of 10-16 NTU decreased pH significantly, in which case bicarbonate was mixed into the clay before addition to the system. Light was constituted only during feedings and dimmed to the lowest setting all other times, with filtered ambient overhead lights on from 0800 to 1800 hours (Kestemont 2015). Two sprinklers with angles of 90 and 45 degrees relative to the water surface were fitted to opposing sides in each tank to break surface tension, improve flow, and provide aeration (Wojno et al. 2012). The 45-degree sprinkler was pointed in the same direction as the incoming flow, spray nozzles were checked daily to discourage sprinklers from becoming clogged with incoming clay. Laminar inflow was created using perforated inlet pipes at 2 Liters per minute (Lpm). Flow was increased as larvae became resilient and solids increased, an increase to 3Lpm occurred at dph 16.

At the time of complete dry diet transition the laminar inlets were removed, flow was increased to 5Lpm, air stones added, and standpipe cover screen sizes increased to 1.5 mm. During live feed stages a special dual screen standpipe cover was used. The lower screen (0.5 mm) accounted for the water column depth below the surface, while a slightly larger 1 mm screen at-above the water's surface was used to allow the passage of oils. Tanks were scraped and siphoned daily into a 5-gallon bucket in the absence of light to promote the dispersion of larvae throughout the water column, minimizing the risk of siphoning out live larvae from the bottom of the tank. The 5-gallon bucket of solids was then strained, and mortalities considered as all fish contained within the gallon bucket.

Feeding Trial

Larval fish at 8 days post-hatch (dph) were distributed to the black tanks at a density of 65 larvae per L. The trial was carried out using three replicate tanks for each experimental group. Four dietary regimens were tested in the study: Live feed only (LF reference diet), Commercial starter (CD reference diet; Otohime, Japan), Intact control diet (IC), and Silver Carp hydrolysate diet (SCH). All diet groups were first supplied live food, rotifers *Brachionus plicatilis* in excess, at 9 dph, immediately after swim-up stage. As such, 9 dph was established as first feeding day. Throughout the study all groups were fed ad libitum minimum five times a day. At 11 dph, decapsulated *Artemia* nauplii were introduced before rotifer feedings to encourage transition. Starting at 13 dph, all YP receiving *Artemia* nauplii were fully transitioned. At 16 dph, transitioning to dry diet regimens started with pellets <150um. *Artemia* were always provided after dry diets, and gradually phased out over the course of 3-4 days, to promote high intake of the experimental diets. All fish (aside from the LF group) were completely transitioned to dry diets starting on 19 dph. The trial was conducted until 32 dph when the fish reach a juvenile stage, significantly beyond the critically fragile stages of swim-bladder inflation, and dry feed acceptance. The feeding regime is presented in Figure 2.

Sampling

Twenty larvae were collected from each diet replicate during full diet transition days (Rotifer-Artemia, Artemia-Dry Diet) to track total body length and weight gain. At the end of the study, 100 randomly sampled fish from each tank were used to assess the following parameters: final length, final weight, weight gain, survival, and condition factor. The following parameters were calculated using the formulas below:

Total Mortality (#fish/tank) = Summation of daily mortality counts

Final Length (mm) = (Final length – Initial Length)

Final Weight (g) = (Final body weight – Initial body weight)

Condition = $100 * \text{Weight} / \text{Total} [\text{Length}(\text{cm})]^3$

Survival = (Initial Stocking Count – Total Mortality) / Initial Stocking Count

Histology

Additionally, 25 fish were collected from each tank and placed in formalin for histological analysis. Histological procedures were performed by Saffron Scientific Histology Services, LLC (Carbondale, IL) and in collaboration with Oregon State University. Briefly, the sampled intestinal portions were fixed in formalin, embedded in paraffin, then sectioned into slides and stained with hematoxylin and eosin. These slides were examined under a microscope and camera to assess morphological changes between groups such as length and width of villi, thickening of mucosal and muscle layers, and the extent leukocytic infiltrate. Additional slides were stained with periodic acid Schiff base and alcian blue to count goblet cells.

Statistical Analysis

The average weight, length, body condition, and mortality data were grouped by treatment and then analyzed using a one-way ANOVA to identify any significant differences that may have been present between treatment groups. If so, a least significant difference test was used to determine which groups were significantly different from one another. The same statistical analysis method was used for the histology measurements, using the numerical data provided from intestinal slides (Oregon State University, Corvallis, OR). Statistical analysis was performed with R software (RStudio 4.1, Boston, MA). Results are presented as means (\pm standard deviation).

To compare mortality between all groups the study used a combination of total mortality and survival ratios. The intact control group suffered intense mortality early on in the trial, and the total number of mortalities counted in this group exceeded the initial assumed stocking density. The error was likely a product of initial variation in volumetric stocking or in the subsequent counting of daily mortalities. Total mortality was used in order to make comparisons between all groups, while survival ratios were still determined for all other groups to make broader comparisons with other relevant studies.

Survival

At 32 dph, the average mortality over the entire course of the study was observed lowest in the SCH group (2178 ± 569) and highest in the IC (3668 ± 2026); followed by LF (2888 ± 523), and CD (2345 ± 1168). There were no significant differences ($p > 0.05$) found in the average cumulative mortality among any treatment groups, however strong trends were present (Figure 1).

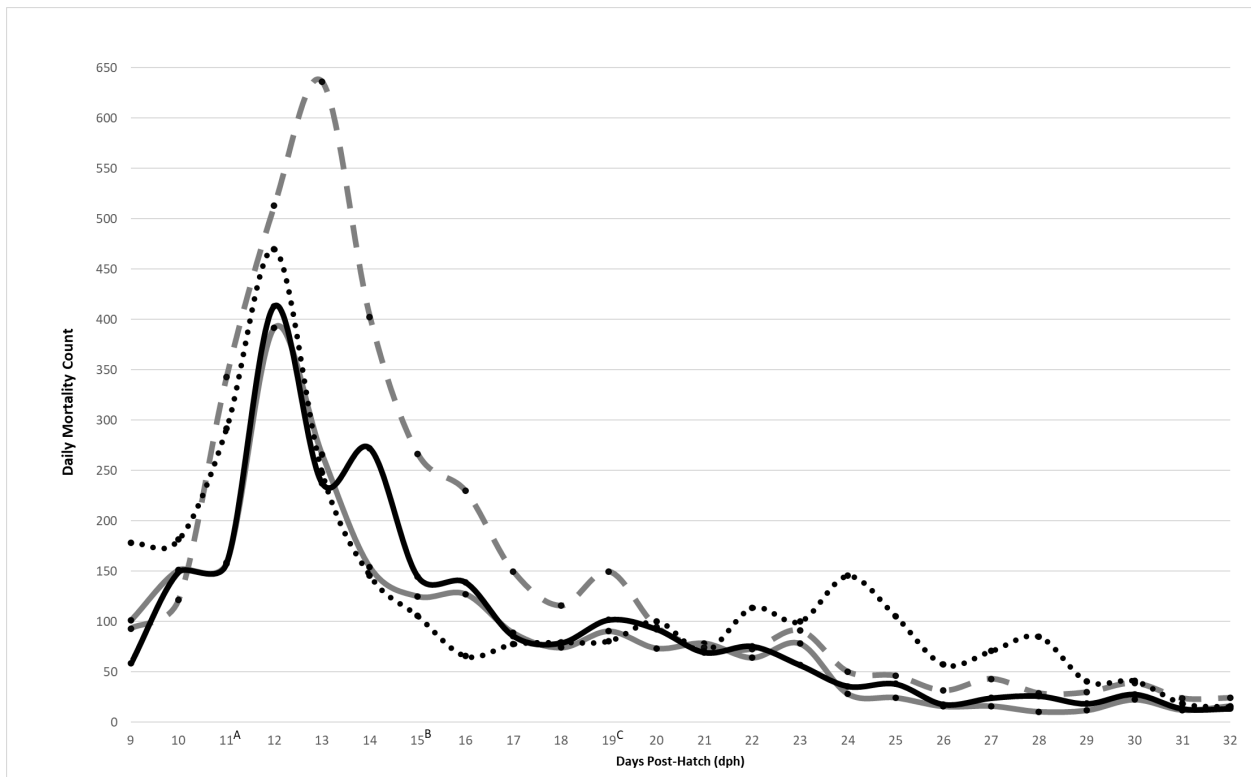
Growth Performance

There were no significant differences detected in the average final body length ($p = 0.06$) or final average fish weight ($p > 0.05$). However, the SCH group tended to have higher total length compared to the ISC but similar

compared to LF and CD. Final average fish weights tended to be highest in the CD and SCH groups, while lowest in the LF and ISC groups, respectively (Table 3).

Figure 1: Average mortality per day, by diet group, SCH (Solid Gray Line), IC (Gray Dash Line), COM (Solid Black Line), LF (Dotted Black Line). Days post-hatch (dph) 11 (A) denotes final day of rotifer feeding. Dph 15 (B) denotes test diet introduction in combination with artemia. Dph 19 (C) denotes full transition to test diet only, no artemia fed to dry diet groups after dph 19.

Body Condition



There were significant differences found in the final average body condition among treatment groups (Table 3). The average body condition of the CD group was significantly higher than that of the SCH group and the LF group ($p < 0.05$), but not significantly different from the intact control group. No other significant differences were found among treatment groups.

Histology

No significant differences were detected when comparing the intestinal villi thickness ratios or lamina propria (LP) thickness ratios ($p = 0.064$) between groups (Table 4). However, average base size and width of the LP tended to be numerically largest in the SCH group and smallest in the LF group (Table 5). Muscular thickness tended to be numerically largest in the CD and SCH groups and smallest in the LF and IC groups, respectively (Table 5).

Figure 1: Average mortality per day, by diet group, SCH (Solid Gray Line), IC (Gray Dash Line), COM (Solid Black Line), LF (Dotted Black Line). Days post-hatch (Ddph) 11 (A) denotes final day of rotifer feeding. Dph 15 (B) denotes test diet introduction in combination with artemia. Dph 19 (C) denotes full transition to test diet only, no artemia fed to dry diet groups after dph 19.

Table 3: Growth performance of study 2 diets. Superscript denotes significant difference ($p < 0.05$).

	Final Length (mm)	Final Weight (mg)	Body Condition (k)
SCH	17.92 (± 0.83) ^a	57.63 (± 10.00) ^a	0.93 (± 0.04) ^b
IC	15.57 (± 2.06) ^a	43.91 (± 22.85) ^a	1.06 (± 0.07) ^{ab}
LF	17.68 (± 1.91) ^a	50.96 (± 18.55) ^a	0.89 (± 0.08) ^b
COM	17.73 (± 1.98) ^a	66.53 (± 24.61) ^a	1.15 (± 0.01) ^a

Table 4: Villus and lamina propria (LP) thickness. No Significant differences detected.

	Villi Thickness Ratio	LP Thickness Ratio
SCH	2.44 (± 0.07)	1.47 (± 0.13)
IC	2.22 (± 0.50)	1.48 (± 0.59)
LF	2.97 (± 0.17)	1.21 (± 0.35)
COM	3.19 (± 1.28)	0.98 (± 0.15)

Table 5: Histological results of study 2 diets. No significant differences detected.

	Villi Height	Villi Width	LP Width	LP Base	Muscular Thickness
SCH	352.39 (± 35.04)	144.52 (± 12.67)	24.35 (± 5.41)	16.44 (± 2.34)	47.21 (± 2.42)
IC	329.24 (± 71.16)	147.48 (± 6.41)	18.61 (± 5.36)	13.63 (± 5.60)	44.93 (± 10.32)
LF	391.56 (± 15.48)	132.32 (± 6.94)	14.07 (± 1.91)	12.66 (± 5.33)	43.84 (± 1.28)
COM	394.89 (± 85.47)	127.91 (± 12.91)	15.92 (± 2.16)	16.28 (± 1.57)	50.32 (± 4.81)

Study 1b – Yellow perch

The focus of this study was to elucidate whether *Brachionus plicatilis* feeding schedules could be completely replaced by introduction of decapsulated A. San Francisco in first feeding larval yellow perch. Specifically, this study aimed to determine if decapsulated A. San Francisco could be captured and utilized by larval yellow perch without any significant reduction in growth or survival. Therefore, two main objectives needed to be accomplished:

- 1) Assess the size discrepancies between decapsulated artemia strains, non-decapsulated strains, and rotifers to ensure larval yellow perch can fit the live foods within their particularly small mouth gape ($< 190\mu\text{m}$) post-hatch.
- 2) Compare growth and survival outcomes of larval yellow perch fed either decapsulated A. San Francisco or rotifers (*Brachionus plicatilis*) from first feeding to assess feed acceptance and utilization.

Ethics

The care and experimentation of animals in this study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Southern Illinois University-Carbondale (SIUC). All protocols (protocol #19-033)

were approved by SIUC Institutional Care and Use Committee (IACUC). Culturing of live food followed procedures in accordance with IACUC Policy #560 “Policy on Live Animals Used as Food Sources”.

Rotifer Culture

A volume of 20L of city water was aerated for 24 hours prior to introducing rotifer culture. 400g of Instant Ocean® sea salt (XXInstant Ocean, Blacksburg, VA) was added to the aerated water or until salinity reached 17.5 (± 2.5) ppt. pH was maintained at 7.5 (± 0.5). The water temperature was then maintained at 25°C ($\pm 3^\circ$). Live rotifer bags were then acclimated by floating them in the culture tank prior to addition to the culture tank. Live rotifers were obtained from Reed Mariculture (Campbell, CA). Culture water was slowly added to the bags prior to adding the rotifers into the culture tank. A 50% water change was performed each day. Daily water quality tests were performed to maintain salinity, temperature and pH. Feeding of rotifers was done as needed, as water became clear colored, 1.5mL of Nanno 3600 (Reed Mariculture Inc., Campbell, CA, USA) was added to the culture tank. Care was taken not to overfeed, and a light green water color was maintained.

Non-Decapsulated Artemia Culture

Brine shrimp eggs were purchased from a commercial vendor (Brine Shrimp Direct, Utah, USA). 6L Macdonald jars or 6L hatching cones were filled with tap water and heavily aerated. 250g of salt was added to each jar under heavy aeration, until salinity reached 35ppt. 5g of sodium bicarbonate was added to maintain a pH above 8. The temperature was maintained at 27°C ($\pm 1^\circ$). 7g (± 1 g) of brine shrimp eggs were then added to the hatching jars. Strong illumination (2000 lux) was used above each hatching jar to trigger embryonic development and later to promote vertical migration of the nauplii. After 24 hours, aeration was removed, and the cysts were allowed to settle on the bottom. Nauplii were then siphoned from the upper column of water and captured in a 125 μ m mesh sieve. Harvested nauplii were rinsed with tap water and immediately fed to the respective treatment.

Artemia Decapsulation

The decapsulation procedure closely followed Delbos and Schwarz (2009). There are five main steps associated with Artemia decapsulation: hydration, decapsulation, deactivation, washing, and incubation. First, the cysts were hydrated by placing them in water (100g/L) with heavy aeration for one hour. The hydration process creates more spherical cysts that improves physical contact with the decapsulation solution. Second, the decapsulation solution was prepared using hypochlorite, alkaline product, and seawater. This study used liquid bleach (0.5g active hypochlorite per gram of cyst). The amount of alkaline product, NaOH (0.15g per gram of cyst), was used to maintain a pH above 10. The amount of seawater (30ppt) used was equal to a final solution of 14ml decapsulation solution/gram of cysts. The hydrated cysts were then added to the decapsulation solution under heavy aeration. The temperature was monitored throughout the reaction, because it is exothermic, to ensure temperature never exceeded 40°C. The reaction was considered complete once the solution turned from brown to yellow, then to orange. Third, the solution was deactivated using 0.1% sodium thiosulfate solution to neutralize the liquid bleach. Fourth, cysts were collected from the solution and strained through a 125 μ m screen. Cysts were washed with water to remove the excess solution as early and quickly as possible, as prolonged time in the solution affects their viability. The decapsulated cysts were stored in an airtight container and stored in a refrigerator for up to two weeks. Cysts that were stored for greater than two weeks were placed in an aerated brine (330g of NaCl per liter of water) at a concentration of 1g of cysts per 20ml of brine. Decapsulated cysts stored in this brine were viable for over 2 months. Fifth, to hatch the decapsulated artemia, 6L McDonald jars were used for incubation. The jars were filled with tap water and salt was added (25-30ppt) under heavy aeration. 0.5g/L of sodium bicarbonate was added, and the pH was maintained at 8.5 (± 0.5). Light (~2000 lux) was placed atop the jars to promote vertical migration of nauplii. The hatching jars were maintained at a temperature of 28°C ($\pm 2^\circ$ C) for 18-24 hours before the nauplii were strained onto a 125 μ m sieve, then fed to the corresponding feed group.

Live Food Measurement

Live food organisms were anesthetized in a Lugol solution prior to measurements. The rotifer and *Artemia* were measured along the length and width body axis using a microscope equipped with a camera (Nikon #XX, Japan).

Culture System

Yellow perch egg ribbons were collected from a farm (Millcreek Perch Farm, Marysville, OH) and transported immediately in aerated, pond water-filled coolers to SIUC. Each egg ribbon was placed into a McDonald-style hatching jar within a UV-treated recirculated water system chilled (Aqualogic San Diego, CA) steadily at 12.1°C ($\pm 0.4^\circ\text{C}$). Water flowed from a rubber inflow pipe down standpipe placed in the center of the jars at 2.8 L – 3 L per minute. After 8 days, once a small proportion of newly hatched yellow perch exhibited the swim up stage, the egg system was gradually raised to 15 degrees C and fish were transferred to the larval rearing system following proper acclimation. The day the majority of larvae (over 90%) in the incubation system had hatched was considered 0 days post hatch (dph). At 6 dph larvae were transferred from the incubation system to the experimental tanks. Six experimental tanks were housed in a semi-recirculating indoor water system with a municipal water source.

The semi-recirculating larval rearing system used two mechanical (sand) filters (Pentair, Minneapolis, Minnesota) and inflow was intensely aerated using trickling tower biofilters. Municipal water was treated and replaced at 3 L/min, or around 4% of total system flow. Six black, 280L tanks were used. An inline chiller (Aqualogic, San Diego, CA) was used to maintain a temperature of 20°C ($\pm 2^\circ\text{C}$). During the live feed study, salinity was kept at 1.49 (± 0.55) ppt to prolong the viability of the live food (Dabrowski and Miller 2018). A pH of 7.2 (± 0.2) was maintained throughout the study. To minimize cannibalism, multiple precautionary measures were taken. Bristow and Summerfelt (1994) demonstrated the use of Clay (up to 50 NTU) as an effective method for reducing cannibalism and creating a contrast in color to aid larvae in foraging. Using clay to keep turbidity at levels of 10-16 NTU decreased pH significantly, in which case bicarbonate was mixed into the clay before addition to the system. Light was constituted only during feedings and dimmed to the lowest setting all other times, with filtered ambient overhead lights on from 0800 to 1800 hours (Kestemont 2015). Two sprinklers with angles of 90 and 45 degrees relative to the water surface were fitted to opposing sides in each tank to break surface tension, improve flow, and provide aeration (Wojno et al. 2012). The 45-degree sprinkler was pointed in the same direction as the incoming flow, spray nozzles were checked daily to discourage sprinklers from becoming clogged with incoming clay. Laminar inflow was created using perforated inlet pipes at 2 Liters per minute (Lpm). Flow was increased as larvae became resilient and solids increased, an increase to 3Lpm occurred at dph 16 dph.

Treatment Groups & Feed Regime

At 6 days post-hatch (dph), approximately 4,150 larvae were volumetrically stocked into each tank with a starting water inflow rate of 2 L/min. Larvae were then randomly separated into two groups, and each group contained three replicates. The rotifer group (RG) followed the traditional yellow perch larvae feeding regime. From first feeding day (7 dph) fish larvae were given *Brachionus plicatilis* ad libitum 4-5 times until 11 dph. At 12 dph the RG larvae were transitioned to non-decapsulated Salt Lake *Artemia* (GSL) and fed solely GSL from 13-17 dph. In contrast, the second group, the decapsulated *Artemia* group (DG), was fed decapsulated San Francisco *Artemia* (SFD) ad libitum from first feeding (7 dph) to 11 dph. At 12 dph, the fish were transitioned to the larger decapsulated Salt Lake *Artemia* strain (GSLD) and fed solely GSLD from 13-17dph. From 17-19 dph, the two groups were transitioned to a commercial larval diet (Otohime, Japan) to assess any differences in dry feed acceptance, which was later elucidated by survival counts. At 20 dph all groups were considered fully transitioned to dry feed (Table 2).

At 11 dph, 20 fish were randomly taken from all six each replicates to measure length (mm) and weight (mg) and later compared for growth differences during the initial first feeding phase (7-11 dph). At 21 dph an additional 20 fish were randomly taken from each replicate to compare final length and weight differences. During sampling, the fish were euthanized with an ice bath until loss of opercular movement was achieved and placed on a paper towel to

remove excess water (Strykowski and Schech 2015). Fish were measured with electronic calipers and weighed on an electronic scale (Mettler Toledo, Columbus, OH, USA).

Statistical Analysis

For total length and body weight, measurements were grouped by treatment and analyzed using a one-way ANOVA followed by a Tukey’s multiple comparison’s test to find any significant differences between groups. A value of $P < 0.05$ was considered significant. Mortalities were recorded daily and used to calculate survival, which is calculated as the percentage of fish remaining from the initial stocking. Significance of survival was calculated using a one-way ANOVA. Total body length, body weight and survival are presented as the average \pm standard deviation of the three replicates. Statistical analysis was performed with R software (RStudio, PBC, Boston, MA).

Live Food Size

Analysis of live feeds under a microscope revealed numerous significant differences in length and width dimensions amongst all tested live feeds (Table 1). Most notably, the decapsulated *A. San Francisco* (SFD) nauplii body width and body length were significantly smaller than its non-decapsulated counterpart (SF); and was also significantly smaller than the *A. Great Salt Lake* strain in both length and width dimensions (Figure 1). Specifically, the SFD body width was on average $157.8\mu\text{m}$ and most similar to the average rotifer body width of $146.3\mu\text{m}$ (Table 1).

Fish Larvae Growth

At 11 dph, the DG group had significantly ($P < 0.05$) greater body weight than the RG group and tended to be larger in total body length (Table 3). This trend continued for the remainder of the experiment. At 21 dph, the DG group tended to be longer in total body length and greater in body weight than the RG group. No significant difference ($P > 0.05$) in survival was found between the RG ($53.64 \pm 9.18\%$) and DG ($55.70 \pm 20.17\%$) groups.

Table 1: Mean length (L; μm) and width (W; μm) relationships amongst rotifers, decapsulated *A. San Francisco* (SFD), *A. San Francisco* (SF), decapsulated *A. Great Salt Lake* (GSLD), and *A. Great Salt Lake* (GSL)

	Rotifers		SFD		SF		GSLD		GSL	
	L	W	L	W	L	W	L	W	L	W
Mean	191.1	146.3	431.2	157.8	469.7	190.6	502.6	188.5	507.6	207.4
SD	20.5	20.6	36.1	16.8	33.5	15.9	27.1	16.7	27.2	18.4
Min	148.2	91.5	273.5	104.0	370.2	138.1	435.7	143.3	436.1	148.1
Max	250.3	198.6	533.1	212.9	593.0	233.1	596.3	254.0	594.3	253.6

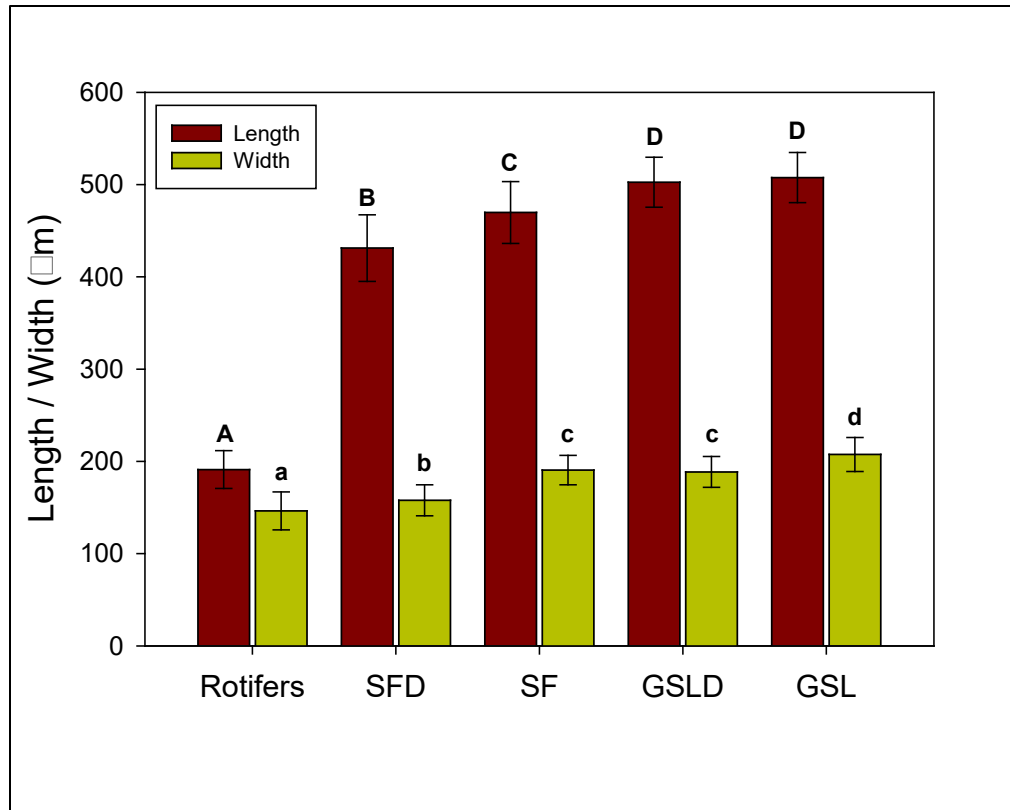


Figure 1: Significant differences amongst live feed groups. Mean length and width relationships amongst rotifers, decapsulated *A. San Francisco* (SFD), *A. San Francisco* (SF), decapsulated *A. Great Salt Lake* (GSLD), and *A. Great Salt Lake* (GSL).

Table 2: Feed schedule of live feed groups.

Treatment	7-11 dph	12 dph	13-17 dph	17-20 dph
Rotifer Group	<i>B. plicatilis</i>	<i>B. plicatilis</i> / GSL	GSL	Dry diet
Decapsulated Group	SFD	SFD / GSLD	GSLD	Dry diet

Table 3: Larval performance of Yellow Perch fed different live feed diets from first-feeding. Superscript denotes a statistically significant difference between groups.

	Weight (g) 11dph	Weight (g) 21dph	Length (mm) 11dph	Length (mm) 21dph
Rotifer Group	1.89 (±0.24) ^a	14.94 (±2.31)	6.36 (±0.60)	12.75 (±0.5)
Decapsulated Group	2.59 (±0.34) ^b	18.15 (±5.16)	7.49 (±0.46)	13.46 (±1.54)

Study 2 – Walleye

Study 2 was conducted in spring of 2023 and aimed to assess the larval response in walleye (*Sander vitreus*) to hydrolysate when introduced at first feeding and when used as a weaning diet (hereafter first feeding trial and weaning trial respectively). Walleye larvae were obtained from eggs obtained from a captive broodstock held at UWSP NADF. At 4 days post-hatch (DPH) larvae were stocked into 240L tanks at a density of 5 larvae/L or 1200 larvae/tank. Tanks were provided with flow-through 20°C water containing clay (KT OM-4, L&R Specialties, MO) delivered via a peristaltic metering pump to increase turbidity and reduce maladaptive clinging behavior and cannibalism (Clayton et al., 2011; Rieger & Summerfelt, 1997). Flow rates began at 2 L/minute with tank stand pipes containing a small screen size. Both flow rates and screen size were increased at regular intervals as the larvae grew and more feed was required (Table 1). Water temperature, dissolved oxygen, turbidity were monitored daily and maintained at values optimal for walleye larviculture (Hauser et al., 2023; Summerfelt & Johnson, 2015). Dim lighting (2 lux) was provided 24h/day and a constant surface spray was maintained to disrupt the surface tension of the water and facilitate gas bladder inflation (Clayton et al., 2011). Tanks were inspected and cleaned daily via siphoning to ensure a small amount of uneaten feed was present each day. During daily cleaning, all mortalities were removed and enumerated (observed mortalities). A random sample of 15 fish per tank were taken every 10 days and examined to estimate gas bladder inflation, feed acceptance, deformity rate, length, and weight. 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: $((P_{\text{initial}} - P_{\text{final}} - P_{\text{sampled}} - \text{Observed mortalities}) / P_{\text{initial}}) \times 100$ where P is the tank population. Deformity rate and gas bladder inflation rates were calculated as a percentage of the final sample at day 30 or 40 (first feeding and weaning trials respectively). Samples were collected for histological analysis and qPCR, but have not been analyzed yet. All data were analyzed using R version 4.3.0 (R Core Team, 2021). Fish weight and survival were analyzed via Kruskal Wallis test and Wilcoxon Rank Sum post-hoc test with $\alpha = 0.05$.

In the first feeding trial, three diets were tested in triplicate: Commercial starter (Otohime, Japan), Intact protein (Control), and Hydrolysate. Diets were fed following size and rate recommendations previously developed for walleye (Table 1; Hauser et al. 2023) and were delivered continuously over a 24-h period with rotary micro-diet feeders. In the weaning trial, all treatments were fed decapsulated artemia nauplii for the first 18 days before transitioning to one of 4 weaning diets (in triplicate): Commercial starter (Otohime), Intact protein (control), Hydrolysate, and CPSP. Artemia were fed 3 times daily at a rate of 1944 nauplii/fish/day based on the initial stocking density. On day 18, the C1 size of the respective diet was introduced following the same feed schedule from the first feeding trial (Table 1).

Results

In the first feeding trial, survival was highly variable at the end of the 30 day larviculture period. The hydrolyzed diet treatment resulted in both the highest (43.5%) and lowest (0%) survival rate. The commercial starter diet had more consistent survival rates, but there were ultimately no significant differences in survival among diet treatments (Kruskal-wallis, chi-squared = 0.62, df = 2, p-value = 0.73; Figure 1). Growth performance did differ significantly among diet treatments (Kruskal-Wallis, chi-squared = 84.244, df = 2, p-value < 0.0001). The commercial starter diet walleye reached the highest average weight at the end of the 30 day larviculture period (0.52g) followed by the hydrolyzed diet fish (0.33g) and intact diet fish (0.12g; Figure 2). Neither gas bladder non-inflation nor lack of feed in the gut were present in fish from any treatment at day 30. Deformities were present in 11.1% of intact treatment fish, but were absent in the commercial starter and hydrolyzed diet treatments.

In the weaning trial, survival was highest in the commercial starter diet treatment (Figure 3), but differences among treatments were not significant (Kruskal-Wallis, chi-squared = 6.5897, df = 3, p-value = 0.086). Average weight at the end of the weaning trial differed significantly among treatments (Kruskal-Wallis chi-squared = 131, df = 3, p-value < 0.0001). Larvae weaned onto the commercial starter diet were significantly larger (1.08g) than larvae

weaned to the hydrolyzed (0.49g; $p<0.0001$), intact (0.55g; $p<0.001$), and CPSP (0.23g; $p<0.001$) diets (Figure 4). The larvae weaned onto the hydrolyzed and intact diets were not significantly different from one another ($p=0.11$), but were larger than those weaned onto the CPSP diet (Figure 4). Gas bladder non-inflation was not present in larvae from any treatment. Feed acceptance was also high in all treatments with lack of feed in the gut only present in 2% of larvae in the intact diet treatment and not present in the other treatments. Deformity rates were high (86.6%) in the CPSP treatment followed by the intact treatment (37.8%), and the hydrolyzed treatment (28.9%). No deformities were present in larvae in the commercial starter treatment.

This study suggests that the introduction of a hydrolyzed diet has considerable advantages for growth performance over intact proteins when fed as a first feeding diet for walleye. However, these advantages were not present when introduced as a weaning diet. Larval fish have physiological restraints on gut absorption capacity and digestion relative to fully developed adults (Kjørsvik et al., 2011), and during the first few weeks of larval development, peptide availability, amino acid availability, and protein solubility are critical for larval digestion (Hamre et al., 2013). These requirements are possibly more critical for walleye prior to the start of weaning to a dry diet in our study (18 days) than they are after that period. Regardless, our results highlight the critical importance of diet formulation in early larval development for walleye. Unsurprisingly, the commercial starter diet outperformed the hydrolyzed diet suggesting that it is adequately meeting the protein and solubility requirements for larval walleye and that a hydrolyzed protein is not the only component required for a successful larviculture feed. With further optimization, the hydrolyzed protein diet we tested may be able to compete with Otohime as a first feeding diet for walleye and at minimum demonstrates the importance of hydrolyzed protein sources in larviculture diet formulation.

Figure 1. Survival rate at 30 days in the larviculture system for the first feeding trial.

Day in System	Feed Rate (gm/1000fish)	Feed Size	Screen Size	Flow rate
1-8	4	100% B1		2LPM
9-10	5	75%B1:25%B2	#1	4LPM
11-13	6	50%B1:50%B2		
14-15	8	25%B1:75%B2		
16-17	12	75%B2:25%C2		
18-19	16	50%B2:50%C1		
20-21	28	25%B2:75%C1	#2	
22	32	25%B2:75%C1		
23-24	50	100%C1		
25-26	55	75%C1:25%C2		6LPM
27-30	55	50%C1:50%C2: Introduce Dressed 1mm**		
32	60	100%C2: Dressed 1mm**	#3	
33-35	20%TBW	75%C2: 25%1mm**		
35-40	20%	50%C2: 50%1mm**		

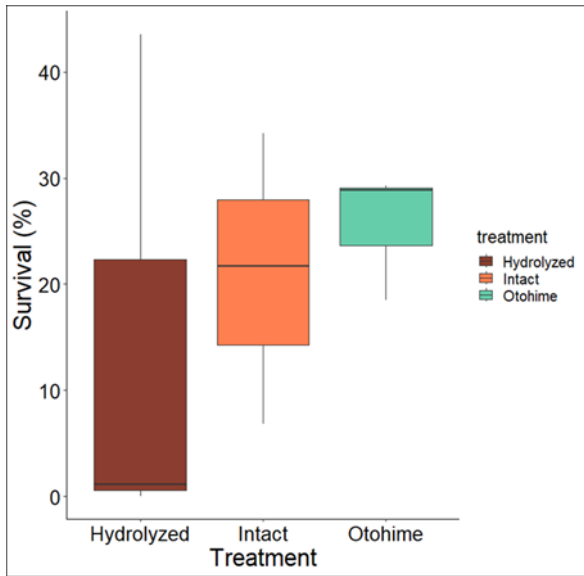


Figure 1. Survival rate at 30 days in the larviculture system for the first feeding trial.

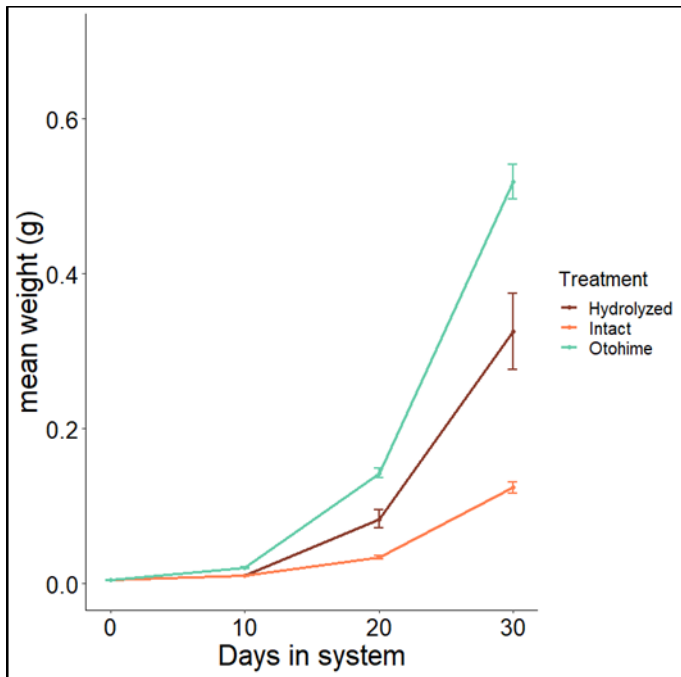


Figure 2. Mean weight of larval walleye in the first feeding trial through 30 days in the larviculture system.

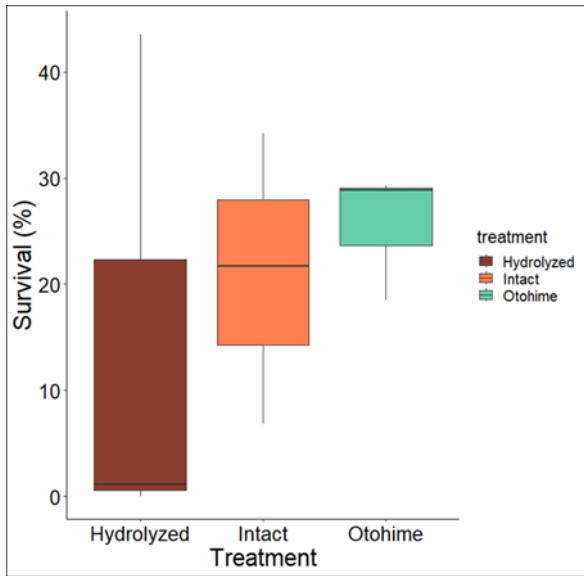


Figure 3. Survival rates at the end of the 40 day larviculture trial.

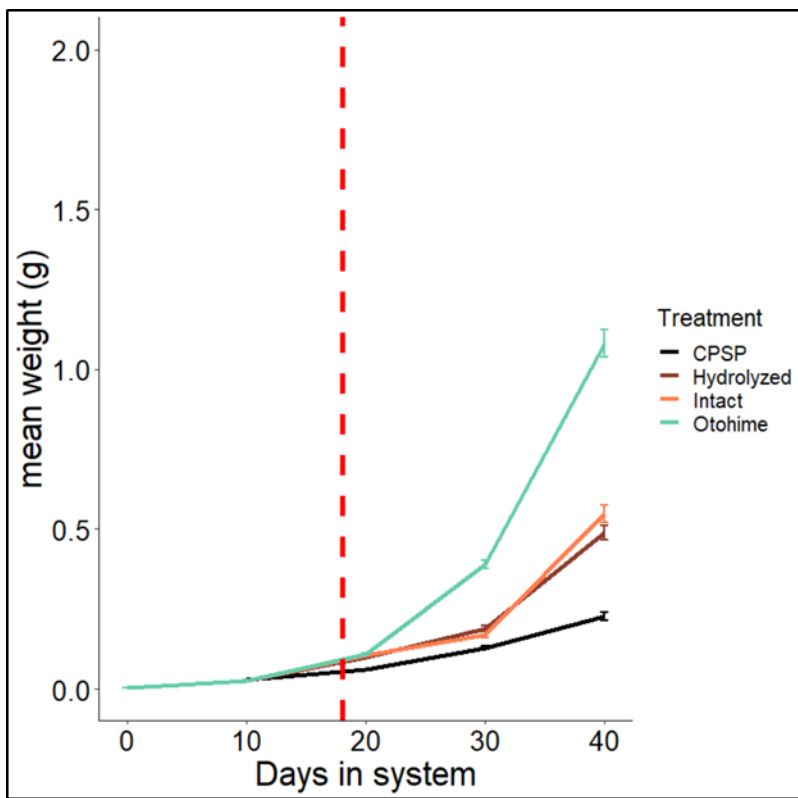


Figure 4. Mean weight of larval walleye during the weaning trial. The dashed red line indicates the beginning of weaning from Artemia to the indicated diet treatment.

Q12 Principal Accomplishments: (Summarize in a concise form the findings for each objective for the duration of the project. Measurement data are to be given in metric units. However, to minimize confusion, a dual system of measurement may be used to express results.)

Study 1a – Yellow Perch

Indoor RAS Percid larviculture, especially that of yellow perch, remains in a state of infancy. Pikeperch *Sander lucioperca*, walleye *Sander vitreus*, and yellow perch are predominantly reared in nursery ponds for 4-6 weeks using a steady supply of live prey. Often, the young juveniles are then transferred to flow-through tank systems for dry pellet habituation (Kestemont et al. 2015). This practice has been adopted by some commercial-scale yellow perch producers, who are met with various challenges and inconsistent results (Shambach 2022). A major issue with this process is the stress induced by moving these fish, especially smaller (less than 17-20mm) which commonly results in significant mortality and heightened susceptibility to disease (Brown et al. 1994). The move to complete intensive tank culture from hatch has been a major focus for natural resource agencies in recent decades and the percid sector has continued to be met with further challenges during early development (<20 dph). Percids reared in tank systems experience cannibalistic rates (3.2-13.7%), clinging behavior, skeletal deformities, and poor swim-bladder inflation, however an advantage of tank systems is the ability to remove or grade disproportionately sized individuals quickly (Bodis et al. 2007; Summerfelt et al. 1994) and proper feed training. Tank systems allow for consistent monitoring of the entire fish population and the ability to provide food in a consistent manner within the individual tanks, generally improving feed efficiency and reducing transport stress. Bristow, Summerfelt, and Clayton (1996) also demonstrated the effective use of OM-4 clay to create a turbid environment which reduced cannibalism to 1.1-1.7% and substantially mitigated clinging behavior with turbidities of 5-23 NTU. Moore et al. (1994) developed a system of surface sprayer orientations which effectively improved gas bladder inflation rates ranging from 90-100%. Malison et al. (1994) demonstrated methods for feed training perch using lights to congregate the photopositive fish within their initial nursery ponds. Stocking density in a pond setting can influence the availability of natural food sources and result in rapid depletion of adequate live prey availability, resulting in an increase of cannibalistic behavior and increasingly greater size variation over time (Kestemont et al. 2015). Furthermore, in this study yellow perch were fully transitioned to dry diet by 20dph and all groups were less than 17mm during this stage and acceptance of dry feed was observed at five days. As suggested by Malison and Held (1992), if perch could be harvested from ponds at a smaller size, then faster habituation time could be achieved in fish smaller than 17mm and would likely result in improved growth and feed conversion later on in the production cycle. Building upon the contributions from these past studies, the indoor system in this study used over-tank lighting to congregate fish during feedings, black colored tanks, perforated drain screens to allow the passage of oils, and OM-4 ball clay (16 NTU), laminar flow, and for the first time 45- and 90-degree surface sprayers to ease gas exchange during swim bladder inflation in each tank. All these modifications of larval perch culture environment likely contributed to the high survival and growth performances of perch in our study with no observable deformities or clinging behavior and 100% swim bladder inflation rates across all groups. This was a success that was never reported before.

Although no statistical differences were detected in mortalities, a strong trend was present when comparing the control diet group to other treatment groups. All groups suffered similar mortality rates during the initial live feed phase, however, it is not uncommon for yellow perch to exhibit early mortality. Traditionally when percids reach fingerling size (16-32mm), transition from live feed to pelleted diets results in average mortalities ranging from 14-59%, especially during the first few generations of domestication and even at low-experimental stocking densities (Kestemont 2015; Molnar et al. 2018). In other percid studies, pikeperch fingerlings transferred to flow through tanks reported survival at 41-52% using daphnia and pelleted feeds, respectively (Bodis et al. 2007). In a previous study with similar tank size and turbidity (23.8 NTU) larval walleye (21dph) were seen to have a survival rate of 44.3% (Bristow et al. 1994). All groups in this study exhibited a similar pattern of mortality peak during the initial live feed stage (9-16 dph), because this trend was seen during a period when the yellow perch were transitioning to exogenous feeding it may explain why the peaks were observed across all groups. Moreover, the silver carp

hydrolysate tended to have the highest survival ($48\% \pm 11\%$) followed by the commercial reference diet ($43\% \pm 28\%$), live feed ($30\% \pm 13\%$), and the intact control diet ($23\% \pm 32\%$).

Early mortality is a common occurrence in larval percid species culture as they are considerably smaller at hatching than other commercial fish species and require vast amounts of live feed to be present in the environment to adequately receive the proper nutrition for growth and survival (Grayson and Dabrowski 2022). Unlike other species which have more complete digestive tracts upon the transition to exogenous feeding, yellow perch may benefit from the enzymes present in live feed during this time directly following swim bladder inflation (9-13dph) (Kolkovski 2001). Many other studies have observed significant mortality patterns during this stage of life, when all fish are only beginning to search for external sources of food (Yufera and Darias 2007). During this transition, every group in the study followed this mortality peak trend, which likely signals the challenge each yellow perch faces as they absorb the last of their highly nutritious yolk and begin to search for food, with varying success (Malison and Held 1992).

It is well established that digestion in larvae depends on activities of pancreatic and intestinal enzymes, and possibly on the contribution of live feed enzymes and intracellular digestion of protein absorbed by pinocytosis (Watanabe 1982; Kolkovski 2001; Zambonino-Infante & Cahu 2001). However, the nutrient composition of live prey diets is suboptimal to promote growth in larvae at their later stages (Planas and Cunha 1999; Conceicao et al 2003). This trend was particularly observed in the live feed group over the course of the study when receiving dry diets. During the transition to dry diets (16-20dph) the intact control group also tended to experience greater mortality compared to all other groups. Alternatively, the other two dry diet groups' (commercial and silver carp hydrolysate) daily mortality closely resembled one another throughout the remainder of the study. The simplest explanation for this trend was the primary difference between the form of protein contained in the silver carp hydrolysate diet and the intact control diet. The absorption of hydrolyzed protein has been observed to be 2.2-3.0 times faster than intact protein in pre-metamorphic Atlantic Halibut *Hippoglossus hippoglossus* (Tonheim et al. 2005). Further, it is becoming more widely accepted that many species at their pre-metamorphosis stage harbor a significantly greater capacity for digesting 5-70 kDa oligopeptides compared to post-larvae which are more efficient at utilizing polypeptides and intact proteins (Canada et al. 2017). Some research also indicates that dietary excess of protein hydrolysates can reduce growth performance in some species (Kolkovski and Tandler 2000). Atlantic cod *Gadus morhua* larvae performed better with up to 40% hydrolyzed protein in the diet, while Atlantic halibut larvae exhibited reduced survival with more than 10% hydrolyzed protein (Kvale et al. 2009).

It is not uncommon for hydrolysate feeds to produce mixed results in larval fish studies, however, the silver carp hydrolysate from this study which was created using our in-vitro hydrolysis method seemed to induce similar growth to that of the commercial reference diet containing marine based hydrolysates (CPSP). In this study, acceptance of dry feed was observed at five days from introduction when the yellow perch were 16 dph. As suggested by Malison and Held (1992), if perch could be harvested from ponds at a smaller size, then faster habituation time could be achieved in perch smaller than 17mm and would likely result in improved fish growth and feed conversion later in the production cycle.

Dietary hydrolysates have shown to influence the transport and efficient uptake of proteins in young fish, particularly since many larval fish can absorb free amino acids, dipeptides, and tripeptides through pinocytosis (Kwasek et al. 2022). Early introduction to the silver carp hydrolysate feed did not yield any significant differences in final body weight of the larval yellow perch compared to other groups, however the intact control diet tended to have numerically the lowest final body weight. The final average weight of the silver carp hydrolysate group also tended to be greater than that of the live feed and intact control groups, but less than that of the commercial reference diet group. Similar trends have been seen in studies such as Kotzamanis et al. (2007) which observed improved weight gain and growth in diets containing CPSP, a similar high-quality hydrolysate mixture to that found in this study's commercial reference diet, compared to that of experimental sardine hydrolysate. Additionally, this study also found that the intact control group tended to have the lowest final weight. Other studies which compared

pre-digested proteins found similar trends in growth, where fish fed moderate levels of protein hydrolysate tended to have greater weight gain than that of solely intact protein diets (Cahu et al. 1999 and Srichanun et al. 2014). Finally, the silver carp hydrolysate group tended to have final total lengths numerically greater than all other groups, but quite similar to that of the reference diet groups. Most notably, the intact control diet was the only group which did not achieve a final total length greater than 17mm. Previous studies like that of Sheng et al. (2022) and Molinari et al. (2021) found that largemouth bass *Micropterus salmoides* fed protein hydrolysates had significantly larger total body lengths than those fed diets containing only intact protein. Since the intact protein might not be as efficiently utilized for growth compared to its hydrolysate form this further supports the notion that large molecular weight protein may be less than ideal during the early stages of yellow perch growth.

Given the high mortality in the intact control group, it is possible that the most fit individuals may be present in the final sampling data. However, even if this were assumed, individuals randomly sampled from the intact control group still tended to be smallest in final weight and length. Evidence that individuals from the intact control group tended to be disproportionately fit can be seen in the body condition parameters. While survival, final total length, and final weight tended to be lowest in the intact control group; body condition was observed to be most similar to the commercial dry diet, which was significantly different from the live feed and the silver carp hydrolysate diets. Body condition is generally accepted as a determinant of fitness and uniformity (Brosset et al. 2023). Although the body condition of the intact control group tended to be higher than the silver carp hydrolysate diet, there were no significant differences found between these two groups.

Alternatively, when considering the surviving larvae in each group, it is possible that the less fit individuals may have survived in the silver carp hydrolysate group that may have not survived within the silver carp intact group. Therefore, it may be possible the less fit individuals from the silver carp hydrolysate group were present in the subsequent samples, while the intact control group may have had disproportionate numbers of fit individuals which overcame the well-known effects of intact protein digestion at an early age. Several studies have reported that moderate levels of fish protein hydrolysate can promote onset of adult mode of digestion (Cahu et al. 1999 and Ovissipour et al. 2012). It is generally accepted that the ability to digest intact protein increases as fish age through a process referred to as enzymatic maturation of the intestine (Lao et al. 2007). The larval digestive capacity of dipeptides, peptides, and FAA are especially high due to increased peptidase activity, which declines during development (Kwasek et al. 2022). Species specific requirements during digestive organ development are still generally unknown, but intestinal health and maturation can be a significant contributor to a fish's ability to digest formulated diet as well as having effective immune response to potential viruses or infections (Holt et al. 2011).

Understanding the morphological changes within larval digestive systems is essential for further optimization of larval rearing and feeding techniques. The feeding habits of fish larvae are intertwined with the development of digestive structures (Moghadam et al. 2014). Percids and many other fishes do not possess a functional stomach at the time of first feeding, which influences their ability to digest and utilize complex dietary proteins (Ostaszewska 2005; Zambonino and Cahu 1994). In pikeperch, the development of the stomach coincides with the appearance of gastric glands, pyloric sphincter, and the elongation or looping of the intestine around 15-20 dph (Hamza et al. 2015). In Eurasian perch *Perca fluviatilis* (15-30 dph) there is a strong increase of brush border membrane enzyme activity that coincides with a decrease of cytosolic enzyme activity (Hamza et al. 2007). This shift in gastric development reflects a point in which larvae acquire an adult mode of digestion (Cahu and Zambino Infante 1994). The maturation of the larval digestive tract in many teleost species coincides with other morphological, histological, and physiological developments (Tanka 1969). For example, sea bass *Dicentrarchus labrax* larvae exhibit scarce and irregular microvilli during their first week of life, when they are around 3-5mm in length (Hernandez et al. 2001). When the larvae have absorbed their yolk sac the intestine begins to form more numerous long microvilli and muscular striations. By 30 dph, the larvae exhibit significantly more complex digestive structures than at the time of hatch. Some of these include a thick developed muscular layer, differentiation of gastric glands, a noticeably thicker

lamina propria, and numerous long microvilli (Herandez et al. 2001). Therefore, using such identifiers through histological analysis can be a useful tool for determining the transition of young fish to adult mode of digestion.

In this study, the two larval diets which contained endogenous enzymes (SCH and IC) tended to have the widest lamina propria and largest lamina propria base compared to the other reference diets. The development of digestive structures and the various enzymatic activities can be modified by the biochemical composition of the diet which, in turn, can affect the gut maturation process. This modification has been demonstrated in percids and other larval species such as common carp and sea bass *Dicentrarchus labrax* (Kestemont et al. 2001; Gisbert et al. 2012; Cahu et al. 1998). While the histological analysis did not yield any significant differences, gastric glands are generally situated in the lamina propria; and the oxynticopeptic cells contained within this region largely contribute to the breakdown of complex proteins (Lazo et al. 2011).

While no significant conclusions could be drawn from the other available gut morphology data in this study, the high total mortality of the control group could be partially linked to the unattractive properties of complex proteins during larval gut development. When additionally considering the high condition factor of the surviving control group it may be possible that the surviving control group fish were more equipped to utilize complex proteins allowing them to achieve adult mode of digestion. The villus ratios of the test diets revealed that the SCH and IC diet were most uniform compared to the reference diets (i.e. closest to 1.0) (Table 4). While other larvae from the control group may have struggled to process larger complex proteins or refused to transition from artemia to dry feed, the control group may represent those individuals which were equipped with genetic advantages over others. The lack of weaning success cannot be solely attributed to the absence of a functional stomach or lower digestive enzyme production, additionally, previous authors have suggested the overall contribution of live prey exogenous enzymes to the total digestive capacity of larvae is negligible in most species (Holt et al. 2011). It seems that the enzymatic capacities of larvae are not necessarily the only limiting factor to digest microdiets and ensure proper growth. Rather, the diet suitability and its ability to effectively stimulate digestive enzymatic secretion may be of greater influence (Hamza et al. 2008; Holt et al. 2011). While the silver carp hydrolysate diet may not be optimized, this study demonstrated that hydrolyzed silver carp (32.1% inclusion) can perform as well as the industry leading commercial diet and standard feeding regimen of live feeds.

Study 1b – Yellow perch

Larvae of most fish are, from an evolutionary perspective, fixed on an early diet of motile prey organisms. Even if larval fish accept inert diets, their poor enzymatic activity and low-function stomach will likely not allow them to digest and effectively utilize existing formulated diets (Pedersen and Hjelmeland 1988; Dhert et al. 2001). It has been suggested that larvae with only a partially developed digestive tract after hatching strongly depend on exogenous enzymes from live food. It has been proposed that rotifers and other live feeds such as *Artemia* are partly digested by their own enzymes by the time they reach the gut of larval fish (Lauf and Hofer 1984; Munilla-Moran et al. 1990; Kolkovski et al. 1993). Previous studies have shown that larvae fed live foods from the onset of feeding could achieve better digestive tract development, improved growth, and reduced mortality (Madkour et al. 2022).

Rotifers (*Brachionus plicatilis*) are the primary choice of many hatchery operators because of their small size (130-320µm), caloric value, relatively low mortality, and rapid reproduction to high densities (Yoshimatsu and Hossain 2013). Extensive culture of rotifer species requires only a few hours of labor but is often inconsistent and limited to the carrying capacity of algal blooms within a given pond. Ponds are often fertilized 1-3 weeks prior to larvae being introduced into the system. Perch larvae remain in fertilized ponds for 30-45 days, with survival ranging from 2.5-32% (Brown and Dabrowski 1996). Successful extensive larval rearing is often dependent on the timing of the algal bloom and subsequent zooplankton grazing. Because of this, larval rearing success can be extremely inconsistent, with factors such as foraging success, cannibalism, and stocking density contributing to the overall challenges of

early larval development. After being in the pond for up to 45 days, yellow perch are traditionally harvested, placed into tanks for feed habituation, then returned to ponds, with success rates from 0-60% (Malison et al. 1994).

However, a great deal of mortality can be attributed to this process due to handling and stocking density stress, water chemistry fluctuations, and diet changes. Therefore, indoor hatching and larval rearing could eliminate many of the early stressors of the traditional larval yellow perch rearing process because yolk-sac fry can be moved easily into a single RAS system and reared beyond acceptance of dry feed, as demonstrated by this study. Introducing larval fish to live feeds has been shown to be beneficial to numerous species as opposed to first feeding of formulated diets (Vanhaecke et al. 1990; Harzeveli et al. 2004; Laczynska 2020).

It was previously unknown whether larval yellow perch could accept *Artemia* nauplii from first-feeding. It is believed that the mouth gape of larval yellow perch is too small to consume and ingest traditionally hatched nauplii. However, body size varies amongst the numerous strains of *Artemia* and nauplii size at hatch can also be influenced by the decapsulation procedure, cyst batch, and hatching environment (Webster and Lovell 1990; Vanhaecke and Sorgeloos 1983). It is critical to measure nauplii after hatch in a timely manner, as the nauplii will molt up to 15 times and reach maturity in 8-20 days (Madkour et al. 2022; Webster and Lovell 1990).

In this study, nauplii body length and body width were measured and significant differences were found between SFD and all other live food sources in the study. In this study it was quickly apparent, through visual observation, that the translucent stomachs of majority of the larval Yellow Perch in the DG were filled with bright orange SFD *Artemia*. While the body length of SFD *Artemia* was well beyond the mouth gape of larval Yellow Perch, the body width was measured on average to be $32\mu\text{m}$ ($\pm 16.8\mu\text{m}$) smaller than the mouth gape capabilities of larval Yellow Perch ($<190\mu\text{m}$) (Table 1). This evidence suggests that larval Yellow Perch can capture prey based on one dimension of the live feed being of proper size to fit the mouth gape.

Feeding ecology has been difficult to observe for Yellow Perch less than 12mm (Graeb et al. 2012). However, it is well established that fish tend to select prey that optimize their growth and survival (Werner and Hall 1974). Furthermore, fish and other predators choose prey based on energetic tradeoffs, prey abundance, and capture efficiency; these tradeoffs will change numerous times as larval fish mature. For example, Graeb et al. (2012) observed a willingness of yellow perch (12-16mm) to consume cladocerans with high capture efficiency and long handling time while other Yellow Perch (12-16mm) consumed copepods with low capture efficiency and short handling times; and ultimately similar net energetic gains were realized between both groups. A similar predator-prey interaction may be present in this study, where energetic tradeoffs of capturing SFD were likely offset by prey abundance, capture efficiency, or handling time. It further highlights the importance of frequent feedings and increased opportunity for prey interactions that are specific to RAS systems and tank rearing of larval fish.

While it may be difficult to further elucidate the feeding behavior of the newly hatched larval yellow perch, this study suggests that larval perch may quickly learn to orient themselves to capture SFD along the length axis and capture prey successfully with no significant difference in survival. In fact, a significant difference ($p < 0.05$) in body weight was observed between the DG and RG group after the initial four day first-feeding stage was complete. Further, body length and weight in the DG group tended to be greater than the RG group for the entirety of the study suggesting that SFD and GSLD can be used as an exclusive diet for first-feeding Yellow Perch. These findings are consistent with other patterns observed in the field, where naupliar copepods dominated Yellow Perch ($<12\text{mm}$) diets when abundant (Schael et al. 1991; Fisher and Willis 1997).

While rotifers remain to be the primary source of live feed for many larval fish, the effectiveness of fish hatcheries can be greatly improved through expanding the knowledge of various *Artemia* strain characteristics (Dhont and Dierckens 2013). It was established shortly after the advent of commercial *Artemia* use, that certain nutrient deficiencies do not satisfy the requirements of some, especially marine, predatory fish (Sorgeloos et al. 2001; Dhont and Dierckens 2013). During the early adoption of *Artemia*, challenges associated with detrimental bacterial loads, deficient levels of DHA/EPA, and a lack of standard propagation protocols were like that of rotifer species (Dhont and Dierckens 2013). However, nauplii can be enriched with HUFAs, immunostimulants, and other microparticulate products because they are non-selective feeders; moreover, the biosecurity risks associated with rotifers can be

minimized in Artemia culture through the decapsulation process and at a level achievable by even novice hatchery producers.

Study 2 – Walleye

Our findings suggest that the use of a diet based on hydrolyzed protein derived from carp muscle tissue using same-species digestive enzymes has considerable advantages for growth performance over intact proteins of the same origin when fed as a first feeding diet for walleye. However, the hydrolyzed protein diet did not significantly improve survival relative to other treatments, and the improved growth performance was not present when the diet was used to wean larval walleye off live feed. These results highlight the potential benefits of protein hydrolysates in larval diets, particularly during early development, but further refinement is necessary before they can match the performance of commercially available diets.

The difference in growth performance for the hydrolyzed diet treatment between the first feeding and weaning trials suggest that the requirement for smaller peptides and free amino acids is more critical in the early stages of walleye larval culture. Larval fish have physiological constraints on gut absorption capacity and the specific dietary requirements of larval fish progressively change during development (Kjørsvik et al., 2011). During the first few weeks of larval development, peptide availability, amino acid availability, and protein solubility are more important for larval digestion than latter weeks of larval development (Hamre et al., 2013). These requirements also appear more critical for walleye prior to the start of weaning to a dry diet in our study (18 days) than they are after that period, which would explain the difference in relative growth performance and incidence of deformities between the hydrolyzed and intact diet treatments during the first feeding trial. The lack of a difference in growth during the weaning trial likely suggests that peptide and amino acid availability is less critical after 18 days.

Our trials highlight the critical importance of diet formulation in early larval development. As expected, the commercial starter diet outperformed both experimental diets in both growth and survival. However, due to the proprietary nature of commercial feeds and therefore, differences in nutritional composition, ingredients type and source, and pellet physical properties direct comparison of the commercial starter with experimental diets might be misleading. Otohime and other commercial starters have undergone extensive testing and refinement that make direct comparison with our trial diets difficult. With further optimization, the hydrolyzed protein diet we tested may be able to compete with Otohime as a first feeding diet for walleye and at minimum demonstrates the importance of hydrolyzed protein sources in larviculture diet formulation. Investing resources into developing alternatives to currently available larval diets is important as the industry has previously suffered from the sudden loss of imported diets due to import bans (Fischer et al., 2022; Johnson et al., 2011).

Outreach and Extension

The primary focus of outreach and extension as described above (See Objectives 5 and 6) was to 1) disseminated practical information regarding successful larval rearing protocols and research findings to stakeholders interested in rearing yellow perch and walleye larvae fingerlings in indoors systems and 2) provide feed manufactures with the knowledge and the tools for the production of high-quality well-digested diets based on research findings. Outreach and extension accomplishments are:

Provided a one-day hybrid workshop to 63 farmers on larval feeds and intensive early life stage fish culture of commercial important NCR fish species—largemouth bass, yellow perch, and walleye. Total number of attendees at the workshop was 71—60 participants, 11 speakers (three speakers were farmers).

Published recorded workshop talks on UWSP-NADF' YouTube channels and created a workshop playlist. Available online: <https://www.youtube.com/playlist?list=PLP8KoWtbBLVy-Zpsxkp1cTQp81VLBP59Y>

Provided online access to PowerPoint Presentation after the workshop. Available online: https://uwspedu-my.sharepoint.com/personal/ehauser_uwsp_edu/_layouts/15/onedrive.aspx?id=%2Fpersonal%2Fehauser%5Fuwsp%5Fedu%2FDocuments%2FPROJECTS%2FWALLEYE%2FWalleye%202023%2FAsian%20Carp%20Hydro%2F

[Workshop%20SIU%2FIntensive%20Larval%20Culture%20Workshop%202023%2FFinal%20Presentations%2FIntensive%20Larval%20Culture%20Workshop%202023&ga=1](https://www.uwsp.edu/nadf/northern-aquaculture-demonstration-facility/initial-dietary-protein-source-and-palatability-enhancer-for-successful-production-of-fingerlings/)

Created a project page on the UWSP-NADF website. Available online: <https://www.uwsp.edu/nadf/northern-aquaculture-demonstration-facility/initial-dietary-protein-source-and-palatability-enhancer-for-successful-production-of-fingerlings/>

Provided the opportunity for 11 farmers to tour SIU's aquatics research lab and see operational incubation, larval rearing, fingerling rearing, and live culture systems.

Provided 108 workshop registries with links for workshop resources, recommended resources, and Q&A posted in the chat feature. Speakers were asked to register.

Shot additional AV assets for a practical video guide on larval and fingerling yellow perch rearing.

Created scripts for ten modules for the video guide. Topics covered by modules are cleaning and disinfection, setting up a incubation system, egg incubation, larval rearing tanks, stocking larval rearing tanks, first feeding, rotifer culture, artemia, cleaning and maintaining larval rearing tanks, and dry feed training.

Informational and instructional based media is currently being developed in conjunction with Purdue University. Media is, so far, planned to be distributed to farmers, public meetings, through Sea Grant, and at national/local aquaculture conferences at least through the year 2023.

The fact sheet entitled *Production and Verification of Fish Muscle Hydrolysate: A Novel Method for Producing Sustainable Dietary Protein* was published.

The ground work has been done for the production of up to 10 video modules on larval yellow perch culture. Production work for Module 1: Clean & Disinfection is complete and Modules 2: Preparing for Eggs: Setting Up Your Incubation Systems, Module 3: Egg Incubation, and Module 4: Larval Rearing Tanks are in progress. Modules 5: Stocking Larval Rearing Tanks and Module 6: First Feeding are in the queue to be worked on next.

The innovative diet formulation and knowledge derived from the study will provide the US industry with new approach for obtaining a high quality cost-effective protein source and development of successful high-quality feeds that will support sustainable expansion of the hatchery sector using RAS systems and consequently contribute to the development of competitive and intensive aquaculture market in the Midwest. These innovative feeds produced using SIUC commercial feed processing method (small scale) will allow for immediate implementation of the formulation by the aquafeed industry.

Increased new knowledge pertaining to rearing of largemouth bass, yellow perch and walleye to NCR residents and non-NCR. Evaluation results indicated that a minimum of 23 NCR residents from seven NCR states (Illinois, Indiana, Kansas, Minnesota, Missouri, Ohio and Wisconsin) attended the workshop. Evaluation response rate was 66.6% based on participant registration (n=60). 39 out of 40 respondents answer the question that asked, "what state do live in". Non-NCR participants were from Poland, New Zealand, Texas, Canada, New York, Maryland, North Carolina, and Maine.

Increased new knowledge pertaining to larval feeds to NCR residents and non-NCR.

Provide new knowledge that fish culturist intended to implement. 22 attendees said that they were likely or highly to use information from the workshop to implement production methods. One commercial producer planned to implement new knowledge in 2024.

Q13 Impacts: (In concise statements (possibly a bulleted list) indicate how the project has or will benefit the aquaculture industry either directly or indirectly and resulting economic values gained (where appropriate).

Study 1a – Yellow perch

This study combined the work of many dedicated authors, scientists, and farmers for the successful commercial production of yellow perch larvae using indoor RAS rearing methods. While the industry should further strive for improved performance, yellow perch cultured extensively often experience mortalities greater than 50% (Kestemont 2015). The culture methods used here including 45- and 90-degree surface sprayers, OM-4 ball clay, overhead lighting of black tanks, laminar flow, and variable outlet screen mesh sizes significantly contributed to the successful performance of the larval yellow perch raised using indoor RAS. The silver carp hydrolysate diet did not appear to inhibit the performance of larval yellow perch compared to the other reference diet standards. In fact, the silver carp hydrolysate group tended to perform best in terms of survival; and body length/weight was not significantly different from other groups. The combined RAS technique and diet regimens further opens the door to possibilities of rearing yellow perch in a space efficient, sustainable, and commercially productive manner. These results highlight the need for further research into species specific larval diets for yellow perch and other percids. It seems a worthwhile endeavor to investigate further the effects which protein hydrolysates can impact juvenile stages of yellow perch and the possible effects which hydrolysates may influence second or third generation larvae. This study demonstrated a practical use for silver carp, an invasive species in the U.S., for commercial yellow perch rearing. In 2023 there were 340 metric tons of silver carp harvested from just one site over 10 days in the Starved Rock pool of the Illinois River (Loos 2023). Similarly, in Missouri there were 47,000 silver carp harvested and brought to the landfill in 2018 (Chen 2018). While regulations on the use of invasive species can differ by state, and harvesting-processing pose a significant challenge, finding new incentives for the harvest of silver carp could be a major contributor to the control of silver carp in the Mississippi river system. Further attention should be brought to the utilization of silver carp muscle as food for farmed fish. While silver carp is one of the most reared fish in the world, and a primary food source for many cultures, its popularity in the U.S is largely negative and has only really been suggested for use in pet foods and fertilizers. Further attention should be given to alternative uses of silver carp, like the optimization of hydrolysates for larval fish. Further understanding of hydrolyzed larval diets could lead to not only more sustainable fishmeal sources, but improvements in sustainable weaning diets often comprised of difficult to digest alternatives.

Study 1b – Yellow perch

Ultimately, dependence on live feeds remains to be a major bottleneck for commercial producers. In 2025, the developments of formulated diets have ventured further from groundbreaking and more closely to stepwise progress, often being species specific. To date, no commercial diet exists for Yellow Perch, and as with many other species, a high protein salmonid diet is the standard. From a management standpoint, the fluctuating prices of live feeds and associated labor hours have forced producers to compromise between known advantages of larval rearing with live feeds and associated mortality losses associated with using formulated diets at first-feeding. While formulated commercial diets remain more stable relative to live feeds, cost and time appears to be the driving force for larval management practices. However, there is considerable concern for ecosystem degradation associated with the harvesting of fish for fish oil and equally associated challenges with alternative protein replacements like soybean meal in formulated diets. As live feed usage continues to increase around the world, it will be important to diversify the resources from which live feeds and formulated diet ingredients originate. Moreover, it will be equally important

to expand the knowledge around live feed propagation techniques on a species/strain-specific level as it relates to the dynamic of each specific fish species. In doing so, hatchery producers may no longer have to make decisions based entirely on operating cost and move toward the techniques that produce abundant-healthy fish cohorts.

Study 2 – Walleye

In addition to showcasing the importance of hydrolyzed protein derived from same-species digestive enzymes, this study also suggests that utilizing fish meal from other sources, such as a non-native carp species, to produce hydrolysate diets may be a more economically and environmentally sustainable option than currently available marine fish hydrolysate ingredients (as tested with the CPSP diet). The improved growth performance of fish weaned onto the carp muscle diets (hydrolyzed and intact) coupled with the lack of a significant difference in survival indicates that there is room for improvement with currently available diet ingredients. Carp muscle tissue (hydrolyzed or intact) may be a suitable alternative to costly marine fish hydrolysate while reducing reliance on marine resources (Bowzer et al., 2014). Furthermore, the use of carp muscle tissue provides a potential mechanism for assisting population management of carp in non-native waters. Despite the potential advantages of carp muscle tissue as a protein source, our findings suggest that further refinement is required before it can be implemented in commercial walleye larval diets.

Q14 Recommended Follow-Up Activities: (State concisely how future studies may be structured.)

Future studies should focus on live food replacement in percid culture, assessment of nutritional requirements of percid larvae, development of optimal formulated feeds for percid larvae, and improvement of current percid larvae rearing practices towards increased survival, growth. Ad reduced skeletal deformity rate.

Q15 Publications, Manuscripts, or Papers Presented: (List under an appendix with the following subheadings: Publications in Print; Manuscripts; and Papers Presented. *For all publications note if NCRAC was acknowledged as a funding source. For the first two subheadings, include journal articles, popular articles, extension materials, videos, technical reports, theses and dissertations, etc. using the format of the American Fisheries Society. Under Papers Presented subheading include the authors, title, conference/workshop, location, and date(s). Please provide web addresses for any on-line publication(s) including theses and dissertations that were the result of this NCRAC-funded project. Format guidelines for publications and presentations are also noted in the NCRAC Proposal Submission Checklist posted on the NCRAC web site.*)

Oral presentations

Boessen P. The use of *in vitro* hydrolysis towards utilization of invasive species as a source of protein for larval yellow perch diets. Aquaculture America, New Orleans, February 2023

Boessen P. First-Feeding of Larval Yellow Perch Using Decapsulated Artemia as a Replacement for Rotifers (*Brachionus plicatilis*). Aquaculture America, New Orleans, February 2023

Thesis

Boessen, P. 2023. Yellow Perch Response to Asian Carp Hydrolysate. Master's thesis, Southern Illinois University, Carbondale, IL. In Progress (anticipated graduation date: December, 2025).

Publications

Amy Shambach, Michal Wojno, Karolina Kwasek, Tyler Firkus, Emma Hauser, Stuart Carlton. Fish Muscle Hydrolysate: A Novel Method for Producing Dietary Protein from Invasive Species for Fish Larvae. 2025. Illinois-Indiana Sea Grant Report IISG24-SFA-BRC-022.

Firkus, Tyler J., Boesen, Peter F., Wojno., M., Kwasek, K., Hauser, Emma M., Neibauer, Jared L., Branville, C. The use of hydrolyzed protein from non-native carp species to improve growth and survival in walleye (*Sander vitreus*) larval aquaculture (*in review*).

Q16 Please provide two or three pictures of your field work on your project in jpeg or png format. Examples include students in the field, workshop pictures, etc. We would like to put these in our annual report.

Technical Update. Provide a table or figure (no more than two pieces of information) that may help convey results of the project to date. Tables need to be provided as separate M.S. Word files. Graphs and figures should be provided as both M.S. Word and JPG files; colors can be used since the NCRAC report will be developed as a PDF file. Keep in mind that once the final NCRAC report is published, all information will become public knowledge.

Tables – provided as an attachment to this report Do not use all capital letters in table headings. Use a period at the end of table title. Table headings should be typed as bold, flush left. Use lowercase letters for superscripts in table footnotes 1.336a . Use solid underlining in tables; one under table heading, one separating table from footnotes. Table font should be 11 pt. Times New Roman.

Figures/pictures - placed at end of article after tables Figures and pictures can be used with your report. Do not use all capital letters with figure captions. Figures transfer best when inserted in document as a JPG.

Figure and picture headings should appear **under** the graphic. Figure number and captions--bold, flush left. Use period at the end.

Send all additional information being used for this section as separate files to ncrac@iastate.edu.