Asian Carp Muscle as an Initial Dietary Protein Source and Palatability Enhancer for Successful Production of Yellow Perch and Walleye Fingerlings

The proposed study addresses two Target Research Areas: TRA A-2: Nutrition (Alternative/Novel Diet Ingredients, First feeding enhancement), TRA A-1: Reproduction/Early Life History (Larval/early rearing)

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Funding Request: $198,614

Duration: 9/1/2021- 8/31/2023

Objectives:

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 a 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.

Deliverables:

1. Fish feeds are a major bottleneck in aquaculture since they constitute up to 70% of total fish production costs and hence, their high quality is critical to achieve maximal growth. The proposed methodology for obtaining the optimal protein hydrolysate for YP and W larvae will become a practical way of attaining, in a controlled way, an innovative, natural, and cost-effective dietary ingredient for larval Percid diets that will meet both the nutritional requirements and functional capacity of the digestive system of larval YP and W. In addition, Asian carp hydrolysate used as a natural attractant for juvenile YP and W will help wean the fish to formulated plant-based diets by improving feed acceptance and its utilization. Finally, SBM hydrolysate will be better utilized by fish in their young stage due to improved digestibility and reduced content of anti-nutritional factors.

2. At the completion of the study we will be able to achieve larviculture of YP and W completely transitioned to formulated diets and presenting positive growth performances, low skeletal deformity rate, and high survival. More specifically, we will be able to observe acceptance of formulated feeds by larval Y and W right at the start of the feeding by providing well-utilized diets based on the right molecular weight and the optimal AA composition which will enhance dietary AA assimilation and utilization for tissue protein synthesis and hence, improve growth and survival of larval YP and W. The proposed study will deliver an innovative dietary formulation, which will replace live food by improving the growth and survival of fish characterized by a challenging and vulnerable larval stage as presented by Percids.

3. We also expect that Asian carp muscle hydrolysate combined with SBM hydrolysate both obtained using YP and W digestive enzymes will allow for successful weaning of the fish to formulated feeds without jeopardizing fish growth and survival. The Asian carp hydrolysate will likely support high feed intake and at the same time the exposure to pre-digested SBM will help adapt the fish to dietary plant protein earlier.

4. This project will also deliver strong outreach component in a form of YP larval rearing fact sheet, larval rearing fact sheet, videos (mostly YP and W first feeding and larval rearing), dietary protein hydrolysate fact sheet (how to make it) for feed manufacturers, a webinar, and a workshop for all stakeholders. This project has strong support from many industry providers as shown by the attached letters of support.

5. 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 will be produced using SIUC commercial feed processing method (small scale) that will allow for immediate implementation of the formulation by the aquafeed industry.

Proposed Budgets

Institution	Principal Investigators	Objectives	Year 1	Year 2	Total
Southern Illinois University-Carbondale (SIU-C)	Karolina Kwasek Michal Wojno	1,2,3,4,5,6	$48,371	$48,211	96,582
University of Wisconsin Stevens Point (UWSP) Northern Aquaculture Demonstration Facility (NADF)	Greg Fischer	2,3,4,5,6	$39,750	$46,680	$86,430
Illinois-Indiana Sea Grant (IISG) Purdue University (PU)	Stuart Carlton Amy Shambach	5,6	$1,150	$14,452	$15,602

TOTAL	**$89,271**	**$109,343**	**$198,614**

Non-funded Collaborators

Facility	Collaborator(s)
University of Wisconsin Stevens Point NADF	Emma Wiermaa

Table of Contents

Project Summary

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.

Justification

Both yellow perch (YP) and walleye (W) have received tremendous interest in the Midwest in the past few decades due to high market demand and the decline of wild populations that can no longer support that demand. Consequently, there has been an increasing pressure on the production of these species to help to satisfy the market as food fish and help to reduce the pressure on natural resources particularly in the Great Lakes (Carlton et al. 2020; Wiermaa et al. 2015). The expansion of YP and W aquaculture industry on a large scale, however, has been constrained by several production barriers including low survival and difficulty in feed training of larval and juvenile stages (Carlton et al. 2020; Wiermaa et al. 2015). For example, the current practices focus on feeding early stages of YP with live food, and then weaning the fish at certain size gradually to formulated dry feeds which are required for YP reared in intensive production systems to provide maximum growth performance (Hart et al. 2006; Summerfelt 2010).

For walleye, some success has been made in raising the larval fish indoors using marine ingredients-based formulated diets from the start (Johnson et al. 2008; G. Fischer, pers. Comm).

Current commercial feeds, however, rely on a large proportion of plant meals and although some Percids have been reported to utilize high quality plant protein concentrates, including wheat gluten, efficiently for growth (Kwasek et al. 2011) they have also been found to be sensitive to dietary inclusion of lower-quality ingredients such as soybean meal (SBM) (Kasper et al, 2007). Consequently, the inability of the fish to grow satisfactorily on lower cost feeds with higher inclusion of SBM has been considered as the major bottleneck to further expansion of aquaculture production in the North Central Region (NCR). Although some progress with utilization of cost-effective fishmeal substitutes, such as SBM, has been made in the aquaculture industry, a number of concerns must be overcome including low palatability, imbalanced amino acid profile, or a presence of anti-nutritional factors responsible for inducing intestinal inflammation, to maintain acceptable growth rates and feed efficiency values at high fishmeal substitution levels. Plant-based diets in the present study will be utilized at juvenile and not larval stage for walleye and yellow perch. Both species have been specifically chosen for this study due to their overall sensitivity to dietary soybean meal. Thus, the aquafeed industry has focused on ways of including some of the more cost-effective alternative sources of protein that will not only help to further replace marine fishmeal but also substitute some of the expensive high-quality plant protein concentrates and provide more flexibility in feed formulations using a wider range of locally available raw materials.There is evidence that the use of hydrolyzed SBM in young fish diets may prepare them to adapt better to plant-based diets (with intact soy proteins) at later stages based on studies which argue that early exposure to dietary plant protein leads to better adaptation of thefish to the same dietary plant protein later in their life (nutritional programming concept). The SBM components responsible for this “imprinting” effect have not been fully identified (Pereraand Yufera 2016) but they are possibly associated with fish appetite regulation (Kwasek et al. 2020) or stimulation of olfactory senses (Balasubramianian et al. 2016) with free amino acids assigned to the olfactory signals (Yamamoto et al. 2010; Ueda et al. 2007). The purpose of usingsoybean meal

hydrolysate in the juvenile fish diets in the proposed study is purely to enhance theutilization of this raw material in those young fish and at the same time expose them to those soybean meal components.

"Asian carp" mostly refers to Silver Carp *Hypophthalmichthys molitrix* and Bighead Carp *H. nobilis* species, which in the last few decades have threatened the Great Lakes via their uncontrollable dispersion from previously established populations. Harvest has been considered as one of the approaches to reducing Asian carp abundance, however, considering that Asian carp are not favored food fish in the US finding a local market for the fish has been a challenge. Because of the high availability of Asian carp (IDNR 2017 Commercial Catch Report Exclusive of Lake Michigan) there has been an incentive, however, in the last few years, to utilize it as fishmeal.

Asian carp as fishmeal has been shown to be highly palatable and suitable replacement for marine fishmeal without compromising growth of Largemouth bass *Micropterus salmoides* (Bowzer et al. 2014). In fact, Asian carp body composition has been reported similar compared to traditional more expensive marine fishmeal sources (Bowzer et al. 2013). Malaypally et al. (2015) suggested potential use of Asian carp as a source of protein hydrolysate and antioxidants, hence providing an alternative application for the use of these invasive fish as "functional" or health-promoting ingredient. Although there have been numerous attempts in the past 20 years to utilize "trash" fish as a source of fish meal, none of the studies performed earlier have utilized byproducts in a form of fish digestive tracts to hydrolyze low economical value invasive fish species in order to generate a high-quality easily digestible protein hydrolysate suitable for larval fish diets. We have also provided a strong preliminary data from our largemouth bass experiment and based on the resultswe are confident of the effectiveness and applicability of the proposed method. This study proposes the next important step, which is evaluation of Asian carp muscle as an initial protein source and dietary attractant for successful larval rearing and juvenile weaning of both YP and W.

Related Current and Previous Work

To increase fish production the aquaculture industry has been moving towards sustainable farming intensification, which utilizes the following solutions with its own challenges: 1) indoor recirculation systems resulting in less land/water use, considerable reduction of effluents, and a more controlled culture environment (where a lack of naturally occurring live food requires establishment of indoor live food culture, which is difficult to control, expensive, needs substantial space, and oftentimes does not provide adequate nutrition); and 2) use of formulated balanced feeds to support optimal performance and feeding efficiency of fingerlings (where suboptimal dietary formulations often result in poor feed intake reducing growth rates and survival during weaning).

Young YP and W grow rapidly and consequently, delivery of protein building blocks - amino acids (AA) - in a highly available form for energy and tissue protein synthesis is critical during larval development (Terjesen et al., 2006). AA in fish diets can be provided in different forms: protein-bound (intact protein - long AA chains, example: protein in fishmeal), free amino acids (single AA; completely broken down protein), or peptides (short, medium and/or long-AA chains; partially broken down protein - hydrolysate) that induce different responses in larval fish. For example, free AA-based diets are not well utilized for tissue protein synthesis and growth (Murai et al., 1984; Ng et al., 1996) while peptide/hydrolysate-based diets seem to support good growth performance compared to intact protein. This has been demonstrated in different species including goldfish *Carassius carassius* (Szlaminska et al., 199?), carp *Cyprinus carpio* (Carvalho et al., 1997), sea bass *Dicentrarchus labrax*. (Cahu and Infante 1995, b; Infante, 1997; Kotzamanis et al. 2007), gilthead seabream *Sparus aurata* (Kolkovski and Tandler, 2000), Asian seabass *Lates calcarifer* (Srichanun et al. 2014; Siddik et al. 2020), Japanese eel *Anguilla japonica* (Masuda et al. 2013), yellow croaker *Larimichthys crocea* (Cai et al. 2015), and Atlantic salmon *Salmo salar* (Egerton et al. 2020).

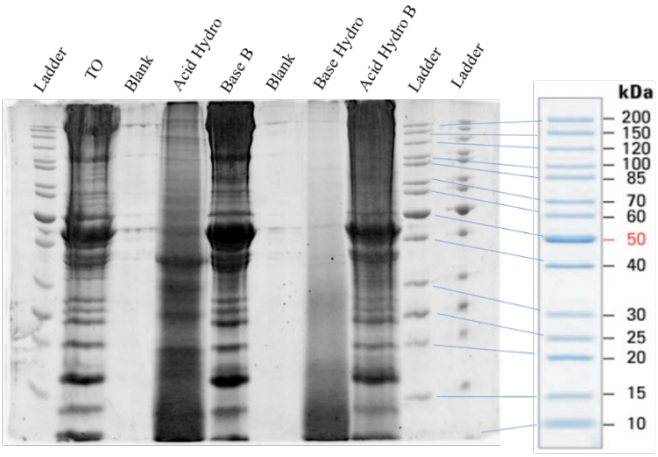

Figure 1. 10% T denaturing SDS PAGE of Bighead carp muscle hydrolysate obtained using adult largemouth bass endogenous digestive enzymes. The treatments presented are as follows: Ladder – protein ladder (marker; 200 – 10 kDa from top to bottom); T0 – muscle homogenates only, no enzymatic treatment; Blank – blank sample; Acid Hydro – muscles enzymatically hydrolyzed in acid pH, not centrifuged; Base B – muscles incubated in acid and alkaline pH without enzymes; Base Hydro - muscles enzymatically hydrolyzed in acid and alkaline pH, centrifuged; Acid Hydro B – muscle incubated in acid pH, no enzymes.

Consequently, predigested proteins have long been introduced in larval feed formulations to ease dietary protein utilization, with the expectation of promoting its absorption and tissue protein synthesis. However, larval capacity to digest dietary components of different molecular weight changes throughout its development. Canada et al. (2017) showed, for example, that Senegalese sole *Solea senegalensis* pre-metamorphic larvae are much better at digesting 5-70 kDa oligopeptides compared to metamorphosing and post-larvae that are more efficient in utilizing polypeptides and intact proteins, respectively. In fact, it has been shown that highly hydrolyzed (< 1.4 kDa) and partially-hydrolyzed (10–75 kDa) proteins are absorbed 3.0 and 2.2 times (respectively) faster than intact protein (> 65 kDa) within the first 2 h after tubefeeding pre-metamorphic Atlantic halibut *Hippoglossus hippoglossus* larvae (Tonheim et al., 2005) suggesting that the molecular size of the protein fraction is critical to support proper development of larval fish. Studies also indicate that dietary excess of protein hydrolysates can reduce growth performance in some species (Cahu et al., 1999; Kolkovski and Tandler, 2000). For example, Atlantic cod

Gadus morhua larvae performed better with up to 40% hydrolyzed protein in the diet, while Atlantic halibut larvae presented reduced survival with more than 10% hydrolyzed protein (Kvåle et al., 2009).

Functional properties of dietary hydrolysates and therefore, differences in responses in larval fish subjected to protein hydrolysate-based diets can vary depending on peptide composition, protein source and digestive enzymes used for the hydrolysis process, duration of hydrolysis and its conditions (Leduc et al. 2020), as well as level of dietary inclusion (Cahu et al. 1999). Various protein hydrolysates have been obtained using *in vitro* methods that have attempted to reproduce the physiological conditions of the digestive tract (Moyano et al., 2015). However, the practical application of *in vitro* hydrolysis has not been routinely used by the aquafeed industry due to complexity and low repeatability. In addition, to date no studies have shown that dietary protein hydrolysates are able to replace live food in larval fish culture.

In Objective 1 we propose to develop the optimal *in vitro* methodology for Asian carp muscle "pre-digestion" (hydrolysis) using digestive enzymes obtained from adult YP and W that can be used as dietary protein source and dietary attractant for larval and juvenile fish, respectively. Specifically, we propose to utilize digestive tracts, obtained from adult YP and W, to hydrolyze/pre-digest adult carp muscle to obtain different protein fractions (hydrolysates) that will correspond to nutritional and physiological requirements of both early stage YP and W, respectively.

In 2019 we ran a preliminary study and found that Asian carp muscle can be easily broken down to smaller fractions (peptides) by using practical and repeatable method with digestive enzymes representing adult fish digestive system (in review). Figure 1 presents 10% SDS-PAGE gel of Bighead carp muscle hydrolysates obtained using endogenous digestive enzymes from adult Largemouth bass digestive tracts. The results indicate that samples treated with digestive enzymes and incubated at both acid and alkaline pH (to mimic digestive process of Largemouth bass; Fig. 1 - Base Hydro) were composed of a wide range of low molecular weight fractions (peptides) as opposed to non-hydrolyzed muscle protein (T0) or muscle treated only with acid pH or alkaline pH without enzymes from Largemouth bass digestive tracts (Fig. 1 - Base B) presenting large molecular weight fractions (polypeptides above 150 kDa).

Furthermore, in a feeding trial conducted later we found that both of our experimental diets: "control diet" (based on intact Asian carp muscle – T0 on Fig. 1) and "hydrolysate diet" (based on Asian carp muscle hydrolysate - Base Hydro on Fig. 1, used as 50% replacement of the intact Asian carp muscle) were actively ingested and consumed by larval bass during first feeding. At the end of our feeding trial we found that bass in the hydrolysate group presented significantly larger final weight, weight gain, and body length compared to the control group (Figure 2).

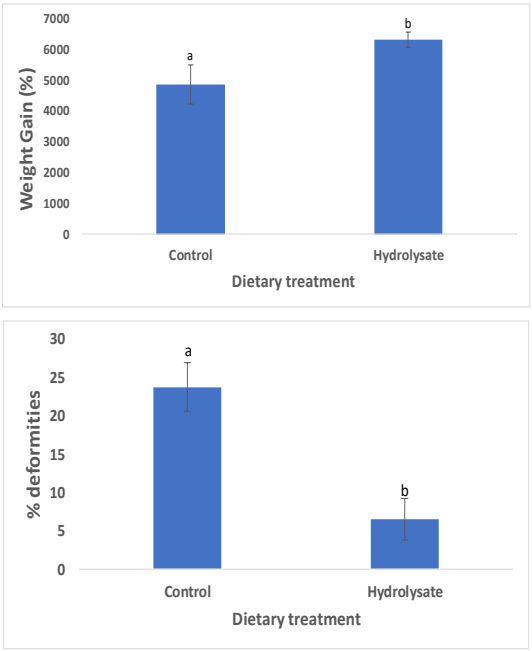

Figure 2. The weight gain (A) and total skeletal deformities (B) of larval Largemouth bass fed control (intact carp muscle-based) and carp muscle hydrolysate-based diet. Different letters indicate statistical difference at $p<0.05$.

Finally, the occurrence of skeletal deformities, another major bottleneck in fingerling production (Fernandez et al. 2008), decreased significantly in the hydrolysate group compared to the control (Figure 2). These preliminary results suggested that dietary protein hydrolysate obtained from Asian carp muscle using our innovative hydrolysis method can be successfully used as a protein source to support high feed intake and optimal performance on formulated feed in larval bass. In the present proposal, we propose to proceed a step further and prove that the same method can be applied to different species and that Asian carp muscle can be hydrolyzed using digestive enzymes from YP and W to produce an ultimate dietary protein source and dietary attractant that will support YP and W larval and juvenile growth, respectively. We also propose that our *in-vitro* "pre-digestion" method can be applied to different feed ingredients, including SBM, to break its protein down effectively, and help reduce or remove completely the anti-nutritional factors within SBM to increase digestibility and acceptance of this ingredient in both species during weaning.

Statement Regarding Duplication of Research

The proposed research is original and does not duplicate any previously published work or projects previously funded by the USDA or NOAA. We have performed a search of the scientific literature (Google Scholar, Web of Science, PubMed) and searched the following sponsor databases: National Sea Grant Office Funding Page, USDA Current Research Information System (CRIS), Sea Grant Program website, and NOAA Office of Aquaculture Funding Opportunities Page. The following keywords were used: Asian carp, muscle hydrolysate, live food replacement, yellow perch, walleye, larval stage, weaning, and soybean meal hydrolysate. There were no current or previously funded projects found in any of the databases that directly overlapped with the proposed project. The *in vitro* hydrolysis method of fish muscle has been investigated by two previous grants that SIUC (PI on both: Dr. Karolina Kwasek) was funded with by Illinois-Indiana Sea Grant. However, those projects focused on Largemouth bass as a model species characterized by relatively easier larval rearing stage as opposed to YP and W and served as preliminary data studies. Only one of those projects have evaluated Asian carp as an initial protein hydrolysate source and none of the studies investigated *in vitro* hydrolysis of SBM and its inclusion in fish diets.

Anticipated Benefits

We expect that the novel dietary ingredient originating from Asian carp muscle digested using YP and W digestive enzymes, characterized by the optimal molecular size of the protein fraction, will induce positive growth responses in YP and W larvae and juveniles, respectively. We also expect that Asian carp muscle hydrolysate combined with SBM hydrolysate both obtained using YP and W digestive enzymes will allow for successful weaning of the fish to formulated feeds without jeopardizing fish growth and survival. The Asian carp hydrolysate will likely support high feed intake and at the same time the exposure to pre-digested SBM will help adapt the fish to dietary plant protein earlier.

Objectives

1. To develop the optimal *in vit*ro 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 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.

Deliverables

1. The proposed methodology for obtaining the optimal protein hydrolysate for YP and W larvae will become a practical way of attaining, in a controlled way, an innovative, natural, and cost-effective dietary ingredient for larval Percid diets that will meet both the nutritional requirements and functional capacity of the digestive system of larval YP and W. In addition, Asian carp hydrolysate used as a natural attractant for juvenile YP and W will help wean the fish to formulated plant-based diets by improving feed acceptance and its utilization. Finally, SBM hydrolysate will be better utilized by fish in their young stage due to improved digestibility and reduced content of anti-nutritional factors.

2. At the completion of the study we will be able to achieve larviculture of YP and W completely transitioned to formulated diets and presenting positive growth performances, low skeletal deformity rate, and high survival. More specifically, we will be able to observe acceptance of formulated feeds by larval Y and W right at the start of the feeding by providing well-utilized diets based on the right molecular

weight and the optimal AA composition which will enhance dietary AA assimilation and utilization for tissue protein synthesis and hence, improve growth and survival of larval YP and W. The proposed study will deliver an innovative dietary formulation, which will replace live food by improving the growth and survival of fish characterized by a challenging and vulnerable larval stage as presented by Percids.

3. We also expect that Asian carp muscle hydrolysate combined with SBM hydrolysate both obtained using YP and W digestive enzymes will allow for successful weaning of the fish to formulated feeds without jeopardizing fish growth and survival. The Asian carp hydrolysate will likely support high feed intake and at the same time the exposure to pre-digested SBM will help adapt the fish to dietary plant protein earlier.

4. This project will also deliver strong outreach component in a form of YP larval rearing fact sheet, W larval rearing fact sheet, videos (mostly YP and W first feeding and larval rearing), dietary protein hydrolysate fact sheet (how to make it) for feed manufacturers, a webinar, and a workshop for all stakeholders. This project has strong support from many industry providers as shown by the attached letters of support.

5. 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 will be produced using SIUC commercial feed processing method (small scale) that will allow for immediate implementation of the formulation by the aquafeed industry.

Procedures

In **Objective 1** (SIU-C) we propose to develop the optimal *in vitro* methodology for Asian carp muscle "pre-digestion" (hydrolysis) using digestive enzymes obtained from adult YP and W that can be used as dietary protein source and dietary attractant for larval and juvenile fish, respectively. There will be three incubation times used to obtain muscle hydrolysates: short, medium,and long, to generate different protein products consisting of short, medium, and long peptides. Based on our preliminary data one protein hydrolysate product consisting of those three (equal) fractions obtained will be utilized in the feeding trial. This is described in more detail below.

Hydrolysate preparation

Adult YP and W will be euthanized after receiving two meals within a two-hour period to ensure release of stomach and pancreatic juices into the digestive tract lumen. Bighead carp muscle and SBM will be processed three times with a meat grinder, diluted with deionized water, and homogenized with tissue homogenizer (PowerGen 1000, Fisher Scientific) on high speed for ten minutes. Digestive tracts of YP and W will be processed similarly and later the homogenates will be centrifuged to separate the supernatant from the solid mass (that includes digested feed, fat, and other tissues). Muscle and SBM homogenates will be moved to separate 12-liter(3.2 qt) containers placed in a water bath, diluted further with deionized water, and stirred using overhead stirrer (VWR VOS 16). After temperature and pH are adjusted to the required level, muscle and SBM homogenates will be mixed with digestive tract supernatants (22°C [72^{o}F]; initial 3-4 pH followed by 7-9 pH to mimic intestinal digestion). For the control, muscle and SBM homogenates and digestive tract supernatant will be both incubated at 90°C for 15 minutes to inactivate enzymatic activity; all will then be mixed, and subsequently incubated in parallel to muscle/SBM hydrolysates in the exact same conditions (pH, temperature, and time duration).

There will be three incubation times: short (30 min stomach and 1 hour intestine digestion), medium (1 hour stomach and 2 hours intestine digestion – as seen in the preliminary Largemouth bass study), and

long (2 hours stomach and 4 hours intestine digestion), to generate different protein products consisting of short, medium, and long peptides. After the incubation, profiles of each protein hydrolysate will be analyzed by gradient gel electrophoresis and quantitative image analysis to identify those hydrolysate fractions with the greatest amount of polypeptides in the range of 1kDa to 7.2kDa. This range is based on the report of Canada et al. (2017) who showed that larval fish preferentially absorbed polypeptides in the 1kDa to 7.2kDa range. Electrophoretic analysis will be performed as previously described (Reddish et al., 2008).

The fillets from Asian carp harvested from the Illinois River will also be tested for presence of PCB's and arsenic, mercury, and selenium, due recent concerns regarding heavy metal bioaccumulation of these heavy metals in bighead and silver carp (Levengood et al. 2014). If concentration of these metals detected is higher than the limit recommended by the US Food and Drug Administration's (USFDA), the inclusion in fish diets will be adjusted in order to reduce the heavy metal level below the USFDA limit.

In **Objectives 2 and 3** (SIU-C and UWSP) we propose to evaluate the effect of Asian carp muscle protein hydrolysate obtained using methodology in Objective 1 as protein source in diets for YP and W when used as first feed; and to evaluate the effect of Asian carp muscle protein hydrolysate obtained using methodology in Objective 1 as an attractant (palatability enhancer) in diets for juvenile YP and W on successful weaning to formulated feeds. Furthermore, in **Objective 4** (SIUC and UWSP), we propose to evaluate the effect of Asian carp muscle protein hydrolysate combined with SBM hydrolysate both obtained using methodology in Objective 1 in diets for juvenile YP and W, on successful weaning to formulated feeds and easier transition to plant-based feeds. We hypothesize that our method of using digestive enzymes derived from digestive tracts of Percids to hydrolyze Asian carp muscle and SBM is a cost-effective and logical approach of reproducibly producing a source of easily digestible dietary protein and dietary attractant that will meet the nutritional requirements, functional capacity of the digestive system, and olfactory preferences of young Percid fish.

Larval YP and W feeding trial

Live food, such as rotifers or *Artemia* nauplii, support positive growth in young fish. This is partially associated with live food containing substantial amount of soluble nitrogen in the form of low molecular weight peptides and free AA (Carvalho et al. 2003; Helland et al. 2000). On the other hand, formulated diets contain higher molecular weight, intact proteins, that are difficult to digest by young fish compared to live food. As a result, poor growth is often associated with low digestion and assimilation of dry feeds. It is widely known that inclusion of pre-digested protein in the form of protein hydrolysates improves palatability of a diet and supports positive growth performance in early developmental stages (Cahu et al., 1999; Infante et al., 1997).

However, the capacity to digest dietary components of different molecular weights changes throughout fish early development (Canada et al. 2017). Therefore, the right balance between different sizes of protein fractions in diets is critical to induce positive growth responses in larval fish. In the present study we will evaluate the effect of "pre-digested" Asian carp muscle for larval and juvenile YP and W on their growth performance when used as a protein source in first feed and/or dietary attractant during weaning. Specifically, for larval diets different protein hydrolysates will be selected based on the most suitable ratios of short, medium, and long peptides to replace dietary intact protein in experimental diets (Canada et al. 2017). The different diets, each composed of the different peptide ratios, will then be tested during the following stages of larval YP and W development: 1) immediately after yolk sac absorption (pre-metamorphic stage), 2) during larval metamorphosis (metamorphic stage), and 3) during post-metamorphosis (post-larval stage). YP larval feeding trial will be conducted at SIUC and W larval trial will be carried out at UWSP. Briefly, right after the swim-up stage and before the first feeding YP and W larvae will be randomly distributed into separate 300 L (79 gal; SIUC) and 240 L (63 gal; UWSP) tanks in density of 20 larvae/L (75 larvae/gal), respectively. Three additional diets will be provided to each

species group that will serve as controls: live food, commercial starter, and a diet similar to the experimental formulation, which will contain Asian carp muscle as an intact protein source instead of protein hydrolysate. All diets will be tested in triplicates. YP and W have been considered challenging species particularly with respect to larval rearing, however, based on PIs' previous experience successfully raising larval YP and W in laboratory conditions, we are confident we will obtain good survival and swim-bladder inflation rates using similar rearing conditions (Grayson et al. 2014).

YP and W weaning trial

For assessment of the weaning success both newly hatched larval YP and W will be raised on live food for approximately one month. The juvenile fish will then be distributed into their experimental systems (YP weaning will be tested at SIUC, W at UWSP) consisting of 1000 L (264 gal) tanks at a density of 1000 juvenile fish per tank. All water quality parameters including temperature, pH, conductivity, will be adjusted to meet optimal water requirements for YP and W. The following weaning diets will be tested in triplicates:

- Asian carp intact muscle and SBM hydrolysate-based diet (50:50),
- Asian carp muscle hydrolysate and intact SBM-based diet (50:50),
- Asian carp intact muscle and intact SBM-based diet (50:50),
- Asian carp muscle hydrolysate and SBM hydrolysate-based diet (50:50),
- a commercial weaning diet.

Larval fish trialwill last until the larvae fully transition into the juvenile stage. The juvenile trial will be terminated after at least one of the groups achieves minimum 1000% weight gain. Larval fish will be fed to apparent satiation to ensure high feed intake of the formulated diets. Restricted feeding will be applied at the juvenile stage. The feeding rate will be originally set by measuring the observed feed intake for each tank and setting the feeding level to the tank with the lowest feed intake. This ensures a consistent feeding rate across all tanks and ensures all foodadded to the tanks is consumed. In addition, the feeding rate will be adjusted daily, using an assumed FCR of 1, and also readjusted through observations of feed intake at each feeding. Also,a biweekly weighing will be conducted during the restricted feeding period in order to determinethe actual biomass in each tank and to readjust the feeding rate accordingly.

Measured responses

At the end of the larval feeding and weaning trials the following measured responses will be assessed:

- Survival (%) = 100 × (final number of fish/initial number of fish)
- Final Weight (g) = Final body weight – initial body weight)
- Weight gain (% of initial weight) = 100 × (final body weight – initial body weight)/initial body weight
- Feed efficiency ratio (FER) = weight gain/feed consumed (weaning trial only)
- Protein retention efficiency (PRE, %) = protein gain/protein intake = 100 × (final body weight × final body protein − initial body weight × initial body protein)/(weight of fed diet × protein content of the diet)

At the completion of both larval feeding and weaning trials, five fish from each tank will be sampled for

whole-body proximate composition, and whole-body free AA levels to assess the availability of dietary AA from the tested protein hydrolysates. These analyses will be carried out by SIUC.

Diet preparation

Previously prepared carp muscle and SBM hydrolysates will be freeze-dried and pulverized (Labconco FreeZone 6) before inclusion in diets. The amino acid composition of protein hydrolysates remain intact after the freeze-drying process. Diets will be produced by grinding, mixing, pelleting, and freeze-drying followed by sieving to appropriate sizes for larval YP and W using a microfeed production system which has been established as a small scale (bench-top) feed processing line delivering high-quality pellet for fish in early ontogeny. Briefly, dry components of the feeds will be ground down to a fine particle size (~0.10-0.15 mm) using a centrifugal mill (Retsch 2M 100). Once ground, the components will be mixed (Farberware Mixer) to achieve uniform dispersion of all ingredients within the mix. After mixing, the feeds will be forced through pharmaceutical-grade extruder (Caleva Extruder 20) and spheronizer (Caleva Multibowl Spheronizer) to obtain solid, spherical particles characterized by high water stability and high nutrient retention. All diets will be later freeze-dried to remove the moisture. The pellets will be separated by size using a vibratory sieve shaker (Retsch AS 200 Basic) to appropriate sizes. The feeds will be stored at -20°C until use. All diets will be formulated and manufactured at SIUC.

All diets will be formulated to be isonitrogenous and isolipidic and the essential amino acids will be included in levels required by YP and W or closely related species if the requirement data for some nutrients are not available (NRC, 2011). Diets will be formulated according to our preliminary Largemouth bass study with some modification to meet both macro- and micronutrient requirements of YP and W (NRC, 2011; refer to Table 2 for an example of diet formulation).

Table 1. An example of a diet formulation that will be used in the study for larval Y and W feeding.

Ingredients (%)	**Control**	**Test diet**
Carp intact muscle	74.00	37.00
Carp muscle hydrolysates		37.00
CPSP 90[a]	5.00	5.00
Krill meal	5.00	5.00
Fish oil	4.00	4.00
Lecithin	4.00	4.00
Mineral mix	3.00	3.00
Vitamin mix	3.00	3.00
$CaHPO_4$	1.00	1.00
Taurine	1.00	1.00
Choline chloride	0.10	0.10
Vitamin C	0.05	0.05
Total	***100***	***100***

[a]*Fish attractant, Roche, France*

The weaning diet will follow commercial diet formulation where the protein source will be replaced by protein obtained from Asian carp muscle (intact/hydrolysate), SBM (intact/hydrolysate), or both.

Biochemical analyses

FAA in whole body fish samples will be analyzed to assess the availability of dietary AA. Samples will

be obtained at the end of the larval YP and W trial 3- and 24-hours after a meal - times considered characteristic for post-prandial and basal levels in fish, respectively (Kaushik and Dabrowski, 1983). FAA will be analyzed according to methodologies from Terjesen et al. (2006) and Kwasek et al. (2009) with some modification. Extracted FAA will be quantified using Shimadzu Prominence Nexera - i LC-2040C Plus (Shimadu, Japan) according to the Shimadzu protocol No. L529 with modifications. FAA concentrations (expressed as μmol/kg wet body weight) will be calculated in LabSolutions software version 5.92 (Shimadzu, Japan) using internal and external standards.

Proximate composition will include quantification of the following: crude protein, crude lipid, moisture, and ash. Briefly, samples will be analyzed for ash by combustion (550 °C for 5 h) (1022 °F for 5 h) in a muffle furnace (Lindberg Blue M, MA); crude protein (N×6.25) using a Leco nitrogen analyser (Model FP-628, Leco Corporation, St. Joseph, MO); and crude lipid from whole fish samples will be extracted with chloroform–methanol (2:1, v/v), as described by Folch et al. (1957). Finally, heavy metals and PCB's in carp muscle will be assessed by Toxicology Laboratory in School of Biological Sciences at SIUC.

Data Management Plan

Expected Data Type

Within the proposed project, multiple methodologies, assays, and platforms will be utilized to generate both qualitative and quantitative data. To further clarify the data generated, techniques are categorized and further explained below:

1) Performance (spreadsheets with primary data);
2) Biochemistry (spreadsheets with primary data);

Data Format

During the generation of data, field and laboratory quality control and quality assurance practices will be utilized to ensure all data are accurate and comprehensive. All data will be well annotated and when applicable metadata will be included to ensure the data yield a complete decription of the research conducted. All numerical datasets will be stored in .xls spreadsheet formats.

Data Storage and Preservation

The data will be preserved in digital format at a dedicated computer server and on external hard drives as backup. Data will be preserved on the computer server for a minimum of 5 years after completion of the project. In addition, raw data will be stored at local repository at Southern Illinois University (OpenSIUC) within two years of the completion of the project.

Data Sharing and Public Access

All data generated within this project will be dissemenated to the public through journal publications, presentations at scientific conferences, and university outreach publications. Accepted journal publications and all associated supplementary materials and methods will be made available through journal subscriptions or provided at request by the PIs according to copyright agreements. All data will be shareable at the completion of the proposed project, with the exception of intellectual properties that may be derived from the research findings. More restricted policies will be implemented for accessing such proprietary data to protect the intellectual properties.

Roles and Responsibilities

The PI will be responsible for fulfilling the objectives of the study. The PI will also be responsible for maintaining and curating the data associated with the objectives in accordance with this DMP. In addition, the PI will be responsible for ensuring all individuals involved in the project (co-PIs,

graduate students, researchers) are adhering to the DMP, as well as mitigating any issues with data management that may arise.

Monitoring and Reporting

Prior to conducting the research outlined in this proposal, PI, and associated co-PIs will meet to layout and review the overall data management plan (DMP), as set forth in this section, to ensure consistency and confluence between individuals. The PI will be responsible for ensuring data from each objective follows the outlined DMP, in addition to synthesizing the combined results for the report. The PI, will continually review and ensure adherence to the DMP, as outlined herein, throughout the grant period.

Research report will be compiled at the completion of the research objectives for submission to NCRAC, under the direction of the PI, to describe the research that has been conducted. As expressed in this DMP, data from this project will be made publicly available in the final report.

Outreach and Evaluation Plan

Outreach is an essential component of the long-term development of an economically and sustainable aquaculture industry in the region. The primary audience for the proposed study is the aquaculture industry. Therefore, within **Objective 5** (SIUC, UWSP, PU) we plan to provide the aquaculture community within the NCR with guidelines on successful larval rearing that will expand upon existing resources for both YP and W in indoor systems (e.g.,,The Walleye Video Manual, The Walleye Culture Manual, and the Yellow Perch Culture Manual). Early rearing of Percid fish has been considered challenging due to the need for cultivation of live food, weaning difficulties and lack of optimal formulated feed, cannibalism control, and swim bladder uninflation, which all might lead to the production of "poorer" quality fingerlings. We therefore propose to develop a workshop for farmers where YP and W larvae rearing methods will be presented and discussed. Objective 5 extension deliverables include a workshop hosted at PU, a minimum of two NCRAC fact sheets on larval rearing, and a series of short videos that will be the foundation of a yellow perch larval rearing video manual. If appropriate, a video on walleye weaning will be produced and added to UWSP NADF's Walleye Video Manual. In addition, to ensure effective communication in **Objective 6** (SIUC, UWSP, PU) we will also provide the farmers and feed manufacturing industry with the knowledge and the tools required for the production of high-quality dietary protein hydrolysate as a source of highly digestible protein for Percid fish feed. Specifically, as part of the workshop, hosted at PU, we will present a descriptive methodology for the production of the dietary protein hydrolysates and its optimal inclusion in larval Percid feeds that will allow for the replacement of live food and easier weaning in hatchery systems. Objective 6 extension deliverables include workshop that focuses on manufacturing high-quality protein hydrolysate, one NCRAC fact sheet, and a webinar. Additional outputs of this study include presentations at professional aquaculture conferences (World Aquaculture Society and Ohio Aquaculture Association) and peer-reviewed publications. The research results will also be disseminated through the NCRAC Annual Progress Reports. In addition, UWSP NADF will utilize their existing outreach and communication platform to disseminate various project updates, results and deliverables. This platform includes an existing website, social networking sites, videos, and quarterly newsletter.

Logic Model

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

Situation:
1) Currently, no diet exists specifically formulated for larval YP or W that would support optimal growth performance and survival and allow for substitution of live food.

2) Juvenile fish raised on live food have difficulties accepting formulated feeds during the weaning period.

3) Current commercial feeds rely on a large proportion of plant meals such as SBM that impair fish growth. The inability of the fish to grow satisfactorily on lower cost feeds with higher inclusion of SBM has been considered as the major bottleneck to further expansion of aquaculture production in the NCR.

Goal:
To replace live food use in larval Percid culture and improve transition of Percid fingerlings to formulated plant-based feeds.

Objectives:
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 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.

Inputs	Outputs		Outcomes		
	Activities	Participation	Short-term	Medium-term	Long-term
Faculty and staff from North Central Region (SIUC, UWSP, IISG) Research funding Novel feed ingredient technology	Development of innovative methodology for obtaining high-quality well-digestible dietary protein source and suitability assessment of the novel dietary protein source on larval and juvenile YP and W growth performance Communication of findings with ADM Animal Nutrition™ Zeigler, Prairie Aquatech, and other commercial feed and hatchery producers via the outreach briefings and workshop Presentation of findings to researchers at professional meetings	Researchers associated with the project Other aquaculture researchers and farmers Feed/additive industry representative, fish producers	Knowledge of suitability of alternative forms of dietary protein in larval and juvenile Percid fish Knowledge and tools required for obtaining the innovative dietary protein source using the new approach Awareness of novel feed ingredients approaches for fish/feed producers and research community	Commercialization of the innovative diet formulation Development of hydrolysate-based feeds for other species	Reduced reliance on live food and increased flexibility in intensive production of YP and W in the NCR Increased YP and W production control Less water/land use in YP and W production Improved weaning to plant-based diets Reduction of costs in YP and W production Improved control over invasive carp populations

Facilities

Activities related to Objectives 1, 2, 3, and 4 will be carried at Southern Illinois University-Carbondale (SIUC). SIUC's facilities are equipped with technology to support fish rearing during complete life cycle. Dr. Kwasek's research facility includes "Feed Processing" laboratory with full production line for manufacturing of high-quality formulated microparticulate diets for young fish (larvae and juveniles), which includes: mortar grinder (Retsch), centrifugal mill, and knife mill (Retsch) used for grinding, pulverizing, ingredients blending, and mixing; pharmaceutical grade extruder and spheronizer (Caleva) for feed particle preparation; vibratory sieve shaker (Retsch) for particle fractionation; and two freeze-drying systems (Labconco) for final feed particle drying process. SIUC's Center for Fisheries, Aquaculture and Aquatic Sciences has nearly 1,115 m^2 (12,000 ft^2) of floor space in the Life Sciences II, III and Annex Buildings located on the SIUC campus. A 770 m^2 (8,300 ft^2) square-foot temperature-controlled wet laboratory houses more than 50 fiberglass tanks ranging in size from 1.2 to 2.4 m (4-8 ft), 3 m (10 ft) fiberglass raceways and numerous smaller tanks and aquaria, feed storage, water chemistry laboratory, and a workshop. Other wet laboratories house experimental systems designed primarily for intensive nutritional research. The new Aquatic Research Laboratory (ARL) and Saluki Aquarium is a state-of-the art 650 m^2 (7,000 ft^2) climate-controlled facility with capabilities for both marine and freshwater recirculating aquaculture. A large space is also specifically designed for culture of rotifers and Artemia to support larviculture research. The Rader laboratory consists of a main laboratory occupying 67 m^2 (724 ft^2) on the SIUC main campus. In addition, three additional spaces consisting of a 34 m^2 (365 ft^2) square feet and two adjoining rooms totaling 15 m^2 (156 ft^2). The Rader laboratory houses the following equipment for molecular work: three Bio-Rad MJ Mini PCR machines, one Bio-Rad Mini-Opticon Real-Time PCR systems, multiple water baths, heat blocks, tabletop centrifuges, an Eppendorf 5804 R tabletop refrigerated centrifuge, three upright temperature incubators, multiple DNA electrophoresis gel boxes and power sources, a station for weighing reagents and pHing solutions and a Thermo Fisher NanoDrop 1000. The Rader laboratory also contains the following imaging, detecting equipment: Shimadzu Spectrofluorophotometer, Thermo Fisher Genesys 10S UV-Vis spectrophotometer, Thermo Fisher NanoDrop 1000, Nikon Eclipse E200 Flourescence Compound scope and Hg light source, a Leica/Wild M3Z stereoscope, an Amscope stereoscope, and a GloMax Luminometer. General microbiology: 37 °C/42 °C (99 °F/108 °F) incubators (separate from the 37 °C (99 °F) shared warm room), a Bio-Rad GenePulser electroporation system, a full sized -20 °C (-4 °F) freezer, a -80 °C (-112 °F) chest, and multiple small refrigerators/freezers.

Activities related to Objectives 2, 3, and 4 will be carried at Northern Aquaculture Demonstration Facility (NADF) University of Wisconsin Stevens Point (UWSP). The UWSP Northern Aquaculture Demonstration Facility is recognized as an international leader in rearing walleye intensively and in recirculating aquaculture system technology. The facility is one-of- a-kind, designed with modern, high tech commercially scaled aquaculture production systems and is equipped to provide a wide range of applied research and demonstration systems to be fully capable of achieving the project deliverables and milestones. The facility has several modern commercially scaled RAS systems capable of rearing cool and coldwater fish as needed. A custom designed Bell jar incubation system and intensive larval rearing system with 50 replicated 240 L (63 gal) tanks and biosecure water supply of appropriate water quality and water temperatures are also available for the project. The professional facility scientists and staff are highly dedicated, well trained, and has over 50 years of combined experience in successfully rearing walleye and are considered experts in this field. The facilities webpage is aquaculture.uwsp.edu for more information.

Activities related to Objective 5 and 6 will be hosted at the J.S. Wright Conference Center of Purdue University. The center has three rooms available for the workshop. The 255 m^2 (2,750 ft^2) conference room holds 120-150 people or 60-75 people when social distancing is required. The conference room is equipped with audio-visual equipment, which includes slide projectors, overhead projectors, LCD projectors, computer hookups, t-1 line, and wireless network connection. Adjacent to the conference room

is a fully equipped kitchen that is available. If the J.S. Wright Conference Center is not available, an alternative location will be secured on the Purdue campus, at SIUC, or at UWSP NADF.

In addition, the UWSP NADF has an extensive, well utilized outreach program already in place. This project will join the existing program that utilizes two active aquaculture and aquaponics websites, social media sites, quarterly newsletters, industry and professional publications, online videos, site visits and workshops to share results and information with stakeholders. The UWSP-NADF has a strong partnership with the Wisconsin Aquaculture Association, National Aquaculture Association, WI Sea Grant, and the USDA North Central Regional Aquaculture Center and frequently provides presentations at state, regional and international workshops and conferences. Our outreach plan for stakeholders will begin at the onset of the project by communicating with aquaculture businesses through conferences, online outlets (using the outreach tools stated above) and direct contacts about the project goals and the possibility of including additional businesses of various scales and production system designs for the grow-out phase. We will engage with stakeholders about the project's progress through both traditional outlets (Wisconsin Aquaculture Association newsletter CREEL, WISG newsletter and fact sheets, UWSP-NADF newsletter) and digital communication (social media: Facebook, Twitter; project website).

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Project Leaders

State	Name/Institution	Area of Specialization
IL	Karolina Kwasek, Ph.D. Southern Illinois University-Carbondale	Aquaculture/Nutrition
IL	Michal Wojno, Ph.D. Southern Illinois University-Carbondale	Aquaculture/Nutrition
WI	Greg Fischer University of Wisconsin Stevens Point	Aquaculture/Larval Fish Culture
IN	Stuart Carlton, Ph.D. Illinois-Indiana Sea Grant Purdue University	Aquaculture Extension
IN	Amy Shambach Illinois-Indiana Sea Grant Purdue University	Aquaculture Extension
WI	Emma Wiermaa University of Wisconsin Stevens Point	Outreach/Communication

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

BUDGET

OMB Approved 0524-0039

ORGANIZATION AND ADDRESS Southern Illinois University - Carbondale 1263 Lincoln Dr, Carbondale, IL 62901 PROJECT DIRECTOR(S) Karolina Kwasek				**USDA AWARD NO.** Year 1: Objective 1, 2, 3, 4 Duration Proposed Months: __ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer	7,479			
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students				18,882			
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages							
B. Fringe Benefits (If charged as Direct Costs)				4,010			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				30,371			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				16,500			
F. Travel				1,500			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)				48,371			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K)							
N. Other							
O. Total Amount of This Request				48,371			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)	Leave Blank	
Cash (both Applicant and Third Party)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039

BUDGET

ORGANIZATION AND ADDRESS Southern Illinois University - Carbondale 1263 Lincoln Dr, Carbondale, IL 62901 PROJECT DIRECTOR(S) Karolina Kwasek	**USDA AWARD NO.** Year: 2 Objective : 1, 2, 3, 4			
	Duration Proposed Months: __ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages — **CSREES FUNDED WORK MONTHS** (Calendar / Academic / Summer)				
1. No. of Senior Personnel				
a. ___ (Co)-PD(s)	7,704			
b. ___ Senior Associates				
2. No. of Other Personnel (Non-Faculty)				
a. ___ Research Associates-Postdoctorates . . .				
b. ___ Other Professionals				
c. ___ Paraprofessionals				
d. ___ Graduate Students	19,448			
e. ___ Prebaccalaureate Students				
f. ___ Secretarial-Clerical				
g. ___ Technical, Shop and Other				
Total Salaries and Wages				
B. Fringe Benefits (If charged as Direct Costs)	4,119			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)	31,272			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				
E. Materials and Supplies	15,440			
F. Travel	1,500			
G. Publication Costs/Page Charges				
H. Computer (ADPE) Costs				
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)				
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				
K. Total Direct Costs (C through I)	48,212			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)				
M. Total Direct and F&A/Indirect Costs (J plus K)				
N. Other				
O. Total Amount of This Request	48,212			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)	Leave Blank	
Cash (both Applicant and Third Party)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE
BUDGET

OMB Approved 0524-0039

ORGANIZATION AND ADDRESS Southern Illinois University - Carbondale 1263 Lincoln Dr, Carbondale, IL 62901 PROJECT DIRECTOR(S) Karolina Kwasek				**USDA AWARD NO.** Year: 1&2 Objective : 1, 2, 3, 4 Duration Proposed Months: __ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. ___ (Co)-PD(s)				15,183			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students				38,330			
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages							
B. Fringe Benefits (If charged as Direct Costs)				8,129			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				61,643			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				31,940			
F. Travel				3,000			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)				96,583			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K)							
N. Other							
O. Total Amount of This Request				96,583			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)	Leave Blank	
Cash (both Applicant and Third Party)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Budget Justification per Institution

Southern Illinois University-Carbondale (SIUC)
Karolina Kwasek

Objectives: 1, 2, 3, 4

A. Salary, Wages, and Fringe Benefits. Funds for approximately 1 summer month salary per year including fringe benefits at 48.8% SIUC rate are budgeted for the PI who will be responsible for fulfilling the project objectives 1 through 4 and overall management of the study. Funds for 12 months Graduate Research Assistant are budgeted for two years at 50% effort including $356 Primary Care Fee. Graduate student will help with ingredient sourcing including Asian carp, hydrolysate preparation, diet formulation, preparation of the larval rearing system, maintenance of experimental fish, live food culture, execution of experimental trials, data collection, and statistical and biochemical analyses. Y1: $30,371; Y2: $31,272.

B. Nonexpendable Equipment. No funding for nonexpendable equipment is requested.

C. Materials and Supplies. Funds for live food and feed ingredients ($5,600), system PVCs and sprinklers and general wet lab supplies (Instant Ocean salt, clay, nets, etc.) ($5,000), live food (rotifers, algae, Artemia cysts, etc.) ($2,000), PCBs, heavy metal and biochemical analyses (ingredients, feeds, and tissues; $8,440); biochemical lab supplies (gloves, mixer probe, pH probe, scalpel blades, centrifuge tubes, ethanol, Eppendorf tubes, plastic bags, etc.; $4,900), proteomic analyses of hydrolysates ($6,000) are budgeted for each year. Y1: $16,500; Y2: $15,440.

D. Travel. Funds for transportation ($500 airfare), lodging ($100 per night, 5 nights), and registration fee ($500) for attending a domestic conference are budgeted for one person. Y1: $1,500; Y2: $1,500.

E. All Other Direct Costs. No other direct costs are requested.

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE
BUDGET

OMB Approved 0524-0039

ORGANIZATION AND ADDRESS University of Wisconsin-Stevens Point 2100 Main St. Stevens Point, WI 54481 PROJECT DIRECTOR(S) PI Name Gregory Fischer				**USDA AWARD NO.** Year 1_: Objective _SIU Diet Study Duration Proposed Months: 12__ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. _1__ (Co)-PD(s)	12			2,500			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)	12			16,283.00			
a. __1_ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				18,783.00			
B. Fringe Benefits (If charged as Direct Costs)				9466.00			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				28250.00			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				0			
E. Materials and Supplies				10,000			
F. Travel				1,500			
G. Publication Costs/Page Charges				0			
H. Computer (ADPE) Costs				0			
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)				0			
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				0			
K. Total Direct Costs (C through I)				39,750.00			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)				0			
M. Total Direct and F&A/Indirect Costs (J plus K)				0			
N. Other				0			
O. Total Amount of This Request				39,750.00			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)	Leave Blank	
Cash (both Applicant and Third Party)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director Gregory Fischer, NADF Assistant Director/Research Program Manager		10/6/2020
Authorized Organizational Representative Katherine P. Jore, Associate Vice Chancellor		10/8/2020
Signature (for optional use)		

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE
BUDGET

OMB Approved 0524-0039

ORGANIZATION AND ADDRESS University of Wisconsin-Stevens Point 2100 Main St. Stevens Point, WI 54481 PROJECT DIRECTOR(S) PI Name Gregory Fischer				**USDA AWARD NO.** Year 2_: Objective _SIU Diet Study			
				Duration Proposed Months: 12__ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. _1__ (Co)-PD(s)	12			5,000			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)	12						
a. __1_ Research Associates-Postdoctorates . . .				16,608.00			
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				21,608.00			
B. Fringe Benefits (If charged as Direct Costs)				12,071.00			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				33,680.00			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				0			
E. Materials and Supplies				10,000			
F. Travel				3,000			
G. Publication Costs/Page Charges				0			
H. Computer (ADPE) Costs				0			
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)				0			
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				0			
K. Total Direct Costs (C through I)							
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)				0			
M. Total Direct and F&A/Indirect Costs (J plus K)				0			
N. Other				0			
O. Total Amount of This Request				46,680.00			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)	Leave Blank	
Cash (both Applicant and Third Party)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director Gregory Fischer, NADF Assistant Director/Research Program Manager		10/6/2020
Authorized Organizational Representative Katherine P. Jore, Associate Vice Chancellor		10/8/2020
Signature (for optional use)		

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

BUDGET OMB Approved 0524-0039

ORGANIZATION AND ADDRESS University of Wisconsin-Stevens Point 2100 Main St. Stevens Point, WI 54481 PROJECT DIRECTOR(S) PI Name Gregory Fischer				**USDA AWARD NO.** Year 1 & 2_: Objective _SIU Diet Study			
				Duration Proposed Months: 24__ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a. _1__ (Co)-PD(s)	24			7,500			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. __1_ Research Associates-Postdoctorates . . .	24			32,892.00			
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				40,392.00			
B. Fringe Benefits (If charged as Direct Costs)				21,538.00			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				61,930.00			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				0			
E. Materials and Supplies				20,000			
F. Travel				4,500			
G. Publication Costs/Page Charges				0			
H. Computer (ADPE) Costs				0			
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)				0			
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				0			
K. Total Direct Costs (C through I)							
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)				0			
M. Total Direct and F&A/Indirect Costs (J plus K)				0			
N. Other				0			
O. Total Amount of This Request				86,430.00			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)	Leave Blank	
Cash (both Applicant and Third Party)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director Gregory Fischer, NADF Assistant Director/Research Program Manager		10/6/2020
Authorized Organizational Representative Katherine P. Jore, Associate Vice Chancellor		10/8/2020
Signature (for optional use)		

Budget justification

University of Wisconsin Stevens Point (UWSP)
Greg Fischer

Objectives: 2, 3, 4

A. Salary, Wages, and Fringe Benefits. Year 1: Aquaculture Technician (Project appt.) 50% effort. Assist in setting up and running research projects. Daily care of fish, systems, and collection of data. Data entry into computer systems. Assist with organizing data.

Salary: $16,283.00 Fringe: $8,206.00

Senior Personnel: $2,500 overload salary
a. (Co) Principal Investigator

Greg Fischer will coordinate and oversee UWSP-NADF efforts and collaborations in this project including personnel and scientific oversight, communication and coordination with the SIU and industry partners, report/publication writing, attending conferences and demonstration workshop.
Fischer will receive $2,500 for YR1 for extra service pay/overload due to significant changes in work activity related to additional responsibilities with project include including additional communications and organization with all partners.

Salary: $2,500.00 Fringe: $1,260.00

Total salary and fringe $ 28,250.00

Year 2: Aquaculture Technician (Project appt.) 50% effort. Assist in setting up and running research projects. Daily care of fish, systems, and collection of data. Data entry into computer systems. Assist with organizing data.

Salary: $16,608.00 Fringe: $9,201.00

Senior Personnel: $5,000 overload salary
a. (Co) Principal Investigator

Greg Fischer will coordinate and oversee UWSP-NADF efforts and collaborations in this project including personnel and scientific oversight, communication and coordination with the SIU and industry partners, report/publication writing, attending conferences and demonstration workshop.
Fischer will receive $5,000 for YR2 for extra service pay/overload due to significant changes in work activity related to additional responsibilities with project include including additional communications and organization with all partners.

Salary: $5,000.00 Fringe: $2,870.00

Total salary and fringe $33,680.00

B. Nonexpendable Equipment. No funding for nonexpendable equipment is requested.
C. Materials and Supplies. Year 1: Oxygen($2000) water quality chemicals($1000), in house water quality testing ($2000), fish feed($1000), egg collection ($500), fish health inspection (1,000) misc. supplies for larval systems($2,500).

Total: $10,000

Year 2: Oxygen($2000) water quality chemicals($1000), in house water quality testing ($2000), fish feed($1000), egg collection($500), fish health inspection ($1,000)misc. supplies for larval systems($2,500).
.

Total: $10,000

D. Travel. a) Year 1: Travel to meetings and to disseminate results of project research with other interested groups at pertinent and related workshops.
1 trip x $600/flight = $600
1 trip x registration fee for conference= $500

1 trip x 2 nights lodging x $120/night= $240
1 trip x 2 days x $60 estimated meal cost per day= $120
1 trip – misc costs, parking, fees etc = $40
Total cost = $1,500

Year 2: Travel to meetings and to disseminate results of project research with other interested groups at pertinent and related workshops.
1 trip x $600/flight = $600
1 trip x registration fee for conference= $500
1 trip x 2 nights lodging x $120/night= $240
1 trip x 2 days x $60 estimated meal cost per day= $120
1 trip – misc costs, parking, fees etc = $40
Total cost = $1,500 x 2 = $3,000

E. All Other Direct Costs. No other direct costs are requested.

ORGANIZATION AND ADDRESS University Purdue University Address 155 S Grant Street City, State, ZIP West Lafayette, IN 47907-2114 PROJECT DIRECTOR(S) PI Name Stuart Carlton				**USDA AWARD NO.** Year 1_: Objective _ Duration Proposed Months: 12__ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS** Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates . . .							
b. _1__ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B)							
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
F. Travel				1,150			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)				1,150			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)				-0-			
M. Total Direct and F&A/Indirect Costs (J plus K)				1,150			
N. Other							
O. Total Amount of This Request				1,150			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** 1,150 **Non-Federal funds: $** **Total $1,150**							
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) . . . . . Non-Cash Contributions (both Applicant and Third Party)						Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

ORGANIZATION AND ADDRESS University Purdue University Address 155 S Grant Street City, State, ZIP West Lafayette, IN 47907-2114 PROJECT DIRECTOR(S) PI Name Stuart Carlton				**USDA AWARD NO.** Year 2_: Objective _			
				Duration Proposed Months: 12__ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. _1__ Other Professionals	1.82			7,705			
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				7,705			
B. Fringe Benefits (If charged as Direct Costs)				2,547			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				10,252			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				250			
F. Travel				850			
G. Publication Costs/Page Charges				3,100			
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)				14,452			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)				-0-			
M. Total Direct and F&A/Indirect Costs (J plus K)				14,452			
N. Other							
O. Total Amount of This Request				14,452			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** 14,452 **Non-Federal funds: $** **Total $14,452**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)	Leave Blank	
Cash (both Applicant and Third Party)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

ORGANIZATION AND ADDRESS University Purdue University Address 155 S Grant Street City, State, ZIP West Lafayette, IN 47907-2114 PROJECT DIRECTOR(S) PI Name Stuart Carlton				**USDA AWARD NO.** Total_: Objective _			
				Duration Proposed Months: 24__ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS** Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. _1__ Other Professionals	1.82			7,705			
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				7,705			
B. Fringe Benefits (If charged as Direct Costs)				2,547			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				10,252			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				250			
F. Travel				2,000			
G. Publication Costs/Page Charges				3,100			
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)				15,602			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)				-0-			
M. Total Direct and F&A/Indirect Costs (J plus K)				15,602			
N. Other							
O. Total Amount of This Request				15,602			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** 15,602				**Non-Federal funds: $**		**Total $15,602**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) . . . . . Non-Cash Contributions (both Applicant and Third Party)						Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Budget justification

Purdue University - Illinois-Indiana Sea Grant (IISG)
Stuart Carlton

Objectives: 5, 6

A. Salary, Wages, and Fringe Benefits. Funds to cover extension specialist's time is budgeted at 15% for year two of the project. The extension specialist will plan a one-day workshop to communicating results and recommendations to stakeholders, develop extension publications, and create a technical outreach video(s). Y1: $0; Y2 $10,252.

B. Nonexpendable Equipment. No request to cover video supplies.

C. Materials and Supplies. Y1: $0; Y2: $250.

D. Travel. Funds for transportation, lodging, and meal expense are budgeted to visit SUI in Carbondale on two occasions. Once for the one-day stakeholder workshop and once to record content for a technical video(s). Y1: $1150; Y2: $850.

E. All Other Direct Costs. Funds are requested for the design, printing of extension publication, video(s) production, and costs associated to hosting a workshop. Y1: $0; Y2: 3100.

Budget Summary

Proposed Summary Budget for Year 1 For All Participating Institutions

	NCRAC Funds				
	Objective #	**Southern Illinois University-Carbondale**	**University of Wisconsin Stevens Point**	**Illinois-Indiana Sea Grant**	**Project Total**
Salaries, Wages, and Fringe Benefits	**1, 2, 3, 4**	$30,371.00	$28,250.00	$0.00	$58,621.00
Nonexpendable Equipment		$0.00	$0.00	$0.00	$0.00
Materials and Supplies		$16,500.00	$10,000.00	$0.00	$26,500.00
Travel		$1,500.00	$1,500.00	$1,150.00	$4,150.00
All Other Direct Costs		$0.00	$0.00	$0.00	$0.00
Total		**$48,371.00**	**$39,750.00**	**$1,150.00**	**$89,271.00**

Proposed Summary Budget for Year 2 For All Participating Institutions

	NCRAC Funds				
	Objective #	**Southern Illinois University-Carbondale**	**University of Wisconsin Stevens Point**	**Illinois-Indiana Sea Grant**	**Project Total**
Salaries, Wages, and Fringe Benefits	**1, 2, 3, 4, 5, 6**	$31,271.00	$33,680.00	$10,252.00	$75,203.00
Nonexpendable Equipment		$0.00	$0.00	$0.00	$0.00
Materials and Supplies		$15,440.00	$10,000.00	$250.00	$25,690.00
Travel		$1,500.00	$3,000.00	$850.00	$5,350.00
All Other Direct Costs		$0.00	$0.00	$3,100.00	$3,100.00
Total		**$48,211.00**	**$46,680.00**	**$14,452.00**	**$109,343.00**

Schedule for Completion of Objectives

Start date: September 2021

Completion date: August 2023

Objectives and Tasks	Year 1						Year 2						Year 3 - not funded	
	S O	N D	J F	M A	M J	J A	S O	N D	J F	M A	M J	J A	S O	N D
Objective 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.														
Hydrolysis ingredients sourcing	■													
Muscle hydrolysis preparation	■	■												
Feed ingredient sourcing		■	■											
Objective 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.														
Experimental diet production			■	■										
Larval feeding trial				■	■									
Objective 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.														
Objective 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.														
Weaning feed trial					■	■	■							
Objective 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.														
Objective 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.														
Communication of results with industry representatives and scientific community									■	■	■	■	■	■
Report preparation and submission												■	■	
Workshop											■	■		
Manuscript preparation										■	■	■	■	

Participating Institutions and Co-Principal Investigators

Institution: Southern Illinois University-Carbondale
Karolina Kwasek, Ph.D.
Michal Wojno, Ph.D.

Institution: University of Wisconsin Stevens Point
Greg Fischer

Institution: Illinois-Indiana Sea Grant Purdue University
Stuart Carlton, Ph.D.
Amy Shambach

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Education

B.S., M.Sc. (University of Warmia and Mazury, 2007, Inland Fisheries)
Ph.D. (The Ohio State University, 2012, Animal Science)

Positions

2018 -Present	Assistant Professor Department of Zoology, Southern Illinois University-Carbondale
2015-2017	R&D Scientist Biomar, Scotland, UK
2014-2015	Research Intern WorldFish, Penang, Malaysia
2013-2014	Postdoctoral Researcher School of Environment and Natural Resources, The Ohio State University
2012-2013	Postdoctoral Researcher University of Insubria, Varese, Italy
2012	Research Associate School of Environment and Natural Resources, The Ohio State University
2008-2012	Graduate Research Associate Department of Animal Science, The Ohio State University
2007-2008	Research Scholar School of Environment and Natural Resources, The Ohio State University

Recent Publications

Kwasek, K., S. Rimoldi, A. G. Cattaneo, T. Parker, K. Dabrowski and G. Terova. 2017. The expression of hypoxia-inducible factor-1α gene is not affected by low-oxygen conditions in yellow perch (*Perca flavescens*) juveniles. Fish physiology and biochemistry 43(3):849-862.

Kwasek, K., G. Terova, B.-J. Lee, E. Bossi, M. Saroglia and K. Dabrowski. 2014. Dietary methionine supplementation alters the expression of genes involved in methionine metabolism in salmonids. Aquaculture 433:223-228.

Kwasek, K., G. Terova, M. Wojno, K. Dabrowski, and M. Wick. 2012. The effect of dietary dipeptide lysine–glycine on growth, muscle proteins, and intestine PepT1 gene expression in juvenile yellow perch. Reviews in fish biology and fisheries 22(3):797-812.

Kwasek, K., K. Dabrowski, K. Ware, J. M. Reddish, and M. Wick. 2011. The effect of lysine-supplemented wheat gluten-based diet on yellow perch *Perca flavescens* (Mitchill) performance. Aquaculture Research 43(9):1384-1391.

Kwasek, K., Y. Zhang, and K. Dabrowski. 2010. Utilization of dipeptide/protein based diets in larval and juvenile Koi carp–post-prandial free amino acid levels. Journal of animal physiology and animal nutrition 94(1):35-43.

Kwasek, K., Y. Zhang, P. Hliwa, P. Gomułka, T. Ostaszewska and K. Dabrowski. 2009. Free amino acids as indicators of nutritional status of silver bream (*Vimba vimba*), when using commercial and purified diets. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 153(2):113-119.

VITA

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Education

Ph.D. The Ohio State University, Environmental Science Graduate Program, 2012
M.Sc., BS. University of Warmia and Mazury, Inland Fisheries, Poland, 2007
M.Sc., BS. University of Warmia and Mazury, Food Science and Technology, Poland, 2005

Postitions

2019 – present	Southern Illinois University-Carbondale Assistant Scientist
2016-2017	Biomar, Scotland, UK UK Product Manager
2015-2016	GlobalFish, Tilapia RAS Facility, Poland Production Director
2014	WorldFish, Penang, Malaysia Volunteer
	The Ohio State University, ESGP:
2013-2014	Graduate Research Assistant
2012-2013	Graduate Teaching Assistant
2011-2012	Graduate Administrative Assistant
2010-2011	Graduate Research Assistant
2008-2010	Volunteer
2007-2010	Research Associate

Recent Publications

Kwasek, K., M. Wojno, G. Terova, V.J. McCracken, G.S. Molinari, and F. Iannini. 2020. ociNutritional Programming Improves Dietary Plant Protein Utilization in Zebrafish Danio rerio. Plos ONE 15(3): e0225917

Dabrowski, K., M. Wojno, M. Miller, K. Kwasek, and J.Grayson. 2018. Continued embryonic development, survival, and growth of walleye larvae following exposure to dewatering and storage in melting-ice temperatures. North American Journal of Aquaculture 80: 404-410.

Kwasek, K., K. Dabrowski, J. Nynca, M. Wojno, and M. Wick. 2014. The influence of dietary lysine on yellow perch maturation and the quality of sperm. North American Journal of Aquaculture 76, 119-126.

Kwasek, K., K. Dabrowski, J. Nynca, R. Takata, M. Wojno, and M. Wick. 2014. The influence of dietary lysine on yellow perch (*Perca flavescens*) female reproductive performance and the quality of eggs. North American Journal of Aquaculture 76:351–358, 2014

Kwasek, K., M. Wojno, G. Terova, T. Ostaszewska T, M. Wick, and K. Dabrowski. 2012. The effect of the dipeptide, Lys-Gly, supplement on growth, muscle proteins and PEPT1 gene expression in juvenile yellow perch. Reviews in Fish Biology and Fisheries 22: 797–812.

VITA

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West Lafayette, IN 47907

Education

A.A., A.S.	College of the Redwoods, 2002, Science and Mathematics, Marine Science Technology
B.S.	Ball State University, 2010, Biology

Positions

2019 – Present	Aquaculture Marketing Outreach Association Illinois-Indiana Sea Grant, Purdue University, Indiana
Oct. 2014 – 2019	Aquaculture Lab Technician RDM Aquaculture LLC, Indiana
Aug. 2014 – Oct. 2014	Consultant Aqua International Corporation, Costa Rica
Jan. 2014 - Aug. 2014	Compliance and Certification Coordinator Bell Aquaculture, Indiana
2012 – 2013	Farm Manager Bell Aquaculture, Indiana
2010 - 2012	Analytical Research Coordinator Bell Aquaculture, Indiana
2010	Undergraduate Intern Oregon State University, Oregon
2007	Farm Worker 1 University of Hawaii, Hawaii
2001 – 2005	Fisheries Technician Pacific States Marine Fisheries Commission, California
2003	Environmental Health Technician Mendocino County Environmental Health Department, California
2002 – 2003	Naturalist Hendy Woods State Park, California

Scientific and Professional Organizations

Indiana Aquaculture Association Inc.

Recent Publications

Carlton, S., A.Shambach, and C. Foley. 2020. Walleye Aquaculture Working Group Workshop: Identifying Walleye Marketing and Production Barriers. Workshop Proceedings Summary IISG20-SAQ-BRC-005. Purdue University, Illinois-Indiana Sea Grant, West Lafayette, Indiana.

Stinton, A., L. Ciannelli, D. Reese, and W. Wakefield. 2014. Using In Situ Video Analysis to Assess Juvenile Flatfish Behavior Along the Oregon Central Coast. California Cooperative Oceanic Fisheries Investigations Reports, 55: 158-168.

VITA

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Education

B.A. Tulane University, 2001, English
M.S. University of Georgia, 2004, Fisheries Biology
Ph.D. University of Florida, 2012, Interdisciplinary Ecology

Positions

2018–Present	Assistant Director, Illinois-Indiana Sea Grant College Program
2014–Present	Healthy Coastal Ecosystems Specialist, Texas Sea Grant College Program
2013–2014	Postdoctoral Research Assistant. Natural Resources Social Science Lab, Purdue University

Scientific and Professional Organizations

International Association for Society and Natural Resources
Sea Grant Association

Selected Publications

Prokopy, L.S., J.S. Carlton, T. Haigh, M.C. Lemos, A.S. Mase, and M.Widhalm. 2017. Useful to Usable: Developing usable climate science for agriculture. Climate Risk Management 15: 1–17.

Church, S. P., T. Haigh, M. Widhalm, S. Garcia de Jalon, N. Babin, J.S. Carlton, M. Dunn, K. Fagan, C.L. Knutson, and L. S. Prokopy. 2017. Agricultural trade publications and the 2012 Midwestern U.S. Drought: A missed opportunity for climate risk communication. Climate Risk Management 15: 45–60.

Carlton, J. S., T. Haigh, C.L. Knutson, M. Lemos, A.S. Mase, D. Todey, and L.S. Prokopy. 2016. The effects of the 2013 drought on climate change beliefs, risk perceptions, and adaptation attitudes. Climatic Change 135: 211–226.

Cook, J., N. Oreskes, P. Doran, W. Anderegg, B. Verheggen, E. Maibach, J.S. Carlton, S. Lewandowsky, A. Skuce, S. Green, D. Nuccitelli, P. Jacobs, M. Richardson, B. Winkler, R. Painting, and K. Rice. 2016. Consensus on consensus: a synthesis of consensus estimates on human-caused global warming. Environmental Research Letters 11: 048002.

Carlton, J. S. and S. K. Jacobson. 2016. Using expert and non-expert models of climate change to enhance communication. Environmental Communication 10: 1–24.

Carlton, J. S., R. Perry-Hill, M. Huber, and L. S. Prokopy. 2015. The scientific consensus about climate change extends beyond climate scientists. Environmental Research Letters 10: 094025.

Haigh, T., E. Takle, J.A. Andresen, M.J. Widhalm, J.S. Carlton, and J. Angel. 2015. Mapping the decision points and climate information use of agricultural producers across the U.S. Corn Belt. Climate Risk Management 7: 20–30.

VITA

Name: Gregory J. Fischer Phone: 715-209-0011
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Education

Assoc. of Arts w/honors, Major-Biology. 1988, Jackson Community College, Jackson, MI.
B. S., Fisheries & Wildlife Management, 1992. Lake Superior State University, Sault Ste. Marie, MI.

Positions

2019-Present	Assistant Director/Research Program Manager, Northern Aquaculture Demonstration Facility, Bayfield, WI. University of Wisconsin Stevens Point
2019-Present	Aquaculture Design Consultant, McMillian and Jacobs Associates, Boise ID (part-time)
2002-2019	Facility Operations Manager, Northern Aquaculture Demonstration Facility, University of Wisconsin-Stevens Point, Bayfield, WI. University of Wisconsin Stevens Point
2000-2019	Fish Hatchery Design Consultant, Fischer Biological Consulting LLC, Washburn WI
1994-2002	Natural Resources/Fish Hatchery Program Director, Red Cliff Band of Lake Superior Chippewa

Scientific and Professional Organizations

American Fisheries Society
World Aquaculture Society/U.S. Aquaculture Society
International Aquacultural/ Recirculating Systems Engineering Society
Wisconsin Aquaculture Association/ Wisconsin Aquaculture Industry Advisory Council
North Central Region Aquaculture Center- Scientific Advisory Council Member
European Percid Aquaculture Group Member

Recent Publications

Davidson, J., C. Grimm, S. Summerfelt, G. Fischer, and C. Good. 2020. Depuration system flushing rate affects the kinetics of geosmin removal from market-size Atlantic salmon Salmo salar. Aquaculture Engineering 90, 102104, ISSN 0144-8609, https://doi.org/10.1016/j.aquaeng.2020.102104.

Tyler J. Firkus, Cheryl A. Murphy, Jean V. Adams, Ted J. Treska, Gregory J. Fischer. Assessing the assumptions of classification agreement, accuracy, and predictable healing time of sea lamprey wounds on lake trout. Journal of Great Lakes Research. July 2020.

Fischer, G.J., K. Holmes, E.M. Wiermaa and C. Hartleb. 2019. Experimental rearing system for the intensive larviculture of walleye (Sander vitreus) and hybrid walleye (S. vitreus x S. canadensis). Aquaculture International. In Review

Davidson, J., R. Summerfelt, F. Barrows, B. Gottsacker, C. Good , G.J. Fischer, and S. Summerfelt. 2016. Walleye Sander vitreus performance, water quality, and waste production in replicated recirculation aquaculture systems when feeding a low phosphorus diet without fishmeal versus a traditional fishmeal-based diet. Aquaculture Engineering 75:1-13, ISSN 0144-8609, https://doi.org/10.1016/j.aquaeng.2016.09.005.

Fischer, G. J., C.F. Hartleb, and K. Holmes. 2011. A commercial demonstration of new technologies for producing walleye and hybrid walleye for stocking and food fish. Final report to the WI Department of Agriculture, Trade and Consumer Protection.

Fischer, G.J., J. Held, C. Hartleb, and J. Malison. 2009. Evaluation of brook trout production in a coldwater recycle aquaculture system. Aquacultural Engineering 41: 109-113.

Fischer, G. J. 2009. Novel approaches for improving perch growth in RAS systems. USDA Agricultural Research Service, Cooperative Agreement CRIS #3655-31320-001-02S Final Report.

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Education

B. S., Ecology and Environmental Biology, 2012. University of Wisconsin-Eau Claire

Positions

2014-Present	Aquaculture Outreach Specialist, University of Wisconsin Stevens Point Northern Aquaculture Demonstration Facility, University of Wisconsin-Stevens Point and Wisconsin Sea Grant Institute
2014	Aquaculture Technician, University of Wisconsin Stevens Point Northern Aquaculture Demonstration Facility, University of Wisconsin-Stevens Point
2013-2014	Program Coordinator, Alliance for the Great Lakes, Duluth, MN
2011	Research Assistant, University of Wisconsin-Eau Claire
2010-2011	Education Program Coordinator, Longfellow Elementary School, Chippewa Falls, WI

Scientific and Professional Organizations

World Aquaculture Society/U.S. Aquaculture Society- Member
Wisconsin Aquaculture Association- Member
North Central Region Aquaculture Center -Technical Advisory Committee for Extension
Sea Grant Fisheries, Aquaculture and Seafood Group
National Aquaculture Extension Steering Committee Member

Recent Publications

Fischer, G.J., K. Holmes, E.M. Wiermaa and C. Hartleb. 2019. Experimental rearing system for the intensive larviculture of walleye (Sander vitreus) and hybrid walleye (S. vitreus x S. canadensis). Aquaculture International. In Review.

Wiermaa, E. M 2018. UWSP NADF: Advancing Aquaculture Education and Outreach. Aquatic Sciences Chronicle. Retrieved from https://www.uwsp.edu/colsap/nadf/Documents/PDF/2018_vol2%20aquaculture%20updates.pdf

Wiermaa, E. M 2018. Ground breaking sea lamprey research happening at UW-Stevens Point Northern Aquaculture Demonstration Facility. Bayfield County Land & Water Conservation Dept.-Aquatic Invasive Species Project. Retrieved from https://www.uwsp.edu/cols-ap/nadf/Documents/PDF/Spring%202018%20AIS%20Newsletter.pdf

Wiermaa, E. M. and G.J. Fischer. 2018. Sustainable land-based Atlantic salmon production using. Xylem Application Note A629. Retrieved from https://www.ysi.com/File%20Library/Documents/Application%20Notes/YSI-Sustainable-Land-based-Atlantic-Salmon-Production-A629_web.pdf