

ATTACHMENT B

**NUTRITIONAL PROGRAMMING OF YELLOW PERCH
LARVAE USING LIVE FOOD AS A VEHICLE**

DETAILED PROJECT OUTLINE FOR THE PLAN OF WORK

FOR GRANT #2018-38500-28887

Nutritional Programming of Yellow Perch Larvae using Live Food as a Vehicle

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Funding Request:	\$123,785
Duration:	2 years (July 1 2019 – June 30, 2021)

Objectives:

1. To determine if Nutritional Programming of yellow perch larvae via live food improves dietary plant protein utilization in yellow perch during later life stages.
2. To determine the mechanism underlying the Nutritional Programming responsible for improved dietary plant protein utilization:
 - a) To assess if Nutritional Programming changes gut microbial communities responsible for improved digestion of dietary plant protein.
 - b) To determine if Nutritional Programming mitigates any inflammatory or morphological changes in the gut responsible for improved digestion of dietary plant protein.
3. To communicate the Nutritional Programming concept via live food, Nutritional Programming feeding strategy protocol, and live food enrichment formulation that could be used by fish farmers and feed manufacturing industry, to improve plant protein-based diets utilization during yellow perch grow-out phase.

Deliverables:

1. We anticipate significant improvement in the growth performance of yellow perch fed plant protein-based diets after Nutritional Programming induced via live food in larval yellow perch. This study will therefore deliver an innovative feeding approach and an enrichment formula for live food (rotifers and *Artemia nauplii*) used for Nutritional Programming of yellow perch that will allow to enhance the capacity of the fish to utilize lower cost plant protein sources.
2. The results derived from the study will be the first evidence for mechanisms behind Nutritional Programming that will help to promote the use of Nutritional Programming in yellow perch and will serve as a base for future studies on other important North Central Region aquaculture species that will focus on further improvement of Nutritional Programming protocols by targeting the specific Nutritional Programming mechanisms including the gut microbiome.
3. This study will also provide a workshop, which will discuss and facilitate an open dialogue regarding live foods and first feeding, and offer the opportunity to “showcase” the results of this proposed project through the facilitation of technology transfer to Midwest fish farmers regarding alternative feeding strategies and live food enrichments. The theoretical and practical knowledge derived from the workshop will build farmers’ awareness around the concept of Nutritional Programming and provide them with tools to use this feeding strategy in the most effective way to enhance utilization of commercial feeds based on plant protein sources.

Proposed Budgets

Institution	Principal Investigators	Objectives	Year 1	Year 2	Total
Southern Illinois University-Carbondale	Karolina Kwasek Bethany Rader	1,2	\$45,156	\$45,001	\$90,157
Southern Illinois University-Edwardsville	Vance McCracken	2	\$0	\$27,860	\$27,860
The Ohio State University Madison County Extension Office	Matthew Smith	3	\$0	\$5,768	\$5,768
TOTAL			\$45,156	\$78,629	\$123,785

Non-funded Collaborators

Facility	Collaborator(s)
National Corn to Ethanol Research	Yan Zhang

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Project Summary

Replacement of fishmeal (FM) in aquaculture diets with plant protein (PP) has been an ongoing challenge. High-quality PP concentrates are widely used since their digestibility can be comparable to FM. However, their price can exceed the cost of marine raw materials. Progress with utilization of lower-quality PP has been made but a number of concerns must be overcome to maintain acceptable growth rates and feed efficiency values at high FM substitution levels. Nutritional Programming (NP) is a promising approach to offset the negative effects of dietary PP by modifying specific physiological responses during early development leading to fish with long-lasting ability to assimilate a previously undesirable PP. We propose an unconventional NP strategy with dietary PP for yellow perch (YP) *Perca flavescens* using live food as a vehicle. We believe this innovative feeding approach will become a practical way for enhancing utilization of diets based on high levels of cost-effective plant raw materials. Consequently, this study will contribute to expansion of YP production and development of competitive aquafeed market within the North Central Region (NCR) by providing feed manufacturers and farmers with possibility of using bigger raw material basket allowing for more flexibility in formulations of diets deprived of FM.

Justification

One of the major goals of worldwide aquaculture is to replace FM with alternative protein sources derived mostly from plants. The estimated global amount of aquafeeds produced by 2020 is predicted to reach 65 mmt with only ~7% of the feed being based on marine raw materials of animal origin including FM and the rest derived from alternative protein sources mostly from plants. High-quality PP sources such as soy or pea protein concentrates, wheat or corn gluten, have been widely used by the feed industry since their digestibility in some species is comparable to FM. However, their price can often exceed the cost of marine raw materials. Although some progress with utilization of lower-quality PP has been made 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 FM substitution levels. 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 FM but also substitute some of the expensive high-quality PP concentrates and provide more flexibility in feed formulations using a wider range of locally available raw materials.

YP has 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 that species to help to satisfy the market as a food fish and help to reduce the pressure on natural resources particularly in the Great Lakes. The expansion of YP aquaculture industry on a large scale, however, has been constrained by several factors including low survival and difficulty in feed training of the larval stages. The current practices focus on feeding early stages of YP with live food such as rotifers and *Artemia* nauplii, 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. Most of the commercial feeds, however, rely on a large proportion of PP meals and although YP has been reported to utilize high quality PP concentrates including wheat gluten efficiently for growth (Kwasek et al. 2011) it has also been found to be sensitive to dietary inclusion of lower-quality PP such as soybean meal (Kasper et al. 2007). Consequently, the inability of the fish to grow satisfactorily on lower cost feeds with higher inclusion of soybean meal and similar quality PP sources has been considered as the major bottleneck to further expansion of YP production in the NCR.

Related Current and Previous Work

Nutritional Programming (NP) has been found as a promising approach to counteract the negative outcomes of PP sources in feeds. NP is a way of modifying or inducing specific physiological responses during vulnerable early developmental stages of an animal that will help to cope with specific challenges in the animal later (adult) life. NP in fish only recently has received more attention and some studies already indicate it is possible to “nutritionally program” fish during their early larval/juvenile age with alternative raw materials or nutrient levels to allow fish to utilize different dietary compounds more efficiently later in their life. Geurden et al. (2013) found that juvenile rainbow trout *Oncorhynchus mykiss* subjected to PP-based diet during the first three weeks of life showed improved acceptance and utilization of the same dietary PP-based diet when given during later life stages. Furthermore, two

other studies reported that early exposure of larval European seabass *Dicentrarchus labrax* to long-chain polyunsaturated fatty acids deficiency induced higher expression of delta-6 desaturase mRNA levels in juveniles – the rate-limiting enzyme involved in biosynthesis of highly unsaturated fatty acids (Vagner et al. 2007, 2009). Other studies also point out the importance of maternal NP and its impact on the well-being of progeny. Izquierdo et al. (2015) found that it is possible to achieve improved growth of 4-month old gilthead seabream *Sparus aurata* juveniles fed low FM and low fish oil diets by previously exposing the broodstock of those fish to high vegetable-based oil feeds. Moreover, those authors later showed that the same fish at 16-month of age were still able to grow on low FM/fish oil diets better compared to control group suggesting positive long-term effect of NP on utilization of vegetable-based diet (Turkmen et al. 2017). Finally, NP with soybean meal-based diets have been shown to successfully adapt YP to utilize the same soybean meal diet during fish adult stage leading to much better growth compared to YP that were not exposed to soybean meal diet during the early developmental stage (Kemski et al. 2018). Most of these studies focus, however, on NP induced during fish juvenile stages. *We believe that earlier exposure to NP induced already in the larval phase via the first feed will further improve capacity of the fish to utilize PP for growth in later life stages.*

Although NP has a great potential for improving fish growth and health performance during the grow-out phase, the mechanism behind the NP phenomenon remains elusive. Studies in other animals including mice (Sanchez-Samper et al. 2017) reported that NP can influence bacterial densities in the digestive tract. Similarly, in fish diet and many dietary components including protein and lipid sources or probiotics can have a substantial impact on bacterial populations of the gut (Bakke-McKellep et al. 2007; Tarnecki et al. 2017). *We believe that NP with dietary PP induces changes in fish gut microbiome and those changes are one of the mechanisms responsible for improving PP digestion and assimilation during fish later life stages.*

Flavor experiences during early life stages have also been shown to be a driver for life-long flavor acceptance in adults' life. For example, citric acid, which has a sour/bitter taste, is known to be an instinctively aversive substance for rats. However, rats exposed/programmed to citric acid during nursing showed increased voluntary ingestion of citric acid compared to rats that were not exposed to citric acid at all (London et al. 1979). Similarly, Balasubramanian et al. (2016) indicated that early PP diet exposure in rainbow trout might mediate feed acceptance of the same diet at a later life stage by affecting pathways regulating the sensory perception of taste, odor, and possibly vision. If NP-induced improved dietary PP utilization is a result of improved palatability towards PP, can the negative effects of anti-nutritional factors present in PP still induce morphological changes in fish digestive tract as seen in other studies (Baeverfjord and Krogdahl 1996; Uran et al. 2008; Hedrera et al. 2013). Dietary PP, such as soybean meal, have been associated with many cases of intestinal inflammations in several fish species limiting their dietary inclusion rates. Typical signs of dietary PP-induced inflammation in the distal intestinal mucosa include shortening of the mucosal folds and hence, reduced absorptive capacity of the enterocytes lining the intestinal epithelium; thickening of lamina propria and sub-epithelial mucosa; infiltration of inflammatory cells; and increased number of goblet cells in the epithelium (Booman et al. 2018). All these processes decrease the capacity of the digestive tract to digest, absorb, and utilize nutrients consequently affecting fish growth and health by diminishing their response to pathogens. The NP has been shown to improve dietary PP utilization, including soybean meal; whether this process is driven solely by increased palatability and therefore improved feed intake, or by specific intestinal responses to dietary PP that lead to morphological adaptations remains unknown. *We believe that NP with dietary PP alters the gut epithelial lining and consequently increases fish resistance to negative side-effects of PP improving fish capacity to cope with those alternative raw materials better.*

Statement of 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. There were no current or previously funded projects found in any of the databases that were similar to this project. Search terms used: nutritional programming, imprinting, yellow perch, soybean, plant protein, microbiome, intestinal inflammation, live food, enrichment.

Anticipated Benefits

We believe that NP induced at first feed is a much more effective way of improving YP acceptance and utilization capacity of dietary PP compared to the “traditional” NP method, which is normally induced with dry feed during later fish stages. The combination of live food and PP will provide all the nutrients required for proper growth and development and at the same time expose the fish to alternative raw materials and/or anti-nutritional factors delivered in low enough concentrations to induce long-lasting adaptation of the fish towards the same dietary components later in their life without impairing the larval well-being. If proven, this feeding strategy will become a feasible and practical way for enhancing YP utilization of diets based on almost any raw material. The outcome of this study will provide the fish farmers and feed industry within the NCR with the possibility of using bigger and more cost-effective raw material basket and hence, allow for more flexibility in formulations of diets deprived of FM. This will consequently lead to the development of competitive aquaculture feed market that will contribute to the intensification of more sustainable production of YP and other important fish species in the NCR. Since NP has been shown effective in a wide range of fish species we believe that NP induced via live food could be applied to other aquaculture species particularly within the *Perciformes* family.

Objectives

1. To determine if NP of YP larvae via live food improves dietary PP utilization in YP during later life stages.
2. To determine the mechanism underlying the NP responsible for improved dietary PP utilization:
 - a) To assess if NP changes gut microbial communities responsible for improved digestion of dietary PP.
 - b) To determine if NP mitigates any inflammatory or morphological changes in the gut responsible for improved digestion of dietary PP.
3. To communicate the NP concept via live food, NP feeding strategy protocol, and live food enrichment formulation that could be used by fish farmers and feed manufacturing industry, to improve PP-based diets utilization during YP grow-out phase.

Deliverables

We anticipate significant improvement in the growth performance of YP fed PP-based diets after NP induced via live food in larval YP. This study will therefore deliver an innovative feeding approach and an enrichment formula for live food (rotifers and *Artemia nauplii*) used for NP of YP that will allow to enhance the capacity of the fish to utilize lower cost PP sources.

The results derived from the study will be the first evidence for mechanisms behind NP that will help to promote the use of NP in YP and will serve as a base for future studies on other important NCR aquaculture species that will focus on further improvement of NP protocols by targeting the specific NP mechanisms including the gut microbiome.

This study will also provide a workshop, which will discuss and facilitate an open dialogue regarding live foods and first feeding, and offer the opportunity to “showcase” the results of this proposed project through the facilitation of technology transfer to Midwest fish farmers regarding alternative feeding strategies and live food enrichments. The theoretical and practical knowledge derived from the workshop will build farmers’ awareness around the concept of NP and provide them with tools to use this feeding strategy in the most effective way to enhance utilization of commercial feeds based on PP sources. Furthermore, the results of this study as well as the feeding procedure for larval yellow perch will be included in NCRAC factsheet and presented in a Webinar in the light of previous NCRAC studies that focused on a similar topic to facilitate the transfer of knowledge and new technology. Since NP has been shown effective in a wide range of fish species we believe that NP induced via live food could be applied to other aquaculture species particularly within the *Perciformes* family.

Procedures

Objective 1 - To determine if NP of YP larvae via live food improves dietary PP utilization in YP during later life stages.

We plan to achieve this by accomplishing the following activities performed in Dr. Kwasek's laboratory at SIUC:

1. Live food, rotifers and *Artemia* nauplii, will be enriched for 8-12 hours with conventional soybean meal (lf-SBM), soy saponin (lf-SS) - an anti-nutritional factor, distiller's dried grains with solubles (lf-DDGS), sunflower expeller (lf-SE), or FM (lf-FM; control). We have done preliminary testing in SIUC laboratories for the use of PP or FM as an enrichment for live food and found no negative effect on the viability of rotifers or *Artemia nauplii* even after 24 hours exposure.
2. The enriched rotifers and *Artemia nauplii* will be provided to corresponding groups of YP larvae distributed randomly into 50 L tanks (80 larvae/L) as first feed for 3 and 7-9 days, respectively.
3. All YP larvae will be weaned to commercial FM-based starter diet.
4. After one month of feeding, all fish from lf-SBM, lf-SS, lf-DDGS, lf-SE, and lf-FM will be provided with corresponding diets based on either SBM (lf-SBM, lf-SS groups, and lf-FM control group), DDGS (lf-DDGS group), or SE (lf-SE group) to assess if NP via live food improved dietary PP utilization short-term.
5. All YP will be subjected to commercial FM-based diet again.
6. After four months, all YP will be provided with the same PP-based diets (as in point 4.) to assess the long-term NP effect and whether it persists to fingerling stage.

YP has been considered a challenging species particularly with respect to larval rearing, however, based on PI's previous experience successfully raising larval YP in laboratory conditions, we are confident we will obtain good survival and swim-bladder inflation rates using similar rearing conditions (Grayson et al. 2014).

Objective 2 - To determine the mechanism underlying the NP responsible for improved dietary PP utilization:

- a) **To assess if NP changes gut microbial communities responsible for improved digestion of dietary PP.**

Changes in fish diet, specifically the inclusion of a higher concentration of plant ingredients, has been shown to alter the proteome of the intestinal mucosa, growth, and resistance to parasites in the gilthead sea bream *Sparus aurata*. These physiological and immunological changes are correlated with changes in the composition of the microbiome, specifically a decrease in microbial diversity (Piazzon et al. 2017). This suggests that changes in the gut microbiota of fish due to changes in diet may underlie physiological and immunological response of the fish to those diets. To determine if the normal (conventional) intestinal microbiome is necessary for improved digestion of dietary PP in YP, we will utilize antibiotics to disrupt the composition and abundance of bacterial species present in the intestine and compare lf-SBM animals to control animals. Sub-clinical doses of broad spectrum antibiotics have been shown to decrease the overall abundance and diversity of the gut microbiota in zebrafish *Danio rerio* (Pindling et al. 2018), and decreasing tolerance to pathogenic bacteria in the mosquitofish *Gambusia affinis* (Carlson et al. 2017). We hypothesize that changes in the PP utilization are due, at least in part, to the function of the gut microbiome. To test our hypothesis we will conduct the following experiments.

Firstly, we will investigate whether NP of YP larvae via live food alters the composition and diversity of the intestinal microbiome in YP by dissecting the intestines out of a subset of YP in Objective 1 at the following time points: i) before the start of the study, ii) after the first feeding with live food, iii) after the first feeding with FM, and iv) after the first and second PP challenge. The intestines will be homogenized, total genomic DNA will be extracted from the homogenates, and the V4 region of the 16s rRNA will be sequenced via next generation sequencing, to be completed at an off-campus facility. The sequences will be computationally analyzed generating phylogenetic profiles of bacterial gut community membership that will be compared within or between animals/treatments for differences in alpha and beta diversity.

Secondly, we will investigate whether the disruption of the microbiome alters the response of YP to NP with live food by altering the composition of the microbiome of YP receiving NP with a constant low dose of antibiotics, and comparing that to YP that receive no antibiotics. As the sample set for this aim is potentially double that of the first part of sub-objective 2a, making experimentation and analysis impractical, we will only compare Lf-SBM animals to FM control animals. Lf-SBM animals were chosen because of reported effect that soybean meal has on intestinal changes in fish. For this part, FM control animals and Lf-SBM animals will be split into two groups, one which will receive low doses of antibiotic in their water throughout the experiment to alter the composition of the intestinal microbiome, and one that will remain without antibiotics and retain the “conventional” intestinal microbial community. Fish intestinal samples will be taken i) before the start of the study, ii) after the first feeding with live food, iii) after the first feeding with FM, and iv) after the first and second PP challenge. To assess changes in the YP intestinal microbiome intestines will be extracted from YP, homogenized, and whole genomic DNA will be extracted from homogenates. The V4 region of the 16s rRNA will be sequenced via next generation sequencing, to be completed at an off-campus facility, and computationally analyzed generating phylogenetic profiles of bacterial gut community membership that will be compared within or between animals/treatments for differences in alpha and beta diversity. The activities related to Sub-Objective 2a will be performed in Dr. Rader’s laboratory at SIUC.

b) To determine if NP mitigates any inflammatory or morphological changes in the gut responsible for improved digestion of dietary PP.

Many dietary PP are characterized by presence of anti-nutritional factors that have been associated with intestinal inflammation in fish, which can decrease the capacity of fish digestive tract to digest, absorb, and utilize nutrients consequently affecting fish growth and health (Uran et al. 2008; Hedrera et al. 2013; Bravo-Tello et al. 2017). To assess if NP mitigates any negative side-effects of PP on the gut health samples of YP intestines will be taken from fingerlings at the end of the study and fish health diagnostics will be carried using histology and molecular biology techniques that will assess expression of cytokines, oxidative stress response, and other inflammation-related genes. Procedures for this Sub-Objective will be performed by Co-I McCracken’s laboratory at SIUE. Specifically, for histological analysis, intestines will be removed upon euthanasia in MS-222. One portion each from the proximal and distal intestine will be fixed in cold formalin, embedded in paraffin, and sectioned. Slides will be stained with hematoxylin and eosin to observe inflammation-induced alterations in morphology, including thickening of the mucosal and muscle layers, villi length and shape, and the vertical and longitudinal extend of leukocytic infiltrate. Separate slides will be stained with periodic acid Schiff base and alcian blue to count goblet cells. Additionally, one portion each from the proximal and distal intestine will be used to assess expression of cytokines, oxidative stress response, and other inflammation-related genes, including interleukin-1 β , serum amyloid a, heat shock protein 70, glutathione peroxidase, and superoxide dismutase; β -action will be used as an endogenous control. RNA will be isolated from homogenized tissues using Trizol, and reverse transcribed using a High Capacity cDNA Reverse Transcription Kit. Real-time PCR will be performed using previously validated primer sets and SYBR Green Master Mix, and relative fold-changes in expression quantified using the $\Delta\Delta C_t$ method.

Objective 3 - To communicate the NP concept via live food, NP feeding strategy protocol, and live food enrichment formulation that could be used by fish farmers and feed manufacturing industry, to improve PP-based diets utilization during YP grow-out phase.

Extension, specifically within the NCR, is an essential component to the long-term development of an economically and sustainable aquaculture industry in our region. The primary audience for the proposed study is the aquaculture industry. Therefore, in Objective 3, we will deliver to the industry a first-generation product formulation that can be used as live food enrichment designated for NP of YP early stages for improved dietary PP utilization during the grow-out phase. Specifically, we will organize a workshop at Millcreek Perch Farm in Marysville, Ohio for Midwest fish farmers to facilitate the transfer of knowledge regarding alternative feeding strategies and live food enrichments to improve growth and feeding efficiencies of important local aquaculture species, including YP. We will introduce the farmers to the concept of NP and how to use this feeding strategy in the most effective way to enhance utilization of commercial feeds based on PP sources. We will also discuss conventional live food feeding techniques currently used by the industry to facilitate an open dialogue between farmers.

We understand that changing a farmer's typical protocols for feed-training YP on the farm will not be easy or immediately adopted by the entire industry. Our desire is for a hands-on workshop to discuss the necessary protocols and how easy this could be adopted into a farmer's typical practices. The co-owner of Millcreek Perch Farm is heavily involved in NCRAC, and we believe that successful demonstrations during the workshop will help OSU Extension in delivering the results from this proposed work. Smith also has camera equipment that can be utilized for recording the workshop, explaining in detail the protocol the farmer would ideally follow, and the benefits of the research results. This work would then be displayed on NCRAC's Vimeo website and several Midwest aquaculture websites. Smith will also be responsible for transferring the results to the rest of the NCRAC Extension/outreach community and beyond. The results will also be presented at the next North Central Aquaculture Conference.

Outreach and Evaluation Plan

Research activities will be conducted at SIUC and SIUE, and extension and outreach activities will be conducted by OSU Madison County Extension Office. In particular, OSU Madison County Extension Office will lead a workshop on first-feeding in the spring/summer of the second year. The workshop will be conducted at a commercial Millcreek Perch Farm in Marysville, Ohio, so that current farmers, interested hatcheries, and raw materials suppliers including ADM Animal Nutrition™ can observe a functioning and real-world hatchery. This workshop will discuss and facilitate an open dialogue regarding live foods and first feeding, but it will also offer the opportunity to “showcase” the results of this proposed project through the facilitation of technology transfer to Midwest fish farmers regarding alternative feeding strategies and live food enrichments to improve growth and feeding efficiencies of important local aquaculture species, including YP. Extension and researchers will discuss the premise behind the project, the key results, and the practical application for farmers who hatch/feed train YP and other popular Midwest species.

Our ultimate goal is that this product or “formula” will be something that SIU and OSU can give to the farmers so that they can purchase the raw materials themselves and not rely on feed manufacturers to enrich the live food. Depending on the results and the conclusions drawn from the results, it is possible that the “formula” or recipe is something that may need to be incorporated into the live feeds prior to distribution. It is not in the interest of the PI nor the collaborators to patent any of the information, and all information will be relayed to the industry through Extension publications (e.g., *Buckeye Aquafarming*), a workshop, discussion with other Midwest Extension personnel to increase transfer to other states, and a YouTube-type video or a Webinar describing results and the workshop to be published on NCRAC's Vimeo channel.

Additional outputs of this study include NCRAC fact sheets as well as 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. These reports will be available on the NCRAC Web site (<http://www.ncrac.org>). This innovative NP approach for YP will become the subject of discussion with fish farmers and other industry representatives including ADM Animal Nutrition™ in respect to production of a new generation of fish capable of efficient utilization of PP. We will seek input from ADM, hatchery managers and extension officers including Paul Hitchens at SIUC, their involvement in reviewing reports and by these means secure an expeditious way to implement the innovative live food enrichment formulation and NP strategy into YP farming. Considering the PI's extensive experience in the private sector, we will ensure that the practical outcome of this research, in a form of new live food enrichment and feeding strategy, will be translated into immediate action and implementation by the aquaculture industry. Finally, we will also communicate these research findings with feed manufacturing industry on how the NP strategy can be used to allow more flexibility in commercial feed formulation using a variety of lower quality PP. The Department of Zoology at SIUC facility is able to accommodate technical and educational seminars, which will also allow for regular updates of the industry representatives, extension officers, and scientific community with any new findings as well as provide more insight to effective feeding strategies in hatcheries.

Logic Model

Title: Nutritional Programming of Yellow Perch Larvae using Live Food as a Vehicle

Situation: Successful expansion of YP production relies on improving utilization of plant protein PP-based diets.

Goal: To improve PP-based diet utilization in YP by inducing Nutritional Programming (NP) with dietary PP in larval YP during the first feeding period using live food.

Objectives:

1. To determine if NP of YP larvae via live food improves dietary PP utilization in YP during later life stages.
2. To determine the mechanism underlying the NP via live food responsible for improved dietary PP utilization:
 - a) To assess if NP changes gut microbial communities responsible for improved digestion of dietary PP.
 - b) To determine if NP mitigates any inflammatory or morphological changes in the gut responsible for improved digestion of dietary PP.
3. To communicate the NP concept via live food, NP feeding strategy protocol, and live food enrichment formulation that could be used by fish farmers and feed manufacturing industry, to improve PP-based diets utilization during yellow perch grow-out phase.

Inputs	Outputs		Outcomes – Impact		
	Activities	Deliverables	Knowledge gain	Behavior change	Conditions
Faculty and staff from North Central Region (SIUC, SIUE, OSU)	Development of innovative live food enrichment formula and feeding strategy for improvement of dietary PP utilization	Significant improvement in growth performance of YP fed PP-based diets after NP induced via live food	Knowledge of suitability of the novel feeding strategy for YP	Commercialization of the innovative feeding strategy	Reduced reliance on FM-based feeds and increased flexibility in feed formulation in feed manufacturing
Research funding	Suitability assessment of the novel feeding strategy on larval and juvenile YP performance	An innovative feeding protocol and live food enrichment formula for successful NP that enhances YP growth on PP-based diets	Knowledge of tools required for the innovative NP feeding strategy via live food	Use of the novel NP feeding approach in other fish species in the NCR	Lower feed costs due to utilization of locally produced and more cost-effective raw material sources

	Outputs		Outcomes – Impact		
Inputs	Activities	Deliverables	Knowledge gain	Behavior change	Conditions
Team’s expertise in aquaculture, nutrition, microbiology, immunology, and aquaculture extension	Assessment of the effect of novel feeding strategy on gut responses in YP	First evidence for mechanisms behind NP that will serve as a base for future studies on other important NCR aquaculture species focusing on further improvement of NP protocols by targeting specific NP mechanisms including the gut microbiome	Awareness of novel NP feeding approaches for feed/fish producers and research community particularly within the NCR		Reduction of costs in YP production
		Workshop which will facilitate an open dialogue with fish farmers regarding live foods and first feeding and allow for transfer of knowledge and technology to build farmers’ awareness around the NP concept and provide them with tools to use this feeding strategy to enhance utilization of commercial feeds based on PP sources Related factsheet(s)			
		Communication of findings to hatchery and feed producers			

Facilities

Activities related to Objectives 1 and Sub-Objective 2a 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 m² (12,000 ft²) of floor space in the Life Sciences II, III and Annex Buildings located on the SIUC campus. An 770 m² (8,300 ft²) 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 m² (7,000 ft²) 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 m² (724 ft²) on the SIUC main campus. In addition, three additional spaces consisting of a 34 m² (365 ft²) square feet and two adjoining rooms totaling 15 m² (156 ft²). 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 Fluorescence 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 Sub-Objective 2b will be carried at Southern Illinois University-Edwardsville (SIUE) in the Department of Biological Sciences, which includes a dedicated microscopy suite that houses inverted Olympus fluorescence microscope; a Leica DM5500B upright microscope with phase/DIC/fluorescence attachments, digital camera, and Applied Fluorescence Suite software; an Olympus SZX16 fluorescent stereomicroscope with CellSens software; and a motorized Olympus 1X81 confocal microscope with Fluoview software. Molecular biology equipment includes a Biotek Synergy Hybrid UV/Vis/Luminescence plate reader with GenV software, a BioTek Select plate washer, a Stratagene MX3000P real-time PCR machine, several standard PCR machines, a Bio-Rad Gel-Doc XR documentation system with Quantity One software, an Accuri C6 flow cytometer, as well as a Bio-Rad ChemiDoc system with ImageLab software, and a Bio-Rad DCode system.

Activities related to Objective 3 will be carried mainly by Madison County Extension Office, College of Food, Agricultural, and Environmental Sciences at The Ohio State University and supported by SIUC. OSU has all of the necessary equipment (cameras, software, and computers) to generate a webinar and record the workshop for further dissemination throughout the Midwest.

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Project Leaders

State	Name/Institution	Area of Specialization
IL	Karolina Kwasek, Ph.D. Southern Illinois University-Carbondale	Aquaculture/Nutrition
IL	Bethany Rader, Ph.D. Southern Illinois University-Carbondale	Microbiology
IL	Vance McCracken, Ph.D. Southern Illinois University-Edwardsville	Immunology
OH	Matthew Smith The Ohio State University Madison County Extension Office	Aquaculture Extension

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

ORGANIZATION AND ADDRESS Southern Illinois University - Carbondale 1263 Lincoln Dr, Carbondale, IL 62901			USDA AWARD NO. Year 1: Objective 1, 2			
			DURATION PROPOSED MONTHS: 12	DURATION PROPOSED MONTHS: _____	Non-Federal Proposed Cost-Sharing/Matching Funds (If required)	Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)
PROJECT DIRECTOR(S) Karolina Kwasek Bethany Rader			Funds Requested by Proposer	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)						
b. ____ Senior Associates						
2. No. of Other Personnel (Non-Faculty)						
a. ____ Research Associates/Postdoctorates						
b. ____ Other Professionals						
c. ____ Paraprofessionals						
d. <u> 1 </u> Graduate Students				19,254		
e. <u> 1 </u> Prebaccalaureate Students				8,580		
f. ____ Secretarial-Clerical						
g. ____ Technical, Shop and Other						
Total Salaries and Wages						
B. Fringe Benefits (If charged as Direct Costs)				322		
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				28,156		
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)						
E. Materials and Supplies				13,500		
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.)				2,000		
K. Total Direct Costs (C through J)				45,156		
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 included in on/off campus bases.)				0		
M. Total Direct and F&A/Indirect Costs (K plus L)						
N. Other						
O. Total Amount of This Request				45,156		
P. Carryover -- (If Applicable) Federal Funds: \$			Non-Federal funds: \$		Total \$	
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)						
Cash (both Applicant and Third Party)						
- Non Cash Contributions (both Applicant and Third Party)						
AME 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

ORGANIZATION AND ADDRESS Southern Illinois University - Carbondale 1263 Lincoln Dr, Carbondale, IL 62901			USDA AWARD NO. Year 2: Objective 2			
PROJECT DIRECTOR(S) Karolina Kwasek Bethany Rader			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. ____ (Co)-PD(s)						
b. ____ Senior Associates						
2. No. of Other Personnel (Non-Faculty)						
a. ____ Research Associates/Postdoctorates						
b. ____ Other Professionals						
c. ____ Paraprofessionals						
d. 1 Graduate Students				19,832		
e. 1 Prebaccalaureate Students				8,837		
f. ____ Secretarial-Clerical						
g. ____ Technical, Shop and Other						
Total Salaries and Wages						
B. Fringe Benefits (If charged as Direct Costs)				332		
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				29,001		
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)						
E. Materials and Supplies				12,500		
F. Travel				1500		
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.)				2,000		
K. Total Direct Costs (C through J)				45,001		
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 included in on/off campus bases.)						
M. Total Direct and F&A/Indirect Costs (K plus L)						
N. Other						
O. Total Amount of This Request				45,001		
P. Carryover -- (If Applicable)Federal Funds: \$			Non-Federal funds: \$		Total \$	
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)						
Cash (both Applicant and Third Party)						
- Non Cash Contributions (both Applicant and Third Party)						
AME AND TITLE (Type or print)				SIGNATURE (required for revised budget only)		DATE
Project Director						
Authorized Organizational Representative						
Signature (for optional use)						

Budget Explanation for Southern Illinois University-Carbondale
(Kwasek/Rader)

Objectives: 1, 2
Years 1 & 2:

C. Salary, Wages, Fringe Benefits: Funds for Graduate Research Assistant are budgeted for two years including a portion of the student medical benefit as required by their contract. The graduate student will help with ingredient sourcing, diet production, live food culture and enrichment, execution of the nutritional trials, and biochemical analyses. Funds are requested to support one undergraduate student for years 1-2 of the granting period. The student will participate in all aspects of the microbial portion of the proposed project including protocol design, implementation, analysis, trouble shooting, presentation (at lab meetings and conference meetings) and writing and editing of manuscripts. Y1: \$28,156; Y2: \$29,001.

E. Materials and Supplies: Funds for raw materials, feed ingredients and other components required for raw materials required for live food enrichment and experimental diet production, and general wet laboratory supplies required for trial execution and sampling, are budgeted for each year. Funds are requested for the purchase of chemicals (\$5,000): Items in this category include the purchase of chemicals as needed for the preparation of general laboratory solutions and media for bacterial growth, and other reagents, such as DNA extraction kits and sample preparation kits for high throughput sequencing. Costs are estimated based on current laboratory expenditures; plasticware (\$5,000): Items in this category include the purchase of routine laboratory supplies including pipet tips, centrifuge tubes, falcon tubes, gloves, paper towels, and other disposable items. Costs are estimated based on current laboratory expenditures. All sample preparation and subsequent bioinformatics analysis will be completed at SIU. Y1: \$13,500; Y2: \$12,500.

Items	Year 1	Year 2	Total
Raw materials, feed ingredients, wet laboratory supplies	\$3,500	\$2,500	\$6,000
Purchase of chemicals as needed for the preparation of general laboratory solutions and media for bacterial growth, and other reagents, such as DNA extraction kits and sample preparation kits for high throughput sequencing;	\$5000	\$5000	\$10,000
Items in this category include the purchase of routine laboratory supplies including pipet tips, centrifuge tubes, falcon tubes, gloves, paper towels, and other disposable items.	\$5000	\$5000	\$10,000
Total	\$13,500	\$12,500	\$26,000

F. Travel: Funds for transportation, lodging, and meal expenses are budgeted for farms visits, attending NCRAC Annual Meeting, and the study's workshop. Y1: \$1,500; Y2: \$1,500.

J. Other Direct Costs: Funds are requested to cover 16S bacterial sequencing to be completed at an off-campus facility. Y1: \$2,000; Y2: \$2,000.

April 11, 2019

Dr. Joseph E. Morris, Director
North Central Regional Aquaculture Center
Iowa State University
339 Science II
Ames, Iowa 50011-3221

SUBJECT: Project entitled: "Nutritional Programming of Yellow Perch Larvae using Food as a Vehicle"

Dear Dr. Morris:

As the Authorized Organizational Representative (AOR) I would like to inform you that Southern Illinois University Carbondale wishes to participate in the above referenced project as a subcontractor to Iowa State University.

Drs. Karolina Kwasek will serve as the Principal Investigator and Bethany Rader will serve as the Co-Principal Investigator of the subcontract and they have access to all of the necessary equipment, laboratory, and office space to successfully undertake this project. I also approve the budget as submitted for Drs. Kwasek and Rader.

Upon issuance of approval to the North Central Regional Aquaculture Center for this project, Iowa State University and Southern Illinois University Carbondale will enter into a formal agreement.

Sincerely,



Sonjie Schwartz
Interim Director

ORGANIZATION AND ADDRESS Southern Illinois University - Edwardsville Campus Box 1651, Edwardsville, IL 62026			USDA AWARD NO. Year 1: Objective 2			
PROJECT DIRECTOR(S) Vance McCracken			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. ____ (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						
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						
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 J)					0	
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 included in on/off campus bases.)						
M. Total Direct and F&A/Indirect Costs (K plus L)						
N. Other						
O. Total Amount of This Request					0	
P. Carryover -- (If Applicable)Federal Funds: \$		Non-Federal funds: \$		Total \$		
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)						
Cash (both Applicant and Third Party)						
- Non Cash Contributions (both Applicant and Third Party)						
AME AND TITLE (Type or print)				SIGNATURE (required for revised budget only)		DATE
Project Director						
Authorized Organizational Representative						
Signature (for optional use)						

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 2: Objective 2
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Southern Illinois University - Edwardsville 1263 Lincoln Dr, Carbondale, IL 62901			DURATION PROPOSED MONTHS: 12	DURATION PROPOSED MONTHS: _____	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)
PROJECT DIRECTOR(S) Vance McCracken			Funds Requested by Proposer	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)						
b. ____ Senior Associates						
2. No. of Other Personnel (Non-Faculty)						
a. ____ Research Associates/Postdoctorates						
b. ____ Other Professionals						
c. ____ Paraprofessionals						
d. <u>1</u> Graduate Students				11,412		
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)				11,412		
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)						
E. Materials and Supplies				12,248		
F. Travel						
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.)				4,200		
K. Total Direct Costs (C through J)						
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 included in on/off campus bases.)				0		
M. Total Direct and F&A/Indirect Costs (K plus L)						
N. Other						
O. Total Amount of This Request				27,860		
P. Carryover -- (If Applicable)Federal Funds: \$			Non-Federal funds: \$	Total \$		
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)						
Cash (both Applicant and Third Party)						
- Non Cash Contributions (both Applicant and Third Party)						
AME AND TITLE (Type or print)			SIGNATURE (required for revised budget only)		DATE	
Project Director					8/28/2018	
Authorized Organizational Representative						
Signature (for optional use)						

Budget Explanation for Southern Illinois University – Edwardsville
(McCracken)

Objectives: 2b
Years 1 & 2:

C. Salary, Wages, Fringe Benefits: Funds are requested for a graduate student who will provide most of the labor for laboratory analyses, including histology and gene expression analysis (RT-PCR). This request is for a one-year, 50% GA appointment. Year 2: \$11,412

E. Materials and Supplies: Funds are requested for laboratory supplies, chemicals, reagents, solvents, supplies for consumables (pipette tips, centrifuge tubes, etc) for histological staining and analysis, RNA extraction, cDNA synthesis, and real-time qPCR. Year 2: \$12,248.

Items	Year 1	Year 2	Total
Laboratory supplies, chemicals, reagents, solvents, supplies for consumables (pipette tips, centrifuge tubes, etc) for histological staining and analysis, RNA extraction, cDNA synthesis, and real-time qPCR; The Developmental Biology Histology Core at the Washington University School of Medicine in St. Louis, contracted paraffin embedding, sectioning, and hemotoxylin and eosin staining; Primers synthesized by Integrated DNA Technologies; biohazardous waste removal	\$0	\$12,248	\$12,248
Total	\$0	\$12,248	\$12,248

J. Other Direct Costs: Funds are also budgeted for The Developmental Biology Histology Core at the Washington University School of Medicine in St. Louis, which will be contracted to perform paraffin embedding, sectioning, and hemotoxylin and eosin staining. Prices include 30% outside user fees charged to non-Washington University users. Primers will be synthesized by Integrated DNA Technologies. In addition, funding is requested to remove biohazardous waste per institutional policy. Y2: \$4,200.



Date April 11, 2019

Dr. Joseph E. Morris, Director
North Central Regional Aquaculture Center
Iowa State University
339 Science II
Ames, Iowa 50011-3221

SUBJECT: Project entitled "Nutritional Programming of Yellow Perch Larvae using Live Food as a Vehicle"

Dear Dr. Morris:

As the Authorized Organizational Representative (AOR) I would like to inform you Southern Illinois University Edwardsville (SIUE) wishes to participate in the above referenced project as a subcontractor to Iowa State University.

Dr. Vance McCracken will serve as the Principal Investigator(s) of the subcontract and he/she/they have access to all of the necessary equipment, laboratory, and office space to successfully undertake this project. I also approve the budget as submitted for Dr. McCracken's involvement in this project

Upon issuance of approval to the North Central Regional Aquaculture Center for this project, Iowa State University and SIUE will enter into a formal agreement.

Sincerely,

A handwritten signature in blue ink that reads "Jerry B. Weinberg".

Jerry B. Weinberg, Ph.D.
Associate Provost for Research and Dean of the Graduate School
Southern Illinois University Edwardsville

ORGANIZATION AND ADDRESS				USDA AWARD NO.				Year 1: Objective 3			
Madison County Extension Office, College of Food, Agricultural, and Environmental Sciences, 217 Elm Street, London, Ohio 43140				DURATION PROPOSED MONTHS: 12		DURATION PROPOSED MONTHS: _____		Non-Federal Proposed Cost-Sharing/Matching Funds (If required)		Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)	
PROJECT DIRECTOR(S) Matthew Smith				Funds Requested by Proposer		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)											
b. ____ Senior Associates											
2. No. of Other Personnel (Non-Faculty)											
a. ____ 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											
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											
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 J)						0					
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 included in on/off campus bases.)											
M. Total Direct and F&A/Indirect Costs (K plus L)											
N. Other											
O. Total Amount of This Request						0					
P. Carryover -- (If Applicable) Federal Funds: \$				Non-Federal funds: \$				Total \$			
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)											
Cash (both Applicant and Third Party)											
- Non Cash Contributions (both Applicant and Third Party)											
AME AND TITLE (Type or print)						SIGNATURE (required for revised budget only)				DATE	
Project Director						Karolina Kwasek				8/28/2018	
Authorized Organizational Representative											
Signature (for optional use)											

ORGANIZATION AND ADDRESS				USDA AWARD NO.				Year 2: Objective 3			
Madison County Extension Office, College of Food, Agricultural, and Environmental Sciences, 217 Elm Street, London, Ohio 43140				DURATION PROPOSED MONTHS: 12		DURATION PROPOSED MONTHS: _____		Non-Federal Proposed Cost-Sharing/Matching Funds (If required)		Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)	
PROJECT DIRECTOR(S) Matthew Smith				Funds Requested by Proposer		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)											
b. _____Senior Associates											
2. No. of Other Personnel (Non-Faculty)											
a. _____Research Associates/Postdoctorates											
b. 1 Other Professionals						2,521					
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)						797					
C. Total Salaries, Wages, and Fringe Benefits (A plus B)						3,318					
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)											
E. Materials and Supplies						950					
F. Travel						600					
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.)						900					
K. Total Direct Costs (C through J)						5,768					
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 included in on/off campus bases.)											
M. Total Direct and F&A/Indirect Costs (K plus L)											
N. Other											
O. Total Amount of This Request						5,768					
P. Carryover -- (If Applicable)Federal Funds: \$				Non-Federal funds: \$				Total \$			
Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)											
Cash (both Applicant and Third Party)											
- Non Cash Contributions (both Applicant and Third Party)											
AME AND TITLE (Type or print)						SIGNATURE (required for revised budget only)				DATE	
Project Director						Karolina Kwasek				8/28/2018	
Authorized Organizational Representative											
Signature (for optional use)											

**Budget Explanation for OSU – Madison County Extension Office
(Smith)**

**Objectives: 3
Years 1 & 2:**

C. Salaries, Wages, Fringe Benefits: Smith will coordinate with OSU’s IT for workshop recording and YouTube-type videoing. Salary for OSU IT (Program Assistant, OSU Madison County Extension Office) for 1.0 month (8.5% effort; \$2,521) and Fringe (31.60%; \$797) for assistance (developing YouTube video, recording/editing workshop, handling registration for the workshop, getting lunch/snacks for workshop) is requested. Year 2: \$3,318.

E. Materials and Supplies: Funds are requested to procure non-feed trained and feed-trained fish for workshop demonstration (\$250). Funds are also budgeted for purchase of live foods and necessary live food materials for workshops demonstration (\$500). Funds for flyers/pamphlets/results printed for dissemination of results and workshop materials (\$200) are also requested. Year 2: \$950.

Items	Year 1	Year 2	Total
Non-feed trained and feed-trained fish for workshop demonstration	\$	\$250	\$250
Live foods and necessary live food materials for workshops demonstration	\$	\$500	\$500
Flyers/pamphlets/results printed for dissemination of results and	\$	\$200	\$200
Total	\$	\$950	\$950

F. Travel: Travel costs are requested for two OSU people [Smith and IT] to drive to the Ohio farm for recording/presenting and Smith to partially travel to another Midwest state association meeting. Year 2: \$600.

J. Other Direct Costs: Funds for workshop meals for workshop attendees, invited speaker funds (travel), and/or fees for hosting workshop are requested. Year 2: \$900



THE OHIO STATE UNIVERSITY

Office of Research
Office of Sponsored Programs
Research Administration Building
1960 Kenny Road
Columbus, OH 43210
614-292-3815 Phone
614-292-5913 Fax
osp.osu.edu

April 16, 2019

Dr. Karolina Kwasek
Southern Illinois University – Carbondale
1125 Lincoln Dr, Life Science II, RM 251
Carbondale, IL 62901

SUBJECT: Project entitled “Nutritional programming of yellow perch larvae using live food as a vehicle”

Dear Dr. Kwasek:

As the Authorized Organizational Representative (AOR) I would like to inform you The Ohio State University wishes to participate in the above referenced project as a subcontractor to Southern Illinois University-Carbondale.

Mr. Matthew Smith will serve as the Principal Investigator of the subcontract and he will have access to all of the necessary equipment, laboratory, and office space to successfully undertake this project. I also approve the budget as submitted for Mr. Smith’s involvement in this project.

Upon issuance of approval for this project, Southern Illinois University-Carbondale and The Ohio State University will enter into a formal agreement.

Sincerely,

Katie Groeniger
Sr. Sponsored Program Officer
The Ohio State University
1960 Kenny Road
Columbus, Ohio 43210-1016
Groeniger.10@osu.edu

Budget Summary

Year 1

	NCRAC Funds				
	Objective #	Southern Illinois University-Carbondale	Southern Illinois University-Edwardsville	OSU Madison County Extension Office	Project Total
Salaries, Wages, and Fringe Benefits	1, 2	\$28,156.00	\$0.00	\$0.00	\$28,156.00
Nonexpendable Equipment		\$0.00	\$0.00	\$0.00	\$0.00
Materials and Supplies		\$15,500.00	\$0.00	\$0.00	\$15,500.00
Travel		\$1,500.00	\$0.00	\$0.00	\$1,500.00
All Other Direct Costs		\$0.00	\$0.00	\$0.00	\$0.00
Total					\$45,156.00

Year 2

	NCRAC Funds				
	Objective #	Southern Illinois University-Carbondale	Southern Illinois University-Edwardsville	OSU Madison County Extension Office	Project Total
Salaries, Wages, and Fringe Benefits	2	\$29,001.00	\$11,412.00	\$0.00	\$40,413.00
Nonexpendable Equipment		\$0.00	\$0.00	\$0.00	\$0.00
Materials and Supplies		\$14,500.00	\$12,248.00	\$0.00	\$26,748.00
Travel		\$1,500.00	\$0.00	\$0.00	\$1,500.00
All Other Direct Costs		\$0.00	\$4,200.00	\$0.00	\$4,200.00
Salaries, Wages, and Fringe Benefits	3	\$0.00	\$0.00	\$3,318.00	\$3,318.00
Nonexpendable Equipment		\$0.00	\$0.00	\$0.00	\$0.00
Materials and Supplies		\$0.00	\$0.00	\$950.00	\$950.00
Travel		\$0.00	\$0.00	\$600.00	\$600.00
All Other Direct Costs		\$0.00	\$0.00	\$900.00	\$900.00
Total					\$78,629.00

Schedule for Completion of Objectives

Start date: September 2019

Completion date: August 2021

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 determine if Nutritional Programming of yellow perch larvae via live food improves dietary plant protein utilization in yellow perch during later life stages.														
Dietary ingredient sourcing														
Development of enrichment formula														
Experimental diet production														
Feeding trial														
Objective 2: To determine the mechanism underlying the Nutritional Programming via live food responsible for improved dietary plant protein utilization:														
Sub-Objective 2a: To assess if Nutritional Programming changes gut microbial communities responsible for improved digestion of dietary plant protein.														
Gut microbiome diversity and composition assessment														
Gut microbiome disruption study using antibiotics														
Microbiome sequencing and analysis of samples														
Sub-Objective 2b: To determine if Nutritional Programming mitigates any inflammatory or morphological changes in the gut responsible for improved digestion of dietary plant protein.														
Intestine sampling														
Histological assessment														
Inflammation markers assessment														
Objective 3: To communicate the Nutritional Programming concept via live food, Nutritional Programming feeding strategy protocol, and live food enrichment formulation that could be used by fish farmers and feed manufacturing industry, to improve plant protein-based diets utilization during yellow perch grow-out phase.														
Workshop														
Deliverables														
Communication of results with industry representatives and scientific community														
Report preparation and submission														
Manuscript preparation														

Participating Institutions and Principal Investigators

Southern Illinois University-Carbondale
Dr. Karolina Kwasek
Dr. Bethany Rader

Southern Illinois University-Edwardsville
Dr. Vance McCracken

The Ohio State University- Madison County Extension Office
Mr. Matthew Smith

VITA

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Carbondale, IL 62901

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E-mail: karolina.kwasek@siu.edu

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

SELECTED PUBLICATIONS

- Kwasek, K., S. Rimoldi, A. G. Cattaneo, T. Parker, K. Dabrowski, and G. Terova. 2017. The expression of hypoxia-inducible factor-1 α 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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Carbondale, IL 62901

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E-mail: Bethany.rader@siu.edu

EDUCATION

Ph.D. (University of Oregon, 2006, Biology)
B.S. (University of Wisconsin-Madison, 1999, Botany)

POSITIONS

2014 - present Assistant Professor
 Department of Microbiology, Southern Illinois University-Carbondale
2013 - 2014 Adjunct Professor
 Department of Biology, University of Oregon
2012 - 2013 Postdoctoral Fellow
 Department of Microbiology and Plant Biology, University of Oklahoma
2010 - 2012 Postdoctoral Fellow
 Department of Molecular and Cell Biology
2007 - 2010 Postdoctoral Fellow
 Department of Medical Microbiology and Immunology, University of Wisconsin, Madison

SELECTED PUBLICATIONS

Rader, B.A. 2017. Alkaline phosphatase, an unconventional immune protein. *Frontiers in Immunology* 8:897.
Kremer, N., E. Philipp, M. Carpentier, C.A. Brennan, L. Kraemer, M.A. Altura, R. Augustin, R. Haesler, E.A.C. Heath-Heckman, S.M. Peyer, J. Schwartzman, B.A. Rader, E.G. Ruby, P. Rosenstiel, and M.J. McFall-Ngai. 2013. A small number of symbionts orchestrate organ-wide transcriptional changes that prime tissue colonization. *Cell Host and Microbe* 14(2):183-94.
Rader, B.A., and S.V. Nyholm. 2012. Host/Microbe interactions revealed through "Omics" in the symbiosis between the Hawaiian Bobtail Squid, *Euprymna scolopes*, and the Bioluminescent Bacterium, *Vibrio fischeri*. *Biological Bulletin* 223(1):103-111.
Collins, A.J., T.R. Schleicher, B.A. Rader, and S.V. Nyholm. 2012. Understanding the role of host hemocytes in a squid/*Vibrio* symbiosis using transcriptomics and proteomics. *Frontiers in Immunology*. 3:91
Rader, B.A., N. Kremer, M.A. Apicella, W. Goldman, and M. McFall-Ngai. 2012. Modulation of symbiont lipid A signaling by host alkaline phosphatase in the squid-*Vibrio* symbiosis. *mBio*. 3(3) doi:10.1128/mBio.00093-12
Rader, B.A., C. Wreden, K.G. Hicks, E.G. Sweeney, K.M. Ottemann, and K. Guillemin. 2011. *Helicobacter pylori* perceives the quorum sensing molecule AI-2 as a chemorepellent via the chemoreceptor TlpB. *Microbiology* 157: 2445-2455.
Rader, B.A., S.R. Campagna, M.F. Semmelhack, B.L. Bassler, and K. Guillemin. 2007. The quorum sensing molecule AI-2 regulates motility and flagellar morphogenesis in *Helicobacter pylori*. *Journal of Bacteriology*: 189:6109-6117.
Mouery, K., B. A. Rader, E. C. Gaynor, and K. Guillemin. 2006. The *Helicobacter pylori* stringent response is required for survival of stationary phase, acid, and aerobic shock. *Journal of Bacteriology* 188:5494 – 5500.

VITA

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44 Circle Drive, SLW 1190,
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Edwardsville, IL 62026

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Fax: (618) 650-3174
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EDUCATION

Ph.D. University of Illinois at Urbana-Champaign, 2001, Animal Sciences/Immunology
M.A. The University of Nevada, Reno, 1991, Spanish Literature
B.A. The University of Tennessee at Martin, 1988, Spanish
B.S. Illinois State University, 1994, Biological Sciences

POSITIONS

2012 - present Associate Professor, Department of Biological Sciences, Southern Illinois University
Edwardsville, Edwardsville IL
2006 - 2012 Assistant Professor, Department of Biological Sciences, Southern Illinois University
Edwardsville, Edwardsville IL
2002 - 2006 Research Associate, Department of Pathology, University of Alabama, Birmingham, AL
2000 - 2002 Postdoctoral Research Fellow, Department of Pathology and Immunology, Washington
University School of Medicine, St. Louis, MO
1994 - 2000 Research Assistant, Department of Animal Sciences, University of Illinois at
Urbana-Champaign
1998 - 1991 Teaching Assistant, Department of Foreign Languages, University of Nevada, Reno

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association for the Advancement of Science
American Society for Microbiology
American Association of Immunologists
Society for Mucosal Biology

SELECTED PUBLICATIONS

Mora, F.D., L. Alpan, N. de Tomassai, V.J. McCracken, and M. Nieto. 2013. New antibacterial germacrene from *Verbesina negrensis*. *Planta Medica* 79:707-710.
Schmitz J.M., C.G. Durham, T.R. Schoeb, T.D. Soltau, K.J. Wolf, S.M. Tanner, V.J. McCracken, and R.G. Lorenz. 2011. *Helicobacter felis*-associated gastric disease in microbiota-restricted mice. *The Journal of Histochemistry and Cytochemistry* 59:826-841.
Schmitz J.M., V.J. McCracken, R.A. Dimmitt, and R.G. Lorenz. 2007. Expression of CXCL15 (lungkine) in murine gastrointestinal, urogenital, and endocrine organs. *The Journal of Histochemistry and Cytochemistry* 55:515-524.
McCracken V.J., S.M. Martin, and R.G. Lorenz. 2005. The *Helicobacter felis* model of adoptive transfer gastritis. *Immunological Research* 343:183-194.
Elson C.O., Y. Cong, V.J. McCracken, R.A. Dimmitt, R.G. Lorenz, and C.T. Weaver. 2005. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. *Immunological Reviews* 206:260-276.
McCracken V.J., T. Chun, M.E. Baldeón, S. Ahrné, G. Molin, R.I. Mackie, and H.R. Gaskins. 2002. TNF- sensitizes HT-29 colonic epithelial cells to intestinal Lactobacilli. *Experimental Biology and Medicine* 227:665-670.
McCracken V.J., J. M. Simpson, R.I. Mackie, and H.R. Gaskins. 2001. Molecular ecological analysis of dietary and antibiotic-induced alterations of the mouse intestinal microbiota. *Journal of Nutrition* 131:1862-1870.
Deplancke B.R., K.R. Hristova, H.A. Oakley, V.J. McCracken, R. Aminov, R.I. Mackie, and H.R. Gaskins. 2000. Molecular ecological analysis the succession and diversity of sulfate-reducing bacteria in the mouse gastrointestinal tract. *Applied and Environmental Microbiology* 66:2166-2174.

