ESTABLISHING LARGEMOUTH BASS STRAINS FOR RAPID GROWTH TO 1.5 POUNDS IN THE NORTH CENTRAL REGION

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Funding Request: $155,000

Duration: 2 years (September 1, 2014 – August 31, 2016)

Objectives:

1. Identify the best genetically distinct largemouth bass populations for fast growth in the NCR.
2. Conduct a meta-analysis using all appropriate data for largemouth bass from both published and non-published sources to identify at minimum three populations of LMB with the potential to exhibit rapid growth to target weight in the NCR.
3. Evaluate the identified populations at two or more latitudes in the NCR to identify the optimal source population

Deliverables:

1. Publication of results in journal articles(s).
2. Extension products, including a selection mix.

Proposed Budgets:

Institution	Principle Investigators	Objectives	Year 1	Year 2	Total
Southern Illinois University	Brian Small Paul Hitchens	1-3	$37,664	$41,505	$79,169
The Ohio State University	Han-Ping Wang David Glover	1-3	$41,031	$34,800	$75,831
		Total	**$78,695**	**$76,305**	**$155,000**

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Project Summary:

Largemouth bass (LMB) is an important aquaculture species in the Midwest and throughout the U.S. Interest in improving commercial culture efficiency has grown due to the great demand and high value compared to other cultured species. While this fish has been extensively investigated for management of the fisheries and hatchery production, little research has been conducted to maximize growth for commercial foodfish production. A NCRAC Priority is to increase the efficiency of LMB growth to market size through means beyond dietary modification. One impediment beyond nutritional insufficiency is the rearing of LMB stocks with little to no domestication or selective breeding for efficient production. Therefore, strain evaluation and identification of the best largemouth bass populations for fast growth would result in an immediate impact on the economic return of many small aquaculture operations in the North Central Region (NCR). At the completion of this project, anticipated outcomes include a description of LMB genetic diversity among commercial and public stocks available to NCR, the development of a selection matrix for fast growing LMB, and the identification of fast growing populations verified in production systems. This will benefit fish farmers by increasing the efficiency and profitability of largemouth bass aquaculture production.

Justification:

Largemouth bass (LMB) have become an economically important and high-value species, both from the perspective of their use in aquaculture, as well as their recreational value. In some Midwest states like Ohio and Michigan, largemouth bass have been listed as one of the top three culture species of fish because of their desirable characteristics for production, and their high demand and value in the marketplace. For these reasons, interest in improving commercial culture efficiency of LMB has grown significantly in North Central Region (NCR). As such, a NCRAC Priority is to increase the efficiency of LMB growth to 1–1.5 pounds through means beyond dietary modification. At the completion of this project, we expect to identify the best LMB populations with the potential to exhibit rapid growth to target weight for the NCR aquaculture industry.

The proposed research will specifically address the needs identified by NCRAC to improve LMB production beyond dietary modification. Largemouth bass have been propagated with little or no genetic control since the 1970's. There are two recognized subspecies of largemouth bass: the Northern largemouth bass *Micropterus salmoides salmoides*, and the southern Florida largemouth bass, *Micropterus salmoides floridanus*, which can only be distinguished through genetic testing. While there may be differences in growth between the two subspecies, the Florida LMB have been shown to have poor winter survival in the NCR, where ice cover occurs and pure non-selected Florida largemouth bass usually winterkill in shallow ponds (Heidinger, 2000). Therefore, there is a technical need for improvement and development of broodstocks with traits amenable to commercial-scale aquaculture in the NCR through strain evaluation, and genetic breeding and selection. Genetic and growth evaluation of different populations of Northern LMB is the first step to developing a genetic improvement program for the NCR. By the completion of the proposed research, we expect to identify the best largemouth bass populations for fast growth and the optimal source population for aquaculture industry in the NCR. This will benefit fish farmers by increasing the efficiency and profitability of largemouth bass aquaculture production in the U.S.

Related Current and Previous Work:

Modern genetic technologies are extensively applied by the diverse aquaculture industries, though not to the same extent for all important aquacultured species (Hulata, 2001). Concentrated breeding efforts have led to significant gains for some species (i.e. common carp, Atlantic salmon, rainbow trout, channel catfish, Nile tilapia), while other major cultured species, such as LBM have received little or no attention and have not been genetically improved at all. Where selection has been applied to LMB, it has focused on improvements in final fish weight and not on rapid growth to market weight. A good example of this is the ShareLunker program in Texas, where the goal is to develop a trophy LMB fisheries. Although a breeding program for trophy size fish may be contrary to the goal of rapid growth to market size for foodfish producers, it does provide us with insight into the possibility for genetic improvement of the species and demonstrates the successful use of genetic fingerprinting techniques in LMB selective breeding (http://www.tpwd.state.tx.us/spdest/visitorcenters/tffc/sharelunker/accomplishments/).

Most of the genetically improved strains of foodfish species were developed for the aquaculture industry through traditional selective breeding (selection, crossbreeding, and hybridization; Hulata, 2001). Even though LMB are an important aquaculture species in the NCR, genetic strains of largemouth bass have not been formally developed (Heidinger, 2000). This is not to say that some degree of selection and domestication has not occurred through an informal selection process since some hatcheries have maintained brood fish for many generations (Heidinger, 2000). For this reason, LMB producers in NCR will be surveyed for their management methods and growth data to be included in the present study.

Genetic fingerprinting of LMB plays an important part in selective breeding. Lutz-Carrillo et al. (2006) examined microsatellite DNA variation at 11 loci in populations of Florida largemouth bass *M. salmoides floridanus* and northern largemouth bass *M. s. salmoides* from northern and southern latitudes in North America and found despite the systematic introduction of Florida largemouth bass into Texas, genetic integrity of a number of populations had not been or only minimally been affected. However, they suggested that a full assessment of the variation throughout the range of each subspecies should be conducted to identify minimally impacted environments and quantify the levels of gene flow from impacted to intact water bodies. Lutz-Carrillo et al. (2008) subsequently developed 52 novel microsatellite markers from *M. salmoides floridanus* for use in population genetics studies and expected them to be helpful for the resolution of hatchery-released individuals in the wild, introgression among *Micropterus* species, and gene flow following stocking and restoration efforts. For the past three years, the Ohio Center for Aquaculture Research and Development (OCARD) has collected a total of 1045 LMB finclip samples from 8 states and 25 populations (n) throughout the US (FL [n=2], TX [n=3], MS [n=6], SC [n=3], AR [n=1], OH [n=7], WI [n=1], and MN [n=2]) including both wild (n=15) and hatchery populations (n=10) (Table 1). Finclips were collected from freshly caught fish and immediately preserved in 95% ethanol. In the present study, additional samples will be collected from populations identified for rapid growth following a meta-analysis of population growth data. Furthermore, eight stocks representing major populations were previously obtained from five states (FL, TX, AR OH, and WI). Eight microsatellite markers have been optimized and selected as a best marker panel for identification of genetically distinct LMB populations for fast growth. These preliminary data and selected markers will be combined with new data and used to identify the best LMB populations for fast growth in Objective 2 of this study.

Table 1 LMB finclip samples from 8 states and 25 populations throughout the US

Populations	State/Country	Abbreviation
Wild populations		
Lake Snowden	OH	LSOH
Nettle Lake	OH	NLOH
Acton Lake	OH	ACLOH
North Reservoir	OH	NROH
Alum Creek lake	OH	ACLOH
Mary Street	OH	MSOH
Pike Lake	WI	PLWI
Spirit Lake	MN	SLMN
Hill Lake	MN	HLMN
Q8TX	TX	Q8TX
Devils Rivers	TX	DRTX
Pascagoula River	MS	PRMS
Columbus Lake	MS	CLMS
Belzoni Old River	MS	BRMS
Ross Barnett Reservoir	MS	RBRMS
Broad River Reach 2	SC	BR2SC
Broad River Reach 3	SC	BR3SC
Copper River	SC	CRSC
Q9 Kissimme	FL	KMFL
St. Johns	FL	SJFL
Hatchery populations		
Guang Zhou	CHINA	GZCH
Lake Kickapoo	TX	LKTX
Turcotle Fish Hatchery	MS	THMS
North MC Fish Hatchery	MS	NHMS
Piketon	OH	PKOH

In addition to growth data generated from producer surveys, data sets of growth and natural mortality developed for fisheries managers exist for hundreds of LMB populations across the United States (e.g. Beamesderfer and North, 1995); other populations exist that do not reach large maximum sizes, yet grow rapidly to target size in their natural environment (e.g., Norris et al., 2010). Given that largemouth bass have a tendency to become locally adapted (Cooke et al., 2001) and exhibit a latitudinal pattern in growth (Garvey et al., 2003), it is likely that growth performance is driven to some degree by genetics as well as environmental conditions. There exists, therefore, a critical need to utilize these data sets developed for fisheries managers to identify LMB populations with ideal growth for commercial culture in the NCR. A meta-analysis using all appropriate data for largemouth bass from both published and non-published sources (e.g. producer and agency surveys) will be conducted in the present study to identify at minimum three populations of LMB with the potential to exhibit rapid growth to target weight in the NCR, which will then be evaluated for actual growth in production trials in Objective 3.

USDA Current Research Information System (CRIS or REEport) was accessed to review any related or relevant research and that the proposed work is original research and does not duplicate any previously funded projects in CRIS. The NOAA databases of previously funded projects relevant to the proposed research, including: 1) National Sea Grant Office Funding Page; 2) website of state Sea Grant Program; and 3) NOAA Office of Aquaculture Funding Opportunities Page were also reviewed to ensure the proposed work is original research and does not duplicate any previously funded projects. Search terms used included: largemouth bass, genetic, selection, and breeding.

Anticipated Benefits:

The great demand for largemouth bass and their high selling price and growth rate (compared to other cultured species) have raised interest in their commercial culture. Differential performance of genetic strains of largemouth bass is an important management consideration for both recreational fisheries and aquaculture. Therefore, strain evaluation and identification of the best genetically distinct largemouth bass populations for fast growth and the optimal source population would result in an immediate impact on the economic return of many small aquaculture operations in the North Central region. A NCRAC Priority is to increase the efficiency of LMB growth to 1–1.5 pounds through means beyond dietary modification. At the completion of this project, we expect to identify the best populations of LMB with the potential to exhibit rapid growth to target weight for the NCR aquaculture industry based on a meta-analysis of available published and unpublished data and demonstrated growth during the first year of production (due to the limitation of only 2-years of available funding).

Furthermore, a description of diversity among commercial and public stocks available to NCR producers will be available. At present there is little consensus regarding the stocks currently produced, with opinions that all the LMB produced are of similar genetic background, coming primarily from a single fingerling producer, to the opinion that many producers have already selected for fast growth on their own. If stock improvement is to be made, a thorough investigation of the genetics and the management must first be assessed. Objectives 1 and 2 will address these issues. Specifically, Objective 2 will take the growth information obtained from the literature, surveys, and public databases to predict stocks of fast growing fish for the NCR. As a result, a selection matrix will be made available to commercial producers. The third objective will validate the results of the meta-analysis used for selecting fast growing stocks by conducting production studies during the first year of growth. Although this project does not provide funding beyond year 2, it is anticipated that the PIs will be able to find funding to continue the production studies to market weight and ultimately present conclusive evidence to the NCR producer. Regardless, workshop materials will be developed to discriminate the results and train producers how to use the genetic data and selection matrix for their own breeding programs. Results will also be published as factsheets and research papers. A listing of anticipated knowledge, actions and conditions are provided in the Logic Model.

Objectives:

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Deliverables:

1. Publication of results in journal article(s).
2. Extension products, including a selection mix.

Procedures:

Southern Illinois University (SIU) Center for Fisheries, Aquaculture & Aquatic Sciences (CFAAS) has been involved in aquaculture research for over sixty years with extensive experience in LMB research and production. Researchers at SIU will oversee the collection of growth and genetic data for the meta-analysis and identification of superior growing populations to be evaluated in Objective 3. The Ohio State University (OSU) Center for Aquaculture Research and Development (OCARD) at Piketon has been conducting stock evaluation of LMB for three years. Eight stocks representing major populations were previously obtained from five states (FL, TX, AK, OH, and WI). Eight microsatellite markers have been optimized and selected as a best marker panel for identification of the best genetically distinct LMB populations for fast growth. These preliminary data and selected markers will be used for this project. Objective 3 will be carried out both SIU and OSU.

We propose to use three ways to identify high-performing LMB stocks for the NCR aquaculture industry: 1) We will conduct genetic analyses of ~30 populations across the US to identify the distinct LMB stocks in Year 1; 2) Simultaneously, we will use both published and non-published growth data (acquired by surveying private and public LMB producers) among LMB populations to identify at minimum three populations of LMB with the potential to exhibit rapid growth to target weight in the NCR; and 3) We will communally raise the identified strains or populations at two sites to identify the optimal source population in Year 2.

Objective 1 - Identify the best genetically distinct largemouth bass populations for fast growth in the NCR.

Population sample collection:

For the past three years, OCARD has collected a total of 1045 LMB finclip samples from 8 states and 25 populations throughout the US (FL [n=2], TX [n=3], MS [n=6], SC [n=3], AK [n=1], OH [n=7], WI [n=1], and MN [n=2]) including both wild (n=15) and hatchery populations (n=10) (Table 1). Additional finclips will be collected from freshly caught fish at commercial producers in the NRC and from populations identified for rapid growth in Objective 2. Finclips will be immediately preserved in 95% ethanol and shipped to OSU for microsatellite analysis. All samples will be used to identify LMB populations distinct for fast growth in Objective 2.

Extract DNA and microsatellite analysis:

Total DNA will be extracted from an optimum volume of fin tissue following a modified version of the Pure gene protocol for extraction from fish tissue (Lutz-Carrillo et al, 2006). Amplification of microsatellite loci will be performed using eight primers optimized by OSU Aquaculture Genetics and Breeding Laboratory (AGBL) at Piketon from those isolated and characterized by Lutz-Carrillo et al (2006, 2008). Each locus was amplified with a three primer system in which only the M13 and CAG primers were fluorescently labeled with FAM, HEX, or NED. Polymerase chain reaction of 6µL will contain 3µL of JumpStart RedMix (Sigma), 1.5 pmol of both nontailed and labelled primers, and 0.1 pmol of the tailed primer, 25 ng DNA, in the presence of 100 µm spermidine. Amplification will be conducted in PTC-100 thermal cyclers (MJ Research) using an initial denaturation at 94ºC for 2 min, followed by 35 cycles of 30 s denaturation at 94ºC, 30 s annealing at a locus-specific temperature, 30 s extension at 72ºC, and a final 5-min extension at 72ºC. Amplification products will be separated using an ABI 3130 Prism DNA genetic analyzer.

Statistical analyses:

Genetic variation will be evaluated according to observed heterozygosity and the number of alleles per locus within populations and regions. Private alleles among populations will be computed using GenAlEx 6 (Peakall and Smouse, 2005). Genetic structure among populations will be analyzed based on a chord distance according to Cavalli-Sforza and Edwards (1967). Genetic structure among individuals will be assessed using the model based Bayesian clustering method according to Pritchard et al. (2000) and Lutz-Carrillo et al. (2006). Analyses of molecular variance (AMOVAS) will be conducted with Arlequin. AMOVAS will examine population structure after combining populations in various ways to test for geographical structure. Allelic richness was calculated with fstat version 2.9.3.2 (Goud et al. 2001). All these tests will be adjusted for multiple simultaneous comparisons using a sequential Bonferroni correction. Animalfarm (Landry et al. 2002) will be used to test for equal contribution of all loci to estimators of genetic distance based on the stepwise mutation model (SMM). We will use bottleneck version 1.2.02 (Cornuet et al. 1999) to test for evidence consistent with recent population bottlenecks or expansions by recognizing significant heterozygosity excess or deficiency for each population using a Wilcoxon sign–rank test. We will conduct the tests using 1000 iterations using the SMM of microsatellite evolution, as well as a two-phased mutation model (TPM). Allele frequency data will be tested for evidence of a ‘heterozygosity excess’ (HE) (Cornuet & Luikart 1996; Luikart & Cornuet 1998) using the program bottleneck (Piryet al.1999). Three statistical tests (sign test, standardized differences test, and Wilcoxen signed-ranks test) will be conducted in order to determine whether there is significant HE, which may indicate that a recent bottleneck has occurred.

Objective 2 - Conduct a meta-analysis using all appropriate data for largemouth bass from both published and non-published sources to identify at minimum three populations of LMB with the potential to exhibit rapid growth to target weight in the NCR.

Population data collection:

Largemouth bass weight-at-age data will be collected from as many LMB populations as possible (no less than 30) throughout the NCR from producers, state and federal agencies, and universities. Attempts will be made to obtain raw weight-at-age data for further analyses from published sources, grey literature, and unpublished sources. To facilitate this process, we have been in contact with state extension agents and permitting departments to compile lists of LMB producers in each NCR state. Furthermore, we have asked that those producers known to produce their own fingerlings be identified. This information will be used by the graduate students on the project to perform a survey of management activities (e.g. selection methods), source of fish, and growth data.

Statistical analyses:

Demographic data of wild LMB populations (e.g., length, weight, age) are typically collected once a year in the fall. The reasoning behind this approach is that the most recent annuli are more clearly defined on the structures used for aging (typically otoliths, but scales are sometimes used in northern regions). Individuals collected during the spring, for instance, may have not laid down their most recent annuli thereby increasing the uncertainty in the age of that individual. As such, priority will be given to demographic data collected during the fall period. The consequence of having demographic data on an annual time step, however, is that the exact time in which individual largemouth bass reach a size that would be considered “market size” is not known for all individuals. It is therefore necessary to use relationships between weight and age to estimate the average time required to reach this market size. The most typical relationships used for wild populations to estimate growth over time are the von Bertalannfy and Gompertz growth functions, which are used for estimating growth in length and weight, respectively, as a function of age. In this study, we are more concerned with growth in weight as opposed to length; thus, the Gompertz growth function is more appropriate.

Estimates of time required to reach market size for the average individual within a population could be used to assess population-specific growth performance, yet is unadvisable because of the inherent individual to individual variability in weight-at-age. Ideally, a candidate population for aquaculture purposes would reach market size within a short amount of time relative to other populations, and also have a low amount of individual variability in growth performance (i.e., the average growth to market size is not influenced by outlier individuals that have a growth advantage relative to other individuals due to earlier hatch dates, or other ecological phenomena). As such, a Bayesian approach will be employed to simultaneously determine both the average time to reach the target weight for each unique population, as well as the individual variability. In cases where multiple sources of collection occurred (e.g., from different agencies), a hierarchical Bayesian approach will be used to account for potential biases resulting from different collection methods. An uninformative prior distribution will be used in all cases such that the posterior distribution of parameter estimates (i.e., the average time required and the individual variability) is driven only by their likelihood. Statistical analyses will be performed using WinBUGS 1.4.3.
The disadvantage of using a growth model to identify candidate populations for growth trials is two-fold. First, the observed growth performance among populations is related not only to genetic influences, but also environmental conditions such that growth performance would likely change in a different growth environment, such as hatchery ponds. Second, the coarse time periods in which size-at-age is assessed requires an estimation of the time required to reach a marketable size. Both growth models assume a constant growth rate over time that slows as the fish approach their maximum size. Given our knowledge of seasonal growth, there will be uncertainty in the accuracy of the estimate of time to market size. Unfortunately, without a great deal of additional population-specific information to inform a bioenergetics model of growth in mass (including average daily temperatures, whole-body largemouth bass energetic density, information concerning largemouth bass prey including the proportions in which they are consumed over seasons and age of the largemouth bass as well as the energetic content of these prey), the Gompertz growth function is the best available option for estimating the time required to reach size-at-age. In addition, the accuracy of bioenergetics models for largemouth bass has been shown to be influenced by the source population, likely due to genetic differences in these populations. Given that parameter estimates for the largemouth bass bioenergetics model were derived from a limited number of populations, a bioenergetics approach to identifying candidate populations would require a great amount of additional population-specific parameter estimates temperature and body-size dependent function of maximum consumption and metabolic rates, as well as proportional energetic losses due to specific dynamic action (i.e., digestion), egestion, and excretion. Such an undertaking would be unreasonable to accomplish the objectives of this study within a timely fashion. That being said, information used in the meta-analysis that are obtained from the same time of year will at the very least provide a measure of the relative differences in the time required to reach market size (i.e., similar bias will be introduced into each population). Unfortunately there is no method to identify a potential source population with ideal growth performance that has no associated risks. This evidence-based approach, however, should be preferred over a trial-and-error approach.

Domestic strains of largemouth bass may be easier to estimate the time required to reach a marketable size, but will be dependent on the quality of record keeping. Based on the temporal nature of the data, growth models may or may not be needed to obtain estimates of time to reach market size. Yet, if the ideal market size was reached between record keeping events, some sort of interpolation estimate will be necessary to provide an estimate of the time required to reach market size, albeit with some degree of uncertainty. Evidence-based identification of candidate strains of largemouth bass for growth trials will be favored over anecdotal information.

The top populations in terms of the time required to grow to the ideal weight with the smallest amount of variation will be ranked for further genetic testing as described in Objective 1; preference will be given to domestic stocks given that wild populations may not readily switch to pelleted feed. Based on the results of Objective 1, this will be narrowed down to no less than three populations to be used for production studies (Objective 3).

Objective 3 - Evaluate the identified populations at two or more latitudes in the NCR to identify the optimal source population

Brood fish collection and selection:

At the end of Year 1, both SIU and OSU will collect broodfish from three to five identified fast-growing populations of LMB based on results from Objective 1 and 2, and will be transported to Piketon aquaculture facility. Eighty broodfish (sex ratio: ~1:1) will be collected from each of the populations and PIT tagged and genotyped at the OSU AGBL using microsatellite markers. Molecular genetic pedigrees will be determined and a genetic relatedness chart will be constructed for mating. Among the 80 fish, at least 30 pairs of the least-related fish will be selected for fry production.

Broodstock genotyping:

All the brood fish candidates will be genotyped using eight microsatellite loci that have been optimized. Microsatellite amplification reactions for each experimental individual will be performed using approximately 100 ng genomic DNA derived from ethanol-preserved tissue. PCR will be performed using BioRad PTC-200 DNA engine thermal cycler to cycle according to Li et al. (2007). Genotyping will be performed using ABI 3130 DNA Sequencing and Genotyping System, and genotypes automatically scored using Genemapper. Individual genotypes will be checked for accuracy and consistency.

Genetic pedigree and relatedness chart construction:

Parentage assignment will be performed using the exclusion-based approach implemented in the program CERVUS 2.0 (Marshall et al. 1998). Also the likelihood-based method described in CERVUS 2.0 will be applied to obtain a probability of the most confident parent as an additional support to the exclusion-based strategy. All brood stock individuals will be included as putative candidates. To ascertain the possible influence of kinship among breeders in the deviation of theoretical and actual exclusion power of microsatellites (Marshall et al. 1998), relatedness coefficients (r) between all pairs of breeders from experimental brood stock will be obtained using the statistical package RELATEDNESS (Queller and Goodnight, 1989). Based on the parentage assignment and relatedness coefficients, a microsatellite pedigree and genetic relatedness chart will be constructed for mating.

Fry and fingerling production:

Fry of identified genetically distinct and fast-growing populations of LMB will be produced at the OSU facility. The broodfish from each population will be stocked into one pond free of predacious insects and other fish. Spawning nests will be placed in ponds. The eggs will be siphoned from the nests and incubated in hatching jars. In order to produce fingerlings of the same size within and among populations, only fry that hatch within a 3-day period will be used. Fry will be pond-reared until they reach 25-35 mm (1-1.4 in; ~6 weeks), at which time they will be moved or shipped to indoor tanks in both locations for feed-training (~3 weeks).

Feed-training will be conducted in 1.5– 3.0 m (5 – 10 ft) tanks at a stocking density of 4 – 5 kg/m^3 (7 – 8.5 lb/ft^3) fingerling at a temperature of 20- 24 ºC for 3-4 weeks at both locations. Fish will be fed AquaMax Fry Powder and Starter 100 with high protein using automatic feeders. Feeding rates will be about 5% of body weight (BW) for the first 2 days, and then be increased 7-8% BW. Tanks will be siphoned once a day. Daily temperature, dissolved oxygen (DO), and mortality will also be recorded for each tank. Each location will produce ~10,000 - 20,000 feed-trained fingerlings based on needs for the pond grow-out experiments.

Grow-out and evaluation of the identified populations:

Experiments will be conducted in both pond and flow-through/recirculating tank systems after feed-training at two sites.

Pond Test:

Each site will raise the identified stocks communally in replicated ponds to avoid pond-specific environmental effects. This communal rearing is necessary in order to avoid environmental effects (pond environment, different densities and feeding rations resulting from differential survival, water quality, etc.). The common environmental effects on fish growth can account for up to 30% (Winkelman and Peterson 1994). If survival is different among ponds because of genotype by environment interaction (Ernst et al., 1989; Wang et al, 2009, 2010), the fish growth would be significantly different due to different densities with fish at the lower density growing much faster. In that situation, it would be difficult to compare growth data. Therefore, communal rearing is an effective and necessary design, which can increase testing validity and power. Molecular markers will be used to assign different populations of LMB to their family of origin for trait comparisons using methods previously published by OCARD. All fish will be raised using consistent rearing protocols (i.e. feeding frequency, feeding amount, and seasonal aeration) briefly as followings:

Stocking and feeding: Respective feed-trained populations of LMB fingerlings will be stocked into each pond at 15,000 - 20,000/acre. AquaMax Starter 100 to AquaMax Grower 400 feed will be used for all experimental ponds with a feeding rate of 5% (before 5 g BW) - 3% BW based on weather. Feeding amount and rates will be adjusted monthly based on an assumed survival of 80% and calculated biomass using mean weight.

Water quality and management: Daily temperature, DO, and mortality will be recorded for each pond; pH, ammonia and nitrite will be monitored monthly for each pond. Water exchange rate and feeding regimes in all ponds will be kept the same at each testing site.

Sampling and harvest: Before stocking at Day 0, five-hundred feed-trained fingerlings will be randomly sampled for weight and total length, and preserved individually with 95% alcohol in small vials for future identification of family composition by genotyping. Thereafter, 100 fish from each pond will be randomly sampled monthly for weight and feeding ration adjustment. At the end of year 1, fish will be harvested, and 1,000 fish from each of communal ponds will be individually weighed, measured, and finclipped. Finclip samples will be preserved individually with 95% alcohol in small vials. Using the data, production data including survival, growth rate, feed efficiency, fillet yields, and time to market size will be compared among the identified stocks of LMB to identify the optimal source population.

Tank test:

Five hundred feed-trained LMB from each population will be stocked into each of three 3 - 4 ft-diameter production tanks (replicates) at each site. When the fish reach the mean size of 10 cm, 1000 fish from each population will be tagged with Visible Implant Elastomer (VIE) color tags, combined, restocked at reduced density, and communally raised in 3 large tanks with 7’ to 10’ diameter under similar environmental and management conditions for an accurate comparison.

Throughout the experiment fish will be fed high protein feed (>40%) using automatic feeders. Feeding rates will be about 5-6% of BW at beginning, and then be reduced 1% body weight every two months until reaching 3% BW. Feeding amount and mortality will be recorded on data sheets in order to calculate feed conversion ratio (FCR) and survival rate for each tank. Feeding ration will be adjusted monthly based on new weights and survival. Daily temperature, DO and mortality will also be recorded for each tank. PH, ammonia and nitrite will be monitored monthly for each tank. Water exchange rate in all tanks will be kept the same.

During the experiment, 100 fish from each tank will be sampled for individual weight and length monthly, and their tag color will be recorded. At the end of the test, fish will be harvested, and production data including survival, growth rate, feed efficiency, fillet yields, and time to market size will be compared among the identified stocks of LMB to identify the optimal source population based on VIE markers.

Data analysis:

Microsatellite markers will be used for genotyping and for family identification for communal rearing practices. Parentage assignment will be performed using the program CERVUS 3.0. Differences in the mean responses among populations in survival, growth rate, feed efficiency, fillet yields, and time to market size will be determined with one-way ANOVA followed by Duncan's test using SAS program for performance evaluation test.

Logic Model:

Situation → Inputs → Activities → Outputs → Outcomes (Knowledge, Actions, Conditions)

Situation	Inputs	Activities	Outputs	Outcomes: Knowledge	Outcomes: Actions	Outcomes: Conditions
Description of problem: - Slow growth of LMB to market size - Lack of knowledge regarding genetic variability among commercial LMB stocks in the NCR. -No formal selective breeding program for LMB foodfish market. - Data regarding growth rates of LMB populations has not been collected and analyzed to identify fast growing stocks for NCR.	**What we invest:** - NCRAC funding for data collection and performance testing - Faculty - Postdoctoral Fellow - Aquaculture specialist for extension/outreach - Graduate students - Technicians - Growth data from commercial and public LMB production facilities - Microsatellite markers developed for LMB	**What we do:** - Collect, compile, and analyze microsatellite data to determine genetic variability among LMB populations - Collect, compile, and analyze LMB growth data from different source populations, industry and public, using a meta-analysis - Assess growth performance of highest ranked available LMB populations at two sites in the NCR, Piketon, OH and Carbondale, IL - Train graduate students -Conduct workshop to disseminate genetics variability in NRC LMB populations, selection matrix, LMB population rankings, and growth trial results. **Who we reach:** - NRC LMB producers - Researchers & extension agents - LMB fingerling producers	**Products, services and events that are intended to lead to the program's outcomes:** - Description of LMB genetic variability among NRC populations - Selection matrix for fast growing LMB - Identification of fast growing populations verified in production systems - Extension factsheet - Peer-reviewed publications - Workshop materials - Presentations at scientific meetings - Reports for NCRAC - Graduated Master's students	**Anticipated knowledge learned:** - Amount of genetic diversity among NCR LMB populations for genetic selection - Predictions of which populations of LMB will grow the fastest to market size in the NCR - Predicted verses actual growth of the highest ranked populations - Improved skills for technicians and students	**Expected changes resulting from new knowledge:** - Development of selective breeding programs for LMB in NCR - LMB producers utilize results in deciding what fish to acquire, breed, or purchase. - Technicians' and student's application of new skills toward a career or graduate degree	**Anticipated improvements:** - Improved LMB production efficiency through integration of results into management and breeding decisions - Rural communities receive economic benefit related to improved production efficiency of LMB - LMB production is increased in the NCR - Increased career and graduate degree options for students and technicians

Assumptions -
1) LMB cultured in the NCR exhibit significant genetic variability
2) LMB producers, private and public, will share growth data and fin clips.
3) LMB production efficiency will be increased by developing a selection matrix through meta-analysis of available growth data and identification of fast-growing populations.
4) The LMB populations predicted to grow the fasted will be available for growth trials and utilization by the industry.

External Factors -
1) Management differences have a significant effect of production efficiency
2) Informal selection at LMB farms in the NRC may have limited the ability to further improve growth of some populations due to inadvertent inbreeding.

Facilities:

SIU CFAAS has 90 research ponds (0.05-0.1 acre), some of which will be used for performance evaluation test for this project. FIAC's 8300 ft^2, temperature-controlled, wet laboratory houses three production systems (33 total 400-gallon circular tanks) available for LMB feed-training and production evaluation. CFAAS has over 15,000 ft^2 of floor space on SIU campus for conducting basic and applied research in the aquatic sciences. The PI's have approximately 800 ft^2 of dedicated research space for the proposed project and the necessary computing equipment and software to conduct the meta-analysis.

The OSU-OCARD has 16 research ponds (0.25-1.0 acre), some of which will be used for performance evaluation test for this project. Up to sixteen 10'-diameter tanks and hatching facilities in wet laboratory will be available for tank tests.. A new facility with sixty 6-ft tanks for housing brood stocks and feed training was completed last year. This new tank system will be used for housing LMB brood stocks and feed training for this project. The OSU aquaculture genetic laboratory at Piketon is equipped with a variety of equipment including a high throughput ABI Genetic Analyzer for genetic analysis. Genotyping and parentage analyses will be conducted in this lab.

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Project Leaders:

State	Name/Institution	Area of Specialization
Illinois	Brian Small/Southern Illinois University	Fish culture, physiology & genetics
Illinois	Paul Hitchens/Southern Illinois University	Aquaculture extension/outreach
Ohio	Hanping Wang/The Ohio State University	Fish culture & genetics
Ohio	David Glover/The Ohio State University	Fish bioenergetics & and population modeling

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

OMB Approved 0524-0039

ORGANIZATION AND ADDRESS Board of Trustees of Southern Illinois University c/o Office of Sponsored Projects Administration, Mail Code 4709, Woody Hall C-206, Southern Illinois University Carbondale, 900 S. Normal Avenue, Carbondale, IL 62901 PROJECT DIRECTOR(S) Brian Small Paul Hitchens				USDA AWARD NO. Year 1: Objectives 1-2 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. 1.083 Graduate Students				19923			
e. _____ Prebaccalaureate Students							
f. _____ Secretarial-Clerical							
g. _____ Technical, Shop and Other							
Total Salaries and Wages ☐				19923			
B. Fringe Benefits (If charged as Direct Costs)				232			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) ☐				20155			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				13009			
F. Travel				3500			
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.)				1000			
K. Total Direct Costs (C through J) ☐				37664			
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) ☐				37664			
N. Other ☐							
O. Total Amount of This Request ☐				37664			

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)		

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

Form CSREES-2004 (12/2000)

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

OMB Approved 0524-0039

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 2: Objectives 1 & 3			
Board of Trustees of Southern Illinois University c/o Office of Sponsored Projects Administration, Mail Code 4709, Woody Hall C-206, Southern Illinois University Carbondale, 900 S. Normal Avenue, Carbondale, IL 62901 PROJECT DIRECTOR(S) Brian Small Paul Hitchens	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)

	CSREES-FUNDED WORK MONTHS Calendar	Academic	Summer	Funds Requested by Proposer	Funds Approved by CSREES	Non-Federal Proposed	Non-federal Approved
A. Salaries and Wages............................							
1. No. Of Senior Personnel				5076			
a. ___1_ (Co)-PD(s)	1						
b. ____ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ____ Research Assistant							
b. ____ Other Professionals							
c. ____ Paraprofessionals							
d. __1__ Graduate Students				18942			
e. ____ Prebaccalaureate Students................................							
f. ____ Secretarial-Clerical................................							
g. ___ Technical, Shop and Other................................							
Total Salaries and Wages .... ☐				18942			
B. Fringe Benefits (If charged as Direct Costs)				2793			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) ☐				26811			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				10606			
F. Travel				3588			
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.)				500			
K. Total Direct Costs (C through J).................. ☐				41505			
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) ☐				41505			
N. Other .... ☐							
O. Total Amount of This Request...................... ☐				41505			

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)		

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 Board of Trustees of Southern Illinois University c/o Office of Sponsored Projects Administration, Mail Code 4709, Woody Hall C-206, Southern Illinois University Carbondale, 900 S. Normal Avenue, Carbondale, IL 62901 PROJECT DIRECTOR(S) Brian Small Paul Hitchens				USDA AWARD NO. Years 1& 2: Objectives 1-3 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	5076			
a. ____ (Co)-PD(s)							
b. ____ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ____ Research Assistant							
b. ____ Other Professionals							
c. ____ Paraprofessionals							
d. ____ Graduate Students				38865			
e. ____ Prebaccalaureate Students							
f. ____ Secretarial-Clerical							
g. ____ Technical, Shop and Other							
Total Salaries and Wages ☐				43941			
B. Fringe Benefits (If charged as Direct Costs)				3025			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) ☐				46966			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				23615			
F. Travel				7088			
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.)				1500			
K. Total Direct Costs (C through J) ☐				79169			
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) ☐				79169			
N. Other ☐							
O. Total Amount of This Request ☐				79169			
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)							

NAME 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
(Small and Hitchens)

Objectives 1-3

A. SALARIES AND WAGES:

YEAR 1:

- Salary for one 12-month graduate research associates (RA) and 1-month salary for another RA is requested for sample collection and preparation for genotyping, industry and public hatchery surveying, and data collection, interpretation and compilation of meta-analysis results, maintaining fish and performing experimental procedures. The RA salaries represent 50% time.

YEAR 2:

- One month salary for the Extension Liaison (Hitchens) is requested for extension/outreach activities.
- Salary for one 12-month graduate research associates (RA) is requested for maintaining fish, performing experimental procedures, analyzing data and compiling results. The RA salary represents 50% time.

B. FRINGE BENEFITS:

RA: A graduate student primary care fee (primary health service and major medical insurance) is budgeted for each semester the student will be employed during the life of the grant. This fee is $116 for spring and fall semesters during the 2014 academic year. The fee increases to $120 per semester for the 2015 academic year. Year 1: $232 Year 2: $240. (Two students year 1, but one is only one month; one student year 2.)

co-PI: Fringe Benefits are calculated as 50.3% of salary . Year 1: $0; Year 2: $2,553.

E. MATERIALS AND SUPPLIES:

Items	Year 1	Year 2	Total
Feed for feed training, and growth test and broodstock	$2,000	$4,000	$6,000
Lab chemicals and supplies for genetic sampling, sample storage, and shipping	$6,109	$3606	$9,715
Water quality reagents	$500	$500	$1,000
Belt feeders x 8 for feed training and growth test	$2,400	$0	$2,400
Pond and tank supplies (nets, bucket et al)	$2,000	$2,000	$4,000
Workshop materials	$0	$500	$500
Total	$13,009	$10,606	$23,615

F. TRAVEL (DOMESTIC):

Year 1:

- One trip to OSU for project collaboration: $1000 - Air ticket: $380; Travel to Airport and hotel: $132 (round trip from Carbondale to St. Louis: 234 miles x 0.565 = $132); rental car: $48; Hotel: 2 x 140=$280; Food per Diem: 5 days x $32=$160.
- Multiple trips to NRC LMB producers and public hatcheries to collect samples for genotyping and collect broodfish for spawning and progeny testing: $2,500 - Auto travel: (2725 miles x 0.565=$1540); Per diem: $960 (30 days x $32= $960)

Year 2:

- One trip to OSU for project collaboration: $1000 - Air ticket: $380; Travel to Airport and hotel: $132 (round trip from Carbondale to St. Louis: 234 miles x 0.565 = $132); rental car: $48; Hotel: 2 x 140=$280; Food per Diem: 5 days x $32=$160.

- One trip to OSU for fish: $884 - Auto travel: $476 (round trip from Carbondale to Piketon: 842 miles x 0.565 = $476); Hotel: 2 x 140=$280; Food per Diem: 4 days x $32=$128.
- One professional meeting to report on project findings - for one person to report of project findings: $1,704 - Air ticket: $300; registration-$430; Hotel: 5 x $130=$650; Food per diem: 6 x 32=$192; Travel to Airport and hotel: $132 (round trip from Carbondale to St. Louis: 234 miles x 0.565 = $132).

J. OTHER INDIRECT COSTS:

Shipping of samples to OSU, gas cylinder rental, contractual fees. Year 1: $1000; Year 2: $500

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

OMB Approved 0524-0039

ORGANIZATION AND ADDRESS Ohio State University South Centers 1864 Shyville Rd Piketon, OH 45661 PROJECT DIRECTOR(S) Han-Ping Wang David Glover	USDA AWARD NO. Year 1: Objectives 1 - 2 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: Calendar / Summer				
1. No. Of Senior Personnel				
a. ___ Co-PD (Calendar: 1)	3,917			
b. ____ Senior Associates				
2. No. of Other Personnel (Non-Faculty)				
a. _0.17_Research Associates/Postdoctorates (Calendar: 2)	8,000			
b. ____ Other Professionals				
c. ____ Paraprofessionals				
d. ____ Graduate Students				
e. ___ Prebaccalaureate Students				
f. ____ Secretarial-Clerical				
g. 0.25 Technical, Shop and Other	6110			
Total Salaries and Wages ☐	18027			
B. Fringe Benefits (If charged as Direct Costs)	6508			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) ☐	24535			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				
E. Materials and Supplies	15,496			
F. Travel	1000			
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) ☐	41031			
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) ☐	41031			
N. Other ☐				
O. Total Amount of This Request ☐	41031			

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)		

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

Form CSREES-2004 (12/2000)

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

OMB Approved 0524-0039

BUDGET

ORGANIZATION AND ADDRESS Ohio State University South Centers 1864 Shyville Rd Piketon, OH 45661 PROJECT DIRECTOR(S) Han-Ping Wang				USDA AWARD NO. Year 2: Objectives 1 and 3 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	1						
b. ____ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. 0.17_ Research Associates/Postdoctorates	2			8,346			
b. ____ Other Professionals							
c. ____ Paraprofessionals							
d. ____ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ____ Secretarial-Clerical							
g. _0.25_ Technical, Shop and Other				6200			
Total Salaries and Wages ☐				14546			
B. Fringe Benefits (If charged as Direct Costs)				5251			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) ☐				19797			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				13503			
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.)							
K. Total Direct Costs (C through J) ☐				34800			
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) ☐				34800			
N. Other ☐							
O. Total Amount of This Request ☐				34800			

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)		

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

Form CSREES-2004 (12/2000)

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

OMB Approved 0524-0039

ORGANIZATION AND ADDRESS Ohio State University South Centers 1864 Shyville Rd Piketon, OH 45661 PROJECT DIRECTOR(S) Han-Ping Wang				USDA AWARD NO. Years 1 & 2: Objectives 1 - 3 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	**CSREES-FUNDED WORK MONTHS**						
1. No. Of Senior Personnel	Calendar	Academic	Summer				
a. _ Co-PD	1			3917			
b. ____ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates/Postdoctorates				16346			
b. 0.17_ Other Professionals				0			
c. ____ Paraprofessionals							
d. ____ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ____ Secretarial-Clerical							
g. 0.25 Technical, Shop and Other				12310			
Total Salaries and Wages ☐				32573			
B. Fringe Benefits (If charged as Direct Costs)				11759			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) ☐				44332			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				0			
E. Materials and Supplies				28999			
F. Travel				2500			
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 J) ☐				75831			
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) ☐				75831			
N. Other ☐				0			
O. Total Amount of This Request ☐				75831			

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)		

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

BUDGET EXPLANATION FOR OHIO STATE UNIVERSITY
(Wang and Glover)

Objectives 1-3

A. SALARIES AND WAGES:

One month co-PI's (Glover) salary is requested in year 1 for the meta-analysis in Objective 2.
Two months' salary for a research associate (RA) is requested for lab genotyping and pedigree analyses.
Three months salary for a hatchery technician is requested for feeding fish and managing experiment.

One month co-PI's salary for year 1: $3,917.
Two months salary for a RA for year 1: $8,000 and year 2: $8,346.
Three months salary for a technician for year 1: $6,110 and year 2: $6,200.

B. FRINGE BENEFITS:

PI, RA, Tech: 36.1%
Year 1: $6,508; Year 2: 5,251.

E. MATERIALS AND SUPPLIES:

Items	Year 1	Year 2	Total
Feed for feed training, and growth test and broodstock	$3,000	$4,624	$7,624
PIT tags for broodfish	$1,000	0	$1,000
Lab chemicals and supplies for genotyping and genetic analyses	$4,996	$4,279	$9,275
Water quality kit	$300	$300	$600
Heaters x 10 for heating water for nursery and growth test	$1,800	$1,800	$3,600
Belt feeders x 8 for feed training and growth test	$2,400	$0	$2,400
Pond and tank supplies (nets, bucket et al)	$1,000	$1,000	$2,000
Power and water costs	$1,000	$1,500	$2,500
Total	$15,496	$13,503	$28,999

F. TRAVEL (DOMESTIC):

Year 1: One trip to SIU for project collaboration: $1000 - Air ticket: $380; Travel to Airport and hotel: $160 (round trip from Piketon to Columbus: 170 miles x 0.65 = $110); Hotel: 2 x 140=$280; Food per Diem: 3 x 60=$180.

Year 2: One professional meeting to report on project findings - for one person to report of project findings: $1,500 - Air ticket: $300; registration-$430; Hotel: 4 x $105=$420; Food per diem: 4 x 60=$240; Travel to Airport and hotel: $110 (round trip from Piketon to Columbus: 170 mile x 0.65 = $110).

Budget Summary:

YEAR 1

	OSU	**SIU**	**Total**
Salaries & Wages	$18,027	$19,923	$37,950
Fringe Benefits	$6,508	$232	$6,740
Total Salaries, Wages, and Fringe Benefits	$24,535	$20,155	$44,690
Nonexpendable Equipment	$0	$0	$0
Materials and Supplies	$15,496	$13,009	$28,505
Travel	$1,000	$3,500	$4,500
All Other Direct Cost	$0	$1,000	$1,000
Totals	$41,031	$37,664	$78,695

YEAR 2

	OSU	**SIU**	**Total**
Salaries, Wages, and Fringe Benefits	$14,546	$24,018	$38,564
Fringe Benefits	$5,251	$2,793	$8,044
Total Salaries, Wages, and Fringe Benefits	$19,797	$26,811	$46,608
Nonexpendable Equipment	$0	$0	$0
Materials and Supplies	$13,503	$10,606	$24,109
Travel	$1,500	$3,588	$5,088
All Other Direct Cost	$0	$500	$500
Totals	$34,800	$41,505	$76,305

Schedule for Completion of Objectives:

Start date: September 1, 2014
Completion date: August 31, 2016

Objectives and Tasks	**Year 1**						**Year 2**					
	S O	N D	J F	M A	M J	J A	S O	N D	J F	M A	M J	J A
Objective 1												
Sample collection												
Microsatellite and data analysis												
Objective 2												
Survey, collect and compile LMB growth data												
Conduct meta-analysis and identify fast growing population												
Objective 3												
Brood fish collection, genotyping and pedigree												
Fry and fingerling production												
Grow-out and evaluation of the identified populations												
Delivery												

PARTICIPATING INSTITUTIONS AND CO-PRINCIPAL INVESTIGATORS

Southern Illinois University-Carbondale

Paul Hitchens
Brian Small

The Ohio State University

Han-Ping Wang
David Glover

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EDUCATION

Ph.D.-Fish Physiology/Aquaculture, University of Maryland, 1998
B.A.-Marine Science (minor chemistry), Kutztown University, 1992

RESEARCH AND PROFESSIONAL EXPERIENCE

2009-Present	Associate Professor; Southern Illinois University Carbondale (SIUC), Fisheries and Illinois Aquaculture Center and Department of Animal Science, Food, and Nutrition (ASFN)
2007-2009	GS-14 Research Physiologist; United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Catfish Genetics Research Unit
2008	Interim Center Director/Research Leader (60-day detail); USDA, ARS, National Center for Cool and Cold Water Aquaculture, Leetown, WV
2003-2007	GS-13 Research Physiologist; USDA, ARS, Catfish Genetics Research Unit
2000-2003	GS-12 Research Physiologist; USDA, ARS, Catfish Genetics Research Unit

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society - Fish Culture (1998-present) and Physiology (2000-present) sections; United States Aquaculture Society (1998-present); World Aquaculture Society (1998-present); Sigma Xi (1998-present); Catfish Farmers of Arkansas (2000-2010); Society for Integrative and Comparative Biology (2000-2010), North American Society for Comparative Endocrinology (2011-present)

RELEVANT AWARDS

2007 Distinguished Early Career Award in U.S. Aquaculture, United States Aquaculture Society

RECENT PUBLICATIONS (PAST 5 YEARS)

Kittel, E.C. and B.C. Small. 2014. Growth performance and body composition of juvenile pallid sturgeon, *Scaphirhynchus albus*, fed diets containing differing protien:energy ratios. N. Amer. J. Aquacult. 76:28-35.

Nelson, L.R. and B.C. Small. 2014. Stress response in pallid sturgeon *Scaphirhynchus albus* exposed to high ammonia levels, low dissolved oxygen concentrations, and crowding stress. N. Amer. J. Aquacult. 76:170-177.

Pohlenz, C. A. Buentello, T. Miller, B.C. Small, D.S. MacKenziee, and D. Gatlin III. In review. Effects of dietary arginine on endocrine growth factors of channel catfish, *Ictalurus punctatus*. Submitted to Comparative Biochemistry and Physiology Part B: Biochemistry & Molecular Biology, April 2013.

Fenn, C.M., D.C. Glover, and B.C. Small. In press. Efficacy of Aqui-S® 20E as a sedative for handling and cortisol suppression in pallid sturgeon. Submitted to North American Journal of Fisheries Management, January 2013.

Tapia, P.J., M.C. Puebla, A. Muñoz, E. Rojas, C.M. Marchant, M.A. Cornejo, M. Futagawa, and B.C. Small. 2012. Evaluation of the cortisol stress response in a marine perciform fish, the San Pedro *Oplegnathus Insignis*. North American Journal of Aquaculture. 74:438-442.

Peterson, B.C., B.G. Bosworth and B.C. Small. 2010. Comparison of growth, body composition, and stress response of three select lines and industry channel catfish. J. World Aquacult. Soc. 41:156-162.

Peterson,B.C., G.C. Waldbieser, L.G. Riley Jr., K.R. Upton, Y. Kobayashi, B.C. Small. 2012. Pre- and postprandial changes in orexigenic and anorexigenic factors in channel catfish (Ictalurus punctatus). General and Comparative Endocrinology. 176:213-239.

Wang, S., Peatman, E., Abernathy, J., Waldbieser, G.C., Lindquist, E., Richardson, P., Lucas, S., Wang, M., Li, P., Thimmapuram, J., Liu L., Vullaganti, D., Kucuktas, H., Murdock, C., Small, B.C., Wilson, M., Liu, H., Jiang, Y., Lee, Y., Chen, F., Lu, J., Wang, W., Xu, P., Somridhivej, B., Baoprasertkul, P., Quilang, J., Sha, Z., Bao, B., Wang, Y., Wang, Q., Takano, T., Nandi, S., Liu, S., Wong, L., Kaltenboeck, L., Quiniou, S., Bengten, E., Miller, N., Trant, J., Rokhsar, D., Liu, Z, and the Catfish Genome Consortium. 2010. Assembly of 500,000 inter-specific catfish expressed sequence tags and large scale gene-associated marker development for whole genome association studies. Genome Biology 11:R8.

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EDUCATION

Bachelor of Science-Environmental Biology, Eastern Illinois University, 1981

RESEARCH AND PROFESSIONAL EXPERIENCE

2005-	Aquaculture Specialist/Researcher II, Illinois Aquaculture TechSERV; Fisheries and Illinois Aquaculture Center-Southern Illinois University; Carbondale, IL
2003-2005	Technical Service Agent, Illinois Fish Farmers Co-op; Pinckneyville, IL
2003	Independent Private Consultant, Inter Sea Farms De Venezuela C. A.; Maracaibo, Venezuela
1990-2003	Technical Manager, Larfico S.A., Penaeid Shrimp Laboratory, Ayangue, Ecuador
1984-1990	Technical Manager, Langomorro Cia., Ltd.; affiliate of Larfico S.A., Guayaquil, Ecuador
1982-1984	Pond/Hatchery Biologist, Inducam S.A., Guayaquil, Ecuador
1982	Technical Assistant II, Texas A&M University / National Marine Fisheries, Galveston, TX
1982	Independent Private Consultant, Market Facts, Inc., Chicago, IL
1981	Staff Biologist, King James Shrimp, Inc., Park Forest South, IL

PUBLICATIONS

Laramore, R., S. Allen, P. Hitchens, A. Romero, and A. Schuur. 2000. Artificial induction of active accommodation for White Spot Syndrome Virus (WSSV) in Penaeid vannamei with tolerine products. Proceedings of the World Aquaculture Society, Latin American Conference. Panama City, Panama.

Blogoslawski, W.J., C. Perez, and P. Hitchens. 1992. Ozone treatment of seawater to control vibriosis in mariculture of penaeid shrimp, Penaeus vannamei. Pages 131-141. Proceedings of the Third International Symposium on the use of Ozone in Aquatic Systems. International Ozone Association, Pan American Group. Stamford, Connecticut, USA.

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EDUCATION

B.S. Central China Agricultural University (CCAU), 1982, Fisheries Science
M.S. equivalent Yangtze River Fisheries Institute (YFI), 1987, Aquaculture Science
Ph.D. CCAU and University of Missouri-Columbia (a joint training program), 2001, Aquaculture Science

Positions

Adjunct Professor (2005–present), Department of Animal Science, Senior Research Scientist (2010–present) and Director, Ohio Aquaculture Research and Development Integration Program (2008-present), Ohio State University South Centers.

Adjunct Professor (2005-present), Key Laboratory of Fish Genetic Resources and Biotechnology, Chinese Ministry of Agriculture

Research Associate Professor (2003) and Senior Research Associate (2000-2003). Department of Fisheries & Wildlife, University of Missouri-Columbia

Associate Professor (1993–2000), Assistant Professor (1987-1993), Research Associate (1984-1986), Department of Aquaculture & Environment, YFI, Chinese Academy of Fisheries Sciences.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society, World Aquaculture Society, Asian Fisheries Society.

RECENT PUBLICATIONS (PAST 5 YEARS)

Shen, Z. G and H. P. Wang. 2014. Molecular players involved in temperature-dependent sex determination and sex differentiation in fish. *Genetics Selection Evolution*, DOI: 10.1186/1297-9686-46-26.

Wang, H.P., Z. Gao, P. O'Bryant, J. D. Rapp, and H. Yao. 2014. Effects of temperature and genotype on sex determination and sexual size dimorphism of bluegill sunfish *Lepomis macrochirus. Aquaculture,* 420–421: S64–S71.

Eissa, N., and H.P. Wang. 2013. Physiological stress response of yellow perch subjected to repeated handlings and salt treatments at different temperatures. *North American Journal of Aquaculture* 75:449–454.

Cao, X, H.P. Wang, H. Yao, P. O'Bryant, J. D. Rapp and G. K. Wallat. 2012. Evaluation of one-stage and two-stage selection in yellow perch I: genetic and phenotypic parameters for growth traits of F_1 fish reared in ponds using microsatellite parentage. *Journal of Animal Sciences* 90:27–36.

Xu, Y.J., X.Z. Liu, M.J. Liao, H.P. Wang, and Q.Y. Wang. 2012. Molecular Cloning and Differential Expression of Three GnRH Genes during Ovarian Maturation of SpottedHalibut, Verasper variegates. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 317:434-44.

Wang, H.P., H. Yao, P. O’Bryant, D. Rapp, G.K. Wallat, L.G. Tiu and R. MacDonald. 2011. Family-tank interactions on early growth performance of yellow perch reared in single family tanks versus mixed-family tanks as inferred using molecular pedigrees. *Aquaculture research* 42:1694-1702.

Cao, X, H. P. Wang, H. Yao, W. M. Wang, P. O’Bryant and D. Rapp. 2010. Correlations between growth traits and heterozygosity, allelic distance (d2) at microsatellite loci in the yellow perch, *Perca flavescens. Journal of Biotechnology* 150:2-3.

Gao, Z., H.P. Wang, H. Yao, L.G. Tiu, W. Wang, R. MacDonald and L.G. Tiu. 2010. No sex-specific markers detected in bluegill sunfish *Lepomis macrochirus* by AFLP. *Journal of Fish Biology,* 76: 408 - 414.

Wang , W.J., H.P. Wang, L. Li, G.K. Wallat, L.G. Tiu, H.Yao, and Q.Y. Wang. 2010. A first genetic linkage map of bluegill sunfish (*Lepomis macrochirus*) using AFLP markers. *Aquaculture International*, 18:825–835.

Gao, Z., H.P. Wang, G.K. Wallat, D. Rapp, P. O’Bryant, H. Yao, R. MacDonald and W. Wang, 2010. Effects of a nonsteroidal aromatase inhibitor on sex reversal, gonadal differentiation and growth performance of bluegill sunfish *Lepomis macrochirus. Aquaculture research*, 41: 1282-1289.

Wang, H.P., L. Li, G.K. Wallat, B. Brown, H. Yao, Z. Gao L.G. Tiu, P. O’Bryant, D. Rapp and R. MacDonald. 2009. Evaluation of relative growth performance and genotype by environment effects for cross-bred yellow perch families reared in communal ponds using DNA parentage analyses. *Aquaculture Research*, 40: 1363 - 1373.

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EDUCATION

Ph.D.-Fish Ecology (minor in statistics), Auburn University, 2010
M.S.-Fish Ecology, University of Illinois at Urbana-Champaign, 2005
B.S.-Fisheries Management, The Ohio State University, 2001

RESEARCH AND PROFESSIONAL EXPERIENCE

2013-	Senior Research Associate, Aquatic Ecology Laboratory, Department of Evolution, Ecology, and Organismal Biology, The Ohio State University
2010- 2013	Postdoctoral Fellow, Center for Fisheries, Aquaculture, and Aquatic Sciences, Southern Illinois University
2005-2010	Graduate Research Assistant, Department of Fisheries and Allied Aquacultures, Auburn University
2002-2005	Graduate Research Assistant, Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign
2001-2002	Research Assistant, Limnology Laboratory, The Ohio State University
1998-2001	Research Assistant, Aquatic Ecology Laboratory, The Ohio State University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society, Southern Division of the American Fisheries Society, American Association for the Advancement of Science, Ecological Society of America, International Association of Great Lakes Research

RECENT PUBLICATIONS (PAST 3 YEARS)

Bowker, J.D., J.T. Trushenski, D.C. Glover, D.G. Carty, and N. Wandelear. In review. Sedative options for fisheries: a brief review with new data on sedation of warm-, cool-, and coldwater fishes and recommendation for evaluating sedative effectiveness. Transactions of the American Fisheries Society. Submitted on June 6, 2013.

Farmer, T.M., D.C. Glover, D.R. DeVries, and R.A. Wright. In review. Mercury bioaccumulation in estuarine fish populations: A bioenergetics-based case study. Intended outlet: Canadian Journal of Fisheries and Aquatic Sciences. Submitted on August 1, 2013.

Fenn, C., D.C. Glover, B. Small. In press. Efficacy of Aqui-S 20E as a sedative for handling and cortisol suppression in pallid sturgeon. North American Journal of Fisheries Management.

Tew, K.S, P.J. Meng, D.C. Glover, J.T. Wang, M.Y. Leu, and C.C. Chen. In press. Characterizing and predicting algal blooms in a subtropical coastal lagoon. Marine and Freshwater Research.

Kuo, J., K.S. Tew, Y.X. Ye, J.O. Chen, P.J. Meng, and D.C. Glover. In press. Picoplankton dynamics and picoeukaryote diversity in a hyper-eutrophic subtropical lagoon. Journal of Environmental Science and Health, Part A.

Tsehaye, I., M. Catalano, G.G. Sass, D.C. Glover, B. Roth. In press. Prospects for fishery-induced collapse of invasive Asian carp in the Illinois River. Fisheries. Accepted for publication on April 10, 2013.

Bowzer, J., J. Trushenski, and D.C. Glover. 2013. Potential of Asian carp from the Illinois River as a source of raw materials for fish meal production. North American Journal of Aquaculture 75: 404-415.

Glover, D.C., D.R. DeVries, and R.A. Wright. 2013. Growth of largemouth bass in a dynamic estuarine environment: an evaluation of the relative effects of salinity, diet, and temperature. Canadian Journal of Fisheries and Aquatic Sciences 70:485-501.

Sammons, S.M., and D.C. Glover. 2013. Summer habitat use and habitat availability of large adult striped bass in Lake Martin, Alabama. North American Journal of Fisheries Management 33: 762-772.

Glover, D.C., D.R. DeVries, and R.A. Wright. 2012. The effect of temperature, salinity, and body size on routine metabolism of coastal largemouth bass *Micropterus salmoides*. Journal of Fish Biology 81: 1463-1478.

Stoeckel, J.A., J. Morris, E. Ames, D.C. Glover, M.J. Vanni, W. Renwick, and M.J. Gonzalez. 2012. Exposure times to the spring atrazine flush along a stream-reservoir system. Journal of American Water Resources Association 48: 1-19.