Development of an all-female yellow perch population: a strategic approach using thermal manipulation, sperm selection, and genomic data analysis

Theme A: *Aquaculture production* - ***Targeted Research area A-1:*** *Reproduction/ early life history*

Chairperson:	Osvaldo J. Sepulveda Villet
Industry Advisory Council Liaison(s):	Amy Stinton
Extension Liaison(s):	James Held
Funding Request:	$162,261
Duration:	07/01/2017- 06/30/2019 (24 months)

Objectives:

1. To determine the influence of temperature on gonadal differentiation in yellow perch of Ohio origin raised at low 14ºC (57ºF) or high 24ºC (75ºF) water temperature from fertilization until completion of sex differentiation.
2. Examine the sex ratio and growth rate of progenies sired by potentially sex reversed males (obtained from objective 1) reared in parallel groups (OSU) and separately in a "common garden" design by factorial crossing (UW-M). Additionally, outcross performance (fertilization, survival, growth rates at 30 and 90 days, feed efficiency) will be evaluated among crosses of Ohio strain and hybrids between UW-M genetically improved yellow perch x Ohio perch sperm.
3. To determine if the use of a flow-cytometry-based cell sorting method will correctly identify and segregate "Y"- and "X"-sperm, using a fluorescent nuclear tag and differential fluorescence as separation criteria (UW-M).
4. To characterize DNA from "X"-sperm and utilize a novel yellow perch genome to identify putative sex-linked markers that can be used to increase efficiency of cell-sorting or other molecular-based sperm selection methods (UW-M).
5. To optimize high-throughput cryopreservation methods for yellow perch sperm and develop a pilot cryo-bank of sex-reversed ("XX") male yellow perch sperm, which will be immediately available for use by fish farmers in the North-Central region to produce all-female progenies for grow-out (OSU).

Deliverables:

1. The development of standardized methods for collection, extension, cryopreservation and distribution of yellow perch semen.
2. A technique of thermal manipulation that will result in sex-reversed male yellow perch, which produce all-female progenies when crossed to female yellow perch.
3. The identification of putative sex-determining gene(s) for yellow perch.
4. A method to screen and select sperm, as a strategy to produce monosex lines.
5. Primary, peer-reviewed literature highlighting our research products.
6. Technical white paper(s) on collection techniques and use of cryopreserved semen in commercial fish farms.
7. A web-based outreach and training program for the use of cryopreserved semen in commercial farms.

Proposed Budgets:

Institution/Company	Principal Investigator(s)	Objective(s)	Year 1	Year 2	Total
UWM	Osvaldo J. Sepulveda Villet	/2/3/4	$45,664	$49,079	$94,743
OSU	Konrad Dabrowski	1/2/5	$33,859	$33,659	$67,518
		Totals	$79,523	$82,738	$162,261

Non-funded Collaborators:

Facility	Collaborator(s)
Wisconsin Sea Grant Program	Titus Seilheimer

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PROJECT SUMMARY

Strong consumer demand and high fillet value ($30-$40/kg; $14-$18/lb.) support the development of yellow perch *Perca flavescens* aquaculture. However, commercial perch production suffers highly variable sizes, due to females growing larger and faster than males. Collaborative efforts developed genetically-improved yellow perch broodstocks with faster growth, but while mean growth to market size has been reduced from 26 to eight months, size variability persists. One approach to eliminating this variability is mono-sex culture. Current methods are inefficient and typically involve the controversial application of exogenous steroids. A promising alternative technique is the combination of thermal manipulation, sperm selection (separating "X" and "Y" sperm), and genomic identification of sex-linked markers. We propose thermal manipulation-based male sex-reversal, using progeny sex ratio to identify neo-males ("XX"-males). We will analyze sperm from these males using flow-cytometry to verify exclusive production of "X" sperm. DNA from "X" sperm will be analyzed against an annotated yellow perch genome, to characterize sex-determining genes. Finally, these selected sperm samples will be cryopreserved and stored in a pilot cryo-bank. The ability to culture all-female yellow perch will directly benefit commercial producers (20-30% increased growth) and increase profitability. Cryopreserved sperm will be available to research laboratories (OSU and UWM) and fish farms.

JUSTIFICATION

Aquaculture is the fastest growing food-producing sector in the world and now accounts for over 50% of the seafood consumed throughout the world (FAO 2009). While the aquaculture industry has grown significantly in the U.S. over the past 40 years, North America produced only about 1.3% of the total world seafood production in 2004 (FAO 2006). From 1910 until 2007, NOAA (NOAA 2008) reports that per capita consumption (edible kilograms) of seafood in the U.S. rose from 5 to 7.4 (11.2 to 16.3 lb). Given the increase in seafood consumption and the relative low aquaculture production, this means that the U.S. is also one of the largest importers of seafood in the world and, therefore, has not only opened itself to many problems concerning food safety and product authenticity, but has also lost the economic benefits attendant with expansion of domestically-produced seafood product. The increase in seafood consumption in the U.S. and concerns with food safety underscore the need to increase domestic production of safe, nutritious, and desirable seafood such as yellow perch.

Yellow perch are an ecologically and economically important food fish in the Midwestern United States. Perch are the traditional backbone of the regional "Friday night fish fry" in many Great Lakes communities. Historically, the supply of perch for this market came primarily from commercial fisheries in the U.S. and Canada. Those markets saw peak harvests of >15,000 metric ton/yr (>16,500 US ton/yr) in the 1950s and 60s, but by the 1980s and 90s, wild harvests declined to 5,000-8,1000 metric ton/yr (5,500-8,928 US ton/yr) or less (Malison 2000). With the exception of Lake Erie and Green Bay, commercial fishing of perch has been terminated in the Great Lakes and quotas for sport fishing have also been greatly reduced. However, consumer demand for yellow perch remains high, which has driven the development of yellow perch aquaculture. In addition to longstanding consumer fidelity, the bases for this demand are high-quality flesh characteristics such as firmness, high protein and low fat content (Stuiber 1987). The latter characteristics are conducive to the product having a long shelf life, resistance to freeze damage and minimal off-flavor. The yellow perch product is typically sold to retailers as scaled, skin-on fillets, and it continues to command a high retail value of $30-$40/kg($14-$18/ lb). Over the past few decades, advancements in techniques for incubating and hatching perch eggs and improvements to the process of habituation of post-larvae to artificial feeds have greatly facilitated development of the industry. **However, there are still several bottlenecks that exist with yellow perch aquaculture and commercial perch production is hampered by the intensive nature of large-scale broodstock operations, and the lack of technologies that would allow efficient transfer of high-quality gametes from one facility to another.**

While yellow perch are harvested and marketed at a smaller size (~150 g; 5.3 oz) than other commercially-important fish such as rainbow trout and striped bass (450 , and 700 g, respectively; 16 and 25 oz), perch still require the same amount of time to reach market size. A major obstacle to perch aquaculture has been the lack of genetically-improved broodstocks that display improved, and consistent, production traits. Indeed the FAO identified the lack of genetically-defined broodstocks, and the molecular genetic tools with which to manage them, as being two critical problems to the expansion of world-wide aquaculture production (FAO 2009). Selective breeding for enhanced growth has been successful with other farmed fish species and recently two programs (UWM/ARS/USDA and The Ohio State University) have undertaken efforts to develop genetically-improved yellow perch broodstocks Wang et al. 2009; (Rosauer et al. 2011;). Even though genetic gain for growth has been achieved and reported by both

programs, and mean growth to market size has been reduced from ~13 months to ~8 months within our UW Sea Grant/ARS/USDA improved perch broodstocks (Choptank and Perquimans strains), as well as for Ohio State University/ ARS-USDA pond-reared stocks, there is a persistent need for broodstock operations to be optimized. Current challenges include high larval and early life mortalities, occurrence of disease and physical deformities, and the logistical burden of maintaining numerous duplicated broodstock cohorts to enable year-round fingerling production using out-of-cycle spawning techniques.

Such logistical burdens dramatically impact the production cycle (profitability) by requiring time-intensive environmental manipulations for each cohort, the associated feed and manpower expenses, and the increased risks of disease and broodstock loss derived from maintaining multiple cohorts. Before this industry can fully utilize the genetically-improved yellow perch that are being developed, broodstock operations need to be optimized. Consequently, we need to think more critically about developing and implementing novel ways in which to further increase the efficiency and productivity of these genetically-improved yellow perch stocks. One approach to resolve these issues would be the development of cryopreservation protocols for yellow perch semen. Increasingly, semen cryopreservation is considered the best choice for preservation of genetic resources and increasing aquaculture production efficiency (Betsy & Kumar 2013). However, exogenous factors such as diet composition (Kwasek et al. 2014), seasonality and maturation cycles (Christ et al. 1996), differential techniques of spawning and gamete extraction (Ward et al. 2012) and the combination of different extenders and freezing rates (Gaitan-Espitia et al. 2013), can all influence the efficacy and success of cryopreservation. Thus, it is necessary to develop a standardized, repeatable method to cryopreserve yellow perch sperm. The development of these techniques will allow hatchery operations and genetic material transfer to be optimized, thereby facilitating expansion of the domestic yellow perch aquaculture industry.

With effective sperm cryopreservation, broodstock numbers can be greatly reduced, as there would only be a need for maintaining female cohorts in out-of-cycle regimes. Thus, feed, veterinary, and technical costs would be reduced. Cryopreserved semen of known pedigree and quality could be easily transported between facilities as well as being commoditized and converted into an additional income stream, such as those found in the horse, cattle and porcine industries. To enable year round larvae/fingerling production of yellow perch, there is a pressing need optimize methods of collection, cryopreservation and distribution to farmer communities of high value sperm of neomales.

During the commercial grow out phase of yellow perch culture, production is hampered by the slow and highly variable (sexually-dimorphic) growth of this species, wherein females grow larger and faster than males. For instance, well documented growth of European perch in intensive culture conditions resulted in female growth 20-30% better than males after 360 days (Stejskal et al. 2009). Such growth variation dramatically impacts profitability by requiring time-intensive (stressful) grading, increased rearing time and increased feed and veterinary costs, which decrease production and increase costs.

One approach for reducing this variability is through the development of mono-sex culture (all-female), which would eliminate all problems caused by the sexually-dimorphic growth rate in yellow perch. All-female perch would have faster and uniform growth, and recent research suggests that female yellow perch may have enhanced disease resistance as well (Shepherd et al. 2012). According to Rougeot (2015) and Stejskal et al. (2009), an all-female population of European perch (*P. fluviatilis*), the sister species of yellow perch, weighs approximately 30% more than a 1:1 male to female population after the first year. At this time, the mean body weight of the all-female population is 140 g (4.9 oz.) and that of the mixed-sex population is 100 g (3.5 oz.). Therefore, 1,000 female fish at a price of $30/kg($14/lb) . for fillets (Frank's Fish and Seafood Market, Columbus, Ohio, April 2016) and a roughly 45% fillet yield will gross about $1,050 more than the mixed sex population. Current methods for producing mono-sex finfish, such as Nile tilapia, involve the controversial use of hormones to induce phenotypic sex reversal. Hormones can be used to directly control the sex of fish for grow out, or they can be used to produce sex reversed broodstock which then produce genetically monosex progenies for grow out. For example, a sex reversed yellow perch male ("XX") can be mated to a female yellow perch ("XX") and the resulting progeny will be all "XX" females.

Hormonal methods for sex-reversal have been developed for a number of finfish species, including yellow perch (Malison and Garcia-Abiado 1996; Piferrer 2001; Devlin and Nagahama 2002; Strussmann and Nakamura 2002), but these methods are difficult for producers to take advantage of due to regulations preventing the use of hormones in food fish without special permission. There are also environmental concerns about residual hormones and metabolites being present in farm effluent, as well as a negative consumer perception of the process (Beardmore et

al. 2001). However, research conducted at The Ohio State University suggests that environmental temperature during the sex differentiation period may influence the phenotypic sex of yellow perch**, thereby giving the possibility to produce sex-reversed fish without hormonal manipulation**. Sex reversed males, produced by rearing larvae at a specific temperature, could be used as broodstock to produce all-female progenies. The economic gains of this approach **would be significantly increased by adequate methods for the collection and use of cryopreserved sperm, as well as analytical techniques enabling genetic sex identification.**

RELATED CURRENT AND PREVIOUS WORK

Strong consumer demand and high fillet value have provided a strong impetus for the development of yellow perch aquaculture. However, commercial perch production is hampered by the slow and highly variable growth of this species, and the fact that wild populations exhibit a single yearly spawning event in early- to mid-spring. Under collaborative efforts with UWM and USDA-ARS, we have developed the third generation (F_3) of yellow perch broodstock (two geographic strains) that have been selected for faster growth. Although mean growth to market size has been reduced from 14 months to approximately 8 months (Rosauer et al. 2011), genetic selection has not reduced the overall size variation, and the year-round production of fingerlings depends on the extensive manipulation of large numbers of broodstock that spawn at different times of the year. Before this industry can fully utilize the genetically-improved yellow perch that we (or others) are developing, hatchery management will need to be improved by incorporation of cryopreservation technology.

There is currently a large knowledge gap in the development of monosex fish populations. The direct application of hormones (methyltestosterone) has been shown to improve production in tilapia via masculinization of genetic females to phenotypic males (Shepherd et al. 2006); however, direct use of hormones has resulted in negative consumer perceptions. The indirect use of hormones, which involves the production of sex-reversed broodstocks (separating progeny from exposure of steroids) is a valuable method for producing mono-sex progenies (Piferrer 2001) in aquaculture. For yellow perch, Malison and co-workers (1986) demonstrated that the dietary application of 17-methyltesosterone (MT) to yellow perch at 20-35 mm (0.78- 1.37in) total length produced adults that displayed a wide range of gonadal phenotypes (as determined by gonadal histology, but not functional testing) consisting of ~ 5% with normal ovaries, ~45% with ovotestes and ~50% with normal testes. Use of sperm from fish that possessed ovotestes to fertilize normal egg strands, was reported to yield 100% female progeny, which suggest that these animals were neo-males that produced "XX" sperm. Despite the promise of this approach, these yellow perch neo-males possessed reproductive defects that prevent the normal release (spawning) of sperm, which necessitated the killing of these neo-males (a valuable resource) to obtain the sperm for breeding (Malison and Garcia-Abiado 1996). These results indicate that this practice is neither practical nor sustainable on a commercial scale as it requires, 1) the continuous production of new neo-male broodstock to replace neo-males that have been sacrificed for breeding, and 2) the parallel maintenance of an overly large broodstock population of varying phenotypes (normal testes, ovotestis and ovaries). Additionally, this technique is difficult for producers to utilize, particularly small-scale, family-run operations, due to the highly regulated nature of the necessary hormones (methyltestosterone).

Another promising method for mono-sex production is via temperature-dependent sex determination (TSD), which is a wide-spread phenomenon among vertebrates (Devlin and Nagahama 2002; Nakamura 2010; Angelopoulou et al. 2012). TSD has been described in greater than 50 fish species ranging over seven taxonomic orders, suggesting it is also wide-spread phenomenon in finfish (Conover 2004). While genomics techniques are contributing to our understanding of the bio-molecular pathways involved with TSD in finfish, the primary effect of temperature seems to be regulation of the estrogen synthesis rate-limiting enzyme cytochrome P450 aromatase. Higher temperatures result in reduced aromatase activity and thus estrogen levels, yielding predominantly male (masculinized) progeny. (Baroiller et al. 2009; Blazquez and Somoza 2010; Guerrero-Estevez and Moreno-Mendoza 2010; Nakamura 2010). Although TSD can result in masculinization in several teleost species, this phenomenon not been investigated in yellow perch or other closely related species, until our recent efforts at the Ohio State University. Preliminary studies to determine the success of hormone treatment for sex reversal in two size classes of yellow perch resulted in a significantly higher proportion (89%) of spermiating males in the control groups than the expected 1:1 ratio, indicating the potential influence of high water temperature on sex differentiation at the juvenile stage. During the time of gonadal differentiation, these fish were kept at a water temperature of 21.4 ± 1.3°C (75°F) to maximize fish growth. In contrast, Lake Erie water temperature during yellow perch larval stages is 12-16°C (53- 61°F; Reichert et al. 2010). Therefore, we hypothesize that the high temperatures during the period of gonadal differentiation induced masculinization. To the best of our knowledge, there are no studies that have attempted to determine the influence of

temperature on sex differentiation in perch.

Molecular or fluorescent markers that enable the identification of genetic sex in fish or gametes would accelerate efforts to produce all-female (mono-sex) yellow perch by reducing the need identify sex-reversed males through progeny testing. A number of recent studies have begun characterize the expression patterns for several candidate genes, known to be involved with male sex determination in mammals, in gonochristic teleosts. This includes amh, dmrt1, dax1 and sox6 in rainbow trout (Marchand et al. 2000; Brunelli et al. 2001; Alfaquih et al. 2009), amh, dmrta2, sox9 and sox17 in turbot (*Scophthalmus maximus*), and amhrII in fugu *Fugu rubripes* (Kikuchi et al. 2007), which have been mapped to genetic linkage groups involved in sex determination in these fish. This information represents a substantial leap in our understanding of sex determination in fish, however, most teleosts, including yellow perch (Becak et al. 1973, Danzmann 1979), lack a morphologically distinct sex chromosome, which has made the task of developing sex-linked molecular markers difficult. Quoting Weber (2009), "…indirect approaches include treating a mixed sex population to obtain the first generation of fish where one of the sexes has a phenotype that is opposite to its genotype. These individuals must be identified for use in the next generation. Identifying these individuals requires a generation of progeny testing, which amounts to crossing the individuals with the homogametic sex to identify those whose progenies are all of the expected sex. Sex markers would eliminate this need for progeny testing, but they are not widely available for fish". The doublesex and mab-3 related transcription factor 1 (dmrt1) gene, in the medaka fish (*Oryzias latipes*), as well as the *sdY* gene in rainbow trout (Yano et al. 2012) are the only known examples of identified sex-determining genes in teleosts, but they have only been demonstrated as such in these two species (Matsuda et al. 2002; Matsuda et al. 2007; Guerrero-Estevez and Moreno-Mendoza 2010). Given the lack of an applicable candidate gene that can be used as a sex-specific marker in fish, researchers have begun to develop genomic DNA-based markers for identifying genetic sex in finfish. Although few tests exist, the most prevalent types of markers developed for determination of genetic sex, in commercially-important finfish, involve (sex-linked and non-sex-linked) DNA markers such as Amplified Fragment DNA Polymorphisms (AFLPs) and Random Amplified DNA Polymorphic (RAPD) markers (Devlin and Nagahama 2002; Ezaz et al. 2004; Chen et al. 2007; Chen et al. 2008; Koshimizu et al. 2010). The expansion in ability to screen for genomic information, and our current efforts to create an annotated nuclear genome for the yellow perch, provide our impetus to determine the genetic determinant for sex in this species, and to harness this information as a tool to rapidly and efficiently screen and select gametes (sperm selection), or as a directing tool for the creation of all-female lines.

Cryopreservation of yellow perch and European perch semen have been successfully performed using a simple, low-throughput technique (Ciereszko et al 1993, Glogowski et al. 1999; Rodina et al. 2008). These methods entail diluting the semen in an extender and cryoprotectant solution, and then freezing the mixture on dry ice or in fumes of liquid nitrogen. While this is practical on the laboratory scale, the commercial deployment of a cryobank requires the use of high-throughput computerized controlled-rate freezing. Protocols for controlled-rate freezing must be optimized for each species, and while this has been accomplished for several farmed species (Viveiros et al. 2000; Christensen and Tiersch 2005), it has not been attempted in yellow perch. Thus, for the yellow perch industry to make use of cryopreservation technology, optimum cryoprotectant/extender formulations and freezing rates must be determined.

ANTICIPATED BENEFITS

The problem that is being addressed by this research is the lack of analytical and research tools needed for development of a sustainable method to produce all-female fingerlings, and to reliably preserve semen from genetically improved and sex-reversed yellow perch. The technologies and resources gained from these efforts will benefit the aquaculture industry by increasing hatchery efficiency and enabling the production of all-female populations for grow out. This research proposal directly addresses USDA/ NCRAC targeted research area (TRA) A-1: "Reproduction/ Early life history", with activities for "Broodstock quality/ management" and "Monosex production", as well as TRA A-5: "Enhanced Growth Technology" through activities for "Improved strains". This project will increase the number of aquaculture facilities dedicated to yellow perch aquaculture by increasing the year-round availability of yellow perch gametes, and will reduce the scope and size of broodstock operations by reducing the number of male breeder fish required to supplement out of cycle-spawning in commercial facilities. The increased availability of yellow perch gametes will also develop a commodity product, similar to that found in porcine, cattle and poultry industries, with the sale of high quality, pedigreed and validated gametes for commercial hatchery use. The development of molecular sex markers will allow further development of mono-sex yellow perch

strains, which will reduce operational variation in fish size ranges, and reduce operation costs through the abatement of labor-intensive processes, such as size grading and sorting. Finally, this project will secure the availability of yellow perch fingerlings by facilitating the storage and availability of locally-adapted genetic resources found in the Great Lakes region and beyond.

The impact and benefits of an efficient sperm extender, that preserves short-term sperm viability, are:

- An optimized sperm extender will maintain/prolong sperm viability during the breeding season, ensuring sperm is available when eggs are obtained and enabling planned male x female crosses for genetic management and improvement of broodstock.
- An optimized sperm extender will facilitate systematic evaluation and surveillance of sperm quality in broodstock. If sperm quality is known during instances of low fertilization success, then the breeder can address issues of egg quality.

The impact and benefits of an efficient sperm cryopreservation protocol and pilot cryobank are:

- Cryopreservation of sperm from genetically-improved animals ensures that valuable genetic material is protected and backed-up in the event of catastrophic loss of broodstocks that can arise from loss of life-support, physical damage to facilities, disease outbreak or inbreeding depression.
- Cryopreserved sperm can ensure availability of gametes from both sexes for breeding without synchronizing male and female fish.
- Cryopreserved sperm can ensure a stable supply of sperm for optimal hatchery operations, thus reducing the size of the broodstock population.
- Cryopreserved sperm can enable producers to effectively transport genetic resources between facilities without having to move animals, thus reducing animal handling and transportation costs.
- Cryopreserved sperm from select broodstocks can enable crossbreeding to evaluate performance benefits resulting from heterosis.
- Cryopreserved sperm greatly increases flexibility and capacity (synchronization) for year-round, and out-of-cycle, breeding.
- Cryopreserved sperm of known lineage, pedigree and performance can provide an additional income stream possibility as a broodstock commodity in a similar manner to the lucrative cattle and horse semen industries.
- Cryopreserved sperm from sex-reversed males (all X-sperm) can be produced at a licensed farmer facility and easily distributed to producers

The impact and benefits of a reliable marker for genetic sex identification, are:

- A molecular marker for genetic sex will accelerate efforts to develop sex-reversed broodstock for the production of monosex yellow perch.
- Availability of a molecular marker for sex genetic sex determination will immediately impact efforts to develop monosex yellow perch, via use of thermal manipulation, by identifying optimal parameters for induction of sex-reversed (neo-male) yellow perch, and by proving the performance of sex-reversed neo-male broodstock.
- Availability of a molecular test for genetic sex identification will enable rapid and non-destructive (using non-lethal fin clip biopsies) surveillance of sex ratios during the on-going production of sex-reversed broodstock and their progeny.

OBJECTIVES

1. To determine the influence of temperature on gonadal differentiation in yellow perch of Ohio origin raised at low 14oC (57oF) or high 24oC (75oF) temperature during sex differentiation.
2. Examine the sex ratio and growth rate of progenies sired by potentially sex reversed males (obtained from objective 1) reared in parallel groups (OSU) and separately in a "common garden" design by factorial crossing (UW-M). Additionally, outcross performance (fertilization, survival, growth rates at 30 and 90 days, feed efficiency) will be evaluated among crosses of Ohio strain and hybrids between UW-M genetically improved yellow perch x Ohio perch sperm.
3. To determine if the use of a flow-cytometry-based cell sorting method will correctly identify and segregate Y- and X-sperm, using a fluorescent nuclear tag and differential fluorescence as separation criteria (UW-M).
4. To characterize DNA from X-sperm and utilize an annotated yellow perch genome to identify putative sex-linked markers that can be used to increase efficiency of cell-sorting or other molecular-based sperm selection methods (UW-M).
5. To optimize high-throughput cryopreservation methods for yellow perch sperm and develop a pilot cryo-bank of sex-reversed ("XX") male yellow perch sperm, which will be immediately available for use by fish farmers in the North-Central region to produce all-female progenies for grow-out (OSU).

DELIVERABLES

1. The development of standardized methods for collection, extension, cryopreservation and distribution of yellow perch semen.
2. A technique of thermal manipulation that will result in sex-reversed male yellow perch, which produce all-female progenies when crossed to female yellow perch.
3. The identification of putative sex-determining gene(s) for yellow perch.
4. A method to screen and select sperm, as a strategy to produce monosex lines.
5. Primary, peer-reviewed literature highlighting our research products.
6. Technical white paper(s) on collection techniques and use of cryopreserved semen in commercial fish farms.
7. A web-based outreach and training program for the use of cryopreserved semen in commercial farms.

PROCEDURES

Research Objective 1: To test the influence of temperature on sex differentiation, the OSU group will perform the thermal manipulation technique. Full-sib progenies will be split into two groups, one low temperature (14°C; 57°F) and one high temperature (24°C; 75°F). Progenies obtained from twelve males (six from each thermal treatment, 14 and 24°C (57 and 75°F), will be used to raise individually in the system described previously by OSU investigators (Grayson et al. 2014). The fish will be raised at these temperatures from the time of fertilization until a size at which sex differentiation is completed (20 mm; 0.78in; approximately 14-30 days), and then all fish will be raised at 24°C (75°F) until sexual maturity. The sex ratio in each group will be determined based on morphology of the gonads at 6 months of age, and by a non-invasive technique (Shepherd et al. 2013), and compared between temperature groups.

Research Objective 2: Males from high temperature groups with male-skewed sex ratios obtained in Objective 1 will be bred to unrelated females of the Ohio strain and UW-M genetically improved strain. The resulting progenies will be raised in parallel groups (OSU) and in a common garden design (UW-M), after which parentage assignment will be made based on microsatellite genotyping. Fertilization efficiency, sex ratio, growth rate, and other production statistics will be analyzed. Based on the sex ratio of progenies from different males, sex-reversed ("XX") males will be identified and reserved for Objective 5.

Research Objective 3: Recently, the use of flow cytometry and derived cell-sorting techniques have matured and reduced in price point to a viable range for the characterization and segregation of "X"- and "Y"- sperm from semen samples. These techniques have been successfully developed for cattle insemination programs (Garner et al. 2013; Steinhauser et al., 2016), for assisted reproduction in humans, and have been further for other laboratory animal models, including fish (Yang et al., 2015). Cell sorting has also provided the practical basis for other methods, such as magnetic separation, antibody-based recognition and sedimentation of non-target sperm, among others. We propose the modification of the total nuclear fluorescent sorting method developed by Johnson et al. (1989) to identify and segregate "X"- and "Y"- sperm from extended and immobilized yellow perch semen samples, initially on regular semen, and then on neomale-derived semen. A simplified hyperosmotic extender (300mM glucose, pH 8.0) will be used as a carrier buffer, and a nuclear stain (Hoechst 33342) and the selective quencher propidium iodine, will allow the identification of viable and non-viable sperm. Overall values of nuclear fluorescence will be analyzed to determine the cutoff values to segregate "X"- and "Y"- sperm. We will utilize a 4-color BD FACSCalibur flow cytometer, a 10-color FACSAria III sorter, and FlowJo analysis software available at the Biotechnology facility of the University of Wisconsin-Milwaukee. Sorted "X"-sperm will be cryopreserved for research objective 4, and a small sample will be used for research objective 3.

Research Objective 4: As previously mentioned, androgen treatment can yield sex-reversed neo-male yellow perch that produce "XX" sperm, however, reproductive defects prevent normal release (spawning) of sperm, which necessitates killing neo-males (a valuable resource) to obtain the sperm (a crude testicular macerate that cannot be cryopreserved) for breeding (Malison & Garcia-Abiado 1996). Additional drawbacks to sex-reversal are the continual need for regeneration of neo-males, verification of effectiveness, and the lag time needed for the animals to grow and reach maturity. Even though sperm selection is more direct and promising approach that does not involve altering the sex of any animal, one common factor needed for the expeditious development and maintenance of any approach to mono-sex production is access to a rapid diagnostic method that enables the identification of genetic sex well before sexual maturation. Recently, a number of candidate genes responsible for development of the male phenotype (e.g., *amh*, *dmrt* & *sox9*), and DNA-associated sex markers, have been mapped to genomic linkage groups that control sex determination in fish. Researchers have begun to develop genomic DNA-based markers for identifying genetic sex in finfish. Although few tests exist, the most prevalent types of markers developed for determination of genetic sex, in commercially-important finfish, involve (sex-linked and non-sex-linked) DNA markers such as Amplified Fragment DNA Polymorphisms (AFLPs) and Random Amplified DNA Polymorphic (RAPD) markers (Devlin and Nagahama 2002; Ezaz et al. 2004; Chen et al. 2007; Chen et al. 2008; Koshimizu et al. 2010). Accordingly, we shall utilize proprietary transcriptomic data for yellow perch along with standard molecular techniques to clone, sequence and test whether *amh*, *dmrt* or *sox9* can be used as molecular markers for sex identification. We will use our novel in-house annotated genomic DNA constructed utilizing an *in silico* genome assembly and annotation, focusing on rapid throughput methods available through our internal partner the Great Lakes Genomics Center at UWM-SFS. A yellow perch genome (est. 1.2 Gbp) is resulting from ongoing 30X genome coverage sequencing, and *de novo* assembly pipelines from the Pacific Biosciences RSII sequencing platform. This ongoing genome sequence is the current product of a collaboration between UWM-SFS, USDA-ARS, and Mississippi State University - Center for Nanotoxicology. We will additionally use small fragment data generated from Illumina Hi-Seq platform, which is provided by the parallel Mississippi State University project, as part of a coordinated effort to create higher sequence coverage depth in the annotated genome of yellow perch. We will additionally use transcriptome archives from our genetically-improved broodstocks (known sex) for screening and validation. Those markers shown to be effective will be used to develop and validate a PCR-based testing platform, and then to eventually develop a single nucleotide polymorphism (SNP)-based test, cross validated to our ongoing annotated yellow perch genome. Alternatively, a Taqman approach to detect sex-linked markers will be developed, following Larson et al. (2016).

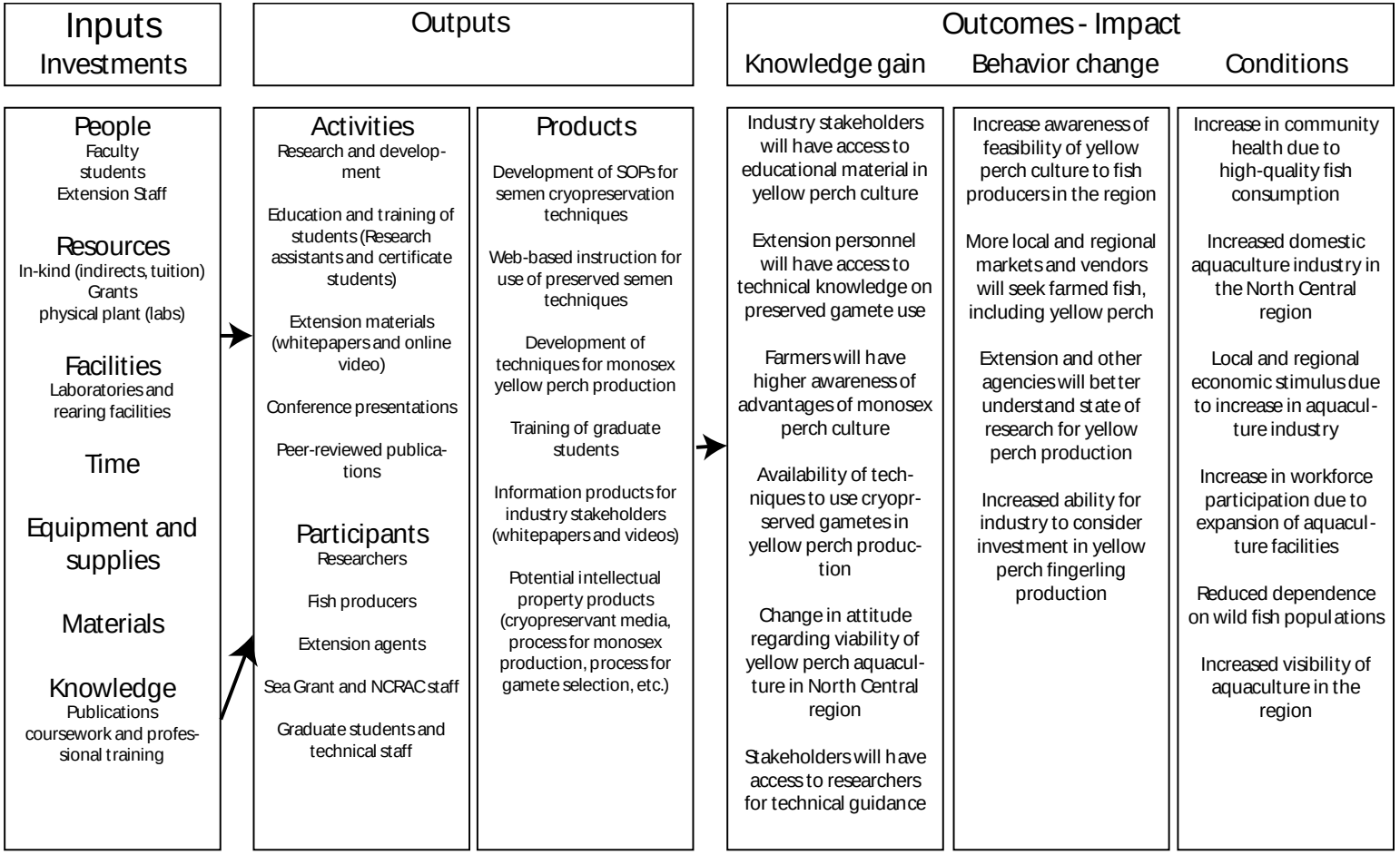
Research Objective 5: A protocol for high-throughput cryopreservation of yellow perch sperm will be developed by optimizing extender formulation, cryoprotectant, and freezing rate, initially on regular semen, and then on neomale-derived semen. An optimum extender will first be developed by examining several formulations based on Lahnsteiner (2000) and Glogowski et al. (1999). Cryoprotectant choice will determined by comparing the efficacy of DMSO and methanol at concentrations from 5 - 25% v/v (Vivieros et al. 2000). Optimum freezing rate will be determined by empirically testing freezing rates between -1°C/min and -45°C/min using our Planar Kryo 10 controlled-rate freezer. Protocols will be tested by collecting semen for yellow perch males, freezing semen using

the different methods to be tested, and then thawing and comparing sperm motility and fertilization capacity between treatments. After the optimum protocol is developed, semen from sex-reversed males obtained in Objective 1 will be cryopreserved, along with semen from their genotypic male siblings. The quality of cryopreserved semen from sex-reversed and genotypic males will be compared to determine if semen from sex-reversed males is more or less resistant to damage from cryopreservation. Cryopreserved semen from the sex-reversed males will be made available to other researchers and producers in the North-Central Region. Cryopreserved sperm from sex-reversed males, as well as cell-sorted specimens, will also be stored at the University of Wisconsin-Milwaukee, as a parallel, long-term storage point for a pilot cryo-bank of yellow perch sperm in the North Central region.

Outreach and Extension: Mr. James Held is our extension liaison (formerly of UW-Extension). Mr. Held will co-develop media (including an instructional video on how to properly use cryopreserved semen to fertilize yellow perch eggs at a hatchery facility) and outreach information products, present at the 2019 Wisconsin Aquaculture association, and at the 2019 NCRAC regional meeting. Other information products develop by Mr. Held will also be incorporated into the class curriculum of the Applied Urban Aquaculture undergraduate certificate at UW-Milwaukee. Other primary literature products (journal articles, posters and/or oral presentations) will be produced by the PIs and their graduate students. Additional interaction with NCRAC, Wisconsin Aquaculture Association, Wisconsin Sea Grant personnel, and with UW-M and OSU personnel will increase our dissemination of the material to stakeholders.

Logic Model goal: successful use of cryopreserved gametes, sperm sorting and identification, and monosex lines of yellow perch

Objective: Develop efficient techniques to preserve gametes and use in commercial facilities, develop markers and technology towards monosex lines.



Assumptions

-Producers are interested in expanding into yellow perch aquaculture
-Producers and stakeholders want to learn and use new techniques to improve production efficiency
-A market and consumer demand exists for yellow perch in the North Central region
-Both industry stakeholders and Federal and state agencies have an interest in promoting and expanding aquaculture industry in North Central region

External factors

-Dwindling number of extension agents in region; reduced operating budgets
-Other species and markets already exist in region; yellow perch demand only partially filled by wild fishery (but not domestic)
-Industry stakeholders lack access to improved yellow perch fingerling production
-Perception from agencies and granting institutions that yellow perch is a "minor species" or not a priority for funding/ research

FACILITIES

Overall facility use strategy: Research will be jointly conducted at the Ohio State University and at University of Wisconsin-Milwaukee. At the Aquaculture Laboratory on the campus of The Ohio State University in Columbus, Ohio, procedures to be conducted at this facility are related to broodstock maintenance, spawning of yellow perch, larval rearing, sperm cryopreservation technology, and histological methods to verify fish gender. Yellow perch sperm will be cryopreserved following the OSU laboratory's simple cryopreservation protocol optimized for yellow perch and modified from Glogowski et al. (1999). Progenies obtained from each thermal treatment, 14°C (57°F) and 24°C (75°F), will be raised individually in the system described previously by OSU investigators (Grayson 2014).

Any larval rearing at UW-M will be carried out in a "common garden" design with factorial crossing under flow-through conditions. Facilities at UW-M available for the fulfillment of objectives 3 and 4 include a larval rearing laboratory (SFS 169) which contains five replicated blocks of four recirculating tanks, and associated filters and support components. A high density continuous rotifer culture system is present to sustain larval perch feeding. Additionally, flow-through grow out facilities (SFS 173), and quarantine bays (SFS 1024) are available for common garden trials. An ultra-low freezer (-150°C; -238°F) is available for long term storage of cryopreserved sperm. The Great Lakes Genomic Center located in UW-M is available for all microsatellite and genomic analyses. Finally, the biotechnology facility at the Department of Biological Sciences, University of Wisconsin-Milwaukee, will be used for all flow cytometry methods.

The Ohio State University: Dr. Dabrowski is in-charge of three research laboratories (analytical, histology/histochemistry, and radioactive laboratories; Fig.1) and two experimental fish facilities at Kottman Hall, The Ohio State University, Columbus, Ohio.

Laboratory: The analytical laboratory is equipped with two biofreezers (-85°C; -185°F), two refrigerated centrifuges, a freeze-drier, a computer controlled cryogenic freezer for sperm cryopreservation (Planer Kryo 10 Series III; Fig. 2), accessories for sperm cryopreservation, and other facilities for biochemical research studies and formulation of specialized feeds. The histology and histochemistry laboratory is equipped with standard facilities for histology, histochemistry, and photomicroscopy. The radioactive laboratory is equipped with a Beckman scintillation counter, Stratagene Stratalinker for UV-irradiation of sperm and eggs, equipment and accessories for cell transplantation studies (Zeiss stereomicroscope with camera, 3 Narishige micromanipulators and 2 microinjectors, Pharmacia chilling chamber) and other facilities necessary for conducting steroid radioimmunoassays and routine studies on radioactive markers.

Temperate fish rearing facility (260 m2; 2798 sq. ft) is equipped with two dechlorination systems for city water, an egg incubation system, a series of tanks for rearing newly-hatched embryos, juveniles, and adult fish, and a light-temperature control chamber. Chromosomal set manipulation techniques can be performed in the lab through the application of thermal shocks (Figure 1 C) or a hydraulic pressure chamber for applying pressure shocks (Figure 1 F). The egg incubation system consists of 10 McDonald jars and 20 California-type hatching trays that can be operated as either recirculating or flow-through systems. There are ninety small tanks (20-60 L; 5.2 – 16 gal) and 20 large tanks (400 L; 105 gal) on a flow-through system and 36 aquaria on a recirculating system. The lab also contains an intensive rotifer culture system (Figure 1 B) for producing rotifers to be used as a live feed for yellow perch larvae. A series of forty 60-L (16 gal) capacity conical bottom tanks are available for testing the interactions between water temperature and hyperoxia, normoxia, and hypoxia in juvenile fish. Hyperoxia conditions are achieved by running fresh, dechlorinated water through a sealed, packed column supplied with pure oxygen. Hypoxia conditions are achieved by supplying pure nitrogen gas into a second, identical packed column. The gas exchange column ensures that the dissolved gas levels can be altered without creating total gas supersaturation. Oxygen levels are monitored using a dissolved oxygen monitoring system and oxygen levels are automatically recorded in a portable computer (Fondriest Environmental, Alpha, Ohio). The fish rearing facility is equipped with a multi-probe (six probes) oxygen monitoring system from YSI (Yellow Springs, Ohio). This system will allow 24-hr automatic recording of oxygen, temperature and conductivity from 6 conical-bottom tanks. Although not part of this proposal, hypoxia has been previously implicated as a factor impacting sex differentiation in fish (Lu et al. 2014), and may be considered for evaluation as part of a future project.

Tropical fish facility: A part of the greenhouse complex in Kottman Hall, consists of 44 glass aquaria (28, 50-L; 13.2 gal, and 16, 35-L; 9.2 gal), six polypropylene plastic tanks (150-L; 39.6), a separate intensive larval rearing recirculating system (Figure 1 E) which allows the manipulation of water temperature, turbidity and salinity, and a large three compartment fiberglass broodstock tank (3,000 L; 795gal) (Figure 1 A).

Figure 1. Dabrowski Facilities and Equipment – A. 3000L (795gal), 3 compartment fiberglass broodstock tank that allows large broodstock fish to be separated by sex for maintenance or for hormonal injection; B. Intensive rotifer culture system that produces large amounts of rotifers to be used as a high-quality, live feed; C. Heat shock setup that will allow heat shocks to be given to embryos in order to induce polyploidy, gynogenesis and androgenesis; D. McDonald jars in hatchery used to provide developing embryos with constant clean water; E. intensive larviculture recirculating system that allows large quantities of larvae to be produced efficiently; F. Hydraulic pressure chamber used for delivering pressure shocks to early embryos in order to induce polyploidy, gynogenesis and androgenesis

Figure 2 - Planer Kryo 10 Series III Computer Controlled Cryogenic Freezer for sperm cryopreservation

University of Wisconsin-Milwaukee, School of Freshwater Sciences: The School of Freshwater Sciences (SFS) comprises 12,000 square meter (130,000 sq.ft.) assignable research space with ~8,000 sq. meter (88,000 sq.ft.) used for laboratories, office space, and storage for the handling and rearing of aquatic animals. All participants have office and laboratory space. Additionally, UWM-SFS has recently annexed a newly completed facility with an additional 4,600sq. meter (50,000 sq.ft.) used for office space, laboratories, genomics core lab, aquatic BSL-2 quarantine labs and a BSL3 aquatic disease challenge facility (Fig. 3).

Laboratory: The laboratory of the PI, Osvaldo J. Sepulveda Villet, is located in the School of Freshwater Sciences. This laboratory comprises a wet laboratory with five recirculating/ flow-through aquaculture systems, and a partitioned space outfitted for general molecular biology prep/ methodologies. Approximate area of this lab is 79 square meter (850 sq. ft.). The wet lab contains a direct-vented fume hood cabinet, large wash-down sink, 50 linear feet of laboratory countertop and cabinets. Major equipment located in this laboratory includes two ultra-low temp freezers, a freezer/ refrigerator, standalone refrigerator, PCR prep cabinet.

Wet Lab Space: The SFS has a 1,394 m^2 (15,000 ft^2) aquaculture workspace with both flow-through and recirculating systems. An automated system supplies dechlorinated water as hot water, ambient cold water and chilled water to the fish rearing tanks at a capacity of 4,500 liter/min (1,200 gal/min). Flow-through rearing tanks, including several large 2.44-m (8-ft) diameter circular, 1.22-m (4-ft) diameter circular, banks of smaller rectangular, round and oval fiberglass and small glass aquaria, are available to support fish culture investigations. Recirculating systems include one 37,854-liter (10,000-gal) commercial-scale system. The entire facility is equipped with real-time dissolved oxygen monitoring system (Water Management Technologies) and a web-interface temperature monitoring/alert system (AvTech). Also available are ~93 square meter (1,000 sq. ft.) of assigned space consisting of 4 x 1.8 meter (6 ft.) diameter stock tanks equipped for flow-through, 4' diameter tanks, and a smaller 24 x 115 liter (30 gal) tank system for controlled experimental studies. The 24 x 115 liter system is designed for replicated feed studies and is comprised of 6 separate (juxtaposed) systems with 4 tanks each. Each system has its own water supply, temperature control and photoperiod control (via custom LED lights). Each of these systems has full automated life-support control and monitoring for temperature and dissolved oxygen. UWM-SFS has analytical laboratories and shop facilities to support a wide variety of aquatic research investigations.

Shared use BSL3 Aquatic Pathology Lab*:* We have recently established a shared use facility for working with selected agents for aquatic species, and is equipped with the following items of equipment: LabConco 5' Type II/A2 Biosafety Cabinet, Binder 140 lt (5 cu. ft.) refrigerated incubator, 85 lt (3 cu. ft.) -80 C Revco chest freezer, Eppendorf Gradient (96-well) Thermalcycler, Beckman Allegra X-22 Refrigerated Centrifuge with various rotors, Fisher Scientific table top microcentrifuge, Steward Stomacher Digester (2 each), Omni Tissue Homogenizer with

generator, 311 lt (11 cu.ft). refrigerator/ freezer, Stainless steel tables, Lab chairs, Misc. manual pipettes, top-loading and analytical balances and pH meter. This facility also contains a separate *in vivo* suite, for pathogen challenges and other studies that have high infective risk. All effluents and liquid waste generated within the facility is chlorine/ acid treated. The in vivo suite has adjacent animal receiving areas, a necropsy room, and a wash-down/ autoclave room with a pass-through autoclave.

BSL2 quarantine suites: Adjacent to the aBSL3 facility, there are five quarantine bays available for medical separation, inspection and receiving of aquatic animals. Each quarantine bay has independent light, photoperiod, air and water temperature controls. The PI has one quarantine assigned for exclusive work in this proposed project. This bay includes two flow-through rack systems for housing aquatic species, with each rack containing 20 polycarbonate self-cleaning tanks (22 liter size; 5.8gal). Additionally, adult broodstock are held in three, 170 liter (45-gal.) fiberglass insulated oval tanks under flow-through conditions. Automated process controllers maintain water quality parameters, and a monitoring system for dissolved oxygen, temperature, and power status is available to protect against equipment failure or water quality fluctuations.

Instrumentation/Machine Shop: The SFS maintains a complete machine and fabrication shop, which is staffed by two full-time machinists.

Genomics Core Facility: The SFS maintains a genomics core sequencing facility that has a PacBio RS II sequencing system for deep sequencing and epigenomic analyses, an Illumina MiSeq for short fragment analysis, and an ABI 3730S sequencer and ancillary equipment (e.g., centrifuges and thermal cyclers) for in-house, high-throughput plasmid preparation, nucleic acid sequencing and genotyping by microsatellites. Additional equipment include, an MJ DNA Engine Opticon Real-time PCR machine, MJ tetrad thermo-cycler, Perkin Elmer Scan Array ExpressHT Microarray Scanner, Perkin Elmer (Wallac) Victor^2 1420 Multi-label Plate Reader with PC, Bio-Rad Gene Pulser II Electroporater, Nano drop UV detector with PC, and a Labconco Freezone-12 Freezedryer. "Standalone BLAST" with complete NCBI protein, nucleotide and EST databases, together with investigator-derived sequence databases, are maintained on a 4-node *Apple Workgroup Cluster for Bioinformatics* along with *BioTeam's INquiry* software. Customized programs developed by bioinformatics personnel at the SFS are also maintained on the cluster for automated analysis of sequences including assembly and RNAseq analysis of large pyrosequence datasets. We also have access to a new 100 node Dell cluster that is housed and maintained by the University of Wisconsin computing services for research applications. A SFS bioinformatics technician maintains the Apple cluster and is in charge of processing and archiving data, and interfacing with the new Dell cluster. Additional available Hardware includes a High-Performance Computing Cluster; the bioinformatics team has unlimited priority access to the local high performance computing cluster (HPC) located at the campus of the University of Wisconsin Milwaukee. Finally, a SlipStream Appliance – Galaxy Edition (http://bioteam.net/slipstream/galaxy-edition/). The Great Lakes Genomics Center owns its own high memory server running the Galaxy software package (512 Gigabytes RAM, 32 CPUs)

Analytical Facility: SFS houses a shared-use, analytical lab facility with a Beckman DU-7400 UV-VIS

Figure 3. Overview of available facilities at the School of Freshwater Sciences, University of Wisconsin-Milwaukee. **A**) one of five BSL2 quarantine bays, **B**) the personal wet lab of Dr. Sepulveda Villet, **C**) The main aquaculture research facility, and **D**) the Great Lakes Genomics Center.

spectrophotometer with PC, a Perkin Elmer Lambda 24 spectrophotometer with PC, a Shimadzu total organic carbon analyzer (TOC 5000) with an autosampler and PC, a Dionex IC25 ion chromatography system with PC, and a TJA Unicam 969 Atomic Absorption Spectrophotometer (graphite furnace) with PC. There are also fume hoods, work benches and miscellaneous ovens/furnaces for sample drying and ashing. The Institute also maintains an Agilent Systems 1100 LC/MSD (ion) Trap mass spectrometer.

Centrifugation Facility: The SFS also houses a Beckman J2-21 high-speed centrifuge with rotors and a Beckman/coulter Optima XL-100K ultra-centrifuge with various rotors and adapters.

Imaging Facility: The SFS houses a microscope/imaging core facility that is equipped with a UVP Epi Chem II Gel Doc Station, an Inverted Microscope (Olympus IX70) with confocal and fluorescence capabilities and digital interface (CCD camera and PC), an Upright Compound Microscope (Olympus BX60) with fluorescence and a CCD camera (with PC) and a Stereo Microscope (Olympus SZX12) with fluorescence, a CCD camera (with PC) and micromanipulator.

Sterilization: SFS houses four separate sterilization facilities with Steris autoclaves and a Heinicke Glassware washer.**Computer:** Personal computers are available in the laboratory of the PI which are linked to the University computer system by wireless or ethernet to access e-mail, library resources, WW Web, and shared server databases.

STATEMENT OF DUPLICATION

Statement of search and review of previously funded research to avoid duplicative work.

The principal investigators searched for duplicative work on the themes proposed in this outline, using the USDA Research, Education, and Economics Information System (REEIS http://reeis.usda.gov/) on March 9, 2017. No funded projects were found to overlap with the methods and scope of this proposal, and thus the work is original research.

Additionally, the principal investigators searched for duplicative work on the themes proposed in this outline, using the NOAA databases for the National Sea Grant Office Funding Page

(http://www.seagrant.noaa.gov/funding/rfp.html), and the website of state Sea Grant Programs (http://www.seagrant.noaa.gov/other/programsdirectors.html. No funded projects were found to overlap with the methods and scope of this proposal, and thus the work is original research.

Term searches for previously NCRAC-funded works on methyltestosterone resulted in three matches, however this approach is one we specifically avoid due to pitfalls of hormone use and market/consumer perceptions, thus, the following projects are not duplicative of our original efforts:

- Drug approval research on 17"-methyltestosterone (2004)

-Drug approval research on 17"-methyltestosterone (2009)

- Environmental assessment of use of 17”-testosterone for sex inversion of newly hatched tilapia (1996)

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PROJECT LEADERS

State	Name	Institution	Area of Specialization
Wisconsin	Osvaldo J. Sepulveda Villet	University of Wisconsin-Milwaukee	Larviculture, genetics/ genomics, selective breeding
Ohio	Konrad Dabrowski	The Ohio State University	Fish physiology and reproduction, nutrition, semen cryopreservation

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

OMB Approved 0524-0039
Expires 03/31/2004

ORGANIZATION AND ADDRESS University of Wisconsin-Milwaukee P.O. Box 340 Milwaukee, WI 53201-0340 PROJECT DIRECTOR(S) Osvaldo J. Sepulveda Villet				**USDA AWARD NO.** Year: 1 Objective:			
				Duration Proposed Months: _12_ **Funds Requested by Proposer**	Duration Proposed Months: _ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
No. of Senior Personnel	Calendar	Academic	Summer	$8,167			
			1				
a._____(Co)-PD(s)							
b. Senior Associates.							
No. of Other Personnel (Non-Faculty)							
_____Research Associates-Postdoctorates. . .							
c._____Paraprofessionals							
d.1_____Graduate Students				$19,000			
e.1_____– Prebaccalaureate Students							
f._____Secretarial-Clerical				$3,600			
g._____Technical, Shop and Other							
Total Salaries and Wages D							
				$30,767			
B. Fringe Benefits (If charged as Direct Costs)				$7,897			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) D				$38,664			
Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$7,000			
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) D				$45,664			
F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) D				$45,664			
N. Other D							
O. Total Amount of This Request D				$45,664			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)						Leave Blank	
Cash (both Applicant and Third Party) D							
Non-Cash Contributions (both Applicant and Third Party) D							

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

Expires 03/31/2004

ORGANIZATION AND ADDRESS University of Wisconsin-Milwaukee P.O. Box 340 Milwaukee, WI 53201-0340 PROJECT DIRECTOR(S) Osvaldo J. Sepulveda Villet				**USDA AWARD NO.** Year: 2 Objective:			
				Duration Proposed Months: _12_ **Funds Requested by Proposer**	Duration Proposed Months: _ **Funds Approved by CSREES**	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
No. of Senior Personnel	Calendar	Academic	Summer	$8,412			
			1				
a._____(Co)-PD(s)							
b._____Senior Associates.							
No. of Other Personnel (Non-Faculty)							
_____Research Associates-Postdoctorates. . .							
c._____Paraprofessionals							
d.1_____Graduate Students				$19,000			
e.1_____Prebaccalaureate Students				$3,600			
f._____Secretarial-Clerical							
g._____Technical, Shop and Other							
Total Salaries and Wages D				$31,012			
B. Fringe Benefits (If charged as Direct Costs)				$8,067			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) D				$39,079			
Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$3,000			
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$7,000			
K. Total Direct Costs (C through I) D				$42,079			
F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) D				$49,079			
N. Other D							
O. Total Amount of This Request D				$49,079			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)						Leave Blank	
Cash (both Applicant and Third Party) D							
Non-Cash Contributions (both Applicant and Third Party) D							
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 UNIVERSITY OF WISCONSIN-MILWAUKEE
(Osvaldo J. Sepulveda Villet)

Objectives 2, 3, 4

SALARIES AND WAGES:

We request a total of $77,743 for *Salaries and Wages over the 2-year project.*

Funds are requested for support of a master's-level graduate research assistant who is needed to successfully complete the proposed studies at the UWM School of Freshwater Sciences, Milwaukee, Wisconsin location. The PI and Co-PI will be responsible for overseeing all aspects of the proposed studies and coordinating the activities of the supervised personnel and collaborative personnel. The PI, however, will be ultimately responsible for the successful completion of the proposed studies. The following justification relates to the main budget sheets for this awarding institution (UWM).

Funds are requested for 9 calendar months of salary & fringe benefits for a masters graduate research assistant for year 1 ($19,000 + $4,503 = $23,503), and year 2 ($19,000 + $4,541 = $23,541). Salary increases reflect an annual inflation rate of 3%. The PI requests 1 summer month and fringe benefits at 100% effort for year 1 ($8167 + $3,275= $11,442), and year 2 ($8412+ $3,407= $11,819). The UWM fringe benefit rate for faculty is 40.5%, and the rate for research assistants is 23.9%. Summer hourly student work is requested to support the graduate student at an hourly rate of $12.00, 300 hours per year ($3,600 + $119 = $3,719 per year; fringe rate of 3.3%).

FRINGE BENEFITS:

Fringe benefits as previously describe will total $15,964 over two years.

MATERIALS AND SUPPLIES:

We request a total of $10,000 for materials and supplies for the 2-year project. Expendable supplies and equipment will include expenses for liquid nitrogen, dry ice, reagents to formulate semen extenders, cryopreservants, disposable pipettes, vials, centrifuge tubes, etc. Prep materials for semen extraction will include reusable and disposable beakers and vials for semen collection, iodine sanitizers and cleaning wipes, etc. Supplies needed for genomic and sperm selection trials will include nuclear stains and carrier solvent for flow cytometry, enzyme and reagents for DNA extraction and PCR amplification, and materials including SMARtCells for molecular marker identification. A small portion of the supplies budget will be used to purchase larval feeds used during the cross trials. Other consumables and bioinformatics expenses are also included.

Items	Year 1	Year 2	Total
Flow cytometry staining chemicals & supplies		$400	$400
Sperm cryopreservation supplies (liquid nitrogen, cryoprotectants, extenders)	$400	$300	$700
Commercial diets for adult broodstock & juveniles	$1,400	$1,000	$2,400
Live feeds (*Artemia* cysts & rotifers)	$550	$550	$1,100
Supplies for larval rearing (salt, filter medium, etc.)	$550	$550	$1,100
Enzymes and consumables for PCR and DNA extraction	$1,300	$200	$1,500

Consumables for marker identification	$1,800		$1,800
Bioinformatics expenses (licenses, cluster time, etc.)	$1,000		$1,000
Total	$7,000	$3,000	$10,000

TRAVEL (DOMESTIC):

No travel funding requested.

OTHER DIRECT COSTS:

We request $7,000 (1.5 months, 75% effort, year two) for a consultant subaward (administered by UWM) to James Held, Extension liaison (formerly of UW-Extension). Mr. Held will develop media (including an instructional video on how to properly use cryopreserved semen to fertilize yellow perch eggs at a hatchery facility) and outreach information products, present at the 2019 Wisconsin Aquaculture association, and at the 2019 NCRAC regional meeting. Other information products develop by Mr. Held will also be incorporated into the class curriculum of the Applied Urban Aquaculture undergraduate certificate at UW-Milwaukee.

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

OMB Approved 0524-0039
Expires 03/31/2004

ORGANIZATION AND ADDRESS The Ohio State University 2021 Coffey Road Columbus, OH 43210 PROJECT DIRECTOR(S) Konrad Dabrowski				**USDA AWARD NO.** Year: 1 Objective:			
				Duration Proposed Months: _12_ **Funds Requested by Proposer**	Duration Proposed Months: _ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
No. of Senior Personnel	Calendar	Academic	Summer				
a._____(Co)-PD(s)							
b. Senior Associates.							
No. of Other Personnel (Non-Faculty)							
_____Research Associates-Postdoctorates. . .							
c._____Paraprofessionals							
d.1_____Graduate Students				$22,700			
e.1_____Prebaccalaureate Students							
f._____Secretarial-Clerical				$1000			
g._____Technical, Shop and Other							
Total Salaries and Wages D							
				$23,700			
B. Fringe Benefits (If charged as Direct Costs)				$2,417			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) D				$26,117			
Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$6,992			
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$750			
K. Total Direct Costs (C through I) D				$33,109			
F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) D				$33,859			
N. Other D							
O. Total Amount of This Request D				$33,859			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)						Leave Blank	
Cash (both Applicant and Third Party) D							
Non-Cash Contributions (both Applicant and Third Party) D							

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

Expires03/31/2004

ORGANIZATION AND ADDRESS The Ohio State University 2021 Coffey Road Columbus, OH 43210 PROJECT DIRECTOR(S) Konrad Dabrowski				**USDA AWARD NO.** Year: 2 Objective:			
				Duration Proposed Months: _12_ **Funds Requested by Proposer**	Duration Proposed Months: _ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
No. of Senior Personnel	Calendar	Academic	Summer				
a._____(Co)-PD(s)							
b._____Senior Associates							
No. of Other Personnel (Non-Faculty)							
_____Research Associates-Postdoctorates . . .							
c._____Paraprofessionals							
d.1_____Graduate Students				$23,381			
e.1_____Prebaccalaureate Students				$1,000			
f._____Secretarial-Clerical							
g._____Technical, Shop and Other							
Total Salaries and Wages D				$24,381			
B. Fringe Benefits (If charged as Direct Costs)				$2,487			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) D				$26,868			
Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$5,041			
F. Travel				$1,000			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$750			
K. Total Direct Costs (C through I) D				$32,909			
F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) D				$33,659			
N. Other D							
O. Total Amount of This Request D				$33,659			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) D Non-Cash Contributions (both Applicant and Third Party) D						Leave Blank	
NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)						**DATE**
Project Director							
Authorized Organizational Representative							
Signature (for optional use)							

BUDGET EXPLANATION FOR THE OHIO STATE UNIVERSITY
(Konrad Dabrowski)

Objectives 1, 2, 5

SALARIES AND WAGES:

We request a total of $48,081 for Salaries and Wages over the 2-year project.

YEAR 1:

We request $23,700 for *Salaries and Wages* during the first year of the project. Specifically, we request $22,700 to provide 12 months of Graduate Research Assistantship stipend to a graduate student who will devote 50% FTE to this project. The Ohio State University will cover the cost of tuition for this student. We request $1,000 to provide hourly wages at a rate of $10 per hour for 10 hours per week over a period of 10 weeks to a prebaccalaureate student who will assist the graduate student with fish husbandry and reproduction aspects of this budget.

YEAR 2:

We request $24,381 for *Salaries and Wages* during the second year of the project as well. Specifically, we request $23,381 to provide 12 months of Graduate Research Assistantship stipend to a graduate student who will devote 50% FTE to this project. The Ohio State University will cover the cost of tuition for this student. We request $1,000 to provide hourly wages at a rate of $10 per hour for 10 hours per week over a period of 10 weeks to a prebaccalaureate student who will assist the graduate student with fish husbandry and reproduction aspects of this budget.

FRINGE BENEFITS:

We request $4,904 to cover fringe benefits at a rate of 10.2% ($2,417 for Year 1 and $2,487 for Year 2).

MATERIALS AND SUPPLIES:

We request a total of $12,033 for materials and supplies for the 2-year project.

Items	Year 1	Year 2	Total
Histological staining chemicals & supplies	$636	$636	$1,272
Sperm cryopreservation supplies (liquid nitrogen, cryoprotectants, extenders)	$693	$493	$1,186
Replacement parts and straws for Planar Kryo 10 controlled rate freezer	$1,473	$675	$2,148
Commercial diets for adult broodstock & juveniles	$1,297	$996	$2,293
Live feeds (*Artemia* cysts & rotifers)	$798	$499	$1,297
Nannochloropsis algae paste & yeast (maintain rotifer culture)	$299	$298	$597
Activated charcoal & sodium thiosulfate (dechlorinate city water)	$650	$450	$1,100
Salt (for live feed culture & maintenance of 3ppt salinity in larval rearing systems & during transport to reduce fish stress)	$129	$128	$257
General laboratory consumables (plastic & glassware, micropipette tips, calibration services for micropipettes, disposable gloves, etc.)	$462	$311	$773
General fish husbandry supplies (nets, water quality analysis reagents, mechanical filter pads, dissolved oxygen meter probe (YSI) replacement, calcium carbonate, etc.)	$555	$555	$1,110
Total	$6,992	$5,041	$12,033

TRAVEL (DOMESTIC):

We request $1,000 for travel expenses.

YEAR 1: No funds requested

YEAR 2:

We request $800 for the graduate student assigned to the project to attend and present findings from the second year of the project at Aquaculture America 2019. Presentations at these two national conferences will allow us to contribute to improvement of aquaculture on the national scale, as the techniques we develop could easily be applied to species cultured in other regions of the nation.

We request $200 for the PI to attend the Ohio Aquaculture Association 2019 meeting and disseminate results and practical recommendations gained from this project to regional producers.

These two presentations will help contribute to the Regional Aquaculture Centers' mission of supporting aquaculture research and development by disseminating our results to both scientists and producers on the national and regional scales.

OTHER DIRECT COSTS:

We request $1,500 to rent a greenhouse unit managed by The Ohio State University Department of Plant Pathology. This greenhouse unit will house the recirculation system used to culture yellow perch larvae raised as part of this project. Approximately 50% of the greenhouse unit will be devoted to this project, and so we request $750/year to rent the unit, which costs $1500/year.

BUDGET SUMMARY

YEAR 1

Institution Name	The Ohio State University	University of Wisconsin-Milwaukee
Salaries & Wages	$23,700	$30,767
Fringe Benefits	$2,417	$7,897
Total Salaries, Wages, and Fringe Benefits	$26,117	$38,664,
Nonexpendable Equipment		
Materials and Supplies	$6,992	$7,000
Travel		
All Other Direct Cost (Greenhouse Rental)	$750	
Totals	$33,859	$45,664

YEAR 2

Institution Name	The Ohio State University	University of Wisconsin-Milwaukee
Salaries, Wages, and Fringe Benefits	$24,381	$31,012
Fringe Benefits	$2,487	$8,067
Total Salaries, Wages, and Fringe Benefits	$26,868	$39,079
Nonexpendable Equipment		
Materials and Supplies	$5,041	$3,000
Travel	$1,000	
All Other Direct Cost (Greenhouse Rental)	$750	$7,000 (subaward to James Held, Extension)
Totals	$33,659	$49,079

SCHEDULE FOR COMPLETION OF OBJECTIVES (UWM)

Start date: 7/2017

Completion date: 6/2019

Year 1 activities (7/2017-6/2018)	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**
Conduct extended sperm strain trials				**x**								
Analyze data for extender performance trials				**x**								
Prepare manuscript/ report for extender trials					**x**	**x**	**x**					
Conduct fertilization trials				**x**								
Preliminary work data for fertilization/ thermal trials				**x**	**x**							
Conduct thermal trials					**x**	**x**						
Analyze data for thermal trials							**x**	**x**	**X**			
Prepare manuscript/ report for thermal trials and techniques									**x**	**x**	**x**	**x**

Year 2 activities (7/2018-6/2019)	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**
Collect and preserve sperm samples for sorting trial	**x**	**X**										
Prepare samples for selection trial Use flow cytometer to perform selection trial	**x**	**x**	**x**	**X**								
Collect and analyze data for selection trial			**x**	**x**	**x**	**X**						
Prepare manuscript for sperm selection technique					**x**	**x**	**x**	**x**				
Extract gDNA from fish samples	**x**	**X**										
Conduct gene expression trials		**x**	**x**	**X**								
Identify sex-linked markers from gDNA			**x**	**x**	**x**	**X**	**X**					
Analyze differential gene expression data				**x**	**x**	**x**	**x**	**x**				
Prepare manuscript/ report for sex linked markers								**x**	**x**	**x**	**x**	**x**
Prepare white papers and outreach activities (w/ extension liaison James Held)										**x**	**x**	**x**
Prepare final report											x	**x**

SCHEDULE FOR COMPLETION OF OBJECTIVES (OSU)

Start date: 7/2017

Completion date: 6/2019

Year 1 activities (7/2017-6/2018)	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**
Preparation of materials and equipment	x											
Screening broodstock for sex ratio	x	x	x									
Establish light and temperature regimes			x	x								
Conduct gender evaluation, spermiation			x	x								
Analyze data for sperm volume, density, motility				x	x							
Prepare materials and equipment for cryopreservation				x								
Conduct optimization of sperm cryopreservation					x	x	x					
Preliminary data analysis of cryo work							x	x				
Conduct female maturity evaluations, induced ovulation					x	x						
Preliminary induced ovulation, egg quality analysis data for thermal trials							x	x	x			
Conduct experiments on low and high temperature effect on embryonic development									x	x	x	x

Year 2 activities (7/2018-6/2019)	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**	**6**
Continue larval/juvenile rearing in low/high temperatures	x	x	x	x	x	x						
Perform preliminary evaluation of sex ratio, gonad development	x	x	x	x								
Collect and analyze data for sex determination			x	x	x	x						
Evaluate sperm quality from thermally sex reversed fish					x	x	x	x				
Perform fertilization trials with control males and sex reversed								x	x	x		
Prepare manuscript/ report for sex determination								x	x	x	x	x
Prepare white papers and outreach activities										x	x	x
Prepare final report											x	x

PARTICIPATING INSTITUTIONS AND CO-PRINCIPAL INVESTIGATORS

Institution: School of Freshwater Sciences, University of Wisconsin- Milwaukee

PI Name: Osvaldo Jhonatan Sepulveda-Villet

Institution: The Ohio State University, School of Environment and Natural Resources

PI Name: Konrad Dabrowski

VITA

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EDUCATION

Post-Doctoral	Finfish Nutrition, USDA/ARS/UW-Milwaukee-School of Freshwater Sciences – April, 2012 – January 2014
Graduate	Ph.D. in Biology (Ecology Track). The University of Toledo, May, 2011.
Graduate	M.S. in Biology. Central Michigan University, May, 2002.
Undergraduate	B.S. in Aquaculture Engineer, Universidad Autónoma de Guadalajara, México. 1999.

POSITIONS

2014- to date	Assistant Professor, School of Freshwater Sciences, University of Wisconsin – Milwaukee.
2012 - 2014	USDA/ARS Head Quarters Funded Post-Doctoral Fellow. School of Freshwater Sciences/Agricultural Research Service/USDA, Milwaukee, WI.
2011 - 2012	Sea Grant Knauss Fellow, Off. Habitat Conservation, NOAA, Silver Spring, MD.
2005 - 2010	Ph.D. Research Assistant, Lake Erie Center, University of Toledo, Toledo, OH.
2002 - 2004	Scientist, Pfizer Pharmaceuticals, Ann Arbor, MI.
1999 - 2002	Graduate Research Assistant, Central Michigan University, Mt. Pleasant, MI

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society, World Aquaculture Society, Wisconsin Aquaculture Association, International Association for Great Lakes Research

SELECTED PUBLICATIONS:

Stepien, C.A., Sepulveda-Villet, O.J. and Haponski, A.E., 2015. Comparative genetic diversity, population structure, and adaptations of walleye and yellow perch across North America. *In* Biology and Culture of Percid Fishes (pp. 643-689). Springer Netherlands.

Sepulveda-Villet, O.J., Barrows, F.T., Sealy, W., Gaylord, G., Palmquist, D.E., Binkowski, F.P., Hill, H., and Shepherd, B.S. *In* Review. Influence of mineral supplementation using soy-based diets on sex-specific growth and performance in yellow perch. North American Journal of Aquaculture.

Shepherd, B.S., Rees, C.B., Sepulveda-Villet, O.J., Palmquist, D.E. and Binkowski, F.P. 2013 Identification of Gender in Yellow Perch by External Morphology: Validation in four Geographic Strains and Effects of Estradiol. *75:361-372*.

Sepulveda-Villet, O.J., Shepherd, B.S., Binkowski, F.P., and Sealey, W. 2013. Live feed enrichment with probiotics: perspectives on increasing finfish larviculture success. Global Aquaculture Advocate, July/ August 2013: 71-74.

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Sepulveda-Villet, O.J., and Stepien, C.A. 2011. Fine-scale population genetic structure of the yellow perch *Perca flavescens* in Lake Erie. Can. Journal of Fisheries and Aquatic Sciences 68: 1435-1453.

Stepien, C.A., Murphy, D.J., Lohner R.N., Haponski, A.E., Sepulveda-Villet, O.J. 2010. Status and delineation of walleye genetic stock structure across the Great Lakes. *In* Status of Walleye in the Great Lakes: proceedings of the 2006 symposium. Great Lakes Fish. Comm. Technical Report 69: 189-223

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EDUCATION

Ph.D. Fisheries; Agriculture University, Olsztyn, Poland, 1976
D.Sc. Fish Physiology, Agricultural University, Szczecin, Poland, 1984
M.Sc. Inland Fisheries; Agriculture University, Olsztyn, Poland, 1972

POSITIONS

Professor, School of Natural Resources, The Ohio State University, Columbus, Ohio (1989-present)
Visiting Professor, University of Ghent, Belgium (2009)
Visiting Professor, Institute of Zoology, University of Innsbruck, Innsbruck, Austria (1987-1989)
Visiting Professor, Department of Biology, University of Paris VII, Orsey, France (1985)
Visiting Professor Department of Aquaculture, University of Fisheries, Tokyo (1984-1985)
Assistant/Associate Professor, Inland Fisheries and Water Protection, Agriculture University, Olsztyn, Poland (1972-1987)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society World Aquaculture Society

SELECTED PUBLICATIONS

Kestemont, P. Dabrowski, K. and Summerfelt, R. 2015. Biology and Culture of Percid Fishes. Principles and Practice. 35 Chapters Monography on North American and European Percids. Springer, 901 pp.

Farmer, T., Dabrowski, K., Marschall, E., Ludsin, S. 2015. Short, warm winters threaten temperate fish populations. Nature Communications 6: 7724 (available on line).

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

Kwasek, K., Dabrowski, K., Takata, R., Wojno, M., Wick, M 2014b. The influence of dietary lysine on yellow perch female reproductive performance and quality of eggs. North Am. Journal of Aquaculture 76: 351-358.

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Hussein, E.E-S., Dabrowski, K. El-Saidy, D.M.S.D., Lee, B-J. 2013b. Enhancing the growth of Nile tilapia larvae-juveniles by replacing plant (gluten) protein with algae protein. Aquaculture Research 44: 937-949.

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Arslan, M., Dabrowski, K., Ferrer, S., Dietrich, M. and Rodriguez, G. 2013. Growth, body chemical composition and trypsin activity of South American catfish, surubim (*Pseudoplatystoma* sp.) juveniles fed different dietary protein and lipid levels. Aquaculture Res. 44: 760-771.

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EDUCATION

B.S. Zoology; University of Wisconsin-Milwaukee 1986

POSITIONS

Owner (2015-Present), Jim Held Consulting LLC

Aquaculture Outreach Specialist (2007-2015), University of Wisconsin-Extension

Senior Research Specialist (2003-2007), University of Wisconsin-Madison Aquaculture Program, University of Wisconsin-Madison

Research Specialist (1995-2003), University of Wisconsin-Madison Aquaculture Program, University of Wisconsin-Madison

Associate Research Specialist (1988-1995), University of Wisconsin-Madison Aquaculture Program, University of Wisconsin-Madison

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Wisconsin Aquaculture Industry Advisory Council

SELECTED PUBLICATIONS

Held, J.A., S.N.M. Mandiki, C. Rougeot, and P. Kestemont. 2015. Performance of Hybrid Percids. Chapter 24 In Biology and Culture of Percid Fishes -Principles and Practices (P. Kestemont, K. Dabrowski and R.C. Summerfelt, eds.) Springer Dordrecht, Heidelberg New York London

Zarski, D., J.A. Horvath, J.A. Held and D. Kucharczyk. 2015. Artificial Reproduction of Percid Fishes. Chapter 4 In Biology and Culture of Percid Fishes -Principles and Practices (P. Kestemont, K. Dabrowski and R.C. Summerfelt, eds.) Springer Dordrecht, Heidelberg New York London

Kestemont, P., C. Melard, J.A. Held and K. Dabrowski. 2015. Culture Methods of Eurasian Perch and Yellow Perch Early Life Stages. Chapter 9 In Biology and Culture of Percid Fishes -Principles and Practices (P. Kestemont, K. Dabrowski and R.C. Summerfelt, eds.) Springer Dordrecht, Heidelberg New York London

Hartleb, C.F., Johnson, J.A. and J.A. Held. 2012. Walleye and Yellow Perch Pond fertilization. In Pond Fertilization: Impacts of Nutrient input on Aquaculture Production (C.C. Mischke, ed.) Wiley-Blackwell Publishing, Ames, IA.

Malison, J.A., and J.A. Held. 2008. Farm-based production parameters and breakeven costs for yellow perch grow-out in ponds in southern Wisconsin. 12 pp. North Central Regional Aquaculture Center Fact sheet series 121. Ames, IA. http://www.ncrac.org/Topics/ypproductionparameters.htm.

Held, J.A., J.A. Malison, and T.P. Barry. 2004. Production characteristics of hybrid walleye (*Sander vitreus* female x *S. canadensis* male) reared to food size in ponds. In Proceedings of Percis III: The Third International Percid Fish Symposium (Barry, T.P., and J.A. Malison, Eds.), pp. 33-34. University of Wisconsin Sea Grant Institute, Madison, WI.

Malison, J.A., A.B. Head, J.A. Held, and T.P. Barry. 2004. Onset of sex-related dimorphic growth in juvenile hybrid walleye (*Sander vitreus* female x *S. canadensis* male). In Proceedings of Percis III: The Third International Percid Fish Symposium (Barry, T.P., and J.A. Malison, Eds.), pp. 41-42. University of Wisconsin Sea Grant Institute, Madison, WI.