Ohio Aquaculture Research and Development Integration Program (OARDIP) Newsletter
The Ohio State University

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Inside this issue:

The Ohio Aquaculture Research and Development Integration Program Newsletter is published biannually to share the Program highlights.

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OARDIP Achievements and Impacts 2012

In 2012, in collaboration with the OSU Department of Animal Sciences, the OSU Department of Food Science, the OSU South Centers Business Development Network, the Ohio Soybean Council, and several international institutions, OARDIP accomplished:

- 8 research studies and projects
- published 4 journal articles
- published 6 proceedings papers
- received 3 grants
- trained 4 graduate students, post doctoral fellows and scholars
- generated 6 new grant proposals
- conducted 5 workshops
- conducted 2 on-farm research projects
- conducted a state-wide bus tour
- made 24 presentations to audiences around the state and at national and international conferences
- received funding from the USDA young farmer and rancher development program to conduct an Aquaculture Boot Camp program, and have successfully launched the project.

For more information on Aquaculture Boot Camp, see page 3.

Ohio aquaculture has an estimated impact of $50 million annually. Aquaculture sales in Ohio have tripled from $1.8 million to $6.6 million in recent years. Nationally, Ohio ranks first in sales of yellow perch for food and is the number one bluegill producing state. Ohio also ranks fourth in sales of baitfish and largemouth bass sold for sport, and fifth in number of baitfish farms. Sixty-four journal articles and proceedings papers have been published by OARDIP, including four journal publications and six proceedings papers in 2012.

Twenty-six participants were selected for the intensive Aquaculture Boot Camp program and meet monthly for hands-on training and education about aquaculture production techniques and business management practices that are essential to having a successful aquaculture production business.

On-farm tests show higher production, growth rate and survival of improved yellow perch

On-station and on-farm tests of genetically improved yellow perch were conducted on three sites at different latitudes in the North Central Region (NCR). The aim of the project was to assess production parameters of selected yellow perch lines as compared to fish from local brood stock at commercial densities in ponds. The third-generation of selected yellow perch lines were used for the tests to best examine genotype by environmental interactions and obtain sound results applicable across the NCR. Both on-farm and on-station tests of selected lines with local control lines were designed at four locations using both separate rearing and communal rearing methods. *(continued on page 2)*

On-farm tests show higher production, growth rate and survival of improved yellow perch, continued

(continued from page 1) Ponds were used at four geographic locations at different latitudes: two demonstration/research stations at Ohio Center for Aquaculture Research and Development (OCARD [39°N]) at Piketon and the University of Wisconsin-Stevens Point (UW-Stevens Point) Northern Aquaculture Demonstration Facility (NADF [46° N]), and two commercial farms at Mill Creek Perch Farms (MCPF, Ohio [40.5°N]) and Coolwater Farms, LLC (CWF, Wisconsin [43°N]). Consistent rearing protocols were developed and used at each of the selected sites.

OSU South Centers, Piketon, OH testing site

Year 1 rearing: 1) at the Piketon station, the selected line of yellow perch and a local-strain (control) were reared in separate ponds, each having two replicates, with a density of 69,300 fish/ha (28,000/acre); 2) at MC Perch Farm, the selected line and a local-strain were raised communally in two 0.2-acre ponds at a density of 232,900 fish/ha (94,300/ acre). 3) at the NADF, the selected line of yellow perch and a local-strain (control) were reared in four 0.4 acre separate ponds, each having two replicates, with a density of 62,500 fish/ha (25,000/acre), and 4) at CF Perch Farm, the selected line and a local-strain were not raised communally in two 0.2-acre ponds because only 5 fingerlings were recovered for feed-training. AquaMax Starter 100 to AquaMax Grower 400 feed was used for all experimental ponds with a feeding rate of 3% BW (Piketon Station and NADF) and satiation feeding (MC Perch Farms). Feeding amount and rates were adjusted monthly based on an assumed survival of 75% and calculated biomass using mean weight at the Piketon site.

Year 2 grow-out: At the end of Year 1 in October 2011, all the fish in Year 1 ponds were harvested in Mill Creek (MC) Perch Farms and Piketon station in Ohio, and re-stocked into Year 2 grow-out ponds (NADF site will conduct this in 2013). The two test sites conducted replicated tests of the improved fish vs. the local-strain using two types of rearing tests: 1) at the Piketon Station, the selected line of yellow perch and a local-strain (control) were re-stocked and reared in separate 0.19-acre ponds, each having two replicates, with a density of 30,263 fish/ha (12,105/ acre); 2) at MC Perch Farms, at the harvesting, commercial grading practice was performed. Each pond of yellow perch was size-graded and the largest fish with mean size of 40.2g and 40.9g were re-stocked into two 0.75 acre ponds, respectively. The selected line and a local-strain were raised communally in the two ponds at a density of 25,000 fish/ha (10,000/ acre). AquaMax Starter 400 to AquaMax Grower 600 feed was used for all experimental ponds with a feeding rate of 2-3% BW (Piketon Station) and satiation feeding (MC Perch Farms) from May to October. Feeding amount and rates were adjusted monthly based on an assumed survival of 80% and calculated biomass using mean weight at the Piketon site. Fish were fed once a day to satiation when water temperature is above 8C (43F) in the afternoon during winter. All of the ponds were harvested in early (MC Perch Farms) and at the end (Piketon Station) of October, 2012.

Year-1 results: At MC Perch Farm, improved yellow perch grew significantly larger than local yellow perch native to the farm in two communal ponds, where both improved and unimproved fish grew in the exactly same environment. The improved line outweighed the local strain by 32.00% on average at the end of the Year 1 test (October). Fingerling survival in the MC Perch Farm's communal ponds with improved fish was as high as they have ever experienced.

In the Piketon ponds, improved fish exhibited a 22.01% higher production and a 27.16% higher survival rate than the local Ohio strain by the end of October of Year 1. Although the 27.16% higher survival rate of the improved fish resulted in a significantly higher density and lower feed rations (rations were calculated based on the same assumed survival rate for all the ponds), the improved line still had a higher mean body weight than the local Ohio strain. A significantly greater reduction of CV_{wt} was observed for the improved line than the unimproved fish, indicating the size variation of the improved fish was smaller, and their

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Soy-Aqua Fish Farm Tour

The Ohio State University South Centers Aquaculture Program and the Ohio Soybean Council partnered to host the 2012 Soy-Aqua Fish Farm Tour on Saturday, October 20, 2012. Thirty people participated. The first stop was Millcreek Perch Farm near Marysville. This farm is a cooperator with two area fish farms. Owner/operator Bill Lynch discussed how this arrangement works to reduce costs and manage labor. The group learned about the construction of his five one-acre ponds, the business side of running an aquaculture cooperative, as well as valuable tips and techniques for farming perch.

Stop number two was RainFresh Harvests in Plain City, an aquaponics and greenhouse facility operating on alternative and sustainable energy. Owner Barry Adler demonstrated the solar and wind energy system that allows him to operate his greenhouse off the grid. He grows high-value specialty crops and sells wholesale to restaurants.

Then it was on to Fresh Harvest Farm, a new aquaponics facility near Richwood that is growing perch and specialty vegetables. Next was Fredericktown Fish Farm, a large commercial trout operation. Then, it was on to Catch of the Day fish farm outside of Galena. This farm has over 100 ponds of all sizes stocked with a variety of fish for pond stocking.

All participants responding to the follow-up survey agreed or strongly agreed that the Soy-Aqua bus tour of farms worth their investment of finances and time.

On-farm tests show higher production, growth rate and survival of improved perch, continued

(continued from page 2)

percentage of marketable size fish would be higher by the end of Year 2.

At the NADF, improved fish exhibited a 27.60% higher production and a 27.80% higher survival rate than the local WI strain by the end of October of Year 1. Although the 27.80% higher survival rate of the improved fish resulted in a significantly higher density and lower feed rations (rations were calculated based on the same assumed survival rate for all the ponds), the improved line still grew 26.60% faster than the unimproved fish.

Year-2 results: In the Piketon ponds, improved fish exhibited a 12.30% higher survival rate and a 42.07% higher production than the local Ohio strain by the end of October of Year 2. Although the 12.30% higher survival rate of the improved fish resulted in a significantly higher density and lower feed rations (rations were calculated based on the same assumed survival rate for all the ponds), the improved line still had a significant higher mean body weight than the local Ohio strain. The improved line grew 25.50% faster than the unimproved fish. There was no significant difference in dress-out percentage between improved line and local Ohio strain (42.0% vs. 42.9%).

In MC Perch Farms, communally reared perch from two ponds averaged 21.7 cm and 141.1 g and 20.8 cm and 122.6 g, respectively. Eight molecular markers are being used to assign selected and local-strain yellow perch to their family of origin for communal rearing. Family identification for fish collected in October 2012 from MC Perch Farms is still ongoing. The **NADF site** will complete the Year 2 grow-out test in 2013.

University of Wisconsin-Stevens Point (UW-Stevens Point) Northern Aquaculture Demonstration Facility

Aquaculture Boot Camp Platoon of 2013

After weeks of hard work and organization, the Aquaculture Boot Camp opened its doors to 26 recruits on Saturday, January 12, 2013. The Ohio State University South Centers serves as headquarters and was the venue for the first training session. We had great weather for January, so our facility tour went perfect and the agenda was followed as planned.

Dr. Tom Worley, the OSU South Centers director, welcomed our recruits with warm words of encouragement and explained the efforts of the USDA to support the development of this unique program for training new and beginning aquaculture farmers in production and business in Ohio. Dr. Hanping Wang, the OARDIP director, reinforced the importance of the program in the industry. Dr. Laura Tiu, Extension Aquaculture Specialist and ABC Commanding Officer, was joyful to share her aquaculture experiences and expectations for the 2013 Platoon. She also explained the levels of participation in the program. ABC offers the integrated training with "3-I" levels: **Intensive**, an in-depth level involving immersion in a year-long hands-on training and mentoring program; **Intermediate**, a mid-level involving participation in a variety of learning activities; and **Introductory**, a general level where sharing of information is the goal.

Our business team emphasized that a business plan is vital to succeed in an aquaculture venture. The aquaculture staff

Aquaculture Boot Camp participants toured the aquaculture genetics laboratory at the OSU South Centers.

The weather was perfect for touring the OSU South Centers aquaculture facilities on the first day of Boot Camp.

presented their professional backgrounds to our recruits. Everyone was amazed to hear the ABC recruits' backgrounds and expectations from our program. We are building a strong network of enthusiastic and committed farmers who later will become mentors of aquaculture in Ohio.

We would like to encourage our recruits to keep their enthusiasm and interest throughout the year. We also would like to invite everyone who wants to learn about the program to visit our website at http://go.osu.edu/abc.

The ABC program has begun with marvelous success thanks to the team work of the Aquaculture, Business and OSU South Centers teams, along with the Ohio Aquaculture Association. We appreciate everyone's efforts and dedication to this new project.

Acknowledgements

OARDIP is currently supported by these major contributors:

- USDA National Institute of Food and Agriculture (NIFA)
- North Central Region Aquaculture Center (NCRAC)
- Ohio Agriculture Research and Development Center (OARDC)
- The Ohio State University Extension
- Ohio Sea Grant
- Ohio Soybean Council

Recent Publications

Genetic improvement of yellow perch through commercial-scale marker-aided cohort selection for growth

Han-Ping Wang, Hong Yao, Paul O'Bryant, Dean Rapp, Laura Tiu and Geoff Wallat

Yellow perch *Perca flavescens* is a particularly important aquacultural and ecological species in the Great Lakes Region (GLR)and the Midwest USA. The demand for this species has remained very high in the GLR. Due to the relatively slow growth of currently cultured populations of yellow perch, the development of the yellow perch aquaculture industry has been hindered.

The goal of this project was to use a marker-aided cohort selection to improve the growth rate of the species and reduce their grow-out time to market.

To achieve this goal, approximately 800 broodfish from eight stocks representing major populations were obtained from eightstates (NC, NY, PA, ME, OH, MI, WI and NE). Those fish served as a base population for the breeding program. By performing cross-breeding of the base population, approximately 1500 fast-growing broodfish candidates were selected as a base generation from more than 100 families that were reared in communal ponds, based on both phenotypic and genetic data in two overlapping generations. More than 100 microsatellite markers were developed for the breeding program. Parentage analysis techniques using eight molecular makers in yellow perch have been developed. Five improved lines have been developed through three rounds of selections.

For each generation, approximately five hundred fish were selected and genotyped using microsatellites to construct a pedigree. Among the 500 fish, at least 100 pairs of the least related, with highest breeding value were selected and divided into five cohorts based on their pedigree. If the constraint on the rate of inbreeding could not be achieved, another batch of fish was genotyped and included in the total number of candidates. The selection lines were created by pair-mating 20 pairs within each cohort to found the next generation of improved lines. The individuals of average weight were chosen for the control line. This cohort selection has the advantage of preventing the top fish only from limited families.

On-farm tests of the improved lines of the third generation are being performed. Current research data shows that the improved lines grew 32.2% faster than unimproved fish in Year 1 in commercial farms. This improvement would make it feasible for yellow perch to reach market size within 14 months in pond culture conditions and in 9 months in recirculating aquaculture systems. In addition, two mapping families have been established and reared to maturation in tanks for future QTL mapping.

Abstract in the Proceedings of the World Aquaculture Society Conference. Page 1142. February 22-25, 2013. Nashville.

Physiological and molecular stress response of yellow perch subjected to handlings at different temperatures

Nour Eissa, Han-Ping Wang, Zhigang Shen, Hong Yao, Paul O'Bryant and Dean Rapp

Two experiments were conducted to explore the effect of handling stress on yellow perch (*Perca flavescens*) subjected to different water temperatures. The first experiment was to determine the optimum temperature for handling of yellow perch with minimal stress. Yellow perch were divided into three groups with four replicates and subjected to water temperatures

14, 20, and 26°C, then, acute handling was performed twice within separate intervals, in addition to a salt treatment at salinity of 5 ppt for each of the fish groups. Plasma samples were taken periodically for plasma cortisol concentration analysis, with cortisol used as a stress indicator. The second experiment was to explore the effect of handling stress on yellow perch larvae under different water temperatures and the ability of probiotics to reduce the stress. Three groups of yellow perch larvae were raised at three different water temperatures, 14, 20, and 26°C for 45 days. Each group was divided into three subgroups, the first subgroup was fed a probiotics incorporated diet, the second group was fed a basal

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Recent Publications - Cont.

Physiological and molecular stress response of yellow perch subjected to handlings at different temperatures, *continued*

diet, and the third subgroup was fed a basal diet and served as the control. All subgroups except the control group were subjected to handling stress for 10 minutes.Initial weight, final weight, weight gain, and mortality rates were recorded for all groups. For both experiments, total RNA was individually extracted from liver and muscle samples and the expression level of genes associated with stress response were quantified using quantitative real-time PCR.

In Experiment 1, the most important response was an increase in plasma cortisol after handling for all groups, but there was a minimal level of plasma cortisol in fish groups subjected to 20°C water temperature. It was concluded that the optimum water temperature for yellow perch handling and minimal stress was 20°C. Salt treatment after handling further stimulated the stress response and increased cortisol levels. In Experiment 2, the mortality rates after larvae handling stress were highestamong subgroups raised at water temperature 26°C, which reached 46.66% among stressed subgroup fed on basal diet, 16.66% mortality in those fed a probiotic incorporated diet and 23.33% in the unstressed control group. And lowest mortality rates among stressed subgroups exposed to 20°C were 3.33% and 16.66 % in the subgroup fed the probiotic incorporated diet and basal diet respectively. Stressed subgroups exposed to 14°C had mortality rates of 21.66% and 26.66 % in subgroups, which received the probiotic incorporated diet and basal diet subgroups respectively, in comparison to the control group, which showed zero mortality.

The experiment concluded that the best temperature for yellow perch larvae rearing and handling is 20°C as the results exhibited higher weight gain and lowest mortality rates under handling stress effect. Probiotics administration had marked effect to increase weight gain and decrease mortality rates in all groups, which can be considered an ideal alternative method to relieve stress instead using chemotherapy. The expression level of genes associated with stress response (heat shock proteins-70 and heat shock proteins-90, heat shock protein transcription factor 1, insulin like growth factor-1, myostatin,glutathione synthase, glutathione S-transferase, metallothioneins and calnexin) were analyzed.

Abstract in the Proceedings of the World Aquaculture Society Conference. Page 300. February 22-25, 2013. Nashville.

Genotype by temperature and hormone interactions on sex determination in bluegill *Lepomis macrochirus*

Zhigang Shen, Hanping Wang, Hong Yao, Nour Eissa, Paul O'Bryant, Dean Rapp

Bluegill sunfish (*Lepomis macrochirus*) is an important recreational and aquaculture fish species in US. Much interest has been generated concerning the development of monosex male populations of bluegill due to their more rapid growth capacity relative to females. The methods involved to develop monosex population require a comprehensive understanding of the underlying basis of sex determination and gonadal function with development of monosex male populations. Thermal and hormonal effects on sex determination in bluegill were observed in our previous studies. The present study was conducted to further determine the genotype by temperature and hormone interactions on sex determination in bluegill.

Three experiments were conducted using four different strains of bluegill sunfish: Hebron (HN), Dave (DE), Hocking (HG)and Jones (JS). Experiment 1 was to determine genotype by temperature interaction on sex determination in bluegill by treating those four strains with three temperature regimes of 16℃, 24 ℃, and 32℃ from 6 days post-hatching (dph) to 90 dph, each having three replicates. Experiment 2 was to determine the labile period of thermal treatments on sex differentiation and determination in bluegill by treating the HN strain with four different thermal times and durations: 0-90 dph, 6-90 dph, 25-90 dph and 40-90 dph, each having three replicates. Experiment 3 was to determine genotype by hormone interaction on sexreversal in bluegill by treating those four strains with two dosages of estradiol-17b (100 mg kg-1 and 150 mg kg-1) from 29 dph to 90 dph, each having two replicates. For experiments 1 and 2, larvae were reared in 25 L round tanks with flow water and aeration. For experiment 3, 29-day-old fry were reared in 400 L round tanks with flow water and aeration. Genotypes of the four strains were analyzed using microsatellite markers. After the experiments, sex ratios were determined by macroscopic and microscopic examination of gonadal tissue using the gonad squash or histological methods. Genotype by temperature and hormone interactions on sex determination in bluegill was systematically evaluated.

Abstract in the Proceedings of the World Aquaculture Society Conference. Page 1015. February 22-25, 2013. Nashville.

Recent Publications - Cont.

Evaluate accuracy of microsatellite DNA marker-based pedigree using known pedigree in yellow perch

Hong Yao, Han-Ping Wang, Aibin Zhan, Paul O'Bryant and Dean Rapp

Accurate pedigree information is essential for determination of genetic relationship among individuals over multiple generations for selective breeding programs. Lack of accurate pedigree information can potentially confound the effects for which a breeding program will be designed. Clear pedigree information permits to maintain the maximum of genetic diversity and genetic response for economic traits and minimize the potential detrimental effects of accumulated inbreeding. Recent works have demonstrated the potential of a panel of polymorphic microsatellite DNA markers in yellow perch (*Perca flavescens*) for analyzing genetic variability and structure of wild stocks and cultured populations. The aim of this study was to evaluate accuracy of the panel of polymorphic microsatellite DNA markers for parentage analysis in yellow perch by comparing the known pedigree.

A total of 69 sets of matings were designed from March to May in three different years (2006-2008). The female in each set was mated to one or two males, which made the maximum number of potential families 138 in total. For all parents, a fin clip was collected and stored in 95% ethanol at -80°C prior to further analyses. All parents and 100 5-day-old progeny from each set were sampled and stored in 95% ethanol for parentage analysis using the microsatellite panel. Software P-Loci was used to determine how many and which marker loci are required for accurate parentage assignment. All the male and female breeders were designated to be mated randomly to produce offspring for simulation using P-Loci. The number of offspring to generate for each cross was set at 100. The mating files were checked visually to rule out the duplicated crosses produced by randomly mating. All the data from all loci was input for computer simulation and the Min % Assigned to Parents was defined as 99. To determine how many loci can aid to assign offspring back to their families, the simulation was run in 10, 20, 50, 100, 150, 200, and 250 families randomly produced. For each family number, simulations were conducted 5 times. After simulation, the best marker panels were selected for true pedigree re-construction.

Using P-Loci, a large number of broodstock individuals were involved to assess the combined probabilities of offspring assigned and calculated over the eight polymorphic loci. The ability of the microsatellite loci to correctly assign parentage was evaluated. When marker data from 4 loci were combined, although a large number of offspring were created by the simulation program, the percent of correct parent-pair assignment was more than 85% in 200 families (20,000 offspring created by simulation program). When marker data from 6 loci were combined, the percentage increased to 95%. The microsatellite DNA marker-based pedigrees were compared with recorded pedigrees to evaluate the accuracy of the panel.

Abstract in the Proceedings of the World Aquaculture Society Conference. Page 478. February 22-25, 2013. Nashville.

OARDIP Economic and Scientific Impact

Ohio aquaculture has an estimated impact of $50 million annually. Aquaculture sales in Ohio have tripled from $1.8 million to $6.6 million in recent years. Nationally, Ohio ranks first in sales of yellow perch for food and is the number one bluegill producing state. Ohio also ranks fourth in sales of baitfish and largemouth bass sold for sport, and fifth in number of baitfish farms. Sixty-four journal articles and proceedings papers have been published by OARDIP, including four journal publications and six proceedings papers in 2012.

Meet our new employees: Estefanía James and Vikas Kumar

Estefanía M. James was hired in October 2012 to be a program assistant for the Aquaculture Boot Camp program. She assists Dr. Laura Tiu and the aquaculture team to design the academic curriculum and with the development of hands-on training modules. Prior to joining Ohio State University South Centers, Ms. James worked for several U.S. financial institutions as a financial advisor.

Ms. James has had a passion for aquaculture since she was a little girl, being influenced by her parents who worked for many years in the Ecuadorian shrimp industry. Estefanía holds two B.S degrees in Aquaculture and Business Administration from Escuela Superior Politécnica del Litoral (ESPOL) and Universidad Tecnológica Empresarial de Guayaquil (UTEG), Ecuador; and two M.S. degrees in Education and Training & Development from Marshall University.

Estefanía has worked in several areas of aquaculture, including as a quality control and handling of aquatic species specialist, culturing tilapia and shrimp in ponds and tanks, and production of microalgae. Ms. James learned very important lessons from one of the most devastating events and industry could face…the white spot syndrome (WSS). Even though this disease destroyed almost all the shrimp industry in Ecuador; it helped farmers to share and invest in technology and research. The industry learned to work as a team and support each other to overcome crisis and enhance the quality of the final products for local and international markets.

Ms. James believes that her prior experience in the aquaculture industry, business background, and academic preparation will highlight the Aquaculture Boot Camp initiatives at The Ohio State University South Centers.

Dr. Vikas Kumar from India is a Post-Doctoral Researcher in nutritional genetics and physiology of fish under Dr. Hanping Wang's supervision at OCARD, OSU South Centers. He received his Master's degree in Fish nutrition and Biochemistry from CIFE Mumbai, India. Thereafter, Mr. Kumar completed PhD thesis entitled *"Jatropha meal and protein isolate as a protein source in aqua feed"* from University of Hohenheim, Germany.

Before he joined the OCARD, he had been working as a Post-Doctoral Research Associate at University of Antwerp and involved with two projects. The first project was "Integrated performance trade-off in cichlid heads: *feeding versus mouth brooding*" and he worked mainly on the metabolic cost for the mouth brooding of Nile tilapia. He was also involved with a project related to combined effects of temperature, hypoxia, carbon dioxide and ammonia on comparative Fish Physiology: *putting feeding into the picture*.

Dr. Kumar has published about 40 papers (research and review) in peer-reviewed international journals and five book chapters, and he has been also acknowledged with many awards, such as: *Best Master Thesis, Best Overseas PhD Thesis and Best Overseas Fisheries Scientist*. Presently, Dr. Kumar's research work is directed toward the *cost effective processed soybean protein as a fish meal replacer in aqua feed* and to evaluate nutritional, physiological and expression pattern of related genes in fish. In addition, he is also involved with another project "*Identify genes which express differentially in response to environmental stress.*"

International Training Program

OARDIP's reputation in aquaculture genetics and breeding has attracted more than twenty scientists and international scholars to work in the Aquaculture Research Center and Genetics Lab at Piketon. Two of them completed and received their PhD in 2012. In 2012, OARDIP trained four PhD students and visiting scholars. They also significantly contributed to OARDIP's success.