Mastering Medaka Culture: Improved Techniques for Increasing Egg and Fry Production in Japanese Rice Fish (*Oryzias latipes*)

ABSTRACT. Aquatic systems are integral to ecosystem function and human health. By trapping sediments and retaining nutrients, wetlands protect our water quality. However, a wetland's capacity to trap sediments and nutrients also means they can capture many contaminants (e.g., heavy metals and radionuclides) released into the environment due to anthropogenic activities. Both heavy metals and radionuclides have detrimental effects on wildlife at acute dosages. However, little is known regarding underlying biological responses associated with chronic low dose exposure to both ionizing radiation and heavy metals. Our long-term research goal is to work to fill this knowledge gap using Medaka (*Oryzias latipes),* small fish amenable to experimental manipulation. However, the current study focused on altering the husbandry protocol of a laboratory colony of Medaka in a way that would increase colony size to at least 400 individual fish over a six-week period. At the beginning of our work, we followed a strict cleaning, feeding and egg harvesting protocol. Two weeks into our work, we suspected that food limitations may be negatively impacting egg production and noticed fungal growth on eggs. Thus, we altered the feeding regime and egg harvesting protocol. The altered protocol more than tripled egg production and almost doubled of fry production. By the end of our study the colony had 673 individuals: 191 adults and 482 fry. Our protocol changes resulted in growth of the colony at sufficient numbers to begin experiments related to investigating sub-lethal impacts of heavy metals and radionuclides on Medaka.

MELISSA THOMPSON is a senior at USC Upstate and is pursuing a Bachelor of Science degree in Biology. Her undergraduate research experience began in the fall of 2013 when she became a volunteer for the North American Amphibian Monitoring Program. In the spring of 2014, she began her research experience working with Automated Recording Systems that are utilized to record anuran populations among four wetlands in the Upstate. In the spring of 2015, she received an opportunity to participate in the first Radioecology Research for Undergraduates at the Savannah River Ecology Laboratory. Melissa spent her summer working under the direction of Dr. Olin E. Rhodes Jr. It was her experience at the Savannah River Ecology Laboratory that led Melissa to the decision of pursuing graduate school to further her endeavors and passion for animals.

MORGAN REED is working on a B.S. in Wildlife and Fisheries Biology at Clemson University. She is one of several students investigating freshwater stream ecology under the direction of Dr. Kanno at Clemson. As part of the REU, Morgan is working with Dr. Rhodes and Dr. Unger on a largemouth bass population and movement study. In addition, she is helping rear and maintain Medaka for use in future studies investigating radionuclide uptake and chronic low dose effects of radiation on wildlife. She hopes to attend graduate school after graduating from Clemson. Her major research interests are in whole system ecology, fisheries biology, and ephemeral wetland nutrient cycling. In her spare time she enjoys recreational fishing, hunting, and 'herping'.

DR. MELISSA PILGRIM is Director of Research and an Associate Professor of Biology at USC Upstate. She joined the faculty at USC Upstate in the Fall of 2006. Her primary research focus involves an integrative approach to investigating how ecosystems respond to environmental change (natural and anthropogenic). Her research program integrates field ecology, biogeochemistry (e.g., stable isotopes), and ecophysiology. She earned her Ph.D. from the University of Arkansas in 2005 and transitioned to a post-doctoral research position at the University of Georgia's Savannah River Ecology Laboratory (SREL). Many of her current research initiatives still involve collaborations with SREL faculty and students. In addition, she has an army of undergraduate students working with her in an undergraduate research group called Upstate Herpetology. Her publications range from book chapters in Herpetology volumes to scholarly articles in isotope, ecological, and herpetological journals. She currently serves on the Editorial Board for Herpetologica, as a Council Member of the South Carolina Academy of Science, and as the Upstate Regional Coordinator for the North American Amphibian Monitoring Program.

DR. SHEM UNGER is an Assistant Professor of Biology at Wingate University in North Carolina. He received his PhD from Purdue University's Forestry & Natural Resources Department in West Lafayette, Indiana. He is originally from the country of Panama where he grew up catching frogs in the tropics. His current area of research focuses on the life history and population genetics of the eastern hellbender populations, across the range of the species, as well as locally in North Carolina. He has presented his research at both national and local herpetological meetings and published in PLOS ONE, Journal of Herpetology, Ethology, and Southeastern Naturalist. While working with Melissa Thompson, he found her exceedingly well focused and committed to conducting original research while at the Savannah River Ecology Laboratory. She maintained a positive attitude even under difficult field conditions, and was an important part of the research team at SREL.

DR. OLIN RHODES, JR. is the Director of the Savannah River Ecology Laboratory where he works for the Vice President of Research at the University of Georgia and is responsible for providing leadership in the development, implementation, and evaluation of strategic goals, objectives, program initiatives and policies for the laboratory. He supervises research faculty and staff in the areas of vertebrate and plant ecology, biogeochemistry, and radioecology as well as all administrative staff in support units for the laboratory. His research interests focus on wildlife ecology and genetics, including studies of the genetic consequences of species reintroduction programs, the application of genetic tools for addressing issues in wildlife management, conservation, and disease ecology, the use of genetic markers to elucidate mating systems, movement behavior, and population structure of wildlife species, and sustainability of wildlife species in human-dominated landscapes with an emphasis on the resolution of human-wildlife conflicts.

1. Introduction

Aquatic systems are integral to ecosystem function and human health. Aquatic systems often harbor high species richness and benefit our communities by providing ecosystem services such as drinking water and food [1]-[2]. By trapping sediments and retaining nutrients, wetlands protect our water quality. However, a wetland's capacity to trap sediments and nutrients also means many contaminants are captured (e.g., heavy metals and radionuclides) and then released into the environment due to anthropogenic activities (e.g., energy production). Both heavy metals and radionuclides have detrimental effects on wildlife, such as developmental and neurological effects in the case of heavy metals [3], and macromolecule damage and evolutionary effects in the case of radionuclides [4].

Historically, research investigating the effect of heavy metals and radionuclides on wildlife has focused on organismal responses to single contaminants at acute dosage levels [5]. However, recent work has demonstrated that heavy metals intensify the effects of radionuclides. For example, a recent study investigating the acute genotoxic effects of ionizing radiation and mercury on earthworms (*Eisenia fetida*) found that DNA damage was increased after the combined treatments of mercury and ionizing radiation [6]. There is also a need to investigate the effect of sublethal exposure to multiple contaminants on wildlife, as such dosages reflect the actual environment exposure experienced by organisms. To help fill this knowledge gap, our long-term research goal is to investigate the sublethal impacts of heavy metals and radionuclides on Medaka (*Oryzias latipes*).

Medaka are small fish that inhabit brackish and freshwaters in Japan, Korea, and eastern China [7]. Medaka have emerged as a model organism well-suited for experimental manipulation in laboratory settings [8]. The fish are easy to maintain in the laboratory [9], have high reproductive output [10] and a known sensitivity to environmental contaminants [11]-[12]. In order to meet our long-term research goal, we needed to establish a large laboratory colony of Medaka. Thus, the focus of the current study was to maintain and expand a breeding colony of Medaka. Specifically, our study goal was to establish a husbandry protocol that would result in a breeding colony with a minimum of 400 Medaka individuals.

2. Methods

A. Research Site and Fish Housing:

We maintained the fish colony at the Par Pond Facility of the Savannah River Ecology Laboratory in Aiken, South Carolina. At the Par Pond facility we housed fish in Pentair Aquatic Habitat Units (AHAB units) and 10 gallon tanks. In total, we housed the fish colony in 3 AHAB units and 11 ten gallon tanks. Each AHAB unit contained 18 individual tanks (Figure 1).

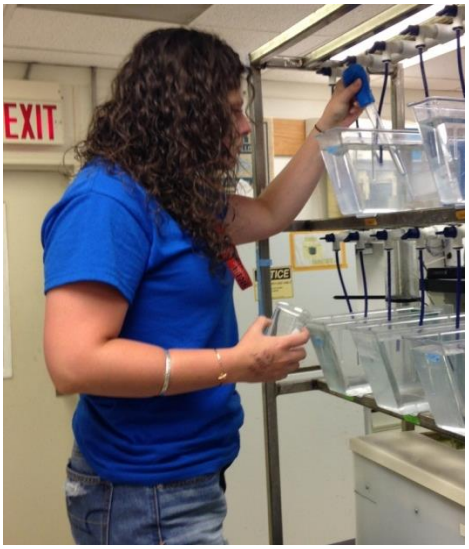

Figure 1. Example of Pentair Aquatic Habitat Units (AHAB units) used to house Medaka colony at the Savannah River Ecology Laboratory.

Proper water quality is essential to the health and growth of a fish culture [13]. The AHAB units and ten gallon tanks were closed systems that accumulated waste products and needed a source of aeration. To maintain the correct water quality in the AHAB units we performed 50/50 water changes weekly, changed filters every other week, and replaced filter media once each month. To maintain the proper water quality in the 10 gallon tanks we performed 50/50 water changes weekly, and 100% water changes bi-weekly. We used Topfin air pumps and stones as a source of fresh oxygen in the tanks; we replaced air stones a minimum of every 3 weeks. We used Simple Green as the cleaning agent for both AHAB units and 10 gallon tanks.
In the event of an individual mortality we followed a series of steps to preserve water quality in both AHAB units and 10 gallon tanks. Specifically, we removed the dead fish, treated the unit/tank with methylene blue for 30-60 seconds, removed tank mates from the methylene blue quarantine and placed them in a clean unit/tank. We performed a 100% water change for the entire system following the disinfectant treatment.

B. Starting Protocol: Feeding Regime and Egg Harvesting:

At the beginning of our study, we fed adult fish flake food daily and frozen brine shrimp every other day. In addition, we collected eggs daily. We checked for eggs in three places: on female bellies, at the bottom of tanks, or as a string of eggs coming out of fish mouths (if left with eggs, females will start eating the egg masses) [13]. Once located, we siphoned eggs from tank bottoms or netted eggs on females. We placed harvested eggs into communal bowls that contained a rearing solution and methylene blue. Use of the rearing solution is standard practice when trying to maintain developing Medaka eggs and encourage hatching [13]-[15], while methylene blue is used to inhibit parasites and fungal growth in fish colonies [13], [15]-[16]. Upon hatching, we transferred fry to separate 2.0 liter containers. Two weeks into our work, we suspected that food limitation may be negatively impacting egg production and noticed fungal growth on eggs (Figure 2). Thus, we changed our feeding and egg harvesting protocol.

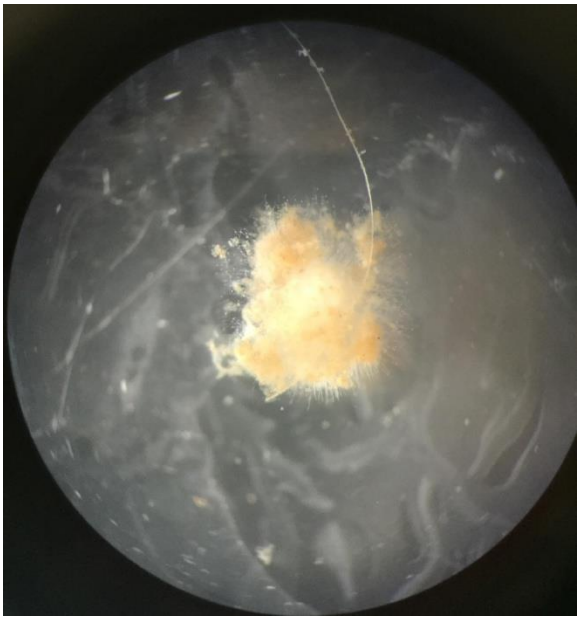

Figure 2. Water mold on an unhatched Medaka egg.

C. Altered Protocol: Feeding Regime and Egg Harvesting

To compensate for the suspected food limitation, we fed adults flake food, fresh brine shrimp and frozen brine shrimp daily. We did not change the protocol for collecting eggs (see above). In an effort to deter fungal growth, we changed how we harvested eggs. Specifically, we placed harvested eggs into a fine mesh net and rinsed the eggs under tap water (Figure 3). In addition, we individually separated eggs from the rinsed egg masses and placed them into daily incubation dishes that contained rearing solution and methylene blue. As with our initial protocol, we transferred fry to separate 2.0 liter containers.

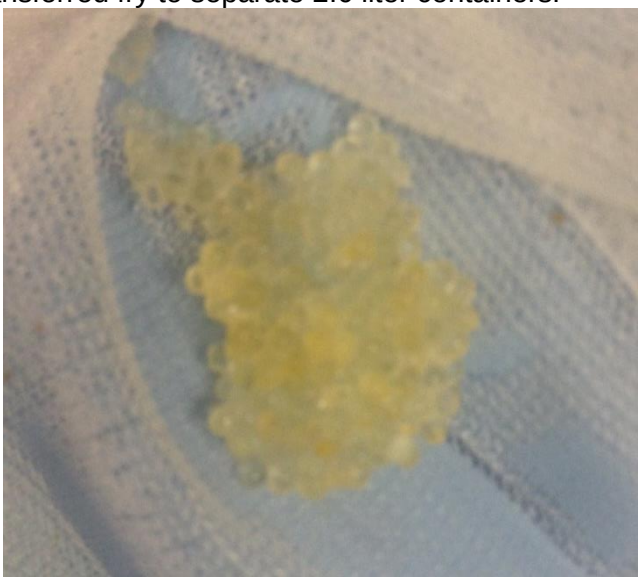

Figure 3. Freshly rinsed Medaka eggs in a fine mesh net.

3. Results

From June 14, 2015 to July 24, 2015 we harvested 7,161 eggs and 482 fry. Both egg production and fry production increased when protocol was changed. We calculated egg production on a per day basis and found that the altered protocol more than tripled egg production (Figure 4). We calculated fry production on a per day basis and found that the altered protocol resulted in an almost doubling of fry production (Figure 5). By the end of our study we were maintaining 673 individuals: 191 adults and 482 fry.

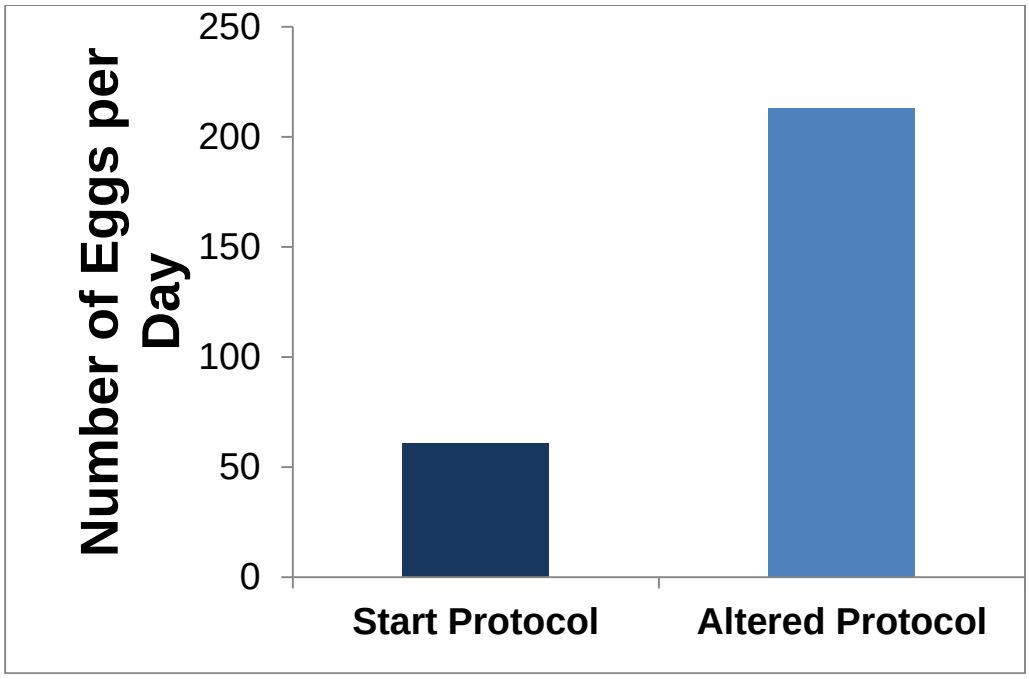

Figure 4. The number of eggs collected per day during starting protocol and altered protocol.

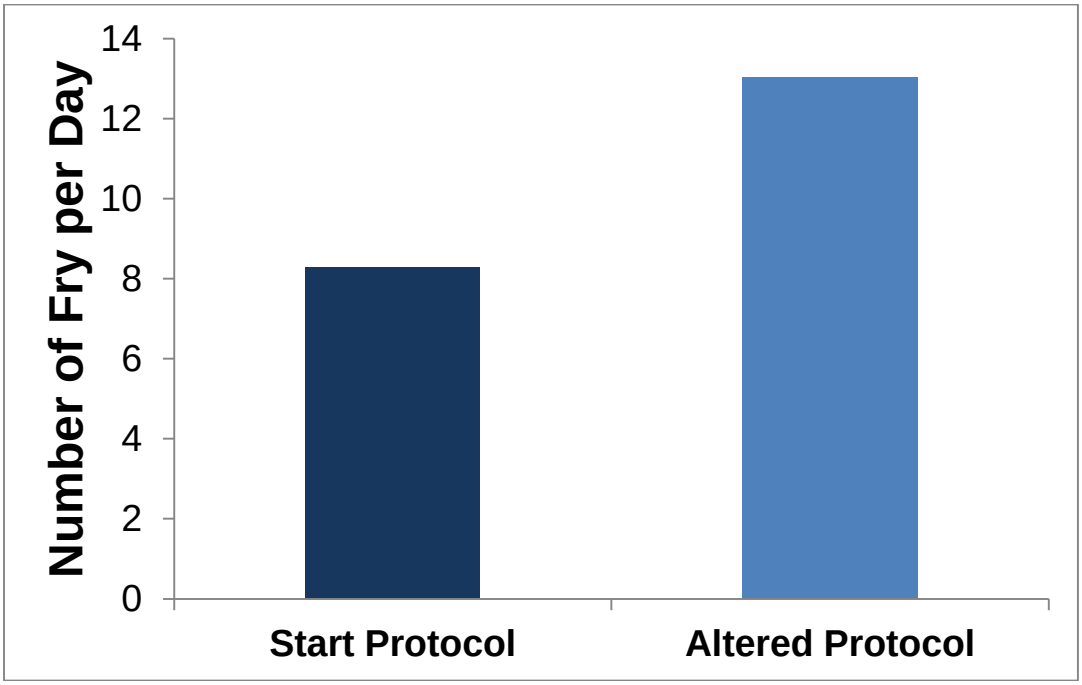

Figure 5. The number of fry hatched per day during starting protocol and altered protocol.

4. Conclusion

We more than met our study goal of increasing the Medaka colony to a minimum of 400 individuals. The increase in both egg production and fry production upon protocol change suggests that Medaka reproduction is dependent on adequate nutrition, while embryo development is sensitive to incubation environment (e.g., water quality and housing density). We were not surprised by our results, as other studies have documented environmental impacts on Medaka population growth and reproduction [10].

For example, [10] investigated the effects of variations in rearing density found that embryo density is inversely related to hatch rate and concluded that 1-2 embryos per incubation well were ideal. Therefore, the low hatch rates we observed using our starting protocol could be due to the high densities of eggs in the communal incubation bowls. As Medaka embryos develop release hatching enzymes to soften the egg envelope [13], [17]. The release of hatching enzymes could soften the envelopes of other embryos at earlier stages in the communal dish, inhibiting their development and reducing overall production of fry. Our switch from communal incubation bowls to daily incubation bowls reduced densities and ensured that Medaka embryos housed together were at similar developmental stages.

By the end of the 5 week period we had established a colony of Medaka large enough for experiments related to our long-term goal to be conducted. However, we think growth of our Medaka lab colony could further be increased by more closely monitoring temperature, photoperiod, and salt concentration of the rearing solution. Previous experiments observed optimal hatching success in embryos developing in a rearing solution with a salt concentration of 20 ppt and a temperature of 28°C [10]. The salt concentration of the rearing solution used in our work had a salt concentration of 1 ppt and the colony was housed at ambient temperature. To further improve the hatching rate the salt concentration of the rearing solution should be increased to 20 ppt and the temperature should be monitored and maintained at 28°C.

Furthermore, [18] observed optimal reproductive success with a 16L:8D photoperiod and water temperature of 25°C. Thus, to further improve egg production we suggest maintaining water temperatures of the broodstock at 25°C. We used a 15L:9D photoperiod during our study. However, AHAB units were located in front of windows that could have affected the actual photoperiod experienced by the broodstock.

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