**Research Proposal to the New York City Science and Engineering Fair**

**(NYCSEF 2015)**

**Submitted by**: Cézanne Bies, Zain Khalid

**Grade:** 11th Grade Students

**Teacher**: Mauricio González

**Research Draft Date**:

**School**: The New York Harbor School

**Mentor**: Prof. Matthew Hare, Cornell University, College of Agriculture and Life Sciences

***EVALUATION OF CRASSOSTREA VIRGINICA FROM THE GENETIC LINES OF CULTURED AND NATIVE LARVAE FOR GROWTH AND SURVIVAL***

***RATE IN THE NYC HARBOR***

**INTRODUCTION**

New York City’s ground and surface waters are being contaminated with heavy metals, persistent organic pollutants (P.O.Ps), and nutrients that have an adverse effect on human health (© TERI, 2007). However, oysters act as nature’s biofiltration system, each providing ~50 gallons of purified water per day (Merrill, 2014).  New York once had up to 350 square miles of oysters (Nigro C., 2011), producing roughly about 300,000 bushels a year. Today, that population has substantially deceased due to the bacterial contamination, overharvesting, pollution and sewage. Only a sparse number of eastern oysters (*Crassostrea virginica*) remain in the NYC harbor, and that damage cannot be fixed without proactive conservation efforts.

**Research Objectives**

The purpose of this study is to conduct a comparative genomic analysis of both the cultured and wild larvae to assess which strain has a better growth rate and mortality rate.

The three specific objectives are:

1. to evaluate genetic variation in an aquaculture strain and native Eastern oysters (*Crassostrea virginica;*
2. to determine which strains mortality rate in the Hudson River has a better growth rate; and
3. understand how *Crassostrea virginica* interacts with stressors in its environment. This involves the monitoring and testing the water quality, salinity, and weather patterns*.*

**Hypotheses**

As major restoration efforts have been made to genetically restore oysters with programs developed by The Urban Assembly New York Harbor, the Hudson River Estuary (HRE) and others, it is believed that there are genetic differences between the wild and hatchery groups.  It is believed that these differences can be used to genetically modify future hatchery oysters, which we believe will make them more durable and resilient to the harsh conditions of the New York waterways. In this project, our team hopes to find differences between the cultured oysters and wild eastern oyster’s barcoding and mortality rates.

We hope our findings will show the diversity in the oyster population, and predict the adaptability of the Eastern oyster species inside the Hudson River ecosystem. This is of great importance because it could be the start of eastern oyster’s survival. With the oyster’s survival, this could also be a vital step to helping the New York Harbor improve from the water pollution it currently faces -- native Eastern oysters are nature’s natural filters. What this means is as the oysters suck in bacteria and plankton for food, they also filter toxins left by trash and harmful chemicals of the city. With this analysis of barcoding, we can be able to change the environment so many call home.

**Research Schedule**

The research project will proceed in a series of five phases. During the first phase, the team will select

**Project Start Date: 9/1/14 Project End Date: 5/15/15**

Detailed chronogram of major due dates – A CAS 110

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| **Milestones** | **Assignment** | **Sept.** | **Oct.** | **Nov.** | **Dec.** | **Jan.** | **Feb.** | **Mar.** | **Apr.** | **May** | **Jun.** | **Action Taken** |
| Phase I | Mentor Search | X | X |  |  |  |  |  |  |  |  | 10/23/14 Email Dr. Matt Hare – Approved request 10/24 |
| Phase I | Formulation | X | X |  |  |  |  |  |  |  |  |  |
| Phase I | IRB - Results | X | X |  |  |  |  |  |  |  |  |  |
| Phase I | 5 PRJA/Presentation | X | X |  |  |  |  |  |  |  |  |  |
| Phase I | Analysis of results | X | X | X | X | X | X | X | X | X | X |  |
| Phase I | 1st draft | X | X |  |  |  |  |  |  |  |  | Completed |
| Phase I | 2nd draft |  |  | X |  |  |  |  |  |  |  | Completed |
| Phase I | 3rd draft |  |  | X |  |  |  |  |  |  |  | Completed |
| Phase I | 4th draft |  |  |  | X |  |  |  |  |  |  |  |
| Phase I | 5th draft |  |  |  |  |  |  | X |  |  |  |  |
| Phase II | 6th draft |  |  |  |  |  |  |  | X |  |  |  |
| Phase II | 7th (final) draft |  |  |  |  |  |  |  |  | X |  |  |
| Phase II | Poster board drafts |  |  |  |  |  |  |  | X | X | X |  |
| Phase III | Digital Presentation |  |  |  |  |  |  |  |  |  |  |  |
| Phase III | Final Presentation |  |  |  |  | X | X |  |  | X |  |  |

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| **Milestones** | **Assignment** | **Start On** | **Due Date** | **Approved/ Completed** | **Status** |
| Phase I | Mentor Search | September | 10/23/14 | 10/24/14 | Completed |
| Phase I | Draft Proposal to M. Gonzalez | September | 10/28/14 |  | Pending |
| Phase I | Proposal Review to M. Hare | October | 11/5/14 |  | Pending |
| Phase I | Anticipated approval of proposal |  | Mid-Nov. |  |  |
| Phase I | Solicit volunteers for project  (3 to 5) | Mid Nov. |  |  |  |
| Phase I | Submit Final Proposal to the NYC Science and Engineering Fair  (NYCSEF 2015 |  |  |  |  |
| Phase II | Collect doz. oysters for each group | March |  |  |  |
| Phase II | Take specimens and place in tanks | March |  |  |  |
| Phase II | Breed Oysters | March |  |  |  |
| Phase III | Transports juvenile oysters from tanks to crates | January |  |  |  |
| Phase III | Deposit crates into in Hudson River (Areas TBD) | March |  |  |  |
| Phase III | Randomly selection of parents of the two larvae strains will undergo genetic analysis | Dec. -  March |  |  |  |
| Phase IV | Water quality, mortality rates, salinity and weather will be surveyed | April - May |  |  |  |
| Phase V | The surviving oysters will be randomly selected for genetic analysis | May |  |  |  |
| Phase V | Submit Final Report and Digital Presentation | May | 5/15/15 |  |  |

**Research Site**

To be determined

**Methods**

Collect mature oysters *Crassostrea virginica* of reproductive age both wildly caught and farm grown. Proceed to breed both types in same salinity tanks heating the water to give a “pseudo-environment” for breeding. Collect both strains after breeding and wait for the larvae to settle (on concrete tiles, dead shells, limestone flakes, etc.). Once in juvenile state (when the oyster has successfully cemented itself), both wild and farmed oysters will be packed into 2 crates and put into the same location in the Hudson

Collect mature oysters *Crassostrea virginica* of reproductive age both wildly caught and farm grown. Proceed to breed both types in same salinity tanks heating the water to give a “pseudo-environment” for River. Throughout the year, mortality, salinity, stressors, and weather will be measured. After, DNA will be extracted and observed to see which population is best suited for the Hudson.

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| **Materials** | **Usage** |
| * Pestles | * To grind up tissue samples |
| * Razors | * To cut a sample of tissue |
| * Forceps | * To retrieve the tissue |
| * Micropipettes, (p1000), (p100), (p10) | * To suck up aliquots |
| * Waste beaker | * To remove possible toxic chemicals from surfaces |
| * Tips for pipettes for each for each size | * Avoids cross contamination |
| * Napkins | * For sterilization |
| * Safety goggles | * To protect eyes from strong UV lights |
| * Tube rack | * To store the micro centrifuge tubes |
| * 1.5 ml micro centrifuge tubes (2 for each sample) | * Contains aliquots and samples of extracted DNA |
| * Water bath (65 and 35 C) | * Heats the tube to a perfect temperature to denture proteins and enzymes to digest DNA |
| * Micro centrifuge | * Spins the tubes for one minute to push insoluble materials down to the bottom of the tube |
| * Nuclei lysis solution | * Dissolves membrane bound organelles including the nucleus, mitochondria and chloroplast |
| * Ice bath | * Chills tubes |
| * RNA Solution | * Degrades RNA that could interfere with PCR |
| * DNA rehydration solution | * Rehydrates aliquot |
| * Protein Precipitation Solution | * Concentrate proteins and purify them |
| * 2 Moshnets screen-boxed bottoms | * To contain Oysters in |
| * Temperature monitors | * To measure temperature of oysters |
| * Rope | * To secure moshnets |
| * 2 Nursery tanks (wild, farmed) (wild, farmed) | * To hold larvae strains and breeding oysters |
| * Water heater | * To incubate Nursery tanks |
| * Micron powder/Dead oyster shells/Tiles | * For the larvae to set and cement properly |
| * Water Quality Testing Kit | * To test the quality of the water |
| * Plankton, algae particles | * Food for the oysters |

**Research Competence of the Student**

**Cézanne Colette Bies**, a junior-year scholar attending The Urban Assembly Harbor School. A recipient of the 2014 Marine Biology Genetics Award. Preparation for this research includes coursework in oyster genetics with Dr. Antonia Florio at The Museum of Natural History and Drs. Cinda Scott and Prof. Matt Hare served as a mentor for independent summer research in intermediate science research earning two college credits with the University of Albany. During Fall 2014, I will take a second course in intermediate methods of research, under the direction of Mauricio Gonzalez and continued guidance of mentor Prof. Matthew Hare with the Natural Resources Department at Cornell University.

Other areas interests include discussions on the *Caulerpa taxifolia* invading the European waters, how we can remove the pollution from the Hudson with the means of oyster beds, how hydropower can replace the means of fossil fuel, and is a strong advocate of genetic engineering in animals. Academic programs attended include: National Hispanic Institute: The Great Debate 2013 (Villanova U) 2014 (Elmhurst College), Brown University Leadership Program (2010-2013), Notre Dame HS. Member of NY Harbor Seals, Atlantic Sailing Team, and the youngest member of the New York Writers Room.

**Zain Khalid** was born in Pakistan and came to the US 2 years later. In elementary school he worked hard and he continued to do so until he got to the 6th grade. During 7th grade his grades dropped, barely passing his classes. Zain has been very active in extracurricular activities such as the art club, sports club, 7th grade VP (vice president)… the list goes on. From these hobbies, he applied to harbor school. On his senior year in middle school, he got accepted and currently goes to the Urban Assembly Harbor high school. The first year was very difficult, being a freshman in high school who had a lot going on in school. When he had to move out in the middle of the school year and get settled into a new house, he worked hard after school with boatbuilding and gardening. His current CTE he studies is the Marine Science Research Lab where the class studies DNA, Water Beds, and the improvement of marine ecosystems. When offered the opportunity to work on the Urban Barcode Project he quickly took the chance. Zain is a hard worker, and takes every project with the upmost seriousness, always being prepared to work.

**Significance of Proposed Research**

This research will contribute to a deeper understanding of how sequencing oyster and will affect present and future conservation efforts. The main objective is to examine and develop a reliable and simple method, using innovative DNA barcodes, for understanding the individual differences between the currently cultured oysters versus the native eastern oyster in the Hudson waters.

By determining and understanding growth and morality changes of the eastern oyster, the study has practical applications for additional research in conservation and restoration effforts of the NY Hudson River.

Our specific aims are to:

* Find if a farmed domestic oyster that has never been outside a laboratory tank and has a different set of DNA than its original native species.
* Show the diversity in the oyster population, and predict the adaptability of the Eastern oyster species inside the Hudson River ecosystem.
* Track changes that may have taken place in its genome.
* Identify the presence or absence of such parasites in order to gain and inform the conservation efforts that are being implemented.

**Works Cited**

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