Roger Williams University
Undergraduate Research Proposal
Due November 15, 2007

Title of Proposed Research Project: Monitoring Predation on Reef Balls® Remote Set with Eastern Oysters (Crassostrea virginica) in the Mount Hope Bay

General Area of Research: Oyster Restoration

Name of Applicant(s): Natalie Huey
Two students maximum — Principle Student Investigator (PSI)

Class Level: Junior
Senior / Junior / Sophomore / Freshman — PSI

Date of Submission: 11/15/2007
mm/dd/yyyy

Faculty Mentor Dr. Timothy Scott

Amount of Funding Requested: $ 603.00 (maximum request: $600)
Round up to nearest whole dollar

Please check all that apply.

Is this a proposal for a senior thesis?	Yes: ☐	No: ☒
Is this a proposal for a CEED student research grant?	Yes: ☒	No: ☐
Is this a proposal for the Mark Gould Fellowship:	Yes: ☐	No: ☒
Are any permits required for the proposed work?	Yes: ☐	No: ☒
Has this proposal been submitted or funded before?	Yes: ☐	No: ☒
Do you grant permission to post your name, abstract, and the award amount on the Internet?	Yes: ☒	No: ☐

Abstract (1200 characters maximum)

The observation of Reef Balls® with oyster restoration has not been studied in the Northeast Atlantic but has had positive effects in their use in other areas. The Eastern Oyster (Crassostrea virginica) is an important fishery in the Northeast Atlantic. Restoration efforts have been underway for many years in order to replenish the natural population which has been declining due mostly to disease. Reef Balls® are a new technology that have shown promise in restoration efforts. This study focuses on the impact of predation on the oysters remote set on the Reef Balls®. The observation of predators will offer more information on the survival of the set oysters. This project will identify and quantify the predators of the oyster Reef Balls® and monitor the survival of the oysters. The location of the Reef Balls® near a large structure will show a larger number in the amount of predators found and therefore, I expected that more predation will occur closer to the learning platform due to the vertical nature of the platform. The Reef Balls® offer a duel benefit because they are a habitat for reef fish as well as offer a cultch for oysters to set on.

Signatures:

Principle Student Investigator Date — Mentor Date

Please email a completed copy of the proposal by November 15 to **jlemire@rwu.edu**.
In addition submit one signed copy to Pat Pimental in MSN office area by November 15.

Project Description:

Eastern oysters (*Crassostrea virginica*) populate an 8,000 km range in the western Atlantic from Brazil northward through the Caribbean and Gulf of Mexico and to the St. Lawrence estuary in Eastern Canada (Carriker and Gaffney 1996). *C. virginica* can live up to ten years and are protandrous hermaphrodites, existing as males until the third or fourth year of life, when about half turn to females. A single female oyster can release up to 10 million eggs at a time. Once fertilized, an egg becomes a swimming larva and about ten days a pediveliger will develop and settlement occurs (figure 1). Once settled it will remain in the same location for the rest of its life (Matthiessen 2001).

Bivalve populations are known to provide numerous ecosystem services such as nutrient cycling and habitat for finfish and other marine organisms (Dame 1979). As filter feeders, bivalve mollusks derive most of their nutrition from filtering particles from the water and can remove phytoplankton, sediment, and detritus, thereby reducing turbidity and improving water quality. Oysters contribute to ecosystems by controlling eutrophication, recycling nutrients, and providing habitat for invertebrates and fish (Soniat et al 2004). Aside from having enormous filtration capacity, oyster beds significantly contribute to biodiversity, food webs, and essential fish habitats (Dame, 1979; Newell, 1988; Mann 2000; Rice et al 2000, Newell 2004; Newell et al 2005; Coen et al 2007). Economically, oysters have been one of the most commercially important fisheries, bringing in 3.6 million pounds valued at $15.5 million in 2000 (NMFS 2001).

Along the Atlantic coast and in Rhode Island waters *c. virginica* populations have dwindled due to protozoan diseases MSX, (*Haplosporidian nelson)* and Dermo, (*Perkinsus marinus)*, habitat degradation, sedimentation, fishing pressure, industrial pollution, harmful algal blooms and hurricanes (Rothschild et al. 1994; Mackenzie 1996; Gomez-Chiarri et. al.,1999; Brumbaugh et al 2000; Rice et al, 2000; Nestlerode et al 2007). Programs conducting oyster restoration for economic and ecological purposes are underway in many Atlantic and Gulf Coast states (Breitburg et al 2000; Office of Water, Rivers Corridors & Wetland Restoration, EPA, Community Based Restoration, NOAA websites).

Remote setting is a common and widely accepted method used in oyster restoration projects. Remote setting is an efficient and cost effective method to produce millions of oysters set onto substrate and shell bags are traditionally used as substrate for oyster attachment (Jones and Jones 1988; Bohn et al 1988; Supan et al 1999). Oyster remote setting began on the west coast of the US and recently has been used for restoration purposes in the Northeast Atlantic including the Narragansett Bay (Marcotti 1997; Tammi 2002; Ryan et al 2004; Gilcrist et al 2004; Marino et al 2005). Single oyster culture is generally not applicable for the purposes of restoring and enhancing the wild population due to cost constraints and lack of sufficient shell material for habitat stabilization. Natural oysters commonly grow on large clusters attaching to hard substrates and other shells. These aggregates form large oyster beds and reef structures providing suitable habitat for other marine organisms as well as providing a continual substrate for the settlement of oyster larvae (Dame, 1979; Mann, 2000).

In order to maintain oyster reefs and large-scale oyster grounds, many state and federal agencies have implemented mandatory clean cultch programs (Volk 1994). Often oysterbeds can experience siltation and sedimentation which may result in the suffocation of adult oysters as well as reduce the availability of suitable habitat for the settlement of larval oysters (Soniet et al. 2004). Newly planted cultch material provides a clean surface for the larvae to settle, attach and grow and improves the overall health and condition of the oysterbed. As a result of the widespread oyster restoration along the Atlantic coast, shell material has become scarce. Furthermore, some states have implemented moratorium on the importation of shell from other states as to prevent the spreading of MXS and Dermo (Busek et al. 2004). As result, many types of shell and other artificial substrates are being used as cultch for oyster restoration efforts (Coen and Luckenbach 2000; Nestelrode et al. 2007).

New artificial reef technology includes Reef Balls®. Reef Balls® are hollow cement rounded balls that are formed using a fiberglass mold with holes in the sides (Reef Ball Foundation® 2007). The initial concept of the Reef Balls® was to provide shelter to reef fish. A relatively new practice is to use this technology with remote setting oysters for restoration projects (Umberger 2000; Maryland Environmental Service 2004; Pohl et al. 2007). Reef Balls® can be used just as easily, and may be more efficient and provide a vertical substrate which is beneficial to both invertebrates and finfish that utilize the habitat (Pohl et al. 2007; Daigle et al. 2007). Soniat et al (2004) showed that vertical substrate allows the oysters to be less affected by sedimentation. It may be that Reef Balls® offer a better habitat for oysters because of the vertical orientation.

Florida and Maryland are two states where oyster Reef Balls® are used for oyster restoration and pollution remediation. In Maryland, Reef Balls® were chosen for their duel benefit of habitat and as settlement substrate for oysters. The objective of this project was to provide habitat, restore fish and fishing areas, increase the oyster population, and the Reef Balls® reduce the demand for shell as cultch. Reef Balls® were installed in 2004 and monitoring of the sites shows that oysters survived and have utilized the Reef Balls® for settlement substrate. After about a year they were covered in oysters making the Reef Ball® unrecognizable (Blankenship 2007). In 2006, monitoring of the Reef Balls® observed that natural oyster spat fall had occurred (Maryland Environmental Service 2006). It was determined that 90% coverage was seen on many of the Reef Balls® and over a year the oysters grew drastically. Also, quantification of the habitat is ongoing but monitoring shows that many species of invertebrates and finfish are utilizing the Reef Ball® habitats (Maryland Environmental Service 2006). The Maryland project is meeting many of its objectives with respect to oyster survival and habitat utilization.

In South Tampa Florida, the oysters were placed in the canals along the waterfront in St. Petersburg in order to filter the water , absorb the nitrogen and ammonia runoff, and also to attract other marine organisms (Umberger 2000). The objectives of the project were to bring back the natural habitat that was destroyed by the building of houses. Oyster Reef Balls® were chosen to bring back sea birds, filtering of sea water and consequently allow light to penetrate and allow the growth of sea grass. The oyster Reef Balls® were also chosen to help stabilize the ground sediment and increase turbidity. These oyster communities also attract fish for local recreational fishing (Clark 2000). Monitoring is ongoing and the benefits from this project are improved water quality, habitat and public awareness (Clark, 2006).

Predation can be a major problem at times and can impact the population significantly. The common starfish (*Forbes asterias*) feed on many benthic marine invertebrates such as mollusks and barnacles (Mackenzie and Pikanowski 1999). Starfish cause a great decrease in the oyster population specifically the spat (White and Wilson 1996). Starfish spawn right before oysters and can wipe out a years worth of oyster spat if there is a good survival rate for the starfish (White and Wilson 1996). Starfish are notorious for their selectiveness when it comes to size because they choose smaller oysters. This relationship between starfish and bivalves play a significant role in marine habitats (Norberg et al. 1994). Oysters that are too small will be ignored by predators and oysters that are too big will not be worth the effort for the predators. Crabs also impact the oyster population significantly as they also consume many oysters, favoring the smaller oysters (White and Wilson 1996). Crabs are responsible for much of the oyster mortality along the Atlantic coast. The potential predators that are expected to be seen are include *Forbes asterias, Panopeus species, Libinia emarginata, Carcinus maenas, Cancer irroratus, Hemigrapsus sanguineus, Urosalpinx cinerea*. To date, predation has not been studied in conjunction with oyster Reef Balls®.

Pohl et al. (2007) experimented with remote setting oysters onto Lo-Pro Reef Balls®. The purpose of this project was to investigate the feasibility of settlement of oysters onto Reef Balls®. The results of this project was that oysters in fact did set on the oysters but due to the low number of larvae used and the lack of following field work oyster survival was not easily determined. However, predators were observed in the vicinity of the study area (Daigle 2007).

My project will identify and quantify the predators (*Forbes asterias, Panopeus species, Libinia emarginata, Carcinus maenas, Cancer irroratus, Hemigrapsus sanguineus, Urosalpinx cinerea*) of the oyster Reef Balls®. Oyster Reef Balls® will be placed at two locations within the vicinity of the RWU learning platform. One will be located close to the learning platform and the other will be placed farther out into the bay as a control site in order to determine if predation varies near manmade structures. There will be three treatments per site with two Reef Balls® per treatment for a total of 12 Reef Balls®.

Will there be a difference in the intensity of predation from one location of the next? The hypothesis to answer this question is that location has an effect on the abundance of predators. The second question is will there be more predators at the Reef Balls® near the learning platform? The hypothesis is that there will be more predators will be observed near the learning platform. This study will also record the success of using remote setting to set the oysters on the Reef Ball® by monitoring the growth and survival of the oysters, which are both dependent on the amount of predation. If Reef Balls® are determined to be very successful; there will likely be widespread application of this method for enhancement projects that involve finfish and shellfish. Additionally, investigation of predation on these structures is necessary before expanding Reef Balls® for large scale oyster restoration.

Materials and Methods:

Oyster Broodstock Conditioning and Larval Care

This project will be executed in the spring 2008 or in the early Fall 2008 semester, provided that Reef Ball® molds can be acquired. Oyster spawning and production begins with the conditioning of the broodstock.

This process will take 6-8 weeks and the net temperature of the water is increased 1ºC per day. During this time they are also being fed a mixed algal diet. They will be fed an algal diet via six 20L drip buckets that will be filled two times daily with a mixed diet of live *Isochrysis*, *Tetraselmis*, and diatoms (on the weekdays) and Reed Mariculture Shellfish Diet 1800® (on weekends). After the conditioning timeframe, each individual oyster will be separated into a glass dish and spawned. The thermal induction process is used, whereby the oysters are submerged into heated filtered sea water and switched between heated and chilled water every thirty minutes. *Chaetoceros* is fed to the oysters at this point. Once the gametes are released they are observed under a microscope in order to determine the sex by viewing eggs and sperm. The gametes are collected and separated into labeled buckets.

Each egg bucket is counted and fertilized with 0.5 mL of sperm per liter of the egg bucket with each bucket containing 20-80 million eggs. Each egg bucket is transferred into a 1000L tank filled with heated filtered sea water. Two air stones are provided but no food is needed. The oysters remain in larval stage for 7-14 days. The water is changed completely on alternating days beginning with day one. A 60 µ screen on top of a 35 µ screen is used to catch the siphoned larvae. The larvae are transferred from the screen and counted and inspected for development. The tank is cleaned, refilled, and the larvae are replaced according to larval size and location that it was siphoned from (top or bottom of tank). For the first five days their diet consists of 100% *Isochysis* and later switched to 50% *Isochysis* and 50% diatom. They are fed twice per day, with a volume calculated by the amount of animals in the tank and the stage of the larvae.

Settlement will occur within 7-14 days after fertilization. The larvae are inspected daily and when a food appears the larvae will soon be ready to set. The larvae are then removed from the tank using the siphon and screen method and are counted. They are placed in the refrigerator overnight. The following day 10 L of filtered sea water will be used to acclimate the larvae. Six Reef Balls® will be placed into 1000 L tanks. The larvae is added and inspected for settlement after 3-5 days. At this time the water is changed and the oysters are fed a mixed algal diet and after about 3 days can consume algal paste.

The Reef Balls® with set oysters will remain in the tank for 14 days post setting until the oysters are about 1 mm and can be easily seen with the naked eye. The Reef Balls® will be placed in a raceway with flowing seawater and more food. The oysters will be counted for an initial number before being moved to the study sites.

Reef Ball Molds

In order to make the Reef Balls®, a Reef Ball® mold will be purchased from the Reef Ball Foundation® (www.reefball.org). Concrete will be poured into this fiberglass mold to create a Reef Ball®. Once the concrete is set the Reef Ball® is removed from the mold and is ready for use. The molds should be purchased and made well in advance of the setting (about 4 months before) because each Reef Ball® needs to set long enough for the concrete to cure.

It is important to assure that all the predators are accounted for when the reef balls are pulled up for monitoring, a mesh sheet will cover the ground below the Reef Ball® weighted down with weights. This mesh will have 1-2 mm holes and will be tethered to a buoy system which will envelop the Reef Ball® when it is pulled up. This mesh will collect any organisms that fall of the Reef Ball®. All of the collected organisms will be identified, measured and counted.

Field Monitoring

Once the oysters are set to the Reef Balls® by the remote setting process, the Reef Balls® will be placed out in the field in two specified locations. These locations are off of the learning platform and farther out in the bay away from the learning platform but manageable by boat. There will be 3 treatments per location with each treatment containing 2 Reef Balls® for a total of 12. They will be monitored daily for the first 30 days and then weekly from spring 2008 to fall 2008. The data collected includes the identification and quantification of each species living on, in, or around the Reef Ball®. The species of interest include *Forbes asterias, Panopeus species, Libinia emarginata, Carcinus maenas, Cancer irroratus, Hemigrapsus sanguineus, Urosalpinx cinerea*, which are all potential predators of the Reef Ball® oysters. Growth and size (in mm) of the oysters that are growing on the Reef Balls® will also be measured. Mortality of the oysters will be measured by counting empty shells and in the case of the shell being missing the white patches from where the oyster had been. The live oysters will also be counted and measured. A qualitative index will be determined to measure macro algae. A YSI will be used to determine the dissolved oxygen, temperature, and salinity of the water during the field monitoring.

Statistical Analysis

I will use a repeated measured analysis of variance (ANOVA) to distinguish differences between locations over a timescale. The oyster survival data and the number of predators data collected will be used in the ANOVA.

Laboratory Space:

I am requesting the use of the Roger Williams University CEED Shellfish Hatchery area, tanks, and materials for the execution of this project. I am also requesting the use of the boat for the conducted field work that is located away from the learning platform.

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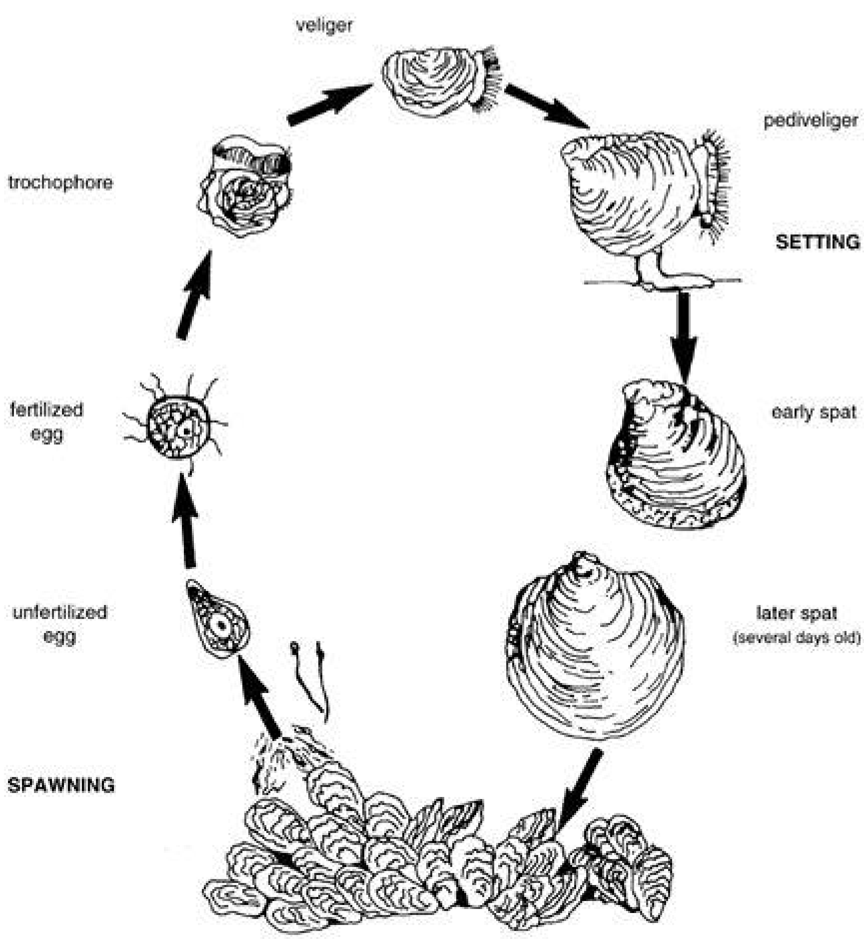
Appendix:



Lorio W.J and Malone S.1994.

Figure 1: The life cycle of the Eastern oyster (*Crassostrea Virginica)*

Natalie E. Huey
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Work Experience

Roger Williams University, Bristol, RI   September 2005 – Present
Shellfish Hatchery/ Wet Laboratory- Cultured and maintained algae for feed source for research projects and shellfish culture; spawned shellfish and took care of larvae, maintained lab tanks, equipment and glassware.

Bath & Body Works, Danbury, CT   Summer 2006
Sales Associate

Ridgefield Academy (Camp Ridgefield), Ridgefield, CT   Summer 2006
Summer Camp Counselor for "Camp Ridgefield"; Watched a group of 20 seven to nine year old children all day including helping them with craft projects, computers, theater, sports and swimming.

Ridgefield Academy, Ridgefield, CT   2004 - 2005
Supervised children in the After School Program including helping with homework subjects.

Education

Roger Williams University, Bristol, RI
Double Major in Marine Biology and German with an Environmental Science minor
Graduate of Ridgefield High School – 2005 GPA of 3.8

Honors and Awards

President's Award for Outstanding Academic Excellence, Honor Roll, Awards for Excellence in Honors Earth Science and Astronomy and awards for achievement in Honors Algebra I, English IV and Psychology from Ridgefield High School.

Community Service

Appalachia Service Project (ASP)
Summer 2004- Member on a church group team that performed home repairs for the needy families in the Appalachia area (Kentucky)

Senior Girl Scout Troop 482
13 years of service with Troop 482
Activities included sewing a quilt for needy infants, creating and selling ornaments to benefit the Ridgefield Animal Shelter, and providing holiday food baskets to needy families.

Activities and Clubs

Member of the Marine Science Club
Campus Entertainment Network – Movie Committee
German Club – Club treasurer

Itemized Budget:

Note: there is a $600 maximum for budget requests

Principle Student Investigator:	Natalie Huey
Title of Proposed Research:	Monitoring Predation on Reef Balls® Remote Set with Easter Oysters (Crassostrea Virginica) in Mount Hope Bay

Itemized List of Supplies:

Item	Cost Per Item	Total Cost
1. Oyster Reef Ball® Mold	$449	$449
2. Cement Addatives	$98	$98
3. Cement Bags	$3	$12
4. 1-Shellfish Diet 1800	$44	$44
5.	$	$
6.	$	$
7.	$	$

For additional items attach another sheet.
Note: Funds will not be allocated for student stipends, housing or travel.

Miscellaneous: $
(If over $50 please attach itemized justification)

Total Funds Requested: $603.00

Required Research Permits (Please List) **No Permits Required** ☒

Permitting Agency	Type of Permit Required