History and Overview

Every spring red knots (*Calidris canuta rufa)* fly from Tierra del Fuego at the southern tip of South America to Delaware Bay where they remain for up to 12 days feeding on horseshoe crab (*Limulus polyphemus*) eggs (Morrison, et al. 2004). These lipid-rich and protein-rich eggs are rapidly converted by the red knots to fat reserves needed to fuel their flight to breeding grounds in the Canadian Arctic (Karpanty, et al. 2006). A combination of factors – loss of habitat, commercial overharvesting of adult horseshoe crabs, and pollution – have resulted in major declines in the adult horseshoe crab populations along the mid-Atlantic and New England coastal regions of the United States (Faurby, et al. 2010). This decline in adult horseshoe crabs has resulted in fewer horseshoe crab eggs available for red knots and other shorebirds to consume during their spring migration, producing a decline in the red knot population (Karpanty, et al. 2006).

The complex relationships between red knots and horseshoe crabs have been studied by numerous researchers over the past several decades (Atkinson, et al. 2005, Botton, et al. 1994, Clark & Niles, 1993, Gillings, et al. 2007, Haramis, et al. 2007, Tsipoura & Burger, 1999). Some of these studies (Karpanty, et al., 2006, Faurby, et al. 2010, McGowan, et al., 2011) examined the relationships between population trends in red knots and horseshoe crabs in the Delaware Bay region. Field investigations (Tsipoura & Burger, 1999) have documented the diets of red knots and the location of their preferred food items. Thousands of red knots have been cannon-netted, sampled, weighed, measured and banded in order to determine the flight patterns, arrival and departure weights of red knots arriving on Delaware Bay beaches (Tsipoura & Burger, 1999, Karpanty, et al., 2011, Haramis, et al., 2007).

Up until the present point in time, the only major attempt to respond to the decline in horseshoe crab eggs on Delaware Bay beaches in the spring has been the regulations adopted by the Atlantic States Marine Fisheries Commission which place limits on horseshoe crab harvests during the breeding season (ASMFC 2007). Despite these

regulations, the population declines of red knots continue unabated (Clark, et al., 1993, Morrison, et al., 2004, Niles, et al. 2011). One current report (Niles, et al., 2011) on red knot numbers overwintering in southern Argentina states that red knot populations have declined another 30% in the last year, falling from ~15,000 birds to ~10,000 birds, an alarmingly low number. It is apparent from this decline that without intervention of some kind the red knot population will continue to decline.

Background & Purpose:

The Red Knot Survival Project was developed by Global Conservation Alliance (GCA) to provide workable, cost-effective methods for increasing horseshoe crab egg availability for red knots. The goal of GCA's Red Knot Survival Project is simple: to increase horseshoe crab egg availability so that shorebirds have more quality food resources available to eat when they reach Delaware Bay on their spring migration. Ingestion of these food resources will increase their fat reserves, allowing more of them to survive their non-stop flight from Delaware Bay to the red knots' Canadian Arctic breeding grounds. Additionally, higher fat reserves should increase year-to-year survival as well as the annual red knot productivity by helping birds buffer the high energy costs of the flight itself, of unpredictable Arctic weather conditions, of lack of food on arrival dates and of the demands of finding and attracting a mate, egg production and other early breeding season activities.

In an unpublished study (Famous, et al. 2010), GCA documented that feeding red knots and other shorebirds were attracted to Delaware beach plots where the upper 15-20 cm of sand were overturned to reveal dense clusters of horseshoe crab eggs compared with spawning beach plots that were left undisturbed.

The work proposed here builds on this previous unpublished work. In 2007, Cuomo reported on her successful research breeding horseshoe crabs in captivity at the First International Horseshoe Crab Symposium (Cuomo, 2007). Her report spanned a decade of research into the problem. Since 2001, she has continued to successfully breed *Limulus* adults in captivity (see Cuomo, 2001, 2002, 2007) as well as conduct research on developing appropriate protocols for rearing the larvae to adulthood, a project she began in 2001. The pilot project proposed here combines Cuomo's work with the work of GCA to achieve the main goal of increasing food resources for the red knot.

Specifically, the work aims to: a) breed captive *Limulus polyphemus* as a source of horseshoe crab eggs b) create a "horseshoe crab egg bank" by developing protocols for non-damaging safe storage of horseshoe crab eggs c) pilot a project that involves placing "feeding stations" filled with the stored horseshoe crab eggs on known red knot feeding beaches during the spring migration in order to supplement the available food resources for the red knot. Should this pilot project prove successful, efforts will be made to seek further funding to scale up the project.

The specific objectives for which funding is sought are:
1) To establish a partnership between Dr. Carmela Cuomo and her *Limulus polyphemus* research lab and aquaculture production and storage facilities with sufficient capability to produce and store Limulus eggs produced throughout the year. Both research and production facilities are expected to be at the same location for the pilot program.

2) To fund three years of the production and storage processes, with the expressed goal of establishing a dedicated production and storage facility at the end of the funding period.
3) To design, test, and firmly establish efficient egg collection, storage, and transport protocols.
4) To design, test, and establish a practical and appropriate egg delivery system.
5) To determine the short-term and potential long-term success and viability of the project and its methodologies in order to ensure the survival and growth of both the red knot and horseshoe crab populations.
6) To disseminate information regarding this project to local conservation groups and interested parties.

Methodological Approach:

Approximately 100 adult *Limulus polyphemus* (~25-50 females, ~75-50 males) will be collected from the shores of Delaware Bay and transferred to an outdoor captive breeding facility established by Dr. Carmela Cuomo. Following protocols developed by Cuomo (2007), captive horseshoe crabs will be induced to breed in the tanks maintained at the breeding facility. Based on previous work (Cuomo, 2007), horseshoe crabs generally can be induced to continuously spawn and produce viable eggs throughout the months of May-October in Cuomo's tanks. Estimates of egg numbers likely to be produced from the captive spawning, based on previous work conducted by Cuomo (2007), range from 2,500,000 – 5,000,000 eggs. All eggs produced from the captive breeding of Delaware Bay crabs for this project will be removed from the breeding tanks and sorted according to viability. Any apparently not-viable eggs will be removed and the rest of the eggs will be stored in an "egg bank". Several different methods of preserving horseshoe crab eggs will be evaluated during the first year of the pilot program, including maintenance of eggs in sterile, filtered, aerated seawater at temperatures of ~ 8^{o}C, Preference will be given to treatments that do not involve the application of any fungicide which is part of an existing established methodology. Once successful protocols are established for the preservation of horseshoe crab eggs, all eggs collected from the captive breeding will be subjected to the protocols and preserved for release onto the beaches at Delaware Bay the following spring when the red knots arrive on the beaches.

Twenty feeding stations will be established within the high tide zone on Delaware Bay beaches during May of the year following the egg production by the captive *Limulus*. Eggs stored in the egg bank will be transported to Delaware Bay beaches and placed within ten of the feeding stations. These will serve as the experimental feeding stations. The remaining ten stations will be filled with sand from the beach and serve as controls. All twenty stations will be observed for 72 hours following egg release. Observations to be made include: number of birds present, number of species present, number of birds/species present, and length of time spent at feeding station. Photographs will also be taken at selected time intervals to further document the feeding activities of shorebirds and red knots at the feeding stations. All data collected will be analyzed using NCSS for significance.

Anticipated Results:

It is anticipated that the research described here will result in the development of a successful protocol for the medium scale production and storage of horseshoe crab eggs, as well as a successful methodology for distributing these eggs onto beaches during the spring migration of red knots in order to enhance red knot survival during and after migration. It is also anticipated that the work proposed here will serve as the basis for the development of a large-scale facility that will specialize in the captive breeding of adult *Limulus polyphemus* for use in egg banks as well as in other conservation projects. It is anticipated that the large-scale storage of horseshoe crab eggs produced via captive aquaculture in an "egg bank" will assure that significant supplies of eggs will be available for later distribution to the red knot and other shorebird species. We propose that aquaculture is the most expedient, cost-effective and environmentally safe option for the emergency provisioning of the red knot.

Summary:

The project proposed here is designed to provide a temporary 'life boat' to help the rufa subspecies of red knot tolerate present food shortages while horseshoe crab populations rebound, which is predicted to take a minimum of 8-12 years (because it takes that long for egg-bearing females to mature). Assuming either a full or partial horseshoe crab population recovery, our food-enhancement methods can be applied over the long-term during years when *Limulus* egg availability is insufficient. What's more, the methodology can be applied anywhere that red knots and other shorebirds depend primarily on horseshoe crab eggs, from Virginia to Japan.

Prepared for the staff of The New England Aquarium, November, 2011 by:

Porter Turnbull porterturnbull@gmail.net
Lanny McDowell blkwtrbrk@comcast.net
Carmela Cuomo ccuomo@newhaven.edu

References:

Atkinson, P.W., A.J. Baker, R.M. Bevan, N.A. Clark, K.B. Cole, P.M. Gonzalez, J. Newton, L.J. Niles, and R.A. Robinson (2005). Unraveling the migration and moult strategies of a long-distance migrant using stable isotopes: Red Knot *Calidris canutus* movements in the Americas
Ibis 147 (4): 738–749.

Botton, M.L., R.E.Loveland, and T.R.Jacobsen (1994). Site selection by migratory shorebirds in Delaware Bay, and its relationship to beach characteristics and abundance of horseshoe crab (*Limulus polyphemus*) eggs. *The Auk: 1*11(3): 605-616.

Clark, K.E., L.J. Niles, and J. Burger (1993). Abundance and distribution of migrant shorebirds in Delaware Bay. The Condor 95(3): 694-705.

Cohen, J.B., S.M. Karpanty, and J.D.Fraser (2010). Habitat selection and behavior of red knots on the New Jersey Atlantic Coast during spring stopover. The Condor 112(4): 655-662

Cuomo, C. (2001). Horseshoe crab aquaculture – results from initial spawning studies, abstract. Proceedings of the 21st Milford NMFS Aquaculture Meeting.

Cuomo, C. (2002) Spawning and rearing of Limulus polyphemus in captivity, abstract. Proceedings of the 22nd Milford NMFS Aquaculture Meeting.

Cuomo, C. (2007). Captive spawning and rearing of larval Limulus in culture, abstract. Proceedings of the First International Horseshoe Carb Symposium, Oakdale, New York.

Faurby, S., T.L. King, M. Obst, E.M. Hallerman, C. Pertoldi and P. Funch (2010) Population dynamics of American horseshoe crabs—historic climatic events and recent anthropogenic pressures. Molecular Ecology 19 (15): 3088-3100.

Famous, N.C., P. Turnbull, and L., McDowell (2010. GCA Final Report 2010, 20 pp.

Gillings, S., P. W. Atkinson, S. L. Bardsley, N.A. Clark, S.E. Love, R.A. Robinson, R. A. Stillman, and R. G. Weber (2007). Shorebird predation of horseshoe crab eggs in Delaware Bay: species contrasts and availability constraints. Journal of Animal Ecology 76(3): 503–514.

Haramis, G. M.,W.A. Link, P.C. Osenton, D.B. Carter, R.G. Weber, N. A. Clark, M.A. Teece, and D.S. Mizrahi (2007). Stable isotope and pen feeding trial studies confirm the value of horseshoe crab *Limulus polyphem*us eggs to spring migrant shorebirds in Delaware Bay. Journal of Avian Biology 38 (3): 367–376

.

Karpanty, S.M., J. Cohen, J.D.Fraser, and J. Berkson (2011). Sufficiency of horseshoe crab eggs for red knots during spring migration stopover in Delaware Bay, USA. Journal of Wildlife Management 75(5): 984–994.

Karpanty, S.M., J.D. Fraser, J.Berkson, L. J. Niles, A. Dey, and E.P. Smith (2006). Horseshoe crab eggs determine red knot distribution in Delaware Bay. Journal of Wildlife Management 70(6):1704-1710

McGowan, C.P., D.R.Smith, J.A. Sweka, J. Martin, J.D. Nichols, R. Wong, J.E. Lyons, L.J. Niles, K.Kalasz, J. Brust, M. Klopfer, and B. Spear (2011). Multispecies modeling for adaptive management of horseshoe crabs and red knots in the Delaware Bay. Natural Resource Modeling 24 (1): 117-156.

Morrison, R.I.G., R. K. Ross, and L.J. Niles (2004). Declines in wintering populations of red knots in southern South America. The Condor 106(1):60-70.

Tsipoura, N. and J. Burger (1999). Shorebird diet during spring migration stopover on Delaware Bay. *The Condor* 101(3): 635-644.