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DATE: April 21, 2011

RE: A report of activities and species collected under a permit to Collect Marine and Estuarine Organisms for Scientific Purposes-Permit No. 1041.

The following is a report on activities and species collected under Permit 1041, Permit to Collect Marine and Estuarine Organisms for Scientific Purposes issued to Norman C. Famous, representing Global Conservation Alliance (GCA), 513 Eight Rod Road, Augusta Maine 04330. This report summarizes methods, invertebrate species collected, their numbers, collecting locations, and sampling dates for The Administrator, Marine Fisheries, P.O. Box 400, Trenton, NJ 08241

GCA's experiments were designed to evaluate several methods for increasing the surface and near-surface density of horseshoe crab eggs for consumption by four species of migrant shorebirds, which are undergoing significant population declines: red knot (*Calidris canutus* ssp. *rufa*), which has been nominated for Federal Endangered Species status; ruddy turnstone (*Arenaria interpres*); dunlin (*Calidris alpina*); and semipalmated sandpiper (*Calidris semipalmatus*). Internationally significant numbers stage for about two weeks in Delaware Bay each spring to feed primarily on HSC eggs, some more than others do.

Delaware Bay is the last stop along their trans-continental migration between the southern tip of South America where they winter and their Arctic and Sub Arctic breeding grounds. This stopover is critically important because their weight must nearly double by accumulating fat reserves needed to fuel their final flight. In addition, body fat is needed to survive during the first week back on their sometimes-frigid nesting habitats and to produce their four-egg clutch.

The purpose of the application was to gain a permit to collect horseshoe crab (HSC) eggs as part of a red knot and other shorebird supplemental provision study being conducted during the spring shorebird migration along the New Jersey shore of Delaware Bay.

The principal investigators included Norman C. Famous, Wetlands and Wildlife Ecologist; Dr. Porter Turnbull, D.C. and Marine Conservation Biologist; and Mr. Lanny McDowell, Field Ornithologist, Avian Photographer and Writer. John Patrick Brown, Field Ornithologist, and Kevin Weir, Conservation Biologist, conducted the field sampling.

The report describes the activities and species collected under the permit. Organization of the report parallels the questions asked in the permit application and includes a general summary of the study design and methods. The study methods we presented in the application are underlined in the text. They are followed by a non-underlined description of the methods we adopted.

1.0 Purpose of Study:

We plan to evaluate in experimental test plots several small-scale mechanical methods of redistributing horseshoe crab eggs buried up to 20 cm to make them more available for consumption by red knots and other Arctic-bound shorebirds. The purpose is to develop, test and refine simple, cost-effective methods to apply at a larger scale next year. Specifically, we want to turn over or stir up the upper 20 cm of middle to upper intertidal sediments.

2.0 Locations(s) where collecting is proposed (Be specific):

We will be working on 3 to 5 Beaches include: Reed's, Moore's, Gandy's, High's, and Cook's. Depending on location of birds and logistics other beaches including Fortescue, Raybin's, Kimble's, Pierce's Point, Rutgers, Surnray, Norbury's Landing, and Villas will be considered. Target or priority beaches will be determined in consultation with Amanda Dey NJ DEP.

Food enhancement experiments were conducted at Reed's Beach and Moore's Beach.

3.0 Time of year permit collecting will take place:

One week between June 6 and June 30, 2010

Samples were collected between June 17 and June19, 2010.

4.0 Equipment proposed to be used in collecting (size of gear, mesh, etc.):

4.1 Digging or sediment mixing method:

We used shovels and garden rakes to dig the trenches and spread the sediments in parallel lines on one side of the trenches. Mixing of sand and HSC eggs occurred during the trench digging and sand spreading activities.

4.2 Horseshoe crab egg sampling method:

Using shovels, trenches were dug to a depth of about 20 cm and the contents (sand/egg mixture) were spread in parallel strips on one side of the ditch. Samples were taken from the upper five centimeters of undisturbed beach surface after the sand/egg mixture from the trenches was spread into 8-foot wide strips. Samples were also taken from the bottom of the freshly dug trenches and from the trench slopes. Trenches typically refilled with sand during the following one or two high tides. Each sample consisted of a 10 cm diameter by 5 cm deep sediment core, extracted using a 10 cm diameter coffee can.

One hundred and twenty core samples were extracted from Reed's Beach and Moore's Beach. Plots were placed in the middle to upper intertidal zone (middle to upper beach) below the previous high tide strand line. Samples were bagged, labeled, refrigerated and shipped to Maine for further cleaning and species identification.

We followed Sekiguchi (1988) HSC egg development scale which is organized into 20 stages with Stage 1 being newly fertilized eggs and Stage 20.1 representing newly hatched 'trilobite larva', the name given to the newly hatched larvae that resemble extinct trilobites in morphology. They are summarized and illustrated in Shuster and coeditors (Shuster et al., 2003). Species identifications of invertebrates were determined using several marine invertebrate identification guides.

Mineral Samples: Mineral or beach sand samples were placed in plastic bags after which the

eggs and other invertebrates were extracted using the floatation method. HSC eggs and other invertebrates were then refrigerated and shipped overnight to Maine for counting.

HSC eggs were counted visually when numbering less than about 150 eggs per sample. For larger samples, eggs were either counted volumetrically or subsamples were counted then extrapolated to the entire sample. Using a dissecting microscope, the growth stages in egg development were classified and the number of eggs in each stage or group of stages (combined stages when differentiation was not possible) were estimated. Egg surface morphology, egg size and shape, and eggshell transparency guided classification into stages.

Detritus Samples: Organic sediments comprised of detritus resting on the surface of the beach near breakwaters were also placed in plastic bags. Detritus samples were refrigerated and shipped to Maine where they were visually counted from subsamples which were extrapolated to the entire sample.

4.3 Number of sediment core samples:

One hundred and twenty sediment core samples were taken.

4.4 Personnel

The principal investigators included:

Norman C. Famous, Wetlands and Wildlife Ecologist

Dr. Porter Turnbull, D.C. and Marine Conservation Biologist

Mr. Lanny McDowell, Field Ornithologist and Avian Photographer and Writer

Supervision by Norman Famous, John Patrick Brown and Kevin Weir carried out Field experiments. John Patrick Brown, a former biology major at Millersville University near Lancaster Pennsylvania, is a Field Ornithologist from Reading, PA. Likewise, Kevin Weir, a resident of Lancaster, PA, studied biology and political science at Millersville University where he developed and supervised recycling and 'green' energy programs.

4.5 Do you intend to collect hard clams, soft clams, mussels, oysters or other bivalve mollusks?

No; there may be a small bi-catch of juvenile bivalve mollusks within the 10 cm by 5 cm deep core samples collected to determine horseshoe crab egg densities; we had no juvenile bivalve bi-catch in 432 samples in 2009.

We collected one juvenile hard clam (*Spisulla solidissima*), one blue mussel (*Mytilus edulis*), *Gemma gemma* (many) and several Tellins (*Tellinus* sp. most likely *Tellinus agilis*)

4.6 List of marine invertebrates sampled:

Horseshoe crab eggs and HSC trilobite larvae were the primary invertebrates collected from sediment samples. Small numbers of 11 other invertebrate species were either collected (8) or observed (3) within the beach and detritus substrata. All species and approximate numbers of individuals counted are presented in Table 1. Outside of the strand lines and detritus fields on the upstream sides of jetties, the upper sand beach, invertebrate fauna was uniformly depauperate.

Two different habitat types were sampled: Open sand beach and detritus deposits on sand substrate.

Open sand beach habitat: Invertebrate samples from open sand beaches were dominated by HSC eggs and low numbers of bivalves. The development status of HSC eggs in sand were primarily Stage 1 through Stages 8 and 9.

Table 1 List of invertebrate species collected in sample plots at Reed's Beach and Moore's Beach on Delaware Bay (HSC eggs and larvae not included)

Gastropods		
Saltmarsh snail	*Melampus bidentatus*	Two individuals
Marsh periwinkle	*Litterina*	
Mud dog welk	*Nassarinus obsoletus*	In detritus over clay
Bivalves		
Gem shell	*Gemma gemma*	1-2 per sample in detritus samples at Reeds Beach
Tellins	*Tellinus* sp.	Most samples at Reed's Beach
(Likely Dwarf Tellins)	*Tellinus agilis*	
Surf clam	*Spisulla solidissima*	Two small individuals
Blue mussel	*Mytilus edulis*	One half cm long individual
Hard tubeworm	*Spirillis spirillum*	Three individuals in detritus samples at Reed's Beach
Crustations		
Scuds	*Gammarus* sp.	Observed only
Beach fleas	*Talorchestia* sp.	Observed only in detritus samples at Reeds Beach
		Marine Nematodes
Marine Nematode	Unidentified	One, extremely small

Detritus-covered beach: HSC egg development ranged from Stage 1 through Stage 21. The well-developed Stages 20 and 21 were scattered in low numbers throughout many of the detritus samples. They were concentrated in large numbers at the base of a detritus sample taken from the upstream side of the rock jetty at Reed's Beach. The distribution of trilobite larvae in a single layer at the base of the organic debris was similar to that reported by Battey in 1883 at Martha's Vineyard, Massachusetts (Shuster et. al 2003). In Delaware Bay, concentrations of trilobite larvae under detritus mats and strand lines may be a localized event in that Robert F. Loveland speculated that this under-detritus phenomenon has not been reported in the Bay (quoted in Schuster et. al 1998). Sampling in appropriate detritus deposits at more locations is needed to determine how widespread the under-detritus trilobite larvae distribution is in the Bay and within and between nesting seasons. Once the outer eggshell is shed, the remaining inner eggshell is clear, revealing the development stages of the embryo.

The most interesting HSC egg embryo development stages were Stages 20.1 where the leg buds elongated inside the capsule and Stage 20.2, the bilobed trilobite larvae stage where the embryo has emerged from the capsule or inner membrane. The trilobite stage looks like a bulbous-shaped mushroom (somewhat like a closed-capped *Agaricus campestris* mushroom) where the two 'segments' have differentiated. The Prosoma portion or the front segment is analogous to the broader concave mushroom cap while the rear segment or the Opisthosoma is narrower and

cylindrical in shape. The thick shedded outer capsules were abundant and clearly visible within the organic matrix. Although biologically interesting, the detritus-covered sections of beach will not be routinely targeted in future HSC egg enhancement investigations.

4.7 Do you intend to retain any organisms after field observation?

No living organisms will be retained.

None were retained.

5.0 Number of individuals:

HSC: egg numbers ranged from 50 to over 75,000 per sample.

6.0 Summary

As expected, spreading a thin layer (2-4 cm) of sand mixed with HSC eggs across undisturbed beach surface dramatically increased egg density within the feeding zones of red knots and other shorebirds. Work in 2009 documented that newly spread sand-egg mixtures attracted large numbers of shorebirds within a half hour after spreading (e.g., 200-300 birds in a small area). Work in 2010 evaluated surface and near-surface egg densities at different distances from the trench, on the trench slope and in the bottom of the trench. Egg densities were highest within four feet of the ditch margins (averaged in the thousands) and at the base of trenches where crab nests were located (more variable ranging up to 10,000). Egg densities on ditch slopes were variable and were related to the distribution of HSC nests (varied from less than 100 to several thousand). To avoid biasing the data, sampling of visible concentrations of eggs on the surface were not sampled. Further work is needed in 2011 to refine minimum ditch widths and the minimum thicknesses (depth) of the sand-egg mixture required to support large flocks of foraging shorebirds. Minimizing both the width of the ditch and the depth of the sand/egg mixture spread on adjacent undisturbed beach surface will minimize impacts to HSC productivity in Delaware Bay.

7.0 Literature sited

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Acknowledgements

Very special thanks go to John Patrick Brown and Kevin Weir for volunteering to conduct the field studies, including the tedious process of removing the invertebrates from samples.