Investigation on the population identity of Indo-Pacific humpback dolphins (*Sousa chinensis)* in the northern Bay of Bengal, Bangladesh and implications for population-level conservation and taxonomy of the species

(Photographs from top left corner clockwise: Main survey vessel with researchers taking photographs and attempting to collect biopsy samples. Indo-Pacific humpback dolphin with a biopsy collection dart in the foreground. Auxiliary vessel during biopsy collection effort. Adult humpback dolphin as evidenced by the absence of spotting and its pinkish/white coloration.)

Interim report to the

International Whaling Commission

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Introduction

The genetic identities of cetacean populations in Bangladesh remain largely unknown. This inhibits the ability of national authorities, regional bodies, and international management organizations to establish science-based priorities for conservation management. At the 2002 International Whaling Commission (IWC) meeting in Shimonoseki, Japan, the Small Cetacean Sub-committee recommended that expanded molecular sampling be carried out for humpback dolphins in areas where samples had not yet been included in genetic analyses, and that hypotheses concerning regional population structure be tested so that gene flow could be evaluated within and between populations. The Sub-committee also recommended surveys, photo-identification and genetic sampling to obtain information on humpback dolphin ranging patterns, population fragmentation and stock structure where their distribution is patchy – such as the situation in Bangladesh where humpback dolphin habitat is closely tied with freshwater inputs.

With this project, the Wildlife Conservation Society (WCS) will address a vital gap of information on humpback dolphins in an area of the eastern Indian Ocean identified as a global hotspot of cetacean diversity and an ecological cul-de-sac with strong implications for climate change impacts. It will provide fundamental knowledge to be incorporated into conservation planning and contribute to increasing national capacity to conduct rigorous molecular research on cetaceans. Knowledge about the true number of species within the *Sousa* species, and population-level differentiation, will be essential in helping to better protect these animals at local, national, regional and international levels.

The specific objectives of this project are to:
(1) Determine the population identity of humpback dolphins in Bangladesh;
(2) Evaluate implications of genetic results on the taxonomy of the species complex;
(3) Build local capacity to conduct molecular studies on cetaceans; and
(4) Contribute scientific information needed for the conservation management of humpback dolphins.

Methods

Biopsy collection was conducted under a permit from the Government of the People's Republic of Bangladesh, Ministry of Environment and Forest. A minimally invasive darting system, that had previously been successfully employed to collect samples from cetaceans in Bangladesh, was used. A Barnett Wildcat III crossbow was employed with a bolt composed of (1) an aluminum shaft (length of the arrow that screws into the tips) with three plastic fins at one end, (2) a torpedo shaped plastic foam float glued near the end of the shaft, opposite the fins for buoyancy and painted bright orange for increased viability, (3) a plastic stopper glued to the end of the shaft to prevent the bolt from penetrating past the tip, and (4) a biopsy tip made from high-grade stainless steel with a diameter of 6 mm on the outside and 5 mm on the inside with a 15 mm penetration. The foam float, plastic stoppers, and biopsy tips were fabricated in Bangladesh.

A precautionary approach was taken. If there was any uncertainty about the safety of the target animal no shot was taken. Biopsy attempts were abandoned if strong reactions were observed to either vessel approach or after a shot was taken. Dolphin groups were approached from an angle and the research vessel attempted to match their travel direction and speed. Special care was taken with groups with calves, and biopsy attempts were immediately stopped if it appeared that a group was tiring out or if there was any possibility of calves getting separated from the mothers. Mothers or calves were not

targeted. Dolphins were targeted in the shoulder area before or just behind the dorsal fin. To avoid injury to the dolphin from the impact of the dart, a minimum firing distance of 5 m was observed.

Information was recorded on biopsy collection effort including the total time spent with the animals, their general behavior, and why the sampling session was stopped. For each shot taken information was recorded on the geographic position and behavioral response (Table 1). Photographs were also taken for identification purposes. Biopsy samples were stored in bar coded vials filled with 80% ethanol solution, kept in a cool dry place, and transferred as soon as possible to a refrigerator at the project office in Khulna.

Two different cross bows were used: one with a 75 pound draw weight for shorter shots and one with a 150 pound draw weight for longer shots. After 11 humpback dolphin sightings, 55 shots taken and only 2 samples obtained, it was realized that information on the distance to the target animal, and whether or not missed shots were long, short, or on target but arriving after the animal was submerged, would be valuable for improving accuracy and better evaluate the pro and cons of using a cross-bow versus alternative biopsy collection system. These data were therefore routinely collected for each subsequent shot.

Table 1. Behavioral reaction codes used to categorize dolphin behavior after each cross bow shot was taken.

Code description	Hits(6) (%)	Misses (272) (%)
1- No noticeable reaction; individual continues to swim in the same direction.	0	36.3
2 - A flinch but individual continues to swim in the same direction.	16.7	8.9
3- Individual accelerates under water and changes direction.	16.7	50
4- Individual accelerates under water followed by a single leap, lobtail or breach.	66.7	4.7
5- Individual accelerates under water followed by a series of leaps, changes in direction and/or leaps, lobtails and breaches.	0	0

Results

For Indo-Pacific humpback dolphins, the research team searched along 1,362 km of systematic trackline during 123 hours of search effort and another 1,011 km of haphazard trackline during 97 hours of search effort conducted over a total of 56 days in 937 km^2 of open estuarine waters of the Bay of Bengal between the Sundarbans mangrove forest and the Swatch-of-No Ground submarine canyon (Figure 1). The transect lines extended out from the shore at depths between 1.7 and 35.9 m (mean=9.6, SD=3.7, n=740) and salinity between 9.1 and 28.2 parts per thousand (ppt) (mean=22.3, SD=4.1, n=548). According to Beaufort sea state sighting conditions were 1-4 during 1%, 25%, 49%, 17%, and 8% of the time, respectively. A total of 48 humpback dolphin sightings were made by the team (mean salinity=22.4 ppt, range=12.5-28.0; mean depth=10.2 m, range=5.3-20), with a mean group size of 16 individuals (SD=13.9, range=1-57).

During 39 sightings, effort was made to collect skin samples for a total of 80.5 hours. Biopsy collection effort was ended 48.2% of the time due to losing the animal group, 37.9% time due to the end of daylight, 10.3% of the time due to the animals fleeing the boat, and 3.4% time due to survey logistics. Despite taking 278 shots (7.1 shots per sampling session, SD=7.8, range=0-40) only six skin samples were obtained during four sightings.

The mean estimated distance to the target dolphin recorded for 214 of these shots was 32.0 m (range=17-50). A total of 77 shots were taken with the cross bow that has a draw weight 75 pounds with a mean estimated distance of 30.1 m (range = 3-50) to target dolphin and 2 samples obtained. A total of 201 shots were taken with the cross bow that has a draw weight of 150 pounds with a mean estimated distance of 36.1 m (range=8-70) to the target dolphin and 4 samples obtained. About the same percentage of shots were short (43.9%) as were long (41.1%) while 13.1% were the correct distance to the dolphin but arrived after the animal was already submerged. Tissue samples were successfully retained for all shots that made contact with the dolphins. The average distance to the dolphins for successful shots was 16.5 m (range=3-30) and all six were made while the dolphins were engaged in traveling or socializing behaviour.

By far the most common reaction (4 out of 6 hits) of a humpback dolphin after it was hit by biopsy dart was to accelerate underwater, followed by a single leap, lobtail, or breach. Although this reaction was energetic it was never repeated and the animals resumed their normal behavior immediately after their single leap, lobtail, or breach response. During one shot the dart stuck in the dolphin. It reacted by making a single leap but then afterwards swam along apparently unbothered. The animal was followed by the team for a short while, whilst they shot bolts near the animal with the hopes of making it flinch, so that the dart would have a better chance of falling off the animals; the dart eventually came loose and fell off the animal and was recovered. The most common reaction of the target dolphin to a missed dart hitting the water surface close to where it surfaced was accelerating underwater or changing its swimming direction.

During 44 sightings, as part of a grant from the Indo-Pacific Cetacean Research and Conservation Fund on the abundance, ranging patterns, habitat selection, and fisheries interactions of Indo-Pacific humpback *Sousa chinensis* and Indo-Pacific bottlenose *Tursiops aduncus* dolphins in coastal waters of the Bay of Bengal, Bangladesh a total of about 23,500 photographs were taken to identify individual humpback dolphins. These photographs are currently being examined to identify individuals for adding to our photo catalogue which presently includes 60 individuals from our first season of data collection in 2010/11 generating a preliminary abundance estimate of 191 individual (SE=27; 95% CI=144-252) using a mark-resign technique. During the same field effort, data were also collected on environmental parameters (depth, salinity, temperature and turbidity) and on the distribution of fishing activities by type and activity according to point-transect sampling of a grid of 39 equidistantly distributed points covering the entire study area.

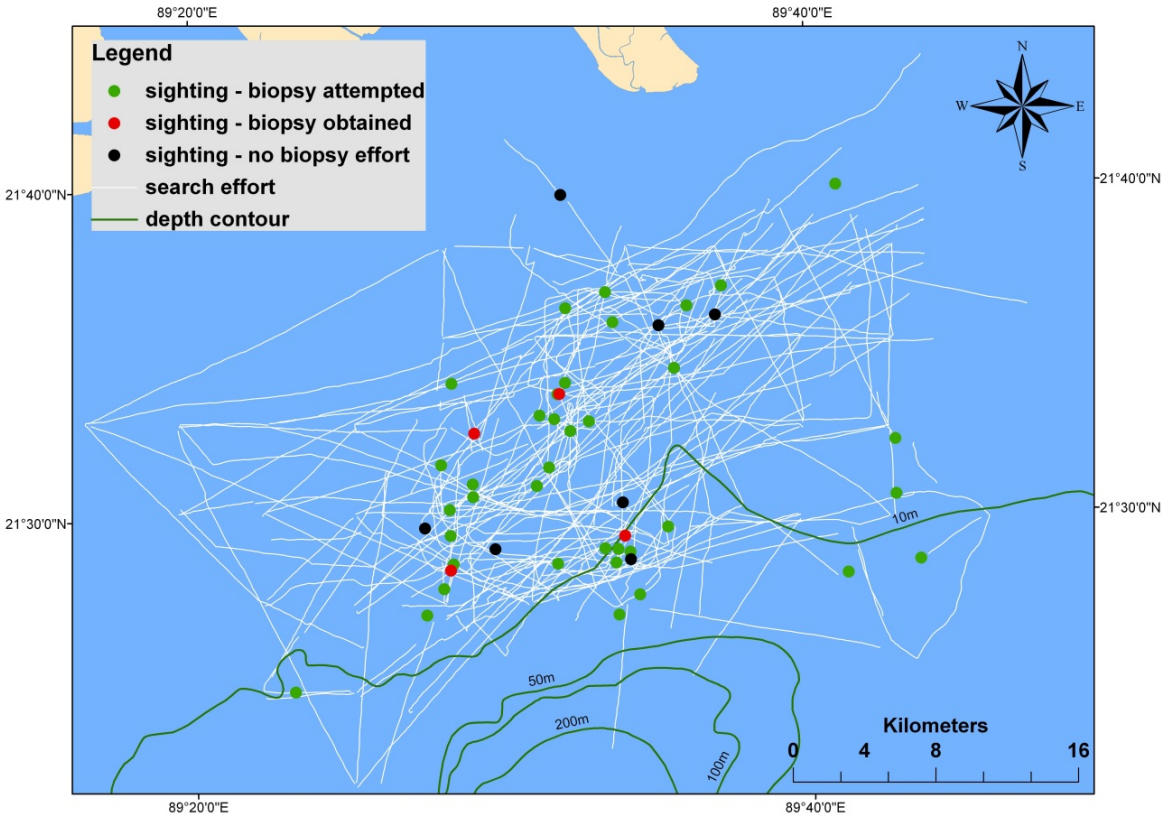

Figure 1. Map showing search effort and humpback dolphin sightings labeled according to biopsy attempted but no sample obtained, biopsy sample obtained, and no biopsy effort.

Discussion

Challenges of biopsy sampling humpback dolphins

The reasons for our limited success in obtaining samples from humpback dolphins include: (1) boat avoidance behavior such that the dolphins generally remained outside of about 50 m from our vessel – thus explaining the long distances of our cross-bow shots and the relatively low number of shots (3.5) taken per hour of biopsy effort, (2) the erratic surfacing patterns of the dolphins and the impossibility of tracking them underwater in the turbid waters of the Bay of Bengal (mean=10.6 nephelometric turbidity units, SD=9.4, range=1.7-32.8, n=29), (3) the long duration it took for the biopsy dart to reach the targeted dolphin at such long distances which meant that the target animal was often submerged before the dart reached it, and (4) the lack of maneuverability of our research vessel in relation to the erratic surfacing behavior of the dolphins.

The incident when a bolt got stuck after hitting a dolphin is reason for concern. Even though the bolt was shaken off, the incident demonstrates the potential danger when the velocity of a dart is insufficient to bounce off the dolphin after hitting it (i.e., during long shots using a cross bow system). A more precise biopsy system that allows the initial velocity of the dart to be increased and decreased according to the estimated distance to group being followed is needed to mitigate the potential for a biopsy dart to become stuck after hitting a target animal.

Next Steps

Due to the low number of samples collected, despite intensive biopsy collection effort, we would like to request that the time schedule of the grant be shifted ahead by one year to conduct an additional season of biopsy collection effort. To improve the efficiency of biopsy collection we will adapt/develop a safe, affordable and effective air gun darting system. A safe elevated platform or crow's nest will also be constructed, where an observer can be stationed to better direct the survey vessel to the dolphin group. Finally, options will be explored to use local set bag net fishing vessels as a platform of opportunity to collect biopsy samples when the dolphins surface close to the fishing nets to feed on falling fish when the fishermen pull their nets up. This effort will require consultation and educational outreach with the fishermen so they clearly understand that the motive is to conserve rather than harm the animals.

The dart design for an air gun system will be developed through collaboration with colleagues from WCS, who have been experiencing similar issues with sampling humpback dolphins in Madagascar (Salvatore Cherchio) and West Africa (Tim Collins), and a cetacean biologist/machinist who has been designing and producing biopsy darts for more than a decade (Jeff Jacobsen). Once the design is finalized, the darts will be fabricated in Bangladesh where we previously made darts for a cross-bow biopsy collection system - 76.7% retention rate for 79 tissue samples from Bryde's whale *Balaenoptera edeni* (38), Indo-Pacific bottlenose dolphins *Tursiops aduncus* (24), pantropical spotted dolphins *Stenella attenuata* (10), spinner dolphins *Stenella longirostris* (5), and Indo-Pacific humpback dolphins *Sousa chinensis* (2). The high retention rate of these locally made darts shows the effectiveness of their design and the quality of their fabrication. This has allowed us to provide darts to other international researchers at a small fraction of the cost available from other sources and, through their feedback, to fine tune our design.

Proposed revision of project work plan

Below is revised project workplan that extends the project from two years to three. This will give us an additional field season (December – February) to collect biopsy samples using an improved biopsy collection system (see above).

Summary of Revised Project work plan			
Activity to be undertaken	Responsibility	Start date (mm/ yy)	Finish date (mm/yy)
Project coordination	Brian Smith & Rubaiyat Mansur	07/11	06/13
Collecting genetic samples	Rubaiyat Mansur & Brian Smith	11/11	02/12
Developing improved air gun biopsy collection system	Brian Smith in collaboration with WCS colleagues and others.	03/12	10/12
Collecting genetic samples with improved biopsy collection system	Rubaiyat Mansur & Brian Smith	11/12	02/13
Obtaining CITES permits and shipping samples	Rubaiyat Mansur & Brian Smith	03/13	05/13
Analysing genetic samples	Howard Rosenbaum, Columbia University Graduate Student & Bangladeshi student (pending recruitment of a suitable candidate and raising additional funds)	06/13	12/13
Capacity building	Brian Smith & Howard Rosenbaum	07/11	06/13
Communication of results	Brian Smith & Howard Rosenbaum	01/14	06/14

Proposed revision of budget (in US$)

Below is a proposed revision of the budget that eliminates funding to support a Bangladeshi geneticist to participate in the genetic analysis conducted at the American Museum of Natural History and uses the balance to support two additional months of salary coverage for the Principal Field Researcher ($1,600) an additional trip for the Principal investigator to travel to Bangladesh for leading the second season of biopsy collection with the Principal Field Researcher ($2,000), six additional days of vessel charter to supplement the number of days paid for by the IPCF grant ($1,800), as well as an airgun, the design and fabrication of appropriate biopsy darts/stoppers/floaters, and hardware for building elevated platform ($2,400). The total amount for the budget remains the same as in the original proposal ($51,400).

Farhana Aktar, the Bangladeshi geneticist who currents works as an Educational Outreach Assistant for WCS in Bangladesh has decided to attend graduate school in India staring in August. We therefore plan to recruit another Bangladeshi genetics student to participate in the analysis of the humpback dolphin samples at the American Museum of Natural History (now planned for the second half of 2013) and to raise additional funds to pay for her/his stipend and travel to New York.

(I) SALARIES/WAGES (INCLUDE NAME/POSITION OF EACH INDIVIDUAL AND BREAKDOWN OF TIME AND DUTIES INVOLVED

Brian D. Smith, Principal Investigator @ $8,800/month X 2 months = 17,600

Rubaiyat Mansur, Principal Field Researcher @ 800/month X 8 months = $6,400

Field Stipend for FD staff @ $200/month X 6 months = $1,200

Columbia University Genetics Student Stipend = $3,000

(II) TRAVEL (BREAKDOWN BY PERSON AND JUSTIFICATION)

Local Travel = $1,200

International Travel for Principal Investigator: two trips to Bangladesh from home in California = $4,000

(III) SERVICES (E.G. AIRCRAFT/VESSEL TIME; CONSULTANCY FEES ETC.)

Vessel charter @ $300/day X 16 days (additional days to be funded through IPCF grant mentioned above) = $4,800

Genetic analyses of samples; laboratory and analytical costs = $10,000

(IV) NON-EXPENDABLE CAPITAL EQUIPMENT (BECOMES IWC PROPERTY UPON COMPLETION)

(V) EXPENDABLE CAPITAL EQUIPMENT

Chemicals, vials, replacement prow for crossbow, razor blades, materials for biopsy tips, airgun, design and fabrication of biopsy darts for airgun, and hardware for building elevated platform - $3,000

(VI) ITEMISED SHIPPING COSTS

Shipping samples from Dhaka to NY - $200

(VII) ITEMISED INSURANCE COSTS

(VII) OVERHEADS **(NOTE: IT IS NOT STANDARD IWC POLICY TO PAY OVERHEADS – HOWEVER, IN SPECIAL CIRCUMSTANCES THESE MAY BE NEGOTIATED ON A CASE-BY-CASE BASIS WITH THE SECRETARIAT. INCLUSION OF OVERHEADS MAY AFFECT THE LIKELY SUCCESS OF THE APPLICATION)**

(VIII) TOTAL BUDGET SUMMARY

Grand Total = US$ 51,400 (Salaries/wages = $28,200; Travel = $5,200; Services = $14,800; Expendable capital equipment = $3000; Shipping = $200)

(IX) OVERALL JUSTIFICATION FOR BUDGET **(NOTE: PLEASE GIVE BRIEF JUSTIFICATIONS FOR EACH OF THE IDENTIFIED COSTS)**

Salaries and stipends need to be covered for the Principal Investigator (2 months), Principal Field Researcher (8 months), Columbia University Genetics Student (3 months), and Bangladesh Forest Department Staff (6 months).

Travel expenses are required for local project staff to get to and from the survey vessel, obtaining vessel and CITES export permits, and organizing project logistics and for the project leader to travel to Bangladesh during both years of data collection.

Services expenses are needed to fund 16 extra days (ten the first year and six the second) for the boat charter, which will supplement the vessel charter funds received through a grant from IPCF for line-transect and photo-identification surveys, and the genetic analyses of humpback dolphin tissue samples at AMNH.

Expendable capital equipment expense are needed to pay for chemicals, vials, replacement prod for crossbow, razor blades, materials for biopsy tips, airgun, redesigned biopsy darts, and hardware for building elevated platform, etc.

Shipping expenses are needed to send the humpback dolphin tissue samples by courier from Dhaka to AMNH.