Enhancing the Visibility of Fishing Ropes to Reduce Right Whale Entanglements

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Abstract

To reduce or eliminate the problem of right whale entanglements in fishing gear, scientists and gear developers have considered the feasibility of enhancing ropes and nets to improve their detection by whales. We conducted laboratory studies which showed that right whale visual sensitivity is tuned to perceive red and orange as high contrast "black" against the ambient blue/green oceanic background light. To determine whether changing the color of the ropes alters the distance at which whales can detect them, we conducted field trials in Cape Cod Bay in the spring of 2013. Rope-mimics were placed in front of right whales that were skim-feeding at the surface, so that changes in behavior (respiration, mouth closures, submergence times, and turning angles) and the distance of that change from the rope mimic (distance of detection) could be documented. Rope mimics were made from rigid PVC pipe, weighted at one end, and attached to a float at the other end, so each whale encountered the equivalent of a vertical buoy line as it swam near the surface. Color selection for the rope mimics included black, green, red, and orange (the first two mimic existing fishing rope colors). A total of 101 encounters between whales and the rope mimics were documented, and analysis indicates that red and orange colored rope mimics were detected by right whales at significantly greater distances than green ones. This suggests that changing commercial fishing rope color to red and/or orange may enhance the whale's ability to visually avoid entanglements in the wild. Field trials of orange ropes by a group of Maine lobstermen indicate that switching rope colors in that industry is feasible.

Introduction

The North Atlantic right whale (*Eubalaena glacialis*) is the most endangered large whale in the north Atlantic, with less than 500 alive today. Population growth is impaired by high levels of human-caused mortalities (Kraus and Rolland, 2007). At least 50% of all deaths in this population are caused by human activities, primarily ship collisions and entanglements in fisheries gear (Moore et al., 2004). Changes in the speed regulations for ships should reduce mortality from that source, but entanglement rates remain high, and claim at least one North Atlantic right whale annually along the east coast of North America (Waring et al., 2012; Kraus, et al. 2005). Approximately 82% of the animals in the Right Whale Catalog carry scars caused by ropes or nets (Knowlton et al., 2012). Fixed fishing gear is distributed very broadly along the coast of North America, and all types of fixed fishing gear have been recovered from entangled right whales (Johnson et al., 2005; 2007). As the right whale-gear entanglement problem continues, the failure to solve it jeopardizes the viability of several fixed gear fisheries, especially the lobster fishery. Other research has indicated that some whale species are aware of ropes in the water column (Kot et al. 2012). This work was to determine if ropes could be developed that would provide whales with a visual cue to avoid entanglements, and it consisted of laboratory work, field experiments, and field trials in the fishing industry.

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Project Objectives

1) Clone, sequence and express the cone visual pigment coding regions from right whale mRNA retinal extracts, and identify the wavelength(s) of light that will provide right whales with the highest level of contrast vision within their photic environments.

2) Develop rope-mimics using a color spectrum matching the spectral sensitivity in right whales and evaluate the effects of colored rope mimics on the behavior of right whales in field trials by measuring the distance at which whales change their behavior near different colored ropes.

3) Test appropriately colored ropes in an active fishery to determine operational characteristics.

Methods and Results

I. Laboratory Analysis of Balaenid Eye Characteristics

From DNA sequence alignments, we designed a set of degenerate and non-degenerate oligonucleotide primers in order to amplify specific regions of cetacean long-wavelength sensitive (LWS) cone opsins. This technique was used previously and proved successful in amplifying cetacean rod opsins which could subsequently be cloned, amplified, sequenced and expressed *in vitro*. PCR reactions were first performed on bottlenose dolphin cDNA to determine correct size and quality of the PCR products. We used a fresh right whale eye (NARW *E. glacialis* calf # CALO 0901) from which we were successful in extracting quality total RNA which was used as template to synthesize cDNA suitable for PCR amplification. Although PCR amplification of the rod opsin cDNA worked flawlessly, amplification of the LWS cone opsin cDNA was not straightforward.

We initially sequenced individual LWS cone exons from right whale genomic DNA in order to estimate the spectral sensitivity of the LWS visual pigment based on the deduced amino acid sequence and a mutagenesis modeling system (Fasick et al., 2011). Total RNA was extracted and cDNA synthesized, and PCR amplification of the LWS opsin resulted in a truncated PCR product when compared to a *Tursiops* control. Sequence analysis revealed three mRNA splice variants in the right whale resulting in various deletions, inversions and substitutions in exon 4 of the LWS opsin cDNA. Figure 1 diagrams the deletions from one splice variant in an alignment of the LWS opsin nucleotide sequences from right whale, along with two positive controls (bottlenose dolphin and cow). Although some of the deletions are in-frame, others are not and result in a premature stop codon at amino acid position 223 of the deduced right whale LWS opsin as shown in Figure 2. These deleterious mutations are not exclusive to only the North Atlantic right whale, but are also observed in two other members of Balaenidae, the Southern Atlantic right whale as well as the bowhead whale (Figure 3). A recent report has observed deleterious mutations in the LWS cone opsins from almost every baleen whale studied to date (Meredith et al., 2013). The deleterious mutations in the right whale LWS opsin gene result in its pseudogenization, or an inability for the visual pigment to function properly. A truncated opsin protein, as observed in the right whale, lacks the ability to covalently bind chromophore and lacks the regulatory carboxyl terminal region of the protein involved with inactivation by the protein arrestin. A protein possessing the mutations similar to those of the right whale LWS opsin would be marked for destruction, and with its destruction we would anticipate the destruction of the LWS cone photoreceptor.

The genetic data presented above leads us to believe that the Balaenid whales are rod monochromats and lack both the SWS and LWS cone photoreceptors used by almost all mammals for dichromatic color vision (note: see Levinson et al 2003 for the loss of SWS opsin genes). However, we first identified the mutations in the LWS opsin cDNA, which means that a LWS cone inner segment with intact nucleus and organelles must exist if the LWS opsin message was transcribed. Our current belief is that a LWS cone inner segment exists although the outer segment, normally extensively packed with membranes containing millions of visual pigments, has atrophied and is missing.

To determine if the Balaenidae are in fact rod monochromats and rely solely on rod photoreceptors for vision, we conducted a series of immunohistochemical and immunocytochemical studies on a single fresh-dead retina from a bowhead whale. Because the LWS opsin genes from both right whale and bowhead share nearly identical sequences and are identical in regards to the mutations, we are comfortable extrapolating what we have learned with the bowhead retina to the North Atlantic right whale. To confirm that the Balaenids are rod monochromats, we performed a series of stainings from bovine (positive controls) and bowhead retinal sections with fluorescently labeled antibodies against rod, LWS cone as well as short-wavelength sensitive (SWS) cone photoreceptor cells. We used MAB5316 anti-rhodopsin for rod labeling and CERN874 and CERN906 anti-M/L-cone opsin for cone labeling with fluorescent secondary antisera. Our results showed rod and cone labeling in the bovine retina, but no cone labeling in the bowhead retina. To confirm the absence of SWS cones, the anti-S cone opsin antibody was used. In both cases, positive control retinae labeled well with the anti-rod, anti-LWS cone and anti-SWS cone antibodies, while only the anti-rod antibody labeled the photoreceptors of the bowhead retina. This data combined with the genetic analysis above provides conclusive evidence that the Balaenid whales, including the North Atlantic right whale, are true rod monochromats and lack photopic cone-based vision.

2) Identify the wavelength(s) of light that will provide right whales with the highest level of contrast vision within their photic environments.

The discovery of rod monochromacy in the right whale has made it very easy to describe the spectral sensitivity of the right whale eye: the spectral sensitivity of the right whale eye is defined by the absorption spectrum of the right whale rod photoreceptor. The right whale rod absorbance spectrum has recently been reported with a wavelength of maximum absorbance at 493 nm (Bischoff et al., 2012) and is shown in Figure 4. As seen in Figure 4, the whale possesses maximum sensitivity to wavelengths in the blue/green region of the spectrum as would be expected for a coastal aquatic marine mammal. The sensitivity of the eye has adapted by matching it sensitivity to the background light to improve the contrast of images against the oceanic color background. Objects with transmission spectra greater than 600 nm would appear black to the whale due to the fact that the rod photoreceptor is insensitive to wavelengths greater than 600 nm, or the red region of the spectrum.

II. Developing Rope Mimics and Testing Whale Responses to Color

To determine whether changing the visual characteristics of the “ropes” might be an effective entanglement avoidance method, we measured the distances at which whales first exhibited a change in behavior in response to the presence of a fake rope. These tests occurred in Cape Cod Bay, following methods developed over the previous two years. The “ropes” were placed along the trajectory of whales feeding in straight lines, and all changes in behavior were recorded by video. Whale and rope distances and separations were recorded by laser range finder, linked

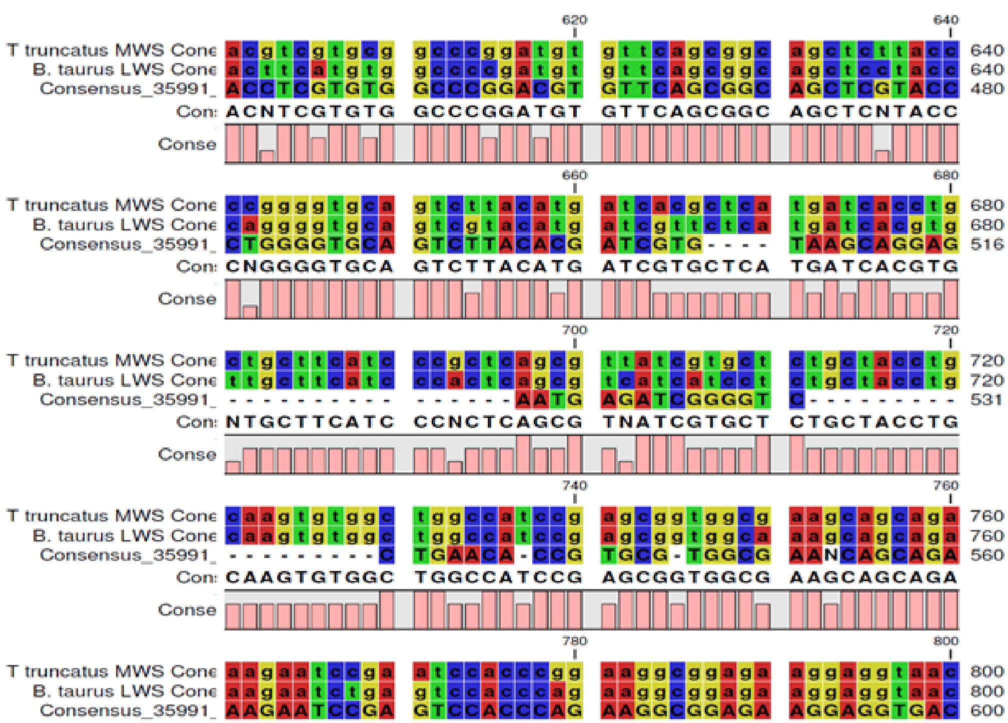


Figure 1. Nucleotide Mutations Observed in Exon 4 of the Long-Wavelength Sensitive (LWS, “Green”) Cone Visual Pigment Opsin in Right Whale. Right whale LWS opsin sequence is compared to sequence from bottlenose dolphin (Tursiops truncates) as well as domestic cow (Bos Taurus). Circled regions mark deleted nucleotide sequence from right whale that ultimately give rise to a premature stop codon in exon 4.

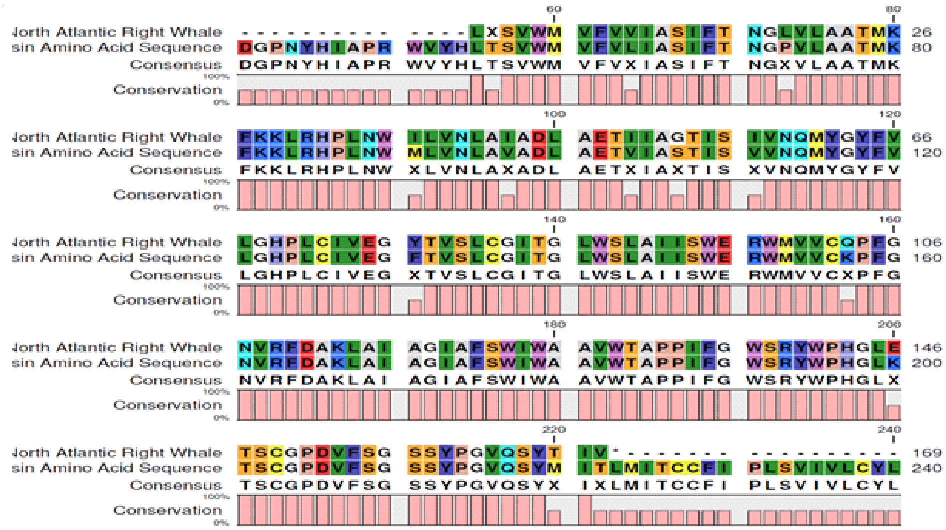


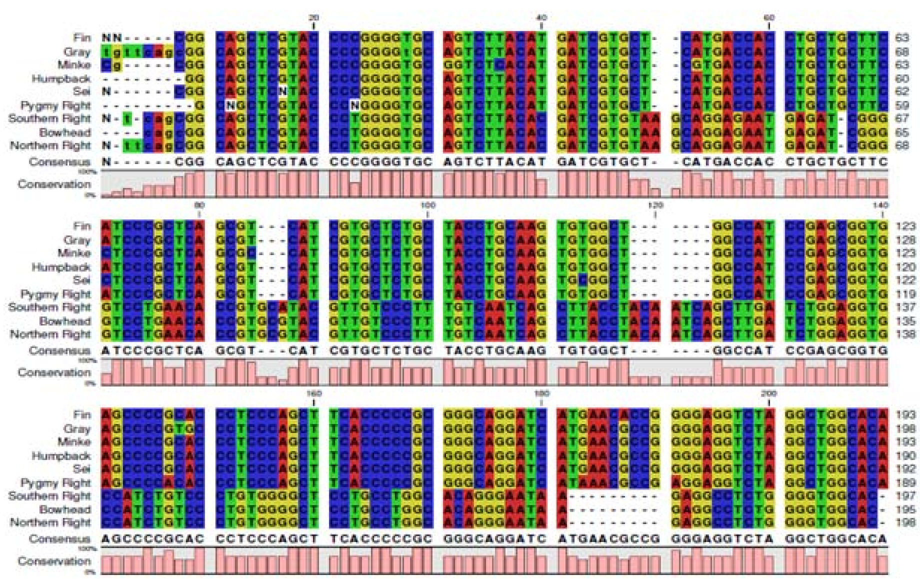
Figure 2. *Deduced Amino Acid Sequence and Resulting Mutations Observed in Exon 4 of the Long-Wavelength Sensitive (LWS) Cone Pigment Opsin in Right Whale. Mutations in genomic DNA result in a premature stop codon at residue position 223 (circled) resulting in a truncated and presumably nonfunctional LWS cone opsin protein.*

to a Panasonic Toughbook and GPS. The observation boat was shut down during trials to minimize any whale response to the boat. Data was collected on respiration rates, submergence time, changes in behavior and/or direction for each whale that approached a rope-mimic within 10 meters (the maximum recorded underwater visibility during these trials). Data collection included the distance at which the first change in behavior occurs, the closest approaches for animals that did not respond to the presence of a rope mimic, and trajectories and behavior for those whales that collided with rope mimics. In addition, we attempted to get underwater visibility estimates using a secchi disk. (see discussion on p. 73)

Fake ropes were constructed from two 10ft sections of rigid PVC pipe approximately the same diameter as 1" rope. The two sections were connected with quick release snap clips, and mechanically scored every 2 to 3 inches so they would shatter if the whale touches them. Ropes were weighted at one end, and attached to a lobster buoy at the other, so that during deployment, whales are presented with the equivalent of a vertical rope in the water column. Ropes were painted with four colors, two of which were commonly used in normal fishing operation (black and green), and two of which appear to fall within the lowest spectral sensitivity for right whales (colors above 600nm, orange and red) (Figure 5), based upon our laboratory studies.

Deployment of the PVC mimics was done in rows of 4 single rope mimics, spaced approximately 25 m apart, depending upon the skim-feeding behavior patterns. This spacing was based upon the underwater visibility, and was designed so that no whale could see more than one rope mimic at a time, eliminating the possibility of multiple color challenges that would confuse the

Figure 3. *LWS Cone Mutations are Exclusive to Balaenidae. Premature stop codon is circled. This specific mutation only arises in the Balaenidae species examined.*



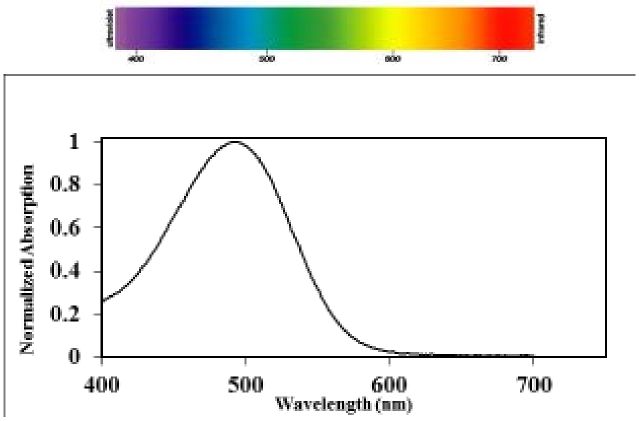


Figure 4. *Normalized absorbance spectrum of the right whale rod visual pigment, showing an absorbance maximum at 493 nm.*

Figure 5. *PVC Rope mimics used in the vision experiments with a color spectrum chart to show color frequencies. (Orange "ropes" not shown)*

interpretation of results. The rope mimics were placed approximately 75-100 m front of the whale in line with its skim-feeding trajectory (see Figure 6). Attempts were made to intercept whales travelling in relatively straight lines, and behavior and turning angles were recorded as they came within underwater visual distance of the rope mimics (ca 10 m).

Right Whale Encounters with Rope Mimics and Estimated Detection Distances

In 2013, we had 101 encounters between right whales and rope mimics in 9 days at sea in Cape Cod Bay. An encounter is defined as the transit of a whale within 30 feet (10 m) of a rope mimic (Figure 7). Previous work indicated that underwater visibility in this area and season is generally around 8 – 10 meters, hence this definition derived from the distance at which a whale may have been able to see a rope mimic underwater. In 2013, we did have periods of lower visibility (ca 5 m), which may have sometimes reduced the effective encounter rates, which will be discussed later.

Of the 101 encounters, whales responded to ropes with a change in behavior 52 times. The distances at which whales responded to rope varied with color. We measured the distance between the whales eye and closest approach to the rope mimic, as well as the distance between the whale's eye and the first change in behavior (COB). In other words, color only can be effective if the whale sees it, so that the eye to rope distance shows detectability distance underwater. The 49 non-responsive whale encounters are currently being analyzed to determine if there are consistent characteristics to those ropes or conditions when whales apparently did not see the rope mimics.

Based on our preliminary analyses on the 52 responding whales, the results consistently indicate the whales respond at varying distances to different colors, presumably because underwater detection is dependent upon color. Using the distance to eye at first COB as the measure of relevance, whales were able to detect red and orange at the furthest distances (averages 3.85 and 4.1 m), black at an intermediate distance (3.1 m average), and green at the closest distance (1.9 m average) (Table 1).

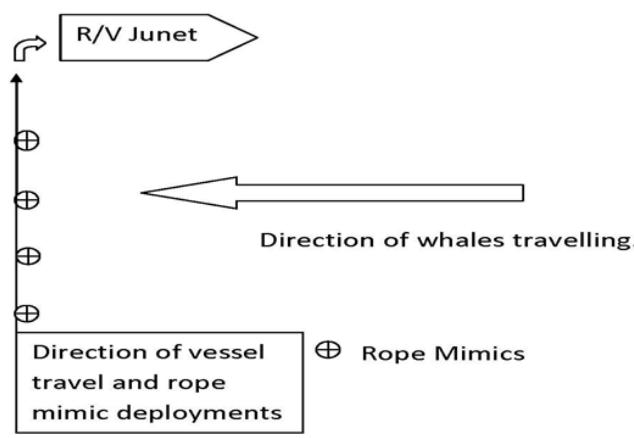


Figure 6. *Experimental design for deploying rope mimics in front of right whales. The vessel would travel at right angles across the estimated trajectory of the skim-feeding whale, deploying rope mimics at regular intervals (generally ca 25 m). At the end of deployment, the vessel would turn to facilitate observations and shut down.*

Figure 7. *Example of a whale encounter with fake ropes. Whales are skim-feeding from right to left. Lobster bouys are connected to 20 feet of scored and colored 1 inch PVC pipe hanging vertically in the water column. These two buoys/ rope-mimics were uncharacteristically close together because of differential drift patterns during a longer deployment.*

An unequal variance t test indicates the difference between the detection distances was significant for green vs red and green vs orange, and approached significance for green vs black (Table 2). This test is done on the closest distance between the eye and the rope-mimic. We are evaluating whether other statistical methods may provide better resolution to the response differences in color.

Collisions with Rope Mimics

In 2013, we had whales run into rope mimics 15 times. These collisions also showed differences by color, with 3 hitting black, 7 hitting green, 3 hitting orange and 2 hitting red. Collisions have may occurred in circumstances with poor underwater visibility, but sometimes the whales appeared to hit the rope mimic head-on, a location where the whale may have poor forward vision.

	Closest approach		Closest eye distance	
color	Mean	Var	Mean	Var
red	1.981	1.477388	3.85	2.558333
orange	2.038538	1.440632	4.142929	5.131231
black	2.545545	3.177178	3.000909	3.394009
green	1.697632	2.544744	1.894895	2.154339

Table 1. Mean and variance of first change of behavior distances for whales and different colored ropes. Both the closest approach and the closest eye distance are given, because the tip of the whales rostrum is ca. 3 m from the eye.

III. Fishermen’s Tests of Orange Rope

Although our research indicates that orange and red colors may be more suitable for reducing the potential for collisions between whales and ropes, changing gear cannot be implemented if fishermen have difficulty with the operational characteristics of the gear. In particular, we wanted to ensure that rope colored in a way to reduce potential entanglements (orange or red) would not foul more than conventional rope, and would have comparable handling characteristics to existing ropes. In partnership with the Maine Lobstermen’s Community Alliance (MLCA), 7 lobstermen were engaged to test the rope during normal fishing operations in 2013 and 2014. The rope was manufactured by Hyliner Rope of St. George, and the color was based upon the preliminary experimental findings suggesting orange was highly detectable by right whales. MLCA purchased 27 coils of 3/8" orange rope; 16 Steel Liner float and 11 Steel Liner sink. The

2 tailed t test unequal variances			
	red	orange	Black
Red			
Orange	0.713954		
Black	0.272467	0.177876	
Green	0.005032	0.003902	0.106424

Table 2. *T-test analyses for differences in whale response to rope colors. Significant differences (< 0.05) are highlighted in yellow.*

color was the same on both the sink and float coils, although the sink rope was distinguished by a white tracer running through it (Figure 8).

The rope was delivered to lobstermen with a logsheet to document the deployment of the rope and its operational characteristics. Six of the seven lobstermen deployed the rope in July or August, one lobstermen deployed the rope in September. Fishermen will provide reports on its operational characteristics in late winter of 2014.

Results

The laboratory work suggests that right whales are rod monochromats, the first mammal so described. This means they effectively have a "black and white" view of their world. Since their peak visual sensitivity is in the blue (493 nm), and blue is the best transmitted and primary ambient light in the ocean, this suggests right

Figure 8. Orange rope developed under this contract and currently being fished by Maine lobstermen.

whales see the oceanic background as bright, and that colors they are not sensitive to appear as high contrast "black" objects or shadows underwater (e.g., red and orange).

Following these findings, our field trials indicate that changes in color can affect the distance at which right whales can detect ropes underwater. Preliminary results indicate that orange and red ropes are detectable at the greatest distances, and that green ropes have poor detectability underwater. Further analyses will examine the distances of the closest approach to rope mimics where the whale did not respond. It is possible that the whales did not respond because the distance of the ropes was just outside of detectable range. These explorations of the data will also include a review of underwater visibility, since that certainly could affect whale abilities to detect ropes of any color. However, the "non-response" analysis may also show difference by color that would be informative to the analysis of detection differences.

To determine if changing rope colors can work for fishermen, we have built ropes of appropriate colors, and are testing them in an operational lobster fishery to determine their handling and fishing characteristics. Given the current findings, it does appear that changing fishing rope colors to red and orange may reduce the probability of collisions between right whales and vertical fishing ropes. Further lab work is needed to determine if such color changes would also be effective for other whale species.

Underwater visibility is likely to be a major factor for whales in detecting objects underwater, regardless of color or other features. We were able to reduce the effect of variability in underwater visibility on the outcome of this experiment by randomizing the color deployments, so that no color was tested more frequently in any particular condition. We used a secchi as a means of testing underwater visibility. ever, during trials conducted in 2011, we vered that secchi depth measurements (the depth iich the white disk disappears from the surface) rally overestimated horizontal visibility (which is a whale may perceive a rope at the surface), ugh the patchiness of plankton can make ontal visibility highly variable. Further, patches ankton in Cape Cod Bay are highly variable in and depth, and measurements taken from the boat l be very different than those taken (theoretically) e whale's feeding path. Therefore, we collected ii depth measurements throughout the season to collect relative visibility information, which can serve as a conservative proxy for horizontal visibility. Secchi measurements ranged from 10 to 60 feet, with most in the 25-30 ft (8-10m) range.

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