**Unoccupied Aerial System assessment of entanglement in Northwest Atlantic gray seals (*Halichoerus grypus*)**

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Unoccupied aerial system (UAS) surveys of large populations of animals that form herds have been used in the context of ecology and conservation (Koh and Wich 2012, Anderson and Gaston 2013, Hodgson *et al.* 2016). UAS can capture high resolution overhead imagery on dense groupings of animals, helping to overcome the limitations of a lateral perspective from boat or land based surveys (Ries *et al.* 1998, Hodgson *et al*. 2016).

UAS can monitor a variety of species, including marine mammals (Hodgson *et al*. 2016, Smith *et al*. 2016, Rhodes and Spiegel 2017, Christiansen *et al*. 2018). While UAS may cause visual and acoustic disturbance of marine mammals, especially pinnipeds that rest on land, if maintained above altitudes where behavioral responses are observed, UAS surveys can be performed without major disturbance (Pomeroy *et al*. 2015, Christiansen *et al*. 2016, Smith *et al*. 2016, Rhodes and Spiegel 2017). However, the physiological effects of UAS flights on seal populations are unknown (Ditmer *et al*. 2013). UAS surveys have been used for marine mammal abundance (Hodgson *et al*. 2016, Sweeney *et al*. 2016) and photographic identification studies (Pomeroy *et al*. 2015), but not extensively for detecting anthropogenic impacts on populations, such as entanglements in pinnipeds.

Marine mammal entanglements are common globally (Laist 1997, Page *et al*, 2004, Boren *et al*. 2006) and were defined by Laist, 1997 as 'interactions between marine life and entanglement material whereby the loops and openings of various type of anthropogenic debris entangle animal appendages or entrap animals'. Entanglements can involve abandoned or 'ghost' fishing gear, active fishing gear in use at the time of entanglement, or nonfishery derived debris (Laist 1997, Raum-Suryan *et al*. 2009, Asmutis-Silvia *et al*. 2017).

Entanglement associated mortality in seals can be acute due to drowning or asphyxiation in gear or chronic through secondary complications of injuries or feeding impairment (Moore *et al.* 2013).

Circumferential entanglements, such as encircling neck wraps of gear, are regarded as serious injuries under NOAA National Marine Fisheries Service Guidelines for Distinguishing Serious from Non Serious Injury of Marine Mammals pursuant to the U.S. Marine Mammal Protection Act, therefore it is likely that these entanglements lead to the animal's mortality (Hanni and Pyle 2000, Date 2012).

Calculating the prevalence of entanglements in pinnipeds has been shown to be challenging, due to the difficulty in monitoring seal interactions with deployed fishing gear, the lack of observed deaths caused by entanglements at sea, and the limitations of observing entanglements during surveys. Existing prevalence estimations are biased towards an underestimation of entanglement (Laist, 1997, Hanni and Pyle 2000, Page *et al*. 2004, Raum-Suryam *et al*. 2009). While acute entanglement mortality is estimated routinely in New England waters, prevalence of longer term gray seal (*Halichoerus grypus*) and harbor seal (*Phoca vitulina*) entanglements on the East Coast of the United States of America have yet to be determined (Hayes *et al*. 2017, Jackman *et al*. 2018).

The primary objective of the study was to assess the prevalence of live entangled gray and harbor seals in discrete haul-outs across Massachusetts and Maine, USA, by analyzing UAS survey data. The study also examined the spatial distribution of entangled seals within discrete haul-outs and the feasibility of identifying entanglement material. Furthermore, the study assessed the efficacy of the UAS method to accomplish the study objectives.

Two types of UAS (Inspire 1RAW dji.com or APH 22 aerialimagingsolutions.com) were used to fly over haul-outs of seals. A 45 mm micro 4/3 1:1.8 Olympus lens or 25 mm micro 4/3 F1.8 Olympus lens was used on each UAS. Serial overlapping still images of the animals were recorded. Video footage was recorded on some of the Inspire flights. On each day sampled, flights were made with the camera angle vertically or obliquely above the seal haul-outs at an altitude sufficient to avoid any sign of disturbance, such as head lifting (Smith *et al*. 2014, Rhodes and Spiegel 2017). The method used to avoid disturbance was commencing the UAS flight at very high altitude (about 55m) and decreasing altitude slowly until head lifting was observed. Once observed, the altitude was increased and kept above that threshold level for the duration of the flight. The altitude at which this occurred varied with location and season. Surveys (see Electronic Supplement, Table 1) were conducted seasonally from June 2017 to August 2018. Time and latitude and longitude for each flight were obtained from the flight logs. Location of seal haul-outs shown where UAS was overflown seen in Figure 1.

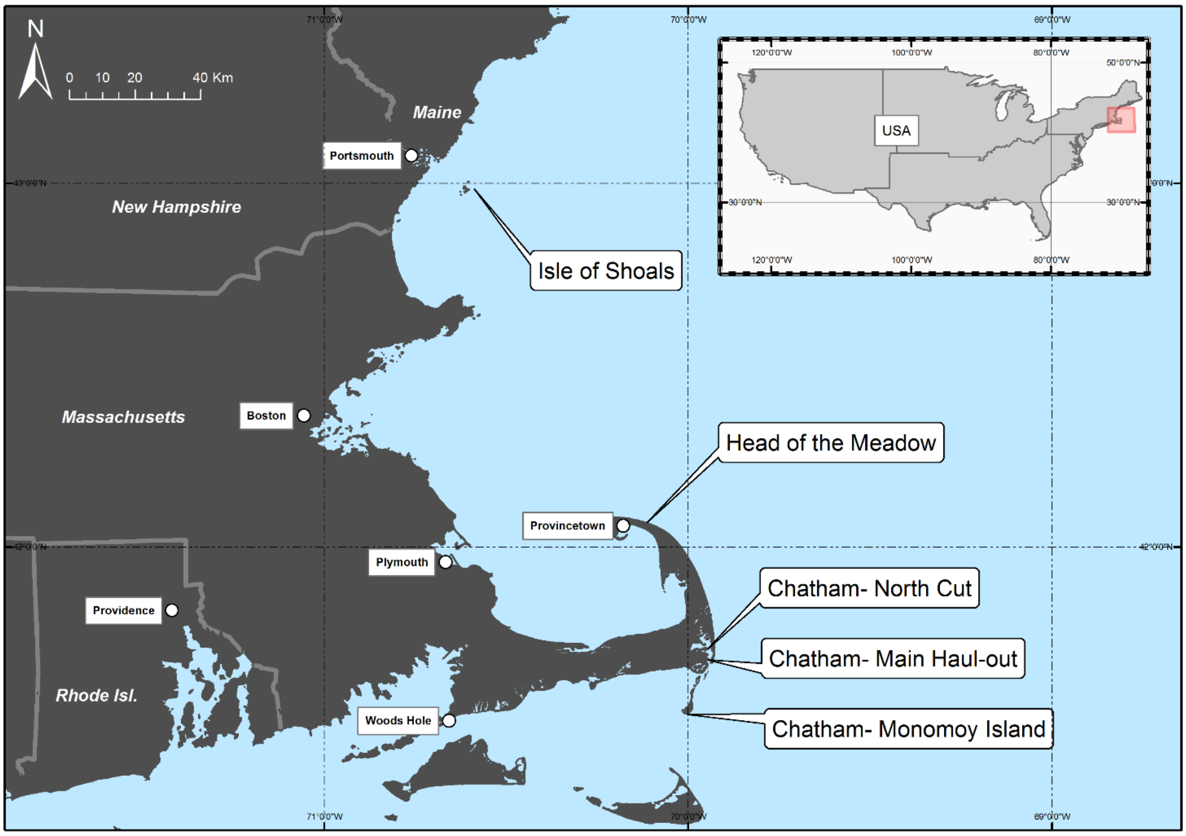


Figure 1- Study areas for UAS gray and harbor seal surveys: Chatham- North Cut (41$^o$42'50''N, 69$^o$56'45''W), Chatham- Main Haul-out (41$^o$40'53''N, 69$^o$56'33''W), Chatham- Monomoy Island (41$^o$32'28''N, 70$^o$0'43''W), Isle of Shoals (43$^o$0'17''N, 70$^o$36'10''W) and Head of the Meadow (42$^o$3'35''N, 70$^o$5'47''W).

For haul-outs to be assessed independently, only 1 flight per site per day could be used due to seal movements between and within haul-outs. This was done to avoid double counting of individuals. For each site, the flight with the best quality images was selected and an image of the whole seal haul-out was constructed by stitching together the serial overlapping images (Fig. 2) using Microsoft PowerPoint. A total count was undertaken using Image-J cell counter plug-in (Scheinder *et al.* 2012, O'Brien *et al*. 2016). Total counts of each haul-out site were made three times. If numbers of total seals differed between the 3 counts, a mean was taken. Only animals where head, neck, and body were fully visible were included in the count and assessed for entanglement (see Electronic Supplement, Table 2).

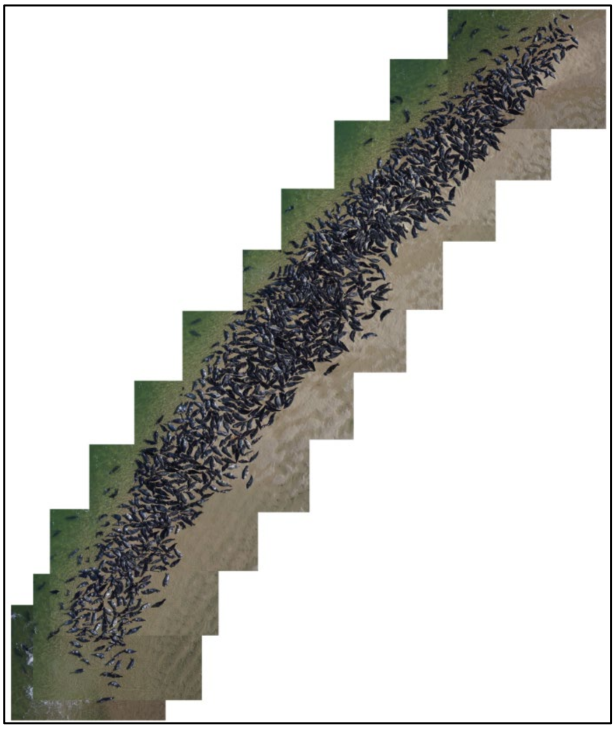

Figure 2-Example of stitch up produced for gray seal counts of total haul-out. Images taken on 26th June, 2017.

Images used to stitch complete haul-outs were then enlarged between 2-4 times in order to examine each individual seal for any sign of entanglement. In this study, entanglement was determined as tight constrictions around neck or body (with or without material or gear visible) or a circumferential wound around the neck or body. Seals with superficial linear impressions and no visible material were not included in the entanglement count as we could not confidently confirm such cases as entanglements. The definition agreed for this study was a suitable description based on the resolution of imagery obtained. Other descriptions from other studies were not considered to avoid overcounting of entanglements. Video footage, when available, was used to confirm entangled individuals and material type. Entanglements were counted per site for any given day, and individual photos of entangled seals were cataloged (Fig. 3). Isle of Shoals, Maine was the only site with a mixture of gray and harbor seal haul-outs, however, only gray seal entanglements were detected. No harbor seals were seen at the Massachusetts sites. For the analyses, due to the lack of harbor seal entanglements, only gray seals were included. Identifying seal species was done using facial characteristics, body shape features, and coat color (Hannah 2006). Prevalence was calculated for each site and day sampled for gray seals only. Prevalence was defined as: number of entanglements/total gray seal count, and converted into a percentage.

Based on the video and still images, only one type of material could be identified and that was monofilament net so each entanglement was categorized as either monofilament net or 'cannot be identified'. Monofilament net is identified from UAS imagery as a laterally extending, 2D knotted structure, often with flaring ends from the entanglement.

Entangled individuals were then analyzed according to their location within the specific haul-outs. Individuals that were within 3 animals from the edge of the haul-out were classified as 'Edge' and those that were more than 3 animals from the edge were classified as 'Middle'. This analysis was done to test whether there was a tendency for entanglements to be found on the edges of haul-outs. Unpublished preliminary studies on boat based methods to detect entanglements, showed a much higher prevalence than obtained through the UAS. Boat based methods cannot detect 'Middle' entanglements or produce a complete total population count. Therefore, if entanglements are shown to be concentrated on the edges, it is possible that boat based methods hyperinflate prevalence.

To test any association between location and entanglement counts, a Pearson's Chi-Squared test was conducted. The mean prevalence for each site per location of entanglement (Edge or Middle) was also calculated. All statistical analyses were conducted on R studio (Rstudio Team 2015).

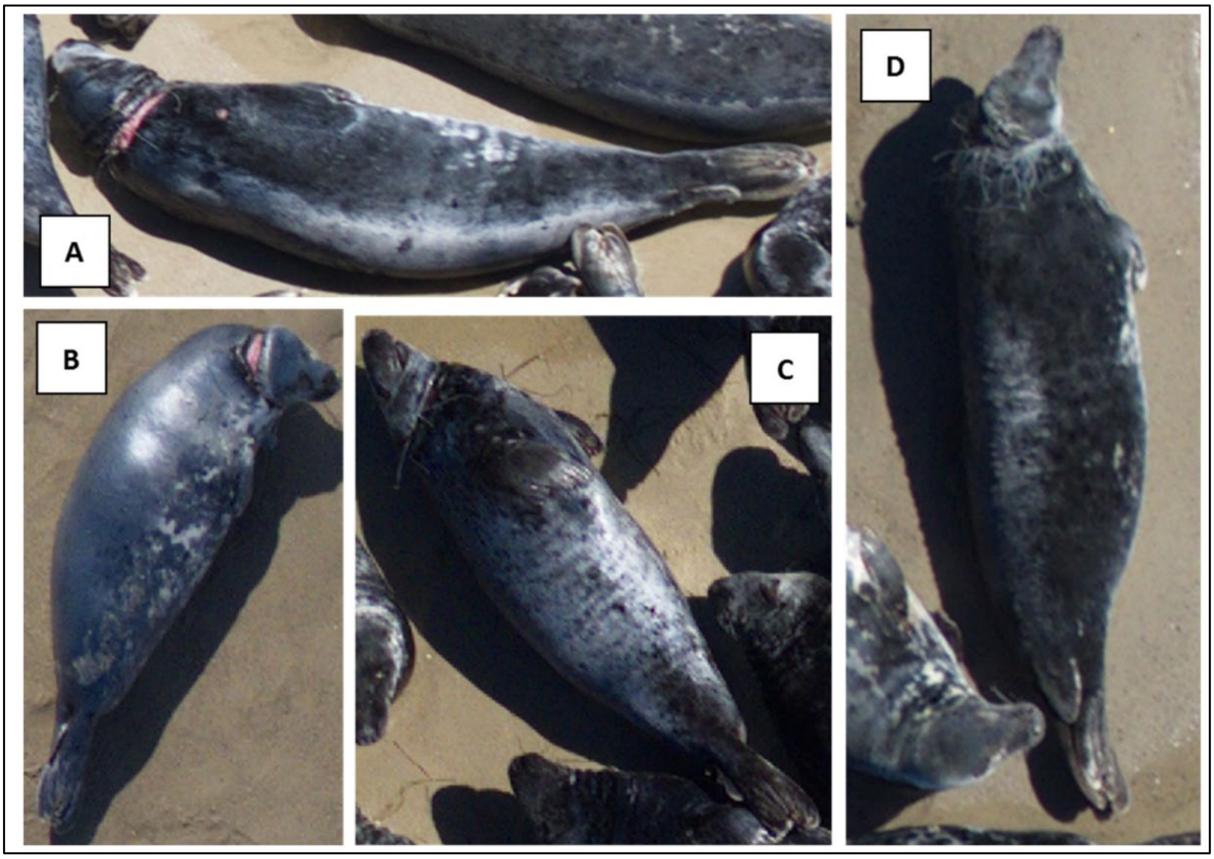


Figure 3- Representative images of entangled individual gray seals. Monofilament net (A, C and D) and entanglement with unidentified material (B). All individuals photographed at Chatham- North Cut.

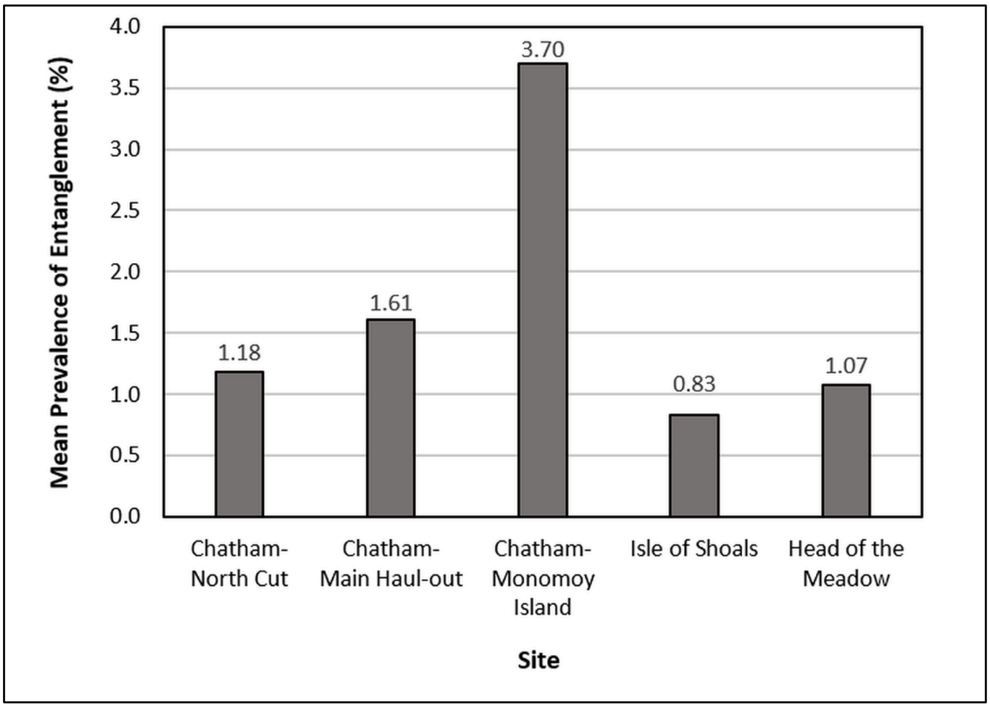


Figure 4-Mean prevalence of gray seal entanglements present at 5 sites (*n*=number of days surveyed at each site): Chatham- North Cut (*n*=7), Chatham- Main Haul-out (*n*=5), Chatham- Monomoy Island (*n*=1), Isle of Shoals (*n*=5) and Head of the Meadow (*n*=3).

Mean prevalence (*n*=number of days surveyed) was calculated for each specific site (Fig. 4). Mean prevalence for each site was calculated using the sum of all entanglement prevalences divided by *n*=number of days surveyed. The mean prevalence ranged from 0.83% at the Isle of Shoals (*n*=5) to 3.7% on Chatham- Monomoy Island (*n*=1), the site with the highest prevalence. Across all sites, the mean prevalence of gray seal entanglement was 1.30% (*n*=21, *s*=1.15). Across all Massachusetts sites (excluding the Isle of Shoals), the mean prevalence of gray seal entanglement was 1.44% (*n*=16, *s*=0.88). The prevalence for all the Chatham sites (North Cut, Main Haul-out and Monomoy Island, *n*=13, *s*=0.92) was 1.54%.

Table 1- Number of gray seal entanglements that where categorized as monofilament net compared with the number of entanglements where material could not be identified per site. Percentage of total entanglements per site in brackets.

| Site | Monofilament Net Entanglements | Unidentified Entanglements |
|---|---|---|
| Chatham- North Cut | 21 (40.4%) | 31 (59.6%) |
| Chatham- Main Haul-out | 3 (10%) | 27 (90%) |
| Chatham- Monomoy Island | 0 (0%) | 3 (100%) |
| Isle of Shoals | 0 (0%) | 2 (100%) |
| Head of the Meadow | 0 (0%) | 20 (100%) |

Only two of the sites (Chatham- North Cut and Main Haul-out) had individuals with identifiable entangling material, which was monofilament net in all cases. Across all sites, 22.4% of all entanglements were classified as monofilament net and in the remaining 77.6%, the type of gear could not be identified. Chatham-North Cut had the highest number of monofilament net entangled individuals identified, with 40.4% being monofilament net and 59.6% being unidentified.

Table 2 shows the number of entanglements classified on the Edge or Middle of a haul-out and the mean prevalence per location and per site. There was a statistically significant difference between location within the herd (Edge or Middle) and entanglement count ($P<0.05$) in two sites: Chatham-North Cut and Main Haul-out, with entangled seals being found more commonly on the Edge. Although not statistically significant elsewhere, this pattern was consistent across all sites (Edge – 1.08% and Middle – 0.22%).

Table 2-Gray seal entanglements on the Edge and Middle of haul-outs: count (% prevalence), *P* value from Pearson's Chi-Squared test. * Significance level $P<0.05$.

| Site | Number of Days Surveyed (*n*) | Edge | Middle | *P* value |
|---|---|---|---|---|
| Chatham- North Cut | 7 | 37 (0.86%) | 15 (0.34%) | 0.00359* |
| Chatham- Main Haul-out | 5 | 22 (1.33%) | 8 (0.29%) | 0.01762* |
| Chatham- Monomoy Island | 1 | 3 (3.70%) | 0 (0%) | 0.24820 |
| Isle of Shoals | 5 | 2 (0.83%) | 0 (0%) | 0.46950 |
| Head of the Meadow | 3 | 14 (0.79%) | 6 (0.28%) | 0.11753 |

Confirmed entanglement cases ranged from obvious monofilament net constrictions to deep and wide wounds that exposed underlying blubber and occasionally muscle. Considering the low probability of marine mammals shedding entanglements over time, our study assumed that all seals with open neck wounds and constrictions, even in the absence of visible gear, were entangled at the time of imaging (Laist *et al*. 1997, Page *et al*. 2004). Entangling material lacking extending strands or trailing gear and/or occurring within a deep wound is difficult to detect from aerial images (UAS or plane), and accompanying videos were useful in improving entanglement evaluations in many of these cases.

Due to seal movements between and within haul-outs, only one flight per site per day could be used, which led to small sample sizes, especially for Chatham- Monomoy Island (*n*=1). Some sites including Isle of Shoals and Chatham-Monomoy Island are difficult and costly to visit which is reflected in the small sample sizes. For future research, more UAS flights are recommended. The extent of movement between and within haul-outs needs to be quantified to better define optimal survey effort on a given day.

It is assumed that the total entanglement count presented here is an underestimation as seals with just linear impressions around neck and body were not confirmed to be entangled. Many entangled seals become lost or die at sea which are also not included in the estimate. The entanglement prevalence was comparable between sites except for Chatham- Monomoy Island. This may be due to the single UAS survey at that site that surveyed only a small portion of the entire haul-out. More UAS flights would be needed on Chatham- Monomoy Island haul-out for a better assessment of the site. The Isle of Shoals site also had limited UAS data, leading to low seal counts and only two detected entanglements. This limited data set does not reflect the high entanglement prevalence for harbor and gray seals assessed with 30-40 boat based surveys per season on the Isle of Shoals between May-August, 2011-2018 (A. Bogomolni pers. com). Additional gray seal haul-out sites, such as Muskeget Island and Nantucket should also be considered in future studies.

Prevalence (0.83% in the Isle of Shoals to 3.7% in Chatham-Monomoy Island) can be extrapolated to current minimum population estimate of gray seals in U.S. Atlantic waters of 23,158 (Hayes *et al*. 2018), the minimum total number of entangled gray seals could be estimated to range from 192 to 857 (0.83% to 3.7%), according to our calculated prevalences. Though this cannot be considered an annual estimate, since a few entangled seals have been known to survive for years, this number can be compared to the annual mortality estimates resulting from bycatch in northeast fisheries. The fisheries bycatch estimates are calculated based on observed rates of mortality extrapolated over the total effort in the fisheries (Chavez-Rosales *et al*. 2017, Orphanides and Hatch 2017). During the years 2011-2015 estimated gray seal mortalities averaged 1,020 (4.33% of the current minimum population estimate) per year in northeast sink gillnet fisheries, and 31 (0.13% of the current minimum population estimate) per year in northeast bottom trawl fisheries (Hayes *et al*. 2018).

During this same period, 4 gray seals or seals of undetermined species reported by fisheries observers were determined to have had sub-lethal interactions with gillnet or trawl fishing gear (Josephson *et al*. 2017). Therefore, the extrapolated estimates from this current study strongly suggest that the incidence of sublethal interactions between gillnets and gray seals in the Cape Cod area is grossly underestimated when relying on observer data alone.


Figure 5- A-Photograph from boat-based survey of an entangled gray seal where monofilament net can be seen in the entanglement but severity of wound is questionable. B-Photograph of the same entanglement wound with monofilament net from physical examination. C- Photograph of the same wound with monofilament net removed. Photographs taken by the International Fund for Animal Welfare.

Whether the prevalence of entangled seals within haul-outs is representative of that of the population at large is unknown. A significant limitation to measuring sublethal entanglement prevalence is that marine mammals tend to be highly migratory and disperse widely, so sampling of entanglements in pinniped populations solely on-shore may not depict true prevalence (Laist *et al*. 1997, Hanni and Pyle 2000). Additionally, these surveys were limited to the summer and early fall, which also may affect perceived entanglement prevalence.

The UAS images did not provide enough resolution to identify the type of material in 77.6% of all entanglement cases. A lens with greater focal length and resolution would be needed to attempt to capture the full detail of the entanglement, given that a lower altitude would result in disturbance and flushing of the hauled out seals. However, a larger resolution lens would need a larger UAS which in turn also causes more disturbance, resulting in the need to fly higher to avoid such disturbance.

Boat based surveys may be able to provide higher resolution images to assist in gear identification, but often with deeply embedded entanglements, only physical examination or necropsy will be definitive in this respect (Fig. 5). Recommendations for future research include conducting boat based surveys simultaneously to UAS surveys to complement the greater spatial coverage that the UAS imagery can provide with the more detailed boat based imagery for better entanglement type identification. If necropsied or stranded animals could be linked to UAS or boat based data, a better understanding of entangling material and wound severity would result.

Gray seal entanglement in monofilament fishing gear reflects the presence of gillnet fisheries in New England (Read *et al*. 2006, Orphanides and Hatch 2017). Recreational fisheries using monofilament lines are also present in New England, but their role in entanglements are unclear due to the cryptic nature of a single line wrap within a wound compared with gillnet monofilament net which extends more laterally given its knotted two dimensional structure. Monofilament line can potentially wrap around and cause a constriction through drag of the trailing line and become multiple simply by the animal turning. However, in 20 yr of documenting live and dead entangled pinnipeds on Cape Cod and Southeastern, MA, the International Fund for Animal Welfare (IFAW), which is the permitted agency for stranded marine mammal response in that area, has never identified a neck entanglement caused by monofilament line (unpublished data, IFAW 2018). Whether these entanglements are caused by active fishing gear or abandoned gear is another uncertainty but in this study, it is assumed to be actively fished gear due to the high prevalence of year-round gillnet fisheries, documented interactions between fisheries and seals, and lack of fouling on the entangling gear as would be expected with 'ghost' gear (Bogomolni *et al*. 2010, Asmutis-Silvia *et al*. 2017, Orphanides and Hatch 2017).

The higher entangled seal prevalence identified at the haul-out Edge *vs*. Middle in Chatham's North Cut, and Main Haul-outs would not have been possible to detect using boat based surveys alone since this method does not have photographic access to seals in the middle of the haul-out. It is also important to note that based on these results, boat based surveys will overestimate entanglement prevalence because they are biased towards the edges of haul-outs. However, due to the relatively small sample size of this study, conducting boat and UAS surveys in tandem are required to investigate this further.

While reasons for the observed marginalization of entangled seals cannot be identified through this study, poorer health may be a contributing factor. Further study on the health implications of chronic entanglements would help to elucidate their impacts on gray seals.

The population of gray seals in Massachusetts and Maine needs regular monitoring and surveying and UAS has shown encouraging results when it comes to estimating prevalence of entanglements. This study improves our understanding of fisheries entanglement within these seal populations and will help better inform potential mitigation efforts to improve their welfare and conservation.

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