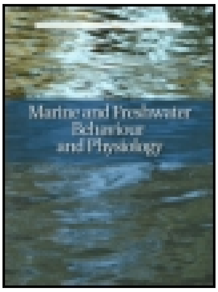

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A trial of acoustic harassment device efficacy on free-ranging bottlenose dolphins in Sardinia, Italy

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Acoustic harassment devices (AHDs) have been deployed to reduce the interaction between different marine mammals and fisheries in many areas. Despite field studies on some marine mammal species, there is a lack of information about their effectiveness on common bottlenose dolphins. The controlled exposure experiment described here is the first practical attempt to assess the influence of an AHD on the behaviour of free-ranging common bottlenose dolphins in association with a marine fin-fish farm. A total of 90.7 h were spent in direct observation of 55 groups of bottlenose dolphins along the north-eastern coast of Sardinia (Italy). Activation of the AHD did not have a significant and immediate effect on bottlenose dolphins' presence, distance from the AHD, group size or time spent in the fish farm area. The AHD alone did not effectively deter bottlenose dolphins, particularly when other motivating factors, such as food, were present. Thus, prior to further employment of AHDs, additional research in their effects on the marine environment is essential for coastal conservation and aquaculture management.

Keywords: bottlenose dolphins; *Tursiops truncatus*; aquaculture; acoustic harassment devices; AHDs; Mediterranean Sea

Introduction

There are two basic categories of electronic acoustic devices that have been deployed to reduce the interaction between different marine mammals and fisheries in many areas. These sound production devices are: low-powered (acoustic deterrent devices (ADDs), commonly referred to as 'pingers') and high-powered (acoustic harassment devices (AHDs), or 'seal scarers'; Haller and Lemon 1994; Milewski 2001). ADDs and AHDs differ in both output source levels and frequency bands and not all cetacean species react to the sounds produced by these devices in the same way (Anderson et al. 2001).

The low-powered ADDs typically operate in the 10–100 kHz band and emit source levels below 150 dB re 1 μPa @ 1 m. They are used to protect marine mammals from potential danger by alerting them to the presence of unnatural structures, such as fishing nets (Northridge et al. 2006). Field studies with pingers in gillnet fisheries have documented changes in the behaviour and local abundance of harbour porpoises (*Phocoena phocoena*; Kraus et al. 1997; Trippel et al. 1999;

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Kastelein et al. 2000; Culik et al. 2001; Kastelein et al. 2001; Olesiuk et al. 2002; Kastelein et al. 2006). Pingers probably also have a deterring effect on tucuxi (*Sotalia fluviatilis*; Monteiro-Neto et al. 2004), short-beaked common dolphins (*Delphinus delphis*; Barlow and Cameron 2003), Franciscana dolphins (*Pontoporia blainvillei*; Bordino et al. 2002) and New Zealand Hector's dolphins (*Cephalorhynchus hectori*; Stone et al. 2000). However, not all field experiments have encountered this level of change. Cox et al. (2001) reported the habituation of free-ranging harbour porpoises to one type of pinger; similar results were observed by Jørgensen (2006) with harbour porpoises that were partially habituated to two different types of pingers. Other studies suggest that some other species such as the Indo-Pacific humpback dolphins (*Sousa chinensis*) in South Africa (Kastelein et al. 2001) and the common bottlenose dolphin (*Tursiops truncatus*) in USA showed little or no response to the alarm (Cox et al. 2003). Similarly, sea lions (*Otaria flavescens*) damaged catches in gillnets containing active pingers more often than those without pingers (Bordino et al. 2002).

The second category involves the high-powered AHDs that mainly operate between 5 and 30 kHz at levels often exceeding 170 dB re 1 μPa @ 1 m (Northridge et al. 2006). These electronic devices have mostly been tested with seals and sea lions and are designed to prevent depredation on farmed fish by causing discomfort to the marine mammal (Reeves et al. 1996; Kraus et al. 1997; Johnson and Woodley 1998; Johnston 2002; Olesiuk et al. 2002). The efficiency of these devices differed depending on the areas and devices tested. In a trial involving one type of harassment device in the Baltic Sea, depredation losses of salmon in traps due to grey seals (*Halichoerus grypus*) were halved, doubling the landed catch (Fjälling et al. 2006). However, Quick et al. (2004) reported survey results indicating that despite the elevated usage of harassment devices, damage to Scottish marine salmon farms by harbour (*Phoca vitulina*) and grey seals increased between 1987 and 2001. Killer whales (*Orcinus orca*) were strongly displaced, as a non-target species, by one type of harassment device in a study conducted in British Columbia (Morton and Symonds 2002). Despite these field studies concerning the efficiency of AHDs on some marine mammal species, there is a lack of information about their effectiveness on common bottlenose dolphins.

Owing to the worldwide growth of intensive fish farming in recent years, new habitats have been created by supplementation of nutrients that attract predator species such as the common bottlenose dolphins (Würsig and Gailey 2002; Díaz López et al. 2005; Díaz López and Bernal Shirai 2007; Bearzi et al. 2008). Bottlenose dolphins are inevitably attracted by high densities of fish concentrated in relatively small areas, such as farmed fish in the cages (Díaz López 2006a). In recent years, aquaculturists have therefore been attempting to dissuade bottlenose dolphins from taking fish that are being grown at great expense. Methods of dissuasion consist mainly of anti-predator nets or other enclosures around finfish aquaculture facilities, but this method can cause concern if there is a high risk of incidental captures (Würsig and Gailey 2002; Díaz López and Bernal Shirai 2007). More recently, AHDs have been used.

The overall objective of this study was to test whether a particular underwater AHD could affect the behaviour of common bottlenose dolphins in association with a marine fin-fish farm on the north-eastern coast of Sardinia (Italy). The controlled exposure experiment described here is the first practical attempt to assess the

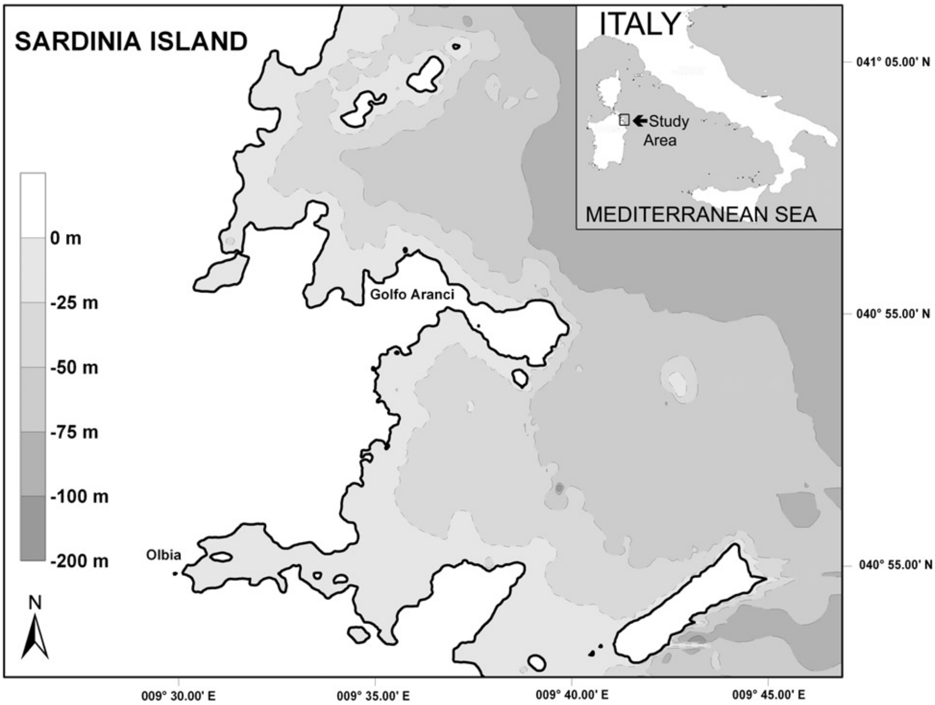

Figure 1. Map of the study area along the north-eastern coast of Sardinia (Italy) showing the location of the marine fin-fish farm.

influence of an AHD on the behaviour of free-ranging common bottlenose dolphins in association with a marine fin-fish farm.

Material and methods

Study area

Field work was conducted over a 20-week period between February and June 2009 on the north-eastern coast of Sardinia (40°59.98′N09°37.09′E), Italy (Figure 1). The marine fin-fish farm in which the experiment was carried out caged sea-bass *Dicentrarchus labrax*, gilthead sea bream *Sparus auratus*, and shi drum *Umbrina cirrosa*. The fin-fish farm consisted of 21 floating cages grouped into three rows of seven cages, totalling in an area of 12,000 m^2. Each floating cage, constructed out of nylon mesh netting, was 22 m in diameter and 15 m deep. The cages were situated approximately 200 m from the shore, with a minimum depth of 18 m and a maximum depth of 26 m. The sea bottom in the study area was characterized mostly by mud with scattered areas of rock and sand.

Experimental procedure and equipment

The experiment was designed and carried out to maximize statistical power and to optimize the likelihood of producing meaningful results in the shortest possible time.

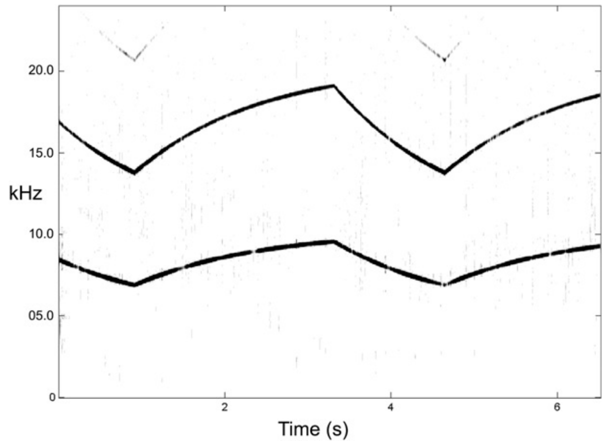

Figure 2. Spectrogram of the AHD frequency curve (FFT = 1024; display frame duration = 10 ms).

To avoid problems associated with stereotyped monitoring times, the experiment was conducted once per trip and the starting time randomly chosen (during daylight hours between 0800 and 1900) before the start of the trip.

The effect of the AHD was assessed by monitoring temporal changes in dolphin presence and group dynamics in the fish farm area. In order to have comparable controls, this was done both with the active and inactive AHDs. Each trial was formed by three sets of the same duration (40 min). The first set was a control, where the dolphins were monitored before the use of the AHD. In the second and experimental set, the dolphins were monitored with the AHD on. The last set was a control set to monitor the dolphins after AHD exposure.

The type of AHD used in this study is developed by the enterprise ICA S.L (Ingenieria y Ciencia Ambiental S.L, Madrid, Spain). It was tested in turn with a single transducer placed 4 m below the surface and attached to a floating fish farm cage. The AHD transmits the sequences of two continuous tonal segments that form a rising and falling frequency contour. These segments last 1200 and 1800 ms, respectively (Figure 2). The fundamental frequencies range from 6.2 to 9.8 kHz with two uniformly distributed harmonics. This AHD further produces a maximum source level of 194 dB re 1 μPa @ 1 m.

In order to avoid variations in the AHD characteristics during the trials, the battery levels were controlled daily. The output from the AHD was detected below the surface via a portable spherical omni-directional hydrophone at an equivalent horizontal range of 1 m. Recordings were made using a professional 2-channel mobile digital recorder (M-Audio) at a rate of 48 kHz and 16 bites, providing a maximum frequency of 24 kHz for all the recordings.

Observation procedures

The observation period (between February and June 2009) corresponds with the peak of common bottlenose dolphin presence in the fish farm area (Díaz López and Bernal Shirai 2007). To minimize the effect of the observer's presence on dolphin occurrence and behaviour, data were collected from a 14 m fish farm boat with the engine turned off. This was done during normal daily farmed fish feeding operations and while the boat remained in the same position for at least 120 min at a time. Two experienced observers were stationed on a 4 m high observation deck, using both the naked eye and 10×50 binoculars to observe dolphin presence and group composition. Both observers made separate tallies and their notes were compared at the end of the trial period. Samples in which the data from the two observers mismatched were excluded in the subsequent analysis.

Any incidental bottlenose dolphin sightings that occurred out of the designated trial period were ignored. During this study, the term group was distinguished as either a solitary animal or any aggregation of dolphins in the visual area, usually involved in the same activity, following Díaz López (2006b). The encounter continued until the focal group changed composition or was lost; a group was considered lost after 15 min without a sighting (Díaz López 2006b).

During a sighting, group size was estimated based on the initial count of individuals that surfaced at one time. The group size and age categories were assessed visually *in situ*, and the data were later verified with photographs and videos taken during each sighting. Group composition was determined by counting the minimum number of adults, calves and newborns present. Age class definitions followed those of Díaz López (2006a), where dolphins were classified as either: (1) calves: dolphins two-thirds or less the length of an adult which consistently swam beside and slightly behind; or (2) adults: those estimated to be longer than 2.5 m.

To score whether or not bottlenose dolphins were present during a set and to define the minimum distance between the AHD and a surfacing bottlenose dolphin, one-zero (categorical) sampling (Altmann 1974) was used. The known distance between the cages and their diameter was used to estimate the minimum distance between the AHD and the bottlenose dolphins. This point of closest approach was subjectively categorized on a four-point scale (1, lower than 50 m; 2, between 50 and 100 m; 3, between 100 and 200 m; and 4, more than 200 m).

Immediately after the beginning and end of each 40 min set, potentially confounding variables that were beyond control but which may have influenced relative abundance, distribution or sightability (environmental and anthropogenic conditions) were recorded. Observations were considered satisfactory when the visibility was not reduced by rain or fog, and sea conditions were < 4 on the Douglas sea force scale (approximately equivalent to the Beaufort wind force scale). Moreover, the number and type of vessels (fishing and motor boats) present in the area during each set were recorded.

Data analysis

A common problem with behavioural studies is the pooling effect, where multiple measurements on the same individual or group are considered independent of each other (Hulbert 1984). To limit the lack of independence arising from repeated sampling of the same individuals, data were randomly selected from all three sets (before, during and after AHD exposure) for all 5 months of data. The target sample size was arbitrarily set at six sets per month for each proposed type of set. Thus, for subsequent statistical analysis, 90 randomly selected samples were used. All variables were tested for normality using Shapiro–Wilks test prior to statistical analysis.

As some data were not normally distributed, the variables were transformed to near-normality by a log 10 transformation.

Principal component analysis (PCA) was conducted to (1) isolate independent and uncorrelated variables from the original set of variables (time spent in the fish farm area, group size, number of adults and number of calves, number of fishing and motor boats) and (2) meet the assumption of independence for subsequent analysis. The principal components method of extraction begins by finding a linear combination of variables (components) that account for as much of the variation in the original variables as possible. All variables were normalized using division by their standard deviations. The eigenvalues represent the variance extracted by each component and are expressed as a percentage of the sum of all eigenvalues (i.e. total variance). Afterwards, PCA components were used in place of the original variables during subsequent statistical analysis (McCowan et al. 1998).

To test if the selected variables would vary with each proposed set (control and experimental; before, after and during AHD exposure), a multivariate discriminant function analysis was conducted on the component loadings from the PCA. Discriminant function analysis identifies a linear combination of quantitative predictor variables that best characterize the differences among groups. For the purposes of this study, discriminant analysis was used in a descriptive manner to reveal major differences between the proposed sets. Variables were combined into one or more discriminant functions. Based on these discriminant functions, the classification procedure assigns each variable to its appropriate group (correct assignment) or to another group (incorrect assignment). The larger the standardized coefficients for each type of variable in each discriminant function are, the greater the contribution of the respective variable to the discrimination between groups will be. For external validation, the cross-validation classification technique was used, in which each case is classified by the functions derived from all cases other than that one.

To test for differences between each proposed set, a multivariate analysis of variance (MANOVA) was conducted. The one-way or nested ANOVA (including Levene's statistic for homogeneity of variances and Welch *F*-test in the case of unequal variances) was conducted to test the equality of means of several univariate samples.

Two contingence table analyses (based on chi-squared test) were used to investigate the effect of the AHD on the occurrence of common bottlenose dolphins (presence *vs*. no presence per set) and the minimum distance of the dolphins from the AHD. All the statistical tests and mathematical analyses were performed with PAST (Hammer et al. 2001) and MINITAB® Release 14.1 software packages. The data are presented as means ± SE. Statistical significance was tested at a $P < 0.05$ level.

Results

Between 2 February and 29 June 2009, the presence of bottlenose dolphins in the fish farm area was noted for 48 days (80% of the total 60 days at sea). A total of 90.7 h were spent in direct observation of 55 groups of bottlenose dolphins in the study area.

To compensate for the lack of independence arising from repeated sampling of the same group of individuals during one trial, 90 sets (55.5% of the 144 trials) were randomly selected from all the three 40 min sets for all 5 months of data. Of these randomly selected sets, a total of 23 different bottlenose dolphin social groups were recorded, corresponding with 17 identified bottlenose dolphins (five males, eight females and four calves). Group size within the sample varied from one to 10 individuals (mean $= 4.6 \pm 0.2$). Samples were composed of either adults only (nine groups) or adults and mother–calf pairs (14 groups).

Bottlenose dolphin presence in the marine fin-fish farm area remained stable throughout the study. Summarized data (mean, standard error, minimum and maximum) for experimental and control sessions are shown in Table 1. The number of sightings per set was not significantly different during AHD activity in either pre-exposure or post-exposure periods (chi-squared contingency table, $P > 0.05$). Likewise, the minimum distance of the dolphins from the AHD was not significantly different between the experimental and control sessions (chi-squared contingency table, $P > 0.05$; Figure 3).

PCA generated six statistically independent components. The first three components accounted for 70% of data variance, suggesting that the complexity

Table 1. Summarized data (*N*, mean ± standard error) for experimental and control sessions.

AHD		No. of sets	Sightings duration	Group size	No. of adults	No. of calves	No. of motor boats	No. of fishing boats
Off	Pre-exposure	30	14.66 ± 3.6	1.9 ± 0.5	1.6 ± 0.4	0.3 ± 0.1	2.1 ± 0.3	1.0 ± 0.2
	Post-exposure	30	22.7 ± 3.6	3.1 ± 0.5	2.6 ± 0.4	0.5 ± 0.1	1.6 ± 0.3	1.1 ± 0.2
On	Exposure	30	19.1 ± 3.3	4.6 ± 0.3	3.8 ± 0.2	0.7 ± 0.1	2 ± 0.3	2.0 ± 0.3
Total		90	18.8 ± 2.0	3.2 ± 0.3	2.7 ± 0.2	0.5 ± 0.1	1.1 ± 1.1	1.9 ± 0.1

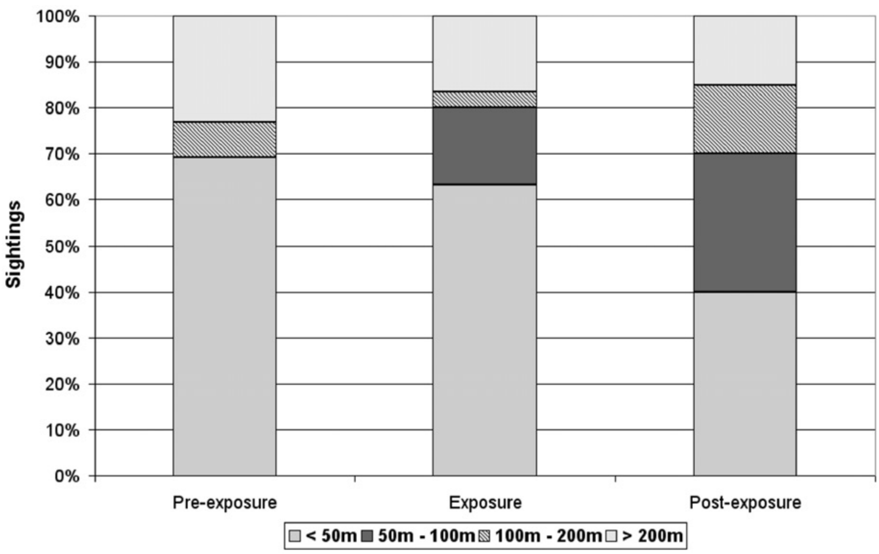

Figure 3. Distribution of sightings as a function of the minimum distance of the dolphins from the AHD between experimental (AHD exposure) and control sessions (pre- and post-AHD exposure).

of the data set can be reduced to three components with a 30% loss of information. Two features combined suggest that the first three components may be used to summarize the data set effectively: (1) the eigenvalues for these components were greater than one (2.0, 1.18 and 1.07, respectively) and (2) the screenplot of all the 10 eigenvalues shows a change in gradient after the third component, suggesting that these additional components are largely redundant.

Component 1 accounts for 33% of data variance and is strongly correlated with the number of dolphins present in the fish farm area. Thus, group size loaded highly with component 1. Moreover, it was observed that the number of adults and group size were strongly correlated.

Component 2 accounts for 19.7% of the variance of the data set and is most closely correlated with a measure of the number of calves present in the group. In addition, component 3 is correlated with the time that bottlenose dolphins spent in the fish farm area during each set and accounts for 17.3% of variance.

Comparisons among experimental and control sessions

A discriminant function analysis was employed to further investigate the relationship between the collected variables and the experimental and control sessions. The percentage of factor scores classified to the correct groups among the experimental and control sessions was 50% overall, and the cross-validation yielded an average correct assignment of 33%. MANOVA supported the results of the discriminant analysis showing that the observed variables were not different among the experimental and control sessions (MANOVA, $F_{3,14} = 0.51$, $P > 0.05$).

In addition, a discriminant function analysis was conducted by the different sets (before, during and after AHD exposure) to evaluate where differences might be found. The percentage of factor scores classified to the correct groups among the three sets was 55.6% overall, and the cross-validation yielded an average correct assignment of only 16.7%. Likewise, MANOVA indicated that the observed variables were homogeneous between the different sets (MANOVA, $F_{6,28} = 1.1$, $P > 0.05$).

Discussion

While AHDs have been found to be effective in some controlled experiments, it is still not clear if they could contribute to solving the problem derived by the interaction between marine mammals and aquaculture. This study shows that a particular AHD device alone does not effectively reduce interactions between common bottlenose dolphins and a marine fin-fish farm, particularly when motivating factors such as food resources are present.

Activation of the AHD did not have a significant and immediate effect on bottlenose dolphin's presence, distance from the AHD, group size or time spent in the fish farm area. The bottlenose dolphins could perhaps weigh the cost of exposure to noise against the benefit of remaining near it. This suggests that the concentration of food resources in the fish farm area (wild and farmed fish species) is more attractive than the quieter waters with fewer preys. These results are similar to those observed with sea lions (Mate et al. 1987; Nash et al. 2000), where the animals got used or were tolerant to the AHD sound from the beginning.

The absence of any response to the AHD used in this study does not mean that bottlenose dolphins could not react in a different way using a different type of AHD. In the same way, sea lions tolerate some types of AHD and are harassed by others (Yurk and Trites 2000).

The presence of bottlenose dolphins in the fish farm area may increase with the use of AHDs, as the animals could learn to associate the sound with a readily available source of food (dinner bell effect: Mate et al. 1987; Olesiuk et al. 1996). Additionally, the behaviour of bottlenose dolphins may be influenced by a learning component that needs to be addressed in further research. For example, in other species such as grey seals, it was observed that they lifted their heads out of the water in response to AHD signals (Bordino et al. 2002; Fjälling et al. 2006). Behavioural (Olesiuk et al. 2002) and masking (Southall et al. 2000) effects have also been observed. Further studies on the interactions between common bottlenose dolphins and AHDs are required to reveal the details of how the animals behave in the proximity of fish farms with and without AHDs.

The mechanisms leading cetaceans and pinnipeds to avoid or become attracted to fishing operations with functional ADDs and AHDs remain uncertain (Kraus et al. 1997; Quick et al. 2004). The degree of effectiveness of the AHDs may be highly dependent on several factors including target species, animals' hearing sensitivity, geographic location, habitat morphology, the time–frequency characteristics of the emitted signals, and the depth of source and receiver (Greenlaw 1987; Shapiro et al. 2009). Furthermore, the acoustic field to which animals are exposed when approaching an acoustic device underwater is complicated. It is not easily described by modelling based on spherical or cylindrical zones of responsiveness which cannot account for masking or discomfort relative to the range from the AHD (Richardson et al. 1995; Shapiro et al. 2009).

The AHD tested in this study was ineffective in controlling bottlenose dolphin depredation on farmed fish; it could however have other effects on dolphin behaviour that ought to be addressed. Important questions remain concerning possible long-term, harmful side effects and the impact of such systems on the target (bottlenose dolphins) and non-target species (other cetaceans, some fish and potentially invertebrates as well) in the surrounding marine environment. In the interests of coastal conservation and aquaculture management, additional research on the effects of AHD on the marine environment prior to further deployment is essential.

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