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Research Article

Evaluating humpback whale (*Megaptera novaeangliae*) behavioral responses to whale pingers in breeding grounds of northern Peru

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ABSTRACT. This study evaluated the behavioral responses of humpback whales (*Megaptera novaeangliae*) to whale pingers (3-20 kHz) in northern Peru during the 2022 breeding season. Land-based observations were conducted from the "La Mesa" rock cliff at 33 m above sea level in the district of Los Organos (4°10'S, 81°8.27'W). Monitoring consisted of observing the behavior of whales that passed within a radius of 1,000 m of a fixed sea-based pinger station. Surface time, breathing frequency, dive time, and movement patterns were analyzed across 104 individual whale trajectories under two treatments, 'pinger on' and 'pinger off.' Results showed a significant reduction in surface time when the pinger was on (from 41 to 32 s, P = 0.004), particularly among mother-calf groups, which comprised 71% (n = 34) of the observed groups within the pinger radius. However, no significant differences were found in breathing frequency, dive time, or directness and deviation indexes. These findings suggest that while humpback whales detected and responded to pinger signals through reduced surface time, they maintained their movement patterns. The study provides insights for developing entanglement mitigation strategies in Peru's artisanal fisheries, where gillnet interactions pose sizeable risks to both whales and local fishing communities. Further research is recommended to more accurately assess its effectiveness in reducing interactions between whales and fishing activities while preserving their critical behaviors in reproductive areas.

Keywords: *Megaptera novaeangliae*; marine mammals; cetaceans; acoustic devices; wildlife management; bycatch

INTRODUCTION

The humpback whale (*Megaptera novaeangliae* Borowski, 1781) is a baleen whale distributed throughout the world's oceans (Clapham & Mead 1999) and known for their aerial displays (Pacheco et al. 2013, Clapham 2018) and complex songs (Mercado et al. 2010, Hawkey et al. 2020). These whales undertake one of the longest migrations among mammals, traveling

over 8,000 km between high-latitude feeding grounds and breeding areas in tropical and subtropical waters (Stone et al. 1990, Rasmussen et al. 2007). The southeast Pacific population, also known as Breeding Stock G (Pacheco et al. 2009, Félix & Guzmán 2014), ranges from northern Peru to the Pacific coast of Nicaragua during the breeding season (i.e. July-October) (De Weerdt et al. 2020, 2023). The estimated population for 2018 was 11,784 ind, with a rate of increase (ROI) of

5.07% (Félix et al. 2021, Seyboth et al. 2023). Although this population has shown signs of recovery after the ban on commercial whaling in 1986, it continues to face various anthropogenic threats (Thomas et al. 2016). One of the most significant threats is entanglement with fishing gear (Félix et al. 2011). A study in Colombia reported that entanglements caused 66% of humpback whale mortalities (Alzueta et al. 2001), and in Peru, entanglements were particularly common with surface gillnets (García-Godos et al. 2013). In Ecuadorian coastal waters, an estimated annual mortality rate of 0.53% (i.e. approximately 15-33 whales yr⁻¹) was recorded between 1996 and 2009 (Alava et al. 2012). Similarly, a 2015 study in Peruvian coastal waters recorded 23 interactions, highlighting the severity of this issue (Torres & Sarmiento 2021). These events have both ecological and socio-economic impacts, affecting cetacean populations and local fishing communities throughout South America. Records of fishing gear interactions have been documented in Chile, Peru, Ecuador, and Colombia (Florez-Gonzalez et al. 2007, Galletti & Cabrera 2007, Félix et al. 2011, García-Godos et al. 2013). In Peru, an increase in whale entanglements has been documented in recent years, with humpback whales being the most affected species (García-Godos et al. 2013). This entanglement can potentially cause severe injuries or death in whales (Chauca et al. 2021). Economic losses in artisanal fisheries of up to US\$20,000 have been reported when an entire net is lost due to entanglement in net panes (Guidino et al. 2022). Such events also pose risks to the physical safety of fishermen (Félix et al. 2011).

To mitigate the impact of the entanglement of baleen whales, various strategies have been explored, including the use of acoustic alarms (Erbe & McPherson 2012, McGarry et al. 2017, Basran et al. 2020), sinking nets in the water column (Kiszka et al. 2021), and spatial closures (Tulloch et al. 2019) or temporal fishing closures (Andrews-Goff et al. 2018). Acoustic alarms have the potential to be one of the most promising strategies if proven effective for reducing entanglements of large cetaceans (Basran et al. 2020). They integrate easily into existing fisheries with minimal operational changes and have little impact on whale behavior (Neumann 2017). Acoustic alarms, commonly referred to as "pingers", are underwater sound-emitting devices designed to alert marine mammals to the presence of fishing gear (Erbe & McPherson 2012). These devices typically emit short, repeated signals at different frequencies, with varying specifications depending on the species of interest (Barlow & Cameron 2003, Dawson et al. 2013, Mangel et al. 2013). For harbor porpoises, *Phocoena phocoena*, commercial pingers operating at frequencies between 110-140 kHz have effectively reduced bycatch (Larsen et al. 2007). In the case of bottlenose dolphins, Tursiops truncatus, pingers operating at lower frequencies, 10-12 kHz, are recommended (Barlow & Cameron 2003). Frequency selection is essential, as it must be within the hearing range of the target mammal species to elicit behavioral responses, such as displacement or habituation (Nowacek et al. 2007). In the case of humpback whales, their auditory system shows maximum hearing sensitivity between 3 and 9 kHz (Tubelli et al. 2018), suggesting that they may be sensitive to the frequencies used in whale pingers, which typically operate between 3-20 kHz (Erbe & McPherson 2012, Harcourt et al. 2014, Pirotta et al. 2016, Basran et al. 2020).

Studies examining the effectiveness of pingers on baleen whales have shown mixed results. Basran et al. (2020) documented that humpback whales exhibited evident behavioral changes when exposed to 3 kHz pingers deployed on a fishing net, specifically reducing their surface feeding behavior in Icelandic feeding grounds. McGarry et al. (2017) reported a significant increase in detection distances and avoidance behaviors exposing minke whales (Balaenoptera acutorostrata) to 10 kHz pingers. Other studies have found limited or no significant changes in the behavior of humpback whales. For instance, Pirotta et al. (2016) found no detectable changes in the direction of travel or surface behavior when testing a single 5.3 kHz pinger on a fixed sea-based mooring station during northern migrations in Australia. Similarly, Harcourt et al. (2014) reported no significant alteration in whale trajectories when exposed to a single 3 kHz pinger in the same study area. In northern Peru, Guidino et al. (2022) conducted trials with 10 kHz porpoise and dolphin pingers on gillnets; however, their findings regarding humpback whale entanglements remained inconclusive due to the limited sample size during the

In this context, the present study aimed to evaluate the behavioral response of humpback whales to a whale pinger under on and off treatments at a fixed coastal station in northern Peru during the breeding season. Specifically, the following behavioral variables were analyzed: a) surface time, b) breathing frequency, c) dive time, and d) deviation and directness indexes. The results of this study can provide crucial information for developing more effective strategies to reduce humpback whale entanglements in the region. Additionally, entanglement events often result in temporary fishing

suspensions, further impacting the livelihoods of local fishing communities (Mangel et al. 2013). Therefore, this research will contribute to both cetacean conservation and the economic sustainability of local fishing communities.

MATERIALS AND METHODS

Study site

The study was conducted in Los Organos (4°10'S, 81°8.27'W) in Piura, Peru (Fig. 1). This area is characterized by the convergence of the cold Humboldt Current and the warm North Equatorial Current, which creates optimal breeding conditions for this species (García-Godos et al. 2013, Félix & Guzmán 2014, Guidino et al. 2014). Monitoring was performed from a rocky cliff known as "La Mesa" (4°10'S, 81°8.51'W), located 30 m above sea level and offering a panoramic view of a transit area for humpback whales (García-Cegarra et al. 2019). This site has been previously used to study whale movements (García-Cegarra et al. 2019, Villagra et al. 2021). A first-order geodetic marker (Pyramid II) established by the Peruvian Navy was used as the reference point for a Sokkia Series 650X total station, ensuring precise spatial measurements within the study area.

Experimental design

A fixed mooring with a buoy and a flag was installed 1.3 km from the coastline, aligned with the former oil platform MX1 (4°10.24'S, 81°8.40'W), to serve as a visual reference for whale observations. The mooring site was at 53 m depth. A Fishtek Marine whale deterrent pinger (3-20 kHz) was attached at 5 m depth, as used in Harcourt et al. (2014) and Pirotta et al. (2016). The pinger (dimensions: 185L×52W×42H mm, 229 g) emits a sound pressure level of 135 ± 3 dB re 1 μPa at 1 m. It activates automatically upon immersion and is powered by a C-type alkaline battery (LR14), providing one month of operation with 50% immersion time. The chosen frequency range (3-20 kHz) was based on prior studies on the auditory sensitivity of humpback whales (2-10 kHz) (Houser et al. 2001, Harcourt et al. 2014, Basran et al. 2020). The installation depth was selected to optimize sound propagation in shallow waters, aligning with the approach of Erbe & McPherson (2012) to reduce cetacean interactions with fishing gear deployed near coastal areas. This depth minimizes interference from surface noise while ensuring the signal remains within the detection range of humpback whales (Erbe & McPherson 2012). To verify pinger functionality and assess its audible range, in situ acoustic monitoring was made following Harcourt et al. (2014). Measurements were taken from a drifting vessel (i.e. engine off) under calm sea conditions (Beaufort 1) using an Aquarian H1a hydrophone submerged to 10 m depth and a Zoom H1 recorder. We tested the pinger sound at distances of 40-50, 90-100, 150, and 200-220 m, with positioning tracked via a handheld GPS. The pinger became inaudible at approximately 200 m, consistent with findings from Erbe & McPherson (2012), who reported a 210 m detectability range for similar alarms in humpback whales. All measurements were conducted when no whale-watching or fishing vessels were present in the area to prevent acoustic masking or whale disturbance, ensuring our recordings reflected true pinger signal propagation without anthropogenic interference.

Data collection

Data were collected during September and October 2022, during the peak of the humpback whale breeding season (Guidino et al. 2014, Pacheco et al. 2021). The study covered 36 days, including 21 days with the whale pinger on and 15 days with the pinger off. Observations were conducted by a land-based team consisting of two observers, a total station operator, and a data recorder from 06:00 to 11:00 h, considering oceanographic conditions and tourist vessel activity (Guidino et al. 2020). The observation period was selected to coincide with the first light of day, maximizing visibility while minimizing interference from whale-watching boats, which increased in number later in the morning. Data collection ceased after 11:00 h due to stronger winds, which, after midday, generated whitecaps that could be mistaken for whale blows, reducing detection accuracy. Optimal conditions were defined as a Beaufort scale <3 and visibility >2 km (García-Cegarra et al. 2019). Monitoring (i.e. an observation period of behaviors recorded for a humpback whale) using Bushnell binoculars (10×50) began when a randomly selected individual from a group of whales entered the 1,000 m radius (i.e. outer circle) around the fixed mooring (i.e. pinger) (Fig. 1). Observation continued as the whale moved within the 500 m radius (inner circle) (Fig. 1). These distances were selected based on Harcourt et al. (2014) and McGarry et al. (2017), considering that whales could perceive the pinger within 500 m, while beyond 1,000 m, detection was unlikely. Individuals were selected from groups approaching the target area in coordination with the entire team. The mother was prioritized due to her proximity to the calf. If no calf was present, a whale was randomly selected. Observers used distinctive

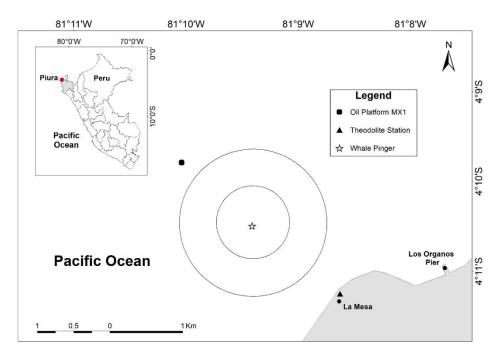


Figure 1. Geographic layout of humpback whale monitoring station along the northern coast of Peru Circles around the whale pinger mark the 500 m (inner circle) and the 1,000 m (outer circle) radii of the likely acoustic detection zone.

physical characteristics, such as body size, scars, and pigmentation patterns, to ensure the same individual was monitored throughout the observation period, minimizing the risk of misidentification. Humpback whales were classified as single whale (S), duo (D), and group (G), consisting of three or more whales. Groups that included a calf were defined as mother and calf (MC), mother, calf and escort (MCE) (escort being a single accompanying whale), mother-calf and >1 escort (MCE+) (Félix & Botero-Acosta 2011, García-Cegarra et al. 2019, Pacheco et al. 2021). The surface behavior response variables of selected individual whales were recorded through land-based observations, including a) surfacing time, b) breathing frequency, and c) dive time. Surfacing time was measured as the proportion of time a whale spent at or above water for <60 s (García-Cegarra et al. 2019). Breathing frequency was recorded as the number of blows per minute. Dive time was the time a whale spent underwater for >60 s between successive surfacing's (García-Cegarra et al. 2019). Data collection continued until the whale left the study area, allowing for the calculation of average values for each variable.

A Sokkia Series 650X total station, positioned at a first-order geodetic marker (Fig. 1), was used to determine the precise geographic location of the selected individuals and track their movement patterns

(i.e. directness and deviation indexes). The total station measured the vertical and horizontal angles of the whale. These angles, combined with the total station's height, allowed for the determination of the whale's position, referred to as a "fix" (Godwin et al. 2015). Fixes were mapped (whale's trajectories) and recorded at the start and end of each surfacing to track movement patterns accurately. The directness index was used to quantify the linearity of whale movement, calculated by dividing the distance between the start and end points of a track by the total surface distance traveled (Schuler et al. 2019). Values range from 0 (i.e. circular movement) to 100 (i.e. straight-line movement) (Williams et al. 2002). Higher values indicate more direct and predictable movement patterns (García-Cegarra et al. 2019). The deviation index measured how much a whale's movement deviated from a straight-line path, calculated by the mean turning angle between consecutive positions in a track. Values range from 0° (i.e. linear movement) to 180° (i.e. erratic movement). A higher deviation index indicates unpredictable movement (Williams 1999, Williams et al. 2002, Schaffar et al. 2013, Schuler et al. 2019).

Data analysis

To evaluate the effectiveness of the whale pinger, behavior response variables recorded in both treatments (i.e. 'alarm on' vs. 'alarm off') of individual humpback whales were compared within the established radius from the pinger (i.e. 1,000 m) based on previous studies (Harcourt et al. 2014, Pirotta et al. 2016). Whale trajectories obtained from the total station were then analyzed using Google Earth and the Kml Creator program (http://www.apps.ingeapps.com/gtools/en/ kml-creator.php) to estimate variations in movement (Williams et al. 2002, Pacheco et al. 2009, García-Cegarra et al. 2019). The closest fix to the pinger was identified for each complete track using the Google Earth measurement tool. These distances were then compared between the two treatments: when the alarm was on and when it was off. The analysis focused on determining which treatment had the shortest average distance, assuming that whales would maintain a greater distance when the alarm was on. Trajectories that met the following criteria were selected: 1) included two or more dives, and 2) location within the 1,000 m radius of the pinger (Harcourt et al. 2014, Pirotta et al. 2016). Four main variables were calculated: (a) mean surfacing time, (b) mean breathing frequency, (c) mean dive time, and (d) deviation and directness indexes.

For data analysis, the Shapiro-Wilk test (n < 50) was applied to assess data normality, and Levene's test was used to evaluate the variance of homogeneity. Response variables were classified based on their distribution characteristics. Surfacing time, which followed a normal distribution with homogeneous variances, was analyzed using parametric methods. In contrast, breathing frequency, dive time, deviation, and directness indexes, which did not meet the normality assumptions, were analyzed using non-parametric methods. Parametric and non-parametric tests were conducted to evaluate the effect of the whale pinger under the treatments (i.e. 'on/off') on the behavioral response of humpback whales. t-Student (n < 30) tests for independent samples were applied to the variable that met the normality assumption. Non-parametric Mann-Whitney (n > 20) tests were utilized for variables that did not follow a normal distribution. The analyses were performed using SPSS (version 29.0.2.0) (IBM Corp. 2023), with a significance level set using an alpha (α) = 0.05.

RESULTS

A total of 104 whale trajectories were obtained over 36 days (162 h), considering both 'pinger on' and 'pinger off' treatments. Of these, 48 observations (46%) were recorded within the 1,000 m radius from the pinger

location and analyzed, while the remaining trajectories outside the range (54%, N=56) were excluded from the analysis (Table 1). More than half (N=34) of the individual whales monitored within the 1,000 m radius were identified as MC groups (i.e. MC, MCE, MCE+) (Table 2).

Distance to pingers: Visual differences in the distance of the trajectories to the pinger between both treatments were apparent (Figs. 2a-b), but no statistical significance was found (t = -1.160, P = 0.252) (Table 3).

Surface time: A significant reduction in mean surface time was observed under the pinger on treatment (t = 3.032, P = 0.004) (Table 3), with shorter time (M = 0.30 ± 0.11 min, standard deviation SD) compared to when it was off (M = 0.43 ± 0.09 min, SD) (Fig. 3a).

Breathing frequency: No significant difference (U = 216.00, P = 0.773) (Table 3) between treatments (M = 2, M = 2 blows per min) (Fig. 3a).

Dive time: No significant difference was found between treatments (U = 84.00, P = 0.051) (Fig. 3a, Table 3).

Directness and deviation: No significant differences were observed between the two treatments (U= 220.00, P = 0.862 and U = 218.00, P = 0.943, respectively) (Table 3). Results indicated that as linearity in whale movement increased, deviations from a direct path decreased (Fig. 3b), implying that the pinger had no measurable effect on these movement parameters.

DISCUSSION

Behavioral responses to whale pingers

This study offers insights into the behavioral responses of humpback whales, particularly those in MC groups, to the presence of a whale pinger. Our primary finding is a significant reduction in surface time within the 1,000 m radius of the active pinger. Although our study area is a breeding ground, it is important to mention that Basran et al. (2020) found surface feeding in a foraging area decreased from 11% pre-exposure to 4% during 3 kHz pinger exposure, highlighting potential behavioral effects. In our study, mean surface time decreased from 43 to 30 s when the pinger was on. This response suggests that whales have an avoidance response, possibly motivated by a perception of the pinger as a disturbance.

On the other hand, Harcourt et al. (2014), using the same methodology, found no significant effect of the same 3 kHz pinger on migrating humpback whales in

Table 1. Total number of humpback whale trajectories recorded within 1000 m whale pinger radius.

Distance range	Whale pinger on	Whale pinger off	Total	%
Inside (<1,000 m)	35	13	48	46
Outside (>1,000 m)	38	18	56	54
Total	73	31	104	100

Table 2. Group composition of monitored individual humpback whales within 1000 m whale pinger radius. MC: mother and calf, MCE: mother, calf and escort, MCE+: escort being a single accompanying whale, mother-calf and >1 escort.

Group composition	Total	%
(MC, MCE, MCE+)	34	71
Duo	7	15
Single	6	13
Groups >3	1	2

Australia, suggesting, as was also found in the present study, that a single pinger may not have been optimal to deter the whales effectively, which highlights the need for more comparative studies to help understand the factors influencing whale responses to pingers. Pirotta et al. (2016) also did not observe a significant effect of a single 5.3 kHz pinger within the same 1,000 m radius distance range of the pinger for migrating humpback whales in Australia, suggesting that the migratory state and social context of the whales (i.e. fewer MC groups compared to southward migration) may influence the limited response observed in the study. Although García-Cegarra et al. (2019) did not study the effect of the pinger sound, they found that MC groups were more sensitive to disturbances than other groups of whales, such as whale-watching vessels, and exhibited reduced surface time (24 to 20 s). The fact that most of our sample comprised MC groups (71%, n = 34) may explain why surface time was significantly impacted, suggesting that the coastal activities in northern Peru may elicit avoidance responses, especially in shallow waters where MC groups prevail (Guidino et al. 2014, Pacheco et al. 2021).

Breathing frequency and dive time

Breathing frequency and dive times remained consistent across pinger treatments in our study. In contrast, Boisseau et al. (2021) found that minke whales in feeding grounds in Iceland exposed to 15 kHz pingers exhibited altered breathing frequencies and increased dive time, highlighting potential species-

specific responses to varying pinger frequencies. These results suggest that while pingers affect surface time, their influence on breathing and dive patterns may be context-dependent.

Directness and deviation

The lack of significant changes in the directness and deviation indices suggests that the sound of one pinger is unlikely to be disruptive enough to alter their movement patterns (Erbe & McPherson 2012), which is consistent with observations by How et al. (2015), who noted that humpback whales continued on their paths on their northern migrations along the coast of western Australia despite exposure to 3 kHz whale pingers placed on fishing gear, particularly in regions with frequent anthropogenic disturbances. In contrast, Dunlop et al. (2013), reporting on southward migrating humpback whales (more presence MC groups in this route) exposed to a 2 kHz pinger on a fixed mooring, showed a course deviation during exposure to the pinger. Specifically, MC groups tended to move inshore, potentially as a protective measure against the threat. Furthermore, as García-Cegarra et al. (2019) and Villagra et al. (2021) observed, the presence of whalewatching vessels in northern Peru, along with reported inadequate whale-watching practices, could further elicit whale responses through habituation, potentially reducing the efficacy of pingers (Erbe & McPherson 2012). Vessel noise could potentially mask pinger signals or influence whale behavior. Whale-watching vessels typically generate underwater noise in the range of 0.01-10 kHz, with sound pressure levels ranging from 130-160 dB re 1 µPa at 1 m (Erbe 2002). These frequencies overlap with the hearing range of humpback whales, which is most sensitive between 0.1-8 kHz (Au et al. 2006). Our study utilized pingers operating at 3-20 kHz (135 \pm 3dB re 1 μ Pa at 1 m) to minimize potential masking from vessel noise while remaining within the auditory detection range of humpback whales. Although our experimental design minimized vessel presence during observations, chronic exposure to vessel noise in this region could influence overall responsiveness to acoustic deterrents (Pirotta et al. 2016).

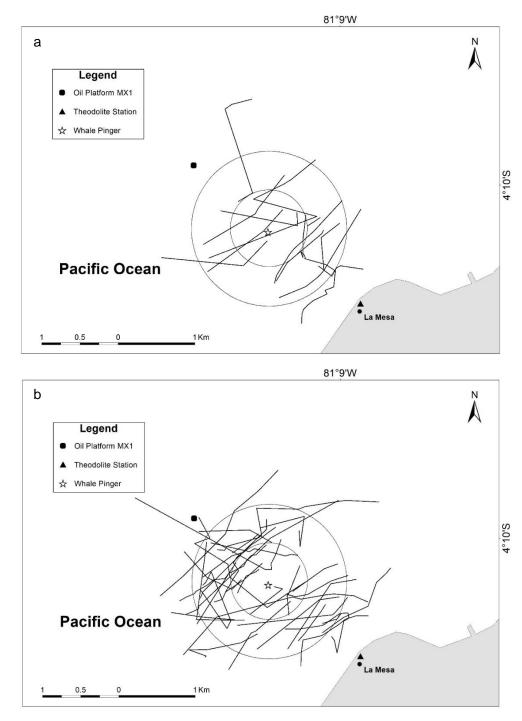


Figure 2. Humpback whale trajectories within a 1,000 m radius of the whale pinger (outer circle). a) Trajectories for conditions of whale pinger off treatment, and b) trajectories with the whale pinger on. Each black line represents the trajectory of a different individual.

Future studies could refine the methodology to better reflect real-world pinger applications, particularly by testing multiple pingers spaced at intervals, as commonly deployed in fisheries (e.g. Mangel et al. 2013). Further research with larger

sample sizes and longer monitoring periods could strengthen the conclusions on the effectiveness of pingers in modifying humpback whale behavior (Dunlop et al. 2013, Basran et al. 2020). Additionally, testing pingers alongside visual cues, such as colored

	Tests of variance homogeneity (Levene's test)				T test (parametric test)			U Mann-Whitney (non-parametric)	
Response variables	Levene statistic	df1	df2	P < 0.05	t	df	P < 0.05	U	P < 0.05
Surfacing time	0.228	1	36	0.636	3.032	36.000	0.004	-	-
Breathing frecuency	0.003	1	46	0.957	-	-	-	216.000	0.773
Dive time	0.560	1	35	0.459	-	-	-	84.000	0.050
Directness index	0.091	1	46	0.765	-	-	-	220.000	0.862
Deviation index	0.585	1	45	0.448	-	-	-	218.000	0.943
Distance to pinger	1.355	1	46	0.250	-1.160	17.613	0.252	-	-

Table 3. Comparative analysis of parametric and non-parametric tests for behavioral responses within 1,000 m radius of the whale pinger. df: degrees of freedom.

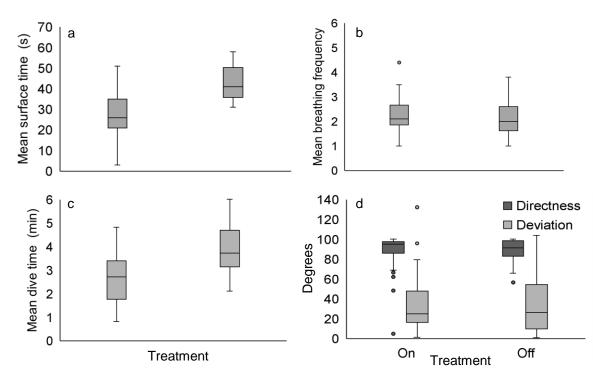


Figure 3. Boxplots representing the response variables for whales passing within a 1,000 m radius of the whale pinger under two treatments (on and off): a) mean surface time, b) mean breathing frequency, c) mean dive time, and d) deviation and directness indexes. The grey boxes indicate the interquartile ranges, while the black lines represent the median values for each condition. The whiskers extend to the lower and upper quartiles, and the dots mark individual outliers.

ropes, may enhance detection in areas with complex fishing gear (How et al. 2015). Evaluating behavioral responses to pinger presence should be complemented by direct testing on fishing nets to accurately assess their effectiveness in reducing entanglements. Examining different group compositions, including MC groups, could also provide insights into potential variations in response (García-Cegarra et al. 2019, Basran et al. 2020).

Credit author contribution

A.S. Galán: conceptualization, validation, methodology, formal analysis, writing-original draft, review and editing; C. Guidino: funding acquisition, project administration, conceptualization, supervision, methodology, review, and editing; J.C. Francia-Quiroz: formal analysis, review, and editing; J. Alfaro-Shigueto: design, methodology, review and editing; J.C. Mangel: formal analysis, methodology, review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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