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Behavioural and physiological responses of captive Antillean manatees to small aerial drones

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Abstract

 Context. Unmanned aerial vehicles or drones are powerful tools for wildlife research. Identifying the impacts of these systems on target species during operations is essential to reduce risks of disturbance to wildlife, to minimise bias in behavioural data, and to establish better practices for their use.

 Aims. We evaluated the responses of captive Antillean manatees to the overhead flight of a small aerial drone.

 Methods. We used aerial and ground videos to compare manatee activity budgets and respiration rates in three 15-min sampling periods: 'before', 'during' and 'after' flights with a DJI Phantom 3 Advanced. The drone was hovered stationary for 3 min at five altitudes (100 m, 40 m, 20 m, 10 m, 5 m) to determine whether manatees display behavioural responses compared with the control period, and whether they respond more at lower altitudes. Only one flight was performed per manatee group to avoid bias owing to habituation to the drone.

 Key results. Manatees responded to drone flights by (1) increasing their activity levels during and after flights, therefore signalling after effects; (2) decreasing their respiration rate during flights; and (3) displaying behavioural reactions including grouping, tail-kicking, fleeing from their original position and moving under submerged structures. From the 11 individuals displaying behavioral reactions, 9 reacted in the first ∼2 min of flight, preventing assessments of altitude effects and suggesting manatees responded to the drone sound at take-off.

 Conclusions. Behavioural changes of responding manatees were similar to previous reports of disturbance responses to boats and drones in this species. Our use of a control period showed shifts in respiration rates and activity budgets that persisted after flights. Several manatees reacted to the drone from the time of take-off and first minutes of flight, indicating that the sound of the electric rotors could be a strong negative stimulus to manatee and highlighting the importance of establishing safe distances for take-off.

 Implications. Future studies should consider that drones could elicit conspicuous and inconspicuous responses in manatees. Our results emphasise the need for control data on animal behaviour to better assess the impact of drones on wildlife and to design non-invasive protocols.

Keywords: behaviour, disturbance, unmanned aerial vehicles, *Trichechus*, UAS, UAV.

Introduction

Unmanned aerial vehicles (UAV), or 'drones', are versatile remote-sensing tools now popular in ecological research, conservation, and the management of wildlife (Koh and Wich 2012; Anderson and Gaston 2013). These tools have many applications, including high-resolution mapping and habitat assessments (Messinger *et al.* 2016; Joyce *et al.* 2019), anti-poaching surveillance (Mulero-Pázmány *et al.* 2014), and wildlife monitoring (Linchant *et al.* 2015; Hodgson *et al.* 2018). Drones are relatively safe for the operator compared with manned aerial surveys (Jones *et al.* 2006) and are less costly to operate (Colefax *et al.* 2018). Additionally, these systems enable access to remote areas (Christie *et al.* 2016) and have the capacity to collect systematic data and permanent high-resolution visual records (Hodgson *et al.* 2013).

In marine mammal research, drones serve as powerful tool to supplement and enhance the capacity to collect important data on wild populations (Hodgson *et al.* 2017; Colefax *et al.* 2018; Raoult *et al.* 2020). The increasing use of drones for wildlife research (Linchant *et al.* 2015) emphasises the need for regulations guiding the safe use of these systems to avoid potential impacts on the target species during operations. Disruption of wildlife can affect vital activities, such as, for example, causing reductions in feeding time (Williams *et al.* 2006), changes in spatial use (Buckingham *et al.* 1999), increases in energetic expenditure (Lusseau and Bejder 2007), and eliciting physiological stress (French *et al.* 2010). Moreover, animal responses to the observation platform create biases in data collection, such as, for instance, skewing detection accuracy during individual counts (Brisson-Curadeau *et al.* 2017) or causing behavioural shifts that can bias studies of natural behaviour. Thus, guidelines and protocols for the use of drones are needed to reduce the risks they pose to wildlife and to minimise bias in data collection (Smith *et al.* 2016; Raoult *et al.* 2020).

A critical component for evaluating the effects of disturbance from drones is to report and understand the spectrum of responses displayed across a range of species (Bevan *et al.* 2018) and in different environments. Raoult *et al.* (2020) reviewed and outlined operational protocols for using drones to study marine megafauna and identified inter-specific variation in marine animal physiology (e.g. auditory capacity) and ecology (e.g. predation by aerial animals) as relevant factors in determining the impact of drones on these species. Numerous studies have directly assessed the risk of disturbance of drones on marine mammals such as grey seals (*Halichoerus grypus*, Pomeroy *et al.* 2015; Arona *et al.* 2018), blue whales (*Balaenoptera musculus*, Domínguez-Sánchez *et al.* 2018), humpback whales (Fiori *et al.* 2020), bottlenose dolphins (*Tursiops truncatus*, Ramos *et al.* 2018; Fettermann *et al.* 2019), and Antillean manatees (*Trichechus manatus manatus*, Ramos *et al.* 2018). However, most studies investigating behavioural responses of marine mammals to drones lack control data where animal behaviour can be observed without the presence of the platform, data essential for determining the risk of disturbance of different drone systems and flight methods (Bevan *et al.* 2018). Thus, experiments quantifying disturbance using captive animals, where control data are less challenging to obtain, are ideal for developing species-specific protocols (Hodgson and Koh 2016).

Sirenian research using aerial drones has been conducted only with dugongs (*Dugong dugon*, Hodgson *et al.* 2013) and West Indian manatees (*Trichechus manatus*; Jones *et al.* 2006; Ramos *et al.* 2018; Landeo-Yauri *et al.* 2020). Of the two subspecies of the West Indian manatee, namely, the Florida manatee (*T. m. latirostris*) and the Antillean manatee (*T. m. manatus*), the latter is distributed throughout at least 19 countries across the Caribbean Sea, and Central and South America (Reynolds 1999). Most of these nations are small, low-income countries that would benefit from the development and adaptation of inexpensive tools to monitor and study wild manatee populations. With the expanded accessibility to drone technology, the use of these systems for manatee research will likely increase in these areas. Thus, exploring the effect of drones on manatees and using this information to design protocols for non-invasive data collection is necessary to mitigate their potential negative effects (e.g. physiological and behavioural stress responses, changes in group cohesion, among others).

Only one study has attempted to assess the disturbance responses of free-ranging Antillean manatees to small drones. Manatees responded to the overhead flight and vertical approach of a small quadcopter (DJI Phantom 4 Pro) in a quarter of all flights, with more responses occurring at lower flight altitudes. Given no vessels or other plausible sources of disturbance were near any of the responding manatees, the reactions of the individuals were likely caused by the sound or visual stimulus associated with drone flight (Ramos *et al.* 2018). In the present study, we tested whether captive manatees respond to experimentally controlled drone flights at different altitudes. We recorded ground-based footage before, during, and after the flights, and aerial footage during flights, to compare activity budgets and respiration rates across sampling periods.

Methods

Drone flights

All experiments were performed with 25 captive manatees (12 females and 13 males) of different age classes (calves, juveniles, adults) housed at eight pools located in six different aquarium facilities in the state of Quintana Roo, Mexico (Table 1, Fig. 1). A DJI Phantom 3 Advanced quadcopter (P3A, DJI Technology Co., San Diego, CA, USA) was flown for drone exposure experiments in the daytime (from 0900 hours to 1830 hours). The aircraft was equipped with a 12.4 MP camera (FOV 94°, 20 mm, f/2.8 lens) filming in 2700 dpi. The drone was flown manually with a remote control with a mounted iPad tablet (Apple Inc., Cupertino, CA, USA) and visually monitored via the live video stream to correctly orient over aquarium pools (*n* = 8, Table 1).

The pilot (SL) positioned the drone over each manatee pool, starting at an altitude of 100 m and descending the drone with stationary hovering for approximately 3 min at altitudes of 100 m, 40 m, 20 m, 10 m and 5 m. Aerial videos were recorded for the 15-min duration of each flight. Only one flight was performed per pool to avoid introducing bias due to the possible habituation or sensitisation of the manatees to the presence of the drone. Seven flights were conducted in total, six flights over one pool each, and one flight was conducted over two adjacent pools (G and H).

Table 1. Study locations and manatee groups used to test for responses to small aerial drones

Sex: M, male; F, female; Age class: A, adult; J, juvenile; C, calf; R, rescued, C, captive-born

Flight date	Facility	Pool	Manatees per pool	Individuals
2 May 2017	DD. Puerto Aventuras	A	8	Pablo (MAR), Quijote (MAC), Nohoch (MCC), Julieta (FAR), Dorothy (FAR), Conchis (FJC), Claudia (FJC), Bombon (FCC)
26 July 2017	DD. Dreams	B	2	Roberto (MAR), Lorenzo (MJC)
27 July 2017	DD. Isla Mujeres	C	4	César (MAR), Fabián (MAC), Sabina (FAR), Africa (FCC)
9 May 2017	DD. Cozumel	D	3	Yoltzin (MAR), Angel (MAR), Edgar (MAC)
26 July 2017	Xel-Há	E	2	Tunich (FJC), Nikté (FJC)
		F	3	Baxal (FAR), Dayami (FJR), Pompom (FAR)
25 July 2017	Xcaret[A]	G	2	Mach (MAR), Buul (MAC)
		H	1	Nohoch (MAR)

[A]At Xcaret, two flights were performed: one over the female pool (F) and one over the male pools (G and H).

Fig. 1. Aerial view of the facilities and pools where our drone tests were conducted. Pools are outlined in yellow and labelled according to facility in Table 1.

To determine the effect of the drone on manatees and to rule out alternative variables possibly influencing their behaviour (e.g. pool structure, vegetation presence, number of manatees present), we used the same sampling periods across all pools. In that way, uncontrolled variables were maintained constant across sampling periods within each pool.

Each experimental exposure involved a pre-exposure period of video-recorded ground observations of manatees, followed by a single flight, and a post-exposure period of ground observations. Ground video and drone-based observations were used to examine the activity of manatees. One to two ground observers filmed manatee behaviour with digital video cameras (Sony

DSC-HXA50 cyber-shot and Fujifilm FinePix XP130). Three 15-min videos were recorded in each pool corresponding to the three sampling periods: 'before', 'during', and 'after' the drone flights. The pre-exposure 'before' period was considered the control. Aerial drone videos also provided information in the 'during' sampling period. All behaviour events considered by observers as a visible response displayed from drone take-off through drone flights (i.e. behaviour reactions) were noted and described, as well as the flight altitude during which they occurred.

Data analysis

Aerial and ground videos were reviewed in VLC Media Player to determine individual activity budget (i.e. time each manatee spent in different behaviours) and the respiration rate (number of breaths per minute) of each manatee. The following behavioural states were used to sample the behaviour of each focal manatee with-continuous sampling: *locomotion* (*L*), which includes swimming movement displayed at any level of the water column and diving; *bottom resting* (*BR*), which includes lying near or at the bottom, and exhibiting minimal movements, and/or rotations along their axes; and *surface resting* (*SR*), which includes resting near the surface and exhibiting minimal movements and/or rotations along the axes. Videos of manatee behaviour throughout experiments were reviewed to detect behavioural reactions to the drone that could be interpreted as avoidance, fear or stress.

Manatees were considered out-of-sight (OS) when the target individual was not visible in the video. The proportion of time each manatee spent in different behavioural states was calculated to determine individual activity budgets. OS periods were discarded to standardise observations among individual manatee activity budgets.

Manatee respiration rates and activity budgets were compared among sampling periods ('before', 'during' and 'after' drone flight) using the Wilcoxon matched-pairs tests in STATISTICA 0.7 (StatSoft, Inc. Tulsa, Oklahoma, USA, 2004). Individuals with more than 10 min of OS time in each sampling period were not included in this analysis. We considered that manatees responded to the drone if changes were detected in their respiration rate and activity budgets from the 'before' sampling period compared with the 'during' or 'after' sampling periods. Flight altitude was not considered as a category for manatee respiration rate or activity budget comparisons, because each flight altitude was maintained for no more than 3 min. This time lapse is insufficient for adequate comparisons regarding activity budgets, or to assess the breathing interval of manatees (2–3 min for low-energy activities according to Hartman 1979). The flight altitude at which behavioural events were detected was identified to determine whether lower flights altitudes had a higher likelihood of causing responses than did higher altitudes.

Results

In total, 660 min of video observations were analysed, including 105 min from aerial videos and 555 min of ground-based videos. Of all focal manatees evaluated, 18 individuals (72%) met the condition of having less than 10 min of OS time to compare their
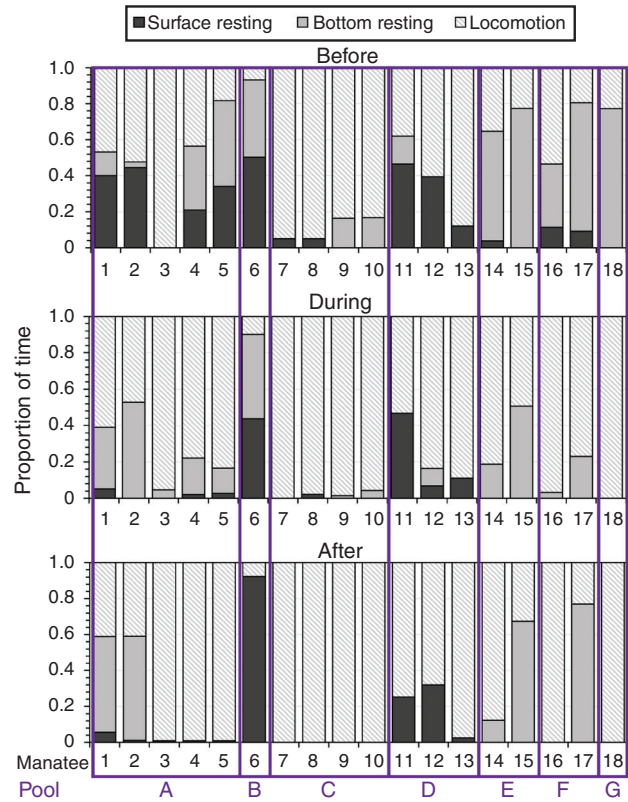

Fig. 2. Activity budgets of exposed Antillean manatees (*n* = 18) for the three sampling periods. Individual numbers correspond to 6 = Lorenzo, 7 = Sabina, 8 = Africa, 9 = César, 10 = Fabian, 11 = Angel, 12 = Yoltzin, 13 = Edgar, 14 = Nicté, 15 = Tunich, 18 = Nohoch. We were not able to identify individuals 1–5, 16 and 17.
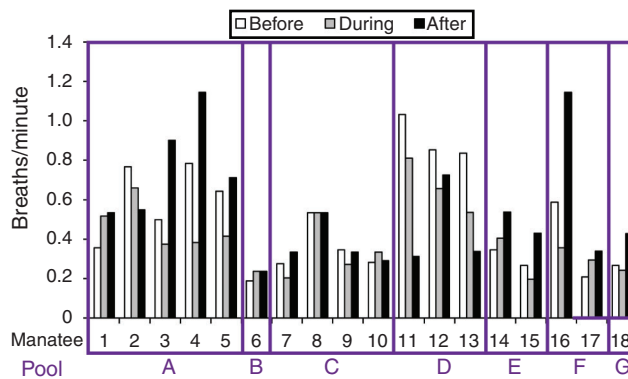

Fig. 3. Respiration rates of exposed Antillean manatees (*n* = 18) grouped by pool for each of the three sampling periods. Individual numbers correspond to 6 = Lorenzo, 7 = Sabina, 8 = Africa, 9 = César, 10 = Fabian, 11 = Angel, 12 = Yoltzin, 13 = Edgar, 14 = Nicté, 15 = Tunich, 18 = Nohoch. We were not able to identify individuals 1–5, 16 and 17.

respiration rate and activity budget among sampling periods. Individual data recorded from these are presented in Fig. 2 (activity budgets) and Fig. 3 (respiration rates).

Manatees (*n* = 18) spent most of their time in locomotion (Fig. 4). During drone flights, manatees increased their activity
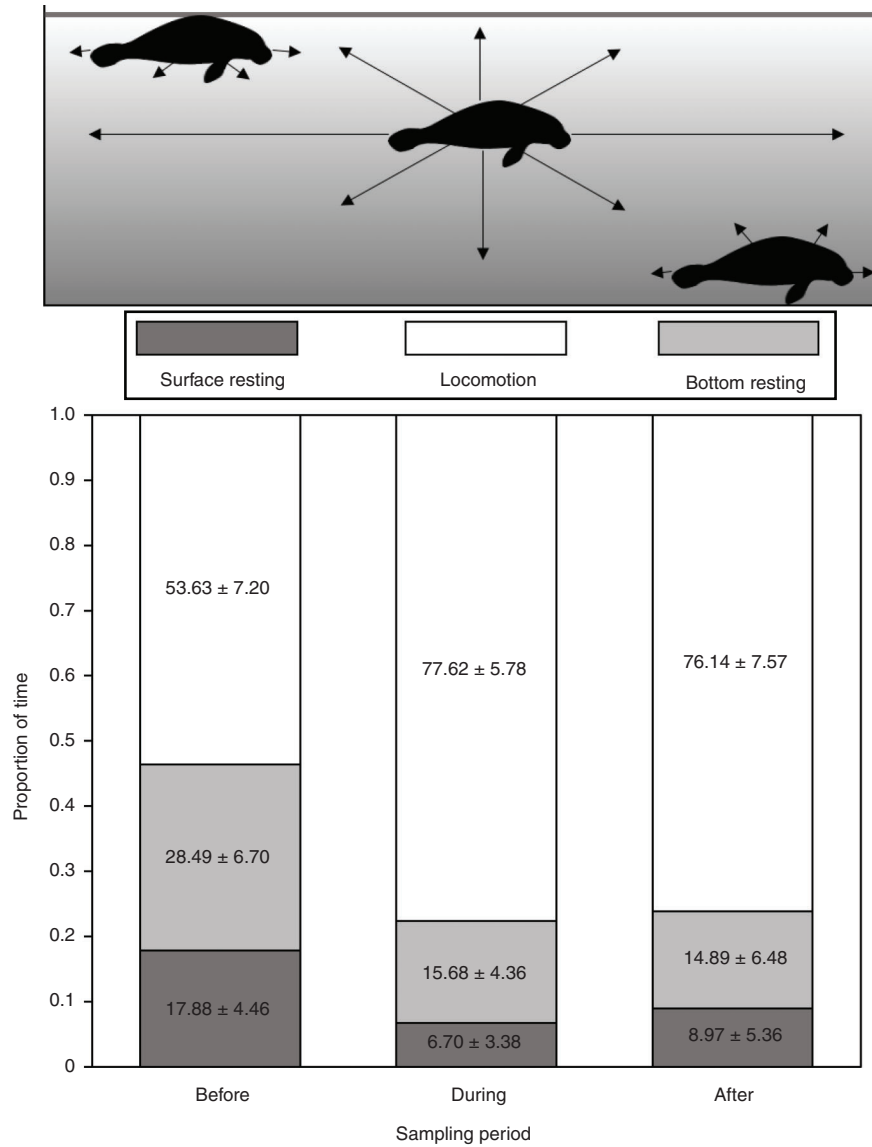
Fig. 4. Average proportion of time (±standard error) Antillean manatees ($n = 18$) spent in different behavioural states (surface resting, locomotion, bottom resting) for all three sampling periods.

levels and spent less time at the surface than before drone exposure (locomotion: $t = 10$, $Z = 3.288$, $P = 0.001$; surface resting: $t = 1$, $Z = 3.110$, $P = 0.002$). Increased activity levels were also observed after flights when comparing to before flights (locomotion: $t = 17$, $Z = 2.983$, $P = 0.003$; surface resting: $t = 14$, $Z = 2.417$, $P = 0.016$). There were no significant differences among sampling periods in time spent bottom resting (before vs during: $t = 28$, $Z = 1.817$, $P = 0.069$; before vs after: $t = 21$, $Z = 1.712$, $P = 0.087$). The average respiration rate of manatees ($n = 18$) decreased 'during' the drone flight when compared with 'before' and 'after' flight sampling periods (Fig. 5). Manatee respiration rate increased significantly during flight compared with before flights ($t = 28$, $Z = 2.296$, $P = 0.022$), but not between 'before' and 'after' periods ($t = 52$, $Z = 1.159$, $P = 0.246$), or between 'during' and 'after' periods ($t = 36$, $Z = 1.917$, $P = 0.055$).

Four behavioural events detected in manatees were considered reactions to drone. *Grouping* involved several manatees aggregating in a small area, sometimes close enough to be in physical contact. *Take cover* involved individuals going under submerged structures, where the body can be entirely or partly out of sight, but head is hidden; *Fleeing* involved individuals swimming away from previous position; and *Tail-kick* was a rapid tail flap, generally as a result of startling. These reactions to the drone were observed at three different aquarium facilities during experiments and/or confirmed during the review of videos. Manatees responded to the drone in three of the seven flights (43% of total), including the flight over two adjacent pools. Of the 25 manatees exposed to overhead drone flight, at least 11 (44%) displayed visible reactions.

Most reactions (e.g. *fleeing*, *grouping*) started within the first 2 minutes of the flight and continued throughout the flight,
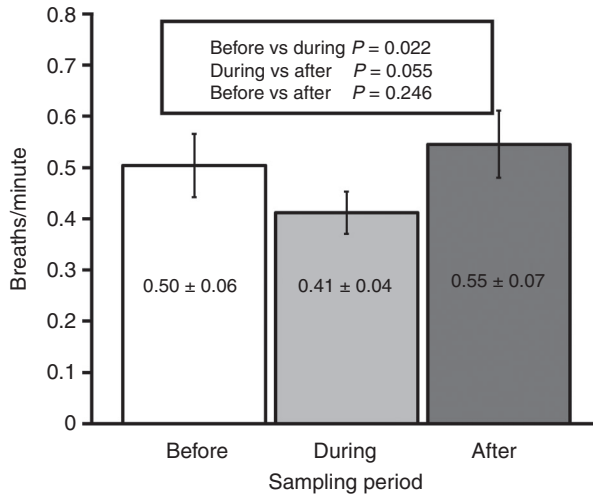
Fig. 5. Average respiration rate of individual Antillean manatees ($n = 18$) and standard error for sampling time periods: before, during and after drone flight. Results of the Wilcoxon matched pair tests between sampling periods are shown. Respiration rate was significantly lower 'during' the flights compared with the 'before' sampling period ($P = 0.022$). No differences were found between during and after ($P = 0.055$) nor between before and after ($P = 0.246$).

making it infeasible to examine the disturbance effects of drone flight at a specific altitude owing to the responses that occurred. Lower flight altitudes did not elicit more responses than did higher flight altitudes (Fig. 6, Table 2).

Fleeing was observed in one manatee in pool G, where at the beginning of test (flight altitude of 100 m), the male 'Mach' fled from its previous position and remained in the opposite end of the enclosure during the flight. After the flight ended, 'Mach' returned to his previous position. *Tail-kick* was observed in the same facility during the drone take-off, but the responding individual was not identified. *Take cover* was observed in two manatees in pool E, where females 'Tunich' and 'Nikté' went under structures (platform and stairs) unused by the animals in the sampling period prior the flight. This behaviour lasted less than a minute and was performed by the females on at least five occasions during the drone flight. *Grouping* was observed in pool A, during which all individuals left their previous positions and aggregated in close proximity to each other at the beginning of flight (at a flight altitude of 100 m). During the flight, the group separated into two subgroups, which regrouped three times at flight altitudes of 40 m, 20 m and 10 m. During this process, the male 'Pablo' circled the group(s) multiple times. When the drone was closer to the water surface at a flight altitude of 5 m, the subgroups grew separated.

Discussion

Our experimental design allowed us to compare the behaviour of captive Antillean manatees before, during and after drone flights. Ground behavioural observations prior launching the drone ('before') were critical to ensure that manatees were not evidently stressed before the experiment, and therefore, that the behaviours suggesting stress observed during and after flight were likely to have been triggered by the presence of the drone.

Hence, in the present study, we demonstrated that drones have the potential to affect manatee behaviour and cause shifts in their behavioural activity and physiological responses such as their respiration rate. Manatee respiration rate is reported to increase with the activity level (Hartman 1979); thus, we expected that the increased activity during drone flights would correlate with an increased respiration rate. In contrast, the average respiration rate of manatees decreased during drone flights. Decreased respiration rates may represent a stress response to a negative stimulus. For example, during the capture and handling of wild manatees and dugongs, animals can enter apnoea or decrease their respiration rate (Lanyon *et al.* 2010; Wong *et al.* 2012). Additionally, the manatees in the present study decreased their time at the surface during and after drone flights. Reduced blow rates and time at the surface are documented as an avoidance strategy on fin whales (*Balaenoptera physalus*) responding to watercraft (Jahoda *et al.* 2003). In manatees, this avoidance response may result in animals spending extended periods of time hidden underwater from a source of disturbance, causing decreases in respiration rate.

Changes in manatee activity budgets resulted in increased energetic expenditure (more time spent in locomotion) during drone flights. This effect lasted for at least 15 min after exposure. In contrast, average respiration rates after the drone flight did not differ from those before flight. This suggests that drones can elicit physiological changes, but these changes may have shorter recovery times than do behavioural changes of manatees. These findings closely parallel those of Ditmer *et al.* (2015) who reported median recovery times of less than 16 min for the increased heart rates of black bears (*Ursus americanus*) in response to drone flight.

Most of the manatee reactions reported during our drone flights (*fleeing, grouping, take cover, tail-kick*) involved avoidance and the seeking of refuge following disturbance. The exception is *tail-kick*, which seems to be a typical reaction of manatees when startled, a movement facilitating propulsion before fleeing (Hartman 1979). *Fleeing* and *tail-kick* were the primary reaction behaviours observed by Ramos *et al.* (2018) in wild manatees exposed to a multirotor drone. *Grouping* and *take cover* behaviours are probably reactions influenced by the presence of other individuals and/or the characteristics of their enclosure. Particularly, *take cover* is a response that depends on the presence of structures in the manatee pools. However, regardless of the environmental characteristics, this reaction suggested that manatees try to avoid a disturbance by seeking refuge (Nowacek *et al.* 2004; Ramos *et al.* 2018). In natural habitats, this behaviour may involve diving into deeper areas or using submerged vegetation so as to evade other manatees or boats (Hartman 1979). *Grouping* could have been influenced by the enclosure size and the manatees' inability to flee from it, thus associating in a safer space, seeking refuge. In a sense, the reaction of grouping involves fleeing previous positions and associating, likely as threat avoidance.

Studies of sources of disturbance for manatees have primarily focussed on the effects of motorised watercraft as the primary anthropogenic stressor to manatees. Responses of manatees to boat disturbance include increasing their swimming speed and orienting to deep waters (Nowacek *et al.* 2004) and an increased variability in respiration rates during vessel approaches (Miksis-
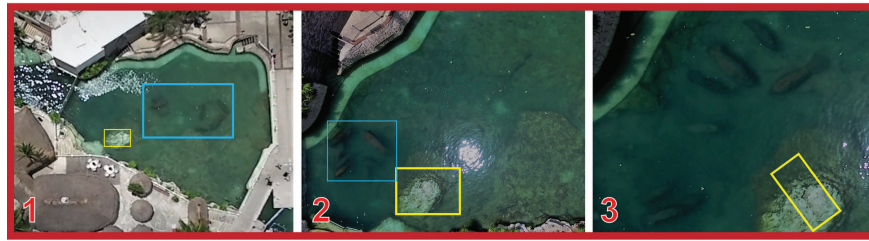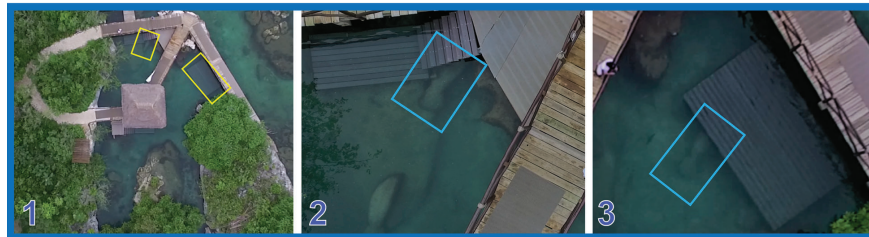
Grouping

Fleeing
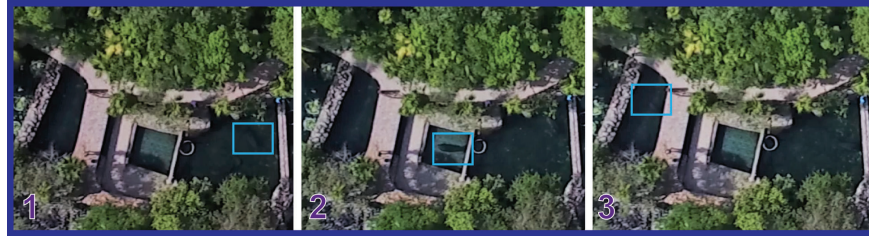
Take cover

Fig. 6. Aerial imagery of the reactions of captive Antillean manatees to the small drone (DJI Phantom 3). Blue squares point to manatees' position. Grouping: Panel 1 shows manatee's starting to leave the previous area towards reference object and grouping (recording at 1:00 min), panel 2 shows manateés still grouping near reference object around middle of flight (recording at 7:52 min), panel 3 shows in detail the 8 manatees near reference object. On a yellow square reference object. Fleeing: From 1 to 3, sequence of positions of the manatee during the first 150 s of recording, panel 3 shows the position the individual occupied during the rest of drone flight. Take cover: Panel 1 shows yellow squares framing panel 2 and 3 positions within pool, panel 2 shows manatees under stairs and panel 3 shows manatee under platform.

Table 2. Individual manatee reactions to drone in relation to flight altitude

Transitions involved steady vertical descents to the next lower altitude. The unknown manatee could not be identified to an individual. X represents a response behaviour detected in each listed manatee. Age Class: A, adult; C, calf; J, juvenile. T, transition

Manatee name	Sex	Age class	Pool	Reaction	Flight altitude (m)								
					100	T	40	T	20	T	10	T	5
Julieta	F	A	A	Grouping	X		X		X		X		
Dorothy	F	A	A	Grouping	X		X		X		X		
Conchis	F	J	A	Grouping	X		X		X		X		
Claudia	F	J	A	Grouping	X		X		X		X		
Bombon	F	C	A	Grouping	X		X		X		X		
Pablo	M	A	A	Grouping	X		X		X		X		
Quijote	M	A	A	Grouping	X		X		X		X		
Nohoch	M	C	A	Grouping	X		X		X		X		
Tunich	F	J	E	Take cover		X					X		
Nikté	F	J	E	Take cover			X	X					X
Mach	M	A	G	Fleeing	X								
Unknown	M	A	G or H	Tail kick	[A]								

[A]The individual that tail-kicked during drone take-off could not be identified.

Olds *et al.* 2007). The responses displayed by manatees in our experiment can be interpreted as stress responses, and agree with observations on wild manatee behaviour under situations of disturbance such as boat approaches (Nowacek *et al.* 2004; Miksis-Olds *et al.* 2007), human presence (Abernathy 1995) and drone exposures (Ramos *et al.* 2018).

Some of the manatees did not visibly react to the drone exposure. This was also reported by Ramos *et al.* (2018) who detected visible manatee responses to drone on 24% of all exposures. Moreover, they noticed differences in responsiveness of individuals during repeated drone exposures. This highlights that the individual response can be influenced by an array of factors, including a manatee's personality and life experience, and how manatees differ across individuals (Sorice *et al.* 2003; Ramos *et al.* 2018). Furthermore, animals of the same species may respond to drones on different ways depending on their age, sex, and biological state (e.g. breeding; Pomeroy *et al.* 2015).

The absence of visible behavioural reactions in manatees does not rule out an effect over the animals: differences in activity budgets and respiratory rates with and without the presence of drone are also relevant evidences of the drone's effect. The captive manatees with which we conducted our experiments are individuals under constant monitoring and sometimes trained to interact with visitors, so their personalities are well known by their trainers. The animal caretakers noticed that some individuals were 'distracted' and 'uncooperative' to instructions after drone flights. Although these observations are anecdotal, they support the fact that some effects of disturbance are not visible or may not be easily measurable using short-term behavioural observations.

The ability to detect the visual and acoustic disturbance of an aerial drone, and the degree to which these are associated with a threatening stimulus (e.g. predator), is related to a given species disturbance threshold (Bevan *et al.* 2018). Manatees do not have any natural predators and there are no reports of manatees being attacked or harassed by flying animals such as seabirds. Thus, it is possible that the sound of the approaching drone is the main source of disturbance to manatees.

The noise perceived by manatees and the disturbance level associated can be influenced by physical factors. Environmental noise has the potential for masking drone noise (Christiansen *et al.* 2016) and it is likely that it was variable among experiment facilities (e.g. mechanical noise from nearby buildings). Additionally, the received noise levels underwater from an overhead aircraft typically decrease as aircraft flight altitude increases. The reception of the sound also depends on the orientation and position of the animal in relation to the source because physical factors from the environment can cause sound interference (Erbe *et al.* 2017). Finally, because of the structure of facilities we conducted our work at, in most cases, the drone was launched near manatee enclosures. The *tail-kick* reaction was reported during a drone take-off, when drones are typically louder (Arona *et al.* 2018). Interestingly, this response occurred when the drone was in audible range but not visible to the manatee, thus, strongly pointing to the drone's noise as the disturbance stimulus. *Grouping* and *fleeing* also occurred during the first 2 min of the flight recording. This suggests that some individuals were already aware of the drone and modified their behaviour from its launch or when the drone was at 100 m above them.

Manatee responses to take-off operations made it infeasible to isolate an effect of flight altitude on manatee reactions, but indicated that the noise of aircraft rotors represents a strong negative stimulus to manatees. Ramos *et al.* (2018) reported that lower flight altitudes increased the likelihood of disturbance in wild Antillean manatees exposed to drones. However, in some cases, animals continued to respond to the aircraft at >100 m. The reactions of manatees to the sound of drone take-off in our study indicated that increasing the distance of the launch site to the animals is a key consideration to reduce possible impacts and tests for responses.

Manatee behaviours we documented in response to the drone could have been influenced by the presence of the other individuals within the enclosure. For example, one startled animal could have driven the *Grouping* response observed in multiple individuals. However, this was not always the case, because in one response, *fleeing* was observed only on one of the two manatees sharing the same pool. This is similar to reports by Ramos *et al.* (2018) who observed multiple manatees in one group flee in response to drone flight, whereas in other groups, only one animal responded. Individual experiences and personalities are likely to influence the response we observed, and future experiments conducted with isolated individuals would be needed to determine whether there are group effects in manatee responses to drones.

Recommendations for using drones with manatees

Our study has provided evidence of small drones causing disturbance in Antillean manatees, by eliciting behavioural and physiological responses (i.e. change in respiration rate), likely signalling temporary stress. It is important to balance the quality and type of data needed with the potential level of disturbance inflicted (Bevan *et al.* 2018).

Visible reactions to drone flight, such as those observed during our experiments (e.g. tail-lick, fleeing), can be used as an indicator of stress. These indicators can lead the operator to suspend, redirect or continue the approach on a case-by-case basis. These measures may be feasible when collecting data for photogrammetry, photo-ID and behaviour studies, in which the operator manually controls the drone and is able to observe the manatee's reactions in real time. Precautionary principles should be adopted for individuals sensitive to disturbance impacts, such as mother–calf pairs, because they could separate during fleeing. For detection and density estimation studies, the high flight altitudes in which drones typically operate (>100 m) should prevent the disturbance of individuals (Ramos *et al.* 2018; Raoult *et al.* 2020).

Behavioural studies of manatees using drones should consider the potential bias owing to the effect of the method on manatee behaviour. For example, studies aiming to assess behavioural budgets would not be recommended because of important potential effects of the drone on activity rates. The increases in manatee activity levels observed after drone flights should be considered when recording further data after flights. Including no-fly periods (30–60 min) after each drone flight could help minimise biased assessments when recording behaviour-related information (e.g. bioacoustics, ground observations) after these flights. Also, limiting the number of low flights per day on the study area should reduce the possible

pressures on targeted individuals. Future studies should perform additional drone-exposure experiments to obtain more information regarding the after-effects and recovery times of manatees exposed to drones.

The manatees in our study exhibited physiological responses to the drone, with changes in their respiration rate. Thus, we advise caution when using drones for precise data recording of manatee respiration rates unless associated information such as surface behavioural observations or animal-borne tag data is available to identify changes in respiration rate related to drone operations. If control data are available, respiration rates during drone operations could be monitored to detect stress in manatees.

The behavioural and physiological responses we detected in captive manatees in response to small drones support the validity of previous recommendations for the use of drones in wildlife research (Hodgson and Koh 2016; Mulero-Pázmány *et al.* 2017) and emphasises the need for careful guidance in their use with manatees. An important first step is the selection of smaller and quieter drones with suitable data-capture capabilities (e.g. DJI Mavic Pro) to minimise visual and auditory stimuli (Hodgson and Koh 2016). New commercial drone models are emerging regularly with a smaller visual and acoustic profile than in previous models, likely reducing the probability of manatees detecting the aircraft. Data acquisition needs and preliminary assessments of the disturbance levels caused by different systems should be considered during the conceptual stages of a study, at each study site and with each study population (Fettermann *et al.* 2019). For example, testing behavioural responses of the same and multiple species with different types of drones is important because each may differ in their level of noise output (Erbe *et al.* 2017). In general, pilots should fly drones at the highest altitudes feasible for obtaining sufficient-quality data (Ramos *et al.* 2018) and permitted according to local law. Aircrafts should not directly approach animals in vertical descents and target-oriented flight patterns (Mulero-Pázmány *et al.* 2017; Ramos *et al.* 2018). Finally, minimum distances for a launch site should be implemented because multirotor aircrafts are louder during take-off (Brisson-Curadeau *et al.* 2017). The manatee responses we observed at take-off and within minutes of the first flight position at 100 m suggest that the drone operators should not launch aircrafts near manatees.

In conclusion, our results have provided support for previous findings about manatee reactions to drones, demonstrating responsivity to drone flights in the species, and present additional evidence for the behavioural and physiological responses of manatees to overhead drone flights. Further studies are needed to better understand the physiological responses of manatees to drones and the effect of other sources of behavioural differences, including manatee group interactions, age, sex, personality and habituation.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

References

Abernathy, J. (1995). 'Time-activity budgets and displacement rates in Florida manatees (*Trichechus manatus*) in the absence and presence of humans.' (Florida Atlantic University: Boca Raton, FL, USA.)

Anderson, K., and Gaston, K. J. (2013). Lightweight unmanned aerial vehicles will revolutionize spatial ecology. *Frontiers in Ecology and the Environment* **11**, 138–146. doi:10.1890/120150

Arona, L., Dale, J., Heaslip, S. G., Hammill, M. O., and Johnston, D. W. (2018). Assessing the disturbance potential of small unoccupied aircraft systems (UAS) on gray seals (*Halichoerus grypus*) at breeding colonies in Nova Scotia, Canada. *PeerJ* **6**, e4467. doi:10.7717/peerj.4467

Bevan, E., Whiting, S., Tucker, T., Guinea, M., Raith, A., and Douglas, R. (2018). Measuring behavioral responses of sea turtles, saltwater crocodiles, and crested terns to drone disturbance to define ethical operating thresholds. *PLoS One* **13**, e0194460. doi:10.1371/journal.pone.0194460

Brisson-Curadeau, É., Bird, D., Burke, C., Fifield, D. A., Pace, P., Sherley, R. B., and Elliott, K. H. (2017). Seabird species vary in behavioural response to drone census. *Scientific Reports* **7**, 17884. doi:10.1038/s41598-017-18202-3

Buckingham, C. A., Lefebvre, L. W., Schaefer, J. M., and Kochman, H. I. (1999). Manatee response to boating activity in a thermal refuge. *Wildlife Society Bulletin* **27**, 514–522.

Christiansen, F., Rojano-Doñate, L., Madsen, P. T., and Bejder, L. (2016). Noise levels of multi-rotor unmanned aerial vehicles with implications for potential underwater impacts on marine mammals. *Frontiers in Marine Science* **3**, 277. doi:10.3389/fmars.2016.00277

Christie, K. S., Gilbert, S. L., Brown, C. L., Hatfield, M., and Hanson, L. (2016). Unmanned aircraft systems in wildlife research: current and future applications of a transformative technology. *Frontiers in Ecology and the Environment* **14**, 241–251. doi:10.1002/fee.1281

Colefax, A. P., Butcher, P. A., and Kelaher, B. P. (2018). The potential for unmanned aerial vehicles (UAVs) to conduct marine fauna surveys in place of manned aircraft. *ICES Journal of Marine Science* **75**, 1–8. doi:10.1093/icesjms/fsx100

Ditmer, M. A., Vincent, J. B., Werden, L. K., Tanner, J. C., Laske, T. G., Iaizzo, P. A., Garshelis, D. L., and Fieberg, J. R. (2015). Bears show a physiological but limited behavioral response to unmanned aerial vehicles. *Current Biology* **25**, 2278–2283. doi:10.1016/j.cub.2015.07.024

Domínguez-Sánchez, C. A., Acevedo-Whitehouse, K. A., and Gendron, D. (2018). Effect of drone-based blow sampling on blue whale (*Balaenoptera musculus*) behavior. *Marine Mammal Science* **34**, 841–850. doi:10.1111/mms.12482

Erbe, C., Parsons, M., Duncan, A., Osterrieder, S. K., and Allen, K. (2017). Aerial and underwater sound of unmanned aerial vehicles (UAV). *Journal of Unmanned Vehicle Systems* **5**, 92–101. doi:10.1139/juvs-2016-0018

Fettermann, T., Fiori, L., Bader, M., Doshi, A., Breen, D., Stockin, K. A., and Bollard, B. (2019). Behaviour reactions of bottlenose dolphins (*Tursiops truncatus*) to multirotor Unmanned Aerial Vehicles (UAVs). *Scientific Reports* **9**, 8558. doi:10.1038/s41598-019-44976-9

Fiori, L., Martinez, E., Bader, M. K. F., Orams, M. B., and Bollard, B. (2020). Insights into the use of an unmanned aerial vehicle (UAV) to investigate the behavior of humpback whales (*Megaptera novaeangliae*) in Vava'u, Kingdom of Tonga. *Marine Mammal Science* **36**, 209–223. doi:10.1111/mms.12637

French, S. S., DeNardo, D. F., Greives, T. J., Strand, C. R., and Demas, G. E. (2010). Human disturbance alters endocrine and immune responses in the Galapagos marine iguana (*Amblyrhynchus cristatus*). *Hormones and Behavior* **58**, 792–799. doi:10.1016/j.yhbeh.2010.08.001

Hartman, D. S. (1979). 'Ecology and behavior of the manatee (*Trichechus manatus*) in Florida.' Special Publication No. 5. The American Society of Mammalogists, Lawrence, KS, USA.

Hodgson, J. C., and Koh, L. P. (2016). Best practice for minimising unmanned aerial vehicle disturbance to wildlife in biological field research. *Current Biology* **26**, R404–R405. doi:10.1016/j.cub.2016.04.001

Hodgson, A., Kelly, N., and Peel, D. (2013). Unmanned aerial vehicles (UAVs) for surveying marine fauna: a dugong case study. *PLoS One* **8**, e79556. doi:10.1371/journal.pone.0079556

Hodgson, A., Peel, D., and Kelly, N. (2017). Unmanned aerial vehicles for surveying marine fauna: assessing detection probability. *Ecological Applications* **27**, 1253–1267. doi:10.1002/eap.1519

Hodgson, J. C., Mott, R., Baylis, S. M., Pham, T. T., Wotherspoon, S., Kilpatrick, A. D., Raja Segaran, R., Reid, I., Terauds, A., and Koh, L. P. (2018). Drones count wildlife more accurately and precisely than humans. *Methods in Ecology and Evolution* **9**, 1160–1167. doi:10.1111/2041-210X.12974

Jahoda, M., Lafortuna, C. L., Biassoni, N., Almirante, C., Azzellino, A., Panigada, S., Zanardelli, M., and Di Sciara, G. N. (2003). Mediterranean fin whale's (*Balaenoptera physalus*) response to small vessels and biopsy sampling assessed through passive tracking and timing of respiration. *Marine Mammal Science* **19**, 96–110. doi:10.1111/j.1748-7692.2003.tb01095.x

Jones, I. G. P., Pearlstine, L. G., and Percival, H. F. (2006). An assessment of small unmanned aerial vehicles for wildlife research. *Wildlife Society Bulletin* **34**, 750–758. doi:10.2193/0091-7648(2006)34[750:AAOSUA]2.0.CO;2

Joyce, K., Duce, S., Leahy, S., Leon, J., and Maier, S. (2019). Principles and practice of acquiring drone-based image data in marine environments. *Marine and Freshwater Research* **70**, 952–963. doi:10.1071/MF17380

Koh, L. P., and Wich, S. A. (2012). Dawn of drone ecology: low-cost autonomous aerial vehicles for conservation. *Tropical Conservation Science* **5**, 121–132. doi:10.1177/194008291200500202

Landeo-Yauri, S. S., Ramos, E. A., Castelblanco-Martínez, D. N., Niño-Torres, C. A., and Searle, L. (2020). Using small drones to photo-identify Antillean manatees: a novel method for monitoring an endangered marine mammal in the Caribbean Sea. *Endangered Species Research* **41**, 79–90. doi:10.3354/esr01007

Lanyon, J. M., Sneath, H. L., Long, T., and Bonde, R. K. (2010). Physiological response of wild dugongs (*Dugong dugon*) to out-of-water sampling for health assessment. *Aquatic Mammals* **36**, 46–58. doi:10.1578/AM.36.1.2010.46

Linchant, J., Lisein, J., Semeki, J., Lejeune, P., and Vermeulen, C. (2015). Are unmanned aircraft systems (UAS s) the future of wildlife monitoring? A review of accomplishments and challenges. *Mammal Review* **45**, 239–252. doi:10.1111/mam.12046

Lusseau, D., and Bejder, L. (2007). The long-term consequences of short-term responses to disturbance experiences from whale watching impact

assessment. *International Journal of Comparative Psychology* **20**, 228–236.

Messinger, M., Asner, G. P., and Silman, M. (2016). Rapid assessments of Amazon forest structure and biomass using small unmanned aerial systems. *Remote Sensing* **8**, 615. doi:10.3390/rs8080615

Miksis-Olds, J. L., Donaghay, P. L., Miller, J. H., Tyack, P. L., and Reynolds, J. E. (2007). Simulated vessel approaches elicit differential responses from manatees. *Marine Mammal Science* **23**, 629–649. doi:10.1111/j.1748-7692.2007.00133.x

Mulero-Pázmány, M., Stolper, R., Van Essen, L., Negro, J. J., and Sassen, T. (2014). Remotely piloted aircraft systems as a rhinoceros anti-poaching tool in Africa. *PLoS One* **9**, e83873. doi:10.1371/journal.pone.0083873

Mulero-Pázmány, M., Jenni-Eiermann, S., Strebel, N., Sattler, T., Negro, J. J., and Tablado, Z. (2017). Unmanned aircraft systems as a new source of disturbance for wildlife: a systematic review. *PLoS One* **12**, e0178448. doi:10.1371/journal.pone.0178448

Nowacek, S. M., Wells, R. S., Owen, E. C., Speakman, T. R., Flamm, R. O., and Nowacek, D. P. (2004). Florida manatees, *Trichechus manatus latirostris*, respond to approaching vessels. *Biological Conservation* **119**, 517–523. doi:10.1016/j.biocon.2003.11.020

Pomeroy, P., O'connor, L., and Davies, P. (2015). Assessing use of and reaction to unmanned aerial systems in gray and harbor seals during breeding and molt in the UK. *Journal of Unmanned Vehicle Systems* **3**, 102–113. doi:10.1139/juvs-2015-0013

Ramos, E. A., Maloney, B. M., Magnasco, M. O., and Reiss, D. (2018). Bottlenose dolphins and Antillean manatees respond to small multi-rotor unmanned aerial systems. *Frontiers in Marine Science* **5**, 316. doi:10.3389/fmars.2018.00316

Raoult, V., Colefax, A. P., Allan, B. M., Cagnazzi, D., Castelblanco-Martínez, N., Ierodiaconou, D., Johnston, D. W., Landeo-Yauri, S., Lyons, M., and Pirotta, V. (2020). Operational protocols for the use of drones in marine animal research. *Drones (Basel)* **4**, 64. doi:10.3390/drones4040064

Reynolds, J. E. (1999). 'Efforts to Conserve the Manatees. In 'Conservation and Management of Marine Mammals'. (Eds J. R. Twiss and R. R. Reeves.) pp. 267–295. (Smithsonian Institution Press: Washington and London.)

Smith, C. E., Sykora-Bodie, S. T., Bloodworth, B., Pack, S. M., Spradlin, T. R., and LeBoeuf, N. R. (2016). Assessment of known impacts of unmanned aerial systems (UAS) on marine mammals: data gaps and recommendations for researchers in the United States. *Journal of Unmanned Vehicle Systems* **4**, 31–44. doi:10.1139/juvs-2015-0017

Sorice, M. G., Shafer, C. S., and Scott, D. (2003). Managing endangered species within the use/preservation paradox: understanding and defining harassment of the West Indian manatee (*Trichechus manatus*). *Coastal Management* **31**, 319–338. doi:10.1080/08920750390232983

Williams, R., Lusseau, D., and Hammond, P. S. (2006). Estimating relative energetic costs of human disturbance to killer whales (*Orcinus orca*). *Biological Conservation* **133**, 301–311. doi:10.1016/j.biocon.2006.06.010

Wong, A. W., Bonde, R. K., Siegal-Willott, J., Stamper, M. A., Colee, J., Powell, J. A., Reid, J. P., Deutsch, C. J., and Harr, K. E. (2012). Monitoring oral temperature, heart rate, and respiration rate of West Indian manatees (*Trichechus manatus*) during capture and handling in the field. *Aquatic Mammals* **38**, 1–16. doi:10.1578/AM.38.1.2012.1

Handling Editor: Aaron Wirsing