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METHOD

Novel attachment methods for assessing activity patterns using triaxial accelerometers on stingrays in the Bahamas

Chris R. E. Ward[1,2] · Ian A. Bouyoucos[3] · Edward J. Brooks[1] · Owen R. O'Shea[1,4]

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Abstract

The use of bio-logging devices is important for describing behaviour, energy expenditure and activity budgets of cryptic marine organisms. In stingrays, the physical deployment of bio-logging devices is challenging due to their lack of raised structures or hard tissue for attachment. Previous studies have used a range of attachment techniques on various locations, including the pectoral musculature of the discs, spiracular cartilage or tail musculature. For devices such as accelerometers that capture precise animal movement, appropriate attachment and retention are important for collecting data that are representative of animal movement. Here, we detail a novel attachment method for bio-logging devices on stingrays using triaxial accelerometers that were attached through the musculature at the base of the tail of ten wild southern stingrays (*Hypanus americanus*). Data returned upon recapture suggest that stingrays exhibited active and non-active states and had the highest activity levels (vector sum acceleration) during the night with no apparent tide-associated activity patterns. Tag retention was 100% for all recaptured individuals ($n=8$), with deployments lasting from 13 to 212 days. Wounds associated with the tagging process were completely healed for individuals that were recaptured after tag removal ($n=3$). High rates of tag retention, usability and ecological significance of retrieved data, and complete healing following tag removal suggest that the methods described herein should be considered when attaching small bio-logging devices to large demersal rays for short- (weeks)-to-medium-term (months) studies.

Introduction

The dynamic nature of marine ecosystems often creates challenges for conservation planning, particularly for free-ranging or cryptic species where patterns of habitat use and activity are poorly documented. Evaluating mechanisms that underpin the energetic and spatial requirements of marine species are critical when considering life-history and effective species management (Connell et al. 2000; Lowe and Goldman 2001; Schindler et al. 2002; Lewison et al. 2004; Lowe and Bray 2006; Whitney et al. 2007). Advances in technology over the past 50 years have provided a means for many studies to deploy data-logging ("bio-logging") and transmitting ("biotelemetry") devices on free-ranging aquatic animals (Payne et al. 2014; Cooke et al. 2016). Specifically, accelerometers are well-established tools for generating fine-scale data on activity budgets, movement, behaviour and energy expenditure in marine organisms (e.g., Yoda et al. 2001; Wilson et al. 2006; Whitney et al. 2007, 2010; Thiem et al. 2015; Stehfest et al. 2015), realized over extended temporal scales (weeks-to-months). However, researchers must take care when attaching electronic tag packages to wild animals so that recorded data are not confounded by behavioural modifications and stress responses. Furthermore, one must take practical experimental factors into consideration, such as tag retention and retrieval.

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✉ Chris R. E. Ward
chris.ward@unb.ca

Owen R. O'Shea
owen@coresciences.org

[1] Shark Research and Conservation Program, The Cape Eleuthera Institute, Rock Sound, Eleuthera, Bahamas

[2] Department of Forestry and Environmental Management, University of New Brunswick, Fredericton, NB, Canada

[3] Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD, Australia

[4] Centre for Ocean Research and Education (CORE), Gregory Town, Eleuthera, Bahamas

Rays (Batoidea) occur in a broad range of marine ecosystems, yet they are most commonly found in shallow coastal and shelf regions (McEachran and Fechhelm 1998; McEachran and Aschliman 2004) where they can make up a significant portion of the fish community biomass (O'Shea 2012). Rays present a unique problem when attaching bio-logging devices: being dorso-ventrally compressed, many rays lack any prominent features (e.g., dorsal fins, caudal peduncle) to which devices can be attached. Further, any obvious tagging locations (e.g., the disc, dorsal musculature) are mainly composed of soft tissue with small, fine denticles; undulation of the discs for movement can aggravate or open attachment wounds that may become prone to necrosis. This has led researchers to use varying methods to attach devices to rays. Hunter et al. (2005) attached electronic data storage tags to 197 thornback rays (*Raja clavata*) from the Thames Estuary using a nylon thread passed through the pectoral musculature and fixed with a disc attached the ventral surface. Collins et al. (2007) affixed acoustic transmitters to cownose rays (*Rhinoptera bonasus*) using cinch tags inserted through the spiracular cartilage. Le Port et al. (2008) used monofilament to suture pop-up satellite archival tags (PSATs) through the musculature of the tail in short-tailed stingrays, with retention of 62 and 151 days, respectively, and further reported re-sightings of one animal that had completely healed 7 months later. Finally, Speed et al. (2013) used steel-headed dart tags fired from a pneumatic spear gun to attach active acoustic transmitters to the pectoral fins of cowtail rays (*Pastinachus sephen*) and porcupine rays (*Urogymnus asperrimus*).

To our knowledge, only two published studies have deployed accelerometers on rays, which involved a dorsally mounted package immediately posterior to the spiracles in a small coastal ray from Japan (Otaki et al. 2015), and a tag package attached to the pectoral fin of a myliobatid ray (Whitney et al. 2012). The former study used a mesh netting and tape to attach the device for periods of up to 4 h, whereby a release mechanism allowed the package to float to the surface. It is unclear how much of the package was left on the animal (*Hemitrygon akajei*), and caution must be exercised on smaller bodied individuals. The latter study sutured a data-logging accelerometer through the tip of the pectoral fin of a captive eagle ray (*Aetobatus narinari*) to detect wingbeats and identify feeding activity. Indeed, best-practice techniques for deploying accelerometers have been previously established for other aquatic organisms, including sharks (Gleiss et al. 2010), bony fishes (Wright et al. 2014), jellyfish (Fossette et al. 2016), and diving sea birds (Halsey et al. 2011); there is a need for minimally invasive tagging methods for rays (Speed et al. 2013).

Therefore, the overall objectives of this study were to (1) develop a resilient, reliable and ethically responsible method for attaching bio-logging packages to stingrays, and (2) measure ecologically meaningful differences in activity levels of tagged stingrays using accelerometer bio-loggers, thus demonstrating our methods as sound.

Methods

Study site and capture

Southern stingrays (*Hypanus americanus*) were targeted for sampling at The Schooner Cays, north of Powell point on the island of Eleuthera in the Bahamas. These islands are situated on the Great Bahama Bank and are homogeneous limestone cays with extensive and shallow (< 1 m) soft sediment flats giving way to deeper channels (< 10 m) that eventually flow into Exuma Sound; a deep-water inlet of the Western Atlantic Ocean, to the west of these islands. For this study, two islands were chosen due to ease of access and stingray abundance: Water Cay (24.908608°, −76.354762°) and Buttonwood Cay (24.920424°, −76.384696°). Sites were chosen based on previous studies (O'Shea et al. unpublished data) that demonstrated strong site attachment and, therefore, high recapture rates. Individual rays were captured by spot seining using a combination of barrier and hand nets following methods outlined by O'Shea et al. (2017). All rays were kept in shallow water during tag mounting, which lasted no longer than 10 min.

Tag package and mounting

The tag package included a HOBO pendant triaxial accelerometer (product # UA-004-64) mounted on a flexible, fine plastic backing plate and sealed in a waterproof external rubber sealant to create a uniform hydrodynamic surface. The tag packages had a mass of 28*g*, an apparent submerged weight of 12.6*g* (negatively buoyant) and had approximate dimensions of 80 mm × 48 mm × 25 mm (length × width × height). Upon capture, stingrays were restrained in large hand nets and their barb secured with a soft, non-abrasive cloth. During tagging, stingrays were physically restrained by hand and net in shallow water. Size (disc width W_D, in mm), weight, sex and maturity of individuals were recorded before base tail width was measured to assess an individual's suitability for tag deployment (criterion: base tail width > 45 mm). The tag was mounted by first creating four identical holes through the ventral surface of the stingray's tail at the base, through to the dorsal surface using sterile hypodermic needles embedded in a rigid foam block. The tag package was secured via four stainless steel annealed locking wires connected to each corner of the backing plate, that were passed into each hypodermic needle, before removing the foam block, allowing the wire to travel through the stingray's tail (Fig. 1). These wires were then locked to a second backing plate and tied to ensure rigidity

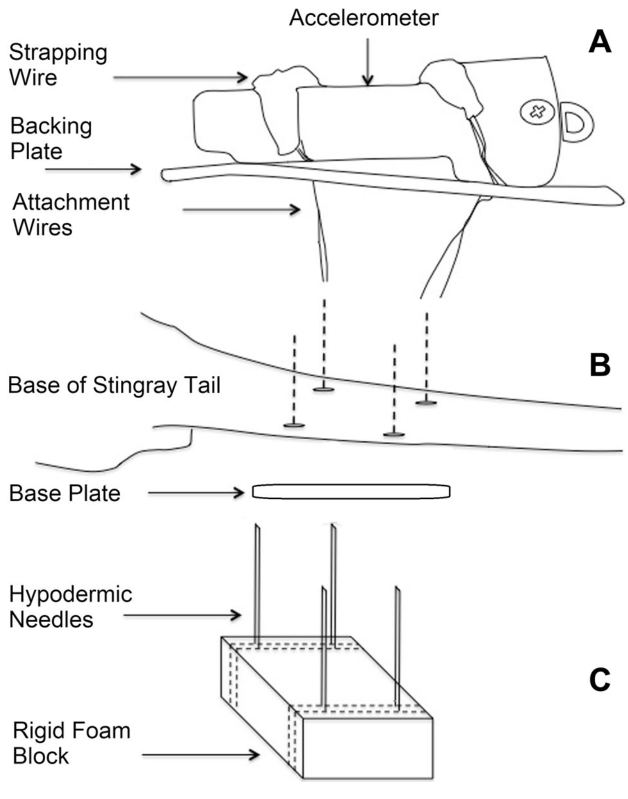

Fig. 1 Schematic of accelerometer tag package used on southern stingrays (*Hypanus americanus*) in the wild. The entire contents in **a** are sealed in three layers of Performix Plasti-dip to further waterproof and improve hydrodynamics, with attachment wires going through the stingray's tail on either side of the vertebra (**b**) guided by hypodermic needles in **c**

with little to no loose movement (Fig. 2). Each tag was pre-programmed for a total logging time of 60 consecutive hours, recording acceleration every 10 s in three axes, and measuring within a range of $\pm 3g$ with $\pm 0.105g$ accuracy and $0.025g$ resolution (where $1g = 9.81$ m s^{-2}). Indeed, data-logging tags, such as the package used in this study, are typically deployed with higher logging frequencies to provide precise estimates for field metabolic rate estimation or behavioural identification, neither of which were objectives of this study (Shepard et al. 2008; Brownscombe et al. 2018). The acceleration logging frequency used in this study, however, was more representative of acceleration transmitting tags (e.g., O'Toole et al. 2011; Payne et al. 2010; Wilson et al. 2013). Accelerometers were programmed to activate 24 h post-release, a conservative estimate to avoid recording post-release behaviours that may have resulted from the stress of capture and handling. A 24-h delay was considered appropriate because other studies on sharks have suggested that animals resumed consistent behaviour within 10 h of hook-and-line capture (e.g., Whitney et al. 2016). Areas where individuals were tagged were revisited 7 days after tagging for recapture and were revisited on a weekly basis until tags were retrieved throughout the following year.

Analytical methods

A linear mixed-effects model was applied to the raw accelerometer output (vector sum $= \sqrt{x^2 + y^2 + z^2}$) to define variation in activity levels by diel period (day/night) and tide state (ebbing/flooding). A vector sum value of approximately $1g$ represents a stationary tag or an animal at rest, because the only acceleration the tag is experiencing is acceleration due to gravity that is recorded by the only vertically oriented axis. All other values should be indicative of animal movement, although acceleration data recorded for an active animal or animal at rest may be influenced by water currents, especially in shallow habitats (Whitney et al. 2010). This analysis was conducted as a proof of concept that the tagging location could yield ecologically meaningful data. Sunrise, sunset, and tide times were collected online from an on-island monitoring station (http://www.eleuthera-map.com/). The mean vector sum per hour of deployment was modelled as a function of diel period, tide state, and their interaction, including stingray ID as a random effect. All analyses were conducted in R using the "R Stats Package" (R Core Team 2018) and "nlme" package (Pinheiro et al. 2018).

Results

Ten mature females ($W_D > 750$ mm) had accelerometer packages attached representing sizes classes between 998 and 1112 mm W_D and weighing between 13 and 44 kg. Two tagged individuals evaded recapture, and at this time have not been observed since. The remaining individuals ($n = 8$) were recaptured 13–212 days after tagging. Recaptured individuals had bruising and abrasions immediately near wire entrance points (Fig. 3a, d), one individual was further recaptured 14 days after initial tag removal and showed rapid healing (Fig. 3b, e), and one individual was recaptured 1 month after initial tag removal and showed almost no signs of tagging (Fig. 3c, f). Of the eight accelerometers retrieved, five returned usable data and three accelerometers failed to record data. Vector sum acceleration values ranged from 0.979 to $1.032g$, and multi-modal distributions of acceleration data were apparent from vector sum frequency histograms (Fig. 4). Furthermore, vector sum acceleration was significantly higher during the night with no effect of, or interaction with, tide state (Table 1; Fig. 5).

Discussion

Through the acquisition of meaningful—albeit short-term—data and general healing of individual rays used in this study, this novel method for short-term attachment of bio-logging

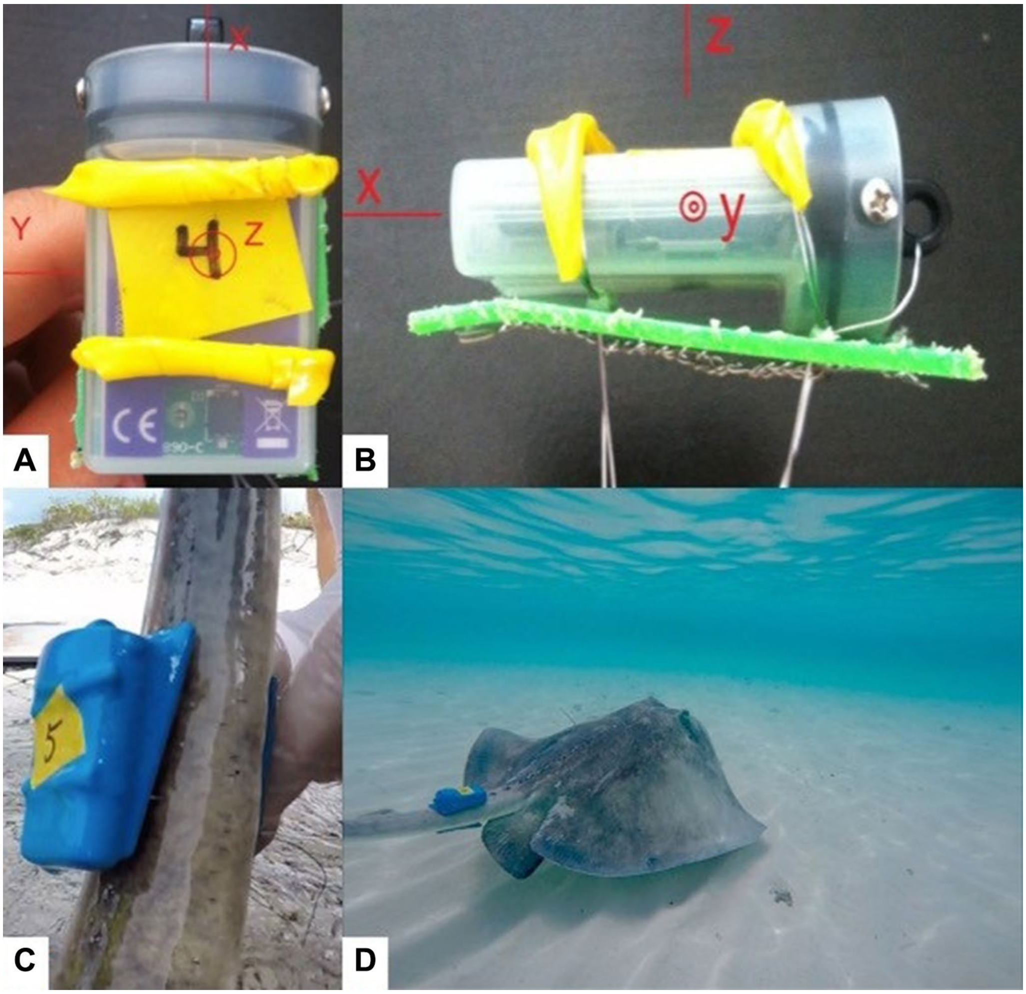

Fig. 2 Accelerometer tag package without external sealant (**a**, **b**) used to record activity of southern stingrays (*Hypanus americanus*) off the Schooner Cays, the Bahamas. The tag package was sealed with Performix Plasti-dip and attached at the base of the tail (**c**, **d**)

devices is well suited to large demersal rays, particularly where site fidelity enhances recapture rates. Submerged weight of similar species of demersal batoids has been reported as 3–5% of the individual's mass in air (*Dasyatis pastinaca*, Bone and Roberts 1969; *Urobatis jamaicensis*, Sherman and Gilliam 1996; *Narcine brasiliensis* Rosenblum et al. 2011). If southern stingrays have a similar submerged weight (i.e., submerged weight = 3–5% of mass in air) then our tag package increased the submerged weight of southern stingrays in this study by a range of 1–3.2% (Blaylock 1990). Of the eight recaptured individuals here, all showed varying degrees of scarring, bruising and abrasions around the tag; however, data presented were typical of anticipated and previously observed wild behaviours and all were similar to each other. In addition, time at liberty (regardless of the 60-h tag life) ranged from 13 to 212 days, demonstrating longer term mounting may be possible without further hindrance to behaviour. Anecdotal evidence from ongoing studies by O'Shea et al. on southern stingrays at the same study site have since noted the continued recapture of several individuals used in this study, with three individuals that were recaptured > 4 weeks post tag removal having their tagging wounds fully healed.

Deployment location on the tail of the stingray allowed for quantification of animal activity and identification of activity states. Indeed, we were only able to test values of relative activity level and discern three potential activity states (i.e., resting values of approximately 1*g* and two non-resting states). A drawback to tagging the tail of individuals is the inability to quantify wingbeat frequency with the current study's sampling frequency. One approach to quantify wingbeat frequency would be to tag one side of the disc with an accelerometer. Acceleration data that captures movement of the disk could then be used to calculate wingbeat frequency and thus energy expenditure through the movement of one disc fin (e.g., Whitney et al. 2012). However, large devices secured to one side of the locomotion structure of a symmetrical marine animal may have energetic

Fig. 3 Bruising and abrasions at tagging location on a southern stingray (*Hypanus americanus*) immediately after tag removal (**a** dorsal; **d** ventral). Representative photos of healing are presented from a separate individual 14 days after tag removal (**b** dorsal; **e** ventral), and from a third individual 1 month after tag removal (**c** dorsal; **e** ventral)

consequences that could confound energy expenditure calculations, as well as affecting the ability of individuals to undulate their discs for movement (Di Santo et al. 2017). Further studies using this tagging method and a higher sampling frequency may be able to detect wingbeat frequency. Alternative tag locations and methods (e.g., gastric insertion, tethering to the dorsal musculature) used on sharks have also yielded reliable estimates of tailbeat frequency, which is analogous to wingbeat frequency in batoids (Whitney et al. 2007, Jorgensen et al. 2015). Regardless, the current study demonstrated that accelerometers mounted on the base of the tail of a large demersal batoid fish could measure animal activity, which is relevant to future endeavours to estimate field metabolic rates through animal activity metrics like dynamic body acceleration. Based on the 100% tag retention rate of the eight recaptured individuals over periods from 2 weeks to 6 months, this study suggests that deploying tags using the anchoring methods presented here over similar durations is an acceptable practice for small bio-logging devices.

Acceleration data retrieved from tag packages suggest that individual activity by *H. americanus* was significantly influenced by diel period, but not tide. Increased activity at night agrees with previous radio-tracking studies of this species by Corcoran et al. (2013) and Tilley (2011). Tilley (2011) tracked juvenile southern stingrays and suggested that stingrays may forage at night when they reach a size threshold, as he observed increases in movement at night as the size of stingrays increased. As the present study was restricted to mature females (> 750 mm W_D), our results correspond with those of Corcoran et al. (2013) and the hypothesis of Tilley (2011). Previous studies have suggested that many species of batoid forage according to tide, where individuals exploit the increased availability of foraging habitat at higher tides (Gilliam and Sullivan 1993, Carvalho et al. 2010). Data presented here, however,

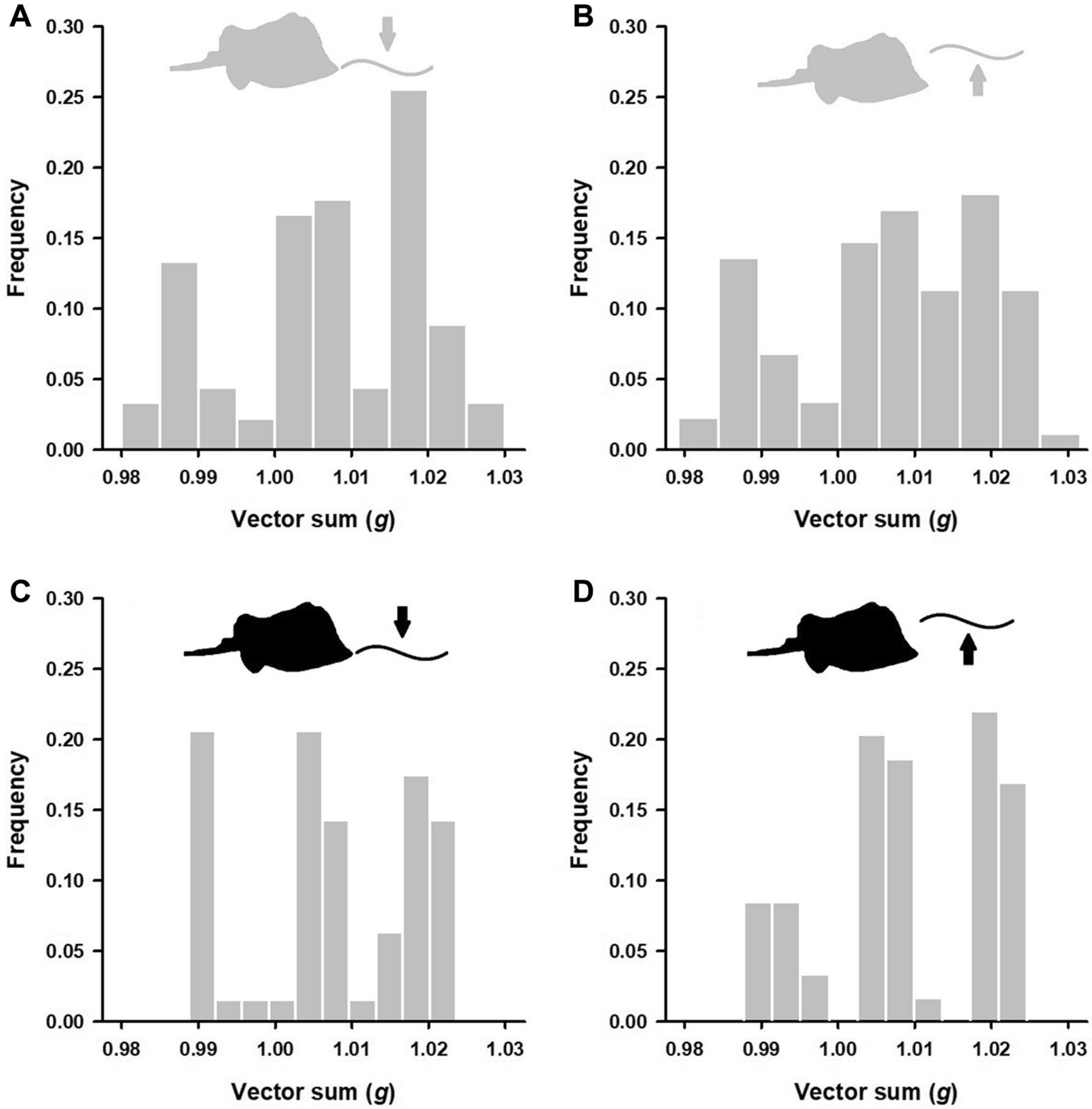

Fig. 4 Histograms of vector sum acceleration of five southern stingrays (*Hypanus americanus*) in the wild. Panels are separated by diel period (day = grey stingrays, night = black stingrays) and tide state (flooding tide = upward facing arrow, ebbing tide = downward facing arrow). Acceleration data are presented in g, where $1g = 9.81$ m s^{-2}

Table 1 Linear mixed-effects model output for the effects of tide state and diel period on vector sum acceleration of five southern stingrays (*Hypanus americanus*) in the wild

Response	Parameter	*df*	*F*	*P*
Vector sum	Tide state	1, 292	0.04	0.844
	Diel period	1, 292	9.39	0.002
	Tide state × diel period	1, 292	0.03	0.858

do not suggest that stingrays in the Exuma Cays modulate activity with the tides. Our data contrast Tilley's (2011) findings where rate of movement in juvenile southern stingrays was found to be significantly higher at low slack tide. As mesobenthic predators, foraging and predator avoidance could be driving factors underlying observed diel activity patterns. Although individuals in the present study were captured in shallow, near-shore habitats, it is unclear whether rays used tidal flats during the observation period. Establishing animal location concurrently with acceleration data during observation would further elucidate environmental drivers of activity. Given the agreement between our results and similar studies of southern stingrays, we are confident in the ability of accelerometers mounted using the methods described herein to generate ecologically meaningful data.

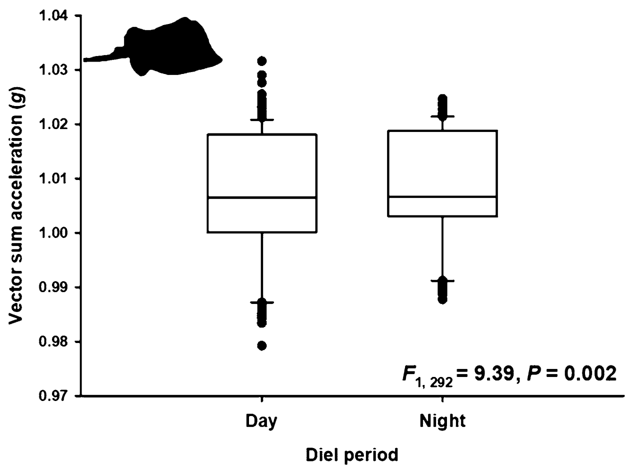

Fig. 5 Vector sum acceleration of five southern stingrays (*Hypanus americanus*) during day and night in the wild. Acceleration data are presented in *g*, where $1g = 9.81\ \mathrm{m\ s^{-2}}$

This study presents a novel and minimally invasive attachment method for bio-logging devices and is the first successful attempt at using accelerometers to determine activity in southern stingrays. Future studies should tag captive and wild individuals to validate specific behaviours against acceleration data so that behaviour can be inferred in situ. In addition, telemetry devices used in conjunction with accelerometers would be useful for defining relationships between environmental conditions (e.g., temperature), physiological performance (e.g., field metabolic rate), and behaviours (e.g., foraging, reproduction). These data will assist in identifying important habitats for stingray, leading to more informed and robust conservation measures for batoid species.

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Compliance with ethical standards

Data availability statement The datasets during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest All authors declare they have no conflict of interest.

Ethics statement Research was conducted under permits MAF/FIS/17 and MAF/FIS/13, issued by the Bahamian Department of Marine Resources. Animal care protocols were based on guidelines from the Association for the Study of Animal Behaviour and the Animal Behaviour Society (Rollin and Kessel 1998).

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