

**Discovering behaviours in captive Port Jackson sharks
(*Heterodontus portusjacksoni*) using accelerometers**

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Declaration

I declare that this thesis, as a whole or in parts, has not been submitted for a higher degree to any other university or institution.

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All other research described in this report is my own original work.

JULIANNA KADAR

12 November 2016

GENERAL ABSTRACT

Uncovering how animals utilise their time in the wild is vital to understanding their ecology. Continuous observation in a marine environment is not feasible, but telemetry devices provide an opportunity for fine-scale analysis of movement and behaviour. We used accelerometers to investigate activity patterns and behaviours of the Port Jackson shark (*Heterodontus portusjacksoni*) with the aim of building a model from captive sharks that can be applied to wild populations. We developed techniques to identify behaviours from two types of accelerometers - high and low resolution. To validate the accelerometer data, we identified shark behaviours from multi-angle, high definition video that was time-matched to both types of accelerometer data. The low-resolution accelerometer (Vemco V13AP) was used to identify diel patterns and migratory restlessness in captive sharks. Wild shark diel patterns and presence data were used as a comparative measure to validate findings. The high-resolution accelerometer (Cefas G6a+) tested the application of a classification tree method in classifying four behaviours: resting, swimming, vertical swimming against the wall of the enclosure and chewing on prey. Through evaluating the ability of two types of accelerometers to identify captive shark behaviour, we are validating methods for long-term (low resolution - looking for trends) and short-term (high resolution- detailed behaviours) observation of wild shark populations.

GENERAL INTRODUCTION

Due to the difficulty of observing species within the marine environment there is a lack of detailed information about many marine predators with respect to their behaviour and ecology (Wilson and McMahon, 2006). Time-intensive techniques such as direct observation, through SCUBA diving and snorkelling have been used since the beginning of marine research. Since then, more advanced means have been developed like remote operated underwater vehicles (ROVs) or baited remote underwater video (BRUVs). Direct observation methods offer an understanding of animal movements that contribute to the basis of informed research studies. However, direct observation techniques can be limited by observer bias and the large spatial scales marine predators traverse (Brown et al., 2013). Bio-loggers afford the opportunity to follow animals without direct observation. The ability to see in greater detail what marine species are doing gives us insight into migration patterns, foraging habits, and reproductive strategies among other important processes (Wilson, 2013, Brownscombe et al., 2014, Fossette et al., 2012). Deeper understanding of these habits help us understand the ecological role of a given species and can play a significant role in management.

Sharks play important ecological roles as apex predators and have become the focus of many accelerometry studies aimed to gain insight into shark behaviour and movement. In order to record behaviours, accelerometers quantify animal movement on three orthogonal axes that represent both body orientation, known as static acceleration, and change in velocity over time, known as dynamic acceleration. Static acceleration is recorded through the accelerometer's orientation in relation to the gravitational pull of the earth (Shepard et al., 2008a) while dynamic acceleration represents locomotion along each axis (Wilson et al., 2006). Accelerometers can be deployed with a suite of other sensors that include magnetometer, gyroscope, temperature, depth and conductivity, creating a detailed picture of the environment and an animal's reactions to factors within it (Whitney et al., 2012, Wilson et al., 2008). Accelerometry data from sharks facilitates insights into energetics, movement ecology and the wider fields of behaviour and movement ecology (e.g.

Gleiss et al., 2011a, Bouyoucos et al., 2017, Whitney et al., 2007). In behavioural observations, for example, the frequency and amplitude of accelerometer measurements are usually consistent and when separated into distinct classes, they represent activity patterns on a broader scale and the signatures for each behaviour observed on a finer scale (Shepard et al., 2008c). Parametrisation of behaviours as identified via accelerometry data in captivity can subsequently be used for *in situ* studies (Campbell et al., 2013).

In part due to their difficulty to observe, shark species have been the subject of several studies using tri-axial accelerometry that have aimed to find out more about shark movement patterns. Diel patterns investigated in whitetip reef sharks (*Triaenodon obesus*) have shown a nocturnal rhythm (Whitney et al., 2007) while study of whale shark (*Rhincodon typus*) diel vertical migration shows a crepuscular pattern in habitat selection (Gleiss et al., 2013). Relatively finer-scale behaviours have also been quantified to examine the composition of sub-second behaviours in sharks. Quantifications of behaviours such as swimming and diving as a foraging strategy (Nakamura et al., 2011), feeding (Jorgensen et al., 2015), mating (Whitney et al., 2010) and sociality (Wilson et al., 2015) continue to offer insight into the fine-scale difference in behaviours. Additionally, quantifications of these fine-scale behaviours inform research into activity (Murchie et al., 2011) and energy budgets (Gleiss et al., 2011b) as well as habitat use (Sims et al., 2005).

Thesis Rationale

Port Jackson sharks (*Heterodontus portusjacksoni*) have been widely studied, research of their migratory movements, ecology and behaviour form a strong knowledge base for this species (Bass et al., 2016, Byrnes and Brown, 2016, Powter and Gladstone, 2008). However, detailed views of their activity patterns and fine-scale behaviours have not been investigated. Without these more detailed views into frequently-occurring behaviours, we will lack understanding of fine-scale movement patterns and behaviours occurring along movement trajectories for this species. In order to examine movement patterns and behaviours this thesis employs

two types of accelerometers within a captive environment. The findings contribute to Port Jackson shark movement patterns on both a broad and fine scale. Additionally, they provide a further basis for elucidating movement patterns within wild Port Jackson shark populations.

Chapter 1 investigates diel activity patterns and migratory restlessness in Port Jackson sharks using root mean square acceleration extracted from low-resolution accelerometers (Vemco V13AP). The validation of the findings is achieved by comparison with wild shark behaviour obtained from acoustic telemetry.

Chapter 2 examines fine-scale behaviours of the Port Jackson shark using classification trees as a method to distinguish four distinct behaviours based on the output of high-resolution accelerometers (Cefas G6a+) sampling at 10Hz. I presented tri-axial acceleration characteristics and summary features that held the most relative importance for classification of four key behaviours (swimming, resting, chewing and vertical swimming). A combination of random forest and classification tree analysis were used to establish what features are the most important in the accelerometer output to distinguish between behaviours.

Sharks are fundamental to marine ecosystems and our understanding of them has increased dramatically over the last decades. In order to move toward an increasingly detailed understanding of shark movements, fine-scale behaviours that are exhibited along movement trajectories must be examined. Examination of these quickly-occurring behaviours requires the development of methods that can be applied to wild individuals and thereby can offer insight into the dynamics of wild Port Jackson shark populations. A lack of detailed information regarding the movements of this species leaves us with a gap in understanding of the requirements and ecological impacts of a highly prevalent shark species. At present, habitat use and foraging behaviour during both migration and breeding season are unknown. Additionally, these methods provide a template for accelerometry studies of other species that aim to examine fine-scale behaviours.

Note to reviewers: This thesis is submitted as a thesis by publication and formatted for *The Journal of Experimental Biology* with the exception that line numbers have been removed, margins adjusted, tables and figures are embedded in the text, references are combined for both chapters and supplementary materials for both chapters are listed together in the appendix

Chapter 1

Accelerometry reveals diel activity patterns in Port Jackson sharks, *Heterodontus portusjacksoni*

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SUMMARY

Distinguishing the factors that influence movement variation within a species advances understanding of their behaviour and ecology. Acoustic accelerometers were used to investigate variations in activity patterns of male and female Port Jackson sharks (*Heterodontus portusjacksoni*) early and late in their breeding season. HD video was used to assign behaviour to root mean square (RMS) acceleration recorded by accelerometers. In order to quantify diel patterns of the sharks, we used linear regression to relate RMS acceleration output to the previously time-matched behaviours on video. To validate captive data, diel patterns from captive sharks were compared with diel movement data from free-ranging sharks. We then used linear mixed effects models to determine factors most influencing activity levels. Captive sharks showed nocturnal diel patterns, peaking in activity during the late evening before midnight and decreasing in activity before sunrise. Free-ranging sharks displayed comparable diel patterns showing captive studies can be used to understand behaviour in the wild. By directly relating RMS acoustic acceleration output to activity we show the diel, seasonal and sex-specific movement variation within this species and the corresponding activity levels of sharks in captivity.

INTRODUCTION

Activity patterns are a key component of animal fitness shaped by endogenous and exogenous factors that dictate behaviours and determine movement patterns within species. Animals display movement patterns in response to endogenous circadian rhythm (Nelson and Johnson, 1970), to biotic factors such as the risks and benefits of finding prey and avoiding predators (Iwasa, 1982, Neilson and Perry, 1990) and to abiotic environmental factors such as temperature (Sims et al., 2006, Andrews et al., 2009) and light intensity (Bohl, 1979, Clark and Levy, 1988, Appenzeller and Leggett, 1995, Nelson et al., 1997). Many activity patterns in the marine and terrestrial environments are dictated by light intensity. The dawn chorus of birds is a prime terrestrial example (McNamara et al., 1987). In the marine realm, the steepest vertical movement patterns of a megamouth shark (*Megachasma pelagios*), for example, also correspond most closely to changing light levels (Nelson et al., 1997).

Similarly, bigeye thresher sharks (*Alopias superciliosus*) choose to hunt prey in periods of the day when silhouettes are most visible to their upward-facing eyes (Nakano et al., 2003). Nocturnal and diurnal activity patterns can be subject to high plasticity even though circadian systems are reasonably robust (Reebs, 2002). A number of factors may cause activity patterns to stray from their normal daily patterns, such as reproductive behaviour. Female dogfish (*Scyliorhinus canicula*) seasonal movements, for example, are both spatially and temporally separated from males as a means for females to conserve energy during the costly time of parturition (Sims et al., 2001).

Continuing advances in technology have resulted in a golden age for bio-logging extending the limits of ecological research examining animal activity patterns (Wilmers et al., 2015). Bio-logging has been widely applied to elasmobranchs because of the challenges involved with observing them in the marine environment (Hussey et al., 2015). Tri-axial accelerometers in particular have answered questions in regard to shark physiological efficiency (Whitney et al., 2012), energetics (Gleiss et al., 2010), foraging habits (Nakamura et al., 2011), vertical migrations (Sims et al., 2006), swimming efficiency (Payne et al., 2016), and sociality (Wilson et al., 2015). Earlier studies on marine animal movement ecology used visual observations and radio technology, however, these methods are not applicable to species that remain submerged for long periods of time or hardly ever surface (Brown et al., 2013). Although radio telemetry can show a movement trajectory in some species, the small datasets they create do not provide sufficient insight into what the animal does along their path (Rutz and Hays, 2009). Accelerometers have been included as part of sensor packages to overcome this problem and allow finer-scale documentation of movements along an animal's trajectory, even when the subject is completely out of sight (Wilmers et al., 2015, Brown et al., 2013). It is now possible to identify important behaviour patterns such as discriminating between periods of rest and activity (Whitney et al., 2007), identifying crepuscular fluctuations (Gleiss et al., 2013), and identifying times of peak activity (Gleiss et al., 2013). In addition to these insights, accelerometers can quantify fine-scale movements such as tailbeat

frequency (Gleiss et al., 2013, Watanabe et al., 2012), feeding events (Jorgensen et al., 2015) and mating behaviour (Whitney et al., 2010).

Captive studies have become the standard when attempting to apply accelerometer values to movement patterns and observed behaviours (Whitney et al., 2007, Viviant et al., 2010, Mooney et al., 2015, Graf et al., 2015, Houghton et al., 2009). They allow close observation of the animal and thereby enable the calibration of accelerometer data with behaviour. As part of a long-term study on horseshoe crab rhythms, for example, captive trials determined that the threshold for movement in relation to root mean square (RMS) acceleration was $>0.1 \text{ m s}^{-2}$ (Watson et al., 2016).

Observing squid in captivity allowed for classification of finning, jetting, direction reversal and turning behaviours within tri-axial accelerometry (Mooney et al., 2015). These studies are key to interpreting the output of accelerometers once they are deployed in the field.

Sharks are key components of marine ecosystems and through advances in technology the movements of sharks can be examined in increasingly precise detail. There is a clear need to examine the movement patterns of small, temperate elasmobranchs more-closely since they represent the majority of shark diversity (Chapman et al., 2015). This is particularly the case for common species that likely play important roles in shaping their surrounding ecosystem. Port Jackson sharks, for example, are known to prey on echinoderms which play key roles as ecosystem engineers on rocky reefs (Harrold and Reed, 1985). Port Jackson sharks are often the subject of research owing to the ease of studying them, and their movement patterns have been examined for some time using traditional approaches (McLaughlin and O'Gower, 1971, O'Gower and Nash, 1978, O'Gower, 1995, Powter and Gladstone, 2008). A primarily nocturnal species (O'Gower, 1995), the Port Jackson shark has recently been found to migrate great distances and display sex-specific migration patterns (Bass et al., 2016). This species is an ideal model for accelerometry owing to the fact that as a benthic, non-obligate ventilator it is not difficult to differentiate between phases of activity (i.e. swimming and resting). For other pelagic species, swimming and resting become more difficult to define and

separate. Gaining a better understanding of Port Jackson shark activity patterns, in particular the variation between sexes and seasons, is crucial to understanding their behaviour and fitness profiles in a changing wild environment.

Here we tested the viability of an energy efficient accelerometer utilising a novel attachment method to examine the relationship between RMS acceleration and activity patterns in the Port Jackson shark. Specifically, we aimed to: (1) assess if RMS acceleration (10Hz) could accurately relay activity patterns, (2) examine sex, time of day and time of year as factors potentially influencing Port Jackson shark activity patterns and (3) compare the accelerometer activity pattern data from the captive study with activity patterns of free ranging sharks.

MATERIALS AND METHODS

Captive experiments were conducted with eight adult Port Jackson sharks at Taronga Zoo in Sydney, Australia ($-33^{\circ}50'N$, $151^{\circ}14'E$) from July-October in 2015 and 2016. Sharks were captured from Balmoral ($33^{\circ}49'S$, $151^{\circ}15'E$) or Fairlight Beach ($33^{\circ}48'S$, $151^{\circ}16'E$) in Sydney Harbour and transported to an open-roof enclosure at Taronga Zoo. The enclosure measured 17.8 x 8 m with a depth of 3.3 m and received constant water flow that was mechanically filtered. To replicate caves that the sharks are found naturally inhabiting, three hides were placed on the floor of the enclosure. All individuals were measured for total length (TL) and weighed upon arrival. Sharks were allowed to acclimate to the enclosure for one week prior to experiments. During experimentation, two sharks were tagged at a time and allowed to swim freely in the enclosure. Temperature and water quality parameters were measured daily. The sharks were fed daily at 15:00 with squid, crab or mussels ad libitum.

Three female and five male sharks participated in the experiments (Table 1). In two cases, data were obtained for less than 24 hours and were therefore excluded from the final analysis (ID 4763 and ID 4769), thus data was obtained for six sharks. This study was carried out under permits from Macquarie University ethics committee

(ARA-2014/015) and Taronga ethics committee (3a/06/15). Wild sharks were captured under NSW DPI Fisheries Scientific Collection Permit P08/0010-4.2.

Table 1. Shark characteristics and deployment details for 8 accelerometer deployments. Two sharks were excluded from the study as a result of < 24 h of data collection. TL: total length, EB: early breeding season, LB: late breeding season.

Individual	Sex	Length (TL) (cm)	Attachment method	Attachment duration (days)	Month/Season	Year
5539	M	102	dorsal	13.38 d	Sep – Oct (LB)	2015
5537	F	114	dorsal	17.83 d	Oct – Nov (LB)	2015
5540	F	117	dorsal	27.83 d	Sep – Oct (LB)	2015
4768	F	121	harness	2.96 d	Aug (EB)	2016
4764	M	99	harness	5.75 d	Aug (EB)	2016
4751	M	104	harness	5.75 d	Aug (EB)	2016
4769	M	97	harness	(15 hours)-excluded	Aug (EB)	2016
4763	M	95	harness	(18 hours)-excluded	Aug (EB)	2016

Accelerometer attachment

Tri-axial accelerometers measuring at 5Hz (Vemco V13AP; Vemco, Nova Scotia, Canada) were attached to eight individual sharks using two methods; – dorsal spinal needle (dorsal) and harness. For the dorsal attachment method, two stainless steel 14-gauge Sureflo IV surgical catheters (Terumo, Tokyo, Japan) were used to puncture the anterior end of the first dorsal spine. Suture thread, Prolene size 1 (Ethicon, New Jersey, USA), was then threaded through the catheters and used to secure the accelerometer against the skin. The alternative method used a commercially available small-animal harness (Petbarn, Sydney, Australia) that was adapted to fit around the body of the sharks, over the first dorsal fin and behind the pectoral fins. The accelerometer was attached to the harness in close proximity to the dorsal spine. The accelerometers transmitted data acoustically to a receiver (VR2W 69kHz) within the enclosure.

Sampling

The accelerometers were pre-programmed to measure activity 8.3% of the time. These low-resolution settings were chosen to mimic long-term deployment methods. These methods are applicable in a wild setting where low resolution measurement at 5Hz has been used for measurement of general activity levels (Watson et al., 2016). Every ~120 seconds the three axes were measured for 20 seconds and then converted into a RMS value that summarized movement for the previous 20 seconds of measurement. The axes were converted using the following equation:

$$m\ s^{-2} = \frac{\sqrt{(x^2+y^2+z^2)}}{T},$$

where x, y and z represent the axes and T represents time. During the first year of experimentation in 2015 multiple sharks were already fitted with high powered acoustic tags (Vemco, V16) prior to adding the accelerometers. Due to the small size and concrete walls of the enclosure this resulted in an overload of acoustic pings, which caused the 69 kHz receiver to record 40-43% of pings. Accelerometer data was received at an average of ~300 seconds instead of ~150 seconds. This reduction in recordings did not affect overall analysis of activity patterns as recordings were still relatively evenly spaced over a large number of days (14-29 d). In the second year of experiments, only two acoustic accelerometers were deployed in the enclosure at any one time resulting in 100% receiver recording rate (Table 2).

Table 2. Number of detections for each shark within each year of experimentation. In 2015, frequency of accelerometer recordings was reduced due to acoustic collisions and receiver overload within the shark enclosure. This resulted in a doubling of the average delay and fewer mean pings per hour each day.

Shark ID	Year of trial	Attachment method	Average delay (seconds)	Days recorded	Total pings	Mean pings per hour/day
5539	2015	dorsal	~300 s	13.38 d	1846	5.75
5537	2015	dorsal	~300 s	17.83 d	3514	8.21
5540	2015	dorsal	~300 s	27.83 d	3770	5.64
4768	2016	harness	~150 s	2.96 d	957	13.47
4764	2016	harness	~150 s	5.75 d	1804	13.07
4751	2016	harness	~150 s	5.75 d	1812	13.13

Video analysis

In order to obtain as much consecutive video footage as possible, video cameras (GoPro Hero4, California, USA) were submerged in three locations in the enclosure to record movements of the tagged sharks during daylight hours. 22 ~1-hour (h) videos (recorded at random times of the day from sunrise to sunset) were edited and exported at 50 fps using Final Cut Pro Software (Apple, California, USA). The behaviour of the sharks was scored minute by minute. If a shark was swimming for more than 10 seconds during the minute interval it received a score of 1 and if it was resting it received a 0 (as per Watson et al., 2016). The scoring methodology was applicable because once a period of activity was initiated, the shark usually remained swimming for longer than 10 seconds. If the subject animal swam out of view of the cameras during a minute interval, the data was excluded from analysis. In order to calibrate the accelerometers, the video footage of the sharks was time-matched with the accelerometry data. In this way the accelerometer data could be converted to the proportion of time spent active based on the correlation with observed behaviour. For each individual we then calculated the mean proportion of time spent active for each hour over the 24 h diel cycle.

Calculating proportion of time active

At the end of the experiments the acoustic receiver was removed from the enclosure and raw data was downloaded using VUE software (Vemco, Nova Scotia, Canada). Using this software the raw acceleration values were converted to m s^{-2} and the time was adjusted to local Sydney time (UTC+10). For each individual, the mean was taken for each 1-h block of acceleration data that corresponded to the behaviour-coded video footage. Binning the data into 1-h blocks was necessary because the sampling occurred at irregular intervals. 1-h blocks created a window long enough for multiple transmissions to be detected while still depicting a detailed picture of activity throughout the day. The activity scores and acceleration data were combined in a simple linear regression using R (Version 0.99.902; R Development Core Team, 2016). The output from the regression enabled us to directly interpret the accelerometer data.

Generating the linear mixed effects model

The 24 1-h bins of mean accelerometer values minimised serial autocorrelation within the activity data. The bins were transformed for normality using Box-Cox power transform (MASS package in R; Venables and Ripley, 2002). Multiple linear mixed-effects models (LME) with restricted maximum likelihood (REML) estimated the sources of variation that accounted for changes in activity levels (NLME package in R; Pinheiro et al., 2014).

An information theoretic approach was applied to build candidate models. Candidate models consisted of individual shark ID and hour of the day as random effects to account for non-independence of the data. Predictor variables included: sex, time of day: day/night, and time of year: early breeding season/late breeding season. Sex was included as variable due to previous research indicating differences in male and female movement patterns in the Port Jackson shark (Bass et al., 2016). Within the 1-h bins, time of day was determined according to local times of sunrise and sunset. The fixed effects including interactions between sex * time of year, time of year * time of day, and sex * time of day were included in candidate models. The R function dredge from package MuMIn was used to run all combinations of

variables (Barton and Barton, 2013). Models were ranked using (AICc) (Appendix Table 1A) (Sugiura, 1978). The lowest AICc was used to select the best fit model. Ability of random effects to strengthen the model was determined by the comparison of the final model to a null model in which random effects were excluded. Difference between the final and null models was determined by parametric bootstrap analysis applying exact likelihood ratio tests (RLRsim Package in R; Scheipl et al., 2008).

To explain the amount of variance in activity patterns caused by the random effects of individual shark/hour of the day, marginal (fixed effect) and conditional (fixed and random effects) R^2 values were compared. Assumptions were tested by plotting predicted vs fitted residuals, QQ-plots, Cleveland dot-plots and ACF plots to examine homoscedasticity, normality, homogeneity and independence (Zuur and Ieno, 2016). To examine significant differences in mean active time between shark activity and the predictor variables, we used post-hoc general linear hypothesis applying the Tukey method with the function `glht` from the `multcomp` package (Hothorn et al., 2008). Analysis was completed in R (Version 0.99.902; R Core Development Team, 2016) and R Studio (Version 3.2.1; RStudio Team, 2016). Values are reported as means ($\pm$ SD) and significance was set at alpha 0.05.

Data from the dorsal attachment method suggested that activity patterns were disturbed by the application of accelerometers for the first 24 h, and were not significantly changed in the second cycle of 24 h. Thus for this attachment method we excluded the first 24 h. For harness attachment, there was no notable change in the first 24 h compared to background activity post-tagging, therefore no data was excluded.

Wild activity patterns

Omni-directional submersible ultrasonic receivers (miniSUR, Sonotronics Inc, Tuscan, Arizona, USA) are fixed acoustic receivers that record the identity of tagged individuals with acoustic transmitters (Bass et al., 2016). These receivers had a detection range set to shift every 5 minutes between 18 dB and 36 dB

(corresponding to ~10 m and 60 m respectively). The receivers were able to record data sent from acoustic transmitters (Vemco V16, Halifax, Nova Scotia, Canada, 69 kHz, 90 s transmission delay) implanted into 39 Port Jackson sharks (Bass et al., 2016). Eleven miniSURs (Sonotronics) were deployed in August-September 2016 on a breeding reef (Orion) in Jervis Bay, New South Wales (Appendix Fig. 1A). For this analysis, we restricted the recorded data to those recorded within a 10 m radius of the 11 receivers. Diel activity patterns were calculated based on the number of hourly movements between receivers. For each shark, we counted every movement in and out of any receiver and determined the frequency of movements for each hour and each shark. The frequency of movement was then entered as a variable in a model.

The frequency of movement model was constructed with the same approach as the captive activity model. Data was transformed ($\log(x) + 2$) for normalisation. LME with REML estimated the factors influencing variation that dictated the frequency of wild shark movements ($n = 12$) (NLME package in R; Pinheiro et al., 2014). Random effects included: shark ID and hour of the day, and fixed effects included: sex, time of day, and sex * time of day interaction.

Consistent with the captive activity model, the random effects ability to add strength to the model was determined through comparing the final model with a null model that had no random effects. Parametric bootstrap analysis was used once more to determine the difference between the null and final models (5000 simulations, Package in R: RLRsim; Scheipl et al., 2008). Examination of R^2 values determined the variance within the wild shark movements which were caused by random effects. Assumptions were tested using the same methods as the captive model.

RESULTS

Predicting proportion of time active

Low resolution accelerometers allowed for the prediction of amount of time each shark ($n = 6$) spent active per hour of the day ($R^2 = 92.1$) (Fig. 1). Video recording paired with accelerometry show three distinct levels of activity; 1) Low level activity: the majority of time spent resting with occasional movement ($16.97 \pm 13.23\%$ of time active), 2) intermediate level activity: swimming on the floor or foraging ($56.67 \pm 2.10\%$), and 3) high activity: swimming in the water column or vertically against the enclosure wall ($99 \pm 1.32\%$).

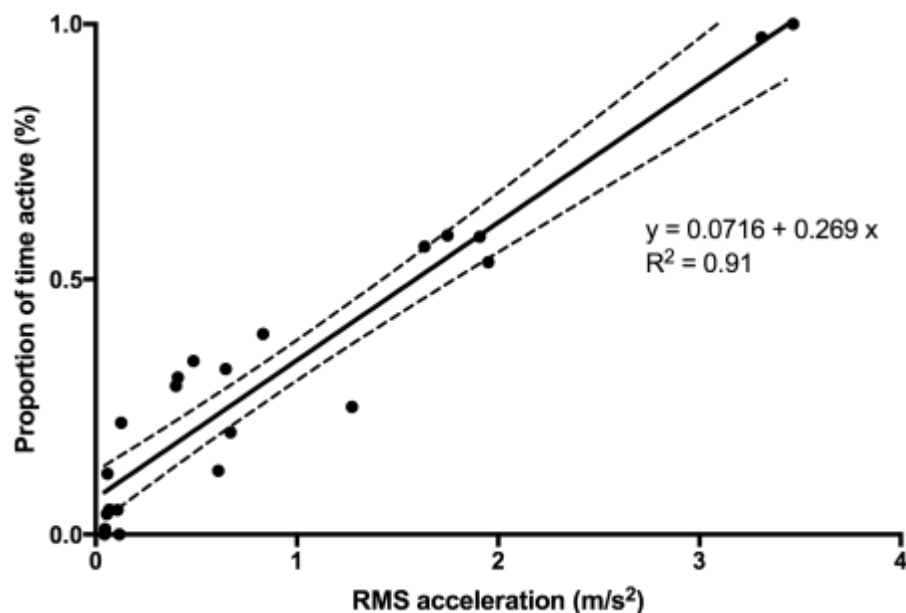


Fig. 1. Simple linear regression comparing proportion of time sharks were active and RMS acceleration output.

Activity ranged from 0.085 to 0.787 proportion of time active per hour. Overall the sharks spent an average of 0.24 ± 0.118 of their time active, either swimming on the enclosure floor, in the water column, foraging or vertical swimming. The sharks spent 74% of their time within the low activity range (0 - 0.17), 10% within intermediate activity range (0.18-0.57), and 16% within the high activity range (0.58-

1.0). Sharks in late breeding season spent 8% more time engaged in high activity behaviours (Fig. 2).

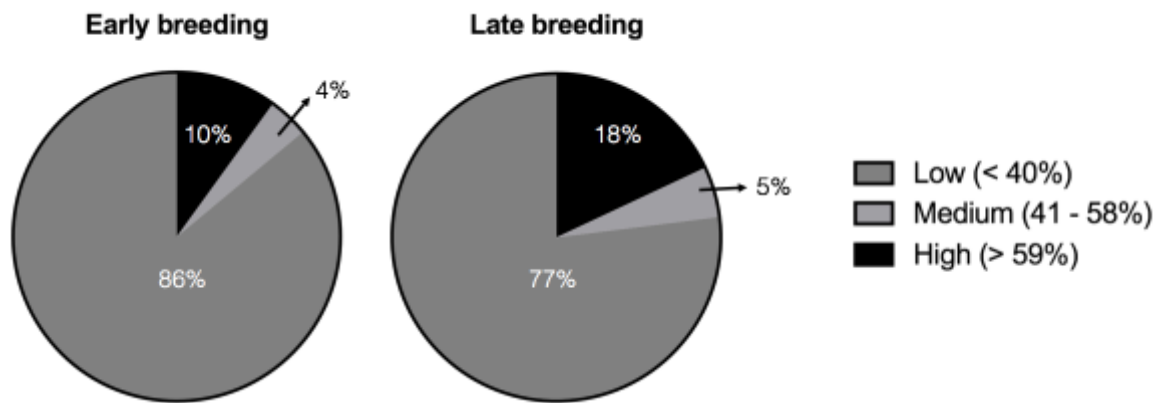


Fig. 2. Proportion of time spent at three levels of activity (%) for sharks in early and late breeding season shown as parts of a whole. Low activity is 0 – 17% proportion of time active, medium activity is 18 – 57% proportion of time active and high activity is 58 – 100% proportion of time active.

The diel pattern of the sharks in captivity consisted of low level activity in the morning with a secondary peak in activity from 15:00 to 19:00 (daily feeding occurred at 15:00), a peak in activity from 18:00 to 22:00 and a decline of overall nocturnal activity from 00:00 to 04:00 (Fig. 3). The lowest activity levels for all sharks ranged from 04:00 to 17:00 (i.e. early morning and daylight hours).

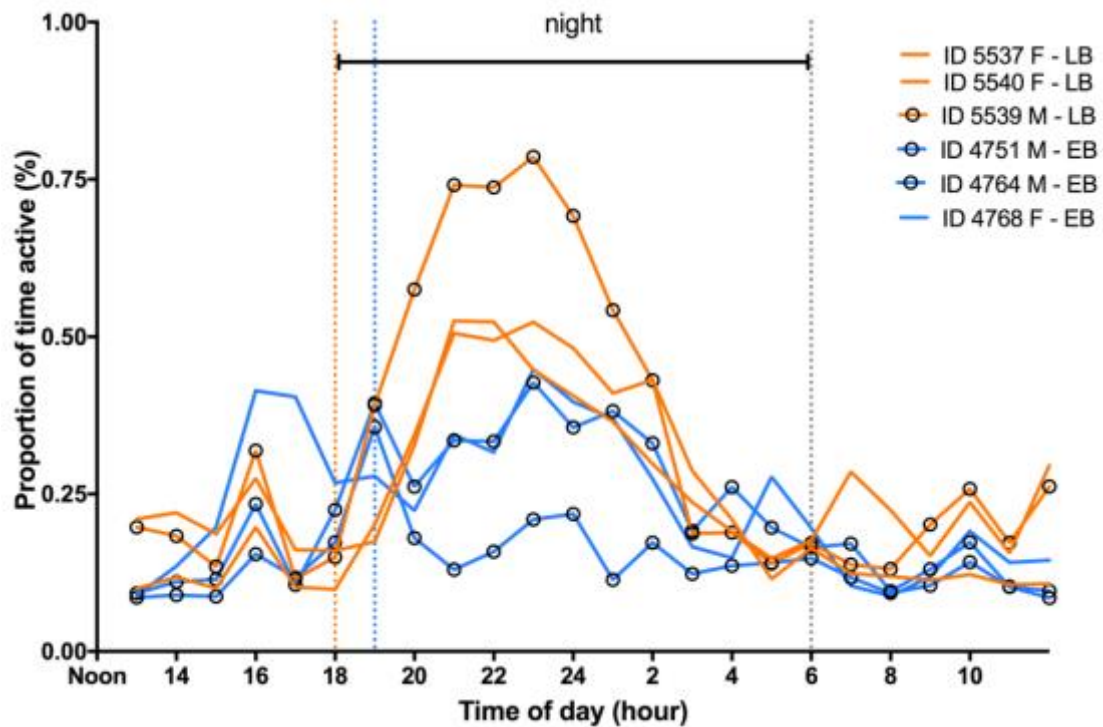


Fig 3. Mean diel level of activity of each individual in captivity (n = 6). Group early in the breeding season in blue and late in the breeding season in orange. Lines with open circle pattern indicate males and without open circle pattern indicate females. Dashed lines indicate sunset and sunrise, two lines for sunset indicate shifting sunset times over early and late season. M: male, F: female, EB: early breeding season, LB: late breeding season.

Factors influencing activity in captivity

The best fit activity model consisted of the fixed effects: time of day (day/night), sex, time of year (early/late breeding season), and sex * time of year interaction.

Individual ID and hour of the day were included as random effects (AIC 292.56, BIC 318.97, logLik -137.28).

Table 3. Best fit model summary shows the relationship between RMS acceleration and explanatory factors. SE: standard error, *: interaction.

Factors	Intercept	SE	t value	P value
Sex	-0.43	0.14	3.18	0.0019
Time of day	1.05	0.17	6.00	< 0.0001
Time of year	0.12	0.14	0.87	0.3874
Sex * time of year	0.74	0.19	3.87	0.0002

We found a significant relationship between RMS acceleration and time of day ($P < 0.0001$; Table 3). The sex of the sharks also had a significant effect on acceleration ($P = 0.0019$; Table 3). There was no effect of time of year ($P = 0.387$; Table 3), however the interaction between sex and time of year was significant ($P = 0.0002$; Table 3).

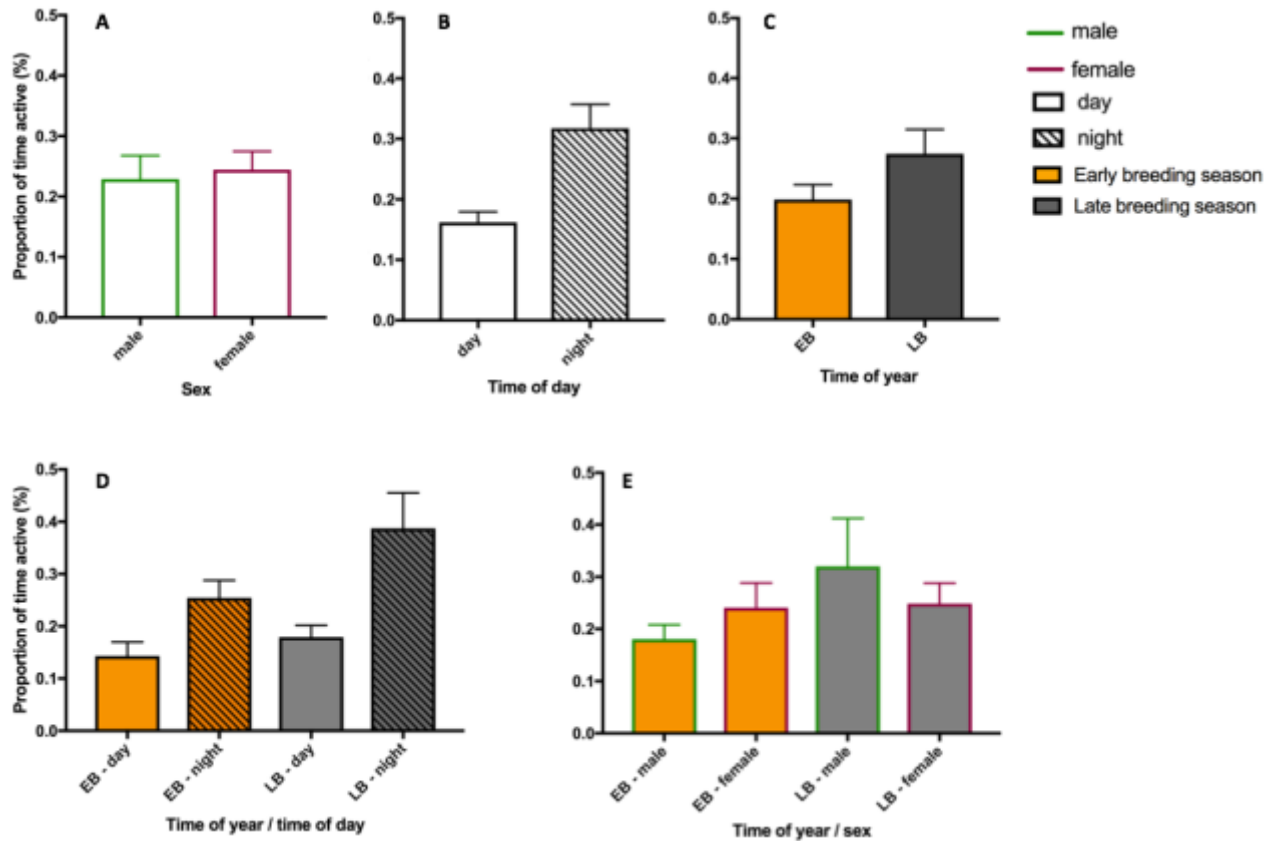


Fig. 4. Mean (SE) proportion of time Port Jackson sharks spent active in captivity. A) males and females, B) day and night, C) early (EB) and late (LB) breeding season, D) day and night within early and late breeding season, E) males and females within early and late breeding season.

The strong interaction between sex and time of year was primarily driven by male behaviour in October which displayed the highest level of activity. The mean peak hour activity for this male was 0.786 ± 0.313 (ID 5539) while the next nearest individual's peak in activity was 0.523 ± 0.417 (ID 5540), both at 22:00. The most active male's (ID 5537) mean time spent active was 0.327 ± 0.221 which was substantially higher than the group mean (0.240 ± 0.118).

Further post-hoc analysis of the interaction effect (sex * time of year) showed a significant difference between males and females in early breeding season ($P = 0.0099$), and a strong contrast in activity levels between males in early versus late breeding season ($P = < 0.001$). No significant difference was detected between females in early and late breeding season ($P = 0.822$) (Appendix Table 2A).

Parametric bootstrap analysis showed that including individual ID as a random effect improved model strength significantly (LRT= 11.092, $P = < 0.001$), suggesting that individuals have unique activity patterns. Including hour of day also added greatly to model strength (LRT = 19.807, $P = < 0.001$), which is generated by the strong nocturnal activity pattern (Fig. 3). Comparisons of marginal and conditional R^2 values for the final activity model (activity ~ time of day + time of year + sex + (time of year * sex)) showed high reliance on random effects within the model. Fixed and random effects explained 72.7% (marginal R^2) of the variance in activity and random effects alone accounted for close to half of that variance (conditional $R^2 = 0.332$).

Wild activity patterns

We found that the activity in wild sharks, as measured by the frequency of movements within a network of short range receivers covering their home reef, mirrored activity patterns measured on captive sharks using accelerometers (Fig. 5). The activity model for wild sharks consisted of the fixed effects: time of day (day/night), sex, and the sex * time of day interaction (AIC -1813.06, BIC -1784.57, logLik 914.53). As per the captive model, Individual ID and hour of the day were included as random effects. The effect of time of day (day or night) on wild activity patterns was significant ($P = 0.0044$, Table 4), while both sex and the interaction sex * time of day were not found to be significant (sex: $P = 0.4367$; sex * time of day: $P = 0.237$; Table 4).

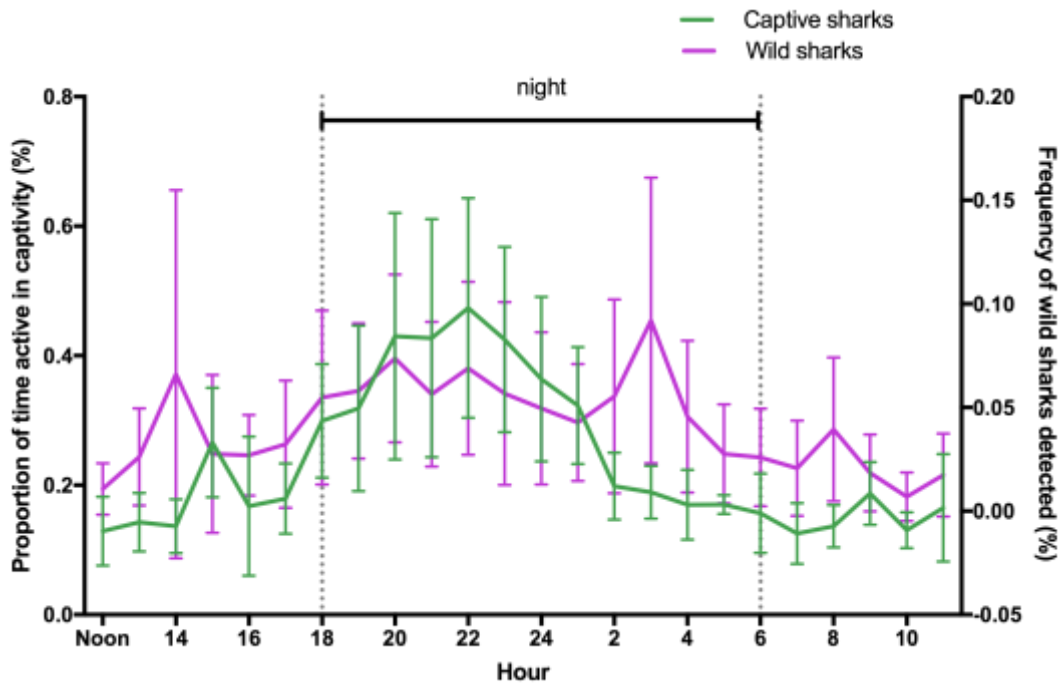


Fig. 5. Mean diel level of activity of captive (green) and wild (purple) sharks ($\pm$ SD). Dashed lines indicate sunset and sunrise.

Parametric bootstrap analysis showed that while hour of the day improved model strength significantly (LRT = 4.496, $P = 0.058$), individual ID was not significant (LRT = 1.3304, $P = 0.3108$) suggesting most animals in the wild had similar activity patterns. Both the captive and wild shark activity models were improved by hour of the day, highlighting the strength of the nocturnal pattern. Comparing marginal and conditional R^2 for the wild activity model (activity $\sim$ time of day + sex + (time of day * sex)) showed that 49.1% (marginal R^2) of the variance could be explained. Random effects composed $\sim 15\%$ of that variance (conditional $R^2 = .1469$).

Table 4. Model illustrates the dependence between frequency of detection in wild sharks and factors. (* = interaction)

Factors	Intercept	SE	t value	P value
Sex	-0.001	0.001	-0.78	0.4367
Time of day	0.006	0.001	3.17	0.0044
Sex * Time of day	0.002	0.002	1.18	0.237

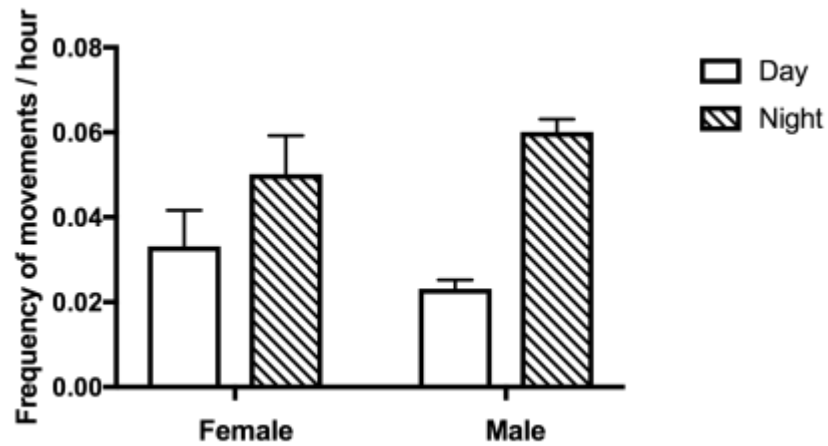


Fig 6. Mean (SE) frequency of movements per hour for male and females Port Jackson sharks during the day and at night on a breeding reef in the wild from August to early October.

We also found that the overall increase in activity patterns in captive sharks between the two periods corresponded to a drop in the proportion of sharks detected by the network of receivers (Fig. 7), suggesting that increase in activity was linked to the beginning of migration.

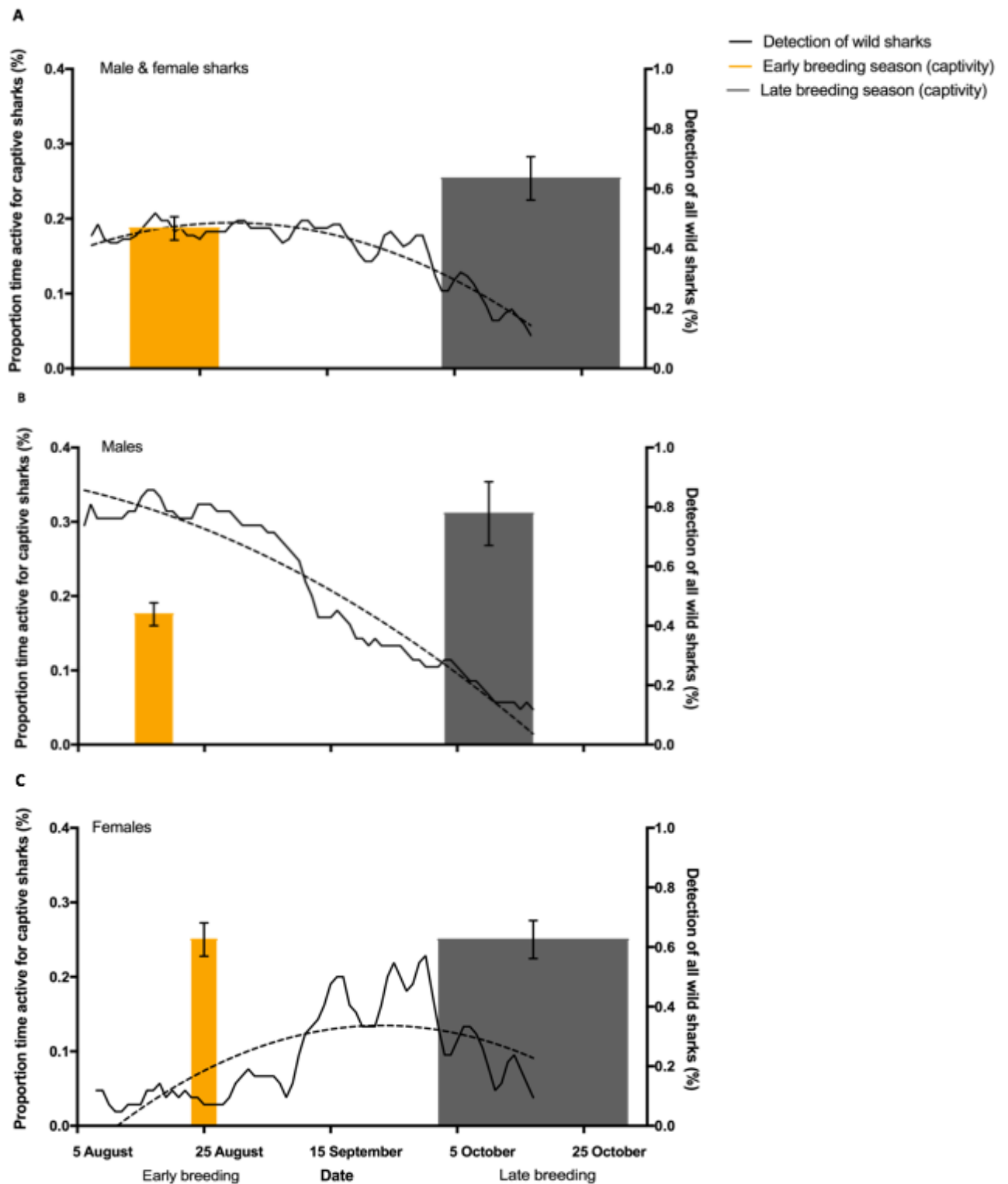


Fig. 7. A comparison between detection of wild sharks (dashed line shown as polynomial best fit for moving average of raw detection) and captive shark activity levels (orange: early breeding season, grey: late breeding season). Male and female sharks are combined (A), then males (B) and females (C) separately.

DISCUSSION

Findings from this study show that acoustic accelerometers can be applied to benthic elasmobranchs and return accurate information regarding their movement patterns. All Port Jackson sharks exhibited nocturnal activity patterns, peaking during late-evening hours and exhibited the lowest activity levels during early morning and daylight hours (Fig. 3). There was little difference in activity during daytime for both early and late breeding season, however the trend was towards an increase in activity at night in late breeding season. Although time of year alone was not found to influence activity, captive males in the late breeding season showed considerably higher activity levels as an effect of migratory restlessness. The activity results obtained by accelerometers in the captive setting corresponded closely with those obtained by acoustic telemetry in the wild (Fig. 5) suggesting the behaviour exhibited in captivity mirrored that in the wild.

Port Jackson shark activity rose in the hours just before and during sunset and declined before sunrise. Although artificial feeding times during daylight influenced natural activity in captive sharks, the majority of their activity took place during hours of darkness. This corroborates information from previous studies where juvenile Port Jackson sharks spent the least amount of time active during the day and large amounts of time active during the night, but more time was spent inactive than active overall (Powter and Gladstone, 2009). Nelson and Johnson (1970) found similar patterns in horn sharks (*Heterodontus francisci*), which remained inactive during most of the day, residing in caves. Recent studies have found increased movement during crepuscular periods than at night for most elasmobranchs, along with increased foraging during dark hours (Hammerschlag et al., 2016). Port Jackson sharks may show greater preference for nocturnal activity than other elasmobranchs due to their strongly nocturnally geared visual ecology (Schieber et al., 2012, Hart et al., 2006). This characteristic aides in the occupation of sensory niches made possible through visual adaptations for darkness (Hueter, 1990).

While diel patterns for this species occurred nocturnally the majority of the time, they displayed shifting activity patterns depending on early and late periods within

their breeding season. Sharks observed in October, late in the breeding season, spent more time active and engaged in high activity level behaviours than sharks early in the breeding season. Overall, males and females in October spent 18% of their time engaged in high activity behaviours such as vertical swimming and swimming in the water column, while the group earlier in the year spent 10% engaged in the same levels of activity. These times of year reflect differences in environmental cues such as temperature and length of day that might influence movement patterns, though some environmental influences can occur alongside endogenous rhythms (Takemura et al., 2010). Differences in activity between early and late breeding season is most likely due to increasing migratory pressure occurring in late breeding season, just before the scheduled southern migration from the breeding grounds (Bass et al., 2016).

High levels of activity, especially by males, late in the breeding season is likely due to migratory restlessness. Migratory restlessness exhibits itself as increased activity in captive individuals during the time of departure for migration and has been known to predict departure times in wild individuals (Eikenaar et al., 2014). This type of behaviour has been extensively studied in birds and provides insight into the mechanisms that may be behind elevated activity patterns at this time (Liedvogel et al., 2011). Birds that migrate over long distances compared to middle-distance migrators show greater levels of migration restlessness in controlled settings (Berthold, 1973). Moreover, species that follow different migratory routes display different restlessness patterns in captivity and the resulting restlessness appears to be most influenced by individual genetic factors (Liedvogel et al., 2011). Observation of Port Jackson shark migration patterns showed that males leave breeding grounds sooner than females to migrate south (Bass et al., 2016). It is expected therefore, that males should show signs of migration restlessness earlier than females. Females typically lay two eggs every two weeks and may stay within breeding sites longer than males to reduce egg mortality (Bass et al., 2016). The females' departure from breeding sites in particular has been correlated with rising sea-surface temperatures (Pini-Fitzsimmons et al., unpublished data). In the captive setting, both water temperature and day length could provide reliable cues to initiate migration. In

salmon, for example, day length is a key cue for initiating migration and spawning (Liedvogel et al., 2011). Environmental and endogenous cues triggering restlessness may serve to optimize energetic costs. For example, movements made to avoid higher temperatures have been shown to play a role in mitigating energetic costs for male dogfish (*Scyliorhinus canicula*) (Sims et al., 2006). Furthermore, the activity patterns observed here suggest that migration movements are most likely to occur at night. When in captivity, these migration pressures still exhibited themselves in the form of higher levels of activity and behaviours like vertical swimming, particularly against the southern wall of the enclosure. To our knowledge, this study presents the first evidence of migratory restlessness in fish species.

Further variability in movement is suggested by elasmobranchs that are known to have less predictable seasonal movements (Papastamatiou and Lowe, 2012). For example, there is evidence that Port Jackson sharks are partial migrators, with individuals following different routes while a small number do not migrate at all (Bass et al., 2016). Individual strategies that exist within a population may evolve to lessen intraspecific competition (Papastamatiou and Lowe, 2012) or may simply reflect consistent individual variation in behaviour (Byrnes and Brown, 2016). Quantification of captive migratory restlessness in non-migratory sharks would expect to see little or no rise in activity within those individuals late into breeding season. In addition, there is evidence that some Port Jackson sharks may use the East Australian current to facilitate their southern journey (Bass et al., 2016). That variation in northerly versus southerly activity patterns should be detectable using these methods. Thus there is potential to use acoustic accelerometers to identify instances where environmental flow affects migrating sharks (Hays et al., 2016). Ultimately, these methods can be applied to quantify the energetic costs and benefits of migration.

In summary, the use of accelerometers deployed on Port Jackson sharks in captivity showed high correlations between observed behaviour and acceleration data. These sharks were found to be nocturnal with sex-specific seasonal activity patterns. The captive activity data matched the behaviour observed in free-ranging sharks as

determined by acoustic telemetry. Our results showed that increasing activity patterns in captivity corresponded to the timing of seasonal migration of Port Jacksons along the New South Wales coast. Future work should consider deploying these tags on wild sharks to examine their activity patterns in more detail. We suggest further study on free-ranging sharks to collect information over a longer temporal scale particularly in regards to seasonal and sex-specific movements, the variations within them and routes taken during migration.

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AUTHOR CONTRIBUTIONS

All authors were involved with the design of the study and drafting of the journal article. J.K. and J.M. carried out the data collection from the animals and all authors contributed to analysis and editing.

COMPETING INTERESTS

No competing interests declared.

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Chapter 2

Tri-axial accelerometers classify fine-scale behaviours in the Port Jackson shark (*Heterodontus portusjacksoni*)

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SUMMARY

Movement ecology has traditionally focused on the course movements of animals over large time scales, but with advancements in technology the focus can become increasingly fine-scale, examining specific behaviours and environmental factors that influence them. Currently, habitat use and foraging habits of the Port Jackson shark (*Heterodontus portusjacksoni*) are largely unknown. In order to gain understanding of their role in surrounding ecosystems, an ethogram developed in conjunction with modern movement technology would inform habitat use on a fine scale. We used fine-scale tri-axial accelerometers (10Hz) to quantify four behaviours (resting, swimming, swimming vertically, and chewing) in Port Jackson sharks. To validate the accelerometer data we identified shark behaviours in captivity from multi-angle, high definition video that was time matched to the accelerometry output. We calculated summary statistics from the tri-axial acceleration data to find signature features that allowed for differentiation between the four behaviours. Two testing sets were created: one and two second epochs consisting of 10 and 20 samples from each behaviour event, respectively. Predictor variables from the x, y, and z raw axes were assessed for relative importance and standard deviation was identified as the most important characteristic. The decision tree with a longer time epoch (2 seconds) classified the four behaviours more accurately (87.82%) than the 1-second epoch (82.13%). The interpretable framework of the classification tree allowed for identification of the signature features responsible for separation of the four behaviour classes. Thus we were able to show that tri-axial accelerometers are an effective tool for classifying fine-scale Port Jackson shark behaviour. The decision guidelines developed here can be applied to classify behaviour in free-ranging sharks that are unable to be observed directly.

INTRODUCTION

Understanding the fine-scale behaviour of sharks is important for understanding habitat selection and use over a range of spatio-temporal scales. Examining these behaviours closely is difficult, owing to the aquatic environment and cryptic nature of benthic shark species that rarely surface. Advances in biotelemetry and biologging

have started to elucidate the behaviour of many species throughout the marine environment, extending the limits of observation and deepening our understanding of previously unobserved animals (Wilmers et al., 2015). Acoustic telemetry, for example, has been widely applied to examine the broad-scale movements of marine animals, but does not have the capability to examine important fine-scale behaviour patterns such as foraging or mating. All types of fine-scale movements are difficult to observe and quantify in wild marine animals, nevertheless, they have great applied significance. The movements an animal makes and the associated fine scale behaviour are determinant of individual fitness, but may also influence the structure and functions of communities and populations (Turchin, 1998, Swingland and Greenwood, 1983). By understanding fine-scale movements we can build a picture of how each movement contributes to behaviour and ultimately survival. Biologging is starting to fill in this knowledge gap. The development of increasingly efficient storage capacity and batteries has enabled the miniaturization of biologging technology and made analysis of movement on a fine-scale possible (Brown et al., 2013). A growing problem, however, is that these techniques result in the collection of huge data sets that require novel analytical approaches. These large data sets have necessitated the application of statistical algorithms to differentiate and classify behaviours according to their individual characteristics or “signatures”.

Analysis of fine-scale accelerometry data is still in its infancy and there have been a number of approaches developed. In some cases, features based on a single variable are used (Watanabe et al., 2005), while in others a number of characteristics describing behaviour signatures are used to tell them apart (Laich et al., 2008). The application of these summary features has been completed using both computationally heavy methods (i.e. random forests (Wang et al., 2015); artificial neural networks (Nathan et al., 2012); hidden Markov models (Leos-Barajas et al., 2016); stochastic gradient boosting (Ladds et al., 2016: in press)) and light methods (i.e. k-means clustering (Sakamoto et al., 2009); classification trees (Nishizawa et al., 2013)). Computationally light approaches have potential for on-board tag analysis that can compress raw acceleration into behavioural classifications in real-time (McClune 2014). Classification trees in particular balance classification and

computational power and are useful in identifying important descriptive variables that represent behavioural kinematics (Nathan et al., 2012).

A number of fine-scale movements have been quantified in sharks resulting in a diverse collection of behaviours: resting and resting in a surge zone (Whitney et al., 2010) as well as swimming, and temporally fine-scale swimming events such as burst swimming (Watanabe et al., 2012) and chafing (Bullock et al., 2015). Within the swimming class of behaviour, the quantification of tailbeat frequency has allowed for comparison of activity levels (Watanabe et al., 2012, Gleiss et al., 2010). Foraging strategies have been observed in the form of surface-feeding (Gleiss et al., 2013) and yo-yo diving (Nakamura et al., 2011). Social behaviours in the lemon shark (*Negaprion brevirostris*) (Wilson et al., 2015) and mating in nurse sharks (*Ginglymostoma cirratum*) have also been quantified (Whitney et al., 2010) from acceleration data, providing insight into the social and reproductive dynamics that govern conspecific interactions. Though, because movement observations alone are not sufficient to link them to ecological processes, information regarding the surrounding environment (e.g. temperature) can be analysed in unison for a more complete picture (Nathan et al., 2012). These observations are bridging the understanding between shark behaviour, ecology and temporal and spatial population dynamics and producing data that can directly contribute to conservation and fisheries management.

Port Jackson sharks are a benthic species endemic to Australia with comparable fine-scale movements to many other shark species. They play an ecologically important role within benthic ecosystems, feeding on a broad variety of prey. Juveniles typically feed on benthic invertebrates like echinoderms, polychaetes, molluscs and crustaceans and adults tend to feed at a higher trophic level on teleosts and cephalopods (Last and Stevens, 2009, Powter et al., 2010). Port Jackson sharks are non-obligate ram ventilators, making them an ideal model compared to actively swimming sharks to separate behaviours within accelerometry, especially fine-scale feeding events. Along the east coast of NSW, Port Jackson sharks aggregate in coastal embayments during the winter breeding season (Bass et al., 2016), however,

it is unknown whether foraging occurs during this period. Their feeding habits are also unknown during their southern migration around October each year. This information could help us understand larger scale movement patterns and the foraging habits within them.

In addition to being an important part of wild ecosystems, Port Jackson sharks are also commonly kept in captivity. Behavioural effects of captivity on this migratory species are periodically externalised as increased levels of activity and vertical swimming against upright surfaces in captive environments that are less frequently found in wild habitats (Chapter 1). Vertical swimming has been previously observed during swimming when obstacle avoidance is necessary and during foraging when egg predation occurs (J.P. Kadar unpublished observations). Though this behaviour occurs more frequently in captivity, its occurrence reflects an internal cue which is reflective of wild shark movements. In addition, vertical and near vertical posture of the shark occurs on a fine temporal scale and is reflective of a high activity level (e.g. costly) behaviour. Quantification of migratory restlessness within captivity would inform the effects of captivity upon migrating shark species and have considerable welfare implications.

In this study, we assessed the classification accuracy of a decision tree algorithm to identify tri-axial acceleration data collected from captive Port Jackson sharks. We aimed to classify four behaviours: resting, swimming, vertical swimming, and chewing. Two of these occurred at a fine temporal scale (vertical swimming and chewing) and two occurred at a relatively broader temporal scale (resting and swimming). Alongside the classification process, we aimed to determine whether a 1 or 2 second sampling epoch generated a higher overall accuracy. We identified the most important predictor variable characteristics for classification of behaviours for both epochs of time. In addition, we aimed to identify the specific predictor variables responsible for separating behaviour classes within the most appropriate epoch.

MATERIALS AND METHODS

Captive experiments were conducted with four adult female Port Jackson sharks (Appendix, Table 1A) at Taronga Zoo in Sydney, Australia ($-33^{\circ}50'N$, $151^{\circ}14'E$) from October-November in 2015. Sharks were captured from Balmoral ($33^{\circ}49'S$, $151^{\circ}15'E$) or Fairlight Beach ($33^{\circ}48'S$, $151^{\circ}16'E$), Sydney Harbour and transported to an open-roof enclosure at Taronga Zoo. All individuals were measured for total length (TL) and weighed upon arrival. The enclosure measured 17.8 x 8 m with a depth of 3.3 m and received constant water flow sourced from Sydney Harbour that was mechanically filtered. Temperature and water quality within the enclosure was measured daily. The group of sharks was fed daily at 15:00 with squid, crab or mussels ad libitum. To replicate caves that the sharks are found naturally inhabiting, three hides were placed on the floor of the enclosure. Sharks were allowed to acclimate to the enclosure for one week prior to experiments.

This study was carried out under permits from Macquarie University ethics committee (ARA-2014/015) and Taronga ethics committee (3a/06/15). Wild sharks were captured under NSW DPI Fisheries Scientific Collection Permit P08/0010-4.2.

Accelerometers

Accelerometers were attached to the first dorsal fin at two points to prevent instability and shifting. Two stainless steel 14-gauge Sureflo IV surgical catheters (Terumo, Tokyo, Japan) were used to puncture the anterior end of the first dorsal spine. Suture thread, Prolene size 1 (Ethicon, New Jersey, USA), was then threaded through the catheters and used to secure the accelerometer against the skin.

During experimentation, two sharks were tagged with accelerometers at a time and allowed to swim freely in the enclosure. We used a tri-axial accelerometer (CEFAS G6a+: 40mm x 28 mm x 16.3 mm and weigh 18 g in air and 4.3 g in seawater, CEFAS technology Ltd, Lowestoft, UK) to measure the movement of the sharks on three axes: x-axis, y-axis and z-axis (Fig. 1). Accelerometers were set to record at +2g and record 10 samples per second (10Hz). Data were recorded at random blocks of time

during daylight with a minimum recording time of 2 hours and maximum of 6 hours. The accelerometers remained on the animal for 10 days.

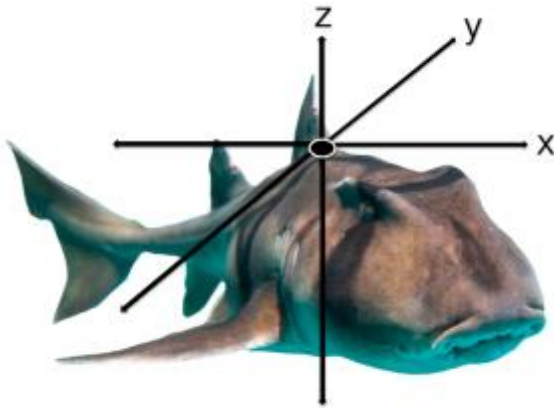


Fig. 1. Representation of x, y and z axes reflective of accelerometer attachment on each Port Jackson shark.

Video observations

Video cameras (GoPro Hero4, California, USA) were submerged in three locations in the enclosure to record movements of the tagged sharks during daylight hours for 10 days. Videos were scored according to an ethogram (Appendix Table 1A) developed from the video footage of four unique behaviours; resting, swimming (in the water column or on the floor), vertical swimming (against a vertical of the enclosure) and chewing on prey. The videos were coded continuously, however, periodically the sharks swam out of camera; as a result those sections were labelled as 'out of camera' within the behaviour coding and excluded from the final dataset. Following behaviour coding, videos and acceleration output were matched to create annotated datasets.

Data analysis

Following removal of the accelerometers from the sharks, data from the trials were downloaded using G5 Host software (version 6.7.0, CEFAS technology Ltd, Lowestoft, UK). Framework 4 was used to visualise the acceleration signatures for the four behaviours (Framework 4, Swansea UK). In order to avoid abnormal behaviour

resulting from catch and handle effects, the first 24 h of data were excluded from analysis.

1 s and 2 s epochs of time were chosen to classify each of the four behaviour classes. These epochs were chosen due to the fact that two behaviours (vertical swimming and chewing) took place over relatively finer temporal scales (i.e. ~1-3 seconds). The 2 s epoch resulted in 20 samples for each behaviour event and the 1 s epoch resulted in 10 samples per event. These different time scales resulted in a different number of behaviours and samples for each epoch (Table 2).

To begin analysis, static and dynamic acceleration were calculated from the raw acceleration data. To obtain static acceleration, raw axes were smoothed by applying a moving average across five samples due to the relatively small body size of the sharks and the partial focus on classification of finer-scale behaviours that occur within power strokes (Shepard et al., 2008a). Dynamic acceleration was calculated by subtracting static acceleration from raw acceleration (Wilson et al., 2006). The four characteristics calculated were the mean, standard deviation (SD), minimum and maximum for the features: x-axis, y-axis, z-axis, overall dynamic body acceleration (ODBA), movement variation, energy, pitch, and roll (Table 1). This resulted in 32 summary features to potentially describe each behaviour.

Table 1. Equations used to calculate five of the features compared for relative importance.

Feature	Equation
ODBA	$ X_i + Y_i + Z_i $
Movement variation	$ X_{i+1} - X_i + Y_{i+1} - Y_i + Z_{i+1} - Z_i $
Energy	$(X_i^2 + Y_i^2 + Z_i^2)^2$
Pitch	$\tan^{-1} \left(-\frac{X_i}{\sqrt{Y_i^2 + Z_i^2}} \right) \times 180 / \pi$
Roll	$\text{atan2}(Y_i, Z_i) \times 180 / \pi$

The R package ‘randomForest’ was used to select the most important features relative to one another (Liaw and Wiener, 2002, Alvarenga et al., 2016). Higher

values of mean decrease in accuracy suggested features that are most important to behaviour classification. The relative importance may vary between separate models created for time epochs, however, this selection can be used as an over-arching guide to feature importance (Alvarenga et al., 2016). For each epoch, the most important features were selected based on a combination of the relative Gini coefficient and information theoretic approach. The model was optimized for classification of the relatively finer-scale behaviours.

The behaviours were classified using classification trees through the rpart library in R (Therneau et al., 2014). This classification algorithm employs recursive partitioning to build a tree with a general structure, first identifying the variable that best splits the data into two groups and then continuing the partitions until no further advancements are possible. The resulting classification trees were re-sampled and pruned using 10-fold cross validation. Classification trees were chosen because of their easily interpretable structure of classification, allowing for the identification of specific features that are used to differentiate between discrete behaviours (Nathan et al., 2012). The trees were assessed for their effectiveness to distinguish between the four behaviours of interest using tests for accuracy, recall, precision and *F* measure as follows; where TP = true positive, TN = true negative, FP = false positive, and FN = false negative:

$$\text{Accuracy} = (\text{TP} + \text{TN}) / (\text{TP} + \text{FP} + \text{TN} + \text{FN})$$

$$\text{Recall} = \text{TP} / (\text{TP} + \text{FN})$$

$$\text{Precision} = \text{TP} / (\text{TP} + \text{FP})$$

$$F\text{-measure} = 2 \times (\text{precision} \times \text{recall}) / (\text{precision} + \text{recall})$$

Kappa values were compared between the epochs to assess influence of random chance upon accuracy (Viera and Garrett, 2005).

RESULTS

Sampling

All acceleration data loggers (*n* = 4) remained attached for ten days before they were manually removed from the dorsal fin of each female shark. In total, 5.6 hours of video were captured and coded for behaviours. The footage equated to 394 and 414

behaviour events captured for the 2s and 1s epochs respectively (Table 2). A total of 4,140 data points were used for classification in the 1s epoch and 7,880 for the 2s epoch. Chewing and vertical swimming behaviours contained the least number of examples owing to their difficulty to capture on video footage (Table 2). Swimming had the most number of examples for both epochs of time (Table 2).

Table 2. The numbers of behaviour events and data points per behaviour event for each of the four behaviour classes within the 1 and 2 second epochs.

	Epoch			
	1 second		2 second	
Behaviour	Behaviour events	Data points	Behaviour events	Data points
Resting	121	1210	113	2260
Swimming	181	1810	183	3660
Vertical swimming	41	410	41	820
Chewing	71	710	57	1140
Total	414	4140	394	7880

Description of behaviours

Resting behaviour displayed little to no change across the three axes (Fig. 2C) and exhibited the lowest gravity (g) values. Infrequently, resting events showed small peaks in cases of jostling due to group resting behaviour. Chewing behaviour took place over short durations, ($\sim 1-3$ s) and was most distinctly visible over the x and y-axes (Fig. 2D). Compared to resting, the x-axis for chewing was further below the y-axis and exhibited small, quick rhythmic peaks for each chew (Fig. 2D). Swimming events were most variable on the y-axis with an increase in magnitude during the undulation of the body from left to right (Fig. 2E). The clearest 3-axis movement and highest variation in g values occurred for vertical swimming behaviour, during which the tailbeats were more pronounced to move the body in a vertical direction (Fig. 2B).

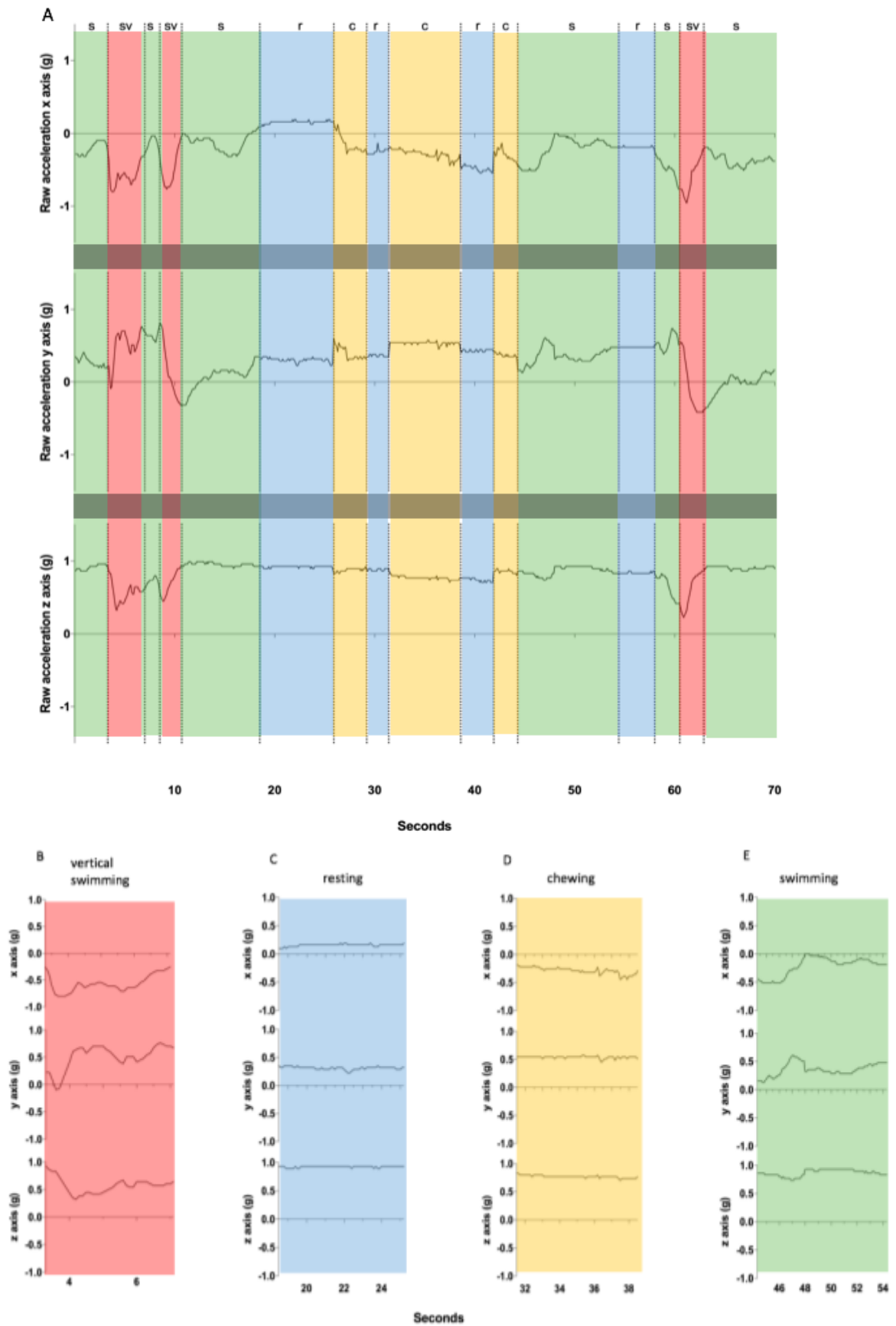


Fig. 2. An example of raw acceleration data over a 70-second time window (shark ID 4765) engaging in each of the four behaviour classes (A). Vertical dashed lines

indicate transitions between behavioural states where r: resting (blue), s: swimming (green), sv: vertical swimming (red), and c: chewing (yellow). B-E represent magnifications of each behaviour from the same 70-second time window (A).

Feature importance

Feature importance for both the 1 s and 2 s epochs suggested SD was the most valuable characteristic (Appendix Fig. 2A-3A). Based upon Gini coefficient, the 2 s epoch suggested the 8 predictor features of most importance were as follows: SD movement variation, SD pitch, SD y-axis, SD x-axis, SD z-axis, SD roll, mean ODBA and SD energy. The 1 s epoch required nine predictor features to perform at a comparable accuracy, with 6 out of 9 predictors the same as the 1 s epoch. Final selection was based upon mean decrease in Gini coefficient and an information theoretic approach that accounts for the visually observed kinematics of chewing. To gear the model toward chewing accuracy, SD energy was replaced by SD ODBA with the understanding that ODBA was the most descriptive feature for the separation of chewing and all other classes.

Model classification

The classification tree demonstrated the best results for resting for the 1 s epoch (90.91%; Table 3) and vertical swimming for the 2 s epoch (95.12%; Table 3). The 2 s epoch resulted in a higher rate of classification for all individual behaviours. Resting remained comparably well classified in the 1 s epoch (1 s: 90.91% / 2 s: 91.53%; Table 3), however the remaining three behaviours reached higher levels of classification in the 2 s epoch classification. Chewing was most often misclassified overall and often classified as swimming or resting. By increasing the epoch length from 1 to 2 s, chewing was misclassified as resting at a considerably lower rate (1 s: 14%, 2 s: 2%; Table 3). Misclassification for chewing as swimming remained consistent in both epochs (misclassified in 1 s: 32%, misclassified in 2 s: 30%; Table 3).

Table 3. Confusion matrix for both 1 s and 2 s epoch lengths including the four behaviour classes: resting, swimming, vertical swimming and chewing. Total refers to the number of behaviour events within each behaviour class. The numbers in bold show correct classification instances.

Observed behaviour (%)	Predicted behaviour (%)			
	Resting	Swimming	Vertical swimming	chewing
1 s Epoch				
Resting	90.91	4.13	0	4.96
Swimming	6.63	87.85	0	5.52
Vertical swimming	0	2.44	87.8	9.76
Chewing	14.08	32.39	1.4	52.11
2 s Epoch				
Resting	91.53	5.1	0	3.39
Swimming	2.37	92.9	0	4.73
Vertical swimming	0	0	95.12	4.88
Chewing	1.52	30.3	3.03	65.15

The decision tree for the 2 s epoch classified behaviours at the highest accuracy (87.82%). Kappa values confirmed the better success rate of the longer time epoch in classification of the four classes.

Table 4. Accuracy, Kappa value, precision recall and *F* measure for each behaviour class within the 1 and 2 second epochs.

Behaviour	Accuracy (%)	Kappa	Precision	Recall	<i>F</i> – Measure
<i>1 s Epoch</i>	82.61	.74			
Resting			.83	.91	.87
Swimming			.85	.88	.86
Vertical swimming			.97	.88	.92
Chewing			.65	.52	.58
<i>2 s Epoch</i>	87.82	.82			
Resting			.96	.92	.94
Swimming			.86	.93	.89
Vertical swimming			.95	.95	.95
Chewing			.75	.65	.70

Vertical swimming returned the highest recall rate for the 2 s epoch and overall (.95; Table 4). Recall for chewing behaviour was most significantly improved by increasing the epoch length to 2 s. Resting behaviour events had the highest precision for the 2 s epoch. Vertical swimming demonstrated a higher precision for the 1 s epoch and was the only behaviour class this was true for (1 s epoch precision: .97, 2 s epoch precision: .95; Table 4).

Behaviour separation

Through the visualisation of the classification tree, observations could be made regarding specific features that separate behaviour classes (Table 5, Fig. 3). SD y-axis performed the first separation decision between swimming and resting (Table 5, Fig. 3). In order to differentiate between swimming and vertical swimming as well as resting and chewing, a greater SD pitch indicated transition to the shorter temporal scale behaviours (vertical swimming and chewing) (Table 5, Fig. 3). Separation between swimming and chewing was accomplished through the use of SD ODBA, mean ODBA, and SD x-axis, indicating that ODBA and the x-axis are key features to the kinematics of chewing (Table 5, Fig. 3). The decision tree also had to differentiate between swimming behaviours that belonged to the same class. In other words, because of a variation in swimming behaviour events, multiple branches of the tree

were used to differentiate them (Fig. 3). Variation within the swimming classes was observed as swimming on the floor versus swimming in the water column, and swimming at different speeds. The decision tree identified SD y-axis and mean ODBA as variation between swimming behaviours in the same class (Table 5, Fig. 3).

The 1 s epoch used different features to separate all branches of the tree, except for vertical swimming which maintained SD pitch as the separation feature from swimming (Appendix Fig. 4A).

Table 5. Features used for separation of behaviour classes at each branch of the classification tree (2 s epoch).

Behaviour class decision	Separation features
Swimming / resting	SD y-axis
Swimming / vertical swimming	SD pitch
Swimming / chewing	SD ODBA, mean ODBA, SD x-axis
Resting / chewing	SD pitch
Swimming / swimming	SD y-axis, mean ODBA

DISCUSSION

We can use accelerometry data analysed through the use of classification trees to clearly differentiate between four important behaviours in Port Jackson sharks with a high degree of confidence. The application of the classification tree method returned a high level of accuracy (87.82%) when distinguishing the four behaviour classes using the 2 second epoch of time. Application of classification trees using tri-axial animal accelerometry have returned comparable accuracies achieved in previous studies (85.95%, Nathan et al., 2012; 92.5%, Alvarenga et al., 2016; 86.21%, Nishizawa et al., 2013). The four behaviours classified represent parts of an activity budget that are associated with feeding and migration.

Classification of behaviour was affected by the chosen epoch length and there was a clear separation in higher classification success and accuracy for the 2 s epoch, which is consistent with other findings using comparable methods (Alvarenga et al., 2016, Robert et al., 2009). In the 2 s epoch, swimming and resting (behaviours occurring at relatively coarser scale) were classified with a high accuracy (swimming: 89%; resting: 94%; Table 4), whereas behaviours occurring at a relatively finer scale differed in their classification success based on their characteristics. Chewing behaviour signatures varied in subtle rhythmic peaks within the x-axis and were most often misclassified with swimming behaviour thereby reducing classification accuracy (70%; Table 5). Vertical swimming, the other temporally fine-scale behaviour, had the most distinct variation across all three axes and was classified with the highest accuracy across both epochs (1 s epoch: .92; 2 s epoch: .95; Table 5). The application of random forest feature importance analysis revealed SD was the most important characteristic applied to predictor variables across both epochs of time. SD has been established as an important characteristic for classification by previous studies (Rekvik, 2016, Nishizawa et al., 2013, Wang et al., 2015, Martiskainen et al., 2009). Whereas the mean of total acceleration has been shown to be representative of static acceleration, SD was shown to be a good measure for dynamic acceleration (Soltis et al., 2016). SD accounts for the oscillating nature of the acceleration signals in that it creates a truer representation of the waveforms

around 0 g within a behaviour signature. Identification of specific features responsible for separating behaviours within the decision tree gave a finer understanding of behaviour kinematics than less transparent analysis methods (e.g. random forest).

The guidelines developed here for separation of behaviour classes within this benthic shark species will be useful for investigation of fine-scale behaviours occurring at both relatively broad (e.g. traveling) and fine (e.g. foraging) temporal scales in this and other similar species. Swimming behaviour was most dynamic on the y-axis, in corroboration with the observation that the y-axis is most important for fish locomotion owing to the undulation of the body from side to side (Shepard et al., 2008b). For this reason, the first decision rule was the separation between resting and swimming based upon the SD of the y-axis. Vertical swimming was differentiated from swimming by the SD pitch feature alone, which has also proven to be an important defining feature in shark mating (Whitney et al., 2010). The upward tilt of the head and body at a $\sim 90^\circ$ angle as the shark meets the vertical surface of the enclosure was the most distinct behaviour signature.

The subtlest differences occurred between swimming and chewing behaviours in which chewing was misclassified as swimming $\sim 30\%$ of the time (Table 4). Crushing during feeding resulted in small ripples in the body and corresponding small movements of the tail along the y-axis. This was quantified as small peaks within the x and y axes for chewing signatures (Fig. 2D), resulting in separation of chewing and swimming behaviours through the application of SD x-axis, SD ODBA and mean ODBA. Misclassifications between the two behaviours most likely resulted from timing discrepancies between acceleration data and validation videos due to the very fine temporal scale of the behaviour (~ 1 -3 seconds) or the relatively small number of chewing behaviour events. Feeding habits of sharks have been explored using a variety of methods in an effort to better understand shark foraging (e.g. Marshall et al., 2007, Heithaus et al., 2002, Bethea et al., 2004, Lucifora et al., 2006, Papastamatiou et al., 2010, Sims and Quayle, 1998). Nelson and Eckert (2007) visually observed distinct behaviours characterized as passive, vertical and active

feeding in whale sharks (*Rhincodon typus*) providing further insight into the complexity of movements behind feeding patterns in this species and those with similar movement patterns. Defining this movement by Port Jackson sharks furthers the understanding of foraging behaviour within this species and allows for the development of more detailed quantification methods as well as the basis for detection of foraging events in free-ranging Port Jackson sharks. In particular, it enables the identification of feeding events during different parts of the migration path, and in combination with acoustic telemetry, can identify the location, and hence microhabitats, that are associated with feeding behaviour.

While Port Jackson sharks have been the subject of multiple studies focusing on their behaviour and ecology in the wild (McLaughlin and O'Gower, 1971, Powter and Gladstone, 2008, Powter and Gladstone, 2009) this species is also studied in captivity as well as kept in aquariums for recreational and educational purposes. Little is known about the effects of captivity upon migratory sharks, however in accordance with other migratory species, it has been shown that sharks display elevated activity patterns in captivity during migration periods (Chapter 1). Elevated activity and physiological response resulting from capture and handling stress is well-documented in shark species (Mandelman and Skomal, 2009, Skomal and Bernal, 2010, Hoffmayer and Parsons, 2001). The long-term behavioural response to confinement is less known and represents a facet of shark behaviour with means for accessible observation (Cliff and Thurman, 1984). In the present study, vertical swimming behaviour was observed and quantified using accelerometers in pre-migratory Port Jackson sharks. The short duration of this behaviour suggests its energetically costly nature, sustained duration of which would result in exhaustion. Application of the quantification of this temporally fine-scale behaviour could be used in combination with other methods to assess fitness in captive sharks and the associated welfare concerns (e.g. frustration).

In addition to captive sharks, these quantifications can be applied to wild sharks and provide the basis for the building of activity and energy budgets in wild populations (Murchie et al., 2011, Gleiss et al., 2011b). Accelerometers are important tools for

the quantification of fine-scale energetics and can be used to determine energy expenditure during different behavioural states such as during the breeding season (Payne et al., 2011). Moreover, these techniques can be applied to investigate the potential effects of different temperatures on energetic expenditure across wide-ranging habitats. Considering that little is known about the spatial foraging habits of the Port Jackson sharks, their consequent ecological effects upon the coastal embayments and migratory routes they use are also largely unknown. In Jervis Bay, for example, tag and recapture estimates suggest that tens-of-thousands of sharks are present during the breeding season (Brown, unpublished data) and must have significant impact on the local ecology. Longer-term tracking is required to ascertain knowledge of migratory foraging habits in this species, requiring long-term biologging solutions. The computationally low power of the decision tree method lends it to on-board tag analysis algorithms that can classify behaviours in real-time, making best use of valuable memory space and battery life within tags deployed long-term on free-ranging animals (Soltis et al., 2016, Spink et al., 2013).

In conclusion, this study represents the basis for quantification of the Port Jackson shark ethogram through tri-axial accelerometry. We found the decision tree was a viable, low-computational power method for classification of four ecologically important behaviours for the Port Jackson shark. The selected 2 second epoch and behaviour classification rules based upon specific features can inform detection of behaviours in wild sharks. In combination with other telemetry and biologging techniques, this approach could provide an important window through which to elucidate the behaviour and ecology of cryptic marine species.

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AUTHOR CONTRIBUTIONS

All authors were involved with the design of the study and drafting of the journal article. J.K. carried out the data collection from the animals and all authors contributed to analysis and editing.

COMPETING INTERESTS

No competing interests declared.

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GENERAL DISCUSSION

The results presented here improve understanding of Port Jackson shark behaviour and ecology. The evaluation of activity patterns and fine-scale behaviours provide further detail of movement within this common benthic shark species. In the first chapter, analysis of factors influencing movement corroborates the findings of previous papers by identifying this species as largely nocturnal (O'Gower, 1995). Additionally, it is shown that time of year considering the sex of the animal, plays an important role in dictating activity within these sharks and that captive shark activity levels are reflective of movement patterns in wild shark populations. Further, we found that these annual movements indicate that captive sharks exhibit a form of migratory restlessness. In the second chapter, high resolution accelerometer data analysed using classification tree methods were able to classify resting, swimming and vertical swimming behaviours with high accuracy and chewing behaviour with moderate accuracy. Ideal feature characteristics, epoch length and summary features lead to the best possible classification of behaviours.

The first chapter adds to knowledge of Port Jackson shark movement patterns and identifies the preference for nocturnal activity within this species as well as the seasonal and sex specific differences. Previous research on horn sharks (Nelson and Johnson, 1970) and dogfish (Sims et al., 2001) has shown that nocturnal rhythms are key to both foraging and reproductive fitness. Although there are knowledge gaps surrounding increased elasmobranch behaviour during crepuscular or nocturnal periods (Hammerschlag et al., 2016), the identification of ecological adaptations for selective activity during times of day could reveal important ecological niches. While we found Port Jackson sharks to be influenced by time of day and time of year, we did not examine environmental cues such as temperature or length of day that play a large role in the offset of migrations for fish species (McCleave, 1978). To deepen understanding of these processes, endogenous rhythms, such as blood parameter and metabolic shifts, can be examined in conjunction with environmental factors (Dye et al., 1986, Miller et al., 2009). Endogenous cues may differ by sex, especially during the reproductive season. For example, over two years, sharks captured within

Sydney harbour for this experiment were almost all male in July (9 males / 1 female, early breeding season) and almost all female in September (9 females / 1 male, mid-late breeding season). Similar biases in population structure occur during the breeding season at Jervis Bay (Bass et al., 2016). Repeating the methods we employed here over the entirety of the breeding season (variable: mid-July – late October) would create a better representation of activity levels over the course of the breeding season. This adjustment would also lend to a more detailed view of the range of migratory restlessness and associated behaviours exhibited in captivity.

After looking at broader scale movement patterns in the first chapter, the fine-scale behaviours there informed aims for the second chapter. For example, using the underwater viewing window at the zoo enclosure greatly increased the quality of visual observations. Although we did not look at a comprehensive ethogram of Port Jackson shark behaviours, the use of certain summary features and characteristics as well as descriptions of behaviour signatures will inform further study and be applicable to wild sharks in assessing their basic kinematics. Nakamura et al. (2011) used time-series analysis such as wavelet transform in combination with a K-means clustering algorithm to predict behaviours such as diving and burst swimming with high accuracy over shorter durations. Analysis methods using comparable algorithms have led to remote observation of behaviours that were previously unidentified in some cases (Nakamura et al., 2011, Payne et al., 2016). In order to look more closely at fine-scale behaviours in the wild, overall accuracy of the method employed here could be increased by taking a few measures. The inability to classify chewing behaviour with an accuracy comparable to the other three behaviours suggests that behaviours which occur on a very fine temporal scale with less distinct signatures require more detailed quantification methods. Employing different summary features such as power spectrum density with a more powerful analysis method, such as random forest, will likely be able to identify the subtle chewing behaviour with higher accuracy. Increasing the sampling rate of accelerometers from 10 Hz to 25 or 50 Hz would likely also lead to higher classification accuracy, but would suffer in terms of the length of time these methods can be deployed. Some of these adjustments would compromise the low-computational power approach of the

method used here, therefore a balance should be found between analysis method and field deployment requirements if the aim is to focus on real-time behaviour classification (McClune et al., 2014, Soltis et al., 2016). These adjustments for future study will provide an important addition to knowledge of this species and inform movements of benthic shark species overall.

Further understanding of Port Jackson shark behaviour is key to the awareness of their role in surrounding ecosystems. This common, benthic species displays movements and behaviours common to other benthic species and simultaneously has a unique life history including seasonal migrations across large spatial scales and annual group aggregations (Bass et al., 2016, Powter and Gladstone, 2008). Thus it is recommended that broad scale trends between seasons and sexes are further examined and fine scale patterns including a comprehensive ethogram of behaviours is developed. Activity levels across an annual cycle would be a valuable contribution to determining the requirements and ecological impact of Port Jackson shark communities and populations. In regard to fine-scale patterns, the development of a comprehensive ethogram could inform detailed use of habitat along broader scale movements, for example, to determine when and where sharks are foraging during their breeding season and along their migration routes. The identification of additional key behaviour signatures such as foraging within substrate, feeding on different prey items, mating and egg-laying are recommended for validation in captivity and application to wild shark populations. Furthermore, as indicated by these studies, impacts of migratory restlessness should be considered in the captive environment and examined for physiological stress response in order to define the effects of captivity upon migrating shark species. Continued research into activity on a broader temporal scale and refinement of tri-axial accelerometer analysis methods will provide invaluable insights into shark behaviour and ecology.

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APPENDIX

Chapter 1

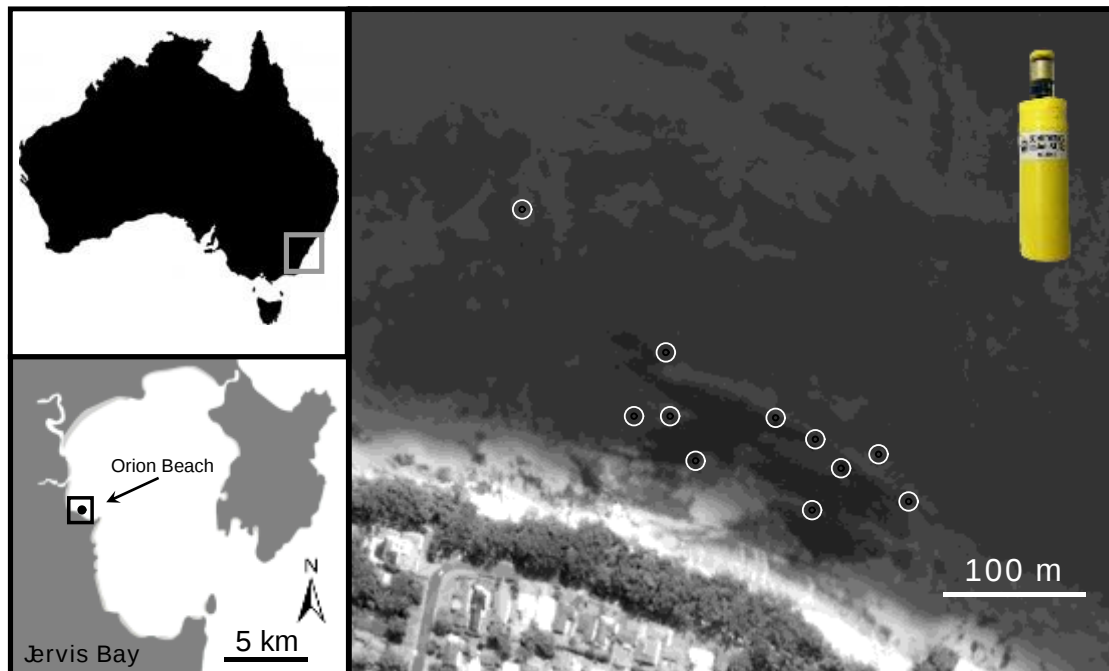


Figure 1A. Map of receivers in Jervis Bay, NSW used to detect wild shark movement frequency and presence.

LME model selection

Table 1A. Ten best fit models based on AICc score. TD: time of day, S: sex, TY: time of year, *: interaction.

Model rank	Intercept	TD	S	TY	S * TY	TD * S	TD * TY	logLik	AICc
1	-1.31	+	+	+	+			-137.28	293.90
2*	-1.60	+		+				-142.46	299.75
3	-1.60	+		+			+	-143.18	303.42
4	-1.56	+	+	+				-143.65	304.36
5	-1.56	+	+	+			+	-144.36	308.06
6	-1.55	+	+	+		+		-144.37	308.07
7	-0.79		+	+	+			-148.89	314.84
8	-1.08			+				-153.85	320.31
9	-1.32	+						-153.86	320.34
10	-1.21	+	+					-152.93	320.68

* The model with the lowest AICc score (activity ~ time of day + time of year + sex + (time of year*sex)) compared to the second scoring model (activity ~ time of day + time of year) had a difference in AICc score of 5.84. This resulted in a 0.0015 probability that the second-highest model minimized information loss.

Post hoc pairwise analysis

Table 2A. Post hoc pairwise comparison: Sex * Time of year (interaction). M: male, F: female, EB: early breeding season, LB: late breeding season.

Contrasts	Estimate	SE	t value	P value
F / EB – M / EB	0.434	0.136	3.185	0.0099
F / EB – F / LB	-0.118	0.136	-0.868	0.8215
F / EB – M / LB	-0.429	0.157	-2.728	0.0365
M / EB – F / LB	-0.552	0.111	-4.954	< .0001
M / EB – M / LB	-0.863	0.136	-6.329	< .0001
F / LB – M / LB	-0.311	0.136	-2.284	0.1077

Chapter 2

Table 3A. Individual and experiment details for four female sharks within the fine-scale accelerometer study.

Individual ID	Sex	Length (TL) (cm)	Attachment method	Duration of attachment (days)	Month of experiment	Year of experiment
5541	F	107	dorsal	10 d	Oct - Nov	2015
5542	F	115	dorsal	10 d	Oct - Nov	2015
5544	F	114	dorsal	10 d	Oct - Nov	2015
5545	F	119	dorsal	10 d	Oct - Nov	2015

Table 4A. Ethogram for behaviour coding each behaviour used for analysis within the classification tree.

Behaviour	Description	Coding begins	Coding ends	Usually followed by (behaviour)
Resting	Not moving with both pectoral fins and tail on the floor	Begins when shark stops swimming and lower lobe of caudal fin first touches the ground	Ends when tail first begins to undulate from straight resting position	Swimming, chewing
Swimming	Moving over the floor or in the water column	Begins when tail first begins to undulate from straight resting position	Ends when movement stops and lower lobe of caudal fin first touches the ground	resting, chewing, vertical swimming
Swimming vertically	Moving in the water column with head upward	Begins when body is near perpendicular to floor at $\geq 45^\circ$ angle	Ends when body falls out of $\geq 45^\circ$ degree angle with floor	swimming (water column)
Chewing	Masticating/moving jaw up and down (does not include coughing behaviour)	Begins when prey item first touches mouth	Ends when last mastication is complete (i.e. jaw moves up and does not move down again)	resting

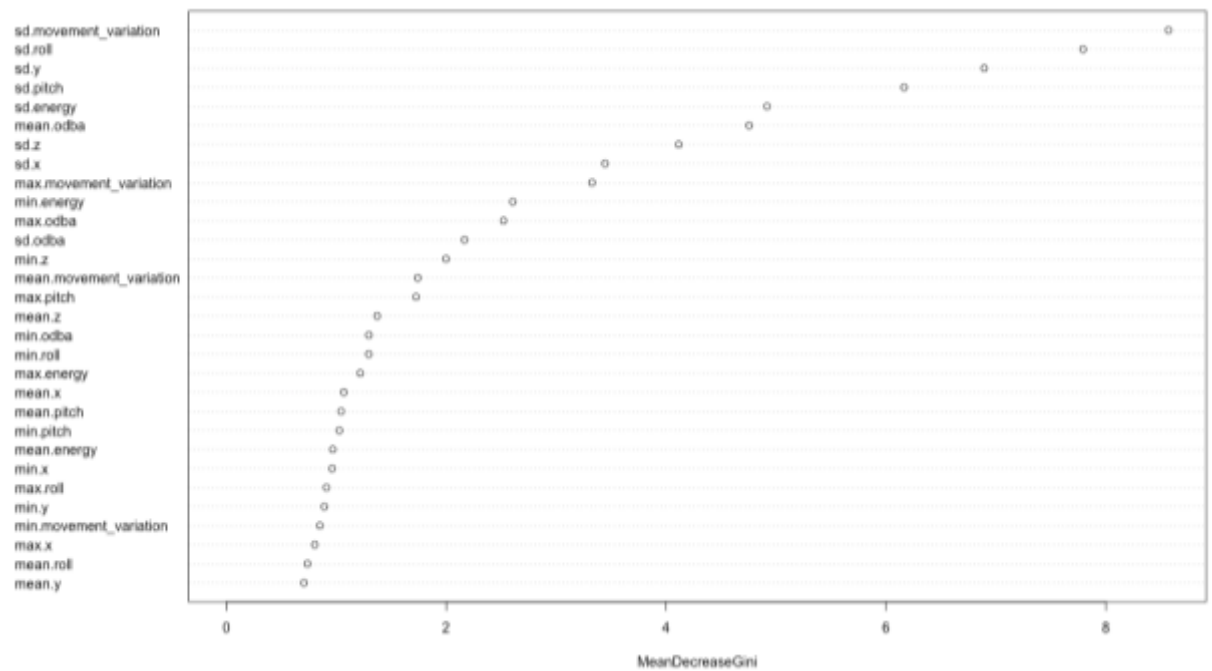


Fig. 2A. 1 s epoch feature importance selection based upon 32 summary features (y axis) and mean decrease in Gini index.

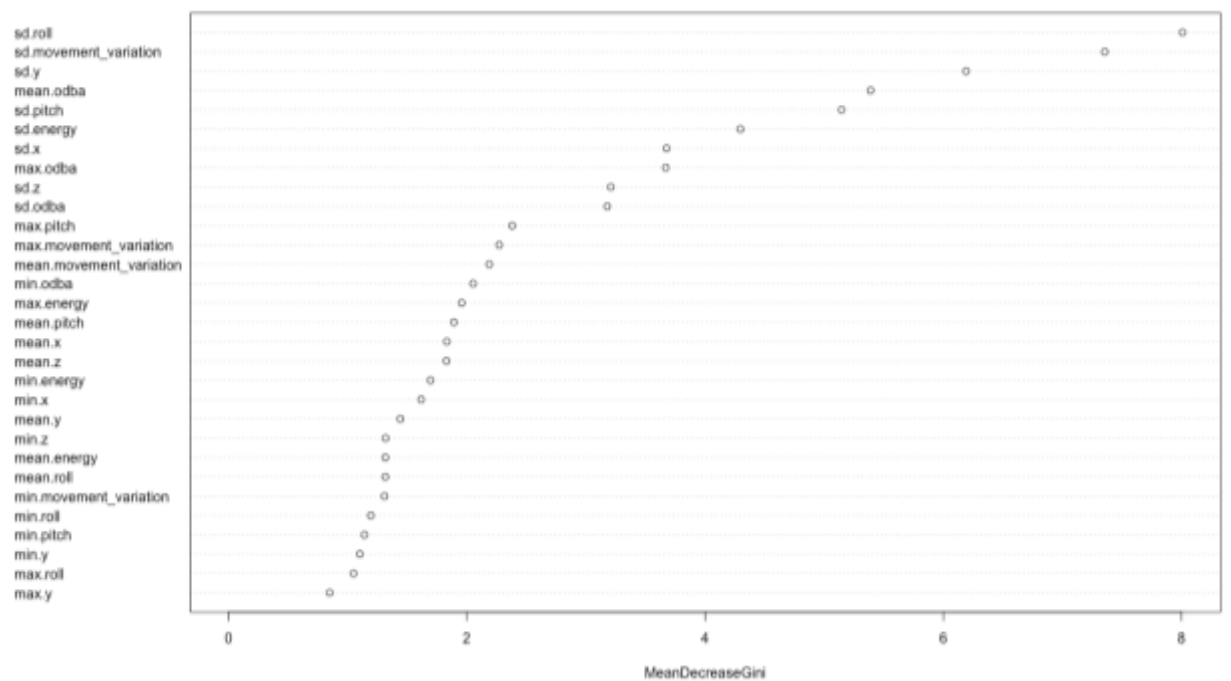


Fig. 3A. 2 s epoch feature importance selection based upon 32 summary features (y axis) and mean decrease in Gini index.

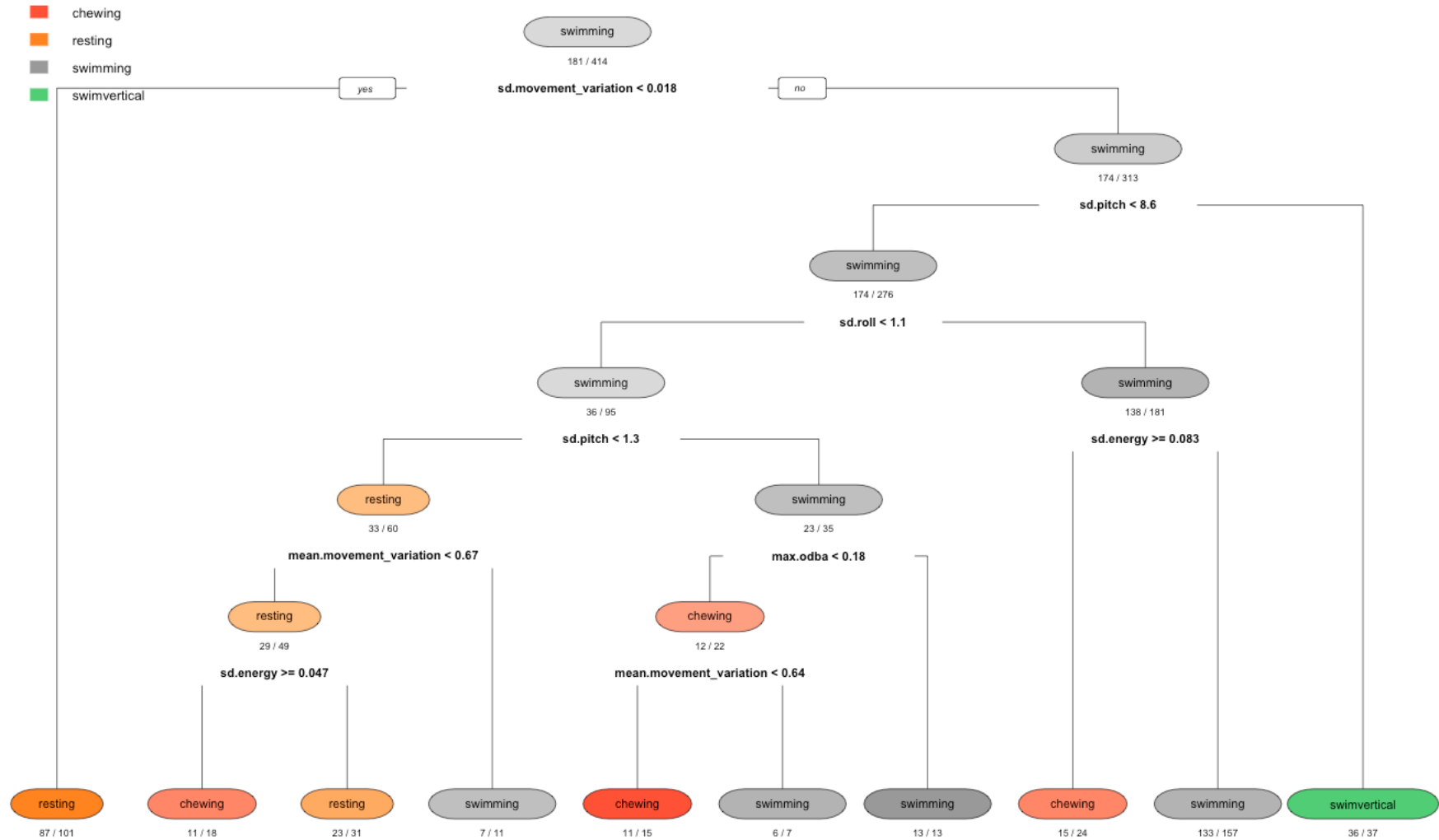


Fig. 4A. 1 s epoch classification tree distinguishing between Port Jackson shark behaviours (resting, swimming, vertical swimming and chewing). The numbers by each node and coloured bubble represent the number of behaviour events that were correctly classified / the number of behaviour events that were misclassified.