REGULAR RESEARCH PAPER

Behavioural sleep in two species of buccal pumping sharks (*Heterodontus portusjacksoni* and *Cephaloscyllium isabellum*)

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Abstract

Sleep is known to occur in most, if not all, animals studied thus far. Recent studies demonstrate the presence of sleep in flatworms and jellyfish, suggesting that this behaviour evolved early in the evolution of animals. Sharks are the earliest known extant, jawed vertebrates and may play an important role in understanding the evolutionary history of sleep in vertebrates, and yet, it is unknown whether they sleep. The Port Jackson (*Heterodontus portusjacksoni*) and draughtsboard (*Cephaloscyllium isabellum*) sharks are both benthic, buccal pumping species and remain motionless for extended periods of time. Whether these periods of prolonged inactivity represent sleep or quiet wakefulness is unknown. A key criterion for separating sleep from other quiescent states is an increased arousal threshold. We show here that inactive sharks of both species require significantly higher levels of electric stimulation before they show a visible response. Sharks deprived of rest, however, show no significant compensatory increase in restfulness during their normal active period following enforced swimming. Nonetheless, increased arousal thresholds in inactive animals suggest that these two species of shark sleep – the first such demonstration for members of this group of vertebrates. Further research, including electrophysiological studies, on these and other sharks, is required for a comprehensive understanding of sleep in cartilaginous fishes.

KEYWORDS
arousal thresholds, cartilaginous fishes, elasmobranchs, evolution, homeostatic regulation

1 | INTRODUCTION

Sleep is a conspicuous and prominent behavioural state found across the animal kingdom (Ungurean, van der Meij, Rattenborg, & Lesku, 2020). It is often associated with behavioural correlates, such as quiescence in a species-specific posture, which is rapidly reversible to wakefulness, and a decrease of awareness of the local environment resulting in an increased arousal threshold. Sleep is also regulated by two processes: (a) homeostasis, whereby sleep loss increases sleep need, causing animals to sleep more and/or more

intensely (Tobler, 2011), and (b) a circadian process, whereby an internal clock is entrained by environmental *zeitgebers*, such as 24-hr natural light–dark cycles, to influence the adaptive timing of sleep and wakefulness (Kazimi & Cahill, 1999). In many organisms, once a circadian clock is set, circadian rhythms are (largely) maintained even under constant conditions, such as constant light or dark (Falcon, Besseau, Sauzet, & Boeuf, 2007). These behavioural features of sleep can be used to test for the presence (or absence) of sleep in a diversity of animals in a comparative framework aimed at understanding the function of sleep (Cirelli & Tononi, 2008; Lesku, Aulsebrook,

Kelly, & Tisdale, 2019). As most studies of sleep have focussed on mammals (Campbell & Tobler, 1984; Lesku, Roth, Rattenborg, Amlaner, & Lima, 2008) and birds (Lesku & Rattenborg, 2014; van der Meij, Ungurean, Rattenborg, & Beckers, 2020; Roth, Lesku, Amlaner, & Lima, 2006), and to a lesser extent on non-avian reptiles (Kelly, Peters, Tisdale, & Lesku, 2015; Libourel et al., 2018; Libourel & Herrel, 2016; Shein-Idelson, Ondracek, Liaw, Reiter, & Laurent, 2016), there is a dearth of sleep data outside of these taxa. The recent discovery of two sleep states in larval zebrafish (*Danio rerio*) (Leung et al., 2019) highlights the merit of conducting research on sleep in more "ancient" groups of vertebrates in order to trace the evolutionary history and ontogeny of sleep (Blumberg, Lesku, Libourel, Schmidt, & Rattenborg, 2020).

Sharks (Selachii) are members of the Chondrichthyes, which represent the earliest extant group of jawed vertebrates (Gnathostomata), yet little is known about sleep in this taxon (Kelly, Collin, Hemmi, & Lesku, 2019). Of the *c.* 450 living species of sharks, only 30 species have been observed to show any sleep-like behaviour, including periods of immobility or the circadian organization of activity. Only the nurse shark is known to meet multiple behavioural criteria of sleep, including the anecdotal observation of a single animal exhibiting reduced responsiveness to divers (Weber, 1961). No study has systematically explored the existence and form of sleep in sharks.

Although some sharks seemingly do not have conspicuous periods of restfulness (the so-called ram ventilators owing to their method of respiration, which involves forward movement to push oxygenated water over their gills), many species are known to remain stationary for extended periods of time, in the same manner as many teleost species (Carlson, Goldman, & Lowe, 2004; Kelly et al., 2019; Roberts, 1978; Kelly et al., 2020). During periods of inactivity, these sharks facilitate gas exchange by opening their mouth and lowering their mandible to draw oxygenated seawater into the mouth under negative pressure, followed by closing their mouth and raising the buccal cavity floor to push oxygen-rich water over their gills under positive pressure (Carlson & Parsons, 2001). These are buccal pumping sharks and include the Port Jackson (*Heterodontus portusjacksoni*) and draughtsboard (*Cephaloscyllium isabellum*) sharks; both species are nocturnal and are inactive for much of the daytime (Figure 1) (Horn, 2016; Kadar, Ladds, Mourier, Day, & Brown, 2019; Powter & Gladstone, 2009; Kelly et al., 2020). The diel activity patterns of these sharks show evidence of circadian organization and light plays an integral role in regulating periods of inactivity (Kadar et al., 2019; Kelly et al., 2020). Whether these periods of sustained immobility reflect sleep, however, is unclear. Describing the presence, or absence, of sleep in sharks would shed light on questions regarding the evolution of sleep, such as how sleep manifests in constantly swimming species and whether sleep evolved once early in the evolution of vertebrates and has persisted over evolutionary time, or whether some animals have lost the need for sleep throughout evolution.

In this study, we investigated whether inactivity represents sleep in two species of buccal pumping sharks. Firstly, we tested for the presence of an increased arousal threshold in restful sharks by titrating the intensity of an electrical stimulus to evoke a response. Secondly, we explored whether rest is homeostatically regulated in these sharks by encouraging inactive animals to swim during half of
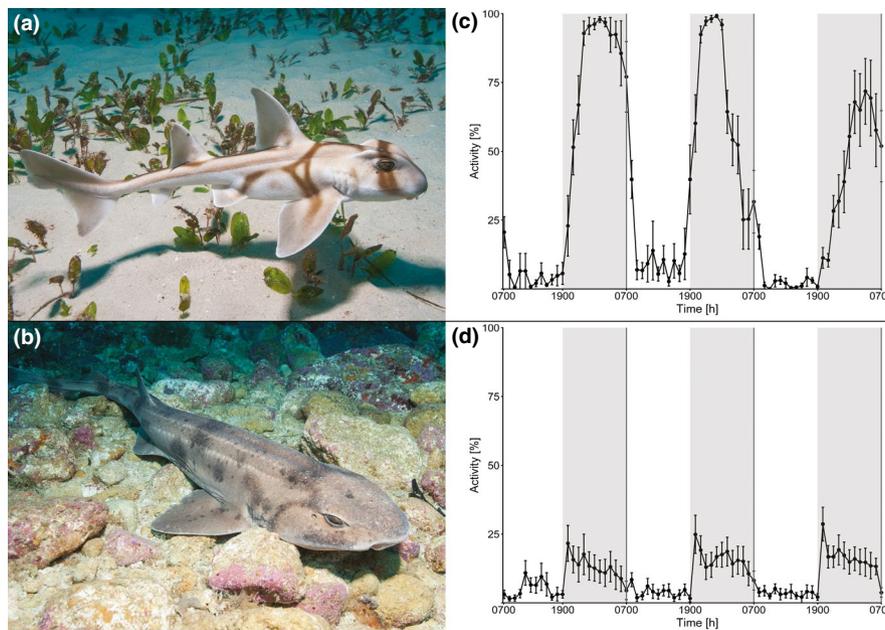
FIGURE 1 (a) The Port Jackson (*Heterodontus portusjacksoni*) and (b) draughtsboard (*Cephaloscyllium isabellum*) sharks are both demersal, buccal pumping sharks known to remain motionless for extended periods of time. Furthermore, both the (c) Port Jackson and (d) draughtsboard sharks are nocturnal, as revealed by a 3-day protocol showing percent of each hour spent swimming across three 24-hr days denoted by black dots (hourly mean ± standard error [*SE*]). Reprinted with permission from *Journal of Biological Rhythms*, Kelly M. L., Murray E. R. P., Kerr C. C., Radford C. A., Collin S. P., Lesku J. A., & Hemmi J. M. (2020) Diverse activity rhythms in sharks (Elasmobranchii). Photos (a) by Andy Murch/Bigfishexpeditions.com; (b) by Iain Anderson

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the day when they would otherwise be immobile; we then looked for a compensatory increase in restfulness when animals were left undisturbed to behave freely.

2 | MATERIALS AND METHODS

2.1 | Experimental animals and housing

Experiments were conducted between September 2017 and March 2019 at The University of Auckland, New Zealand (animal ethics #001983) and at The University of Western Australia (Indian Ocean Marine Research Centre at Watermans Bay, animal ethics #RA/3/100/1506). All work was carried out in strict accordance with the guidelines of the New Zealand Code of Ethical Conduct (CEC) and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th Edition 2013). Animals were monitored daily before, during and after behavioural testing to ensure their good health and well-being.

Ten juvenile draughtsboard sharks (six females and four males; 320–450 mm in total length) were collected from the waters around Leigh, New Zealand (36°04′02.3S, 175°19′24.2E), and were transported to the Leigh Marine Laboratory. Seven juvenile Port Jackson sharks (four males and three females; 270–320 mm in total length) were collected from waters around Perth, Australia, and transported to the Indian Ocean Marine Research Centre at Watermans Bay.

Both species were group-housed (in groups no larger than six individuals) indoor in round, plastic housing tanks (diameter 1,800 mm, depth 1,600 mm, 4,000 L capacity at Leigh Marine Laboratory; diameter 1,000 mm, depth 600 mm, 500 L capacity at Watermans Bay). Both housing tank systems were flow-through, receiving fresh seawater from the ocean (complete water change every hour), and were temperature controlled (16–18°C at Leigh Marine Laboratory; 18–20°C at Watermans Bay) and filtered (50 μm at Leigh Marine Laboratory; 30 μm at Watermans Bay). Water quality and environmental conditions were maintained and monitored continuously. Animals were fed every 72 hr and were kept under natural light conditions for a minimum of 2 weeks prior to the commencement of experiments.

### 2.2 | Experimental tanks and video recording setup

Animals were housed individually in rectangular, glass tanks (900 mm long, 500 mm wide, 500 mm deep, 225 L capacity for Port Jackson sharks; 1,200 mm long, 600 mm wide, 540 mm deep, 390 L capacity for draughtsboard sharks), which were compartmentalized with curtains to remove any uncontrolled sources of light and to prevent visual stimulation from adjacent tanks and the research team. The floor of each tank was installed with waterproof (silicone injected), near-infrared (IR) LED strip lights (12 V DC, 850 nm single chip, 120 LEDs 9.6 W/m), fixed 100 mm apart from one another

with aquarium-grade silicone, which were left on at all times during experiments. These provided near-IR illumination (invisible to sharks; Hart, Theiss, Harahush, and Collin (2011)) to enable tracking of the natural movements of the sharks using IR-sensitive cameras (Altronics, 4.0 megapixel, weatherproof varifocal Internet Protocol, Power over Ethernet) under dark conditions. A diffuser (3-mm-thick, opal-coloured, acrylic sheet) was placed into the tanks, spaced 100 mm off the tank floor and held in place by nine evenly spaced clear, acrylic blocks, which were fixed to the tank floor with aquarium-grade silicone. A programmable (via a Zetlight I200 Lancia ZP-4000 controller), marine spectrum, aquarium LED light (Zetlight Lancia ZP4000 Marine Light, 1,200 mm, 46 W) was centrally fitted 980 mm above each tank to provide a 12:12 light:dark photoperiod. A diffuser of 1-mm polytetrafluoroethylene (PTFE) sheeting was placed over each aquarium LED light. In order to capture video footage of the sharks, an IR-sensitive camera fitted with a photographic red gel filter (Lee Filters, Pop Red) was placed centrally above each tank. The red gel filter and IR sensitivity of the cameras ensured that video footage was unaffected by changes in photoperiods set by the overhead aquarium light. This enabled the capture of clear footage in both light and dark conditions. Personal video surveillance software (Security Monitor Pro, Deskshare, Plainview, USA) was used to record video footage and a live feed was observed on a desktop computer in an adjacent room.

Animals were placed in individual experimental tanks and left to acclimatize under 12:12 light:dark conditions for 72 hr before the commencement of all experiments. Food was withheld for 30 hr prior to the commencement of behavioural tests; this did not affect the well-being of the sharks as they were accustomed to eating on every third day.

2.3 | Arousal threshold protocol

Experimental tanks were fitted with two stainless-steel sidewalls (plates) at each end of the tank, which acted as electrodes (Figure 2). Experimental animals were placed individually into the body of water between the two electrodes, where they were free to move throughout the tank. The electrodes were connected to a digital, programmable power supply (0–15 V DC 0–40 A Regulated Switchmode Laboratory Power Supply, PowerTech), located in an adjacent room, via insulated cables. Electrical pulses (stimulus events) of 0.5 s duration (between 0.5 and 11 V) were produced by the power supply via an experimenter-controlled switch, and passed between the two electrodes, completing a capacitor circuit. An IR light, attached to the power supply circuit, flashed in view of the overhead IR-sensitive camera (observable by the experimenter) in synchrony with the stimulation events to aid with analysis. As the applied voltage caused a linear voltage gradient across the two large electrodes, which covered both ends of the tank, animals experienced approximately the same level of stimulation, irrespective of their position and orientation within the tank. However, the orientation of each shark was recorded at the time of stimulation for later analysis. Initial

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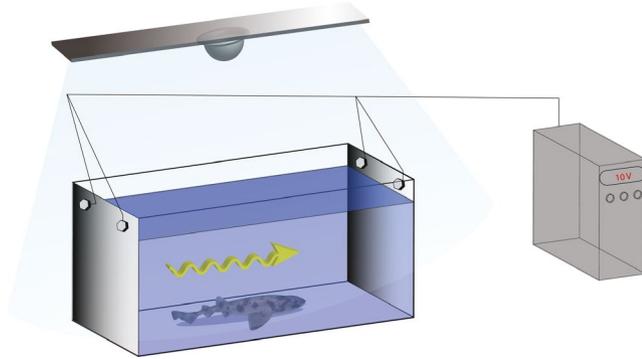
FIGURE 2 Experimental apparatus used to assess stimulus suprathreshold levels in active and inactive animals. A programmable power supply sent electrical pulses (stimulus) of varying intensities, via insulated cables, between steel plates (electrodes) fitted to each end of a glass tank filled with seawater. A shark in the tank (orientated here as parallel to the electrical field) experienced the stimulus as it passed between the two electrodes, completing a capacitor circuit. Animals were monitored from an adjacent room, via an overhead infrared (IR)-sensitive camera, to deliver each stimulus at the appropriate time. An overhead aquarium LED light provided a 12:12 light:dark cycle, and the behavioural responses to stimulus events were recorded on a surveillance system. Photo by Richard Taylor

stimulation began at 0.5 V and was gradually increased in increments of 0.5 V. Stimulus events did not exceed 11 V. The electrical pulses were monitored via an oscilloscope (ADS1000 Series Digital Storage Oscilloscope, Atten Electronics Co., Ltd).

Scoring of animal behaviour was performed in real time to ensure sharks met the criteria for stimulation (see below). This was achieved remotely via the aforementioned surveillance system, which provided a live feed of animals at two frames per second. Active animals were either swimming or inactive for no more than 5 s; inactive sharks were restful for at least 5 min. In both behavioural states, animals were subjected to stimuli of increasing intensity between 0.5 and 11 V until a startle response was elicited. A paired design was used in which each animal was tested under both conditions (active and inactive) in a random order. The interstimulus interval was at least 5 min. Resting/stationary animals responded with movement (swimming), whereas active/recently active animals responded with changes in swimming trajectory, speed and body position. In the case where an animal did not respond to the stimulus, the voltage was increased by 0.5 V and after an interval (break) of 5 min the stimulus was presented. This process continued until a response was observed or the maximum stimulation level was reached (11 V), after which point the result was recorded and the experiment ended. This process was repeated three times per individual, with an interval of at least 48 hr between trials, at three different times of the day (07:00 hours, 15:00 hours and 23:00 hours), delivered in random order. Threshold stimulus levels were defined as the lowest voltage that elicited a response in each behavioural state.

A control protocol was conducted concurrent to the treatment protocol in an adjacent tank in which an animal experienced the identical conditions (including the IR flash at time of the stimulation event in the treatment protocol tank) without the stimulus presentation. Control animals showed no measurable response. At the end of the experimental period, sharks were returned to their housing tanks. A recovery period of 7 days was given to the animals before the commencement of the sleep homeostasis experiment.

2.4 | Homeostatic regulation protocol

After being allowed to acclimatise for 72 hr under 12:12 light:dark conditions, all sharks were left undisturbed for the first 6 hr of the experimental protocol (under light conditions). For the following 6 hr (under light conditions), swimming behaviour was promoted every 10 min via gentle tactile stimulation with a soft rubber net. Each stimulus event ceased after 5 s or as soon as the animal started swimming. Upon the onset of dark, the deprivation protocol ended and the sharks were left undisturbed for 12 hr. Control protocols were conducted, concurrent with the experimental protocols, in adjacent tanks in which sharks were left undisturbed to behave freely. Activity rates and distance travelled by animals during experimental and control protocols were recorded via overhead IR-sensitive cameras.

2.5 | Video analysis

Video clips of homeostatic regulation trials were analysed with the video analysis software Digi developed by JMH (e.g. Hemmi & Tomsic, 2015) in MATLAB (MATLAB Release 2015b, The MathWorks, Inc., Natick, USA). Shark position was automatically determined with a time resolution of two frames per second by comparing each video frame with a background image that did not contain a shark. In the resulting difference matrix, using a blob analysis in MATLAB, the largest blob closest to the position of the shark in the previous image was taken as its new position. Distance travelled per minute was calculated by filtering the data in time with a boxcar filter of 1-min lengths (120 frames) and then subsampled to one sample per minute.

2.6 | Statistical analysis

All statistical analyses were completed with the statistical software programs MATLAB, R (R Core Team 2017, R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria) or SYSTAT (SYSTAT Software, Inc., Tools for Science Version 13.2, Chicago, USA). Data on the responsiveness to electrical pulses of draughtsboard sharks was not normally distributed. Consequently, a non-parametric Wilcoxon signed rank test was used to determine whether active sharks responded to lower levels of stimulation compared to inactive sharks. A paired t test was used to analyse arousal threshold data in Port Jackson sharks as those data were normally distributed. Independent sample

t tests and mixed effects models, in which *orientation* (parallel and perpendicular to the electric field) and *individual* were set as a random effects and *stimulus intensity* was set as a fixed effect, were used to determine whether the orientation of animals had an effect on the sensitivity to stimulation. Finally, mixed-effects models, in which *individual* was set as a random effect, and *time* (time of day/ night) and *treatment* (control or sleep deprivation) were treated as fixed effects, were used to compare activity rates and distance travelled between rest-deprived and control animals in order to determine the presence of homeostatic regulation of sleep in both species of shark. We conducted post hoc tests (Tukey's post hoc test) to determine at which hourly time-points significance was reached.

3 | RESULTS

3.1 | Arousal threshold experiments

Sharks of both species required stronger stimulation to respond when immobile for at least 5 min (Figure 3). Specifically, inactive Port Jackson sharks had significantly higher arousal thresholds (mean minimum response level: 6.6 ± 0.9 V) relative to active Port Jackson sharks (4.5 ± 0.5 V) (paired t test: $t_6 = -6.04$, $p < .01$). Active draughtsboards sharks showed a more dramatic difference in arousal thresholds, reacting to stimulus events six times lower (1–3 V; median 1.5 V) relative to inactive draughtsboard sharks, which often did not respond at all (3.5–11 V; median 11 V) (Wilcoxon signed rank test: $v = 0$, $p < .01$). In both species, control animals in adjacent tanks, which experienced the identical experimental conditions without the stimulus presentation, never reacted during control trials. No habituation to electrical stimulation levels was observed between trials.

The orientation of the animal relative to the electrical field (parallel or perpendicular) had little effect on the arousal threshold (Table 1). As expected, there was no correlation between Port Jackson shark orientation within the tank and stimulus thresholds; that is, the number of times sharks reacted to stimulation did not differ significantly between parallel (2.71 ± 3.57 events per animal) and perpendicular (3.29 ± 3.57) orientation (independent sample t test: $t_{12} = -0.56$, $p = .58$). Furthermore, stimulation intensity was similar between orientations of reactive animals (5.65 ± 3.21 V during parallel orientation; 5.47 ± 1.96 V during perpendicular orientation) (independent sample t test: $t_{40} = 0.36$, $p = .72$). A correlation between animal orientation and stimulus-reactive events was present in draughtsboards sharks, with a significantly higher number of stimulus reaction events occurring in animals perpendicular to the electric field (4.30 ± 3.34 events per animal) compared to those parallel (1.70 ± 3.34 events per animal) (independent sample t test: $t_{18} = 3.17$, $p < .01$). Animals in the perpendicular orientation also reacted to stimulus levels more than two times higher (6.76 ± 17.51 V), relative to parallel-oriented animals (3.06 ± 9.71 V) (independent sample t test: $t_{40} = 3.84$, $p < .01$). However, this appeared to be an artefact of the animals' preference for resting in this orientation, as
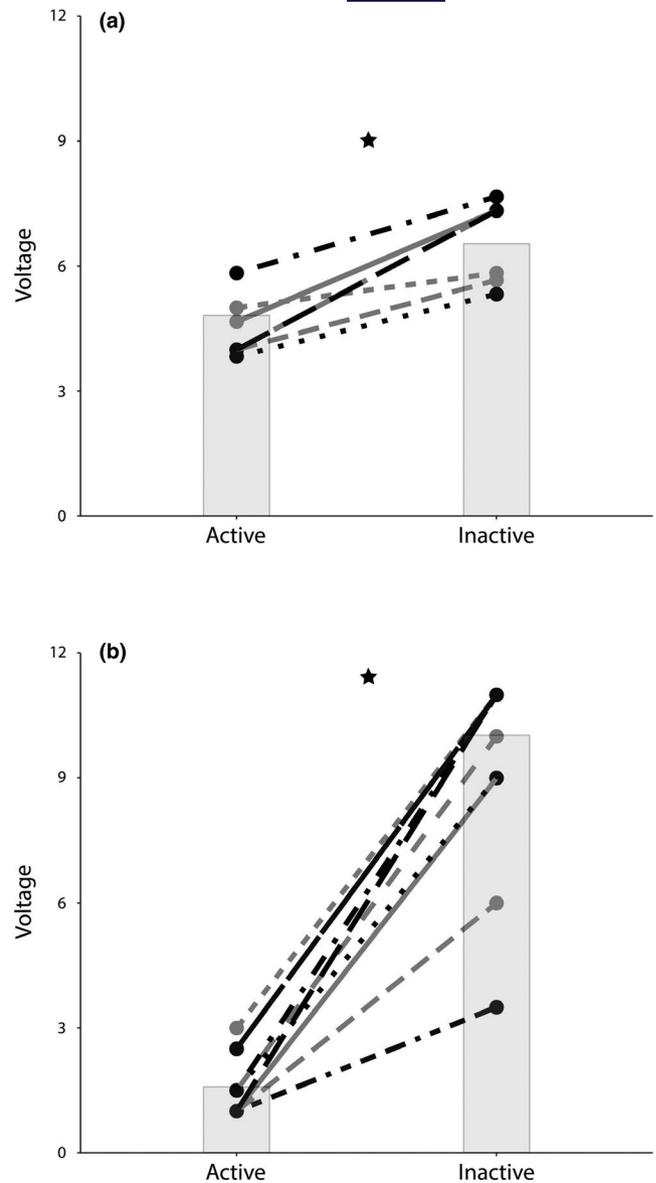
FIGURE 3 Arousal thresholds in (a) Port Jackson and (b) draughtsboard sharks. Lines reflect individual data (the mean from three trials; variation in spacing between dashed lines denotes a different individual); shaded bars show the mean (in Port Jackson sharks owing to normally distributed data) or median (draughtsboard, non-normal data) group values. Stars denote significance between stimulus intensity in active and inactive animals

92% of stimulus events were presented to animals in this orientation. Accordingly, when we statistically controlled for this preference, the effect of orientation on stimulus level was non-significant (Table 1).

3.2 | Homeostatic regulation experiments

Immobile sharks that were stimulated responded rapidly and switched to an active state, whereas mobile animals responded to the stimulation by increasing activity rates. In practice, the amount of time spent encouraging a shark to move was low, as sharks

TABLE 1 Results of mixed-effect models for the arousal threshold experiment.

Arousal threshold protocol	Effects	Nominator *df*, denominator *df*	*F*-ratio	*p*-value
Port Jackson	Activity state	1; 32	39.74	<.01
	Orientation	1; 32	0.72	.40
	Orientation * Activity state	1; 32	0.17	.69
Draughtsboard	Activity state	1; 47	153.90	<.01
	Orientation	1; 47	1.34	.25
	Orientation * Activity state	1; 47	1.34	.25

Note: In each model, *individual* and *orientation* (parallel and perpendicular to the electric field) were set as random effects; *activity state* (active and inactive) was treated as a fixed effect. We also tested whether the arousal threshold depended on the orientation and activity state considered. *df*, degrees of freedom.

reacted quickly to stimulation and continued to move upon removal of the stimulus. Draughtsboard sharks, however, returned to a state of inactivity quickly following the end of each stimulation event (generally within 10 s). Port Jackson sharks showed greater variation with the amount of time they continued swimming after stimulation events (between 10 s and 3 min), although no consistent trend was observed towards faster or longer returns to inactivity following stimulation events as the experiment progressed. In the case of the Port Jackson sharks, we were able to significantly increase the amount of swimming and distance travelled relative to the same circadian time of the control shark for 5 of the 6 hr of the stimulation period (Table 2; Figure 4). Draughtsboard sharks reacted less to the stimulation events and only increased their swimming activity over the first hour of stimulation, after which they appeared to habituate quickly to the stimulation. However, we successfully increased the distance the sharks swam for 5 of the 6 hr of the stimulation period. The increase in distance travelled, without an increase in overall swimming, confirms that draughtsboard sharks acutely increased swimming for short periods at the time of stimulation, rapidly becoming inactive thereafter. An increase in distance travelled was, therefore, achieved without affecting the overall activity rate. There was no significant compensatory increase in restfulness (from swimming or distance travelled) following enforced swimming detected in either species during the following 12 hr of the dark photoperiod.

4 | DISCUSSION

Sleep is a prominent behaviour, characterized by restfulness in a species-specific posture, often aligned more to one part of the 24-hr day, rapid reversibility to an awake condition, increased arousal threshold, and homeostatic regulation. Here, we find evidence for three of these four criteria. Our results suggest that bouts of inactivity longer than 5 min reflect periods of sleep in Port Jackson and draughtsboard sharks. In both species, quiescence would quickly give way to swimming with sufficient stimulation.

We found no evidence that sleep is homeostatically regulated in these animals, as the sharks did not recover lost sleep by sleeping more when allowed to behave freely. Both species, however, were nocturnal, a pattern of activity that persists in the absence of light cues (Kelly et al., 2020).

Reduced responsiveness to stimuli is one of the most important criteria to distinguish sleep from other quiescent states. In this study, we used an electrical pulse stimulus to successfully identify arousal thresholds in inactive sharks. Such methods have also been used by Yokogawa et al. (2007) to describe the same phenomena in zebrafish. We found no correlation between animal orientation and arousal thresholds in the Port Jackson shark, whereas draughtsboard sharks showed a general preference for a perpendicular orientation to the electrical field, laying against the side of the tank when immobile. This may be due to the fact that, in this orientation, an animal has more body-surface-area contact with the corners of the tank, which may have been perceived as a safer sleep site than a more exposed, central area of the aquaria (Lima, Rattenborg, Lesku, & Amlaner, 2005). Moreover, this relatively protected site might more closely resemble their natural daytime habitats, resting in caves and crevices on reefs (Awruch, Frusher, Stevens, & Barnett, 2012; Horn, 2016; Nelson & Johnson, 1970). As the Port Jackson sharks were smaller in size, this orientation did not provide them with the same level of contact with the tank corners, which may explain the difference in behavioural choices between the two species. Although anecdotes of increased arousal thresholds have been described in sharks (Clark, 1973; Kelly et al., 2019; Ritter, 2020; Weber, 1961), this is the first quantitative evidence of this phenomenon in any cartilaginous fish.

Sleep homeostasis has been demonstrated in a plethora of vertebrates (Rattenborg, Martinez-Gonzalez, & Lesku, 2009; Tobler, 2011). For example, flatworms (*Girardia tigrina*) and jellyfish (*Cassiopea spp.*) kept awake during their normal rest period showed evidence for the homeostatic regulation of sleep (Nath et al., 2017; Omond et al., 2017). In both Port Jackson and draughtsboard sharks, we were successful in increasing the amount of activity. However, increased restlessness was not followed by increased restfulness

TABLE 2 Results of mixed-effect models for the homeostatic regulation plots shown in Figure 4 for the amount of activity, and the distance travelled over time.

Homeostatic regulation protocol	Effect	Nominator df; denominator df	F-ratio	p-value
(a) Port Jackson				
Activity (day)	Time	11; 88	7.43	<.01
	Treatment	1; 88	36.83	<.01
	Time * Treatment	11; 88	8.14	<.01
Activity (night)	Time	11; 88	2.57	.01
	Treatment	1; 88	0.29	.64
	Time * Treatment	11; 88	2.18	.02
Distance (day)	Time	11; 88	7.67	<.01
	Treatment	1; 88	21.99	<.01
	Time * Treatment	11; 88	8.47	<.01
Distance (night)	Time	11; 88	2.51	.01
	Treatment	1; 88	0.01	.92
	Time * Treatment	11; 88	2.89	<.01
(b) Draughtsboard				
Activity (day)	Time	11; 198	6.30	<.01
	Treatment	1; 198	1.16	.28
	Time * Treatment	11; 198	2.91	<.01
Activity (night)	Time	11; 198	9.36	< .01
	Treatment	1; 198	0.07	.79
	Time * Treatment	11; 198	1.18	.30
Distance (day)	Time	11; 198	7.28	<.01
	Treatment	1; 198	7.44	<.01
	Time * Treatment	11; 198	6.65	<.01
Distance (night)	Time	11; 198	8.43	<.01
	Treatment	1; 198	0.25	.61
	Time * Treatment	11; 198	1.39	.18

Note: Each 24-hr protocol was divided into two 12-hr blocks (day 07:00–19:00 hours and night 19:00–07:00 hours), with a model produced for each block. In each model, *individual* was set as a random effect; *time* (time of day/night) and *treatment* (control, sleep deprivation) were treated as fixed effects. We also tested whether the treatment effect depended on the time considered.

when the animals were left undisturbed. A similar result was found in zebrafish (Yokogawa et al., 2007). Similar to the protocol for sleep deprivation of zebrafish, our experimental design left the sharks to recover at the start of the photoperiod when they would have been most active; in this case, at night. Consequently, it is possible that light-mediated or circadian-regulated activity patterns served to override homeostatic pressure for sleep at this time, as observed in activity patterns of Port Jackson sharks (Kelly et al., 2020). Tench (*Tinca tinca*), zebrafish, goldfish (*Carassius auratus*) and some cichlids (*Amatitlania nigrofasciata*) have also shown extreme, activity-based sensitivities to light, which can overcome sleep homeostasis (Campbell & Tobler, 1984; Tobler & Borbély, 1985; Yokogawa et al., 2007). Other animals favour wakefulness over sleep, such as in white-crowned sparrows (*Zonotrichia leucophrys gambelii*) exhibiting migratory restlessness (Rattenborg et al., 2004), male pectoral sandpipers (*Calidris melanotos*) trying to secure paternity during the breeding season (Lesku et al., 2012), or great frigatebirds (*Fregata minor*) engaged in long-distance foraging sojourns over the open ocean (Rattenborg et al., 2016). Nonetheless, in these instances, increased wakefulness is due to competing waking demands taking precedence over sleep, rather than a diel drive for activity.

Our deprivation method prevented periods of quiescence longer than 10 min. In doing so, we increased swimming rates of Port Jackson sharks by 44%, but by only 12% in draughtsboard sharks even while the distance travelled increased. A similar method of intermittent rest deprivation highlighted the homeostatic regulation of sleep in jellyfish (Nath et al., 2017). The absence of an incurred sleep debt in sharks in our study, however, may suggest that sharks sleep on shorter timescales (<10 m), akin to the short temporal structure of avian sleep (Lesku & Rattenborg, 2014; Lesku et al., 2012; Rattenborg et al., 2016). Future work should investigate whether more rigorous deprivation protocols (more intense or longer periods of stimulation) could induce a homeostatic response following extended periods of wakefulness.
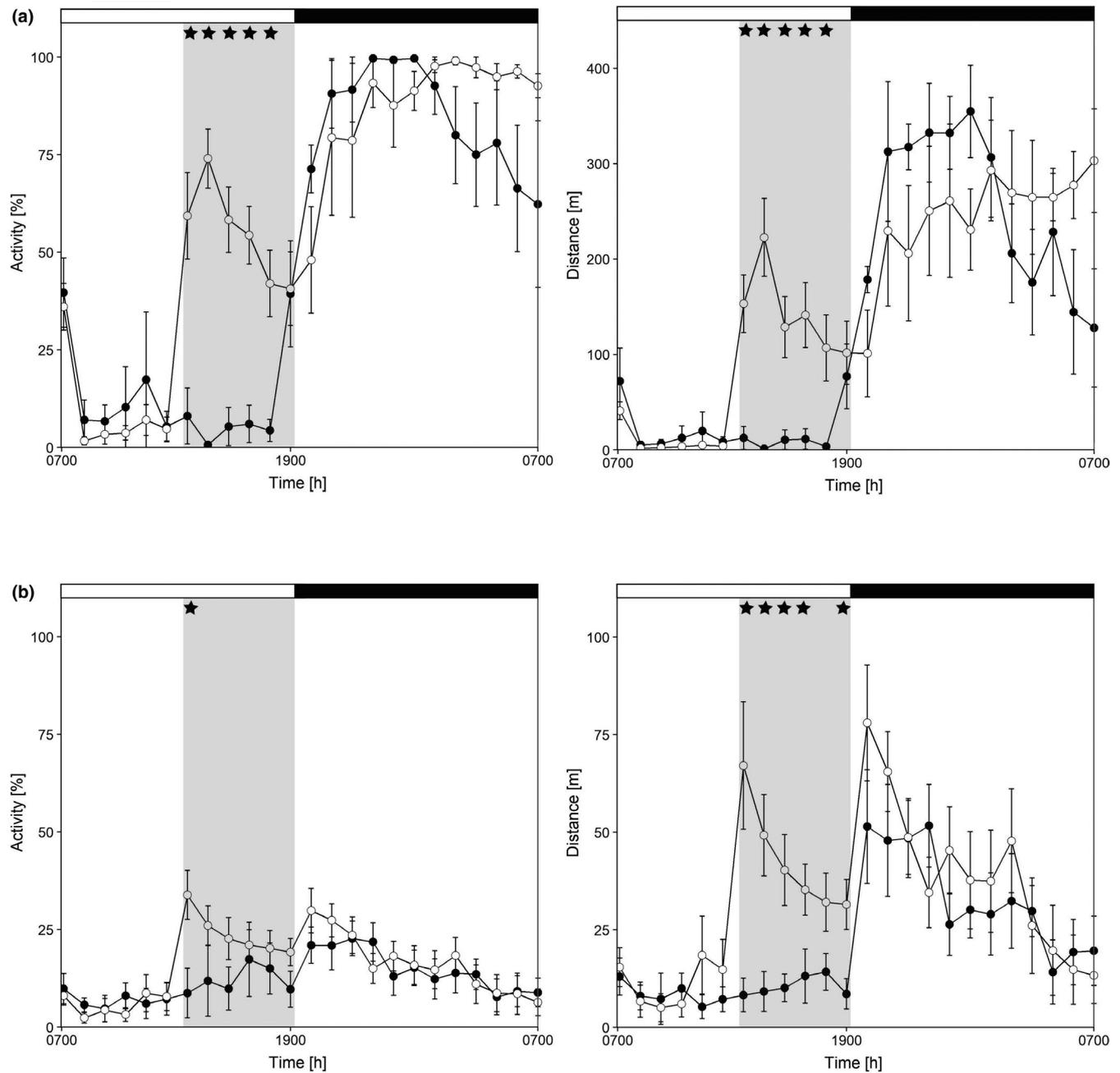
FIGURE 4 Effect of inactivity deprivation (or forced locomotion) on the subsequent amount of activity (left) and distance travelled (right) in (a) Port Jackson sharks and (b) draughtsboard sharks. Data from concurrently run control animals (filled circles) and stimulated animals (open circles) are plotted as hourly means (± standard error of the mean [*SEM*]) at the start of each hour; grey shading reflects the timing and duration of the inactivity deprivation protocol. The black horizontal bar at the top of the plot denotes the 12-hr night; the open bar reflects the 12-hr day. Stars indicate statistically significant differences between time-points of the control and stimulated animals

Our finding of spontaneous and reversible periods of quiescence, which coincide with reduced responsiveness to electrical stimulation, is the first direct evidence for the presence of sleep in any cartilaginous fish. Further investigation is needed to fully understand how such sleep is regulated in these animals and what role light might play. Furthermore, nothing is known about sleep in continuously swimming sharks, including great white sharks (*Carcharodon carcharias*) and other large, pelagic species. These animals have secondarily evolved the need for constant swimming to ventilate their gills; as such, they may have also evolved interesting adaptations to permit sleep under this unusual lifestyle (Kelly et al., 2019). Here, electrophysiological studies of brain activity may provide comprehensive insight into the form sleep takes in these animals, and what this means for the evolution of sleep, sleep states and sleep function in other vertebrates.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

MLK, SS, SPC, CCK and CAR collected all animals and were responsible for the husbandry and maintenance of animals and experiments; MLK conducted all experiments; JMH wrote and provided video analysis software and programs; MLK, SS and JAL produced all figures; MLK wrote the first draft; all the authors then contributed to editing the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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