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## [SLEEP IN CAPTIVE BOTTLENOSE DOLPHINS]

*Sleep identification in bottlenose dolphins, *Tursiops truncatus*, using mammalian behavioral sleep criteria.*

## Abstract

Sleep can be identified using electrophysiological and/or with behavioral criteria. Both methods have been proven valid throughout numerous mammalian sleep studies. If the animal exhibits all mammalian sleep criteria (provided by numerous mammal sleep research studies over the past years), then sleep is present. The behavioral sleep criteria, however, have been founded on bi-hemispheric sleeping mammals. Studies on Cetaceans have proven they rely on uni-hemispheric sleep instead. The bottlenose dolphin (*Tursiops truncatus*), for instance, shows constant movement throughout the night. This constant seemingly alert state is argued to be wakefulness. But what if we were to apply existing mammalian behavioral sleep criteria on their resting behavior? How well would these criteria apply to them, if at all?

The captive bottlenose dolphins exhibit two types of rest during low activity hours (18:00 – 06:00). Slow Circular Swimming (SCS) and logging behavior, recognized by *v.d. Klij* (unpubl.) during a previous study, has also been acknowledged by numerous other behavioral studies on bottlenose dolphins.

*Mukhametov 1985* drugged the dolphins to sleep, measured EEG, and recorded similar behavioral patterns of a logging dolphin. This observation allows us to combine both EEG, which proposed bilateral desynchronization, with a behavioral state which had been repetitively described as passive hanging of the bottlenose dolphin (logging behavior). He also concluded longer periods of unilateral desynchronization, lasting up to 2.5 hours, with similar behavioral description of a dolphin in SCS. We therefore assume that these 2 behaviors, observed only during low activity time, are in fact rest and perhaps even, sleep.

With the predetermined mammalian sleep criteria we wish to conclude whether this established rest is in fact sleep in our captive bottlenose dolphins. With 24-hour behavior analysis we attempted to establish whether they exhibit a resting pattern. This combined with arousal threshold analysis and anticipatory feeding behavior, which is driven by an internal clock, enabled us to conclude if they exhibit two very important behavioral criteria: Namely, increased arousal threshold and a circadian rhythm.

The bottlenose dolphin fits extremely well in the given mammalian behavioral criteria. They exhibit two types of rest during the night, Slow Circular Swimming (SCS) and Logging behavior. Both states exhibit an increase in arousal threshold, a circadian rhythm, a quiescent state with a certain posture and location. Their logging behavior fits most perfectly in the mammalian sleep model, their SCS behavior fits 6 of the 8 criteria. Because of this, we conclude sleep to be present in our captive bottlenose dolphins.

**Keywords:** *Sleep, Uni-hemispheric sleep, Captive bottlenose dolphins, Tursiops truncatus, Mammalian behavioral sleep criteria, Arousal threshold, Anticipatory feeding behavior, Circadian rhythm, Slow Circular Swimming, SCS, Logging, Surface rest.*

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## Introduction

Sleep is a complex process that appears to be present throughout the entire animal kingdom.

Overall, sleep studies rely either on electrophysiological or behavioral criteria (*Rechtschaffen et al. 1966, Mukhametov et al. 1988, Neckelmann & Ursin 1993, Ridgeway 2002, Casagrande & Bertini 2008*). Electrophysiological sleep criteria are EEG and/or EMG recordings which determine brain and muscle activity, respectively. In cetaceans for instance; EEG and EMG recordings can be a challenge or even impossible to perform. In such cases, behavioral criteria are applicable to identify sleep. In total, there are eight behavioral sleep criteria (*Rattenborg et al. 2000, Lyamin et al. 2002, Casagrande & Bertini 2008, Rihel*

*et al. 2010*). These criteria can help to confirm the presence of sleep.

Mammalian sleep studies have collected and fine-tuned the behavioral criteria (*Halász et al. 2004, Kato et al. 2004, Blumberg et al. 2005, Gennaro et al. 2000, Rechtschaffen et al. 1966, Neckelmann & Ursin 1993*). These studies concluded that there is a correlation between behavioral sleep criteria and electrophysiological measurements. If a mammal shows all the desired behavioral sleep criteria further research with EEG confirms the presence of sleep. The behavioral criteria are:

1. **Quiescence:** *subject is in a calm and quiet state, doesn't produce sounds.*
2. **Posture:** *a species specific posture of the body coincides with the period of 'rest'.*
3. **Immobility** *subject is motionless during the period of 'rest'.*

4. **Typical nest/place:** *resting/sleeping behavior usually occurs within or at a certain location.*
5. **Circadian rhythm:** *the resting/sleeping behavior occurs at a typical time of day*
6. **Reversibility:** *subject can be aroused from resting period. If this is not the case, the subject might be dead, in a coma or hibernating.*
7. **Increased arousal threshold:** *a sleeping subject loses touch with the environment. It is no longer fully responsive to its' environment, unless the subject experiences a certain type of stimulus that exceeds/matches its' arousal threshold. The deeper the sleep, the higher the threshold.*
8. **Homeostatic regulation:** *subject shows an increase of sleep after being deprived of sleep. This strongly suggests the subject requires a minimum amount of sleep to function properly and thus that it is a necessary process.*

Thus if the animal satisfies all of the criteria above, we are able to conclude that the animal is sleeping, without adding EEG measurements.

However, most of these sleep research studied bi-hemispheric sleep; this is a complete shutdown of both hemispheres during sleep, which is the most common type of sleep. This leaves to wonder whether these behavioral sleep criteria are applicable to mammals that do not have bi-hemispheric sleep?

Uni-hemispheric sleep is a continued activity of at least one brain hemisphere throughout the night (Nicolau et al. 2000, Lesku et al. 2009). It is proposed that uni-hemispheric sleep allows the animal to remain in control, be able to swim/fly, whilst sleeping (Rattenborg et al. 2000, Lima & Rattenborg 2007, Lyamin et al. 2008).

Lilly 1964 was the first to suggest that dolphins have uni-hemispheric sleep. Lilly 1964 concluded that this type of sleep would allow them to sleep with one eye open, which in turn assures them they were always able to

scan the environment with at least half of their 'afferent input'.

Years later, studies proposed uni-hemispheric sleep to have evolved to fit in extreme habitats (Tobler 1995, Madan & Jha 2012). In such a habitat, e.g. living in the ocean, breathing is only possible if the dolphin is in constant motion to keep its operculum (breathing hole) at the surface (Mukhametov 1977, Tobler 1995).

Dolphins are uni-hemispheric sleepers and according to the hypothesis; this allows them to remain afloat and continue to breathe while resting (Rattenborg et al. 2000, Lyamin et al. 2008).

A study by v.d. Klij (unpubl.) studied the dolphins behavior throughout the day. These observations resulted in to 4 possible categories, of which 2 were presented as sleep; Slow Circular Swimming & Logging behavior.

Both of the rest behaviors only occurred during low activity hours (from 18:00 - 07:00). These two 'sleep' categories were identified with the following criteria;

*SCS:* when the dolphin swims slow circles and does not make sounds. It comes up at regular intervals and shows overall lack of wakefulness. The dolphin often swim in groups during this state. This behavior could continue for longer periods of time throughout the night. Occurred only during low-activity time.

*Logging:* the dolphin hangs, completely motionless, in the water column with its operculum above the water. Breathing does not occur during this state. This behavior was short and did not last longer than 60 seconds. Occurred only during low-activity time.

Mukhametov 1985 found intermediate bilateral synchronization, its occurrence ranging from 2-10% of total recording time in their different dolphins. The rest of the uni-hemispheric slow wave sleep occurred approximately 30-40% of their recording time. The dolphins were thus found committing to USWS for most of their sleep duration. The maximum length of USWS

measured in their dolphins was 2.5 hour. This observation coincides with the observations made by *v.d. Klij* (unpubl.) in which case SCS occurred much more frequent and for longer periods of time compared to the sporadic and short logging episodes.

*Sekiguchi & Kohshima 2003* analyzed the behavior of 16 captive bottlenose dolphins. They measured two parameters, swimming speed and breathing frequency. The parameters were lowest from 0:00 to 03:00 (low activity time) and highest from 13:00 to 16:00 (high activity time). These parameters were significantly higher during high activity time than the daily mean. The opposite is true for the parameters during the low activity time.

They analyzed the behaviors observed during low activity time and compared them with those observed during the high activity time. They identified three forms of rest behavior that occurred mainly during the low-activity time. These accounted for 86.6% of the total observation time.

1. **Bottom-rest:** lay still on the bottom of the tank (0.5-6.5 minutes, n=189) without surfacing for air. Bottom-rest was always interrupted by surfacing for air. This behavior always followed by surface-rest or swim rest, never by another bottom-rest.
2. **Surface-rest:** (*Our logging behavior*) lay still at the surface of the tank (5 sec. – 55 min, n= 290), blowhole and tip of dorsal fin at the surface and bending posterior part of the body towards the bottom of the tank.
3. **Swim-rest:** (*Our Slow Circular Swimming behavior*) kept swimming slowly (less than 2 m/s (n=28) in a long circular course along the tank wall (0.5-30.5 min, n=175). For high-activity time, the dolphins eye(s) are closed 93.2 % (n=162) of the time, with 31.5% of the time only one eye, 61.7% both eyes. Dolphins with higher (more active swimming speeds) swam 92.3% (n=104) of the time with both eyes open. For low-

activity time, 100% (n=51) of the time, at least one eye was closed.

They measured two types of sound emission in this state, although less frequent when compared to behaviors with high speed swimming.

*McCormick 1969* did similar behavioral research and concluded 2 types of rest behavior to be present in their bottlenose dolphins. Surface and bottom rest.

*Flanigan 1974 & Gnone et al. 2001* found 2 types of rest behavior as well, only they found surface rest (logging) and slow circular swimming.

Other studies have also found similar resting behaviors (SCS and logging) in other sea-mammals, for instance; Pacific white-sided dolphin which shows both logging and SCS behavior (*Flanigan 1975*). In both killer whale & Chinese river dolphin logging behavior was confirmed (*Flanigan 1974, Renjun et al. 1980*). Studies found SCS present in the Amazon river dolphin (*Oleksenko & Lyamin 1996*), harbor porpoise (*Mukhametov 1984, Oleksenko & Lyamin 1996*).

All these studies assume the inactive behaviors that they observe during nightly observations (low-activity time) as rest behavior or sleep. So far no quantitative analysis has been done with the same subjects. How well does our assumption that these behaviors that occur, mainly during low activity time, are rest/sleep?

*Mukhametov 1985* applied sleep drug injections to its dolphins. They demonstrated that the uni-hemispheric sleep is connected with respiration. The dolphins autonomous breathing appears to be incompatible with bilateral delta-waves. With the drug, which provoked maximum bilateral delta waves, breathing stopped completely. They also found that before a breath of air, the EEG of one or both hemispheres always became desynchronized. Behavioral studies agreed that during both bottom and surface (logging) rest, no breathing occurred (*Sekiguchi &*

*Kohshima 2003*). Because their logging behavior matches a state in which they were drugged to sleep, it is plausible that the natural occurrence of such a state is in fact sleep.

Next to breathing frequency, eye closure links behavioral rest observation to USWS. *Gnone et al. 200, Sekiguchi & Kohshima 2003 & Ridgeway et al. 2006* all concluded that the dolphins closed one eye during their swim-rest (SCS). *Mukhametov et al. 1985* reported that 98.1% of EEG sleep of captive bottlenose dolphins were unihemispheric slow wave sleep. They also found that they closed the eye at the opposite side of the resting hemisphere during their USWS. The eye condition of the swim-rest behavior suggests a relationship between swim-rest and unihemispheric sleep.

Because of these previous studies on behavioral sleep and electrophysiological sleep, it is possible to use the behavioral sleep criteria to continue to decipher the function and form of their proposed sleep. How well do the behavioral sleep criteria apply to our captive bottlenose dolphins?

An increase in arousal threshold could reveal whether their perceived rest behaviors are in fact sleep. It could also reveal whether their motionless and breathless logging behavior is a deeper form of rest/sleep than their swim-rest. Is there a link between SCS and logging behavior, could it be that SCS is a precursor to sleep?

The presence of a circadian rhythm proposes that the observed behavior occurs only at certain moments of the day. Their perceived rest behavior should not occur as frequent during high activity hours as it would during low activity hours, for it to be able to be rest. To establish the presence of a circadian clock, the basics of a circadian rhythm, we shall test their anticipatory behavior with the reward for food at a certain location and time.

An alliance between the University of Groningen, Netherlands and the Dolphin

Academy, Curacao allowed the behavioral observations/experiments to continue. The experiments were conducted from January until May 2014.

The goal of this study is **(i)** to identify sleep in captive bottlenose dolphins using mammalian sleep criteria and **(ii)** determine whether anticipatory feeding behavior is present in the bottlenose dolphins. Thus establish the presence of an internal clock.

**Research question:** Can we identify sleep in captive bottlenose dolphins with the help of mammalian behavioral sleep criteria?

## Methods

The Dolphin Academy Curacao (DAC), located in the southern Caribbean Sea, has 21 dolphins. Most of the dolphins are a part of the day-to-day trainer & audience programme; a few are only part of the 'therapy' programme (CDTC, Curacao Dolphin Therapy Centre). The dolphins were in good health and able to join the experiment. There were no invasive procedures during the experiment, thus, no ethical clearance was necessary. It was important that our tests and behavioral analysis did not affect the dolphin in any negative way. Therefore most of our studies were purely observational and required very little interference.

There is little variation in temperature (aerial or sea) throughout the year. Average aerial and sea temperatures are 26-30°C & 25-29°C respectively. Day and night cycles are on average 12:12 hours.

The Dolphin Academy Curacao is open from 08:00 until 17:00, shows occur at 10:30, 13:30, 16:30 and other swim-with-dolphins programs such as; 'encounters' and 'swims' started at 08:00, 11:00, 14:00. The dolphins are engaged in different programs and trainers throughout the day, to protect them from being over stimulated or bored.

## The Academy Dolphins

In total, 14 dolphins (5 male & 9 female) were a part of the experiments and included in our observations (Table 1). The pools are in constant contact with the Caribbean Ocean and are naturally shaped by rocks. The pools have a variety of depth and all have the ocean's natural flora and fauna in their environment.

The Dolphin Academy of Curacao has to 3 pools (East, Main and North pool). These pools can be connected or separated with gates. The dolphins are often switched between these spools to promote variability and equal socialization. Roxette and Ukit were paired with one other mother/calf combination and kept in the East pool (Annie & Machu, **Table 1**). This is similar with the set-up of last year, where the youngest mother/calf was paired with an older mother/calf combination. It was necessary for another mother & calf to be present in the pool to allow for 'aunt-behavior' to occur. Aunt-behavior: the other mother dolphin allows for the calf to stay with her when the calf's' mother is resting. The 'Aunt', in this case, continues with SCS or wakeful swimming with the calf while the mother experiences logging or SCS behavior.

Name	Sex	Age (year)	Note
GeeGee	F	30	
DeeDee	F	20	Pregnant (due December 2014)
Annie	F	17	
Tela	F	13	
Caiyo	M	9	
Ritina	F	9	Pregnant (due July 2014)
Romeo	M	9	
Roxette	F	9	
Pasku	M	5	Son of Tela
Tikal	M	5	Son of DeeDee
Alita	F	2	Born March 2012 (Ritina)
Machu	M	2	Born April 2012 (Annie)
Serena	F	2	Born April 2012 (Tela)
Ukit	F	<1	Born June 2013 (Roxette)

**Table 1** Basic information of the dolphins used for the experiments. Both Ritina and DeeDee were pregnant during the experiments.

## Around-the-clock observations of rest and activity behavior

We performed the behavioral rest and activity observations according to the methods of the study of *v.d. Klij (unpublished)* in 2013. Daily observation shifts were set to 3-hour time periods during 3 consecutive days over 3 weeks. First week; 08:00-11:00, 14:00-17:00 & 23:00-02:00, second week: 05:00-08:00, 11:00:14:00 & 17:00-20:00 and third week: 20:00-23:00 & 02:00-05:00. This was repeated 3 times, giving a total of 9 observational days (**Appendix I**).

The observations took place during the end of February and beginning of March 2014. This is the same period of time in which *v.d. Klij* observed the dolphins previous year, thereby we aimed to minimize possible seasonal variation in their rest behavior.

We used the following four categories to log their activity/inactivity throughout the day.

**0/NA:** No observation made, dolphin out of sight.

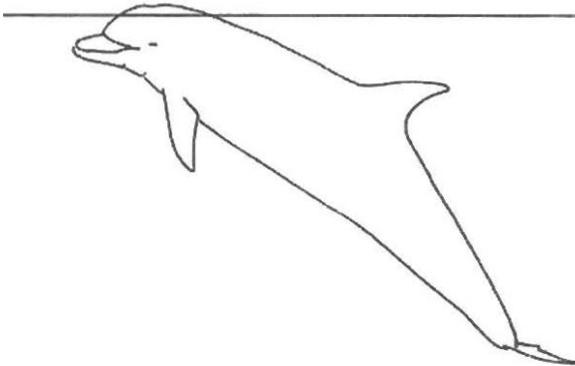
**1:** Active swimming with direct human interaction (show, swim, encounter, feeding time).

**2:** Active swimming without direct human interaction. All 'natural' active behavior falls within this category; e.g. active swimming,

mating, playing, sex, fighting.

**3: Slow circular swimming (SCS)**, most of the dolphins' body remains underwater, they move with slow and quiescent strokes and their eyes stay underwater. The dolphins are synchronized with each other when they swim in groups, they resurface together. They swim in circles, mostly counter-clockwise, but this is subject to change throughout the night. If 'they swam at least one complete circle without evidence of active behavior (eating, sound emission, or playful/sexual/interaction with others, variable speed or irregular trajectory), they were scored with behavioral category 3 (Lyamin *et al.* 2007). This behaviour is categorized as an inactive state, thus one of the two resting states of the captive bottlenose dolphin.

**4: Resting/floating at water's surface** is recognized as *logging* (**Figure 2**). Their blowhole is at the surface while their lower body hangs below the dolphin. They bob up and down the water column (no active movement). Their eyes remain under water during this behaviour (identical to category 3). Overall, logging is their most inactive state.



**Figure 2** Adult bottlenose dolphin logging at the surface (from: *Gnone et al.* 2001)

We recorded the dolphins' behavior every five minutes. We used half values (e.g. 1.5, 2.5 & 3.5) if the dolphins exhibited two distinct behavioral categories within our five minute measurement. Thus if the dolphin exhibited slow circular swimming for three minutes

and followed that behaviour with active swimming, the behavioral category was 2.5. Logging does not last long enough to represent the full five minutes of observation, thus, if the dolphin shows three or more logging periods within our five minutes, only then will we note category 4 behavior.

The pools were lit by several bright flood lights, during the night. These were set to turn on before sun set (18:45, sunset at 18:50, and full dark by 19:10) and turn off just before sunrise (06:10, sunrise at 06:15, full light at 06:30).

We also scored the duration of the dolphins' logging behaviour. The observer noted: which dolphin was logging, in which pool it was present, when the log started and the duration. The log is terminated once the animal breathes and switches back to SCS or another behavioural activity.

The dolphins were identified by their dorsal fins; each one has a unique feature, which could be linked to the individual. Most of the time they made formations based on sex. The males and females form separate groups and become fully synchronized with each other. In some cases the dolphin in question could not be identified, this problem occurred mostly during the night. We relied on their groups to remain constant over a night, if a dolphin was out of sight for over 5 minutes, then we did not include that dolphin for that particular time frame (falls into category NA).

### *Arousal threshold measurements*

Arousal threshold is an important behavioral criterion to be able to confirm or reject the presence of sleep. Are the behavioral states 'logging' and 'slow circular swimming' of our captive bottlenose dolphins, associated with an elevated arousal threshold? This would support the hypothesis that these behavioral states represent sleep. Or, do dolphins, in contrast to all other mammals, never experience an elevated arousal threshold because at least one hemisphere is always awake?

Our study identified two forms of behavioral rest, slow circular swimming and logging behavior. We expected increased arousal threshold to be most visible in a logging dolphin. We investigated both 'resting' behavioral states in our captive bottlenose dolphins and compared them to responsiveness when they were wakeful.

*Reynolds et al. 2000* suggests that bottlenose dolphins, during rest, primarily rely on auditory/acoustic stimuli to scan their environment. Their sensory system acts as a selective filter, this avoids the dolphins being hassled by non-threatening environmental stimuli during the night.

There are other methods of waking the dolphins, such as mechanical stimulation (e.g. electricity). However auditory stimuli are most appropriate, as the bottlenose dolphin vigilance relies mostly on its' hearing. Auditory threshold also has the advantage of being able to be set in predetermined levels and allows for easy (measurable) increase in sound level. These levels (in order of magnitude) describe the depth of the dolphin rest/sleep. The louder the noise necessary to wake the dolphin, the greater its' sleep depth is. The intensity of the stimuli can cause either an arousal or a shift to a lighter sleep stage without immediate arousal.

We applied 6 different stimuli, 0.5, 1, 6, 10, 14 & 18 kHz. These stimuli are set to increase in volume (dB) over 15 seconds time (actual volume of the stimuli can't be determined. Unfortunately, this varied per stimulus and per day). On average, dolphin logging behavior lasted 15 seconds (**Appendix II**). Therefore we set the auditory stimulus to emit an increasing sound volume up to 15 seconds in duration. This would make it easier to distinguish an arousal from our applied stimulus as opposed to an internal cue.

We used an underwater speaker for the 6 stimuli and a hydrophone, for recording vocal responses and check/examine the applied stimuli.

The 24 hours observation study reveals that the dolphins are least active between 19:00-

01:00. Thus that is the perfect time to perform arousal threshold measurements.

We performed the arousal threshold measurements at the cat-walk (bridge between main pool and east pool, **Figure 1**). This location provides easy access to the water (to install both the hydrophone and speaker) and this is where we found the dolphins to log during previous behavioral observations. Stimuli analysis and dolphin reactions (clicking, whistles and background noise) were measured in Wavesurfer® version 1.6.2.

A response to stimulus was present if the dolphin:

We noted the presence of a 'response' when the dolphin stopped its resting behavior combined with one of the criteria:

1. Moves towards the speaker.
2. Changes direction (only applicable for SCS behavior).
3. Starts whistling/barking at the speaker as they approached.
4. If the switch from rest to wakefulness occurred hastily/suddenly. This emphasizes the 'scare' response of the dolphins (similar to them reacting to a sudden shadow on the water). This was the easiest response to identify.

The second behavior especially makes the observer aware that the dolphin registers the sound and from which direction it came from. These criteria were established through 'control experiments' with wakeful dolphins during the day.

If the dolphin stopped logging with the presence of a stimulus however continued with SCS, no response was recorded. This is also true for a dolphin in SCS suddenly reverting to logging behavior with the applied auditory stimulus. Although there is a change in behavior, it reverts to a resting state, thus the animal is not awakened by the arousal stimulus.

The auditory stimuli are randomly applied over time to the nearest dolphins, thus preventing them from 'learning' when the

stimulus would be presented. If an animal were aroused by the stimulus, the next arousal stimulus was not applied for at least 5 minutes.

*Nachtigall et al. 2004* concluded that bottlenose dolphins exhibit Temporary Threshold Shifts (TTS), although in their case, the dolphins were exposed for a significant amount of time ( $\pm 20$  minutes) as well as volume, which is a very different set-up from ours. Eventually, not regarding this effect would be unwise, TTS result in a dolphin non-responding to a stimulus it might respond to in different circumstances. These TTS's are reversible; they concluded that their hearing improved with every 5 minutes that passed (*Nachtigal et al. 2004*). Because our stimuli only lasted for maximum 15 seconds, we found 5 minutes of non-stimulation be sufficient.

Only the dolphins within vicinity of the speaker (between 0 and 10 meters, no information gathered on relative distance of dolphin to speaker) were used in the observations.

An increase in reaction time (compared to full wakefulness) to the stimulus confirms the presence of a sleeping state.

### *Food anticipatory activity & Time-place association*

*Challet et al. 2009* states that 'circadian clocks enable the organisms to anticipate predictable cycling events in the environment.'

The internal process that couples the biological rhythms and meal cycles is called the feeding entrainment system (*Sanchez-Vasquez & Madrid 2001*).

This system is particularly useful if food availability is restricted to a temporal window. The individual displays an increase in locomotor activity, body temperature and corticosterone release, prior to the presentation of food. This process is called anticipatory behavior.

We tested whether the dolphins can learn to anticipate a meal at a certain location, purely on the basis of the time of day. Thus we tested if they have an endogenous timing system and/or if they show the presence of a biological clock.

The dolphins were fed at 19:00 and 21:00 at two fixed places (**Figure 3**). The experiment was conducted after their 'normal' feeding hours (between 08:00 and 17:00). They are fed every night until the observer confirms the presence of anticipatory behavior.

The observer is removed from visual and auditory field of the dolphins and remains inside behind glass for the remainder of the time, the observer can't be considered a cue for the dolphins.



**Figure 1** The 'north' pool, contains two male dolphins; Romeo and Caijo (both 9 years old). Left arrow shows 19:00hrs feeding area (rock), with feeder arriving from the (viewers) left side of the pool. Right arrow shows 21:00hrs feeding area (bridge) with feeder arriving from the (viewers) right side of the pool. This way, if they show anticipatory behavior combined with location, they will be looking in opposite directions at both 19:00 and 21:00. The feeding areas are approximately 12 meters removed from each other.

Anticipatory behavior is described as increased activity prior to the feeding event, as well as looking/spying behavior of the dolphin even prior to the arrival of the trainer. Looking/spying behavior is identifiable by the way the dolphin seems to scan its environment. If the dolphin tilts to one side, looking upwards with one eye, it is considered searching for the trainer, thus in this case, searching indirectly for its food. In another case, when the dolphin lifts its' entire head out of the water, it's also considered to be searching for food. The area in which it is looking for food also affects the noted amount of anticipatory behavior. Thus an animal looking at the 19:00 feeding area at 21:00 has a less complete anticipatory behavior than one that has both the time and location correct. Although the dolphins (n=2, both male) exhibit anticipatory behavior, only one has both timing and location combined for optimal result.

The 24-hour study shows that the dolphins are watching their trainers waiting for them to arrive at their pool. Similar behavior is expected to be presented during this experiment.

Once the observer identifies anticipatory behavior, the following night will be without interaction or food. In this case they will be tested if they continue to search for food and/or if the dolphins continue to wait at the 19:00 feeding area even though that time has long expired.

There were a few behavioral patterns visible during these trials. These behavioral patterns were numbered from 0 – 3 accounting for amount of anticipatory behavior present. 0 is the lowest amount of anticipatory behavior (for instance sleeping) and 3 is the highest amount of anticipatory behavior (actively looking around in the pool in the right direction). These criteria gave us the following behavioral values:

Behaviors with value **0**; dolphin exhibits SCS, when the trainer arrives at feeding area at feeding time, the trainer needs to call the dolphin to him/her.

Behaviors with value **1**; Active swimming, the dolphin arrives at feeding area only after a period >10 seconds.

Behaviors with value **2**; Spying behavior and actively searching through the pool, dolphin responds immediately when trainer arrives (no whistle or other stimuli necessary)

Behaviors with value **3**; The dolphin is already waiting at feeding area when trainer arrives, spying behavior is directed towards the right area before feeding.

## Results

### *24 hour observations*

In total, 72 hours of behavioral observations were conducted over a total of 9 days. There is no significant difference between the 3 observational days. Therefore we rely on the 'average' behavior of those three days for further investigation to their behavioral pattern.

Rest/activity analysis shows a very distinct diurnal activity rhythm, in these captive bottlenose dolphins. Their slow circular swimming occurred mostly from 17:00 up until 06:00 (dusk until dawn) and therefore show a particular time of day resting behavior (**Figure 4 & 5**).

Logging 'inactivity' occurred only after 17:00 and before 21:00, it has several peaks of presence throughout the night (**Figure 8**).

We compared 2013 and 2014 rest-activity patterns as well as; average total resting time, number of resting bouts and average bout duration (**Table 2, Figure 6**).

Average number of resting bouts in 2014 was significantly smaller than that of 2013 ( $11.5 \pm 4.1$  &  $20.2 \pm 4.5$  for 2014 and 2013 resp.).

Average bout duration increased in 2014 ( $51.1 \pm 65.2$  &  $25.6 \pm 36.5$  min. for the years 2014 and 2013 resp.). Although 2014 had decreased number of resting bouts, with increased duration, there is an increasing trend in average resting duration per night.

In 2013 average rest duration was: 519±118 minutes compared to & 587±78 minutes per night in 2014 (**Figure 6, Appendix II**).

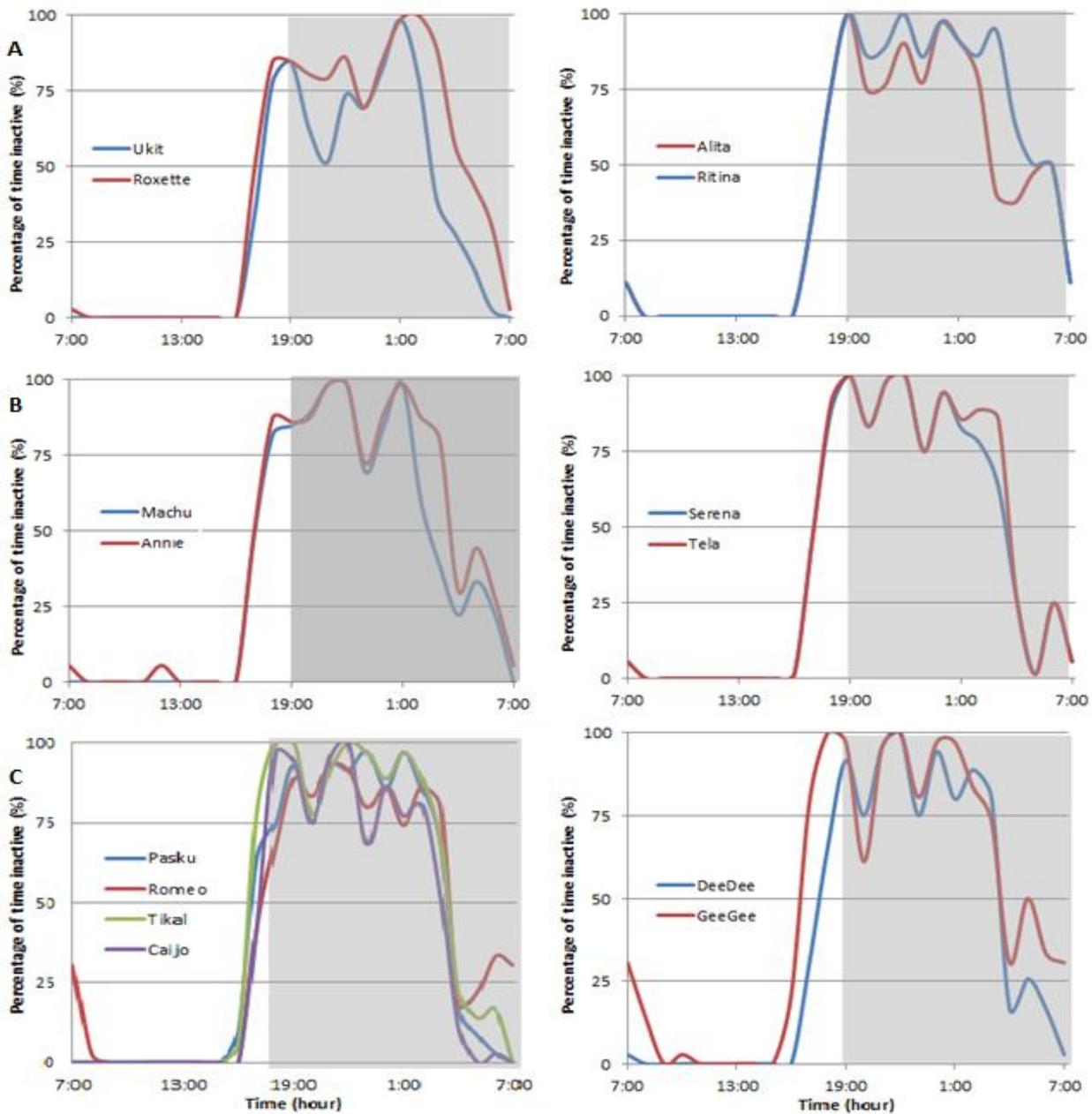
Observational data also reveals patterns of the behavioral categories. A logging dolphin only rarely (5.9%) became fully active (category 2) right after logging (category 4). The remaining 94.1% of the time the dolphin would continue with SCS right after logging.

The logging behavior of the dolphin is always preceded by slow circular swimming.

There have been no cases of an active and in human contact dolphin (category 1) to continue with SCS or logging behavior in the following 5 minute time frame.

	2014			2013		
	Average resting time (hrs)	# resting bouts	Average bout duration (min)	Average resting time (hrs)	# resting bouts	Average bout duration (min)
<b>GeeGee</b>	11.4 ± 1.7	12.0 ± 4.6	56.8 ± 57.0	12.2 ± 0.2	14.7 ± 2.5	49.1 ± 57.9
<b>Annie</b>	10.4 ± 1.0	12.0 ± 4.0	51.8 ± 78.1	9.7 ± 0.7	20.0 ± 1.7	29.1 ± 42.0
<b>Machu</b>	9.2 ± 1.2	10.7 ± 4.2	51.6 ± 77.1	6.7 ± 0.7	24.0 ± 2.0	16.9 ± 22.0
<b>Tela</b>	10.0 ± 0.7	9.3 ± 2.1	60.9 ± 77.6	9.4 ± 0.4	20.3 ± 3.8	27.7 ± 36.3
<b>Serena</b>	9.6 ± 0.5	11.7 ± 5.7	49.4 ± 71.4	5.3 ± 0.6	22.0 ± 1.0	14.5 ± 21.0
<b>Ritina</b>	10.9 ± 0.4	9.3 ± 3.8	69.8 ± 80.0	9.9 ± 0.1	19.0 ± 5.6	31.2 ± 46.4
<b>Alita</b>	9.6 ± 0.4	14.0 ± 4.0	42.7 ± 49.2	6.3 ± 0.1	24.3 ± 0.6	17.9 ± 24.7
<b>Roxette</b>	10.3 ± 1.5	13.0 ± 5.0	47.6 ± 52.4	11.1 ± 0.9	13.3 ± 3.8	49.9 ± 59.0
<b>Ukit</b>	7.8 ± 1.9	14.3 ± 4.2	31.6 ± 37.2	NA	NA	NA
<b>DeeDee</b>	9.3 ± 0.9	11.3 ± 3.8	48.8 ± 57.8	NA	NA	NA
<b>Caijo</b>	8.8 ± 0.9	11.3 ± 5.1	53.7 ± 58.5	7.6 ± 0.8	23.7 ± 1.5	18.9 ± 25.5
<b>Tikal</b>	10.4 ± 0.1	8.7 ± 4.2	60.5 ± 70.4	8.6 ± 0.4	21.3 ± 2.9	24.1 ± 28.6
<b>Pasku</b>	9.6 ± 1.0	10.7 ± 2.3	54.1 ± 87.0	7.1 ± 0.1	20.7 ± 1.2	20.7 ± 25.0
<b>Romeo</b>	9.7 ± 2.0	11.7 ± 4.9	50.0 ± 62.6	7.4 ± 0.4	25.0 ± 2.6	17.7 ± 22.1
<b>Kayena</b>	NA	NA	NA	10.1 ± 0.7	14.3 ± 4.5	41.6 ± 47.9

**Table 2** The number of resting bouts per night, average resting bout duration and resting time per night for both the year 2013 and 2014. The average number of resting bouts per night (#); 11.5 ± 4.1 & 20.2 ± 4.5 and the average bout duration (minutes); 51.1 ± 65.8 & 25.6 ± 36.5 (for 2014 and 2013 resp.).



**Figure 4.** The behavioral rest/activity pattern depicted as average percentage rest (inactivity) per hour per dolphin, set against time of day. Complete rest during that hour resembles a 100% value. The grey area indicates the dark period of the day.

(A, Left) Ukit and Roxette show great differences in measured behavioral pattern. Ukit remains more active than her mother throughout the night. Ukit reaches 50% rest level at 01:30hrs while Roxette continues to climb up until she reaches a 100% inactivity/rest level.

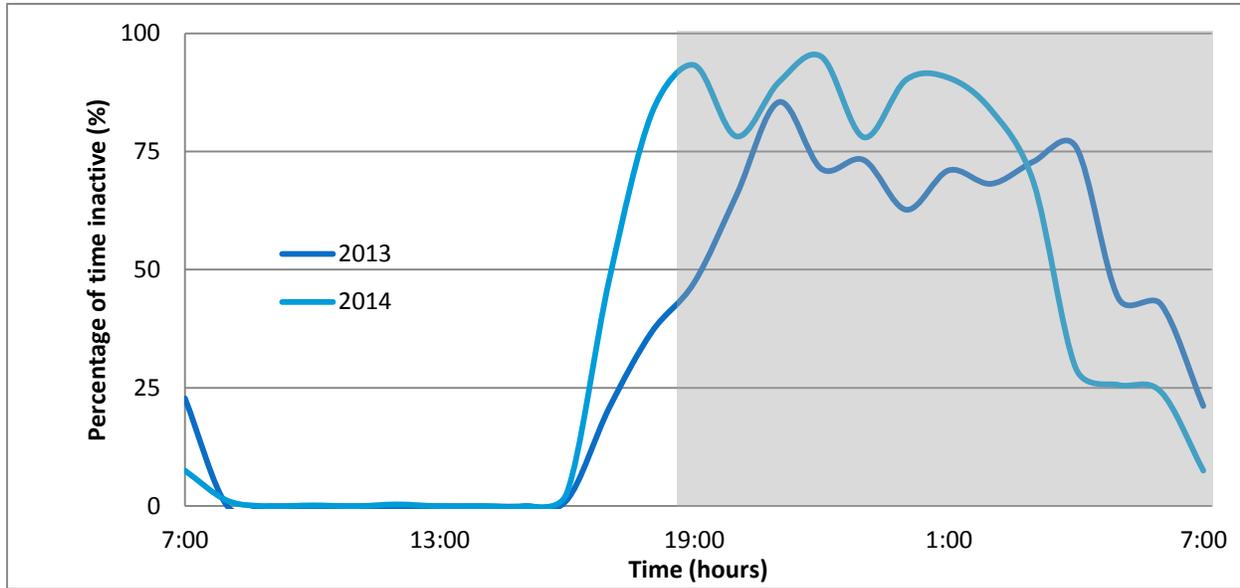
(A, Right) Alita and Ritina show a somewhat similar pattern, however Alita lacks a 4<sup>th</sup> rest peak. And as Ukit shows, Alita too has decreased rest levels. During Ritina's final peak, Alita's rest percentage per hour dropped to 37.5 %.

(B, Left) Annie and Machu show a strikingly similar activity/rest pattern, along with the same amount of peaks. They both start and end their resting period at similar times. Though near the end of the night Machu shows decreased rest percentages, he follows the same pattern as his mother, yet at a decreased level.

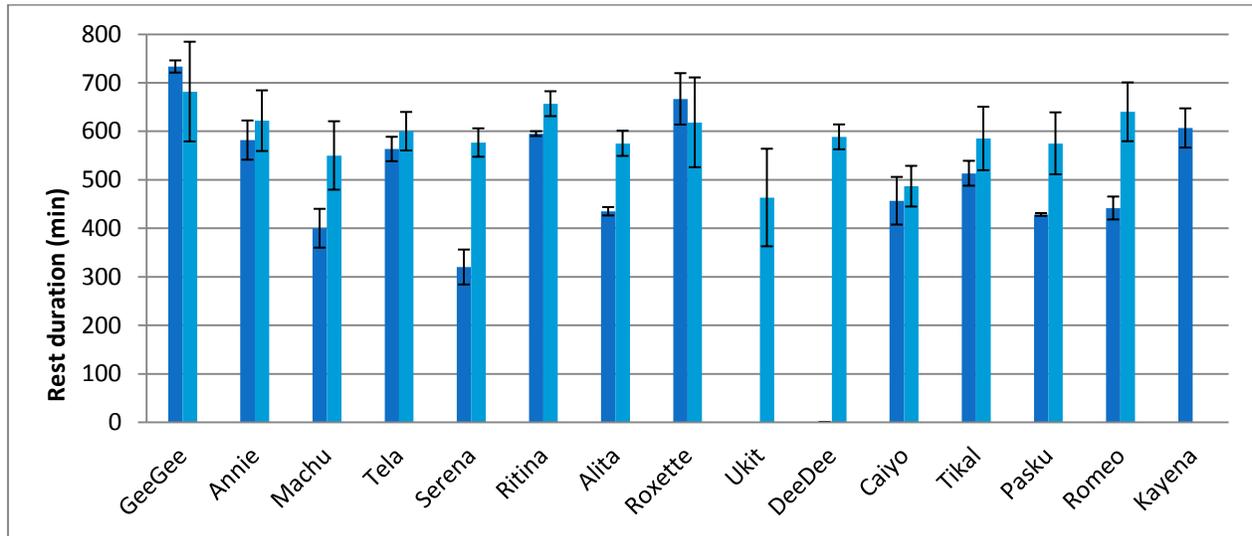
(B, Right) Tela and Serena are almost completely synchronized in their rest and activity behavior. Only the last behavioral rest peak is not present in Serena's behavioral pattern. This small period of time does, however, match GeeGee's behavioral pattern.

(C, Left) Graph of the four adult males. They all show an increase in rest before the sun sets (19:00). Activity decreases and increases throughout the night. Romeo continues resting for small periods of time, even when all the other males are fully active. All the males (except for Tikal) show 4 peaks of increased rest during the night.

(C, Right) Graph of the two females without calves. GeeGee is the eldest dolphin (30 years of age) and exhibited rest behavior right after the final session (17:00). GeeGee and DeeDee have similar peaks of increased rest behavior during the night and these peaks coincide with that of the 4 males (See C left).



**Figure 5.** The percentage of time in rest averaged over all the dolphins per year. The onset of rest behavior occurs both in 2013 and 2014 (dark and light blue resp.) at the same time namely at 17:00. However in 2014, this onset is stronger and remains at higher levels of rest behavior during the beginning of the night and remains higher as the night continues. There are three clear peaks of increased inactivity in 2014, this pattern is not present in 2013. It appears that, on average, 2014 resulted in higher percentages of rest behavior per hour.



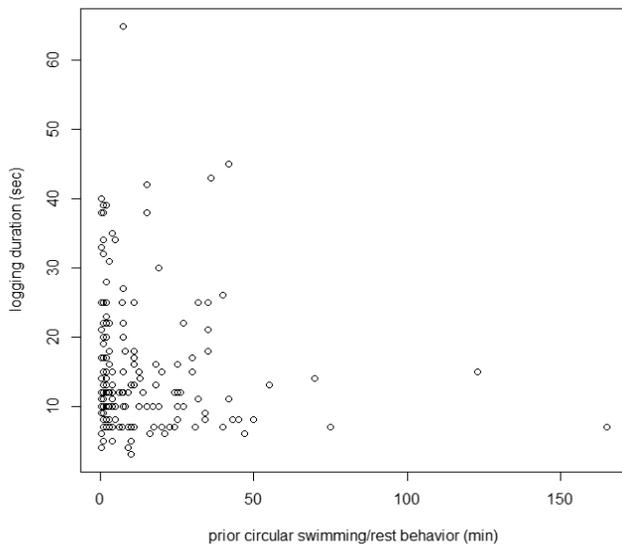
**Figure 6.** Total average resting duration in minutes per dolphin per year (dark blue: 2013, light blue: 2014). DeeDee, Kayena and Ukit were only present in one of the two observational years. Average rest duration per night:  $519 \pm 118$  &  $587 \pm 78$  min. (for 2013 & 2014 resp.) All three babies in 2013 rest an average of 400, 320 & 435 min (Machu, Serena and Alita resp.) and show a significant increase of total rest in 2014 (550, 577 & 575 resp. t-test  $p < 0.05$ ). Their resting time in 2014 resembles more that of the ‘adult’ dolphins. Only three of the adult (>4 years old) dolphins show a significant increase in rest duration in 2014, Ritina ( $\pm 62$  min), Pasku ( $\pm 147$  min), Romeo ( $\pm 198$  min). Overall there is a significant difference between the two study years; in 2014 the dolphins were resting for a longer period of time when compared to 2013 (t-test,  $p < 0.05$ ). Even when only considering average rest duration of the adult dolphins (>4 years) there is still a significant difference between 2013 and 2014.

The dolphins at the Dolphin Academy changed back and forth between anticlockwise and clockwise swimming direction. On a purely observational note, there seemed to be a difference in swimming direction between the east pool and the main pool. We found clockwise swimming behavior more often in the east pool than the main pool. Actual amount of clockwise vs. anti-clockwise swimming was not registered. Both pools swam near the catwalk and their circles during SCS often did not go further than half of the pool.

### Logging duration

Initiation time and duration of their logging behavior were noted during the 24-hours of observation. Measured logging durations were rounded up to whole seconds.

Logging is always preceded by SCS (100%,



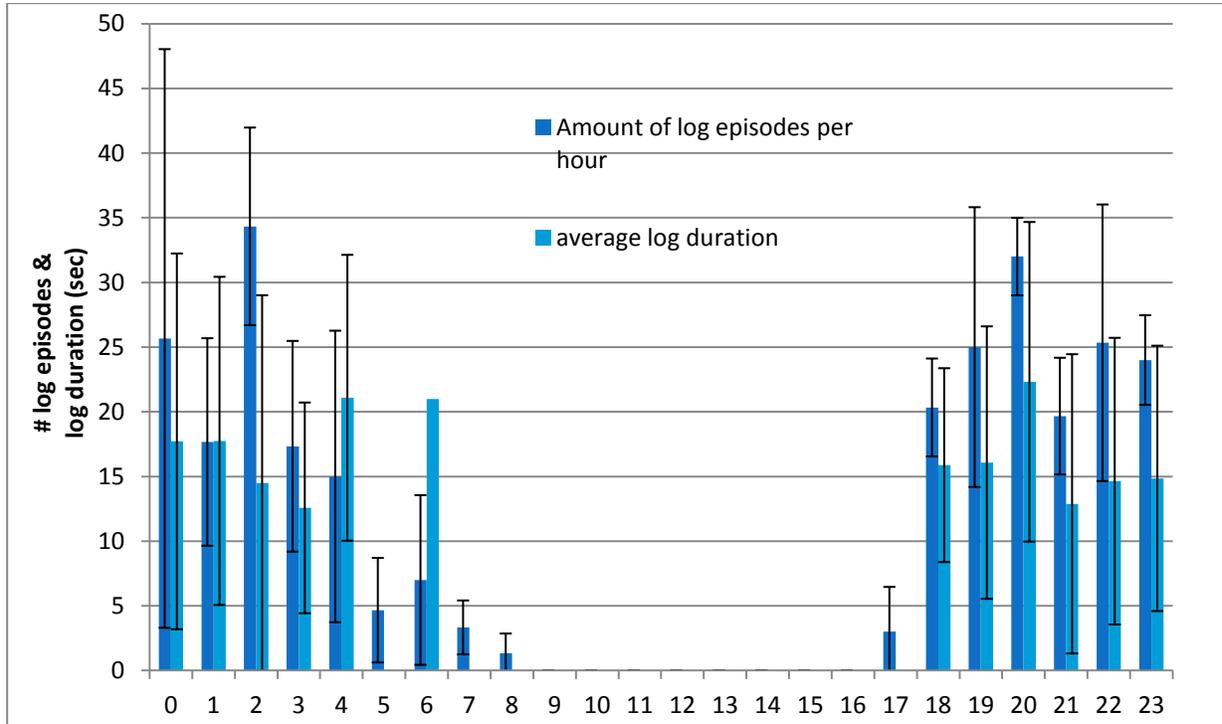
**Figure 7** Logging duration (in seconds) set to prior circular swimming/rest behavior (in minutes) (n=210). There is no correlation between the logging duration and time of SCS prior to logging. There is no correlation between logging duration and the individual dolphin, age or time of night.

n=827). Therefore we assume that SCS is a form of pre-sleep that must be fulfilled prior to starting logging (presumably full sleep) behavior. Because of this notion, we assumed that the duration of the SCS preceding the logging behavior has an effect on the duration of the logging behavior as well. If not, we expected at least to find a SCS duration which correlated with the onset of a logging episode. We do not expect this prior SCS duration to be identical between the dolphins, however we did expect to find a pattern for the individuals.

We calculated duration of slow circular swimming prior to log with the 24-hour observations. This prior SCS is the duration the dolphin spent in SCS behavioral state before switching to logging behavior.

There is no correlation between prior SCS behavior and logging duration (**Figure 7**). Nor is there a correlation between logging events and time of day. Although logging behavior was only recorded during low-activity hours (19:00-05:00), thus limited to the lowest activity hours. There appears to be a slight difference between the amount of logging behaviors from 18:00-23:00 and 0:00 – 05:00 (**Figure 8**). The dolphins might log more often during the beginning of the night as opposed to near the end of the night. Although there is a slight increase in logging episodes at 02:00. This is significantly different from the hour before and after it. What causes this sudden increase has yet to be determined. There is no correlation between dolphin (neither age nor sex) and the duration of logging behavior. Overall, average duration of a logging episode is  $15.0 \pm 9.8$  seconds.

Most of the recorded logging behavior (69%) occurred within 0 to 12 minutes of prior SCS durations (**Figure 7 & Appendix III**).



**Figure 8** The total amount of logging behavior episodes per hour averaged for the three measured days. Hours were rounded up, 00:05 – 01:00 = 1 etc. Combined with average log duration per hour. Only one log episode duration was measured at hour 6. Amount of logging durations measured, n = 42, 16, 8, 28, 40, 27, 22, 44, 47, 61 for hours 0, 1, 2, 3, 4, 6, 18, 19, 20, 21, 22 & 23 respectively.

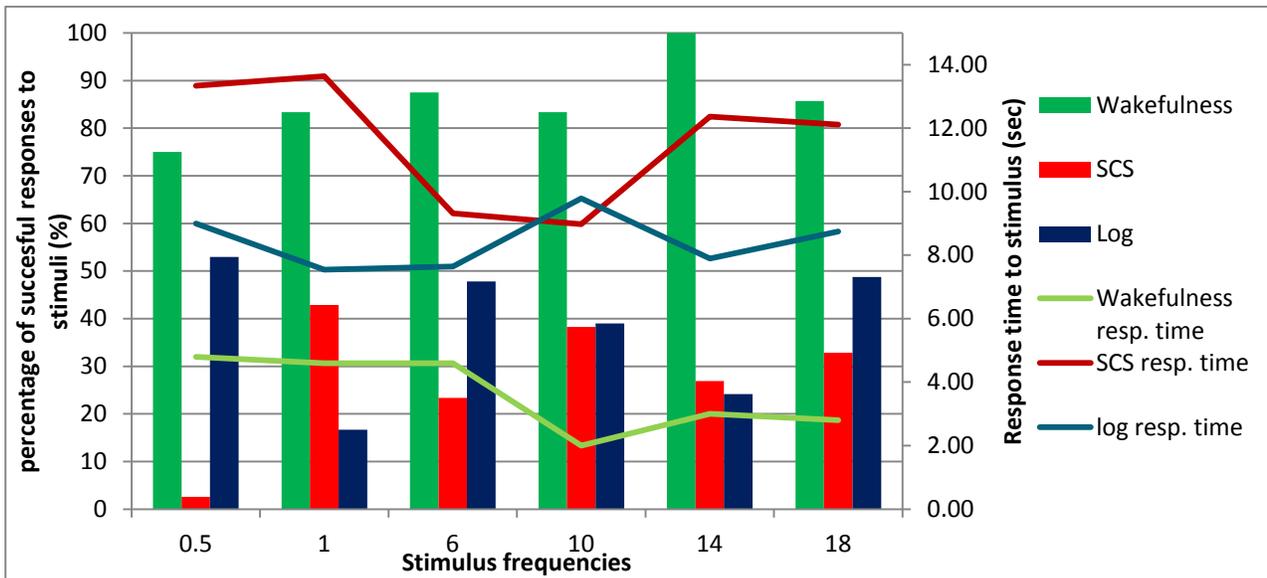
Our low-activity time, assuming logging behavior is a form of rest and thus low in activity, lies between 17:00 and 08:00. This coincides with the activity hours of the Dolphin Academy. During these hours it is nearly impossible for the dolphins to exhibit resting behavior because it is a part of a scheduled program. At hour 2 there appears to be the most amount of logging episodes, however the duration of these logs varies the most out of all observational hours. There is a decreasing trend visible from hour 2 onwards to hour 8 in the amount of logging episodes. Average logging duration appears to be relatively even throughout the night, there are no significant differences between the different hours.

### Arousal threshold measurements

Our 24 hour observations revealed highest inactivity levels ( $\geq 75\%$ ) between 19:00 and 01:00 (**Figure 4 & 5**). Thus this is the perfect time to measure arousal threshold in our captive bottlenose dolphins (n=13).

The dolphins exhibiting logging behavior and were non-responsive to the arousal stimuli

have, on average, longer logging durations than those with logging behavior that did respond ( $p < 0.05$ , average logging duration,  $10.7 \pm 9.5$  and  $18.5 \pm 14.2$  seconds respectively). Thus the auditory stimulus had an effect on their logging duration, we initiated an arousal response and that the dolphins were not awakened by an internal cue instead.



**Figure 9.** The percentage of responses per activity (Wakefulness (without human interaction), Slow Circular Swimming & Logging behavior; green, red and blue bars resp.) set to the six auditory stimuli frequencies. The n differs per behavioral state and stimulus (see **Table 3**).

Response time to stimulus (for wakefulness, SCS and logging; green, red and blue lines resp.) set on the secondary axis (black line). The response time to stimulus accounts only for a dolphin who responded to the stimulus. Those that did not respond are only included in the analysis of percentage of responses to stimuli.

The percentage represents the ratio of responses per auditory stimulus. There is a significant difference between the response ratio's of wakefulness compared to both SCS and logging behavior. The response time of a wakeful individual is also significantly lower than that of an individual in SCS or logging position. The average response ratio of a dolphin in wakefulness is 84.4 %, compared to 29.5 & 37.0% for SCS and logging respectively.

Auditory stimulus 0.5 kHz represents both the highest and lowest response percentages of logging and SCS behavior, resp. The opposite is true for 1 kHz. Both 10 and 14 kHz have similar response percentages in both behavioral states. Overall logging duration is constant for frequencies 6, 10 and 14 kHz, but is significantly lower at 1 kHz. Thus when the dolphins did respond to 1 kHz during logging behavior they responded quicker than to any other stimulus (t-test,  $p < 0.05$ ).

The 0.5 kHz stimulus evokes the most response when the dolphins are logging and the least when they are performing SCS (with response percentages: 52.9 & 2.6% respectively, **Figure 9**). The opposite is true for the 1 kHz stimulus evokes the most response when the dolphins are in SCS, and the least response when the dolphin is logging (with corresponding percentages of 42.9 & 16.7% respectively, **Figure 9**). If the logging dolphins responded to the arousal stimuli they exhibited a quicker response than the dolphins in SCS to the arousal stimuli,  $8.4 \pm 3.8$  seconds and  $11.8 \pm 4.0$  seconds respectively (t-test,  $p < 0.05$ ).

There were two types of clicking frequencies registered during their wakeful and SCS behavior. There was a lower frequency, which averaged at  $0.44 \text{ kHz} \pm 0.075$  (at 29.5 dB), and a higher frequency which averaged at  $1.92 \text{ kHz} \pm 0.90$  (at 7.4 dB). The lower frequencies were significantly higher in volume compared to the higher frequencies. No further analysis can be done, with our current data set, on the differences between the two behaviors and their clicking sounds. The dolphin did not always produce these sounds after being awakened via stimulus. However, only through a few observations, they did seem to prefer to use their lower range clicks (0.44 kHz) when they approached the speaker.

Dolphins	0.5 kHz	1 kHz	6 kHz	10kHz	14kHz	18kHz	Overall
<b>Annie</b>	1/1	0/0	0/0	0/0	1/1	1/1	3/3 (1.00)
	9/0	17/7	9/0	5/1	8/2	12/5	50/15 (0.30)
	5/1	3/1	6/2	2/0	4/4	2/1	22/9 (0.41)
<b>DeeDee</b>	1/1	0/0	1/1	1/0	0/0	0/0	2/3 (0.67)
	8/2	11/4	7/3	5/3	6/3	5/1	36/16 (0.44)
	7/4	7/4	8/3	5/3	3/1	4/2	34/17 (0.50)
<b>GeeGee</b>	1/0	1/0	1/1	1/1	1/1	1/0	3/6 (0.50)
	11/0	15/5	12/1	13/3	8/1	14/4	73/14 (0.19)
	7/0	10/5	11/2	6/4	5/2	10/5	49/18 (0.37)
<b>Machu</b>	1/1	0/0	0/0	0/0	1/1	0/0	2/2 (1.00)
	9/0	18/8	9/0	6/2	8/2	12/5	62/17 (0.27)
	1/0	0/0	2/1	0/0	1/1	1/1	5/3 (0.60)
<b>Pasku</b>	2/1	1/1	2/2	1/1	1/1	1/1	8/7 (0.88)
	12/0	19/8	12/2	13/4	7/1	13/4	76/19 (0.25)
	4/0	4/2	5/0	4/2	4/2	4/1	25/7 (0.28)
<b>Ritina</b>	1/1	0/0	1/1	1/1	0/0	0/0	3/3 (1.00)
	7/1	14/6	5/1	5/2	5/2	5/1	41/13 (0.32)
	11/1	8/4	10/2	5/2	8/4	7/4	49/17 (0.35)
<b>Roxette</b>	1/1	1/1	1/1	0/0	1/1	0/0	4/4 (1.00)
	12/0	21/9	14/6	15/8	11/5	14/5	87/33 (0.38)
	1/0	0/0	2/1	5/3	1/0	0/0	9/4 (0.44)
<b>Serena</b>	1/1	1/1	0/0	1/1	0/0	0/0	3/3 (1.00)
	12/0	24/12	14/4	13/5	8/1	14/5	85/27 (0.32)
	0/0	1/1	0/0	1/0	1/1	0/0	3/2 (0.67)
<b>Tela</b>	1/0	1/1	0/0	1/1	0/0	1/1	4/3 (0.75)
	12/0	24/12	14/4	13/5	8/1	14/5	85/27 (0.32)
	1/0	2/1	3/0	5/1	3/1	1/0	15/3 (0.20)
<b>Tikal</b>	1/1	1/1	1/1	0/0	0/0	2/2	5/5 (1.00)
	12/0	16/6	12/2	10/2	8/1	12/3	70/14 (0.20)
	5/1	10/4	10/2	6/4	3/1	11/2	45/13 (0.29)
<b>Ukit</b>	1/1	1/0	1/0	0/0	1/1	1/1	5/3 (0.60)
	12/0	21/9	14/6	15/8	11/5	15/6	88/34 (0.39)
	1/0	0/0	1/1	2/1	1/1	0/0	5/3 (0.60)

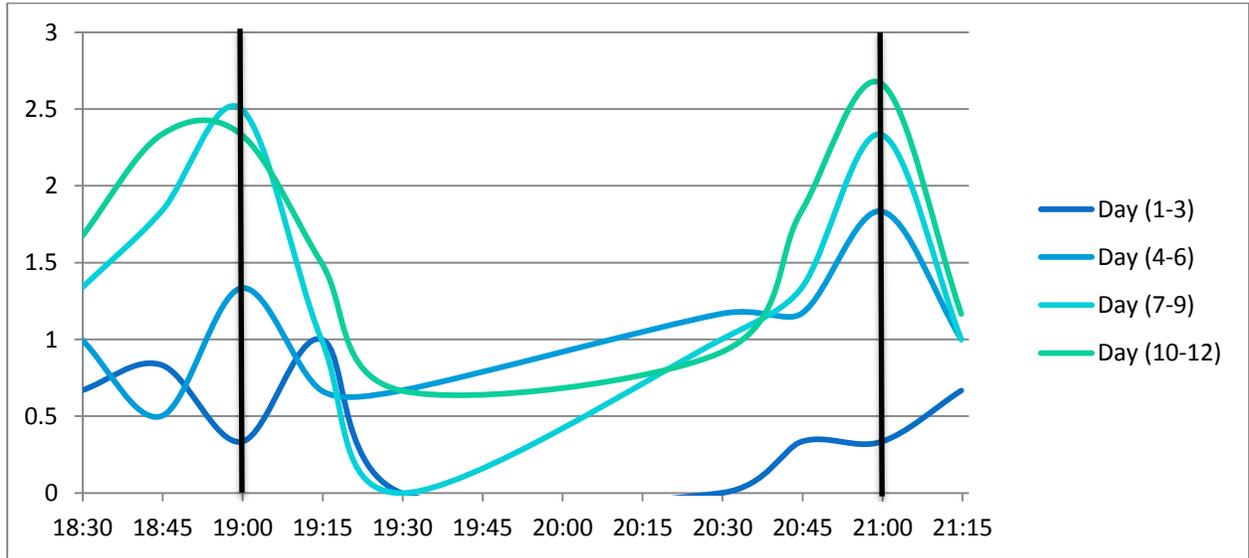
**Table 3.** Number of times stimuli applied/amount of responses per dolphin, top row shows wakeful (control) data, middle row shows SCS data and bottom row shows logging data. Though, ideally, every dolphin would have been stimulated an equal amount of times, this is not the case for our current data set. Wakeful stimulation events per stimulus type (0.5, 1, 6, 10, 14 & 18 kHz resp.) n= 12,6,8,6,6,7 (45 stimulation events in total) Stimulation events for SCS and logging behavior resp. n=117, 203,124, 115, 93, 137 (789 stimulation events in total) & n=34, 42, 46, 41, 58, 41 (stimulation events in total).

Dolphin whistles are complicated multi-level sounds. A whistle could consist of multiple frequencies which varied between 6 and 18 kHz. The higher frequencies were perceived to have a higher volume. On average, whistles were  $8.41 \text{ kHz} \pm 3.45 \text{ kHz}$  at 11.5 dB.

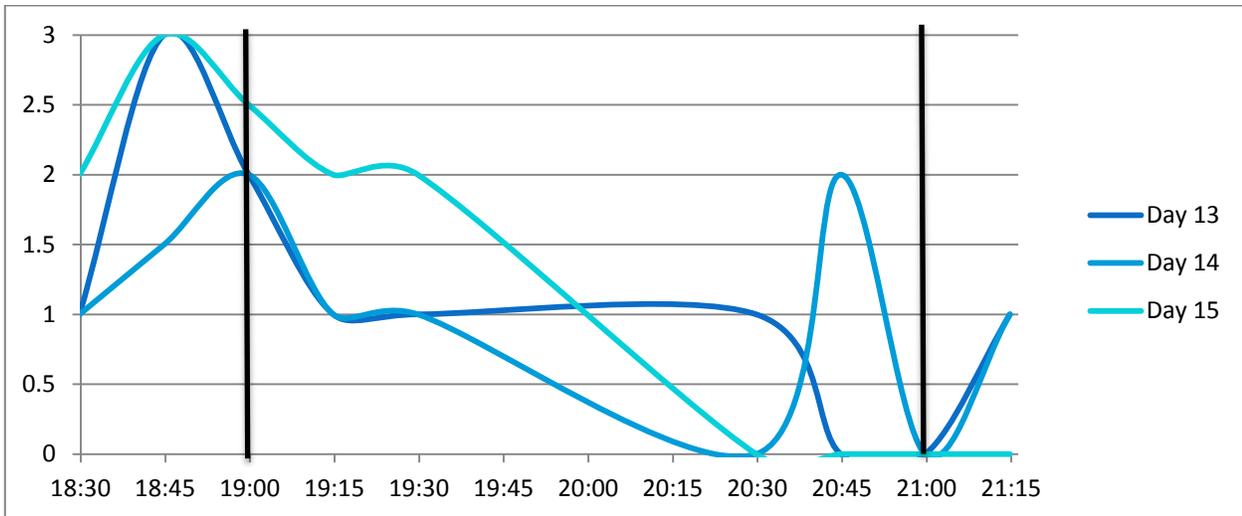
Because the dolphins are in a 'natural' pool, many creatures inhabit the area as well. Especially the snapping shrimp (*Alphaidae spp.*) caused for a great deal of the background noise. Other contributors were e.g. wind and crabs. Overall average background noise resulted in a pitch of 0.55 kHz at 28.6 dB. These noisy contributors, however, were much closer to the hydrophone than the dolphins, thus it is very

likely that their sound was recorded much louder than what the dolphins were exposed to. How great this increase in sound was, has not been taken into account.

*Time and Place association*



**Figure 10.** Anticipatory behavior set out to time. Results averaged per 3 days (t-test  $p > 0.05$ ). The two black lines show the feeding moments during these nights, 19:00 at the rock and 21:00 at the bridge. First 3 nights show very little anticipatory behavior, Nights 4-9 show strong anticipatory behavior. There is a significant difference between the first 3 nights and the rest of the observational nights. The dolphins needed at least 3 nights of fixed feeding before they started to show signs of anticipatory behavior. The first night of non-feeding (day 12) is not significantly different from night 10 and 11. This proves that the presence of the trainer did not work as a cue for the dolphins.



**Figure 11.** Anticipatory behavior averaged for the two dolphins per day, set out to time. The two black lines show the 'trainers' previously 'trained' feeding times. They were not fed during these three days. For all three days there appears to be moderate to relatively present anticipatory behavior prior to the supposed 19:00 feeding time. This effect has nearly disappeared and only shows a small peak of anticipatory behavior during night 14, at 21:00 feeding time. Though there is a peak present at 20:45 on day 14, the second night of non-feeding. What caused this is uncertain, and might be due to another environmental stimuli. Their wakefulness only lasted for maximum of 15 minutes after which they continued with resting. Their behavior on day 14 is still very different from those of days 1 to 12.

There is an overall increase in activity found as anticipatory experiment continues. On the first day the dolphins were in SCS state. As the experiment progressed the dolphins switched to actively moving through the pool, playing/fighting and watching/spying their area (**Figure 10**). However, the second night of non-feeding resulted in the dolphins no longer searching for food after 19:30 (**Figure 11**). These days show similar low levels of activity/searching behavior, at 21:00, as they did during the first 3 days of the experiment. Unfortunately, calculating exact maximum volume is not available. Our applied stimuli varied strongly in perceived dB over time (as recorded with the hydrophone). And unfortunately, not all stimuli were recorded. In some cases stimulus volume would increase one second and decrease the following second, in other cases the volume would hit a platform within 10 seconds of stimulation. Whether this is due to the set-up or equipment failure, we do not know. However results show that the dolphins wake within a certain window of time (between 7.6 & 9.8 seconds) thus we've assumed that the inconsistency in volume does not affect the outcome of the arousal threshold.

## Discussion

### 24-hour observations

Comparing the two years, 2013 & 2014 (**Figure 5**), concludes that the dolphins have begun resting earlier and longer in 2014, even although both years have been tested in the same period of time (end of February and beginning of March). This shift in time might be caused by environmental factors/-cues or it is possible that their resting patterns are subject to continuous change. There have been no changes in daily routines of the DAC nor of their programs. Temperature and seasonality have not been recorded during both the studied years. In order to document this, further research is required. It remains a possibility that, the differences in their rest-wake cycle between the two years, is due to

observer bias. 2013 was only observed by one, while 2014 is a combination of two observers. However, the shift of when resting starts is apparent over all the observed dolphins. Neither one of the observers had fixed dolphins during the experiment. The observers switched pools per day and time of day. Thus I would not expect a shift in onset of rest for all of the dolphins, if this was only caused by observer bias.

Average resting duration does not differ significantly between the consecutive nights thus we averaged the three nights to show their overall rest-activity pattern for a 24 hour period (**Figure 4**). Because they show a clear rest/wake pattern it emphasizes the necessity of the rest behavior. This leads to conclude that their rest is in fact sleep.

*Sekiguchi & Kohshima* concluded that 86.6 % of the time their resting behaviors were observed during their set 'low-activity time' (from 01:00 to 03:00). However they also recorded their resting behaviors 38.5% of the high-activity time (12:00 to 16:00). That is a significant amount of rest behavior measured during the non-resting time of the day. Unfortunately, they do not mention the schedule of the captive bottlenose dolphins, and thus if they are subject to training or shows or anything else that might have an effect on their daily resting behavior. Their observation stands in contrast to our observations, because none of this was recorded with our captive bottlenose dolphins. However, our dolphins were busy during those 'high'-activity' hours and might therefore be unable to rest during that time. Perhaps our dolphins increase their rest time during the night in order to compensate for the lack of rest during the day? Or the dolphins of *Sekiguchi & Kohshima* are not stimulated enough and opt for sleep whereas they would, under natural circumstances, exhibit wakefulness.

Of the calves in 2013 (Machu, Serena and Alita) all have increased in rest duration in 2014 (**Figure 4**). This trend, however, is also present in Pasku and Romeo who both are

mature dolphins (5 & 8 years respectively). Our data can not conclude why they experience a significant increase in sleep duration. Location is not an issue, because both dolphins were in the main pool during the experiments, this is similar to last year. When comparing the activity/rest cycle of Romeo and Ritina, it shows a similar pattern. Ritina has increased rest behavior, but both show inactivity at the same time. Perhaps Romeo attuned himself more to his female, he impregnated Ritina (she's due in July). But neither of these account for Pasku's increase in sleep duration. An extra sleep study regarding individual sleep behavior would be necessary to shed light on this subject.

From all the calves the youngest calf, Ukit, varies the most in complete activity/rest cycle compared to her mother (**Figure 4A**). Serena is the eldest calf and fits almost perfectly with her mothers' resting pattern. It is expected that as the calves grow older, they start maturing and perhaps fitting themselves to their mothers' resting cycle. Last year observations also show varying cycles for mothers and calves. The calves are much more active throughout the night. As the calves age, they continue to shape their rest cycles according to their mothers.

Overall, rest duration slightly increased in 2014 (519±118 & 587±78 min. for 2013 & 2014 respectively). There is a significant difference in duration and amount of resting bouts between 2014 and 2013 (t-test,  $p < 0.05$ ). The dolphins had fewer but longer resting bouts compared to 2013 (**Table 2 & Appendix VI**). There were three calves in 2013, all under the age of 1 year old. Removing the calves out of the analysis minimally reduces the amount of resting bouts and increases resting duration. There is still a significant difference between 2013 and 2014.

*Sekiguchi & Kohshima 2003* concluded significantly longer logging (they refer to it as surface rest) duration when compared to our data. Their results varied from 5 seconds to

55 minutes (12.75 min ± 20.25 and n = 290), our data averaged at 15.0 seconds ± 9.8 (n=210, **Figure 7**). They did not reveal the average amount of log episodes during one night, thus it might be that they exhibited far fewer logging episodes than our bottlenose dolphins, which results in different overall logging behavior. This might, in part, be due to differences in criteria, or perhaps we are dealing with significant differences in rest behavior between these two dolphin groups. *Sekiguchi & Kohshima* used different criteria for rest recognition/determination. They acknowledged 3 types of resting behavior, while we only found 2 types.

Their resting behaviors were: **Bottom rest** (long immobile stay on the bottom), **surface rest** (long immobile stay at the water surface, similar to our logging) and **swim-rest** (slow circle swim near the bottom along a fixed trajectory, comparable to our slow circular swimming). Both their bottom rest and surface rest suggest something similar to our logging behavior (although our behavior only occurred on the surface). Their bottom rest, however, matches our logging duration more correctly (2.23 min ± 1.61 and n = 189). This behavior is still significantly longer than our data suggests. Why do their dolphins exhibit bottom rest, while our dolphins have nothing like this? And how is it possible that their actual surface resting durations are a factor longer than our logging durations? It might be due to the Dolphin Academy's environment that the animals experience a more natural setting and perhaps show a more natural cycle of rest. We will need more studies like this, occurring in both natural and artificial type tank set-ups, in order to establish if there is a difference between the two.

Logging episode durations prove to be even more enigmatic as they show no correlation with the individual dolphin, age, time of day or prior SCS duration. The duration of a logging episode might be of matter of some internal cue, however this remains unanswered with or current data. Perhaps logging episodes and duration occur at random? This seems in contrast with current

beliefs regarding sleep. If an aspect of sleep is to occur at random, this would imply that this aspect has a trivial function, which can be avoided. Although the dolphins commit to this type of rest every night and continue to do so (highest amount of logging durations occur between 20:00 and 01:00).

The environment, however, does seem to impact their logging behavior. Visual observations of the dolphins during windy nights show that they commit far fewer logging behaviors. Also when they log, the logging durations last for a shorter period of time. Thus due to 'extreme' weather/ocean conditions, they are more reluctant to log. Although more data is necessary to confirm this hypothesis.

There is also this interesting aspect of a lone logging dolphin joining a group that just passes it, putting its log to a halt. This observation will need more back-up for further conclusions to be drawn. However, so far, it seems that the logging dolphin is waiting for another group to pass which he/she joins in SCS. When a group does not pass the logging dolphin it seems to log for a much longer period of time. However, again, this is a mere observation and would need exploratory work before concluding anything out of it.

*Stafne & Manger 2004* categorized their resting behaviors as; counterclockwise swimming, clockwise swimming, counterclockwise hanging and clockwise hanging. They stated that clockwise swimming lasted, on average, 44.7 seconds. This varied from 32.9 – 52.1 seconds between individuals. Overall they found that the dolphins spend 41.1 seconds on any given behavior before switching to the next one.

The dolphins at DAC, on the other hand, were capable of continuing their circular swimming behavior for over hours at a time. Average circular swimming bout lasted  $51.1 \pm 65.2$  minutes (**Table 2**). This duration coincides more with the study performed by *Mukhametov et al. 1988*. They concluded that USWS episodes lasted 42.5 minutes (with a range of 3.5 – 131.5 minutes). It is staggering

that multiple articles show multiple possible outcomes, some so distinctly different from one another. Arguably, all these differences are caused by location and environment, rather than the species, the bottlenose dolphin.

*Stafne & Manger 2004* report a link between breathing and change in behavior. With every breath the dolphin took it would change its' behavior. Again, this aspect is very different from what we observed, namely, synchronized breathing during SCS. They show no signs of changing their behavior, and our behavioral analysis implies that they are still resting. Such stark difference between two studies on the same species of dolphin suggests that rest cycles are perhaps very different between groups and subject to change. The dolphins of *Stafne & Manger 2004* are also in an artificial tank, as opposed to our natural tank. Might it be that this difference causes the dolphins to rest differently? Would the DA dolphins exhibit more natural sleep? We definitely need more studies regarding captive bottlenose sleep, perhaps their resting behavior is a fluid one and easily adapted to its environment.

### **Arousal threshold**

We hypothesized that logging behavior is a deeper form of rest when compared to SCS. Predominantly because *Mukhametov 1985* found similar behavioral pattern when a dolphin was drugged to sleep as a logging dolphin. Combined with the lack of breathing occurring during a logging episode, led to believe it to be more of a shutdown mechanism as opposed to the clear uni-hemispheric sleep type SCS.

The results, however, conclude that the logging dolphins responded more often to the presented arousal stimuli than SCS dolphins (37.0% and 29.5% response rate respectively, **Figure 9**). The logging dolphin, which simply hangs in the water column, might be more alert to its surroundings than a seemingly wakeful dolphin in SCS.

The dolphins in SCS were continuously checking their environment with their echolocation (observation through sound

analysis, because of the relative distance between a logging dolphin and the hydrophone it remains uncertain whether they perform echolocating clicks during their logging behavior). There are records for wakeful echolocation sounds of the dolphins, thus far they seem to be similar to the sounds produced by an apparently resting dolphin.

Considering logging behavior only occurred after the presence of SCS, predominately after 6 to 13 minutes of prior SCS behavior (**Appendix III**). It was hypothesized that SCS might be a precursor to logging behavior, presumably the deepest form of sleep. Similar to *Duboué et al. 2011*, they concluded that their zebrafish need more than 60 seconds of prior inactivity before falling asleep. Which they determined by a significant increase in arousal threshold after 60 seconds of inactivity. The dolphins do not show a correlation between previously circular swimming time and stimulation duration (arousal threshold). Neither is there a correlation between SCS duration prior to the logging behavior and the duration of the log (**Figure 5**). Thus far, it seems that both their SCS duration, logging episode and depth (or rather stimulus responsiveness) are random.

The 1 kHz stimulus yields the shortest logging duration, although this stimulus is also the least response inducing stimulus, thus if the dolphins responded, they responded quicker than other stimuli (**Figure 10**). Only responsive dolphins' logging duration was taken into account.

10 kHz has, on average, the longest logging duration (9.8 seconds) but shows a high response rate when applied (39% of all the 10 kHz stimulations applied).

Logging dolphins are more attuned to 0.5 kHz than SCS dolphins (opposite is true as well, **Figure 9**). A dolphin using echolocation transmits a sound of varying frequencies (0.3 – 1.5 kHz), the most prominent (in volume) one is at around 0.44 kHz. And on numerous occasions we observed the logging dolphin to terminate its log right when a

group/individual dolphin swam past it. The dolphin in question would join in and continue SCS with them. This might explain their preference for certain stimuli frequencies. The exact reason for them to react so strongly to the 18 kHz stimuli has yet to be determined.

The whistles of the dolphin, which were recorded during these sessions, were extremely dense in pitches. Some of the whistles contained over 5 different strands of pitch. These whistles varied between 6 and 18 kHz, our applied arousal threshold stimuli were mostly within this range, with 0.5 kHz and 1 kHz being the exceptions. The whistles were far more complex than our applied stimuli, but perhaps certain pitches are more susceptible to the sleeping dolphin than others.

The logging dolphins react more often to the provided stimuli compared to dolphins in SCS. This is not due to speaker/stimuli distance, because the logging dolphin was further removed from the speaker than the SCS dolphin.

It was impossible to keep the distance between the two types of rest identical. The foremost reason for that was due to their logging behavior. This would take place at either the center of the pool or, sporadically, near the catwalk. Whether the logging dolphins were present in the center or near the catwalk during the arousal stimulation had no effect on their reaction (or reaction duration).

The logging dolphin also reacted sooner to applied stimuli, compared to a dolphin in SCS. The applied stimuli all increase in volume over time, thus the dolphins in SCS require louder volumes of similar stimuli to show a response (**Figure 9**).

Unfortunately the direction of the dolphins head (with respect to the underwater speaker) was not noted during the experiment. *Bullock et al. 1968* stated the importance of the bottlenose dolphins' melon and lower jaw for receiving sound (required for their echolocation). Perhaps a dolphin

facing the other direction would be less keen to catch the auditory stimulus. Future research would be well to include position of the head relative to their arousal stimulus.

During the 24-hour behavioral observations, the dolphins showing SCS had slow but steady breathing frequencies. During the logging behavior, dolphins seized breathing all together (or when logging durations superseded 40 seconds, took a single breath of air, visual observation only).

This decrease in breathing frequency coincides with the conclusion of *Mukhametov & Supin 1974*, *Sekiguchi & Kohshima 2003*, However, as our current results show, the dolphins exhibiting logging behavior were not necessarily showing an increased arousal threshold. They reacted sooner and more often than dolphins exhibiting SCS. Whether we can conclude that their theory of breathing frequency and depth of sleep is incorrect, is yet to be determined.

There is too little data per dolphin, especially during their wakeful state, to establish individual differences in arousal threshold. We are unable to conclude whether the individuality has an effect on their arousability and hence the outcome of our analysis. There is a trend visible with regards to the 'responsiveness' of the animals (as seen in **Table 3**). The animals respond more often when in a logging state, but there are already a few exceptions. Overall we can conclude a great significant difference between the two states and the states compared to wakefulness (t.test  $p < 0.05$ ). Although further study is required to examine the effect of individuals responsiveness.

Dividing the group into three age classes 0 – 2 years (n=3), 5 – 9 years (n=4) and 13-30 years (n=4), their patterns look similar compared to analysis without taking age class into account. All age classes show no to very little amount of response to the 0.5 kHz stimulus and high amount of response to the 1 kHz when in SCS (**Appendix IV**). It appears, from our analysis, that 1 kHz invokes a

stronger response than the 0.5 kHz. What the exact reason is for this phenomena, has yet to be established.

### *Anticipatory feeding*

It seems that there is a large individual difference. In all cases, Caijo seems to be picking up the fact that there is food involved and that there is a certain place attached to that reward. Both dolphins started looking for food well before actual feeding time. And in cases of no reward, they continued looking well after this feeding time was supposed to occur.

Of the two feeding times, the rock at 19:00, seems to be the most distinct moment of the day. It is just before the sun sets, a clear visual for the dolphins to use as a time indicator. The 21:00 (bridge) feeding time is during full darkness thus less visual feedback for them to rely on. Overall there is very little temperature variation throughout the day and night. After the sun sets the dolphins rely fully on their internal clockwork for any time guidance. Even so, the dolphins handled this remarkably well (**Figure 10**). They learned both times within the same amount of time. It seems that the sunset might be a very visual indicator, however not necessary for them to use.

Observations also show that the dolphins were more reluctant to search for prolonged periods of time after 21:00 (when no food was provided). It seems that they are eager to continue their resting behavior, normally they would be in SCS for a few hours. We will need more research to conclude whether that is truly the case. For instance add an extra feeding time, at 23:00. If they continue to show a sudden decrease in activity (within 15 minutes) at both 21:00 and 23:00, then perhaps their rest is more essential then we assumed before.

Both feeding areas were relatively close by, thus the dolphins had the opportunity to swim back and forth between the two feeding areas. Perhaps in future studies they should focus on doing this in separate areas. Whereas it would take them a relative

amount of time to go from feeding site 1 to feeding site 2.

The anticipatory behavior for food was soon present in their rhythm. The first three nights show very little searching activity or any type of anticipatory behavior. However the 4<sup>th</sup> – 6<sup>th</sup> night show a clear increase in activity levels over the entire measurement, as well as increased levels at 19:00 and 21:00. They remain relatively active after the 19:00 feeding. This is interesting, because the 7<sup>th</sup> until the 9<sup>th</sup> night there is a visible decrease in activity right after the 19:00 feeding time. It seems that they were ‘perfecting’ their behavior accordingly. This however suggests that their rest is essential and that they need to optimize this to fit their resting schedule.

The first ‘food’-restricted night was on the 12<sup>th</sup>, looking back at the gathered data, this night could have taken place sooner. The 4<sup>th</sup> until the 6<sup>th</sup> night they are already exhibiting higher activity levels, especially around the feeding times. The dolphins learned the anticipatory relatively quick but then ‘forgot’ this behavior even quicker. After the first food-restricted night, the following night the dolphins were much less alert. They did respond relatively soon to the presence of a trainer, but no longer displayed the spying behavior as seen during previous nights.

The potential cues that might cause the dolphin to sense a meal is coming, were eradicated as much as possible. The buckets were prepared beforehand, thus we only required the trainer to come in and grab the bucket. Then, according to the feeding time, they would come in either from the left side of the pool or right side of the pool. This does provide a cue for the dolphins,. However, if the dolphins were exhibiting spying behavior/anticipatory behavior beforehand, they could see the trainer come their way. If, however, they were exhibiting spy behavior in the wrong direction, they might miss the trainer arriving. This allowed us to establish whether they look in the right direction at the right time. This allows us to differentiate

between anticipatory behavior and enhanced anticipatory behavior.

### *Individual factor*

Ritina is pregnant and due to deliver in July. Her pregnancy could affect her ‘rest’ and ‘activity’ pattern, as well as any responses to arousal threshold stimuli. The same goes for DeeDee, who is due in December.

*Sekiguchi & Kohshima 2003* concluded that mothers change their resting behavior at least 12 weeks prior to delivery. This behavior continues up until 12 weeks after delivery. This explains why Ritina has one of the highest resting duration (**Figure 5, Table 2**). Her resting duration is not much higher when compared to her previous year (10.9 & 9.9 hours for 2014 & 2013 respectively). So it is questionable how much effect her pregnancy had on her resting behavior.

Ritina did not have increased arousal threshold levels, when compared to the other dolphins, thus, presumably her pregnancy didn’t affect the ‘depth’ of her rest.

There is no clear correlation between age and resting duration, however the data shows GeeGee (30 years, eldest dolphin) has the longest resting duration and the youngest dolphins; Ukit, Machu, Serena & Alita, have the shortest resting duration.

### *Mammalian sleep criteria applied to captive bottlenose dolphins*

Homeostatic regulation is the most difficult criteria to recognize. The animal in question could either be sleeping for longer periods of time, to regain lost sleep, or it could increase its sleep depth.

Arousal threshold stimuli should be able to conclude whether they increase their sleep depth or not. Our data, however, was inconclusive. There were two nights at which arousal threshold was significantly higher, but whether this proves the presence of sleep rebound, or this effect is due to environmental factors (e.g. increase in wind) remains to be examined.

Although the main characteristic of SCS is swimming, it is clearly a less active behavior compared to a wakeful dolphin. I therefore suggest that, in the case of uni-hemispheric sleeping mammals, immobility should be rephrased to decreased activity. Dolphins in SCS were non responsive to observers passing their pool. This is distinctively different from daily/wakeful behavior. If then an observer passed the pool the dolphin would immediately respond and swim towards the observer.

The reason why SCS occurred more often and at longer intervals, than logging, has yet to be determined. It seems that the dolphins need the logging to switch their resting brain hemisphere. This process however, seems completely random, as there is no correlation with time of day, age, duration or pre-SCS duration. Further investigation into this process is necessary.

**Conclusion**

*With the help of mammalian behavioral sleep criteria, can we conclude dolphins to sleep?*

So far, (most) mammalian behavioral sleep criteria apply to a dolphin in observed rest. I conclude that our captive bottlenose dolphins do sleep and that they have two types of sleep, SCS and logging.

	<b>Stage 3 (Slow circular swimming)</b>	<b>Stage 4 (Logging)</b>
<b>Quiescence</b>	Partially true, they do exhibit echolocation clicking	True, no sounds nor breathing during logging
<b>Immobility</b>	False, slow swimming	True
<b>Reversibility</b>	True	True
<b>Circadian Rhythm</b>	True	True
<b>Increased Arousal Threshold</b>	True	True
<b>Homeostatic regulation</b>	Inconclusive	Inconclusive
<b>Posture</b>	True, it is similar to wakeful swimming only slower	True
<b>Typical place/nest</b>	True, continuous circular movements	True, either center of pool or near bridge

**Table 4** Rest/sleep behavior observed in the dolphins combined with behavioral sleep criteria and whether the criteria fit the observed behavior. Both 'stages' 3 and 4 (slow circular swimming & logging resp.) appear to fit most of the behavioral sleep criteria. The slow circular swimming, however, lacks the immobility criteria. SCS does include decreased activity, but only logging shows clear immobility.

## References

1. **Blumberg M.S., Karlsson K.A., Seelke A.M.H., Mohns E.J.** (2005) The Ontogeny of mammalian sleep: a response to Frank and Heller (2003). *Journal of Sleep Research*, Vol. 14, pp. 91-101
2. **Bullock T.H., Grinnell A.D., Ikezono E., Kameda K., Katsuki Y., Nomoto M., Sato O., Suga N., Yanagisawa K.** (1968) Electrophysiological studies of Central auditory mechanisms in Cetaceans. *Zeitschrift für vergleichende Physiologie*, Vol. 59, pp. 117-156
3. **Casagrande M., Bertini M.** (2008) Laterality of the sleep onset process: Which hemisphere goes to sleep first? *Biological Psychology*, Vol. 77, pp. 76-80
4. **Challet E., Mendoza J., Dardente H., Pévet P.** (2009) Neurogenetics of food anticipation. *European Journal of Neuroscience*, Vol. 30, pp. 1676-1687
5. **Cirelli C., Tononi G.** (2008) Is Sleep Essential? *Plos Biology*, Vol. 6, pp. 1605-1611
6. **Duboué E.R., Keene A.C., Borowsky R.L.** (2011) Evolutionary convergence on Sleep Loss in Cavefish Populations. *Current Biology*, Vol. 21, pp. 671-676.
7. **Flanigan Jr W.F.** (1974) Nocturnal behavior of captive small cetaceans I. The bottlenosed porpoise. *Sleep Research*, Vol. 3:84
8. **Flanigan Jr W.F.** (1974) Nocturnal behavior of captive small cetaceans II. The Beluga whale, *Delphinapterus leucas*. *Sleep Research*, Vol. 3:85
9. **Flanigan Jr W.F.** (1975) More nocturnal behavior of captive small cetaceans I. The Killer whale, *Orcinus orca*. *Sleep Research*, Vol. 4:139
10. **Flanigan Jr W.F.** (1975) More nocturnal behavior of captive small cetaceans II. The Pacific white-sided dolphin, *Lagenorhynchus obliquidens*. *Sleep Research*, Vol. 4:140
11. **Flanigan Jr. W.F.** (1975) More nocturnal observations of captive small cetaceans III. Further study of the Beluga whale, *Delphinapterus leucas*. *Sleep Research*, Vol. 4:141
12. **Gennaro de L., Ferrara M., Bertini M.** (2000) The relationship between frequency of rapid eye movements in REM sleep and SWS rebound. *Journal of Sleep Research*, Vol. 9, pp. 155-159
13. **Gnone G., Benoldi C., Bonsignori B., Fognani P.** (2001) Observations of rest behaviours in captive bottlenose dolphins (*Tursiops truncatus*). *Aquatic mammals*, Vol. 27, Issue 1, pp. 29-33
14. **Goley P.D.** (1999) Behavioral aspects of sleep in Pacific white-sided dolphins (*Lagenorhynchus obliquidens*). *Marine Mammal Society*, Vol. 15 (4), pp. 1054-1064
15. **Halász P., Terzano M., Parrino L., Bódizs R.** (2004) The nature of arousal in sleep. *Journal of Sleep Research*, Vol. 13, pp. 1-23
16. **Kato T., Montplaisir J.Y., Lavigne G.J.** (2004) Experimentally induced arousals during sleep: a cross-modality matching paradigm. *Journal of Sleep Research*, Vol. 13, pp. 229-238
17. **Klij v.d. M.** (unpubl.) Sleep and Circadian rhythms in the bottlenose dolphin (*Tursiops truncatus*).
18. **Lesku J.A., Roth II T.C., Rattenborg N.C., Amlaner C.J., Lima S.L.** (2009) History and future of comparative analyses in sleep research. *Neuroscience and Biobehavioral Reviews* Vol. 33, pp. 1024-1036
19. **Lilly J.C.** (1964). *Animals in aquatic environments: adaptations of mammals to the ocean*. In: Dill, D.B. (Ed.), *Handbook of Physiology—Environment*. American Physiology Society, Washington, DC, pp. 741-747
20. **Lima S.L., Rattenborg N.C.** (2007) A behavioral shutdown can make sleeping safer: a strategic perspective on the function of sleep. *Animal Behavior*, Vol. 74, pp. 189-197
21. **Lyamin O.I., Mukhametov L.M., Siegel J.M., Nazarenko E.A., Polyakova I.G., Shpak O.V.** (2002) Unihemispheric slow wave sleep and the state of the eyes in a white whale. *Behavioral Brain Research*, Vol. 129, pp. 125-129
22. **Lyamin O.I., Shpak O.V., Nazarenko E.A., Mukhametov L.M.** (2002) Muscle jerks during behavioral sleep in a beluga whale (*Delphinapterus leucas* L.). *Physiology & Behavior*, Vol. 76, pp. 265-270
23. **Lyamin O., Pryaslova J., Kosenko P., Siegel J.** (2007) Behavioral aspects of sleep in bottlenose dolphin mothers and their calves. *Physiology & Behavior* Vol. 92, pp. 725-733
24. **Lyamin O.I., Manger P.R., Ridgway S.H., Mukhametov L.M., Siegel J.M.** (2008) Cetacean sleep: An unusual form of mammalian sleep. *Neuroscience and behavioral reviews*, Vol. 32, pp. 1451-1484
25. **Madan V., Jha S.K.** (2012) Sleep alterations in mammals: Did aquatic conditions inhibit rapid eye movement sleep? *Neuroscience Bulletin*, Vol. 28, Issue 6, pp. 746-758
26. **McCormick J.G.** (1969) Relationship of sleep, respiration, and anesthesia in the porpoise: a preliminary report. *Proc. Natl. Acad. Sci. USA*, Vol 62, pp. 697-703
27. **Mukhametov, L.M., Supin A.Y.** (1974) EEG study of different behavioral states in free moving dolphin (*Tursiops truncatus*). *Journal of Higher Nervous Activity*, Vol. 25, pp. 396-401
28. **Mukhametov L.M., Supin A.Y., Polyakova I.G.** (1977) Interhemispheric asymmetry of the electroencephalographic sleep patterns in dolphins. *Brain Research*, Vol. 134, pp. 581-584
29. **Mukhametov L.M.** (1984) Sleep in Marine Mammals. *Experimental Brain Research*, Suppl. 8, pp. 227-238
30. **Mukhametov L.M.** (1985) Unihemispheric slow wave sleep in the brain of dolphins and seals. *Endogenous sleep substances and sleep regulation*, Inoué S., Borbély A.A. Publ: CRC-press. ISBN-13: 978-9067640589 . Pp. 67-75
31. **Mukhametov L.M., Oleksenko A.I., Polyakova I.G.** (1988) Quantification of ECoG stages of sleep in the

- bottlenose dolphin. *Neurophysiology*, Vol. 20, pp. 398-403
32. **Nachtigall P.E., Supin A.Y., Pawlowski J., Au W.W.L.** (2004) Temporary Threshold shifts after noise exposure in the Bottlenose dolphin (*Tursiops truncatus*) measured using evoked auditory potentials. *Marine Mammal Science*, Vol. 20, pp. 673-687
  33. **Neckelmann D., Ursin R.** (1993) Sleep stages and EEG power spectrum in Relation to Acoustical stimulus Arousal threshold in the Rat. *Sleep*, Vol. 16 (5), pp. 467-477
  34. **Nicolau M.C., Akaârîr M., Gamundí A., González J., Rial R.V.** (2000) Why we sleep: the evolutionary pathway to the mammalian sleep. *Progress in Neurobiology*, Vol. 62, pp. 379-406
  35. **Oleksenko A., Mukhametov L.M., Polyakova I.G., Supin A.Y., Kovalzon V.M.** (1992) Unihemispheric sleep deprivation in bottlenose dolphins. *Sleep Research*, Vol. 1 (1), pp. 40-44
  36. **Oleksenko A.I., Lyamin O.I.** (1996) Rest and active states in female and baby harbor porpoise. *Journal of Sleep Research* Vol. 5 (Suppl. 1), p. 318
  37. **Rattenborg N.C., Amlaner C.J., Lima S.L.** (2000) Behavioral, neurophysiological and evolutionary perspectives on uni-hemispheric sleep. *Neuroscience and Biobehavioral Reviews*, Vol. 24, pp. 817-842
  38. **Rechtschaffen A., Hauri P., Zeitlin M.** (1966) Auditory awakening thresholds in REM and NREM sleep stages. *Perceptual and Motor Skills*, Vol. 22, pp. 927-942
  39. **Reynolds III J.E., Wells R.S., Eide S.D.** (2000) *The Bottlenose dolphin: biology and conservation*. University Press of Florida, Gainesville, Florida, xv + 288p. ISBN: 0-8130-1775-0 (alk. Paper)
  40. **Rial R.V., Nicolau M.C., Gamundi A., Akaarir M., Aparicio S., Garau C., Tejada S., Roca C., Gené L., Moranta D., Esteban S.** (2007) Theoretical review: The trivial function of sleep. *Sleep Medicine Reviews*, Vol. 11, pp. 311-325
  41. **Rial R.V., Akaârîr M., Gamundí A., Nicolau C., Garau C., Aparicio S., Tejada S., Gené L., González J., De Vera L.M., Coenen A.M.L., Barceló P., Esteban S.** (2010) Review: Evolution of wakefulness, sleep and hibernation: From reptiles to mammals. *Neuroscience and Biobehavioral Reviews*, Vol. 34, pp. 1144-1160
  42. **Ridgway S.H.** (2002) Asymmetry and Symmetry in Brain Waves from Dolphin Left and Right Hemispheres: Some Observations after Anesthesia, during Quiescent Hanging Behavior, and during Visual Obstruction. *Brain Behavior and Evolution*, Vol. 60, pp. 265-274
  43. **Ridgway S., Carder D., Finneran J., Keogh M., Kamolnick T., Todd M., Goldblatt A.** (2006) Dolphin continuous auditory vigilance for five days. *The Journal of Experimental Biology*, Vol. 209, pp. 3621-3628
  44. **Ridgway S., Keogh M., Carder D., Finneran J., Kamolnick T., Todd M., Goldblatt A.** (2009) Dolphins maintain cognitive performance during 72 to 120 hours of continuous auditory vigilance. *The journal of experimental biology*, Vol. 212, pp. 1519-1527
  45. **Rihel J., Prober D.A., Schier A.F.** (2010) Monitoring Sleep and Arousal in Zebrafish. *Methods in Cell Biology*, Vol. 100, pp. 281-294
  46. **Sekiguchi Y., Kohshima S.** (2003) Resting behaviors of captive bottlenose dolphins (*Tursiops truncatus*) *Physiology and Behavior* vol. 79, pp.643-653
  47. **Siegel, J. M.** (1999) The evolution of REM sleep. In: R. Lydic and H. A. Baghdoyan (Ed.) *Handbook of behavioral state control*. CRC Press, Boca Raton, pp. 87-100
  48. **Siegel J.M.** (2005) Clues to the functions of mammalian sleep. *Nature*, Vol. 437, pp. 1264-1271
  49. **Siegel J.M.** (2008) Review: Do all animals sleep? *Trends in Neurosciences*. Vol. 31, No. 4, pp. 208-213
  50. **Stafne G.M., Manger P.R.** (2004) Predominance of clockwise swimming during rest in Southern Hemisphere dolphins. *Physiology & Behavior*, vol. 82, pp. 919-926
  51. **Tobler I.** (1995) Is sleep fundamentally different between mammalian species? *Behavioral Brain Research*, Vol. 69, pp. 35-41
  52. **Twyer H. van, Garrett W.** (1972) Arousal Threshold in the Rat Determined by "Meaningful" Stimuli. *Behavioral biology*, Vol. 7, pp. 205-215

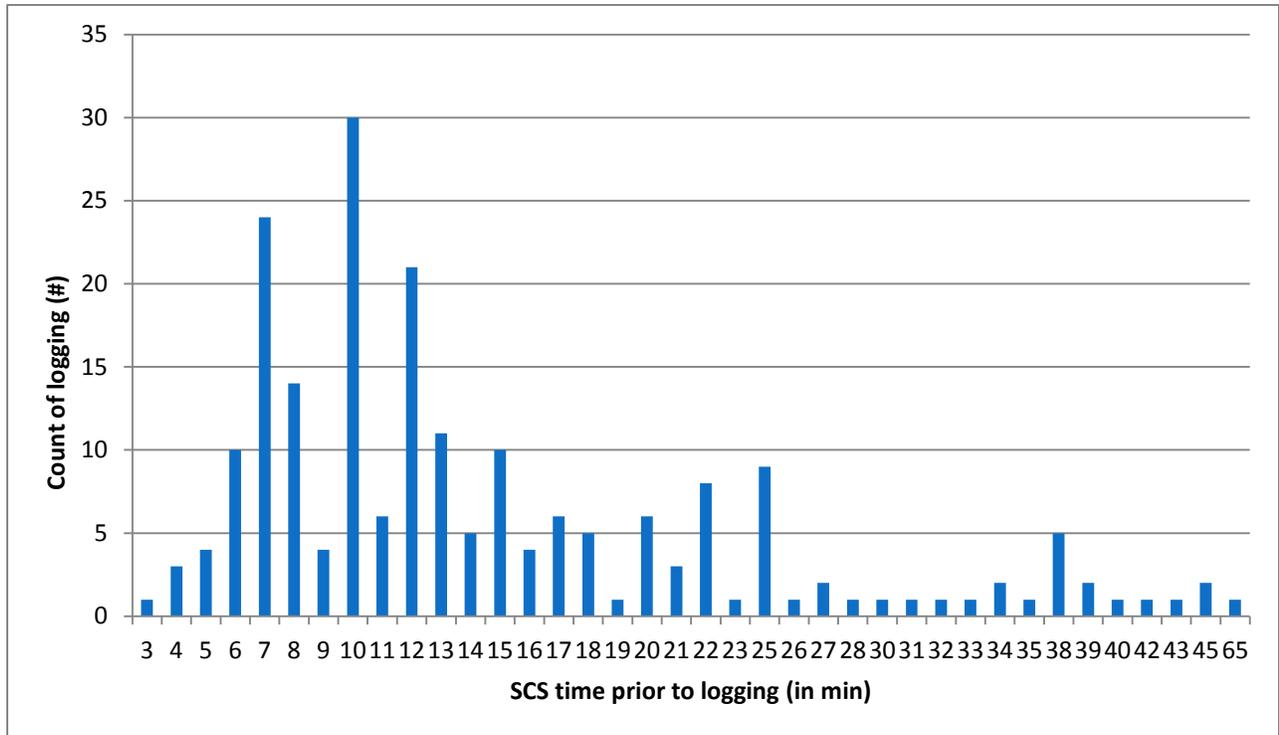
## Appendix

Day	Time 1	Time 2	Time 3
25-02-2014	2300-0200		
26-02-2014	0800-1100	1400-1700	2300-0200
27-02-2014	0800-1100	1400-1700	2300-0200
28-02-2014	0800-1100	1400-1700	
02-03-2014	1700-2000		
03-03-2014	0500-0800	1100-1400	1700-2000
04-03-2014	0500-0800	1100-1400	1700-2000
05-03-2014	0500-0800	1100-1400	
09-03-2014	2000-2300		
10-03-2014	0200-0500	2000-2300	
11-03-2014	0200-0500	2000-2300	
12-03-2014	0200-0500		

**Appendix I** The 24-hr observation analysis time schedule. One day of 24 hours was divided into 8 groups of 3 hours each, these hours were repeated over three following nights. There were always two observers present during these watches.

Dolphin	Average logging duration (sec)	N (#)	Maximum logging (sec)	Minimum logging (sec)
DeeDee	9.7±3.6	6	15	6
GeeGee	15.9±8.9	10	34	7
Annie	16.7±10.9	48	45	5
Machu	16.1±12.0	20	45	6
Tela	11.2±4.9	6	18	5
Serena	10.0±7.0	3	18	5
Ritina	15.9±6.3	15	27	8
Alita	11.2±6.2	9	22	3
Roxette	13.1±9.0	26	43	4
Ukit	13.0±10.4	8	38	6
Caijo	10.5±6.4	4	20	7
Pasku	11.6±5.3	10	22	4
Tikal	17.9±11.3	42	65	4

**Appendix II** On average, the longest logger is Tikal, with a maximum log duration of 65 seconds. In total 209 logging durations were measured, which resulted in an average logging duration of 15 ( $\pm 9.8$  seconds). The minimum duration of a log is 3 – 8 seconds. Their logging is independent of age, sex, dolphin, time of day, resting time prior to logging and pool in which the dolphin was present. Mother and calves have similar logging durations, a part from Alita and Ritina. Certain dolphins have been measured more often than others, this is due to the fact that some of the dolphins tended to log more often than others. Romeo is completely missing from this list, either he was not within the observers' visual reach for observation or he did not log during the entire experimental process. There is a remarkable large variation in log durations per dolphin, which furthermore, emphasizes the possibility of a random process.



**Appendix III** Count of logging behaviors (#) set to prior SCS time. After 25 minutes of prior SCS there is a decreasing trend in the observed number of logging behavior. Most of the logging behaviors occurred at 10 minutes of prior SCS.

Age class (yrs)	0.5 kHz	1 kHz	6 kHz	10 kHz	14 kHz	18 kHz
0-2	1.00	0.50	0.00	1.00	1.00	1.00
	0.00	0.46	0.27	0.44	0.30	0.39
	0.00	0.50	0.67	0.33	1.00	1.00
5-9	0.80	1.00	1.00	1.00	1.00	1.00
	0.02	0.41	0.26	0.37	0.29	0.30
	0.10	0.48	0.19	0.53	0.44	0.32
13-30	0.50	0.50	1.00	0.67	1.00	0.67
	0.05	0.42	0.19	0.33	0.23	0.33
	0.25	0.50	0.25	0.44	0.53	0.47

**Appendix IV** percentage of responses for applied stimuli per group (divided in age, n =3, 4 and 4 for classes: 0-2, 5-9 & 13-30 resp.) So far little data has been collected for wakeful response to stimuli. Overall there appear to be no significant differences between amount of responses and age. Although our data does reveal a trend for higher amount of responsiveness present in the older dolphins (13-30) compared to the younger dolphins, current data can not conclude this effect to be significant. Thus the age is not considered to determine responsiveness to arousal stimuli in our experiments.