Developing welfare parameters for bottlenose dolphins (Tursiops truncatus) under human care

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THESE

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Developing welfare parameters for bottlenose dolphins (*Tursiops truncatus*) under human care

Présentée par **Isabella Clegg**

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Et le co-encadrement de: Fabienne Delfour

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Developing welfare parameters for bottlenose dolphins (*Tursiops truncatus*) under human care

Isabella Clegg

A collaboration between Parc Astérix and the Laboratoire d'Ethologie
Expérimentale et Comparée at Université Paris 13.

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Acknowledgements

"The pessimist sees difficulty in every opportunity. The optimist sees the opportunity in every difficulty."

Winston Churchill

I would like to begin and end my acknowledgments with a quote. I believe measuring animal welfare an inherently difficult task, but, as a self-proclaimed optimist, I have been inspired over the last years to see what opportunities lie in this field in terms of better understanding dolphins. To this end, I would like to thank all the people and animals who have supported me along the way.

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To my friends: I have realised that similar to the dolphins, strong social bonds and support seem to be of particular importance in my life, so thank you for celebrating the good times and carrying me through the difficult periods. Whether in Paris, London, the US or elsewhere, you know who you are and I am so grateful for you.

My family have been and always will be my rock and were my first port of call during the highs and the lows of the last three years- Mum, Dad, Eloisa, Hugh and Tom, you have helped me so much and sorry for all the rants/excess of dolphin pictures on WhatsApp! More profoundly, my family's influence on my personality and capabilities are the real reason I was able to complete thesis: my Mum taught me true compassion, my most valued quality above all else; my Dad gave me an indomitable work ethic and my optimism; to my Grandfather, I learnt from you how to be passionate and spirited about work and life; and to my Grandmother, you instilled in me a deep love and interest for animals.

Lastly, and linked to the final quote, I would like to say thank you to the animals. My first dog Tilly showed me just how strong a human-animal bond can be, and the dolphins I have studied continue to amaze and fascinate me, as much now as when I first laid eyes on them. To the Parc Astérix dolphins (especially Femke, Ekinox and Baily- sorry for having favourites!), thank you for providing such meaningful interactions over the years. True to form, I have found the more I study dolphins the more I realise I don't know, but luckily I see this as a lifetime of more opportunities!

"...To the Dolphin alone, beyond all other, nature has granted what the best philosophers seek: friendship for no advantage"

Plutarch

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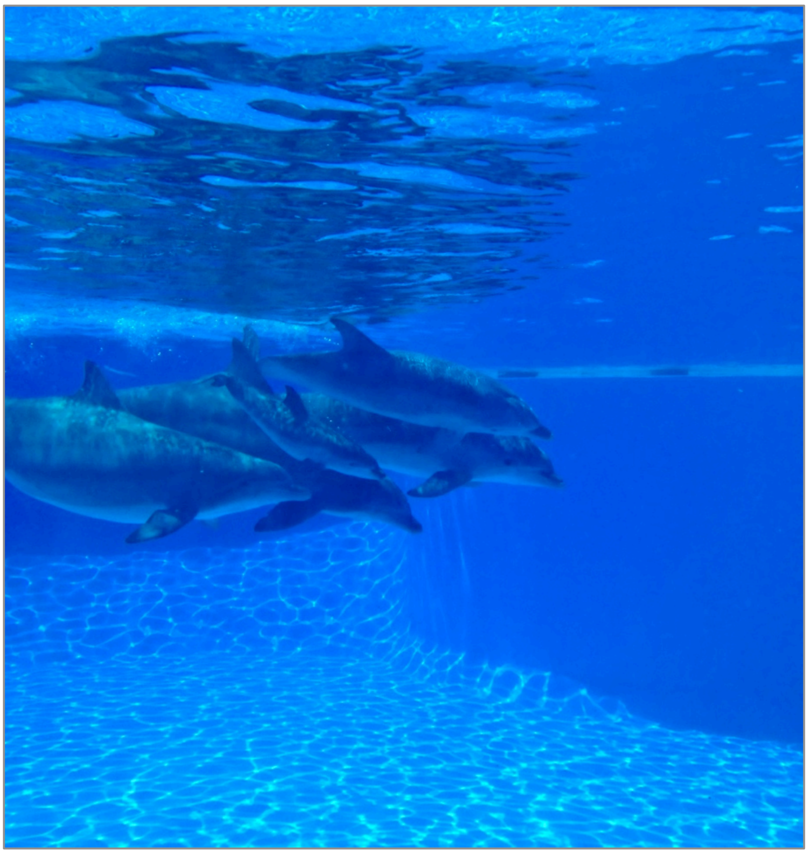

Chapter 1
General Introduction

Chapter 1: General Introduction

The study of animal welfare is now an established discipline and has tasked itself with measuring how an animal is feeling in its environment (Boissy et al., 2007; Désiré et al., 2002; Mendl and Paul, 2004; Yeates and Main, 2008). This has been achieved through measuring aspects of health, behaviour, physiology and cognitive processing in order to arrive at an accurate estimation of the animal's internal subjective state (Boissy et al., 2007; Webster, 2005). Animal welfare science was born out of concerns regarding conditions for farm animals as described in the 1960s Brambell report (Veissier et al., 2008), and although the research effort remains focussed on this industry, welfare studies are increasingly being conducted on laboratory, companion, and recently zoo-housed animals (Webster, 2005; Whitham and Wielebnowski, 2013). Dolphins (family *Delphinidae*) are among those animals often maintained for public display, i.e. in zoological parks and aquaria (hereafter zoos), and of them the most common species kept is the bottlenose dolphin (*Tursiops truncatus*) (Wells and Scott, 1999). Bottlenose dolphins are gregarious mammals with purported advanced cognitive abilities, and are relatively adaptable towards life in a captive environment (Mason, 2010; Schusterman et al., 2013; Wells and Scott, 1999). Their existence in zoos worldwide has captured the interest of the public, media, politicians, and scientists alike, but unfortunately there is increasing discordance among the many stakeholders about this species' and other delphinids' level of welfare (Brando et al., 2016; Jiang et al., 2007; Marino et al., 2007), perhaps due to the industry's past links with circus-style attractions (Maple and Perdue, 2013) and/or how the general public perceives these animals (Servais, 2005). However, this collective concern over dolphin welfare has not yet translated into scientific effort: very little research exists on this topic (Clegg et al., 2015).

The motivation behind this thesis was to contribute to emerging field of dolphin welfare research, through providing the first building blocks of knowledge and stimulating future studies in the multidisciplinary topics involved. Bottlenose dolphins are the most common delphinids found in zoo collections (Wells and Scott, 1999) and the most often studied in scientific terms (Hill and Lackups, 2010), and thus are used as the model species in this first in-depth exploration of cetacean welfare. While bottlenose dolphins were the only species used in the practical experiments, the lack of welfare studies on this species meant that findings from other delphinids were sometimes used to support reviews, theoretical discussions and study design when research was not available for bottlenose dolphins (it is specified when concepts apply only to *T. truncatus*). The focus during the thesis remained on animal-based measures (those assessing aspects of the animal itself e.g. behaviour) of positive and negative affective states, where at first, theoretical and practical investigations were needed to identify potential measures of bottlenose dolphin welfare. Subsequently, the potential indicators were tested within well-established welfare test paradigms validated in other species. The indicators with the most potential for welfare measurement were then investigated further, principally in terms of their inter-individual variation and what they might tell us about how the animals perceive their environment. Lastly, the applications of welfare tools in dolphin institutions are explored, with an international multi-facility project launched to establish whether behavioural scoring by animal trainers could predict welfare changes. Hereto, this introduction describes current animal-based approaches to measuring animal welfare, existing dolphin welfare research, as well how indicators of affective state might be used to understand the animals' perception of their environment.

Methods of assessing animal welfare

Defining welfare

Since the genesis of animal welfare research (see Paper 1 for full review) there has been much debate over how to define an animal's welfare state, and in general proponents tended to support one of three types of definition: welfare based on health status, welfare based on whether the animal was living a "natural life", or welfare based on what the animal was feeling (Fraser et al., 1997). Although some discussions are still on-going, researchers have generally settled on the latter "feelings-based" definitions (reviews by Broom and Fraser, 2015; Dawkins, 2015), which follow the premise that welfare is solely determined by the animal's subjective feelings and emotions. This is concurrent to general movement away from how an animal is 'coping' in its environment (Broom, 1991) to how it might thrive and experience positive states (Boissy et al., 2007; Yeates and Main, 2008). A typical "feelings-based definition" was followed throughout this thesis, chosen for its equal consideration of positive and negative states and its concurrence with theories of measuring complex emotions and affective states in animals (such as Mendl et al., 2010). This definition describes welfare as the *"…balance between positive* (*reward, satisfaction*) *and negative* (*stress*) *experiences or affective states. The balance may range from positive* (*good welfare*) *to negative* (*poor welfare*)*"* (Spruijt et al., 2001). Affective states are thought of as emotions grouped together, and they themselves combine over time to constitute overall welfare (Boissy et al., 2007; Mendl et al., 2010; Panksepp, 2005). Welfare could then imaginably be investigated and measured for any animal, including those in the wild (Papastavrou et al., 2017), but of course the vast majority of past work has focussed on those in captivity since they are under our more direct responsibility (Butterworth, 2017). A few studies differentiate between animal well-being and welfare, with some researchers preferring to use 'animal well-being' due to the fact that the term 'animal welfare' became attached to animal rights activism, especially in the US (Clark et al., 1997). However the majority use the terms interchangeably (Maple and Perdue, 2013; Mason and Veasey, 2010) and 'animal welfare' remains the central concept in considering the quality of life of animals (Appleby and Sandøe, 2002).

Animal vs. resource-based measures

There are two types of welfare measures generally used in assessments: firstly, animal-based measures are those that assess aspects of the animal itself, i.e. its behaviour, disease status, or body condition. In contrast, resource-based measures aim to evaluate welfare by assessing the resources provided to the animal (e.g. pool size) (Whay et al., 2003). Whereas resource-based measures were exclusively used in past welfare discussions, it is now agreed that animal-based measures are more accurate reflections of welfare, mainly since they are able to take into account an individual's responses to the environment and indicate the resulting emotional states, where resource-based measures cannot (Roe et al., 2011). However, it is often more difficult to develop animal-based measures and find ways to standardise and apply them *in situ* (Roe et al., 2011; Rushen et al., 2011; Whitham and Wielebnowski, 2009). Nevertheless, the preference of animal-based assessments is considered an achievable task and significant advance in the field, along with the recognition that welfare policy should not only prevent suffering but also promote positive emotions and affective states (Boissy et al., 2007; Yeates and Main, 2008). Welfare assessments aiming to determine an animal's welfare state should be comprehensive in nature i.e. they should capture the overall, holistic state through a large group of measures, as opposed to just selected elements (Dawkins, 2006; Pritchard et al., 2005). Comprehensive welfare assessments based on these principles have been developed for farm animals and have started to influence industry regulations, such as those from the WelfareQuality® project (e.g. WelfareQuality®, 2009a,b,c). Some frameworks have been updated to stay in line with the latest thinking, for example the Five Domains model which makes inferences about negative and now also positive affective states (Domain 5) by using a combination of animal- and resource-based measures (Domains 1-4) (Mellor and

Beausoleil, 2015). Given the dearth of dolphin welfare research, we might assume that the approaches described above are not feasible or are inapplicable to marine mammals. However this is not the case: many welfare assessments are based on general paradigms which are not species-specific (e.g. the ‘Five Freedoms’, FAWC, 1992, and the Five Domains model, Mellor and Beausoleil, 2015). Consequently, assessment frameworks are often described as highly adaptable to other species than those for which they were originally designed (Botreau et al., 2012; Veissier and Miele, 2015). In recent years such adaptions have taken place, such as with the well-received WelfareQuality® assessment and its application to fur-farm animals (Mononen et al., 2012), dogs (*Canis familiaris*) in shelters (Barnard and Ferri, 2015), horses (*Equus caballus*) (Dalla Costa et al., 2014), and in the years since this thesis started, to bottlenose dolphins (Clegg et al., 2015). While it is ideal to conduct a welfare assessment on an individual animal if the goal is to improve its welfare (Barber, 2009), this is not always feasible for species living in large groups on farms and may also be the case in some zoo settings. Nevertheless, steps can be taken to assess welfare at the group-level where valuable data can be gathered on a sample of animals and results extrapolated to the rest of the group (as in WelfareQuality® 2009 a,b,c), and with some modifications animal-based measures can be performed at the group-level as well (Johnsen et al., 2001).

Strong support has been given for adapting farm animal welfare principles to zoo settings (Barber, 2009; Hill and Broom, 2009; Whitham and Wielebnowski, 2013), where long-term, focal animal studies would be feasible and very useful for the field (Maple, 2007), and thus all that remains is for the approach to be fully embraced by scientists and the industry’s stakeholders. Zoo welfare studies are increasingly being conducted and are becoming a central research activity in many organisations (Baird et al., 2016; Maple and Perdue, 2013). Initial work has investigated indicators of welfare such as stress hormones (e.g. Menargues Marcilla et al., 2012; Pifarré et al., 2012), response to environmental enrichment (Carlstead et al., 1993; Meehan and Mench, 2007) or presence of stereotypic behaviour (Montaudouin and Le Pape, 2005; Shepherdson et al., 2013). The power of conclusion from zoo research is often hampered by small sample sizes and difficulties in standardisation, and as a result multi-institutional studies have proved very valuable (Baird et al., 2016) and should be greatly encouraged in future endeavours (Maple and Perdue, 2013). There is also an emerging focus involving zoo research where organisations representing different stakeholders are realising that objective, animal-based welfare measures developed in captive studies can be used in conservation and management projects in the wild (Maple and Perdue, 2013;

Ohl and van der Staay, 2012). Several experts have recently voiced their support for the application of welfare assessment frameworks specifically to marine mammal conservation (Papastavrou et al., 2017; Seuront and Cribb, 2017).

Measuring emotional states

When validating the animal-based measures used in assessments, i.e. verifying that certain indicators are indeed reflective of emotions or larger affective states/welfare, correlating at least behavioural and physiological data is superior to single or one-dimensional parameters (Boissy et al., 2007; Désiré et al., 2002; Mason and Veasey, 2010). If possible, cognitive parameters should also be measured to increase accuracy further: changes in emotion (and thus affective states) are typically accompanied by neural, behavioural, physiological and cognitive adjustments (Mendl et al., 2010; Paul et al., 2005) and therefore the associated fields of study are the ones most often implicated in welfare research. Emotions have two dimensions, arousal and valence, and while the level of arousal is often easily measured through behavioural and physiological measures, valence is hard to discern without cognitive parameters (Paul et al., 2005). Webster (2005) explained this concept elegantly using the principle of ‘Triangulation’, where he labels the three categories of welfare measures as points of a triangle: behaviour, physiology and neurobiology. The true centre is the animal’s actual welfare and the predicted welfare state starts on one of the points with a certain category, and adding second and third bearings (categories) brings the predicted welfare ever closer to the centre. In order to develop welfare measures for any species for which there is little explicit research, potential indicators of emotions, structured into these broad categories, should first be identified (Mendl et al., 2010; Veissier et al., 2012).

The two main approaches to studying emotions are labelled as the *discrete emotion* or *dimensional emotion* theories where in the former, emotions are measured using the already-established emotions categorised in human language (Scherer, 2005). This approach on its own is not overly conducive to animal models, since it is not yet clear whether and to what extent they experience the same types of emotions as humans, and (thus far) verbal reports are not possible (Mendl et al., 2010). *Dimensional theories* from human psychology propose that emotions are all found within quadrants bisected by two axes: arousal and valence (Burgdorf and Panksepp, 2006; Russell and Barrett, 1999). Therefore measuring the degree of arousal (higher or lower energy) and valence (pleasant or not pleasant) can differentiate the many different types of emotions felt, and thus their related behaviours and physiological indicators can also be considered in terms of these two dimensions. Although always kept separate in the past, several social science scholars combined the two approaches and found they complimented each other well: the discrete emotions were overlaid onto the arousal and valence dimensions (Burgdorf and Panksepp, 2006; Russell and Barrett, 1999; Scherer, 2005). Recently, for the first time, this combined framework (Fig. 1) was proposed for the study of animal emotions (Mendl et al., 2010), and is likely to be very useful in encouraging "bigger picture" discussions on the affective states of non-human animals, allowing moods and complex emotions are able to be logically investigated (Panksepp, 2011).

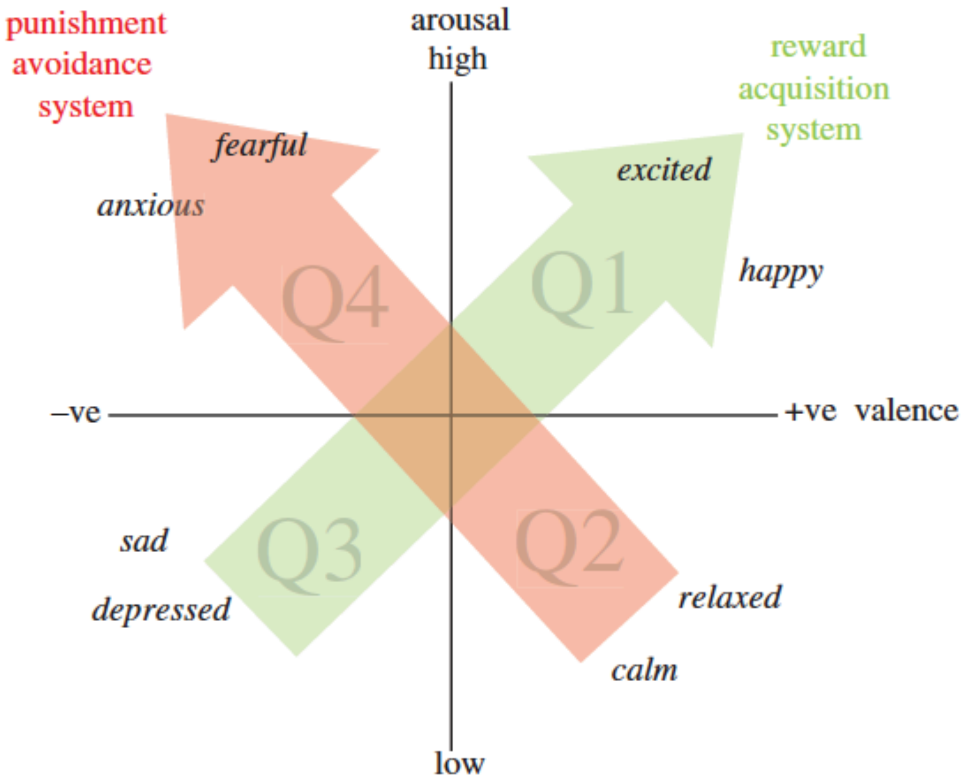

Fig. 1 *Taken directly from Mendl et al. (2010): Core affect represented in two-dimensional space. Words in italics indicate possible locations of specific reported affective states (including discrete/basic emotions). Positive affective states are in quadrants Q1 and Q2, and negative states in quadrants Q3 and Q4. Arrows indicate putative biobehavioural systems associated with reward acquisition and the Q3–Q1 axis of core affect (green), and punishment avoidance and the Q2–Q4 axis of core affect (red). Adapted from Russell (e.g. Russell and Barrett, 1999) and Panksepp (e.g. Burgdorf and Panksepp, 2006).*

Following on from this model (Fig. 1), Mendl and co-authors (2010) also proposed how overall core affective state might change with time, which they explain by differentiating between the “emotion-eliciting situations” and “free-floating moods” (i.e. longer-term, and not directed at particular objects or events) which make up such states. Despite time being an important factor in measuring core affect, it is likely that longer-term core affect can still be inferred from shorter-term measures. Since moods are thought to be culminations of shorter-term emotional responses to situations, an individual’s core affective state, which we take to be synonymous with overall welfare state, will always be closely linked to the rewards and threats present in the animal’s environment (Mendl et al., 2010). If we want to measure this somehow in a welfare assessment i.e. capture all the different aspects of an individual’s response to its environment, many and varied parameters must be used to allow such evaluations to be as holistic and comprehensive as possible (Dawkins, 2006; Pritchard et al., 2005). This is echoed in the other principal concept followed throughout this thesis: the ‘Triangulation’ of different welfare measures (Webster, 2005). In order to visualise the concepts studied in this thesis, I combined the principles put forward by Mendl and co-authors (2010) with the concurring welfare definition of Spruijt and co-authors (2001) to create a schematic showing how welfare measurement can be broken down (Fig. 2). The objectives of the studies within this thesis are to identify potential indicators of positive and negative emotions and possibly affective states in dolphins, through the combination of different categories of measures ('Triangulation': Webster, 2005), with the goal of facilitating evaluations of the “tilt of the balance” and thus overall welfare in these animals. As a starting point, the next section reviews the few previous studies on dolphin welfare that have had similar objectives to the one described above.

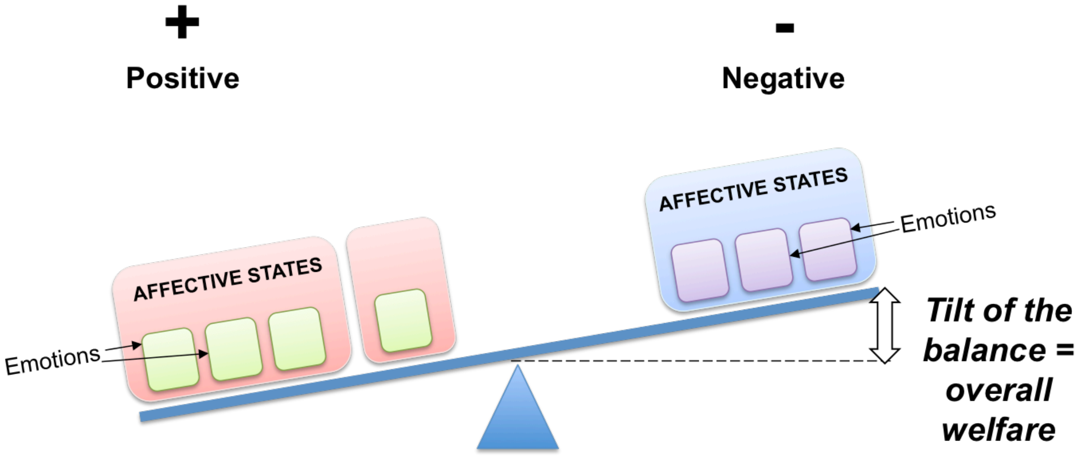

Fig. 2 *A simplified schematic showing a bottom-up structure of how emotions (smaller squares) combine to form affective states (larger rectangles). The overall welfare of the animal is the balance of affective states i.e. the angle of the seesaw, and can move between negative, neutral and positive (Mendl et al., 2010; Panksepp, 2011; Spruijt et al., 2001).*

Research on bottlenose dolphin welfare in captivity

The number of studies that have directly investigated the welfare of bottlenose dolphins in captivity could almost be counted on one hand: we have very little knowledge on how to measure these animals' welfare or what factors might have an influence (Clegg et al., 2015; Ugaz et al., 2013). Given this fact, the review of the literature here has referenced any studies which are available on the topic in other dolphin species. There are 250 bottlenose dolphins in *European Association of Zoos and Aquaria* facilities (EAZA, 2015), and 444 in the US and Canada (Ceta-base, 2011), with many in other countries not on official registers, and all kept in a wide range of facilities which are likely to differentially impact welfare (Joseph and Antrim, 2010). Current laws regulate the resources provided, for example the pool size or water quality (Animal Welfare Act, 1966; EC Council Directive, 1999), but are supported by very little scientific evidence and thus viewed as minimum requirements (Joseph and Antrim, 2010). Clearly, objective research is needed to answer questions and aid policy makers on dolphin welfare in captivity, but up until now there has been very little conducted.

The first work directly posing questions on bottlenose dolphin welfare were two behavioural investigations conducted over two decades ago, where it was suggested that dolphins may be performing stereotyped swim patterns (Gygax, 1993) and that behaviours such as play frequency and behavioural diversity might be indicators of welfare (Galhardo et al., 1996). Around the same time, two studies specifically questioned the welfare impacts of *Swim-with-the-dolphin* (SWTD) programs, and found potential signs of disturbance to the animals but both concluded further work was needed (Frohoff and Packard, 1995; Samuels and Spradlin, 1995). A decade later, another study using similar parameters would conclude that SWTD programs were not detrimental to dolphin welfare (Trone et al., 2005), and results on this aspect of captivity remain sparse yet conflicting. Although only consisting of case studies, the first detailed investigation into social stress and its welfare implications for captive dolphins was published a few years later (Waples and Gales, 2002). In terms of physiological welfare indicators, a first study used salivary cortisol to suggest that space of enclosure might impact bottlenose dolphin welfare (Ugaz et al., 2013), and similar research was conducted using serum levels of stress hormones in belugas (*Delphinapterus leucas*) where it was found that out-of-water veterinary examinations caused elevations in multiple stress hormone levels but SWTD programs did not (Schmitt et al., 2010). An assay for faecal corticosteroid metabolites was recently developed with captive bottlenose dolphins and was shown to be a viable indicator of adrenal activity, and thus could be useful in future welfare investigations (Biancani et al., 2017).

Although welfare questions and conclusions were made in these previous studies, none of them followed the principles, approaches or terminology used by the general animal welfare science field, e.g. as used in farm animal studies. After the start of this thesis, a study published a comprehensive, animal-based dolphin welfare framework (the “C-Well© Assessment”) (Clegg et al., 2015) that was adapted from a well-established farm animal assessment (WelfareQuality®, 2009a,b,c). Thirty-six measures were developed and tested in three dolphin facilities, but the assessment was not fully validated in terms of testing the relationship between the parameters and emotions or affective states. This study represented the first application of welfare science principles to dolphins in captivity and aimed to stimulate further work in the area (abstract provided in Appendix A.2.i).

Of course, the field of cetology has a vast knowledge base and studies are often published on aspects related to dolphin welfare, but do not mention the term or specifically focus on it (Hill et al., 2016). This past cetology research will aid in the advancement towards measuring dolphin welfare, but as yet a comprehensive review of the related topics that would facilitate this has not been conducted. In the following sub-section, I discuss the cetology disciplines that are likely to be most relevant by placing them in the Triangulation framework proposed for assessing welfare in other species (Webster, 2005), while also highlighting techniques from terrestrial research that have aided in discovering different types of welfare measures. As the first step of this project, a review of cetology knowledge relevant to welfare was published (Paper 1, Chapter 2) in order to identify gaps and set foundations for this topic (as recommended by Hill and Lackups, 2010).

Potential health, behavioural and cognitive welfare measures

For the much-needed review of potential dolphin welfare indicators, which became the first project of this thesis, we adapted the ‘Triangulation’ concept slightly by expanding the categories of measures to health, behaviour and cognition, for several important reasons. First, health influences welfare, and if we follow current thinking and only consider conditions where the animal actually “feels ill” (Boissy et al., 2007; Mason and Veasey, 2010; Veissier et al., 2012), using more than just physiological indicators may aid in assessing welfare holistically and move away from measuring only discrete emotions (Mendl et al., 2010). Second, broadening the neurobiology category to all cognitive processing allows the inclusion of extremely useful welfare test paradigms developed from experimental psychology (chiefly cognitive bias, Mendl et al., 2009). Lastly, since our work represents the first steps in dolphin welfare, remaining conservative and not excluding potential influencing factors through using limited categories would be prudent. Dolphins and their environment are significantly different from terrestrial animals and our existing knowledge, and thus there may be many other variables, unknown and intangible to us, which affect dolphin welfare (Delfour, 2006).

Among the fields of health, behaviour and cognition of cetaceans, there is a relatively large knowledge base concerning the various diseases and medical conditions that has resulted from years of wild and captive studies (e.g. Reif et al., 2008; Venn-Watson et al., 2012). Nevertheless, dolphins tend to mask symptoms of poor health (Castellote and Fossa, 2006; Waples and Gales, 2002), so physiological parameters are likely to also be informative about welfare states. For example cortisol levels have potential to indicate welfare state, as has been shown with terrestrial animals (e.g. Palme, 2012; Pifarré et al., 2012), but the early stage of this research with dolphin species has led to cautious interpretations thus far (Atkinson et al., 2015; Biancani et al., 2017; Ugaz et al., 2013). Inappetence is commonly correlated with poor health in dolphins and could be a useful welfare measure in the absence of other symptoms: it is a basic indicator of dolphin ‘sickness behaviour’ (Johnson et al., 2009; Schmitt and Sur, 2012) and is easy to monitor, but further work is needed to disentangle the causes which can be behavioural as well as health-related (Waples and Gales, 2002). Similar to the general poor health indicator of inappetence, Body Condition Scoring (BCS) can be a useful welfare tool which captures longer-term states of poor (or good) health: it is commonly used in farm animal studies (e.g. Roche et al., 2009; WelfareQuality®, 2009a) and is starting to be applied to wild and captive dolphin species (Clegg et al., 2015; Joblon et al., 2014). Other external physical measures such as presence of bodily injuries are often used in farm animal welfare assessments (Mononen et al., 2012; WelfareQuality®, 2009b), since they simultaneously measure pain/infection levels as well as aggression rate; similar measures have been proposed for dolphin welfare evaluations and warrant further investigation (Clegg et al., 2015).

Behaviour measurement and thus ethology is essential to welfare research (Dawkins, 2004; Maple and Perdue, 2013), and the information yielded is generally thought to be more revealing about welfare state than health parameters (Gonyou, 1994; Joseph and Antrim, 2010). This is likely to be extremely applicable to dolphins due to their complex and flexible social structures increasing the occurrence of social stress as a welfare-reducing factor: this has been reported anecdotally but merits in-depth investigation as it could have a major influence in captive dolphins' lives where group composition is artificial and less changeable (Barrett and Würsig, 2014; Clegg et al., 2015; Waples and Gales, 2002). Estimating the frequency of aggressive behaviour is often part of terrestrial welfare assessments, for example in farmed pigs (*Sus scrofa*) through the occurrence of tail-biting lesions (Temple et al., 2011). Aggressive behaviour in dolphins is often hard to observe (often occurs at fast speeds underwater) and so indicators such as the extent of rake marks (from conspecifics' teeth) have been shown as useful proxy measures for aggression (Marley et al., 2013; Scott et al., 2005), and were recently proposed as a measure of welfare (Clegg et al., 2015). Dolphin species' capacity for close social relationships could equally provide a buffer towards stress (Barrett and Würsig, 2014) and thus lead to improved welfare (Brando et al., 2016), as well as potentially compromising it through sustained aggression. Social behaviours linked to positive welfare might be gentle tactile behaviours, synchronous swimming and play (respective examples: Connor et al., 2006a; Kuczaj et al., 2013; Paulos et al., 2010), all of which have been described as affiliative but have not yet been studied empirically as emotional indicators. Inter-specific social behaviours, such as Human-Animal Interactions (HAIs) for captive dolphins, could also be very influential for their welfare, based on terrestrial zoo animal research: positive HAIs can significantly improve welfare and *vice versa* (Hosey and Melfi, 2010; Whitham and Wielebnowski, 2013). However this has hardly been studied with dolphins and proposed methods such as an approach-avoidance test adapted from farm animals (Clegg et al., 2015) should be developed further, especially given that compared to other non-domesticated captive animals, trainer-dolphin interactions are likely to be unique in terms of the level of physical contact, the time spent in proximity each day, and the simulated working relationship (Galhardo et al., 1996; Maple and Perdue, 2013). In general, positive intra- or inter-specific social interactions have the potential to be indicators of positive emotions, but also to move an animal's balance of affective states towards the positive side (Rault, 2012); therefore, increasing opportunities for these behaviours to occur can be thought of as a tool to improve welfare (Boissy et al., 2007). Another behaviour warranting investigation is abnormal repetitive behaviour, which is not often studied and poorly understood in dolphins, despite anecdotal evidence supporting its occurrence (review by Clark, 2013). Lastly, evidence from wild and captive studies show that certain parameters of the vocal behaviour of dolphin species are undoubtedly linked to their emotional state (e.g. Dibble et al., 2016; Herzing, 2000) and thus could be invaluable to welfare studies, but so far we know relatively little about which emotions certain vocalisations might indicate due to problems with identifying the sound-emitting dolphin (Herzing, 1996). Fortunately, new technology and etho-acoustical approaches are actively addressing this issue and the first results look promising in terms of discovering the meaning behind dolphin vocalisations (Lopez Marulanda et al., 2016, 2017).

The third category within which dolphin welfare measures should be established is cognition i.e. the cognitive components of emotional responses, which significantly help to decipher the valence of emotions (Paul et al., 2005). Cognitive processes have been shown to influence emotions through the appraisal of external stimuli, and emotional states can impact cognitive functioning by inducing attentional, memory and judgement biases (collectively ‘cognitive bias’) (Mendl and Paul, 2004; Paul et al., 2005). Cognitive biases represent perhaps the most promising tool for measuring welfare in recent years, since testing animals’ biases, where ambiguous cues elicit either positively or negatively biased decisions, has proven to be a reliable measure of an individual’s affective state (latest reviews by Baciadonna and McElligott, 2015; Roelofs et al., 2016). Cognitive bias testing will be discussed further in terms of its applicability to dolphins in the following sub-section. The other main cognitive phenomenon discussed in relation to welfare is laterality, since many studies have found that due to hemispheric specialisations individuals show preferences towards the left or right eye or body part in different situations, with some theories going further to suggest that this is related to emotional regulation and thus welfare (Leliveld et al., 2013; Rogers, 2010; with *Tursiops truncatus*: Delfour and Marten, 2006; Sakai et al., 2006). More work is needed before standardised welfare conclusions can be drawn, but lateralised behaviours are certainly worth investigating as the data are feasible to collect and could be correlated with other potential parameters.

After this overview of the types of measures likely to be relevant to dolphin welfare, it can now be considered how they might be experimentally tested in order to prove their accuracy and validity.

Welfare tests applicable to dolphins

Cognitive bias testing

In the early days of animal welfare science, perhaps due to resistance in accepting the presence of emotions in animals (Shettleworth, 2001), welfare measures were identified by exposing subjects to unquestionably negative situations or making pharmacological modifications, and then recording behavioural and physiological parameters as indicators of poor welfare (Boissy and Bouissou, 1995; Makowska and Weary, 2013). As demonstrated by these relatively uncomplicated approaches, it is easier to measure indicators of 'discrete' emotions like fear and anxiety than more complex or positive states (Mendl et al., 2010; Paul et al., 2005). Complex emotions e.g. depressed, and affective states (combinations of emotions) are measured using detailed experimental protocols and require consideration of cognitive processes: the goal here is to measure the valence dimension of affect, whereas with the simpler, discrete emotions the level of arousal is usually sufficient for evaluations (Mendl et al., 2010).

Tests of cognitive biases allow measurement of the valence of affective states: results from numerous judgement bias studies show that when humans or animals are placed in poorer environments or chronic stress is stimulated they make more pessimistic judgements, and when they experience for example enriched surroundings or positive social groupings they judge more optimistically (reviews on humans: MacLeod and Byrne, 1996; Wright and Bower, 1992; and other animals: Baciadonna and McElligott, 2015; Mendl et al., 2009; Roelofs et al., 2016). While research on this topic has increased exponentially in the last few years, there are still gaps in the knowledge: for example, there have been cases where the directionality of results is not as predicted (i.e. optimistic judgements are not correlated to better welfare) (Bethell, 2015; Mendl et al., 2009). A common explanation is that the animal's current mood and affective state, occurring independently of any experimental manipulations, is the cause for the unexpected biases (Baciadonna and McElligott, 2015; Wichman et al., 2012). Instead of viewing this as a problem in cognitive bias studies, future work may be able to help identify measures of affective state by just studying the animals in their "home environment". For example it was suggested recently to use bias tests to fully explore correlations with social behaviours, which for social species are likely to significantly impact affective state (Wichman et al., 2012). Results from the first few studies investigating this already show a clear link: domestic canaries (*Serinus canaria*) judged more optimistically when pair-housed (as opposed to those housed singly) (Lalot et al., 2017), and optimistic judgements in two primate species were likely linked to dominance behaviour (Bateson and Nettle, 2015; Schino et al., 2016). Cognitive bias testing had not yet been applied to any marine mammal or zoo-housed species prior to the publication of our study (Chapter 3, Papers 3 and 4): bottlenose dolphins are highly trainable using positive reinforcement methods (Brando, 2010) and in the wild live in complex fission-fusion social networks (Connor et al., 2001), therefore representing an interesting model for cognitive bias questions.

Anticipatory behaviour and reward sensitivity

Although it has garnered less research thus far, another current welfare paradigm which seems to indicate affective states is the performance of anticipatory behaviour before a predictable reward. This is defined as the behavioural patterns established in preparation for a predictable, upcoming positive event, and is linked to affective states in two ways. Firstly, it has been shown in many species that animals will perform a certain level of anticipatory behaviour before a reward and that this anticipation in itself stimulates positive emotional states (van der Harst and Spruijt, 2007; Watters, 2014), as evidenced through behavioural (e.g. Anderson et al., 2015; McGrath et al., 2016) and physiological (e.g. Gimsa et al., 2012; Opiol et al., 2015) indicators. Secondly, it has been theorised that an animal's affective state can further be deduced by the connection between anticipatory behaviour and the reward-sensitivity system (Spruijt et al., 2001; van der Harst and Spruijt, 2007). This theory was developed from multidisciplinary research into the brain's reward-evaluating mechanisms, where it was found that an individual's sensitivity (i.e. 'want'/desire) regarding a reward is continuously changing (adaptive response to help fulfil needs and increase positive experiences) (van der Harst and Spruijt, 2007). Using the simple example of food acquisition, if a food reward appears reliably after a cue, an animal will show some level of anticipatory behaviour after perceiving the cue, assuming that it is not fed *ad libitum* and thus not satiated. However, if the animal has not been fed for days and is very hungry and thus in a negative affective state, it will place more value on the food reward ('incentive value') and therefore perform more anticipatory behaviour before its arrival. This also applies for negative and positive experiences unrelated to each other: for example, an animal in a barren, under-stimulating environment would be predicted to anticipate food rewards more. This has been proven with laboratory rats (*Rattus norvegicus*) (Makowska and Weary, 2016; van der Harst et al., 2003a), and furthermore it was shown that socially isolated rats anticipate food rewards as well as social contact more than group-housed conspecifics (van den Berg et al., 1999). In terms of the relationship between the levels of stress and anticipatory behaviour for a reward, and how we might use this to predict welfare, a recent study proposed that an animal with positively-balanced welfare and low stress will still perform a certain level of anticipation, and as stress increases so does anticipatory behaviour, until a threshold of chronic stress is reached beyond which anticipatory behaviour is almost totally suppressed as the animal experiences a state of apathy and helplessness (Watters, 2014).

More cross-species research into anticipatory behaviour as an indicator of affective state is needed, since many elements remain unclear: for example, does increased anticipation result in increased participation or consummatory activity in the reward once it arrives? This question has not yet been the focus of any study, and past descriptive results would suggest that this is the case (Anderson et al., 2015; McGrath et al., 2016), but perhaps situations where animals anticipate a reward strongly, but then do not exploit or utilise it, have different implications for the individual's welfare state. In addition, the act of anticipating can itself be rewarding, and thus should be investigated in terms of interaction with the reward's incentive value and the deduced affective state (van der Harst and Spruijt, 2007; Watters, 2014). Nevertheless, anticipatory behaviour is relatively simple to measure and thus should be investigated in a variety of species and settings: including dolphin species in captivity. The only study in captive dolphins on this behaviour empirically confirmed the presence of anticipatory behaviour, where a group of *T. truncatus* performed more surface vigilance behaviours in response to the upcoming public presentation sessions during which their food was provided (Jensen et al., 2013). Since anticipatory behaviour levels can be used to understand which objects and events might be rewarding for captive animals (van der Harst and Spruijt, 2007), it could also be applied to bottlenose dolphins in order to discover what elements of their environment they find rewarding: so far, we know only that they will voluntarily interact with enrichment items (Delfour and Beyer, 2012; Kuczaj et al., 2002) but not whether and how they value such items (Delfour et al., 2017). One study has shown that captive bottlenose dolphins voluntarily sought tactile contact from familiar humans (Perelberg and Schuster, 2009) but again more information on how the animals perceive Human-Animal Interactions (HAIs), perhaps through their levels of anticipation for such events, would be instrumental to welfare discussions.

Practical application of welfare tools

The cognitive bias and anticipatory behaviour 'tests' of affective state presented above require time and effort since they must be conducted as part of rigorously controlled studies. In general, feasible and inexpensive methods to regularly monitor behaviour or health are relatively more useful to animal managers in terms of making changes to the system to improve welfare (Maple and Perdue, 2013; Napolitano et al., 2010), and I discuss one such approach here. Qualitative Behavioural Assessments (QBA) are conducted when an observer makes a qualitative evaluation of an anima's emotional expressivity by considering and integrating many aspects of its behaviour, which in conventional quantitative approaches are recorded separately or not at all (Wemelsfelder, 2007). In practice QBAs ask questions about the expressive demeanour of the animal either using freechoice profiling, where observers provide their own terms regarding the animal's behaviour, or consist of fixed rating scales with pre-determined descriptions where the observer chooses a grade (Wemelsfelder and Lawrence, 2001). Although seemingly very subjective, such observer ratings can amalgamate multimodal information across time and situations in a way that one-dimensional quantitative data does not (Meagher, 2009; Wemelsfelder and Mullan, 2014). Numerous studies have reported strong inter-observer concordance (even with different levels of expertise, Wemelsfelder et al., 2012), and it was found that the QBA results can be correlated with quantified behaviours as well as physiological markers of stress (Rutherford et al., 2012; Stockman et al., 2011; Wemelsfelder and Mullan, 2014; Wickham et al., 2012). QBAs have been successfully applied in zoo settings to measure personality traits and welfare of zoo animals, as measured through significant correlations of the QBA results and other objective welfare indicators (Carlstead et al., 1999; Meagher, 2009; Weiss et al., 2006; Whitham and Wielebnowski, 2009). Outside of being used for ratings in personality studies, QBA-style approaches have not been conducted in dolphin emotion studies. Since animal caretakers are widely considered to be most knowledgeable about their charges' behaviour and spend the most time with them (Meagher, 2009; Whitham and Wielebnowski, 2009), and dolphin trainers are no exception, there is the scope to establish a qualitative tool to monitor dolphin behaviour and/or overall welfare. Furthermore, an easily applicable record-taking practice such as this would be valuable for the zoo management and scientists alike. For example, a dolphin's behaviour during the multiple daily training sessions they experience is likely to reflect their affective state in measureable ways, but as yet no studies have investigated this (Brando et al., 2016). Recently a QBA was designed to answer a similar question on horse welfare during endurance riding (Fleming et al., 2013) and thus the same approach could be applied to dolphins during training sessions.

Although the animal welfare literature presented above covers many disciplines, being rich in some areas but significantly lacking in others, its underlying principles are applicable to all species and therefore bottlenose dolphins are more than viable candidates for such investigations. There may even be potential for dolphin welfare studies to enhance general animal welfare knowledge: their capabilities in learning trained tasks and their wide range of inter- and intra-specific social behaviours could allow the development of paradigms applicable to other species. There is a strong need for dolphin welfare research in terms of its applications to the management of animals in public display facilities, as well as the subsequent transmission of information to the general public and regulatory bodies (Clegg et al., 2015). Progress on dolphin welfare assessment techniques would also be applicable to some contexts with wild populations and could aid in conservation programs (Papastavrou et al., 2017; Paquet and Darimont, 2010).

Aim and outline of the thesis

The aim of this thesis was to develop animal-based measures of bottlenose dolphin welfare in captivity. This project was facilitated through a collaboration between Parc Astérix and the Laboratoire d'Ethologie Expérimentale et Comparée (LEEC), Université Paris 13, with the support of a CIFRE scholarship, Association Nationale de la Recherche et de la Technologie) and a Universities Federation for Animal Welfare (UFAW) grant. The studies were conducted at Parc Astérix (France), Planète Sauvage (France) and Dolfinarium Harderwijk (the Netherlands). Three main questions, and a fourth concerning the implementation of a welfare tool, were asked (corresponding to Chapters 2, 3, 4 and 5; see Fig. 3). Since very little data existed on dolphin welfare, the first question was:

Which behavioural and other parameters are likely to have the most potential for measuring welfare? Firstly, a comprehensive review was conducted of all cetology sub-topics related to dolphin welfare, since this was not present in the literature (Paper 1). A study was then carried out among four groups of dolphins in international facilities to investigate the variation of selected potential behaviours welfare measures in response to the timings of daily training sessions (Paper 2). Chapter 2 is comprised of these two studies, where the common goal was to highlight the most likely areas where welfare measures might be found. Using these findings, Chapter 3 aimed to answer the question of:

Are frequencies of potential behavioural measures of welfare correlated to individual differences in cognitive biases? A spatial location judgement task for terrestrial species was adapted for the first time to dolphins, and cognitive bias results were correlated to the frequency of social behaviours, selected for their potential links to welfare, observed around the time of testing and in the few months before (Paper 3). We expected that synchronous swimming and social play might be correlated to optimistic judgements (considered affiliative, Kuczaj et al., 2013; Paulos et al., 2010), and that agonistic behaviour might link to pessimistic judgements if indeed such behaviour indicates reduced welfare (as proposed for terrestrial species, (Broom and Johnson, 1993). In a follow-up study, the frequency of anticipatory behaviour towards upcoming training sessions was also measured and correlated to cognitive bias results (Paper 4): based on the reward-sensitivity theory (Spruijt et al., 2001; van der Harst and Spruijt, 2007) it was predicted that the highest levels of anticipation would be linked to the most pessimistic biases. Continuing with our investigation of anticipatory behaviour, the third question of the thesis and the subject of Chapter 4 was:

What events do the bottlenose dolphins anticipate in their environment, and does anticipation reflect event participation? Due to purported links with affective state and earlier results in this thesis, anticipatory behaviour showed potential as a measure of welfare and a certain level is thought to indicate what the animal “wants” in its environment (Watters, 2014). Furthermore, it had not yet been studied in any species whether the level of anticipation reflects participation in the predicted event, but we predicted that this would be the case. For this study, a Pavlovian conditioning paradigm was used to test the dolphins’ anticipation before the occurrence of toys in the pool, a Human-Animal Interaction (HAI), or a feeding session, and their subsequent participation in these situations (Paper 5).

Lastly, Chapter 5 includes a long-term study that was carried out in five facilities housing six bottlenose dolphin groups where qualitative ratings of dolphin motivation during training and other welfare data were taken daily over a year (Paper 6). This was conducted to answer the question of: *Can qualitative ratings of motivation, social behaviour markers and health-related data be correlated to develop an applicable, holistic welfare tool for dolphins?* Due to time constraints and the participation of as many facilities in the project, the results are not yet available and a short methodology only will be presented in this thesis. There follows a discussion of the work in Chapter 6 where the likely indicators of welfare for dolphins are discussed, and future directions highlighted.

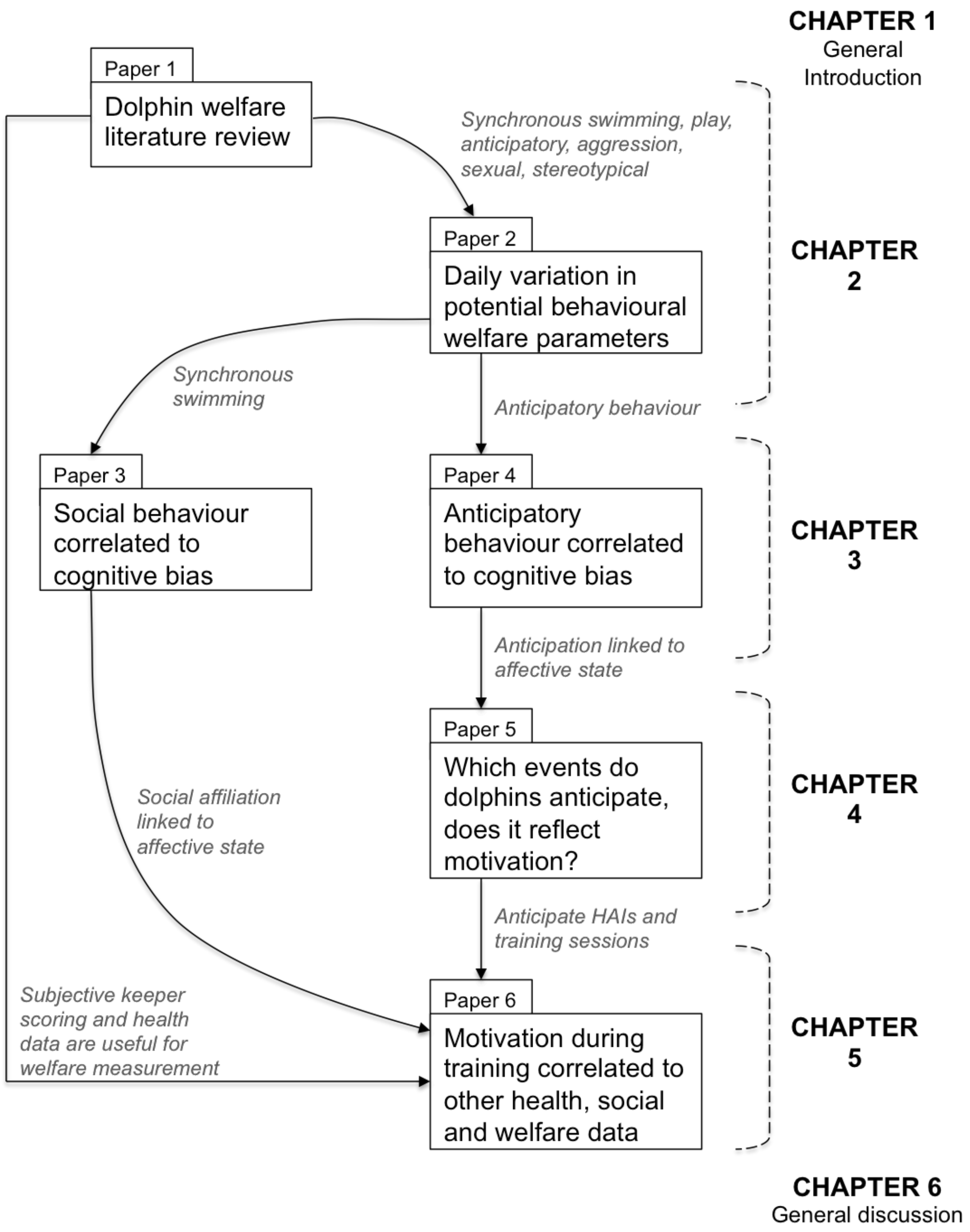
CHAPTER 1
General Introduction
Paper 1
Dolphin welfare literature review
Synchronous swimming, play, anticipatory, aggression, sexual, stereotypical
Paper 2
Daily variation in potential behavioural welfare parameters
CHAPTER 2
Synchronous swimming
Anticipatory behaviour
Paper 3
Social behaviour correlated to cognitive bias
Paper 4
Anticipatory behaviour correlated to cognitive bias
CHAPTER 3
Anticipation linked to affective state
Paper 5
Which events do dolphins anticipate, does it reflect motivation?
CHAPTER 4
Social affiliation linked to affective state
Anticipate HAIs and training sessions
Subjective keeper scoring and health data are useful for welfare measurement
Paper 6
Motivation during training correlated to other health, social and welfare data
CHAPTER 5
CHAPTER 6
General discussion

Fig. 3 *Flow diagram of the evolution of studies within the thesis, which then evolved into scientific papers published in peer-reviewed international journals. Papers 1, 2, 3 are published, papers 4 and 5 have received first-round revisions, and paper 6 is in preparation. Grey text accompanying the arrows shows the main potential welfare measures identified in the previous study, which are then used in the following study. Thesis chapters group together papers with interconnected aims, and are denoted by dotted lines on the right-hand side. The ascending chapter and paper numbers reflect the chronological progression of the thesis. The references for each paper are listed below.*

Paper 1: Clegg, I. L. K., van Elk, C. E., and Delfour, F. (2017). Applying welfare science to bottlenose dolphins *(Tursiops truncatus). Animal Welfare, 26,* 165-176.

Paper 2: Clegg, I. L. K., Rödel, H. G., Cellier, M., Vink, D., Michaud, I., Mercera, B., Böye, M., Hausberger, M., Lemasson, A. and Delfour, F. (2017). Schedule of human-controlled periods structures bottlenose dolphin *(Tursiops truncatus)* behavior in their free-time. *Journal of Comparative Psychology.* 131, 214-224.

Paper 3: Clegg, I. L. K., Rödel, H. G. and Delfour, F. (2017). Bottlenose dolphins engaging in more social affiliative behaviour judge ambiguous cues more optimistically. *Behavioural Brain Research, 322,* 115-122.

Paper 4: Clegg, I. L. K. and Delfour, F. (2017). Cognitive judgement bias is associated with frequency of anticipatory behaviour before training sessions in bottlenose dolphins. *Submitted to Zoo Biology.*

Paper 5: Clegg, I. L. K., Rödel, H. G., Boivin, X. and Delfour, F. (2017). Looking forward to interacting with familiar humans: dolphins' anticipatory behaviour indicates their motivation to participate in specific events. *Submitted to Applied Animal Behaviour Science.*

Paper 6: Qualitative ratings of bottlenose dolphins' motivation during training sessions in five facilities: practical indicators of health-related and social welfare? *In preparation*.

Chapter 2
Identifying potential welfare parameters

Chapter 2: Identifying potential welfare parameters

Paper 1: Applying welfare science to bottlenose dolphins (*Tursiops truncatus*).

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Animal Welfare (2017), 26, 165-176.

Résumé

La science du bien-être animal est un domaine en pleine expansion, mais la recherche sur les cétacés (baleines, dauphins et marsouins) fait défaut. Les grands dauphins (*Tursiops truncatus*) sont les cétacés les plus connus et les plus étudiés, particulièrement en captivité, et ils sont utilisés dans cette revue de la littérature comme un modèle pour d'autres espèces de cétacés. Malgré l'intérêt du public et le besoin d'une telle recherche, des études qui spécifiquement examinent le bien-être des dauphins manquent. Cette revue utilise les trois catégories générales du comportement, de la santé et de la cognition, pour discuter comment le bien-être des dauphins a été évalué jusqu'à présent, et comment il pourrait être évalué à l'avenir. Nous présentons des indicateurs de bien-être validés dans d'autres espèces qui pourraient être appliqués aux dauphins, y compris des mesures innovantes comme l'évaluation cognitive des émotions. Nous fournissons un résumé de recommandations pratiques pour valider les indicateurs de bien-être des grands dauphins. Ce papier vise à stimuler des recherches plus approfondies sur le bien-être des dauphins qui pourraient améliorer la vie des animaux eux-mêmes et étayer des décisions réglementaires. Nous recommandons d'unir l'expertise en cétologie avec la science du bien-être animal pour développer une approche holistique de l'évaluation du bien-être des dauphins.

Abstract

Animal welfare science is a burgeoning field, but research on cetaceans (whales, dolphins and porpoises) is lacking. Bottlenose dolphins (*Tursiops truncatus*) are the most well-known and studied cetaceans, particularly in captivity, and thus are used in this review as a model for other cetacean species. Despite the public interest and need for such research, studies specifically investigating dolphin welfare are lacking. This review uses the three broad categories of behaviour, health, and cognition, to discuss how dolphin welfare has been assessed thus far, and could be assessed in future. We present welfare indicators validated in other species that could be applied to dolphins, including innovative measures such as cognitive appraisal of emotions. We provide a summary of practical recommendations for validating the indicators of bottlenose dolphin welfare. This paper aims to stimulate further research into dolphin welfare which could improve the lives of the animals themselves and ultimately support regulatory decisions. We recommend uniting expertise in cetology and welfare science in order to develop a holistic approach to dolphin welfare assessment.

Keywords:

Affective states, animal-based measures, animal welfare, bottlenose dolphins, cetaceans, welfare assessment

1. Introduction

Research into welfare assessments for zoo and aquarium (hereafter referred to as 'zoo') animals is increasing as farm animal welfare assessment is proven to be feasible and valid (Barber, 2009; Whitham and Wielebnowski, 2013). There is much support for the adaptation of farm animal measures to zoo animals (Hill and Broom, 2009; Mason and Veasey, 2010; Swaisgood, 2007), and zoological institutions are well set-up for conducting measures due to the individualised care and multiple daily interactions (Barber, 2009).

In this review, we conceptualise welfare using Spruijt et al.'s (2001) description of a "…*balance between positive* (*reward, satisfaction*) *and negative* (*stress*) *experiences or affective states. The balance may range from positive* (*good welfare*) *to negative* (*poor welfare*)*".* This "feelings-based" definition aligns with positions taken recently by many others (e.g. Fraser and Duncan, 1998; Mason and Veasey, 2010; Watters, 2014; Yeates and Main, 2008), and specifies measurement of both positive and negative welfare. Our review also prioritises animal-based over resource-based measures, since they are more likely to accurately reflect welfare (Roe et al., 2011; Webster, 2005; Whitham and Wielebnowski, 2013). We also give equal consideration to indicators of positive and negative welfare (Boissy et al., 2007; Désiré et al., 2002; Paul et al., 2005; Yeates and Main, 2008).

There is very little existing research on the welfare of cetaceans (Clegg et al., 2015; Ugaz et al., 2013), in captivity or the wild. Given that public interest often stimulates research in the associated areas (e.g. with farm animal welfare, Rushen et al., 2011), cetacean welfare studies are likely to increase markedly in the near future since the public's interest is at a high level and intensity (Grimm, 2011; Ventre and Jett, 2015). Although many questions posed are in fact ethical dilemmas (e.g. *"Should we keep dolphins in captivity?"),* objective data on the animals' welfare state would aid in these personal decisions (Clegg et al., 2015; Jiang et al., 2007). Published farm welfare assessments have shown how this type of data can be gathered, for instance the WelfareQuality® project for farm animals (WelfareQuality®, 2009a,b,c) and its subsequent cross-species adaptations (e.g. Barnard and Ferri, 2015; Clegg et al., 2015; Mononen et al., 2012;).

While cetology, the study of cetaceans, has burgeoned (Hill and Lackups, 2010), there are very few studies on cetacean welfare and methods of assessment (Clark, 2013; Clegg et al., 2015; Galhardo et al., 1996; Ugaz et al., 2013). Bottlenose dolphins (*Tursiops truncatus*) are the most studied cetacean species (Hill and Lackups, 2010), and the most common in captivity (Pryor and Norris, 1998; Wells and Scott, 1999), thus we choose them as the focus species for this review of how welfare science could be applied to cetaceans. We arrange the relevant cetology knowledge into behaviour, health-related and cognition research, all well-established specialities (Wells, 2009). These three categories are derived from Webster's (2005) 'Triangulation' principle for the measurement of welfare, where accumulating information from each specialty increases overall validity. Importantly, while our review includes wild research and welfare applications (wild animal welfare should be measured: Jordan, 2005; Ohl and van der Staay, 2012), our discussions orientate towards captive dolphins since their environment is closely controlled by humans. The necessity for dolphin welfare research is clear: there are 250 bottlenose dolphins in the European Association of Zoos and Aquaria facilities (EAZA, 2015), 444 animals listed in US and Canadian dolphinaria (Ceta-base, 2011), and many others worldwide not registered on an official record, all maintained in a huge range of facility types that differentially impact welfare (Joseph and Antrim, 2010).

This review compiles what we believe to be literature on wild and captive bottlenose dolphins most relevant to welfare, suggesting some farm and zoo animal approaches which could be adapted to cetaceans, with final recommendations on initial studies and how the dolphin welfare discipline might evolve. A strong focus is maintained on those areas of cetology that merit further investigation to answer questions on bottlenose dolphins' quality of life.

2. Published Work on Dolphin Welfare

There are very few studies of dolphin welfare, either in captivity or the wild (Clegg et al., 2015; Ugaz et al., 2013). Thus there are no validated measures, i.e. ones that we know are linked to positive or negative affective states, as yet. Given the dearth of welfare research, in some cases findings from other cetacean species are extrapolated to bottlenose dolphins.

2.1 Studies of wild dolphin welfare

Very few studies discuss the concept of wild cetacean welfare, but there are a handful which essentially aim to assess welfare even if direct mentioning of the word is rare. A popular topic has been assessments of the impacts of tourist boats on various cetacean species (e.g. Christiansen and Lusseau, 2015; Stockin et al., 2008), although the focus remains at population-level indicators. Long-term data revealed that rate and repetitions of wild bottlenose dolphin whistles were potential indicators of short-term stress (Esch et al., 2009). A more recent study suggested that an upward shift in whistle frequency was linked to increased emotional arousal (Heiler et al., 2016). Butterworth et al. (2013) empirically evaluated dolphin welfare in the Taiji drive hunts, an annual harvesting of dolphins in Japan, but this research only concentrated on welfare at the point of death. In the first and only teaming of wild marine mammal research with animal welfare science to our knowledge, Butterworth et al. (2012) used the five freedoms to discuss how entanglement affects individual animal welfare in a number of species, including dolphins.

2.2 Studies measuring captive dolphin welfare

Similarly, there are only a handful of captive dolphin studies that have endeavoured to develop welfare measures. Ugaz et al. (2013) correlated salivary cortisol to behavioural parameters in 23 *T. truncatus*, concluding that welfare was better in open (enclosed area of the sea) than closed (artificial water and pool) facilities due to lower cortisol levels and less floating and circular swimming. Castellote and Fossa (2006) suggested acoustic activity as a welfare measure for belugas (*Delphinapterus leucas*) and found it dropped to low levels during stressful events, but they did not correlate it with other parameters and only studied two animals. In a multidisciplinary approach, Waples and Gales (2002) looked at inappetence, social behaviour, lethargy, weight loss and blood parameters in three *T. truncatus* with substantially deteriorating welfare likely due to social stress, revealing useful associations although again limited by sample size.

2.3 The C-Well© Assessment: Clegg et al., *(2015)*

In the first development of a welfare assessment for dolphins, Clegg et al., (2015) studied 20 *T. truncatus* in three facilities and adapted a well-established farm animal assessment (WelfareQuality®, 2009a,b,c) to this species (the C-Well© Assessment). The research used 36 multi-dimensional measures, 58% of which were animal-based, to yield individual welfare scores comparable on many different levels (e.g. by measure, criteria, in total; among pools, sex, age class, facilities). Although the measures were unweighted, they were validated through expert opinion and application in specific contexts, and have associated standardised methods and thresholds. Some are reviewed in the relevant categories of section 3.

Given the lack of existing studies on dolphin welfare indicators, the next section is a review of cetology disciplines relevant to welfare. We expanded Webster's (2005) welfare measurement categories to behaviour, health (from Webster's 'physiology') and cognition (from 'neurobiology'). Health, while still including physiology, encompasses longer-term welfare indicators, and cognition includes experimental psychology methods potentially valuable for assessing welfare.

3. Research Relevant to Dolphin Welfare

3.1 Health

3.1.1 Health-Welfare interface

Health and welfare interact directly and indirectly as part of a complex relationship (Walker et al., 2012). A reasonable level of health is considered a prerequisite for good animal welfare (Hill and Broom, 2009; Webster, 2005), while poor health is a likely contributing factor to poor welfare (Boissy et al., 2007; Dawkins, 2004; Fraser et al., 1997; Mason and Veasey, 2010). But do all components of poor health affect welfare? We refer back to our definition of welfare (Spruijt et al., 2001) to address this: the balance of affective states and health and should only be impacted when poor health either directly impacts affective state through, for example, nausea, lethargy or pain, or indirectly through loss of function. Poor health (e.g. an asymptomatic tumor) does not always affect emotional state and hence welfare, as we define it (Fraser et al., 1997; Mason and Veasey, 2010).

3.1.2 Health parameters in dolphins

An infection or disease can cause pain and/or ‘sickness behaviour’, which includes inappetence, lethargy, depression, and anti-social behaviours, all of which have direct or indirect effects on affective state (Broom, 1991; Millman, 2007; Sneddon et al., 2014). Dolphins tend to mask symptoms of pain and disease as a survival adaptation (Castellote and Fossa, 2006; Waples and Gales, 2002), which therefore may only become obvious when the health problem is severe. Perhaps as a consequence, little is published about indicators of pain in dolphins, with exceptions for extreme situations such as their behavioural response to killing methods in the wild (Butterworth et al., 2013). Weary et al. (2006) and Sneddon et al. (2014) provide cross-species advice for identifying behavioural and physiological pain markers, such as studying behavioural differences after analgesia administration. Inappetence and lethargy in dolphins have been correlated with many different diseases and together are generally reliable as poor health indicators (Johnson et al., 2009; Joseph et al., 1986). However they can also be caused by social stress or even reproductive events such as oestrus (Waples and Gales, 2002), where the associated affective states may vary from negative to positive. Studies on the behavioural and haematological characteristics of inappetence, where differentiations are made depending on whether it was caused by poor health or social stressors, are much needed.

In lieu of reliable pain indicators, physical bodily damage has been used as a health-related welfare measure in other captive species (Broom, 1991; Mononen et al., 2012; WelfareQuality®, 2009a,b,c), and for wild animals as well (e.g. Cattet et al., 2008; Jordan, 2005). Clegg et al. (2015) proposed the percentage of rake marks (superficial lesions and scars caused by conspecifics in play, sexual and aggressive behaviours, Scott et al., 2005) on the body as a welfare measure for bottlenose dolphins, since such marks can be used as a proxy indicator of aggression levels and social stress (Orbach et al., 2015; Scott et al., 2005). However, this measure requires further investigation for example to differentiate rake mark levels due to high levels of play and aggression while controlling for age and sex differences.

Longer-term measures of dolphin health could also be useful for assessing welfare. Body condition scoring (BCS), an assessment of the extent of body fat present (Roche et al., 2009), has been favoured as a general welfare measure (e.g. wild: Cattet et al., 2008; Mann and Kemps, 2003; Pettis et al., 2004; captive: Mononen et al., 2012; Roche et al., 2009; WelfareQuality®, 2009a,b,c;) and it has already been used in wild health assessments of *T. truncatus* (Fair et al., 2014; Schwacke et al., 2014). Joblon et al. (2014) produced a standardised protocol using stranded short-beaked common dolphins (*Delphinus delphis*), and Clegg et al. (2015) developed a standardised BCS graphic for *T. truncatus* but did not test its reliability. The next step for these BCS tools is to correlate the results to other measures of affective state: Roche et al. (2009) conducted this with cows, concluding that BCS may serve as a proxy indicator for hunger, satiety or feeling ill (leading to inappetence). Other health-related conditions such as diarrhoea, skin inflammation, eye condition, and coughing, have been used as farm animal welfare measures (Mononen et al., 2012; WelfareQuality®, 2009a,b,c), some of which were proposed for *T. truncatus* welfare (skin and eye condition, coughing; Clegg et al., 2015), but have not been studied in relation to affective states. Haematological indices can be measures of disease states, especially when the pathology is advanced, but so far have had limited use in welfare assessments due to potential high inter- and intra-individual variation. Although wild dolphin health assessments have published their data and established baselines (Dierauf and Gulland, 2001; Thomson and Geraci, 1986; Wells, 2009), and captive dolphin voluntary blood sampling is readily achievable using positive reinforcement training (Brando, 2010), studies have not yet linked ranges of blood values to health-related welfare.

Population measures of longer-term health and welfare such as longevity and reproductive rate should also be considered (Barber, 2009; Dawkins, 1998). However as with farm animals, parameters, such as high reproductive success, do not necessarily indicate that welfare is good (Dawkins, 1980). For captive dolphin populations, baselines are being established by projects (notably in the US) allowing access to their valuable multi-species databases (Innes et al., 2005; Small and Demaster, 1995; Venn-Watson et al., 2011). Welfare conclusions from fitness measures should be supported by other data (Swaisgood, 2007), such as in Christiansen and Lusseau's study (2015) linking disturbance behaviour from whale-watching boats, body condition and fetal growth rate in minke whales (*Balaenoptera acutorostrata*). Data on the incidence and severity of diseases can also be used as population-level health parameters: such wild studies are available (e.g. Reif et al., 2008; Schwacke et al., 2014), but data are not published for captive dolphins. However, extensive records are kept for most captive dolphins (personal communication, C van Elk, September 2016) and thus peer-reviewed publications on the nature of their diseases would be beneficial for establishing standardised health assessments.

3.1.3 Physiological parameters

Measures of physiological responses can contribute to assessments of emotions and affective states (Boissy et al., 2007; Désiré et al., 2002; Webster, 2005). Endocrine (hormonal) responses to stressors are most commonly used (e.g. Moberg and Mench, 2000), but as interest increases in positive welfare, other markers are being considered: for example the balance of sympathetic and parasympathetic systems (review by Boissy et al., 2007), and indicators of eustress (positive stressors, e.g. mate acquisition, experienced by the animal, Selye, 1975). Within dolphin physiology research, numerous studies of physiological measures of stress for wild *T. truncatus* (e.g. Fair et al., 2014; Ortiz and Worthy, 2000; Schwacke et al., 2014) have provided useful baselines, which will start to elucidate individual variation and repeatability questions (Atkinson et al., 2015). Sample collection in the wild is challenging since taking blood is not possible without restraint and faecal samples are difficult to obtain (Atkinson et al., 2015). This area specifically is where training for voluntary samples in captivity has exceptional advantages; for example, voluntary saliva collection is feasible and can provide accurate cortisol measurements in *T. truncatus* (Pedernera-Romano et al., 2006; Ugaz et al., 2013). Other sampling protocols are also possible with training, such as blood, faecal, blow (expiration of air) and biopsy collection. As for terrestrial animals, marine mammals experience diurnal and seasonal variation in cortisol levels (see Atkinson et al., 2015 for review), which would need to be taken into account in any welfare assessment and suggests that a conservative range would need to be used in any conclusions made, as opposed to a single threshold. Additionally, a recent review advised caution when applying terrestrial animal stress models to marine mammals. While corticosteroid pathways seem to be similar, evidence indicates other neuroendocrine hormones (e.g. catecholamines) may be regulated very differently (Atkinson et al., 2015). Further, long-term studies on cetaceans in captivity could start to answer such questions on hormone regulatory systems. Innovative new collection techniques enabling accurate animal identification (example with cetaceans: whale blow), and insightful behavioural correlations, must also guide future progress (Möstl and Palme, 2002): advice very applicable to dolphin studies.

3.2 Behaviour

3.2.1 Social behaviour

Behavioural measures are an important component in welfare frameworks (Dawkins, 2004; Maple, 2007), with some believing that they are more informative about welfare than health since behaviours are likely more indicative of emotional state (Gonyou, 1994; Joseph and Antrim, 2010). There have been a number of long-term studies of wild dolphin behaviour (Mann et al., 2000; Parsons et al., 2006; Wells, 1991), including social relationships within their fission-fusion societies (Mann et al., 2000; Wells, 2009). Surprisingly, ethological studies have not been commonplace for captive populations until recently (Dudzinski, 2010). Social behaviour measures will foreseeably be one of the most important tools in assessing dolphin welfare: as highly social mammals (Mann et al., 2000; Pryor and Norris, 1998), they are susceptible to social stress. Sudden changes in conspecific associations, aggression levels and social isolation have been correlated with declines in welfare (Waples and Gales, 2002). Excessive or abnormal aggression levels are used as farm animal welfare measures (Mononen et al., 2012; Webster, 2005; WelfareQuality®, 2009b), and using existing ethograms of aggressive behaviours to analyse frequencies over time could reveal dolphins' 'excessive' and 'normal' thresholds (Samuels and Gifford, 1997; Scott et al., 2005). Increased quantity and severity of rake marks could serve as a proxy indicator for levels of aggression and social stress (Scott et al., 2005; Waples and Gales, 2002). Clegg and co-authors' (2015) rake mark assessment, currently using very conservative thresholds, might be validated to allow monitoring of aggression levels. Rake mark quantification is an example of a method where collaboration between wild and captive researchers might be fruitful, since aggression is harder to observe in the wild and rake mark levels could be used as proxy measures (Clegg et al., 2015; Scott et al., 2005).

While social stress is a negative consequence of being a social mammal, there are also positive effects of their highly social life of dolphins. Positive social (i.e. affiliative) behaviour has been proposed as an indicator of good welfare in other species (Boissy et al., 2007; Buchanan-Smith et al., 2013). Affiliative behaviour has been well-documented in wild *Tursiops* spp. (Connor et al., 2000; Connor et al., 2006b; Herman and Tavolga, 1980 (early review); Sakai et al., 2006), and a little less so in captivity (Dudzinski, 2010; Tamaki et al., 2006). Gentle rubbing behaviours between dolphins are thought to be analogous to allogrooming in terrestrial mammals (Kuczaj et al., 2013; Tamaki et al., 2006) and may have potential as a measure of goof welfare (Boissy et al., 2007), along with synchronous swimming (thought to reflect social bonds, Connor et al., 2006b). Dudzinski (2010) and Kuczaj et al., (2013) reviewed both wild and captive social affiliative behaviour, agreeing that in both settings tactile behaviours seem to be used to express emotions.

3.2.2 Play

Play behaviour is one of the strongest potential welfare indicators for animals, mainly because it is more likely to occur in the absence of threats and utilitarian needs (Bel'kovich et al., 1991) and is linked to positive emotions (Held and Špinka, (2011) reviewed link with welfare). Play is also likely to improve long-term fitness and health, as well as being socially contagious and therefore capable of spreading good welfare in groups (Held and Špinka, 2011); these less-acknowledged benefits are especially relevant to the welfare of group-living dolphins. Despite this, play is not commonly used as a measure in welfare assessments (WelfareQuality® 2009a,b,c), most likely because of its inherent variability (Held and Špinka, 2011) and difficulty of measurement, as well as evidence in some species that it may not always be linked to a positive emotional state (Blois-Heulin et al., 2015).

Evidence for wild and captive dolphin play is abundant (reviews by: Kuczaj and Eskelinen, 2014; Paulos et al., 2010), including copious examples of object play (recent papers: Delfour and Beyer, 2012; Greene et al., 2011; Kuczaj and Makecha, 2008; Paulos et al., 2010), and evidence of inventing games (Pace, 2000). McCowan et al. (2000) showed that captive dolphins monitored their bubble quality as well as "plan" for the behaviour: this suggests involvement of conscious thought and appraisal, strengthening the notion that play impacts affective state. Hill and Ramirez (2014) studied play in 14 belugas (*Delphinapterus leucas*) over three years, revealing differences between adult and young preferences and showing that bouts were longer and more diverse when enrichment devices were involved. Where play is studied in captivity, the influence of any prior conditioning should be noted: Neto et al. (2016) showed that when trainers positively reinforced dolphins' interactions with toys, their interest in the objects increased outside of the sessions. This technique could be used to increase the benefits of the toys to the dolphins, but until we have other measures of positive affective states available in this situation the motivation to play may be influenced. In any case, as with all species, standardised measurements are needed. A study on African elephants (*Loxodonta africana*) addressed this using a play index (Vicino and Marcacci, 2015), and a similar approach might be possible with dolphins. Such a behavioural measure could easily be applicable to wild dolphin welfare assessments, for example to investigate whether exposure to more environmental or social stressors show reduced play frequencies.

3.2.3 Abnormal behaviour

Abnormal behaviours are most often studied in the context of stereotypic behaviour, which has been most recently defined as "*…repetitive behaviour induced by frustration, repeated attempts to cope, and/or CNS dysfunction"* (Mason and Rushen, 2008). Abnormal behaviours are seldom observed in wild animals, although Miller et al. (2011a) suggest they observed stereotypic swimming in lemon sharks (*Negaprion brevirostris*). In one of the only studies describing abnormal behaviour in wild dolphins, the causes and effects of solitary living for *T. truncatus* were investigated, and certain aspects were concluded as abnormal (e.g. behaviour oriented excessively towards humans, Muller and Bossley, 2002). Stereotypic behaviour is commonly investigated as a welfare measure for captive animals (review by Mason and Rushen, 2008). There are scarcely any published studies with captive dolphins, and the small handful existing are out-dated (Clark, 2013 and Gygax, 1993 describe this literature), making it hard to identify any common explanatory variables. Stereotypic swimming has been discussed in the literature as a concern for captive dolphins. There are disparities among definitions of this behaviour (Gygax, 1993; Miller et al., 2011b), but terrestrial animal studies also suffered similar problems with pacing behaviours and found that the variability and possible functions must be meticulously analysed for it to be defined as a stereotypy (Rushen and Mason, 2008). Sobel et al. (1994) observed preferences in the directionality of circular swimming, but did not measure whether the route around the pool was fixed and whether the animal was vigilant at the time. There is little evidence correlating stereotypic swimming with other potential factors of welfare: for example, although Ugaz et al. (2013) found that in closed facilities there were higher rates of circular swimming as well as higher cortisol levels, they did not statistically test for a correlation between these two factors. Clegg et al. (2015) included a stereotypy measure but based thresholds on terrestrial animal frequencies, since there was no data from dolphin species. Since there are on-going questions about whether higher stereotypy frequency infers poorer welfare even in terrestrial animals (Dawkins, 2006; Mason and Latham, 2004; Rushen and Mason, 2008), future studies on this phenomenon in dolphins should aim to correlate suspected stereotypic behaviour with other indicators of welfare to validate it as an indicator. Basic work regarding the appearance of stereotypies is also needed, for example the two main types of stereotypy defined in terrestrial mammals are ‘oral’ and ‘movement’ (Mason and Rushen, 2008; Webster, 2005), so a fundamental investigation would be whether the same is true for dolphins.

3.2.4 Anticipatory behaviour

Recently, anticipatory behaviour (a measure of “reward-sensitivity”, Spruijt et al., 2001) has been used as a welfare measure in farm and zoo animals; low intensity anticipatory behaviour is thought to reflect positive affective states and high intensity anticipation indicates poorer welfare as the animal is heavily dependent on the reward (Spruijt et al., 2001; Watters, 2014). While one preliminary study focussed on anticipatory behaviour in captive bottlenose dolphins (Jensen et al., 2013), further work is necessary given the different training methods (i.e. conditioning to ‘rewards’) in dolphin facilities, which might ultimately be closely linked to welfare. Based on the reward-sensitivity paradigm it is likely that dolphins showing moderate anticipatory behaviour might experience positive affective states, while those that perform it for excessive amounts of time might be frustrated or have little other stimulation (Watters, 2014). In order to evaluate its utility for welfare assessment, future studies with dolphins and other species should investigate the association between anticipatory behaviour frequencies and other welfare indicators, in order to understand what might qualify as ‘excessive’ levels.

3.3 Cognition

3.3.1 Emotions

Emotions are defined as intense, short-lived affective responses to an event, usually associated with specific physiological changes (Danzter, 1988). Animals with higher cognitive abilities may be capable of more complex emotions (e.g. guilt) (Paul et al., 2005), and while this might result in increased chances of suffering, it could also lead to higher potential for positive affective states. Research beyond the 'basic emotions' (e.g. fear, rage, play; Mendl et al., 2010) is essential to understand the valence and arousal levels of affective states which make up overall welfare (Leliveld et al., 2013; Siegford, 2013). Boissy et al. (2007) and Désiré et al. (2002) provide reviews on measuring animal emotion and the relevance to welfare.

Although dolphin emotion studies are scarce, there have been more studies on negative than positive ones. Most studies have looked at how sounds might reflect emotions, for example burst pulse sounds have been associated with agonistic and aggressive behaviours (Overstrom, 1983), and long-term etho-acoustical projects have made headway in pairing sounds to behaviour (e.g. Herzing, 1996; Janik and Sayigh, 2013). Animal emotion research is now widening to measure positive emotions as well (Boissy et al., 2007), but there are no strongly supported indicators as yet in dolphins (Kuczaj et al., 2013). Tactile behaviour was suggested by Dudzinski (2010) and Kuczaj et al. (2013) to be linked to positive emotions in dolphins, but has not yet been analysed in conjunction with other indicators. Motivation and preference testing are applicable to captive dolphins and could reveal indicators of emotion (Gonyou, 1994; Mendl et al., 2010).

3.3.2 Environmental enrichment

This sub-section is applicable to dolphins under human care only. Environmental enrichment is any technique designed to improve biological functioning of captive animals through environmental modification (Newberry, 1995). Bottlenose dolphins are good candidates for enhanced welfare through enrichment due to their cognitive abilities (Schusterman et al., 2013) and propensity to, and creativity within, play (Kuczaj and Eskelinen, 2014). Enrichment has been provided to captive dolphins for several decades, but there are few published studies describing the animals' responses (for a review see Clark, 2013). Furthermore, providing enrichment is often assumed to automatically enhance welfare even if it is unclear whether the animal's affective state will be improved (see reviews by Swaisgood, 2007; Würbel and Garner, 2007). Enrichment should be kept enriching by monitoring the animals' responses and looking for signs of habituation, allowing management teams to form a feedback loop which influences when, where and how the enrichment is presented again (Hoy et al., 2010; Kuczaj et al., 2002; Siegford, 2013).

Recently, cognitive challenges have been presented as enrichment, with positive results as long as the animals possess the resources and skills to solve the problem (Meehan and Mench, 2007; Siegford, 2013). Clark (2013) supports cognitive enrichment with dolphin species, hypothesising that floating, simplistic objects are not sufficient to hold the dolphins' interest in the long-term. However, behaviour should be monitored to investigate whether this is indeed the case (Hill and Broom 2009), and such data, which shows responses to definable, repeatable contexts, could also aid in finding welfare indicators (Delfour and Beyer, 2012). The Human-Animal Relationship (HAR) is only just beginning to be investigated in other species in relation to cognitive enrichment and welfare (Whitham and Wielebnowski, 2013) and, due to the multiple, daily, and often close-contact interactions, is very likely to contribute to the welfare state of captive dolphins (Brando, 2010; Clegg et al., 2015). Future investigations assessing the HAR might aim to disentangle the effects of food reinforcement with the dolphins' attitude towards the humans themselves. An example of such an approach is shown by Perelberg and Schuster (2009), who demonstrated that outside of feeding sessions, a captive bottlenose dolphin group approached humans to receive rubs and petting in the absence of any other rewards. Given that many cetacean species show much tactile behaviour during intra-specific social affiliation (Dudzinski, 2010; Kuczaj et al., 2013), investigating the frequency and dimensions of voluntary human contact by the animals, during and outside of training sessions, might be a meaningful measure of their affective states.

3.3.3 Cognitive measures of dolphin welfare

Dolphins' cognitive abilities are frequently compared to those of great apes (Delfour, 2010; Schusterman et al., 2013), and allow them to thrive in their fusion-fission society (Connor et al., 2000; Maze-Foley and Wursig, 2002), display cooperative hunting (Connor et al., 2000), use tools (e.g. Smolker et al., 1997), and recognise their mirror image (Delfour, 2006; Reiss and Marino, 2001). Studies of cognitive bias, which investigate how emotional experiences affect cognitive processes, may aid in our interpretation of welfare, and constitute measures themselves (Mendl et al., 2009; Paul et al., 2005). Given the dolphins' learning capabilities (Brando, 2010), many of the non-invasive cognitive bias methods reviewed in Mendl et al., (2009) used with other species could be adapted. Paul et al., (2005) also reviewed evidence for memory and attention bias processes in animals, concluding that if confirmed they could have implications for measuring welfare.

In humans as well as non-humans the brain hemispheres process information differently, producing lateralised behaviours, i.e. a preference for either the left or right eye or body part (Rogers, 2002). It seems that animals may predominantly use the right hemisphere when stressed (see Rogers, 2010), with Leliveld et al.'s (2013) review going further to conclude that negative emotions are managed by the right hemisphere and positive emotions by the left ("emotional lateralisation"). Examples of lateralised behaviours in wild and captive cetaceans are common e.g. during foraging (Clapham et al., 1995; Silber and Fertl, 1995), flipper-rubbing (Sakai et al., 2006), and visual discrimination tasks (Delfour and Marten, 2006; Yaman et al., 2003). Most notably, Karenina et al. (2010, 2013) showed that belugas and killer whales (*Orcinus orca*) placed calves on their right side in non-threatening situations, with killer whales preferring the left when the situation became increasingly threatening (in this case proximity to boats). Sakai et al. (2006) suggested a link with positive affective state since the left pectoral fin and eye were favoured during affiliative flipper rubbing behaviour in Indian Ocean bottlenose dolphins (*Tursiops aduncus)*. These last examples concerning lateralised behaviour and affective states should form a basis for future research into welfare implications of this phenomenon.

In the field of cognition in particular, but within health and behaviour as well, researchers notably either study wild or captive dolphins but rarely both, resulting in a skewed perspective of particular topics in certain environments, and leading us to an initial recommendation to increase collaborative efforts and reviews (agreeing with Hill and Lackups, 2010; Pack, 2010). Finally, although we must understand the dolphins' cognitive abilities, we should do so bearing in mind their *umwelt,* i.e. their "subjective universe", and the focus of etho-phenomenological studies (Delfour, 2006, 2010). For example, an intermodal associative task was completed very differently by bottlenose dolphin subjects due to the dominance hierarchy at the time dictating participation and mode of learning (Delfour and Marten, 2006). Being cognisant of the dolphins' *umwelt* may help in determining what is important to the dolphins, and thus how to provide them with a good quality of life.

4. Considerations for Developing Dolphin Welfare Measures

In this section we review recommendations on design of studies that choose to investigate the measures discussed above. Welfare measures should be developed *in situ*, thus ensuring applicability to the dolphins and their environment (Dawkins, 2006; Maple, 2007). The measures must also be species-specific (Barber, 2009; Blokhuis, 2008; Hill and Broom, 2009), examining welfare on an individual level where possible (Siegford, 2013). Zoological institutions have been advised to employ scientists specifically dedicated to assessing welfare (Maple, 2007; Barber, 2009), and facilities maintaining dolphins should take this step too.

The first proposed measures for *T. truncatus* should be validated through correlations with other parameters. Our review pools together potential measures for validation: those which we have identified in cetacean health, behaviour and cognition (Table 1). Captive dolphin research should take the lead in establishing welfare measures due to greater access to the subjects, their history, and their environment than with wild populations. International, inter-facility collaborations are vital to combat problems of low sample sizes and to control for inevitable environmental variation. For wild dolphin welfare indicators, long-term studies are the natural starting point since most have individual behavioural, physiological, as well as life history data (Fair et al., 2014; Wells, 2009). While it would be inaccurate to apply all measures for wild and captive animals without validation (Jordan, 2005), it is likely that many welfare indicators, at least behaviourally, will be consistent between wild and captive *T. truncatus* since their repertoires show similarities (Mann et al., 2000; Dudzinski, 2010).

Table 1 *Summary of the welfare-related topics in dolphin health, behaviour and cognition which merit further investigation in order to develop measures of welfare. Evidence supporting each topic has been taken from bottlenose dolphins (*Tursiops

Category	Aspects meriting further investigation as dolphin welfare measures
Health	Epidemiological measures (e.g. mortality, reproductive success) Disease prevalence Body Condition Scoring Cortisol (and other stress hormone) levels Rake mark percentage cover
Behaviour	Excessive aggression Affiliative behaviour Play Anticipatory behaviour Abnormal and stereotypic behaviours
Cognition	Emotions linked with sound production Indicators of basic emotions (e.g. fearful, playful, rage) Indicators of more complex emotions (e.g. contentedness, depression) Cognitive bias testing Visual and behavioural laterality

truncatus*) where possible, but where these were lacking studies from other cetacean species had to be used.*

When validating the first groups of measures, contexts should be used where it is likely the animal has very good or poor welfare (Castellote and Fossa, 2006; Jordan, 2005; Whitham and Wielebnowski, 2013). Transportation offers opportunities to assess welfare as it is assumed to induce a substantial, but short-term, welfare change for captive cetaceans (e.g. Castellote and Fossa, 2006). Long-term states associated with social contexts may be more salient for welfare measurement: for example the period after transport when the animals are introduced to a new group. Group changes are frequent enough in dolphinaria networks to provide adequate sample sizes for analysis. The selected behaviours and physiological parameters should then be measured during these events (and cognitive data if possible), with focal qualitative data (e.g. trainer ratings) taken concurrently to support the presumed change in welfare. Welfare measures should be conducted regularly, and also separately from full assessments. For example, behavioural measures of welfare could be applied on a weekly basis to dolphin groups since behavioural monitoring has been advised as essential for ensuring good welfare (Maple 2007), and especially with captive dolphins (Clegg et al., 2015; Waples and Gales, 2002). Eventually, comparing results from measures and assessments between individuals can highlight associations with good or poor welfare, thus indicating where changes in management protocols should occur and stimulating improvements in welfare of the animals themselves.

5. Conclusions

We have reviewed the literature on animal welfare science and cetology in order to identify the most successful intersections for developing bottlenose dolphin welfare measures. A general theme is that collaborations, whether wild-captive, across different cetology fields, or between multiple captive facilities, are necessary if we want to address this multi-dimensional concept.

We suggest that indicators such as cortisol levels, inappetence and bodily injuries, as well as body condition and population fitness measures in the longer-term, may help us assess health-related welfare. Behavioural measures are likely to be the most informative for dolphin welfare, and we have shown evidence that tactile affiliation, play, anticipatory behaviour and stereotypic behaviours may be closely linked to affective states. Cognitive measures reflect how behavioural and physiological components are integrated to form the affective states experienced by the animals, and thus recent techniques such as cognitive bias testing hold much promise for welfare assessment.

Lastly, we identified practical recommendations for validating the first measures, concluding that although captive studies should take the lead, long-term wild studies are also rich sources of potential indicators. Any proposed measures should be tested in situations likely to elicit changes in welfare with adequate sample sizes to allow the major environmental variations to be controlled for. Established measures would allow facility managers to monitor and improve the dolphins' welfare, aid in regulatory decisions, and could enrich wild dolphin research by revealing changing affective states. This review's findings are species-specific to bottlenose dolphins, but the general principles and selected measures could be adapted to other cetacean species. Our overall aim was to present current cetology knowledge in terms of measuring welfare, with the hope of stimulating researchers globally to take up the challenge.

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Thesis ethogram

Following Paper 1, and in preparation for Paper 2 and indeed any other behavioural studies that were going to be conducted during the thesis, a comprehensive ethogram was developed of bottlenose dolphin behaviours. The ethogram was constructed using six months of observations at Parc Astérix, Planète Sauvage and Dolfinarium Harderwijk (October 2014 – March 2015), and the behavioural definitions were adapted from those found in the literature (Connor et al., 2006b; Dudzinski, 1996; Galhardo et al., 1996; Holobinko and Waring, 2010; Miller et al., 2011b; Sakai et al., 2010; Samuels and Gifford 1997; Trone et al., 2005).

BEHAVIOUR	DESCRIPTION
Surface rest	Stationary at water surface, eye(s) half or fully closed, minimal head orientation movements
Bottom rest	Stationary at bottom of pool, eye(s) half or fully closed, minimal head orientation movements
Slow swimming	Swimming with small tail beats, eyes generally open, head may orientate to conspecifics, may change position around other dolphins if synchronous, speed generally <2m/s
Medium swimming	Swimming with moderate tail beats, eyes generally open, alert to stimuli, speed roughly 2-4 m/s
Fast swimming	Swimming with vigorous tail beats (c. 3 beats/s), head moves up and down, eyes open, speed is around 4 m/s
Social play	Dolphin engaged in rubbing, nudging, chasing, attempting to bite, pushing, jumping, all more gently and at lower speeds (< 4m/s) than agonistic interactions. There are few prolonged instances of aggressive postures such as “S” posture (arched back, head and tail lower) or jaw clapping, but they may occur very briefly during intense play.
Object play	Play behaviours (e.g. chasing, biting, pushing, jumping) involving a toy/bubble/part of the environment, can be solitary or part of a group
Locomotor play	Play behaviours involving body movements and manipulation of body parts (without object or other dolphin moving them). Usually solitary, but can be social if other dolphin imitates or watches closely. Includes carrying (but not playing with) the object.
Tail chasing	Dolphin is open-mouthed and trying to bite or chase the tail, and sometimes then dorsal and pecs, of another dolphin, who responds neutrally i.e. does not respond with play behaviour or aggression; recipient dolphin often tries to avoid these threats but is not aggressive at this point towards actor.
Rubbing	Dolphin rubs body or part of body clearly back and forth against another object, and not incidentally in an play bout: can be social i.e. with another dolphin (even with teeth if very gently and slowly), or solitary i.e. against the environment, but is non-sexual (not focussed on genitals, no obvious arousal)
Pectoral rubbing	Dolphin moves pectoral fin(s) back and forth to rub the body of another. Non-sexual (i.e. not concentrated on genitals). Note who is the actor and recipient.
Nudging	Dolphin pushes another dolphin (any body part but genitals, otherwise is GNG), usually with rostrum but also could be with melon/pec fin. If at fast

	pace and very directed, defined as aggressive Body Hit.
Synchronous swimming	Dolphin swimming within 1 body length of another dolphin, showing parallel movements and body axes, with only a few seconds delay at most between movements
Contact synchronous swimming □	Dolphin is swimming while touching another dolphin. To be defined as contact the animals must touch/be touching at least every 4-5 seconds. If more time elapses, the contact swimming bout is over. The contact could be minimal (just pectoral tips, dorsal) or maximal (full belly-melon).
Within touching synchronous swimming □	Dolphin is swimming within touching distance ((less than 0.5m) of another animal i.e., if it made a small movement it could conceivably touch its partner.
Close synchronous swimming □	Dolphin is swimming out of touching distance with another animal but still within 1m (therefore distance is between 0.5 - 1m away)
Distant synchronous swimming □	Dolphin is swimming more than 1m away from the next dolphin but within 1 body length (further than 1 body length would not be synchronous swimming).
Biting †	An aggressive behaviour where dolphin bites/rakes or tries to do this to another dolphin: may swim along with mouth open
Jaw pop †	Dolphin gestures with an open mouth, may open and close rapidly; may also be a bubble stream
Chasing †	Dolphin chases another aggressively at high speed (roughly 4 m/s), may see S posture
Body hit †	Dolphin uses body/body part to hit another dolphin with force
Tracking †	Dolphin orientates head towards another dolphin obviously while swimming next to it, but not touching (note if actor or recipient). Preceding or succeeding aggressive behaviour, may be showing S-shape. Not as fast as chasing behaviour, and not oriented towards genitals.
Genital-Genital sexual contact	Dolphin engages in genital to genital contact, with or without full penetration, with both dolphins' bodies generally on the same axis i.e. belly to belly
Genital-Non Genital sexual contact	Dolphin positions own body part (e.g. fin, rostrum) in contact with conspecific's genitals, or projects genitals onto other body part of conspecific
Side mount	Dolphin directs genitals towards genitals of another dolphin, with body axis at perpendicular angle to the target dolphin's body, usually in a forceful manner
Genital tracking	Dolphin orientates head towards genitals of another obviously while swimming next to it, but not touching (note if actor or recipient)
Bubble blow	Dolphin blows a bubble ball or stream (note which) while orientating its head and/or body towards another dolphin
Nursing	Dolphin positions rostrum against mammary slit of lactating female for >2 secs
Beaching	Dolphin deliberately beaches itself on side of the pool *(Note whether other dolphins near or not)*
Side breach*	Dolphin purposefully leaps out of water and lands on side, making a loud slapping sound.
Tail slap* †	Solitary context: dolphin swims parallel (dorsally or ventrally) to surface and lifts tail out of water and slaps on surface, making a distinct sound and sometimes repeating, and usually near the area where an anticipated event will commence. Social context: dolphin uses tail to hit body part of another dolphin.

Spy hop	Dolphin propels itself vertically out of the water with the eyes directed to a point above the water's surface, usually as far as the pectoral fins, and then descends vertically. Often repeats this several times consecutively.
Head slap	While swimming, dolphin propels head and sometimes pectoral fins out of the water, to allow a few seconds suspended above the surface where is looking at a point above the surface, before a forceful slap is made as its head re-enters the water.
Surface look	Dolphin lifts head out of the water while on the move, or head is held fixed while floating stationary, and eyes are directed towards a point above the surface. If a dolphin is swimming at the same time, a surface look can be distinguished by the fact the head may be held suspended for a second or so above the water, the animal may not necessarily take a breath, and the eyes are clearly above the surface line (a distinct "slap" sound is not made on re-entering the water).
Leap*	Dolphin performs solitary aerial behaviour where body is fully out of the water and head and eyes are directed towards the area where the anticipated event usually commences.
Abnormal repetitive behaviour	Dolphin is solitary and repeatedly performs a behaviour which does not vary and appears to have no function. **Note** the type of stereotypy e.g. fence biting (only if invariant), swim patterns (only if using specific part of pool in repeated, invariant path), stone chewing
Throat pop	Dolphin manipulates the throat back and forth swims while in a ventral position, with or without snapping mouth open and closed
Bubble Snap	Animal repeatedly blows bubbles from blowhole and snaps at them as they move past the mouth, always in very similar pool and body positions. Not a play behaviour
Water throw	Dolphin has head out of water and throws water up in the air and catches it. Not conducted in a playful or variable manner, often precedes or follows other abnormal repetitive behaviours
Mouthing object	Dolphin uses mouth to manipulate part of environment in a non-playful manner, more focused, clear objective of the behaviour e.g. chewing fence, but with some variation in movements (otherwise likely to be stereotypic)
Underwater look	Dolphin focuses eyes and body axis towards environmental stimuli underwater, including activity at windows *(note if obviously directed at observer)*

** For starred behaviours to be recorded as "anticipatory behaviour" towards upcoming training sessions, the dolphin must be focussed on the beach area of the pool, with its eyes and normally head orientated towards this area. If play, aggression or sexual behaviour is occurring between the focal and other dolphins and the focal is not looking at the beach area while performing these behaviours (e.g. leaping), this should not be classified as anticipatory behaviour.*

[†] These behaviours are generally considered as agonistic in nature, and have been classified as such in the thesis studies (Paper 3, Chapter 3).

□ This symbol indicates the variants of synchronous swimming dependent on speed and distance to partner. Therefore synchronous swimming can either be measured globally, with no variants and using the "synchronous swimming" definition, or a higher level of detail can be recorded by using the four definitions of the variants.

Paper 2: Schedule of human-controlled periods structures bottlenose dolphin *(Tursiops truncatus)* behavior in their free-time.

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Résumé

Des modèles comportementaux sont établis en réponse aux signaux environnementaux prévisibles. Tous les jours, les animaux en captivité vivent fréquemment des événements prévisibles et contrôlés par l'homme, mais très peu d'études se sont intéressées à comment leurs rythmes comportementaux sont affectés par de telles activités. Les grands dauphins (*Tursiops truncatus*) présentés au public sont de bons modèles pour étudier de tels rythmes puisqu'ils vivent de nombreuses périodes contrôlées par l'homme chaque jour (par exemple des spectacles, des entrainements aux spectacles ou aux soins vétérinaires). Ainsi nous avons examiné l'effet des horaires des sessions d'entrainement sur leur comportement 'de temps libre', en étudiant 29 individus dans quatre groupes hébergés dans trois delphinariums européens. Nos analyses initiales du budget temps ont révélé que parmi les comportements étudiés, les dauphins ont passé la plupart du temps engagés dans la nage synchronisée, et dans cette catégorie ils ont nagé le plus souvent à faible vitesse et tout près les uns des autres. La nage synchronisée 'lente-proche' atteignait un niveau maximal peu de temps après des sessions d'entrainement et était basse peu avant la session suivante. Le comportement de jeu avait des fréquences significativement plus hautes pour les animaux adolescents que pour les adultes, mais l'effet a été seulement vu pendant la période entre les sessions (pas dans l'intervalle un peu avant, ni un peu après des sessions). Le comportement anticipatoire envers les sessions était significativement plus haut peu avant les sessions et plus bas ensuite. Nous concluons que deux comportements des dauphins non liés aux périodes contrôlées par l'homme ont été tout de même modulés par elles : la nage synchronisée 'lente-proche' et le jeu (dépendant de l'âge), qui ont des dimensions sociales importantes et sont liés au bien-être. Nous discutons des parallèles potentiels relatifs aux périodes contrôlées par l'homme pour d'autres espèces, y compris pour les humains eux-mêmes. Nos découvertes pourraient être prises en compte lors de la conception des évaluations de bien-être animal et elles pourraient contribuer à gérer la provision d'enrichissement et l'efficacité des programmes quotidiens bénéfiques aux animaux eux-mêmes.

Abstract

Behavioral patterns are established in response to predictable environmental cues. Animals under human care frequently experience predictable, human-controlled events each day, but very few studies have questioned exactly how behavioral patterns are affected by such activities. Bottlenose dolphins (*Tursiops truncatus*) maintained for public display are good models to study such patterns since they experience multiple daily human-controlled periods (e.g. shows, training for shows, medical training). Thus we investigated the effect of training session schedule on their 'free-time' behavior, studying 29 individuals within four groups from three European facilities. Our initial time budget analyses revealed that among the behaviors studied, dolphins spent the most time engaged in synchronous swimming, and within this category swam most at slow speeds and in close proximity to each other. 'Slow-close' synchronous swimming peaked shortly after training sessions and was low shortly before the next session. Play behavior had significantly higher frequencies in juveniles than in adults, but the effect was only seen during the in-between session period (interval neither shortly before nor after sessions). Anticipatory behavior towards sessions was significantly higher shortly before sessions and lower afterwards. We conclude that dolphin behaviors unconnected to the human-controlled periods were modulated by them: slow-close synchronous swimming and age-dependent play, which have important social dimensions and links to welfare. We discuss potential parallels to human-controlled periods in other species, including humans themselves. Our findings could be taken into account when designing welfare assessments, and aid in the provision of enrichment and maintaining effective schedules beneficial to animals themselves.

Keywords: animal welfare, anticipatory behavior, human-controlled periods, play, synchronous swimming

Introduction

In humans and non-human animals the performance of a behavior might appear arbitrary when viewed discretely, but in general repeatable and identifiable patterns are present across different time-scales. Our knowledge of behavioral patterns in a number of animal species is well-established (Finn et al., 2014; Mcclintock et al., 2013; Stamps, 2016), with notable developments made in circadian and ultradian rhythms (or lack thereof) in general activity and feeding (e.g. Bloch et al., 2013; Mistlberger and Skene, 2004; Storch and Weitz, 2009), and aided by new technology (e.g. accelerometers: Robert et al., 2009; Shepard et al., 2008). Behavioral patterns in animals or indeed humans are effectuated in response to the 'time-structure' of the surrounding environment (Daan, 1981), where common and predictable salient events entrain the performance of certain behaviors, for example food-searching activity in response to the daily appearance of a food source (Storch and Weitz, 2009; Bloch et al., 2013). Those individuals who use the available environmental cues to structure their behavior around the fulfilment of their needs are considered to be well-adapted to their surroundings (McEwen and Wingfield, 2003; Wingfield, 2005).

Behaviors relating to food acquisition are among the most fundamental to survival, and thus their daily rhythms and '*Zeitgebers*' (entraining stimuli; Aschoff, 1965) have logically been popular research topics for many years. One phenomenon under this umbrella is Food-Anticipatory Activity (FAA), which describes animals' increased arousal and locomotory behavior before food events that are available on a restricted schedule (Mistlberger, 1994). FAA has been shown to be robust, stable over many daily cycles, and not always within the circadian rhythm (Storch and Weitz, 2009). It has been well-studied in laboratory rodents (see review by Storch and Weitz, 2009) and as result progress has been made into the emotional value of FAA and other types of anticipatory behavior, where it is thought to be a reflection of the 'balance of reward systems' experienced by the animal (Spruijt et al., 2001). Animals in zoos and aquariums tend to have strongly structured daily schedules of food provision and other events, which are usually highly predictable through numerous environmental cues (Waitt and Buchanan-Smith, 2001), promoting the occurrence of anticipatory behavior (Watters 2014): however, it has not been well-studied in this setting (Anderson et al., 2015; Watters, 2014).

Another element of daily behavioral rhythms that has interested researchers is social interaction. Although much less is known about what drives patterns of social behavior (Krause et al., 2013), this topic has recently experienced a surge in interest (see review by Panksepp et al., 2008). Social behavior can be entrained to circadian and ultradian rhythms, and there is also likely to be a prominent genetic influence (Panksepp et al., 2008). Social cues can also be *Zeitgebers* themselves where they stimulate certain patterns of behaviors to occur (Mistlberger and Skene, 2004). Human research has shown that work schedules impact the frequency and type of social behavior conducted in after-work hours (Ilies et al., 2007; Sonnentag and Bayer, 2005). Investigating animals' social behavior patterns is not only desirable for its fundamental research value but also in terms of the many applications to management practices (Krause et al., 2013; Mistlberger and Skene, 2004).

Behavioral patterns are more strongly linked to predictable and frequent events occurring in the surroundings, and for animals maintained under human care, the environmental time-structure is largely dictated by human-controlled events or periods of time (Watters 2014). However, there are only a small number of studies focused on animal behavior in response to human-controlled schedules (Waitt and Buchanan-Smith 2001). Some animals are subject to regular, controlled, non-alimentary events, e.g. cows (*Bos taurus*) being milked (Ketelaar-De Lauwere et al., 1999), visitors present in proximity to zoo animals (Hosey, 2005), periods of exercise and being left alone for domestic pets (Lund and Jørgensen, 1999), but nearly all animals are subject to a schedule of food provision by humans. FAA and anticipatory behavior in response to other events is present and increasingly studied in captive animals, particularly in relation to welfare (Anderson et al., 2015; Jensen et al., 2013; Spruijt et al., 2001). Very few studies have looked at the variation of social behavior or general activity patterns in response to different management schedules (Storch and Weitz, 2009). However, limited results thus far indicate that as well as food-related behavior, social and other behavioral activity unrelated to human-controlled periods can vary as a result of the time-structure (with primate species: Ulyan et al., 2006; Waitt and Buchanan-Smith, 2001).

Bottlenose dolphins (*Tursiops truncatus*) under human care are a striking example of animals that inhabits an environment closely controlled by humans, and their days are generally structured by a number of training sessions. These may constitute shows, show training, medical training, guest interactions, "play" sessions, cognitive tasks (all described hereafter as 'training sessions'), but all within which they receive their food as reinforcement after participating in exercises chosen by their care-takers (Brando, 2010). The dolphins and this environment represent a good model for the study of behavioral patterns since they experience repeated and controlled daily events, are easily observable, and are gregarious animals, therefore providing the opportunity to analyze the impact on social behaviors as well. Only a few studies have focused on behavioral patterns in captive dolphins (Galhardo et al., 1996; Nelson and Lien, 1994; Saayman et al., 1973; Sekiguchi and Kohshima, 2003), and thus even fewer look at the effect of training sessions. One study found that as the latency to and from sessions varied, the occurrence of dolphins' behaviors within affiliative, aggressive and repetitive categories also varied, although the authors concluded they were likely *not* caused by the sessions since notable individual behavioral differences were observed (Miller et al., 2011b). In addition, anticipatory behaviors were not investigated and one category was used for all social interactions. In a study on three Indo-Pacific humpback dolphins (*Sousa chinensis*) individual differences in behavior were found before and after guest interactions (Sew and Todd, 2013), and thus collective findings were limited. Recently, it was shown that bottlenose dolphins significantly increased the frequency of anticipatory behavior in relation to upcoming training sessions (Jensen et al., 2013). Thus far there have been no findings showing how the daily behavioral pattern of captive dolphins might be structured around the multiple, human-controlled training sessions.

The aim of our study was to investigate how scheduled human-controlled activities modulate animals' behavior in their 'free-time', through observations of multiple groups of bottlenose dolphins in different facilities. Our focus was on behaviors related to the sessions (i.e. anticipatory) and social behaviors. Zoo and aquarium environments promote the development of anticipatory behavior and evidence already supports its presence in bottlenose dolphins (Jensen et al., 2013). We predicted that our defined anticipatory behaviors would increase in the period before the next scheduled session. In a previous similar study, data on synchronous swimming was not taken (Miller et al., 2011b), and in another it was chosen to consider any type of swimming as the 'default' behavior (Sew and Todd, 2013). As a consequence, a concurrent aim of our study was to investigate, in much more detail than previously, the dolphins' behavior of swimming synchronously. Other salient social behavior categories (play, aggression, and sexually motivated) were chosen as the most often seen in dolphin interactions (Galhardo et al., 1996; Samuels and Gifford, 1997; Shane et al., 1986). Due to the lack of previous studies *a priori* predictions were not possible, but we expected that at least some social behaviors would vary in relation to the session schedule.

Since daily, human-controlled periods are a common phenomenon for many captive animals, the results of our study could extend to forming cross-species parallels, including behavior of working animals and even regarding the daily routines of humans.

Materials And Methods

Study Animals and Facilities

Our study included 29 Atlantic bottlenose dolphins (*Tursiops truncatus*) housed at three European zoological facilities and maintained in 4 groups, all kept in artificial pool complexes. There were eight dolphins at Parc Astérix (Plailly, France) living in an outdoor pool conjoined to two indoor pools with a total volume of 3790 m^3 of water, where there was always free access to all pools (see Table 2 for age and sex characteristics of the study population). At Planète Sauvage (Port-Saint-Père, France), 7 dolphins inhabited four inter-connected pools which together contained 7490 m^3 of water. In general the gates to all pools were left open but sometimes access was prevented to pools for maintenance. At the start of the study there were six dolphins in this group but after two weeks a 25-year-old female arrived: on her first night she stayed in the medical pool with one other male before being mixed with the group the next day. Dolfinarium Harderwijk (Harderwijk, The Netherlands) housed 14 dolphins in a network of seven pools interconnected by gates and sluice channels, with a total volume of 2743 m^3. The animals at this facility were split into groups of six and eight animals, where the group of eight participated in the shows while the other six dolphins primarily conducted guest interactions. Of the 29 dolphins in the study population, 25 were captive-born and 4 originally wild caught. Planète Sauvage and Dolfinarium Harderwijk were open daily to the public for the whole of the study period, and Parc Astérix opened two weeks into data collection. At all parks, the dolphins' diets consisted of a variety of fish and squid species being fed to them at multiple sessions during the day, with the total amount per day for each dolphin ranging between 5 and 12 kg. Also in all parks, during the day there were generally three public presentations (a type of training session but with an audience present) and between two and five other training sessions (which could include training for shows, medical training, play sessions and research sessions), and always having two short feeding-only sessions at the beginning and end of the day. All these types of sessions were considered under the umbrella of 'training session' for our methods and analysis, but the potential variance was addressed by testing for effects of type and duration of sessions in our analyses.

Table 2 *Age and sex characteristics of bottlenose dolphin study population.*

Group	Facility	*N* total	*N* females (juvenile/adult)	Age range females	*N* males (juvenile/adult)	Age range males
1	Parc Astérix	8	0 / 4	15 to 42	2 / 2	3 to 33
2	Planète Sauvage*	7	1 / 2	6 to 25	2 / 2	4 to 15
3	Dolfinarium Harderwijk	8	-	-	1 / 7	10 to 41
4	Dolfinarium Harderwijk	6	-	-	2 / 4	4 to 22

Juveniles: 0 ≤ 10 years old; Adults: 11 years old or more.

*One dolphin (female, 25 years old) joined the group in second week of observation period

Data Collection

Study period

The study took place at all three parks from April to June 2015, and observations were taken at random times of the day between 07:00 and 21:00.

Behavioral observations

We established a five-minute focal observation protocol with scan sampling every 15s where the behavior being performed was noted down (Martin and Bateson, 1986). Scans where the animal was not visible were recorded so that a percentage of total scans (maximum of 21 scans) could be calculated.

At Parc Astérix, the positioning of underwater windows allowed observations to always be conducted from this location since a large proportion of the pool could be seen at all times, whereas at Planète Sauvage and Dolfinarium Harderwijk the view from underwater windows covered 50% of the pool or less, so observations were conducted above water. Both observation positions were adopted since the pools' water was always clear, the background color contrasted well with the dolphins' bodies, and the behaviors were clearly recognizable above and below water. Above and below-water observer positions were at inconspicuous locations to limit the effects on the dolphins' behavior.

We developed a behavioral repertoire containing 22 behaviors within five categories (synchronous swimming; play; overt aggressive: sexually motivated; anticipatory) (Thesis ethogram, Chapter 2), with the aim of including the most common anticipatory and social behaviors for this species. The behaviors were taken directly or adapted from published repertoires (Clegg et al., 2015; Galhardo et al., 1996; Holobinko and Waring, 2010; Samuels and Gifford, 1997). In regard to measuring synchronous swimming behavior, Connor and co-authors (2006b) used a differentiation of distance between partners, but here we went a step further and also took into account the speed of the behavior, since it is likely synchronous swimming at different speeds has different functions (e.g. faster in aggressive contexts; Herzing, 1996). Therefore, we defined synchronous swimming by proximity and speed (*slow*: around 2 m/s or less, minimal tail beats; *fast*: more than 2m/s and stronger tail beats which may cause head to move up and down; *close*: contact to partner or within touching distance [≤ 0.5 m]; *distant*: partner is 0.5 m – one body length away) in order to investigate the variation in each sub-category's occurrence. This yielded four categories of the behavior: slow-close, slow-distant, fast-close and fast-distant synchronous swimming.

Individual recognition of study animals

No individual tagging or marking was used. Each dolphin could be recognized individually by a combination of: distinct patterns of notches on the dorsal fin and tail, patches of permanent skin discoloration on the body, size and shape of the eyes, and general coloration of the body. It was verified that each observer could identify the dolphins with 100% accuracy, multiple times each, before data collection began.

Observers and inter-observer concordance

There were three different observers at each park (IM, MC and DV), who were all trained by the same person (IC) to use the same data collection techniques and accompanying behavioral repertoire. Prior to the start of data collection an inter-observer reliability test was conducted using five randomly chosen video footage samples of 5 minutes each stemming from different animals. An intra-class correlation based on 1,000 permutations (library *rptR*; Schielzeth and Nakagawa, 2013) revealed a very high concordance in the time budgets of the different behaviors quantified by the 3 observers with respect to slow-close ($R = 0.999$, $P < 0.001$), slow-distant ($R = 0.992$, $P < 0.001$), fast-close ($R = 0.999$, $P = 0.013$) and fast-distant synchronous swimming ($R = 0.999$, $P = 0.012$), and also with respect to sexually motivated behavior ($R = 0.833$, $P = 0.012$) and anticipatory behavior ($R = 0.904$, $P < .001$). Play behavior and aggression in these videos was quantified by all observers to be zero, and thus the inter-observer agreement was 100% with respect to these behaviours (not tested statistically).

Timing of training

No observations were taken during the training sessions, and the time delay since the last session and until the next one was recorded by the observer for each observation. Subsequently we defined the timing of training variable into three periods: “shortly before”: ≤ 15 min before the next session (*n* = 145 observations); “shortly after”: ≥ 15 min after the last session (*n* = 157 observations); and “In-between”: the intermediate period more than 15 min since the last and before the next session (*n* = 724 observations). It was verified that the data included only observations done when training sessions were at least 30 minutes apart to ensure these categories were mutually exclusive.

Ethics Note

Behavioral observation was the only means of data collection for this study and the ASAB/ABS Guidelines for the Use of Animals in Research (2012) were consulted and followed. As a result of this study all routines remained unchanged for the animals and the only difference to their environment was an observer standing either by the poolside or at the underwater window for a maximum of two hours per day. This study, which was purely observational, was reviewed and accepted by the pluridisciplinary scientific committee of the company representing the facilities.

Data Analysis and Sample Size

Statistical analyses were done with R, version 3.1.1 (R Core Team, 2016). We applied generalized linear mixed-effects models GLMM for proportional data, using a model structure for binomial distribution with a logit link. Calculations were based on Laplacian maximum likelihood estimates using the package *lme4* (Bates et al., 2015). *P*-values were calculated by likelihood ratio tests based on the changes in deviance when a factor was removed from the full main effects model, or an interaction was added to it (Faraway, 2006).

A total of 1026 five-minute observations from 29 different animals (6 juvenile males and 16 adult males; 1 juvenile female and 6 adult females) from 3 different facilities and kept in 4 different groups were available for this study (details on study animals in Table 2). The age class of juveniles included all animals younger than 10 years; older animals were considered as adults (Smolker et al., 1992). All juveniles had stopped nursing at the time of the study. Data were expressed as percentage (for analysis: proportion) of scans (per total visible scans) of the different behaviors, used as dependent variables in our models. Independent variables were the animals' age class, sex (factors with 2 levels), and the timing of training (factor with three levels), see above for a definition of levels. Due to notable collinearities between sex and age class, these two factors were never tested within the same model. We considered 2-way interactions between sex or age class and the timing of training. See Fig. 1 for sample sizes within the different levels of the factors.

Models included individual identity as a random factor to account for repeated measurements from the different animals. Overall, we obtained 11 to 57, on average 35, 5-min observations per dolphin. In addition, we included group identity as a random factor to account for the different origin of the animals and for the dependencies in behavior within groups. We also considered the identity of the facility as an additional random factor which, however, did not account for any additional variation, as it was strongly collinear with the identity of the groups. Thus, this random factor was omitted from the model and these were re-calculated. This procedure did not change any of the results obtained. The content of training sessions could vary between free-feeds (no behaviors asked, fish given consecutively until ration is finished), training (rehearsal of known or teaching of new behaviors, husbandry tasks, play or fun sessions) or show/guest interactions (either a show with public present or an interaction session with guests, who remain out of water). As the type of the prior as well as the upcoming session might have potentially and differentially influenced the behavior of the animals, we included "session type" as an additional random factor.

Models were checked for homogeneity of variances and goodness of fit by plotting residuals versus fitted values. As models showed signs of over-dispersion, we included a case-level random factor (Browne et al., 2005). Furthermore, we calculated variance inflation coefficients (VIF) for all models with multiple independent variables (sex and timing of training, or age class and timing of training) to check for (multi)collinearities among them (Zuur et al., 2010). VIF were always lower than 2, indicating no interfering effects of multicollinearities.

Results

Time Budgets of Different Behaviors

The different behaviors observed differed significantly in their occurrence (χ^2_4 = 11951.03, $P < 0.001$). The behavior which was the most frequent i.e. in terms of percentage of scans out of total visible scans per observation (hereafter described as frequency) was synchronous swimming, displayed for an average of 35.2% ($CI_{95\%}$: 33.0%, 37.4%) of scans per observation (Fig. 4a).

Synchronous swimming was categorized into slow-close, slow-distant, fast-close and fast-distant. These categories also differed significantly in their occurrence (χ^2_3 = 115.05, $P < 0.001$) with slow-close synchronous swimming displayed most often at an average of 22% of scans per observation, followed by slow-distant at 14%, then fast-distant at 6%, with fast-close swimming occurring in only 1% of observation scans (Fig. 4b).

Effects of Different Factors on Behavior

Slow-close synchronous swimming differed significantly with respect to the timing of training (Table 3a). Animals showed significantly lower frequencies of this behavior 15 minutes before and the highest occurrences 15 minutes after the training. During the time in-between, i.e. between the periods shortly after and shortly before the training, this behavior was seen at intermediate levels (post-hoc comparisons in Fig. 5a).

Slow-distant synchronous swimming did not significantly differ between the three time periods considered, although the frequencies were significantly higher by 2.0% in males than in females (Table 3b). There were no significant effects of any of the factors considered on the frequencies of fast-close and fast-distant swimming (Table 3c,d).

The frequency of play behavior depended on the timing of training session; however, this effect was modulated by the animals' age (see significant interaction in Table 3e). Juveniles showed significantly more play behavior than adults in-between the training sessions, which was on average 2.7 times that of adults. However, differences were not statistically significant during other time periods (post-hoc comparisons in Fig. 5b).

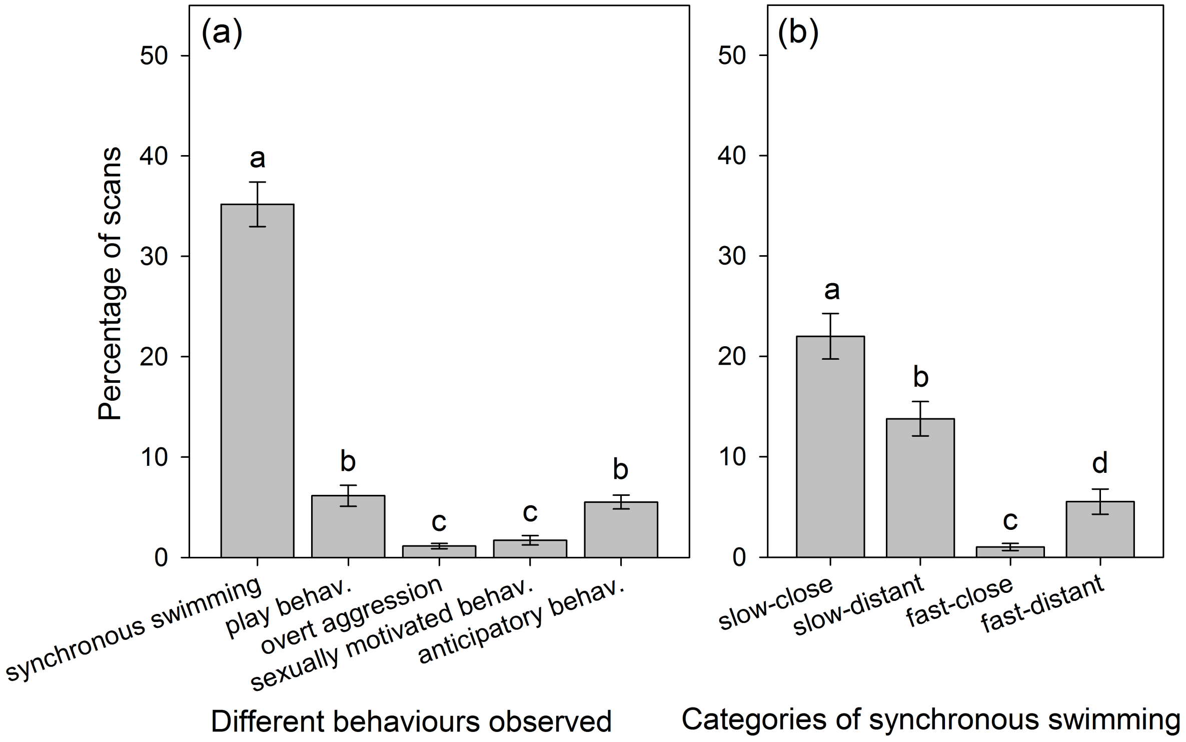

Fig. 4 *Comparison of different behaviors that bottlenose dolphins displayed during repeated 5-min daylight observations, showing average percentage of scans per 5 min observation spent on* **(a)** *the five behavioral categories studied (definitions in Thesis ethogram, Chapter 2) and* **(b)** *the four variants of synchronous swimming. Percentage scans denotes the scans where a certain behavior was performed out of the total visible scans in the focal 5 min observation. Data are based on 1026 observations from 29 dolphins kept in 4 different groups. Different letters indicate significant differences between groups, tested by pair-wise comparisons using GLMM for proportional data with sequential Bonferroni correction (Holm, 1979).*

Anticipatory behavior was seen significantly higher by on average 5.8% of scans per observation shortly before the training sessions when compared to the period shortly after and in-between (Table 3h; post-hoc comparisons in Fig. 5c).

There were no significant effects of any of the factors tested on the frequencies of overt aggressive or sexually motivated behavior (Table 3f and 3g). Additionally, none of the factors or interactions considered were significant when separately analyzing offensive and defensive overt aggression, or acting and receiving sexually motivated behaviors (all *P* > 0.10).

Table 3 *Effects of sex, age class (juvenile vs. adult) and the time delay to training (split into: ≤ 15 min before the session; ≥ 15 min after the session; and the period in-beetween, i.e. more than 15 min since the last and before the next) on the occurrence of different behaviors of bottlenose dolphins under human care. Behaviors were measured as percentage of scans out of total visible scans in the 5 min focal observations, and definitions of behaviors in each category are found in the Thesis ethogram (Chapter 2). Data stem from 1026 observations from 29 animals, kept in 4 groups at 3 different facilities. Analysis conducted by GLMM for proportional data, including individual identity, group identity and the type of the previous and following session as random factors. Only statistically significant interactions are given ($P < 0.05$), and significant differences are highlighted in bold.*

Response variable	Predictor variable	χ^2	*df*	*P*
(a) Synchronous swimming: Slow-close	Sex	0.05	1	0.82
	Age class	0.65	1	0.42
	Timing of training	33.82	2	**< 0.001**
(b) Synchronous swimming: Slow-distant	Sex	8.45	1	**0.004**
	Age class	2.60	1	0.11
	Timing of training	5.42	2	0.066
(c) Synchronous swimming: Fast-close	Sex	0.13	1	0.72
	Age class	0.38	1	0.54
	Timing of training	1.68	2	0.43
(d) Synchronous swimming: Fast-distant	Sex	1.63	1	0.20
	Age class	1.73	1	0.19
	Timing of training	4.40	2	0.11
(e) Play behavior	Sex	1.80	1	0.18
	Age class	1.40	1	0.24
	Timing of training	0.41	2	0.82
	Age class × Timing of training	8.13	2	**0.017**
(f) Overt aggressive behavior	Sex	0.89	1	0.35
	Age class	2.38	1	0.12
	Timing of training	0.70	2	0.71
(g) Sexually motivated behavior	Sex	1.75	1	0.19
	Age class	2.40	1	0.12
	Timing of training	0.01	2	0.99
(h) Anticipatory behavior	Sex	1.75	1	0.19
	Age class	0.67	1	0.41
	Timing of training	9.83	2	**0.007**

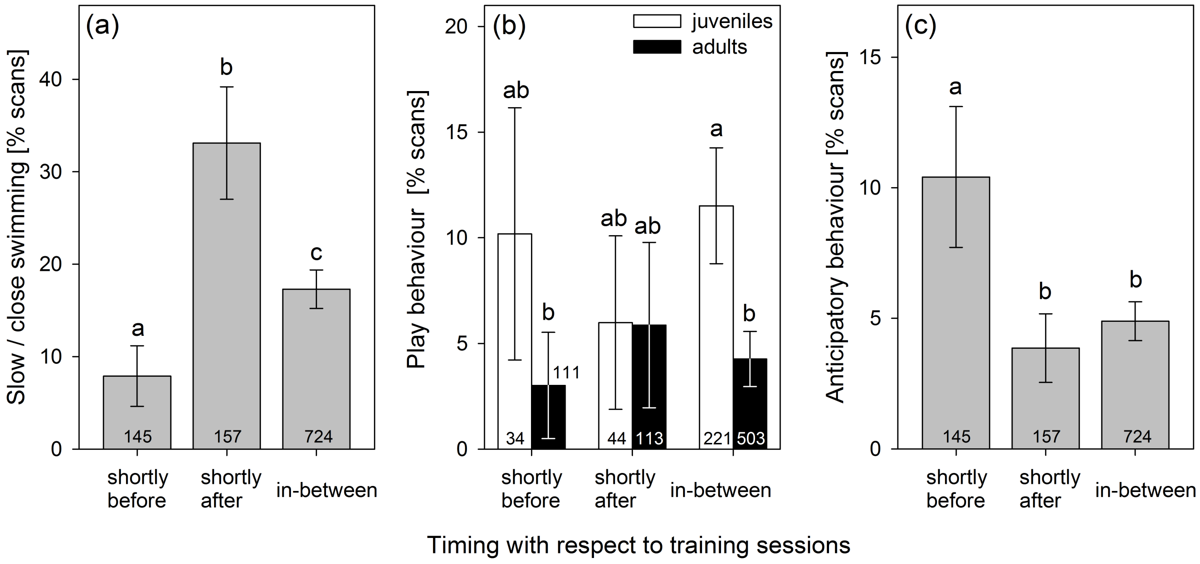

Fig. 5 (a) *Effects of the timing of training sessions on the percentage of scans per observation of slow-close synchronous swimming,* **(c)** *anticipatory behavior, and* **(b)** *play behavior where differences were seen between juveniles and adults during the different time periods (significant interaction, see Table 3). Percentage scans denotes the scans where a certain behavior was performed out of the total visible scans in the focal 5 min observation. Sample sizes (total number of observations per group) are given in the bars; see Table 3 for details on statistics. Different letters indicate significant differences between groups, tested by pair-wise comparisons using GLMM for proportional data with sequential Bonferroni correction (Holm, 1979).*

Discussion

Overall, we found that the schedule of sessions significantly modulated behaviors in the three most commonly shown behavioral categories that we studied: synchronous swimming, play and anticipatory behavior. Our results showed a peak in slow-close synchronous swimming after sessions, higher frequencies of age-dependent play in the in-between session period, and high levels of anticipatory behavior shortly before training sessions.

Veasey (2006) emphasized how evaluating time budgets of captive animals is fundamental for investigations on how behavioral patterns are affected by management protocols. We used the percentage of scans out of the total visible in 5 min focal observations to formulate a type of time budget, which showed that synchronous swimming was by far the most frequent behavior seen, with play and anticipatory behavior the next most common but relatively at lower levels. This study is the first to separate synchronous swimming by speed as well as by distance to partner (Connor et al., 2006b defined distances), and the different rates of occurrence indicate that each swimming topography may have a different role in social interactions of dolphins.

Behaviors Modulated by Schedule of Sessions

The frequencies of slow-close synchronous swimming, play and anticipatory behavior observed in our study were influenced by the timing of sessions. In dolphin species, synchronous swimming has often been suggested as an affiliative behavior which helps to maintain bonds, and is thought to be an indicator of positive emotions (Connor et al., 2006b; Holobinko and Waring, 2010). Since the proximity between partners is likely to be salient in relation to the different functions of synchronous swimming (Connor et al., 2006b), we chose to study four variants of this behavior and found that only slow-close swimming was modulated with timing to or from sessions. Synchronous swimming in close proximity starts at birth and although the spatial arrangements of the partners develops with age (among other factors), it is thought that dolphins swimming closer together have a stronger relationship (Gubbins et al., 1999; Holobinko and Waring, 2010). Therefore our results of slow-close synchronous swimming is likely to reflect the formation and/or maintenance of social bonds in the group, and may be seen more frequently following the sessions since the dolphins are reunited after a human-controlled period of separation. A recent study at Parc Astérix showed that the emission rate of signature whistles increased after the training sessions, and the authors postulated that they function as cohesion calls and affiliative signals: this concurs strongly with the behavioral results in our study (Lopez Marulanda et al., 2016). Examples can also be found in other species: working donkeys (*Equus africanus asinus*) gathered together to socially interact immediately after finishing their working period, even if they were fatigued and dehydrated, and water and food were available (Swann, 2006). Our results with the dolphins' slow-close synchronous swimming concur with the increase in general affiliative behaviors seen after sessions by Miller and colleagues (2011b). However, the component of *synchronicity* of this behavior may have a stronger significance than other affiliative behaviors: a recent review concludes that activity synchrony in animals, where behaviors are performed in unison, is itself likely to represent close social bonds between individuals (Duranton and Gaunet, 2016). A recent study found links between the level of synchrony during food provision and milking of cows, to their lying and grazing behavior in their free time (Flury and Gygax, 2016): a similar investigation with dolphins' synchrony during and outside of training sessions might shed further light on this element of their behavior patterns.

An alternative explanation to increased synchronous swimming after sessions might be that it functions as a rest or sleep period. The training sessions are a form of exercise (Brando, 2010), and it is thought that dolphins need to sleep from 4-6 hours per day (range taken from wild and captive research: Goley, 1999; Lilly, 1964; Lyamin et al., 2008). Their decision to conduct sleep behavior synchronously with others could be an adaptive strategy to improve vigilance (Goley, 1999). However, dolphins perform slow-wave unihemispheric sleep and thus often do not close both eyes, rendering their sleep behavior "indistinguishable from that of quiet wakefulness" (Oleksenko et al., 1992). Therefore from our results we cannot conclude for certain whether this might have been the true function of the behavior; this might be clarified in further studies.

Play is usually considered an affiliative behavior (Bateson, 2014; Boissy et al., 2007; in dolphins: Kuczaj and Eskelinen, 2014; Neto et al., 2016; Paulos et al., 2010; but see Blois-Heulin et al., 2015; Hausberger et al., 2012) and was performed in our study at higher frequencies in the in-between session period, but only in juveniles. On average there were indications of a similar tendency for shortly before the sessions as well, but this effect was not significant, perhaps due to our smaller sample size of juvenile dolphins. There is evidence that aerial behaviors, a likely component of play, occur more frequently in wild and captive dolphins after a feeding event (Paulos et al., 2007), and it is well-accepted that play occurs mostly when other needs, such as food acquisition, are satisfied (Baldwin and Baldwin, 1976; Bateson, 2014; Boissy et al., 2007). However while evidence supporting this is available for juveniles in many species, the association between adult play and affective state remains unclear (Blois-Heulin et al., 2015). Our results suggest that juveniles may be more sensitive to the environmental time-structure, leading them to play mostly in the times farthest from the sessions. Alternatively, or in addition, as play is often considered an indicator of positive emotions (Boissy et al., 2007; Held and Špinka, 2011), it could be hypothesized that the juveniles either experience more positive affective states and/or are more aroused/excited in-between the sessions as compared to adults. Further work on the daily patterns of dolphin play with large sample sizes are needed to fully understand this age-dependent element.

The higher occurrence of anticipatory behaviors observed shortly before sessions was the result we most expected to see; a recent first study with bottlenose dolphins found similar results (Jensen et al., 2013) and there is much anecdotal evidence from the animals' care-takers. It was no surprise that dolphins can predict imminent start of training sessions, especially since the timings were fairly regular and environmental cues were available (e.g. sound of buckets) in the three parks in our study. Anticipatory behavior reflects the ability of animals to respond to predictable daily events, but it has also recently been proposed as an indicator of the balance of reward systems (Spruijt et al., 2001). A certain level of anticipatory behavior is thought to reflects positive expectation of the event, but excessive anticipation, either in terms of duration or intensity, may reflect negative affective states such as frustration, perhaps due to lack of other stimulation in the environment (Spruijt et al., 2001; Watters 2014). Our study provides preliminary results concerning this behavior in dolphins which could then be developed upon in order to investigate certain frequencies relevant to the balance of reward systems.

Our results show that three different dolphin behaviors occurred at predictable points in relation to the session schedule, and that this was not significantly influenced by individual or facility differences. Thus we might say that these behaviors are entrained in an ultradian rhythm (recurrent cycle repeated within 24 hours) by the timings of the training sessions. Feeding times and rest-activity cycles have been shown to act as *Zeitgebers* as well as light and dark phases (Flury and Gygax, 2016). However, we can only postulate this for our study population due to some limitations which must be discussed. Firstly, much further work would be needed to understand whether it is the food provision or exercise/cognitive element that entrains the rhythm, how easy it is to disrupt, and whether in fact it is social cues that prompt the group to perform a certain behavior in synchrony. Bloch and colleagues (2013) recently postulated that highly social animals, and those in a constant environment, are more likely to have ultradian behavioral patterns. Further studies are needed before this can be confirmed for dolphins, where the timings of sessions, environmental cues, and light and dark cycles would need to be controlled for as was not possible in our study.

Relevance to Dolphin Welfare and Implications for Other Species

Overall, our results contribute to improving our knowledge of how the dolphins respond to the captive environment, since an individual's behavioral pattern is a fundamental indicator of how it is adapting to its environment (Eagle and Pentland, 2009; McEwen and Wingfield, 2003; Wingfield, 2005), and thus is highly relevant to evaluating overall welfare (Broom, 1991; Dawkins, 1998; Veasey, 2006). The relatively large sample size of dolphins and observations allowed us to see past short-term individual behavioral differences, which limited conclusions in the previous studies on this topic (Miller et al., 2011b; Sew and Todd, 2013; Trone et al., 2005). Although not the objective of our study, some possible implications for dolphin welfare and its assessment can be seen in the results.

Firstly, those designing welfare assessments should take into account daily rhythms of behaviors and their likely cues. Here, the dolphin behaviors modulated by the schedule of training sessions had all previously been suggested as emotion or welfare indicators (Connor et al., 2006b; Holobinko and Waring, 2010; Jensen et al., 2013; Neto et al., 2016), and thus in the future might be measured as part of a comprehensive assessment. If this is the case, the timing of human-controlled periods must be taken into account when quantifying such behaviors in dolphins, or indeed other species: the time when a certain welfare measure is conducted in relation to these periods might affect the data collected and conclusions made (e.g. frequency of play behavior).

Our findings also allow us to suggest some more specific implications for dolphin welfare. The performance of synchronous swimming peaked shortly after training sessions and thus in order not to disturb this behavior pattern, it could be beneficial for the management team to not provide environmental enrichment immediately following sessions (as some facilities do) but instead after a short delay. In general, affiliative behaviors (such as synchronous swimming for the dolphins) and juvenile play in animals are thought to be naturally rewarding and induce positive affect in animals (Bateson, 2014; Boissy et al., 2007). Indicators of affective state in dolphin species are highly sought after (Clegg et al., 2015) and we show here that, as well as play behavior, synchronous swimming when delineated by speed and proximity represents a strong potential indicator and deserves further research in this context.

We provide definitions of multiple anticipatory behaviors in dolphins (validated by the significant increase in this activity shortly before sessions), and the fact that this was a concurrent finding despite facility and management differences, for on average 6% of observation time, could provide a starting point for further work on this phenomenon which is thought to indicate welfare state (Spruijt et al., 2001, Watters, 2014). The next step would be to test different frequencies of anticipatory behavior in conjunction with other physiological and cognitive data (Anderson et al., 2015) to investigate whether, and to what extent, it represents either positive affect in the dolphins as they await their ‘reward’ (Spruijt et al., 2001), or whether in some contexts this activity can induce negative emotions such as frustration (Boissy et al., 2007).

There are many other animal species that experience daily human-controlled sessions. Our results correlate to other similar studies: Baldwin and Baldwin (1976) found that feeding ecology and play behavior were closely linked in squirrel monkeys (*Simia sciureus*) since even changing the form of the food given decreased play behavior significantly, and a few other studies of mammalian species have found that social play increased post-feeding sessions (Cordoni, 2009; Pellis, 1991). The frequency of play and/or affiliative behaviors before or after feeding sessions may be worth investigating as a measure for motivational state of hunger (Pellis, 1991). This could aid management of routines on the commercial scale, for example milking and grazing schedules affecting cow behavior and productivity (Ketelaar-de Lauwere et al., 1999), and would be particularly pertinent as the industry continues to trial automated milking systems (entry and timings under animal control, Flury and Gygax, 2016). Elements of the dolphins' behavioral patterns could also be likened to findings on human routines. For instance Stevenson and Lee (1990) used differences in school schedules to highlight the children's need for recess (unstructured free-time) since it gives "opportunities for play", something which we found juvenile dolphins to engage in specifically in-between controlled sessions. This would suggest that children might also be motivated to interact socially shortly after the lessons end, and therefore planning spaces where children can interact socially and freely in recess times might be beneficial and improve their attention spans (Holmes et al., 2006; Stevenson and Lee, 1990). Without doubt further research with human subjects is needed to investigate this, especially given that the dolphins' sessions included food provision, but the similarities could be worth considering from both sides.

Conclusions

Through observations of captive bottlenose dolphins we found that slow-close synchronous swimming, age-dependent play and anticipatory behavior were modulated by the timing of daily human-controlled periods. That social behaviors such as synchronous swimming and play were affected, which would seem to not have as clear, direct connection with the occurrence of training sessions, suggests that the schedule of sessions acts as an entraining cue for the general daily behavioral patterns. We use these preliminary findings to tentatively suggest implications for dolphin welfare, such as the provision of enrichment following a short delay after sessions and the consideration of daily behavioral rhythms when conducting welfare assessments. Furthermore, the three modulated behaviors have all been previously suggested as indicators of dolphin welfare or affective state, but further investigation is needed to discover the actual link between each behavior and welfare and we suggest potential directions to achieve this. Parallels from our findings can be drawn to controlled periods experienced by other species and even humans, providing further evidence that daily schedules are closely linked to behavior in an individual's free-time.

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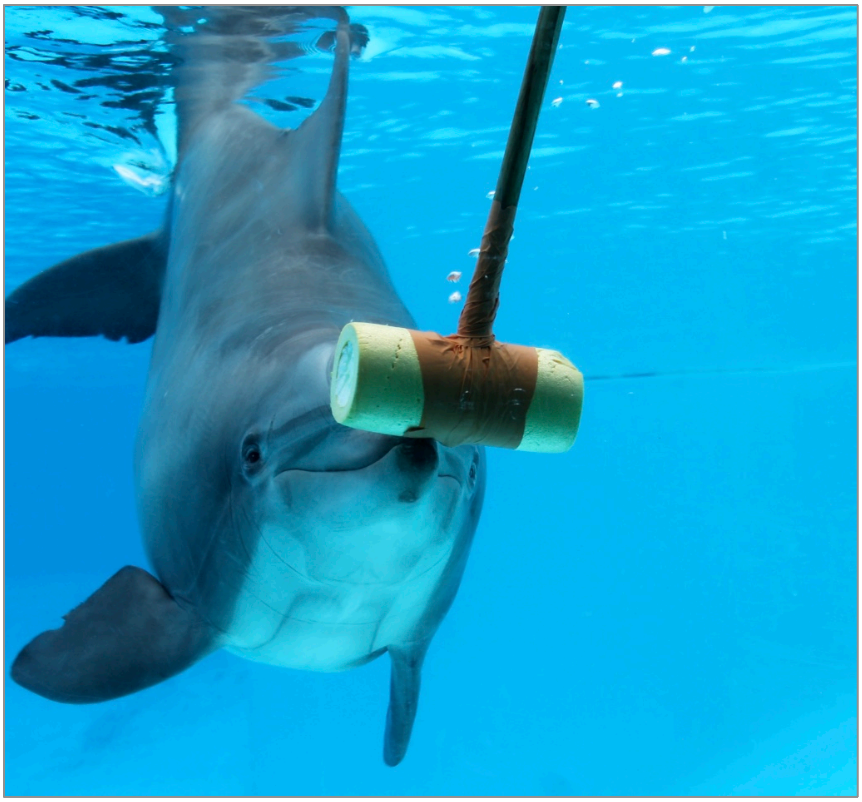

Chapter 3
Cognitive bias testing

Chapter 3: Cognitive bias testing

Paper 3: Bottlenose dolphins engaging in more social affiliative behaviour judge ambiguous cues more optimistically.

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Résumé

Les tests de biais cognitif mesurent la variation dans les évaluations émotionnelles et sont des méthodes validées pour évaluer les états affectifs des animaux. Cependant, le lien entre des comportements sociaux et les biais cognitifs n'a pas encore été examiné. Les grands dauphins sont une espèce sociable pour qui la recherche de bien-être est importante, ils sont ainsi un bon modèle pour tester une telle association. Nous avons adapté un test de biais de jugement d'emplacement spatial sur huit grands dauphins captifs pour examiner le lien entre le biais cognitif et le comportement social, au cours duquel nous avons conduit des observations comportementales en dehors des sessions d’entrainement et nous n'avons pas expérimentalement initié d'état affectif. Les sujets ont montré des différences individuelles stables de biais au cours des trois jours de test. En outre, les dauphins qui montraient le plus de nage synchronisée, un comportement affiliatif fondamental, ont jugé des signaux ambiguës significativement plus avec optimisme. Nos données sur le long terme ont montré que le biais cognitif et la fréquence de nage synchronisée ont été significativement associés jusqu'à deux mois précédant le test, mais pas avant cela, suggérant qu'ici les différences de biais cognitif ont été reflétées par des états affectifs transitoires plutôt que par des traits à plus long terme. Nous formulons une hypothèse selon laquelle la réalisation de nage synchronisée peut initier des états affectifs et-ou être initiée par eux; de manière générale, la nage synchronisée montre un fort potentiel pour être un indicateur d'état affectif dans cette espèce et dans d’autres.

Abstract

Cognitive bias tests measure variation in emotional appraisal and are validated methods to evaluate animals' affective states. However, the link between social behaviours and cognitive bias has not yet been investigated. Bottlenose dolphins are a gregarious species for whom welfare research is increasing in importance, and thus are a good model to test such an association. We adapted a spatial location judgement bias test for eight captive bottlenose dolphins to investigate the link between cognitive bias and social behaviour, where we conducted behavioural observations outside of training sessions and did not experimentally induce an affective state. Subjects showed stable individual differences in cognitive biases across the three test days. Furthermore, dolphins showing more synchronous swimming, a fundamental affiliative behaviour, judged ambiguous cues significantly more optimistically. Our longer-term data showed cognitive bias and synchronous swimming frequency were significantly associated for up to two months preceding the test, but disappeared prior to that, suggesting that here cognitive bias differences were reflected by transitory affective states rather than longer-term traits. We hypothesise that conducting synchronous swimming may induce affective states and/or be induced by them; either way, it has strong potential as an indicator of affective state in this species and beyond.

Keywords: affiliative behavior, animal welfare, Bottlenose dolphins, cognitive bias, social behavior, synchronous swimming

1. Introduction

Cognitive bias has been the subject of recent interest due to its successful application to animal welfare investigations, and describes the effects of emotional experiences on cognitive functioning (chiefly attention, memory and judgement, Mendl et al., 2009). In many animal species there is evidence to support the experience of emotions, which are then thought to form various affective states (Boissy et al., 2007; Mendl et al., 2009; Panksepp, 2005). Welfare can be generally described as the balance between positive and negative affective states (Spruijt et al., 2001) and welfare indicators are sought in order to measure characteristics of these states (Mendl et al., 2010): therefore the fields of animal emotion, welfare and cognitive bias research are all closely interlinked.

Cognitive biases are most likely adaptive: for example, individuals in environments, which induce anxious or fearful emotions may enhance their fitness through biased attention or judgement towards negative stimuli (Mendl et al., 2009). In humans, certain cognitive biases in perhaps a more complex form are known as optimism and pessimism (Paul et al., 2005), and a congruent finding is that being more optimistic is correlated to better subjective well-being (see reviews by Carver et al., 2010; Peterson, 2000). Soon after the first animal judgement bias paradigm was applied to laboratory rats (*Rattus norvegicus*, Harding et al., 2004), a handful of other mammalian and bird species were tested, and in the last few years the number of studies has increased exponentially (latest reviews by Bethell, 2015; Roelofs et al., 2016). In the vast majority of studies an experimental condition was imposed to induce a certain affect, with results convincingly showing that animals with induced negative affective states/ poorer welfare judge ambiguous stimuli more “pessimistically” (Mendl et al., 2009) (hereafter discussed as animal optimism and pessimism without forgetting the caveat that this is an anthropocentric concept).

Many cognitive bias studies have induced affective states through imposing conditions involving physical stress (Bateson et al., 2011), pharmacological treatments (e.g. Enkel et al., 2010), and chronic environmental and psychosocial stress (e.g. Doyle et al., 2011; Papciak et al., 2013). Although past results have mostly concurred with the predicted affect being induced, some studies have reported surprising directionality in cognitive biases (Bethell, 2015; Mendl et al., 2009). It has recently been asserted that the individual’s moods and affective state, occurring independently from the affect induced experimentally, might also be impacting cognitive bias results (Baciadonna and McElligott, 2015; Wichman et al., 2012). Affective states are defined as combinations of discrete emotions which result from the opportunities for threats or rewards in the surrounding environment, and moods are the longer-term result of experiencing affective states (Mendl et al., 2010; Nettle and Bateson, 2012). Since performance of behaviour is in response to current threats or rewards (Webster, 2005), measuring an animal's behaviour in its home environment might indicate its affective state/mood and thus also be correlated with cognitive bias results (Bateson and Nettle, 2015). A strong candidate for such behaviours would be those involved in social interactions: for example, affiliative social behaviour (e.g. gentle tactile interactions, play, allogrooming) is thought to be rewarding and associated with long-term positive affective states (Boissy et al., 2007; Mendl et al., 2010; Yeates and Main, 2008). Furthermore, it was recently recommended that cognitive bias tests be used specifically to investigate the contribution of social interactions to affective state (Wichman et al., 2012). When investigating the correlations between behaviours and cognitive biases, longer-term data would be invaluable for conclusions on whether temporary affective states or more stable behavioural traits are being seen (Roelofs et al., 2016): very few past studies have tested the long-term persistence of their results (Bethell, 2015).

Dolphins are gregarious marine mammals with complex societies and supposedly advanced cognitive abilities, and thus have long stimulated the interest of cognition researchers (Marino et al., 2007; Schusterman et al., 2013). Thus far the meaning and effect of social behaviours on the dolphins themselves has not been explored: for example the influence of dolphin play on affective state (Kuczaj and Eskelinen, 2014; as with other species, Held and Špinka 2011). The relationship between agonistic behaviour and affective state is thought to be complex in dolphin societies, with certain emotions often hard to pinpoint in the animals' multi-modal displays (Kuczaj et al., 2013). Dolphin behaviours such as gentle tactile interactions and synchronous swimming are some of the more direct indicators of social affiliation (Holobinko and Waring, 2010; Kuczaj et al., 2013), and therefore could be linked to positive emotions as well. There is very little research available on emotions and their indicators in dolphin species (Kuczaj et al., 2013), but interest for their discovery in other animals (Boissy et al., 2007; Mendl et al., 2010) is likely to stimulate an analogous increase in such studies, fuelled further by questions over their welfare status in captivity (Clegg et al., 2015; Gygax, 1993).

Despite bottlenose dolphins (*Tursiops truncatus*) being the most studied cetacean species (Hill and Lackups, 2010), and more pertinently the most commonly kept in captivity (Pryor and Norris, 1998), cognitive bias tests have not yet been conducted with them, or indeed any marine species or animals kept in zoos. Such tests could increase our knowledge of dolphin affective states and how social behaviours might impact them. In the only previous study linking cognitive bias and social behaviour, Lalot and colleagues recently found that pair-housing was linked to optimistic judgements in domestic canaries (*Serinus canaria*) (Lalot et al., 2017), while two other studies using cognitive bias tests have suggested links between dominance rank and optimism in two primate species (Bateson and Nettle, 2015; Schino et al., 2016). These studies have started to provide evidence of the 'emotional consequences of social behaviour' (Schino et al., 2016), but it would be useful to delve deeper into which aspects of social behaviours are linked to emotions, for example through studying the opportunistic performance of various behaviours in the social repertoire. Cognitive bias testing in dolphins would be readily applicable and useful for research on this group for a number of other reasons: conditioning to the chosen task would likely be feasible since captive dolphins are highly trainable using positive reinforcement methods (Brando, 2010), it has the potential to validate potential welfare indicators, and the results may enhance our knowledge of dolphin emotions and affective states, which is lacking at present.

Consequently a study was designed to investigate cognitive bias in a group of Atlantic bottlenose dolphins at Parc Astérix dolphinarium (Plailly, France). The two aims of this research were: (*i*) to test whether individual differences in judgement biases were present and repeatable over testing days. Although this has not been investigated with dolphins before, we expected cognitive biases to be present since they have been found in many other species. Finding individually repeatable responses would show that the methodology is eliciting more than a chance phenomenon. In part (*ii*) we wanted to test whether the cognitive bias results were correlated with measures of social behaviour taken around and prior to the testing period. The social behaviours were chosen to reflect the most common social interactions likely to take place, and included social play, synchronous swimming, and agonistic behaviour. We predicted that a higher frequency of synchronous swimming may be associated with optimistic judgements: it is a common social behaviour where two animals or more swim in (near) unison with each other (Connor et al., 2006a; Sakai et al., 2010) and is likely a proxy indicator for higher affiliation and social bonding in the group (Connor et al., 2006a). Social play is generally affiliative and thus higher levels might also be linked to more optimistic judgements, with agonistic behaviour perhaps correlating with pessimistic judgements if it is indeed an indicator of stress (Broom and Johnson, 1993). The results could make headway towards understanding dolphin affective states, and would integrate data from cognitive and behavioural measures which is a more accurate approach to assessing emotions, and therefore welfare, as opposed to using just one category (Boissy et al., 2007; Désiré et al., 2002). We also took social behavioural data in the months prior to testing, since persistence of links to cognitive bias results would reveal importantly whether transitory affective states, or stable behavioural traits, were being measured.

2. Material and methods

2.1 Study animals and facility

Our study involved eight Atlantic bottlenose dolphins (*Tursiops truncatus*) housed at Parc Astérix (Plailly, France) in an outdoor pool conjoined to two indoor pools, with a total volume of 3790 m^3 of water where access was always free between pools. The age range of the study subjects was from 4 to 43 years old, and consisted of 4 females: all adults of 11 years or over (age classification taken from Smolker et al., 1992), and 4 males: 2 adults and 2 juveniles; not all animals were related, and three were wild caught while the remaining five were captive born. A female calf of 6 months was also present in the group but not included in the study as she was too young to participate in training sessions. The dolphins' diets consisted of a variety of fish and squid species, and during multiple sessions each dolphin received between 5 and 12 kg per day depending on individual needs. The park was closed to the public for the duration of the experiment. "Training sessions" involved completing tasks conditioned using positive reinforcement (see Laule et al., 2003) for explanation), and could involve medical training, show practice, novel behaviours, free-feeds, and play sessions.

2.2 Cognitive bias test methodology

2.2.1 Test protocol: a Go-Go spatial task

The task required the dolphins to "touch the target with rostrum and return to the trainer", where the target position (the "cue") would move between the extreme left and right of a semi-circle, and eventually to the ambiguous positions along the arc in-between (Fig. 6). Once the animal returned to the trainer, they received either a "Positive" or "Less-positive" reward. This is a Go-Go task, where a response is required for both extreme cues, and thus avoided potential problems with generalisation gradients (discussed in detail in Roelofs et al., 2016). This spatial location task was adapted closely from Burman and co-authors' studies (Burman et al., 2011, 2009, 2008) with laboratory rats and domestic dogs (*Canis familiaris*), and was chosen here because spatial localisation is a salient feature in dolphin ecology (Shane et al., 1986), and a simple locomotory behaviour permitting the differential responses (latency to return to trainer, measured in s) to be seen would be relatively easy for dolphins to learn.

A 3-step protocol was developed with strict criteria regulating progression to the next stage. The animals first participated in trials involving only the conditioned (Positive and Less-positive) cues until they showed pronounced, sustained differences in return latencies. They then entered the true testing phase where each ambiguous cue (Near-positive, Middle, Near-less-positive) was presented among the conditioned cues once per day, over three consecutive test days, and the return latencies measured. The 5 cues (i.e. target positions) were set in an arc shape, all equidistant from each other and the opposite 'beach' area of the pool where the dolphin would start each trial (Fig. 6). The test was completed by the eight dolphins (4 males, 4 females) during January and February 2016.

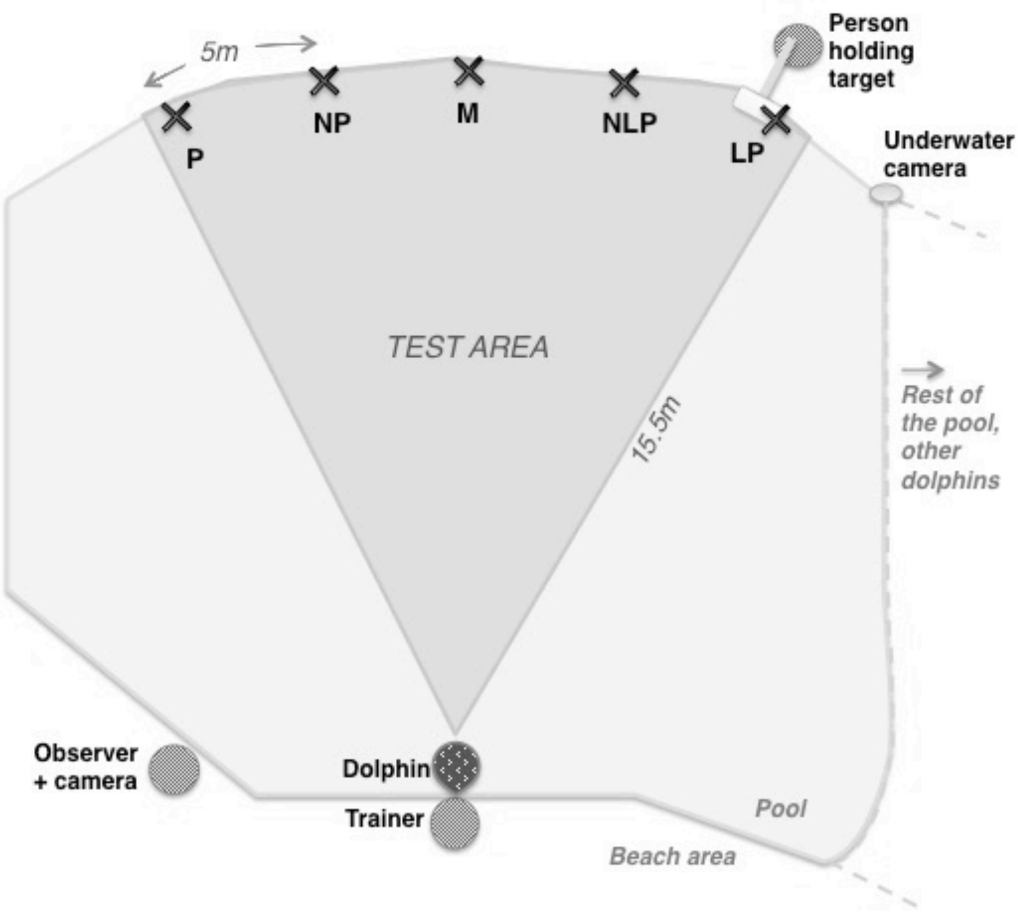

Fig. 6 *Schematic of cognitive bias test area. A diagram (not to scale) of the test area within the pool at Parc Astérix, utilised for tests with 8 bottlenose dolphins. Positions*

are shown of the conditioned (Positive, P; Less-positive, LP) and ambiguous (Near-positive, NP; Middle, M; Near-less-positive, NLP) cues, test subject, and personnel involved. Note that for analyses an average of return latency times from all ambiguous cues (NP, M, NLP) was used.

2.2.2 Positive and Less-positive rewards

When returning from touching the target at the “Positive” position, the dolphins received a large reward of one herring as well as a few seconds of applause and eye contact, and from the “Less-positive position” they received a small reward of just the applause and eye contact, as well as for the ambiguous cue positions (Near-positive, Middle and Near-less-positive). Eye contact and applause on its own is already used in dolphin training techniques as a secondary reinforcer (Neto et al., 2016), where it has “acquired reinforcing value through learning by being paired with events that are already reinforcing” (Brando, 2010). The test’s Positive and the Less-positive rewards were verified by animal care staff at Parc Astérix as representing larger and smaller reinforcement respectively, and being clearly distinct. All dolphins seemed to prefer herring from the other types of fish (herring are relatively larger and have a high fat content, Fisher et al., 1992), and reacting positively (e.g. approaching and contacting trainers) in response to applause and eye contact (dolphins have been shown to closely monitor attentional state through human gaze, Pack and Herman, 2006).

Differential positive reinforcement for the extreme cues has been applied before in cognitive bias testing and offers advantages including less risk of extinction of behaviour and frustration, avoidance of difficult analysis of trials with no response, and a generally more positive experience for the animals (Keen et al., 2014).

2.2.3 Testing within the social group

There are no pre-established methods for testing cognitive bias in dolphin species, and since separating the dolphins in Parc Astérix would have caused undue stress, focal cognitive bias tests were conducted during training sessions when the animals could be segregated behaviourally i.e. under stimulus control. Testing within the social group was recently conducted for the first time in cognitive bias research (Nogueira et al., 2015), and the several advantages were highlighted in a recent review (Roelofs et al., 2016). Since our test was conducted on each dolphin in the presence of all group members, our choice of a spatial discrimination task was conducive to reducing the likelihood of conspecifics learning about the test: for example, using underwater sound cues would have been inappropriate since they would be heard in all areas of the pool and therefore accessible to all dolphins during each trial.

2.2.4 The 3-step progression of the cognitive bias test

Step 1: Teaching the task behaviour

In the first step of the process, the animals were taught to respond to the trainer's signal (hand gesture) by swimming to the opposite side of the pool, touching the target submerged 50cm under the water's surface, and returning to the trainer. The target was made out of an orange foam cylinder attached securely to a wooden shaft. The person holding the target would 'bridge' the behaviour (a whistle which lets the animal know it has completed the correct behaviour (Laule et al., 2003)), which signalled to the dolphins that they should return to the original trainer. The addition of the person whistling to signal the correct behaviour was necessary to encourage the dolphin to return to the original trainer, allowing them to be the one to provide reinforcement and meaning only one trainer was needed for the tests. During this first step of the test, the "Test area" (Fig. 6) was avoided and the target placed at different positions around the entire pool in order to not establish any conditioned responses in the Test area. The criteria to confirm that the dolphins had learnt the behaviour was that they must complete the behaviour correctly in at least 11 out of 12 trials (92%): once this was achieved they passed on to Step 2.

Step 2: Conditioning the responses to Positive and Less-positive cues

In Steps 2 and 3, the structured setting of the trials commenced: 12 trials (1 set) were conducted in one session, with three brief pauses after every three trials. During the pauses, the trainers asked the animal a few simple, low-energy, known behaviours and reinforced them equally (always with one small fish). The pauses were necessary to maintain the interest in the cognitive bias tests: the dolphins each know around one hundred different conditioned behaviours and are accustomed to being asked a variety of them in their regular training sessions. In prior trial runs of the test method, when there were not any pauses, the animals left the trainer frequently and showed more refusals to conduct the task. When the standardised pauses were introduced the animals stayed with the trainers for the entirety of the test. No more than 1 set of 12 trials were conducted per dolphin per day, and there was no more than three days between consecutive testing days. The response variable for cognitive bias analysis was the latency to return to the trainer (in s).

Subjects were randomly assigned either the extreme left or right of the arc as their “Positive” reinforced position. For the first trail of each session, the trainer and person holding the target were blind to which were the Positive and Less-positive positions, since the experimenter (IC) relayed the target position just before each trial and the reinforcement type during the animal’s return to the trainer: however, once the first trial of the set was completed the cue values were unavoidably revealed. For both Steps 2 and 3, a pseudorandom order of the positions was used where there were no more than two consecutive Positive or Less-positive trials (as conducted in Burman et al., 2011). If the animal performed the incorrect behaviour, this was noted and the behaviour was asked again; in practice, never more than two non-consecutive refusals occurred during a set of trials. Also noted was whether any other dolphin came within a body length of the test subject, and if this caused a distraction where the subject changed trajectory, the trial was repeated. All trials were filmed from both a GoPro® HD Hero 2 at the surface and a GoPro® Hero 4 (GoPro, Inc., San Matteo, CA, USA) placed underwater to measure return latencies. Videos were synchronized *a posteriori*.

In order to be sure that the animals were responding differently to the two extreme cues, criteria were adapted from the similar spatial task used with domestic dogs (Burman et al., 2011). Here, to continue to Step 3, the return latency of the dolphins in Step 2 had to be two seconds longer from the Less-positive cue than from the Positive cue, for five consecutive trials.

Step 3: Presenting the ambiguous cues

In Step 3 the ambiguous cues at the Near-positive, Middle, and Near-less-positive positions were presented in the sets of 12 trials. Each ambiguous cue was presented once each per 12 trials, and with at least two conditioned trials in between, following the same pseudorandom order as in Step 2. The ambiguous cues were reinforced similarly to the Less-positive cue: with a few seconds of eye contact and applause from the trainer. The 12 trials for Step 3 were conducted three times (to avoid loss of stimulus ambiguity, Roelofs et al., 2016), once per day on three consecutive days for each dolphin.

2.2.5 Calculation of cognitive bias response variables for analysis

The dolphins’ return latency (in s) from the target to the trainer, where reinforcement was given, was used to analyse cognitive bias. Return latencies were calculated from the video footage taken and were measured from the instance the animal broke contact with the target to when its head surfaced above water in front of the trainer. We calculated an average of the return times from all ambiguous cues (Fig. 6) to be able to have one measure of the response to ambiguous positions, since past studies have shown varying associations in relation to each ambiguous cue when analysed separately, without being able to attribute meaning to the differences (Mendl et al., 2009; Salmeto et al., 2011). We then used as our response variable the percentage deviation of this ambiguous cue average from each individual's average return latency from the Positive and Less-positive (conditioned) cues. This allowed us to control for the different swim speeds of the dolphins, and produced a measure of individual response to ambiguous cues relative to the conditioned ones.

2.3 Collection of social behavioural data

During the 3-day cognitive bias testing period for each dolphin (January/February 2016) there were also five behavioural observations (5-min focal observations, scan sampling every 15s; Mann, 1999; Martin and Bateson, 1986) conducted per day during the dolphins' "free-time" between training sessions, for a total of 15 observations per animal. In order not to bias the observations towards times where certain behaviours would be more likely, one observation was taken shortly before (≤ 15 min) a training session, one shortly after (≤ 15 min), and the remaining three were taken at randomly chosen times in-between sessions.

For analysis the scans were converted to a percentage of total scans per observation (i.e. to correct for non-visible scans), and are therefore described as frequencies hereafter. Observations were all carried out by the same person (IC), using a behavioural repertoire containing nine behaviours classified within three categories (synchronous swimming, social play, and agonistic; see definitions and their sources in Thesis ethogram, Chapter 2). Synchronous swimming (as we define it) between mothers and dependent calves is very frequent in the first 6 months of life (Gubbins et al., 1999), and since one dolphin had a 6 month old calf during the study, synchronous swimming between her and the calf was not recorded, unless there was a third dolphin involved. As well as collecting these social behaviour measures during the days where cognitive bias was tested, they were also taken on a longer-term basis: near-daily observations were taken for the four months preceding testing. These data were organised into 2-month long periods preceding the first day of testing, i.e. 0 to 2 months and 2 to 4 months before.

Data analysis

Statistical analyses were done with R, version 3.1.1 (R Development Core Team, 2016), and were guided by advice from a recent review concerning analysis of cognitive bias data (Gygax, 2014). For our first question regarding whether test criteria were maintained, we used the Fisher-Pitman permutation test (package *coin*, Hothorn et al., 2006).

To test for the repeatability of individuals' latency times over the three test days we applied an intra-class correlation based on a linear mixed effects model with permutation tests (package *rptR,* Nakagawa and Schielzeth, 2010), where we calculated the repeatability (R; based on 10,000 bootstrap runs) of the individual return latencies from ambiguous cues (in s) measured during the 3 days of testing. In order to correct for different swim speeds of individuals we used the relative latencies, which were the percentage deviation of the ambiguous cue latencies from the averaged conditioned cue (Positive and Less-positive) latencies. In doing so, we used individual identity as a random factor (Nakagawa and Schielzeth, 2010). Furthermore, the model included sex as a further between-subject effect. However, this factor was not significant ($p > 0.10$) and was removed from the model before this was re-calculated.

In order to calculate the associations between cognitive bias test results and the three different social behaviours, we used linear regression models with permutation tests (package *lmPerm,* Wheeler, 2010). Such permutation tests are particularly adequate when sample sizes are moderate and do not have any assumptions regarding normal distribution of residuals (Good, 2005). The behaviours were each tested separately as independent variables. Furthermore, we included sex of the animals as a factor in all models. However, this factor (including an interaction with the respective behaviour of each model) was never significant (all $p > 0.10$), and thus was always reduced from the models before these were re-calculated.

3. Results

3.1 Maintenance of test criteria

During the 3-day test period where ambiguous cues were presented in addition to the conditioned cues, the return latency from the Less-positive cue was significantly longer, on average by 2.0 seconds (min: 1.5 s, max: 2.4 s) than from the Positive position (Fisher-Pitman test with 10,000 permutations: $Z = 11.72$, $p < 0.001$).

3.2 Repeatability in individual judgement patterns

The latencies to return from the ambiguous cues (averaged over the Near-positive, Middle, and Near-less-positive) were significantly repeatable across the three days of testing. This was the case with respect to the absolute latencies in seconds (intra-class correlation with 10,000 permutations: $R = 0.888$, $p = 0.001$) as well as when using the relative values, calculated as the percentage deviation from the averaged return latency from the conditioned cues ($R = 0.466$, $p = 0.015$).

3.3. Association between different behavioural parameters and optimistic judgements

3.3.1 Short-term associations

There was a significant and negative correlation between the frequency of synchronous swimming and the averaged, relative latency to return from the ambiguous cues (linear regression with 10,000 permutations: $R^2 = 0.679$, $\beta = -0.419$, $p = 0.013$; Fig. 7a). That is, dolphins showing more synchronous swimming around the testing period returned more quickly, which signifies a more optimistic-like judgement. The other behavioural parameters, social play and agonistic behaviour, were not significantly associated to return latency from the ambiguous cues ($p > 0.10$).

3.3.2 Longer-term associations

When considering the behaviours quantified during regular observations over a period of 0 to 2 months prior to the testing period, we found similar results. Again, there was a significant and negative correlation between the frequency of synchronous swimming and the animals' averaged, relative latency to return from the ambiguous cues ($R^2 = 0.541$, $\beta = -0.371$, $p = 0.044$; Fig. 7b). Social play and agonistic behaviour were not significantly associated to the animals' latency to return (all $p > 0.10$).

During the period of 2 to 4 months prior to testing, there were no significant associations between any social behaviour quantified and the animals' averaged latency to return from the ambiguous cues (all $p > 0.10$).

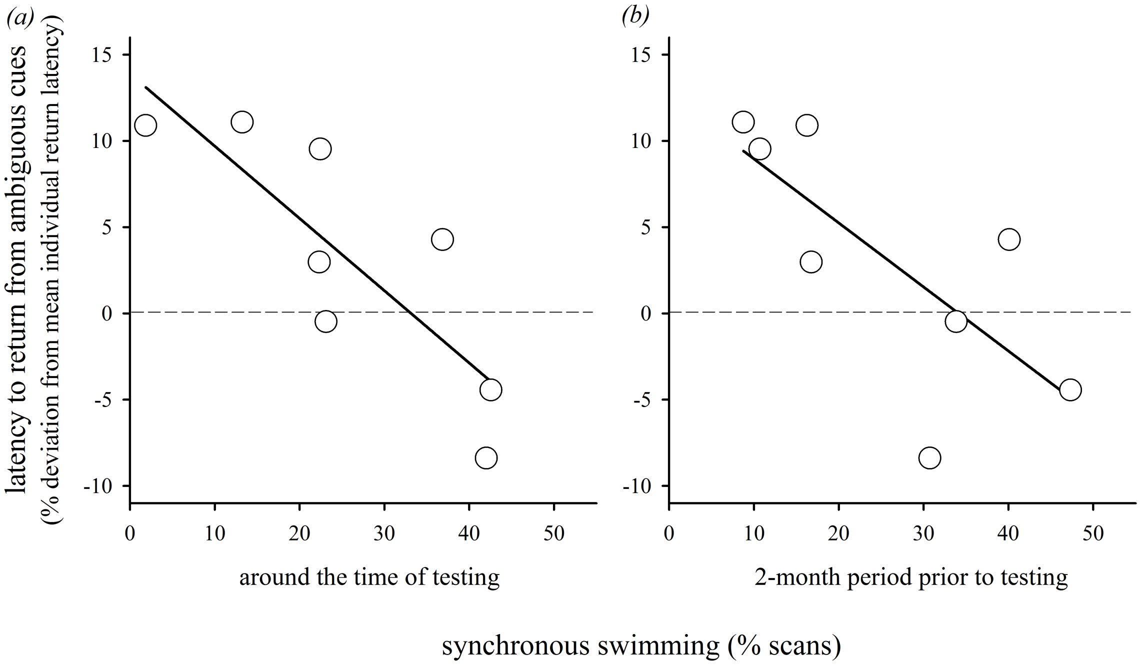

Fig. 7 *Cognitive bias results predicted by synchronous swimming frequency in bottlenose dolphins.* **(a)** *The animals' return latencies to ambiguous cues were significantly correlated with the frequency of synchronous swimming behaviour during the 3-day test period, where those fastest to return also showed the highest frequency of synchronous swimming. This same relationship was present* **(b)** *with behavioural data taken up to 2 months before the testing period, where those who were more frequently synchronous swimming also returned significantly more quickly in the subsequent cognitive bias tests. The dotted line represents individual average return latencies, and thus low and negative y-axis values correspond to faster return times and optimistic-like judgements, and the higher and positive deviations to pessimistic-like judgements. See text for statistics.*

4. Discussion

We found that captive bottlenose dolphins showed stable individual difference in judgement biases across three testing days. These differences were correlated with the frequency of synchronous swimming, a parameter reflecting social affiliative behaviour, shown around and up to two months prior to the time of testing, but for which the association disappeared when studying behaviour between two and four months prior to testing. Dolphins who conducted more synchronous swimming in the time outside of training sessions made more optimistic-like judgements of ambiguous cues.

4.1 Test protocol with dolphins

There have been no previous cognitive bias studies in zoo or aquarium facilities (Bethell, 2015), and few where testing involves positive reinforcement only and occurs within the animals' social groups (Keen et al., 2014; Nogueira et al., 2015). Roelofs and co-authors (2016) recommended that testing in social groups should be a future direction of cognitive bias experimentation to facilitate application for welfare assessment. A number of initial validations for a judgement bias test with dolphins can be taken from our results: the animals successfully progressed through the stages of the test; they maintained their responses to the conditioned cues throughout the presentation of ambiguous cues; and, the clearest indication that the test was functional, the dolphins had individual profiles of judgement biases, which were significantly repeatable over the three test days.

4.2 Cognitive bias and social behaviour

Higher frequencies of synchronous swimming in the dolphins' free-time were associated with more optimistic-like judgements towards the ambiguous cues, and such a relationship persisted up to two months prior to testing days. Synchronous swimming is generally considered an adaptive (Fellner et al., 2013) and affiliative behaviour (Connor et al., 2006a; Sakai et al., 2010), as well as a foundation for other social behaviours (Fellner et al., 2013). Social support is thought to significantly buffer stress in dolphins (Fellner et al., 2013; Waples and Gales, 2002) and thus unsurprisingly higher levels of conspecific bonding have been shown to increase survivability (Frère et al., 2010; Stanton and Mann, 2012), similar to other animals (e.g. DeVries et al., 2003; Rödel and Starkloff, 2014). The lack of dolphin emotion research (Kuczaj et al., 2013) means we cannot make easily speculate about the presence of positive emotions, but evidence in other mammalian species suggests affiliative behaviours are indeed linked to positive affective states (Boissy et al., 2007). Our results are correlative and thus cannot confirm the causality between synchronous swimming and optimistic-like judgements. However, since past cognitive bias testing has shown that more optimistic judgements generally reflect more positive emotional states (Mendl et al., 2009; Roelofs et al., 2016), one explanation for our findings might be that conducting more synchronous swimming likely results in higher social bonding and affiliation, and this may in turn lead to a more positive internal affective state as evidenced by the cognitive bias results. Those animals who had less affiliative social interactions through synchronous swimming may have experienced more negative affective states, making more pessimistic judgements. This hypothesis is supported by findings from other species and humans: domestic canaries housed together made more optimistic judgements (Lalot et al., 2017), and a study on zoo-housed Rhesus macaques (*Macaca mulatta*) found the more sociable subjects had higher subjective well-being, as assessed by their keepers (Weiss et al., 2011). Therefore with dolphins, we might assume that those conducting more synchronous swimming are actively choosing to be in the company of conspecifics, inducing positive affective states and leading to more optimistic judgements. In the future, synchronous swimming frequency might therefore merit investigation as an element of overall welfare assessments.

4.3 Longer-term persistence of cognitive bias predictor, and future directions

As humans we can have a consistent, personality-level degree of optimism, termed “dispositional optimism” (Peterson, 2000), as well as shorter-term optimistic or pessimistic biases induced by temporary mood states and salient changes in environment (Carver et al., 2010; Segerstrom, 2007). In our study we took longer-term behavioural data to see whether any associations with cognitive bias were longer-lasting (i.e. stable behavioural traits) or more transitory in nature (i.e. reflecting transient affective states). Our results showed that the correlation between cognitive bias and synchronous swimming was still present when using observational data taken in the two months prior to testing, but disappeared when going two to four months back in time. Therefore synchronous swimming frequency, a measure of affiliative behaviour and social context (Connor et al., 2006a; Holobinko and Waring, 2010; Kuczaj et al., 2013), is correlated with certain affective states which persist for a few weeks or months (Mendl et al., 2010; Nettle and Bateson, 2012). Further investigations into this relationship over a longer period of time are needed to illuminate the true mechanisms in play. As social animals with complex group networks, it would make sense adaptively if the social situation and resulting behaviours did indeed influence the affective states of dolphins. There are a few limitations with our results, including the fact that our moderate sample size may not have allowed us to detect all effects, and thus non-significant results must be treated with caution. Studies with higher sample sizes in a larger range of facilities would be valuable, as well as insight into the repeatability of cognitive biases. In general more work is needed on the occurrence of longer-term mood states in dolphins and other species, and cognitive bias testing such as that used here must start to fill the gaps and stimulate future investigations.

4.4. Conclusions

We conducted the first cognitive bias tests in a zoo setting, and found that bottlenose dolphins seem to show differing levels of "optimism" in captivity. The subjects' cognitive biases were repeatable over several testing days, and the bias differences could be explained by the frequency of synchronous swimming observed outside the test sessions. To our knowledge, our results are the first to suggest empirically that higher social affiliation in animals is associated with positive affective states. Since describing the causal relationship is out of our scope, we support the hypothesis that the performance of synchronous swimming either induced a certain affective state in the dolphins, and/or was induced by it. Our longer-term data showed that the relationship between synchronous swimming and cognitive bias exists two months prior to testing but disappears after that, indicating that we have measured transitory affective states as opposed to stable behavioural traits. Synchronous swimming has been cited as a measure of affiliative behaviour and social bonding, and our results show that it is indeed likely to be associated with affective state. Further studies on such behaviours and cognitive biases over a longer timeframe are needed, and which might also provide support for the tests' use in measuring overall welfare.

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Compliance with ethical standards: Ethics. This study adhered to the ASAB/ABS Guidelines for the Use of Animals in Research (Association for the Study of Animal Behaviour, 2012), and was reviewed and accepted by the scientific and animal welfare committees of Parc Astérix. Our pre-established protocol dictated that if the dolphin showed signs of frustration or annoyance, the trial was stopped.

Competing interests. We have no competing interests.

Paper 4: Cognitive judgement bias is associated with frequency of anticipatory behaviour before training sessions in bottlenose dolphins

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Résumé

Beaucoup d'animaux montrent une vigilance et/ou une activité accrue par rapport aux événements à venir, ces comportements sont regroupés sous le terme de « comportement anticipatoire ». Le comportement anticipatoire à l’égard d’événements positifs a été proposé comme une mesure d'état affectif trans-espèce puisqu'il reflète probablement l'équilibre du « reward sensitivity system »: des études diverses suggèrent que dans des situations de mal-être, les animaux montrent une anticipation accrue envers des événements positifs à venir. Un autre outil pour évaluer l'état affectif des animaux sont les tests de biais cognitif, et bien qu'il ait été tenté, aucun lien n'a encore été fait entre le biais cognitif et des niveaux de comportement anticipatoire. En captivité les grands dauphins (*Tursiops truncatus*) produisent plus de comportements tels que ‘surface-looking’ et ‘spy-hopping’ en attendant des sessions de dressage aux cours desquelles ils vont recevoir de la nourriture. Les tests de biais cognitif ont été récemment appliqués avec succès à cette espèce et l'étude actuelle utilise le même ensemble de données, mais inclut des données comportementales supplémentaires prises les jours de test à l'extérieur des sessions de biais cognitif, pour mesurer la fréquence de comportement anticipatoire et examiner s'il est associé aux biais cognitifs des dauphins. Nous avons trouvé que les fréquences les plus hautes de comportement anticipatoire avant des sessions étaient significativement associées aux jugements plus pessimistes dans des tests de biais cognitif, ce résultat est en accord avec des découvertes précédentes liant une plus haute sensibilité à la récompense à des états affectifs négatifs. D’autres études sont nécessaires pour déterminer le seuil de comportement anticipatoire au-dessus duquel l'état affectif est probablement négatif et le seuil au-dessous duquel l'animal anticipe positivement l'événement. Le comportement anticipatoire est une activité facilement mesurable qui pourrait représenter un indicateur de bien-être pour des dauphins ainsi que pour d'autres animaux dans des environnements captifs.

Abstract

Many animals display a suite of increased vigilance and/or activity responses in relation to upcoming events, termed ‘anticipatory behavior’. Anticipatory behavior towards positive events has been suggested as a cross-species measure of affective state since it likely reflects the balance of the reward-sensitivity system: various studies suggest that animals in poorer welfare situations show increased anticipation for positive events. Another tool for evaluating animals’ affective state is cognitive bias testing, and although it has been attempted, a link has not yet been made between cognitive bias and anticipatory behavior levels. Bottlenose dolphins (*Tursiops truncatus*) in captivity increase the performance of behaviors such as surface-looking and spy-hopping in anticipation of training sessions during which food is provided. Cognitive bias testing was recently applied with success to this species, and the current study uses the same dataset but includes additional behavioral data taken on the testing days but outside of cognitive bias sessions, to measure anticipatory behavior frequency and investigate whether it is associated with dolphins’ cognitive biases. We found that higher frequencies of anticipatory behavior for training sessions was significantly associated with more pessimistic judgements in cognitive bias tests, agreeing with previous findings linking higher reward sensitivity with negative affective states. Further work is needed to determine the threshold of anticipatory behavior above which negative affect is likely, and below which the animal is positively anticipating the event. Anticipatory behavior is an easily measured activity and could represent a welfare indicator in dolphins as well as other animals in captive environments.

Key Words: Animal welfare, anticipatory behavior, Bottlenose dolphin, cognitive bias, reward sensitivity

Introduction

Anticipatory behavior describes the activity performed by an individual in expectation of an upcoming event (Spruijt et al., 2001). The ability to anticipate predictable events extends to the vast majority of species, and so far anticipatory behavior has most often been documented as increased activity, vigilance and/or increased transitions from one behavior to another (van den Bos et al., 2003; van der Harst et al., 2003a). The most often studied anticipatory behaviors have been in relation to expected food events (Mistlberger, 2009; Storch and Weitz, 2009), but anticipation has also been shown towards for example access to play opportunities (Anderson et al., 2015), enriched housing (van der Harst et al., 2003b), sexual interactions (van der Harst et al., 2003b) and positive human-animal interactions (Krebs et al., 2017).

Anticipatory behavior has recently garnered interest in animal welfare research (van der Harst and Spruijt, 2007; Watters, 2014) because it is thought to reflect underlying affective states (combinations of discrete emotions Mendl et al. 2010), through its links with the reward sensitivity system (Spruijt et al., 2001; van der Harst and Spruijt, 2007). A handful of studies have demonstrated that animals in poorer welfare conditions show a significantly higher level of anticipation to positive events than conspecifics in better welfare conditions (e.g. laboratory rats, *Rattus norvegicus*, in standard *versus* enriched cages, van der Harst et al., 2003a, 2003b); or social isolation *versus* group housing (van den Berg et al., 1999). Animals experiencing negative affective states place more “incentive value” on acquiring positive rewards, and thus anticipate their arrival more intensely (e.g. a hungrier animal will place more incentive value on a food item) (Spruijt et al., 2001). However the link between anticipatory behavior and affective state does not seem to be a straightforward linear relationship: farmed silver foxes (*Vulpes vulpes*) increased anticipatory as well as stereotypical behaviors before a positive reward, but did not show stereotypical behaviors before negative events (Moe et al., 2006), while rats in depressed-like states did not anticipate positive rewards at all (von Frijtag et al., 2000). More work on this topic is necessary to understand the underlying motivational systems, and a recently published paradigm (adapted from van der Harst and Spruijt 2007) provides some direction: it predicts that the intensity of anticipatory behavior will increase with poorer welfare up to a point where the animal has entered chronic stress, after which it drops dramatically (Watters, 2014).

In parallel to anticipatory behavior being proposed as a welfare measurement tool (van der Harst and Spruijt, 2007), another approach under investigation is cognitive bias testing (Mendl et al., 2009; Wichman et al., 2012). Cognitive biases reflect the effect of emotions on an individual's cognitive functioning, and numerous tests with a wide range of species have shown in general that animals in poorer welfare conditions will judge ambiguous cues more pessimistically, and *vice versa* (latest reviews by Baciadonna and McElligott, 2015; Roelofs et al., 2016). Recent evidence supports a link between affiliative social behavior and biases: a study on canaries (*Serinus canaria*) found pair-housing led to more optimistic judgements (Lalot et al., 2017), and bottlenose dolphins conducting more synchronous swimming in their free-time also judged more optimistically (Clegg et al., 2017a). One study (with laying hens, *Gallus gallus domesticus*) aimed to correlate anticipatory behavior frequency to cognitive bias but found no associations, however they recommended further attempts be conducted to try and relate these two potential welfare tests (Wichman et al., 2012).

In order to examine the potential links between anticipatory behavior frequency and cognitive bias, we analyzed further the behavioral data from Clegg and co-authors' study (Clegg et al., 2017a [Paper 3, Chapter 3]) by measuring the frequency of anticipatory behaviors of captive bottlenose dolphins towards the upcoming training sessions. In general it is thought that dolphins view the multiple, daily training sessions positively as opposed to negatively, due to the anticipatory behaviors towards them demonstrated in previous studies (Clegg et al., 2017b; Jensen et al., 2013), the fact that the sessions constitute the provision of food, and their potential for cognitive stimulation (Brando, 2010; Laule et al., 2003; Miller et al., 2011b). Therefore we hypothesized that in accordance with the reward-sensitivity theory (Spruijt et al., 2001), those dolphins showing the higher frequencies of anticipatory behavior would also make more pessimistic judgements in the cognitive bias task.

Materials And Methods

For the full methodology, equipment and test protocols please refer to Clegg and co-authors' study (Clegg, et al., 2017a; [Paper 3, Chapter 3]). Short, summarized descriptions, and any precisions in this study's approach, are given below.

1. Study Animals and Facility

Eight Atlantic bottlenose dolphins (*Tursiops truncatus*) housed at Parc Astérix (Plailly, France) were used in this study (4 males, 4 females, age range: 4-43 years). The dolphins participated in multiple daily training sessions, which could involve tasks conditioned using positive reinforcement (Brando, 2010) such as medical training, show practice, novel behaviors, free-feeds, and play sessions. During the experiment, almost all training sessions began with the trainers approaching the outside beach area, and the start time did not vary by more than half an hour, with the intervals in-between sessions varying very little. Furthermore, acoustic and visual environmental cues (e.g. trainers preparing and setting up the food buckets) were accessible to the animals for distinguishing the start time of the upcoming training session (as with other zoo animals' anticipatory behavior; Krebs et al., 2017; Watters, 2014). Since this management practice had been in place for the last several years, the dolphins had been conditioned to cues signalling the imminent start of training sessions. Fixed feeding schedules and predictable environmental cues have been shown to elicit anticipatory behavior in many species in zoo and other captive settings (Krebs et al., 2017), and such classical conditioning has also been deliberately applied in other anticipatory behavior studies i.e. pairing experimental cues to rewards (e.g. Wichman et al. 2012). The park was closed to the public for the duration of the experiment.

2. Cognitive bias testing

2.1 Test protocol: judgement bias task from Clegg et al. 2017a [Paper 3, Chapter 3]

Individual cognitive biases were measured using a spatial judgement Go/Go task adapted by Clegg and co-authors (Clegg et al., 2017a). A three-step procedure was designed with conservative criteria that had to be fulfilled in order to pass to the next stage: first the animals were taught the task behavior, which was to touch a target on the other side of the pool and return to the trainer. Once the animal touched the target with their rostrum, a whistle ("bridge") was blown by the target-holder which indicated to that the correct behavior had been performed and they should return to the trainer. In Step 2, repeated trials were conducted where the target was placed on either of the extreme positions (far left or right of an arc, see Fig. 1 in Clegg et al., 2017a), which were differentially rewarded with either a herring, applause and rubs (the "Positive" (P) cue reward) or only applause and eye contact (the "Less-positive" (LP) cue reward). The animal care staff at Parc Astérix confirmed that all dolphins would view the Positive and the Less-positive rewards as representing larger and smaller reinforcement respectively. Herring is larger and has a higher fat content than the other types of fish fed at Parc Astérix (Fisher et al., 1992), and while the dolphins react positively (by approaching and contacting trainers) to applause and eye contact, this is considered a secondary reinforcement whereas food items are stronger, primary reinforcers (Brando, 2010; Neto et al., 2016). The animals were not separated from their regular social groups during testing (advantages discussed in (Roelofs et al., 2016)), in order to avoid inducing stress from social isolation, which has often been recorded with dolphins (e.g. Waples & Gales, 2002).

Once consistent differences in speed were seen (criteria: ≥ 2s faster to return from the Positive than the Less-positive cue), the animal progressed to Step 3, where the ambiguous cues were presented: the target was placed at three points along an arc shape between the Positive and Less-Positive positions. The ambiguous cue positions were termed Near-positive (NP), Middle (M), and Near-less-positive (NLP), and were equidistant from the start/finish position of the animal, and from each other. Within Step 3, each animal completed three sets of testing, with 12 trials in each, conducted on three consecutive days. In each set of trials each ambiguous cue was presented once, following a pseudorandom order where a maximum of two positively (i.e. P) or less-positively reinforced cue positions (i.e. NP, M, NLP, LP) could follow one another. The reward given when the target was at the ambiguous positions was applause and eye contact, and the fact that the whistle was blown when the target was touched continued to let the animal know it had performed the correct behavior (Laule et al., 2003). The return times to the trainer from each of the ambiguous cues were used as measures of cognitive bias. The full tests were completed by each animal during January and February 2016, and the fact that all animals progressed to Step 3 (i.e. showed ≥ 2s difference between the Positive and Less-positive cue) validated our choice of reinforcement for these two extreme cues.

2.2 Cognitive bias response variables

The overall response variable reflecting cognitive bias was the dolphins' return latency (in s) from the target to the trainer, where it would receive the reward (the same as in Clegg et al., 2017a, and measured from video footage). For the current study, the return latencies of each dolphin were averaged for each of the Near-positive, Middle and Near-negative cues (instead of taking an average of all three ambiguous cues as in Clegg et al., 2017a). This approach was chosen to fully investigate any associations between our one behavior variable (as opposed to multiple behaviors tested in Clegg et al., 2017a) and cognitive bias. If correlations are present it would be fruitful to be able to see whether they are conserved among the different ambiguous cues, yielding as much information as possible on the link between cognitive bias and anticipatory behavior since it is clearly a species-independent topic of interest (Wichman et al., 2012).

For each of the three ambiguous cues, the final response variable, (as in Clegg et al., 2017a), was the percentage deviation of the ambiguous cue average return latency from the individual's average return latency from the Positive and Less-positive (conditioned) cues. This allowed us to control for the different swim speeds of the dolphins, and produced a measure of individual response to each ambiguous cues relative to the conditioned ones.

3. Behavioral data collection

During the 3 days when the final cognitive bias tests took place for each animal, behavioral data was also taken during their "free-time" in order to assess the level of anticipatory behavior. Focal observations of 5 minutes with scan sampling for behaviors every 15s (Mann, 1999) were conducted once a day within the 15-minute period before one of the five main training sessions (between 10:00 and 16:30), in order to capture an accurate reflection of anticipation for these events specifically. These training sessions could either include the cognitive bias testing or not (see description of training sessions earlier in the methodology), but these were conducted at random times of the day and there was no way for the animal to discern the contents of the session before it started, they were only able to predict the start time using environmental and time-delay cues. The same person (IC) always conducted the observations using a behavioral repertoire of the two most commonly described anticipatory behaviors in the literature (Clegg et al., 2017b; Miller et al., 2011b): surface-looking and spy-hopping (Table 4). Both of these behaviors were found to be among those that increased before training sessions in two previous studies at Parc Astérix (Clegg et al., 2017b; Jensen et al., 2013). For analysis, the scans of both anticipatory behaviors were summed and then converted to a percentage of total scans per observation (i.e. to correct for non-visible scans), and are therefore described as frequencies hereafter.

Table 4 *Behavioral repertoire used for observations of anticipatory behavior taken directly or adapted marginally from (Clegg et al., 2017b [Paper 2, Chapter 2]; Jensen et al., 2013; Miller et al., 2011b).*

Anticipatory behavior	Description
Surface look	Dolphin lifts head out of the water while swimming, or head is held fixed while floating stationary, with an eye/eyes directed towards a point above the surface where a predictable event usually commences. A surface look can be distinguished from simply taking a breath by the fact that in the former, the head may be momentarily suspended above the water, where the eyes are clearly focussing above the water surface line.
Spy hop	Dolphin propels itself vertically out of the water with the eyes directed to a point above the water's surface, usually as far as the pectoral fins, and then descends vertically. Often repeats this several times consecutively.

4. Data Analysis

Statistical analyses were done with R, version 3.1.1 (R Development Core Team, 2016). To test the associations between cognitive bias test results and anticipatory behavior frequency, we used linear regression models. P-values were calculated by Monte Carlo sampling with 10,000 permutations, using the R package *pgirmess* (Giraudoux, 2016). Such permutation tests are especially appropriate for moderate sample sizes and do not have any assumptions regarding normal distribution of residuals (Good, 2005). The anticipatory behavior frequency represented the independent variable in three separate models with the relative return latency for the NP, M and NLP ambiguous cues as the response variable in each. The Bonferroni correction was not applied to P-values because it was felt that the three variables, i.e. the three ambiguous cues NP, M and NLP, were measuring a facet of the same phenomenon and thus not truly independent (the correction should be applied when the *"variables being tested are independent"*, Garcia, 2004). We also used linear models and permutation tests to investigate whether anticipatory behavior frequencies and relative return latencies from each ambiguous cue differed with the sex of the animals (included as a factor in each model).

Results

Absolute return latencies for all cues

A descriptive graph showing the absolute return latencies for each dolphin over all the cues in the final testing phase (Fig. 8) allows us to verify that the animals maintained their differentiation between the conditioned cues (P and LP, on average 2.0s difference, Clegg et al., 2017a). We can also see that in general the latencies for all dolphins increase incrementally as we move from further away from the Positive and closer to the Less-positive position.

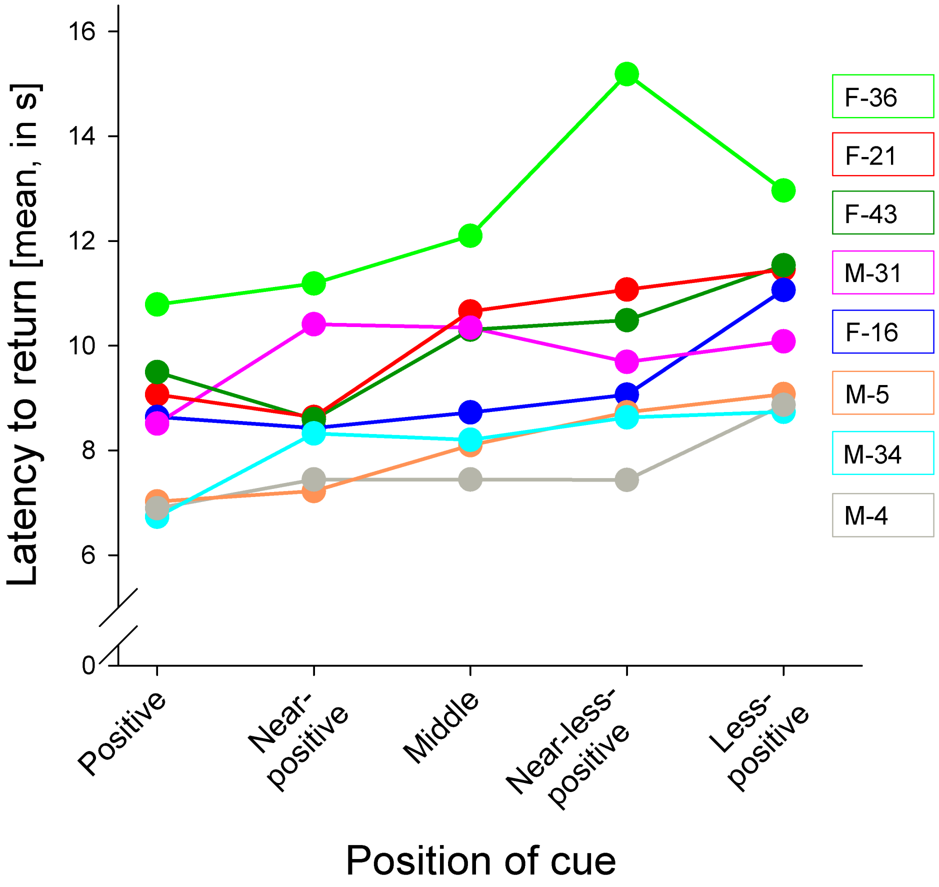

Fig. 8 *Individual dolphins' absolute latencies (in s) to return from the conditioned (Positive and Less-positive) and ambiguous (Near-positive, Middle, Near-less-positive) cues. Labels on the right hand side indicate the sex (M/F) and age of each of the 8 dolphins. When all animals' data were averaged, the latency to return from the Positive versus the Less-positive cue was 2.0 seconds (min: 1.5 s, max: 2.4 s) (Clegg et al., 2017a), demonstrating that the dolphins had continued to differentiate between the two conditioned cues.*

Association between anticipatory behavior and ambiguous cue return latencies

There was a positive, significant correlation between anticipatory behavior frequency and the relative return latency from the Near-positive cue (linear regression with 10,000 permutations: R^2 = 0.593, P = 0.034; Fig. 9a), and a similar, although non-significant, tendency with respect to return latency from the Middle cue (R^2 = 0.410, P = 0.087; Fig. 9b). Those animals performing more anticipatory behavior also returned slower from the ambiguous cues i.e. made more pessimistic decisions. However, there was no significant correlation between anticipatory behavior frequency and return latency from the Near-negative cue (R^2 = 0.023, P = 0.749).

There were no significant differences between males and females with respect to the frequency of anticipatory behavior, nor their return latencies from the Near-positive, Middle or Near-less-positive cues (all $P > 0.05$).

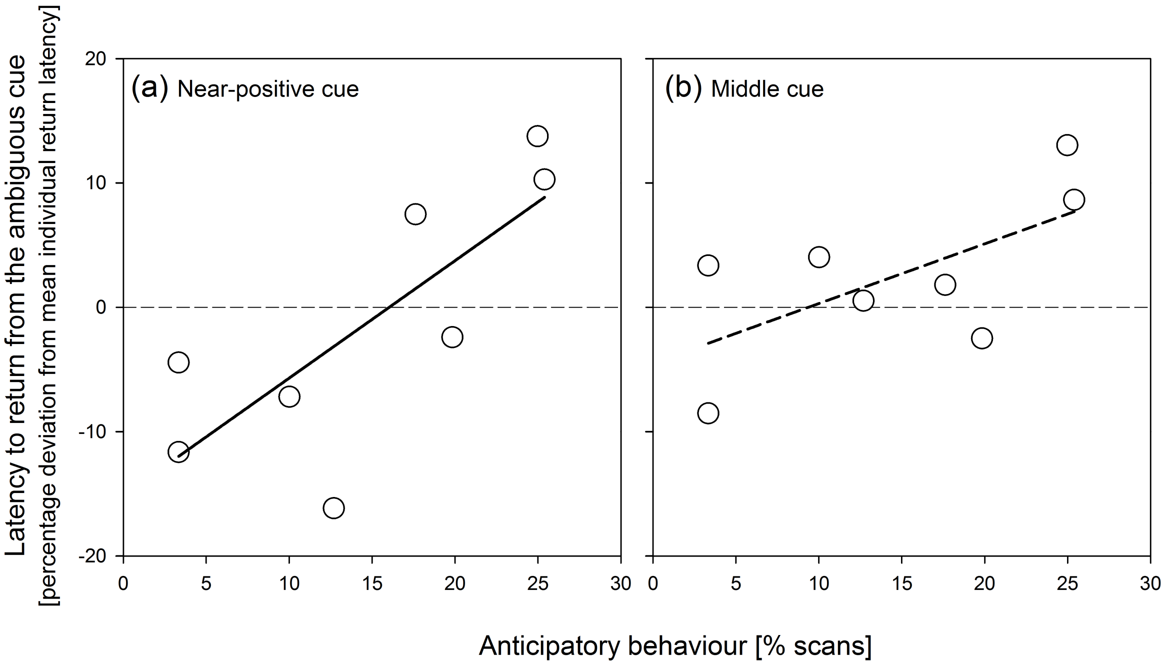

Fig. 9 *Cognitive bias test results predicted by anticipatory behavior. (a) The dolphins' relative return latencies to the Near-positive cue were predicted by the frequency of anticipatory behavior toward the training sessions during the 3-day test period: those slowest to return (pessimistic-like judgement) also showed significantly highest frequencies of anticipatory behavior, and this same relationship was present (b) with respect to the Middle cue, although the association was a statistical tendency only. Note that high and positive y-axis values correspond to an optimistic-like judgement,*

and the lower and negative to pessimistic-like judgements. See text for details on statistics. Anticipatory behavior in relation to upcoming training sessions was the combined frequencies of two behaviors, surface-looking and spy-hopping (see Table 4 for definitions).

Discussion

We found that the frequency of anticipatory behavior towards upcoming training sessions predicted cognitive bias in bottlenose dolphins. Dolphins showing the higher frequencies of anticipatory behavior also made more pessimistic judgements in relation to the Near-positive ambiguous cue, and tended towards the same association for the Middle cue.

Our findings are the first to provide evidence for a link between anticipatory behavior and cognitive bias in any species, both of which have been proposed as tools to measure animals' affective states and thus having implications for welfare (Mendl et al., 2009; van der Harst and Spruijt, 2007). A recent study with laying hens predicted that results of cognitive bias and anticipatory behavior tests would be related, but were not able to prove this (Wichman et al., 2012). Here, increased anticipatory behavior was associated with longer return times i.e. more pessimistic judgements: significantly for the Near-positive ambiguous cue, a tendency for the Middle cue, but not for the Near-less-positive position. This discrepancy between cues is congruous to previous cognitive bias animal studies, where focus is placed on any significant result found among the ambiguous cues: the difference between results from each ambiguous cue is thus far unclear (Mendl et al., 2009; Salmeto et al., 2011).

The direction of our results agrees with our hypothesis: those dolphins that anticipated the training sessions more also had more pessimistic cognitive biases, which have been associated with negative affective states in many species (Baciadonna and McElligott, 2015; Mendl et al., 2009; Roelofs et al., 2016). According to the reward-sensitivity theory, the dolphins experiencing more negative affective states (as indicated by the pessimistic bias) increase their anticipatory behavior for positive events because they place a higher value on the reward to come, i.e. they desire it more, perhaps due to a lack of other positive events in their environment (Spruijt et al., 2001). Conversely, the dolphins that made more optimistic judgements may have been in more positive affective states, and thus were less sensitive to the upcoming reward and anticipated it less. However, as discussed in the previous paper on this dataset (Clegg et al., 2017a), the design of the study means that causal relationships between the behaviors and affective states represented by the cognitive bias results can not be confirmed. Another alternative hypothesis is that even though the literature regarding dolphins and other species suggests otherwise, it could be that the dolphins judging pessimistically and who were potentially in more negative affective states in fact anticipated and viewed the feeding/training sessions as negative events (Frohoff and Packard, 1995) (but note that the reward-sensitivity theory does not apply to negative events, Spruijt et al. 2001). Although anticipatory behavior of captive dolphins is still a very new research area, current evidence suggests that we might reject this alternative hypothesis: firstly, it has been shown with other species that the type of anticipatory behavior differs in response to upcoming positive *versus* negative events (e.g. Moe et al. 2006; Zimmerman et al. 2011). Therefore if some dolphins were anticipating the training sessions in a negative light, we would not have likely seen such a convincing and significant association since the anticipatory behaviors measured would have likely been different types (e.g. those with links to fear or frustration, as with hens, Zimmerman et al., 2011). Finally, there is some evidence from measures of physiological stress during or after training/show/guest interaction sessions: the breathing rate (St. Aubin and Dierauf, 2001) of bottlenose dolphins did not differ between the periods before and after show sessions (Jensen et al., 2013), and three stress hormones of belugas (*Delphinapterus leucas*) did not change from baseline levels during guest interaction sessions (Schmitt et al., 2010).

The anticipatory behaviors of captive dolphins studied here, surface looking and spy-hopping, are direct products of the environment and management of humans since the animals are using cues to prepare for an event that will occur in the near future (Jensen et al., 2013). Therefore, it is hard to make comparisons with any potential anticipatory behaviors in wild dolphin populations, since the context, cues and rewards are completely different. Further investigations with controlled variation of these components would help us to better understand anticipatory behaviors, as well as for example basic experiments with captive animals to discover whether increased anticipatory behavior means the animal is more motivated for the reward i.e. do anticipation levels predict behavior during the reward's acquisition? It could be that animals that strongly anticipate a positive reward, but once it arrives are not motivated to exploit it, are in poorer welfare than animals who highly anticipate the reward and then actively exploit it (perhaps similar to a stereotypy, since there would appear to be no function to the behavior, Mason and Rushen, 2008). Our study has shed some light on the frequencies of anticipatory behavior in captive dolphins which might be linked to positive and negative affective states, and thus could start to fill in the gaps for this species (i.e. thresholds on the proposed models: van der Harst and Spruijt, 2007; Watters, 2014). However our work is only the first step and used a moderate sample size of animals: more work is needed on multiple groups to uncover the real significance and utility of anticipatory behavior measurement in captive bottlenose dolphins.

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Compliance with Ethical Standards

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The experimental protocol followed the ASAB/ABS Guidelines for the Use of Animals in Research (Association for the Study of Animal Behaviour, 2012), and was accepted by the scientific and animal welfare committees of Parc Astérix.

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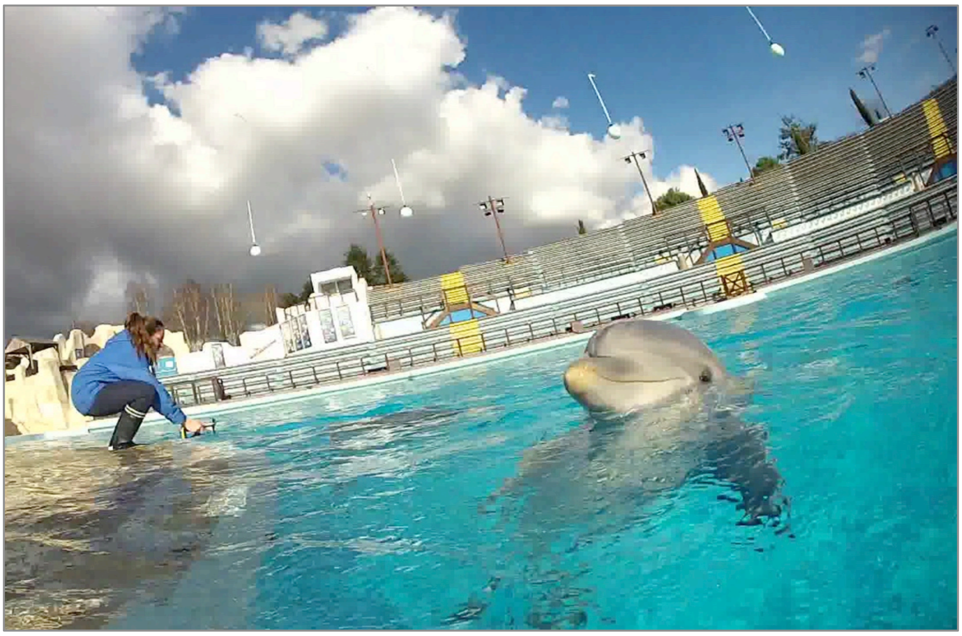
Conflict of Interest. Isabella Clegg and Fabienne Delfour declare that they have no conflict of interest.



Chapter 4
Further investigation into anticipatory behaviour

Chapter 4: Further investigation into anticipatory behaviour

Paper 5: Looking forward to interacting with familiar humans: dolphins' anticipatory behaviour indicates their motivation to participate in specific events.

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Résumé

Le comportement anticipatoire décrit les actions prises pour se préparer à un événement à venir. Des grands dauphins (*Tursiops truncatus*) en captivité montrent des comportements anticipatoires avant des sessions alimentaires, mais nous ignorons s'ils vont anticiper des événements non-alimentaires. En outre, il n'y a aucune information publiée disponible sur n'importe quelle espèce pour savoir si le niveau de comportement anticipatoire est lié à la participation réelle d'un animal dans l'événement suivant ou à la récompense : répondre à cette question nous aiderait à comprendre ce comportement et les états affectifs qui lui sont liés. Dans cette étude, nous avons utilisé des signaux sonores pour conditionner des dauphins à l'arrivée de jouets dans leur bassin ou d'une Interaction Humain-Animal positive (HAI) avec un soigneur familier et nous avons mesuré leur comportement anticipatoire avant chaque événement. Le protocole a été validé lorsque les dauphins ont montré significativement plus de comportement anticipatoire avant l'arrivée des jouets et de l'HAI que lors dune situation contrôle, des fréquences accrues de 'surface-looking' et 'spy-hopping' ont été mesurées. En outre, nous avons trouvé que les dauphins ont montré plus de comportement anticipatoire avant l'HAI que lors du contexte de jouets. Dans la deuxième partie de l'étude, le comportement anticipatoire plus élévé avant l'introduction de jouets, l'HAI et des sessions alimentaires étaient significativement corrélées aux niveaux les plus hauts de participation à l'évènement lui-même (mesuré par le temps passé avec des humains/jouets et le nombre de fois que les dauphins ont quitté les soigneurs pendant des sessions alimentaires). Nos résultats suggèrent que les jouets et l'HAI ont été perçus comme des événements positifs par les dauphins, et nous proposons que des interactions humaines non-alimentaires jouent un rôle important dans la vie de ces animaux. Nous apportons aussi la première preuve empirique que le comportement anticipatoire est corrélé au niveau de participation à l'événement suivant, soutenant que le comportement anticipatoire est une mesure de motivation, et nous espérons que ce résultat stimulera de nouveaux travaux sur l'utilisation de ce comportement pour évaluer et améliorer le bien-être animal.

Abstract

Anticipatory behaviour describes the actions taken to prepare for an upcoming event. Bottlenose dolphins (*Tursiops truncatus*) in captivity are known to display anticipatory behaviours before feeding sessions, but it is unknown whether they would anticipate non-alimentary events. Furthermore, there is no published information available for any species on whether the level of anticipatory behaviour is predictive of an animal's actual participation in the following event or reward: answering this question would bring us closer to understanding this behaviour and its related affective states. In this study, we used sound cues to condition dolphins to the arrival of toys in their pool or a positive Human-Animal Interaction (HAI) with a familiar trainer, and measured their anticipatory behaviour before each event. The protocol was validated since the dolphins performed significantly more anticipatory behaviour before the toys and HAI contexts than a control situation, by means of increased frequencies of surface looking and spy hopping. Furthermore, we found that dolphins showed more anticipatory behaviour before the HAI than the toys context. In the second part of the investigation, higher anticipatory behaviour before toy provision, HAIs, and feeding sessions was significantly correlated to higher levels of participation in the event itself (measured by time spent with humans/toys, and number of times dolphins left during feeding sessions). Our results suggest that toys and HAIs were perceived as rewarding events, and we propose that non-food human interactions play an important role in these animals' lives. We also provide the first empirical evidence that anticipatory behaviour is correlated to the level of participation in the following event, supporting anticipatory behaviour as a measure of motivation, and hope that this stimulates further work regarding the use of this behaviour to assess and improve animal welfare.

Keywords: animal welfare, anticipatory behaviour, Bottlenose dolphins, enrichment, human-animal interactions; positive reinforcement training

1.Introduction

Anticipatory behaviour performed in expectation of predictable events is well-documented in wild and captive animals, and consists of behavioural patterns that prepare the animal for the upcoming situation (Spruijt et al., 2001). Such anticipatory behaviours often manifest as increased activity, vigilance, and/or behavioural transitions (Makowska and Weary, 2016; van den Bos et al., 2003; van der Harst et al., 2003a). Studies on anticipatory behaviour have revealed links with captive animals' affective states, and thus suggest implications for welfare (see reviews by Van der Harst and Spruijt, 2007; Watters, 2014). However, although resource provision in captive environments is often highly predictable and thus anticipatory behaviour is nearly always present and obvious (Waitt and Buchanan-Smith, 2001; Watters, 2014), there is still a lack of knowledge on how anticipatory behaviour reflects animals' motivational and affective states.

Anticipatory behaviour towards a positive event is adaptive since it is associated with the motivational system that directs the animal from an aversive state (e.g. hungry) to a reinforcing state (e.g. food acquisition; see Spruijt et al. 2001, for full explanation). Animals use a variety of environmental cues to predict when rewards will become available and thus make a contingent associations between the cue and following event (Anderson et al., 2015): such contingencies can also be experimentally induced using the Pavlovian paradigm to measure anticipatory behaviour. The link between this behaviour and affective states is not linear however, since anticipation levels have been found to vary with reward sensitivity. Again, this is adaptive: a more food-deprived animal is in a more negative affective state where the reward (food) will have a higher value, and thus more anticipatory behaviour is performed (termed "incentive value" by Spruijt et al., 2001).

Thus far, anticipatory behaviour has principally been used to test whether an animal perceives a certain predictable event as a reward (or not), and what the current affective state is likely to be based on the anticipatory behaviour frequency (van der Harst and Spruijt, 2007). Anticipatory behaviour before food acquisition has been extensively studied and shown to be robust and stable over multiple cycles (Mistlberger, 2009; Storch and Weitz, 2009). Since anticipatory behaviour reflects a reward's value (Anderson et al., 2015; van der Harst et al., 2003b), studies have also used it to test relative values placed on certain non-alimentary events, in multiple species: for example, laboratory rats (*Rattus norvegicus*) anticipated access to sexual contact (van der Harst et al., 2003b); laying hens (*Gallus gallus domesticus*) valued a dusty substrate more than a food reward (McGrath et al., 2016); and lambs

(*Ovis aries*) anticipated opportunities to play (Anderson et al., 2015). Environmental enrichment seems to be a notable context that stimulates anticipation in various species (e.g. McGrath et al., 2016; van der Harst et al., 2003b), and such studies are applicable to improving welfare as they reveal what the animal “wants” most in its environment (Dawkins, 2006). A few past studies with rats have successfully shown that anticipatory behaviour can predict affective states: animals in more enriched cages conducted less anticipatory behaviour than those in standard cages (Makowska and Weary, 2016; van der Harst et al., 2003a), and those who experienced social isolation anticipated food rewards and social contact significantly more than group-housed conspecifics (van den Berg et al., 1999).

However, these are the first studies using anticipatory behaviour as a measure of affective state and there are still many unknowns (Watters, 2014). There seems to have been a slight overlook in regards to what this behaviour is representing, and we suggest that a much needed line of research is whether the levels of anticipation actually correspond to the animal’s participation in the reward once it has access to it i.e. the consummation of the anticipated goal (Watters, 2014). For example, are the animals that show much anticipatory behaviour for enrichment provision also those that interact with the enrichment the most? A few studies have presented incidental data suggesting that this might be the case: for example some anticipatory behaviours were correlated in lambs with subsequent play behaviour (but only at some points of the test, Anderson et al., 2015), and in laying hens with the latency to approach the reward (McGrath et al., 2016). However this question has not yet been directly posed in such studies, and discovering whether frequency of anticipatory behaviour predicts interaction with the reward would surely be a step towards revealing the function of these behaviours.

Bottlenose dolphins (*Tursiops truncatus*) have been shown to display anticipatory behaviour in response to training sessions during which they receive their food (Clegg et al., 2017b; Jensen et al., 2013). Apart from food provision, there are other events occurring in the captive environment which are thought to be rewarding for dolphins, but for which there are only a few studies: all we know is that the animals will voluntarily interact with toys and other enrichment items and so seem to view them positively on the whole (Clark, 2013; Delfour and Beyer, 2012; Kuczaj et al., 2002). However, we have little to no knowledge on dolphins’ actual motivation or “want” for non-food enrichment events, and the literature suggests that measuring anticipatory behaviour could help in answering this question, which is of interest to researchers and managers of zoo collections alike (Watters, 2014). It has been suggested that positive Human-Animal Interactions (HAIs), e.g. those involving stroking, play, voluntary contact, might also be rewarding for dolphins like they are for other species (Perelberg and Schuster, 2009). Positive HAIs in domesticated species generally incite affiliation, have calming effects, and stimulate approach behaviour (e.g. Coulon et al., 2015; Handlin et al., 2011). Even for non-domesticated animals, positive HAIs can reduce stress (Hosey and Melfi, 2014; Whitham and Wielebnowski, 2013), represent gratifying events (Claxton, 2011; Hosey, 2008) and develop into strong, welfare-enhancing bonds (Hosey and Melfi, 2010). Wild and captive dolphins frequently engage in intra-specific tactile and play behaviour (Dudzinski et al., 2012; Kuczaj et al., 2013), which could tangibly be translated to inter-specific relations (Perelberg and Schuster, 2009), and case-level evidence suggests they can view humans as play partners or objects (review in Paulos et al., 2010). Mixed results from studies on dolphins' reactions to swim or touch interactions with unfamiliar guests suggest these are viewed both positively and negatively (Frohoff and Packard, 1995; Kyngdon et al., 2003; Trone et al., 2005). However, zoo animal research suggests that they significantly prefer interactions with familiar humans (Martin and Melfi, 2016; Melfi and Thomas, 2005; Mitchell et al., 1991). In the only study on HAIs with familiar humans and captive cetaceans, it was shown that outside of food-related training sessions bottlenose dolphins voluntarily chose to receive petting from their trainers in seemingly positive interactions (Perelberg and Schuster, 2009). However, no studies have used anticipatory behaviour measurement as a means to determine the value that dolphins or other species place on HAIs with familiar humans. Only one related study has been published previously, using case studies with two individual zoo animals, where anticipatory behaviour increased before predictable visual HAIs (no contact) with an unfamiliar human (Krebs et al., 2017).

The lack of knowledge regarding supposed positive events for captive dolphins in particular, coupled with the potential for anticipatory behaviour to answer this, stimulated a study to be conducted on the bottlenose dolphins at Parc Astérix (Plailly, France). Two main questions were established: 1) Does the dolphins' anticipatory behaviour differ in relation to predictable upcoming contexts: the provision of toys, an HAI with a familiar person, versus a control context? To test this, these events would be paired with different predictor cues signalling a certain event will occur; anticipatory behaviour duration in the period after the cues would then be measured. We predicted that dolphins would anticipate the toys and HAI events significantly more that the control context, and that they might anticipate toys and

HAIs similarly. The second question addressed an as yet unanswered element of anticipatory behaviour research: 2) Is the duration of anticipatory behaviour before an event correlated with dolphins' level of participation in the event itself? To answer this, anticipatory behaviour before the event would be correlated with measures of interaction during it i.e. with the toys or familiar humans, and also in a third context of food provision within positive reinforcement training sessions. Based on the literature discussed above the provision of food, toys, and positive HAIs were thought to be rewarding events for the dolphins, although no *a priori* assumptions were made which might have influenced our experimental design. We predicted that animals who anticipated the signalled events the most would also participate the most during the event itself: if confirmed, these results could validate dolphins' anticipatory behaviour as a reflection of the intrinsic value they place on aspects of their environment.

2. Material and methods

2.1 Study animals and their daily routine

Our study was conducted at Parc Astérix on a group of seven Atlantic bottlenose dolphins (*Tursiops truncatus*), who were living in an outdoor pool joined to two indoor pools, with a total volume of 3790 m^3 of water with continuous free access between pools. The study subjects ranged in age from 1.5 to 43 years old and consisted of 5 females: 4 adults and 1 juvenile, and 2 males: both adults (11 years or over, age classification taken from Smolker et al., 1992). Not all animals were related, and three were wild caught (the oldest) and four were captive born. The park was closed to the public for the duration of the experiment. The animals' daily routine was structured through multiple "training sessions" where they received their food on a positive reinforcement basis (see Laule et al., 2003, for detailed explanation), supplemented by free feeds as first and last sessions of the day (Table 5a). At least once a day but more often 2 or 3 times (outside of this experiment's implementations), both enrichment items and HAIs would be offered to the animals in their "free-time" between training sessions. The enrichment was provided three times a day (morning, lunch-time period, and afternoon; Table 5a), using a random selection from a list of about 40 objects ranging in size, material and buoyancy, and could be combined with water jets and hoses. HAIs occurring outside this study's protocol were not planned as such: at random times throughout the day the dolphin trainers would approach and sit/stand by the side of the pool and interact with the dolphins who decide to approach. These spontaneous HAIs consisted of strokes, cuddles, playing with water and toys, running back and forth, with the trainers using their hands, feet and voices.

Table 5 a) *General daily schedule for the dolphins when the study was not taking place and* **b)** *daily schedule of the dolphins during the study, showing times when data were taken in relation to Question 1: Does the dolphins' anticipatory behaviour differ in relation to predictable upcoming contexts, and Question 2: Is the duration of anticipatory behaviour before an event correlated with dolphins' level of participation in the event itself. All data for both questions were taken between December 2016 and February 2017.*

Time	**a)** General daily schedule **OUTSIDE** of study period		**b)** General daily schedule **DURING** the study period		*Type of data taken for Question 1 & 2 (**Q1, Q2**)*
09h00	Free-feed 1	*	Free-feed 1	*	*For **Q2,** before and during **randomly-selected** training sessions (not free-feeds)**:** anticipatory behaviour and participation behaviour measured*
10h00	Training 1	ENRICHMENT	Training 1	ENRICHMENT	
11h00	Training 2	*	Training 2	*	
12h00	Training 3		Training 3		
13h00		ENRICHMENT		ENRICHMENT	
14h00	Training 4	*	Training 4	14h45 : Sound cue, then Control/Toys/ HAI context	***14h45**, for **Q1** and **Q2 :** anticipatory behaviour frequency measured after sound cue played. For **Q2** : participation behaviour measured during context*
15h00		ENRICHMENT			
16h00	Training 5	*	Training 5		
17h00	Free-feed 2		Free-feed 2	*	

*represents spontaneous, non-food Trainer-Dolphin Interactions, which occurred at random points of the day apart from in the 30 min before and after the sound cue and context trials at 14h45 daily.

2.2 Question 1: differential anticipation of Control, Toy and HAI contexts?

2.2.1 The cues (Unconditioned Stimuli, US)

In order to measure the dolphins' anticipation for the provision of toys and the opportunity for an HAI, we needed to pair these contexts with cues that reliably signalled their imminent arrival. We also needed to include a Control context where a cue would signal the arrival of nothing i.e. no change in the environment, in order for us to verify that it was not simply the cues themselves that were causing anticipatory behaviour. We chose to use sounds as the principal cues because this is a predominant and highly salient sense for dolphins (Janik, 2009), and included visual cues as supplementary signals to be sure that the animals could distinguish the upcoming context, in case they had for some reason not perceived the sound cue (for example if they were engaged in active play or aggression at the time it was played, or their head was out of the water). During the experiment, the dolphins would have free access between the inside and outside pools, and sound cues would ensure that even if they happened to be inside and out of sight of the area where the cues and contexts were to be provided (the main beach area of the outside pool), they would still be able to hear the cues each time they were played.

Three different sounds were needed (to pair with the three contexts) which had not been heard before by the dolphins, and we synthesised them using AVID Pro Tools software (Avid Technology, Inc., Burlington, MA, USA). In order to create unique, non-aversive sounds which were not completely counter-intuitive to the dolphins, we referred to the literature: the acoustic repertoire of *Tursiops truncatus* is characterised by frequency-modulated vocalisations (Janik, 2009), and studies have shown these animals are able to successfully pair artificial frequency-modulated sounds with objects (Reiss and McCowan, 1993; Richards et al., 1984). The three sounds created for this experiment (Fig. 10) were narrow-bandwidth, frequency-modulated signals, each having a duration of 2s, where the frequency was kept well-within reported limits of this species (Janik, 2009). The sounds were played to the animals using a Lubell Lab underwater speaker (Lubell Labs Inc., Columbus OH, USA; LL916C, frequency response: 200 Hz–20 kHz) connected to a TAG Premio 8 (Techniques Audio Groupe, France) via an AC203 transformer box, with the sound files being played from an iPhone SE (Apple Inc., Cupertino, CA, USA). The underwater speaker was held at 50cm below the water's surface at the edge of the pool (always in the same place on the main beach area), and each sound was repeated three times with a 1s pause in-between so that the total sequence for each sound type was around 8s in duration. In a preliminary test, using hydrophones, we verified that when the sound was played from the outside pool beach area, it was perceivable at all points of the inside pools.

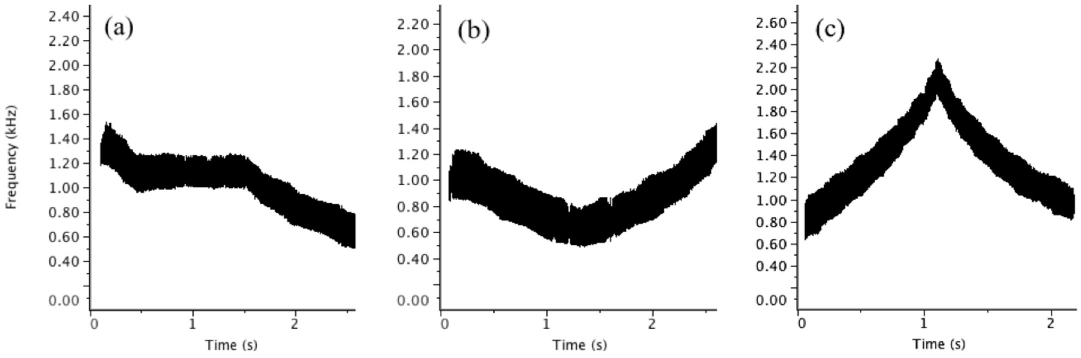

Fig. 10 *Spectrograms (Raven Lite 1.0, Cornell University, NY, USA) of the three sound cues used to announce the three different contexts which were presented after a 5 min delay;* **(a)** *was the sound for the Control context,* **(b)** *indicated the arrival of toys in the pool, and* **(c)** *signalled an Human-Animal Interaction (HAI). Recordings of these sounds were made using a CRT hydrophone C54XRS (frequency response: 0.016–44 kHz ± 3 dB) plugged into a TASCAM HDP2 recorder (acquisition rate: 96 kHz; samples coded on 24 bits). Fast Fourier transformation = 1.024, Hanning window, overlap = 50%.*

In order to teach the dolphins that each sound signalled the arrival of a certain context, Pavlov's classical conditioning paradigm was used, as in previous anticipatory behaviour studies (e.g. van der Harst et al., 2003a; Wichman et al., 2012). Before any conditioning occurred, the Sound Pressure Level (SPL) of the sounds emitted was verified in case it might be aversive to the dolphins, who had never experienced underwater playback experiments before. Based on established hearing thresholds for *Tursiops truncatus* (Johnson, 1967), and the reported SPLs of dolphin echolocation and whistling (Janik, 2009), the SPL of the sounds played from the underwater speakers was set at 130 dB re 1µPa. A few habituation trials were conducted to get the dolphins used to the speaker playing sounds (using those of a similar type but not the same as the test sounds), after which there were no aversive or avoidance behaviours seen. Lastly, for each context and in addition to the sound cues, we also placed a visual cue- a laminated piece of coloured cardboard- vertically on the main beach area which remained visible to the dolphins for the duration of each trial. For the Control context the cardboard positioned on the beach was 100% black, for the Toys it was 100% white, and for the HAI it was mid-grey (50% black) with white diagonal stripes. In the following methods description it should be assumed that references to presentation of "sound cues" refers simultaneously to these supplementary visual cues.

2.2.2 The contexts (Conditioned Stimuli, CS)

For all trials (presentation of sound cues followed by a context) in each stage of the experiment, the context always occurred for a duration of 10 min, after which it was fully removed/terminated from the animals' environment.

A trial with the 'Toys' context consisted of the addition of seven non-novel, floating objects which differed in size, shape and texture. This was a slightly more limited selection of enrichment than the dolphins received in their normal daily schedule (Table 5a) i.e. no sinking objects or water jets. Toys had to be floating to allow accurate measurement of the interaction duration times, and all toys were removed from the pool at the end of the trial. Furthermore, a recent study on the same group of dolphins has shown that dolphins played more with simple-floating, as opposed to complex-sinking, objects (Delfour et al., 2017). The seven toys were selected in a pseudo-random fashion from a list of 40 where the same toy could not be selected for more than three consecutive days, in order to avoid a loss of interest in the toys for the dolphins.

The HAI context was defined as a familiar trainer (worked with the dolphins for ≥ 2 years, n = 7) approaching the pool side and playing with the dolphins. This was an adaptation of the farm animal Approach-Avoidance tests used to measure Human-Animal Relationships (Waiblinger et al., 2006), and suggested previously for assessing dolphin welfare (Clegg et al., 2015). A consensus-taking session with all participating trainers occurred prior to the experiment in order to determine a number of common elements of a Trainer-Dolphin interaction which they believed the dolphins seemed to enjoy the most. Through a system of voting rounds, it was decided that during the HAI context trials the trainers could interact with the dolphins using some or all of the following five elements: eye contact; strokes and rubs; use of the voice; use of hands and feet (but not full body) in water for tactile interactions; moving around in a follow/chase-me type of game. We standardised the HAIs to a certain level since trainers were not permitted: to leave the poolside of the main beach area (arc with circumference of around 10m); to fully enter the water; to use any hand signals resembling show cues; to use any toys, ice-cubes or other objects to play with the dolphins; to call the dolphins to the beach, for example by slapping the water. Therefore in any given HAI trial, the trainer would present themselves at the pool's side and interact with whichever dolphins decided to approach them, using the five pre-determined elements and refraining from any of the prohibited actions; at the end of the trial, they would simply leave the poolside and move out of sight. The trainer who would take part in each trial was selected pseudo-randomly in that they could not be chosen for more than two consecutive trials.

A Control context trial entailed the sound cue being played and then nothing in the environment changing. Throughout the experiment for all of the contexts, no external events were allowed to take place around the poolside, including but not limited to: staff conducting cleaning, maintenance work, divers cleaning underwater, or any other toys or water jets in the pool.

2.2.3 Measurement of anticipatory behaviour (Conditioned Response, CR)

Using previous studies documenting anticipatory behaviour, we defined the two main behaviours thought to indicate dolphins' anticipation of a positive event (Table 6). Anticipatory behaviour was the CR in the Pavlovian paradigm, and thus was measured during the time after the sound cue (CS) was played and up until the selected context (US) was presented. Video footage was taken with a GoPro® HERO 4 (GoPro, CA, USA) secured to a vantage point 5m above the surface of the water where all animals in the pool could be seen (apart from when they were in the inside pool). Employing focal individual sampling, the duration (in s) of the two defined behaviours was measured using the video footage and the response variable was the cumulative durations as a percentage of visible time.

Table 6 *Behavioural repertoire used for anticipatory behaviour data collection (adapted from Clegg et al., 2017b; Jensen et al., 2013).*

Anticipatory behaviour	Description
Surface look	The dolphin's head is out of the water while swimming, or head is held fixed while floating stationary, and eyes are directed towards a point above the surface. A surface look can be distinguished from simply taking a breath by the fact the head may be momentarily suspended above the water, where the eyes are clearly above the surface line looking towards the area where the predictable event usually commences.
Spy hop	The dolphin propels itself vertically out of the water with the eyes directed to a point above the water's surface, usually as far as the pectoral fins, and then descends vertically. Often repeats this several times consecutively.

2.2.4 Sequence of trials

Learning phase: Pairing sound cues (US) to contexts (CS)

The learning phase took place over four weeks (November-December 2016) with two trials (sound cue and presentation of context) at set times every weekday.

The order of trials was a fixed consecutive pattern of the three contexts (Control, Toys and HAI). In the trials at the start of this phase, the sound cues were played and the context was presented immediately afterwards with no delay. Then, in small increments and over 34 trials, the time delay was increased gradually from 0 s to 5 min (similar to previous anticipatory behaviour study protocols, e.g. Wichman et al., 2012). Once this stage was reached, a set of trials (*n* = 9, three repeats of each context) was conducted where the sound was played and 5 min later the context was presented, and additionally the anticipatory behaviour was measured during the 5 min intermediate period. This data showed that on average over all individuals and for the three repeats, the dolphins were performing anticipatory behaviour for twice as long in the 5 min before the Toys and HAI context as before the Control context. This provided the evidence that they had successfully made the association between the CS and US, and thus the experiment advanced to the data collection phase.

Data collection phase: Measuring the anticipatory behaviour (Conditioned Response, CR)

The data collection phase ran from mid-December 2016 until February 2017, and comprised of one trial per day (*n* = 47; 16 Control trials, 15 Toys, 16 HAI). Trials were always conducted between 14:40 and 15:00 each day, since the previous training session finished at around 14:30 and the next did not start until 15:30, so this would reduce as much as possible the potential confounding effect of anticipatory behaviour occurrence in relation to the training sessions (see Table 5b). The delay between the sound cues and the arrival of the context remained fixed at 5 min, the context was always presented to the dolphins for 10 min, and the same person (IC) always played the sound cues and added the toys to the pool.

2.3 Question 2: link between anticipatory behaviour and participation in upcoming event?

2.3.1 Measuring the dolphins' anticipatory behaviour

Our goal was to compare an individual dolphin's anticipation with the extent that it participated in the event once it became available i.e. its level of interaction or motivation during the event. In order to cover a range of events, we used two non-food and one food situation: the animals being presented with Toys, the opportunity for an HAI, or receiving food rewards within a training session (hereafter referred to as "feeding session"). The first element needed to answer this question was data on the level of anticipatory behaviour before the event appeared. For the contexts represented by Toys and HAI, the protocol conducted for Question 1 (described in section 2.2.3) was also used here: Pavlovian conditioning trials allowed the anticipatory behaviour to be recorded after the sound cue and in the 5 minutes before the context arrived. Concerning the animals' anticipation of the feeding session context, inadvertent classical conditioning had taken place over several years due to the fixed feeding schedules and multiple cues signalling the start of sessions (common in zoo environments, Krebs et al., 2017). The dolphins were engaged in five feeding sessions each day during which they participated in medical training, show practice, novel behaviours, research tasks, and play sessions, all conducted by the trainers and using positive reinforcement techniques (as well as two "free-feeds" at start and finish of the day) (Table 5). During this study and for several years before, feeding sessions almost always began by the trainers walking out to the main beach area, with the start times never differing by more than 30 minutes, and the intervals in-between sessions varying very little. In addition, acoustic and visual cues (such as trainers preparing and setting up the food buckets) were easily perceptible to the dolphins, allowing them to predict the start time of the upcoming feeding session. This was confirmed by a previous study on anticipatory behaviour in this group of dolphins (Jensen et al., 2013), as well as in other facilities following similar fixed schedules (Clegg et al., 2017b; Miller et al., 2011b). Therefore, for this study, observations of anticipatory behaviour before feeding sessions were conducted 5 min before the session start. Observations were made in person (always IC) and the observer was elevated in the stands above the pool, hidden from the dolphins.

For all contexts in Question 2 (Toys, HAI and feeding sessions), our response variable for analysis was the same: focal individual sampling was used to measure the duration (in s) of the two principal anticipatory behaviours (Table 2) before summing them and calculating the duration as a percentage of observable time.

2.3.2 Measuring dolphins' participation in Toy and HAI contexts

Regarding the trials with the Toys and HAI reward contexts, the methodology and data collection was exactly the same as for Question 1, and is described throughout section 2.2 above. The only added element was that video footage (same equipment) was also taken during the 10 min that the context was presented (equipment and position detailed in 2.2) which enabled us to collect data on the animals' level of interaction with or interest in either the toys or during the HAI (Table 5b). The same response variable was used during both situations: the duration (in s) of the focal dolphin's interaction or time spent with the object/human, defined as not only physical contact but also when the animal was investigating and focussing its attention on the object/human (Neto et al., 2016) (Table 7).

Table 7 *Definitions of behaviours used for measurement of participation in the three events (adapted from Brando, 2010; Delfour and Beyer, 2012; Eskelinen et al., 2015).*

Anticipatory Behaviour	Measured during which context	Description
Interacting with object	Toys, HAI	Focal dolphin is within 1m of the toy or the human (the "object") and making almost constant eye contact with it. The animal may also be touching, rubbing, mouthing or vocalising in the direction of the object.
Session break	Feeding session	The dolphin voluntarily moves away more than 2m from the trainer, and leaves this area for more than 5 seconds. Such breaks are similarly defined when another dolphin is involved i.e. another animal may chase the focal away from the trainer, or the focal may leave the trainer to interact with another nearby animal.

2.3.3 Measuring dolphins' participation in feeding sessions

The dolphins in this study participated in positive reinforcement feeding sessions where they received fish after performing conditioned behaviours, and where there is no punishment or negative outcome for their leaving the trainer's presence (Laule et al., 2003). The trainer represents the primary reinforcement of food provision, as well as secondary reinforcers (e.g. offering play or rubs, Brando, 2010), and all trainers conducting feeding sessions during this study were experienced staff members and familiar to the animals. Thus we might assume that the decision of the dolphin to swim away from the trainer and the fish reflects that the animal is not motivated to access these reinforcements. This reluctance may be due to the dolphin feeling uncomfortable with the situation, bored, fearful, frustrated, more interested by other activities in the pool, or a combination of all of these (Brando, 2010). Therefore, as a measure of participation in feeding sessions, we observed how many times the focal dolphin took a "break" from the session i.e. it voluntarily swims away from the trainer, either as a solitary action or in response to other dolphins' social behaviours (Table 7). This variable was calculated and used in analysis as the number of breaks per minute, in order to account for the slight variation in feeding session duration. Observations of feeding sessions were made daily, in person (always by IC), and the specific session to be observed (including the 5 minutes before to collect the anticipatory behaviour data) was chosen at random (Table 5b). These observations were conducted between December 2016 and February 2017.

2.4 Data analysis

Statistical analyses were done with R, version 3.1.1 (R Development Core Team, 2016). To test the hypothesis in our first question of whether the dolphins performed less anticipatory behaviour before the Control context than the Toys and HAI contexts, and whether the frequency differed between these latter situations, we constructed linear mixed effect models using the R package *nlme* (Pinheiro et al., 2016). *P*-values were calculated by Monte Carlo sampling with 1000 permutations, using the R package *pgirmess* (Giraudoux, 2016). Such permutation tests are particularly adequate when sample sizes are moderate and do not have any assumptions regarding normal distribution of residuals (Good, 2005). Homogeneity of variances was verified by plotting fitted values versus residuals (Faraway, 2006). The response variable was anticipatory behaviour duration as a percentage of visible time, and averaged per individual for each context. First, the three level factor of "Context" would be included in the model as a predictor of the duration of anticipatory behaviour. If this yielded a significant result, post-hoc tests consisting of pairwise linear mixed effect models (again, with permutation tests to extract *P*-values, 1000 runs) between the Control, Toys and HAI contexts would be conducted. All models included dolphin identity as a random factor and sex as a fixed factor. However, sex was never significant ($P > 0.10$) and thus was reduced from the models before these were recalculated.

Our second question asked whether the animals' participation in the upcoming, signalled event was predicted by the anticipatory behaviour duration in the period preceding access to it. First, we investigated whether the averaged values of anticipatory behaviour and the behaviours reflecting the level of participation (see sections 2.3.2 and 2.3.3) were correlated, using Spearman's rank correlation tests. We also tested for an association between anticipation and participation while taking into account intra-individual variation: for these two behaviours, the deviation from the individual means for each context was calculated, and used as variables in linear mixed effect models LMM (with permutations), with one model for each context where participation was measured (Toys, HAI and feeding sessions).

To enhance our analysis and thus conclusions about the dolphins' anticipatory behaviour, we also calculated the repeatability of the performance of this behaviour by each individual. Data on the frequency of anticipation in the 5 min before all four different types of contexts were used: Control, Toys, HAI, and training sessions. We used intra-class correlation based on a linear mixed effects model with permutation tests (package rptR, Nakagawa and Schielzeth, 2010), where we calculated the repeatability (R; based on 10,000 bootstrap runs) of the individuals' anticipatory behaviour (duration as a percentage of observable time), including sex and "Context" as additional factors.

3. Results

3.1. Comparison of anticipatory behaviour duration before the Control, Toys and HAIs

Anticipatory behaviour duration was significantly different between the 3 contexts (LMM with 1000 permutations: $P < 0.001$). Post hoc comparisons revealed that anticipatory behaviour duration before both the Toys and HAI contexts was significantly higher than before the Control context, and anticipation duration before the HAI was significantly higher than for before the Toys context (Fig. 11).

3.2. Repeatability of anticipatory behaviour

Anticipatory behaviour was significantly repeatable at the individual level across the three months of the study, indicating that individual dolphins showed a tendency to either display higher or lower levels of anticipation for upcoming events ($R = 0.192$, $P < 0.001$).

3.3. Association between anticipatory behaviour and participation in the upcoming event

There were no significant associations between the averaged values in individual anticipatory behaviour and the averaged individual participation in the provision of toys (Spearman rank: $r_s = 0.428$, $P = 0.354$), the HAI ($r_s = 0.200$, $P = 0.571$), and the feeding sessions ($r_s = -0.286$, $P = 0.556$). That is, inter-individual differences in anticipation did not explain inter-individual differences in participation.

However, when considering intra-individual variation across the different trials, we found significant and positive correlations between the anticipatory behaviour and subsequent participation in the Toys (LMM with 1000 permutations: $\beta = 0.216 \pm 0.100$ SE, $P = 0.039$; Fig. 12a) and HAI events ($\beta = 0.274 \pm 0.097$ SE, $P = 0.008$; Fig.

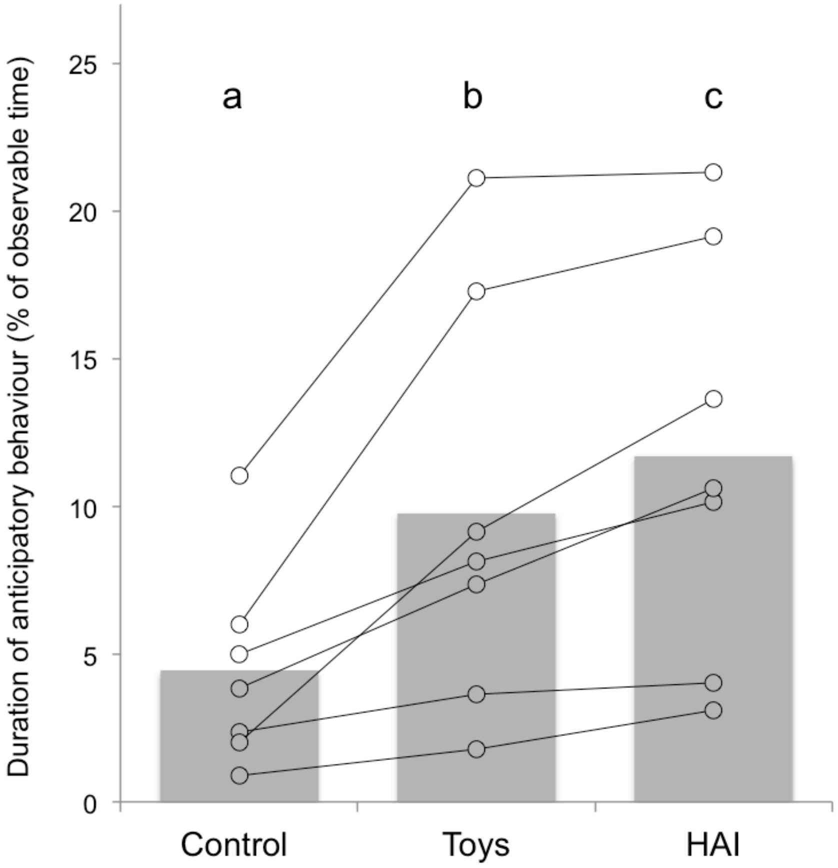

Fig. 11 *Duration of anticipatory behaviour shown by bottlenose dolphins ($n_{individuals}$ = 7) in the 5 min after a sound cue and before the presentation of one of three contexts: either a control, addition of toys to the pool, or a Human-Animal Interaction (HAI). Data points represent averages of all repeated tests for each individual. Grey bars indicate the average anticipatory behaviour per context over all dolphins. Different letters indicate significant differences between groups, tested by pairwise comparisons using LMM (with 1000 permutations) with Bonferroni correction; see text for details on statistics.*

12b). Correspondingly, there was a significant and negative correlation between the anticipation behaviour and the number of breaks (measure of participation) displayed during subsequent feeding sessions (β = –0.169 ± 0.080 SE, P = 0.045; Fig. 12c), where those dolphins showing more anticipatory behaviour also took less voluntary breaks during the following session. That is, intra-individual variation in anticipatory behaviour significantly explained the dolphins' day-to-day variation in their participation during the events.

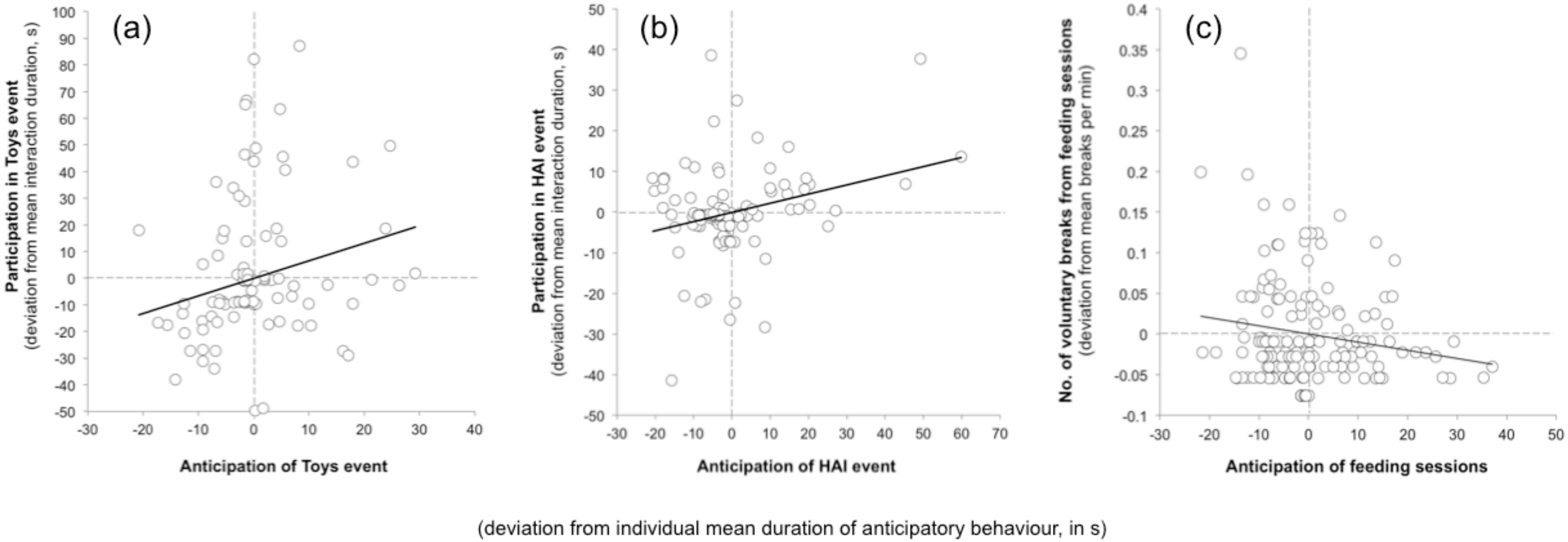

Fig. 12 *Anticipatory behaviour shown by bottlenose dolphins ($n_{individuals} = 7$) in relation to measures of participation in three types of upcoming event. For all events, the duration (in seconds) of anticipatory behaviour was measured and then deviations from individual means for each context were used as the predictor variable. (a) The first context consisted of the addition of toys to the pool ($n_{average\ trials\ per\ dolphin} = 14$) where participation was measured as the duration (s) of interaction with the toys (see Table 7 for definitions), and then deviation from individual means for each context was used as the response variable. (b) The second context tested was a Human-Animal Interaction (HAI) ($n_{average\ trials\ per\ dolphin} = 15$), where participation was measured in the same way as the Toys context. (c) The event in the third context was feeding sessions ($n_{average\ trials\ per\ dolphin} = 22$), with participation being measured as the number of voluntary breaks the animal took per minute (full definition in Table 7), which then was again calculated as deviation from individual means. Associations were tested using LMM (with 1000 permutations); see text for details on statistics.*

4. Discussion

Dolphins showed significantly more anticipatory behaviour before the opportunity for a non-alimentary HAI than before the arrival of toys (and before both contexts performed significantly and considerably more anticipatory behaviour than before the control situation). Furthermore, it was also found that the more anticipatory behaviour performed before the event, the more the dolphins subsequently participated in the event (in both food and non-food contexts).

4.1 Anticipatory behaviour reflects motivation to participate in event

We will first discuss our results showing that anticipation levels are correlated to subsequent participation, since this novel finding aids our later discussions on the dolphins' anticipation towards non-alimentary events. Perhaps due to the fact that most anticipatory behaviour research has been conducted in relation to food rewards (Storch and Weitz, 2009), no previous studies have expressly looked at whether an animal's anticipatory behaviour predicts motivation to exploit the reward/event once it arrives. Using the dolphin model, we were able to measure the animals' participation in two non-food (HAI and toy provision) and one food-related (feeding during positive reinforcement training session) event, and found that in all contexts anticipatory behaviour predicted participation. In addition this relationship was shown using intra-individual data, where even if the dolphins' anticipation and participation varied from day-to-day the correlation between the two variables remained present and significant. Our findings here are important because we can make inferences on the meaning of anticipatory behaviour: those dolphins performing the most anticipatory behaviour beforehand were also those who participated the most in the event, therefore suggesting that they were positively anticipating and placed intrinsic value on the three contexts tested (Spruijt et al., 2001). Anticipatory behaviour towards negative events also occurs (e.g. Moe et al., 2006) and this could be an alternative explanation for our results: however, if this was the case here and the dolphins viewed the events as negative, we might have seen high levels of anticipation but low participation. We therefore propose that the study's dolphins likely perceived non-food HAIs with familiar trainers, the arrival of toys, and feeding during positive reinforcement training sessions, as positive or rewarding events overall and that their anticipatory behaviour represented a measure of motivation for resources in the surrounding environment.

4.2 The dolphins' perception of enrichment and HAIs with familiar trainers

Firstly, our experimental protocol was validated in terms of the events being conditioned to the different cues: the average levels of anticipatory behaviour before both toy provision and HAIs was more than double when compared to the control context (10.7% *versus* 4.4% of observable time). Given that interacting with the toys and familiar trainers was an entirely voluntary choice for the dolphins in our experiment, and given that more anticipatory behaviour beforehand was correlated to higher participation in the event, we concluded that they viewed both toys and HAIs with familiar trainers as positive and rewarding: this is something that has not been demonstrated empirically before. Concerning environmental enrichment, it was not a surprise that the dolphins performed anticipatory behaviour before the provision of toys since the literature shows they interact willingly with toy items (e.g. Clark et al., 2013; Delfour and Beyer, 2012). However, although research shows domesticated animals likely view positive HAIs as rewarding (Schmied et al., 2008; Tallet et al., 2005; Waiblinger et al., 2006), very few studies have investigated non-domesticated species' motivation towards or enjoyment of interactions with humans (Baker, 2004; Carlstead, 2009). A single study on captive dolphins (*T. truncatus*) showed that they voluntarily approached familiar trainers to be rubbed, outside of food provision sessions (Perelberg and Schuster, 2009). Therefore, our results provide the first convincing evidence that a non-domesticated zoo species looks forward to and thus positively anticipates HAIs with familiar caretakers. Unlike many past HAIs studied in zoos (Krebs et al., 2017; Whitham and Wielebnowski, 2013), the HAIs studied here were "free contact" situations i.e. there were no barriers and the animal could contact the trainer however they liked (although for standardisation purposes trainers were never fully swimming in the water). The unique trainer-dolphin relationship may explain the animals' motivation to interact with their caretakers: relative to other zoo animals and their keepers, dolphins spend more time in proximity with the trainers since they participate in multiple daily training sessions (Clegg et al., 2017b; Hosey and Melfi, 2010) which often involve physical contact and working together to complete tasks (Brando et al., 2016). This habitual interaction in the animals' lives, as well as the fact that they conduct high levels of tactile behaviours solitarily and with conspecifics (e.g. Dudzinski et al., 2012), suggests that our results show the dolphins' motivation to conduct such affiliative inter-specific behaviours with the trainers. Finally, it could be argued that the dolphins automatically associate the trainers with food provision: however, given that the dolphins had experienced the type of non-food HAIs in our study for many years and which were very different from the context of feeding sessions, we believe it is unlikely that they interacted with trainers with the hope of acquiring food. Nevertheless, future studies might aim to disentangle the effects of food conditioning with affinity towards the caretaker, as has been conducted with lambs, *Ovis aries* (Boivin et al., 2000; Tallet et al., 2005).

There is much need for further research into the broader topic of anticipatory behaviour. This is well-demonstrated by our results on the repeatability of anticipatory behaviour, which has not yet been investigated in other studies. We found that dolphins' anticipatory behaviour is individually stable in the longer-term (over our 3-month study), but that it varies day-to-day and between contexts. Therefore if using as a measure of affective state/welfare, individual tendencies might need to be taken into account, and perhaps measures of variance of the behaviour should be favoured.

Implications and conclusions

The findings of this study have significant implications for the captive management of cetaceans and other species. As mentioned in many previous studies, in order for an environmental enrichment program to be effective in stimulating activity and positive behaviours, data must be collected on the animals' interactions with the objects and fed back to influence future protocols (Clegg et al., 2015; Delfour and Beyer, 2012). Our study suggests a valid tool- frequency of anticipatory behaviour- that can indicate the motivation levels for enrichment items. To use this tool, zoo facilities would simply condition animals to cues which signal varying forms of enrichment, and use the varying levels of anticipatory behaviour preceding the different enrichment types as a measure of their "want" for each one. Another, more direct implication of our study concerns the opportunity for non-food HAIs for captive cetaceans, which our results suggest are events that they look forward to and want to participate in. For example, the welfare impacts of the regulation passed by the US Department of Labor banning trainers at SeaWorld from swimming with killer whales, *Orcinus orca* (US Department of Labor, 2010) may need to be considered again in light of our results.

The anticipatory behaviour of dolphins has successfully revealed preferences of events provided to them: the dolphins performed more anticipatory behaviour before the opportunity for an HAI than the provision of toys to the pool. For the first time, it was been shown that an animal's anticipatory behaviour intensity is linked to subsequent participation in the upcoming event. Our results highlight the importance of enrichment programs and HAIs in zoos as drivers of positive welfare. There are many practical implications of these findings, but in general, anticipatory behaviour can be measured by zoo managers or scientists to closely monitor enrichment and

HAI protocols (as well as others) to verify the animals' motivation. Nonetheless, more investigations are needed on anticipatory behaviour in all species in order to understand its intra-individual stability, context-dependent variability, and the thresholds reflecting different affective states. Despite these many unknowns we hope our findings demonstrate the significant value of this behaviour both in welfare research and as an applied management tool, thus providing the impetus for future work.

Acknowledgements

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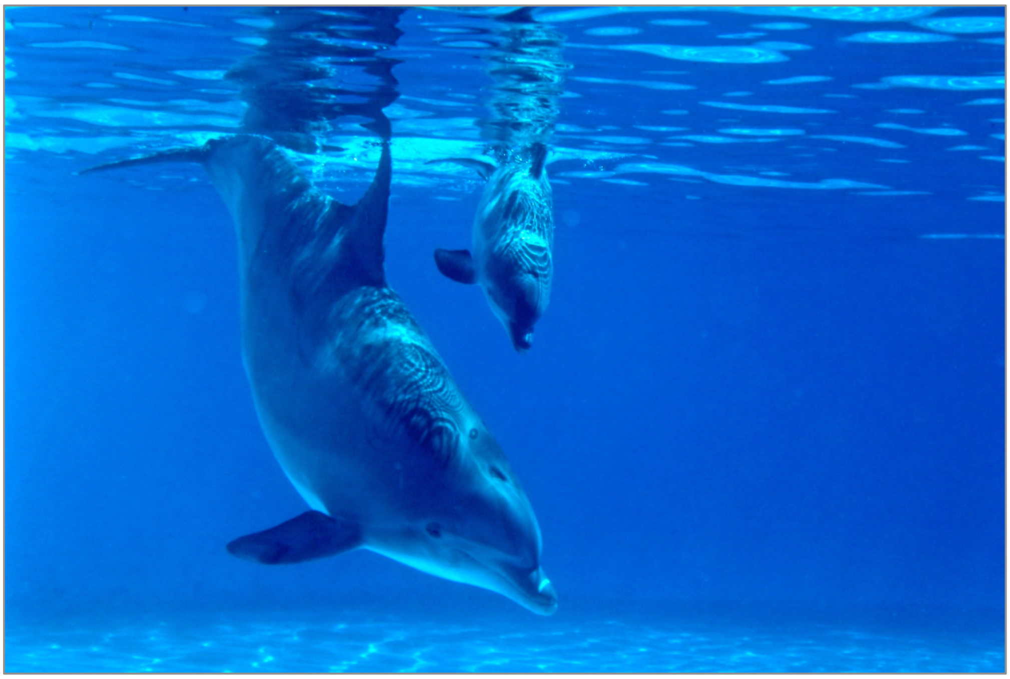
Compliance with ethical standards

Ethics. This study adhered to the ASAB/ABS Guidelines for the Use of Animals in Research (Association for the Study of Animal Behaviour, 2012), and was reviewed and accepted by the scientific and animal welfare committees of Parc Astérix. Our pre-established protocol dictated that if the dolphin showed signs of frustration or annoyance, the trial was stopped.

Competing interests. We have no competing interests.



Chapter 5

Behavioural and health measures within a practical tool

Chapter 5: Behavioural and health measures within a practical tool

Paper 6: Qualitative ratings of bottlenose dolphins' motivation during training sessions in five facilities: practical indicators of health-related and social welfare?

Study in data collection phase.

In collaboration with:

Parc Astérix (FR)

Planète Sauvage (FR)

Dolfinarium Harderwijk (NL)

Attica Park (GR)

Boudewijn Seapark (BE)

Rationale for the project

The findings described in the above chapters show that conducting well-designed experiments can lead to successful advances in the measurement of bottlenose dolphin emotions and affective states. However, due to time and geographical limitations, these studies are based on moderate sample sizes of animals and thus further work is needed to confirm the results apply to other groups of animals. Furthermore, while there are many advantages to conditioning the dolphins to certain cues or tasks so that questions can be asked of them, such work may not have the most direct benefits to animal managers who are looking for simple, feasible welfare indicators to measure on a daily basis in order to monitor the positive or negative states of their animals (Napolitano et al., 2010; Whitham and Wielebnowski, 2009). Qualitative Behavioural Assessments (QBAs) are often used as valid indicators of certain behavioural dimensions or welfare in farm species (Rutherford et al., 2012; Wemelsfelder, 2007), and lately similar qualitative approaches are being used with zoo animals as well (Phillips et al., 2017; Whitham and Wielebnowski, 2009). Situations where captive animals participate in training or interactive sessions with their caretakers can provide an opportunity for assessment of the animals' demeanour and emotions (Fleming et al., 2013; Phillips et al., 2017). It has been shown in many terrestrial species that motivation to perform operant tasks significantly changes if the animal is in pain (e.g. Brando, 2012; Nagaraja and Desiraju, 1994) or experiencing social stress (e.g. Pedersen et al., 2002; Søndergaard and Ladewig, 2004): the same has been reported in one dolphin study (Waples and Gales, 2002) but is often described anecdotally (personal communication). The protocol in many facilities is to take notes on the dolphin's behaviour after every training sessions (personal communication), but unfortunately this valuable data is difficult to use as it is taken differently between parks, and among different trainers within parks. Therefore the goal of this project was to develop a standardised method for collecting regular qualitative and quantitative data on the motivation for training, social situation, inappetence and health status of bottlenose dolphins and apply it in multiple facilities.

Research question

Can qualitative scoring scales be developed to measure motivation for training, new rake marks and health status in captive bottlenose dolphins, and can this feasibly be applied in multiple, international facilities?

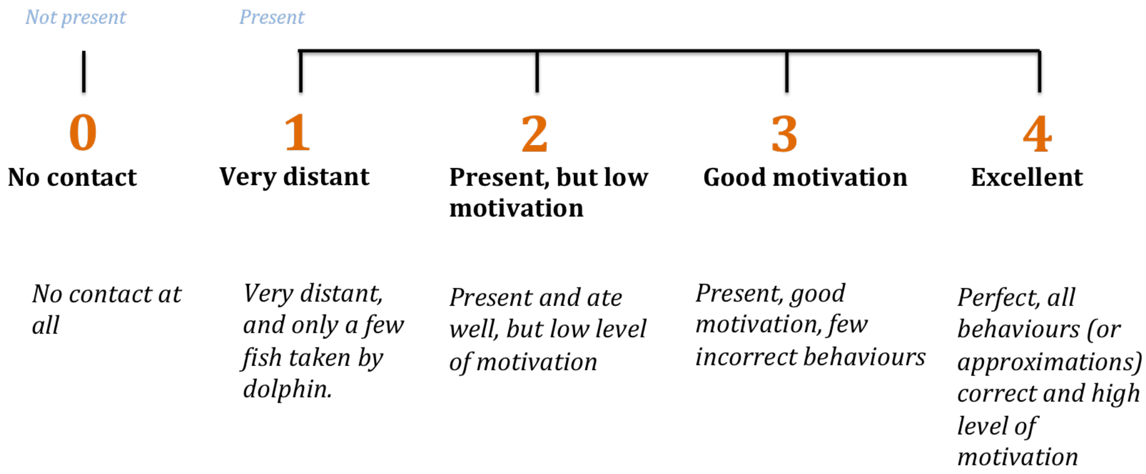
Methodology

Given that the principal aim of this project was to develop a simple and applicable tool to indicate the welfare of dolphins in captive facilities, the data collection techniques had to remain uncomplicated. In order to capture as much of the variation between individuals and facilities as possible the study needed to be conducted over a substantial time-frame with a large sample size of animals: this further rendered the need to keep the separate welfare-related measures simple. Five facilities (Parc Astérix, Planète Sauvage, Dolfinarium Harderwijk, Attica Park and Boudewijn Seapark) from four countries are participated in the project, resulting in an average of 60 bottlenose dolphins involved over the course of the study period. Data collection was conducted from around October 2016 until September 2017 but some facilities started later than others, resulting in an average number of 11 months of data collection. Due to the time-frame and sample size, the study required that data was collected by the personnel at each facility, where steps were taken to standardise and ensure inter-observer agreement (see details below). The four data elements collected were: a "Trainer score" of each dolphin's behaviour during each training session; a "Vet score" where the resident veterinarian scored the general health status of the animal; a "Social score" of the extent of new rake marks on the animal to indicate social stress; and an "Inappetence score", where the number of kg of fish eaten that day out of the total offered was noted (a quantitative score, whereas the others are qualitative). The trainer score was hypothesised to be the predictor variable in terms of reflecting changes in the other measures, and the process to test its validity was similar to Whitham and Wielebnowski's (2009) study testing zoo animal welfare scoring by keepers. We first developed the species-specific scoring scale for the "Trainer score", and plan to correlate it with other welfare-related items (i.e. the other measures), before ensuring its application to the facility's management system.

Trainer scores: 5-point Likert scale of dolphin's motivation during training sessions

Keeper scoring of animals' behaviour and emotions has been shown to be a valid measure of welfare in zoo research (e.g. King and Landau, 2003; Robinson et al., 2017; Whitham and Wielebnowski, 2009). The dolphins' motivation during positive reinforcement training sessions (including shows and all other types of session, training techniques described in Brando, 2010) was already being measured by the trainers at two of the study facilities using a 3-point Likert scale. These existing scores were based on elements including, but not limited to, the number of times the animal chose to leave the trainer (such “breaks” are never punished), the dolphin’s enthusiasm for tasks, vocalisations indicating excitement, and the attention of the animal on the trainer. For this project, we developed similar scales but established 5-point scores and structured the scale descriptions using principles similar to Qualitative Behavioural Assessments (QBAs). QBAs have often been used to successfully assess terrestrial animal emotional state and welfare through strong correlations with physiological and other quantitative measures of welfare (Fleming et al., 2013; Stockman et al., 2011; Wemelsfelder, 2007). The strength of QBAs lies in the fact that they can incorporate multiple, subtle indicators of welfare simultaneously to yield a holistic evaluation, whereas choosing certain quantitative measures might not always capture such underlying states. (Wemelsfelder, 2007; Whitham and Wielebnowski, 2009). The qualitative Trainer score here can be defined as in the style of QBAs but nonetheless slightly different as Visual Analog Scales (VAS) were not used and we aim to measure motivation, as opposed to emotional expressivity.

The reference scale that was established to score the dolphins’ behaviour during sessions (Fig. 13) was developed using the previous scales of the two facilities, the consensus of the experienced trainers at each facility, and behavioural data collected almost daily over two years (by myself) during all types of training sessions at Parc Astérix. Once developed, the scale was tested for over six months at Parc Astérix before small adjustments were made based on suggestions from the trainers. In the actual data collection phase at all facilities, the scores were noted per dolphin per session by the trainer who had primarily interacted with the animal for that session. In order to increase standardisation of the scoring between trainers, reference videos were made for each of the 0-4 scores and were given to the facilities. The videos included footage showing typical examples of behaviours indicative of each score, accompanied by on-screen text explanations of why the behaviour was linked to the score (versions in both English and French). In order to increase accuracy and concordance between the trainers, before data collection started I visited each park at the beginning period of the project in order to provide these videos and complete some examples of the scoring with the staff. To assess inter-observer reliability, a representative selection of trainers from each park will score several video examples taken of dolphins’ behaviour during sessions and the Intra-class Correlation Coefficient will be calculated.

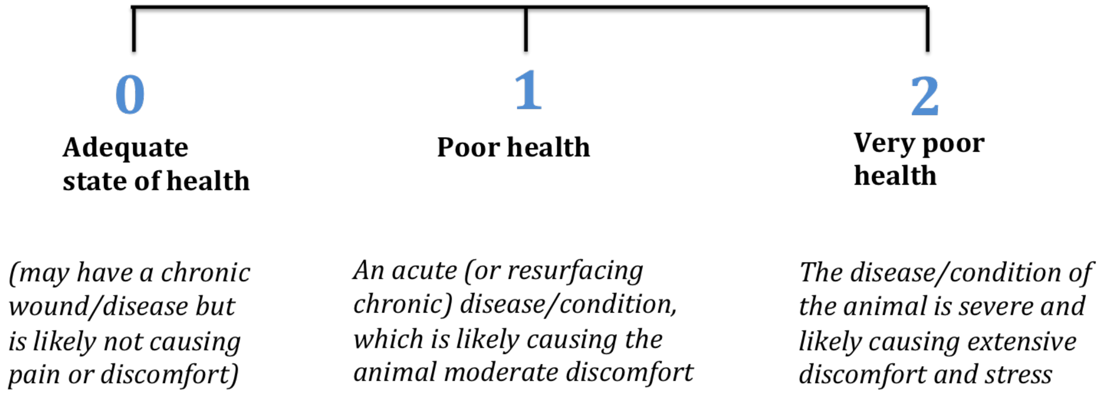


0	1	2	3	4
No contact	**Very distant**	**Present, but low motivation**	**Good motivation**	**Excellent**
No contact at all	*Very distant, and only a few fish taken by dolphin.*	*Present and ate well, but low level of motivation*	*Present, good motivation, few incorrect behaviours*	*Perfect, all behaviours (or approximations) correct and high level of motivation*

Fig. 13 *A 5-point Likert scale used by the trainers to give a score of an individual dolphin's motivation during each training session. A 0 is given if the animal does not approach the trainer at all (come to a stop within 1 m), and scores 1-4 are given if the dolphin is "present" at the session where trainers should correspond as closely as possible the dolphin's behaviour with the scale descriptions and reference videos provided.*

Vet scores: 3-point Likert scale of dolphin's overall health as related to welfare, conducted by resident veterinarians

Each facility had an associated veterinarian who conducted examinations of the animals at least once a week, and for this project they agreed to also take a general score of health at the same time. The developed 3-point Likert scale (Fig. 14) was designed to capture, very generally, whether the animal was likely to have any health issues which would result in negative emotions/feelings and sometimes loss of function. This stipulation was important to define for the veterinarians since it stemmed from on our "feelings-based" welfare definition of the thesis (Spruijt et al., 2001), where health does not impact welfare unless it has an impact on affective state i.e. an asymptomatic tumour would not be classified as causing poor welfare (Mason and Veasey, 2010). Thus the three points on the scale were simply labelled as adequate health, poor health and very poor health, and again during the consultation pilot phase of the project where some veterinarians provided feedback, small adjustments were made to the descriptions for the scores. The Vet score was taken once per week for each animal during the data collection phase.



(may have a chronic wound/disease but is likely not causing pain or discomfort)

An acute (or resurfacing chronic) disease/condition, which is likely causing the animal moderate discomfort

The disease/condition of the animal is severe and likely causing extensive discomfort and stress

Fig. 14 *A 3-point Likert scale of dolphin's overall health as related to welfare, used by resident veterinarians at each facility to score the animal's health once per week.*

Social scores: 3-point Likert scale of the new rake marks seen on dolphins each day

As mentioned previously, the limitation of this study is that complex data cannot be taken reliably in a standardised fashion at each facility. Therefore although there surely exists better measures of social stress and disturbance in dolphins, we needed to establish a relatively simple indicator with a practical method of measurement. We used a Likert scale to measure three levels of the extent of new rake marks on an individual's body, using a human hand as a simple reference to increase standardisation (Fig. 15). Rake marks have been shown to be proxy measures of aggression in dolphins (Marley et al., 2013; Scott et al., 2005), and it has been proposed that extensive marks are indicators of poor welfare (Clegg et al., 2015; Waples and Gales, 2002). Rake marks can also sometime result from high-arousal play interactions but rarely would appear as extensive when compared to those due to truly agonistic encounters (Scott et al., 2005; personal observations). During the visit conducted to all facilities at the beginning of the study, photographs were provided to clearly differentiate between old and new rake marks. This measure was taken by the trainers for each dolphin on a daily basis.

Inappetence scores: kilograms of fish eaten that day out of total offered to dolphin

It has been described previously in this thesis how inappetence is likely to be a measure of poor welfare in dolphins, and may be caused by either health or social reasons (Johnson et al., 2009; Waples and Gales, 2002). Therefore in this project

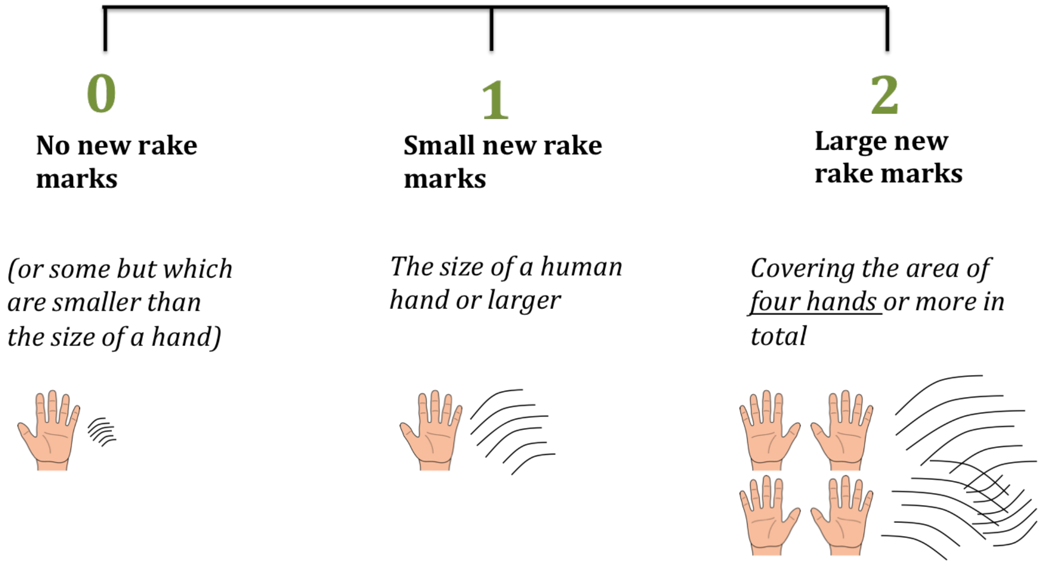


Fig. 15 *A 3-point Likert scale of the new rake marks seen on an individual dolphin, measured each day by a trainer.*

the number of kilograms of fish that the dolphin ate that day was noted down, as well as the total amount offered so that a percentage of what the dolphin ate could be calculated. Since all facilities started and ended the day with a “free-feed”, where fish was given without asking conditioned behaviours and at the end of day this included all the fish not eaten in the previous sessions, food was never withheld from the animals and thus any inappetence could be assumed to be voluntary refusal.

Next steps for the project

After data collection is finished in September 2017 the next months will be occupied by integrating it together, and analysis will begin soon after depending on its distribution and the quality of the data.

The overall aim is to investigate whether the dolphins’ behaviour during training is correlated to measures of social stress and health-problems. By considering the timeframe of the scores collected, we will also be able to ask whether behaviour during training can act as an early predictor of these welfare-related problems. Only one study has directly shown that behaviour during training might indeed be a salient welfare indicator in bottlenose dolphins (Waples and Gales, 2002). A multidimensional approach to measuring welfare such as this is supported by the Triangulation principle, since combining different categories of measures is the best way to identify changes in an animal’s affective state (Webster, 2005).

Chapter 6
General Discussion

Chapter 6: General Discussion

The goal of this thesis was to identify and develop potential welfare parameters for bottlenose dolphins. The initial studies of the project facilitated a deeper understanding of the literature and diurnal variance in the dolphins' behaviours. As a result, we were then able to suggest welfare measures for this species (synchronous swimming, anticipatory behaviour, play), tools to measure affective states (cognitive bias testing, qualitative ratings), and resources/management protocols that seem to promote positive welfare (HAIs, toy enrichment). This progress should stimulate further studies in the field, continuing to fill the gaps in the knowledge regarding measuring the welfare of dolphins.

The key, underlying attribute of 'welfare' is its holistic nature: it can only be discussed in a multi-factorial, all-encompassing manner (Broom and Fraser, 2015; Dawkins, 2006). In order to understand and study welfare in a manageable fashion, we can consider it as a bottom-up structure of simpler elements that combine over a number of steps, finishing with overall welfare (Fig. 2). The discussion of this thesis is organised in this way: after presenting the main findings and highlighting their significance as some of the first steps in the field, behaviours are presented that we can now say are likely linked to dolphins' emotions and possibly affective states, before it is considered how the findings might contribute to overall welfare assessment. The discussion finishes by highlighting some much-needed lines of future research that would continue to expand our understanding of how dolphins experience their captive environment.

Main findings of the thesis

Research efforts into animal welfare first focussed on farm animals, before spreading to companion and laboratory species, and, only very recently, have been increasing for zoo animals (Whitham and Wielebnowski, 2013). Therefore as a single species found in zoo collections, there were very few studies on the welfare of bottlenose dolphins before this thesis (Clegg et al., 2015; Hill et al., 2016). Of course, the field of cetology covers many different topics with wild and captive cetaceans, but this information had never been brought together in terms of animal welfare principles and questioning (Hill and Lackups, 2010; Hill et al., 2016).

Overview of results

The thesis started by addressing the non-existence of a dialogue between cetology and welfare science, and a review paper was written (Paper 1) in order to lay the foundations for cetacean welfare assessment. The principle of 'Triangulating' welfare measures (Webster, 2005) guided the review as it did the entire thesis, and current knowledge in cetacean behaviour, health and cognition was evaluated and used to propose potential welfare measures in each category. While the review identified numerous health- and cognition-related potential measures, it became apparent that behavioural measures represented the most promising and feasible category of welfare parameters, based on the available literature from wild and captive settings. In the past, before the realisation of emotion research with animals, health measures were thought to be the most closely associated to welfare state (Dawkins, 2006). However, it is now agreed upon that behavioural measures are crucial to comprehensive evaluations, and may even be more informative about welfare than health indicators (Gonyou, 1994; Joseph and Antrim, 2010; Maple, 2007). In response to this, and in order for behavioural data to be collected in a standardised way during the different studies and among the parks, a comprehensive ethogram of all bottlenose dolphin behaviours was constructed for this thesis using definitions from the literature and *in situ* observations (Thesis ethogram, Chapter 2).

The review paper and supposed significance of behavioural measures prompted the first empirical study of this thesis, which explored the daily variation in dolphin behaviours in relation to the time-schedule of training sessions (Paper 2). In order for meaningful conclusions to be made, it was imperative that such a study used a larger and more diverse sample than had been conducted before (see Miller et al., 2011b), and thus 29 bottlenose dolphins from three international facilities were recruited for the project. The main findings, that synchronous swimming, play and anticipatory behaviour were modulated by timing of training sessions, provided the premise for the following experiments of the project.

In another first for the field of dolphin welfare, cognitive bias tests were developed and applied to the bottlenose dolphins at Parc Astérix (Papers 3 and 4). This was also the first time such tests had been conducted in a zoo setting or on a marine mammal species (Bethell, 2015; Roelofs et al., 2016). Thus far, measuring cognitive bias is one of the only valid tests of animals' affective state and welfare, with numerous cross-species results showing that more "optimistic" judgements (ambiguous stimuli judged positively) are correlated with better welfare (Mendl et al., 2009; Roelofs et al., 2016). Given that very little is known about dolphins' emotions and affective states (Kuczaj et al., 2013), and that these animals in captivity are highly trainable for such tasks (Brando, 2010), the application of cognitive bias testing within this thesis was welcome progress (see Appendix A.4 and A.5 for media articles). It was previously suggested that the link between animals' social behaviour and cognitive biases should be explored (Wichman et al., 2012), and taking measurements from spontaneous social interactions is a means of investigating this (instead of trying to manipulate social welfare conditions experimentally, which is unlikely to be reliable). Our study correlated cognitive bias results with spontaneous behaviours (social and anticipatory) observed outside of test sessions, an approach which has not commonly been adopted in the field but which yielded thought-provoking results. We were able to show that higher frequencies of synchronous swimming (Paper 3) and lower frequencies of anticipatory behaviour (Paper 4) were correlated to dolphins' optimistic biases.

Following the results linking anticipatory behaviour to cognitive bias, and its proposed use as a measure of affective state (van der Harst and Spruijt, 2007; Watters, 2014), we decided to further investigate it in more detail. In Paper 5, we wanted to see whether dolphins would also anticipate non-alimentary events, and chose two common contexts: toy enrichment and positive Human-Animal Interactions (HAI) with familiar trainers. HAIs with trainers and the impact on welfare had been the subject of early discussions in the thesis (see model in Appendix B.1), as this inter-specific relationship had been little investigated in the past (Clegg et al., 2015) and it was unknown whether dolphins viewed such events positively/as rewards, or not. Our study (Paper 5) also investigated a new question concerning anticipatory behaviour which had not yet been addressed in other species: is the level of anticipatory behaviour correlated to participation in the expected event? We were able to show that participation was indeed reflected by anticipation beforehand, and that the dolphins anticipated positive Human-Animal Interactions more than toy provision, but that both were likely to be seen as rewarding.

In situ feasibility of the project

It has been argued that conducting welfare research *in situ* i.e. at the farm or zoo as opposed to a laboratory, is much more likely to be successful since the expertise of the animal caretakers can be exploited, the animals are in their "real-life" environment, and afterwards the results will be more applicable for improving welfare (Dawkins, 2006). A gratifying but unforeseeable component of this thesis was revealed when it became clear that welfare research in the dolphins' day-to-day environment could indeed simultaneously benefit the animals, caretakers, and scientists. For example, in the cognitive bias experiments (Papers 3 and 4) it was decided not to separate the animals in order not to cause isolation stress (seen in this species, Esch et al., 2009), and despite initial misgivings the dolphins all learnt to discriminate the cues and completed the test with no problems. More studies may also start to take this approach, since a recent review emphasised the advantages of non-isolation during cognitive bias testing (Roelofs et al., 2016). The cognitive bias study involved teaching the dolphins a new behaviour using positive reinforcement training (shown to enhance welfare, Laule et al., 2003), and they seemed to enjoy learning and performing this new task as exemplified by their continued participation and motivated attitude (personal observations of multiple dolphin trainers). Furthermore, once the research was completed, the results were able to inform the animal care team about individual dolphins' judgement tendencies and the potential meaning behind synchronous swimming and anticipatory behaviour. The study on anticipatory behaviour and non-food events (Paper 5) was able to inform the facility management that the dolphins viewed positive HAIs as a rewarding event, and of course the animals benefitted since throughout the experiment they were given access to these HAIs and toys as well. A last example of the compatibility of ethological/welfare research and captive dolphins is reflected by the final study in the project (Paper 6), where it is planned to correlate qualitative ratings of motivation during training to social and health-related welfare data to discover whether this feasible scoring system can indicate changes in welfare. It is hoped that this data facilitates the development of a practical welfare tool, which would give facility managers an overall picture of the dolphins' state as well as detecting more subtle changes in welfare.

Practical limitations

Concurrent to the successes of the project, there were also many limitations given that it represents the first steps into dolphin welfare research. Limitations of the reasoning used to interpret the results are discussed in the following sections, and the limitations regarding designing and executing the experiments are highlighted here. For example, although multi-facility studies (Papers 2 and 6) allowed an increased sample size of animals and broader conclusions to be made, the geographical distance between facilities and the different management protocols between them meant that to ensure standardisation, only relatively simple data could be taken. This led to limitations in our discussions, for example in Paper 2 the frequency of slow-close synchronous swimming peaked after training sessions, but we were unable to deduce whether the type of training session differentially affected this behaviour. In the cognitive bias experiments at Parc Astérix (Papers 3 and 4), the test subject was not permitted to be isolated from the rest of the group and thus testing occurred in the presence of all dolphins. We limited interference as best we could, principally by using a separate section of the pool (Fig. 6, Paper 3, Chapter 3), but we could rule out the possibility of interference to an animal's return latency from the cues which could lead to non-independence of results. Non-independence could potentially have been a factor in our results for Paper 5 where we were measuring individual anticipatory behaviour towards predictable contexts, again tested in a group setting. Anticipatory behaviour performed by certain dolphins could have influenced the levels seen in others, but the alternative situation where dolphins would have to be socially isolated could have equally confounded the results through increased stress to the animals. Ultimately, studies should develop and test welfare measures *in situ* since this is where the animals spend their lives (Dawkins, 2006), while at the same time, as with most zoo research, controlling as much as possible for problems of experimental design and independence and formulating conservative conclusions (Whitham and Wielebnowski, 2013).

Findings and pertinence to measuring emotions

The main findings regarding potential indicators of emotion mostly concerned dolphin behaviour, although some health-related and cognitive measures were investigated as well. This may be due to the fact that behavioural measures are relatively feasible, often making up a large proportion of terrestrial welfare assessments, and that there is an apposite level of existing behavioural knowledge on dolphins (Maple, 2007; Wells and Scott, 1999). This section follows the schematic of the breakdown of overall welfare (Fig. 2) and here I start with the basic elements: by reviewing how the behavioural parameters discovered may be linked to dolphin emotions, and thus might be eventually useful in measuring affective states and overall welfare (Mendl et al., 2010).

Play behaviour

Although often observed more frequently in young animals, play occurs during adulthood in a number of species (e.g. Bradshaw et al., 2015; Cordoni, 2009; Palagi and Paoli, 2007), including cetaceans (Hill and Ramirez, 2014; Paulos et al., 2010). In general, play behaviour occurs in the absence of fitness threats and as a result has long been considered an indicator of good welfare (review by Held and Špinka, 2011). However its inclusion in standardised welfare assessments has been somewhat limited due to problems over defining the behaviour and the large interand intra-individual variation (Boissy et al., 2007; Held and Špinka, 2011). Difficulties with finding a definition of dolphin play are no exception, and thus far research has remained largely descriptive (Kuczaj and Eskelinen, 2014; Paulos et al., 2010). Nevertheless, such studies have revealed important fundamentals of patterns of play in cetaceans: it seems that while both adults and young play, it is the calves that produce the innovative and novel play behaviours, and in doing so they can also increase the levels of play in the group as a whole (Hill and Ramirez, 2014; Kuczaj and Eskelinen, 2014). Similar to synchronous swimming, play can spread through behavioural contagion, and thus the emotions linked to the activity can surely transfer as well (Kuczaj and Eskelinen, 2014; Špinka, 2012) making it a worthy candidate for investigations into its utility for measuring dolphin welfare.

As part of this thesis, the frequency of play (social and object) behaviour was investigated in relation to the timing of training sessions, in four different groups of dolphins (*n* = 29, Table 2, Paper 2). It was found that play frequency differed significantly with the timing of sessions, but in an interaction with age: post-hoc testing revealed that juvenile dolphins played more than adults in the time in-between the sessions (at other points the difference was not significant). While this difference is likely due to the higher rates of play seen in younger dolphins (Paulos et al., 2010), it also shows that elements of the captive environment can significantly impact the occurrence of dolphin play. Following this line of research, a parallel study (which was not part of this thesis) was conducted at Parc Astérix and found that factors such as noisy construction work significantly decreased dolphin play, and providing environmental enrichment increased social play (Serres and Delfour, 2017). The authors thus concluded that social play has much potential as a welfare indicator for dolphins.

Concurrent to the study by Serres and Delfour (2017), the frequency of social play was investigated for associations with cognitive bias (Paper 3). We predicted that higher play frequencies might be associated with higher optimism, but no significant results were found: that is, a dolphin's individual judgement bias did not seem to affect/be affected by the performance of social play. Of course, the non-significant result here cannot be concluded as the absence of an association, but equally we can tentatively explore some possible contributing factors. The study's time frame may have played a role: the behavioural data represented 75 minutes of observation time over three days, and this may not have been a large enough window to capture accurate individual play frequencies. Alternatively, it could be that bottlenose dolphin play may not have a strong correlation to positive affect. Although increased play seems to be linked to better welfare and positive emotions in many species (Held and Špinka, 2011) there are also some contrasting findings. One such study with domestic horses found that higher frequencies of social play were linked with multiple signs of poorer welfare (Hausberger et al., 2012). In dolphin species, play fights escalate into real fights increasingly with age (review in Kuczaj and Eskelinen, 2014) and therefore it could be that social play behaviour in this group of animals is a double-edged sword with regards to the influence on emotional states.

Following on from this study and using indications from the literature, it was investigated whether object and interspecific play (with a familiar human) could also be useful in welfare evaluations. Given that our time limitations may have prevented us capturing the true levels of social play in Paper 3, our approach for the next study was to induce play behaviours by systematically providing the dolphins with the relevant opportunities. The experiment (Paper 5) studied the dolphins' responses to signalled opportunities for interacting with a familiar trainer (HAI) or accessing toy enrichment. We found that they performed anticipatory behaviour before both events but anticipated HAIs significantly more, and that in this study anticipation was correlated to subsequent participation in the event i.e. object play with the enrichment, and inter-specific play with the humans. We therefore concluded that these events, and thus the opportunity to perform these types of play behaviour, were perceived as rewarding. In terms of bottlenose dolphin object play, the many examples in wild and captive settings (e.g. Delfour et al., 2017; Kuczaj and Eskelinen, 2014; Paulos et al., 2010), along with our study's findings that play with objects was positively anticipated, suggest that this type of play is also a likely indicator of positive emotions and possibly welfare in this species.

Another interesting finding from Paper 5 was that significantly more anticipatory behaviour was displayed before a positive HAI with a familiar trainer than before enrichment provision, and furthermore every dolphin in the group individually showed this trend. This could reflect a lack of interest in the enrichment provided at this facility and/or a desire to interact with the trainers: our results do not allow us to distinguish which factor was predominant (if either) in this "preference". However, in terms of motivation for the event, anticipatory behaviour was also shown to predict participation levels (time spent with the trainer) and so it seems that in general the dolphins in this group "wanted" to interact with the trainers (no aggressive or sexual behaviour towards humans was seen: interactive behaviours included visual and tactile play). Therefore, similar to other types of play, it could be that interspecific play between dolphins and familiar humans at least is also a potential measure of positive emotions and welfare. However, apart from this thesis, there are no previous studies on dolphin emotions or motivation towards HAIs, with only a few descriptive studies existing about play or tactile behaviour during interactions with humans (Kuczaj et al., 2006; Paulos et al., 2010; Perelberg and Schuster, 2009). Many dolphin species are regularly observed conducting intra-specific tactile and play behaviour (Dudzinski et al., 2012; Kuczaj et al., 2013), and thus in captivity the animals might transfer these behaviours to the familiar humans present in their environment (Paulos et al., 2010), possibly viewing them as heterospecific social partners (Perelberg and Schuster, 2009; Servais and Delfour, 2013). To fill the gaps in our knowledge, studies should delve deeper into the HAIs between trainers and dolphins, as very little is known about how profound these keeper-animal relationships are (Hosey and Melfi, 2010) and how they might influence dolphin welfare. Furthermore, dolphin species have been shown to react differently to familiar versus unfamiliar humans (e.g. Thieltges et al., 2011), and it would be interesting to see whether similar levels of anticipatory behaviour are shown towards humans they are not familiar with, or to familiar humans who are not involved with providing food to the animals.

Synchronous swimming

Synchronous swimming is a common, affiliative behaviour seen in many dolphin species in both wild and captive settings (Connor et al., 2006b; Harvey et al., 2017; Sakai et al., 2010). The exact definition used throughout this thesis is where two or more animals swim within one body length of another, corresponding their movements and body axes, with 0-2 seconds delay between each individual surfacing for breathing (based on Connor et al., 2006a; Harvey et al., 2017; Holobinko and Waring, 2010). The review paper (Paper 1) at the start of the project briefly highlighted synchronous swimming as a possible indicator of positive welfare states, and therefore it was included in the suite of behaviours studied in relation to timing of training sessions in Paper 2. In addition, in this paper we went a step further and delineated the behaviour by speed and distance to partner which resulted in four variants of synchronous swimming: slow-close, slow-distant, fast-close, fast-distant (see Thesis ethogram, Chapter 2, for definitions). This was conducted in order to start investigating whether different variations of synchronous swimming might indicate different emotions and possibly welfare. This step turned out to be a prudent choice: the results showed that slow-close synchronous swimming was the only variant to significantly change in frequency, where it increased shortly after training sessions. It was suggested that this increase of a calm, socially affiliative behaviour showed that the dolphins were motivated to be reunited after being under human control (although not physically separated) during training sessions. This hypothesis is supported by acoustic results from the dolphin group at Parc Astérix taken at a similar time, where it was found that the rate of signature whistles also increased significantly shortly after training sessions: such whistles are often considered as social cohesion calls so these results also suggest an increase in social affiliation after training sessions (Lopez Marulanda et al., 2016).

Although the causes of synchronous swimming frequency changes were not confirmed in Paper 2, such findings are important in terms of uncovering the intention behind these social behaviours for the dolphins, and thus the link this may have to their emotions. For example, it has often been reported that during intra- or inter-specific aggression and 'intense' social behaviour, the level of synchronous swimming or at least synchronous behaviours is increased (e.g. Connor et al., 2006b; Herzing and Johnson, 1997). In trying to explain the behaviour in this context, the utility of adding the variable of speed and distance is evident: it can be imagined that the type of synchronous swimming seen in 'intense' social behaviour might be at higher speeds than during relaxed, low-energy affiliation.

In Paper 3, cognitive bias paradigms were used to test for correlations between biases and social behaviours, such as synchronous swimming. Due to the shorter behavioural data collection phase (over 3 days, compared to 2 months for Paper 2), synchronous swimming was not differentiated by distance and speed (the implications of this will be discussed later). Those dolphins that made more optimistic judgements were also those that conducted more synchronous swimming in their 'free-time'. Elsewhere, over one hundred studies on many different species have shown that optimistic judgements are correlated to positive affective states or welfare conditions (most recent review by Roelofs et al., 2016). Therefore we could conclude from our cognitive bias tests with the dolphins that higher synchronous swimming frequencies were likely to be correlated to more positive emotions, such as 'relaxed' or 'happy' (Mendl et al., 2010). The influence of social behaviour on cognitive biases has only been studied a few times, where laboratory rats were more pessimistic after experiencing chronic social stress (Papciak et al., 2013) and canaries judged more optimistically when pair-housed (Lalot et al., 2017). These two examples support the notion that affiliative social behaviour is linked to optimistic judgements and positive affective states, but more studies in this area are crucial and have been specifically recommended (Wichman et al., 2012).

The findings concerning social affiliation and emotions within this thesis are just a part of the vast topic of positive emotions and measuring welfare (Boissy et al., 2007). More questions can immediately be asked regarding the dolphins: our previous study (Paper 2) showed that more work on the speed and distance between the dolphins is needed to be able to fully understand the link between synchronous swimming and positive emotions. This was not possible during the cognitive bias experiment due to time constraints and the fact that the hypotheses were focussed on a range of social behaviours, not just synchronous swimming. However, such a protocol could be used again to test whether all variants of synchronous swimming are indeed linked to positive affective states. The problem of distance between animals also arises for farm animal researchers trying to establish affiliative behaviour as an positive emotion indicator, since these animals may also decrease the distance from their partner when faced with an environmental threat (Boissy et al., 2007). However this does not confound the use of affiliative behaviour as a welfare measure by any means: although it may be displayed in stressful as well as non-stressful circumstances, the result of increasing social cohesion and support is clearly the same. Affiliative behaviours in more stressful situations are thought to function by moving the animal's emotional state back towards the positive side, so that homeostasis is not unfavourably impacted (Rault, 2012). Therefore those animals that have diminished social support, as shown by no or low levels of affiliative behaviours during stressful times, would likely be in a worse state than those who have such a 'stress buffer' available to them (Rault, 2012; Waples and Gales, 2002). Stress buffering and terrestrial animal welfare has been studied with findings showing that social support can greatly mitigate the effects of stress (latest review by Rault, 2012): hence, an opportunity exists to investigate the same concepts with captive dolphins, where evidence already suggests that stronger social bonds can ensure better overall health and welfare (Brando et al., 2016; Hoffland et al., 2017; Waples and Gales, 2002). Based on our results and the available literature, synchronous swimming, as an affiliative, bond-enhancing activity, may be one of the behaviours which increases social support, and thus we recommend further investigations to fully understand its relevance to dolphin welfare.

Anticipatory behaviour

Anticipatory behaviour describes the activity conducted in response to the predicted occurrence of an upcoming event (Spruijt et al., 2001). Its performance is thought to reflect the individual's sensitivity to the reward. For example, if an animal is consistently deprived of a desired resource it is likely to experience a more negative affective state, leading to a higher reward sensitivity (more 'incentive value' placed on the resource) and therefore more anticipatory behaviour is performed before the reward finally arrives (van der Harst and Spruijt, 2007; see Fig. 16 below, taken from Watters, 2014). Inducing anticipatory behaviour may also function as a tool to improve welfare since 'looking forward' to an event can be as rewarding as having access to the resource itself (Moe et al., 2009; Watters, 2014). Reviews of the literature supporting the link between anticipatory behaviour and affective states can be found in Papers 4 and 5 (and in: van der Harst and Spruijt, 2007; Watters, 2014).

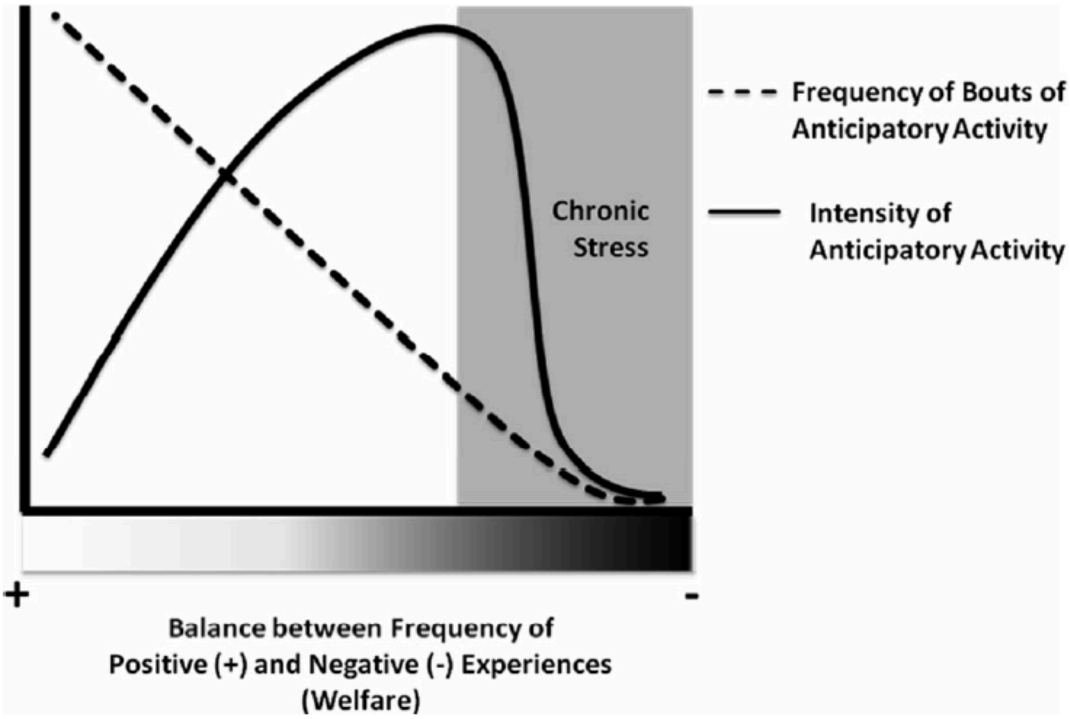


Fig. 16 *Predictive model of anticipatory behaviour from Watters (2014), modified from van der Harst and Spruijt (2007). The x-axis represents the animal's welfare state as indicated by the balance between positive and negative experiences. Here, there is specific reference to the frequency of these experiences and, for the sake of explanation, an assumption that they all have the same value. In other words, a positive event is equally as positive as a negative event is negative. When positive events are common, the opportunity to anticipate them is also common and the frequency of anticipatory events is high. However, because of frequent occurrence, positive events do not generate incredibly intense anticipation. As positive events become rarer, they also become more important to the animal. Here, the intensity of anticipatory behaviour increases as a result of the animal's increased reward sensitivity. Also, the frequency of individual bouts decreases because fewer bouts are initiated by relevant cues. The shaded grey area represents chronic stress. This is the area where negative outcomes are so much more common than positive ones that the animal's normal functions are disrupted. Here, low intensity of anticipation results from disrupted homeostatic systems. Here also, a low frequency of anticipatory bouts results from rare opportunities to express the behaviour.*

The first study to explicitly investigate anticipatory behaviour in cetaceans was conducted on the bottlenose dolphin group at Parc Astérix a few years prior to this thesis, and found that dolphins increased certain vigilance-related behaviours before the start of training sessions or shows (Jensen et al., 2013). Since then, one other has been conducted where false killer whales (*Pseudorca crassidens*) seemed to vocally anticipate food-provision training sessions (Platto et al., 2015). Due to the lack of available studies, the behavioural repertoire used in Paper 2 of this thesis included anticipatory behaviours based on previous studies' descriptions of vigilance behaviour towards above-surface events (Jensen et al., 2013; Miller et al., 2011b). Indeed, we found that our proposed anticipatory behaviours collectively increased in frequency shortly before food-provision training sessions (occurring on a fixed, predictable schedule). We did not study the number of behavioural transitions as a measure of anticipation, as has been conducted in some other studies (Moe et al., 2006; van der Harst et al., 2003a), due to the fact that dolphins were able to be vigilant to poolside activity while swimming or even social swimming. Further studies that validate methods to measure cetaceans' behavioural transitions would be useful.

The results from Paper 2, and the growing body of literature supporting its links to welfare measurement, prompted us to focus further on anticipatory behaviour: as part of the subsequent experiment, we aimed to correlate anticipatory behaviour to individual cognitive bias (Paper 4). The two most commonly described surface vigilance activities were chosen as reflecting the dolphins' "anticipation", based on the findings in Paper 2 and the literature: surface looking (animal orientates head and eye(s) towards poolside area, either while swimming forward or while floating stationary) and spy-hopping (vertical movements, often repeated, above water's surface that allow viewing of poolside area). These two behaviours were defined in this study as 'anticipatory behaviour' towards food-provision training sessions. The sessions occurred on a fixed schedule, almost always started from the same area, with various environmental cues available to predict the arrival of the trainers (e.g. buckets being prepared) for the start of the session. Considering that both cognitive bias testing and anticipatory behaviour are thought to be linked to affective state, a previous study has already aimed to correlate results from the two phenomena (Wichman et al., 2012). However, in this investigation on laying hens (*Gallus gallus domesticus*) there were no significant correlations found, and the authors put this down to individual differences. In our study which led to Paper 4, we wanted to test for such an association in captive dolphins and we based our predictions on the reward sensitivity theory: we expected higher levels of anticipatory behaviour to be correlated to more pessimistic judgements, since the literature suggests these both reflect negative affective states. Our results showed that the more optimistic dolphins anticipated the sessions less (significant association for the Near-positive cue and a tendency for the Middle cue), and we thus provide the first evidence for the link between cognitive bias and anticipatory behaviour. Of course, there is much more work needed in order to fully understand the meaning of these results and our experiment had limitations: for example, anticipatory behaviour may vary in appearance among individuals and we did not take this into account (in this study, but see repeatability tests in Paper 5). We were not able to control for the exact nature and timing of the cues signalling the start of training sessions which may have resulted in some inaccuracy regarding the animals' actual anticipation of the training, and possibly could account for the tendency towards significance for the Middle cue results, or the lack of correlation to the Near-less-positive cue. There is also the possibility that the animals view the training sessions as aversive and thus are negatively anticipating them, although this is unlikely since such responses are usually behaviourally distinct from positive anticipation (e.g. Zimmerman et al., 2011). Furthermore, participation in training sessions is voluntary for the animals (Brando, 2010), where the only consequence of not attending is that they receive the food planned for that session at the end of the day instead.

In response to the novel findings of Paper 4 that show the potential for dolphin anticipatory behaviour as a welfare measure, Paper 5 used the behaviour to investigate whether the animals seem to view the training sessions and other non-food related events as positive or negative, as well as test whether those animals anticipating the event the most would also show higher levels of participation. Since few studies existed on anticipatory behaviour of non-food events in all animals (Anderson et al., 2015; McGrath et al., 2016; van der Harst et al., 2003b), our first question concerned testing the dolphins' response to the signalled provision of toys or a positive Human-Animal Interaction with a familiar trainer (HAI). As expected, the animals successfully paired the cues with the contexts and showed significantly more anticipatory behaviour before the non-food events than before the control context. There was no existing knowledge regarding whether the animals might anticipate toy or HAI opportunities more, given that there are only a few studies which even consider measuring HAIs with dolphins (Clegg et al., 2015; Perelberg and Schuster, 2009; Servais and Delfour, 2013). Interestingly, we found that all dolphins performed more anticipatory behaviour before the HAI context than the toys, which resulted in a significant difference when data were averaged. Nevertheless, the same alternative hypotheses as in Paper 4 could be posed here, where we cannot be sure that the behaviour being shown reflects positive or negative anticipation. It is here that an important second question was asked in Paper 5, and aided greatly in the explanation of results of question one.

No previous anticipatory behaviour studies had tested whether the level of anticipation beforehand corresponded with the individual's participation in the event itself i.e. the consummation of the goal (whether food- or non-food related). A few studies included peripheral results which indicated that there should indeed be a correlation between animals' appetitive and consummatory behaviour (Anderson et al., 2015; McGrath et al., 2016). Therefore, we decided to test this fundamental question with dolphins and the results were convincing: across three difference cued contexts (toy provision, HAIs, and food-provision training sessions) it was shown that the level of anticipatory behaviour was correlated with participation in the event (different behaviours used for participation measures, see Table 7 of Paper 5). Furthermore this significant correlation concerned intra-individual data and not averages per individual, indicating that although the duration of anticipatory behaviour is likely to vary from day-to-day, it remains indicative of the animal's participation in the event. This relationship surely merits further investigation, since in terms of welfare it could be more informative about specific affective states than the one-dimensional data on levels of anticipation. It could be imagined that an animal that spends much of its time anticipating a positive upcoming reward, but then does not extensively exploit or engage with it once it arrives, might be in a state of boredom or frustration. More work is needed to test this hypothesis, but for the moment it is reasonable to conclude that an animal that anticipates an event and then shows subsequent keen participation during it, is motivated, or has a "want", for that resource (Spruijt et al., 2001). We therefore tentatively suggest that dolphins' anticipatory behaviour could be used as a measure of motivation towards certain resources and events, i.e. as a type of "*in situ* measure of preference" which indicates what the animal might 'want' in its environment (Dawkins, 2004). When this finding is coupled with the results of question 1 in Paper 5, it can be further concluded that the dolphins viewed both toy and HAI opportunities positively and that positive emotions were likely associated with the acquisition of the rewards. Even before a reward arrives, a certain level of anticipation is known to induce positive affective states in animals (Kamal et al., 2010; Spruijt et al., 2001), with some evidence that these can exceed the positive affect resulting from the reward itself (Dudink et al., 2006). In order to fully understand the findings with the dolphins, in the next section I explore further the specific "levels" of anticipation which might represent differing affective states, where the multiple studies must be considered together (Papers 2, 4 and 5) and in relation to the reward-sensitivity theory.

Findings in relation to affective states and overall welfare

The discussion is continued by moving up a level in the bottom-up structure of welfare evaluation: the thesis findings are now discussed in terms of what types of affective states they might relate to, and how in turn this might impact overall welfare. Firstly, I delve deeper into what the performance of anticipatory behaviour might mean for the dolphins, and evaluate its potential as an applicable welfare assessment tool. In order to collectively consider the findings regarding the potential dolphin welfare indicators in terms of affective states, different frequencies of play, synchronous swimming and anticipatory behaviour are loosely placed within Mendl and co-authors' (2010) framework. Lastly, since the welfare definition followed in this thesis describes a balance of positive and negative affective states, only taking into account one affective state at a time may inadvertently lead to incorrect welfare assessments. Therefore, I briefly discuss approaches to assessments of overall welfare, and propose how these might be applied to dolphins in captivity.

Proposed relationship between anticipatory behaviour and welfare

As is likely to be evident following the previous discussion of anticipatory behaviour measured in Papers 2, 4 and 5, the frequency at which the behaviour is performed is likely to be crucial for deducing the type of associated affective state. Given that the thesis included multiple studies which yielded anticipatory frequencies within different contexts, I decided to explore whether our data on this concept could be more informative if it was all considered together. A schematic was constructed (Fig. 17) to map the relationship between the level of anticipatory behaviour and dolphin welfare through adapting an existing predictive model based on the reward sensitivity theory (Fig. 16, from Watters, 2014). Van der Harst and Spruijt (2007) and Watters (2014) have constructed and discussed general models of anticipatory behaviour and welfare, but this schematic is the first endeavour to apply these principles to a certain species and attribute specific behavioural frequencies. However, it is essential to keep in mind that this is a very preliminary exploration which includes many limitations, primarily that the behaviours defined as anticipatory behaviour differed slightly between Papers 2, 4 and 5, as well as their units of measurement (either as percentage of visible scans or percentage of visible time in seconds). Similar to the original model by Watters (2014) (Fig. 16), this adapted schematic collectively considers anticipatory behaviour to different positive rewards. The schematic is not intended to be used for detailed predictions and the absolute values should not be focussed upon: moreover it is simply a visual representation of this project's findings, and what they might indicate about welfare if the reward sensitivity theory is applied to dolphins. If extensive further work was to be conducted, the schematic also shows how measuring the frequency of anticipatory behaviour might be a practical tool to aid in welfare assessment. Details of how the schematic was constructed, including justifications for the welfare designation for each group of results, are also given below (Table 8). The model that it was based on (from Watters, 2014) can be found earlier in the manuscript (Fig. 16).

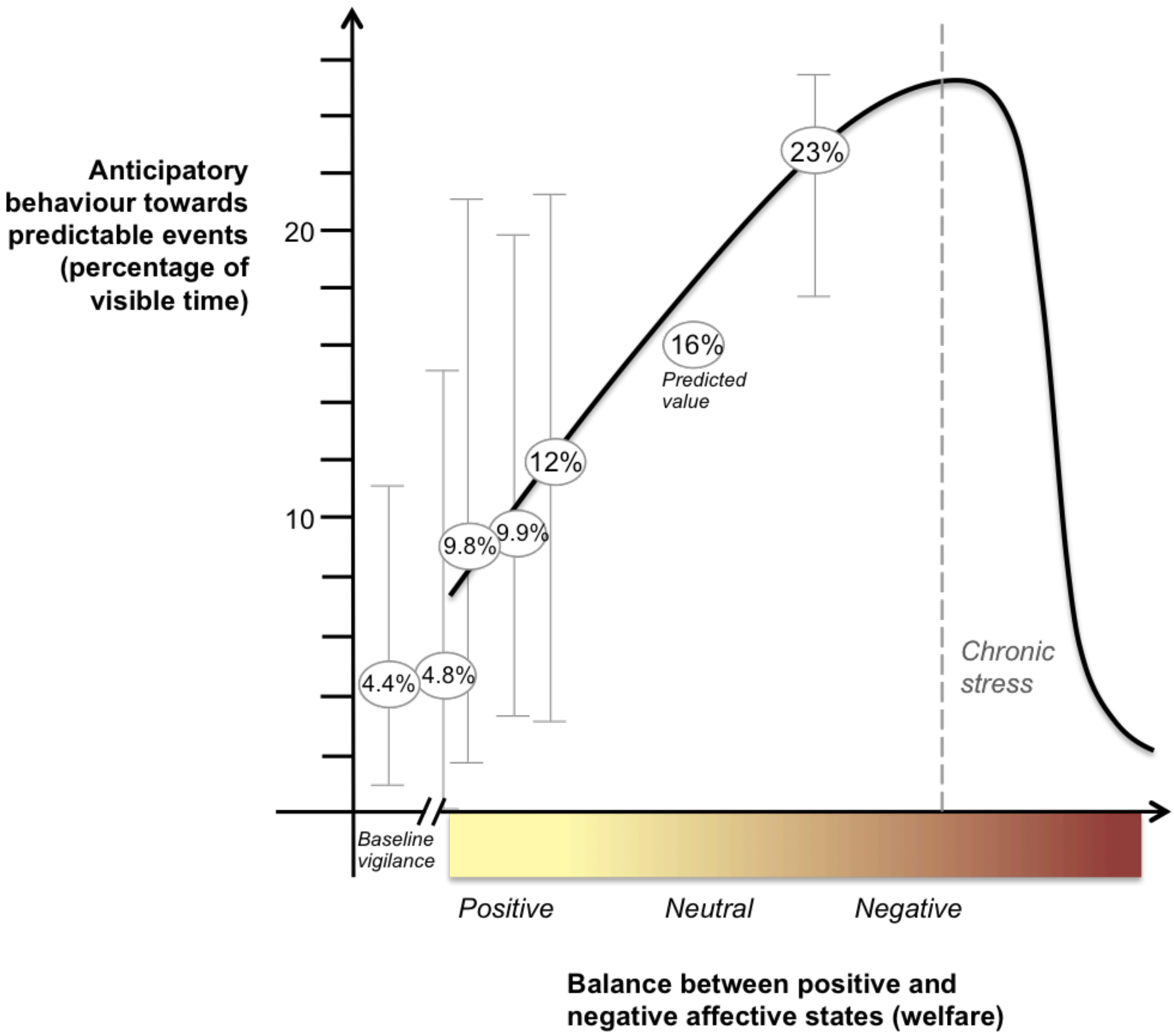


Fig. 17 *Proposed schematic showing relationship between anticipatory behaviour and dolphin welfare, based on Watters (2014) and van der Harst and Spruijt (2007). The x-axis depicts the balance of affective states and thus ranges from positive to negative overall welfare (reflecting the Spruijt et al., 2001, welfare definition followed in this thesis). The y-axis shows anticipatory behaviour towards various predictable*

events, which was measured as percentage of visible time (either as percentage of visible scans or percentage of visible time in s) and defined as the behavioural patterns established in preparation for a predictable, upcoming event (Spruijt et al., 2001). Based on Watters' (2014) predictive model (Fig. 16), the dotted grey line represents a threshold above which such levels of anticipatory behaviour may indicate chronic stress. Filled white circles represent anticipatory behaviour averages over all animals, while the grey lines indicate maximum and minimum averages among individuals: Table 8 details the sources, calculation and welfare designations of these data. The averages of 4.4 and 4.8 represent data taken when no predictable event was scheduled to occur, and so it is suggested that these frequencies represent baseline vigilance activity by the animals.

Table 8 *Source of the data displayed in Fig. 17, an exploratory schematic proposing a link between anticipatory behaviour and dolphin welfare. The anticipatory behaviour data is explained in terms of its source and welfare designation. Welfare designations (i.e. whether the level of anticipatory behaviour in that context is likely to be linked to positive, neutral or negative welfare states) are given based on conclusions from each study and the current literature, and the reasoning is explained in the last column. A "-" indicates that the level of anticipatory behaviour have no clear link to welfare: they are included nonetheless as they could indicate baseline vigilance levels.*

**Denotes that value was predicted from regression line; all other values are raw data.*

Paper	Reference to source in this thesis	Mean anticipatory behaviour of all animals (% of visible time)	Range of individual anticipatory behaviour (% of visible time)	Welfare designation of context	Justification for welfare designation
2	Fig. 5c "Shortly after" and "In-between"	4.8	0.11 – 15.4	-	Shortly after and in-between training sessions, dolphins had no predictable events to anticipate, therefore this level of the behaviour represents baseline vigilance and is likely not related to affective state.
4	Fig.9a Dotted line on Near-positive cue graph	16.0*	-	Neutral	The point where the dotted line (mean individual return time, i.e. no bias, neutral affective state) crosses the regression line corresponds to 16% of anticipatory behaviour. (Near-positive cue data used as Middle cue association was only a tendency).
4	Fig.9a "Optimistic" dolphins	9.9	3.3 – 19.8	Positive	Average of the anticipatory behaviour frequencies correlated to "optimistic" judgements (below dotted line), known to be associated with positive welfare.
4	Fig.9a "Pessimistic" dolphins	22.7	17.6 – 25.4	Negative	Average of the anticipatory behaviour frequencies correlated to "pessimistic" judgements (above dotted line), known to be associated with negative welfare.
5	Fig.11 Anticipation before Control context	4.4	0.9 – 11.0	-	No event occurred in the Control context, and a low level of anticipatory behaviour was conducted anyway, possibly as a baseline vigilance activity.
5	Fig.11 Anticipation before HAI context	11.7	3.1 – 21.3	Positive	Anticipatory behaviour before an event they are motivated to participate in (“rewarding”), and where play behaviour is performed (i.e. usually in the absence of other needs).
5	Fig.11 Anticipation before Toys context	9.8	1.8 – 21.1	Positive	Anticipatory behaviour before an event they are motivated to participate in (“rewarding”), and where play behaviour is performed (i.e. usually in the absence of other needs).

The schematic (Fig. 17) uses the data yielded from the studies in this thesis (Table 8) to hypothesise that a dolphin experiencing more positive affective states will likely perform a certain level of anticipatory behaviour for predictable rewards (conservative range might be between 5 and 12% of the time). As the balance of affective states moves towards neutral, the animal might conduct more anticipatory behaviour (around ~16% of the time), and then as the balance tips towards negative it may perform the behaviour at levels around 23% of the time. No data is yet available but if the case with dolphins is similar to terrestrial animals that have been studied, once a chronic stress stage is reached (i.e. very negative affective states), anticipatory behaviour levels would decrease sharply to 0 (Kamal et al., 2010; von Frijtag et al., 2000). The proximate causation behind the theory as it translates to dolphins suggests that a lack of stimulation in their pools and daily lives would cause them to place higher values on the rewards that they *do* have, and thus they anticipate them more. An advantage of integrating the findings of this thesis in such a way is that it clearly highlights the gaps in the knowledge and thus future directions. For example, our results showed that a certain level of ‘anticipatory-like’ behaviour occurs when there are no predicted upcoming rewards (e.g. Control context in Paper 5), and this was tentatively labelled as “baseline vigilance” activity (Fig. 17). This baseline level of ‘anticipatory-like’ behaviour also occurred in other studies (e.g. Anderson et al., 2015), but possible functions have not been readily discussed nor has it been recognised in previous models (van der Harst and Spruijt, 2007; Watters, 2014). One line of questioning which might provide information about baseline frequencies of the behaviour, and was touched upon in Paper 5, is the individual repeatability of anticipatory behaviour. This had not yet been tested in terrestrial species (Watters, 2014), but with the dolphins’ data we found that anticipatory behaviour was significantly repeatable at the individual level across the 3-month study. Considering the other results from Paper 5 where anticipation levels were also context-dependent, it is likely that individuals show a tendency to conduct either higher or lower levels of anticipatory behaviour, which are nonetheless subject to intra-individual variation.

A major limitation of the schematic is that we cannot be sure about some of the ‘welfare designations’, that is, whether the anticipatory data really taken in situations where the dolphins experienced positive or negative states. With the cognitive bias data of Paper 4, we can be much more confident of the welfare designations due to the fact that we “Triangulated” cognitive and behavioural welfare measures, which is a more accurate approach than using data from a single category (Dawkins, 2006; Webster, 2005). In Paper 5 we did not correlate multidimensional measures: our controlled conditioning paradigm allowed the dolphins to predict enrichment and HAIs, where their anticipation reflected their voluntary participation and thus suggested that they found the two events rewarding. Given that simply the act of anticipating something rewarding can induce positive affective states (Dudink et al., 2006; Kamal et al., 2010; van der Harst and Spruijt, 2007), and that play behaviours (i.e. as seen with enrichment and in HAIs) are thought to be conducted only in the absence of other primary needs (Boissy et al., 2007; Held and Špinka, 2011), a "positive" welfare state was designated to these levels of anticipation (Fig. 17, Table 8). However, the same level of certainty could not be attributed to the affective states of the dolphin anticipating the feeding sessions and so these data from Papers 2 and 5 were not included in the model. In those experiments, many other uncontrolled factors could influence whether individuals were in a generally positive or negative state e.g. training methods, presence of visitors, other environmental cues. These questions regarding the underlying welfare state, which are to be expected since very little is known about dolphin affective states, demonstrate how valuable and essential it is to correlate multidimensional welfare measures wherever possible.

Recently, another angle was proposed from which anticipatory behaviour could be linked to welfare: it could be used as a tool to measure boredom, a phenomenon which has been severely understudied in animals (Burn, 2017). Time seeming to pass slowly is a known indicator of boredom in humans and since anticipation of an event signals a sense of time passing, Burn (2017) suggested anticipatory behaviours and conditioning paradigms could be used to study the presence and implications of boredom in animals. Much future work is needed into anticipatory behaviour, from its stability over time, its utility as a welfare indicator, and the significance of measuring the frequency versus intensity of the activity. With cetaceans, species-specific elements are also likely to influence on the behaviour, for example acoustic signalling (Platto et al., 2015), and it is hoped that the findings here stimulate an increase in such studies and on anticipatory behaviour as a whole.

Thesis findings within a core affect framework

As a result of combining the two most popular theories of emotion and using well-accepted human psychology principles, Mendl and co-authors' (2010) framework to measure animal affective states (Fig. 1) has been well-received in the animal welfare field. It has been applied conceptually to several different species and for different purposes (e.g. in multiple presentations at the Universities Federation for Animal Welfare Symposium, 15-17th May, Egham, UK). Here, I use the framework to display the findings of this thesis in the same domain which allows them to be considered collectively as indicators of dolphin affective states (Fig. 18). Similar to the schematic proposed for anticipatory behaviour and dolphin welfare (Fig. 17), the purpose of this exercise was not to empirically position the results or establish

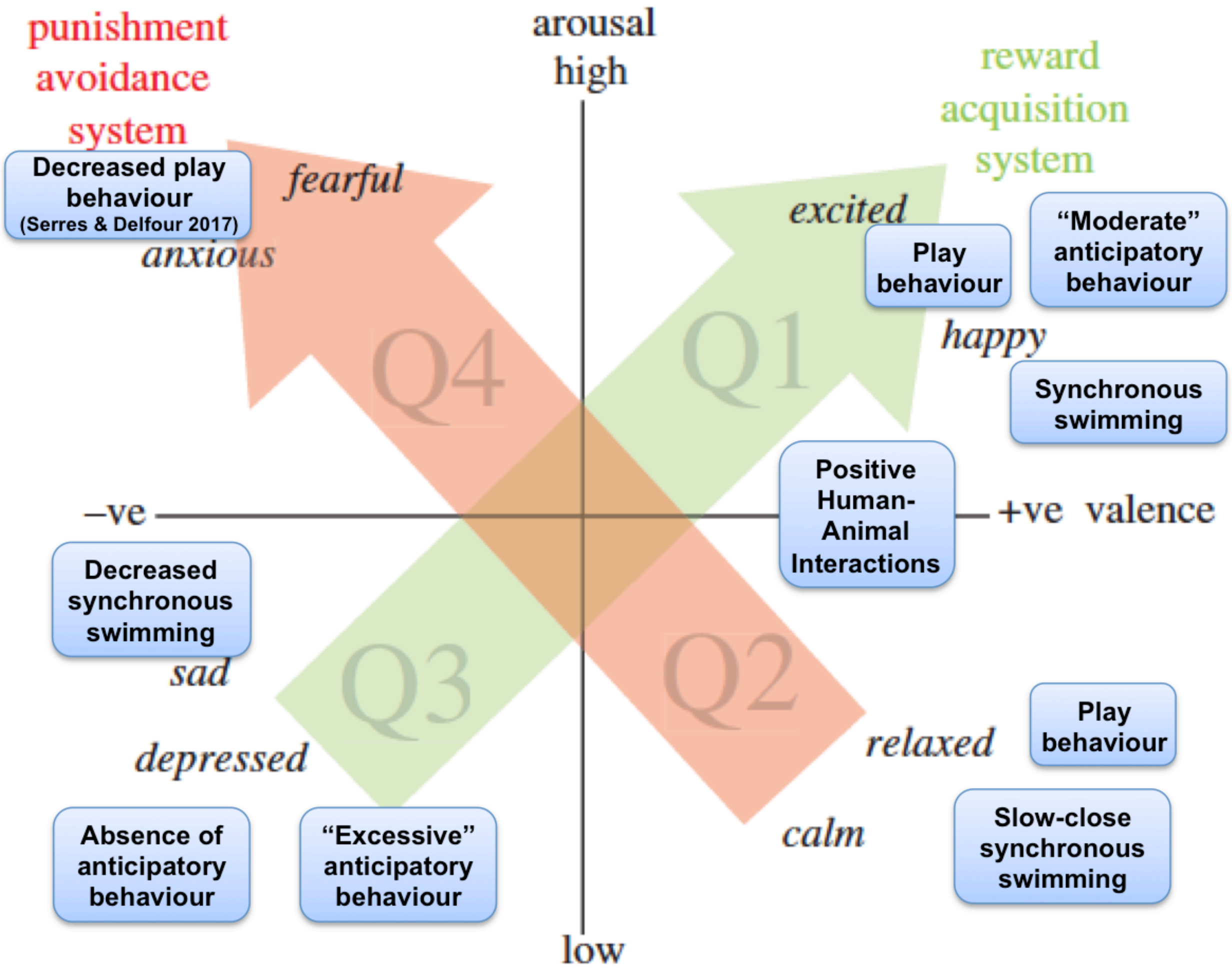


Fig. 18 *Adapted framework of core affect from Mendl and co-authors (2010) showing suggested behavioural indicators (in rectangular boxes) of bottlenose dolphin affective states, based on the results of this thesis (original framework: Fig. 1 in Chapter 1 of this manuscript). See text for full explanations of behavioural indicators. Description taken directly from Mendl and co-authors (2010): words in italics indicate possible locations of specific reported affective states (including discrete/basic emotions). Positive affective states are in quadrants Q1 and Q2, and negative states in quadrants Q3 and Q4. Arrows indicate putative biobehavioural systems associated with reward acquisition and the Q3–Q1 axis of core affect (green), and punishment avoidance and the Q2–Q4 axis of core affect (red).*

absolute values on the valence and arousal axes, but instead to provide a visual summary of my results and what I concluded about them in terms of welfare. The indicators displayed on the adapted framework are discussed in full in the following sections, and represent the main findings of the thesis (with one addition of play behaviour results from Serres and Delfour, 2017, conducted at Parc Astérix). However this framework is by no means comprehensive, and could be used as a ‘working document’ where multidisciplinary indicators could be added and removed as research progresses. The concept of Triangulating (combining) behavioural, health-related and cognitive parameters results in a more accurate and valid evaluation of affective states (Désiré et al., 2002; Paul et al., 2005; Webster, 2005), and while this thesis yielded some behavioural measures, the next steps could be to investigate health, physiological and cognitive indicators of dolphins’ affective states.

Play behaviour

Social play was found to increase in the period in-between training sessions (though only significantly in juveniles), and it was tentatively concluded that the animals may have chosen to play at that time since other needs i.e. hunger and social bonding (the latter of which we found to occur shortly after sessions, Paper 2) were fulfilled. Supporting this, Serres and Delfour (2017) found that play behaviour decreased in bottlenose dolphins when noisy construction work or aggressive social interactions were occurring, presumably because other behavioural needs (i.e. finding safety) were more important to the dolphins. In Paper 5, it was found that the dolphins performed anticipatory behaviour in response to upcoming object and inter-specific play opportunities and, furthermore, that these were highly likely to be rewarding as higher levels of anticipation were correlated to amount of time spent with the object or familiar human. Play behaviour was placed in both quadrants 1 and 2 of the framework (Fig. 18) since performance of play in our studied contexts was always deemed to be positive, but the arousal level of the animals likely varied. The dolphins seemed to perceive play as a rewarding context (Paper 5) and thus it belongs in Q1 as part of the reward acquisition system, but was also present in-between sessions where the animals had no other primary needs to attend to (Paper 2) and so also fits in Q2. Play is a commonly used indicator of positive welfare, but different types of play (object, social, solitary etc) may be linked to slightly different emotions. The thesis findings regarding this differentiation were limited, and further work is needed in bottlenose dolphins as well as other species (Boissy et al., 2007; Held and Špinka, 2011).

Synchronous swimming

On the adapted framework (Fig. 18), it is proposed that slow-close synchronous swimming might be linked to emotions found in Q2, leading to 'calm' or 'relaxed' overall affective states. In contrast it is possible that fast-close synchronous swimming, as a behaviour shown by alliances during defensive or aggressive situations, would indicate emotions in either Q1 or Q4, although this is not shown on the framework since this project's studies did not investigate this. The emotions linked to synchronous swimming at faster speeds would depend on whether the dolphin was ultimately trying to acquire a reward (e.g. mate with a female), in which case the corresponding emotion might be excitement (Q1), or to avoid a punishment (e.g. defence against a predator), where the synchronous swimming would indicate an emotion such as fear (Q4). However, given the lack of dolphin emotion research and that only one previous study has investigated synchronous swimming and included distance to partner as a variable (Connor et al., 2006b), we can only hypothesise that slow-close synchronous swimming is an indicator of positive emotions and must wait for future studies to continue the progress. Nonetheless, the short example above shows that Mendl and co-authors' (2010) framework could indeed serve as an insightful structure for behavioural indicators of emotions, in dolphins at least, and the example with synchronous swimming variations would be applicable to research in both wild and captive contexts.

Slow-close synchronous swimming peaked shortly after food-provision training sessions within the four dolphin groups studied in Paper 2, and it was proposed that, similar to play, after the behavioural need of hunger was satisfied the dolphins engaged in this affiliative behaviour to reaffirm their bonds (Boissy et al., 2007; Connor et al., 2006b). Therefore slow-close synchronous swimming was placed in Q2 where it could be associated with emotions such as 'relaxed', conducive to the low speed of this behaviour (and thus perhaps lower arousal levels of the dolphins). Due to synchronous swimming (without delineations in speed or distance) being correlated to optimistic judgements (Paper 3), the occurrence of this behaviour as a whole was placed in Q1, and decreased levels were thought to be associated with Q3 emotional states, since our cognitive bias paradigm was based on the acquisition of rewards. However, the placement of the decreased levels of synchronous swimming indicator should technically be in both Q3 and Q4; our analysis did not distinguish which of these was more likely to be the case. Such differentiation is possible and has been recommended as a future use of cognitive bias testing in animals, since meticulously analysing the responses to the different ambiguous cues can reveal whether the animal showed enhanced expectation of a negative event (Q4) or a decreased expectation of the positive reward (Q3) (Mendl et al., 2010).

While theoretically it is easy to explain how affiliative behaviours among social, group-living animals are linked to positive emotions and affective states, it is relatively difficult to demonstrate experimentally (Boissy et al., 2007). Synchronous swimming in bottlenose dolphins is an indicator of social bonds and serves to reinforce them while also serving an adaptive role in terms of maintaining group cohesion for hunting, defence and social interactions (Connor et al., 2001; Duranton and Gaunet, 2016; Fellner et al., 2013). Emotions in themselves are adaptive as well, functioning to drive the individual's behaviour towards seeking rewards and avoiding threats (Boissy et al., 2007). Therefore in dolphin populations we can imagine that positive emotions might have evolved in association with behaviours that enhance group cohesion, and the cognitive bias testing allowed us to suggest that synchronous swimming is indeed linked to more positive affective states in the study group of dolphins. We also went a step further and took behavioural data from the 4 months preceding the bias tests, in order to investigate the persistence of such proposed affective states and question whether the correlation of bias and synchronous swimming might represent more of a stable behavioural tendency. This question is often posed by researchers in the field (Roelofs et al., 2016), since there are a few studies that suggest cognitive bias may be linked to personality traits (Cussen and Mench, 2014; Gordon and Rogers, 2015), but there are also others who find no effect (Lalot et al., 2017). In our cognitive bias investigation, we thus conducted a second analysis using synchronous swimming frequencies from 0-2 and 2-4 months before the test, applying the same approach as for the behavioural data taken in the present. We found that in the 0-2 months before the test, a higher synchronous swimming frequency significantly predicted more optimistic biases in the test, but that this association disappeared when using data from 2-4 months before the test. This suggests that a transitory phenomenon, persisting from a few weeks to months, was causing the differences in dolphins' biases. The association between synchronous swimming and bias is unlikely to be due to personality factors in this case, as then we would have expected to see a significant correlation when using 2-4 month-old data. This additional, longer-term data indicates that group dynamics and social standings, which presumably last from between weeks to months, were more likely to have driven the affective states experienced by the animals and influenced their judgements (i.e. positive social relations that led to more optimism). This is in line with current literature discussing the duration of affective states, and the role of social behaviour in overall welfare (Boissy et al., 2007; Mendl et al., 2010). However, given our inability to determine the causal relationship and that an individual's performance of synchronous swimming is likely dependent on others' behaviour in the group, we do not conclude here that dolphins' personality-like traits do not influence cognitive bias or indeed synchronous swimming tendencies: further work is needed to investigate this. Synchronous swimming, as a behaviour conducted between one or more conspecifics, showcases the potential role of emotional contagion and transfer in establishing affective states and thus in welfare measurement (Held and Špinka, 2011; Špinka, 2012). Positive feedback of emotions between partners conducting such behaviours is thought to occur, reinforcing affiliative bonds, but so far few studies exist in any species (Špinka, 2012).

Anticipatory behaviour

The acquisition of rewards was labelled as one of the two biobehavioural systems that drives the manifestation of affective states (Mendl et al., 2010), and anticipatory behaviour is a key component in reward acquisition (Spruijt et al., 2001; Watters, 2014). The studies in this thesis concerning anticipatory behaviour in bottlenose dolphins (Papers 2, 4 and 5) allowed us to propose a relationship between this activity and welfare (Fig. 17). This is extremely useful for plotting where anticipatory behaviour might be placed on the adapted framework of core affect (Fig. 18). Three designations are used for three indicators in this framework: "moderate", "excessive" and the absence of anticipatory behaviour. These are intended to reflect to the frequencies of anticipatory behaviour correlating to positive welfare, negative welfare, and chronic stress as shown in Fig. 18 and supported by Watters' (2014) original model (Fig. 16). As has been discussed in the previous section, a certain ("moderate") level of anticipatory behaviour, such as that shown by the dolphins before toy enrichment, HAIs and training sessions, might be linked to positive emotions associated with reward acquisition, so this indicator was placed in Q1 (e.g. "excited", "happy" emotions). "Excessive" anticipatory behaviour, such as those levels shown by the dolphins that also judged more pessimistically, is therefore likely an indicator of emotions in Q3. Animals in such negative states are much more focussed on the reward's arrival since they have fewer other rewarding experiences available in their current environment (van den Berg et al., 1999; van der Harst et al., 2003a,b). With the dolphins, this may have manifested as having less access to positive social interactions. Although we did not find any evidence of chronic stress in our studies, Watters' (2010) model and my adapted version (Fig. 17) are used to propose that the absence of anticipatory behaviour in dolphins would be an indicator of an extremely negative and chronic Q3 state, where it is suggested the animals enter a state of apathy and anhedonia (van der Harst and Spruijt, 2007; Watters, 2014).

There is much to learn about the utility of anticipatory behaviour within this type of framework: for example, human psychology studies have suggested that reward sensitivity “may underlie individual predispositions to particular mood states” (Corr, 2004; Mendl et al., 2010). This means that anticipatory behaviour could potentially indicate longer-term moods, and not just shorter-term affective states. To conclude their theories of core affect in animals, Mendl and co-authors (2010) also link optimistic and pessimistic decision-making with the prevailing environmental conditions and core affective states (overall welfare) and suggest that moods may influence cognitive biases differently to shorter-term affective states. Since we tested cognitive bias with the dolphins (Papers 3 and 4), our results could be learned from and built upon in terms of measuring longer-term affective states in this species.

Lastly, although we can spend time considering these thought-provoking frameworks and schematics, it goes without saying that the projections are based on moderate sample sizes, many of the specifics are as yet unsubstantiated, and much more work is needed to validate anticipatory and the other behaviours as reliable indicators of bottlenose dolphin affective states.

Overall welfare assessment in practice

Mendl and co-authors’ (2010) framework and discussion highlights *theoretically* how emotions, affective states and longer-term “free-floating” moods might interact to impact core affect i.e. overall welfare. The underlying common denominator, as shown by the systems (arrows) in the framework (original: Fig. 1; my adapted version: Fig. 18), is that core affect is always linked to the rewards and threats present in the animal’s environment (Mendl et al., 2010). It is with this information that we can progress from the theoretical domain towards practical welfare assessments in the environments that they inhabit. Of course, we can use such theoretical frameworks to input different indicators and determine the different affective states the animal might be experiencing, and thus make predictions about its welfare state at a certain point in time. But, it is more likely that comprehensive assessments which collect longer-term data using both animal and resource-based measures, i.e. some associated with emotional states and some indirect measures of the environment, will help us to improve the welfare of captive animals (Roe et al., 2011; Veissier et al., 2008).

Measuring an animal's overall welfare is often the ultimate goal for those managing and regulating animal-use industries since such information can be used to maximise efficiency, attract consumers, and benefit the individual animals. Welfare assessments are groups of separate, multidimensional measures (Veissier et al., 2012; Whay et al., 2003) and are not yet established in zoo settings (Bloomsmith, 2009), although there has been one proposal for an assessment for bottlenose dolphins (Clegg et al., 2015). While not yet tested in many groups and over time, the measures in Clegg and co-authors' C-Well© Assessment are being validated as the studies increase in this field (e.g. Paper 5 of this thesis endorses positive HAIs as a rewarding situation for the dolphins). An often-cited concern with measuring welfare is the time-frame, where taking data at one point in time using a few measures might mean that a significant problem is missed due to time limitations (Roe et al., 2011). Farm animal research has shown that well-designed holistic assessments can combat this through being "strategically redundant" (term from Clegg et al., 2015) i.e. a welfare issue has the opportunity to be identified in multiple measures during the assessment. The results from the welfare-based studies in this thesis may also have been limited by time, for example the lack of correlation between social play and cognitive bias in Paper 3 as discussed earlier. Furthermore, the potential behavioural indicators of welfare found here (play, synchronous swimming, anticipatory behaviour) can only be measured in different time-frames themselves. To progress past this seemingly difficult obstacle in welfare research, we should first accept that although there exists an overall welfare state of an individual human or animal at any moment in time (Mendl et al., 2010), without a shared language we are unlikely to be able to determine what this is for animals instantaneously. So, to try and assess the welfare of an individual animal accurately (Barber, 2009), we can collect data based on the animal as well as its environment (Veissier et al., 2008), with multidimensional measures covering many aspects of their life (Pritchard et al., 2005), and sometimes repeating welfare measures if the original time-frame is inadequate (Clegg et al., 2015).

The concept of integrating many measures to be able to capture a certain welfare issue spurred the establishment of the final project of this thesis, where qualitative ratings of dolphin motivation during training sessions were collected with basic health and social behaviour data to investigate how changes in welfare manifest over time, and how this might be revealed in the chosen measures. Qualitative rating approaches such as QBAs are a method for confirming face validity specifically and are intended to encompass the holistic and multidimensional nature of an animal's response to its environment (Stockman et al., 2011; Wemelsfelder et al., 2012), and so this final study's goal was to test their utility for the first time in dolphin welfare research. It is hoped that the results of this multi-facility study will reveal the links between behavioural and health-related measures of welfare, and stimulate progress towards the application of standardised tools to measure overall welfare in captive dolphin facilities.

Future directions

The novelty of dolphin welfare as a subject area means that there are countless future channels of research worth exploring. The approach for this thesis was to focus strongly on behavioural studies and what they could reveal, but other studies could and should use other disciplines e.g. health, cognition and personality research, as their foundations. Our behavioural investigations revealed some potential welfare measures for captive dolphins, chiefly synchronous swimming, anticipatory behaviour and play but, as discussed above extensively, these should be studied further in many facilities and in more detail. We were able to correlate behavioural and cognitive measures together in the cognitive bias studies, but apart from that our time and sample size constraints limited our attempts to correlate different types of measures together. Further, multidisciplinary research will continue the progress towards identifying indicators of emotions and affective states, and ultimately overall welfare (Fig. 2: breakdown of overall welfare).

There are elements from this thesis with bottlenose dolphins for which our data on novel welfare concepts could inform studies on farm or other terrestrial species, e.g. the finding that anticipatory behaviour predicted levels of reward participation, and that undomesticated animals raised by humans anticipated interactions with them (Paper 5). Positive HAIs have been shown to promote positive emotions and improve welfare in many domesticated species (e.g. Handlin et al., 2011; Schmied et al., 2008; Waiblinger et al., 2006), but our findings provide another model for research on HAIs and the domestication process, which up until now has almost solely focussed on comparing domestic dogs (*Canis familiaris*) and wolves (*C. lupus*) (Anderson and Serpell, 1996; Topál et al., 2005). With dogs, inter-specific play is suggested to reflect the quality of the relationship with the human, but that this is an adaptive trait selected for over the domestication process (Bradshaw et al., 2015). While very different to other zoo animals' interactions with their keepers, the positive reinforcement training and close, regular human contact which is common with captive dolphins seems to foster positive HAIs, which in themselves have much potential as welfare indicators and enhancers (Bloomsmith et al., 2003; Brando, 2012; Laule et al., 2003). As the facilitator of these findings concerning Trainer-Dolphin interactions, anticipatory behaviour should be much further investigated in order to reach its potential as a welfare assessment tool: for example, the proposed model linking anticipatory behaviour and welfare (Fig. 17) could be further tested and developed. Although only touched upon in the above sections, the ability to anticipate a reward or event is closely linked with the concept of increased control over the environment, something which is thought to increase welfare in terrestrial species (Bassett and Buchanan-Smith, 2007; Dudink et al., 2006) and likely also in captive dolphin species (Brando et al., 2016). There is much to be further investigated regarding how HAIs, anticipatory behaviour and control over the environment can influence dolphin welfare.

Over the course of the thesis, some health-related and cognitive measures of welfare were investigated such as breathing frequency, health status as scored by veterinarians, and cognitive bias. But, due to limited time and moderate sample sizes, these parameters (especially physiological measures) did not take precedence. Thus we propose that an effective approach for future dolphin welfare studies would be to use the near-validated behavioural measures found here to identify corresponding health/physiological and cognitive parameters. For example, if the validity of cortisol measurement was being investigated, levels of the hormone (which can be measured from blood, faeces, urine or saliva, Atkinson et al., 2015) could be correlated to the occurrence of social affiliative or anticipatory behaviour, as this might explain some variation. Cognitive bias testing is one of the few available validated tools to indicate affective states in animals (Mendl et al., 2010), and since our adapted test was very feasible and yielded valuable results with the dolphins, similar methods should be applied further to cetaceans and in a variety of contexts.

The studies conducted in this thesis show that, in agreement with the literature (Barber, 2009; Whitham and Wielebnowski, 2013), research into zoo animals' welfare is indeed possible and fruitful in terms of basic and applied science. In general it has been highly recommended that more zoos collaborate with ethologists and welfare scientists to conduct such studies as were completed here (Hopper, 2017; Maple, 2007). A noteworthy difference at the present time between dolphins and other zoo animals is their "trainability": the fact that the bottlenose dolphins in our studies were habituated towards and responded so well to positive reinforcement training (Laule et al., 2003) meant that this was an invaluable tool for conducting the more complex studies (Brando et al., 2016) , such as the cognitive bias testing. Our experiments have directly addressed specific recommendations that the trainability of captive cetaceans should be harnessed to study their cognition as well as provide stimulation and enrichment for the animals themselves (Brando et al., 2016; Maple and Perdue, 2013). It is also possible that the animal-based measures of dolphin welfare proposed here could be used to evaluate the impact of specific resources or management decisions on these animals. This research should be multi-institutional to be able to control for the variation found between programs, such as in Baird and co-authors' (2016) study where they used behaviour and glucocorticoid levels to evaluate the impact of education programs on a number of zoo species within many facilities; the same questioning could be applied to dolphins facilities using the animals' behaviour and participation in shows/guest interactions.

A new branch of work that, in many cases, has been facilitated by captive animal welfare research is the application of welfare science principles to wild animals (Jordan, 2005; Ohl and van der Staay, 2012). Recently, experts have specifically recommended that welfare assessments of wild marine mammals take place in order to better understand the anthropogenic and other effects on individual animals (Butterworth, 2017; Butterworth et al., 2012; Papastavrou et al., 2017). Therefore, although this was not a primary aim of the overall project, our findings could also be used to support welfare research on wild dolphins. For example, measuring the frequency of synchronous swimming is relatively simple as it can be completed from afar and from above or below water, and thus could be applied as a measure of social cohesion in the group. If further studies elucidate the meaning of differences in proximity and speed for this behaviour, it could be used as an even more specific measure of an individual's affective state. Wild dolphins' affective states and moods are just as likely to vary with the strength of social relationships, as our findings with captive dolphins suggest, and thus a measure like synchronous swimming could potentially reveal the animals' emotions towards anthropogenic events such as the presence of whale-watching boats.

General conclusion

Welfare science is a recently established discipline which aims to reveal the core affective states of animals, but which had not yet been applied to dolphins. Using bottlenose dolphins as the study species, this thesis aimed to develop the first objective indicators of welfare. "Dolphin welfare" was a phrase very rarely used in the literature, but as a result of the review and empirical studies conducted here the foundations for the field are underway. Validated tools used in terrestrial animal welfare research were applied here: chiefly cognitive bias testing and a Pavlovian conditioning paradigm used to study anticipatory behaviour. Synchronous swimming, an affiliative social behaviour of dolphins, was identified as a likely measure of positive emotions/affective states due to its association with optimistic judgements during cognitive bias testing. We showed for the first time that anticipatory behaviour predicts the level of participation in the upcoming event and, since the dolphins performed most anticipatory behaviour before positive, non-alimentary Human-Animal Interactions (HAIs), we proposed that the animals place intrinsic value on these inter-specific interactions and find them rewarding. The cognitive bias study enriched the analysis of anticipatory behaviour: the dolphins who performed higher levels of anticipatory behaviour before training sessions also made more pessimistic judgements (linked to negative affect). This finding agrees with the reward sensitivity theory, which proposes a non-linear relationship between anticipatory behaviour and welfare, where excessive anticipation reflects negative affective states. We suggested a model that applies this theory to the dolphins' data, thus proposing a relationship between reward sensitivity and dolphin welfare and demonstrating that anticipatory behaviour may have great potential as a non-invasive welfare parameter. The lack of previous dolphin welfare studies meant that, although the studies in this thesis were able to identify potential measures of emotions and some affective states, more research is needed before the dolphins' overall welfare can be more accurately assessed. The discussion suggests important lines of future research, such as cross-validating the behavioural measures found here with physiological indicators of affective state, and further investigating the intriguing finding that dolphins, as an undomesticated species, seem to significantly value positive HAIs. In regard to the applications of this thesis, the ultimate goal should be to develop a comprehensive welfare assessment for bottlenose dolphins as this is the most accurate way to measure overall welfare. Despite constraints with sample sizes and studying the animals out of a laboratory environment, this project has reinforced the notion that welfare research on dolphin species is feasible and can yield results which are valuable to managers, scientists, regulators and the general public. The inherent subjectivity of welfare will forever remain a caveat in this discipline, but well-designed studies and conservative conclusions such as those generated within this thesis can only enhance our knowledge, helping us to better protect and improve the lives of the animals we are responsible for.

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Figure and Table legend

Figures and Tables are labelled continuously throughout the thesis and their details are shown in this legend.

Declaration

I, Isabella Clegg, confirm that the content of this thesis is the result of my personal endeavour. I confirm that any data, reasoning and conclusions sourced from existing literature are reported exactly as they were found and are cited in full in the References. Finally, I confirm that this thesis has never been reviewed nor published elsewhere.

Isabella Clegg

Isabella Clegg
Paris, 25th July 2017

Abstract

Developing welfare parameters for bottlenose dolphins (*Tursiops truncatus*) under human care

Résumé

La science du bien-être animal est une discipline bien établie qui permet de faire des mesures objectives. Les grands dauphins (*Tursiops truncatus*) sont une espèce de cétacés communément présente en captivité, et bien que des questions se posent sur la qualité de leur vie dans cet environnement, très peu d'études ont porté sur la mesure objective de leur bien-être. Cette thèse répond à ce manque de données en développant des indicateurs de bien-être basé sur l'animal, ici le grand dauphin. Une revue bibliographique initiale a identifié des mesures potentielles de bien-être, avant que des indicateurs comportementaux choisis aient été mesurés par rapport aux sessions d'entrainement. Un test de biais de jugement a alors été adapté aux dauphins, où des biais optimistes ont été significativement liés aux fréquences les plus hautes de nage synchronisée durant leur 'temps libre' et aux fréquences les plus basses de comportement anticipatoire avant les sessions d'entrainement. Une avant dernière étude a montré que le comportement anticipatoire a prédisait la participation à l'événement à venir, et que des Interactions Humaines-Animales positives étaient anticipées plus que l'introduction de jouets. Une dernière expérience en cours a développé un protocole standardisé pour mesurer la motivation des dauphins pendant des sessions d'entrainement par rapport aux problèmes de bien-être sociaux et de santé. Bien que le bien-être global soit toujours difficile à mesurer, cette thèse propose des premières mesures d'émotions et d'états affectifs chez le dauphin. La nage synchronisée est un indicateur probable d'émotions positives, bien que plus de recherches doivent examiner la variabilité entre divers contextes. Le comportement anticipatoire semble témoigner de la motivation pour des événements à venir et nous suggérons qu'il reflète une sensibilité à la récompense comme chez d'autres animaux: des travaux ultérieurs portant sur des seuils de fréquence le transformerait en indicateur de bien-être pertinent. Un objectif majeur de la thèse est de stimuler plus de recherches sur des mesures de bien-être chez des grands dauphins et d'autres espèces de cétacés en captivité.

Mots-clés : biais cognitif, bien-être animal, émotions, états affectifs, mesures à base d'animal, grands dauphins

Abstract

Welfare science is now an established discipline which enables objective measurements of animal welfare to be made. Bottlenose dolphins (*Tursiops truncatus*) are a common cetacean species kept in captivity, and although questions are arising over their quality of life in this environment, very few studies have focussed on objectively measuring their welfare. This thesis aimed to address this lack of data by developing animal-based indicators of bottlenose dolphin welfare. An initial review identified potential dolphin welfare measures, before selected behavioural indicators were measured in relation to training sessions. A judgement bias test was then adapted to dolphins, where optimistic biases were significantly linked to higher frequencies of synchronous swimming in their 'free-time' and lower frequencies of anticipatory behaviour before training sessions, (concurring with the reward-sensitivity theory). A penultimate study showed that anticipatory behaviour predicted participation in the upcoming event, and positive Human-Animal Interactions were anticipated more than access to toys. A final, on-going experiment has developed and applied a standardised protocol for measuring dolphins' motivation during training sessions in relation to social and health-related welfare problems. Although overall welfare is still difficult to measure, this thesis has proposed some first measures of dolphin emotions and affective states. Synchronous swimming is a likely indicator of positive emotions and social support, although more research should investigate variability between contexts. Anticipatory behaviour seemed to indicate motivation for events, and we suggest it reflects reward sensitivity as in other animals: further work into frequency thresholds would render it a valuable welfare indicator. A major objective of the thesis is to stimulate more research on welfare measures for bottlenose dolphins and other cetacean species in captivity.

Key words: affective states, animal-based measures, animal welfare, bottlenose dolphins, cognitive bias, emotions

Discipline: Ethology

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Appendix

A. Publications of the candidate

1. Peer-reviewed journals and books

Clegg, I. L. K. & Delfour, F. (2017). Cognitive judgement bias is associated with frequency of anticipatory behaviour before training sessions in bottlenose dolphins. *Submitted to Zoo Biology.*

Clegg, I. L. K., Rödel, H. G., Boivin, X. & Delfour, F. (2017). Looking forward to interacting with familiar humans: dolphins' anticipatory behaviour indicates their motivation to participate in specific events. *Submitted to Applied Animal Behaviour Science.*

Clegg, I. L. K. & Butterworth, A. (2017) Chapter 12: Assessing the welfare of Cetacea. In (A. Butterworth, ed) *Marine Mammal Welfare: Human induced change in the marine environment and its impacts on marine mammal welfare*, pp. 183-205. ISBN 978-3-319-46994-2. Springer International Publishing AG, Cham, CH.

Clegg, I. L. K. & Butterworth, A. (2017) Chapter 16: Assessing the welfare of Pinnipeds. In (A. Butterworth, ed) *Marine Mammal Welfare: Human induced change in the marine environment and its impacts on marine mammal welfare*, pp. 273-295. ISBN 978-3-319-46994-2. Springer International Publishing AG, Cham, CH.

Clegg, I. L. K., Rödel, H. G., Cellier, M., Vink, D., Michaud, I., Mercera, B., Böye, M., Hausberger, M., Lemasson, A. & Delfour, F. (2017). Schedule of human-controlled periods structures bottlenose dolphin *(Tursiops truncatus)* behavior in their free-time. *Journal of Comparative Psychology.* Early view, doi: 10.1037/com0000059

Clegg, I. L. K., van Elk, C. E., & Delfour, F. (2017). Applying welfare science to bottlenose dolphins *(Tursiops truncatus). Animal Welfare, 26,* 165-176*.*

Clegg, I. L. K., Rödel, H. G. & Delfour, F. (2017). Bottlenose dolphins engaging in more social affiliative behaviour judge ambiguous cues more optimistically. *Behavioural Brain Research, 322,* 115-122.

2. Papers not discussed in thesis and published during the time-frame

2. i. Clegg, I. L. K., Borger-Turner, J. L., & Eskelinen, H. C. (2015). C-Well: The development of a welfare assessment index for captive bottlenose dolphins (*Tursiops truncatus*). *Animal Welfare, 24*(3), 267-282.

Abstract

The field of welfare science and public concern for animal welfare is growing, with the focus broadening from animals on farms to those in zoos and aquaria. Bottlenose dolphins (*Tursiops truncatus*) are the most common captive cetaceans, and relevant regulatory standards are principally resource-based and regarded as minimum requirements. In this study, the farm animal WelfareQuality® assessment was adapted to measure the welfare of bottlenose dolphins, with a similar proportion of animal-based measures (58.3%). The 'C-Well®' assessment included eleven criterion and 36 species-specific measures developed in situ at three marine mammal zoological facilities, tested for feasibility and accuracy, and substantiated by published literature on wild and captive dolphins and veterinary and professional expertise. C-Well® scores can be calculated for each measure or combined to achieve an overall score, which allows for the comparison of welfare among individuals, demographics, and facilities. This work represents a first step in quantifying and systematically measuring welfare among captive cetaceans and can be used as a model for future development in zoos and aquaria, as well as a means to support benchmarking, industry best practices, and certification.

2. ii. Clegg, I. L. K., Borger-Turner, J. L., & Eskelinen, H. C. (2017). Measuring Cetacean Welfare. *Soundings- Magazine of the International Marine Animal Trainers' Association.* Volume 42, No. 2

2. iii. Clegg, I. L. K. & Butterworth, A. (2017) Chapter 12: Assessing the welfare of Cetacea. In (A. Butterworth, ed) *Marine Mammal Welfare: Human induced change in the marine environment and its impacts on marine mammal welfare*, pp. 183-205. ISBN 978-3-319-46994-2. Springer International Publishing AG, Cham, CH.

Abstract

Most of the species from the order *Cetacea* appear to possess advanced cognitive abilities and close social networks, and are also likely to experience different affective states comprising of more than just basic emotions. Welfare describes a balance of positive and negative affective states experienced by an individual, and this balance is a good indicator of how it perceives the surrounding environment. In this chapter, we discuss how the first steps in cetacean welfare science are being taken to establish this as a discipline. We discuss how there are pertinent areas of cetology that merit investigation to form the basis of possible cetacean welfare measures. In this arena of welfare assessment, much of the existing work comes from farm animal science, and this previous work also offers potential tools and techniques, which could be adapted for cetaceans. In this chapter we review these sources of information, make suggestions for potentially relevant investigations, and discuss how assessment of cetacean welfare might be accomplished.

2. iv. Clegg, I. L. K. & Butterworth, A. (2017) Chapter 16: Assessing the welfare of Pinnipeds. In (A. Butterworth, ed) *Marine Mammal Welfare: Human induced change in the marine environment and its impacts on marine mammal welfare*, pp. 273-295. ISBN 978-3-319-46994-2. Springer International Publishing AG, Cham, CH.

Abstract

Recent collaborative and independent studies on sea lions, seals and walruses have advanced our knowledge and sustained interest in pinniped welfare. Nevertheless published discussions of the welfare of pinnipeds, and secondly of potential measures to assess their welfare, are, respectively, very few and non-existent. This chapter aims to make first steps in the discussion on assessing pinniped welfare, with the goal of stimulating future welfare investigation. Pinniped species are able to thrive in two opposing environments, the land/ice margin at the coast, and in the sea, and these animals use these two 'domains' for different functions. Welfare measurement is concerned with the outcome of an animal's internal and external responses to its environment, and pinniped species' evolutionary biology may be especially important in this respect, in terms of our understanding of the animals' responses and interactions within their two domains. Pinnipeds are being directly impacted by serious anthropogenic disturbances in the wild, including human interference at established feeding and breeding grounds, hunting, entanglement and climate change, and are also often kept in captive collections. Feasible evaluations of welfare can therefore be assumed to have potential widespread utility, including applications benefitting the animals themselves.

3. Oral presentations and posters

3. i. Presentations

Clegg, I. L. K., Mercera, B., Van Elk, C. E., Rödel, H. G. & Delfour, F. (2017, May). *Bottlenose dolphins conducting more social affiliative behaviour made more optimistic judgements of ambiguous cues.* Presented at the 47th Société Francaise pour l'Etude du Comportement Animal (SFECA), Gif-sur-Yvette, France.

Álvarez I.*, Martín Y., Clegg I. L. K., López-Béjar M., Monreal Pawlowsky T. & Almunia J. (2017, March). *Multi facility study to determine a salivary cortisol baseline in Tursiops truncatus*. European Association of Aquatic Mammals 45th Conference, Genova, Italy.

Clegg, I. L. K., Rödel, H. G., Mercera, B., Van Elk, C. E. & Delfour, F. (2017, March). *Are all dolphins really smiling? More social dolphins judged ambiguous cues more optimistically.* Presented at the 45th European Association of Aquatic Mammals Conference, Genova, Italy.

Clegg, I. L. K. (2016, November). "Grand dauphins dans des Delphinariums". Presented to the Club de Robotique, Espace Multimédia. 5 Rue Paul Demange, Meudon la Foret.

Clegg, I. L. K. (2016, May). Keynote speaker: "The C-Well® assessment as a dolphin welfare framework and Conducting research to find objective measures of dolphin welfare". At Nuremberg Zoo, in collaboration with the European Association for Aquatic Mammals, European Association for Zoos and Aquariums, and Verband der Zoologischen Gärten (VdZ), and the European Commission Directorate General for Health and Food Safety: "Scientific and Practical Development of Objective Indicator Approach to Assessment of Welfare of Marine Mammal Species in Zoological Parks". 4 May 2016.

Clegg, I. L. K., Rödel, H. G., Vink, D., Michaud, I., Cellier, M., Mercera, B., Böye, M., van Elk, C., Hausberger, M., Lemasson, A. & Delfour, F. (2016, March). *Schedule of training sessions affects specific dolphin behaviours.* Paper presented at the European Association of Aquatic Mammals 44th Conference, Benidorm, Spain.

Alvarez I*, Martin Y, Clegg, I. L. K., Lopez-Bejder M, Monreal-Pawlkowsky T, Alumnia J (2016, March). *Multi facility study to determine a salivary cortisol baseline in Tursiops truncatus.* Paper presented (by I Alvarez) at the European Association of Aquatic Mammals 44th Conference, Benidorm, Spain.

Clegg, I. L. K., Borger-Turner, J. L., and Eskelinen, H. C. (2015, September). *C-Well: The development of a welfare assessment index for captive bottlenose dolphins* (*Tursiops truncatus*). Paper presented at the 43rd annual conference of the International Marine Animal Trainers Association, Nassau, Bahamas.

Clegg, I. L. K., Rödel, H. G., Van Elk, C. E., Mercera, B., Delfour, F. (2015, April) Developing measures for dolphin welfare. In V. Deiss (Chair) *Conférence Enrichissement du Milieu d'Elevage, Comité d'Ethique du Institut Nationale de la Recherche Agronomique,* Clermont-Ferrand, France.

Clegg, I. L. K., Rödel, H. G., Van Elk, C. E., Mercera, B., Delfour, F. (2015, April) *Measuring dolphin welfare*. Presented at the Ecole Nationale Véterinaire de Toulouse, France.

Clegg, I. L. K., Rödel, H. G., Van Elk, C. E., Mercera, B., Delfour, F. (2015, March). *The science behind dolphin welfare.* Paper presented at the European Association of Aquatic Mammals 43rd Conference, Kolmarden, Sweden.

3. ii. Posters

Clegg, I. L. K., Rödel, H. G., Van Elk, C. E., Mercera, B., Delfour, F. (2017, June). *Animal-based welfare measures in zoos: the case of captive dolphins*. Poster to be presented at the Universities Federation for Animal Welfare (UFAW) symposium "Measuring animal welfare and applying scientific advances - why is it still so difficult?". 27th-29th June 2017, Royal Holloway, University of London, Surrey, UK.

Clegg, I. L. K., Rödel, H. G., Van Elk, C. E., Mercera, B., Delfour, F. (2017, June). *Difficulties in interpreting the first indicators of positive welfare in dolphins*. Poster to be presented at the Universities Federation for Animal Welfare (UFAW) symposium

"Measuring animal welfare and applying scientific advances - why is it still so difficult?". 27th-29th June 2017, Royal Holloway, University of London, Surrey, UK.

Clegg, I. L. K., Rödel, H. G., Vink, D., Michaud, I., Cellier, M., Mercera, B., Hausberger, M., Lemasson, A. and Delfour, F. (2015, October). *Variation in synchronous swimming in bottlenose dolphins (Tursiops truncatus) under human care.* Poster presented at the annual Journées du Groupement de Recherche en Ethologie (GDR) et de l'Institut Francilien d'Ethologie (IFE), Villetaneuse, France.

Clegg, I. L. K., Rödel, H. G., Van Elk, C. E., Mercera, B., Delfour, F. (2015, April). *Developing measures for dolphin welfare.* Poster presented at the annual conference for the Société Francaise pour l'Etude du Comportement Animal (SFECA), Strasbourg, France.

3. iii. Invited attendee

"Reflexions sur des rencontres entre des animaux et des humains : mediations, parc de loisir, laboratoire". Dans le cadre de l'action de recherche "Humanité", Université Paris Ouest Nanterre, 2nd November 2015.

"Le bien-etre animal de la science au droit". Colloque organisée par La Fondation Droit Animal, Éthique et Sciences (LFDA). UNESCO, 10 and 11 December 2015.

4. Non-refereed first author articles

4. i. “Synchronised swimming makes dolphins more optimistic”. The Conversation. 21st February 2017. Written by Isabella Clegg, edited by Clea Chakraverty. https://theconversation.com/synchronised-swimming-makes-dolphins-more-optimistic-73209

Synchronised swimming makes dolphins more optimistic

21 février 2017, 08:46 CET

Isabella Clegg
PhD student in Dolphin Behaviour and Welfare, Université Paris 13 – USPC

Some people say the glass is half-empty, some say it’s half-full – but can animals also be optimistic or pessimistic?

Recent studies show that certain animals do make more positive or negative judgements depending on the situation and their emotional state, just like humans. This phenomenon is called cognitive bias.

Cognitive bias is present in many aspects of our lives, whenever we make decisions about events with an unknown outcome. It has been shown that our current emotional state can influence whether decisions are more positive or negative in nature: either we expect the best or prepare for the worst.

Thanks to recent cognition research, we can test this in animals by training them in a judgement task.

Measuring optimism and pessimism

A judgement task works like this: first the animal is taught what will happen when certain cues appear.

For instance, if we place a bowl in the left-hand corner of a room, it means they will receive a big reward. When the bowl is in the right-hand position, this means the animal gets no reward, or something bad will happen (for example, a loud sound is played). Logically, the animal will run faster towards the positive cue and much slower towards the negative cue.

After this priming, the bowl is placed in the middle of the room. If an animal still runs fast to the bowl, it is thought to be more "optimistic", since it expects something positive to occur from an unknown event.

Past studies involving many species (for example rats, dogs and bees) have used this approach and shown that animals in poorer welfare situations, such as those in barren cages, or those subjected to veterinary exams or social isolation, make more pessimistic judgements. Those in enriched environments make more optimistic judgements.

These experiments lead scientists to believe that cognitive bias testing is a valid way to discover the emotional state of the animal. However, these tests had never been applied to captive dolphins before.

Optimistic dolphins

At the Parc Astérix dolphinarium in France, I led a study to find out whether dolphins also had cognitive biases, and what might influence them.

We taught the parks' eight dolphins to touch a target and return to their trainer. The dolphins then learnt that if the target was presented on one side of the pool, they would get a big herring (their favourite fish). If the target was on the other side of the pool, they would receive only applause and eye contact from the trainer.

The dolphins were soon swimming faster when the target was in the "herring position". It was then placed in the middle position and we measured the level of optimism of each dolphin by their swimming speed as they returned to the trainer. Those swimming faster back to the trainer were thought to be more optimistic as they are were probably expecting to receive a herring, while the slower swimmers were not as hopeful about getting a reward.

The results showed that indeed, the dolphins had different levels of optimism and pessimism, which remained the same over repeated days of testing.

But the most interesting discovery came when we compared the cognitive bias with individual observations of behaviour taken in the dolphins' "free-time", in between the sessions.

In both the wild and captive environments, dolphins engage in social behaviour. Swimming in synchrony is thought to be an important affiliative behaviour which reinforces the bonding between individuals.

In the park, we observed that those dolphins who swam in synchrony more often were also the ones who made the most optimistic decisions. For example, a 16-year-old female dolphin was seen very often swimming in synchrony with other partners, especially her mother, and during the judgement tests she swam the fastest back from the middle target, thus making an optimistic judgement.

As highly social animals, this isn't entirely surprising, but the link between optimism, positive emotions and social behaviour has proved difficult to measure so far. Positive social behaviour is an adaptation that is thought to help the dolphins survive in the wild, for example in the cooperative hunting behaviours seen in Florida.

Sociability and emotions

The findings of the cognitive bias study suggest that synchronised swimming is linked to positive emotional states, which for the first time gives us an insight into the emotions linked to dolphins' social interactions.

Intrigued by the results, our team went one step further and compared optimism levels to the social behaviour seen in the four months preceding the test. We had taken daily observations of the dolphins' social behaviour, and measured the amount of time they spent swimming synchronously during the weeks before the test.

We found that the most optimistic dolphins were also those who had performed most synchronised swimming in the two months prior to the test, but that there was no relation between optimism and the behaviour before that. This suggests that the optimism levels are linked to emotional states, as opposed to fixed personality characteristics. The emotional states are likely driven by the positive social behaviour occurring within the group at that time.

Dolphins' emotional states, and their overall welfare in captivity, have recently incited much interest for scientists and the public. The authors of this study believe that the level of synchronised swimming could be used as an indicator of emotional state, and thus could help to monitor and improve the animals' social dynamics.

Our study was small, and more work is needed to investigate the link between welfare and positive social behaviour, but it is encouraging that these types of studies can yield such fruitful results and enhance our knowledge of dolphins' social lives.

4. ii. "Synchronised swimming makes dolphins more optimistic". The Huffington Post. 21st February 2017. Isabella Clegg, linked from The Conversation Global. http://www.huffingtonpost.com/entry/synchronised-swimming-makes-dolphins-more-optimistic_us_58ac8669e4b0ead5f0d41e71

4. iii. "Why Synchronized Swimming Makes Dolphins More Optimistic" Inner Self. Isabella Clegg, linked from The Conversation Global. http://innerself.com/content/personal/relationships/14953-why-synchronized-swimming-makes-dolphins-more-optimistic.html

5. Media articles and interviews

5. i. “Synchronised swimming seems to make dolphins more optimistic”. New Scientist. Ramin Skibba, 9th February 2017. https://www.newscientist.com/article/2120805-synchronised-swimming-seems-to-make-dolphins-more-optimistic

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NewScientist

DAILY NEWS

9 February 2017

Synchronised swimming seems to make dolphins more optimistic

By **Ramin Skibba**

Bottlenose dolphins that engage in synchronised swimming with their peers tend to see the glass as being half full.

Some of these dolphins frequently swim in tight-knit groups, and they’re the ones who appear the most optimistic, according to a study of eight captive animals.

In the experiment, individual dolphins were trained to swim towards one of two targets. They were taught that when they reach the left one, they receive applause and eye contact, while the one on the right delivers herring – the jackpot – and dolphins swim faster towards it.

When presented with a new and ambiguous middle target, some dolphins still swim rather fast, presumably hoping they’ll receive another tasty herring, although it’s only a 50/50 chance.

Those were dubbed the "optimistic" dolphins, and the analysis found that they were the same animals who had participated in the most synchronised swimming recently: moving closely alongside their fellow dolphins and matching their movements.

Social swimming

Dolphins continue to make these optimistic judgements up to two months after frequent synchronised swimming with their friends, but the boost fades after that.

Swimming together is an important social activity for dolphins that increases bonding between them, and the researchers argue that it could be linked to positive emotions.

"I think it's the social behaviour that drives the dolphins' optimistic decisions," says Isabella Clegg, a zoologist at the University of Paris-North and lead author of the study. Social interactions are thought to be rewarding and associated with positive views, and she believes her study of synchronised swimming confirms that.

"We know that dolphins in the wild and in captivity tend to use synchronous swimming as a form of bonding, such as between pairs of adult dolphins or mothers and calves," says Adam Pack, a psychologist at the University of Hawaii at Hilo. "People interpret these behaviours as reinforcing close relationships." The dolphins' optimistic behaviour resembles "cognitive bias": how humans judge situations differently depending on their social environment. People's social activity affects their outlook on the world, and something similar may happen among some animals, too.

Kinder zoos

Cognitive bias has been studied with laboratory rats, for example, by comparing those residing in enriched and barren cages. The dolphin study says it is the first test of cognitive bias in a marine mammal or a zoo-housed species.

"This study could be used as a tool to probe aspects of animal welfare in captive environments," says Lori Marino, a behavioural biologist at Emory University in Atlanta, Georgia. Zoo animals, for instance, don't choose many aspects of their lives, but they can benefit from opportunities for social activities with their peers, she says.

Clegg agrees. Zookeepers and aquarists could use this to monitor how many dolphins often swim together, and manage their practices accordingly. "In better welfare situations, animals judge [things] more optimistically," she says.

5. ii. “Swimming together lifts dolphins' spirits”. New Scientist magazine Issue 3113, 18th February 2017, Page 19.

5. iii. “Dolphins that swim on the bright side of life”. Metro newspaper, London. 16th February 2017.

Thursday, February 16, 2017 METRO

outlook is windy with a chance of virtual rain

fool your eyes and ears – k your other senses with lates weather. ombines a VR headset rated wind module and g temperature that attaches to the back of the neck. Developer Nimesha Ranasinghe at the National University of Singapore says it can simulate the wind in your face 'as you ski down a mountain' or the harsh sun 'when walking through a virtual desert'.

Dolphins that swim on the bright side of life

SYNCHRONISED swimming gives bottlenose dolphins a more positive outlook on life.

In a study of eight captive animals, individual dolphins had two targets they could swim towards. Only the one on the right delivered a herring meal, and once the dolphins learned this, they swam faster towards it.

When presented with a new and ambiguous middle target, some dolphins still swam fast, presumably hoping for herring. Those were dubbed the ‘optimistic’ dolphins.

When researchers analysed the animals’ movements before the experiment, they found the optimistic dolphins had been most in sync with their peers – swimming close to others and matching their movements.

Swimming together is considered important for increasing bonding between the animals, and researchers at the University of Paris-North argue it could be linked to positive emotions.

Study author Isabella Clegg said: ‘I think it’s the social behaviour that drives the dolphins’ optimistic decisions.’

ms ■ The universe's hidden patterns revealed

5. iv “Dolphins Who Hang With Mates Display a Positive Spin on Life” Psychology Today. 22nd February 2017. Mark Bekoff. https://www.psychologytoday.com/blog/animal-emotions/201702/dolphins-who-hang-mates-display-positive-spin-life

Dolphins Who Hang With Mates Display a Positive Spin on Life

Study shows dolphins who swim together display positive emotions and optimism

Marc Bekoff Ph.D.
Animal Emotions

Posted Feb 22, 2017

A few hours ago, when I was looking for some information on another topic, I came across an essay by Ramin Skibba called "Synchronised swimming seems to make dolphins more optimistic." The caption to an image accompanying the article reads, "Hanging with your mates may put a positive spin on life."

Mr. Skibba's essay focuses on a research paper published in *Behavioural BrainResearch* by Dr. Isabella Clegg and her colleagues titled "Bottlenose dolphins engaging in more social affiliative behaviour judge ambiguous cues more optimistically." I honestly didn't see the main message of this extremely interesting and intriguing study until I saw Mr. Skibba's summary.

Cutting to the chase, this study shows "Bottlenose dolphins that engage in synchronised swimming with their peers tend to see the glass as being half full." When dolphins swam toward an object on the left they received eye contact and applause, and when they swam toward an object on the right, they received much-loved herring. When they were offered an ambiguous target, the researchers showed that the dolphins who swam together were the more optimistic individuals and swam faster than other dolphins. The effect lasted around two months, after which it declined.

Mr. Skibbas writes, "Swimming together is an important social activity for dolphins that increases bonding between them, and the researchers argue that it could be linked to positive emotions," and lead researcher Isabella Clegg notes, "I think it's the social behaviour that drives the dolphins' optimistic decisions." And, it's known that wild dolphins bond as a result of synchronous swimming. Along these lines, Mr. Skibba writes, "The dolphins' optimistic behaviour resembles 'cognitive bias': how humans judge situations differently depending on their social environment. People's social activity affects their outlook on the world, and something similar may happen among some animals, too."

What I really like about this study in addition to showing that dolphins who swim together display more optimism, is that it also has practical applications in that social interactions might make captivity less stressful for individuals who are able to socialize with peers. Mr. Skibba concludes, "Clegg agrees. Zookeepers and aquarists could use this to monitor how many dolphins often swim together, and manage their practices accordingly. 'In better welfare situations, animals judge [things] more optimistically,' she says."

I really like this study and I hope other researchers will follow up with additional comparative studies on other species. Social interactions can be very positive for individuals in a wide variety of species, and not only can we learn about wild animals, but also those who are forced to live in captive conditions where they have little to no freedom to make choices about how they want and need to spend their time.

5. v “Retrouvez le LEEC au Parc Astérix: Interview d’Isabella Clegg, doctorante au LEEC”. 6th April 2017, Edouard Cid. https://www.univ-paris13.fr/retrouvez-le-leec-au-parc-asterix/

Retrouvez le LEEC au Parc Astérix

Suite à la présentation du LEEC (Laboratoire d’Ethologie Expérimentale et Comparée) et de l’éthologie, nous interrogeons aujourd’hui Isabella Clegg qui réalise sa thèse sur le bien-être des dauphins en captivité au Parc Astérix.

Doctorante au LEEC, Isabella est co-encadrée par madame Fabienne Delfour, responsable scientifique au Delphinarium du Parc Astérix, et Heiko .G. Rödel, Directeur du LEEC. Notons qu’elle bénéficie d’une bourse CIFRE, ce qui signifie que le Parc Astérix finance sa thèse, une première pour une structure animalière. > En savoir plus sur la bourse CIFRE

Dans la présente interview, Isabella Clegg nous partage ses premiers résultats sur le lien entre l’optimisme et le niveau de sociabilisation. Un lien pour la première fois mesuré et prouvé chez le dauphin.

Interview d’Isabella Clegg, doctorante au LEEC

Qu’avez-vous fait avant d’être inscrite au LEEC ? J’ai d’abord fait une licence en Angleterre pendant laquelle j’ai étudié le comportement et le bien-être animal, puis un Master à l’université de Miami dans laquelle j’ai étudié la biologie marine. Six mois après, je suis arrivé au Parc Astérix pour réaliser ma thèse.

Cela fait trois ans que vous êtes au LEEC, quel est l’objet de votre thèse ? Mon objectif est de trouver des mesures objectives du bien-être des dauphins en captivité. L’approche est la suivante : on combine la mesure du comportement, de la physiologie et de la cognition. L’utilisation de l’ensemble de ces mesures permet une meilleure fiabilité des résultats, contrairement à une seule.

Comment procédez-vous à la mesure du bien-être animal ? J’ai de la chance d’être au Parc Astérix puisque je peux effectuer des observations quotidiennes auprès des dauphins. J’ai pu ainsi mieux prendre en compte leur état de bien-être, état que l’on détermine sur une période de longue durée.

L’objet de la première étude dans le cadre de ma thèse a été d’identifier les comportements des dauphins. Nous voulions connaître leurs habitudes avant et après les entraînements, le matin et l’après-midi. Après plusieurs observations, nous avons pu constater que les dauphins anticipent l’entraînement et qu’à la suite de ce dernier ils pratiquent des comportements sociaux positifs. Autrement dit, ils nagent de façon synchronisée en groupe.

Pour la suite de ma thèse, je me suis inspirée d’approches du bien-être appliquées à des animaux fermiers. Nous avons réalisé le test du « Biais cognitif », habituellement appliqué aux chiens, avec les dauphins du parc. C'est une première dans une structure animalière. Ce test nous a permis de mesurer l'émotion du dauphin, nous avons donc pu connaître son niveau d'optimisme et constater qu'il est élevé lorsqu'il pratique la nage synchronisée avec ses pairs. Ce qui revient à dire que le dauphin le plus optimiste est également celui qui nage le plus avec les autres.

Quels sont les moyens dont vous disposez au Parc Astérix ? J'ai le droit d'accéder tous les jours au delphinarium du Parc Astérix. De ce fait, je peux observer longtemps les dauphins afin de mieux comprendre leurs comportements. De plus, grâce aux soigneurs et à leur proximité avec les dauphins, je peux mettre en place différents exercices avec un système de récompense à la clef. Le test d'optimisme (précédemment cité) n'aurait pas été possible sans le travail des soigneurs. C'est avec eux que nous avons adapté le test au dauphin.

Quel est le but de ces recherches ? Autrement dit, quelles seraient les applications dans la société ? J'espère que d'autres delphinariums verront les résultats de mes recherches et les utiliseront pour le bien-être de leurs dauphins. Par exemple, en les observant simplement, en regardant s'ils pratiquent la nage synchronisée, les delphinarium peuvent désormais mesurer leur niveau d'optimiste. Ils pourront donc connaître l'impact des exercices et des autres activités sur le bien-être des dauphins et ainsi, les ajuster de façon à ce que leurs dauphins se sentent mieux.

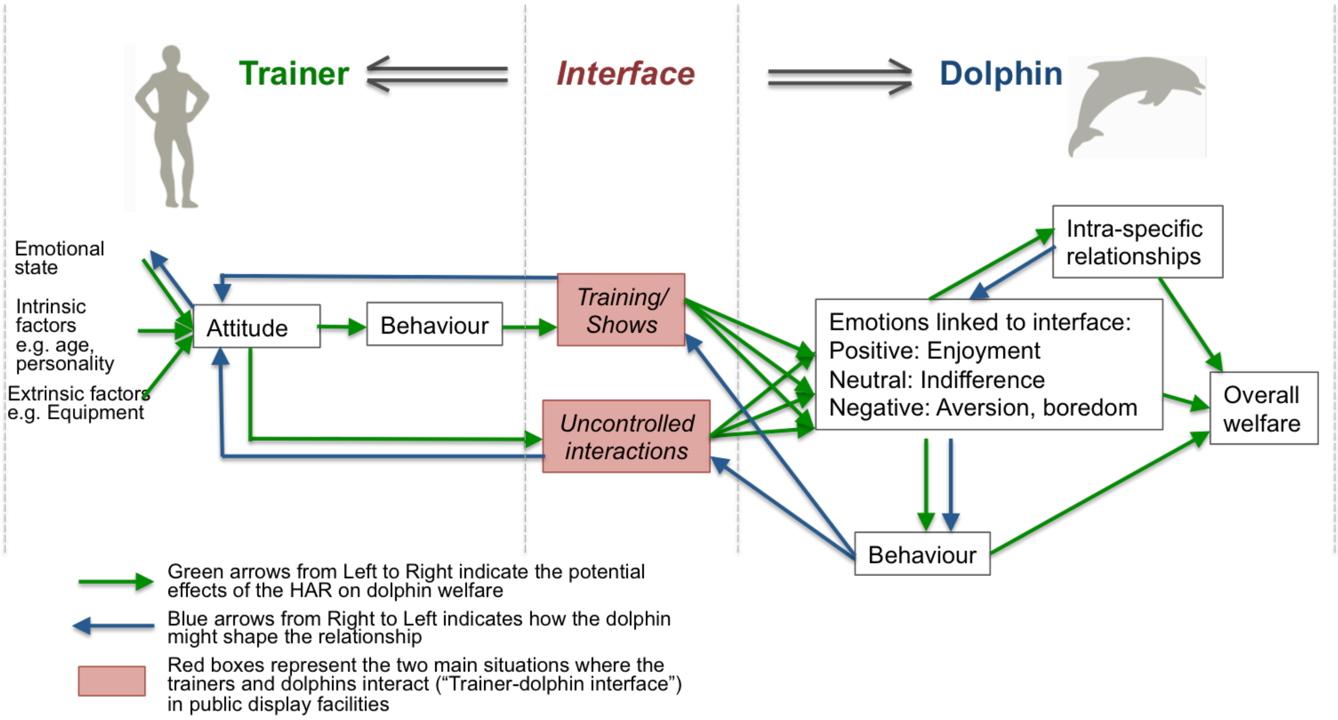
En ce moment, nous menons une autre expérience avec les soigneurs. Nous souhaitons savoir si le dauphin préfère jouer dans le bassin ou interagir avec un soigneur. C'est l'exemple de recherche dont les résultats peuvent servir à d'autres delphinariums, mais également au grand public qui souhaite mieux connaître la relation entre le dauphin et l'être humain.

Allez-vous continuer à étudier les dauphins ? L'intérêt pour moi dans la recherche se porte notamment sur la transmission du savoir vers le grand public. Ma thèse se termine à la fin de cette année universitaire, suite à cela j'aimerais continuer mes recherches que ce soit avec des dauphins en captivité ou dans leur milieu naturel.

B. Output of discussions early in the project

1. Trainer-Dolphin relationship and its links with dolphin welfare

A theoretical model constructed by I. Clegg (following discussions with X. Boivin and others) proposing how the trainers (animals caretakers) and dolphins might interact, and how these intraspecific encounters might impact the animals' welfare. Several sources from farm and zoo Human-Animal Relationship research were used to structure the elements (Boivin et al., 2003; Hemsworth, 2003; Hosey, 2008; Waiblinger et al., 2006). This model was not published but aided in the development of the study that resulted in Papers 5 and 6.



This work should be cited as:

Clegg, I. L. K. (2017) Developing welfare parameters for bottlenose dolphins (*Tursiops truncatus*) under human care. 217 pp, Doctoral dissertation. Université Paris 13, Villetaneuse, France.

All photograph credit to Isabella Clegg.