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C-Well: The development of a welfare assessment index for captive bottlenose dolphins (Tursiops truncatus)

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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 Welfare Quality® 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.*

Keywords: *animal-based measures, animal welfare, bottlenose dolphins, C-Well® assessment, dolphin welfare, welfare assessment*

Introduction

Animal welfare research is expanding globally, and welfare assessments are now applied in a variety of forms to improve welfare and management protocols in animal use industries (Webster 2005; Dawkins 2006; Blokhuis 2008). Although there are comprehensive, widely supported welfare frameworks employed in Europe's farming industry (see Veissier *et al* 2008), including but not limited to the 'Five Freedoms' model (FAWC 1992) and the Welfare Quality® (WQ) effort (Welfare Quality® 2009a,b,c), non-farm animal welfare is under-reported, particularly within the zoological industry (Reade & Waran 1996; Jiang *et al* 2007; Barber 2009). Welfare assessments, by design, involve objective and quantitative measures of physical and mental well-being (Barnard & Hurst 1996; Hosey 2005; Dawkins 2006; Whitham & Wielebnowski 2013) and are, ideally, developed *in situ* (Dawkins 2006). Most welfare assessments strategically incorporate both resource-based (ie input measures, resources provided to animals) and animal-based (ie outcome-based, parameters of animals' behaviour/physiology) measures (Veissier *et al* 2008; Whitham & Wielebnowski 2013), with animal-based measures regarded as a more direct indication of welfare, though often limited in practical applicability (Whitham & Wielebnowski 2009; Roe *et al* 2011).

More than 700 million people visit zoos and aquaria worldwide each year (Gusset & Dick 2011), contributing to increased public awareness regarding animal welfare, and concurrent with an increase in welfare studies of captive animals (Rushen *et al* 2011; Whitham & Wielebnowski 2013). The American Zoological Association (AZA 2013) recently initiated the development of welfare assessment tools (Barber 2009; Whitham & Wielebnowski 2009), and Kreger and Hutchins (2010) designated captive animal welfare assessments as a research priority. Additionally, Barber (2009) described the structure and functionality of most zoos and aquaria as being well-suited for welfare assessments, as there are sufficient opportunities to assess individuals (versus herd assessments typical on farms) using animal-based measures. Yet, to date, there are no comprehensive, practically applicable welfare efforts in place for zoological animals.

Initial efforts to measure zoo animal welfare identified keeper assessments as a potential, yet limited, tool (Whitham & Wielebnowski 2009), and most of the progress in zoo welfare science is focused on captive elephants (*Elephantidae*) (Maple 2007; Mason & Veasey 2010). Salivary cortisol was highlighted as a practical and useful welfare measure for elephants (Menargues *et al* 2008), and faecal corticosteroid levels were correlated with relevant behavioural parameters in an Asian elephant (*Elephas maximus*) case study (Laws *et al* 2007). Additionally, Vanitha *et al* (2011) correlated stereotypy frequency and social grouping among 140 captive Asian elephants as a measure of animal welfare, and Mason and Veasey (2010) summarised and proposed potentially useful welfare indices specific to elephant behaviour, cognition, reproduction, and physiology. Welfare assessments designed to address other zoo animals are limited, but the farm animal welfare frameworks, including the WQ, have been noted as adaptable for use in zoos (eg Hill & Broom 2009; Mason & Veasey 2010; Botreau *et al* 2012). Mononen *et al* (2012) applied the WQ measures to farmed foxes (*Vulpes* spp) and mink (*Neovison vison*) (ie the 'WelFur' assessment), indicating that the framework can be adapted to a variety of other species and animal management goals.

Although cetaceans have been maintained in managed care for over 150 years, with bottlenose dolphins (*Tursiops truncatus*) identified as the predominant species held in captivity (Wells & Scott 1999), there are no comprehensive welfare assessments for bottlenose dolphins and a paucity of peer-reviewed literature on the welfare of captive cetaceans in general (Gygax 1993; Galhardo *et al* 1996; Ugaz *et al* 2013). Dolphinaria exist worldwide and are regulated by governmental bodies, including only resource or management-based measures in their assessments (US: Animal Welfare Act [AWA 1966]; EU: Council Directive 1999). The goal of most marine mammal facilities is to exceed the resource-based standards (McBain 1999), which are largely regarded as minimum requirements (Conley 2008; Joseph & Antrim 2010). Published analyses of cetacean welfare are limited to two studies of bottlenose dolphins (Waples & Gales 2002; Ugaz *et al* 2013) and one evaluation of belugas (*Delphinapterus leucas*) (Castellote & Fossa 2006). Both Waples and Gales (2002) and Ugaz *et al* (2013) focused on stress in bottlenose dolphins as a metric for welfare using behavioural and physiological parameters, with the former study limited to three subjects and the latter focused largely on the relationship between different enclosure types and salivary cortisol levels. Castellote and Fossa (2006) assessed the vocal behaviour of two belugas as an indicator of animal welfare, utilising acoustic data collected before and after transport but did not correlate the results with other parameters.

Although significant advances have been made in bottlenose dolphin husbandry (for a review, see Wells 2009), there are few to no standardised methods to quantify and address cetacean welfare (Hill & Lackups 2010). This study represents the first effort to develop assessment indices and protocols to quantify the welfare of bottlenose dolphins in captivity, utilising the WQ framework as a model, with an emphasis on animal-based measures. This comprehensive assessment, along with the relevant data, will contribute to a better understanding of cetacean health, care, and management, as well as support informed public awareness regarding the maintenance of marine mammals in captivity (Webster 2005; Maple 2007; Broom 2010).

Materials and methods

The bottlenose dolphin welfare assessment, named the 'Cetacean Welfare Assessment' or 'C-Well®', was developed at three, semi-open, seawater facilities in Florida (Dolphins Plus, Dolphin Cove and Island Dolphin Care), with eight male and 12 female subjects ranging from 1–36 years, following Dawkins' (2006) recommendation that welfare measures be developed *in situ*. Measures were applied and tested opportunistically between June and November 2013, and a full pilot C-Well® assessment was conducted at all facilities in December 2013 to ensure the functionality and practicality of the measures and complete assessment.

The WQ framework (Welfare Quality® 2009a,b,c) (Table 1 shows the framework for fattening cattle) is considered both practical and successful in assessing and measuring farm animal welfare (Blokhuis 2008). In this study, the WQ framework was adapted for use with *Tursiops truncatus* due to its widely accepted structure and standards, comprehensive design, as well as the noted and evidenced potential for application to other species in managed care. This study and manuscript followed the developmental structure and considerations described by Mononen *et al* (2012) relevant to adapting the WQ framework to a novel species. Additionally, utilising the WQ framework as a first model for dolphin welfare was ideal, because it does not require the use of expensive or specialised equipment and was purposefully established to transfer information from animal use industries to the public (Welfare Quality® 2009a,b,c). By using a standardised model covering all aspects of the subject animal's life, there is the potential for constructive and collaborative input and future development across many, relevant disciplines.

When developing an assessment, welfare criteria should be selected that are exhaustive, minimal (limited to 12 or less criteria), and mutually independent (Botreau *et al* 2007). When applying WQ to other species, Botreau *et al* (2012) emphasised that the principles and criteria should remain consistent. Therefore, the welfare assessment for bottlenose dolphins incorporated all four principles and eleven criteria of the WQ (Table 1 shows how the WQ framework corresponds to the adapted model for dolphins). The 12th WQ criteria, 'positive emotional state', was omitted due to the paucity of data regarding emotional state in cetaceans. Following the WQ model, 36 practical and objective welfare measures were developed, prioritising the application of animal-based measures over resource-based measures whenever possible (Table 1). Some of the measures were developed specifically for *T. truncatus*, but others reflect direct adaptations of the farm animal measures (eg Body Condition Scoring), and the WQ's logical bottom-up approach was preserved (Figure 1).

The measures in the assessment were developed predominantly using published literature, describing wild and captive bottlenose dolphin health, behaviour, physiology, anatomy, cognition, and ecology, focusing on normal and abnormal standards and conditions. Additionally, professional insight

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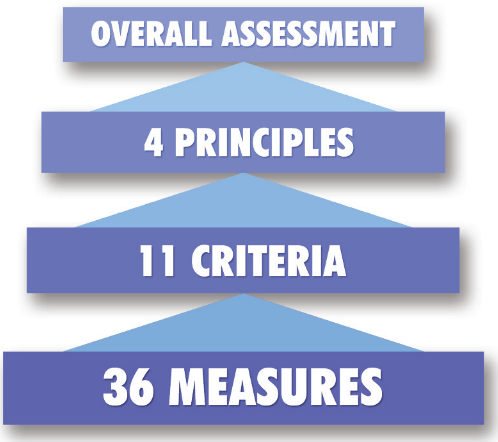
Table 1 Criteria and measures of the Welfare Quality® framework adapted to establish the C-Well® assessment.

Category	Welfare Quality® Criterion	Welfare Quality® Measure*	C-Well® Criterion	C-Well® Measure
Good Feeding	1 Absence of prolonged hunger	Body Condition Score	1 Absence of prolonged hunger	1.1 Body Condition Score 1.2 Frequency of weight measurements 1.3 Dietary records
	2 Absence of prolonged thirst	Water provision, cleanliness of water points, number of animals using water points	2 Absence of prolonged thirst	2.1 Capillary refill time 2.2 Hydration protocol
Good Housing	3 Comfort around resting	Time needed to lie down, cleanliness of animals	3 Resting comfort	3.1 Time budget
	4 Thermal comfort	No current measure	4 Thermal comfort	4.1 Frequency of water temperature testing 4.2 Water temperature and diet 4.3 Shade
	5 Ease of movement	Pen features according to live weight, access to outdoor loafing area in pasture	5 Appropriate environment	5.1 Topography 5.1.1 Echolocation 5.1.2 Complexity of enclosure 5.2 Ability to exhibit complex movements 5.2.1 Swim speed 5.2.2 Aerials 5.3 Water quality 5.3.1 Salinity 5.3.2 Coliform 5.3.3 pH 5.3.4 Chlorine 5.3.5 Frequency of water quality testing 5.4 Enrichment 5.4.1 Application of enrichment
Good Health	6 Absence of injuries	Lameness, integument alterations	6 Absence of injuries	6.1 Total wound threshold 6.2 Wounds from enclosure
	7 Absence of disease	Coughing, nasal discharge, ocular discharge, hampered respiration, diarrhoea, bloated rumen, mortality	7 Absence of disease	7.1 Respiratory system 7.1.1 Frequency of coughing 7.1.2 Inhalation duration 7.2 Eye diseases 7.2.1 Discolouration 7.2.2 Squinting 7.3 Skin diseases 7.3.1 Skin abnormalities 7.3.2 Mouth abnormalities 7.4 Blood parameters 7.4.1 Blood sampling protocol
	8 Absence of pain induced by management procedures	Disbudding/dehorning, tail docking, castration	8 Absence of pain induced by management procedures	8.1 Blood draw 8.2 Gastric tubing 8.3 Voluntary restraint 8.4 Emergency containment training
Appropriate Behaviour	9 Expression of social behaviours	Agonistic behaviours, cohesive behaviours	9 Expression of social behaviours	9.1 Presence of social behaviours
	10 Expression of other behaviours	Access to pasture	10 Absence of abnormal behaviours	10.1 Stereotypic behaviour
	11 Good human-animal relationships	Avoidance distance	11 Positive human-animal relationship	11.1 Response to trainer while not under stimulus control 11.2 Non-food tactile interactions
	12 Positive emotional state	Qualitative behaviour assessment		

* Measures from Welfare Quality for fattening cattle (Welfare Quality® 2009a).

Figure 1



Bottom-up approach and integration of data from measures to overall assessment, adapted from WQ documents (Welfare Quality® 2009a,b,c).

was obtained, particularly when supporting literature was not available, through collaborative efforts of three marine mammal facilities, multiple universities, and the collective expertise of four DVMs (Doctors of Veterinary Medicine) with specialisations in marine mammal medicine, three PhDs, an animal welfare scientist (DVM, PhD, who participated in the development of the farm animal WQ), and two curators with ten or more years of field and clinical experience. Information was collected through *ad hoc* meetings, with veterinary expertise thoroughly integrated into each measure, since welfare science originated within the discipline of veterinary medicine (Dawkins 2006).

For each of the 36 measures, data were collected to test the applicability and practicality of the protocols and the quantitative scoring system, and to ensure that they were reflective of welfare. This was achieved by conducting the welfare assessment opportunistically using animals known to be healthy and members of stable social groups and animals known to be unhealthy and/or likely to be experiencing reduced welfare (eg animals with illnesses, excessive tooth-rake marks, those exhibiting prolonged inappetence, animals in confined medical enclosures, and during animal introductions, the latter of which can cause social stress in managed care [Waples & Gales 2002]). As a means of reporting on the process of adapting the WQ for use with *T. truncatus*, this study followed the structure and format of the WelFur assessment described by Mononen *et al* (2012), which includes considerations relevant to the development of the framework and the general methods but excludes data collected *in situ* to refine and validate the measures.

Welfare measures in the C-Well® assessment

The C-Well® assessment includes 36 measures, which constitute eleven welfare criteria and four welfare principles (Table 1). Thus, results can be analysed as a cumulative score or separated by principle, criteria, or measure. Of the 36 measures, 21 are animal-based (58%), and of the eleven criteria, nine included at least one animal-based measure (82%). A scoring system, based on a 0–2 scale (0 = good welfare; 1 = adequate or sub-optimal welfare, 2 = poor welfare), was developed and justified using data collected *in situ* (ie facility records and practical applications), published literature describing normal and abnormal trends in both captive and wild dolphins, and personal communication with professionals. The C-Well® assessment protocols and descriptions of each measure were outlined in a detailed, comprehensive handbook, reflecting the format of WQ application guidelines. This handbook was designed to be utilised by a trained assessor and includes step-by-step guidelines describing how to conduct each measure, how to analyse the data collected (if necessary for scoring), and how to award scores (including visual references). Video references, additional photographic aides, definitions of keywords, and datasheets were also developed to accompany the handbook to ensure accuracy and facilitate execution.

Absence of prolonged hunger

Hunger is a subjective sensation of appetite and difficult to assess in non-human animals, but well-designed measures can indicate probable hunger or satiation (Broom 1991; Barnard & Hurst 1996; Dawkins 2006). Three measures were developed to assess the absence of hunger, including a Body Condition Score (BCS), frequency of animal weighing, and the degree of detail included in dietary records. Following the WQ model, the latter two measures evaluate protocols in place to assess and prevent hunger, with the highest welfare scores assigned when animals were weighed regularly (supported by Cheal & Gales 1991; Worthy 2001), since weight loss is an indication of reduced welfare (Broom & Johnson 1993; Dawkins 1998). A good welfare score was given if the dolphins' diet was species-specific (see Worthy 2001; Kastelein *et al* 2002; Caut *et al* 2011) and if dietary records included information about species, calories, mass, protein, and fat and water content, which was used to indicate that the facility considers appropriate nutrition as part of a broader animal management plan. Body Condition Scoring (BCS) is a well-established welfare

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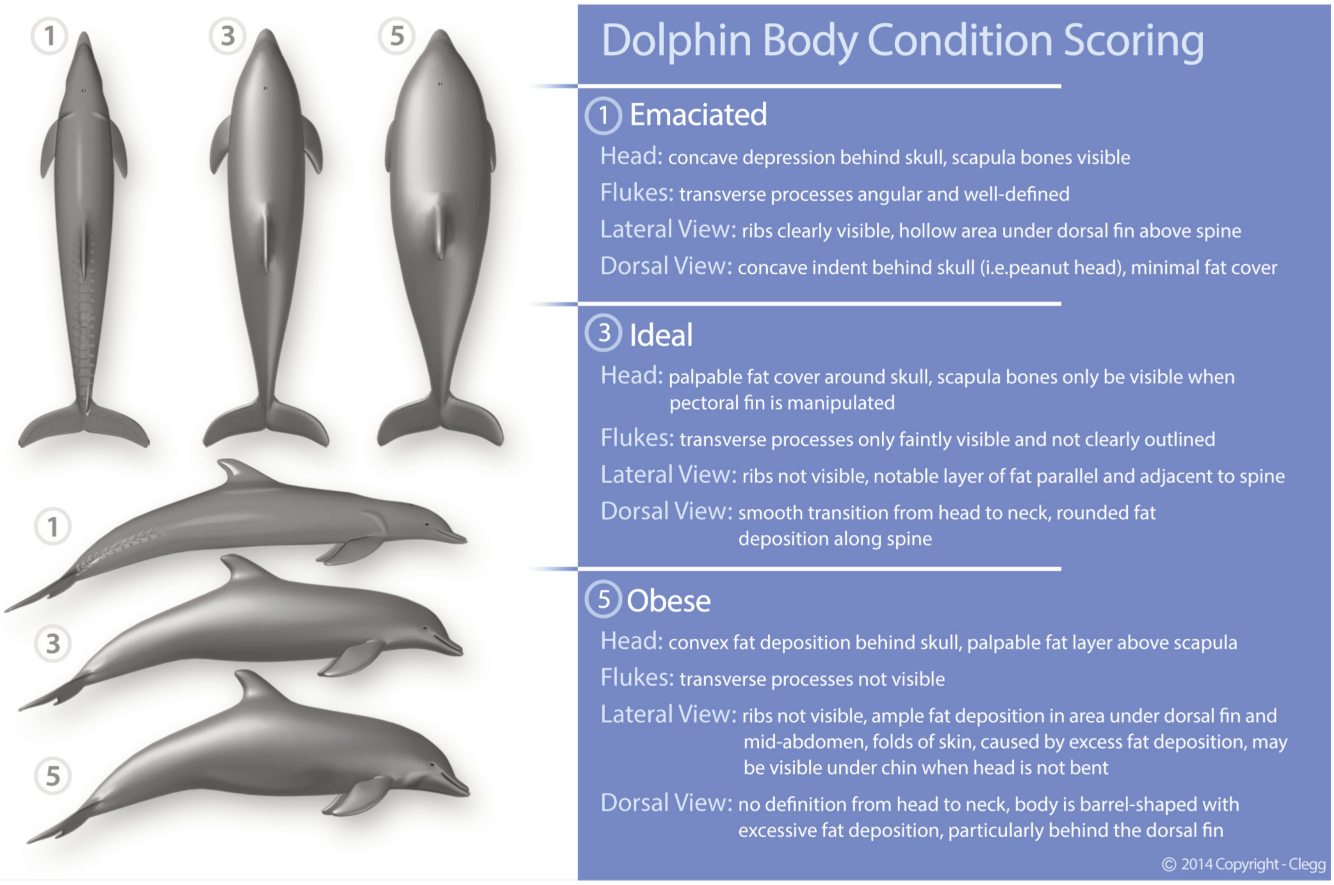
Figure 2



C-Well® assessment Body Condition Score graphic for *Tursiops truncatus*.

tool applied to farm animals, domestic pets and, more recently, research (Ullman-Culleré & Foltz 1999; Roche *et al* 2009; Welfare Quality® 2009a,b,c). Some zoos use BCS as an indication of animal health (EAZA 2011), but at the time of this study the only published marine mammal applications involved an evaluation of wild right whale (*Eubalaena glacialis*) body and skin condition (Pettis *et al* 2004) and an assessment of the post-nuchal depression in wild *T. truncatus* neonates (Gryzbek 2013). A C-Well® BCS schematic was developed, which considered the extent of bony prominences and fat depositions relevant to *T. truncatus* (ie the skull, scapula, peduncle, and dorsal spine) and across the whole body on a scale of 1 to 5, where: 1 = emaciated; 2 = underweight; 3 = ideal; 4 = overweight/fleshy; and 5 = obese (Figure 2). The level of detail in the schematic facilitated objective evaluation of body condition, and along with the scoring system, is considerably more comprehensive than the analogous WQ BCS measures.

Absence of prolonged thirst

Two measures were developed to assess the absence of thirst in *T. truncatus*: presence of supplemental hydration protocols and the Capillary Refill Time (CRT). Wild cetaceans obtain freshwater primarily from their food as pre-formed and metabolic water (eg Worthy 2001; Ballarin *et al* 2011). In captivity, marine mammal food items are commonly frozen and thawed, leading to dehydration and drip loss (Crissey 1998; Ngapo *et al* 1999). Therefore, supplemental, controlled hydration can allow osmoregulation to occur similarly to that of wild dolphins (Ballarin *et al* 2011), prevent dehydration, and alleviate health conditions, such as kidney stones, which are common in both wild (Miller 1994) and captive *T. truncatus* (Venn-Watson & Ridgway 2007; Wells 2009), particularly among older animals (Ridgway & Schroeder 1989). The Capillary Refill Time (CRT) test was utilised in place of the skin turgor test; although both tests can indicate dehydration in humans and animals (eg Kasari & Naylor 1984; Saavedra *et al* 1991; Palmer 2009), the latter requires skin elasticity, not present in dolphins. The CRT assesses intravascular volume and can indicate the absence of thirst. The test has been successfully applied to captive cetaceans (Butterworth *et al* 2004) using the soft tissue at the back of the throat inside the mouth (Figure 3). The thresholds and relevant scoring were established according to published CRT values for six species of cetaceans (Butterworth *et al* 2004) and veterinary expertise.

Figure 3

A Capillary Refill Time (CRT) evaluation: the skin blanches immediately following depression (circle), and the time required for normal colouration to reappear is measured using an electronic timer.

Resting comfort

A time budget metric was used to establish resting comfort in terms of animal welfare (Dawkins 1988; Veasey *et al* 1996), defined as the time an animal devotes to certain activities in a fixed period. Time budgets have been documented in both wild and managed dolphin populations (eg Galhardo *et al* 1996; Neumann 2001), with recent focus on how tourism activities affect group behaviour (Stensland & Berggren 2007; Stockin *et al* 2008). In managed care, activity (eg training, shows, and guest interactions) should be balanced with rest. However, since studies of the behaviours related to cetacean sleep are scant (eg Goley 1999; Sekiguchi & Kohshima 2003), thereby obscuring definitions of 'rest' behaviour, the only feasible way to measure if the dolphins experience appropriate rest is through assessing the opportunities they have to rest. Dolphins have been shown to exhibit unihemispheric, slow wave sleep (eg Lilly 1964; Supin *et al* 1978; Ridgway 1986; Mukhametov 1987) for approximately 4–8 h in a 24-h period (combined range taken from both wild and captive research: Lilly 1964; Norris & Dohl 1980; Goley 1999; Lyamin *et al* 2008). Therefore, the C-Well® measure for rest included balanced thresholds for time spent in training, show and/or guest interactions (ie activity) and the daily operational times of the facility, and the good welfare score was associated with at least 8 h of non-operation (no presence of public or animal care staff), which was implied as time available to the animals for sleep and rest. This was a conservative welfare goal, given that animals have been shown to rest during hours of operation (Galhardo *et al* 1996; Sekiguchi & Kohshima 2003).

Thermal comfort

Bottlenose dolphins are widely distributed between 45°N and 45°S, occupying tropical, temperate, coastal and pelagic environments (see Wells & Scott 1999). Their distribution is affected by temperature, either directly as a function of thermal stress or indirectly as it influences prey abundance (see Shane *et al* 1986). Blubber and fat stores increase during colder, winter months and decrease during the summer, which aids in thermoregulation and supports varying energetic demands (eg Meagher *et al* 2008; Wells 2009). Three measures were developed to assess thermal comfort, adapted from the WQ model: availability of shade, frequency of water temperature measurements, and whether the animals' diets varied systematically with temperature variation within the documented species-specific range. Given the ease of measuring water temperature, the best welfare score was assigned when enclosure temperatures were recorded daily (Joseph & Antrim 2010), with the range for *T. truncatus* established as 10–33°C (using Sweeney & Samansky 1995; Kastelein *et al* 2002; Joseph & Antrim 2010; EAAM 2013). Where water temperatures vary naturally or systematically (ie in closed systems), optimum welfare scores were assigned to facilities that alter animal diets accordingly (Piercey *et al* 2013). The third measure assessed whether all dolphins in an enclosure could occupy shaded areas simultaneously; provision of shade is also a USDA requirement (AWA 1966).

Appropriate environment

This was the only criterion altered from the WQ protocols, adapted to account for dolphins' advanced sensory systems in a dynamic aquatic environment, and was changed from 'Ease of movement' (Welfare Quality® 2009a,b,c) to 'Appropriate environment'. Ten measures were developed

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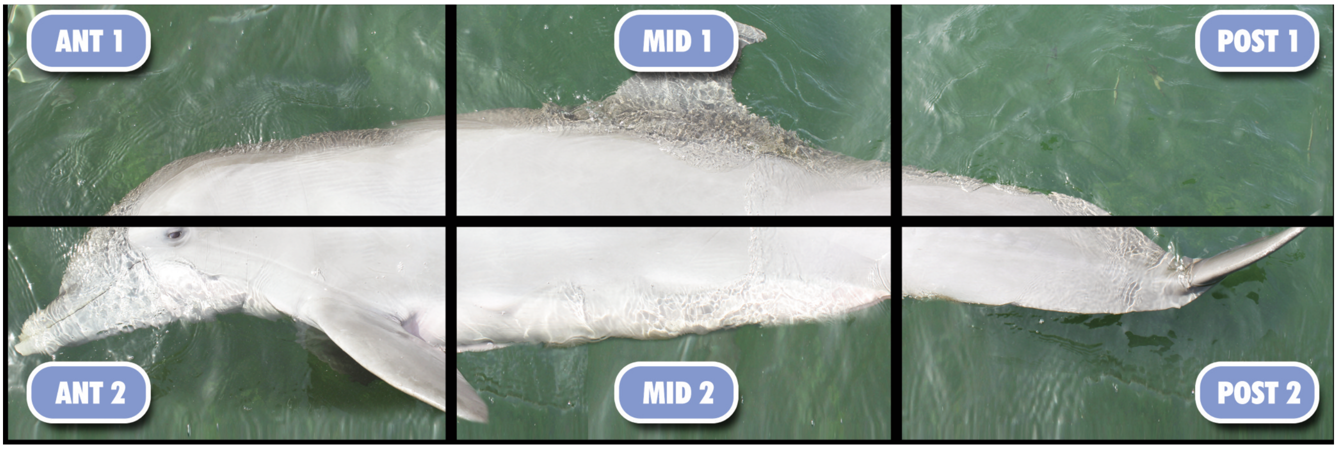
Figure 4



Sample photographs and grid used in wound assessment tool applied to left lateral regions. The grid indicates the six body regions utilised in the calculation, including anterior dorsal (ANT1), anterior ventral (ANT2), mid-dorsal (MD1), mid-ventral (MID2), posterior dorsal (POST1), and posterior ventral (POST2).

to assess enclosure topography, the ability to exhibit complex movements, water quality, and enrichment.

The topography of zoo enclosures has been associated with animal well-being (Hosey 2005; Hoy *et al* 2010), with variability and complexity of space considered critical to improving welfare (Hosey 2005; Maple 2007). Marine mammal enclosures have evolved from simple, practical exhibits to resembling natural habitats using behavioural, visual, and acoustic stimulation (Arkush 2001). Thus, enclosure complexity was assessed, whereby more topographically complex and dynamic enclosures achieved better welfare scores. This resource-based measure was supplemented by testing for the presence/absence of echolocation, which is an animal-based measure that might reflect acoustic stimulation as a function of topographic complexity (eg Akamatsu *et al* 1998; Harley *et al* 2003), and the lack of echolocation may indicate sub-optimal captive environments (Sobel *et al* 1994). Echolocation is a prominent and important behaviour for dolphins, as it facilitates interactions between the animal and its environment (Akamatsu *et al* 1998; Herzing 2000), and a recent study suggests that echolocation may also be fundamental to social interactions (Yoshida *et al* 2014).

The 'ability to exhibit complex movements' was assessed using two measures: the presence of 'high-speed' swimming (defined as > 4 m s^{-1}) and aerial behaviours greater than 1 m above the water surface. Conley (2008) recommended daily opportunity for exercise among captive dolphins, specifically in reference to enclosure design and space available. Thus, high energy swimming and aerial behaviours were measured either opportunistically or under stimulus control, since they are both natural behaviours exhibited in many, different functional contexts (eg Fish & Hui 1991; Galhardo *et al* 1996; Samuels & Gifford 1997; Lusseau 2006) and potentially limited by poor enclosure design.

Water quality can affect wild (Thompson & Hammond 1992) and captive (Arkush 2001) marine mammal health. The C-Well® assessment included a measure for salinity, total and faecal coliform, pH, and chlorine, and the frequency of water quality testing. Acceptable and ideal ranges for each parameter were established using the literature and existing marine mammal facility standards (AWA 1966; Gage 2009; Crane 2013; EAAM 2013). Water quality should be tested regularly, with specific schedules for each parameter, since anomalies can occur quickly and may have serious effects on animal health and welfare (eg Arkush 2001; Gage 2009).

The last measure in this criterion evaluated enrichment and how it was applied. Activities and objects perceived as enriching may be ineffective, aversive and could even decrease animal welfare (Hoy *et al* 2010; Delfour & Beyer 2012). Therefore, enrichment must be planned, variable, and implemented utilising knowledge about the reinforcing value to individual animals (Kuczaj *et al* 2002; Delfour & Beyer 2012), and behavioural records for each individual should be used to evaluate the efficacy of applied enrichment, avoiding potentially aversive stimuli, as part of a sound maintenance and development plan (Hill & Broom 2009; Hoy *et al* 2010). The measure assessed three, specific enrichment criteria: consideration of the safety of enrichment; existence of records to document enrichment interactions; and utilisation of those data to optimise subsequent enrichment for individual animals. If these three criteria were fulfilled, the provision of enrichment was considered likely to promote good welfare.

Absence of injuries

Among *T. truncatus*, the most common, visible injuries are caused by the teeth of conspecifics, called 'rake marks', characterised as superficial, parallel lesions on the skin; they are considered a normal facet of social behaviour in wild and captive populations and were

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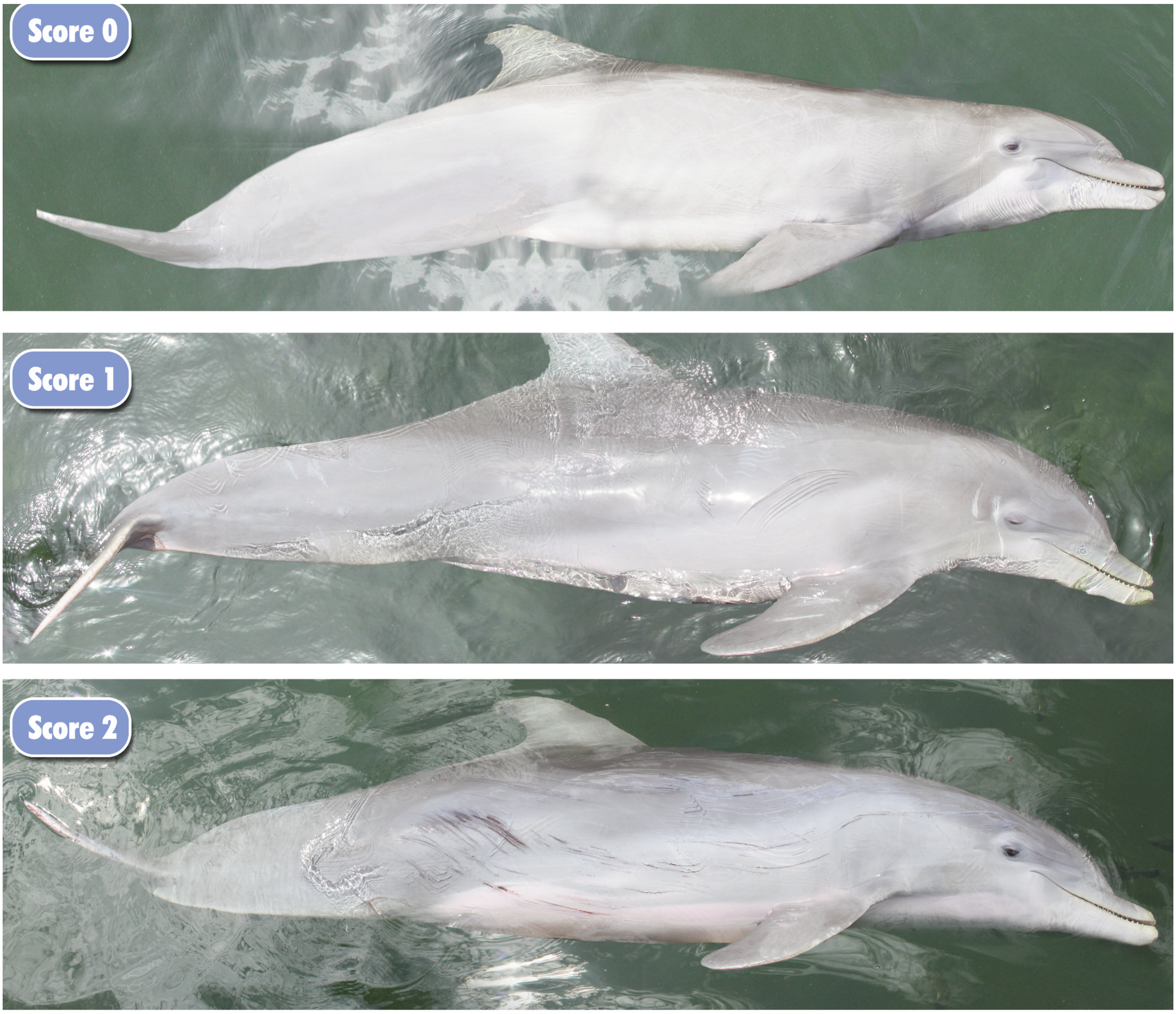
Figure 5



The wound percent cover range and relevant welfare score for the dolphin in each image. Score 0: < 15% new wounds and < 30% old wounds; score 1: < 10–15% new wounds and > 30% old wounds; and score 2: > 15% new wounds.

Figure 6

Image reference scale, from 0–2, for intensity of squinting. Score 0: no squinting; score 1: moderate squinting; and score 2: eye fully closed.

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utilised in this assessment to measure aggression (see Waples & Gales 2002; Scott *et al* 2005; Marley *et al* 2013). Environmental injuries are caused by an animal's surroundings but often occur indirectly because of intraspecific aggression, play, or the avoidance of aversive stimuli. In accordance with recommendations made by Marley *et al* (2013), a standardised tool was developed to quantify scarring as percent cover of the total body surface, including new and old wounds (characterised by depth and colouration), using a series of contiguous images (anterior, mid, and posterior), a grid to divide the body into six sections (Figure 4), and step-wise percent cover calculations. Welfare thresholds for wound percent cover were conservative (Figure 5) and established qualitatively using veterinary welfare evaluations of dolphins with sustained, higher levels of rake marks. Systematically monitoring aggression can improve management, including promoting pro-social behaviour, managing animal introductions, and evaluating the success of strategies implemented to reduce aggression. Furthermore, results can be compared among age classes, sex, enclosures, and facilities. The second measure in this criterion included a threshold for environmental wounds, which are distinct in that they are often wider and deeper than rake marks and environmental injuries (Scott *et al* 2005; Marley *et al* 2013), making them more likely to become infected (Gulland *et al* 2001) and impact welfare.

Absence of disease

Many emerging, resurging, and enzootic diseases affect cetaceans worldwide (see Miller *et al* 2001), and C-Well® focused on the commonly observed chronic and acute diseases of captive dolphins, specifically respiratory (Ridgway 1972; Venn-Watson *et al* 2012), ocular (Ridgway 1990; Joseph & Antrim 2010), and skin diseases (Gulland *et al* 2001), as well as haematologic disease indicators (Waples & Gales 2002; Castellote & Fossa 2006).

Dolphin respiratory anatomy enables large volumes of air to be exchanged rapidly, resulting in a higher susceptibility to respiratory infections (Ridgway 1972; Venn-Watson *et al* 2012). The absence of respiratory disease was assessed by measuring the frequency of coughing, which can indicate upper respiratory tract disease (Dunn *et al* 2001), and inhalation duration, where the full exhalation and inhalation duration was timed repeatedly to obtain a mean value. Longer durations may indicate lower respiratory tract disease (Anonymous DVM, personal communication, November 2013), and thresholds were established using veterinary data and expertise.

Ocular disease was measured by the degree of squinting and ocular discolouration, common indicators of ocular anomalies in captivity (Ridgway 1990; Joseph & Antrim 2010). Each of these measures was assessed utilising image reference scales (eg Figure 6), with three levels of severity and relevant scoring.

A variety of skin lesions, excluding rake marks and wounds caused by the environment (addressed above), have been documented in wild and captive dolphins (see Van Bressem *et al* 2008), with water quality, stress and depressed immune function identified as common correlates (Harzen & Brunnick 1997; Gulland *et al* 2001; Van Bressem *et al* 2008). Cutaneous and oral lesions were measured separately, with the former characterised by extensive sloughing (see Gulland *et al* 2001), plaque-like patches, erupting blisters, and lesions characteristic of well-documented cetacean diseases, including erysipelas, papilloma virus, pox virus, and dermatitis (see Van Bressem *et al* 2008 for morphologies). Oral anomalies were identified as general lacerations, cracked/broken teeth, discoloured teeth, and lesions characteristic of papilloma virus on the tongue, gums, and/or throat tissue (Brooks & Anderson 1998).

The last measure evaluated the facility's blood-sampling protocol, which is critical to acute and prophylactic care, particularly since dolphins may delay external signs of disease (Waples & Gales 2002; Castellote & Fossa 2006). Haematologic parameters are useful, objective disease indicators (eg Waples & Gales 2002; Brando 2010), and leukocyte count, endocrine hormones (specifically cortisol, see Schmitt *et al* [2010] and Fair *et al* [2014]), and haematocrit were selected as primary indicators of external and internal stressors. The measure assessed whether each dolphin's values were within specified ranges, established by the *CRC Handbook for Marine Mammals* (Bossart *et al* 2001), and expanded to account for individual variation (Engelhardt 1979; Wells *et al* 2004).

Absence of pain and distress during management procedures

The WQ assessments include a criterion for pain associated with management procedures, such as de-horning, castration, and tail docking (Welfare Quality®2009a,b,c). Acute, invasive procedures are rare at dolphin facilities, but the criterion was applied to common husbandry practices, including blood draws, beaching, and gastric tubing, and altered to assess the potential 'distress' of these interactions by monitoring the procedure success and voluntary participation (Ridgway 1993; Brando 2012; Crane 2013). In the C-Well® assessment, each of the three husbandry procedures was tested on 20% of the total captive population, selected randomly, and two or less failures to achieve the desired outcome in a single trial with voluntary participation, defined explicitly for each procedure, resulted in the highest welfare score.

The fourth measure assessed the presence (yields a good welfare score of 0) or absence (poor welfare score of 2) of 'emergency containment training', defined as either net avoidance or gate training. Marine mammals are commonly moved within and among enclosures for feeding, training, medical care, or in emergency situations where rapid access or separation must occur (Bloomsmith *et al* 2003; Colahan & Breder 2003); the ability to achieve this with voluntary animal participation is critical to animal health, safety, and welfare.

Expression of social behaviour

When measuring welfare, psychological well-being should be considered (Broom 1991; Dawkins 2006; Brando 2012), although difficult to define and measure in animals. However, assessments of behaviour, specifically social interactions, may provide as much or more information about welfare than physiological measures (Waples & Gales 2002; Hill &

Table 2 Social behaviour categories, behaviours and definitions used in the C-Well® assessment.

Category	Behaviours	**Definition** (adapted from Galhardo *et al* 1996; Samuels & Gifford 1997; Kyngdon *et al* 2003; Trone *et al* 2005)
Affiliative	Play	Focal dolphin and conspecific(s) participate in low-speed chasing (less than 4 m s^{-1}), pushing, object passing, all at lower energy levels than agonistic behaviours
	Rubbing	Focal dolphin and conspecific(s) engage in prolonged (3+ s) body contact while stationary or during a slow-moving swim, often with back and forth movements
	Tactile	Close proximity swimming of focal dolphin and conspecific(s) with one dolphin's body part (non-genital) touching the other (mother-calf pairs must include 3rd dolphin to count as tactile)
	Synchronous swimming	Two or more animals in the enclosure swimming < 2 m apart, horizontally or vertically. Mother-calf pairs do not qualify unless joined by a 3rd dolphin
Agonistic	Raking/biting	Focal dolphin's teeth make contact with another animal
	Chasing	Focal dolphin in a high energy pursuit of another dolphin(s) with swim speeds of approximately 4 m s^{-1} or higher (see reference speed video)
	Jaw pop	Focal dolphin gestures towards another with an open mouth, may open and close rapidly; sometimes accompanied by bubble stream
	Body slam	High energy contact of focal dolphin's full body or tail/peduncle region with another dolphin, either during a leap or underwater
Sexual	Genital-genital contact	Focal dolphin and conspecific engages in genital-to-genital contact, with or without full penetration
	Genital-non genital contact	Focal dolphin participating in genital-to-non-genital contact with conspecific
	Prolonged belly-to-belly contact	Focal dolphin and conspecific(s) engage in prolonged (3+ s) ventral-to-ventral contact

Broom 2009; Joseph & Antrim 2010). Furthermore, terrestrial mammal studies have shown that social stress can increase risk of injury, disease, and decrease immune function (eg Barnett *et al* 1975; Cohen *et al* 1997), and stress may cause morbidity and even mortality in captive bottlenose dolphins (Waples & Gales 2002). *T. truncatus* exhibits a complex social structure in the wild, characterised as fluid associations where members and group size change frequently, as well as some long-term associations (Galhardo *et al* 1996; Wells & Scott 1999; Waples & Gales 2002). Group dynamics are somewhat artificial in captivity due to behaviour management (or lack thereof), managerial decisions regarding housing, and the limited means of avoidance or escape. Therefore, this criterion included a systematic measure for the presence of agonistic, affiliative, and sexual behaviours, similar to those exhibited by wild *T. truncatus*. Although animal well-being is verified by comparing captive behaviour to that of wild conspecifics (see Maki & Bloomsmith 1989), all behaviours are stimulus-driven, and captive animals may not need to perform all wild behaviours to achieve good welfare (Veasey *et al* 1996). Therefore, each of the three behavioural categories was defined by characteristic and mutually exclusive responses (Table 2; definitions from Galhardo *et al* 1996; Samuels & Gifford 1997; Kyngdon *et al* 2003; Trone *et al* 2005) and measured only as present or absent using six repeated 10-min ethogram surveys conducted focally. There is little published research regarding the 'normal' frequency of affiliative, agonistic, and/or sexual dolphin behaviour, only that welfare is likely to be reduced with the expression of agonistic behaviour in high frequencies (Galhardo *et al* 1996; Waples & Gales 2002; Scott *et al* 2005) and possibly reduced in association with low frequencies of affiliative behaviours (Tamaki *et al* 2006). Thus, given our limited understanding of the 'normal' frequencies of dolphin behaviours over time, only presence/absence was recorded, and for each animal, a good welfare score was assigned when at least one behaviour in each category was observed during the sampling period.

Absence of abnormal behaviour

Abnormal behaviour is any behaviour not observed in a wild setting and with no obvious function (Rushen & Mason 2008) and may indicate a failure to cope with the environment and sub-optimal welfare (Mason 1991; Hill & Broom 2009). Stereotypic behaviour is a type of abnormal behaviour, defined as a repetitive sequence of invariant motor acts providing no obvious benefit (Mason 1991), and has been broadly applied as an indicator of poor welfare (Welfare Quality® 2009a,b,c; Draper & Harris 2012; Mononen *et al* 2012). Some animals may exhibit stereotypic behaviour as a coping mechanism (Mason 1991; Dawkins 2006; Rushen & Mason 2008) when exposed to inappropriate conditions (Broom 1991).

A series of ethograms were developed and utilised to measure the frequency of stereotypic behaviours exhibited by individual animals. Potential stereotypies of *T. truncatus* were described in the C-Well® document and included self-mutilation (eg repetitive head/genital scraping, tooth rubbing, intentional and repeated collisions with enclosure structures), pattern swimming, and repetitive vocalisations (see Krishnamurthy 1994), which required meticulous analyses of the established criteria and contexts (Gygax 1993; Clark 2013). For example, repetitive vocalisations were evaluated for social relevance in terms of eliciting a response from conspecifics and thus, fulfilling a purpose, and subsequently excluded as a possible stereotypy. Pattern swimming was evaluated using direction, speed, and

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sociality, and if the repeated movements varied, utilised all available habitat, and/or served a social function (see Smith & Litchfield 2010), the behaviour was not characterised as stereotypic. Stereotypic behaviours observed more than three times per hour for an individual animal were designated as a sign of poor welfare, because stereotypic behaviours constituting 10% or more of waking behaviours are likely to be detrimental to welfare (Broom 1983).

Positive human-animal relationship

Human-animal relationships (HARs) have been studied extensively (for a review, see Hemsworth 2007), and most HAR research focuses on companion and farm animals, predominantly highlighting the relationship between aversive and threatening HARs and poor animal welfare (see Waiblinger *et al* 2006). However, more recent work has shown that reinforcing and affiliative relationships can improve welfare, established through positive reinforcement training, familiarity of handlers, gentle handling, and an increase in interactions (Waiblinger *et al* 2006; Hosey 2008; Carlstead 2009; Brando 2012). Hosey (2008) proposed an adapted model for HAR research in zoo settings and suggested that the optimum HAR should be reinforcing, or at the very least neutral. In most cases, dolphins interact more frequently with trainers than animals in zoos, making HAR assessments particularly relevant as a welfare metric, and two C-Well® measures were developed to assess HARs. The first measure recorded the avoidance, approach, or lack of response of a focal dolphin to a randomly selected animal care staff member positioned unmoving (eye contact permitted) on a surface near the water, *sans* food or enrichment, during a non-session/interaction time for 90 s. The focal dolphin's response was recorded during a 60-s period after an initial 30-s lapse to allow the animal time to recognise the absence of food. The test was repeated three times with different staff to calculate a mean response, with approach behaviour assigned the good welfare score. The second measure simply noted the presence (good welfare)/absence (poor welfare) of affiliative tactile interactions between dolphins and humans without the delivery of primary reinforcement. Non-food tactile interactions are a common HAR test with animals in farms and zoos (Waiblinger *et al* 2006; Hosey 2008; Carlstead 2009) and used in the WQ assessments (Welfare Quality® 2009a,b,c).

Discussion

The C-Well® assessment is a practical adaptation of the farm animal Welfare Quality® framework to a novel species, stimulated by the first interspecies adaptation of WQ (ie WelFur; Botreau *et al* 2012; Mononen *et al* 2012). C-Well® includes 58% animal-based measures (a high proportion relative to other WQ frameworks and WelFur) and the ability to simultaneously analyse behavioural and physiological parameters, which is considered the most effective approach to accurate measures of welfare (Broom & Johnson 1993; Castellote & Fossa 2006). Since there are no published, comprehensive welfare assessments for zoo animals that include animal-based measures, the WQ for farm animals was identified as the optimal model for adaptation. The C-Well® assessment is strategically redundant, such that, if a certain measure fails to detect poor welfare, it is likely to be identified in other measures. Additionally, C-Well® allows for measurements of the welfare of individual animals (ideal when assessing welfare; Barber [2009]), meaningful demographics, and entire facilities, all of which would allow for the development of 'best practices' for the industry as a whole. Systematic application of C-Well® animal-based measures on different demographics and group compositions could be used to elucidate harmonious social relationships that are likely to promote good welfare, since group structure is considered to be extremely important to cetacean welfare (Galhardo *et al* 1996; Waples & Gales 2002; Wells 2009). Given that current marine mammal regulations and welfare considerations are principally resource-based and out of date (Conley 2008), the C-Well® assessment can contribute to the improved care of marine mammals worldwide.

The C-Well® assessment was designed to be practical and can be fully executed in two days per ten dolphins. The measures can be broadly separated as those conducted while animals are under stimulus control, those assessed when animals are not under stimulus control, measures evaluated in part by querying the facility curator and records, and those measured opportunistically. While the literature suggests that *in situ* assessments are both reliable and feasible (Botreau *et al* 2007; Roe *et al* 2011), single assessments may be biased by seasonal changes in physiology, behaviour, sexuality, aggression, and inappetence common to *T. truncatus* both in managed care and the wild (eg Wells 2009). Thus, with an attitude of 'collective responsibility' (Roe *et al* 2011) and a shared goal of good welfare and a solution-driven approach, repeated assessments may be required during periods of anomalous conditions. According to Maple (2007), systematic behaviour monitoring is critical to achieving the highest welfare standards and, as such, C-Well® measures can be applied regularly as a management tool (eg for aggression management), with or without a full assessment. Furthermore, Waples and Gales (2002) stated that quantitative behaviour monitoring is crucial for managing *T. truncatus* in captivity and ensuring good welfare, and the animal-based C-Well® measures have the practicability to be used for such purposes.

Initially, C-Well® should be executed by a trained individual, familiar with the methodology, metrics, and relevant evaluation tools, with the intent to expand training to allow for in-house evaluations and applications, such as improved animal management, collaboration, and research. C-Well® is simple, systematic, and accompanied by a comprehensive instructional handbook, complete with information regarding chronological considerations, step-by-step instructions for each measure and relevant calculations, datasheets, and both video and photographic references. The assessment allows for the quantification of a cumulative score for each individual and for the facility as a whole, which allows for intra- and inter-group comparisons to be made, as well as comparisons between and among facilities, and the potential to establish best practices.

The current, functional limitations to the C-Well® assessment include its restricted applicability to very young dolphins (ie neonates and first-year calves) that are still developing physiologically and behaviourally and geriatric dolphins. Age-related limitations have not been addressed by farm animal assessments, since these animals do not reach geriatric ages, and geriatric dolphins will inevitably experience health deterioration and weakening (Galhardo *et al* 1996; Brando 2010), which may confound welfare measures (Gulland *et al* 2001). Additionally, C-Well® was developed at three natural seawater facilities (n = 20), which limited investigations regarding the efficacy of some assessments relevant to different enclosure and facility types. A small sample size also resulted in the absence of weighted scores, which would increase the validity of the overall assessment score (see Botreau *et al* 2012). Furthermore, non-weighted scoring implies that all measures have equal effects on animal welfare, when it is likely that poorer welfare scores in some measures are more wholly detrimental. Thus, future C-Well® developments should include further validation utilising different zoological models, weighted scores, separate measures for calves and geriatric animals, and the potential for expansion to include other cetacean species and animals in rehabilitative care.

Although the C-Well® assessment was designed to be robust, yet conservative and objective, there may be inherent limitations regarding the conclusions made about dolphin welfare in cases where research is limited and/or conflicting. For example, stereotypic behaviour was utilised as a metric for welfare in the 'Absence of abnormal behaviour' criterion. Current research suggests that stereotypic behaviour indicates reduced welfare in many cases but, in some cases, animals exhibiting stereotypies as a coping mechanism may have better welfare than their non-stereotyping conspecifics (Mason 1991; Dawkins 2006; Rushen & Mason 2008). Mason and Latham (2004) reviewed the many, significant correlations between sub-optimal environments and stereotypic behaviours and recommended future studies involving combined measures of stereotypic behaviour and other behavioural or physiological parameters. As such, C-Well® was strategically designed to be comprehensive, with 36 measures of welfare used collectively to evaluate individuals and groups, thereby creating a more holistic and reliable measure of animal welfare. As the fields of marine mammal science and medicine progress, including more investigations of dolphin welfare, these data are likely to contribute to improved C-Well® measures and scoring.

The C-Well® assessment represents the first, documented effort towards producing a standardised welfare assessment tool for bottlenose dolphins in captivity and should be regarded as an initial step in a larger effort to improve marine mammal husbandry, with ample opportunities for cross-disciplinary collaboration and refinement. As is encouraged in the field of welfare science (Broom & Johnson 1993; Webster 2005; Castellote & Fossa 2006), future studies should correlate behavioural and physiological parameters to validate measures of welfare with additional animals in an effort to refine the measures included in C-Well® and those described in other welfare studies.

A major objective of this study was to stimulate discussion and continued research on welfare indicators for cetaceans, which can be used to monitor and subsequently improve their quality of life. A supplemental function of the C-Well® assessment, similar to the WelFur project (Mononen *et al* 2012), is its potential use for certification, benchmarking, and advisory purposes. As the first assessment of its kind, it could also be used as a model for the development of welfare assessments for other cetacean species and terrestrial zoo animals.

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