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Monitoring Bottlenose Dolphin (*Tursiops truncatus*) Welfare During a Functional Neuroimaging Study

Audra E. Ames,[1, 2] Clara Agustí,[3] Frederic Dick,[4] Oriol Talló-Parra,[3]
Anthony Price,[5] Teresa Álvaro Álvarez,[1] Daniel García Párraga,[1]
Julieta Arenarez Lozano,[1] Adrian González Quintero,[1] Alba Rincon Monne,[1]
Amadeo Ten-Esteve,[6, 7] Africa Almendros-Riaza,[6] Alena Uus,[5] Maëlina Gomez,[3]
Luis Marti-Bonmati,[6, 8] Barbara Shinn-Cunningham,[9] and Peter L. Tyack[10]

[1]*Fundación Oceanogràfic de la Comunitat Valenciana, Valencia 46013, Spain*
[2]*Hubbs SeaWorld Research Institute (HSWRI), San Diego, CA 92109, USA*
E-mail: aames@hswri.org
[3]*Animal Welfare Education Centre (AWEC) and Department of Animal and Food Science,*
School of Veterinary Science, Universitat Autònoma de Barcelona, Barcelona 08193, Spain
[4]*Birkbeck/UCL Centre for NeuroImaging (BUCNI), Department of Experimental Psychology,*
University College London, London WC1H 0AP, UK
[5]*School of Biomedical Engineering and Imaging Sciences, King's College, London WC2R 2LS, UK*
[6]*Biomedical Imaging Research Group (GIBI230), La Fe Health Research Institute,*
Imaging La Fe node at Distributed Network for Biomedical Imaging (ReDIB),
Unique Scientific and Technical Infrastructures (ICTS), Valencia 46026, Spain
[7]*Universitat Politècnica de València, Valencia 46022, Spain*
[8]*Radiology Department, La Fe University and Polytechnic Hospital, Valencia 46026, Spain*
[9]*Neuroscience Institute, Carnegie Mellon University, Pittsburgh, PA 15213, USA*
[10]*Biology Department, Woods Hole Oceanographic Institution (WHOI), Woods Hole, MA 02543, USA*

Abstract

The development of non-invasive methods to study brain structure and function has enabled a flowering of cognitive neuroscience in humans and nonhuman species. Herein, we describe the development of protocols for functional magnetic resonance imaging (fMRI) of a bottlenose dolphin (*Tursiops truncatus*), including protocols to monitor the health and welfare of the subject over the course of our five-year study. A Welfare Control Plan (WCP) was designed to monitor, enhance, and protect our subject's welfare throughout the course of the study. The WCP was developed so our team of marine mammal veterinarians, trainers, and researchers could (1) identify study procedures that might negatively impact the individual's welfare and propose measures to mitigate them, (2) define and implement protocols for monitoring the individual's welfare throughout the study, and (3) determine the study's temporary or final endpoints. Overall, behavioral, physiological, and health welfare indicators showed that the dolphin's quality of life was not negatively impacted by participating in our functional neuroimaging study. Our study provides an example of how innovative, ambitious, and logistically complex animal studies can successfully be performed while protecting the welfare of participating animals through adequate planning, enough human and economic resources, and full human/institutional commitment to animal welfare.

Key Words: bottlenose dolphin, fMRI, welfare, behavior, scan, cortex, sensory projection

Introduction

Many factors have driven neuroscience to focus increasingly on a few rodent taxa as model mammals (Manger et al., 2008). This narrowing enables the development of standardized research tools and protocols, increases efficiency, and facilitates collaboration and replication (Yartsev, 2017). Applying results from rodents to other mammals, including humans, relies on the assumption of common mechanisms across mammals, however. Failures of this assumption are one reason for repeated failures of translational research (Nestler & Hyman, 2010; McGonigle, 2014). Testing this assumption requires a comparative approach that involves studying phylogenetically diverse species (Carlson, 2012).

We know that many different skills requiring specialized mechanisms exist across mammals. The approach of carefully selecting the best species for studying a specific scientific problem suggests benefits of doing extra work to broaden the diversity of animal models (Brenowitz & Zakon, 2015). For example, vocal learning is a specialized skill that is important for human language but is rare among mammals. There is some equivocal evidence for and against vocal learning in mice (Kikusui et al., 2011; Arriaga & Jarvis, 2013; Hammerschmidt et al., 2015) and evidence for limited changes in acoustic features of species-specific sounds in other species (Tyack, 2019), but stronger evidence for imitation of novel sounds in other species suggests considering their selection for comparative studies on vocal learning. The strongest evidence for nonhuman mammals able to imitate new sounds comes from cetaceans (e.g., Richards et al., 1984), seals (e.g., Ralls et al., 1985), and elephants (e.g., Stoeger et al., 2012).

Herein, we explore the potential for leveraging non-invasive neural measures and experiments designed for cognitive neuroscience studies in humans to new species, especially those that require heightened attention to welfare. We chose the bottlenose dolphin (*Tursiops truncatus*) as a species with complex vocal learning capabilities and a cortical organization that is thought to differ strongly from terrestrial mammals. Measuring evoked potentials from electrodes implanted into the cortex, Ladygina & Supin (1977) reported visual and auditory projection areas on the dorsum of the cortex, with the visual area shifting from the occipital and the auditory from the temporal lobe to the parietal. Bullock & Ridgway (1972), using similar methods, reported two separate auditory areas, one with rapid (~1 ms) responses to short, high-frequency sounds like dolphin echolocation clicks, and the other with longer (100s of ms) responses to tonal signals like dolphin whistles that are used for communication. This earlier research on the dolphin brain suggests unusual locations of receptive fields for auditory and visual stimuli, with different regions specialized for processing echolocation clicks vs communication whistles. While these findings are intriguing, they are not replicable as such methods are invasive and not ethical by modern animal welfare standards, especially for a taxon with special protected status in many jurisdictions. Thus, the goal of the larger study outlined here is to use functional MRI (fMRI) scanning as a non-invasive method to identify activation differences within brain areas of a dolphin listening to clicks vs whistles.

There are many challenges to developing fMRI methodology for dolphins, especially those related to animal welfare and training. For example, bottlenose dolphins have a brain size similar to humans, but the adult body size is larger, so many animals cannot fit into standard scanners designed for humans. The single MRI scan reported for a living dolphin (Ridgway et al., 2006) was designed to study unihemispheric slow wave sleep, also first demonstrated in bottlenose dolphins through invasively implanted electrodes (Mukhametov et al., 1977). Ridgway et al. (2006) used MRI for a structural scan of the brain while combining other methods to register functional scans. Houser et al. (2010) used positron emission tomography (PET) coupled with images from Ridgway et al. (2006) to measure blood flow and glucose uptake in brain tissue. No study to date has used fMRI with a living dolphin. Consequently, prior to answering broader questions related to signal processing in the dolphin brain, we first explored whether, and how, it might even be possible to develop such non-invasive methods to study brain function in living dolphins while still maintaining high standards appropriate to the elevated welfare requirements of this protected species. We present our approach to these challenges here. Namely, we detail protocols we developed to (1) desensitize the dolphin to MRI scanning, (2) safely transport the dolphin from Oceanogràfic's dolphinarium to inside the scanner and back, (3) train the dolphin for relaxed behavior and apnea during scanning sequences, and (4) monitor the health and welfare of the dolphin subject over both the short- and long-term to determine potential impacts of the study to our subject's welfare status.

Methods

Subject, Facilities, and Materials

This study's subject ("NEP") was an adult male bottlenose dolphin housed at Oceanogràfic Aquarium in Valencia, Spain. NEP was 14 y old at the onset of scans in 2019; he was born at Oceanogràfic's dolphinarium on 21 September 2004. Oceanogràfic's dolphinarium is a six-pool system housing 18 dolphins (Figure 1). All pools are 11 m in depth and connected via a visually and acoustically transparent gating system. Three specific pools (P1, P2B, and P2C; Figure 1) were used for NEP's training during the study as these pools each provided access from the pool to a flat area along one poolside (hereon referred to as a "slide-out"). This allowed NEP to voluntarily remove himself from the water, a necessary behavior for the study's progression (see "Animal Training"). Note that NEP could exit from the slide-out back into the pool, which provided him the choice to voluntarily stop a training session at any time.

All scans for this study were conducted using a 3T Philips Achieva (Philips, Amsterdam, Netherlands) 60-cm bore scanner with a torso XL receive coil. Scans were conducted at La Fe University and Polytechnic Hospital (Valencia, Spain). Recordings for NEP's desensitization

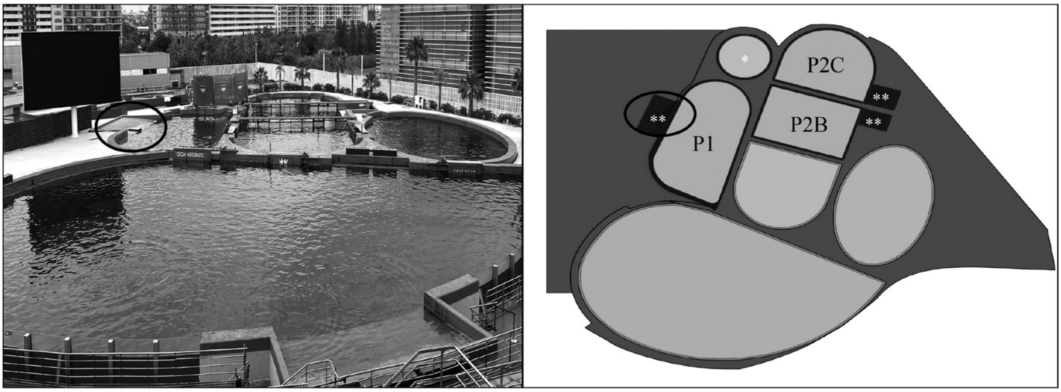

Figure 1. Oceanogràfic's Dolphinarium stadium view (left) and overhead schematics (right) with study pools labeled. * denotes the medical pool; the black ovals denote the slide-out of P1 in both panels; and ** denotes the slide-outs for all the pools. (Photo courtesy of Audra E. Ames)

to scanner noise (see "Animal Training") were taken in-air with an iPhone, Version 11 (recordings taken near slightly ajar scanner room door) and played back to NEP in-air using a Woxter Dynamic Line DL-410BT speaker (Frequency range: 40 to 20,000 Hz; Woxter, Leganés, Spain).

Individual Selection—As evidenced in other studies with marine mammals in managed care (e.g., Clark, 2017; Ortiz et al., 2017; Brando et al., 2018), engaging in research that involves learning and training can provide positive mental stimulation through cognitive enrichment for some individuals. NEP was chosen as the subject of the current study by Oceanogràfic's training team because of his obvious motivation to participate in research-related tasks and his capacity to learn new behaviors quickly. From early life, NEP was continually socialized with his human trainers and is thus habituated to husbandry and additional training procedures.

Animal Training

Training with NEP began in 2017 and was gradually adapted over the course of the study to fit evolving behavioral criteria necessary for successful MR image acquisition. For example, dolphins breathe with an explosive exhalation and rapid inhalation, which causes more movement than typical for human breathing. Thus, the final version of NEP's scan-ready behavior requested that NEP hold his breath while remaining as motionless as possible for short durations during functional and structural scans. Approximations towards this scan-ready behavior included modifying some previously existing behaviors in NEP's repertoire as well as the training of some novel behaviors.

Desensitization of NEP to scanner noise began in 2019. All noise types produced by the scanner during the sequences used in this study were recorded and played back to NEP in-air during a progression of session types. Trainers began by introducing the noises to NEP's daily husbandry sessions. Once NEP was desensitized to the scanner noise within his daily routine, scanner noises were played back during sessions related to scanning objectives (e.g., beaching behavior). The speaker output level was at its maximum setting for all sessions involving noise desensitization, with trainers moving the speaker closer in proximity to NEP over time. Final speaker positioning was 1 m in front of NEP at a 45° horizontal angle with respect to his body axis. It should be noted that received levels of the scanner noise during desensitization would have varied from final conditions inside the scan room as noise propagation would occur differently from a speaker playback in open air vs inside an enclosed room.

From 2020 to 2022, MR functional imaging was attempted with NEP during sessions in which he was not asked to breath-hold or refrain from moving. Functional scanning would occur in four runs, each lasting between 3 to 4 min, during which acoustic stimuli were played to NEP intermittently with short silences. A 30-s food reward break occurred in the middle of each run during which acoustic presentation would stop. NEP was not rewarded during the rest of the run outside of this 30-s break time. In 2022, the protocol was updated to include a discriminatory stimulus (S^D) that requested apnea and for NEP to refrain from movement. For this S^D, we used an annulus with a handle attached (Figure 2) that was presented to NEP by placing it around his rostrum. We found that tactile presentation of this S^D was successful in circumventing potential limitations to NEP's field of vision while in the scanner. We also modified the annulus with white tape to increase S^D visibility in the scanner room (Figure 2).

The final scan-ready behavior was trained in approximately 1 y. First steps towards this final behavior focused on NEP's apnea when presented with the annulus. The first approximation for this apnea behavior used differential reinforcement of a behavior incompatible with breathing. NEP was asked to station with his blowhole underwater (i.e., the position incompatible with breathing) and was given food rewards while the S^D was presented until NEP began associating the S^D with breath-holding. NEP was then asked to station with his body parallel to the side of the pool; and while his blowhole was above the water's surface, he was presented with the annulus target again to ensure that he was correctly associating apnea with the S^D. Sessions in which NEP's blowhole was underwater or above water for presentation of the S^D alternated until NEP completely associated the S^D presentation with apnea. Following this association, NEP was then asked to "beach" himself (i.e., slide his body partially or entirely out of the water onto one of the training pool slide-outs and into a position parallel with the edge of the pool; Figure 3A). This beaching behavior was a previously trained behavior for NEP that was modified to include presentation of the annulus.

Figure 2. (A) The annulus which was used as a discriminatory stimulus for the final scan-ready behavior; and (B) presentation of the annulus S^D by trainer. (Photos courtesy of Audra E. Ames)

While NEP was beached, the apnea S^D was first presented to NEP with no motion restriction. Once NEP performed the apnea behavior successfully while beached, the training team began to ask NEP to rest his fluke and rostrum while holding his breath, simulating NEP's position in the scanner (Figure 3A). The training team accomplished this position by applying the annulus to NEP's rostrum and then slowly lowering it so that NEP's head would come to lie flat on the slide-out. During this approximation, the training team simultaneously supported the rest of NEP's body so that he could relax his fluke (see Video S1 for full behavioral sequence; the supplemental video and tables for this article are available on the *Aquatic Mammals* website). NEP then began to associate the annulus S^D with apnea and assumption of this resting position out of the water; and later, NEP began to assume the simulated scanner position without the physical support of the trainers. Once this association occurred, the training team then worked on prolonging the duration of stillness in this position with simultaneously occurring apnea for up to 30 to 40 s. NEP was trained to assume the final scan-ready position for 30 s for scans in the early part of 2023 (see "Results"). In some sessions, an additional black mat was curved over his body to simulate the scanner (Figure 3B). Later in

Figure 3. (A) NEP in relaxed, "scan-ready" position with jawphones and annulus applied; and (B) NEP in "scan-ready" position with jawphones and annulus applied, as well as the addition of the folding mat (underneath NEP) and black mat as a mock scanner. Note that NEP is in P2C in panel 3A and in P1 in panel 3B. (Photos courtesy of Audra E. Ames)

2023, the duration of NEP's scan-ready breath-hold increased to 40 s to better accommodate presentation of acoustic stimuli during the functional scanning. Training to increase the apnea duration by 10 s began approximately 2 mo prior to the scans at the end of 2023.

During scans, presentations of acoustic stimuli were shortened to 30 to 40 s blocks to accommodate the final scan behavior, after which NEP rested and was rewarded. Once NEP was rewarded, trainers would pause for NEP to inhale and then present the annulus S^D again, signaling researchers in the scan and control rooms to start the next acoustic block presentation (see "MRI Protocol").

From 2020 to 2022, animal training included behaviors which allowed removal of NEP from the water via the dolphinarium medical pool (Figure 1), a pool with a maximum depth of 2.5 m and a false metal bottom that could be lifted to create a shallower pool. NEP was asked to enter the medical pool wherein he would be isolated physically from other group members by closing an acoustically and visually transparent gating system. During 2022, however, the medical pool of Oceanogràfic's dolphinarium was used for quarantine of a different animal, so NEP's extraction method was adapted using the previously trained beaching behavior. NEP was asked to beach onto a folding mat laid flat on the P1 slide-out (Figure 1). Once NEP was fully on the mat, the training team pulled the mat away from the pool's side to load NEP onto the stretcher used for transport. NEP was also asked to beach on the folding mat during other session types, including sessions with the annulus S^D, to decrease association of the mat with transportation. Mat training for annulus presentation began by asking NEP to remove himself completely from the pool so that he was perpendicular to the pool on the slide-out with his fluke resting on the edge (Figure 4A). Then NEP was manually adjusted by the training team so that his body was parallel with the pool's side, at which point NEP could assume the rest position described above (Figure 4B).

Animal Welfare

A Welfare Control Plan (WCP) was designed to monitor, enhance, and protect NEP's welfare throughout the course of the study. To achieve its goals, the WCP worked at three levels: (1) identification of all study procedures with a potential negative impact on the individual's welfare as well as proposing measures to mitigate them (Table 1), (2) definition and implementation of a protocol for monitoring the individual's welfare throughout the study, and (3) determination of the study's temporary or final endpoints. The following subsections identify key welfare concerns related to each level of the WCP and how they were addressed during the study.

The WCP was developed through review of the published literature and through consultation with accredited welfare specialists and researchers, as well as Oceanogràfic's own veterinary and animal care staff, to customize the WCP to NEP's life history. The WCP was then overseen by the welfare management team (i.e., authors of this report affiliated with the Animal Welfare Education Centre [AWEC] of the School of Veterinary Science, Universitat Autònoma de Barcelona, Spain) but included the participation of Oceanogràfic veterinarians, curators, and dolphin trainers in all

Figure 4. (A) Mat training for annulus presentation in which NEP has beached from the perpendicular to the pool on the slide-out with his fluke resting on the edge; and (B) adjustment of NEP by training team to be parallel with the poolside. (Photos courtesy of Audra E. Ames)

welfare-related decisions. The welfare management team consisted of an onsite animal welfare officer (author OTP), who was responsible for coordinating animal welfare for all Oceanogràfic, and a team of welfare monitors (authors CA and MG) supervised by the animal welfare officer and dedicated specifically to NEP.

Oceanogràfic is accredited by the American Zoo and Aquarium Association (AZA), European Association of Zoos and Aquaria (EAZA), American Humane Association (AHA), Iberian Association of Zoos and Aquaria (AIZA), and European Association for Aquatic Mammals (EAAM), which demonstrates the aquarium's continued excellence in animal care. The current study was approved by Oceanogràfic's Animal Care and Welfare Committee (Protocol Number OCE-10-20), the University of St Andrew's Animal Welfare and Ethics Review Body (Protocol Number SEC17017), and the Institutional Animal Care and Use Committees for the Woods Hole Oceanographic Institution (Number 26107) and for Carnegie Mellon University (Protocol Number PROTO201900022).

Identification of Welfare Threats and Refinement of Procedures—Potential risks to NEP's welfare were identified in the first step of the WCP (Table 1).

Pre-Scan Welfare Protocol—Desensitization of NEP to new stimuli and study materials at the edge of the pool was critical in familiarizing the animal with the process and eliminating potential aversive responses (Mineka & Cook, 1986; Bassett et al., 2003). Desensitization to scanner noise was discontinued following the third scan, but reintroduction of some stimuli, materials, or contexts (e.g., folding mat, black mat, medical pool

Table 1. Summary of identified procedures with a potential impact on the individual's welfare and measures to mitigate or refine the impact

Potential welfare threat	Rationale and animal responses	Mitigation and refinement measures*
Social isolation (medical pool, transport, and scan session)	Entails a lack of space for free movement, an invariant environment, and infeasibility of animal-to-animal interactive activity. Potential responses include decreased or increased states of arousal and lack of response to stimulation, enrichment, or reinforcement.	• Previous training and desensitization • Animal accompaniment by a known trainer • Request animal to voluntarily enter the medical pool • Context-adapted and individualized environmental enrichment plan • Frequent positive reinforcement • Increase in number of play and feeding sessions • Alternative voluntary beaching behavior from social pool instead of isolation in medical pool
Removal of animal from water (during transport and scan session)	Animal is removed to an environment where the body is no longer supported by water; thus, breathing is constrained and physiological stress is likely to increase. Additionally, removal enables a restriction of free movement, choice, and control, and inescapable sensory impositions (e.g., noise). Potential responses include animal agitation, perceived threat avoidance, escape, defensive behavior (e.g., open mouth, biting, raking), auditory discomfort, thermal discomfort (e.g., hyperthermia), physical discomfort (e.g., pain, skin irritation, muscle tension), dizziness, fear response and neophobia.	• Previous training and desensitization (e.g., beaching behavior) • Animal continuously accompanied by a known trainer and veterinarian • Frequent positive reinforcement • Best handling practices* • Continuous application of water to animal • Implementation of anti-anxiety medication
• *Animal transportation*		• A short and pre-defined itinerary • Use of an adapted cushioned vehicle with refrigerated cabin and refinement in driving style (smooth drive with low and constant speed, and gentle cornering and braking)
• *Animal handling while loading into and while inside the scanner*		• Previous training and desensitization • Dim light and climate-controlled room • Best handling practices* • Frequent positive reinforcement
• *Animal exposure to scanner noise and magnetic fields*		• Previous training and desensitization • Frequent positive reinforcement

*Best handling practices as defined by the *Cetacean and Pinniped Transport Best Practices* (National Marine Fisheries Service [NMFS], 2022) and the *Standards and Guidelines for the Management of Aquatic Mammals Under Human Care* (European Association for Aquatic Mammals [EAAM], 2019).

access) occurred at least 1 mo to 2 wks prior to each scan session. Specific desensitization and training steps for pre-scan periods are described in detail above (see "Animal Training"). Additionally, protocols for transportation, access to hospital premises, and scanning were practiced by the trainers, animal care staff, and researchers prior to every scan to minimize NEP's time out of the water.

Transportation and Scan Welfare Protocol—Approximately 1.5 h and again 20 min prior to NEP's transport to La Fe, diazepam (Valium®) was administered orally (via fish) in a dose of 0.2 mg/kg of body weight to reduce anxiety levels during all scan sessions. Diazepam is an effective anti-anxiety drug for bottlenose dolphins, which is commonly used to manage potential short-term stressful and transitional circumstances (Gulland et al., 2018; Kastelein et al., 2023). Some slight sedation but no other evident physiological or behavioral changes occurred upon administration of this medication and dosage to NEP prior to scans.

The maximum time limit for NEP to be out of the water was 2 h, with 1 h allotted for NEP to be inside the scanner. Establishing this 2 h limit was conservative and based on reports that show dolphin stress indicators return to baseline quickly following removal from water for 2 h (Champagne et al., 2018). A qualified team of (at minimum) seven dolphin trainers well known to NEP, four veterinarians, and one animal welfare supervisor (the animal welfare officer or another member of the welfare monitoring team) remained with NEP from 1 h prior to and following transportation from the dolphinarium pools at Oceanogràfic to La Fe, including the full duration of time NEP was in the scanner. During each scan, this team focused on recognizing any abnormalities in NEP's behavior indicative of distress or discomfort, identifying emerging health concerns, preventing and detecting potential risks, maintaining NEP's position in the scanner, and administering treatments if necessary. If signs of distress were noted by the team, the protocol called for ending the session and returning NEP to Oceanogràfic's dolphin pools.

For scan sessions that occurred from 2019 to 2022, NEP was asked to enter the medical pool 2 to 3 h prior to transport. During this period, environmental enrichment was provided, and the frequency of play and feeding sessions with trainers was increased to manage the effects of NEP's temporary physical isolation from other social group members. Immediately before transport, the medical pool platform was lifted while the animal was positively reinforced. NEP's body was then lifted by the trainers so that a stretcher could be placed underneath. For scans completed in 2023, NEP was moved from the folding mat to the transportation stretcher using the same lifting technique as above.

After the animal was centered in the stretcher, he was rapidly lifted and moved to the transport van's cargo hold (~10 m from the medical pool or P1 poolside; Figure 1), outfitted to meet EAAM transport recommendations. Animal transportation followed a predefined route (~3 km from the Oceanogràfic to the Hospital La Fe). Because transportation of NEP to La Fe was brief (approx. 22 min ± 2 [SD] from pool to van arrival at La Fe), NEP was placed on a 20-cm open foam mat inside the van's hold, which was also coated with cell foam pads and made free from protrusions. A veterinarian and two trainers remained with NEP in the hold to monitor the animal during transport and to apply water continuously to NEP's skin via a sprayer. Cold bags (bags full of ice or frozen gel) placed on the peduncle near the fluke covered with a damp towel were also used during transport or scans as needed to prevent overheating. No desensitization of water or cold bag application occurred prior to transports. No straps or other immobilization devices were used during either transport or scanning. Handling methods listed here followed best handling practices as defined by the *Cetacean and Pinniped Transport Best Practices* (National Marine Fisheries Service [NMFS], 2022) and the *Standards and Guidelines for the Management of Aquatic Mammals Under Human Care* (European Association of Aquatic Mammals [EAAM], 2019).

Once the van reached La Fe, trainers and care staff who had arrived in alternate vehicles met with the transport van to load NEP from the vehicle onto a wheeled stretcher and move him to the MR scanner room—reaching the room within 5 to 10 min of arrival. Upon reaching the scanner room, NEP was loaded onto the scanner table with his head centered in the bore. A trainer or veterinarian signaled to begin the MRI protocol once NEP was positioned correctly in the scanner. Trainers continuously applied water to areas of NEP's body outside of the bore while he was in the scanner and positively reinforced NEP throughout the scan session and transportation protocol using tactile and food reinforcements. All food rewards consisted of dead fish consumed as part of NEP's regular daily diet requirements. Once the MRI protocol was completed, the transportation protocol was then reversed to return the animal to Oceanogràfic's dolphin pools.

Post-Scan Welfare Protocol—On return to Oceanogràfic's dolphinarium, NEP was immediately reintroduced to the water either via lowering the medical pool's adjustable bottom or by sliding him from the folding mat into P1 once the mat was positioned poolside (Figure 1). NEP then received a short, positive training session before fully returning to his social group. Behavioral observations of NEP occurred opportunistically for 1 to 2 h following reintroduction to the water post-scan to monitor for short-term aversive effects potentially related to the scan session (see below).

Monitoring Protocol for Short- and Long-Term Effects—Short- and long-term indicators of welfare were categorized as behavioral, health-related, or physiological, and were used to monitor NEP's welfare status on the day of scans, in the days immediately before and after scans, during periods between scan sessions, and over the full course of years while the current scan protocols have been developed and refined. The welfare indicators selected for each context are shown in Table 2.

Monitoring of indicators occurred in one of four contexts (A through D) designed to maximize efficiency of the dolphin training and welfare monitoring teams and to take advantage of the existing welfare monitoring strategies already in place as part of daily animal care routines for Oceanogràfic's dolphins (e.g., veterinary and training welfare-related records, welfare-related research projects). Not all study-related activities had the same potential for adverse effects on NEP's welfare. Thus, for the four WCP identified contexts, different strategies and indicators to monitor and manage NEP's welfare were implemented:

- *Context A (baseline*) – NEP during periods without training or husbandry activities, including daily normal free swim time and normal social activity with other dolphins, and nonsupervised enrichment sessions.

- *Context B* – NEP during daily regular interaction with trainers or veterinarians without any relation to current study activities, including regular husbandry or other research sessions, dolphin presentations for the public, regular medical monitoring, or other contexts involving direct interaction with trainers or veterinarians.

- *Context C* – NEP during any training or desensitization sessions directly relating to the current study (e.g., scan-noise and stimuli desensitization, beaching, transport training, etc.), excluding activities on days of scans.

- *Context D* – Activities conducted during the day of the scan, including transportation preparation (e.g., animal isolated in medical pool), transportation, loading into and positioning of NEP inside the bore, scanning, and return of animal to the pool.

The veterinary staff and training team were responsible for interpreting short-term welfare indicators that could result in a study endpoint (especially in Context D), and for measurement of long-term physiological and health indicators. The animal welfare monitoring team was primarily responsible for monitoring long-term behavioral welfare indicators. Observations conducted by the welfare management team were approximately 15 min in duration and occurred opportunistically within the periods defined below (see "Results"). It should be noted that these observations sampled varying conditions based on variability in NEP's husbandry schedule, pool requirement for the study, and social group integrations.

Four to six hours of behavioral observations were collected opportunistically during periods when no training sessions occurred (Context A) for each of 41 mo within the total study period (62 mo from October 2018 to November 2023). All behavioral observations were made poolside from the surface by an experienced behavioral observer from the welfare monitoring team based on an ethogram developed for the current study (Table S1) that included behavioral events and predominant activity sampling for behavioral states (Mann, 1999). The ethogram was created based on behaviors commonly reported in the literature for dolphins in managed care (Dudzinski, 2010; Von Streit et al., 2011; Harvey et al., 2017; Hill et al., 2017; Clegg et al., 2018) and through conducting a preliminary observational study of NEP to determine NEP's baseline behavior prior to implementation of study activities. During each session, observers recorded the most predominant behavioral state noted in each 1-min interval and in all discrete behavioral events (Martin & Bateson, 2007).

Trainers' Behavioral Observations—To evaluate short-term effects in Contexts A through C, welfare indicators (Table 2) were identified in trainers' daily reports recorded over the study's 62 mo. To conserve trainer effort, observations reported by trainers were made only during NEP's training sessions. Trainers' daily reports were comprised of behavioral welfare indicators noted as either absent (0) or present (1) as these reports were originally designed to quickly identify animal health and behavioral concerns, not behavioral frequencies. These reports were subsequently organized by the welfare management team according to the study's ethogram (Table S1) for analyses presented below. Coded behaviors were organized as (1) socio-sexual behaviors, (2) positive social integration (observations of affiliative social state[s] and/or event[s] following successful integrations with a novel social group), (3) negative social integration (lack of

Table 2. Welfare indicators as defined by the Welfare Control Plan (WCP) for each monitoring context

Indicator type	Indicator	Method of monitoring	Response type	Indicator validity	WCP context
Behavioral	Affiliative behavior	Behavioral observations completed opportunistically by an experienced observer (welfare management team) using a customized ethogram (Table S1) Additionally, trainers' reports of specific behaviors outlined here or their outcomes (e.g., new rake marks due to aggressive behavior) in NEP's daily general records if abnormalities observed	Short-term, long-term	Kuczaj et al., 2013; Clegg et al., 2017; Serres et al., 2020	Contexts A (for long-term responses) and D (for short-term responses)
	Anticipatory behavior		Short-term, long-term	Clegg & Delfour, 2018; Clegg et al., 2018	
	Socio-sexual behavior		Short-term, long-term	Mann, 2006; Clegg et al., 2015; Harvey et al., 2017	
	Agonistic behavior causing injuries, social isolation, or inappetence		Short-term, long-term	Waples & Gales, 2002; Clegg et al., 2015; Serres & Delfour, 2017	
	Play behavior		Short-term, long-term	Paulos et al., 2010; Kuczaj et al., 2013; Serres & Delfour, 2017	
	Exploratory behavior		Short-term, long-term	Maple & Perdue, 2013; Clark, 2017	
	Stereotypic behavior		Short-term, long-term	Kastelein et al., 2016; Serres, 2019	
	Apathetic behavior (floating)		Short-term, long-term	Waples & Gales, 2002; Johnson et al., 2009	
	Regurgitation		Short-term, long-term	Shyne, 2006; Lauderdale, 2017	
	Foreign body ingestion	Trainers' and veterinarians' daily general records of NEP noting ingestion of a foreign body or the item's removal	Short-term, long-term	Couquiaud, 2005	All contexts
	Changes in food consumption	Trainers' daily nutritional records of the percentage of fish that NEP ingests out of the total fish offered (recorded in kg)	Short-term, long-term	Waples & Gales, 2002; Johnson et al., 2009; Clegg et al., 2019	Contexts B, C, and D
	Willingness to participate in training sessions	Trainers' daily records of NEP's willingness to participate in sessions as rated on a 5-point scale (0 to 4) representing incremental motivation and enthusiasm during training sessions	Short-term, long-term	Shyne, 2006; Lauderdale, 2017; Clegg et al., 2019; Delfour et al., 2020; Huettner et al., 2021	Contexts B and C
	Willingness to participate during scan sessions	Animal responsiveness to trainer commands as monitored by trainers	Short-term	Couquiaud, 2005	Context D
	Aggressive behaviors towards trainers (e.g., hit, bite)	Trainers' daily records of aggressive behaviors towards trainers	Short-term, long-term	Waples & Gales, 2002; Johnson et al., 2009; Clegg et al., 2019	Contexts B, C, and D
	Trembling	Trainers' reports and behavioral observations during the scan sessions	Short-term	Câmara et al., 2020; Boys et al., 2022	Contexts C and D
	Body movements (e.g., pronounced body rocking, tail slapping, tail movement side-to-side, tail arch, whole body arching/thrashing, movement in lower jaw)		Short-term		

Physiological	Heart rate	Monitored by veterinarians in the presence of additional indicators suggesting the animal's health is deteriorating (e.g., breath rate, body temperature, behavioral indicators)	Short-term	Linnehan et al., 2020	Context D
	Breath rate	Regularly monitored by trainers during scans	Short-term	Broom & Johnson, 2019; Serres & Delfour, 2019	Context D
	Blood cortisol concentrations	Opportunistically if blood sampling were required for another reason (e.g., regular veterinary monitoring)	Short-term	Fair et al., 2014	Context B
	Body condition	Weekly weighing of the individual (in trainers' daily records)	Long-term	Clegg et al., 2015; Brando et al., 2018	Context B
	Pain (arching, squinting, breaching, staying at depth)	Trainers' daily general records and veterinary records	Short-term, long-term	Waples & Gales, 2002; Johnson et al., 2009	Context A (short- and long-term responses) and D (short-term responses)
	Abnormal swimming movement	Trainers' daily general records and veterinary records	Short-term, long-term	Fish, 1993; Sneddon et al., 2014	
	Shock	Regular monitoring during scan sessions by veterinarians	Short-term	NMFS, 2022	Context D
Health	Blood count and indicators of immune system function (total white cell count, neutrophil/lymphocyte proportion, serum protein electrophoretogram)	Regular veterinary evaluations and veterinary records	Long-term	Gulland et al., 2018; Nollens et al., 2020; Lauderdale et al., 2021	Context B
	Incidence of respiratory diseases	Regular veterinary evaluations and veterinary records	Long-term	Venn-Watson et al., 2012; Clegg et al., 2015	
	Incidence of gastrointestinal ulcerations	Regular veterinary evaluations and veterinary records	Long-term	Sapolsky, 2004; St. Leger et al., 2018	
	Incidence of chronic or recurrent diseases potentially associated with stress	Regular veterinary evaluations and veterinary records	Long-term	Gulland et al., 2001; Clegg et al., 2015	
	Generalized weakness	Regular veterinary evaluations and veterinary records	Short-term, long-term	Waples & Gales, 2002; Johnson et al., 2009	
	Dental wearing	Regular veterinary evaluations and veterinary records	Long-term	Graham & Dow, 1990; Clegg et al., 2015	Context A

engagement with a novel social group or agonistic social state[s] and/or event[s] after social integration that necessitates separation), (4) agonistic behaviors (e.g., observations of agonistic social state[s] and/or event[s] [Table S1] or presence of recent and superficial injuries or rake marks), (5) not present (NEP maintaining distance from trainers, not responding to requests, or with low perceived interest in specific training sessions), (6) difficulty gating (challenging request of pool change as indicated by multiple attempts or reluctance to enter requested pool), and (7) regurgitation (observations of NEP ejecting an entire fish or a mix of water and fish debris out of the mouth). Items 5 "not present" and 6 "difficulty gating" from the trainers' reports were used in analyses as indicators that NEP lacked willingness to participate in training sessions. Regurgitation events were integrated with veterinary observations over the lifetime of the animal to determine whether changes in this behavior were abnormal considering NEP's life history. Items 2 and 3 were compared statistically (see "Statistical Analyses") as positive and negative responses to integration with a new social group. Items 1 and 4 were analyzed as separate welfare indicators. As with data collected by the welfare management team, trainers' observations were made under variable conditions. Behavioral welfare indicators not listed here were not reported as occurring in any training sessions (Table 2).

The welfare indicator "daily willingness to participate" also included a 5-point Likert scale designed to quantitatively assess NEP's motivation and performance during daily training sessions (both routine and for study purposes). This scale, adapted from Clegg et al. (2019), ranged from 0 = No contact, indicating an absence of motivation and interaction, to 4 = Excellent, denoting high levels of motivation and correct responses to trainers' behavioral requests. The score was assigned by NEP's main trainer for the day. Additionally, during training sessions that were only related to study activities (Contexts C and D), trainers included more detailed comments regarding their perception of NEP's willingness to participate in study-related tasks. Changes in NEP's daily food consumption were measured using trainers' daily nutritional records of the percentage of diet that NEP consumed from the total offered (recorded in kg).

Temporary or Final Endpoints—The third and ultimate goal of the WCP was to determine whether the project reached welfare-related temporary or final endpoints. Endpoints (i.e., thresholds of welfare indicators measured by the WCP) are referred to here as the pre-established criteria that indicate when a scan (temporary endpoint) or the study (final endpoint) should be ended to avoid (or limit) adverse welfare impacts. Established endpoints (Table 3) were subjected to review and refinement in the event of unexpected outcome(s).

MRI Protocol

Preparation for Scanning—To prevent contamination and damage from salt water, the scanner was prepared for the dolphin by wrapping the bore in tear-resistant plastic sheeting. The dolphin entered the scanner room on a detachable Philips non-ferromagnetic patient bed, accompanied by the research and veterinary team who were all pre-screened multiple times for metallic objects before arrival and entry to the scanner room. The patient bed could not be used to move the dolphin into the bore isocenter as NEP's dorsal-ventral dimension was too great.

Before loading, the Philips Torso XL 16-channel 2-part chest surface coil array was wrapped in protective plastic and placed by MRI techs on the left and right sides of NEP's head, with the coil ends providing a gap for the blowhole. MR compatible ECG leads were also attached, with leads safely kept uncrossed and away from NEP's body.

The Philips Achieva 3T 60-cm diameter inner bore is smooth and cylindrical with a flared front opening. This permits manual loading of the dolphin with surface coils into the isocenter of the bore via an underbelly cushion and pulley arrangement, with support from dolphin handlers on both sides of the scanner table. For NEP, the brain is at the approximate isocenter of the magnet bore when the dorsal fin is 1 to 2 cm from the bore.

For auditory stimuli delivery, custom-made jawphones (Figure 3) were developed and fabricated from MRI-safe piezoelectric ceramic elements. These were potted in soft silicone suction cups and carefully attached with insulated cables leading to an encased cable trap and joint, which, in turn, was connected to a shielded cable. The cable termination was fed through an in-line RF filter and attached to a BNC connector to the audio equipment, which was isolated from main power by using a portable battery power supply.

The trainers leading the session interacted with NEP at all times at the scanner end where NEP was facing, such that he could see the trainers and they could monitor and be guided by his breathing, movements, and vocalizations. NEP's head, the trainers, and the annulus presentation could also be seen on the console room monitor from the rear-wall-mounted video camera.

In-air acoustic scanner noise is always a concern for MRI. Thus, all people within the scanner room wore high-quality earplugs during scanning. To test for the potential for noise-induced hearing effects on the dolphin, we measured the sound

Table 3. Study's temporary and final endpoints based on the indicators used in the Welfare Control Plan (WCP) for each welfare control context

Indicator type	Endpoint	Type of endpoint
Behavioral	Individual's refusal to voluntarily enter the medical pool (for initial scan sessions) or beach onto the folding mat the day of the scan session	Temporary
	A long-term 20% reduction or cessation of affiliative, socio-sexual, play, or exploratory behaviors (unexplained or related to the project)	Final
	Long-term (≥ 3 times) aggressive behaviors causing injuries, social isolation, or inappetence (unexplained or related to the project)	Final
	Appearance of stereotypic (i.e., repetitive locomotion or movement exhibited three times in succession that does not seem to have an apparent goal or function) behaviors (unexplained or related to the project)	Final
	Appearance of apathetic and unresponsive behavior (unexplained or related to the project)	Final
	Changes to regurgitation and/or reingestion that are inconsistent with the life history of the animal	Final
	Long-term unwillingness to participate in routine training sessions (median score below 3; Table 2) unexplained or related to the project	Final
	Long-term low willingness to participate in the project's training or scan sessions (e.g., animal does not appear motivated, maintaining distance from trainers and/or not taking food)	Final
	Unambiguously directing aggressive behaviors towards trainers in contexts related to the project	Temporary
	Long-term (i.e., multiple occurrences) trainer-directed aggressive behaviors in contexts related to the project	Final
	Appearance of trembling during transport and/or scan	Temporary
	Exhibiting frequent and dangerous bodily movements during transport and/or scan (e.g., pronounced body rocking, tail slapping, tail movement side-to-side, tail arch, whole body arching/thrashing, movement in the lower jaw)	Temporary
Physiological	Heart rate out of range (60 to 120 bpm) during transport or scan	Temporary
	Spontaneous breath rate out of range (6 to 30 breaths/5 min) during transport and/or scan	Temporary
Health	Loss of 20% of peak pre-study weight	Final
	Long-term pain (assessed using behavioral or physical indicators of pain—e.g., body or tail arching, body rocking)	Final
	Appearance of abnormal swimming movement (unexplained or related to the project)	Final
	Shock/collapse	Final
	Long-term blood count and indicators of immune system function (total white blood cell drop below 4,500 wbc/µl, persistent neutrophilia > 85% WBC, globulins < 1g/dl)	Final
	Long-term incidence of respiratory diseases, gastrointestinal ulcerations, or other chronic or recurrent diseases potentially associated with stress and immunodepression (unexplained or related to the project)	Final
	Long-term generalized weakness	Final
	Severe dental wearing (unexplained or related to the project)	Final
Cumulative	Integration of multiple short-term indicators that together are interpreted as a state of distress, discomfort, and a reduction in welfare	Temporary
	Integration of multiple short- and long-term indicators that together are interpreted as a reduction in quality of life	Final
	Reaching of temporary endpoints in several scan sessions	Final

levels in this kind of scanner and compared them to noise levels for hearing impact on dolphins established by Southall et al. (2019). Acoustic noise measurements were made on a matching spec 3T Achieva system (Phillips) using an MR compatible sound level meter (OptiSLM; Optoacoustics Ltd., Mazor, Israel) in-air. Sound pressure level (SPL) measurements were taken with the microphone at scanner isocenter in the z-axis unobstructed by the coil and recorded as ~112 dBA SPL during the fMRI protocol. A second sound meter (Casella CEL-63X; Casella UK, Kempston, UK) was used to determine the frequency spectrum of scanner noise. This instrument was placed at the bore's edge to minimize RF interference from the scanner. The peak SPL (L_{eq}) centered on the 1 kHz octave band was 117.3 dB re 20 µPa, which was dropped consistently for each higher octave band, with a level of 76 dB at 16 kHz. By contrast, dolphin hearing gets more sensitive as one increases frequency over this region, with the frequency range of best hearing much higher than most of the scanner noise.

Dolphin hearing is well studied underwater, but not for sound in-air. Cetacean ears evolved for hearing in water rather than in-air, and scanner noise transmission is strongly attenuated by the impedance mismatch between air and soft tissue. Glover et al. (1995) reported a 30 dB reduction in scanner noise intensity recorded in fluid-filled tissue over the frequency band of concern, with a potential additional 10 dB increment coming from transmission through the scanner platform, leading to a 20 dB reduction when this pathway is not blocked. The dolphin did rest on the scanner platform, so we subtracted 20 dB from the in-air measurements. All measurements of effects of sound on dolphins are made with the underwater reference of 1 µPa, so to compare sound levels to thresholds of effects, we corrected for this different reference by adding 20 dB to the airborne sound levels that were reported in dB re 20 µPa. Southall et al. (2019) estimated effects on dolphin hearing by applying a dolphin-weighting function, similar to A-weighting for humans. Applying this weighting to each octave band of scanner noise from 16 to 16,000 Hz, the decrease in scanner noise higher than 1 kHz is shown to be strong enough to outweigh the increasing sensitivity of dolphin hearing. Following the procedure outlined by Southall et al., we integrated the sound energy across these octave bands and calculated a cumulative sound exposure level from 16 to 16,000 Hz, assuming a maximum exposure duration of 30 min. This sound exposure level of 128.6 dB re 1 $\mu Pa^2 s$ is well below the threshold for onset of temporary effects on hearing of 178 dB re 1 $\mu Pa^2 s$ (Southall et al., 2019).

Anatomical and Body Motion Considerations for MRI—The size, shape, and tissue/air/bone composition of the bottlenose dolphin head presents unique challenges to MRI scanning, particularly as tissue and air compartments are under volitional control. The acoustic melon is a large fat mass anterior to the brain that moves with sound production. The massive upper respiratory tract, which includes sacs and the blowhole, lies just anterior to the frontal cortex and is dynamically modulated for producing tonal whistles and echolocation clicks in a lateralized manner (e.g., Madsen et al., 2013). All these structures are embedded in complex arrangements of bone, muscle, and liquid or air. In addition, the > 200 kg muscular and fat-encased dolphin body also rests close to the transmit and receive RF coils throughout the bore. All these factors non-uniformly affect the magnetic field and do so dynamically if the blowhole, melon, or jaw are moved during image acquisition. Relative to other mammals, the cerebrum is also quite wide and is embedded deep in the head, much further from the receive coil elements than in humans and other primates. Finally, when a breath is taken, the entire head and body move, severely corrupting any image acquired during this period and limiting the duration and type of MR sequence that can be used (see below). Thus, the extensive training to establish comfortable periods of apnea was crucial for imaging.

Unlike the human brain, the largest dimension of NEP's brain is right-left (~170 mm), with 140 mm from the most superior cortex to the most inferior part of the cerebellum, and 120 mm anterior to posterior (e.g., the 50th percentile-sized male adult brain—measured when aligned along the anterior-to-posterior commissures—is 136 mm left-right, 176 mm front-back, and 132 mm top-bottom, including the cerebellum; Mennes et al., 2014). The dolphin brain is also positioned in the body and scanner bore such that the superior-inferior axis is along a rotated coronal axis and is anterior-posterior along the axial axis.

Functional MRI Protocol—After a standard 3-plane T1-weighted localizer to visualize the boundaries and orientation of the brain within the head, and to position the subsequent image volumes, a rapid motion-tolerant static magnetic field (B0) map is acquired during a 30-s apnea. The B0 map is used to calculate a 3D shim field using "image-based" (IB) shimming, which makes the magnetic field within the brain volume as homogeneous as possible, essential for good quality echo-planar imaging (EPI).

Following this, the EPI volume is positioned in the unrotated transverse slice plane to minimize potential image wrap given that NEP's head cannot be repositioned automatically by the scanner to isocenter. The 128 × 128 in-plane imaging matrix is acquired with 2 × SENSE right-left acceleration, 53 × 3 mm slices, a FOV of 350 × 350 mm, and 35 mm (10%) phase oversampling on both sides to allow for small variations in position between apneas. The repetition time (TR) = 3.304 s, flip angle = 90, and TE = 30 ms. The SENSE coil reference was acquired at the start of an initial short EPI calibration scan, also collected during an apnea, which is acquired with reverse phase encoding to enable later B0-inhomogeneity-related image unwarping.

Subsequently, a series of fMRI runs were performed, each for 4 min 30 s, with 82 EPI volumes. For scans during which stimulus blocks were 40 s in duration, scan times were extended to collect > 82 volumes to allow complete sets of stimulus blocks to be performed at a pace determined by the dolphin and trainers (i.e., scans were stopped as required rather than run for a predetermined length). Representative images for EPI volumes are shown in Figure 5C.

Structural MRI Protocol—Structural MRI is challenging because the dolphin's head moves substantially with each breath, making traditional T1-weighted 3D acquisitions infeasible. To address this, approaches developed by Kuklisova-Murgasova and colleagues (2012) for *in utero* imaging of fetal brains were used. Initially, single-shot Fast Spin-Echo (FSE) imaging was employed during spontaneous breathing, with the slice-to-volume motion correction method applied to the image data. Although this method works well on fetal brains, this did not result in good quality

Figure 5. (A) The Oceanogràfic team preparing NEP for scanning; (B) *in-vivo* structural scan (T2 axial and coronal view; contrast inverted) for localization of function; and (C) sagittal view of functional data during NEP apnea, with time resolution 3.3 s per whole-brain EPI volume. (Photo for [A] courtesy of Frederic Dick)

images of NEP's brain due to the shorter T2 relaxation times as is observed in mature adult human brains; this thus limited SNR and resolution. We then transitioned to using a series of fast (30 to 40 s) multi-shot T2-weighted FSE acquisitions, with each package of 20 to 25 slices (1 × 1 × 2 mm acquired voxel size; TE = 82 ms) acquired during a single breath-hold (3 to 4 packages were required to image the full brain). The orientation of the imaging volume was changed to create multiple views of the brain; a 3D image was reconstructed to 0.8 mm isotropic resolution using the slice-to-volume registration (Kuklisova-Murgosova et al., 2012) method in the SVRTK toolbox (Uus, 2020) from seven stacks (Figure 5B).

Statistical Analysis

Behavioral Observations (Welfare Monitoring Team)—All analyses were performed in *R*, Version 4.3.1 (R Core Team, 2021). Behavioral observation data were divided into five time periods related to scan sessions (Table 4). For social play and synchronous swimming, data from the immediate pre-scan period were excluded from analyses when transportation began from the medical pool because of NEP's physical isolation from other dolphins.

Behavioral states were quantified as the percentage of time spent in each observed behavioral state relative to the total observation time of the session's segment (i.e., ~15 min). Enrichment play was treated as the percent of time spent interacting with enrichment devices from total time enrichment devices were offered to NEP within an observation session. Behavioral event frequency was measured by summing events according to each behavior and dividing by the total number of observation minutes for the corresponding session segment. Behavioral state percentages and event frequencies were then averaged across observation sessions within each observation period and reported as the means ± standard deviations (SDs) below.

Only behavioral states that were observed for at least 1% of total observation time and behavioral events that occurred at a rate of more than one time per hour were included in the following analyses (see Table S1 ethogram for behavioral states and events). Kruskall-Wallis H-tests were used (*R* package "coin"; Hothorn et al., 2008) to compare the distribution of behavioral states across the five behavioral observation periods listed in Table 4 as data were not normally distributed. Pairwise comparisons were performed using Dunn's test with adjusted *p* values (*R* package 'FSA'; Ogle et al., 2023). Additionally, nonparametric Mann-Whitney U tests (*R* package "coin"; Hothorn et al., 2008) were used to compare NEP's behavior in the immediate pre-scan period between the two different pools (medical pool and P1).

Trainers' Reports—As with behavioral observations, all analyses were completed using *R*, Version 4.3.1. Data from daily trainers' reports were divided into five time periods related to scan sessions (Table 5): (1) non-scan, (2) 7 to 2 d pre-scan, (3) day of scan, (4) 1 d post-scan, and (5) 2 to 7 d post-scan. Trainers' reports that were incomplete were excluded from analyses. Note that the periods forming the basis of the trainers' reports need not be identical with those sampled by the welfare team.

Kruskall-Wallis H-tests were used (*R* package "coin"; Hothorn et al., 2008) to compare potential changes in total presence/absence of reported behavioral indicators as well as food consumption across the five observation periods. A one-tailed Fisher's exact test (R Core Team, 2021) was used to test whether social integration outcome was more likely for particular study contexts. Daily percentage of food consumption was averaged within each observation period and reported as the mean ± SD below.

Table 4. Welfare monitoring team behavioral observation periods defined

Observation period	Definition
Non-scan	Behavioral observations during periods without scheduled activities and not within 48 h of a scan
Pre-scan	Behavioral observations recorded in the 48 h preceding scan, excluding the immediate 2-h pre-scan window
Immediate pre-scan	Behavioral observations conducted during isolation in the medical pool prior to scan (from 3 to 1.5 h before scan), or in P1 during the 2 h prior to scan
Immediate post-scan	Behavioral observations conducted within the 2-h window immediately following scan
Post-scan	Behavioral observations during the 48 h subsequent to scan, excluding the immediate 2-h post-scan window

Results

Neural scanning of NEP began in 2019 and is still ongoing as part of a larger imaging study on acoustic signal processing (Table 6). Four scans using the apnea methodology presented herein occurred in 2023 (Table 6). NEP was trained to breath-hold for 30-s blocks during the March and May 2023 scans. This duration was increased to 40 s for the October and November 2023 scans.

Behavioral Welfare Indicators (Welfare Team Behavioral Observations: Context A)—Over the study period, the animal welfare team completed 825 behavioral observation sessions across five observation periods. NEP was isolated for 66% of immediate pre-scan observations, excluding these data from synchronous swimming and social play analyses below (see Table 7 for descriptive statistics for each period).

Affiliative, agonistic, socio-sexual, and stereotypic behavioral states were observed in less than 1% of the observations (Figure 6). No significant differences were found across the five time periods for floating behavior ($p = 0.648$). Significant differences were found, however, for synchronous swimming, social and enrichment play, and exploratory and anticipatory behaviors ($p < 0.05$). Post-hoc comparisons showed that synchronous swimming notably increased on NEP's return to the pool immediately post-scan when compared to pre-scan ($p = 0.038$) and immediate pre-scan ($p < 0.001$) periods. There was also significantly less synchronous swimming in the immediate pre-scan period compared to non-scan periods (p

Table 5. Trainers' reports of behavioral observation periods

Observation period	Definition
Non-scan	Daily trainers' behavioral observations during periods of at least 1 wk prior to and 1 wk following scheduled scan activities
Days 7 to 2 pre-scan	Daily trainers' behavioral observations recorded on the 6 d preceding the day of transport and scan
Day of scan	Daily trainers' behavioral observations recorded on the day of transport and scan
Day post-scan	Daily trainers' behavioral observations recorded on the day following transport and scan
Days 2 to 7 post-scan	Daily trainers' behavioral observations recorded on the 6 d remaining following the day after transport and scan

Table 6. Session specifics for all scans; N/A = Not available.

Scan date	Pool prior to transport	Pool removal type	Approx. time out of water (h:min)	Approx. time inside MRI (h:min)	Apnea requested?	Apnea duration (s)
21 June 2019	Medical	False bottom lifted	1:44	0:45	Procedure test; no scan	N/A
13 Dec. 2019	Medical	False bottom lifted	1:26	0:22	Structural scan only	N/A
20 July 2020	Medical	False bottom lifted	2:00	0:51	Structural scan only/ fMRI procedure test	N/A
10 Nov. 2020	Medical	False bottom lifted	1:00	0:30	No	N/A
19 May 2021	Medical	False bottom lifted	1:42	0:45	Structural scan only	N/A
20 Sept. 2021	Medical	False bottom lifted	1:59	1:00	No	N/A
4 May 2022	Medical	False bottom lifted	1:50	0:57	Structural scan only	N/A
7 June 2022	Medical	False bottom lifted	1:38	0:53	No	N/A
9 Nov. 2022	Medical	False bottom lifted	1:59	1:02	No	N/A
27 March 2023	P1	Mat removal	1:39	0:46	Yes	30
23 May 2023	P1	Mat removal	1:47	0:47	Yes	30
30 Oct. 2023	P1	Mat removal	1:58	0:57	Yes	40
3 Nov. 2023	P1	Mat removal	1:59	0:57	Yes	40

Table 7. Descriptive statistics for behavioral observation periods (welfare team behavioral observations)

Behavioral observation period	Total observation sessions (*n*)	Duration of observation sessions (Mean ± SD min)	Total duration NEP observed each period (approx. h)
Non-scan	555	14.8 ± 1.1	137.3
Pre-scan	89	14.95 ± 0.4	22.2
Immediate pre-scan	53	14.2 ± 2.2	12.6
Immediate post-scan	38	14.8 ± 1.3	9.4
Post-scan	90	14.8 ± 1.2	22.2
Total	825	14.8 ± 1.2	203.7

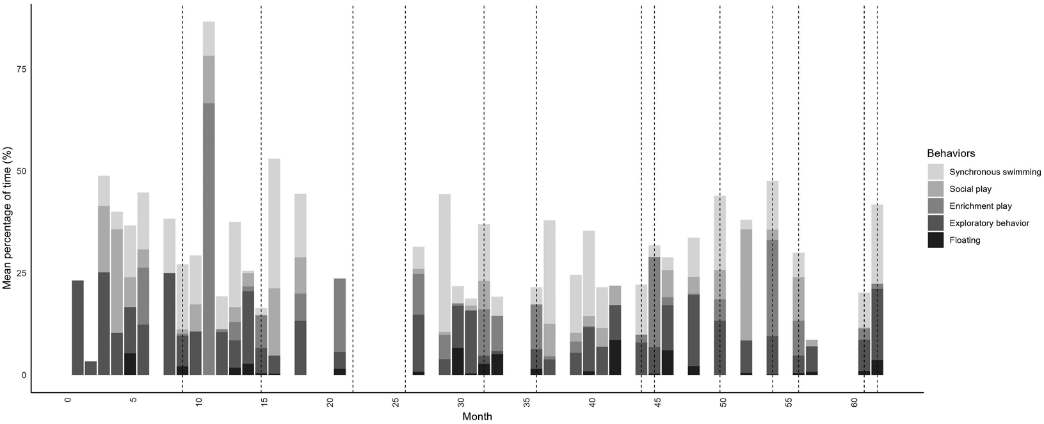

Figure 6. Trends in welfare-related behaviors (%) observed by the welfare management team over the course of the study. Note that vertical, dotted lines indicate scans ($n = 13$).

< 0.001). The Kruskall-Wallis H-test showed a significant difference for social play ($p < 0.001$), with a significant decrease occurring between non-scan and immediate pre-scan periods ($p = 0.035$). Enrichment play was significantly more frequent during pre-scan ($p = 0.008$) and immediate pre-scan ($p = 0.012$) periods while enrichment devices were present when compared to non-scan periods with devices. It should be noted that, when present, the same enrichment devices (e.g., water, buoys) were used within the different study periods. Exploratory behaviors were observed significantly more often during non-scan periods in comparison to immediately prior to scans ($p = 0.012$). From those behaviors measured as events (events/h), anticipatory behaviors were the only behaviors to occur at a rate more than once per hour (Figure 7). Anticipatory behaviors were significantly highest in immediate pre-scan periods ($p < 0.05$) and were significantly lower in immediate post-scan periods when compared to non-scan ($p = 0.023$) and pre-scan ($p = 0.007$) periods (see Table 8 for means ± SDs).

In examining behavioral differences in the immediate pre-scan period between the medical pool and P1 (Table S2), no differences were observed for floating and anticipatory behaviors ($p > 0.05$). However, enrichment play was significantly more frequent in the medical pool ($p = 0.004$), while exploratory behavior was significantly higher in P1 ($p < 0.001$).

Behavioral Welfare Indicators (Trainers' Reports)—Trainers rated NEP's willingness to participate in training sessions on the Likert scale for 814 d of the 1,887 d total across the study (Table 9). The overwhelming majority of scores (92.6%) were 3 out of 4, with little indication of lower scores being associated with scan activities.

Trainers included more detailed comments on NEP's willingness to participate in sessions when the session was study related. From 82 recorded study-related training sessions (Context C), in two sessions (3 and 4 November 2020), trainers reported that NEP appeared disinterested in study-related activities, voluntarily returning himself to the water. Trainers reported no indications of

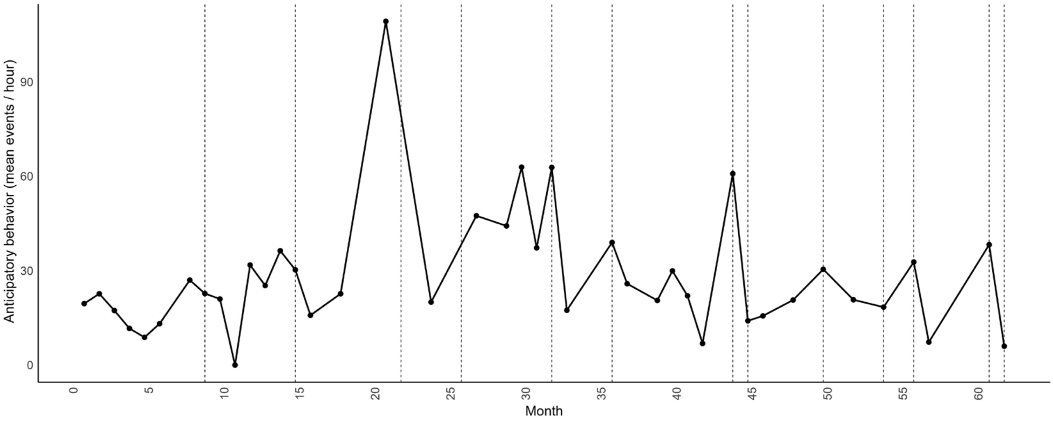

Figure 7. Anticipatory behaviors (events/h) observed by the welfare management team over the course of the study. Note that vertical, dotted lines indicate scans (*n* = 13).

Table 8. NEP's observed behaviors (welfare team behavioral observations) during non-scan periods, prior to scans, and following each scan

Behavioral observation	Non-scan	Pre-scan	Immediate pre-scan	Immediate post-scan	Post-scan
Synchronous swimming (%)	11 ± 23.8	9.8 ± 25.1	0.0 ± 0.0	29.3 ± 42	8.7 ± 22
Social play (%)	4.7 ± 14.5	0.8 ± 5.8	0.0 ± 0.0	0.0 ± 0.0	7.3 ± 23.4
Enrichment play (%)	16.1 ± 30.7	54.7 ± 46.8	22.4 ± 36.1	56.7 ± 43.5	26.7 ± 40.6
Exploratory behavior (%)	9.7 ± 16.9	8.5 ± 16.6	4.8 ± 13.1	4.6 ± 10.5	8.6 ± 15.8
Floating (%)	1.4 ± 6.3	0.8 ± 4.5	2.1 ± 9.9	0.2 ± 1.1	0.7 ± 4.5
Anticipatory behavior (events/h)	27.9 ± 39.8	36.3 ± 49.5	48.1 ± 43.5	14.5 ± 28.2	25.7 ± 40.5

*Data are presented as mean ± SD.

Table 9. NEP's daily willingness to participate in training sessions and daily percentage of food consumption during non-scan periods, prior to scan, and following each scan

	Daily willingness to participate in training sessions (Likert score)						Daily percentage of food consumption	
Behavioral observation period	Total observations (*n* = 814)	0	1	2	3	4	Total observations (*n* = 1,809)	Mean ± SD
Non-scan	747	0	2	27	694	24	1,638	99.6 ± 3.4
Days 7-2 pre-scan	26	0	0	1	24	1	72	98.8 ± 7.5
Day of scan	6	0	0	0	6	0	13	100.0 ± 0.0
Day post-scan	6	0	0	0	5	1	13	94.8 ± 18.8
Days 2-7 post-scan	29	0	0	3	25	1	73	100.0 ± 0.0

NEP's unwillingness to participate during scans (Context D). The trainers' reports noted that NEP continued to respond to requested behaviors despite a decrease in acceptance and/or refusal of food reinforcement during the May and October 2023 scans. Trainers made no additional notes regarding changes to NEP's acceptance of food reinforcement during scans; and after all scans were completed, NEP began accepting reinforcement on return to the van and/or pool.

For daily percentage of food consumption, reports were completed for 1,809 of 1,887 d. A significant difference was found across study periods in NEP's percentage of food consumed daily (p = 0.042)—that is, the mean percentage of diet that NEP ingested of total diet offered. NEP's percentage of diet ingested was significantly lower during days 7 to 2 prior to scans compared to non-scan periods (p = 0.044), although a greater decrease occurred the day of the scan. For instance, during non-scan days (n = 1,706 d), the mean percentage consumed was 99.6% (± 3.7) of 100% of diet offered, while the mean percentage consumed in days 7 to 2 pre-scan (n = 72 d) was 98.8% (± 7.5). Reports from the day immediately following scans (n = 13 d) showed a decrease in percent of NEP's daily diet consumed to 94.8% (± 18.8). During days 2 to 7 following scan (n = 78 d), NEP's trainers reported that 100% (± 0.0) of diet was consumed for all days in this behavioral period. There was also no gradual decrease in consumption observed over the study.

When integrating veterinary records of NEP's life history and trainers' reports of his regurgitation behavior during the study, we found no changes to regurgitation that were inconsistent with NEP's life history. Out of a total of 13 scans, regurgitation was observed on five occasions on the scanning day and/or on the following day (Table S3). This was proportionally more than expected based on the other study periods (Table 5), so it is possible that activities related to scanning increased regurgitation on the day of and day following transport. However, regurgitation may have appeared to increase on these days due to sample size differences in observations between the day of and day following scans and the other remaining periods (Table S3). Ultimately, this behavior was infrequent, inconsistent, not correlated with other welfare-related measures, and did not increase overall during the study.

During eight of the 13 scans, trainers reported that NEP consumed > 85% of fish rewards offered during the scans. For four scans, NEP consumed between 50 to 85% of fish offered; and in one scan (30 October 2023), NEP consumed less than 50% of fish offered. Despite this decrease in food acceptance during some scans, NEP still responded to all trainer requests. Likert scoring totals for each period and means ± SDs of percentage of food consumed within each study period are presented in Table 9.

When comparing behavioral indicators from the trainers' reports, we first explored NEP's response to novel social groupings (i.e., positive and negative social integration indicators). NEP had 82 positive social integrations and 11 negative integrations during 1,787 non-scan days, and six positive integrations and one negative integration during the two post-scan periods combined (n = 87). There was no significant association between context and outcome of a social integration (p = 1.0), which suggests that the likelihood of either a positive or negative outcome to a social integration was just as likely in either non-scan or post-scan contexts. Occurrences of positive and negative social integrations are depicted per month over the course of the study (Figure 8).

The total number of occurrences and proportion of time observed for socio-sexual and agonistic behaviors per observation period were examined, and no significant differences were found when comparing the proportion of days during each behavioral observation period in which socio-sexual (p = 0.845) and agonistic (p = 0.703) behaviors were observed (Table 10; Figure 9).

Remaining Behavioral Indicators—On 9 November 2022, NEP directed two jaw claps towards trainers when in the medical pool as the false bottom was being raised. This was interpreted as NEP responding to the false bottom floor rising, not as trainer-directed aggression, especially because NEP continued responding to trainer behavioral requests. Also, no aggression towards trainers or animal care staff followed these jaw claps or were reported during any other instances over the study period.

Tremors were reported on two occasions. On 13 December 2019, while NEP was being loaded into the van on return from a scan, a little tremor was noticed in the fluke. This stopped as soon as the van started moving. Once returned to the pool, NEP's breathing rates were normal, and NEP consumed offered food and responded correctly to trainer requests. Trainers' Likert evaluations of NEP's daily willingness to participate in sessions during the following days showed no decrease in the quality of NEP's session participation. On 10 November 2020, after ~30 min of scanning, trainers noticed a slight tremor throughout NEP's body. This was treated as a temporary end point; thus, the rest of the scan was canceled, and NEP was transported back to the pool. No other indicators were noted by trainers or veterinary staff present in the scan room at the time, and

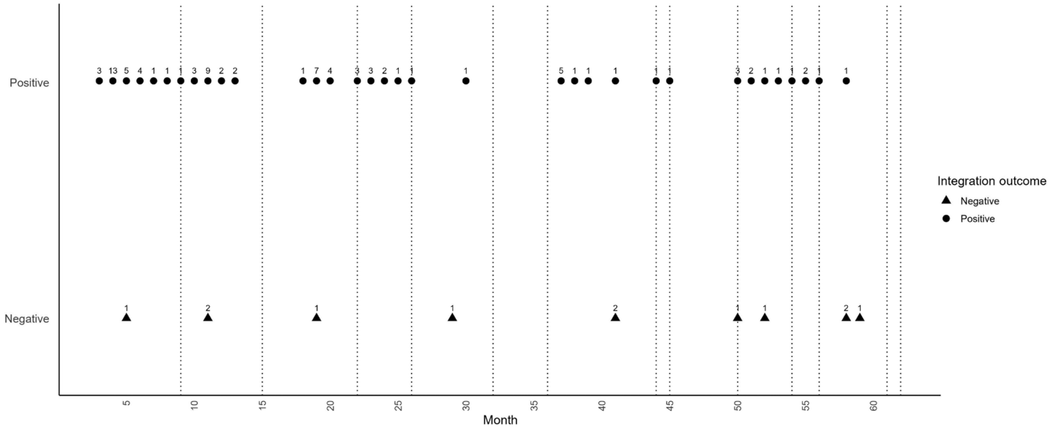

Figure 8. Total occurrences of positive and negative social integrations per month over the course of the study. The value above each marker represents the total number (n) of interactions for that month. Note that vertical, dotted lines indicate scans ($n = 13$).

Table 10. Socio-sexual and agonistic welfare indicators (trainers' reports) during non-scan periods, prior to scans, and following each scan

Behavioral observation period	Total observations (n)	Socio-sexual behavior		Agonistic behaviors	
		Total observations present	% of total observation time	Total observations present	% of total observation time
Non-scan	1,715	10	0.58	32	1.86
Days 7 to 2 pre-scan	72	1	1.39	0	0.00
Day of scan	13	0	0.00	0	0.00
Day post-scan	13	0	0.00	0	0.00
Days 2 to 7 post-scan	74	0	0.00	2	2.70

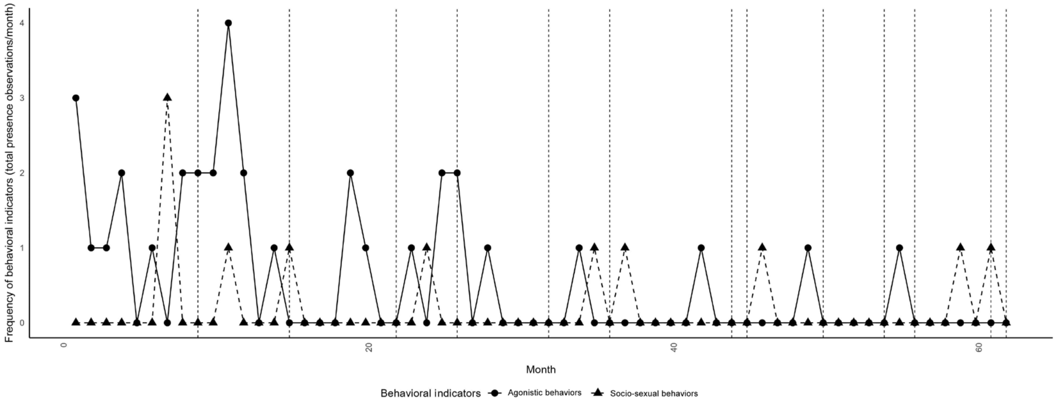

Figure 9. Total number of occurrences of socio-sexual behaviors and total number of occurrences of agonistic behaviors per month of the course of the study. Note that vertical, dotted lines indicate scans ($n = 13$).

no further tremors were reported following the scan's cessation. Again, no changes were apparent in NEP's willingness to participate in sessions following this scan. Additional body movements (e.g., pronounced body rocking, tail slapping, tail movement side-to-side, tail arch, whole body arching/thrashing, movement in lower jaw) were not reported.

Physiological Welfare Indicators—NEP's heart rate was assessed occasionally during transport by palpation but was largely monitored during scans by an MRI-compatible ECG sensor. During scans, NEP showed normal respiratory sinus arrhythmia, remaining within normal heart rate ranges reported for dolphins (e.g., Fahlman et al., 2020). Breath rate during the first scan (21 June 2019) ranged from 14 to 22 breaths/5 min. Breath rates reported for NEP during most transport and scanning ranged from 15 to 20 breaths/5 min period, falling within the interquartile range that Fahlman et al. (2021) described for beached dolphins in managed care. On 9 November 2022, NEP's breath rate was 25 breaths/5 min, but this decreased to 16 breaths/5 min while in the scanner.

Prior to the first scan, cortisol concentrations averaged 0.78 ± 0.31 μg/dl (range: 0.29 to 1.26). For all subsequent blood samples made following the first scan, an average of 0.75 ± 0.26 μg/dl (range: 0.24 to 1.45) was measured. Hart et al. (2015) measured 2.46 ± an SD of 1.14 μg/dl with reference interval of 0.91 to 4.21 for wild *Tursiops*, so NEP's cortisol levels were within the normal reference interval before and after scanning started. Over the scanning study period (2019 to 2023), NEP's weight varied from 167 to 216 kg, within normal body condition for a dolphin of his size and age. No behavioral observations, veterinary records, or trainer reports had any indications of abnormal swimming movement, pain (e.g., arching, squinting, breaching, staying at depth), or shock. Radiographs collected for detection of metal indicated no evidence of foreign body ingestion during the study.

Health Indicators—During the study period, results of NEP's blood tests were generally stable; only some mild (nonsignificant), sporadic changes occurred to some parameters such as white blood cells, hemoglobin, iron, and reticulocyte count that could be due to non-study related contexts (e.g., breeding season with sexually receptive females and aggressive episodes with other males, some of which resulted in minor skin injuries). In 2021, NEP also had an isolated respiratory episode of a fungal nature, which resolved with treatment in 1 mo. Additionally, during the study period, a generalized digestive episode in the whole dolphin group was resolved with medical treatment. There was no indication that any of these reported health issues were related to scanning sessions, nor were any other health indicators reported during the study.

Welfare-Related Temporary or Final Endpoints—On 29 June 2020, a scan was canceled and rescheduled (20 July 2020) due to integration of multiple-short-term indicators (i.e., agonistic behaviors [Table S1] causing injuries and inappetence, and low willingness to participate in training sessions). Early morning on 29 June 2020, an aggressive interaction occurred between NEP and another social group member during which it was unknown which animal was the aggressor as the event occurred prior to the trainers' arrival for the day. The interaction resulted in signs of stress and discomfort for both animals (e.g., new rake marks present, maintenance of distance from trainers, lack of response to trainer requests); and while NEP reportedly improved throughout this day, it was decided that a temporary endpoint had been reached, and the scan was moved to 20 July 2020. On 10 November 2020, a second temporary endpoint was reached, and the scan was terminated early due to NEP's tremor. No other temporary or final endpoints were reached during the study period (Table 3).

Discussion

The use of MRI methodology to study dolphin anatomy and physiology is far less invasive than intracranial electrophysiology in living animals, but it still requires behavior that is not natural for a dolphin (e.g., removal from water). Further, unlike human subjects, dolphins cannot report on their experience, so we must rely on species-specific welfare indicators to inform whether an animal's participation in a study is detrimental to that animal's welfare. In this study, we developed a Welfare Control Plan (WCP) composed of behavioral, physiological, and health welfare indicators that are well-established for interpreting dolphin welfare (e.g., Clegg et al., 2015, 2017a; Lauderdale et al., 2023). We also report all steps in developing our final protocol in attempts to shorten this process for future research teams interested in obtaining MRI data from live dolphins. Through protocol implementation detailed herein, we found little change to NEP's welfare status overall. Our results are organized chronologically by study activities in the following discussion.

Training and Desensitization Sessions

Motivation reflects an animal's interest and engagement in an activity, and is linked to emotional states (Manteuffel et al., 2009). For instance, chronic stress, social isolation, and poor health have been linked to reduced engagement in tasks for rewards in mammals (Larson, 2002; Pedersen et al., 2002; De La Garza, 2005; Kleen et al., 2006), but positive welfare states in animals may be indicated through increased task engagement. Willingness to participate in training sessions has been recognized as a reliable and practical welfare indicator for the bottlenose dolphin, correlating with their health status, daily food intake, absence of new rake marks, or level of excitement during training sessions (Clegg et al., 2019; Delfour et al., 2020). This metric takes advantage of the caretakers' profound knowledge of each dolphin's behavior and is based on the idea that a dolphin's willingness to participate in positive reinforcement training sessions relates to both task performance and reward acquisition (de Jonge et al., 2008; Brando, 2010). NEP had some autonomy during study-related training sessions as the choice to return to the pool was always available to him. NEP appeared consistently engaged during study-related sessions as trainers reported that for only two sessions (on consecutive days), NEP appeared disengaged with study-related activities. In both sessions, NEP chose to return to the pool from the beached position during the session. It can be inferred from NEP's continued willingness to participate in research tasks that these sessions did not negatively impact NEP's welfare. It should be noted that it is difficult to disentangle engagement during training sessions from additional factors as participation may be influenced by the type of session itself (i.e., the animal does not appear enriched by the task through consistent lack of engagement/interest) or by a complex interplay of internal factors (e.g., affective state, seasonal reproductive patterns) and external factors (e.g., temperature, session duration, keepers involved) as described for other species (Hosey & Melfi, 2012).

Pre-Scan and Immediate Pre-Scan Periods

Enrichment behaviors significantly increased in both the pre- (i.e., the 48 h before scan, excluding the 2-h immediate pre-scan window; Table 4) and immediate pre-scan periods, while anticipatory behaviors increased in the immediate pre-scan period. During the pre-scan period, welfare team observations indicated that NEP engaged in more enrichment play when enrichment devices were available compared to non-scan periods. Play behavior is widely considered an indicator of positive welfare status, primarily due to its association with positive emotional states and its reduction in response to welfare challenges (Held & Špinka, 2011; Maple & Perdue, 2013; Ahloy-Dallaire et al., 2018). Play has also been recognized as an indicator of positive welfare in bottlenose dolphins (Kuczaj et al., 2013; Clegg et al., 2015), declining with aggression or noise exposure (Serres & Delfour, 2017), and increasing as stereotypic behaviors decrease (Perez et al., 2018). The observed increase in NEP's enrichment play could thus reflect maintenance of NEP's positive welfare status during pre-transport periods. This may be related to increased trainer interactions during desensitization or transport preparation as such interactions are considered pivotal for captive dolphin's welfare (Clegg & Delfour, 2018; Platto & Serres, 2023). However, the presence or increase of play behaviors does not necessarily signify positive welfare as play can sometimes intensify in adverse conditions (Held & Špinka, 2011).

NEP may have anticipated upcoming transports and scans during the week prior to and during the pre-scan period, influenced by alterations in daily routines and environmental changes, such as the visibility of the transport van and mats, along with increased staff presence and activity around the pool, which is especially heightened on the scan day and in the immediate pre-scan period. NEP exhibited higher levels of anticipatory behavior during pre-scan periods than in any other period observed. Common anticipatory behaviors included increased attention towards trainers or staff that were poolside, often accompanied by increased activity and jumping, most often near areas where staff typically congregated prior to initiation of a training session. This pattern aligns with the specific dynamics of anticipatory behaviors—how they are exhibited in relation to spatial, temporal, and contextual factors—as detailed in a thorough review of existing research (Krebs et al., 2022). Anticipatory behaviors are exhibited in expectation of either a forthcoming rewarding event (Spruijt et al., 2001; Watters, 2014) or aversive stimuli (Spruijt et al., 2001; Moe et al., 2006), and can be cued by external and/or internal factors (Balsam et al., 2009).

Bottlenose dolphins in managed care frequently display anticipatory behaviors in expectation of activities they find rewarding (Clegg et al., 2018). The frequency and intensity of anticipatory behaviors have been observed to align with the degree of individuals' participation in the activity (Clegg et al., 2018), reinforcing the link between anticipatory behavior and motivation in this species (Spruijt et al., 2001). Some anticipatory behaviors have been linked to positive welfare (Jensen et al., 2013; Clegg et al., 2017b, 2018; Clegg & Delfour, 2018). NEP's increased anticipatory behavior during the pre-scan periods may thus suggest that he associated scan-related activities with reward. Although anticipation of aversive stimuli might offer an alternative explanation for NEP's behavior, such behavior would be more likely expressed through defensive or avoidance behaviors rather than behaviors characterized by approach and engagement (Higgins, 2006). Moreover, as suggested by Clegg et al. (2018), if NEP had associated transport and scan activities as threatening, we would have expected to see high levels of anticipatory behavior in combination with unwillingness to participate in study tasks. Given NEP's willingness to participate throughout all study periods, we suggest that NEP's increased anticipatory behavior indicated interest in the task rather than negative association to transport and scan activities.

Uncontrolled factors such as changes to the pre-scan pool (i.e., medical pool vs P1) and social group configuration (see "Challenges of Dolphin-Related Research") in the periods prior to transport and scan may have also contributed to differences in behaviors between periods. For example, NEP's isolation in the medical pool could also account for elevated anticipatory behavior observed in the immediate pre-scan period. Animals lacking access to play or social interaction may become more responsive to rewarding stimuli and increase their anticipatory behavior (Ahmed et al., 1995; Van den Berg, 1999; Spruijt et al., 2001). During the immediate pre-scan period, there was also a decrease in NEP's exploratory behavior compared to non-scan periods. This decrease in exploratory behavior may be explained by the static environment offered by the medical pool in which NEP was isolated prior to scan for most scans. In directly comparing immediate pre-scan behaviors between the medical pool and P1, some behaviors remained consistent across environments, while others varied significantly. Enrichment play, when enrichment devices were available, was higher in the medical pool, suggesting this behavior acted as a buffer against conditions imposed by isolation (Held & Špinka, 2011). Moreover, NEP exhibited increased exploratory behaviors while in P1 immediately prior to the scan, potentially indicating more stimulation in this habitat. The most current study protocol that avoids NEP's isolation in the medical pool prior to scan would thus eliminate concerns of stimulus deprivation in this period.

Scan Sessions

Scans were the most critical study component while also being the most likely to affect NEP's welfare. Behaviors required for scan sessions did not appear to pose any immediate threat to NEP's health. NEP's breath rates during scans were within normal ranges reported for dolphins (Fahlman et al., 2021). NEP's heart rate, which was monitored through ECG during scans, also maintained normal respiratory sinus arrhythmia (Fahlman et al., 2020). Because NEP's autonomy is heavily restricted in scan sessions, we relied on indicators of immediate changes to NEP's welfare. One such indicator (trembling) resulted in a WCP temporary endpoint in which the team response was to rapidly terminate the session and return NEP to the pool. Despite the trembling, NEP still accepted reinforcement prior to, during, and following the session, and there were no other apparent indicators of negative welfare status.

Overall, trainers' reports showed no indications of NEP's unwillingness to participate in any scan sessions, and NEP continuously responded to trainer requests while in the scanner, despite a decrease in acceptance of food rewards during some scans. As discussed above, willingness to participate in training sessions is a recognized reliable indicator of positive welfare in bottlenose dolphins (Clegg et al., 2019; Delfour et al., 2020). Food intake and anorexia (i.e., the lack or loss of appetite for food) have also been accepted as consistent indicators of animal welfare (Broom, 1991; Millman, 2007). Considering that inappetence in dolphins often indicates stress or health problems (Waples & Gales, 2002; Johnson et al., 2009; Clegg et al., 2019), examining food intake in this period was considered especially relevant. Rates of food intake during all study periods were consistently high, and NEP consumed all fish offered on each scan day, including those days in which there was a decrease in accepted food rewards during scans. NEP resumed consuming fish normally once returned to the pool on these days, so changes to NEP's food intake during scan activities were not considered concerning by trainers or veterinary staff. Thus, NEP's continued participation in scans regardless of food acceptance could be interpreted as continued interest in study activities.

Scan sessions did not appear to negatively affect NEP's welfare status long-term when interpreted alongside data from other study periods. In integrating all behavioral, physiological, and health indicators reported by veterinarians and trainers during scans, NEP's response to scans was not indicative of severe distress or welfare impairment, despite the potential risks to the animal's welfare we outline here (Table 1). Instead, he appeared to effectively cope with the study challenges.

Immediate Post- and Post-Scan Periods

In the immediate post-scan period, a significant increase in synchronous swimming was observed. Synchronous swimming was the only social behavior that increased during this period. In dolphins, affiliative behaviors, including synchronous swimming, are associated with positive welfare (Kuczaj et al., 2013; Clegg et al., 2017a). However, this behavior's increase within this context could be interpreted as a coping mechanism in response to stress experienced during the scan. This observation corresponds with previous research that highlights the importance of social support in modulating stress responses among animals (Rault, 2012). Synchronous swimming, as a form of affiliative behavior, may improve dolphin welfare in stressful conditions for bottlenose dolphins as has been reported for other species (Taylor, 1981; Boissy & Le Neindre, 1997; da Costa et al., 2004).

In the post-scan period (i.e., observations spanning the 48 h after scan, excluding the 2-h immediate post-scan period), NEP exhibited behavioral patterns that did not significantly deviate from baseline (non-scan or pre-scan) levels, indicating rapid behavioral stabilization following scans. A nonsignificant reduction (to ~95%) in daily diet consumed by NEP was recorded on the day immediately post-scan. However, in the 6 d following scans (78 d in total), NEP's food consumption was 100% every day. The integration of different observations, therefore, supports that while transportation and scanning may have induced temporary behavioral changes in NEP, the dolphin's behaviors generally returned to baseline levels the following day.

Welfare-Related Temporary or Final Endpoints

A final endpoint has not yet been reached over the 5-y, ongoing study. Temporary endpoints were only met twice. Once was due to a culmination of factors resulting from a negative social interaction between NEP and another male on the morning of a planned scan. Although aggressive interactions are common in male bottlenose dolphins in both the wild (Parsons et al., 2003; Scott et al., 2005) and managed care (e.g., Lauderdale et al., 2023), and occur more often in the morning for other dolphin social groups in managed care (Lauderdale et al., 2023), we cannot rule out that this negative social interaction happened in relation to pre-scan activities as it occurred the morning of a scheduled scan. NEP's behavior returned to normal throughout that day, however, and no other instances were reported throughout the study that indicated NEP was the continued recipient of aggression from other animals.

A second temporary endpoint was reached during a scan session when NEP's trainers reported trembling during fMRI acquisition. The scan was immediately terminated, the team returned NEP to the pool, and no other negative welfare indicators were reported at the time of the scan or in the hours and days following the incident. It is unknown whether NEP's trembling was an early symptom of a larger health concern, potentially averted due to termination of the scan, or whether the trembling was an isolated behavior. Trembling is an indicator associated with negative welfare in stranded animals but is most common in animals that have stranded for the first time (Boys et al., 2022). Such a novel, drastic change to an animal's environment may contribute to additional behavioral and physiological welfare indicators not experienced when an animal strands again. In our study, NEP had already participated in multiple scans by the time the trembling event occurred; however, this was the first playback of auditory stimuli during the study. Perhaps the additional environmental information presented on this day resulted in NEP's trembling behavior. Trembling was also reported one other time while NEP was being loaded into the van to return to the pool, but this behavior appeared isolated as it terminated once the van began the drive back to Oceanogràfic.

Challenges of Dolphin-Related Research

Welfare indicators such as those reported herein are critical as dolphins cannot report on their experience, so we must rely on observable indicators to address both immediate and long-term health and welfare concerns for dolphin participants in our research. Marine mammal experts from different arenas of animal care—including trainers, veterinarians, animal care staff, and researchers—were assembled as a team early in this study with the focus of maintaining NEP's welfare during study participation through selection and monitoring of welfare indicators over time. These indicators were assessed before, during, and after each study activity and compared over the entire study.

Differences in NEP's behavior across study periods were likely influenced by environmental and social changes within or across study periods related to the same scan, as well as across scans. For example, NEP could not engage in social behaviors when isolated during pre-scan, transport, and scan periods. This not only affected the range of behaviors available to him during these periods but may also have influenced his behaviors before and after these periods. Moreover, behavioral differences were observed in pre-scan periods when NEP was isolated in the medical pool vs when he was allowed continued access to his social group while in P1 immediately prior to transport. These behavioral differences may have influenced behavior in later periods of the same scan.

Dolphin trainers simulate the fission-fusion social structure known for bottlenose dolphins in the wild (e.g., *Tursiops* spp.; Connor et al., 2000) by changing the social group membership for managed-care animals in a similar manner. Different social group compositions likely impacted NEP's behavior in study periods as well. For example, synchronous swimming might not have increased in the immediate post-scan period if NEP had been returned to the pool system with animals in which there was no close affiliation. Given the trainers' extensive knowledge of NEP's life history, the social group composition chosen for NEP throughout the study consisted of dolphins closely affiliated with him (as is with management of all Oceanogràfic dolphins).

The trainers' reports did not list any positive welfare indicators on any of the 13 scan days. However, trainers' reports were biased towards negative indicators as positive welfare is the standard and the primary objective of these reports was to improve on NEP's training and welfare in the immediate future. For instance, "difficulty in gating" was marked as a negative indicator, whereas "gating success," representing the majority of routine gatings, was not cataloged as a positive indicator because it does not appear in trainers' reports. We still did not see a significant increase in negative welfare indicators reported for NEP in immediate post- and post-scan periods, and detailed behavioral observations of the welfare management team, often completed at different times than trainers' observations within a study period, confirmed the presence of positive welfare indicators within these periods.

Sample sizes of study observation periods from both trainers' reports and the welfare management team's observations likely influenced our results. We would have expected NEP's percentage of food consumption to have been significantly different during the immediate post-scan period as this was the time with the lowest reported food consumed by NEP; however, no significant difference existed for this behavior in this period, possibly due to the lower sample size for this period than all other periods. Results for synchronous swimming were also likely affected by sample sizes as we would have expected the immediate post-scan and post-scan periods to be significantly different as well as the non-scan period in comparison to the pre- and post-scan periods. Welfare observation procedures were not designed to sustain rigorous scientific testing, but, rather, the primary objective of gauging these welfare indicators during our study period was to ensure that our larger study was not impacting NEP's quality of life in real-time.

Changes in observer effort and opportunistic data collection are also a limit to animal behavior research. For example, the COVID-19 pandemic limited the welfare management team's effort during 2020 and 2021. Moreover, observations completed by the welfare team were limited to surface observations conducted poolside. As most of Oceanogràfic's dolphinarium pools are up to 11 m deep, behaviors occurring beneath the surface were sometimes missed, biasing our data towards what could be observed at the surface. Thus, more surface-level events like anticipatory behaviors or synchronized swimming were observed, and behaviors such as affiliative interactions, which can occur underwater, may not always have been observed and, thus, were potentially underreported.

Animal behavior research is often limited by the number of study participants available. In MRI studies, however, one individual can provide the biological blueprint for answering neurobiological research questions as most animals within a species would have similar neuroanatomy. Our goal here was to use one subject to develop a protocol suitable for others. We cannot assume that results from our WCP will be consistent in other animals, however, so it is critical to the welfare of MRI study participants that the study individual is selected carefully and monitored thoroughly as we have done here.

Weighing Benefits for Research and Veterinary Care Versus Study Risks and Costs

The cost in human effort for MRI studies can be extensive as training is a lengthy process and regular veterinary health monitoring is not sufficient in monitoring animal welfare alone, as demonstrated here. Observations made from our study's training and welfare management teams were critical in elucidating NEP's welfare status within study periods and overall. Additionally, there is always significant risk to a dolphin's health when removing the animal from its environment. The invasiveness of this action is tempered by maintaining the animal's autonomy at the start of removal. By requesting that the animal beach itself, the animal voluntarily removes itself from the water onto land when interested in the research task(s); and by having the animal station near to the pool edge, this allows the animal to return to the pool whenever the research task is uncomfortable or no longer interesting.

We focused here on assessing the effects of a neuroimaging study on our subject, but it is important to weigh these against the reasons and potential benefits of the research. The logic behind many welfare laws is to protect species that are judged to be sentient and conscious enough to experience pain and suffering (Blattner, 2019). This means that there is an intersection between research on animal cognition and policies for animal welfare. In addition to the benefits of basic research, the development of non-invasive methods to study neuroanatomy and neural function of animals subject to stringent welfare protections will be important for improving policies regarding the welfare of animals with diverse sensitivities. Future directions of this research could illuminate critical information regarding dolphin sensory processing and signal production, as well as advance technology towards wider use of MRI for veterinary and dolphin care purposes in managed-care facilities.

Conclusions Regarding Short-Term Study Effects

There were no physiological or health indicators to suggest that NEP's immediate health and safety were at risk due to study activities, and there were no study-related health incidents of concern reported by trainers or veterinary staff. Any short-term changes to NEP's weight and diet were due to husbandry activities unrelated to our study as reported by NEP's trainers and Oceanogràfic's veterinary staff. Behavioral observations by NEP's welfare management team showed no clear signs that NEP's status was negatively impacted by activities related to any of the study periods. Changes in behaviors reported for some study periods may reflect NEP's association of study tasks with rewards when interpreted alongside additional measures. NEP appeared engaged and interested in the study-related tasks pertaining to each period, even during scan sessions.

Conclusions Regarding Long-Term Study Effects

Analyses of welfare indicators over time showed consistency in expression of NEP's behavioral patterns over the study period. In instances where behavioral differences were observed by the management team, the difference was likely the product of a change to NEP's environment or an indicator of potential positive welfare status, or the behavioral change may have reflected NEP's interest in study activities. NEP's behavior overall was largely unaffected by the introduction of the study to his routine according to trainers' reports. Health and physiological welfare indicators also showed no signs of persistent abnormality or concern to NEP's welfare during the study (e.g., NEP's weight remained in the normal range for a dolphin of his size and age) let alone any detrimental health or physiological event that could be tied to study activities. Integration of all measures considered, therefore, suggest that NEP's quality of life was not negatively impacted by the introduction of this study to his daily life.

Note: The supplemental materials for this article are available in the "Supplemental Material" section of the *Aquatic Mammals* website: https://www.aquaticmammalsjournal.org/supplemental-material.

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Literature Cited

Ahloy-Dallaire, J., Espinosa, J., & Mason, G. (2018). Play and optimal welfare: Does play indicate the presence of positive affective states? *Behavioural Processes*, *156*, 3-15. https://doi.org/10.1016/j.beproc.2017.11.011

Ahmed, S. H., Stinus, L., Le Moal, M., & Cador, M. (1995). Social deprivation enhances the vulnerability of male Wistar rats to stressor- and amphetamine-induced behavioral sensitization. *Psychopharmacology*, *117*, 116-124. https://doi.org/10.1007/BF02245106

Arriaga, G., & Jarvis, E. D. (2013). Mouse vocal communication system: Are ultrasounds learned or innate? *Brain and Language*, *124*(1), 96-116. https://doi.org/10.1016/j.bandl.2012.10.002

Balsam, P., Sanchez-Castillo, H., Taylor, K., Van Volkinburg, H., & Ward, R. D. (2009). Timing and anticipation: Conceptual and methodological approaches. *European Journal of Neuroscience*, *30*, 1749-1755. https://doi.org/10.1111/j.1460-9568.2009.06967.x

Bassett, L., Buchanan-Smith, H. M., McKinley, J., & Smith, T. E. (2003). Effects of training on stress-related behavior of the common marmoset (*Callithrix jacchus*) in relation to coping with routine husbandry procedures. *Journal of Applied Animal Welfare Science*, *6*(3), 221-233. https://doi.org/10.1207/S15327604JAWS0603_07

Blattner, C. E. (2019). The recognition of animal sentience by the law. *Journal of Animal Ethics*, *9*(2), 121-136. https://doi.org/10.5406/janimalethics.9.2.0121

Boissy, A., & Le Neindre, P. (1997). Behavioral, cardiac and cortisol responses to brief peer separation and reunion in cattle. *Physiology & Behavior*, *61*(5), 693-699. https://doi.org/10.1016/S0031-9384(96)00521-5

Boys, R. M., Beausoleil, N. J., Pawley, M. D., Betty, E. L., & Stockin, K. A. (2022). Evaluating potential cetacean welfare indicators from video of live stranded long-finned pilot whales (*Globicephala melas edwardii*). *Animals*, *12*(14), 1861. https://doi.org/10.3390/ani12141861

Brando, S. I. C. A. (2010). Advances in husbandry training in marine mammal care programs. *International Journal of Comparative Psychology*, *23*(4), 777-791. https://doi.org/10.46867/IJCP.2010.23.04.03

Brando, S., Broom, D. M., Acasuso-Rivero, C., & Clark, F. (2018). Optimal marine mammal welfare under human care: Current efforts and future directions. *Behavioural Processes*, *156*, 16-36. https://doi.org/10.1016/j.beproc.2017.09.011

Brenowitz, E. A., & Zakon, H. H. (2015). Emerging from the bottleneck: Benefits of the comparative approach to modern neuroscience. *Trends in Neuroscience*, *38*(5), 273-278. https://doi.org/10.1016/j.tins.2015.02.008

Broom, D. M. (1991). Animal welfare: Concepts and measurement. *Journal of Animal Science*, *69*(10), 4167-4175. https://doi.org/10.2527/1991.69104167x

Broom, D. M., & Johnson, K. G. (Eds.). (2019). *Stress and animal welfare: Key issues in the biology of humans and other animals* (2nd ed.). Chapman & Hall.

Bullock, T. H., & Ridgway, S. H. (1972). Evoked potentials in the central auditory system of alert porpoises to their own and artificial sounds. *Journal of Neurobiology*, *3*(1), 79-99. https://doi.org/10.1002/neu.480030107

Câmara, N., Sierra, E., Fernández, A., Arbelo, M., Andrada, M., Espinosa de los Monteros, A., & Herráez, P. (2020). Increased plasma cardiac troponin I in live-stranded cetaceans: Correlation with pathological findings of acute cardiac injury. *Scientific Reports*, *10*, 1555. https://doi.org/10.1038/s41598-020-58497-3

Carlson, B. A. (2012). Diversity matters: The importance of comparative studies and the potential for synergy between neuroscience and evolutionary biology. *Archives of Neurology*, *69*(8), 987-993. https://doi.org/10.1001/archneurol.2012.77

Champagne, C. D., Kellar, N. M., Trego, M. L., Delehanty, B., Boonstra, R., Wasser, S. K., Booth, R. K., Crocker, D. E., & Houser, D. S. (2018). Comprehensive endocrine response to acute stress in the bottlenose dolphin from serum, blubber, and feces. *General and Comparative Endocrinology*, *266*(15), 178-193. https://doi.org/10.1016/j.ygcen.2018.05.015

Clark, F. E. (2017). Cognitive enrichment and welfare: Current approaches and future directions. *Animal Behavior and Cognition*, *4*(1), 52-71. https://doi.org/10.12966/abc.05.02.2017

Clegg, I. L. K., & Delfour, F. (2018). Cognitive judgement bias is associated with frequency of anticipatory behavior in bottlenose dolphins. *Zoo Biology*, *37*(2), 67-73. https://doi.org/10.1002/zoo.21400

Clegg, I. L., 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. https://doi.org/10.7120/09627286.24.3.267

Clegg, I. L., Rödel, H. G., & Delfour, F. (2017a). Bottlenose dolphins engaging in more social affiliative behaviour judge ambiguous cues more optimistically. *Behavioural Brain Research*, *322*, 115-122. https://doi.org/10.1016/j.bbr.2017.01.026

Clegg, I. L. K., Rödel, H. G., Boivin, X., & Delfour, F. (2018). Looking forward to interacting with their caretakers: Dolphins' anticipatory behaviour indicates motivation to participate in specific events. *Applied Animal Behaviour Science*, *202*, 85-93. https://doi.org/10.1016/j.applanim.2018.01.015

Clegg, I. L. K., Rödel, H. G., Cellier, M., Vink, D., Michaud, I., Mercera, B., Böye, M., Hausberger, M., Lemasson, A., & Delfour, F. (2017b). Schedule of human-controlled periods structures bottlenose dolphin (*Tursiops truncatus*) behavior in their free-time. *Journal of Comparative Psychology*, *131*(3), 214-224. https://doi.org/10.1037/com0000059

Clegg, I. L. K., Rödel, H. G., Mercera, B., van der Heul, S., Schrijvers, T., de Laender, P., Gojceta, R., Zimmitti, M., Verhoeven, E., Burger, J., Bunskoek, P. E., & Delfour, F. (2019). Dolphins' willingness to participate (WtP) in positive reinforcement training as a potential welfare indicator, where WtP predicts early changes in health status. *Frontiers in Psychology*, *10*. https://doi.org/10.3389/fpsyg.2019.02112

Connor, R. C., Wells, R. S., Mann, J., & Read, A. J. (2000). The bottlenose dolphin. In J. Mann, R. C. Connor, P. L. Tyack, & H. Whitehead (Eds.), *Cetacean societies: Field studies of dolphins and whales* (pp. 91-125). University of Chicago Press.

Couquiaud, L. (2005). Whales, dolphins, and porpoises: Presentation of the cetaceans. *Aquatic Mammals*, *31*(3), 288-310. https://doi.org/10.1578/AM.31.3.2005.288

da Costa, A. P., Leigh, A. E., Man, M-S., & Kendrick, K. M. (2004). Face pictures reduce behavioural, autonomic, endocrine and neural indices of stress and fear in sheep. *Proceedings of the Royal Society of London B: Biological Sciences*, *271*(1552), 2077-2084. https://doi.org/10.1098/rspb.2004.2831

de Jonge, F. H., Tilly, S. L., Baars, A. M., & Spruijt, B. M. (2008). On the rewarding nature of appetitive feeding behaviour in pigs (*Sus scrofa*): Do domesticated pigs contrafreeload? *Applied Animal Behaviour Science*, *114*(3-4), 359-372. https://doi.org/10.1016/j.applanim.2008.03.006

De La Garza, R. (2005). Endotoxin- or pro-inflammatory cytokine-induced sickness behavior as an animal model of depression: Focus on anhedonia. *Neuroscience & Biobehavioral Reviews*, *29*(4-5), 761-770. https://doi.org/10.1016/j.neubiorev.2005.03.016

Delfour, F., Monreal-Pawlowsky, T., Vaicekauskaite, R., Pilenga, C., Garcia-Parraga, D., Rödel, H. G., García Caro, N., Perlado Campos, E., & Mercera, B. (2020). Dolphin welfare assessment under professional care: "Willingness to participate," an indicator significantly associated with six potential "alerting factors." *Journal* *of Zoological and Botanical Gardens*, *1*(1), 42-60. https://doi.org/10.3390/jzbg1010004

Dudzinski, K. M. (2010). Overlap between information gained from complementary and comparative studies of captive and wild dolphins. *International Journal of Comparative Psychology*, *23*(4), 566-586. https://doi.org/10.46867/IJCP.2010.23.04.13

European Association of Aquatic Mammals (EAAM). (2019). *Standards and guidelines for the management of aquatic mammals under human care*. EAAM.

Fair, P. A., Schaefer, A. M., Romano, T. A., Bossart, G. D., Lamb, S. V., & Reif, J. S. (2014). Stress response of wild bottlenose dolphins (*Tursiops truncatus*) during capture-release health assessment studies. *General and Comparative Endocrinology*, *206*, 203-212. https://doi.org/10.1016/j.ygcen.2014.07.002

Fahlman, A., Cozzi, B., Manley, M., Jabas, S., Malik, M., Blawas, A., & Janik, V. M. (2020). Conditioned variation in heart rate during static breath-holds in the bottlenose dolphin (*Tursiops truncatus*). *Frontiers in Physiology*, *11*, 604018. https://doi.org/10.3389/fphys.2020.604018

Fahlman, A., Brodsky, M., Rocho-Levine, J., Garcia-Parraga, D., Ivančić, M., Camarena, C., Ibarra, L., & Rocabert, J. (2021). Respiratory changes in stranded bottlenose dolphins (*Tursiops truncatus*). *Journal of Zoo and Wildlife Medicine*, *52*(1), 49-56. https://doi.org/10.1638/2020-0033

Fish, F. E. (1993). Power output and propulsive efficiency of swimming bottlenose dolphins (*Tursiops truncatus*). *Journal of Experimental Biology*, *185*(1), 179-193. https://doi.org/10.1242/jeb.185.1.179

Glover, P., Hykin, J., Gowland, P., Wright, J., Johnson, I., & Mansfield, P. (1995). An assessment of the intrauterine sound intensity level during obstetric echo-planar magnetic resonance imaging. *British Journal of Radiology*, *68*(814), 1090-1094. https://doi.org/10.1259/0007-1285-68-814-1090

Graham, M. S., & Dow, P. R. (1990). Dental care for a captive killer whale, *Orcinus orca*. *Zoo Biology*, *9*(4), 325-330. https://doi.org/10.1002/zoo.1430090408

Gulland, F. M. D., Dierauf, L. A., & Whitman, K. L. (Eds.). (2018). *CRC handbook of marine mammal medicine* (3rd ed.). CRC Press.

Gulland, F. M. D., Lowenstine, L. J., & Spraker, T. R. (2001). Noninfectious diseases. In L. A. Dierauf & F. M. D. Gulland (Eds.), *CRC handbook of marine mammal medicine* (2nd ed., pp. 521-547). CRC Press. https://doi.org/10.1201/9781420041637.ch23

Hammerschmidt, K., Whelan, G., Eichele, G., & Fischer, J. (2015). Mice lacking the cerebral cortex develop normal song: Insights into the foundations of vocal learning. *Scientific Reports*, *5*, 8808. https://doi.org/10.1038/srep08808

Hart, L. B., Wells, R. S., Kellar, N., Balmer, B. C., Hohn, A. A., Lamb, S. V., Rowles, T., Zolman, E. S., & Schwacke, L. H. (2015). Adrenal hormones in common bottlenose dolphins (*Tursiops truncatus*): Influential factors and reference intervals. *PLOS ONE*, *10*(5), e0127432. https://doi.org/10.1371/journal.pone.0127432

Harvey, B. S., Dudzinski, K. M., & Kuczaj II, S. A. (2017). Associations and the role of affiliative, agonistic, and socio-sexual behaviors among common bottlenose dolphins (*Tursiops truncatus*). *Behavioural Processes*, *135*, 145-156. https://doi.org/10.1016/j.beproc.2016.12.013

Held, S. D., & Špinka, M. (2011). Animal play and animal welfare. *Animal Behaviour*, *81*(5), 891-899. https://doi.org/10.1016/j.anbehav.2011.01.007

Higgins, E. T. (2006). Value from hedonic experience and engagement. *Psychological Review*, *113*(3), 439-460. https://doi.org/10.1037/0033-295X.113.3.439

Hill, H. M., Dietrich, S., & Cappiello, B. (2017). Learning to play: A review and theoretical investigation of the developmental mechanisms and functions of cetacean play. *Learning & Behavior*, *45*, 335-354. https://doi.org/10.3758/s13420-017-0291-0

Hosey, G., & Melfi, V. A. (2020). Us and them: Human–animal interactions as learning events. In V. A. Melfi, N. R. Dorey, & S. J. Ward (Eds.), *Zoo animal learning and training* (pp. 167-181). Wiley. https://doi.org/10.1002/9781118968543.ch9

Hothorn, T., Hornik, K., van de Wiel, M. A., & Zeileis, A. (2008). Implementing a class of permutation tests: The coin package. *Journal of Statistical Software*, *28*(8), 1-23. https://doi.org/10.18637/jss.v028.i08

Houser, D. S., Moore, P. W., Johnson, S., Lutmerding, B., Branstetter, B., Ridgway, S. H., Trickey, J., Finneran, J. J., Jensen, E., & Hoh, C. (2010). Relationship of blood flow and metabolism to acoustic processing centers of the dolphin brain. *The Journal of the Acoustical Society of America*, *128*(3), 1460-1466. https://doi.org/10.1121/1.3442572

Huettner, T., Dollhaeupl, S., Simon, R., Baumgartner, K., & von Fersen, L. (2021). Activity budget comparisons using long-term observations of a group of bottlenose dolphins (*Tursiops truncatus*) under human care: Implications for animal welfare. *Animals*, *11*(7), 2107. https://doi.org/10.3390/ani11072107

Jensen, A. L. M., Delfour, F., & Carter, T. (2013). Anticipatory behavior in captive bottlenose dolphins (*Tursiops truncatus*): A preliminary study. *Zoo Biology*, *32*(4), 436-444. https://doi.org/10.1002/zoo.21077

Johnson, S. P., Venn-Watson, S. K., Cassle, S. E., Smith, C. R., Jensen, E. D., & Ridgway, S. H. (2009). Use of phlebotomy treatment in Atlantic bottlenose dolphins with iron overload. *Journal of the American Veterinary Medical Association*, *235*(2), 194-200. https://doi.org/10.2460/javma.235.2.194

Kastelein, R. A., Triesscheijn, R. J., & Jennings, N. (2016). Reversible bending of the dorsal fins of harbor porpoises (*Phocoena phocoena*) and a striped dolphin (*Stenella coeruleoalba*) in captivity. *Aquatic Mammals*, *42*(2), 218-226. https://doi.org/10.1578/AM.42.2.2016.218

Kastelein, R. A., Bakker, M. J., Jennings, N., & Covi-Dijkhuizen, J. (2023). Evaluating the use of diazepam in stranded dolphins and porpoises for husbandry and veterinary purposes. *Aquatic Mammals*, *49*(1), 94-103. https://doi.org/10.1578/AM.49.1.2023.94

Kikusui, T., Nakanishi, K., Nakagawa, R., Nagasawa, M., Mogi, K., & Okanoya, K. (2011). Cross fostering experiments suggest that mice songs are innate. *PLOS ONE*, *6*(3), e17721. https://doi.org/10.1371/journal.pone.0017721

Kleen, J. K., Sitomer, M. T., Killeen, P. R., & Conrad, C. D. (2006). Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. *Behavioral Neuroscience*, *120*(4), 842-851. https://doi.org/10.1037/0735-7044.120.4.842

Krebs, B. L., Chudeau, K. R., Eschmann, C. L., Tu, C. W., Pacheco, E., & Watters, J. V. (2022). Space, time, and context drive anticipatory behavior: Considerations for understanding the behavior of animals in human care. *Frontiers in Veterinary Science*, *9*, 972217. https://doi.org/10.3389/fvets.2022.972217

Kuczaj II, S. A., Highfill, L. E., Makecha, R. N., & Byerly, H. C. (2013). Why do dolphins smile? A comparative perspective on dolphin emotions and emotional expressions. In S. Watanabe & S. A. Kuczaj II (Eds.), *Emotions of animals and humans* (pp. 63-85). Springer. https://doi.org/10.1007/978-4-431-54123-3_4

Kuklisova-Murgasova, M., Quaghebeur, G., Rutherford, M. A., Hajnal, J. V., & Schnabel, J. A. (2012). Reconstruction of fetal brain MRI with intensity matching and complete outlier removal. *Medical Image Analysis*, *16*(8), 1550-1564. https://doi.org/10.1016/j.media.2012.07.004

Ladygina, T. F., & Supin, A. Ya. (1977). Localization of the sensory projection areas in the cerebral cortex of the dolphin, *Tursiops truncatus*. *Zhurnal Evolyutsionnoy Biokhimii Fiziologii*, *13*, 712-718.

Larson, S. J. (2002). Behavioral and motivational effects of immune-system activation. *Journal of General Psychology*, *129*(4), 401-414. https://doi.org/10.1080/00221300209602104

Lauderdale, L. K. (2017). *Efficacy of cognitive enrichment for bottlenose dolphins (*Tursiops truncatus*): Evaluation of planning abilities through the use of a novel problem solving task* (Doctoral dissertation). The University of Southern Mississippi, Hattiesburg. https://aquila.usm.edu/dissertations/1399

Lauderdale, L. K., Mellen, J. D., Walsh, M. T., Granger, D. A., & Miller, L. J. (2023). Behavior reference intervals and activity budgets of bottlenose dolphins in accredited zoos and aquariums. *Applied Animal Behavior Science*, *10*, 105968. https://doi.org/10.1016/j.applanim.2023.105968

Lauderdale, L. K., Walsh, M. T., Mitchell, K. A., Granger, D. A., Mellen, J. D., & Miller, L. J. (2021). Health reference intervals and values for common bottlenose dolphins (*Tursiops truncatus*), Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), and beluga whales (*Delphinapterus leucas*). *PLOS ONE*, *16*(8), e0250332. https://doi.org/10.1371/journal.pone.0250332

Linnehan, B. K., Hsu, A., Gomez, F. M., Huston, S. M., Takeshita, R., Colegrove, K. M., Rowles, T. K., Barratclough, A., Musser, W. B., Harms, C. A., Cendejas, V., Zolman, E. S., Balmer, B. C., Townsend, F. I., Wells, R. S., Jensen, E. D., Schwacke, L. H., & Smith, C. R. (2020). Standardization of dolphin cardiac auscultation and characterization of heart murmurs in managed and free-ranging bottlenose dolphins (*Tursiops truncatus*). *Frontiers in Veterinary Science*, *7*. https://doi.org/10.3389/fvets.2020.570055

Madsen, P. T., Lammers, M., Wisniewska, D., & Beedholm, K. (2013). Nasal sound production in echolocating delphinids (*Tursiops truncatus* and *Pseudorca crassidens*) is dynamic, but unilateral: Clicking on the right side and whistling on the left side. *Journal of Experimental Biology*, *216*(21), 4091-4102. https://doi.org/10.1242/jeb.091306

Manger, P., Cort, J., Ebrahim, N., Goodman, A., Henning, J., Karolia, M., Rodrigues, S. L., & Strkalj, G. (2008). Is 21st century neuroscience too focussed on the rat/mouse model of brain function and dysfunction? *Frontiers in Neuroanatomy*, *2*. https://doi.org/10.3389/neuro.05.005.2008

Mann, J. (1999). Behavioral sampling methods for cetaceans: A review and critique. *Marine Mammal Science*, *15*(1), 102-122. https://doi.org/10.1111/j.1748-7692.1999.tb00784.x

Mann, J. (2006). Establishing trust: Socio-sexual behaviour and the development of male-male bonds among Indian Ocean bottlenose dolphins. In S. Volker & P. L. Vasey (Eds.), *Homosexual behaviour in animals: An evolutionary perspective* (pp. 107-130). Cambridge University Press.

Manteuffel, G., Langbein, J., & Puppe, B. (2009). Increasing farm animal welfare by positively motivated instrumental behaviour. *Applied Animal Behaviour Science*, *118*(3-4), 191-198. https://doi.org/10.1016/j.applanim.2009.02.014

Maple, T. L., & Perdue, B. M. (Eds.). (2013). *Zoo animal welfare*. Springer. https://doi.org/10.1007/978-3-642-35955-2

Martin, P., & Bateson, P. (2007). *Measuring behaviour: An introductory guide* (3rd ed.). Cambridge University Press. https://doi.org/10.1017/CBO9780511810893

McGonigle, P. (2014). Animal models of CNS disorders. *Biochemical Pharmacology*, *87*(1), 140-149. https://doi.org/10.1016/j.bcp.2013.06.016

Mennes, M., Jenkinson, M., Valabregue, R., Buitelaar, J. K., Beckmann, C., & Smith, S. (2014). Optimizing full-brain coverage in human brain MRI through population distributions of brain size. *NeuroImage*, *98*, 513-520. https://doi.org/10.1016/j.neuroimage.2014.04.030

Millman, S. T. (2007). Sickness behaviour and its relevance to animal welfare assessment at the group level. *Animal Welfare*, *16*(2), 123-125. https://doi.org/10.1017/S0962728600031146

Mineka, S., & Cook, M. (1986). Immunization against the observational conditioning of snake fear in rhesus monkeys. *Journal of Abnormal Psychology*, *95*(4), 307-318. https://doi.org/10.1037/0021-843X.95.4.307

Moe, R. O., Bakken, M., Kittilsen, S., Kingsley-Smith, H., & Spruijt, B. M. (2006). A note on reward-related behaviour and emotional expressions in farmed silver foxes (*Vulpes vulpes*)—Basis for a novel tool to study animal welfare. *Applied Animal Behaviour Science*, *101*(3-4), 362-368. https://doi.org/10.1016/j.applanim.2006.02.004

Mukhametov, L. M., Supin, A. Ya., & Polyakova, I. G. (1977). Interhemispheric asymmetry of the electroencephalographic sleep patterns in dolphins. *Brain Research*, *134*, 581-584. https://doi.org/10.1016/0006-8993(77)90835-6

National Marine Fisheries Service (NMFS). (2022). *Cetacean and pinniped transport best practices*. Marine Mammal Health and Stranding Response Program, National Oceanic and Atmospheric Administration. https://doi.org/10.25923/jf71-hd33

Nestler, E., & Hyman, S. (2010). Animal models of neuropsychiatric disorders. *Nature Neuroscience*, *13*, 1161-1169. https://doi.org/10.1038/nn.2647

Nollens, H. H., Haney, N. J., Stacy, N. I., & Robeck, T. R. (2020). Effects of sex, age, and season on the variation of blood analytes in a clinically healthy *ex situ* population of bottlenose dolphins (*Tursiops* spp.). *Veterinary Quarterly*, *40*(1), 342-352. https://doi.org/10.1080/01652176.2020.1845415

Ogle, D. H., Doll, J. C., Wheeler, A. P., & Dinno, A. (2023). FSA: Simple fisheries stock assessment methods. R *package Version 0.9.5*. https://CRAN.R-project.org/package=FSA

Ortiz, S. T., Maxwell, A., & Hansen, K. A. (2017). Research as an enrichment tool to improve welfare in captive animals. *Animal Husbandry, Dairy and Veterinary Science*, *1*, 1-6. https://doi.org/10.15761/AHDVS.1000124

Parsons, K. M., Durban, J. W., & Claridge, D. E. (2003). Male-male aggression renders bottlenose dolphin (*Tursiops truncatus*) unconscious. *Aquatic Mammals*, *29*(3), 360-362.

Paulos, R. D., Trone, M., & Kuczaj II, S. A. (2010). Play in wild and captive cetaceans. *International Journal of Comparative Psychology*, *23*(4), 701-722. https://doi.org/10.46867/IJCP.2010.23.04.06

Pedersen, L. J., Jensen, M. B., Hansen, S. W., Munksgaard, L., Ladewig, J., & Matthews, L. (2002). Social isolation affects the motivation to work for food and straw in pigs as measured by operant conditioning techniques. *Applied Animal Behaviour Science*, *77*(4), 295-309. https://doi.org/10.1016/S0168-1591(02)00066-7

Perez, B. C., Mehrkam, L. R., Foltz, A. R., & Dorey, N. R. (2018). Effects of enrichment presentation and other factors on behavioral welfare of pantropical spotted dolphin (*Stenella attenuata*). *Journal of Applied Animal Welfare Science*, *21*(2), 130-140. https://doi.org/10.1080/10888705.2017.1383161

Platto, S., & Serres, A. A. (2023). Quantitative assessment of trainers-dolphins' interactions in the absence of food reward. *Animals*, *13*(10), 1651. https://doi.org/10.3390/ani13101651

R Core Team. (2021). R*: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Ralls, K., Fiorelli, P., & Gish, S. (1985). Vocalizations and vocal mimicry in captive harbor seals, *Phoca vitulina*. *Canadian Journal of Zoology*, *63*(5), 1050-1056. https://doi.org/10.1139/z85-15

Rault, J. L. (2012). Friends with benefits: Social support and its relevance for farm animal welfare. *Applied Animal Behaviour Science*, *136*(1), 1-14. https://doi.org/10.1016/j.applanim.2011.10.002

Richards, D. G., Wolz, J. P., & Herman, L. M. (1984). Vocal mimicry of computer-generated sounds and vocal labeling of objects by a bottlenosed dolphin, *Tursiops truncatus*. *Journal of Comparative Psychology*, *98*(1), 10-28. https://doi.org/10.1037/0735-7036.98.1.10

Ridgway, S., Houser, D., Finneran, J., Carder, D., Keogh, M., Van Bonn, W., Smith, C., Scadeng, M., Dubowitz, D., Mattrey, R., & Hoh, C. (2006). Functional imaging of dolphin brain metabolism and blood flow. *Journal of Experimental Biology*, *209*, 2902-2910. https://doi.org/10.1242/jeb.02348

Sapolsky, R. M. (2004). *Why zebras don't get ulcers: The acclaimed guide to stress, stress-related diseases, and coping* (3rd ed.). Holt Paperbacks.

Scott, E. M., Mann, J., Watson-Capps, J. J., Sargeant, B. L., & Connor, R. C. (2005). Aggression in bottlenose dolphins: Evidence for sexual coercion, male-male competition, and female tolerance through analysis of toothrake marks and behaviour. *Behaviour*, *142*(1), 21-44. https://doi.org/10.1163/1568539053627712

Serres, A. (2019). The frequency of solitary behaviours in captive odontocetes is modulated by environmental and social factors. *International Journal of Comparative Psychology*, *32*, 44338. https://doi.org/10.46867/ijcp.2019.32.00.08

Serres, A., & Delfour, F. (2017). Environmental changes and anthropogenic factors modulate social play in captive bottlenose dolphins (*Tursiops truncatus*). *Zoo Biology*, *36*(2), 99-111. https://doi.org/10.1002/zoo.21355

Serres, A., & Delfour, F. (2019). Social behaviors modulate bottlenose dolphins' (*Tursiops truncatus*) breathing rate. *Animal Behavior and Cognition*, *6*(2), 127-140. https://doi.org/10.26451/abc.06.02.04.2019

Serres, A., Hao, Y., & Wang, D. (2020). Body contacts and social interactions in captive odontocetes are influenced by the context: An implication for welfare assessment. *Animals*, *10*(6), 924. https://doi.org/10.3390/ani10060924

Shyne, A. (2006). Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. *Zoo Biology*, *25*(4), 317-337. https://doi.org/10.1002/zoo.20091

Sneddon, L. U., Elwood, R. W., Adamo, S. A., & Leach, M. C. (2014). Defining and assessing animal pain. *Animal Behaviour*, *97*, 201-212. https://doi.org/10.1016/j.anbehav.2014.09.007

Southall, B. L., Finneran, J. J., Reichmuth, C., Nachtigall, P. E., Ketten, D. R., Bowles, A. E., Ellison, W. T., Nowacek, D. P., & Tyack, P. L. (2019). Marine mammal noise exposure criteria: Updated scientific recommendations for residual hearing effects. *Aquatic Mammals*, *45*(2), 125-232. https://doi.org/10.1578/AM.45.2.2019.125

Spruijt, B. M., Van den Bos, R., & Pijlman, F. T. A. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: Anticipatory behaviour as an indicator for the state of reward systems. *Applied Animal Behaviour Science*, *72*(2), 145-171. https://doi.org/10.1016/S0168-1591(00)00204-5

St. Leger, J., Raverty, S., & Mena, A. (2018). Cetacea. In K. A. Terio, D. McAloose, & J. St. Leger (Eds.), *Pathology of wildlife and zoo animals* (pp. 533-568). Academic Press. https://doi.org/10.1016/B978-0-12-805306-5.00022-5

Stoeger, A. S., Mietchen, D., Oh, S., de Silva, S., Herbst, C. T., Kwon, S., & Fitch, W. T. (2012). An Asian elephant imitates human speech. *Current Biology*, *22*, 2144-2148. https://doi.org/10.1016/j.cub.2012.09.022

Taylor, G. T. (1981). Fear and affiliation in domesticated male rats. *Journal of Comparative and Physiological Psychology*, *95*(5), 685-693. https://doi.org/10.1037/h0077817

Tyack, P. L. (2019). A taxonomy for vocal learning. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1789), 20180406. https://doi.org/10.1098/rstb.2018.0406

Uus, A., Zhang, T., Jackson, L., Roberts, T., Rutherford, M., Hajnal, J. V., & Deprez, M. (2020). Deformable slice-to-volume registration for motion correction in fetal body MRI and placenta. *IEEE Transactions on Medical Imaging*, *39*(9), 2750-2759. https://doi.org/10.1109/TMI.2020.2974844

Van den Berg, C. L., Pijlman, F. T. A., Koning, H. A. M., Diergaarde, L., Van Ree, J. M., & Spruijt, B. M. (1999). Isolation changes the incentive value of sucrose and social behaviour in juvenile and adult rats. *Behavioural Brain Research*, *106*(1-2), 133-142. https://doi.org/10.1016/S0166-4328(99)00099-6

Venn-Watson, S., Daniels, R., & Smith, C. (2012). Thirty-year retrospective evaluation of pneumonia in a bottlenose dolphin *Tursiops truncatus* population. *Diseases of Aquatic Organisms*, *99*(3), 237-242. https://doi.org/10.3354/dao02471

Von Streit, C., Ganslosser, U., & von Fersen, L. (2011). Ethogram of two captive mother-calf dyads of bottlenose dolphins (*Tursiops truncatus*): Comparison with field ethograms. *Aquatic Mammals*, *37*(2), 193-197. https://doi.org/10.1578/AM.37.2.2011.193

Waples, K. A., & Gales, N. J. (2002). Evaluating and minimising social stress in the care of captive bottlenose dolphins (*Tursiops aduncus*). *Zoo Biology*, *21*(1), 5-26. https://doi.org/10.1002/zoo.10004

Watters, J. V. (2014). Searching for behavioral indicators of welfare in zoos: Uncovering anticipatory behavior. *Zoo Biology*, *33*(4), 251-256. https://doi.org/10.1002/zoo.21144

Wright, A., Scadeng, M., Stec, D., Dubowitz, R., Ridgway, S., & St. Leger, J. (2017). Neuroanatomy of the killer whale (*Orcinus orca*): A magnetic resonance imaging investigation of structure with insights on function and evolution. *Brain Structure and Function*, *222*, 417-436. https://doi.org/10.1007/s00429-016-1225-x

Yartsev, M. M. (2017). The emperor's new wardrobe: Rebalancing diversity of animal models in neuroscience research. *Science*, *358*(6362), 466-469. https://doi.org/10.1126/science.aan8865