

Heart Rate and R–R Interval Recording in a Bottlenose Dolphin: Toward a Keeper-Led Framework for HRV-Based Welfare Assessment

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Abstract

Monitoring physiological states in managed animals is important for health management and welfare assessment. Heart rate variability (HRV) may provide welfare-relevant information on autonomic regulation, but practical keeper-led approaches for obtaining cardiac data during routine husbandry remain limited in zoos and aquariums. To address this gap, we propose a keeper-led framework in which aquarium animal-care teams repeatedly collect HR and R–R interval data during routine husbandry. Research partners analyze the cardiac data, using available respiratory, behavioral, and health information as context for interpretation, and return the findings to animal-care practice. We examined whether HR and R–R interval recordings could be obtained from a bottlenose dolphin (*Tursiops truncatus*) during voluntary routine husbandry training. A commercially available belt-mounted sensor was used while the dolphin maintained a stationary dorsal floating posture at the water surface. Usable HR and R–R interval recordings were obtained, demonstrating feasibility in a cooperative husbandry setting. Representative recordings showed beat-to-beat variation and intermittently prolonged R–R intervals, with corresponding fluctuations in derived HR. Synchronized video annotations of respiratory, behavioral, and training-related events provided context. These observations are descriptive and do not establish specific autonomic, affective, or welfare states. However, they provide a basis for HRV analyses and repeated, context-informed investigation of autonomic patterns in relation to stress-related responses, arousal, recovery, and relatively settled physiological conditions. This study presents the initial recording and implementation component of the framework. Repeated recordings under comparable conditions may support development of individual baseline profiles and context-informed HRV-based animal welfare indicators in zoological and aquarium settings.

Keywords: bottlenose dolphin; heart rate variability; heart rate; R–R interval; cooperative care; husbandry training; animal welfare

Introduction

Improving the welfare of animals managed in zoos and aquariums has become an increasingly important goal in modern animal care and research. Zoological institutions are expected not only to maintain animal health but also to provide environments that support appropriate behavioral opportunities and positive welfare states. In some countries, the welfare of animals in zoological facilities is regulated through national legislation and inspection systems. For example, in the United States, the Animal Welfare Act establishes minimum standards for animal management and is enforced through inspections conducted by the United States Department of Agriculture Animal and Plant Health Inspection Service (United States Department of Agriculture, n.d.). In addition, professional organizations such as the Association of Zoos and Aquariums have developed accreditation programs that emphasize systematic welfare assessment as a core component of institutional animal care (Association of Zoos and Aquariums, n.d.).

Alongside regulatory frameworks, animal welfare science has advanced substantially over the past decades (Broom, 2011). Contemporary welfare models emphasize that animal welfare should be evaluated through the integration of behavioral, physiological, and health-related indicators (Mellor, 2016). In the zoo and aquarium field, the World Association of Zoos and Aquariums has also updated its Animal Welfare Strategy to promote evidence-based welfare assessment and the implementation or refinement of formal animal welfare assessment programs in zoos and aquariums (World Association of Zoos and Aquariums, 2025). Within such integrated welfare assessment frameworks, behavioral observations remain one of the most widely used approaches for evaluating welfare, as behavior provides valuable information about how animals interact with their environment and experience their conditions (Dawkins, 2004). In zoological settings, welfare research increasingly emphasizes the need to integrate behavioral observations with physiological indicators in order to obtain a more comprehensive understanding of animal welfare (Whitham & Wielebnowski, 2013).

Physiological indicators can provide valuable complementary information about internal states that may not always be detectable through behavioral observation alone. Among these indicators, heart rate (HR) and heart rate variability (HRV) are widely used measures of autonomic nervous system activity and have been applied across a

wide range of vertebrate species to assess stress responses, emotional states, and recovery processes (von Borell et al., 2007). HRV describes variation in successive cardiac intervals and is influenced by autonomic regulation; it is widely used as a non-invasive measure for examining physiological state in both human and animal studies (Shaffer & Ginsberg, 2017; von Borell et al., 2007). In domestic animals, including horses and livestock species, HR and HRV monitoring have been used to examine autonomic responses associated with stress, activity, and health-related conditions under managed conditions (von Borell et al., 2007; Gehrke et al., 2011; Visser et al., 2002). These studies demonstrate the potential of autonomic indices as practical tools for monitoring animal welfare in controlled environments.

Cardiovascular responses have also been studied in marine mammals, where heart rate dynamics are closely associated with behavioral and physiological states such as diving and activity patterns (Noren et al., 2012). More broadly, cardiorespiratory physiology has been recognized as an important component of understanding the physiological responses of marine mammals to respiratory, environmental, and behavioral stimuli (Fahlman et al., 2020). These studies highlight the potential of cardiovascular measurements as informative indicators of physiological state in marine species.

To translate the potential utility of HR and HRV into practical welfare assessment, physiological monitoring needs to be integrated into routine husbandry practices in a form that can be used in the field. Many physiological studies rely on specialized biologging systems, experimental protocols, or research contexts that are difficult to integrate into everyday husbandry practices. As a result, physiological indicators that could inform welfare assessment are often generated in research contexts but are less commonly translated into routine, field-based welfare monitoring.

Animal welfare management involves stakeholders in animal care, institutional decision-making, regulation, research, and external evaluation. Documented physiological records, interpreted alongside behavioral and health-related information, may provide an additional common evidence base for communication across these roles and support transparent welfare assessment practices.

In applying such physiological indicators within routine management, animal training sessions offer a useful opportunity to integrate physiological monitoring into everyday husbandry practices. In many zoological facilities, animals are accustomed to interacting with keepers during training sessions, potentially allowing physiological data to be collected under relatively standardized and repeatable conditions.

Incorporating HR and R–R interval measurements into these routine interactions may therefore provide an opportunity to obtain repeated physiological recordings alongside

behavioral context.

In cetaceans, previous studies have measured heart rate and cardiac responses in dolphins and other cetaceans in relation to diving, respiration, and behavior (Noren et al., 2012; Fahlman et al., 2020; Blawas et al., 2021). A preliminary report proposed the use of HRV-based physiological monitoring for welfare assessment in managed dolphins and reported the initial feasibility of obtaining HR and R–R interval signals using a belt-mounted heart rate sensor (Shimozato, 2025). However, as noted above, approaches for implementing HRV-based welfare monitoring in husbandry settings remain limited. Building on this background, the longer-term aim of this work is to develop a keeper-led HRV welfare monitoring framework for use during routine dolphin training sessions. This framework is intended to support the development of HRV-based animal welfare indicators through repeated cardiac recordings integrated with behavioral, respiratory, and health-related context. As an initial step toward this aim, the present study examined whether HR and R–R interval recordings could be obtained from a bottlenose dolphin during voluntary routine husbandry training. Repeated recordings obtained under comparable conditions may support future individual baseline characterization and context-informed HRV analysis. Although this study focuses on a bottlenose dolphin, the proposed framework may be adaptable to other trained animals.

Methods

2.1 Study animal and facility

The study was conducted with one male bottlenose dolphin (*Tursiops truncatus*) of estimated age 17 years housed at Kyoto Aquarium, Japan. The dolphin was maintained under professional care as part of the aquarium's regular husbandry program and participated voluntarily in daily training sessions conducted by experienced trainers. Measurements were conducted in either Breeding Pool 1 or the medical pool, depending on the routine management schedule. Breeding Pool 1 is approximately 13.2 m × 6.5 m with a depth of 3.6 m, whereas the medical pool is approximately 9.0 m × 7.4 m with a depth of 3.6 m.

2.2 Heart rate monitoring device

Heart rate was monitored using a chest-belt type sensor (Polar H10, Polar Electro Oy, Finland) attached to an equine monitoring belt (Polar Equine belt, Polar Electro Oy, Finland). The system detects electrocardiogram (ECG) signals through skin-contact electrodes integrated into the belt. The sensor was connected to a mobile device via

Bluetooth for data recording.

2.3 Conceptual framework and measurement overview

In this study, we propose a Keeper-led HRV Welfare Monitoring Framework (K-HRV Framework) to develop physiological indicators for animal welfare assessment by linking field-based data collection with laboratory analysis. In the present framework, “keeper-led” refers to on-site acquisition of HR and R–R interval data by aquarium animal-care staff, without necessarily requiring researcher involvement in each recording. The present study examined the field-recording component of this framework. Physiological data, including heart rate (HR) and R–R intervals, were collected in the field (e.g., an aquarium setting) during routine training sessions. R–R intervals were extracted from the recorded data, from which HR and heart rate variability (HRV) indices (e.g., RMSSD and SDNN) were calculated. In dolphins, interpretation of these indices in relation to autonomic state may require further evaluation of appropriate processing procedures and consideration of repeated within-individual recordings alongside respiratory, behavioral, and other contextual information.

Within the proposed framework, the resulting indicators would be combined with contextual information from the field, such as behavioral observations during training. The longer-term aim is to support integrated evaluation of the animal’s condition, including the potential identification of context-informed patterns associated with stress, relaxation, and arousal, and to inform management decisions and long-term monitoring. Analytical outputs could then be returned to the field to inform monitoring of individual condition, investigation of potential stress-related changes, and refinement of animal care practices, while continued field data collection could support investigation of relationships between behavior and physiological responses. An overview of the framework is provided in Fig. 1.

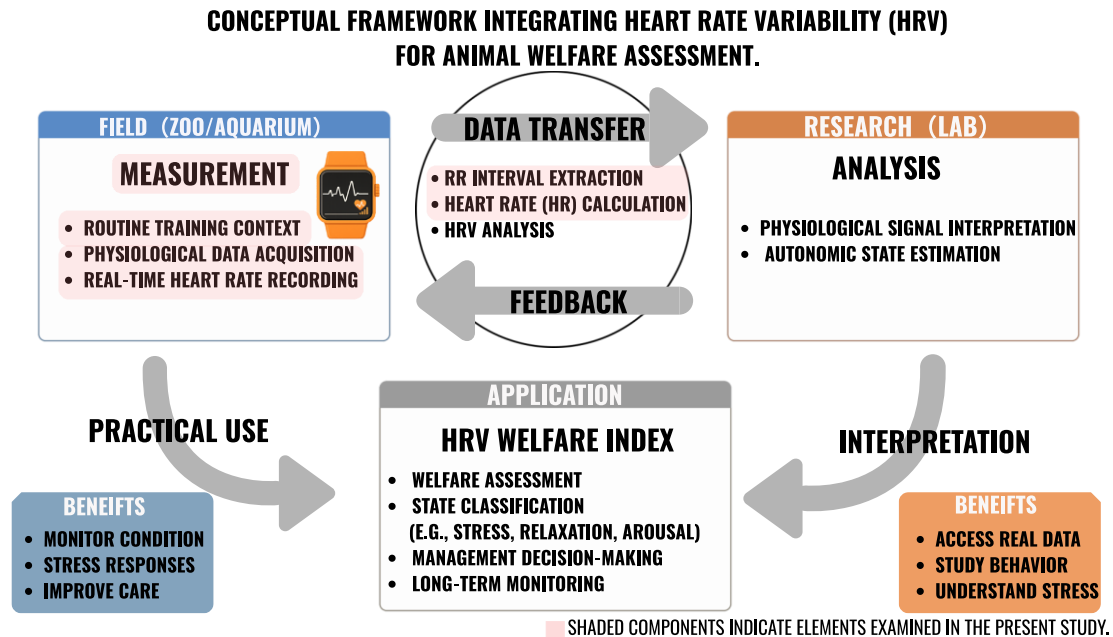


Figure 1. Conceptual framework for HRV-based welfare assessment.

The framework links routine training-based HR and R–R interval recording, HRV analysis, and feedback.

2.4 Sensor attachment during training

The heart rate sensor was attached using a belt designed for equine heart rate monitoring (Polar Equine belt, Polar Electro Oy, Finland). The belt was placed around the trunk so that it passed below the base of the pectoral fins, and the sensor unit was positioned below the left pectoral fin, slightly medial on the left ventral body surface. The approximate location of the heart was confirmed in advance by a veterinarian using ultrasonography to guide sensor placement. Electrodes integrated into the belt were placed in contact with the skin to record ECG signals. ECG signal acquisition was sensitive to sensor placement, and slight displacement could affect signal quality. The belt was adjusted to maintain electrode contact during recording. A Polar H10 sensor was connected to the belt for heart rate and R–R interval recording.

2.5 Habituation, desensitization, and reinforcement procedure

Prior to heart rate monitoring, the dolphin was gradually desensitized and habituated to the belt-mounted sensor and the measurement procedure during routine training sessions. Because the dolphin had already been trained to maintain a stationary dorsal floating posture at the water surface before this study, the newly introduced training in

this study focused on desensitization to the contact and wearing sensation of the belt-mounted heart rate sensor, and habituation to the procedural sequence from belt attachment to the dorsal measurement posture.

The measurement was conducted using the following procedure.

1. **Stationing near the grating**

The dolphin was positioned near the grating in a neutral, upright waiting posture while maintaining attention to the trainer.

2. **Induction of lateral posture by the rollover cue**

Using a rollover cue, the dolphin was guided into a lateral posture, with the body tilted either to the right or left side.

3. **Passing and positioning of the belt**

The trainer lightly held the region near the dorsal fin to stabilize the posture, while the veterinarian passed the belt under the trunk from the caudal side. The belt position was then adjusted so that the electrode area was located near the region below the left pectoral fin.

4. **Confirmation of belt position and electrode contact**

If the sensor was attached after belt positioning, it was attached to the belt at this stage. Belt position and electrode contact were then confirmed.

5. **Transition to the dorsal posture**

The dolphin's left pectoral fin was guided into the trainer's hand while the animal transitioned into the dorsal measurement posture.

6. **Start of heart rate recording**

Heart rate and R–R interval recording were initiated while the dolphin maintained a stable stationary dorsal floating posture.



Figure 2. Sensor attachment procedure in a bottlenose dolphin.

- (a) Rollover cue from the stationing position.
- (b) Drifting in the guided direction in a lateral floating state.
- (c) The sensor belt was passed under the body from the caudal side.
- (d) The sensor belt was adjusted and secured near the left pectoral region.
- (e) Real-time heart rate display on a mobile device.
- (f) Overall view of the measurement posture and setup.

The sensor attachment procedure is illustrated in Fig. 2 and shown in Video 1. From the first attachment session, the dolphin was able to accept the belt while maintaining a stationary dorsal floating posture. In contrast, when the dolphin was allowed to swim freely while wearing the belt, behaviors suggesting discomfort associated with belt displacement or tactile sensation were observed, including body twisting and swimming movements that differed from usual behavior. Therefore, free swimming while wearing the belt was avoided, and movements during measurement were performed, in principle, under trainer cues.

During posture transition, delayed responses to the rollover cue, intermittent vocalizations, and increased body tension during tail fluke handling were occasionally observed in the early phase. These responses were treated as potential indications of discomfort or tension associated with the procedure. When such responses were observed, the reinforcement interval was shortened, and relaxed behavior was

reinforced more frequently before the procedure was continued. Across repeated sessions, the response to the rollover cue improved, and the sequence from belt attachment to presenting the left pectoral fin to the trainer and transitioning into the dorsal measurement posture became more predictive and cooperative.

When behaviors that could displace the belt or responses suggesting discomfort with the attachment were observed, the procedure was not forced and the belt was removed promptly. This was done to avoid negative associations with belt attachment and to prevent the establishment of behaviors directed at removing the belt. After each session, praise and food reinforcement were provided to maintain a positive association with the overall measurement procedure, including belt attachment and approximately five minutes of stationary dorsal floating.

During measurement, reinforcement was provided when the trainer judged that a relaxed and stable posture was being maintained. The trainer assessed relaxation based on whole-body relaxation, such as the tail hanging downward, calm respiration, absence of excessive eye movement or eye widening, and absence of aggression-related responses such as pectoral fin withdrawal or striking movements.

When heart rate signals could not be obtained stably or when belt displacement occurred during measurement, the procedure was temporarily interrupted, and the belt position was readjusted or the posture was reset. In addition, when repeated readjustments resulted in a prolonged period of stationary posture, the trainer sometimes inserted a lap around the pool before resuming the procedure. These interruptions were implemented to stabilize signal acquisition, adjust belt position, and reset the animal's state when necessary.

The procedure was established by combining the dolphin's pre-existing stationary dorsal floating behavior with desensitization to the belt-mounted sensor and habituation to the sequence from belt attachment to the dorsal measurement posture.

2.6 Data collection

Heart rate recordings were obtained during routine training sessions. Each measurement session lasted approximately 5 min. The dolphin was measured while maintaining a stationary dorsal floating posture at the water surface through positive reinforcement training. Recordings were collected over multiple sessions across several days, primarily in the afternoon, with some morning sessions also included. On days when morning sessions were recorded, paired morning and afternoon measurements were available.

In the initial stage of this study, heart rate data were preliminarily recorded using the

Polar Flow application. In subsequent measurements, R–R interval data were acquired using the Polar Equine application, allowing more detailed examination of cardiac interval dynamics.

Because the present study was designed as an initial methodological study, these recordings were used primarily to evaluate the feasibility of obtaining stable HR and R–R interval data during routine husbandry training and to describe representative cardiac interval patterns.

2.7 Data processing

Direct R–R interval data were retained only when values were between 300 and 3000 ms and signal quality (SC) was equal to 1. Time was reset to 0 s at the start of each recording. Heart rate (HR) was calculated from R–R intervals (ms) as $HR = 60,000/RR$. RMSSD and SDNN were calculated from the filtered R–R interval series without additional correction procedures and are reported as descriptive values for the representative segment.

Data filtering and calculation of HR and HRV metrics were performed in Python using custom code. For representative analysis, a continuous segment from approximately 0 to 100 s was selected from the beginning of the recording after sensor attachment.

In sessions where direct R–R interval data were not available, sensor-recorded ECG waveforms were examined to identify stable segments and candidate peaks. Candidate R–R intervals were retrospectively estimated from successive candidate peaks, and values <300 ms or >3000 ms were excluded.

2.8 Video-based event annotation

In selected sessions, synchronized video recordings were used to annotate behavioral and training-related events during heart rate monitoring. Annotated events included respiration, fish reinforcement, and brief interruptions of the dorsal floating posture.

In some sessions, the dolphin temporarily shifted from the dorsal floating posture to a lateral position for sensor adjustment before returning to the measurement posture.

During these periods, respiration behaviors were also observed.

These event annotations were used as reference information for interpreting temporal changes in heart rate and R–R interval recordings. For representative visualization, HR time-series data were presented together with annotated behavioral events to examine temporal relationships between cardiac dynamics and behavior (Fig. 4).

Respiration events included both exhalation-only behaviors and combined exhalation–inhalation behaviors identified from video recordings.

Results

3.1 Initial heart rate recordings (pilot stage)

In the initial stage of data collection, heart rate was recorded using a belt-mounted sensor connected to the Polar Flow application. Although R–R interval data were not available at this stage, continuous heart rate signals were obtained during stationary dorsal floating. Heart rate during the initial recordings ranged approximately from 50 to 70 bpm, and representative stable segments lasted for several tens of seconds. A representative pilot-stage heart rate recording is shown in Supplementary Figure S1. In the selected segment, heart rate gradually decreased over time. Although the biological interpretation of this pattern remains uncertain, these preliminary recordings confirmed that continuous HR signals could be obtained under routine training conditions.

3.2 Representative HR and RR interval dynamics

Representative time-series data of heart rate (HR) and R–R intervals are shown in Fig. 3. Continuous HR and R–R interval signals were recorded during stationary dorsal floating at the water surface. The analyzed segment covered approximately 0–100 s after the start of recording following sensor attachment. HR derived from filtered R–R intervals showed higher and more variable values in the early phase of recording, followed by a gradual decrease and subsequent stabilization. R–R intervals showed the corresponding inverse pattern. Within the analyzed segment, prolonged R–R intervals exceeding 2000 ms were intermittently observed. The lowest HR values derived from filtered R–R intervals were approximately 22–25 bpm, corresponding to prolonged intervals of 2715 and 2391 ms. Overall, mean HR was 65.4 bpm (median: 60.4 bpm), and mean R–R interval was 1056 ms (median: 993 ms). HRV metrics calculated for the representative segment indicated beat-to-beat variability, with an RMSSD of 492.6 ms and an SDNN of 409.2 ms. These HRV values were calculated from a segment that included occasional prolonged R–R intervals. Together, these data indicate that continuous cardiac interval recordings with measurable beat-to-beat variability were obtained under routine training conditions.

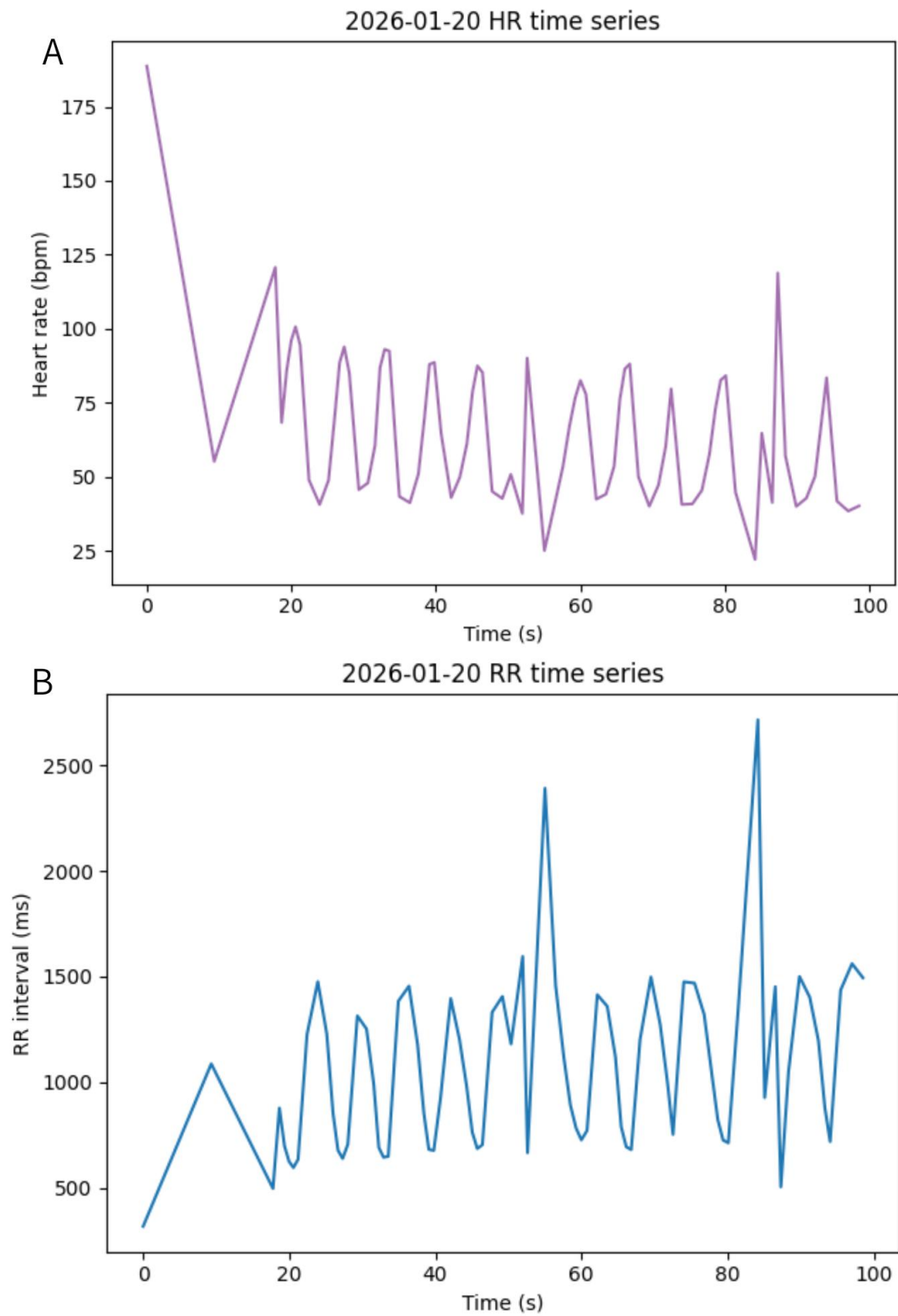


Figure 3. Representative time-series data of heart rate (HR) and R–R intervals recorded from the start of recording after sensor attachment during stationary dorsal

floating.

(A) HR derived from filtered R–R intervals. HR values fluctuated during the early phase of recording, followed by a gradual decrease and subsequent stabilization. Some of the high values observed at the beginning of the recording may reflect transient fluctuations during the initial phase of signal acquisition.

(B) Corresponding R–R intervals from the same continuous segment. Prolonged intervals exceeding 2000 ms were intermittently observed, with the longest intervals reaching 2715 ms and 2391 ms, corresponding to derived HR values of approximately 22 and 25 bpm, respectively.

Both panels represent the same continuous segment (~0–100 s) from the start of recording after sensor attachment. R–R interval data were filtered to retain values between 300 and 3000 ms with signal quality ($SC = 1$), and HR was calculated as $HR = 60,000 / RR$. The time axis was rebased to 0 s at the start of the recording.

3.3 ECG-based estimation of candidate R–R intervals

In one session, direct R–R interval data were not available, although ECG signals were recorded. Representative ECG traces from the recording are shown in Fig. 4. Figure 4A shows a 50-s ECG segment recorded under routine training conditions. Figures 4B and 4C show selected 20-s and 5-s segments, respectively, from the same recording. Candidate peaks were identified in the 5-s segment (Fig. 4C), from which candidate R–R intervals were retrospectively estimated.

These observations indicate that, even in sessions where direct R–R interval data were not available, ECG recordings may allow retrospective identification of candidate peaks and partial estimation of candidate R–R intervals.

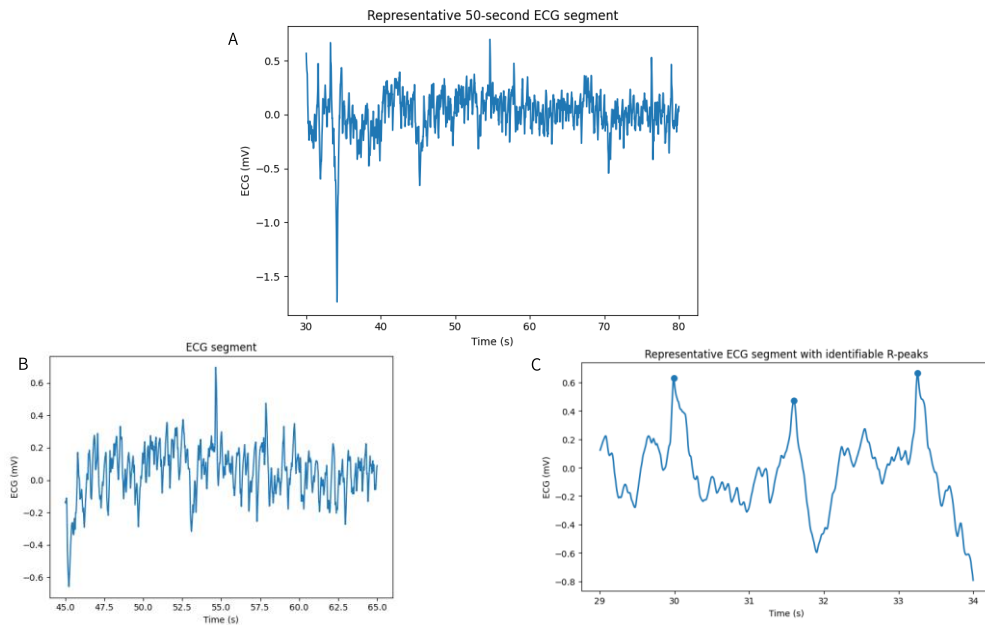


Figure 4. ECG recordings from a recording session in which R–R interval data were not directly available.

(A) A 50-s ECG segment showing continuous waveform acquisition.

(B) A 20-s segment from the same recording session, illustrating a relatively stable portion of the ECG signal.

(C) An enlarged 5-s ECG segment from the same session, with identifiable peaks indicated by dots.

All panels are derived from the same recording session and show ECG waveform data at multiple temporal scales, even when R–R interval data are not directly available.

3.4 Heart rate dynamics in relation to behavioral events

Heart rate (HR) time-series data with annotated behavioral events are shown in Fig. 5. Behavioral events were annotated across the full recording (Fig. 5A). During the earlier portion of the recording, continuous HR data were not consistently obtained, and the sensor was removed once (event ⑥) for readjustment. Following sensor reattachment and adjustment, the dolphin performed a respiration event involving exhalation and inhalation (event ⑦) and then resumed the dorsal floating posture (event ⑧). A later continuous segment (~295–363 s; Fig. 5B) was used for qualitative examination of the temporal alignment between HR data and event annotations. HR values in this segment ranged from approximately 25 to 90 bpm and showed short-term fluctuations. Both feeding events (events ⑨ and ⑩) occurred near local low values in the plotted

HR trace and were followed in the trace by short-term increases in HR. During the interval surrounding the respiration-related event ⑪ (~359 s), HR decreased from approximately 80 bpm to below 30 bpm. Event ⑪ was annotated as exhalation without inhalation. These observations describe temporal proximity between event annotations and HR values and were not subjected to quantitative event-response analysis in the present study.

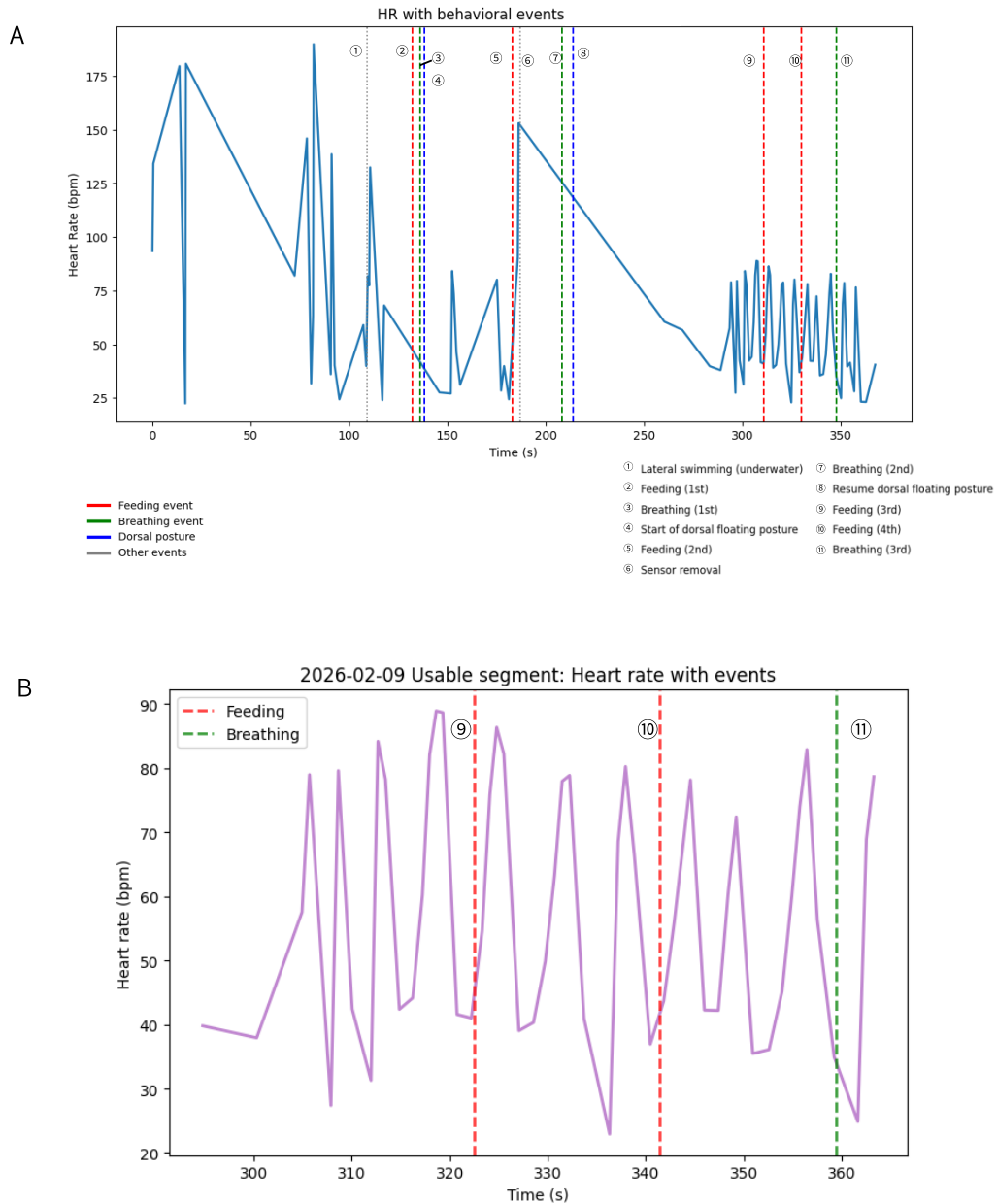


Figure 5. Heart rate (HR) time-series with annotated behavioural events.
 (A) Full recording showing HR variation across the entire session with event

annotations.

(B) Enlarged view of the later segment (295–363 s), highlighting temporal relationships between HR fluctuations and annotated events.

Red dashed lines indicate feeding events, and green dashed lines indicate respiration-related events. Respiration events include both exhalation-only behaviours and combined exhalation–inhalation behaviours.

In the enlarged segment, one respiration-related event was annotated as exhalation without inhalation.

HR was calculated from R–R intervals using filtered data (SC = 1; RR = 300–3000 ms). This figure provides supplementary contextual information for interpreting HR dynamics during the recording session.

4. Discussion

4.1 Feasibility and implementation in routine husbandry

This study demonstrates the feasibility of non-invasive HR and R–R interval recording in a trained bottlenose dolphin using a belt-mounted heart rate sensor during routine husbandry training. Usable HR and R–R interval data were obtained while the dolphin maintained a stationary dorsal floating posture at the water surface. These findings indicate that a wearable heart rate sensor can be adapted for physiological monitoring in managed dolphins under cooperative training conditions.

ECG recordings also retained methodological value in the session where direct R–R interval data were not available. In selected ECG segments, candidate peaks allowed partial retrospective estimation of candidate R–R intervals after exclusion of implausible values. Because agreement between these ECG-derived candidate R–R intervals and directly recorded R–R interval data was not assessed, the estimates should be regarded as exploratory supplementary information rather than validated R–R interval measurements. ECG waveform inspection may nevertheless provide supplementary information when direct R–R interval data are unavailable.

Non-invasive physiological monitoring has the potential to complement behavioral observations and support more comprehensive welfare assessment in managed marine mammals (Whitham & Wielebnowski, 2013). HR and HRV may serve as useful physiological indicators for assessing stress-related responses, arousal, and recovery (von Borell et al., 2007; Shaffer & Ginsberg, 2017). However, practical approaches for incorporating these indicators into routine husbandry-based welfare assessment remain limited. By recording HR and R–R intervals under routine husbandry conditions, the

present study provides a practical basis for future HRV-based welfare monitoring in managed dolphins.

A practical consideration in implementing this approach is that a novel wearable device may itself induce tension, avoidance responses, or increased arousal, thereby influencing the HR values being recorded. In the early phase, delayed responses to cues and behaviors suggesting discomfort while wearing the belt were observed. Therefore, trainers adjusted reinforcement timing and interruption decisions while monitoring the animal's state, and the veterinarian confirmed the safety and overall appropriateness of the procedure while desensitization to the belt progressed.

Across repeated sessions, the dolphin became habituated to the measurement procedure and was able to perform the sequence more smoothly, including presenting the pectoral fin as required during the measurement sequence, transitioning into the dorsal measurement posture, and maintaining that posture. These processes likely supported cooperative participation and stable signal acquisition. Thus, when introducing a new procedure to a trained animal, gradual habituation and desensitization, together with judgment by animal care staff and veterinary oversight, are important foundations for reliable and welfare-conscious physiological monitoring. This is consistent with husbandry training and cooperative care approaches in which animals voluntarily participate in management or veterinary procedures through reinforcement-based training (Brando, 2010; Ramirez, 1999).

4.2 Cardiac interval dynamics and contextual interpretation

The representative HR and R–R interval recordings showed temporal patterns that were not apparent from summary HR values alone. Prolonged R–R intervals exceeding 2000 ms and corresponding low calculated HR values were intermittently observed, forming a transient bradycardia-like pattern in the recorded data. Beat-to-beat variability in R–R intervals was also evident.

These two features should be distinguished. Prolonged R–R intervals and low calculated HR values describe transient slowing of cardiac rate, whereas beat-to-beat variability in R–R intervals describes short-term variation in cardiac intervals. The recording therefore contained both a transient bradycardia-like pattern and beat-to-beat variability, rather than a single stable HR level. The availability of continuous R–R interval data allowed descriptive HRV metrics, including RMSSD and SDNN, to be calculated for the representative segment and may support future examination of HRV measures in managed dolphins.

In dolphins, transient low HR values and beat-to-beat variability in R–R intervals may

occur in relation to respiration, breath-holding, posture, activity, and cardiorespiratory coupling (Noren et al., 2012; Fahlman et al., 2020; Blawas et al., 2021). Accordingly, HR and R–R interval patterns become more informative when considered together with behavioral, respiratory, postural, and training context.

The event-annotated HR recordings provided supplementary contextual information for interpreting these cardiac patterns. Because continuous HR data were not consistently available during the earlier portion of the recording and sensor readjustment was required, interpretation was limited to the later continuous segment (approximately 295–363 s). In this segment, event ⑪ was annotated as exhalation without inhalation and occurred during a decline in the HR trace from approximately 80 bpm to below 30 bpm. This temporal pattern is compatible with known respiration-related cardiac variation in bottlenose dolphins (Blawas et al., 2021; Fahlman et al., 2023), although this single observation does not establish an event-specific effect.

Both feeding events were followed by transient increases in HR. Short-term changes in HR and HRV across food-related cues, food anticipation, and reward-access phases have also been reported in domestic pigs and dogs (Mahnhardt et al., 2014; Zupan et al., 2016). These comparative findings illustrate that cardiovascular responses may differ across components of a feeding sequence, but do not establish the meaning of the present changes in dolphins.

However, because feeding events were time-stamped at fish delivery and the whistle bridge, used as a conditioned reinforcer, was not annotated, the recorded changes could not distinguish responses related to the whistle bridge, anticipation, fish ingestion, or other concurrent factors. Accordingly, event annotations were used as supplementary contextual information rather than as a primary event-response analysis. These observations illustrate the value of recording event context alongside HR and R–R interval data in future repeated recordings.

4.3 Implications for future HRV-based welfare monitoring

The proposed framework relies on the gradual accumulation of repeated HR and R–R interval recordings over time to establish meaningful individual baseline patterns. Such baseline-informed monitoring would provide a reference for describing the usual range and within- and between-day variation in HR and R–R interval measures, against which short-term changes during husbandry or training and longer-term deviations could subsequently be examined in relation to each animal’s usual physiological and behavioral context. This is particularly important because HRV is a sensitive, non-invasive indicator that is widely used to examine autonomic regulation in human and

animal research (von Borell et al., 2007; Shaffer & Ginsberg, 2017). In the present study, RMSSD and SDNN could be calculated for the representative segment. However, the physiological interpretation of these measures in dolphins under the present recording and analytical conditions remains to be established.

In the proposed framework, HR provides practical real-time information for animal care staff in the field, whereas HRV indices derived from R–R interval recordings may provide more detailed insight into autonomic regulation and increase the interpretive value of physiological monitoring for welfare assessment. Over longer time scales, repeated monitoring could help describe seasonal variation or recurrent individual patterns, which may later support interpretation of health- or welfare-related changes. To support such interpretation, HR and HRV data should be considered together with existing health and husbandry records, allowing them to complement behavioral and health-related indicators in welfare monitoring (Mellor, 2016; Whitham & Wielebnowski, 2013).

The stationary dorsal floating posture was selected to provide a controlled baseline-like condition for HR and R–R interval monitoring. Because HRV interpretation can be influenced by methodological conditions such as posture, activity level, and movement-related artifacts, minimizing active movement is useful when obtaining standardized reference recordings for characterizing within-individual patterns in cardiac interval measures (Laborde et al., 2017; Stucke et al., 2015; Kitajima et al., 2021). In the present study, this posture therefore served as a basic reference condition for future HRV-based welfare monitoring. However, because dolphin cardiac patterns are also influenced by respiration and inter-breath intervals, this condition should be understood as a standardized reference condition that includes species-specific respiratory physiology, rather than as a respiration-independent resting baseline (Noren et al., 2012; Fahlman et al., 2020; Blawas et al., 2021).

Together, these elements form the basis of a proposed Keeper-led HRV Welfare Monitoring Framework (K-HRV Framework), in which non-invasive cardiac interval recording would be integrated with individual baseline assessment, behavioral context, health records, and analytical feedback to support routine husbandry-based welfare monitoring. The keeper-led nature of this framework may support long-term implementation in daily husbandry by allowing physiological data to be collected during routine management by animal care staff. These data could then be analyzed in greater depth by collaborating research partners and returned to the field as feedback to support individual monitoring, welfare assessment, and evidence-based adjustment of animal care practices.

The present study should be interpreted as an initial methodological study demonstrating that HR and R–R interval data can be obtained during routine husbandry training in a trained dolphin. Future work should build on this approach through repeated within-individual monitoring and, as data accumulate, extend the framework to additional individuals, facilities, behavioral contexts, and species. Such expansion may help clarify how the K-HRV Framework can support both individual baseline assessment and the development of broader welfare-related physiological indicators in zoos and aquariums. Over the longer term, repeated recordings considered alongside relevant contextual, health, and husbandry information may support the gradual development of context-informed physiological indicators for welfare monitoring and, ultimately, the interpretation of recurring cardiac patterns in relation to stress-related responses, arousal, recovery, or relatively settled physiological conditions.

Conclusion

This study proposes a Keeper-led HRV Welfare Monitoring Framework (K-HRV Framework) and describes its initial recording component in a trained bottlenose dolphin during voluntary routine husbandry training. Non-invasive cardiac recording using a belt-mounted sensor provided usable HR and R–R interval data under a cooperative dorsal floating posture. These findings demonstrate the feasibility of obtaining HR and R–R interval data under routine husbandry conditions in a non-invasive and cooperative manner.

A central practical contribution of this study is the proposal of a keeper-led workflow in which animal care staff familiar with the individual animal can complete HR and R–R interval recordings within routine husbandry using a commercially available belt-mounted sensor. The resulting data could then be shared with research partners for analysis, with the findings returned to the field as feedback. Where available, video recordings or event annotations can be linked with the physiological data during analysis to provide contextual information for interpretation.

Gradual development of HR and HRV measures as context-informed physiological indicators for welfare assessment will require repeated within-individual recordings interpreted alongside relevant contextual, health, and husbandry information. By making such repeated recordings feasible within routine animal care, the proposed workflow provides a practical foundation for accumulating longitudinal HR and R–R interval data that may support the future development and validation of context-informed physiological indicators for welfare assessment.

Overall, the proposed K-HRV Framework provides a practical starting point for

developing HRV-based welfare monitoring under routine husbandry conditions. More broadly, the proposed approach may provide a field-applicable model that other zoos and aquariums can adapt to their own animals, staff, and husbandry routines, potentially contributing to the gradual development of sustainable physiological welfare monitoring across managed animal settings.

Ethical statement

All procedures were conducted under veterinary oversight, with authorization from Kyoto Aquarium, in accordance with Kyoto Aquarium's animal-care guidelines, and as part of routine husbandry and training practices. The heart rate sensor was attached non-invasively and did not involve restraint, invasive procedures, or experimental manipulation of the animal. Participation in the training sessions was voluntary, and the dolphin could terminate participation at any time by leaving the stationing position.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the corresponding author used ChatGPT (OpenAI) to assist with language editing, organization of draft text, and development of Python code for descriptive processing of R–R interval data. The processing criteria and descriptive calculations are described in the Methods section. All authors reviewed and approved the final manuscript.

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References

- Association of Zoos and Aquariums. (n.d.). *Welfare assessments*. Association of Zoos and Aquariums. <https://www.aza.org/welfare-assessments>
- Blawas, A. M., Nowacek, D. P., Allen, A. S., Rocho-Levine, J., & Fahlman, A. (2021). Respiratory sinus arrhythmia and submersion bradycardia in bottlenose dolphins (*Tursiops truncatus*). *Journal of Experimental Biology*, 224(1), jeb234096. <https://doi.org/10.1242/jeb.234096>
- 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>

- Broom, D. M. (2011). A history of animal welfare science. *Acta Biotheoretica*, 59, 121–137. <https://doi.org/10.1007/s10441-011-9123-3>
- Dawkins, M. S. (2004). Using behaviour to assess animal welfare. *Animal Welfare*, 13, S3–S7. <https://doi.org/10.1017/S0962728600014317>
- Fahlman, A., Miedler, S., Marti-Bonmati, L., Ferrero Fernandez, D., Muñoz Caballero, P., Arenarez, J., Rocho-Levine, J., Robeck, T., & Blawas, A. M. (2020). Cardiorespiratory coupling in cetaceans ; A physiological strategy to improve gas exchange? *Journal of Experimental Biology*, 223, jeb226365. <https://doi.org/10.1242/jeb.226365>
- Fahlman, A., Mcknight, J. C., Blawas, A. M., West, N., Torrente, A. G., & Aoki, K. (2023). Cardiorespiratory coupling in the bottlenose dolphin (*Tursiops truncatus*). *Frontiers in Physiology*, 14, 1234432. <https://doi.org/10.3389/fphys.2023.1234432>
- Gehrke, E. K., Baldwin, A., & Schiltz, P. M. (2011). Heart rate variability in horses engaged in equine-assisted activities. *Journal of Equine Veterinary Science*, 31, 78–84. <https://doi.org/10.1016/j.jevs.2010.12.007>
- Kitajima, K., Oishi, K., Miwa, M., Anzai, H., Setoguchi, A., Yasunaka, Y., Himeno, Y., Kumagai, H., & Hirooka, H. (2021). Effects of heat stress on heart rate variability in free-moving sheep and goats assessed with correction for physical activity. *Frontiers in Veterinary Science*, 8, 658763. <https://doi.org/10.3389/fvets.2021.658763>
- Laborde, S., Mosley, E., & Thayer, J. F. (2017). Heart rate variability and cardiac vagal tone in psychophysiological research: Recommendations for experiment planning, data analysis, and data reporting. *Frontiers in Psychology*, 8, 213. <https://doi.org/10.3389/fpsyg.2017.00213>
- Mahnhardt, S., Brietzke, J., Kanitz, E., Schön, P. C., Tuchscherer, A., Gimsa, U., & Manteuffel, G. (2014). Anticipation and frequency of feeding affect heart reactions in domestic pigs. *Journal of Animal Science*, 92(11), 4878–4887. <https://doi.org/10.2527/jas.2014-7752>
- Mellor, D. J. (2016). Updating animal welfare thinking: Moving beyond the “Five Freedoms” towards “A Life Worth Living”. *Animals*, 6(3), 21. <https://doi.org/10.3390/ani6030021>
- Noren, S. R., Kendall, T., Cuccurullo, V., & Williams, T. M. (2012). The dive response redefined: Underwater behavior influences cardiac variability in freely diving dolphins. *Journal of Experimental Biology*, 215, 2735–2741. <https://doi.org/10.1242/jeb.069583>
- Ramirez, K. (1999). *Animal training: Successful animal management through positive reinforcement*. Shedd Aquarium.
- Shaffer, F., & Ginsberg, J. P. (2017). An overview of heart rate variability metrics and

norms. *Frontiers in Public Health*, 5, 258. <https://doi.org/10.3389/fpubh.2017.00258>

Shimozato, Y. (2025). *Feasibility of heart rate variability analysis for welfare assessment in dolphins: A preliminary report*. EcoEvoRxiv. <https://doi.org/10.32942/X20H15>

Stucke, D., Große Ruse, M., & Lebelt, D. (2015). Measuring heart rate variability in horses to investigate the autonomic nervous system activity: Pros and cons of different methods. *Applied Animal Behaviour Science*, 166, 1–10. <https://doi.org/10.1016/j.applanim.2015.02.007>

United States Department of Agriculture. (n.d.). *Animal Welfare Act*. United States Department of Agriculture, National Agricultural Library. <https://www.nal.usda.gov/animal-health-and-welfare/animal-welfare-act>

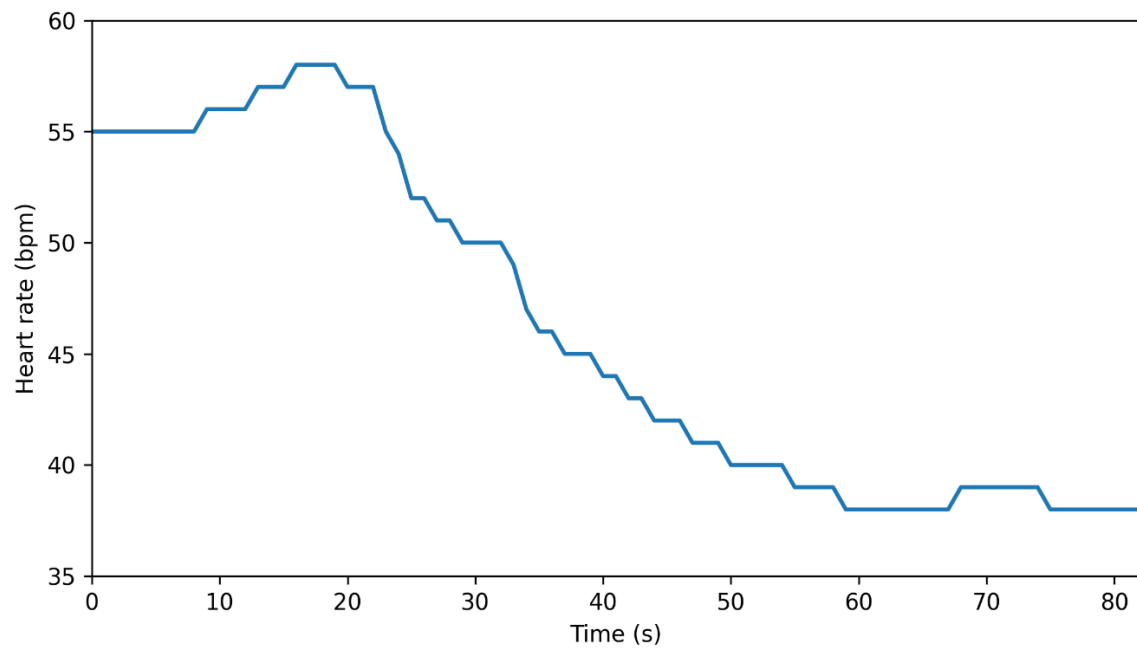
Visser, E. K., van Reenen, C. G., van der Werf, J. T. N., Schilder, M. B. H., Knaap, J. H., Barneveld, A., & Blokhuis, H. J. (2002). Heart rate and heart rate variability during a novel object test and a handling test in young horses. *Physiology & Behavior*, 76, 289–296. [https://doi.org/10.1016/S0031-9384\(02\)00698-4](https://doi.org/10.1016/S0031-9384(02)00698-4)

von Borell, E., Langbein, J., Després, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A., Valance, D., & Veissier, I. (2007). Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals: A review. *Physiology & Behavior*, 92(3), 293–316. <https://doi.org/10.1016/j.physbeh.2007.01.007>

Whitham, J. C., & Wielebnowski, N. (2013). New directions for zoo animal welfare science. *Applied Animal Behaviour Science*, 147(3–4), 247–260. <https://doi.org/10.1016/j.applanim.2013.02.004>

World Association of Zoos and Aquariums. (2025). *Advancing animal welfare: The World Zoo and Aquarium animal welfare strategy*. World Association of Zoos and Aquariums. https://www.waza.org/wp-content/uploads/2025/10/WAZA-AWS_WEB-300DPI.pdf

Zupan, M., Buskas, J., Altimiras, J., & Keeling, L. J. (2016). Assessing positive emotional states in dogs using heart rate and heart rate variability. *Physiology & Behavior*, 155, 102–111. <https://doi.org/10.1016/j.physbeh.2015.11.027>



Supplementary Figure S1.

Initial heart rate recording during pilot measurements. A representative stable segment of heart rate (HR) recorded during the pilot stage using the Polar Flow application is shown. Continuous HR values were obtained while the dolphin maintained a stationary dorsal floating position during routine training. The selected segment shows that continuous HR recording was possible under these routine training conditions, although R–R interval data were not available at this stage. Heart rate showed a gradual decrease across the segment, but the biological interpretation of this pattern remains uncertain.