Physiological responses of narwhals to anthropogenic noise: A case study with seismic airguns and vessel traffic in the Arctic

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Abstract

1. Limited polar geographical range, narrowly defined migratory routes, and deep-diving behaviours make narwhals exceptionally vulnerable to anthropogenic disturbances including oceanic noise. Although behavioural studies indicate marked responses of cetaceans to disturbance, the link between fear reactions and possible injury from noise exposure is limited for most species.

2. To address this, we deployed custom-made heart rate-accelerometer-depth recorders on 13 adult narwhals in Scoresby Sound, East Greenland across a five-year period (2014–2018). Physiological responses of the cetaceans were monitored in the absence (n = 13 animals) or presence (n = 2 animals across 3 acoustic events) of experimentally directed, seismic airgun pulses and associated vessels (full volume source level = 241 dB re 1 μPa·m).

3. We found that anthropogenic noise resulted in marked cardiovascular, respiratory and locomotor reactions by two narwhals exposed to seismic pulses across three acoustic events. The general behavioural response to seismic and vessel noise included an 80% reduction in the duration of gliding during dive descents by seismic-exposed narwhals compared to controls, and the prolongation of high intensity activity (ODBA > 0.20 g) with elevated stroke frequencies exceeding 40 strokes per minute. Noise exposure also resulted in intense (< 10 bpm) bradycardia that was decoupled from stroking frequency. This decoupling instigated increased variability in heart rate, with the heart.switching rapidly between bradycardia and exercise tachycardia during noise exposure. The maximum respiratory frequency following seismic exposure, 12 breaths/min, was 1.5 times control levels.

4. Overall, the effect of seismic/ship noise exposure on Arctic narwhals was a 2.0–2.2-fold increase in the energetic cost of diving, which paradoxically occurred during suppression of the cardiac exercise response. This unusual relationship between diving heart rate and exercise intensity represents a new metric for characterizing the level of fear reactions of wild marine mammals exposed to different environmental stressors. Together, the multi-level reactions...
to anthropogenic noise by this deep-diving cetacean demonstrated how a cascade of effects along the entire oxygen pathway could challenge physiological homeostasis especially if disturbance is prolonged.

**KEYWORDS**  
Arctic, diving, fear, Greenland, heart rate, narwhal, oceanic noise, seismic, stroke frequency

1 | INTRODUCTION

As one of three cetacean species residing year-round in high Arctic waters, the narwhal *Monodon monoceros* has lived in relative isolation from human activities. This is largely due to expansive sea ice cover within its home range (Heide-Jørgensen et al., 2002, 2003; Heide-Jørgensen & Dietz, 1995) making the area inaccessible to wide-spread vehicular traffic. Historically, this has meant a unique level of protection from anthropogenic disturbance including noise that has become pervasive in many other parts of the world’s oceans (Duarte et al., 2021). With the recent, rapid degradation of Arctic sea ice in both volume (Overland et al., 2014) and coverage (Stroeve & Notz, 2018), such protection is now in jeopardy as new northern shipping routes and increased development of Arctic mineral resources are proposed (Heide-Jørgensen et al., 2013, 2021).

Naïveté borne from isolation coupled with a limited geographical range, narrowly defined migratory routes and site fidelity make narwhals exceptionally vulnerable to this future disturbance (Heide-Jørgensen et al., 2015; Laidre et al., 2008). Deep-diving behaviours by narwhals compound the concern. Over the past two decades, anthropogenic noise has been linked to numerous mass stranding events involving cetaceans that routinely exceed 700 m when foraging, most notably beaked whales (Bernaldo de Quirós et al., 2019; Cox et al., 2006; DoN, 2013). Consequently, deep diving odontocete whales like narwhals are considered unusually susceptible to deleterious behavioural and physical impacts when exposed to unanticipated noise (Kochanowicz et al., 2021; Miller et al., 2022).

Despite these concerns, the link between diving behaviour and possible injury from noise exposure is limited for most marine mammals. To date, the connection has been based primarily on the potential for unexpected noise to elicit unusual swimming behaviours and physical exertion (DeRuiter et al., 2013; Goldbogen et al., 2013; Hildebrand, 2005; Richardson et al., 1995; Southall et al., 2016). Controlled exposure experiments involving free-ranging marine mammals have focused on these behavioural responses, demonstrating changes in three-dimensional spatial movements (geographic location and depth), diving profiles, inter-dive intervals as well as changes in vocalizations (Miller et al., 2000). Acoustic, depth, and accelerometer tags deployed on noise-exposed cetaceans have also recorded changes in echolocation and buzzing (Tervo, Blackwell, et al., 2021; Tervo, Ditlivesen, et al., 2021), foraging patterns (Stimpert et al., 2014), inter-dive blow patterns, and stroking movements (Antunes et al., 2014; Harris et al., 2015; Miller et al., 2012, 2015; Southall et al., 2012; Tyack et al., 2011) with consequent disruption in foraging patterns (Silve et al., 2016) and cetacean social structure (Miller et al., 2012; Visser et al., 2016).

It has long been recognized that the integration of behavioural and physiological responses is critical for developing predictive models for anticipating acute and chronic impacts of noise on marine mammals (NRC, 2005). However, the physiological repercussions of the observed behaviours, and how they might instigate eventual injury to individuals or populations are currently uncertain. Leading hypotheses for stranding deaths linked to military sonar suggest that extreme behavioural reactions may instigate physiological instability (Williams et al., 2015; Williams, Blackwell, et al., 2017), which in turn could promote inert vascular bubble formation and decompression syndromes (i.e. Aguilar de Soto et al., 2016; DoN, 2013; Hooker et al., 2012). Although changes along the oxygen pathway and associated energetic costs that define biologically relevant impacts are likely, they remain largely unknown.

To address this, we examined the physiological responses of adult narwhals experimentally exposed to seismic airgun pulses and associated vessels in Scoresby Sound, East Greenland (Heide-Jørgensen et al., 2021). Three major components of the oxygen pathway, cardiovascular, respiratory, and locomotor responses, were monitored with animal-borne tags deployed on free-ranging animals across a five-year period (2014–2018) in the absence (n = 13) or presence (n = 2) of seismic ship activities. In addition to developing new metrics for defining fear responses in a deep-diving odontocete, we found noise-induced changes within each compartment of the oxygen pathway that differed from typical responses associated with intense exercise performance alone. Together, the multi-level reactions to anthropogenic noise by this deep-diving cetacean result in a cascade of behaviorally linked effects, which could challenge physiological homeostasis especially as environmental disturbance is prolonged.

2 | MATERIALS AND METHODS

2.1 | Study area and experimental design of seismic exposure

Narwhals in this study were instrumented and monitored during August-September 2014–2018 in the Scoresby Sound fjord system of East Greenland. These animals represent a subset of a larger...
population monitoring program for narwhals in this area, with details of the location and experimental design for control and seismic tests presented in Heide-Jørgensen et al. (2021). Briefly, from 2014–2016, narwhals were instrumented with heart rate recorders and GPS tags for physiological and population monitoring; no industrial operations were present at this time. In 2017 and 2018, a seismic program was conducted with a ship towing one or two airguns through the area. Physiological data for noise-exposed narwhals were collected in 2018. During this field season, an offshore patrol vessel (HDMS Lauge Koch) towed a cluster of two Sercel G- seismic guns (1,040 in total) with mean pressure = 125 bar (range 115–135 bar) at 6 m depth and a speed of 4.5 knots. At full volume, the source level of the cluster was 241 dB re 1 μPa-m (peak-to-peak, as simulated by the gun manufacturer). The seismic guns were fired synchronously every 80 s as the ship moved in and out of the fjord system; a FastLoc GPS navigation system recorded the location of the ship and each shot. This was used to pair the location of the sound source with the responses of the instrumented narwhals. For the present study, control periods included data from animals tagged in years 2014–2015 in which no seismic operations occurred as well as in seismic years 2017–2018 before the seismic ship arrived in the fjord system. None of the narwhals represented in the control dataset had been exposed to experimental seismic operations associated with the present study. To evaluate maximum responses, data for seismic events are taken from periods when the ship was in close proximity (passing within 1–11 km) of the instrumented narwhals, representing the response zone of the animals. All selected dives occurred >3 hr after the initial capture and tagging period (defined in Williams, Blackwell, et al., 2017) to avoid superimposing reactions to entanglement and noise events. For further information on acoustic measurements and sound source verifications, sound analyses, ship movements, and determination of response zones, see Heide-Jørgensen et al. (2021).

### 2.2 Animals

Physiological and behavioural parameters of 13 wild adult narwhals (5 females, 8 males, Table 1) from the East Greenland population were measured in this study. As detailed in Table 1 and in the Analyses (Section 2.4) below, not all parameters were measured on all animals due to both tag performance and the necessity for using

| TABLE 1 | Morphometrics and Tag Metrics for East Greenlandic narwhals in this study. Body mass was estimated from measurements of total straight-line length based on Garde et al. (2022). Columns without units show the number of dives involved in the analyses for each narwhal via animal-borne instrumentation. Dive number = control in normal type, and seismic events in bold, 2nd seismic event in bold italics. Numbers in parentheses indicate the number of dives from Williams, Blackwell, et al. (2017) used for control comparisons as well as for the regression denoting escape fear responses on stroke frequency and heart rate (Figure 5b). Note that A1 and B1 are the same animal captured in separate years, and are treated as different individuals in the present study. Control values during the seismic year were taken before ship presence. |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| **Individual** | **Year** | **Sex** | **Depth/ GPS (hours)** | **Stroke Freq./ Glide** | **Resp. rate** | **Heart rate** | **Energetic costs** | **Body length (cm)** | **Tusk length (cm)** | **Body mass (kg)** |
| Control | | | | | | | | | | |
| F2 (Mára) | 2014 | F | 8 | 7 (6) | 6 (6) | Control 1 | 390 | N/A | 557 |
| F906 | 2015 | F | 18 | (4) | (4) | Control 1 | 400 | N/A | 898 |
| F907 | 2015 | F | 17 | (5) | (5) | Control 4 | 410 | N/A | 974 |
| F909 | 2015 | F | 58 | (4) | (4) | Control 2 | 400 | N/A | 898 |
| M922 | 2015 | M | 14 | (6) | (6) | Control 2 | 338 | 40 | 557 |
| A1 (Helge) | 2017 | M | 48 | 5 | 8 | 5 | Control 2 | 492 | 207 | 1587 |
| A3 (Bjarne) | 2017 | M | 48 | 5 | 6 | Control 4 | 454 | 195 | 1226 |
| A4 (Hildur) | 2017 | F | 6 | 7 | 8 | Control 4 | 393 | N/A | 849 |
| A5 (Oden) | 2017 | M | 48 | 8 | 7 | 8 | Control 4 | 477 | 198 | 1433 |
| Seismic | | | | | | | | | | |
| B1 (Helge) | 2018 | M | 63.1 | 5, 5 | 7, 6 | 5, 5 | Control 2 | 492 | 207 | 1587 |
| B4 (Nemo) | 2018 | M | 1.2 | 5 | 6 | 5 | Control 2 | 410 | 83 | 909 |
| B6 (Frederik) | 2018 | M | 25.9 | 5 | 6 | 5 | Control 2 | 409 | 73 (broken) | 902 |
| B8 (August) | 2018 | M | 58.3 | 6, 5, 5 | 6, 8, 6 | 6, 5, 5 | IB1 5, 5 | 380 | 97 | 741 |
matched depths for comparing control and experimental treatment groups. The animals had been captured by local hunters using tangle nets during annual indigenous hunts. Following disentanglement, we collected morphological measurements, and then instrumented and released the animals for an ongoing physiological and population monitoring program (Blackwell et al., 2018; Heide-Jørgensen et al., 2015; Williams, Blackwell, et al., 2017). All animals in the study were deemed in excellent condition based on length-girth measurements, behaviour, and general external appearance. Basic vital signs (respiratory rate and heart rates) of the narwhals, and the duration of handling (delimited to include the point of net entanglement, movement to shallow water, disentanglement, and instrumentation periods) were monitored throughout the procedures. Care was taken to keep total handling time to a minimum with instrumentation time for the heart rate recorders ≤20 min during the seismic test years.

Each whale was instrumented with the ECG-ACC tag (described below) for long-term assessment of diving, escape reactions, and recovery responses (Table 1). The water was deep enough to allow for whole body submersion during instrumentation with the animals typically remaining submerged until lifting their heads and surfacing to breathe. Narwhals were released immediately after instrument placement, and their behaviour monitored by observers on shore until the animals submerged on a dive. Released narwhals ranged freely with the instrument package until it fell off within 1–3 days.

Scientific procedures involving the narwhals were approved by institutional IACUCs at the University of California (Santa Cruz) and the University of Copenhagen (Faculty of Health Sciences). Permission for capturing, handling, and tagging of narwhals was provided by the Government of Greenland (Case id: 2010–035453, doc. nr. 429 926).

2.3 | Instrumentation

2.3.1 | Assessment of cardiac responses

Cardiac responses of the narwhals were recorded with a submersible electrocardiograph (ECG) unit. Construction and calibration of the instrument for use on narwhals have been described previously (Williams, Blackwell, et al., 2017). A custom-designed heart rate unit (10 cm or 16 cm long × 3 cm diameter tube; ECG-ACC depth recorder UUB/4-EIAPECG/IBI/acceleration/p Pressure recording system, UFI) was used to simultaneously record ECG waveforms, swimming stroke frequency and body movements from a three-axis accelerometer, and hydrostatic pressure that was converted to water depth (Davis & Williams, 2012; Noren et al., 2012). The latter was used to determine each animal’s position in the water column as well as descent, bottom and ascent portions of each dive. We recorded entire electrocardiographic waveforms at 50–100 Hz to avoid misinterpretation of cardiac events due to potential interference of skeletal muscle noise. The entire instrument package included VHF (VHF 154-155 MHz, atstrack.com) and satellite tags (SPOT5, wildlifecomputers.com) attached to a positively buoyant floatation platform (mikkelvillum.com and Pisces Design). Size of the package represented <5% of the frontal area of the animals to prevent excess hydrodynamic drag, and was slightly positively buoyant to allow retrieval when the tag fell off. The entire package was secured to the animal using two 8 cm diameter suction cups. Once dislodged from the animal, the floating instrument package was located from the VHF and satellite signals, and retrieved by boat.

Two types of surface skin electrodes (2.5 cm silver/silver-chloride disks or Polar Equine-Polar Electro, Inc) encased in 8 cm diameter suction cups were used. We tested a variety of dorsal and lateral surface placements to determine the ideal location for standard ECG signals (Williams, Blackwell, et al., 2017). The best signals were obtained with a midline dorsal placement of both electrodes, the first at the level of the leading edge of the pectoral fins and the second approximately 50 cm diagonally behind (cross-heart placement). Signals from the electrodes were transferred by shielded wire to a waterproof bulkhead connector on the recording unit, with any excess wire length secured to the recorder with waterproof tape to prevent drag and signal noise.

Each dive of the instrumented narwhals was identified from changes in depth, with heart rate and corresponding stroking and post-dive respiratory frequencies visually determined at 20 s intervals for the entire series of dives using UFI ECG-ACC software (UFI) as described below.

2.3.2 | Respiratory responses

Respiration rates during inter-dive periods of the narwhals were monitored with the ACC-ECG instruments during control periods (n = 6 animals) and seismic events (n = 3 events with 2 narwhals). Due to the diagonal cross-heart positioning of the electrodes, respiratory movements of the animals were superimposed on the ECG trace as they surfaced for each breath (Figure S1). Breathing and apneic periods were calibrated and confirmed by synchronous recordings from the on-board depth meter indicating surface intervals, and breath-by-breath analyses from video recordings (GoPro Hero) taken during instrumentation. The number of breaths during each minute of the inter-dive interval was counted manually to determine the recovery decay in respiratory frequency. Mean, minimum and maximum respiratory frequencies of the animals were determined for each recovery minute.

2.3.3 | Locomotor responses

As in previous cetacean studies (Tervo, Ditlevsen, et al., 2021; Williams, Blackwell, et al., 2017; Williams, Kendall, et al., 2017), stroke frequency was determined from changes in the amplitude of the three axes of the accelerometer and recorded as stroke cycles (including the upstroke and downstroke as one cycle). Stroke frequency for each 20 s interval was analysed with respect to the corresponding depth for descent, bottom and ascent periods. A stroke frequency of 0–1 across 20 s was classified as gliding.
2.4 Analyses

2.4.1 Dive classification

To evaluate the effect of seismic pulses on physiological variables, we first determined the range of dive depths and durations that typically occurred during seismic pulse exposures within the response zone of the ship (based on GPS tracks). Using the mean values, we then selected a matched series of control dives in which no ship was present. Control activities consisted of surface swimming periods (maximum dive depth approx. 15–50 m) and non-foraging dives (<300 m with periods of gliding on descent) with foraging dives identified primarily by deeper depths typical of foraging narwhals in this area (Tervo, Blackwell, et al., 2021; Tervo, Ditløv, et al., 2021) and secondarily supported by the absence of 'buzz' (see Blackwell et al., 2018) interference that was periodically detected on the accelerometer recordings (Figure S2). Dives during noise exposure were further classified according to the animals’ avoidance reaction to novel stimuli (here termed startle or stun; see King et al., 2003; Vila et al., 2007; Forkman et al., 2007 for general reviews) using depth, the range of stroking frequencies and the presence/absence of gliding as identifiers. Three dive types specific to noise-exposure were identified, (a) startle dives (high activity dives with maximum depth ≥ 100 m, absence of gliding during rapid descent, prolonged periods with maximum stroke frequency at >34 strokes/min), (b) stun response dives (shorter dives with maximum depth <100 m, absence of gliding during rapid descent, prolonged moderate stroke frequencies with short bursts of maximum stroke frequency at >34 strokes/min) and (3) sequential surface transit dives (<50 m, constant slow to moderate stroking) with directed movements occurring after the other two dive types (Figure 1a,b).

2.4.2 ECG-ACC tag output analyses

Details of the calibration and analysis for the ECG-ACC have been presented previously (Williams, Blackwell, et al., 2017) with special attention paid to the quality of the ECG waveform for representation of each heart beat and for identification of potential artefacts that
may arise from muscle noise during activity. Electrocardiographic wave patterns, three-axes of acceleration, and pressure (depth) from the instruments were digitized and displayed simultaneously at different levels of temporal resolution using UFI (Morro Bay, CA) software. To avoid potential artefacts related to skeletal muscle noise, we visually inspected and manually counted the number of heart beats (R-R intervals) and corresponding fluke strokes at 20 s intervals. In addition, the raw data were downloaded for determination of inter-beat interval from the duration between successive QRS peaks of ECG waveforms.

Cardiovascular analyses included assessments of minimum heart rate (maximum bradycardia), duration of bradycardia, the relationship between instantaneous heart rate and stroke frequency, as well as the determination of heart rate variability from sequential inter-beat interval patterns (see below). Locomotor analyses included the determination of stroke frequency, and the identification of gliding periods throughout the dive. Each parameter was analysed with respect to the activity level, dive duration, dive depth, and presence or absence of a seismic ship. In addition, the respiratory rate was determined per minute of the inter-dive interval.

The total number of swimming strokes taken and heart beats occurring within each 20 s interval was determined on a dive-by-dive basis and analysed with respect to the associated depth. To control for the effects of depth on bradycardia (Davis & Williams, 2012; Noren et al., 2012), we compared control dives to reaction dive types of modest depth ranging from 80 to 200 m. Because of this criterion, startle and stun reaction dives were grouped to allow for a broader general comparison with shallow swimming (control) and transit (noise-exposed) activities. The 20 s interval rates were binned according to the intensity level in beats per minute (bpm) or strokes per minute (spm). The proportion of minutes of the dive comprised of each binned rate was calculated by dividing the bin sum by total dive time. The resulting binned proportional data were then averaged for all dives by individual narwhals collected under control or seismic conditions. The distributions of the raw data comprising the bins were compared for control and seismic periods across the range of stroke frequencies, heart rates and inter-beat intervals for each test condition using Kolmogorov–Smirnov Asymptotic tests (JMP 16.2, jmp.com; See Supporting Information). Averaged data are presented as mean ± 1 SEM unless otherwise noted.

To account for body movements other than stroking (i.e. gliding, rolling), we also calculated the overall dynamic body acceleration (ODBA) of the narwhals. Dynamic acceleration metrics for each axis, partial dynamic body acceleration, and ODBA were initially calculated and compared (see John, 2020 for details of the analyses). In the present study, ODBA based on all three axes is used for evaluating simultaneous changes in both stroking mechanics and rolling manoeuvres, as well as to determine the duration of gliding periods throughout the dive. As for stroking mechanics and heart rates, ODBA was calculated from the unfiltered accelerometer output of the ECG-ACC at 20 s intervals for each dive. The values were converted to ODBA using a 2 s running average of the raw acceleration according to Wilson et al. (2006).

2.4.3 | Variability in heart rate response and bradycardia

Cardiac anomalies, most notably cardiac arrythmias were scored on each digital trace based on sequential changes in inter-beat interval (IBI) for all ECG recordings using UFI software (Morro Bay, CA) and investigated with respect to stroke frequency, dive depth, and ascent or descent trajectory. For this study, a detailed analysis of IBI was conducted for one narwhal (B8) as a demonstration of potential cardiac variability reactions. This required multiple matched-depth dives for both control (n = 5 dives, mean depth = 110.2 ± 17.5 m) and seismic pulse exposure (n = 5 five dives, mean depth = 71.8 ± 12.6 m) periods. Because arrythmias of narwhals typically occurred during initial ascent from the bottom of the dive (Williams, Blackwell, et al., 2017), we compared IBIs of this animal for the period beginning with ascent to the point of anticipatory tachycardia (the increase in heart rate prior to surfacing that occurs at approximately 30–40 m) following protocols from Davis and Williams (2012). The timing of each QRS peak was identified and the duration of each inter-beat period in seconds was calculated using graphing and statistical software (SigmaPlot, Systat Software Inc.). The sequential change in IBI (Δ IBI) was calculated, and the distribution of values used to determine variability in IBI. Individual ectopic beats were identified as IBI values outside of the normal range of average HR responses for the animal during control diving and characterized by ‘spike’ interruptions in IBI sequences between beats (Williams, Blackwell, et al., 2017). This definition enabled us to identify individual cardiac anomalies as opposed to simple adjustments in heart rate that can occur over sequential beats.

2.4.4 | Interrelationship between heart rate and stroke frequency

We developed a new metric for assessing fear responses in narwhals exposed to seismic pulses. Previous research on narwhals from this population demonstrated the potential for a fear response associated with escape reactions during net entanglement (Williams, Blackwell, et al., 2017) based on alterations in heart rate responses during exercise. Here, we used changes in the slope of the relationship between heart rate and stroke frequency for diving narwhals exposed to seismic pulses (n = 2 whales) and for those diving in quiet, control areas where no ships were within line of sight (n = 5 whales) to determine the level of fear. Care was taken to avoid the confounding effects of dive depth on bradycardia (Davis & Williams, 2012; Noren et al., 2012; Williams et al., 2015; Williams, Blackwell, et al., 2017; Williams, Kendall, et al., 2017) by limiting the analyses to moderate dive depths (<214 m).

To establish the datasets for control and noise-exposed groups, we first used a mixed-effects model to account for the potential effects of individual animal and multiple dives by individuals while testing for differences in heart rate as a function
of stroke frequency (JMP 16.2. jmp.com). The model (Table S1) indicated that individual narwhal (2.53 bpsm) and dive number (3.31 bpsm) together contributed ~1/6th of total variance explained by fixed effects (36.69 bpsm). The effect of noise exposure on the interaction between heart rate (in bpm) and stroke frequency (in spm) was determined by testing for similarity in the slope of models generated for depth-matched dives grouped into control (in the absence of a seismic ship) and experimental (in the presence of active seismic airgun pulses with the ship within 1 km of the tagged narwhals) categories. Positions of the animals relative to the seismic ship were based on time-stamped GPS coordinates of each. Note that anticipatory tachycardia associated with depths <40 m was omitted in these analyses as described in Davis and Williams (2012) and Williams, Blackwell, et al. (2017) and above.

2.4.5 | Calculation of energetic costs

Using previously reported relationships between oxygen consumption and stroke frequency (Williams, Kendall, et al., 2017) and ODBA (John, 2020) for cetaceans, we calculated the total energetic cost for each dive by the narwhals during control and noise exposure conditions. A closely related odontocete species, the beluga whale (Delphinapterus leucas), served as a bioenergetics model for narwhals. Care was taken to use similar dorsal placement of accelerometer tags and to account for differences in body mass of individuals. Data for oxygen consumption of beluga whales measured by our lab served as a common metabolic baseline for consistency between energetic costs calculated from the stroke count and ODBA methods.

We used the stroking method from Williams, Kendall, et al. (2017) for determining the energetics of all control dives. These included narwhals from years in which no seismic activities occurred (n = 5 animals), and narwhals in 2018 prior to noise-exposure and the presence of the seismic ship (n = 2 animals). To account for potential increases in body manoeuvres beyond stroking by noise-exposed whales we included the ODBA method from John (2020) for transit, startle and stun dives.

Briefly, the stroke count method (Williams et al., 2004; Williams, Kendall, et al., 2017) involved calculating locomotor costs for individual strokes during preferred swimming gaits (LCPref) and maximum aerobic performance (LCAerobic.Max) based on allometric regressions (Equations 6 and 7 in Williams, Kendall, et al., 2017) using the body mass of each narwhal (Table 1). Distinction between preferred and maximum performance was determined for individual narwhals and based on distribution patterns for each gait level; breakpoints between levels ranged from 34–40 spm depending on the animal. Total energetic cost of a dive, CDive, was then calculated from

\[ C_{\text{dive}} = 20.1 \left( 9.98 \text{ Mass}^{-0.25} \right) D + \left( LCP_{\text{preferred}} \times S_{\text{preferred}} \right) + \left( LCA_{\text{aerobic max}} \times S_{\text{max}} \right). \] (1)

where diving cost is in J/kg, D is duration of the dive in min, and stroking costs are calculated from locomotor costs × S, the number of strokes taken during routine (SPref) and maximum aerobic (SMax) swimming gaits (see Williams et al., 2004; Williams, Kendall, et al., 2017 for details). To account for dynamic movements in addition to stroking (i.e., rolling and spiralling) that occurred during seismic-exposure we also used the ODBA method to determine the cost of transit swimming, startle and stun dives as described above in Section 2.4.2 (ECG-ACC tag output analyses). ODBA from unfiltered recordings was calculated at 20s intervals and converted to oxygen consumption using the predictive equations of John (2020) based on metabolic measurements conducted by our lab on trained beluga whales wearing accelerometers. Note that the placement of the accelerometer was along the dorsal ridge for both the beluga whales and narwhals. These data representing instantaneous energetics were then summed for each dive. A conversion factor of 20.1 J/ml of O2 consumed was used for both methods.

3 | RESULTS

Thirteen heart rate-accelerometer recorders were deployed on narwhals with the resumption of normal movements by the instrumented animals within approximately three hours of tagging (Williams, Blackwell, et al., 2017). Monitoring duration varied for individual animals, with deployment duration for six recorders averaging 2.24±0.11 days. Of the remaining recorders, one fell off within 2 hr, two dropped at approximately 8–10 hr following animal release, and four instruments remained on the narwhals for approximately 24 hr. Data for narwhals were analysed using mixed effects models as described below and in the Supporting Information with details for individuals provided in Table 1. The independent movement of the seismic ship and wild animals resulted in three seismic exposure periods for two animals that form the basis of the noise response dataset. This was compared to data selected from a control group of 11 undisturbed narwhals.

3.1 | General behavioural and physiological responses

Adult narwhals in this study displayed a suite of behavioural and physiological reactions along the respiratory-cardiovascular-cellular/locomotor pathway for oxygen when exposed to seismic pulses from a passing ship. Received airgun pulse sound exposure levels (SEL), as received at 10 m depth during sound source verifications, were approximately 152dB re 1 µPa2·s at 1 km range and decreased to approximately 120dB re 1 µPa2·s at 10 km (see Heide-Jørgensen et al., 2021 and Tervo, Blackwell, et al., 2021 for details). Representative behaviours and daily diving patterns during control and noise exposed periods are illustrated in Figure 1, with movement data for additional animals presented previously (Heide-Jørgensen et al., 2021). In general, the narwhals showed a short (approximately
3 hr) escape response immediately after tagging and release as described in Williams, Blackwell, et al. (2017). This was followed by normal diving behaviours consisting of alternating shallow (<200 m) exploratory dives and transit swimming, as well as presumed foraging dives at 10–17 hr after release that often exceeded 300 m in depth. During exposure to seismic pulses, the animals displayed more continuous stroking activity during each dive (denoted by changes in ODBA and stroke frequency levels) compared to control dives. Dive types included repetitive, shallow stun type response dives (depth <100 m), deeper startle dives (depth ≥100 m) as well as prolonged transit swimming (Figure 1b). The sequence of startle and stun dives varied for individuals but was consistently followed by surface transit dives at 15–50 m with constant, low to moderate level of stroking that often lasted several hours. Diving profiles also showed differences for control and noise exposed periods. For example, the movement from surface to depth (descent) was often more gradual for control dives than for noise exposed dives which showed shorter, more rapid ‘directed’ descents (Figure 1c). For matched dives to approximately 135 m at maximum depth, the descent comprised $62.3\pm3.8\%$ ($n = 10$ dives) and $48.3\pm6.6\%$ ($n = 5$ dives) of the total dive time for control and noise exposed dives, respectively.

General physiological responses also differed for control and seismic exposed periods (Figure 1b,c). During control non-foraging dives, stroke frequency followed the typical marine mammal pattern (Tervo, Ditlevsen, et al., 2021; Williams et al., 2000) with gliding descents (Figure 2), high frequency stroke-and-glide powered ascents, and low stroke frequency inter-dive periods. Changes in heart rate (Figure 3) followed these changes in exercise level with variable bradycardia at depth and a tachycardia of approx. 80 bpm during surface recovery of control dives. The general response to seismic and vessel noise was a marked reduction in gliding descents, and prolonged
periods of high intensity activity (ODBA > 0.20 g) associated with periods of elevated stroke frequencies (>40 spm). Noise exposure also resulted in periods of prolonged and intense (<10 bpm) bradycardia that were decoupled from stroking intensity. An increase in post-dive respiratory rates occurred during recovery from noise-exposed dives compared to control dives (Figure 4).

Details of these responses are presented in the sections below. To account for the effects of depth on cardiovascular responses (Davis & Williams, 2012; Williams, Blackwell, et al., 2017), the physiological data presented below are divided into depth-matched dives for control periods in the absence of seismic or vessel traffic (n = 30 dives by 5 narwhals, mean depth max = 118.3 ± 8.4 m) and seismic exposed periods (n = 15 matched dives by 2 narwhals during 3 sound exposure events, mean depth max = 80.0 ± 11.1 m), unless noted differently.

3.2 | Locomotory responses

The distribution and range of swimming stroke frequencies used by narwhals differed between control and seismic exposed dives (Figure 2a,b; see also Figure S5), with the major differences occurring at both ends of the performance range. On the lower end, the proportion of time spent gliding, particularly during diving descents, was markedly reduced during exposure to noise. In the absence of disturbance, diving narwhals averaged over 2.5 min of gliding on descent; this was reduced to approximately 0.5 min for narwhals on initial exposure to noise (Figure 2b). On the high end of the performance range there was an increased reliance on high frequency stroking during noise exposure, which resulted in a 2.4-fold increase in the percentage use of stroke frequencies from 42 to 55 spm, rates rarely observed during control dives. Median values for stroke frequency were 18 spm (n = 800 samples from 5 narwhals during 30 dives) and 25.5 spm (n = 342 samples from 2 narwhals during 15 dives) for control and 3 seismic-exposure events, respectively. Data for the two treatment groups were significantly different (Kolmogorov–Smirnov Asymptotic test D = 0.1531, p < 0.0001).

3.3 | Cardiovascular responses

Compared to locomotory responses, the range of heart rates of diving narwhals exposed to noise showed little change from control values. Both groups displayed heart rates ranging from 6–60 beats/min (bpm) when submerged (Figure 3a; Figure S6). Mean values were 21.6 bpm (n = 697 samples from 5 narwhals during 30 dives) and 20.3 bpm (n = 320 samples from 2 narwhals during 15 dives) for control and noise-exposed periods, respectively, and were not significantly different (Kolmogorov–Smirnov asymptotic test D = 0.0625, p = 0.3588), although the range of values was less for the seismic-exposed animals.

Detailed comparison of minimum heart rates for individual narwhals exposed to seismic pulses (Figure 3c) showed a 15.4% and 30.3% decrease in mean minimum heart rate with disturbance compared to control values for narwhals B8 and B1, respectively. However, the minimum heart rate values were within the range measured for control animals. Maximum heart rates also showed variable changes during noise exposure that depended on the animal.

Although we did not document differences in interval heart rates, we did observe increased variability in heart rate, including marked changes in cardiac variability with exercise. As reported previously for cetaceans during elevated levels of exercise (Williams et al., 2015; Williams, Blackwell, et al., 2017), we found an increase in cardiac variability in narwhals using faster stroke frequencies during noise exposure. This increased variability in heart rate was reflected in the sequential changes in inter-beat interval (ΔIBI) during dive ascents, with the heart switching rapidly between brief periods of intense bradycardia and exercise tachycardia during noise exposure (Figure 3b ECG traces). The increase in variability of ΔIBI as detailed for an individual narwhal exposed to seismic noise (Figure 3b; Figure S7) resulted in significantly different distributions (Kolmogorov–Smirnov Asymptotic test D = 0.1531, p < 0.0391) for control periods (n = 183 samples) in comparison to the first seismic-exposure event (n = 155 samples), with the range of values being broader during seismic exposure.

3.4 | Respiratory responses

Regardless of experimental condition, the inter-dive interval of narwhals remained stable at 3–4 min. Respiratory frequency during these post-dive intervals was highly variable (Figure 4), especially during the first minute of surfacing for seismic exposed dives (n = 20 dives by 2 narwhals during 3 noise events, mean depth max = 101.3 ± 17.3 m, dive duration = 9.1 ± 1.1 min) compared to control dives (n = 40 dives by 6 narwhals, mean depth max = 105.0 ± 7.1 m, dive duration = 9.8 ± 0.6 min). Mean respiratory frequency was 4.9 ± 0.3 breaths/min immediately after control dives, with recovery to 3.0 ± 0.0 breaths/min within 4 min. The maximum respiratory frequency recorded following control dives was 8 breaths/min and occurred during the first minute of recovery. In comparison, mean respiratory frequency was 6.1 ± 0.7 breaths/min immediately after seismic exposure dives, with little change to 6.0 ± 0.0 breaths/min even after 4 min of recovery before the animals dove again. Maximum respiratory frequency following seismic exposure, 12 breaths/min, occurred during the first minute of recovery and was 1.5 times the maximum level for control dives.

4 | DISCUSSION

Changes in physiological homeostasis provide important benchmarks for objectively assessing the effect of stressors on individual animals and populations living in unpredictable or rapidly changing environments (National Academies of Sciences, Engineering, and Medicine, 2017). Here, we find that one such stressor, unanticipated
oceanic noise from a seismic ship and its towed airguns, resulted in homeostatic disturbance across the entire cardio-respiratory pathway for oxygen in wild narwhals (Figure 5). Ultimately, these changes altered the normal connectivity between organ compartments supporting diving behaviours and energetic costs. Compared to normal diving responses, the noise-exposed narwhals displayed elevated stroke frequencies and post-dive respiration rates typically associated with heightened exercise levels (Figures 2 and 4). Instead of the expected release from bradycardia and concomitant exercise-induced increase in heart rate (tachycardia) as noted in Davis and Williams (2012) and Noren et al. (2012), the cardiovascular response of noise-exposed narwhals evinced a more variable and intense level of bradycardia despite increased levels of exertion (Figures 3 and 5). This is likely due to the integration of neural signals associated with diving, exercise and fear reactions (Williams, Blackwell, et al., 2017), that we term an ‘integrated’ bradycardia to distinguish it from a simple diving bradycardia.

These physiological responses for free-ranging narwhals exposed to seismic pulses were more pervasive than those recorded for trained harbour porpoises (Phocoena phocoena, Elmegaard et al., 2021) and a closely related species, the beluga whale (Delphinapterus leucas, Lyamin et al., 2011), during laboratory sound exposure experiments. In the harbour porpoise study, the animals freely swam and dove in a 15 m × 35 m net pen. When exposed to sonar sweeps and noise pulses [sound exposure level (SEL): 98–131 dB re 1 μPa²·s; sound pressure level (SPL): 103–137 dB re 1 μPa], the small cetaceans initially responded by intensifying the level of bradycardia. However, the response was quickly extinguished as the animals habituated to the noise. Startle reactions in the form of skeletal muscle flinches were often apparent in the porpoises but were decoupled from cardiac responses (Elmegaard et al., 2021). During the beluga study, a young whale was exposed to acoustic noise of varying frequencies (19–108 kHz) and loudness (140–160 dB) while resting on a stretcher in a seawater tub. Unlike the harbour porpoises, the beluga whale showed a marked increase in heart rate upon initial noise exposure followed by a gradual slowing of the heart. Continued noise resulted in eventual bradycardia below control resting values, similar to the intense bradycardia we observed for some dives of wild narwhals during seismic tests (Figure 1c) and contributing to the suppression of exercise-induced heart rate (Figure 5). Respiratory responses of the beluga whale were moderate and only lasted 1 min in contrast to the persistent elevation in respiration rates during inter-dive periods of up to 4 min for the wild narwhals in the present study (Figure 4).
Differences in the physiological responses to noise exposure for captive and wild cetaceans are not surprising given the complexity of fear reactions in swimmers and divers. Opposing autonomic neural signals resulting from the fear-exercise-diving response triad are at play, and often lead to marked cardiovascular variability in marine mammals including narwhals (Williams, Blackwell, et al., 2017). The type of fear stimuli as well as environmental conditions will also affect the response. An extreme level of fear bradycardia has been well-documented for restrained marine mammals during forcible submergence (Scholander, 1940; Murdaugh Jr. et al., 1961; reviewed in Kooymen, 1989, Ponganis, 2016, and Davis, 2019), trained diving (Murdaugh Jr. et al., 1968), and in response to acute aversive stimuli in the lab (Fedak, 1986; Fedak et al., 1988). If divers engage in a flight response, the increased intensity of exercise alters the physiological reaction. During normal aquatic activity, the diving response can override exercise-induced tachycardia in both human (Bergman Jr. et al., 1972; Butler & Woakes, 1987; Foster & Sheel, 2005) and non-human (Davis & Williams, 2012; Williams et al., 2015) swimmers. This is a graded interaction, such that diving bradycardia is progressively relaxed as exercise intensity increases. The addition of fear shifts the cardiac balance back towards a more intense bradycardia. Consequently, the lowest heart rates recorded for wild narwhals (below 4 bpm, representing a >94% decrease from resting heart rate) occur during escape dives from capture and net entanglement (Williams, Blackwell, et al., 2017; Figure 5).

Behavioural decisions by wild narwhals, as occurred when encountering unexpected noise in open water, also modify physiological responses. In the present study, tagged narwhals were free to move relative to a sound source that initially approached the animals and then passed by (Heide-Jørgensen et al., 2021). This would have allowed for anticipation and eventual response to the stimulus. Such mobility between the stressor and subject differs from an acute event as occurs with sudden escape from an immobile, point source like net entanglement; the difference was reflected in the cardiac responses of the narwhals (Figure 5). Likewise, repeated exposures can lead to behavioural habituation, a possible contributing factor to the variable heart rate responses for one narwhal that was exposed to two separate seismic events in the present study (Figure 3c).

Given the suite of mitigating factors and possible physiological responses, it is difficult to determine how noise exposure compares
with other stressors wild animals may encounter. Here we find that the relationship between diving heart rate and stroke frequency represents a new, useful metric for characterizing the level of fear reactions of marine mammals exposed to different environmental stressors including anthropogenic noise. Under quiet, control conditions, the relationship shows a positive trend as described by

\[
\text{Heart rate} = 12.94 + 0.45 \times \text{Stroke frequency} \quad (F_{1,665} = 567.30, p < 0.0001)
\]  

(2)

from 30 dives representing 5 narwhals (n = 668 matched samples), where heart rate is in bpm and stroke frequency is in stroke cycles per minute. In comparison, there is a lack of change in heart rate with elevated stroke frequencies as free-ranging narwhals moved away from seismic pulses and shipping noise (Figure 5). The resulting relationship is described by

\[
\text{Heart rate} = 13.87 + 0.21 \times \text{Stroke frequency} \quad (F_{1,247} = 99.92, p < 0.0001)
\]  

(3)

for 15 matched-depth dives from 3 seismic events for 2 whales (n = 249 samples) with the same units as in Equation 2. The slope of the heart rate-stroke frequency relationship decreased significantly (mixed model interaction \(F_{1,780.6} = 62.57, p < 0.0001\)) with exposure to seismic pulses compared to control dives. Note that average heart rate did not differ between control and noise-exposed groups (see Section 3.3 Cardiovascular responses above).

Overall, the effect of seismic pulse exposure on narwhals was suppression of the cardiac exercise response in a pattern opposite to that reported for freely swimming marine mammals (Davis & Williams, 2012) including narwhals (Williams, Blackwell, et al., 2017). This suppressive neural signal superimposed on intense exercise and diving neural inputs to the heart was coincident with bradycardia-tachycardia switching and an elevation in IBI variability (Figure 3b). Interestingly, acute fear when escaping net entanglement induced an even larger integrated bradycardia than exposure to seismic pulses in the present study. Comparing the heart rate-stroke frequency relationships for each stressor shows that net escape further diminished the heart rate with maximally a 37.5% reduction in integrated bradycardia at the highest comparable stroke frequencies for noise-exposed narwhals (Figure 5).

Thus, minimum heart rates of narwhals exposed to seismic pulses approached 5–10 bpm, a moderate response compared to the extreme bradycardia of <4 bpm during net escape dives.

Common reactions of the narwhals to seismic airguns and vessels included a decrease in foraging rate (Tervo, Ditlevsen, et al., 2021), an increase in swimming transit speed, and active movement from open water to coastal areas (Heide-Jørgensen et al., 2021). All of these behavioural reactions in combination with the physiological responses described here will drive the energetic costs of avoiding the disturbance by narwhals. For example, like beaked whales Ziphius cavirostris exposed to mid-frequency active sonar (DeRuiter et al., 2013), narwhals changed swimming gaits from gliding to nearly constant, high frequency stroking, which instigated increases in body acceleration (ODBA, Figure 1a,b), and instantaneous energetic costs (Williams, Kendall, et al., 2017).

Summing these costs across entire dives, we find that swimming and stun dive activities result in similar costs for both control and noise-exposed narwhals (Figure 6). Higher costs were incurred by the narwhals during transiting and startle dives. The latter was characterized by the absence of gliding and increased reliance on high stroke frequency propulsion. As a result, the cost of a startle dive in response to seismic pulse exposure was 2.0–2.2 times that of a matched control or stun dives (Figure 6). Thus, increased stroke frequencies and associated travel speeds reported for noise-exposed narwhals (Heide-Jørgensen et al., 2021) and beaked whales (DeRuiter et al., 2013; Williams, Kendall, et al., 2017) likely come at a high energetic cost.

In sum, the presence of anthropogenic noise clearly instigated a cascade of physiological consequences in the wild narwhals. Admittedly, this study using controlled noise exposure to assess impacts on individual dives of individual animals only represents the first step in determining the potential effects of oceanic noise on wild populations of deep-diving cetaceans. Further study regarding the role of these physiological responses over long-term exposures, as often occur with anthropogenic noise in the ocean (Duarte et al., 2021), will be needed to differentiate those responses that represent benign physiological adjustments and those that in time and with continued stress will affect the trajectory of wild populations.

**AUTHORS’ CONTRIBUTIONS**

T.M.W. and M.P.H.-J. conceived the ideas and designed the methodologies; all authors contributed to data collection and fieldwork; T.M.W. analysed the data and led the writing of the manuscript. All
of the authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST
No conflicts of interest are noted for the authors of this manuscript.

DATA AVAILABILITY STATEMENT
Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.m0cfxpp69, (Williams et al., 2022).

DATA SOURCES
Original data and sources are included within this manuscript and are also available online through the UCSC Mammal Physiology Project database from the corresponding author. All statistical data are presented in the text and in the online Supporting Information document.

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REFERENCES


SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.