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The impact of sound on the welfare of animals used for experiments

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The aim of these Guidelines is to provide evidence-based recommendations for managing sound in laboratory animal facilities. The recommendations are grounded on current knowledge about auditory perception in target species and a review of literature about the effects of sound on reproduction, immune function, sleep, social behaviour (aggression), and anxiety/fear in the target species.

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dierenwelzijn@vlaanderen.be

Authors
Buddhamas Pralle Kriengwatana – KU Leuven
Esther Van Haver – KU Leuven
Adinda Sannen – Odisee
Leen Verbist – Odisee
Stef Aerts – Odisee
Tomas Norton – KU Leuven

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Contact
For questions or comments about the study, the authors can be contacted via email:
tomas.norton@kuleuven.be

Partners

KU LEUVEN Odisee UNIVERSITY OF APPLIED SCIENCES

INTRODUCTION

Noise is recognised as a major environmental health problem for humans and non-human animals by the Environmental Noise Directive 2002/49/EC (European Parliament and Council of the European Union, 2002), which aims to establish a framework to avoid, prevent, or reduce the harmful effects of exposure to environmental noise. According to the European Environment Agency (EEA) report in 2020 and 2025 (European Environment Agency, 2020, 2025), long-term exposure to environmental noise is a major cause of premature deaths and new cases of heart disease, with millions of people suffering from detrimental psychological effects of noise such as annoyance, sleep disturbance, and learning impairments. The reports also highlighted the impacts of environmental noise on wildlife (both terrestrial and aquatic species), with effects on various physiological and behavioural responses including but not limited to hearing loss, elevated stress, impaired immune function, sleep, reproductive behaviour and success, foraging and territorial behaviour, and communication.

Animals in laboratory and research facilities are also exposed to various levels and sources of noise. Although noise is a potential source of disturbance to laboratory animals, its effects may go unnoticed when it is inaudible to humans or assumed to be imperceptible by animals. The effects of noise on animals may therefore reduce welfare and create unintended variation in research data, thereby affecting reproducibility and undermining the 3Rs goals to reduce and refine research involving lab animals (Turner, 2020; Turner & Manker, 2024). Existing regulations for animals used for scientific research in Directive 2010/63/EU (European Parliament and Council of the European Union, 2019) state that animal welfare should not be adversely affected by noise and should be minimized. However, relationships between different types of sound in research facilities, stakeholder input regarding priorities and feasibility of acoustic monitoring and management have not been synthesized in a single publication.

The aim of these Guidelines is to provide evidence-based recommendations for managing sound in laboratory animal facilities, funded by the Flemish Government, Department of Animal Welfare (specification number OMG_DWZ_2025_001). The recommendations are grounded on current knowledge about auditory perception in target species and a review of literature about the effects of sound on reproduction, immune function, sleep, social behaviour (aggression), and anxiety/fear in the target species. Recommendations for sound management were structured to address specific concerns and incorporate perspectives from relevant stakeholders (i.e. laboratory facility directors and managers).

This guideline is divided into four main sections. The first section provides foundational information about sound and how it is measured, sound perception in laboratory species targeted in these Guidelines (i.e. rats, mice, pigs, chickens, and zebrafish), and sources and levels of sounds in research facilities. The second section paints an unbiased and representative picture of how different types of sounds impact several indicators of animal welfare through a structured literature review. The third section summarises input from stakeholders about their perspectives and priorities regarding noise monitoring and management in animal research facilities. The final section provides recommendations for noise management in animal research facilities. These concepts and approaches can help shape strategies to improve animal welfare through the acoustic environment.

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1 WHAT IS SOUND?

Sound is a mechanical wave that is produced when a source vibrates and disturbs the surrounding medium, such as air or water. This disturbance causes nearby particles to move back and forth, and their motion triggers the movement of neighbouring particles. In this way, the vibration spreads outward as a wave, carrying energy from the source to a receiver, i.e. from an individual to another individual. Hearing begins when this energy is detected by sensory receptors in the ear of the receiver. Due to the greater density of water, sound travels four times faster through water than air.

Two measurable components of sound include sound pressure and particle motion. Sound pressure variations occur when a sound source vibrates, create alternating compressions (regions of higher pressure) and rarefactions (regions of lower pressure) in the medium. Thus, sound pressure level (SPL) represents the magnitude of these pressure variations. Particle motion is the small back-and-forth movements of particles as waves of pressure fluctuations pass (Figure 1). Mammals and birds primarily sense sound pressure levels while particle motion is the primary way many fish and invertebrates detect sound (Hill et al., 2019; Popper & Fay, 2011).

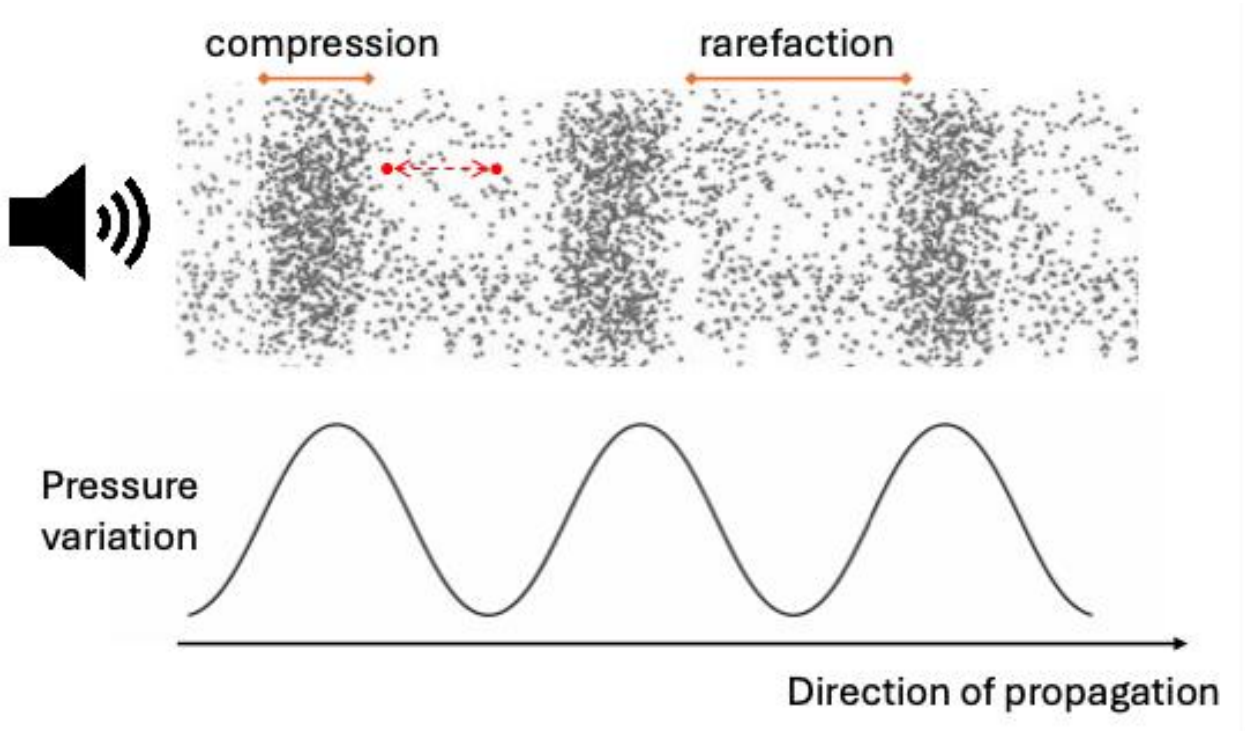

Figure 1. Schematic representation of a longitudinal sound wave. The upper panel shows alternating regions of compression (high particle density) and rarefaction (low particle density) in the medium as the wave propagates from left to right. The lower panel illustrates the corresponding sinusoidal variation in sound pressure over space, with pressure maxima aligning with compressions and pressure minima aligning with rarefactions. The red dot represents particle motion.

The term sound often refers vibrations that travel through air or water and are detected by the ear. Vibration, while sharing many mechanical principles as sound, often refers to vibrations carried through non-air or water mediums that are detected by other receptors in the body.

1.1 PROPERTIES OF SOUND

Sound has several physical properties that are perceived. The most basic ones include frequency, amplitude, and pitch. Frequency refers to how often particles of the medium vibrate back and forth about their fixed position. It is commonly expressed as the number of cycles per second, with Hertz (Hz) as the associated unit. High frequency sounds have a shorter wavelength and are perceived by our auditory systems as having a higher pitch. Compared to low frequency sounds, high frequency sounds are easily degraded and do not travel as efficiently in air and water. Most sounds are composed of many frequencies: sounds with a single frequency, i.e. pure tones, are rare in nature.

Amplitude refers to maximum displacement of a particle from its rest position. Higher amplitude sounds have more energy (because more energy is required to create greater displacement of the particle) and are therefore perceived as louder.

Sound can be visualised as a spectrogram, which represents frequency along the y-axis and time along the x-axis, with colour intensity indicating power (usually expressed in decibels), such that darker colours correspond to higher energy. Figure 2 provides examples of spectrograms recorded under controlled laboratory conditions and in uncontrolled outdoor conditions

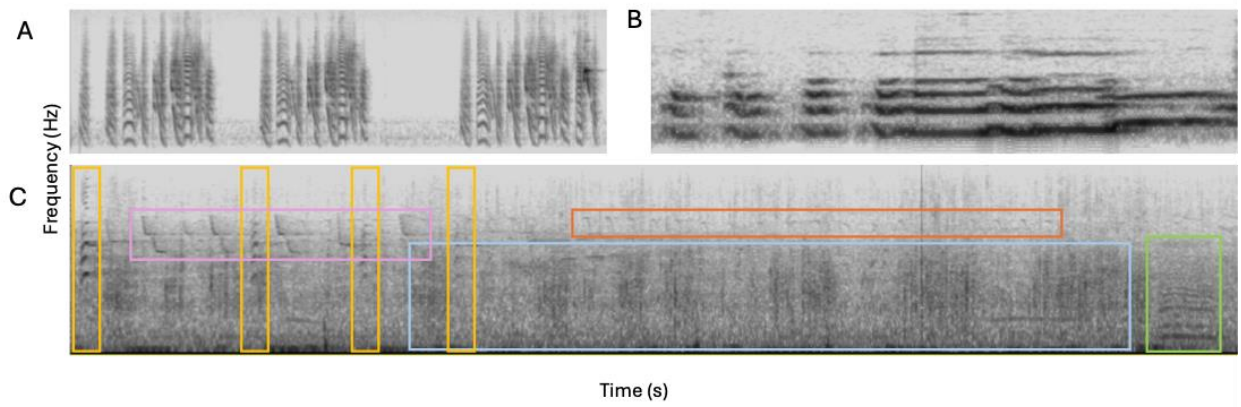

Figure 2. Example spectrograms of natural sounds. (A) Song of a zebra finch, individually recorded in sound-attenuated chambers. (B) Vocalisation of a chicken (laying hen) recorded in a laboratory facility. (C) Outdoor recording with several co-occurring sounds and background noise. Orange, yellow, green, and purple boxes label vocalisations of different bird species while the blue box labels water sounds, with visible amplitude changes caused by animal movement. Vocalisations of yellow and green species occur within the same frequency range as water sounds. Hence, water sounds may mask signals and potentially impair communication in these species, whereas purple and orange species may be unaffected. Spectrograms are not aligned on frequency or time axes.

1.2 HOW IS SOUND MEASURED?

Loudness is a perceptual construct that is based on the physical magnitude of sound and psychological weighting. The physical magnitude of SPL is measured along a linear scale in pascals (Pa).

Decibels (dB) is the logarithmic conversion of this physical magnitude, and expresses how many times larger the sound pressure is compared to a reference pressure. In air, the reference pressure is 20 µPa; in water it is 1 µPa. Hence, the proper notation for SPL is dB re 20 µPa when measured in air, and dB re 1 µPa when measured in water.

The dB scale is expressed logarithmically to better capture the wide range of frequencies humans and animals hear. For instance, the threshold of human hearing is 0 dB re 20 µPa and a normal conversation is typically considered to be 60 dB re 20 µPa, which means it is 106 (1,000,000) times more intense than the threshold of human hearing. Thus, 0 dB does not refer to an absence of sound per se, but at levels below what humans can detect.

Loudness is a perceptual property because the intensity of different frequencies is not represented faithfully but weighted in the brain (i.e. emphasised or de-emphasised) in a species- and experience-dependent manner. Humans hear best at frequencies between 2 -5 kHz, so these frequencies contribute more to perceived loudness than those outside this range. This means that two sounds with the same intensity, but different frequencies will not be perceived as equally loud.

To account for this perceptual weighting, the A-weighting filter is applied when measuring loudness for humans, and is expressed as LA, dB(A), or dBA. In occupational health, C-weighting (LC, dB(C), or dBC) is also used because it represents perceived loudness at high physical sound pressure levels. When inferring perceived loudness in animals, an unweighted, or zero-weighted, filter (LZ, dB(Z) or dBZ) is sometimes appropriate, as it does not assume that animals hear what humans hear, especially when audible and peak sensitivity ranges of the species are unknown. An R-weighting (dBR) has been developed to more accurately represent the auditory sensitivity of rats (Björk et al., 2000) but it faces two major barriers to use: i) no single, formally specified frequency-weighting curve exists so measurements cannot be calibrated or directly compared across studies or instruments; ii) the weighting is not supported by commercially available sound level meters. Note that A, C, Z, and R weightings are based on air reference level (dB re 20 µPa). A comparison between A- and Z-weighting is shown in Figure 3.

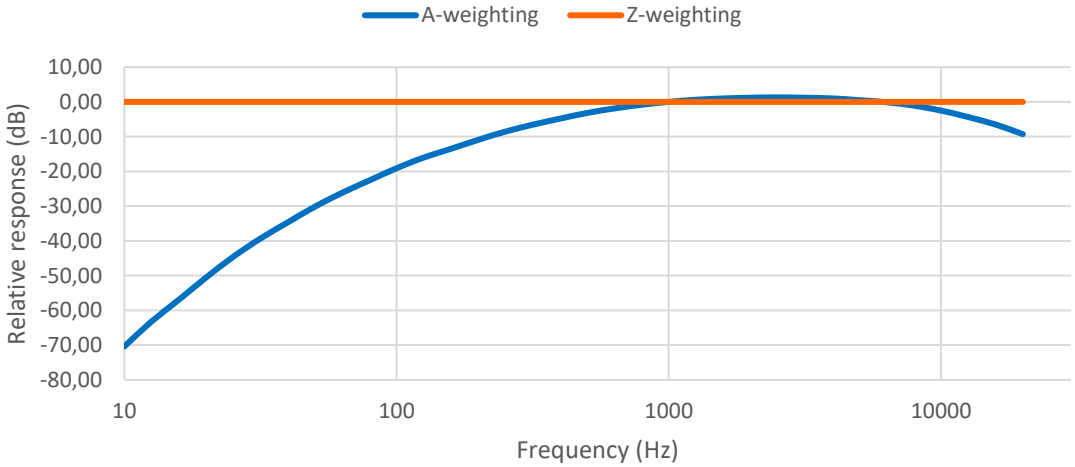

Figure 3. Frequency response curves for A- and Z-weighting. Frequency response curves for A- and Z-weighting. Relative level (dB) is normalised such that both curves equal 0 dB at 1 kHz, meaning sounds at this frequency are neither amplified nor attenuated by the weighting filter. A-weighting attenuates low and high frequencies to approximate human auditory sensitivity at moderate sound levels, whereas Z-weighting applies no weighting (i.e. assumes flat response where sensitivity is equal across frequencies). This means the dB value of low or high frequency sounds will be lower with A-weighting compared to Z-weighting.

The notation dB SPL is sometimes used in scientific literature instead of dB re 20 µPa and is sometimes even denoted as only dB. However, researchers have argued against this convention, as the decibel is a relative unit that does not measure a physical property of sound, hence dropping what the decibel is relative to has led to confusion when comparing measurements made in air and

water (Chapman & Ellis, 1998). In this report, we use the unit and weighting specified in the article; if no weighting was specified, it was assumed to be Z-weighted.

Instruments to measure sound include microphones and hydrophones (Figure 4). Most microphones use the movement of a diaphragm to measure variation in SPL over time. The degree of displacement of the diaphragm over time is converted into an electrical signal, from which properties such as frequency content and energy distribution can be inferred with signal analysis techniques. Hydrophones use two different types of sensors to measure pressure and particle motion (particle velocity, acceleration, and/or displacement) in water.

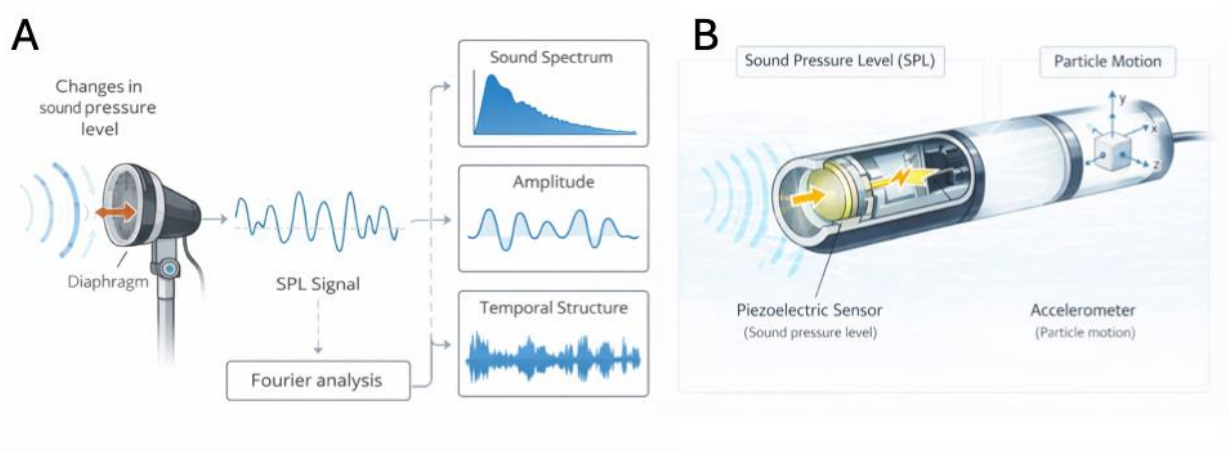

Figure 4. Illustrations of a microphone (A) and hydrophone (B). For microphones, changes in sound pressure levels cause a diaphragm to vibrate back and forth. The microphone converts this movement into an electrical signal (representing SPL over time). From this signal, acoustic properties such as frequency spectrum, amplitude, and temporal structure can be extracted using Fourier analysis. Hydrophones that measure both sound pressure level and particle motion contain two types of sensors (sometimes integrated into a single sensor): a pressure-sensitive (e.g. piezoelectric) and motion sensors (e.g. accelerometers). Sound pressure waves deform the piezoelectric sensor, which is converted into an electric signal, where acoustic properties can be extracted using the same steps as for microphone recordings. Accelerometers pick up the oscillatory movement of water particles and convert this motion into electrical signals, giving measures along three orthogonal axes (x,y,z). Image generated using ChatGPT (OpenAI).

2 SOUND PERCEPTION IN ANIMALS

Sounds play an important role in almost every aspect of the life of most vertebrates, used in communication, foraging, predator/prey detection, navigation and orientation, migration, mating behaviour, and habitat selection. As vertebrates have evolved hearing abilities that are specific to their own species' socio-ecological demands, we should not assume that other species hear what we humans hear. For example, humans cannot hear sounds above 20 kHz (hence called ultrasound) or below 20 Hz (therefore called infrasound), yet animals such as rodents and elephants produce and perceive sounds in the ultrasonic or infrasonic range (Payne et al., 1986; Portfors, 2007).

To gain a better understanding what lab animals might be hearing, this section briefly explains sound perception by describing the auditory system, the range of frequencies that different species can perceive (hearing range), and non-auditory factors that can influence which sounds are perceived and processed.

2.1 THE AUDITORY SYSTEM

The auditory system allows animals to detect sounds in their environment and to use this information to guide behaviour. In most terrestrial vertebrates, it is composed of two parts: the peripheral system (the outer, middle, and inner ear) and the central system (the brain) (Fay, 1988). The outer and middle ear detect sound waves and transmit them to the inner ear (cochlea). Different sound frequencies are processed at different locations along the cochlea, meaning that pitch information is already organised at this very early stage. The cochlea then converts the detected sound into electrical signals and sends them along the auditory nerve to the brainstem (Fay & Popper, 2000).

In the brainstem, basic processing occurs, including sound localisation, separation of pitch and timing information, and reflexive responses to sound (e.g. startle reactions and orienting towards a sound source). The signal is then relayed to higher brain areas, including the primary auditory cortex, where basic sound features such as pitch, loudness, and rhythm are represented, and onward to associative regions where sounds are recognised and interpreted (e.g. species or individual identity). Through connections with movement, attention, and learning systems, sounds can influence how an animal behaves, what it attends to, and how it learns from its environment (Fay, 1988; Fay & Popper, 2000). In this way, sounds in the environment are able to directly influence the welfare of laboratory animals.

Many species of fish use different physical structures to detect sound and vibration in water. Fish generally lack outer and middle ears, so they rely on the inner ear and other specialised structures, such as the swim bladder or lateral line system (Popper & Fay, 2011). The inner ear of fish detects particle motion across a broad range of frequencies. Some species of fish, including zebrafish, possess a swim bladder (a gas-filled organ primarily functions to maintain buoyancy) which is also used in detecting sound pressure levels and particle motion (Popper et al., 2014; Popper & Fay, 2011). The lateral line is a series of sensory receptors along the head and body that is sensitive to low-frequency vibrations near the body, therefore enhancing detection of sound in close range (Popper et al., 2014; Webb et al., 2008). Even though fish brains are structurally different from those of birds or mammals, auditory and vibrational information is still processed and integrated with other sensory inputs and internal states in the brain to influence behaviour (Fay & Edds-Walton, 2008). This means sounds and vibrations present in laboratory facilities (e.g. generated by pumps,

aeration systems, tank walls, and human activity) can be heard by widely used aquatic model species, such as the zebrafish, and may influence their welfare.

2.2 HEARING RANGES

As vertebrates have evolved hearing abilities that fit their specific socio-ecological demands, the range of audible and peak sensitivity frequencies and temporal resolution of sound differs between species. For several species, the audible frequency range and peak sensitivity have been determined with standardised approaches, thus enabling between-species comparisons (Figure 5).

Species	Audible range (kHz)	Sensitive range (kHz)
Human	0.02 – 20	2 – 5
Laboratory rat[1,2]	0.25 – 80*	12 – 24
Laboratory mouse[3]	1 – 100*	8 – 24
Domestic pigs[4,5]	0.042 – 40.5	0.25 – 16
Domestic chicken[6]	0.002 – 7.2**	2 – 4
Zebrafish[7]	0.2 – 1***	0.4 – 0.6

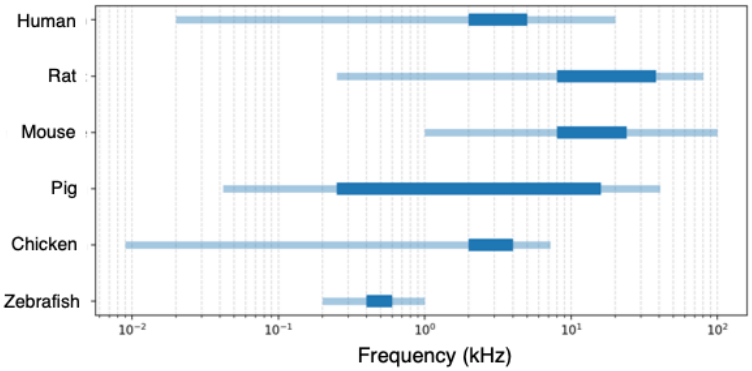

Figure 5. Audible and sensitive ranges of human and laboratory species targeted in these Guidelines, based on behavioural assays. The x-axis is in logarithmic scale. References: [1]Kelly & Masterton (1977), [2]Borg 1982, [3]Radziwon et al. (2009), [4]Heffner & Heffner (1990), [5]Heffner & Heffner (2007), [6]Hill et al. (2014), [7]Cervi et al. (2012). * Threshold curves vary between different strains (Ralls, 1967). ** Young chicks (1-2 days post-hatch) can hear frequencies between 250 Hz to 4 kHz at high thresholds, with peak sensitivity between 750 Hz to 2 kHz (Ordiway et al., 2024). *** Zebrafish larvae can detect frequencies from 100 Hz to 4 kHz, with peak sensitivity between 100–400 Hz (Bhandiwad et al., 2013; Poulsen et al., 2021).

Of the species discussed in these Guidelines, mice have the largest audible frequency range, followed by rats, pigs, chickens, and zebrafish. Like several species of birds, chickens can perceive infrasonic frequencies lower than documented in many mammals, including elephants (Hill et al., 2014; Zeyl et al., 2020). They appear to perceive these low frequencies as sound, not as substrate-borne vibrations (Hill et al., 2014). Although zebrafish show the most restricted hearing range out of the species included here, fish species in general show great diversity in hearing range. Some species can only hear up to 100 or 200 Hz while others can hear over 180 kHz (Popper & Fay, 2011). A comparison of human, chicken, and pig audiograms are shown in Figure 6.

Both frequency and temporal resolution contribute to the ability to quickly and precisely detect and interpret sounds. Temporal resolution refers to how well the auditory system can perceive rapid changes in sound or separate different sound events. A species with high temporal resolution will be able to distinguish between subtle changes in frequency and amplitude characteristics of the sound compared to species with poorer temporal resolution. This means that two species hearing the same sequence of sounds may have very different perception of it, including whether the sounds are continuous or intermittent and whether the sequence is interpreted as a single repeated sound or as multiple distinct sounds. While temporal resolution is known for some species, cross-species comparison is difficult and rare because there are no standard frequencies used to test temporal resolution.

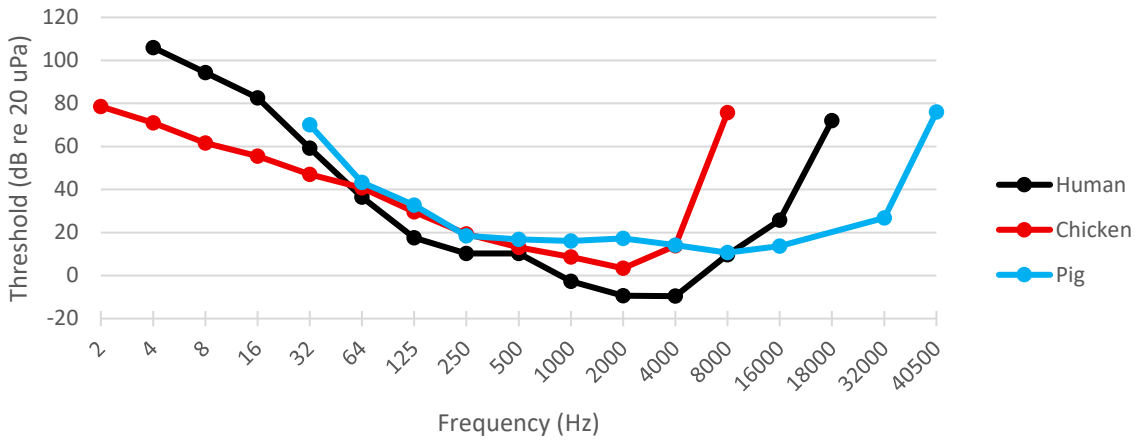

Figure 6. Comparison of human, chicken, and pig audiograms. The vertical axis represents auditory thresholds, i.e. the minimum SPL required for a single frequency to be detected (higher values indicate louder sounds are required). The 0 dB SPL value is a reference level for detection thresholds of tones at ~1000 Hz re 20 µPa, which explains why frequencies within human's peak sensitivity range (~2000 – 5000 Hz) can be detected at thresholds below this reference level. All three species can hear tones between 32 – 8000 Hz, with chickens and pigs being able to detect tones outside of human audible range of 20 – 20000 Hz. However, humans can detect tones between 1000-4000 Hz at lower SPL than chickens or pigs. Adapted from Hill et al. (2014) and Heffner & Heffner (1990).

2.3 FACTORS AFFECTING SOUND PERCEPTION

Breed/strain, age, hormones and auditory and non-auditory experiences (e.g. stress) are among some of the factors affecting auditory perception. In lab rodents, different auditory thresholds have been reported for different strains (Borg, 1982; Koch et al., 2022). Listar hooded rats have worse hearing ability than Sprague Dawley, Wistar, and Long Evans rats (i.e. sounds need to be louder to be detected), and display more intense startle responses than Sprague Dawley and Long Evans rats (Koch et al., 2022). Age also affects hearing: rat pups are born deaf and remain insensitive to sound until approximately two-weeks old (Geal-Dor et al., 1993) and mouse strains differ in progression of age-related hearing loss (Bayne & Turner, 2014). Commercial broilers breeders housed in barns with average broadband noise levels of 90 dB SPL showed anatomical signs of deafness (extensive cochlear damage), although layers of approximately the same age showed no or mild damage (Durham et al., 2002). However, when younger broilers and layers (10-17 weeks) were tested, more layers than broilers showed severely damaged cochlea (Kaiser et al., 2005), which suggests that age and breed may together influence susceptibility to acoustic trauma in chickens.

In some species, hearing ability is affected by seasonality and sex. The plain midshipman fish (Porichthys notatus) male produces a low humming sound to attract females during breeding season. Females in breeding condition show improved hearing: they are able to detect quiet sounds and can hear higher frequencies better than females in non-breeding condition (Sisneros, 2009; Sisneros & Bass, 2003). Hearing loss has also been linked with stress-response systems, as glucocorticoid signalling is involved in susceptibility to and recovery of the auditory system from acoustic trauma (Maniaci et al., 2024).

The animal's acoustic environment also influences what sounds an animal is sensitive to. Rat pups reared with moderate levels (70 dB SPL) of continuous white noise showed retarded development of the auditory cortex (Chang et al., 2024). In birds and mammals, auditory experience with conspecific vocalisations (i.e. vocalisations from other individuals of the same species) guide the development of appropriate neural and behavioural responses towards conspecific signals (Jagersma et al., 2025;

Kim & Bao, 2013; Woolley & Doupe, 2008). Neural plasticity in response to acoustic environments are not limited to developing brains, as exposure to moderate levels of noise also remodels the adult brain, potentially leading to hearing problems without hearing loss (Eggermont, 2017). The relevance of sound also affects its detection: frogs lower their hearing thresholds to improve detection when presented with male frog chorus (Gao et al., 2015), and learning the relevance of different sounds enhances zebrafishes' ability to perceive them (Cervi et al., 2012).

3 NOISE IN LABORATORY FACILITIES

Noise is commonly defined as unwanted sound. Laboratory animal facilities are intrinsically noisy environments with a range of internal and external noise that vary in origin, intensity, and temporal pattern.

3.1 INTERNAL SOURCES OF NOISE

Animal-generated noise includes vocalisations, locomotor activity, interaction with enrichment devices (movement, striking, or rattling of objects) and movement of cages, and is repeatedly identified as a primary internal source of noise. Animal-generated noise can produce substantial short-term peak SPL, particularly in social species (Kroll et al., 2024; Lauer et al., 2009; McLeod et al., 2022; Milligan et al., 1993; Weeks, 2008; Young et al., 2011; Zoontjens, 2012). The intensity and frequency of these activities determine the short-term peak sound levels (McLeod et al., 2022).

Human-related activities include talking, shouting, whistling, walking, opening and closing doors, and performance of routine animal care, cleaning procedures, and experimental procedures, most of which require manual handling, moving, or changing of cages and/or animals (Baldwin et al., 2007; Grandin, 2021; Lauer et al., 2009; McLeod et al., 2022; Milligan et al., 1993; Riegel & Bruce, 2013; Young et al., 2011; Zoontjens, 2012). This is another major source of noise that produces relatively short-term peaks in SPL and can cause animal-generated noise.

Equipment-related noise refers to sounds produced by equipment such as cage washers, decontamination machines, high pressure water and steam cleaners, vacuum hoses, floor scrubbers, and macerators, as well as equipment used in project-specific experimental procedures (e.g. computers with cooling fans). Equipment involved in routine animal care procedures can also produce transient noise when in use, i.e. animal transfer stations, transport carts and racks (often with hard and squeaking wheels), and this also includes metal-on-metal impact caused by manipulation of gates, barriers, or latches (Baldwin et al., 2007; Barabas et al., 2022; Cordingley et al., 2024; Parker et al., 2010; Pate et al., 2013; Reynolds et al., 2010; Sales et al., 1988; Weeks, 2008; Young et al., 2011; Zoontjens, 2012). As metal cages produce higher short-term peak levels during handling or movement compared to alternative materials such as plastic, cage design itself can additionally contribute to noise levels in animal research facilities (Zoontjens, 2012).

Building-related and technical systems further contribute continuous background noise through ventilation and HVAC systems, ventilated rack blowers, air supply systems, watering equipment, climate control installations, elevators, lighting fixtures, and other fixed mechanical infrastructure (Barabas et al., 2022; Lauer et al., 2009; Milligan et al., 1993; Parker et al., 2010; Riegel & Bruce, 2013; Young et al., 2011). For zebrafish, air and water pumps, filtration systems, feeding and maintenance machinery contribute to continuous background noise.

3.2 EXTERNAL SOURCES OF NOISE

Several studies report external and environmental noise sources that penetrate laboratory buildings, including road, rail and air traffic, construction and renovation activities, heavy equipment, sirens, fireworks, adjacent animal facilities or farm animals (Atanasov et al., 2015; Baldwin et al., 2007; Barabas et al., 2022; Bayne & Turner, 2014; Patterson-Kane & Farnworth, 2006; Reynolds et al., 2010; Sobotka et al., 2003).

Together, these sources demonstrate that noise in laboratory settings arises from a complex interaction between animals, humans, equipment, building systems, and external environments, resulting in both continuous background noise and intermittent high-intensity peak events.

3.3 INTENSITY AND RATE OF OCCURRENCE OF NOISE

Animal-generated noise contributes substantially to variability in laboratory sound environments, with around 80 dB SPL in rodents and considerably higher levels in larger animals such as pigs, where average sound levels of 80-103 dB(A) and peaks exceeding 100 dB have been reported (McLeod et al., 2022; Weeks, 2008; Young et al., 2011).

With regards to human and staff-related operations, cage changing, animal transfer, and handling typically generate sound levels between approximately 75 and 85 dB. When turned on, sound levels inside animal transfer stations can increase around 10 dB resulting in levels approximately 65 dB (Barabas et al., 2022; Reynolds et al., 2010). Cage changes can increase average noise levels from about 50 to 60 dB(Z), with peaks at 79 dB(Z) (Barabas et al., 2022). Experimental or handling procedures frequently range from 75 to 100 dB depending on the task (Kroll et al., 2024; Lauer et al., 2009)

Equipment- and material-related sources further contribute to elevated short-duration peaks, particularly metal-on-metal impacts, which can reach approximately 100-112 dB SPL, while transport carts fitted with hard rubber wheels may produce average levels around 86 dB and maximum peaks exceeding 110 dB during movement (Baldwin et al., 2007; Cordingley et al., 2024; Parker et al., 2010; Young et al., 2011).

Cleaning and cage-wash operations add additional intense noise, with vacuum hoses and high-pressure water systems producing levels near or above 100 dB, and alarms or running water reaching 80-95 dB depending on distance and configuration (Pate et al., 2013; Sales et al., 1988). Temporal patterns in laboratory noise show that daytime periods are characterised by highly variable sound levels with frequent transient peaks, whereas nighttime noise is generally lower, more stable, and less frequent (Barabas et al., 2022; Lauer et al., 2009; McLeod et al., 2022). Transport-related noise occurs continuously during movement but is limited to the duration of transport events (Cordingley et al., 2024), while cage wash areas exhibit continuous noise during machine operation with frequent repeated peaks, particularly during dirty-side tasks (Pate et al., 2013).

In contrast, building-related background noise from ventilation systems, infrastructure, lighting, and ventilated racks is typically lower and more stable, generally ranging between 30 and 60 dB SPL or dB(A), with unoccupied or minimally active research facilities averaging around 52-55 dB(A) and occupied rooms around 53-62 dB (Barabas et al., 2022; McLeod et al., 2022; Milligan et al., 1993; Parker et al., 2010; Reynolds et al., 2010; Riegel & Bruce, 2013). In contrast, zebrafish in large-scale housing systems are exposed to much higher continuous background noises, between 122 – 143 dB re 1 µPa; as a reference, boat motor noises are approximately 130 dB re 1 µPa (Lara & Vasconcelos, 2019).

Finally, external sources such as construction activities introduce episodic but sometimes intense noise and vibration that follow daily or weekly cycles and can intermittently influence the acoustic

environment within laboratory animal facilities (Grandin, 2021; Reynolds et al., 2010; Sobotka et al., 2003).

In terms of frequency, most studies describe laboratory noise as a combination of continuous background exposure and intermittent, activity-related peaks. Mechanical systems operate continuously, whereas cage handling, animal movement, procedural tasks, and human activity generate episodic noise that varies with daily work schedules, staffing levels, and visitor presence (Baldwin et al., 2007; McLeod et al., 2022; Milligan et al., 1993; Riegel & Bruce, 2013) Events that produced transient peaks in SPL could occur over 100 times within 24 hours on a weekday in an animal research facility, with a substantial number of these events exceeding 25 dBR over background noise levels in some animal rooms (Glickman et al., 2011).

4 NON-AUDITORY EFFECTS OF SOUND ON ANIMALS

It is well-established that in addition to effects on the auditory system, sound also has non-auditory effects on a wide variety of behaviour, physiological, and cognitive/neural functions in humans and animals (Kight & Swaddle, 2011).

The aim of this literature review is to obtain a representative view of how different types of sounds affect welfare of target laboratory animals: rats, mice, pigs, chickens, and zebrafish.

4.1 OUTCOMES OF INTEREST

The review was commissioned to examine the effect of different types of sounds on reproduction, immune function, sleep, social behaviour, and welfare of the target species. The definitions for the outcomes of interest were operationalised as follows:

- **Reproduction**: litter size, hatching success, and offspring survival
- **Immune function**: glucocorticoids (GC) or GC metabolite levels in blood, feathers, faeces, or hair and differential leukocyte (white blood cell, WBC) counts. These measures are chosen because they are direct or indirect measures of immune function that are affected by psychological and/or physiological stress (Sapolsky et al., 2000). Differential WBC counts were neutrophil-to-lymphocyte (N:L) ratio in mammals and fish, or heterophil-to-lymphocyte ratio (H:L) in chickens.
- **Sleep**: duration and disturbances
- **Social behaviour**: restricted to aggressive behaviour. Measures of aggressive behaviour could be defined in ethograms (e.g. fighting) or species-specific agonistic behaviours, such as injurious feather pecking in chickens
- **Welfare**: behavioural measures of anxiety or fear and abnormal behaviours were used. These could be defined in an ethogram or measured using standardised tests such as the elevated plus maze and open field test for rodents, tonic immobility for chickens, and novel tank diving test for zebrafish.

4.2 LITERATURE SEARCH STRATEGY

To review the effects of noise on target outcomes and species, scientific articles were identified and retrieved from relevant peer-reviewed publications in the Web of Science database using a systematic approach. This approach allows for an unbiased assessment of the current knowledge on a specific topic, including identifying knowledge gaps.

For each study included in the review, the impact of the type of sound (sound category) and sound pressure level (SPL) on outcome variables compared to a control or baseline condition was recorded. The impact of the sound could be positive or negative, or produce no effect or other effects (i.e. effect of sound interacted with a moderator variable such as time of day, strain, age, etc). Some studies compared the effects of multiple types of sounds and thus produced multiple data points.

Increases in GC levels and aggressive behaviour were classified as a negative effect, and decreases as a positive effect. For WBC, an increase in N:L and H:L ratio was classified as negative and a decrease as positive. For sleep, increase in sleep duration was classified as positive and decreases as negative. For reproduction, increases in litter size, survival, or hatchability were classified as positive and decreases as negative.

4.3 QUANTIFYING EVIDENCE

A direction score (DS) was calculated to quantify the evidence that sound was having a positive, negative, no effect, or other effect. DS was calculated based on the number of data points, as (n_positive - n_negative) divided by sum of all effects (n_positive + n_negative + n_none + n_other). Thus, DS ranges from +1 (all studies positive) to -1 (all studies negative). Confidence in DS was classified as 'low' when fewer than three unique studies contributed, and as 'OK' when three or more unique studies contributed data points to the score. This is visually presented in tileplots (e.g. Figure 9), where colours indicate direction of score (negative = red, positive = blue, no effect = white) and where 'OK' confidence in DS is represented as asterisks.

4.4 DESCRIPTIVE STATISTICS

With the search strategy, 5153 articles were initially identified. After removing duplicates, the title and abstract of 3508 articles were screened and 407 articles were retained for full-text screening. The final result was 177 relevant articles included in this review (Figure 7). Plotting all data points for each of the sound categories, species, and outcome variables, it is evident there are several knowledge gaps (Figure 8).

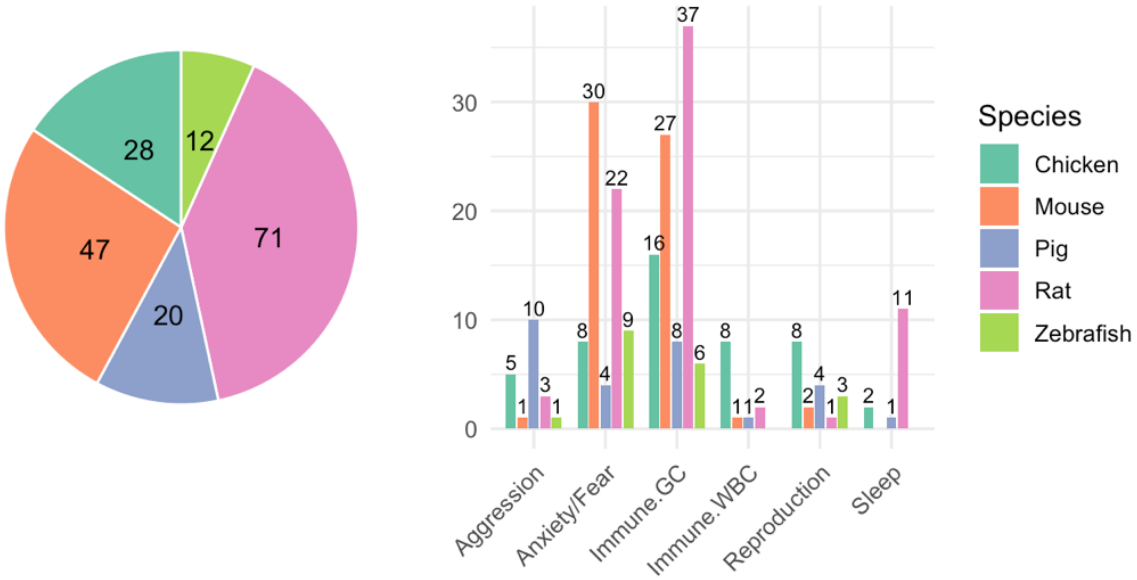

Figure 7: Number of studies identified and included in the review (N = 177), distributed across species and outcomes

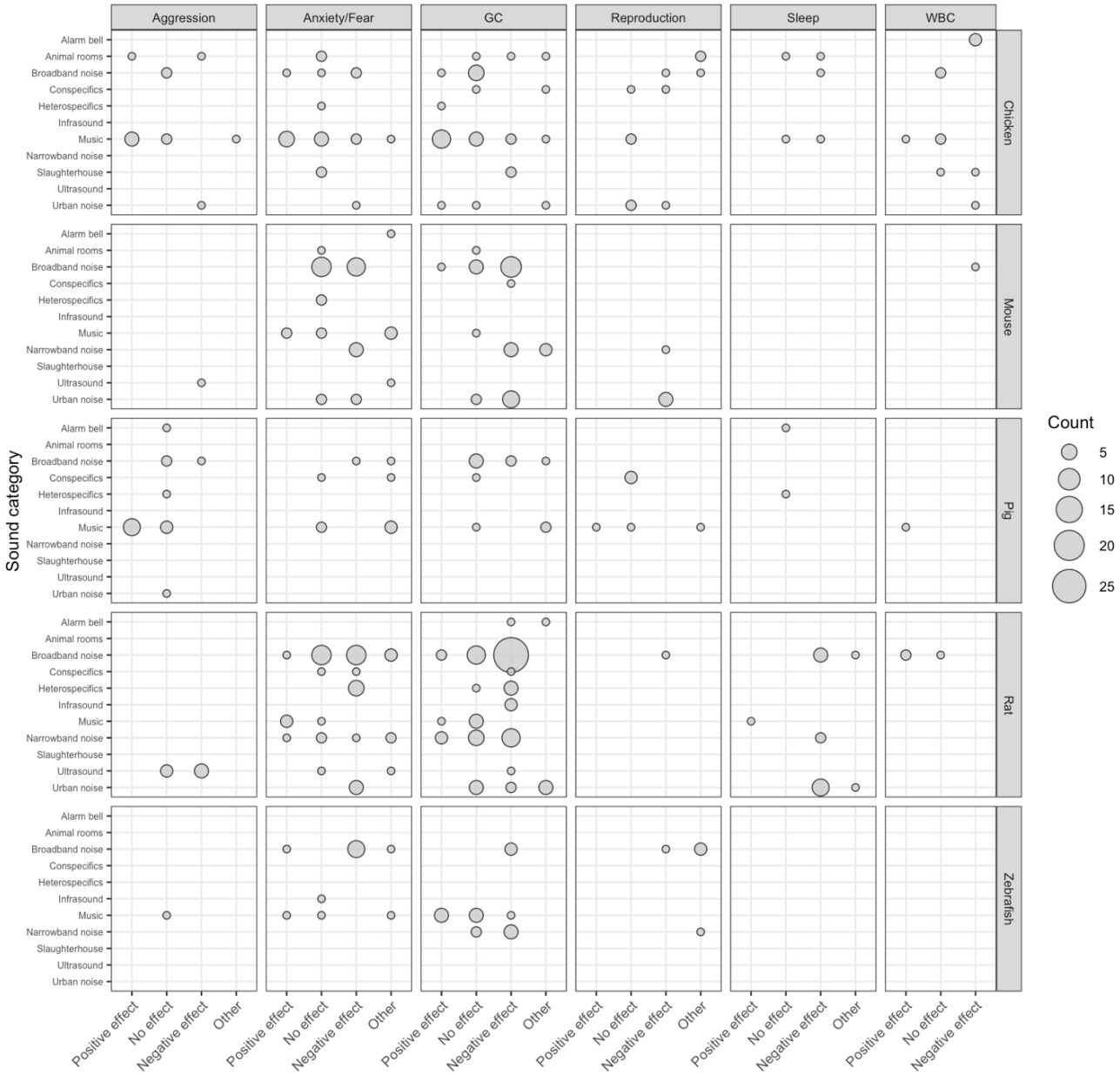

Figure 8. Number of studies per species, outcome, and sound category. Size of circles represent the number of studies (count).

4.5 SOUND CATEGORIES

Broadband noise, music, and urban noise were the sound categories with the greatest number of studies across outcome variables.

Table 1: Description of the categories used in the literature review with the number of datapoints

Category	# data points	Description
Broadband noise	117	Sounds described as broadband noises (i.e. generator, ventilation, fan, pink noise, white noise, vacuum cleaner) that are not classified as urban noise.
Music	61	Sounds described as music or radio
Urban noise	42	Sounds described as vehicle noise, traffic, construction, environmental noise, airport or aircraft noise.
Narrowband noise	36	Sounds composed of a single frequency or a very small range of frequencies
Conspecifics	13	Sounds (vocalisations, movements, etc) produced by animals of the same species as target species
Heterospecifics	11	Sounds (vocalisations) produced by animals of a different species (e.g. human voices, predator vocalisations)
Ultrasound	10	Sounds described as ultrasound (>20 kHz)
Specific equipment	8	Sounds described as MRI pulse/noise, saw cutting, banging bucket, metal turnery, ringtones, etc. This category could contain many different sounds.
Alarm bell	7	Sounds described as alarm bells, including fire alarm
Unknown	7	Does not describe the type of sound used (e.g. only calls it noise)
Animal rooms	6	Sounds described as audio recordings of ambient housing environments. These can include broadband noise from fans or ventilation as well as conspecific sounds.
Infrasound	4	Sounds described as infrasound (< 20 Hz)
Slaughterhouse	4	Sounds described as being recorded from slaughterhouse environments. Similar to animal rooms in that there is a mix of ventilation, machinery, and animal sounds, but the animal sounds may be very different due to distress and pain.

5 EFFECTS OF DIFFERENT TYPES OF SOUND ON ANIMALS

5.1 BROADBAND NOISE

Across all sound pressure levels and duration of exposure, broadband noise produces negative or null effects across almost all outcome variables for which there was data (Figure 9). Broadband noise was associated with increased anxiety/fear across all species, negatively affected reproduction in zebrafish and aggression in pigs, and increased GCs in mice, rats, and zebrafish (but not chicken and pigs). Despite increasing GC levels, broadband noise did not have strong effects on WBC counts.

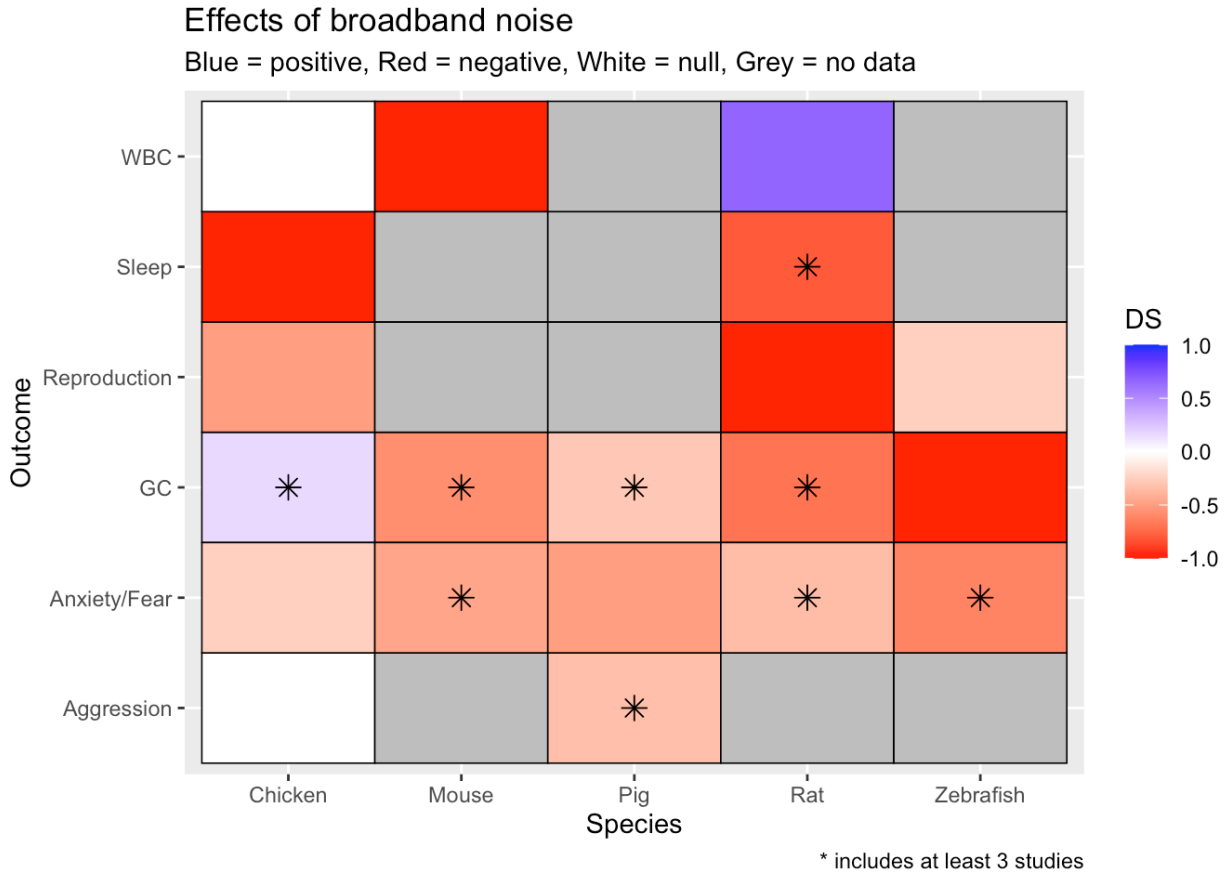

Figure 9. Tile plot of the direction score (DS) for broadband noise per outcome and species. Some studies measure more than one outcome, but the DS is calculated within outcome category. This means the positive effect on GCs but negative effect on anxiety/fear in chickens, for example, may be result from a greater number of studies measuring only GCs than only anxiety/fear.

5.1.1 Mice (n = 19)

Studies examining the effects of broadband noise on mice were concentrated on GC (n = 10) and anxiety/fear outcomes (n = 12), with only one study reporting effects on WBC count. Loud broadband noises over 97 dB for short periods (15 min) or longer periods (three or more hours in one day or across 1-2 weeks) increased GCs in male mice (Farazi et al., 2022; Gao et al., 2015; Kim et al., 2008; Parfitt et al., 2007). GCs were also elevated in male mice when SPLs were lower but over a longer period (i.e. 75 dB for 2h/day over 42 days or 90 dB for 5h/day over 3 or 28 days (Zhang et al., 2025; Zheng & Ariizumi, 2007). On the other hand, female mice appear physiologically more resistant to the effects of broadband noise. Exposure to 100 dB noise intermittently (once every 3 min for 6 hours) over 1 week increased GCs in adult female mice compared to controls, but not

when exposure lasted 4 weeks, suggesting physiological adaptation to noise not seen in males (Kugler et al., 1990). Similarly, exposure to loud or moderate levels (96 dBA, 69 dBC or 80 dBC) of broadband noise for 3 or 14 days had no effect or decreased GC levels in juvenile female mice (Pascuan et al., 2014; Zamora et al., 2009).

Increased GC levels and WBC counts were associated with increased anxiety/fear behaviours in males (females were not tested; Angrini & Leslie, 2012; Farazi et al., 2022; Zhang et al., 2025), suggesting that activation of stress and immune pathways may be behaviourally expressed as heightened anxiety/fear. Male mice exposed to varying levels of broadband noise (75 to 110 dB) over short or long exposure durations (from 1 to 42 days for 2h/day) showed increased anxiety behaviours (Angrini & Leslie, 2012; Farazi et al., 2022; Mahmoodzadeh et al., 2021; Peng et al., 2023, 2024; Samad et al., 2021; Zhang et al., 2025), with one exception where exposure at 90 dB over 30 days had no effect (Mahmoodzadeh et al., 2021). Exposure to 90 dB broadband noise for 15 mins once per day over 15 days had no effect on juvenile female mice (Nishio et al., 2006). Exposure to broadband noise at 70 dB or lower did not affect anxiety or fear in male or female mice (Chikahisa et al., 2007; Hung et al., 2021; Li et al., 2010).

In sum, mice show sex differences in physiological and behavioural responses to broadband noise above approximately 75 dB, with males consistently exhibiting stronger negative outcomes than females. Elevations in glucocorticoids and immune markers co-occur with increased anxiety-related behaviours, indicating that broadband noise is capable of activating physiological pathways that lead to negative affective states. Evidence for sex differences in anxiety and fear responses is suggestive but incomplete due to the limited number of studies in females. Across both sexes, broadband noise exposures at or below 70 dB do not appear to impact anxiety or fear.

5.1.2 Rats (n = 33)

Studies in rats examined the effect of broadband noise on reproduction, GC, sleep, and anxiety (no studies on aggression). The effects of noise on reproduction in male rats was assessed in one study, which showed that litter size, pup survival, and pup birthweight was reduced when males were exposed to broadband noise for 4 h per night over 50 nights (Pirami et al., 2022).

With regards to GCs, 21 studies were conducted. Wistar and Sprague-Dawley strains were used exclusively in studies of GCs and broadband noise in rats, except for one study which used spontaneously hypertensive rat (SHR) strain. Across 15 studies, broadband noise above 85 dB varying from 10 min once a day to several hours per day for 50 days increased GC levels in male and female rats (Abtahi-Eivary et al., 2021; Cui et al., 2016; DeBoer et al., 1988, 1989; Gai et al., 2016; Haleem et al., 2014; Konkle et al., 2017; Morakinyo et al., 2019; Archana & Namasivayam, 1999; Said & El-Gohary, 2016; Sajjadi et al., 2020; Sasse et al., 2008; vanRaaij et al., 1997; Windle et al., 1997) There were some exceptions, where noise above 85 dB had no effect on GC levels (Abtahi-Eivary et al., 2021; Eraslan et al., 2015; Windle et al., 1997) (or even reduced GC levels (Archana, 2014) - these contradictions did not seem to stem from differences in exposure duration, sex, or strain, which varied between studies. Broadband noise presented at 60 dB or less for a prolonged period (at least one hour per day for 21 to 50 days) had no effect on GC levels of male or female rats (Abtahi-Eivary et al., 2021; Chen et al., 2019; Xi et al., 2022), except for one study where exposure to 60 dB for 60 minutes over 50 days increased GC levels in male Wistar rats (Abtahi-Eivary et al., 2021).

Two studies suggested GC adaptation to noise: in one study, levels increased 5-10 mins after acute loud noise exposure (104 dB for 10 mins) and returned to baseline after 40 mins, with GC elevations being attenuated after a second acute exposure more than an hour later (Atkinson et al., 2006).

Acute loud noise exposure at 105 dB lasting 30 mins per day over one week also resulted in dampened GC levels in response to noise compared to noise-naive animals (Campeau et al., 2002).

Changes in GC over the long term were related to changes in white blood cell (WBC) immune markers, whereas acute GC elevations were not. In one study, rats exposed to loud (100 dB) white noise for 4 h/day for 15 or 30 days had lower corticosterone levels and better immune outcomes (N:L ratio) compared to controls (Archana, 2014). On the other hand, samples collected in rats exposed to 100 dB for 4 hours once showed an elevation of GC without a corresponding change in N:L ratio (Archana & Namasivayam, 1999), which is consistent with the broader literature suggesting that acute glucocorticoid elevations are often dissociated from measurable immune changes (Sapolsky et al., 2000). GC changes were also related to anxiety behaviour in one study, where a lack of GC elevations was associated with a lack of effect of broadband noise on anxiety (Chen et al., 2019).

Studies on sleep (n = 3) all showed that constant or intermittent broadband noise above 80 dBA during the resting phase reduces sleep duration and increases sleep disturbance in rats (Nijssen & Snel, 1987; Rabat et al., 2004; Van Twyver et al., 1966). Broadband noise at 90 dBA presented at random intervals for variable durations produced long-lasting sleep disturbance (Nijssen & Snel, 1987; Rabat et al., 2004), indicating that unpredictability of noise exposure prevented animals from habituating to its effects. Broadband noise can also have differing effects on different sleep phases, for example, reducing paradoxical (REM) sleep without reducing slow wave sleep (Van Twyver et al., 1966).

Studies on anxiety in rats (n = 9) shows more mixed effects. Most studies exposed male and/or female rats to broadband noise for 2 minutes to 2 hours once at varying SPLs (65-135 dB). This SPL and duration of exposure was found to increase anxiety or fear in three studies (Gogokhia et al., 2021; Le Moëne et al., 2020; Mzia et al., 2020). Anxiety was increased three days after a 6-min exposure to very loud noise (135 dB but not 105, 115, or 125 dB), but this effect disappeared after 1 month (Song et al., 2025). This indicates that exposure to traumatic levels of noise may have persistent delayed effects that are not long-lasting. Age and repetition of exposure also moderate the effects of broadband noise on anxiety in rats: A single exposure of 96 dB broadband noise lasting 2 h in 7-day-old rat pups had null or positive effects on anxiety while the same exposure in 15 day-old pups increased anxiety (Molina et al., 2016, 2019). Conversely, repeated exposure to the noise (same SPL and duration) had strong negative effects on anxiety in 7-day-old pups but milder anxiety-inducing effects on 15-day-old pups (Molina et al., 2016, 2019). Two studies exposed juvenile or adult male and female rats to 100 dB of broadband noise for 1h per day over 10 days and observed increased anxiety in both ages and sexes (Gogokhia et al., 2021; Mzia et al., 2020).

Overall, anxiety was not uniformly predicted by SPL, as negative, null, or even positive effects were observed when SPL ranged from 65 to 135 dB (Chen et al., 2019; Escribano et al., 2014; Molina et al., 2019). Rather, the effects of broadband noise on anxiety/fear in rats are contingent on several factors (i.e. sound intensity, developmental stage, and exposure history), with brief, extremely loud exposures producing transient effects, whereas repeated or developmentally timed exposures are more likely to result in robust anxiety-related outcomes.

5.1.3 Pigs (n = 9)

Studies in pigs examined effects of noise on GC, aggression, and anxiety/fear. Broadband noise above 90 dB for 5 min or 2h per day over 1 - 4 weeks increased GCs in pigs (Kanitz et al., 2005; Merlot et al., 2011), whereas lower SPL levels between 74 - 85 dB or dBA over similar exposure

durations had no effect (Geverink et al., 1998; O'Connor et al., 2010; Otten et al., 2004). Aggression was unaffected when broadband noise was present at around 80 dB or dBA for 1 month (Nian et al., 2023; Parker et al., 2010) but increased when similar SPL was present for 2 months (Li et al., 2021). This suggests that the effects of broadband noise on aggression may accumulate slowly over time.

The effect of broadband noise on anxiety in pigs was tested in one study. Broadband noise at 80 dBA for five minutes during restraint increased vocalisation rate in piglets (which is used as an indication of distress and/or pain; (Cloutier et al., 2000). Social context modulated the effect of broadband noise on anxiety, as 80 dBA of broadband noise for 20h after weaning increased anxiety in mixed-sex pairs but not same-sex pairs (Cloutier et al., 2000).

In sum, endocrine and behavioural responses to broadband noise are strongly dependent on sound level, exposure duration, and social context in pigs, with GC elevations observed only at SPLs above 90 dB, and aggression emerging only after prolonged exposure. Evidence for anxiety-related effects is limited but suggests that moderate noise levels can increase distress-related behaviours under specific conditions, particularly during restraint or in socially mixed groups.

5.1.4 Chickens (n = 5)

The effects of broadband noise on reproduction, GC, and anxiety were studied in chickens. Adult laying hens exposed to 80 dB(A) of broadband noise for 8 weeks laid fewer eggs compared to controls exposed to 60 dB(A) of broadband noise (O'Connor et al., 2010). Interestingly, younger animals were less affected by noise. GC levels of chicks exposed to 70 or 90 dB broadband noise for 1 to 8 weeks did not differ significantly from controls (McFarlane & Curtis, 1989; O'Connor et al., 2011; Zhao et al., 2022; Zhao, Cui, et al., 2023), although it did reduce amount of time animals spent resting (O'Connor et al., 2011). Furthermore, embryonic exposure to broadband noise at 90 dB compared to 70 dB for the whole or the latter half of the incubation period improved hatching success and decreases GC levels in chicks (Donofre et al., 2020; Zhao et al., 2022) while having no effect on aggressive behaviour (Zhao, Zhang, et al., 2023). The effect of embryonic exposure on anxiety and fear was task-dependent: 90 dB broadband noise decreased anxiety/fear in the tonic immobility task but increased it in open-field tests (Zhao et al., 2022; Zhao, Cui, et al., 2023).

5.1.5 Zebrafish (n = 5)

Studies that exposed zebrafish to very high levels of broadband noise (130 dB re 1µPa) for 1 hour per day over 5 or 14 days showed decreased reproduction in adults and increased GC and anxiety levels in larvae (He et al., 2025; Lara & Vasconcelos, 2021). Adult zebrafish exposed to just one hour of 150 dB re 1 µPa of continuous or intermittent pulsed broadband noise showed greater anxiety/fear in two out of three studies (Shafiei-Sabet et al., 2016; Vasconcelos et al., 2023; Wong et al., 2022). However, broadband noise had anxiety-reducing effects aged zebrafish (over 24 months-old), suggesting age-dependent effects of loud broadband noise on anxiety.

5.2 MUSIC

Overall, music generally produces more positive effects, with only one negative effect on sleep in chickens (although this is a low confidence finding; Figure 10). For studies where we have greater confidence, music reduces anxiety/fear across all species except for pigs (where music does not seem to have measurable effects). Music was often played at lower SPLs than broadband or urban noise.

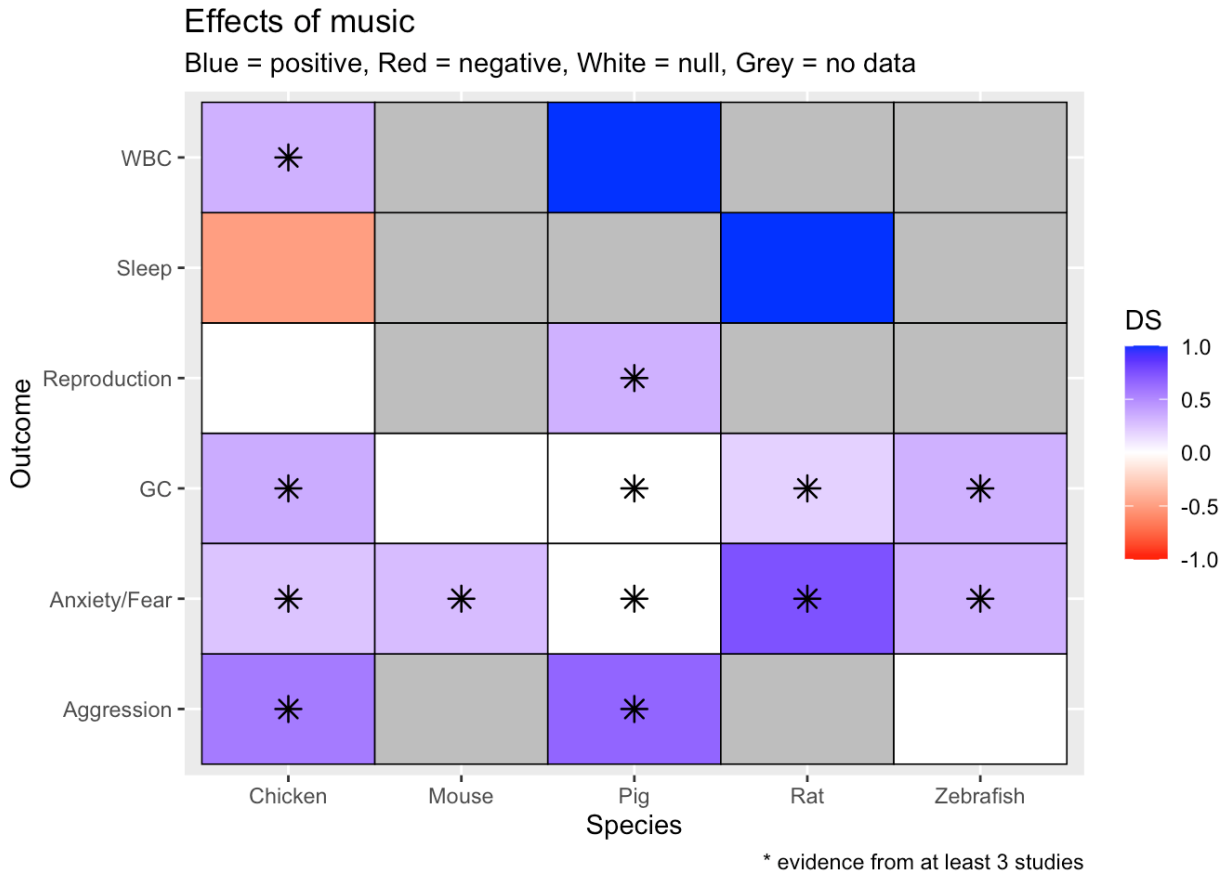

Figure 10. Tileplot of the direction score (DS) for music per outcome and species.

5.2.1 Mice (n = 6)

Studies on music in mice focused heavily on anxiety/fear, with only one study on GC and no studies identified that measured other target variables. Music up to 70 dB had positive effects on anxiety in male and female mice (depending on strain) when exposure duration exceeded 2 weeks (Flores-García et al., 2025; Fu et al., 2025; Li et al., 2010); when exposure durations were shorter, i.e. 3-5 days, no effect on anxiety was observed (Araújo et al., 2020; Hung et al., 2021). Music exposure immediately before standardised tests of anxiety showed sex differences, having stronger anxiety-reducing effects in females than males (Chikahisa et al., 2007).

5.2.2 Rats (n = 8)

The effects of music on GC, sleep, and anxiety/fear in rats were studied. Music for 15 or 30 days at varying intensities (30 - 120 dB) had no effect on rat GC levels in two studies (Dal et al., 2020; Lu et al., 2010), and decreased GC levels in another study (Putra et al., 2023). Music at 70 dB for 12h per day over 55 days increased sleep duration in rats according to one study (Rathod & Vaidya, 2024). Three studies in adult male and female rats found evidence for anxiolytic effects of music between 65-70 dB for 1 day, for 5 hours per day over 15 days, or 30 minutes before an anxiety test (Cruz et al., 2015; de Camargo et al., 2017; Escribano et al., 2014) while one study did not find that music at 70 dB for 12 h/day over 55 days affected anxiety in juvenile rats (Papadakakis et al., 2019).

5.2.3 Pigs (n = 12)

The effects of music on GC, WBC, aggression, and anxiety/fear were studied. Music at 80 dB for 45 mins twice a day over 6 days lead to clearer daily peaks in cortisol patterns in piglets compared to controls, with a concomitant improvement in immune function (lower N:L ratio) and reduction in

aggression (Zapata Cardona et al., 2024). Lower SPL and longer exposure durations (65 dB for 6 h/day over 60 days) reduced free and bound cortisol in piglets after 1 week of exposure, but this effect diminished after 4 weeks, likely due to changes in cortisol binding or clearance (Li et al., 2021). This was also found in another study, where piglets exposed to 75 dB music for 8 h/day over 42 days showed no differences in GC levels compared to controls (Zhao et al., 2021)

Music exposure also influenced aggressive behaviour, although effects were similarly context- and duration-dependent. Music at 70 dB for 4h/day was also found to reduce aggression in young pigs on the day of social regrouping and one day after (Alvarez-Hernandez et al., 2023). Music for 8h/day over 5 days before and during transport decreased aggression in young pigs (Crone et al., 2023). However, music at 65 or 75 dB for 6-8 h/day over 1-2 months in young pigs did not affect aggressive behaviour (Nian et al., 2023; Zhao et al., 2021). In gestational contexts, music at approximately 67 dB for 2 h three times per day over 45–135 days reduced aggressive behaviour in pregnant sows (Lippi et al., 2025; Mendes et al., 2024).

Music for 2h three times a day over 45 days during gestation did not affect the number of piglets born alive but increased the number of weaned piglets and decreased aggressive and stereotypic behaviour of sows (Lippi et al., 2025). However, music during 5 minutes of restraint stress did not affect anxiety, and had similar effects as broadband noise when played for 20h after weaning (i.e. increased signs of distress in piglets; Cloutier et al., 2000).

Altogether, the evidence indicates that music can influence endocrine function and social behaviour in pigs, but effects are strongly contingent on sound level, exposure duration, developmental or reproductive context, and the nature of the stressor; short-term or situational exposure may yield beneficial outcomes, whereas prolonged or poorly timed exposure often leads to habituation or neutral, and in some contexts adverse, effects.

5.2.4 Chickens (n = 16)

Studies examined the effects of music on chicken reproduction, GC, WBC, sleep, aggression, and anxiety/fear. The effect of music on chicken GCs was studied almost exclusively during the prenatal (incubation) phase and immediately after hatching, with exposure durations between 10-42 days and SPL from 65-90 dB. Embryos exposed to music at 90 dB in the incubator did not show increased hatching success (Ahmad-Hanafi et al., 2024; Hanafi et al., 2023), but either showed reduced levels of GCs following food restriction (Ahmad-Hanafi et al., 2024) or temporarily elevated levels later in life (Hanafi et al., 2023).

Music at 70 dB but not 90 dB from hatch to 28 days for 6h/day decreased basal GC levels and lowered fear levels in chicks, but had no effect on aggressive behaviour (Zhao, Zhang, et al., 2023). However, music at 90 dB but not 70 dB from embryonic day 10 until hatching led to decreased basal GC concentrations and fear behaviour compared to control chicks (Zhao et al., 2022) - this higher intensity is probably needed to be heard over the ventilation of the incubator. An additional 3 studies reported that music in early life decreased GC levels in chicks (Ciborowska et al., 2025; Gao et al., 2023; Jin et al., 2025), including one showing that embryonic music exposure temporarily reduced GC levels during social isolation compared to controls (Kauser et al., 2011). One study found that day-old chicks exposed to music at 90 dB for 10h/day over 17 days showed heightened GC responses, whereas exposure at 70 dB had no effect (Chang et al., 2024). In 30-week-old adults, exposure to 75 dB music for 3h twice a day over 24 days had no effect on GC levels (Cao et al., 2024). Postnatal exposure to 75 dB music for 3h/day over 35 days decreased GCs without affecting fear-related behaviours (Tamagi et al., 2024).

Altogether, these studies suggest that the effects of music on glucocorticoids (GCs) and fear-related behaviour in chickens are age- and intensity-dependent, with the strongest and most consistent effects observed following prenatal or early postnatal exposure. Music during incubation or early life (typically 65–90 dB over 10–42 days) frequently reduced basal GC levels and fear responses, although effects on hatching success and aggression were minimal or absent. Importantly, both beneficial (GC reduction) and adverse (heightened GC reactivity) outcomes were reported depending on sound level, timing, and context, while exposure in older chickens generally had weak or no effects, indicating age-related attenuation and possible habituation.

5.2.5 Zebrafish (n = 4)

The effects of music on zebrafish GC, aggression, and anxiety were reported. Exposure to music at 70dB for up to one day decreased GC levels but was not effective at reducing GC levels when fish were socially isolated (dos Santos et al., 2023; Marchetto et al., 2021). Exposure to music at a similar intensity for longer periods lasting 5 to 15 days had no effect on GCs (Barcellos et al., 2018) or affected GCs depending on tempo and instrument: GC was elevated when fast piano music was presented but diminished when guzheng (a traditional Chinese stringed instrument that is plucked) was played slowly, while slow piano or fast guzheng had no effect (Niu et al., 2025).

5.2.6 What kind of music?

Most of the music used in studies was instrumental, i.e. classical Western music from Mozart, Vivaldi, Bach, or Indian Raga, and Chinese classical music. Mozart's K.448 is often viewed has having unique welfare-enhancing properties, however, a recent meta-analysis indicates that this piece does not improve welfare of laboratory rodents, fish, or farmed animals more than any other musical works from Mozart or from other composers (Ilieva, 2025). A few studies retrieved in our search investigated other styles of music and music containing vocals – these generally find that effects are similar to instrumental only. Araújo et al. (2020) used music with a strong percussive component, encompassing many genres/styles (e.g. heavy metal, pop, electronic, country, rap, reggae, reggaeton, romantic, funk) and found that it did not have a significant effect on mouse GC levels or welfare outcomes. Fu et al. (2025) found that Eastern and Western classical music styles, including pieces with vocalisations, had a positive effect on mouse welfare (reduced anxiety). Both instrumental music and Quran recital had positive effects on chicken corticosterone levels (reduced compared to controls) and no effect on welfare (i.e. fear; Tamagi et al., 2024). Pop music reduced aggression in pigs during transport (Crone et al., 2023) and radio improved reproductive outcomes (De Meyer et al., 2020). Different types of music (classical, traditional sufi, and rock) did not affect rat cortisol levels compared to control (Dal et al., 2020). Interestingly, a study on preferences showed that rats avoided radio that included both music and talk shows more than radio segments containing music or talk shows only (Krohn et al., 2011), which could suggest that the transitions between these two types of sounds (music and talk show) is more disturbing.

5.3 URBAN NOISE

Urban noise included sounds described as vehicle noise, traffic or railway noise, construction, environmental noise, airport or aircraft noise. Across species and outcomes, urban noise is relatively consistently associated with negative effects, with some exceptions in chickens (Figure 11). No studies on the effects of urban noise on zebrafish were found.

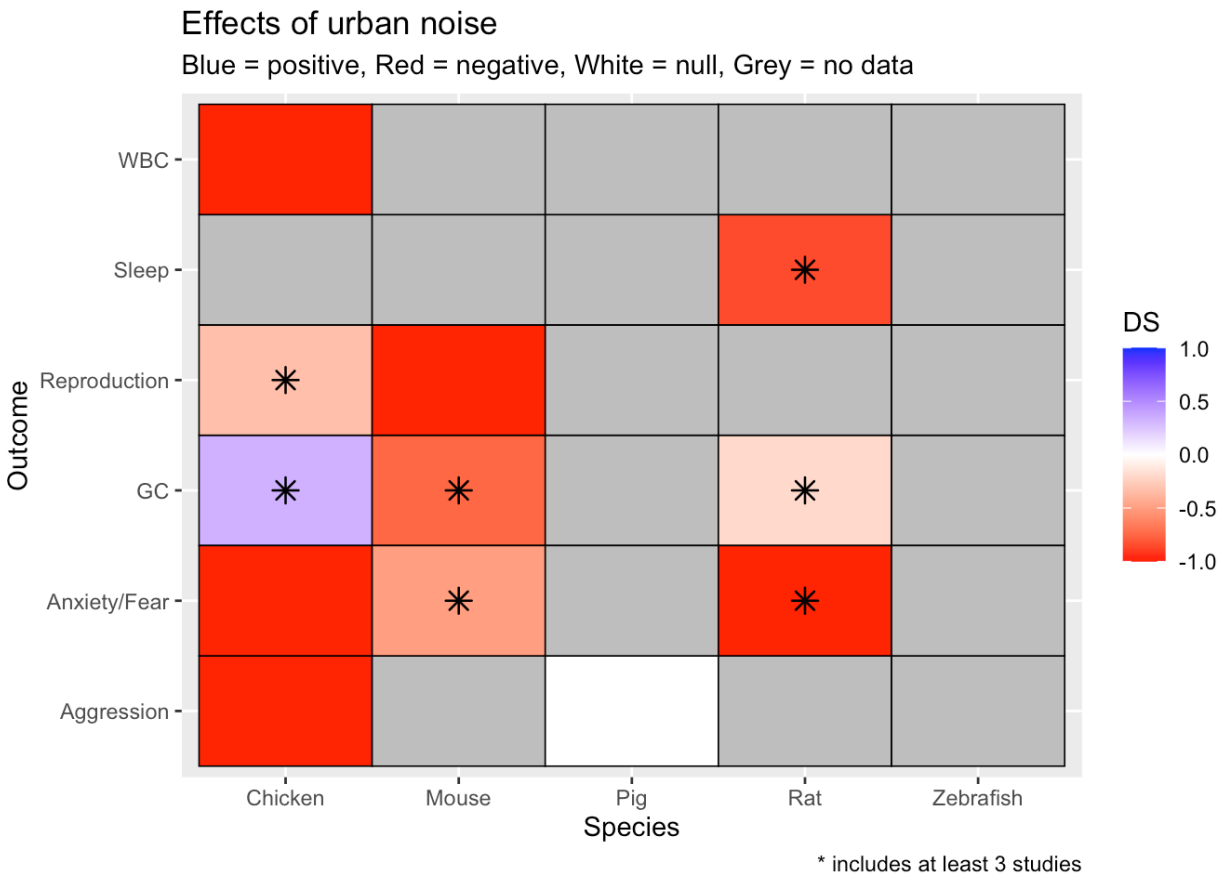

Figure 11. Tile plot of the direction score (DS) for urban noise per outcome and species.

5.3.1 Rats (n = 14)

The effects of urban noise were documented on anxiety, sleep, and GC levels in rats. Chronic exposure (several hours per day for 2 - 4 weeks) to moderate (up to 85 dB) and high (100 dB) levels of urban noise was consistently associated with higher anxiety in male rats (Ardeshiri-Lordejani et al., 2020; Di et al., 2011; Naqvi et al., 2012). Loud urban noise (at least 85 dB or dBA) during the resting phase or continuously for 24 hours for 1 - 9 days also consistently reduced sleep duration and increased wakefulness in male and female rats (Coborn et al., 2019; de Jenlis et al., 2019; Mavanji et al., 2013; Parrish & Teske, 2017; Rabat et al., 2004, 2005). Interesting, some individuals were more vulnerable or resistant to the effects of urban noise on sleep disruption (Rabat et al., 2005), although it is not clear how these phenotypes (vulnerable or resistant) can be identified.

GC responses to loud or moderately loud (70-90 dB) urban noise varied depending on duration of exposure and sex. Male rats exposed to sounds recorded from hospital ICUs for 24h per day for 1, 2, 3, or 7 days only showed an increase in corticosterone after 7 days (Boyacioglu & Ozkan, 2020). In another study, urban noise consisting of sounds such as turbines, horns, hooters increased corticosterone in females after an acute 2 h exposure but not after 12h or 7 or 21 days of continuous exposure (Fernandez-Quezada et al., 2022). On the other hand, the noise increased corticosterone in males only after 7 or 21 days of continuous exposure (Fernandez-Quezada et al., 2022). In an observational study where rats were unintentionally exposed to noise for at least 15 days due to construction near the facility, males and females showed elevated corticosterone responses (Toukh et al., 2014). Finally, a lack of effect of urban noise for 8h/day over 9 days was also observed in males (Houser et al., 2023).

5.3.2 Mice (n = 8)

The effects of urban noise were documented on reproduction, anxiety, and GC levels in mice. Moderately loud to loud construction noise (70 or 90 dBA) for 1h/day for four days during pregnancy increased the number of stillborn pups (Rasmussen et al., 2009). Two studies measured anxiety behaviour of male mice after exposure to combined (highway + high-speed rail) noise or high-speed rail noise alone for 24h noise over 8 weeks at 70 dBA and found that high-speed rail noise increased anxiety but combined highway + high-speed railway noise did not (Di & He, 2013), indicating that noise characteristics such as the maximum sound pressure level, suddenness, and eventfulness of sounds must be taken into account, as well as average sound pressure levels.

Urban noise for 2h per day increased GC levels in mice after 4 days, but not after 1 or 2 days (Münzel et al., 2017). Elevated GCs in males and females were observed when moderately loud (70 dBA or 75 dB) exposure was longer and more chronic, i.e. 8 hours per day for 30 (Jafari et al., 2018; Karem et al., 2021), and associated with higher levels of anxiety in one of the studies (Karem et al., 2021). Male mice exposed to urban noise for 24h per day for 10 days showed a progressive increase in corticosterone levels over the 10 days while animals exposed to urban noise for 12h per day showed an initial elevation during the first five days of exposure, with levels decreasing after 7 days of exposure and resembling the control group after 10 days of exposure (Gonzalez-Perez et al., 2011). A similar finding was reported when mice were exposed to 4 hours of construction noise ranging from 70-100 dB for 50 days: male mice showed an initial increase in corticosterone after the first day of exposure, but not after 15 days (Lee et al., 2024). Unlike males, females in this study did not show an initial increase after the first day, but after 15 days; neither sex had higher corticosterone after 30 days compared to control groups. In both sexes, measures of anxiety were not affected despite the increase in corticosterone levels.

Altogether, results suggest that even moderately loud urban noises are perceived as stressful by male and female mice, reducing reproductive output and can sometimes increase anxiety behaviour. Intermittent but not continuous noise exposure leads to down-regulation of GC response, with sex differences in HPA response. This has implications for where the facility should be located, i.e. away from busy urban areas, and that effort should also be made to reduce this type of noise.

5.3.3 Pigs and chickens (n = 1, 6)

One study exposed young female pigs to 60 dB urban noise for 5 days before transport (8h per day) and during transport (70 mins) but found it did not affect aggressive behaviour during transport compared to controls (Crone et al., 2023). On the other hand, a study in laying hens found that exposure of 75 dB urban noise for 3 hours twice a day for 24 days increased feather pecking duration and frequency (Cao et al., 2024).

5.4 NARROWBAND NOISE

Narrowband noise refers to sounds composed of a single frequency or limited range of frequencies. Our search identified studies on only rats, mice, and zebrafish (none on pig or chicken). For outcomes for which we have data, results show that mice are more sensitive and strongly negatively affected by narrowband noise than rats and zebrafish (Figure 12).

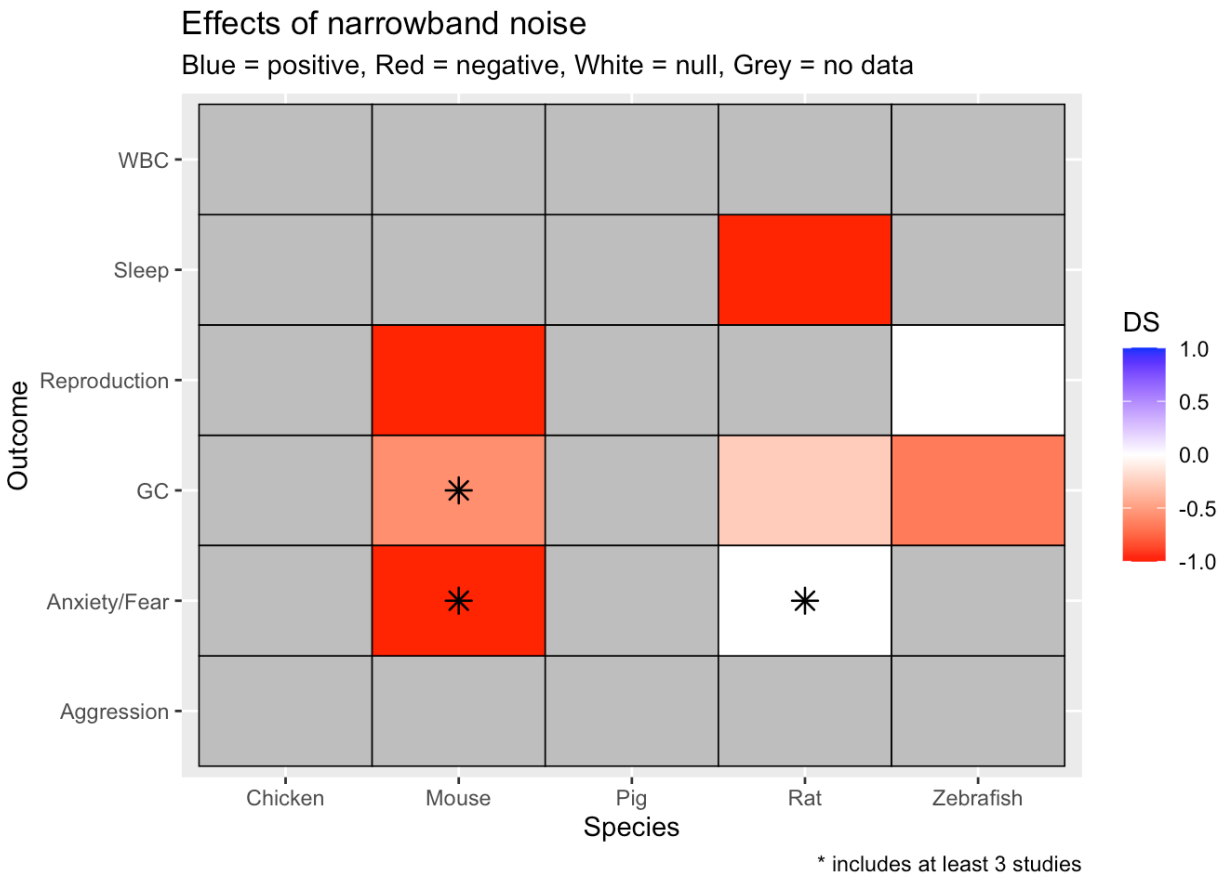

Figure 12. Tile plot of the direction score (DS) for narrowband noise per outcome and species.

5.4.1 Mice (n = 7)

Six studies examined the effects of narrowband noise on mice. Female and male mice exposed to intermittent and unpredictable noise with frequencies between 7-10 kHz at 90 dB for 1 hour showed elevated corticosterone levels compared to controls (Vitale et al., 2005). Pregnant dams exposed to intermittent 3 kHz noise at 90 dB for 24 hours for three days during the last part of gestation also had higher corticosterone levels after the noise treatment and reduced reproductive success (fewer live pups born) and showed increased anxiety/fear behaviours (Jafari et al., 2017, 2019). Furthermore, male offspring of noise-treated dams had significantly higher corticosterone levels and greater anxiety/fear as adults (Jafari et al., 2017). Similarly, 3 kHz noise at 100 dB for 4h per day over 15 days increased anxiety behaviour in young male mice (Samad et al., 2020). Effects may be strain-dependent and circadian-dependent, as exposure to noise at levels that induce acoustic trauma (103 dB of 6-12 kHz noise for one hour) during the resting phase had no effect on GCs of adult male CBA/JRj mice but increased GCs in CBA/CaJ and decreased GCs in CBA/Sca mice (Versteegh et al., 2022). When noise exposure was active phase, GCs increased in CBA/JRj and CBA/CaJ mice but not in CBA/Sca mice that already had high basal GCs. In this case, the immediate elevation of GCs may serve to reduce the damaging effects of noise trauma on the auditory system; hence a lack of elevation in the CBA/Sca mice may be considered maladaptive.

Altogether, these studies suggest that acute and chronic exposure to loud narrowband noise increases anxiety and activation of the stress response system (as measured by GCs) in strain- and circadian-dependent manner, and can have negative transgenerational effects on offspring.

5.4.2 Rats (n = 5)

The effects of narrowband noise on rats are more heterogenous. Four studies provided data points for GC levels, two for anxiety/fear, and one for sleep. Exposure to a 528 Hz noise for 2h per day for 21 days at 100 dB (but not at 80 or 120 dB) had an anxiolytic effect on male rats (Daylari et al., 2019). However, male offspring of pregnant dams exposed to 500 Hz noise at 110 dB(A) for 3 mins per day over 9 consecutive days towards the end of pregnancy showed elevated anxiety behaviours in standardised anxiety tests (Badache et al., 2017). Female rats exposed to continuous loud (130 dB) 2 - 2.2 kHz noise for up to 1h did not show elevated corticosterone; exposure for longer than 2 h for up to 11 hours, however, led to a significant increase (Henkin & Knigge, 1963). At 11.5 to 12h of continuous exposure, corticosterone levels were no longer significantly higher than baseline, and longer exposure for 18, 24, 48h led to a decrease in corticosterone compared to baseline (Henkin & Knigge, 1963). Narrowband noise centred around 8 kHz at 80 or 100 dBA presented under predictable conditions for 3h per day over 30 days transiently reduced sleep in juvenile male and female rats, suggesting that animals were able to habituate to predictable noise disturbance even at high SPLs (Smiley & Wilbanks, 1982).

Together, these results suggests that pregnancy may be a period where females perceive loud narrowband noise as more stressful than other periods, or that repeated loud narrowband noises are more distressing for females than a single longer exposure. The data also suggests that chronic loud noise exposure temporarily reduces sleep and potentially leads to adrenal fatigue (reduced ability to produce testosterone), and downregulation of ACTH receptors in the adrenals or in the brain (reduced sensitivity to signals to produce corticosterone).

5.4.3 Zebrafish (n = 1)

One study reported that zebrafish larvae 3 or 5 days post-fertilisation (dpf) that were exposed to 50 and 2000 Hz noise daily for 90 minutes until 10 dpf showed increased GC levels compared to controls; animals exposed to 432 Hz, however, were no different from controls (Fumakiya & Uggini, 2025).

5.5 ANIMAL ROOMS

Four studies examined how variation in exposure to noise of animal rooms affected health and welfare outcomes: three in chickens and one in rats. Araújo et al. (2018) found that rats housed in noisy rooms (63.4 dBA) for 8 weeks had similar corticosterone levels as rats in quiet rooms (54.6 dBA). Chickens exposed to loud sounds recorded from commercial chicken farms (100 dB) for 30 minutes showed elevated corticosterone levels at 10 and 14 minutes after sound onset but not after (responses measured until 28 minutes; Bedánová et al., 2010). This suggests that noise of animal rooms triggered the HPA axis but did not cause sustained elevation of HPA axis activity.

Chronic exposure to sound of animal housing areas (10h per day for 17 days) at 90 dB, but not 70 dB, increased GC levels in young chicks but had no effect on their fear levels (Chang et al., 2024). Animal room sound at both 70 dB and 90 dB reduced aggression in the chicks, but only 90 dB sound levels decreased feather pecking, while 70 dB sound levels increased it. Sound levels of 90 dB (but not 70 dB) also reduced resting behaviour in the chicks (Chang et al., 2024). Chicken embryos exposed to elevated levels of hatchery sounds (90 dB) showed increased hatching success compared to embryos exposed to lower levels of the sound (70 dB).

Altogether, these findings suggest that lower levels of animal room sounds (up to 70 dB) may not cause prolonged elevations of GCs but could reduce resting behaviour and have nuanced effects on agonistic behaviour. It also suggests that loud sounds may have positive benefits on hatching, although long-term studies are needed to follow up whether exposure to loud sounds has potentially delayed negative effects on health and welfare.

5.6 ALARM SOUNDS

Studies reported playing alarm sounds between 85-97 dB. Acute exposure to alarm sounds for 30 min increased GC levels in rats (Armario et al., 1984). Rats exposed to 3h of alarm sounds for 28 days showed physiological habituation, evidenced by a lower GC response to a 60 min alarm sound compared to naïve controls (Armario et al., 1984). Pigs exposed to alarm and other sounds once every 10 min in the afternoon and night for 4 days did not show higher aggression levels or shorter sleep duration during the night (Gaillard et al., 2023). Exposure to alarm sounds for 2 hours per day for 15 days increased H:L ratio in young chicks, indicating a negative effect on immune function (Lazarevic et al., 2000). Exposure lasting 30 or 45 days continued to produce a negative effect, indicating lack of physiological habituation in this case (Lazarevic et al., 2000). Mice exposed to a 1 min alarm sound during a behavioural test showed signs of startle, but without effects on anxiety or exploratory behaviour (Povroznik et al., 2017).

Altogether, these studies suggest that exposure to loud alarm sounds of at least 30 min over many days has negative consequences, but acute and/or short intermittent exposures may not be perceived as stressful. Nonetheless, more research is needed to determine the threshold at which the alarm duration and frequency begin to be harmful to animals.

5.7 INFRASOUND

Two studies examined the effects of infrasound (frequences below 20 Hz usually imperceptible to humans): one in zebrafish and one in rats. Five minutes of infrasound exposure had no effect on zebrafish welfare (Scatterty et al., 2023), but 1, 2, and 6 weeks of infrasound increased GC levels in rats (Pereira et al., 2021). Thus, prolonged exposures may be required for infrasound to exert negative effects.

5.8 ULTRASOUND

The effects of loud ultrasound frequencies (20-45 kHz at a range of 80-118 dB) were tested in 6 studies (1 mouse, 5 rat). Ultrasound increased aggressive behaviour in mice and altered anxious behaviour depending on strain (i.e. reduced anxious behaviour in CBA mice but increased anxious behaviour in BALB/c mice; Pavlov et al., 2017). Ultrasound increased anxiety in female but not male rats (Abramova et al., 2020; Morozova et al., 2013), showing that there are sex dependent responses to ultrasound. Ultrasound increased aggressive behaviour in rats, but potentially only individuals that have aggressive tendencies: ultrasound did not increase the number of individuals displaying aggressive behaviour but did increase the intensity of aggressive behaviour in animals that did display it (i.e. increased number of attacks, attack duration, decreased latency to attack; Gorlova et al., 2017, 2019). Offspring of pregnant dams exposed to ultrasound for 21 days showed increased aggressive behaviour (Abramova et al., 2020), indicating cross-generational effects of ultrasound exposure in rats. Hence, loud ultrasound appears to increase aggression and anxiety in rodents depending on strain and sex.

5.9 CONSPECIFICS

Five studies examined the effects of conspecific sounds on reproduction and/or GC levels. Squeals of pain and distress produced by pigs presented around 85 dB for 10 mins had no measurable effect on GC levels (Geverink et al., 1998) but in rats sounds of a conspecific distress call (scream) present intermittently 6h per day across 3 weeks elevated GC levels (Xi et al., 2022). Acute playback of a negative emotional sound (22 kHz ultrasonic vocalisations for 3 minutes) also increased anxiety behaviour in rats but playback of a positive emotional sound (50 kHz ultrasonic vocalisation) had no effect on anxiety (Burman et al., 2007). Playback of nursing sounds did not improve reproductive outcomes in piglets (Jeon 2020). Incubation of eggs for 11-12 days with conspecific sounds at 65-72 dB temporarily elevated GCs 72 hours after hatch (Kauser et al., 2011) but did not affect hatching success or GC levels two to three weeks after hatching (Narinç & Aygun, 2023; Tong et al., 2015).

The effects of the sounds of conspecifics in high distress concurrent with broadband and/or urban noise was examined in two studies in chickens. One showed that acute exposure to these sounds at 80 or 100 dB for 10 minutes increased GC levels while chronic exposure for 1 week at 100 dB worsened immune function (increased H:L ratio) but did not affect fear as measured with tonic immobility (Chloupek et al., 2009; Fidan et al., 2015).

Overall, the effects of conspecific sounds were context- and valence-dependent: exposure to negatively valenced conspecific calls (particularly if exposure was long-term) elevated GCs and anxiety and impaired immune function, whereas exposure to positive conspecific sounds generally had no measurable effects. Prenatal exposure to conspecific sounds produced only transiently elevated GC levels.

5.10 HETEROSPECIFICS

Two studies examined the effects of heterospecific sounds on GCs, sleep, aggression, and anxiety. Chen (2019) described the effects of acute exposure to a predator sound in mice and rats at 60 or 80 dB and found that rats reacted anxiously and with elevated GCs to the predator sound at both intensities, while mice did not. Human voices presented intermittently over four days at 85 dB had no effect on sow sleep or aggression (Gaillard et al., 2023).

Together, these studies suggest that responses to heterospecific sounds are species-specific and dependent on the biological relevance of the signal, with predator cues eliciting acute endocrine and anxiety responses in susceptible species, whereas potentially neutral heterospecific sounds such as human voices may have negligible impact on sleep or aggression.

5.11 SPECIFIC EQUIPMENT

MRI pulse sequences presented daily for 90 mins over 1 week had no effects on GC levels or anxiety in male C57BL6/J mice (Almeida et al., 2022). Sounds produced by metallic objects (e.g. metal turnery or machines used to process carcasses) at intensities lower than 105 dB for short (15 mins) or long duration (2 months) also had negligible effects on GC in rats and GC, sleep or aggression in pigs (Farzadinia et al., 2016; Gaillard et al., 2023; Geverink et al., 1998). However, exposure to ringtones from a mobile phone at 76 dBA for 10 mins per day over 4 weeks increased anxiety in male rats (Shehu et al., 2016).

5.12 SUMMARY OF FINDINGS

Across the 177 peer-reviewed studies in rats, mice, pigs, chickens, and zebrafish, the literature review shows that sound has highly context-dependent effects that vary depending on sound category, sound pressure levels, predictability, exposure duration, life-history stage, and timing of exposure (e.g. circadian phase, or proximity to other stressors).

Broadband and urban noise are most consistently associated with adverse welfare-related outcomes. Broadband noise at sound levels commonly used in experimental studies (about 97 dB, which is considerably high) was frequently linked to elevated glucocorticoids, increased anxiety- or fear-related behaviour, disturbed sleep, and, in some cases, reduced reproductive performance. Urban noise (presented at average levels of about 85 dB) also showed negative patterns, particularly in rodents, and was associated with sleep disruption and GC-mediated stress response even when exposure was intermittent. The strong effects of urban noise on GC in mice depended on frequency, timing, and age.

In contrast, music (typically presented at about 70 dB) more often produced neutral or beneficial effects, particularly reductions in anxiety- or stress-related measures. However, these effects were inconsistent and strongly depended on sound pressure level, exposure duration, and timing (e.g. developmental stage or alignment with routine husbandry or testing procedures). Prolonged or poorly timed exposure frequently leads to habituation or null effects. Thus, music cannot be assumed to be universally beneficial.

Evidence for other sound categories (e.g. alarm sounds, conspecific or animal room sounds, ultrasound, infrasound) is limited and uneven, with mixed or context-specific effects. Across categories, the literature does not support a single sound pressure level threshold that reliably predicts welfare outcomes across species.

Taken together, these findings indicate that managing sound in laboratory animal facilities requires an approach that accounts for species-specific hearing, sound characteristics, exposure patterns, and housing or experimental conditions rather than reliance on universal noise limits. Given the heterogeneity of findings and the strong context dependence of findings, stakeholder interviews were conducted to identify practical priorities, constraints, and decision points relevant to sound management in operational laboratory animal facilities.

5.13 LIMITATIONS OF THE LITERATURE REVIEW

5.13.1 Non-specificity of dB weighting.

Many of the studies did not explicitly report dB weighting used; dBZ was assumed unless explicitly stated otherwise. However, it is possible that most values were A-weighted, given that many studies used commercially available sound decibel meters. This lack of reporting has important effects on the interpretation of intensity levels that are harmful, as the difference between dBA and dBZ can be quite large. For example, Araújo et al. (2018) record sound in empty animal rooms and report both A- and Z-weighted dB values: for one of the rooms, it was 63.40 dBA (equivalent to normal conversation) and 87.70 dBZ. Their findings indicate that the physical SPL in the room was high but dominated by frequencies to which humans are less sensitive. As lab facilities contain various types of mechanical equipment and maintain environmental conditions with heating, ventilation, and air conditioning (HVAC) systems, these could contribute both very low or very high frequency

components that increase overall sound pressure level to which measurements using the dBA weighting underestimate.

5.13.2 Choice of outcome measurements.

Results of this review may be biased by the narrowly defined outcomes used in this report, which may bias results. There are numerous other indicators of reproductive capacity, immune function, social behaviour, welfare, and sleep, but papers reporting these outcomes were either not retrieved from the search or excluded in this review because they did not meet inclusion criteria.

5.13.3 Non-specificity of outcomes/indicators.

Sound is only one of many factors influencing reproduction, immune function, social behaviour, sleep, and welfare in laboratory animals. Consequently, these outcomes cannot be mapped one-to-one onto acoustic environments, and changes in any single parameter do not unambiguously indicate a problem with noise in the facility. At the same time, if these parameters deviate from normal in non-experimental contexts, it is important to question whether sound or noise could be a possible cause. Pinpointing whether sound is the cause of welfare concerns is a highly challenging issue. In the Future Directions section, an approach is outlined for devising hypotheses about which welfare indicators are likely to be affected by sound.

The “negative” or “positive” label used to determine the effect of sound in this review should therefore be viewed as non-definitive and context dependent. This is because the outcomes included in this review have pleiotropic effects and because sound may influence distinct processes picked up by behavioural assays designed to measure complex phenomena such as anxiety. For instance, elevated GC levels indicate activation of the HPA axis but this activation is not strictly “stress” related. At SPLs capable of inducing acoustic trauma, elevated GCs can reflect adaptive physiological responses that help cope with the trauma and support homeostasis (Canlon et al., 2007). Thus, elevations in GCs in these contexts could be considered adaptive responses that may or may not also be associated with welfare impairment.

Variation in responses to different standardised tests that capture distinct aspects of anxiety or fear were noted in some studies. Specifically, when anxiety was assessed using both elevated plus maze (EPM) and open field test (OFT), animals sometimes demonstrated clear anxiety responses in one test but not the other. The OFT and EPM both measure anxiety-like behaviour but emphasise different processes, i.e. novelty-driven exploration and arousal versus risk assessment and avoidance of exposed spaces. As a result, sound may increase anxiety in one test but not the other, depending on how it modulates arousal, threat perception, or exploratory motivation (Carter & Shieh, 2015; Kumar et al., 2013).

5.13.4 Heterogeneity in sound categories.

Semantic labels for sound stimuli are useful for categorisation and comparison of the effects of sound in this review, but unavoidably introduce heterogeneity in findings. For example, music as a category contains works that vary immensely in acoustic features (e.g. instrumentation). Even musical works from the same genre or composer may differ substantially and should not be assumed to elicit similar effects in animals (Kriengwatana et al., 2025). Furthermore, distinctions between broadband and narrowband stimuli are to some extent arbitrary because no common standard was applied across studies to define these categories, particularly in the light of species differences in

hearing sensitivity. As a result, studies within a category may be exposing animals to very different sounds, thereby obscuring patterns in the data.

6 STAKEHOLDER INTERVIEWS

Stakeholders were asked about the information they considered essential for inclusion in these Guidelines, existing or potential solutions for noise management in research facilities, the feasibility of such solutions and associated barriers, approaches to noise monitoring and measurement, and unresolved questions relevant to noise management. The interviews identified wide variability in views and highlighted major knowledge gaps regarding management of noise in laboratory animal facilities. Experts agreed that the Guidelines must explain species-specific hearing differences, how animal- and stimulus-based factors influence responses to noise, and a demand for defining loudness limits (potentially frequency- and species-dependent) while also addressing ultrasound detection and the practical use of music or radio.

Main noise sources include trolleys, ventilation systems, human activity, construction, equipment, and vocalisations from conspecifics or other housed species. Sound characteristics relevant to welfare include loudness, frequency (including ultrasound), continuity, predictability, and possibly vibration. Continuous monitoring was generally preferred due to limited baseline knowledge, though opinions varied on optimal locations, weighting scales (A, Z, or R), and whether to track short-term peaks. Maintenance and staff training were considered minimum standards, with other structural or procedural solutions viewed as best practice but often limited by cost and practicality. Experts emphasised that recommendations should allow adaptable ranges with safety margins and noted potential unintended consequences such as staff burden, financial cost, and the risk of music increasing stress. Key research gaps include baseline facility soundscapes, thresholds for negative welfare effects, long-term impacts, masking efficacy, species differences, and strategies for controlling ventilation noise.

7 RECOMMENDATIONS

In this section, previous recommendations are consolidated with the findings of the literature review with regards to the effects of different sound types and intensities on welfare-related outcomes in rats, mice, pigs, chickens, and zebrafish.

7.1 NOISE LIMITS

A sound threshold limit of 85 dBA is likely already implemented by animal research facilities, as this threshold is a part of the occupational health standards for sustained noise exposure for humans in the workplace (Art. V.2-4 of the Belgian Codex on the Welfare at Work). This human-designated limit aligns with thresholds previously suggested for lab animals, as noise levels above the 85 dB threshold are believed to be more likely to induce stress responses or adverse effects (Baldwin et al., 2007; Grandin, 2021; Pate et al., 2013; Sobotka et al., 2003).

The 85 dBA has also been suggested as appropriate for the limits on intermittent, high-intensity peak noise events (Zoontjens, 2012). This is because the 85 dBA reflects limits for human exposure for up to 8 hours per day and may therefore not be suitable as a threshold for 24 hours in animal facilities. Average sound levels below 70 dB over 24 hours are recommended to prevent hearing loss in humans and has been suggested as a goal for animal facilities (Turner & Manker, 2024; Zoontjens, 2012). Chronic exposure to 70 dB noise – well below the “safe” SPL cutoff for humans – produces alterations in the brain that affect sound perception and learning and memory in rodents (Gourévitch et al., 2014; Zheng, 2012), which could motivate a lowering of noise limits for animal research facilities. However, the impacts of low-level noise on animal welfare remain unclear, as the majority of studies identified tested levels at or above this threshold. The average background dB levels reported in studies included in the literature review was 59 dB, yet several reported background SPL exceeding 70 dB. Thus, maintaining average sound levels below 70 dBZ across 24h (Leq 24) is currently advised, although future research may reveal that an even lower limit is desirable.

7.2 FACILITY LOCATION, DESIGN, AND OPERATION

When building a new facility, acoustic considerations should be integrated from the earliest design stages. Location is critical: facilities should be built away from environmental noise sources such as road, rail, or air traffic, sirens, fireworks, or neighbouring animal facilities.

Facility design and structure play an important role in preventing and minimising chronic broadband and urban noises. Within the facility, noisy and quiet zones should be clearly designated and physically separated. For example, cage washing areas should not be adjacent to animal housing rooms. Silent alarms are recommended in place of traditional sound-emitting devices; however, care should be taken to check for unintended ultrasonic emissions.

Within the animal rooms in both new and existing facilities, noise mitigation could target reducing reverberation and sound transmission generated by hard, cleanable surfaces and noise propagation within and between rooms. Research in hospital ICU rooms (which also must comply with strict hygiene standards) shows that installing wall-mounted acoustic panels (designed to be cleanable and adhere to hygiene requirements) and upgrading ceiling material with high performance acoustic tiles substantially reduces reverberation and noise transmission from adjacent rooms and staff

activity in corridors when doors were open (Jonescu et al., 2024). Importantly, these interventions did not necessitate major reconstruction and are therefore likely to be more feasible than soundproofing strategies that require structural modifications (e.g. increasing wall mass through the addition of high-density materials), particularly in older buildings. For older facilities, retrofitting may be necessary: noisy lights, motion sensors, or equipment that produce ultrasonic sounds should be replaced or modified wherever feasible, particularly during renovations or new equipment installations.

In aquatic facilities, reducing noise in tanks may be achieved by increasing the physical distance between fish tanks and pumps, filters, and other noise-generating equipment (e.g. separating tanks and pumps/filters in different rooms), although this may require more powerful pumps that create more noise. Furthermore, this strategy may not reduce vibrations, which for zebrafish may be as important as reducing air-borne sounds because they are sensitive to particle motion as well as sound pressure.

Suitable facility location and design should be combined with appropriate staff training and operational procedures to minimise chronic background noise and intermittent peak events, thereby supporting animal welfare and reliable research outcomes.

7.2.1 Training, education, and communication

As part of facility management strategies, a noise management plan has been recommended (Turner, 2020). In this plan, the institute i) states its position on staff training on how to recognise problematic noise sources and levels and how to mitigate these problems and ii) defines how, by who, when, and how often noise measurements in the facility should be made. The noise management plan should be communicated to staff and assessments of noise levels or characteristics may be reviewed annually to identify problems and determine an appropriate course of action. Examples of practical mitigation measures and operational practices that could be incorporated into the noise management plan are suggested in the following paragraphs.

Limiting loud conversations in animal rooms and corridors, avoiding slamming doors, handling and moving carts in ways that produce less noise are examples of daily practices that may be implemented in the noise management plan that could substantially reduce sources and levels of intermittent noise. Higher compliance with noise mitigation measures identified in the plan may be enhanced by increasing awareness of non-auditory effects of noise on lab animals and species differences in hearing and sensitivity ranges, especially to sounds that fall outside of human auditory thresholds. Explanations of different dB weightings may also help staff to be aware that SPL measurements in the dBA scale reflect perceived loudness in humans but that it may be perceived much louder or quieter by other species. Finally, awareness that sound transmission and attenuation depend on factors such as distance, intensity, frequency, and the properties of surfaces with which sound comes into contact may help staff to make more informed decisions about the placement of cages and sensors for noise monitoring.

Experimental procedures can be distressing for animals, especially if they are unfamiliar with the procedure. As the literature review revealed that repeated exposure to sounds of conspecifics in distress can induce distress in other individuals, animals undergoing procedures should be gradually or incrementally habituated to the protocol when possible. Researchers or animal care staff should be trained to properly guide the animal through habituation protocols.

When noise disturbances are anticipated and cannot be avoided, for example, during construction, facility tours, or maintenance, communication coordination between facility managers, researchers, and external contractors can improve scheduling and reduce unpredictability. Increased predictability may help animals habituate and ensures sufficient recovery periods. Training animals to habituate to routine procedures can further reduce handling-related noise and stress, particularly in social species. In addition, the use of bone-conducting headphones may allow staff to listen to music or communication devices while remaining aware of their own noise production and the broader acoustic environment.

7.2.2 Equipment & facility maintenance

Equipment that generates unnecessary noise or vibration should be replaced or modified wherever possible, for example by fitting trolleys with rubber or neoprene wheels and adding rubber strips to metal-to-metal contact points such as door hinges or gates. Implementing these changes may increase the workload, so roles and responsibilities regarding what will be done, by whom, and when should be clearly discussed and agreed upon with facility staff to ensure coordination and compliance. Conducting noise measurements during routine equipment maintenance may be both convenient and efficient and could be integrated into the noise management plan.

Cage materials and construction can substantially influence the acoustic environment of laboratory animals. While metal cages are durable and widely used, they tend to produce high-intensity, short-duration impact noises during handling or relocation. In contrast, plastic or polycarbonate cages can reduce both impact noise and micro-vibrations. Bedding material can provide indirect cushioning and should be provided to reduce noise if it does not interfere with experimental procedures.

7.3 MONITORING SOUNDS IN THE LAB

Facilities should devise noise management plans that allow identifying the causes of potential disruptive noise and that detail how to best deal with them. Staff discussion and brainstorming of the possible sources of sound within the human hearing range provides a useful starting point. Consideration of sources emanating from animals, humans, equipment, technical systems (e.g. ventilation, alarms) will help to ensure good coverage of different sources. Discussions regarding privacy concerns if continuous monitoring strategies will be used is also essential.

Based on the literature review, long-term, unpredictable sounds appear to have the most adverse effects on animal welfare. It may therefore be advisable to regularly monitor the acoustic environment for these potential sources of noise, including within the ultrasonic range that may be present but unnoticed. Passive acoustic monitoring (PAM) units that continuously record sound pressure levels over a wide range of frequencies (audible and inaudible ultrasonic ranges) can now be purchased at relatively inexpensive prices and operated without specialised knowledge (e.g. Audiomoth or Hydromoth). However, technical expertise is required to analyse and interpret the data collected from recording units. This is probably the largest bottleneck in monitoring acoustic environments of laboratories, where a solution has yet to be reached. If such a system is designed, it should provide insight into the causes of noise as well as indicate whether sounds exceed limits (elaborated in the Future Directions section). Conducting periodic monitoring of acoustic emissions from equipment during routine maintenance may be a useful provisional solution if more continuous and long-term monitoring and analysis systems are not yet in place. Instruments to measure particle motion, on the other hand, are not yet widely commercially available (Popper & Sisneros, 2022).

Sensor placement plays an important role in what sounds are picked up and recorded at a particular location. Stakeholder input and previous recommendations have suggested monitoring noise at room- and/or cage-levels (Turner, 2020). The decision of how many sensors to deploy and where to place the sensors should be up to the facility, given that each facility will face different challenges and room-to-room variation in noise sources and levels. For aquatic facilities, it is important to consider that sound does not transmit well between air and water, hence, measurements of sound in the room will not reflect sounds in fish tanks (Popper & Sisneros, 2022). Monitoring sound at the room level using a single sensor placed in the centre of the room at cage height will provide a coarse idea of what sounds might be reaching animals. Placements of several sensors at cage-level will allow for a more precise reconstruction of the acoustic environment, if desired.However, the amount of work required to analyse the data will also increase substantially. If noise leakage between rooms is suspected, sensors may be placed in adjacent rooms or on opposite sides of shared walls or doors to identify noise transmission pathways and sources.

7.4 ACOUSTIC ENRICHMENT

Even with improvements in facility design and management that reduce chronic and intermittent noise, complete removal of noise will not be possible. Using music to mask residual background noise has been suggested as a method to overcome this problem that has the additional benefit of simultaneously providing sensory stimulation that may be perceived as pleasurable (Kriengwatana et al., 2022).

Based on the literature study, for rats, mice, pigs, and chickens, music can be played between 60-70 dB(Z) on average without negative consequences. The Z-weighting is preferred over the A-weighting because it is less anthropocentric and aligns with other recommendations for noise monitoring (Turner & Manker, 2024). Different styles of music can be played – it is not necessary to play only classical music – but frequent transitions between radio talk shows and music should be avoided for rats. For zebrafish, the data on whether music produces negative or positive effects remains inconclusive, although previous guidelines suggest playing music during isolation for scientific purposes may be acceptable (Vlaamse overheid, Department Omgeving, 2023).

If music is played to mask unwanted background noises, then pieces containing gradual, long, harmonic sounds without sharp attack or sudden onsets may be less disturbing to animals' daily activities (i.e. animals may find these properties easier to habituate to; Blumstein & Récapet, 2009; Snowdon, 2021). There is insufficient evidence that music at night could disturb sleep, or whether its masking effects may be beneficial for nocturnal animals that have their sleep cycle during the day when more noise is expected due to staff activity. Therefore, taking a cautious approach, at present we advise to only play music during animals' active periods to avoid possible sleep disruption, in line with previous recommendations (e.g. National Research Council 2011; Patterson-Kane & Farnworth, 2006).

A final consideration is that music will interact with other sounds present in the animal room, which may produce unintended acoustic effects. This highlights that acoustic enrichment is a dynamic process, and music should not be added without first having a clear welfare goal and taking into account how it might alter the acoustic environment as a whole (Kriengwatana et al., 2022).

8 FUTURE DIRECTIONS

8.1 NOISE MANAGEMENT BEYOND NOISE LIMITS

Although ensuring compliance with noise limits remains the most practical approach for noise management in animal research facilities, it may be worthwhile to consider more nuanced methods of noise management that take into account other acoustic dimensions (Zoontjens, 2012). This is a challenging problem which might be effectively tackled using a soundscape-oriented approach. The term soundscape has been defined in multiple ways across disciplines, ranging from descriptions of the acoustic environment itself to more ecological or perceptual interpretations that emphasise how sounds are organised, used, or perceived (e.g. International Organization for Standardization [ISO], 2014; Farina, 2014). Views of soundscapes as perceptual constructs of a measurable physical phenomenon may be particularly relevant for animal welfare because they emphasise that measurements of sound or SPL at room- or cage-level do not necessarily reflect an animals' acoustic experience (Grinfeder et al., 2022). Therefore, it recognises that reducing or maintaining average noise levels below a specified limit will not necessarily alter animals' perception of the acoustic environment in ways that improve their welfare.

Adopting a soundscape approach and utilising principles from soundscape ecology provides an action plan to determine the relationship between acoustic environments and animal welfare outcomes, potentially leading to the creation of more nuanced management strategies. To move beyond using overall SPL as the main metric of acoustic environment quality, the first step requires identifying sound sources (e.g. sounds originating from human- or animal-related activities), acoustic events that overlap with species-specific hearing ranges and communication signals, and characterising the temporal structure and predictability of the acoustic environment (Farina, 2014). Location, continuity and duration of recordings, and technical limitations of equipment (e.g. whether ultrasonic ranges can be measured) should be planned, considered, and documented. Analysis of recordings at this stage allow inferences to be made about what sounds exist at a particular time and location, but not about what animals perceive or experience.

The next step is to consider the functional relevance, i.e. which ecological functions are plausibly affected by the sound identified or characterised in recordings. This includes masking of communication signals, disruption of daily activity schedules or budget, and the ability to detect acoustic events and use acoustic cues to anticipate events in the environment (Farina, 2014; Kight & Swaddle, 2011). If the identified or characterised sounds are expected to impact functionally relevant activities, then hypotheses about what welfare indicators are likely to be affected can be formed and tested.

In summary, a soundscape-oriented approach provides a methodological blueprint for devising hypotheses about which acoustic signals or features are likely to affect specific welfare indicators. The outcomes of studies following this blueprint may inform the development of complementary evidence-based strategies that extend beyond noise limits and together support improvements in animal welfare.

8.2 INDIVIDUAL-LEVEL FACTORS INFLUENCING RESPONSE TO NOISE

The literature review revealed that the effects of sound were often moderated by strain, sex, and developmental stage. One study even reported noise resistant and vulnerable phenotypes, but did not elaborate on how these phenotypes differed in other characteristic or identifiable ways (Rabat et al., 2005). This suggests that individual characteristics that are relatively stable over time (but not necessarily over life-history stages) could potentially influence how animals perceive and respond to noise and therefore impact their welfare. For example, rats characterised as having active or passive coping styles differ in behavioural and physiological responses to stress-inducing events, including the amount of behavioural flexibility (which is an important determinant of ability to cope with environmental change; Coppens et al., 2010). Zebrafish show stable individual differences in activity levels, which affected their spatial preferences in the tank (Tran & Gerlai, 2013). Research systematically characterising the moderating influences of sex, age, and stable individual differences (personality) are lacking but would provide valuable information on populations and phenotypes that may be more vulnerable or resistant to welfare-diminishing effects of noise.

8.3 VIBRATIONS

Both airborne sound and vibration contribute to the sensory environment of laboratory animals, and may separately affect animal welfare, yet their effects can be difficult to distinguish. In zebrafish, current legislation and guidelines already specify that vibrations should be minimised (Directive 2010/63/EU; Vlaamse overheid, 2023), however, methods to minimise or monitor vibrations and their effects on zebrafish remain largely absent from current guidelines and training programs. Hence, future work is needed to understand how sound and vibration may individually or additively affect animal welfare, and identify suitable ways to measure and monitor vibration sources, pathways, and effects. Practically, clarifying roles and responsibilities regarding noise- and vibration-mitigation within facilities may facilitate implementation and ensure that these issues are embedded in day-to-day operations rather than treated as afterthoughts.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
dB	deciBel
dpf	Days post fertilisation
EPM	Elevated plus maze
GC	Glucocorticoids
H:L	Heterophil-to-Lymphocyte ratio
HPA axis	Hypothalamic-pituitary-adrenal axis
HVAC	Heating, Ventilation, and Air Conditioning
Hz	Hertz
ISO	International Organization for Standardization
n	Sample count
N:L	Neutrophil-to-Lymphocyte ratio
OFT	Open field test
Pa	Pascal
SPL	Sound Pressure Level
WBC	White Blood Cells

APPENDIX

Data used to create tile plots showing the direction score (DS) for broadband noise, music, urban noise, and narrowband noise per outcome and species (Figures 9 – 12).
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