



# Soundscape characteristics of RAS tanks holding Atlantic salmon (*Salmo salar*) during feeding and feed withdrawal

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## ABSTRACT

Behavioural monitoring can provide crucial information on welfare and feeding in aquaculture. Passive acoustic monitoring of behaviour can be particularly useful in recirculating aquaculture systems (RAS), as they often have turbid water that impairs visual monitoring. Currently, little is known about the sounds that make up the soundscapes in RAS tanks holding Atlantic salmon (*Salmo salar*). In this study, hydrophones were used to continuously record the soundscape in eight single tank RAS holding Atlantic salmon parr for 15 days, with the fish in four of the tanks being fasted by feed withdrawal for five days from the sixth to the tenth day. The results show that soundscapes in RAS tanks are affected by feeding. Two main sound sources were identified during feeding in RAS tanks, one related to pellets delivery and the other to fish behaviour. The sound of pellets hitting the water surface had energy concentrated at frequencies between 1.7 and 4.0 kHz, with peak frequency decreasing and amplitude increasing with increasing number of pellets hitting simultaneously. The feeding sounds of Atlantic salmon had energy concentrated at frequencies between 6.5 and 9.4 kHz.

More complex soundscapes were recorded during feeding events. These were characterized by variations in amplitude and frequency that have been described by using acoustic indexes in RAS tanks for the very first time. The Acoustic Complexity Index (ACI), the Acoustic Entropy Index (H) and the Normalized Difference Soundscape Index (NDSI) showed distinct changes in the soundscape related to feeding events; ACI increased while H and NDSI decreased compared to the times in between scheduled feeding times. The sound types identified in this study and the outcomes of the acoustic indices indicate a possibility to monitor system performance as well as fish behaviour in the tank soundscapes in RAS. Soundscape monitoring can contribute to match feeding closer to fish appetite, improve water quality, and reduce risks that deviations in the system performance can have on fish welfare during production.

## 1. Introduction

Feed is an important factor to control in recirculating aquaculture systems (RAS); underfeeding will negatively affect growth and can induce competitive behaviour such as aggression that can lead to poor welfare in juvenile Atlantic salmon (*Salmo salar*) (Cañon Jones et al., 2010; Cañon Jones et al., 2017). Overfeeding on the other hand leads to feed waste (Cho and Bureau, 2001), and can negatively affect the microbial community and a range of water quality parameters (Blancheton

et al., 2013; Rojas-Tirado et al., 2018). As appetite is affected by a series of internal and external factors (e.g. fish size, season, temperature, energetic status, stomach fullness, water quality) (Blanco et al., 2021; Lall and Tibbetts, 2009), it is difficult to accurately provide an adequate amount of feed at the correct time. While models for predicting feed intake exist (Sun et al., 2016), it is particularly difficult to adjust feeding to compensate for short term changes in appetite. A further feed management consideration is feed withdrawal, a procedure where feed is withheld from the fish for varying time periods in order to fast the fish to

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e.g., evacuate the gut or lower metabolic requirements (Noble et al., 2018). This procedure precedes a number of common farming operations during the hatchery phase, including vaccination, splitting of tanks/fish groups, transport between tanks and also transport in relation to smolt transfer (Noble et al., 2018). However, its effect upon various RAS constituents, such as the microbial make-up of the system or bio-filter efficacy, and also the fish at this life stage, are often poorly understood.

Fish behaviour during and around a meal can potentially be used to improve feeding practices in aquaculture as behavioural indicators such as fish orientation, group cohesion or swimming velocity can change depending on feed availability (An et al., 2021; Li et al., 2020; Liu et al., 2022; Zhou et al., 2018). The use of cameras and machine learning show promising results in helping determine the correct time, amount, and duration of feed delivery by monitoring behaviour (An et al., 2021). However, there are potential limitations to this approach, as the high turbidity and high fish density that is common in RAS tanks can make visual monitoring difficult (Zhang et al., 2023).

An alternative to using cameras is to adopt passive acoustic monitoring (PAM), a non-invasive methodology to describe acoustic underwater environments (Lindseth and Lobel, 2018). In aquaculture, feeding systems based on PAM have been developed for several species of shrimp (Reis et al., 2022) and proposed for turbot (*Scophthalmus maximus*) (Mallekh et al., 2003). These systems adjust feed delivery in relation to the known acoustic characteristics (such as duration, frequency range and energy) of sounds produced by the animals while eating. Similarly, sounds associated with feeding have been documented for rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) which both use a combination of ram and suction feeding (Lagardère et al., 2004; Phillips, 1989). In the study by Lagardère et al. (2004), trout produced feeding sounds with the highest signal/noise ratio between 4 and 6 kHz, while Phillips (1989) described short duration “clicking” sounds with energy concentrated below 8 kHz attributed to mastication. Atlantic salmon employ ram feeding for the first 7–10 days after first-feeding before switching to suction feeding (Coughlin, 1991), and it is likely that adult Atlantic salmon exercise a combination of suction and ram feeding when presented with formulated feed pellets (Alfredsen et al., 2007). Given the similar feeding mechanisms, it is likely that feeding sounds of Atlantic salmon resemble those that Lagardère et al. (2004) and Phillips (1989) reported for trout. In Atlantic salmon, suction feeding is associated with pressure transients in the opercular cavity (Alfredsen et al., 2007). This pressure can vary, indicating a capacity to modulate strike intensity depending on the feeding situation (Alfredsen et al., 2007). This in turn could produce sounds with different characteristics. Salmonids have also been documented to produce several other types of sounds linked to specific behaviour such as air gulping (Murphy et al., 2023; Rountree et al., 2018), suggesting that a PAM based monitoring system could yield valuable information.

RAS equipment such as pumps and aerators, as well as human activity near tanks, have acoustic signatures (Craven et al., 2009) that can partially or fully overlap in frequency and time with the sounds related to feeding activity. In addition, sound propagation in tanks is complex (Jones et al., 2019; Okumura et al., 2012; Rogers et al., 2016), and the tank design will affect the sound that is received at a specific point within the tank (Akamatsu et al., 2002; Parmentier et al., 2014). These conditions, with multiple sound sources, resonant frequencies, and reverberations, may represent a challenge to identify the different sound sources that can be recorded in a RAS tank. An alternative to focusing on specific signal types is to adopt an approach that looks at changes in the overall soundscape. For reference, a soundscape is defined as the composition of all sounds of biological, geological or anthropogenic origin, which characterize a specific place in time and space (Pijanowski et al., 2011). Hereafter, the sounds that are recorded within a tank will be referred to as the tank soundscape.

Research on marine soundscapes has mostly focused on biodiversity and on describing the quality of habitats (Lindseth and Lobel, 2018;

Pieretti and Danovaro, 2020), while in aquaculture the focus has been on identifying potentially detrimental noise for the cultured organisms (Bart et al., 2001; Craven et al., 2009; Radford and Slater, 2019; Slater et al., 2020). However, this focus has recently changed. To our knowledge, Rosten et al. (2023) were the first to document that the soundscapes in net-cages change with feeding status of Atlantic salmon, finding significant differences in sound intensity levels between feeding and non-feeding status in the frequency range between 150 Hz and 600 Hz. However, they did not identify the source or production mechanism of the sounds contributing to this difference and suggested future research to look into this, using longer recording periods (>24 h) to extract patterns, and to look into different production phases. Machine learning is also being investigated for determining feeding intensity in aquaculture tanks based on passive acoustic recordings with promising results (Du et al., 2023; Zeng et al., 2023). Nonetheless, studies describing how species-specific sounds and sounds from the rearing environment contribute to the overall soundscape of RAS tanks are still lacking.

The study of soundscapes and its properties may require the calculation of different acoustic metrics and the application of different analytical methodologies (Wilford et al., 2021). Among the acoustic metrics used in soundscape analysis, the power spectral density (PSD) is widely adopted to describe how sound levels varies with frequency (Merchant et al., 2015; Wilford et al., 2021). Additionally, several acoustic indices have been developed to describe specific aspects of the soundscape based on the acoustic properties of a recording (Bradfer-Lawrence et al., 2019). Even though acoustic indices have led to mixed results when used to describe biodiversity metrics (Wilford et al., 2021), they can be adopted to provide an excellent description of soundscape patterns and dynamics (Bradfer-Lawrence et al., 2023). To improve performance consistency of acoustic indices, guidelines have been developed for their application specifically tailored to different terrestrial and aquatic environments (Abrahams et al., 2021; Bradfer-Lawrence et al., 2019; Bradfer-Lawrence et al., 2023; Greenhalgh et al., 2023; Pieretti and Danovaro, 2020). To our knowledge, none of the existing acoustic indices have previously been used to describe variations in soundscapes within an aquaculture setting.

A promising index for analysing changes in the soundscape in fish tanks, including potential signals that can be related to feeding activity, is the Acoustic Complexity Index (ACI; Pieretti et al., 2011), which is based on changes in amplitude from one time sample to the next within a specific frequency band and relative to the total amplitude within that band. ACI has been tested for fish chorusing (Bolgan et al., 2018; Rice et al., 2017), and was recommended by Lindseth and Lobel (2018) to be included in future underwater soundscape analyses.

Another promising index is Acoustic Entropy (H; Sueur et al., 2008b), which calculates a value between 0 (a pure tone with all energy in a single frequency band) and 1 (completely silent or noisy across all frequency bands) to describe specific soundscape. This index indicates how randomly the energy is distributed across time and frequency and has been used to gain insight into daily acoustic activity cycles in temperate ponds (Greenhalgh et al., 2023) and to describe the health status of coral reefs (Williams et al., 2022).

Further, the Normalized Difference Soundscape Index (NDSI) was originally developed to describe the ratio of human-made sounds (anthrophony, theoretically most prevalent between 1 and 2 kHz) to biological sound (biophony, theoretically most prevalent between 2 and 8 kHz) in terrestrial environments (Kasten et al., 2012). It ranges between -1 and +1, with -1 indicating all anthrophony and +1 being all biophony. However, NDSI can be applied to any two frequency bands to compare their relative energy distribution, as shown by Williams et al. (2022) who used it to look at fish sounds compared to snapping shrimp.

A first step towards investigating the eligibility of using acoustic indices to monitor the feeding behaviour of Atlantic salmon in RAS tanks, is to test whether the indices detect changes in the soundscape when feeding stops, and whether these changes are related to specific

sounds. Thus, a controlled study was designed, in which soundscapes of tanks with fish feeding and tanks where feed was not provided were compared. This allowed investigations of how Atlantic salmon feeding affects the soundscapes in RAS tanks by identifying common sound types, and exploring how three acoustic indices (ACI, H and NDSI) correlate with feeding times and times of feed withdrawal.

## 2. Materials and methods

### 2.1. Experimental design and feeding regime

Atlantic salmon juveniles (SalmoBreed SalmoSelect, Benchmark Genetics Iceland) with an average weight of 8.3 g were produced at a commercial RAS facility (Belsvik, Lerøy) from egg prior to their transfer to the Nofima Research Station for Sustainable Aquaculture (Sunnalsøra, Norway) in February 2022. Fish were maintained in a freshwater flow-through system at a temperature between 10 and 12 °C and oxygen saturation above 80%. At the start of the experiment, 2400 individuals with average weight of  $40.1 \pm 0.3$  g (mean  $\pm$  SD) were equally distributed to eight MicroRAS experimental units (300 fish per tank, for further description of the RAS see section 2.2 below). The resulting density equals operationally applicable data for the salmon farming industry. Prior to the start of the experiment, randomly selected individuals ( $n = 5$ ) tested negative for the presence of pathogens (*Candidatus Branchiomonas cisticola*, *Ichthyobo necator*, ILAV, *Piscirikettisia salmonis*, Salmon gill poxvirus). These tests were performed to ensure that these pathogens had no impact on fish appetite and the study outcome.

Fish were acclimatized to the new tanks and RAS for 20 days and kept in freshwater at constant 24 h light during acclimation and the experimental period, following the industry standard for salmon parr production. The fish were fed commercial feed according to their size and expected growth (EWOS Micro, 2 and 3 mm pellet size), using belt feeders (Storvik Akva AS, Norway) that dispensed feed over a period of 42 s every 15 min. Total daily feeding load was calculated based on a growth rate of 1.9%, taken from existing feed tables at the Nofima Research Station for Sustainable Aquaculture (Sunnalsøra, Norway). After the acclimation period, five days (pre-fasting period) were used to establish the baseline soundscapes. Following that period, feeding was stopped in four randomly chosen (lottery) MicroRAS (treatment group, experimental unit  $n = 4$  tanks) for five days (fasting period, 61.3 °C d; 5 D), while feeding continued in the remaining four systems (control group, experimental unit  $n = 4$  tanks). After the fasting period, all eight tanks were again fed for five days (post-fasting period) as outlined in Fig. 1. In each tank, water temperature and oxygen saturation were measured every five minutes during these 15 days, averaging  $12.4 \pm 0.4$  °C (mean  $\pm$  SD) and  $90.3 \pm 3.7\%$  (mean  $\pm$  SD), respectively. Technical staff were aware of tank/treatment allocations during the planning and conduction phases of the experiment, as were the lead and third authors. All authors were aware of tank allocation during data analysis and reporting.

### 2.2. RAS description

The experiment was performed in eight MicroRAS (Landing

Aquaculture, The Netherlands) at the Nofima Research Station for Sustainable Aquaculture, Sunndalsøra, Norway. Each MicroRAS consisted of a single circular Cornell-type dual drain tank made of polypropylene (0.5 m<sup>3</sup>, diameter 97 cm, water level 70 cm) and corresponding water treatment components (Fig. 2), including a drum filter (DF) (Trome, Belgium); moving bed bioreactor (MBBR) placed in two separate chambers, total specific surface area of 750 m<sup>2</sup>/m<sup>3</sup> (RKPlast, Denmark); CO<sub>2</sub>-degasser (Landing Aquaculture, The Netherlands); two Badu 42 pumps (Speck Pumpen, Germany); a TK3000H chiller (Teco, Italy) for temperature regulation; oxygen cone (Landing Aquaculture, The Netherlands) for oxygenation. Systems were primarily operated with the central bottom drain outlet to avoid the sound of running water in the sidewall outlet. An oxygen sensor continuously measured the oxygen levels in the water, which was fitted with a self-cleaning system that released high-pressure air four times a day to avoid fouling of the sensor. The emergency oxygen diffuser was also flushed daily to avoid fouling.

### 2.3. Sound recording

Eight omnidirectional hydrophones (AS-1, Aquarian Audio, USA; linear frequency range of 1 Hz - 100 kHz and response sensitivity of  $-208$  dBV re 1  $\mu$ Pa) were placed singularly in each tank. Hydrophones were routed through a plastic conduit, which was fixed in place through the lid of the tanks and mounted equidistant from the centre and the tank walls, to keep the hydrophones at a specific point 30 cm below the water surface (Fig. 2). The submerged end of the conduits had four slits cut out where the hydrophone was located, allowing the sound to be recorded from all directions. It was also covered with a net to prevent fish from directly impacting the hydrophone. Preamplifiers (PA-6, Aquarian Audio; 26 dB gain) connected the hydrophones to an audio interface (Audiofuse 8Pre, Arturia, France; with 15 dB gain). Audio files (.wav) were continuously recorded (48 kHz sampling rate, 24 bit) using PAMGuard version 2.02.03 (Gillespie et al., 2009). The setup was calibrated before and after the experiment using a pistonphone (Type 42AA, G.R.A.S. Sound and Vibration A/S, Denmark) with a hydrophone adapter (Type RAAQS1, BRC Engineering, USA). Errors in data collection were removed from the dataset before analysis. The same number of recorded minutes from the same time points were removed from all tanks. The remaining minutes that were used for analysis amounted to 27,316, 28,776 and 28,800 min of recording for the pre-, during and post- feed withdrawal periods in each experimental group, respectively.

### 2.4. Identification of soundscape components

To monitor the quality of the recordings, 15 min of audio (starting at 00:00) per tank per day (total 30 h) were visually inspected as spectrograms (FFT size 1024 samples, FFT overlap 50% and Hann window) using the Raven Pro 1.6.4 software (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, 2023), displaying 15 s at a time and zooming or expanding if necessary. Based on these spectrograms, distinct sound types that were commonly present in the soundscape were identified. Sound types were grouped into three categories: i) feeding event (sounds occurring during feeding), ii) RAS (sounds produced by the system), and iii) surface event (sounds occurring in relation to surface activity). Naming of sound types directly related to fish

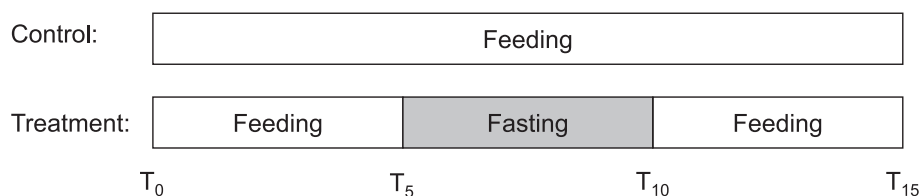


Fig. 1. Overview of experimental design. The control group ( $n = 4$  tanks) was fed throughout the duration of the experiment (15 days,  $T_0$ - $T_{15}$ ). The treatment group ( $n = 4$  tanks) was fasted by feed withdrawal for five days ( $T_5$ - $T_{10}$ ), and were otherwise fed following same feeding regime as the control group.

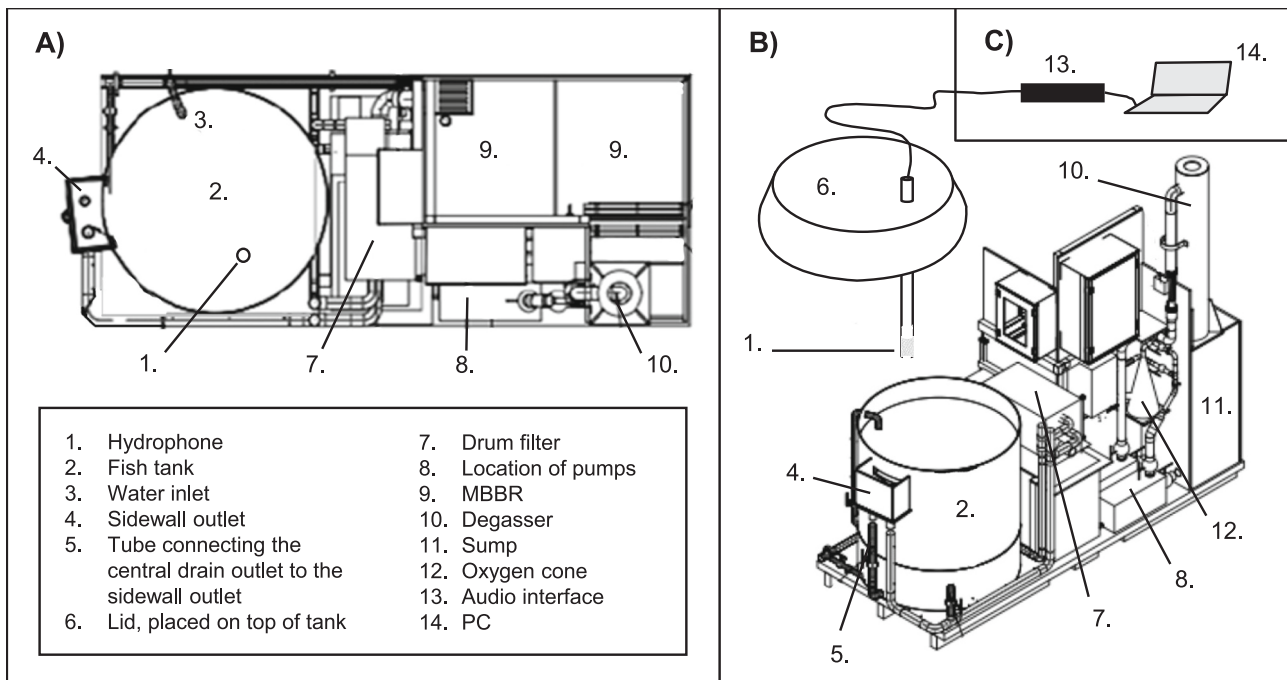


Fig. 2. Schematic overview of the MicroRAS used in the experiment, with the location of key water treatment components highlighted, modified from the operating manual of the Landing MicroRAS. A) Top-down view of the RAS. B) The lid of the tank with plastic conduit through which a hydrophone was installed, and the RAS from a slightly tilted sideways view. C) Room with audio recording equipment.

behaviour was based on similarities shared with the sounds described by Rountree et al. (2018) where applicable, with the following exception: The sound named “surface” or “surface event” is termed “splash” in the current study, while the series of sound named “surface event sound series” is simplified to “surface event” in the current study. The other sounds linked to fish behaviour, which differed from those described by Rountree et al. (2018), were named according to a generic description of the sound.

In addition, after acoustic indices were calculated and plotted (see section 2.5 Data analysis), selected audio files were similarly inspected to confirm which sounds were associated with the trends in the acoustic indices. Three examples per tank of each of the most distinct sound types, with one of the sound types only found in six of the eight tanks, were randomly selected from the pre-fasting and fasting period for analysis of acoustic characteristics. This analysis was performed in Raven Pro and included high-pass filtering (250 Hz) before measuring selected acoustic parameters, following Charif et al. (2010) (Table S1).

To identify sounds related to feed delivery, GoPro cameras were set to film the belt feeders dispensing feed for 48–96 min per tank during the post-fasting period (total 11 h 48 min) while audio was recorded as described in section 2.3. The video was investigated for events where pellets fell into the tank from the feeder. The events were labelled according to the number of pellets falling from the feeder (“Low”, “Medium”, and “High” number of pellets, corresponding to <5, 5–15 and > 15 pellets, respectively). The identified times of the events were subsequently investigated in the audio recordings, by both audio and visual inspection of the spectrograms. Further audio analysis has been performed in Raven Pro to characterize the sound events of the pellets hitting the water surface (Table S2).

## 2.5. Data analysis

Power spectral density (PSD) spectra were calculated in Raven Pro using the “Selection Spectrum” feature on the forementioned spectrogram settings following Charif et al. (2010). These spectra were calculated from four consecutive 15-min sections from each tank per period.

The uncalibrated PSD values were exported from Raven Pro, then calibrated and averaged on a linear scale per group, before being converted to dB re  $1 \mu\text{Pa}^2\text{Hz}^{-1}$  and plotted using R (version 4.2.1). This was to establish how cross-frequency sound levels changed when feeding was stopped.

Means and standard deviations for acoustic characteristics of the identified sound types were calculated in R, using the functions “meandB” and “sddb” from the package seewave version 2.2.0 (Sueur et al., 2008a) for the dB values.

All acoustic indices were calculated using R. Before calculating indices, a 250 Hz high-pass filter was applied to all sound files (using the function “fir” from R package seewave version 2.2.0, modified to maintain 24 bit depth after filtering). The acoustic indices ACI, H and NDSI were calculated with R package soundecology version 1.3.3 (Villanueva-Rivera and Pijanowski, 2018). For calculation of ACI, four frequency bands were chosen based on sounds recorded in the tanks, as well as the acoustic characteristics of feeding activity by brown trout and rainbow trout as reported by Lagardère et al. (2004). Thus, ACI was calculated with a cluster size of 60 and the following minimum and maximum frequencies (in Hz): i) 250 and 1700 ( $\text{ACI}_{\text{low}}$ ), chosen to be below the frequencies of the sounds of pellets hitting the surface and of feeding sounds; ii) 1700 and 4000 ( $\text{ACI}_{\text{pellets}}$ ), selected to include sound energy of the pellets hitting the water surface; iii) 4000 and 8000 ( $\text{ACI}_{\text{feeding}}$ ) expected to contain fish feeding sounds; and iv) 8000 and 24,000 ( $\text{ACI}_{\text{high}}$ ) chosen to include high frequency sounds up until the highest recorded frequency in this study. For all other ACI settings, the default options were used. For the index H, all default settings were used. The index NDSI was calculated with the frequency band for “anthrophony” set at 1700–4000 Hz (corresponding to  $\text{ACI}_{\text{pellets}}$ , see above) and the frequency band for “biophony” set at 4000–8000 Hz (corresponding to  $\text{ACI}_{\text{feeding}}$ , see above), otherwise default settings were used.

All figures featuring acoustic indices were created using R package ggplot2 (version 3.4.0). Spectrograms and linked oscillograms were plotted with Raven Pro 1.6.4 (Hann window, window size = 1024, overlap = 50%, DFT size = 1024).

## 2.6. Ethics statement

Animal use in this experiment was in line with the Norwegian Animal Welfare Act (see <https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/>). This study was approved by the animal experimentation administration (Forsøksdyrforvaltningen) in the Norwegian Food Safety Authority (Mattilsynet), ID: 29322. Personnel involved in conducting the experiment were either certified according to Felasa C requirements and/or mandatory requirements according to the Norwegian Food Safety Authority. The PREPARE guidelines (Smith et al., 2018) were utilised during experimental planning and the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020) were followed in relation to reporting.

## 3. Results

### 3.1. Tank soundscape components

A characteristic part of the tank soundscapes were sounds of a near constant frequency and amplitude that were present when the RAS was running, visible in peaks corresponding to 8000, 12,000, 13,500, 16,000 and 20,000 Hz (Fig. 3). These contributions to the soundscape are also visible as horizontal lines in all spectrograms in this paper (Fig. 4, Fig. 5). The highest sound levels were below 250 Hz (Fig. 3), which were filtered out for all other analyses. During fasting, the treatment group had mostly lower sound levels between 350 and 13,000 Hz (dotted line in Fig. 3). The RAS components that had a distinct impact on the soundscape were the drum filter flushing (DF) (Fig. 4A), which mainly caused the peak at 281.25 Hz as shown by the PSD analysis (Fig. 3), the self-cleaning of oxygen sensors (Fig. 4B), and the emergency oxygen diffuser flushing (Fig. 4C). Sounds related to emergency oxygen flushing were only found in six of the eight tanks. In addition, water running in the sidewall outlet created short duration pulses (Fig. 4D), which had a prominent impact on the PSD of the fed group (control) during post-fasting between 10 and 14 kHz (full line in Fig. 3). When fish breached the surface to jump or eat, they made splashes (Fig. 4E and F), often followed by a combination of sounds such as fast repetitive tick-

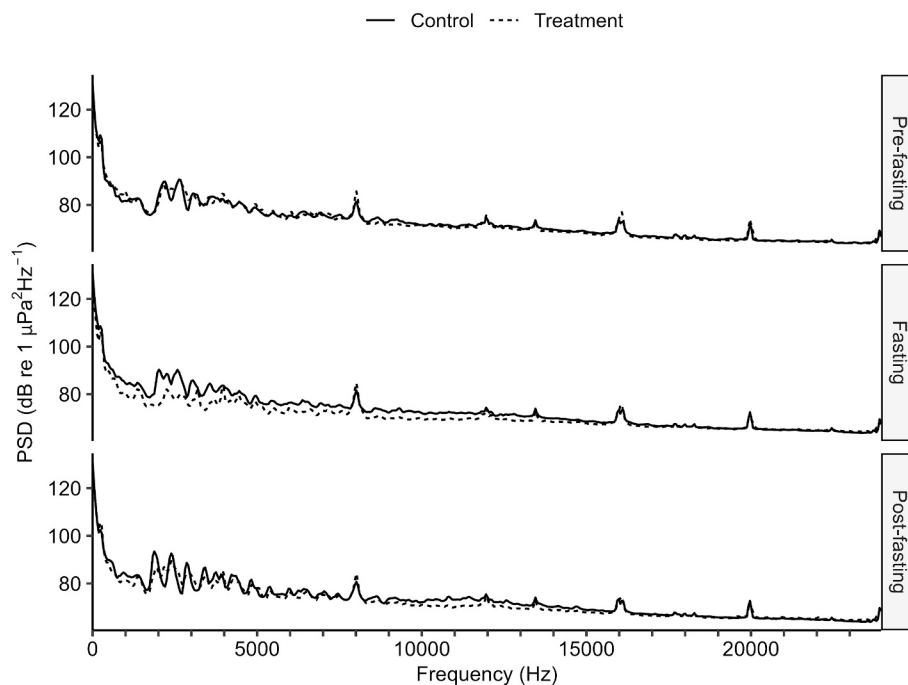
like (FRT) sounds (Fig. 4E), a downsweep (Fig. 4F), miscellaneous air movement sounds (Fig. 4F), and often ending with a snitch (Fig. 4E). The series of sounds starting with a splash are hereafter referred to as surface events.

### 3.2. Feeding events and sounds related to salmon behaviour

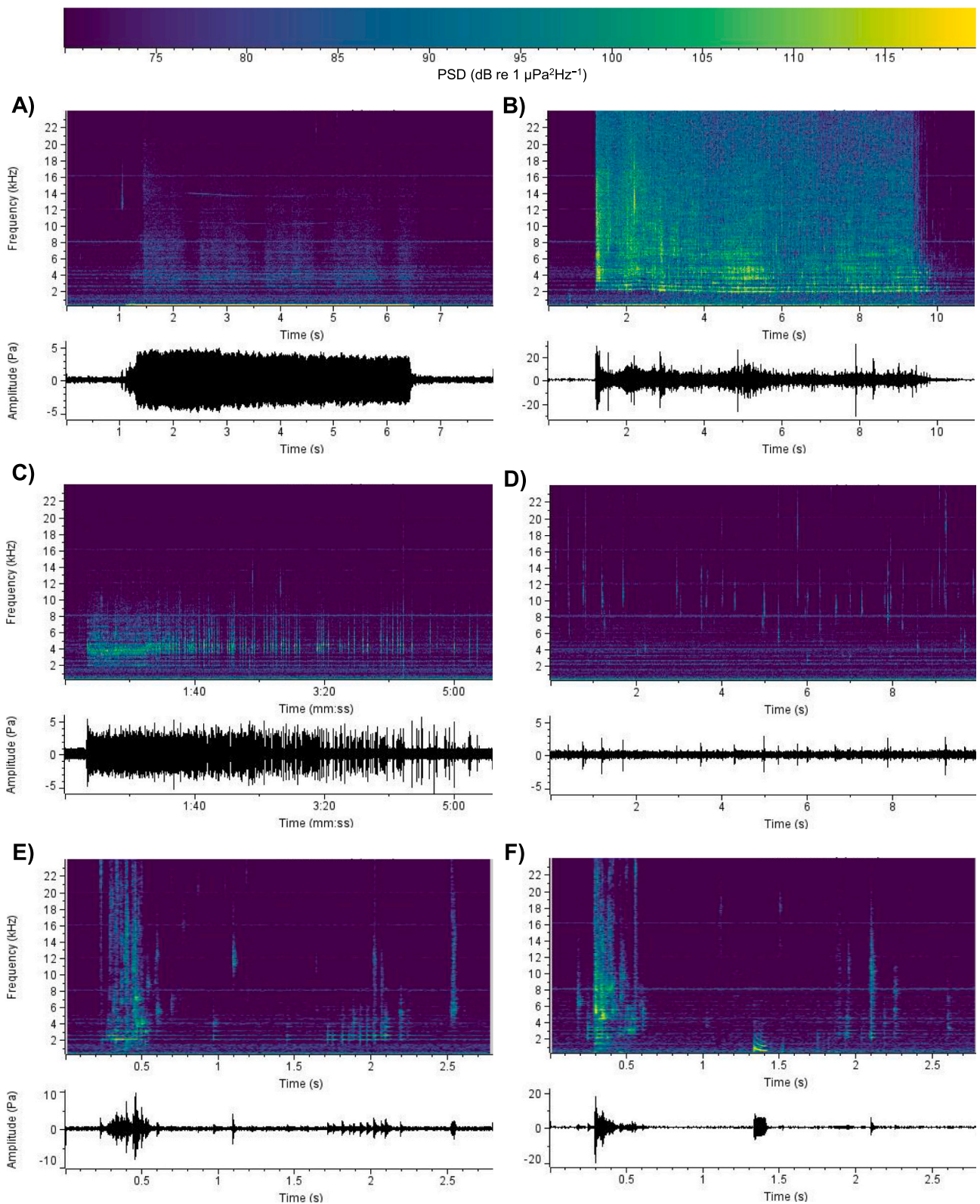
Prior to feeding, the soundscape was often monotonous, and sounds were mostly easy to distinguish. Feeding caused a distinct change in the characteristics of the tank soundscape (Fig. 5A). It started with pellets hitting the water surface (Fig. 5B), followed by a variety of sounds overlapping in time and frequency (Fig. 5C). These series of sounds are hereafter referred to as a feeding event. Many of the sounds during the feeding events were short broadband pulses sounding like snaps or clicks and are assumed to be caused by the fish feeding. Other sounds related to fish behaviour were also present, some of which were easy to distinguish, for instance splash sounds when fish jump or breach the surface (Fig. 5C, between 19 and 19.5 s). The origin of other sounds were difficult to determine with certainty. One such example of a sound of unknown origin is the sound below 1 kHz (Fig. 5C, at 21 s). After feeding, the soundscape reverted to a similar state as before feeding (Fig. 5A, after 41 s).

### 3.3. Sound characteristics

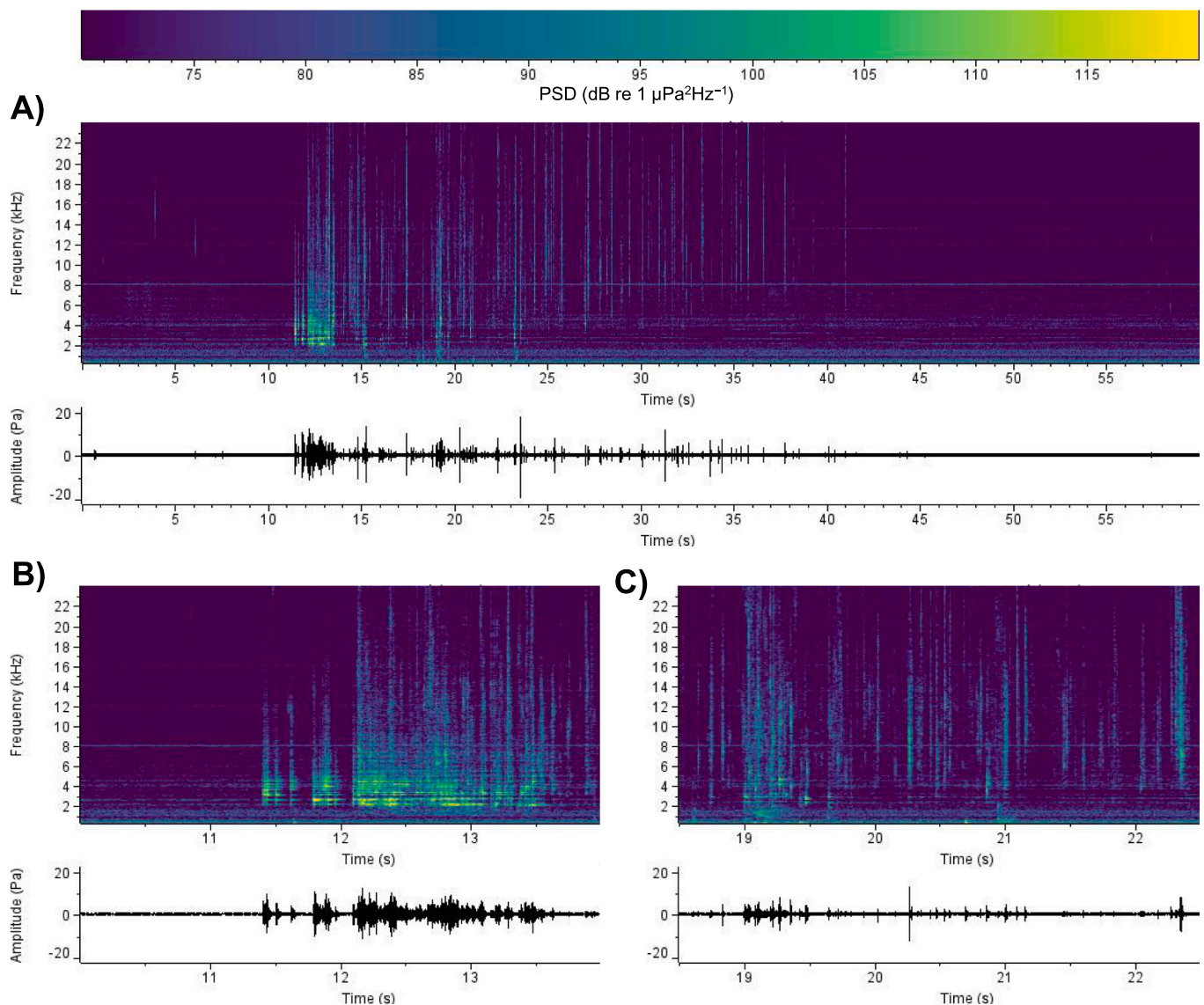
Averages of selected acoustic characteristics of sounds were similar for the control (fed) and treatment group (Fig. 6). Sound characteristics are therefore presented here in general, unless specified by group. Feeding events (FE), starting from the time the first pellet hit the water until the end of feeding sounds, were measured as a single sound to give an overview of the full feeding event (Fig. 6). Two sounds were uniquely associated with feeding events; pellets hitting the water, and short duration pulses named Feeding (Fig. 6). Sounds of the pellets hitting the water surface decreased in peak frequency and incrementally increased in amplitude with increasing number of pellets hitting simultaneously, but at least 70–90% of the energy of the sounds was within the 1700–4000 Hz band (Fig. 6). The Feeding sounds varied in terms of their



**Fig. 3.** Variation in sound levels at different frequencies (Power spectral densities, PSD) in fish tanks averaged over replicates and time before, during and after a period without feeding. Dotted lines indicate the treatment group and solid lines indicate the continuously fed control group.



**Fig. 4.** Spectrograms (250–24,000 Hz, Hann window, window size = 1024, overlap = 50%) with a common colour bar (top) and linked waveforms demonstrating the sound of A) a DF flushing (1.1–6.4 s); B) the self-cleaning of the oxygen sensor by the release of a burst of high-pressure air; C) the emergency oxygen diffuser flushing, starting with a burst of bubbles followed by a tail of bubbles slowly decreasing in occurrence frequency; D) water running in the sidewall outlet; E) a surface event where a fish breached the surface causing a splash (0.2–0.6 s), followed by FRT-like sounds (1.7–2.2 s) and a snitch (2.55 s); F) a surface event where a fish breached the surface causing a splash (0.2–0.6 s), a downsweep (1.3–1.4 s) and miscellaneous air movement sounds (1.7–2.6). For audio visual examples of each figure, see supplementary materials.



**Fig. 5.** Spectrograms of a single feeding event (250–24,000 Hz, Hann window, window size = 1024, overlap = 50%) with corresponding waveform underneath, showing A) a total sound sample of 60 s duration, which includes pellets hitting the surface (from 11.4 to 13.5 s), subsequent feeding activity (around 12.1–40.5 s), and its cessation. Spectrogram B) highlighting part of the same feeding event as in spectrogram A, zoomed in to the onset of the feeding event where the main sound source is pellets hitting the water surface (around 11.4–12 s), and a period with both pellets hitting the surface and the start of fish feeding activity (around 12.1–13.6 s). Spectrogram C) showing part of the same feeding event as in spectrogram A, focusing on fish feeding activity. The short duration pulses with a frequency range between 4 and 24 kHz are likely due to fish capturing pellets. At 19–19.5 s, there is a splash caused by a fish breaching the surface, and at 22.3 s there is a sound that might be a snitch. For audio visual examples of each figure, see supplementary materials.

amplitude and frequency range, were short in duration with most of the energy present at frequencies above 4000 Hz (Fig. 5C, Fig. 6).

Sounds originating from RAS components were present at most frequencies (Fig. 6). >95% of the energy in DF flushing sounds has been recorded below 465 Hz (Fig. 4A, Fig. 6), while for emergency oxygen diffuser flushing, the energy was concentrated mostly between 4000 and 8000 Hz. For the self-cleaning of the oxygen sensor, the energy was concentrated under 14,000 Hz. Self-cleaning of the oxygen sensor and the DF flushing was consistent in duration, while emergency oxygen diffuser flushing duration varied (with a standard deviation of 98 s in the treatment group).

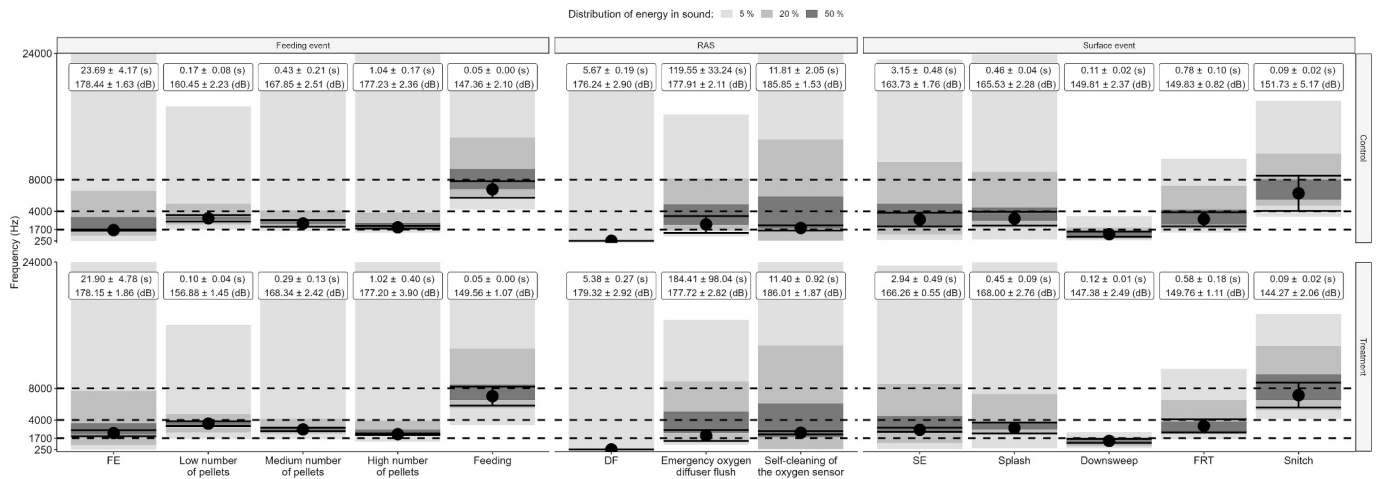
Surface events (SE), including at least a splash, fast repetitive tick, also termed FRT, and a snitch (Fig. 4E), were measured as a single sound in addition to the sounds related to such events (Fig. 6). Downsweeps and miscellaneous air movement sounds sometimes occurred within surface events (Fig. 4F), however not as frequently as FRTs and snitches. The characteristics of sounds occurring within a surface event varied,

with splashes having the highest total energy, downsweeps being concentrated at frequencies below 1700 Hz, fast repetitive ticks occurring mostly between 1700 and 7000 Hz, and snitches varying in amplitude and frequency but concentrated mostly between 4000 and 14,000 Hz.

The only commonly occurring sound types that were not measured and thus not included in Fig. 6 are those of water running in the sidewall outlet (Fig. 4D) and miscellaneous air movement sounds (Fig. 4F) due to inconsistencies in the frequency range and duration between pulses, making it difficult to differentiate them and to ascertain the source of the sound.

### 3.4. Acoustic indices

The four ACI frequency bands were differently impacted by the identified tank sound sources: i) 250–1700 Hz ( $ACI_{low}$ ), was primarily affected by fish activity and DF flushing, ii) 1700–4000 Hz ( $ACI_{pellets}$ )



**Fig. 6.** Energy distribution over frequencies of sound types related to feeding events, RAS and surface events in the control (top row) and treatment (bottom row) groups. Dark grey boxes contain 50% of the sound, mid grey boxes 20%, and light grey boxes 5%, with top and bottom of the bars indicating maximum and minimum frequency of the recorded sound (frequency range: 250–24,000 Hz). Black dots and whiskers indicate peak frequency (mean  $\pm$  SD, Hz). The white boxes contain duration (mean  $\pm$  SD, s) and total energy (mean  $\pm$  SD, dB). Horizontal dashed lines indicate frequency limits used for acoustic index analysis. Sound types are shown on the X-axis and FE = feeding event, Low number of pellets = <5 pellets, Medium number of pellets = 5 to 15 pellets, High number of pellets = >15 pellets, DF = drum filter flushing, SE = surface event and FRT = fast repetitive tick.

was impacted mostly by the energy of the sounds of pellets hitting the water surface, iii) 4000–8000 Hz ( $ACI_{\text{feeding}}$ ) included most of the feeding sounds, and iv) 8000–24,000 Hz ( $ACI_{\text{high}}$ ) was characterized by both the feeding sounds and by the sounds of water running out of a sidewall outlet in the tanks.

All investigated indices showed temporal patterns, with noticeable changes correlated with feeding. In particular, the ACIs increased (Fig. 7) and both the acoustic entropy index (H) and NDSI decreased (Fig. 8) in association with feeding events.

During the pre-fasting period, mean ACI was found to increase during feeding events, for all four frequency bands that were investigated ( $ACI_{\text{low}}$ ,  $ACI_{\text{pellets}}$ ,  $ACI_{\text{feeding}}$ , and  $ACI_{\text{high}}$ ) (Fig. 7). While this increase disappeared in the treatment group during the fasting period, it again reappeared during the post-fasting period when feeding was reintroduced (Fig. 7).

The value of the acoustic entropy index (H) decreased during feeding (Fig. 8A). However, these changes associated with feeding were less pronounced in comparison with the relative changes in ACI, particularly during post-fasting. During fasting, the overall mean H values increased in the treatment group, also in between the scheduled feeding periods. Finally, values of the NDSI index also decreased during feeding times (Fig. 8B), and like H, an overall increase was found for the treatment group during fasting even outside of the scheduled feeding periods.

Other trends, not conclusively correlated to feeding events, also appeared in the analysis of indices. There were indications of a diurnal pattern, with  $ACI_{\text{pellets}}$ ,  $ACI_{\text{feeding}}$  and  $ACI_{\text{high}}$  displaying an increase between 06:00–17:00 particularly during pre-fasting (Fig. 7B, C and D), and H and NDSI displaying a decrease at similar time points (Fig. 8A, Fig. 8B). The control group also had an overall increase in mean  $ACI_{\text{high}}$  from the pre-fasting period until the end of the post-fasting, which was not seen in the treatment group (Fig. 7D, Fig. S1). The acoustic entropy index (H) displayed a decrease till 08:00 and after 20:50 in the post-fasting treatment group (Fig. 8A). NDSI displayed an increase for a few minutes around 04:05, 10:05, 16:05 and 22:05, most prominently in the treatment group (Fig. 8B). Similarly, an increase is present for  $ACI_{\text{feeding}}$  at the same timepoints, also most prominently in the treatment group (Fig. 7C). There was also a concurrent change in all indices for the post-fasting period between ca. 06:30–10:00 (Fig. 7, Fig. 8).

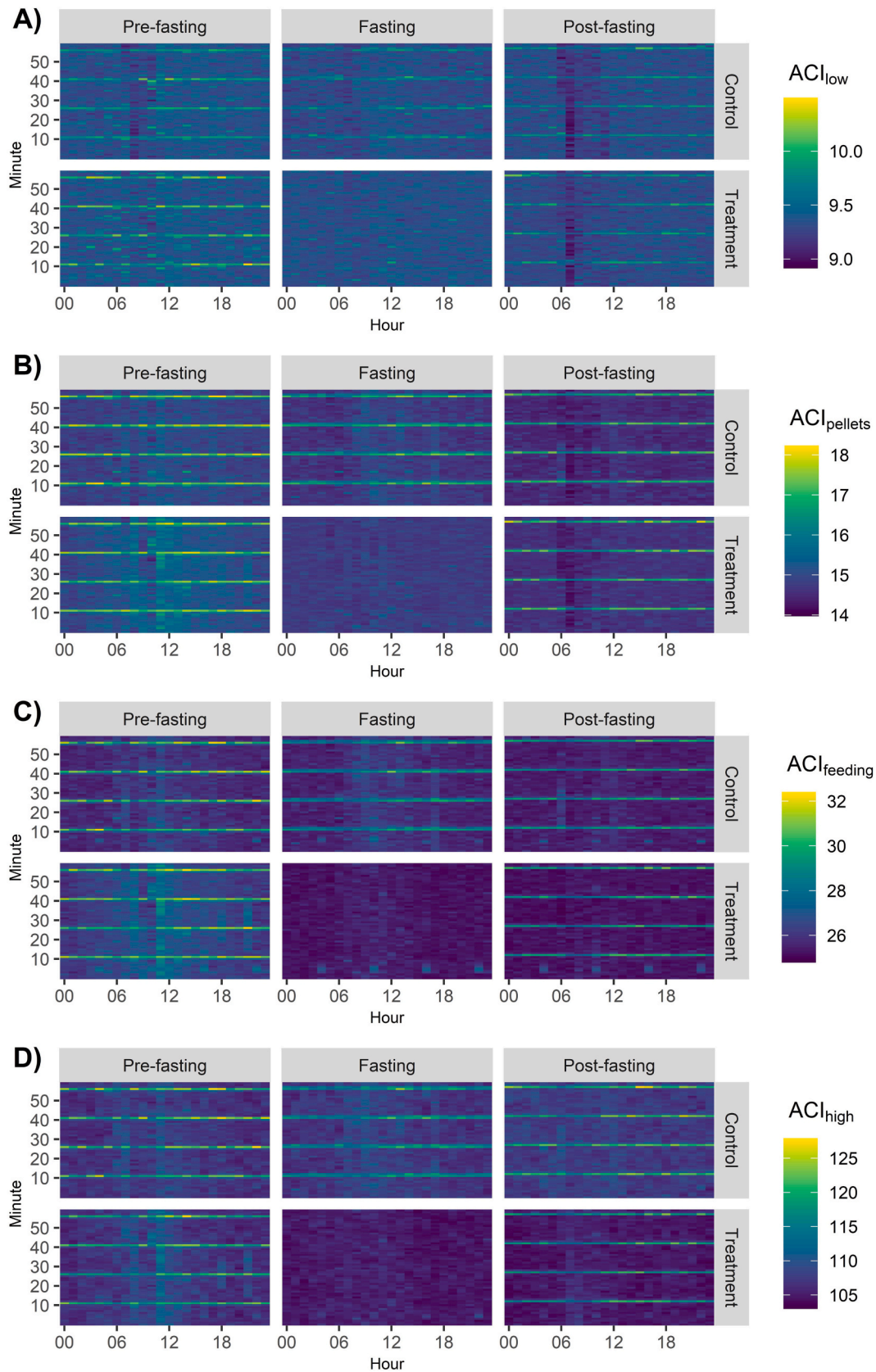
## 4. Discussion

This study shows how feeding affects the soundscapes of active RAS tanks holding Atlantic salmon. As far as the authors are aware, it is also the first to successfully apply acoustic indices to study the characteristics of sounds that comprise RAS tank soundscapes. The results also demonstrate the potential for applying acoustic indices to identify trends in aquacultural soundscapes.

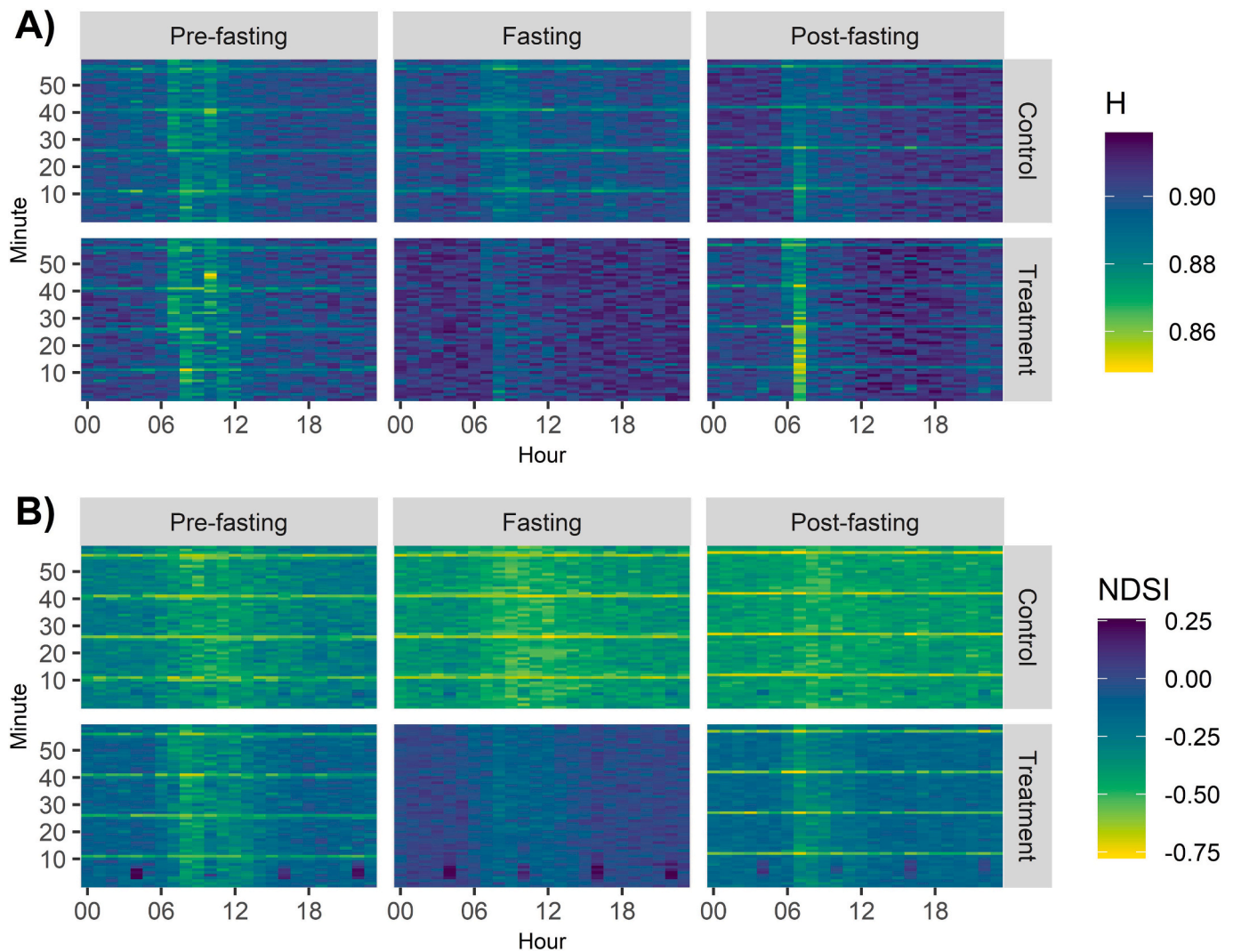
### 4.1. Soundscape characteristics associated with feeding activity

Inspections of spectrograms and waveforms revealed that the feeding sounds produced by Atlantic salmon share similarities with the feeding sounds produced by trout (Lagardère et al., 2004; Phillips, 1989), particularly to being short duration (0.05 s) broadband pulses (ca. 3.3–21.3 Hz, with energy concentrated between 6.5 and 9.4 kHz) that occur after pellets are offered to the fish (Fig. 6, Table S1). Lagardère et al. (2004) pointed out that these sounds might be problematic to use for detecting feeding activity due to a time and frequency overlap with those caused by pellets hitting the water surface. However, they did suggest that the 4–6 kHz frequency band could be the most suitable band for detecting feeding events as it has a weak overlap with sounds of pellets hitting the surface and the low frequency background noise that is common in land based aquaculture tanks (Bart et al., 2001; Craven et al., 2009; Radford and Slater, 2019). Soundscape recordings collected in this study show a prominent background noise in the lower frequencies, particularly below 250 Hz (Fig. 3), and that pellets hitting the water surface have energy mostly concentrated below 4000 Hz (for medium and high number of pellets, Fig. 6, Table S1), supporting the lower limit of 4 kHz suggested by Lagardère et al. (2004). The upper limit of 6 kHz suggested by Lagardère et al. (2004) is however too low to best capture the feeding sounds of Atlantic salmon parr, as around 95% of the energy of feeding sounds was found to be above 5.5–6 kHz in the current study (Fig. 6, Table S1). While 95% of the energy of feeding sounds was below 13,300 Hz (with 75% of energy below 9400 Hz), the upper limit for where feeding sounds were easily discernible was 8 kHz, due to similarities with sounds of water running in the sidewall outlet (Fig. 4D) which impacted frequencies particularly above 8 kHz (control group, Fig. 3). Overall, the 4–8 kHz frequency band was the most suitable to capture feeding sounds in the current study, as it captured around 50–75% of the energy as well as the peak frequency (around





**Fig. 7.** Heatmaps of ACI calculated for specific frequency bands, plotted to visualize the average 24-h cycle per experimental period. The x-axis indicates the hour of the day, and the y-axis indicates minute per hour. Each cell within the heatmaps is coloured according to the calculated mean index value for that minute (averaged over four tanks and five days) for the pre-fasting period (left column), the fasting period (middle column), and the post-fasting period (right column). Different letters show the studied frequency bands: A)  $ACI_{low}$  = 250–1700 Hz; B)  $ACI_{pellets}$  = 1700–4000 Hz; C)  $ACI_{feeding}$  = 4000–8000 Hz; D)  $ACI_{high}$  = 8000–24,000 Hz.



**Fig. 8.** Heatmaps of A) H, and of B) NDSI, plotted to visualize the average 24-h cycle per experimental period. The x-axis indicates the hour of the day, and the y-axis indicates minute per hour. Each cell within the heatmaps is coloured according to the calculated mean index value for that minute (averaged over four tanks and five days) for the pre-fasting period (left column), the fasting period (middle column), and the post-fasting period (right column).

6700–7000 Hz), with minimal impacts of pellets hitting the surface and water running in the sidewall outlet. However, there were no frequencies where feeding sounds were uniquely present, and the sounds of the emergency oxygen diffuser flushing and the self-cleaning of oxygen sensors would impact this frequency band and could mask feeding sounds if they were to coincide with feeding events.

Two main sound sources were always present during feeding events: i) pellet delivery, producing sound as the pellets hit the water surface, and ii) fish, producing sounds during feeding, in addition to splashes when jumping or when breaching the surface, and other unidentified behaviour(s) causing low frequency sound (Fig. 5C). Possible sources of these unidentified low frequency sounds could be fish colliding with the floor or walls of the tank, fish brushing against or colliding with the conduit through which the hydrophone was routed, the oxygen sensor hitting the tank, or fish vocalisations. It is likely that a combination of multiple sound sources was present in recorded soundscapes, and further studies would benefit from filming the feeding events to help ascertain the sources of these sounds if water visibility and experimental designs allows for this.

The energy of the feeding sounds of Atlantic salmon recorded in this study was concentrated at higher frequencies (6.5–9.4 kHz) than those reported for trout and is closer to the values reported for the suction feeding by turbot (maximum signal/noise ratio between 6 and 9 kHz)

(Lagardère et al., 2004). These frequencies also differ from the band where Rosten et al. (2023) observed the main differences (150–600 Hz) in net-cages. Such deviations may be due to different sound analysis procedures, recording conditions, and the life stage of the fish. The sound analysis of Rosten et al. (2023) was based on 10-min averages of sound intensity, which may have contributed to not being able to explicitly attribute their findings to specific feeding sounds. Furthermore, the acoustic conditions of net-cages at sea are not comparable to those occurring in small tanks, due to both sound propagation differences and differences in the potential sound sources affecting the soundscapes (Radford and Slater, 2019). RAS tanks present a significantly more predictable soundscape than net cages, mainly due to the openness of the system, where a higher number of extraneous sound sources can be recorded from the environment. In addition, the soundscape of net cages can be only partly controlled as they are open systems, whilst the sound level generated in RAS by pumps, filters and oxygenation may be mitigated by adjusting their design (Radford and Slater, 2019). These differences make it difficult to compare the outcomes of the current study with the results of Rosten et al. (2023). The observed variation in amplitude, frequency peak and bandwidth of the feeding signals in this study (Fig. 5C, Fig. 6), may partly be explained by the complexity of sound propagation in small tanks fitted with lids. Indeed, the same acoustic stimuli produced at different locations within a tank

could result in a different recorded signal, as shown by Akamatsu et al. (2002) who found significantly distorted dominant (peak) frequency, sound-pressure level and power spectrum, due to reverberations, resonance, and different distances between the sound source and hydrophone. Atlantic salmon are known to feed primarily on pellets in the water column during the light period (Jørgensen and Jobling, 1992), and as pellets follow water flow and sink over time, feeding will happen at different depths and distances to the hydrophone resulting in differences in the recorded signal. Similar signal distortion may likely have influenced other sounds that were produced at varying locations within the tank, such as the snitch sound (Fig. 5E, Fig. 6). Another possibility is that the observed variation in characteristics of feeding sounds is due to a variation in strike intensity, caused by different pressure transients in the opercular cavity of the fish (Alfredsen et al., 2007).

All investigated indices demonstrated distinctive changes that correlated with all scheduled feeding times, with ACIs increasing and H and NDSI decreasing compared to the times in between scheduled feeding times. An increase in ACIs indicates a more complex soundscape where sounds vary in amplitude and frequency over time, which fits well with the changes seen in Fig. 5, where the feeding event causes variation in an otherwise mostly constant soundscape. A decrease in H reflects more energy concentrated in time and frequency, which fits the characteristics of the pellets hitting the surface (Fig. 5B, Fig. 6). A decrease in NDSI shows relatively more energy is in the 1.7–4 kHz band, which is reasonable given the total energy of the sound of pellets hitting the surface is higher than the total energy of feeding sounds (Fig. 6). The sounds of pellets hitting the surface cover a broad frequency range, and likely impacted most of the indices investigated. However, the presence of a change in  $ACI_{low}$ , covering a frequency band below most of the energy from the sound of pellets hitting the surface, indicates that the observable variations in soundscapes during feeding are not only due to the sound of pellets. This is further supported by the fact that the duration of the pellet sounds is only a fraction of the total duration of feeding events, and since ACI is influenced by changes over time, other sounds contributed to the resulting ACI values.

During feed withdrawal, soundscape characteristics of the control (fed) and treatment group were distinctly different. Overall NDSI and H indices increased in the treatment group and ACIs decreased compared to the control group. Interestingly, these NDSI and H indices also detected changes in the tank soundscape *in between* the previously scheduled feeding times in the treatment group. This could be due to a change in the fish behaviour or in the performance of RAS components during the feed withdrawal period but is more likely related to inconsistency of the belt feeders used for all tanks during feeding periods in this experiment. The belt feeders were set to dispense feed for 42 s four times an hour, which should provide feed doses of comparable size at set time points. However, feeders occasionally dispensed a large dose, as some feed falling caused an “avalanche”-effect, pulling more pellets down with it. This in turn caused an uneven distribution of pellets on the belt, resulting in consecutive doses to be either small, large, at some instances non-existent, or even leaving pellets at the edge of the belt which could randomly fall into the tank in between the set feeding times. The assessment of the belt feeder performance indicates that around 12% of the times that pellets were introduced to the tank happened outside of the scheduled feeding regime. Most of those events (84%) were caused by <15 pellets, but since most of the energy of the sound of pellets hitting water is within the 1.7–4 kHz band (Fig. 5B, Fig. 6), the absence of these events in the feed withdrawal treatment group are likely responsible for the increase seen in NDSI and H outside of the previously scheduled feeding times. The fact that the number of pellets being distributed affects their acoustic characteristics when hitting the surface (Fig. 6, Table S2), highlights the need to take feed distribution system design, and also ration size, into account when considering using passive acoustics to monitor feeding.

While the overall feeding events resulted in similar average increases or decreases in the respective indices (Fig. 7, Fig. 8), there is some

variation between feeding times that could be related to the previously described variation in feed doses. This could have affected the appetite and feeding intensity of the fish, which could have resulted in this variation in index values between feeding times. This indicates a potential to use the relative values of  $ACI_{pellets}$  (covering the frequency band of the pellets hitting the water),  $ACI_{feeding}$  (covering the fish feeding) and NDSI (indicating how much energy is present from the sound of fish feeding, compared to how much energy is present from the pellets hitting the surface) to identify different appetite levels of Atlantic salmon in RAS production. For instance, if  $ACI_{feeding}$  values are particularly low while those of  $ACI_{pellets}$  are normal, it could indicate low feeding activity and therefore low appetite. In contrast, relatively high  $ACI_{feeding}$  and normal  $ACI_{pellets}$  values could indicate high appetite. Similarly, low NDSI values could be associated with low appetite (relatively more energy caused by pellets hitting the water surface compared to energy of feeding sounds) while high NDSI values could indicate high appetite (where the energy of feeding sounds are relatively higher in comparison to pellets hitting the water surface). To test this, appetite levels of the fish during feeding events must be known, which requires a controlled study designed to induce low and high appetite, preferably with video recordings to confirm behavioural differences.

#### 4.2. Other sounds

Whilst one of the main aims of this study was to document soundscape characteristics related to feeding activity, a number of other sounds related to system management and fish activity were also identified. Sounds of unknown origin were also detected, highlighting their role as a potential source of error for a PAM based system for feeding activity. Among them, an example is the peaks shown in the power spectrums around 8000, 12,000, 13,500, 16,000 and 20,000 Hz (Fig. 3). While their sound source is unknown, they could be related to resonance frequencies of the tank and/or to the equipment in the RAS that is constantly running, such as pumps. Since these peaks were present at similar magnitudes in both control and treatment groups throughout the experiment (Fig. 3), they are unlikely to be related to feeding.

The sounds associated with surface events are similar to those attributed to Atlantic salmon by Rountree et al. (2018). However, the findings of the current study show a trend towards a shorter duration and higher frequencies for similar sounds. For example, the downsweep recorded here shares similarities with the sound named a “moan” recorded by Rountree et al. (2018), but with a duration around 0.1 s instead of around 0.4 s, while the snitch sound identified in the present study has a peak frequency at 6–7 kHz instead of at 2.3 kHz as documented by Rountree et al. (2018). Bigger individuals have been found to produce sounds of longer duration and lower frequency in other species (Connaughton et al., 2000; Parmentier and Fine, 2016), so potential dissimilarities in fish size between these studies could be a possible explanation for differences in the recorded sounds. Alternatively, they could be due to genetic or behavioural differences between distinctly different populations of Atlantic salmon; Rountree et al. (2018) studied a wild landlocked population, while the current study investigated a farmed strain. It is also possible that the observed differences could be linked to different life stages of Atlantic salmon, which to our knowledge has not been explored in relation to sound production. Further, the effect of the tank environment on sound propagation has also likely impacted the acoustic characteristics of the recorded sounds.

The cause of the diurnal pattern displayed as an increase in  $ACI_{pellets}$ ,  $ACI_{feeding}$ , and  $ACI_{high}$ , and a decrease in H and NDSI, between 06:00–17:00 (Fig. 7B, C, D, 8A, B) is uncertain. It is unlikely to be related to a diurnal rhythm of the fish, since they were held under a 24:0 (LD) light regime and fed every 15 min, and since the pattern is less prominent during the fasting and post-fasting period. Moreover, the time correlates with the working hours of personnel on site, and so the trends seen in the indices could be related to sounds made by people doing routine checks of the systems and the water quality, people working in

neighbouring rooms, building infrastructure that operates according to a schedule (such as ventilation), or fish reacting to any of the forementioned activities. Craven et al. (2009) suggested that sounds produced by personnel would be limited to diurnal periods, which is supported by the patterns seen in the current study.

The increase in NDSI and  $ACI_{\text{feeding}}$  around 04:05, 10:05, 16:05 and 22:05 (Fig. 7C, Fig. 8B) correlates with scheduled events where the sensors measuring oxygen levels in the tanks perform self-cleaning (Fig. 4B) and emergency oxygen diffusers were flushed (Fig. 4C), causing bubbles to be released to minimize fouling of the sensor and diffuser. While these were scheduled events, inspections of audio spectrograms revealed that the time of onset and the duration of these events varied slightly among the tanks, particularly for the emergency oxygen flush which was completely lacking in two tanks in the control group. For the tanks in the treatment group, the onset of the flushing of sensors and diffusers in all tanks was better synchronized in time, and the duration of emergency oxygen diffuser flushing was longer compared to the tanks in the control group, which explains why the mean indices yielded a stronger response for the treatment group (Fig. 7C, Fig. 8B).

The control group displayed a continuous increase in  $ACI_{\text{high}}$  throughout the three periods of the experiment (Fig. 7D, Fig. S1). This correlates with a slight but noticeable increase in water running out of the sidewall outlet in some tanks, which caused an increase in very short duration sounds of running water (Fig. 4D). These sounds share similarities with the sounds of fish feeding, making the 8–24 kHz frequency band challenging for using ACI to assess feeding activity. Further, it shows that the water level in the tanks were not completely stable but increasing slightly over time, which indicates either an increase in water being pumped in, or a reduction of water removed via the central drain.

H was found to be particularly influenced by the sounds of the drum filter (DF), as these were high energy sounds concentrated in time and frequency (Fig. 6) causing a decrease in H. Most of the low index values seen in Fig. 8A outside of feeding times were caused by DF sounds. The rate at which the DFs were flushed varied over time depending on the particles accumulating in the filter and triggering a sensor to initiate flushing, which normally happened 3–6 times per hour. During the post-fasting period, the DF of a MicroRAS in the treatment group had a malfunction, causing it to flush up to 3–4 times per minute from 20:50 on one day to 08:00 the following day. While the malfunction only occurred in one of four RAS and lasted for just over 11 h, it is seen as a decrease in the mean H index (Fig. 8A, Fig. S2). However, this malfunction did not have a similar impact on the other investigated indices. On the other hand, the change seen in all indices concurrently around 06:30–10:00 during the post-fasting period (Fig. 7, Fig. 8A) correlates with a recurring increase in DF flushing in all tanks for all days in this period, which occurred during the daily check of the system. This emphasises the value in using multiple indices in soundscape monitoring, as they describe different features of the soundscape. Further, the impact of system sounds on the soundscape, such as DF flushing, emergency oxygen diffuser flushing, and oxygen sensor self-cleaning, reveal the potential and utility of using passive acoustics for monitoring system performance.

### 4.3. Methodology

Based on the results from this study, 24 h of continuous recording should be enough to detect the main trends of the soundscape in RAS tanks. Since previous research on acoustic indices has focused on natural environments, existing recommendations for their use concerns such as Bradfer-Lawrence et al. (2019) recommends 120 h of continuous recordings to be able to characterize the soundscape when using acoustic indices in environmental research, with indices calculated for each minute. For ponds, Greenhalgh et al. (2023) suggests a minimum of 24 h of continuous recording to capture variations in a pond soundscape, which is in agreement with the finding of this study (Fig. 7, Fig. 8, Fig. S1, Fig. S2). RAS tank soundscapes have fewer unpredictable

variables than many natural ones; indoor RAS tanks are sheltered from weather such as rain and wind; there is only a single cultured species in the tank instead of an unknown quantity of animals and plants; and anthrophony from water treatment components, feeding systems and maintenance work follow organized schedules. However, a single sound source can have a substantial impact on parts of the soundscape (such as the drum filter malfunction affecting the H index in the treatment group during the post-fasting period, Fig. 8A, Fig. S2), so it is important to carry out the recording during a representative period of time, which is also relevant to the choice of research question.

All investigated indices yielded crucial information about tank soundscapes and calculating them for each minute gave good temporal resolution. Recording for a longer time (15 continuous days) and performing the analysis at higher temporal resolution (1 min) allowed us to spot more variation in the soundscapes compared to the findings of Rosten et al. (2023). Nevertheless, while all indices reflected changes correlating with time of feeding, different events induced similar variations in index values. For instance, when using the H index both DF flushing and feeding events caused a decrease in values (Fig. 8A). Similarly, the sound of bubbles from the self-cleaning of the oxygen sensor (Fig. 4B), the emergency oxygen diffuser flush (Fig. 4C) and the feeding sounds (Fig. 5C) led to an increase in  $ACI_{\text{feeding}}$  (Fig. 7C). If these sounds happen simultaneously, a single index value will not be able to disentangle them and determine the underlying sounds causing the specific value. A possible way to mitigate this issue is to utilise multiple indices tailored to the sounds of interest, as shown in the current study; while H index values decreased by both DF flushing and feeding events, the  $ACI_{\text{low}}$  values either decreased (DF flushing) or increased (feeding events). Bradfer-Lawrence et al. (2023) recommends using a suite of indices, with their selection based on expected soundscape patterns, so that multiple facets of the soundscape can be revealed. However, they point out that the interpretation of multiple indices can be complex. A possible way to reduce this complexity is via the use of machine learning, which has been used to combine multiple indices to improve the analysis of coral reef soundscapes (Williams et al., 2022). A similar approach should be explored in future studies to see if it could be utilised to distinguish differences in RAS soundscapes as well.

While the averaged values of the investigated indices were suitable in the current study, other indices or metrics might be more suitable in different environments. Variables such as tank design, the feed delivery system, feeding regime, water treatment components and daily operations can impact upon soundscape patterns and trends, which could result in other frequency ranges, or even other indices, being more suitable than those described in the current study. We recommend that future studies on RAS tank soundscapes consider existing literature on the use of acoustic indices in different environments (Bradfer-Lawrence et al., 2023; Greenhalgh et al., 2023; Pieretti and Danovaro, 2020) and soundscape analysis (Merchant et al., 2015; Wilford et al., 2021), to select the most appropriate methods for the given environment and expected soundscape patterns.

## 5. Conclusions

Soundscapes in RAS tanks are affected by feeding. Both system operation and fish behaviour contribute to RAS tank soundscapes. The acoustic indices ACI, H and NDSI can be adopted to describe how RAS tank soundscapes changes with the feeding activity of Atlantic salmon in a controlled environment. These indices may also be affected by sounds unrelated to feeding, and this must be considered when applying them to feed management. Acoustic indices also have some utility for monitoring RAS performance, and synchronising tank replicates during experimental settings, in addition to monitoring fish behaviour. They can also detect certain deviations in system performance, such as unintentional stops in feed delivery or malfunctions of system components crucial for fish welfare, and can potentially be used as a system management and optimisation tool.

More studies are needed to determine whether the indices and frequency bands describing the feeding activities of salmon explored in this study can be applied in commercial RAS facilities and tanks that have different designs, shapes, materials and sizes. It is also challenging to determine the exact origin of all the recorded sound sources. Future studies should include video to correlate specific sounds with fish behaviour in RAS tanks. More indices should also be explored, to investigate whether they offer additional value to the ones examined in the present work. Lastly, different feeding regimes and feeding systems should also be tested, to investigate if any further soundscape parameters potentially correlate with appetite and known feeding behaviour, which would be an important step towards evaluating the useability of soundscape information for controlling feeding systems in RAS.

### CRedit authorship contribution statement

**Gaute A.N. Helberg:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Marianna Anichini:** Writing – review & editing, Supervision. **Jelena Kolarevic:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Bjørn-Steinar Sæther:** Writing – review & editing, Supervision, Conceptualization. **Chris Noble:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2024.741325>.

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