

**The acoustic repertoire of Atlantic cod  
(*Gadus morhua*) and the effect of feeding  
on broadband clicks and their sound  
characteristics**

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## **Abstract**

Populations of Atlantic cod (*Gadus morhua*) along the Skagerrak coast remain below viable levels today despite various implemented management strategies aimed at their recovery. Augmented management approaches are needed to reverse the population decline and secure sustainable harvesting. Bioacoustical studies such as passive acoustic monitoring, could potentially enhance our understanding of cod behaviour and assist identifying crucial coastal habitats during vulnerable stages of cod development. Additionally, it may be applied in sustainable food production, such as cod aquaculture. However, such automated monitoring systems require baseline studies to increase our known acoustic repertoire of cod sound production. Additionally, a reference library of annotated cod sounds is needed for training deep learning algorithms. Less attention has been given to high frequency broadband clicks emerging from cod. We do not yet understand their behavioural context, let alone their underlying biomechanics. Previously, clicks have only been observed when cod has been threatened or approached by a predator-like figure. Clicks were therefore related to agonistic behaviour and suggested to have a predator startle function.

This study aimed to 1) Catalogue all cod sounds to produce an annotated reference library for future training of a deep learning algorithm 2) Characterise acoustic variation in cod clicks, and 3) Investigate how clicks relate to behaviour by testing the effects of feeding on click count and sound characteristics. The study was conducted during the spawning season in an isolated semi-natural basin in Southern Norway. Sounds from 45 adult cod were manually analysed and annotated from 36 hours of recorded material. This is the first time that all known cod sounds are observed and described in the same study. Knocks, grunts, hums and rumblings were observed in addition to high frequency broadband clicks. Clicks were the second most occurring sound from cod in the recordings, suggesting that clicks are an important part of cod sound production. Contrary to previous reports, the study observed continuous variation in click characteristics rather than discrete categories of click types. The study found that cod clicks occur independent of disturbance and that feeding did not influence the sound characteristics, suggesting that clicks could have an extended function than previously assumed. The study expands our knowledge on the repertoire of cod acoustics and assists in the development of new technology for acoustic monitoring. Further, it advances our understanding of the role of clicking in cod behaviour and ecology.

## Sammendrag

Populasjonene av torsk (*Gadus morhua*) langs Skagerrakkysten forblir under levedyktige nivåer tross en rekke ulike forvaltningstiltak iverksatt for å assistere. Dette indikerer at supplerende tiltak er nødvendig for å snu denne negative trenden og sikre et bærekraftig fiskeri. Bioakustiske studier slik som passiv akustisk overvåkning, kan bidra til å øke vår kunnskap om torskens adferd så vel som bidra til å lokalisere kystområder viktige for sårbare perioder under torskens utviklingsstadier. I tillegg kan det implementeres i bærekraftig matproduksjon, slik som torske-oppdrett. Til gjengjeld krever slike automatiserte overvåkingssystemer grunnstudier som øker dagens referanse database på lyd fra torsk som kan brukes som lydtrainingsmateriale i utviklingen av dyp lærings-algoritmer. Høyfrekvente klikkelyder fra torsk har gjennom tidene fått lite oppmerksomhet. Vi forstår ikke hvordan de er knyttet til adferd og heller ikke hva som produserer lyden. Tidligere har disse lydene kun blitt observert når torsk har blitt skremt eller tilnærmet av en predatorlignende figur. Derfor er de blitt knyttet til aggressiv adferd og er estimert til å fungere som en skremmende teknikk på predatorer.

Målet med studiet var å 1) Etablere en referanse database for annoterte lyder fra torsk for fremtidig trening av dyp lærings-algoritmer 2) Karakterisere akustisk variasjon i klikkelyder fra torsk, og 3) Undersøke hvordan klikkene relaterer til adferd ved å teste effektene av foring på klikkenes forekomst og lydegenskaper. Studiet ble utført igjennom gytesesongen i et avlukket semi-naturlig saltvannsbasseng i Sør-Norge. Lyder fra 45 voksne torsk ble manuelt analysert og annotert fra totalt 36 timer med opptaksmateriale fra perioden. Dette er det første gang hvor alle tidligere kjente lyder fra torsk er observert og beskrevet i det samme studiet. «knocks», «grunts», «hums» og «rumblings» ble observert, i tillegg til de høyfrekvente klikkene. Den nest mest forekommende lyden fra torsk i det analyserte opptaksmateriale var klikk. Dette indikerer at klikke-lyder er en viktig del av lydproduksjon fra torsk. Tidligere rapporterte klasser av klikk ble ikke funnet i dette studiet. Studiet viser at klikke-lyder fra torsk skjer uavhengig av forstyrrelser og at foring ikke hadde en effekt på lydegenskapene. Dette indikerer at lydene kan ha en alternativ funksjon enn tidligere antatt. Studiet utvider vår kjennskap til lydproduksjon fra torsk og assisterer i utviklingen av ny teknologi for akustisk overvåkning. Videre gir det oss et fremskritt i forståelsen for hvilken rolle klikking har i torske-adferd og økologi.

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## **Preface**

Diving into the world of marine bioacoustics has been an incredible journey with a major learning outcome. Firstly, I would like to thank my supervisors Jeppe H. Rasmussen, Rebekah A. Oomen and Halvor Knutsen for the guidance, unlimited assistance, and challenges during this journey. You have been indispensable! Thank you, Jeppe for producing awesome software and assisting me in my steep learning curve in the world of coding. Thank you, Rebekah for the continuous support and excellent discussions. You have both broadened my mind, significantly increased my skills, and introduced me to a new world of biology. Thank you, Halvor for presenting a convincing project description making me choose this subject and providing necessary eyes on the final product.

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Lastly, I would like to thank my family, my friends, the master's office crew, and especially my closest one for your endless support during my journey through academia.

Kristiansand 21.05.2023

A handwritten signature in black ink that reads "Annika Lie". The signature is written in a cursive, flowing style.

Annika Lie

# 1 Introduction

## *1.1 Status*

The development of effective fishing technology and marine overexploitation have resulted in a mosaic of depleted fish populations worldwide (Ding et al. 2017). Moreover, the competition for space along the coast and the need for sustainable resources is ever increasing. However, various marine conservation efforts with successful outcomes, exhibits the potential to rebuild stocks with the right approach (Worm et al. 2009).

The Atlantic cod (*Gadus morhua*; hereafter, ‘cod’) is a demersal predatory fish in the family Gadidae and is found throughout the North Atlantic Ocean. Cod is highly consumed by humans and is an important species both commercially and culturally, making it one of the most exploited fish in the North Atlantic (Rowe & Hutchings 2003, Heath et al. 2014). Consequently, the North Sea stocks gradually declined during the last century and remain below viable levels today (Neuenhoff et al. 2019, Knutsen et al. 2022). Despite the various fisheries management strategies implemented, the stocks still struggle to recover (Knutsen et al. 2022). Along the Skagerrak coast cod constitutes of a mix of two genetically different ecotypes, the more stationary fjord cod and the offshore North Sea cod (Knutsen et al. 2018). The fjord cod is further separated into local subpopulations with an extension up to approx. 30 km or less (Jorde et al. 2007). In addition to anthropogenic influence, the small populations may also be subjected to losses of genetic diversity and demographic- and environmental fluctuations (Rowe & Hutchings 2003, Neuenhoff et al. 2019). All of which accelerates the decline of the populations. This suggests that cod requires augmenting assistance to thrive again. For the cod harvesting to persist on today’s fishery levels, auxiliary management approaches are needed to reverse the population decline and secure sustainable harvesting. Assuring that conservation strategy is effective where and when it is needed most, requires knowledge about the ecology and behaviour of cod.

## *1.2 Studying fish with bioacoustics*

Bioacoustics is a well-recognised approach for a non-invasive and cost-effective method to understand the ecology and behaviour of fishes (Rowe & Hutchings 2006, Hawkins &

Picciulin 2019, Bussmann et al. 2021, Waddell et al. 2021, Looby et al. 2022). Moreover, the long history of bioacoustics (Brawn 1961b) has developed multiple approaches depending on the objectives of the study and the target, such as species or ecosystems. Fish can be challenging to study as they reside in water. However, studying fish by the means of sound and interpreting those sounds in relation to behaviour, allow us to monitor fish without observing them visually. Sound travels as waves through various mediums and sound energy is the vibration of particles. Sound moves faster and longer in water compared to air (Popper et al. 2019). All fish are developed to detect particle motion (Hawkins 2022). However, special adaptations are needed to detect sound pressure (Popper et al. 2019). Cod is a hearing specialist which is able to do both (Hawkins & Popper 2020). Sound from fishes consists of elementary sonic pulses which differ in amplitude, pulse duration and frequency (Kasumyan 2008, 2009). This variation of the amplitude and frequency in addition to duration and structure, is what differentiates species, populations and even individuals (Ladich 2015). As visibility may be limited under water, it is even more important for marine species to use sound to obtain information from their surroundings, especially for bottom dwellers such as cod where the light may be scarce (Skjæraasen et al. 2012).

In addition to perceiving their surroundings by means of sound, fishes are also capable of producing and communicating through sound. Many fishes are known to be soniferous and as many as 989 fish species have so far been found to actively produce sound (Looby et al. 2022). The sounds are produced in varying ways; swimbladder pulsations, stridulation, hydrodynamic movements, substrate body and tendon vibration, and air release (Kaatz 2002, Kasumyan 2008, Ladich 2015). It is used to communicate information among individuals and can include mate attraction, territorial, and agnostic behaviour (Ladich 1997, Kasumyan 2009, Skjæraasen et al. 2012). New fish sounds are continuously being described (Bussmann et al. 2021, Mouy et al. 2023). However, there is still a lot we do not understand about fish acoustics and there is a need for further research. Prior to interpreting and relating sound to fish behaviour and ecology, recording and processing such as listening and annotating is required.

Studies on fish acoustics may impose some challenges which needs to be addressed when choosing the approach. Conducting acoustical studies in the wild can be difficult, expensive, and time-consuming. Therefore, majority of cod vocalisation studies have been conducted during limited hours and recording times in laboratory tanks either in- or outdoors (Finstad &

Nordeide 2004, Vester et al. 2004, Verboom 2005, Rowe & Hutchings 2006, Riera et al. 2018). Studying cod in restricted areas such as laboratory tanks may increase the potential for successful recordings compared to the wild. However, they may also impose sound recording issues like reverberations due to the limiting size of the tank (Hawkins & Amorim 2000, Vester et al. 2004, Riera et al. 2018). These issues may be addressed by using a basin with sufficient size (Hawkins & Amorim 2000). Studies in the open ocean may impose other acoustical issues such as background noise. However, conducting studies in enclosed semi-natural environments might mitigate this (Nilsson 2004). Baseline studies of cod acoustics in such environments may increase recording quality and provide supplementary information to existing knowledge from tank studies. In addition to sound quality, the processing of data is another challenge in bioacoustical studies.

Bioacoustical studies produces massive quantities of data which requires considerable effort for manual processing and analysis. Consequently, the amount of the recorded sound material analysed is limited. Each call must be found and analysed correctly, which is time consuming to do with manual labour. Maximising recording time and data processing is possible by applying continuous recordings to recent technology such as deep learning algorithms (Waddell et al. 2021, Goodwin et al. 2022). Such automated recognition systems allow processing of greater amounts of data, is faster and less subjective. Developing such technology requires training based on preliminary manual annotations of sounds from cod. However, a major bottleneck in the development of these systems today is the lack of access to such reference libraries of cod acoustics. An extended amount of annotated cod sounds is therefore needed. Acquired repertoires of cod sound production may be applied to passive acoustic monitoring (PAM) systems *in-situ* (Goodwin et al. 2022), which could assist identifying crucial coastal habitats during vulnerable stages of cod development (Rogers et al. 2014, Caiger et al. 2020). Additionally, PAM may be applied to sustainable food production such as cod aquaculture, to monitor feeding, activity levels, agonistic interactions, and spawning. Automated recognition programs for cod vocalisation have already been conducted for grunts with success (Urazghildiiev & Van Parijs 2016). Further, it has already been used to located gadoids spawning sites in the ocean (Hernandez et al. 2013, Casaretto et al. 2014). This enlightens the potential and increasing the amount of annotated cod sounds may provide the means for expanding bioacoustical studies and, thus, a greater understanding of cod sound production. However, manual analysis requires good knowledge on cod acoustics.

### ***1.3 Cod sound production***

Gadoids produce sounds in a frequency range of 30-500 Hz (Brawn 1961b, Finstad & Nordeide 2004, Fudge & Rose 2009, Hawkins & Picciulin 2019), and they can discriminate between varying sound pressures and the location the sound is coming from (Hawkins & Popper 2020). The vocal repertoire of cod has previously been estimated to be limited compared to other gadoids such as haddock (*Melanogrammus aeglefinus*) (Brawn 1961b, Hawkins & Amorim 2000, Finstad & Nordeide 2004). The first sound detected and analysed for cod was low frequency grunts (Brawn 1961b), produced by the contraction of the drumming muscle (Hawkins & Picciulin 2019). These have been found to be important for the reproductive success of cod (Rowe & Hutchings 2008), as the drumming muscle mass increases in males before the spawning season (Engen & Folstad 1999, Rowe & Hutchings 2004). The vocal repertoire of cod was later found to be wider than previously expected. Knocks (Midling et al. 2002, Verboom 2005), humming and rumbling (Nilsson 2004, Rowe & Hutchings 2006) were described in addition to the low frequency grunts. Further, high frequency broadband clicks have also been reported (Vester et al. 2004, Verboom 2005). Cod vocalisation increases during spawning, especially during the night (Midling et al. 2002, Finstad & Nordeide 2004, Caiger et al. 2020) when the spawning activity rate increases (Rowe & Hutchings 2006). As cod sound production is related to spawning, it may act as a pre-zygotic barrier for population rebuilding. It may be that the two ecotypes and local fjord populations (Jorde et al. 2007, Knutsen et al. 2018) have their own language and dialects, which is found for other cod species such as the Arctic cod (*Boreogadus saida*) (Riera et al. 2018). It is therefore essential to close the gap in knowledge around the behavioural relationship of all cod sounds and their role in cod reproduction.

Due to its importance, the ecology and behaviour of cod is widely described, especially their spawning. In Southern Norway, cod spawn from February to May (Espeland et al. 2007, Roney et al. 2018b, a). Cod is a broadcast spawner, releasing a large numbers of eggs directly in the water column several times through the spawning season and with no parental care (Rowe & Hutchings 2003). Their spawning fulfils the prerequisites of a conventional lekking mating system where the courtship is driven by female mate choice and sexual selection (Nordeide & Folstad 2000). The male sound performance during courtship may therefore be an important pre-mating mechanism influencing reproductive success (Nordeide & Folstad 2000, Rowe & Hutchings 2004, Rowe & Hutchings 2008). Grunts are directly tied to

spawning where the low frequency grunts are observed during the flaunting display that cod perform during the courtship (Brawn 1961a). Further, the hums have been speculated to be associated with synchronised gamete release (Nilsson 2004, Rowe & Hutchings 2006). The low frequency knocks have not been directly associated to spawning for cod. However, for its gadoid relative haddock, it might be related to pre-spawning mate attraction (Casaretto & Hawkins 2002). On the other hand, they have also been estimated to have a warning function (Hawkins & Amorim 2000). Similarly, the high frequency clicks emitted from cod have been observed in relation to agonistic behaviour (Vester et al. 2004). The low frequency cod sounds are well studied and have been found to be important for spawning. However, the role of clicking in cod sound production and the behavioural context remain less understood.

#### ***1.4 High frequency broadband clicks***

Although described (Vester et al. 2004, Verboom 2005), less attention has been given to the high frequency broadband clicks reported for cod. We do not yet understand the behavioral context of these sounds, let alone its underlying biomechanics. Cod vocalise by rapidly contracting the striated drumming muscle attached to the swim bladder, which compresses the gas-filled bladder and acts as a resonator (Hawkins & Picciulin 2019). Each pulse is likely one muscle contraction and these muscles are among the fastest in vertebrates (Ladich 2015). Despite this, these muscles are not capable of contracting fast enough to produce these high frequency clicks (Ladich 2015). Clicks are characterised as short and abrupt sounds across a wide frequency band. They have a higher frequency than other sounds emerging from cod and the spectra is reported around 1-18 kHz (Vester et al. 2004, Verboom 2005) with a sound pressure of 151 dB (Verboom 2005) and 153 dB re 1 Pa (Vester et al. 2004). Vester et al. (2004) and Verboom (2005) reported multiple classes of clicks based on their interval of occurrence and sound characteristics: single sonic clicks, pre-clicks, double-clicks and series of clicks. Both studies hypothesised that clicks had a protective and predator startling function as the clicks were only heard when cod were threatened or approached by a predator-like figure. Sounds with similar characteristics in other species have been associated to reactions such as fright, appearance of danger, and agonistic behaviour, suggesting they may be used as warning or frightening-off signals (Ladich 1997, Kasumyan 2009, Bussmann et al. 2021).

Clicks produced by cod are outside their hearing capability which is limited to 50-1500 Hz (Hawkins & Popper 2020). On the other hand, they are within the hearing range of cod's

natural predator in Southern Norway, harbour seal (*Phoca vitulina*). Harbour seal's hearing sensitivity ranges from 6-12 kHz (Wolski et al. 2003). Vester et al (2004) found that harp seals (*Pagophilus groenlandicus*) avoided clicking cod and captured significantly less cod when clicks were produced. This suggests that clicking has a predator startling function rather than clicks increasing exposure to predation. Verboom (2005) found that the occurrence of clicking decreased with increasing habituation. Cod in the study reacted strongly to the threat exposure in the beginning of the trials by clicking intensively. However, as they got used to the threat, the clicking decreased. Habituation to threatening experiences was also found when cod was exposed to seismic waves (Walle 2020). Both studies suggests that cod can recognise recurring events and elude them as threats by experience. Cod cannot hear the clicks being produced, however, it is possible that they can feel them through particle motion (Hawkins 2022). Determining if clicks are an active or passively emitted sound (Looby et al. 2022) requires further research on the behavioural context, but also the producing mechanism.

The mechanism producing cod clicks is not known. However, high frequency clicks emerging from other marine species have been related to several production mechanisms. The snapping shrimp (*Alpheus heterochaelis*) produces sounds with similar characteristics as clicks by rapidly snapping its claws creating bubbles which burst (Versluis et al. 2000). Click sounds from fishes have been related to stridulatory sounds (rubbing of bony elements) and are widely described for groups such as catfishes and damselfishes (Kasumyan 2008, Ladich 2015). Further, corkwing wrasse (*Symphodus melops*) produces stridulatory click sounds by rapidly closing its jaw (Bussmann et al. 2021). In contrast, herring (*Clupea harengus*) is found to produce high frequency pneumatic sounds (Kasumyan 2008) by expulsion of bubbles from the anal duct region (Wilson et al. 2004). In addition to diverse sound producing mechanisms, high frequency sounds from fishes may also vary according to temperature (Papes & Ladich 2011, Ladich 2018, Ladich & Maiditsch 2020). The high frequency clicks emitted from cod differentiates from the low frequency cod vocalisations. The behavioural context and the production mechanism is less understood and requires further research.

### ***1.5 Cod clicks in relation to feeding***

Cod clicks have only been investigated in relation to agonistic behavior and predation (Vester et al. 2004, Verboom 2005). Studying clicks in relation to other stimulus such as feeding may provide new insight into the role of clicking in cod sound production. Studying cod in tanks

increases the chance of successfully recording cod sounds, however, captivity may restrict natural behaviour. Comparative studies suggest that cod's repertoire is larger when recorded in the wild compared to captivity (Midling et al. 2002). Studying cod in semi-natural enclosed basins may allow cod to a greater extent express natural behaviour compared to tanks. Regardless, fishes in *ex-situ* studies require feeding, which usually requires human interaction. Clicking increased when cod was approached by a human diver (Vester et al. 2004), suggesting that they perceive humans as a threat. In contrast, cod is a quick learner with a good memory and shows anticipatory behavior when reward is coming and crowd together in that area (Nilsson et al. 2008a). This suggests that feeding is a positive stimulus and clicking has not yet been studied in relation to this. If clicking is related to feeding, it may indicate that cod clicks have a broader function than previously assumed. Studying the effects before and after feeding also allow for investigating clicks when cod are not influenced by a threat or disturbance. Due to competition for food, feeding may also increase the level of agonistic behaviour. In other fishes, sound production has been found to increase in relation to competition for food (Amorim et al. 2004). This should be taken into consideration when assessing the effects of feeding. Studying clicks in relation to feeding may provide new insights in the role of clicking in cod sound production.

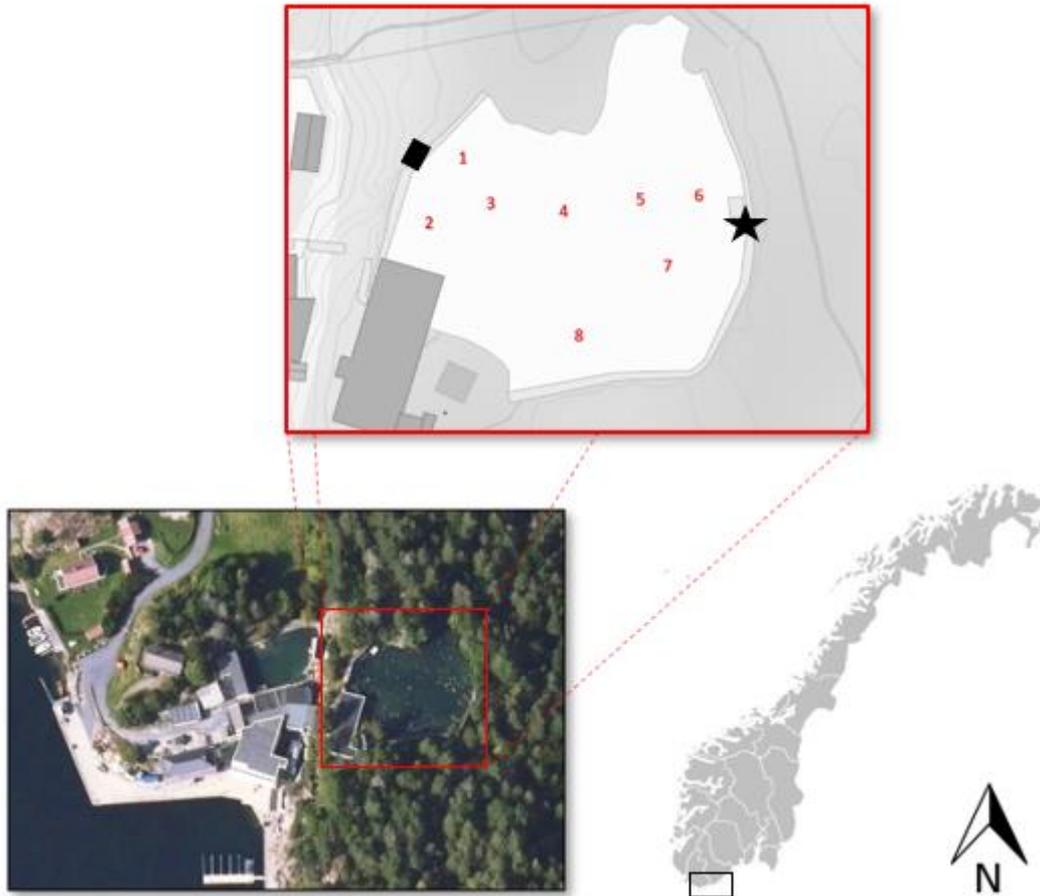
## ***1.6 Aims***

The thesis aims to expand the reference library of annotated cod sounds. Further, to increase our understanding of cod clicks and how they are related to behavior. The objectives were to 1) Catalogue all cod sounds to produce an annotated reference library for future training of a deep learning algorithm 2) Characterise acoustic variation in cod clicks, and 3) Investigate how clicks relate to behaviour by testing the effects of feeding on click count and sound characteristics. The catalogue serves as a resource for the future development of deep learning for automated analysis of cod acoustics. Studying the variation in cod clicking and the effects of feeding will increase our knowledge on cod sound production in relation to key behaviours and activities. Further, expanding our repertoire of cod sounds and applying it to passive acoustic monitoring (PAM) systems *in-situ*, can help supply suitable management strategies for conservation of wild cod. PAM may also be applied to sustainable food production, such as the rising cod aquaculture industry.

## 2 Method

### 2.1 Study area

The basin used for recording was located at the premises of the Institute of Marine Research (IMR) dept. Flødevigen in Arendal, Norway. It is an artificial semi-natural saltwater basin located northeast of the research station (Figure 1). The basin had an area of 1824,07 m<sup>2</sup> and a circumference of 189,62 m. The depths varied throughout the basin, consisting of two deeper sections up to 4.5 m in the east and west, connected by a slightly narrower passage (Figure 1). The saltwater was continuously pumped from 75m depth while simultaneously recording temperature. During the preceding year, the basin was emptied to conduct a lidar analysis. It was then refilled sept 2021, allowing sufficient time for a semi-natural ecosystem with microorganisms and plankton to establish. The volume and semi-natural environment made the basin ideal for sound recording during cod spawning. It was large enough to reduce acoustic disturbance and altered behavior, which can occur in tank-studies (Hawkins & Amorim 2000, Midling et al. 2002). On the other hand, it was small enough to increase the chance of recording of cod compared to *in-situ* as the cod were close to a hydrophone at all times. Additionally, the semi-natural environment with varying depths allowed for multiple sections the cod could reside in. Further, this allowed cod to express natural behavior throughout the spawning period, only limited by the varying depths.



**Figure 1** Study area at the Institute of Marine Research dept. Flødevigen research station, Arendal, Norway. Numbers 1-8 indicate placement of corresponding hydrophones. Black star represents main feeding area and black square the recording station located on land, containing the recording computer and mixer (Figure 2).

## 2.2 Study species

Most of the cod were collected with fyke nets in the nearby ocean outside the research station by personnel at IMR (Figure 1). The rest were caught in fish nets by fishermen outside of Grimstad in Southern Norway. The cod were collected in the period between Nov 2021 and Jan 2022. They were held in an indoor saltwater pool and fed regularly until release into the main experimental basin January 26<sup>th</sup>. The transition was planned such to allow acclimation to the new habitat before the spawning period. The cod were measured for size and weight, and as the primary target was to study sexually mature adults, only cod above 44 cm was selected for the study. The sex-ratio was unknown. All cod were also individually tagged with passive integrated transponders (PIT-tag) on behalf of a telemetry study carried out simultaneously in the basin throughout the study period. Upon release, no pre-determined area- or mating restrictions were imposed on the cod. Throughout the study period, cod were

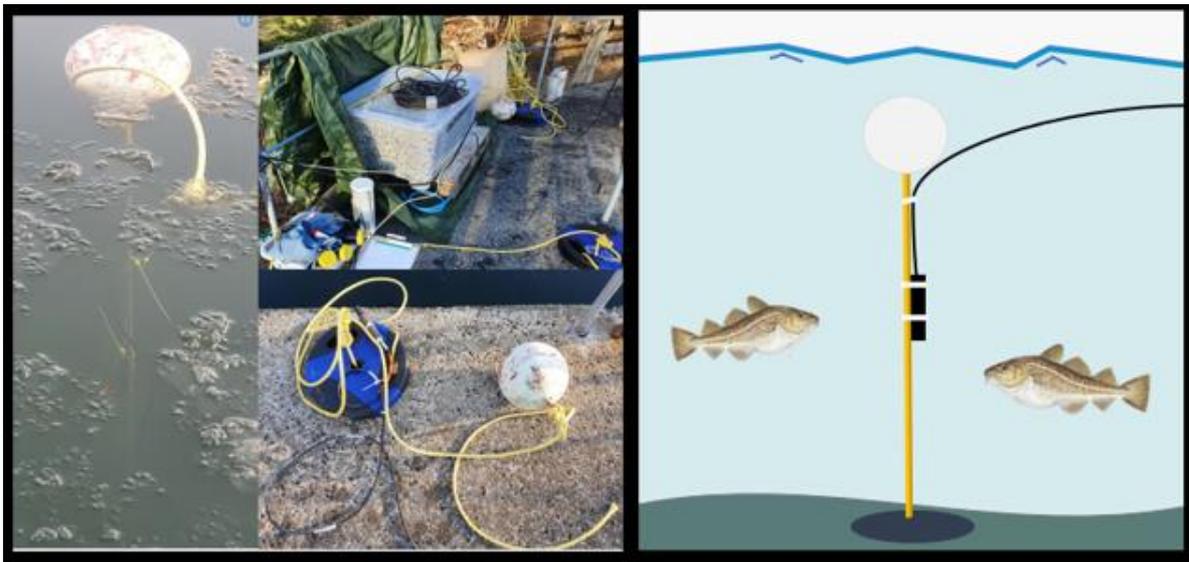
fed one time a day, most frequently at the east side of the basin. The amount of feed varied according to the presence of cod during feeding. Further, a plankton net was pulled across the basin from east to west one time a month to monitor occurrence and number of egg/larvae. These measurements of feeding and net-pulling were manually noted by the personnel at IMR. After the spawning season, the adult cod were measured for length and weight. Additionally, samples were taken for genetical analysis before they were released back into the wild according to laws and regulations imposed by Mattilsynet.

### ***2.3 Sound recordings***

Eight hydrophones (Ambient Recording GmbH, München, Germany, model ASF-1 MKII) were used for the acoustic recordings. Four hydrophones were deployed in January and used as a pilot study for when the remaining four were acquired. It was expected that activity might be centered in the two deeper sections of the basin as cod forms bottom territories and shoals during the spawning period (Brawn 1961a, Rowe & Hutchings 2003). Therefore, a triangular hydrophone formation was placed in each. Additionally, one was placed in the passage between them and one on a shallower area in the south (Figure 1). The strategic placement allowed for maximising acoustical coverage of the basin with emphasis on the deeper areas where the cod was expected to spend most time. Each hydrophone was attached to a submerged rig consisting of a 10 kg anchor on the basin floor with a rope running to a submerged buoy on top (Figure 2). This setup sheltered the hydrophone rig from unnecessary movement caused by wind or shifting ice, which can influence recording quality. Each rig was measured for total depth, distance from hydrophone to surface, hydrophone to buoy and buoy to surface. Additionally, the diameter of the buoy was known. These measurements were used to accurately determine depth of each hydrophone in the water column. The submerged cables ranging from 25 m to 50 m led to the recording station situated on the western shore (Figure 2). Additionally, these were isolated and protected where they resurfaced to avoid damage as well as disturbance during recording.

The recording station was an insulated storage box with a hole in the bottom to accommodate cables. It consisted of the recording computer (*Lenovo, China*), an eight-channel mixer (*MixPre-10 II*, <https://www.sounddevices.com/product/mixpre-10-ii/>), local hard drives (*LaCie 5TB, Seagate, USA*) and the hydrophone cables. Each hydrophone was connected to the channel with the corresponding number in the mixer. Forward, these will therefore be

addressed as channels. The frequency range of cod and the Norwegian AC current system (50 Hz) overlaps and the hydrophone cables are sensitive to frequency disturbance. Therefore, all 230V electrical components such as power extension cords and the computer charger were kept in a separate compartment beside the main box. Due to potential exposure to winter storms and heavy rain the recording station was raised on a podium and protected by a tarp to avoid water damage to electronic gear. The tarp was wrapped over and secured around the box when closed. Additionally, two silica boxes were permanently kept in the box to reduce condensation.



**Figure 2** The recording setup. On the left; Pictures of a hydrophone under water, the main station, and a hydrophone rig. On the right; A vertical representation of a hydrophone rig and its placement under water (Fekjan 2023).

The recordings were set to continuously run day and night from 9<sup>th</sup> of Feb to the 12<sup>th</sup> of May 2022. However, an unexpected update on the recording computer, which was not detected, made a gap in the recording between 7<sup>th</sup> of March and 1st April. Therefore, there was a total cover of 57 days during the recording interval. Until the 1st of March, only the first four channels were active. The recording computer was connected to ethernet to allow external access and downloading. However, all recordings were stored on the local hard drives in the box. They were swapped during the recording interval to secure data and ensure sufficient storage.

The amplification was set to 19 dB on channel 1-4 and 35 dB on 5-8 to amplify weaker sounds, however, adjusted low enough to avoid clipping. The amplification was set higher for

the channels with longer cables (50 m) compared to the shorter ones (25 m) to compensate for the loss of signal (Figure 1). Additionally, the hydrophones had a pre-amplifier built in to even allow for the use of such long cables. The audio bit-depth was set to 32 to assure a high-resolution and an optimisation of the recording ability. The high bit-depth decreased the noise floor, assuring that low frequency cod vocalisations was recorded with adequate quality for detection. The sampling frequency was set to 96000 Hz. According to the Nyquist Frequency, we can record frequencies up to half of the sampling frequency (48 000 Hz) without losing any information. Although cod clicks have been reported to have a considerably lower max frequency than this (Vester et al. 2004, Verboom 2005), the setting allowed for an accurate determination of the timing of the sounds. The length of each sound-file recorded was influenced by sample frequency, bit-depth, and number of channels. When four channels were active each sound file stored had a duration of 43 min and 14 sec. Further, 21 min and 37 sec when eight channels were active.

## ***2.4 Sound analysis***

### *2.4.1 Software development and manual annotation*

An established approach to analyse sound is to use software which allows for manual examination both auditory (through playback), and visually. The analysis of the digitalised sounds and manual annotations were conducted through a software produced in MATLAB (The MathWorks Inc 2022) by supervisor Jeppe Have Rasmussen (see B.1, Appendix B). The annotation software consisted of a custom made user-friendly graphical user-interface, with emphasis on optimised workflow and open accessibility. It was developed in collaboration between Rasmussen and the student, where the latter functioned as an alpha- and beta tester. This was conducted simultaneously as a sound training period using both previously and new recordings of cod acoustics. The training was initiated to assure accurate manual annotation in the analysis by establishing good knowledge on the auditory and visual characteristics of cod sound production. The latter can be conducted through timeseries presenting amplitude as a function of time or through spectrograms presenting the relationship between frequency, time, and energy. Adding the visual aspect to the analysis might reveal characteristics not audible, such as harmonics and pulses with close proximity. For instance, the inter call interval (ICI) between some classes of cod clicks can be as short as 22 ms (Vester et al. 2004) while the estimated ability for separating calls are 150 ms (Hawkins & Amorim 2000). During the

developing stage, the software and approach was modified to optimise adaptation to cod sound production. For example, by adding high pass filter-options and adjusting Fast Fourier Transform (FFT)-window sizes (see Appendix A). Further, the initial approach was to determine classes of clicks before the sound analysis and categorise them thereafter. However, to maintaining objectivity, the approach settled entailed annotation of all individual calls as regions of interest (ROI) in main categories based on their acoustic characteristics. Meaning that all clicks were annotated in one main category. Additionally, a “suspect click” category was used to annotate calls which seemingly looked like clicks but needed further examination.

#### *2.4.2 File processing, resampling and feeding times*

The thesis aimed to compare clicking in relation to the effects of feeding which occurred regularly with fixed times, throughout the spawning period. The manually noted feeding times from the personnel at Flødevigen was digitalised and converted to serial date-time (numbers that represents date and the decimal fraction that represents time from a fixed, preset date) in MATLAB (The MathWorks Inc 2022). Each feeding day within the recording interval was further analysed to validate the times and duration of each feeding. The aim was to include  $\pm$  30 minutes from before and after the feeding event which required extraction of the corresponding sound files. As the total recorded material composed of 26 TB of data, these were cut and processed by Rasmussen to ease data logistics. A list was created of all sound files with start time and duration. This was used to find the sound files within the interval for each feeding day. The sound files were resampled with a sampling frequency of 15 000 Hz. This was equivalent to sampling with an accuracy of 0.0015 ms, sufficient for examining clicks (Vester et al. 2004, Verboom 2005). This limited the observable sounds to the half of sampling frequency, meaning that sounds above 7500 Hz were not visible. However, this information was later extracted from the original sound files. After resampling, nine spectral features efficient for vocal analysis in noisy environments (The MathWorks Inc 2023) were extracted. This resulted in two corresponding files for each sound file, one wav-file and one feature-file.

The accurate feeding times were determined by auditory and visual analysis in the software. Recording days where no feeding was executed, days where feeding was not possible to determine due to background noise, and days where no feeding events were found, were

excluded from the analysis. Concurrently, 36 recorded days with feeding events were found between the 13<sup>th</sup> of Feb to 11<sup>th</sup> May. The new start- and end times for feeding were used to calculate what further sound files were required to include  $\pm 30$  min of each feeding event. As the sound files had two distinct durations, a feeding event to sound file – list was made in Excel to assist the calculation. A total of 133 sound files were included in the sound analysis to cover the 36 days with feeding events. They were listed by date and each file was assigned its start and end time of analysis, feeding time and its occurrence before- or after the feeding event. All time values were set with an accuracy of one millisecond.

#### *2.4.3 Restrictions and randomisation*

An analysis plan was designed to assure requirement of sufficient data for the thesis and avoid bias. This included a restriction of the analysis as well as randomisation of analysis order. First, the thesis aimed to investigate variation throughout the spawning period. It was therefore decided to include the whole recording period in the analysis. Second, as it was difficult to pre-determine how long it would take to manually annotate the recording period, only one channel was chosen and analysed at a time (Channel 4 and then channel 6). The channels were chosen based on their distance to the feeding area (Figure 1) and the good recording quality and limited background noise found during the sound training period.

As an auditory learning curve in the ability to annotate sounds could potentially act as a covariate and influence the data, a randomisation of the data was performed before analysis. To assure sufficient randomisation, the list of sound files was sorted by date and an empty column was added in front in Excel. A random number generator was coded in MATLAB (The MathWorks Inc 2022), which produced a list of random numbers ranging from 1-133. These were exported to Excel and copied into the empty column. Lastly, the list was sorted by least-largest random number and acted as the order for the analysis.

#### *2.4.4 Sound analysis*

The 133 sound files were manually examined visually and aurally in the annotation software (see B.1, Appendix B), based on the randomised analysis plan. An FFT-window of 500 was used for the spectrogram (see Appendix A). All ROIs were annotated in categories based on previously reported sound characteristics for cod. The ROIs for cod sounds were knock

(Midling et al. 2002, Verboom 2005), click (Vester et al. 2004, Verboom 2005), grunt (Brawn 1961b, Rowe & Hutchings 2006), humming and rumbling (Nilsson 2004, Rowe & Hutchings 2006). Additionally, the category “suspect click” contained ROIs which seemingly looked like clicks but needed further examination. Lastly, disturbance and splash were used as categories to describe other sounds. Each ROI contained information on name of the sound file, start and end times, start date and end date times, class, channel and before or after feeding. The ROIs were concatenated in a ROI-masterfile through a script written in MATLAB (The MathWorks Inc 2022) by Rasmussen.

A total of 106 hours (2.9 hours for each feeding day) was used to manually annotate 11972 ROIs from the 36 hours of the recorded material. Of these, 2229 were pre-categorised as clicks and 661 as suspect clicks. Clicks were defined as short, abrupt, and transient calls with or without reverberations. They were additionally defined with a duration of approx. 1-300 ms and with a wide frequency bandwidth above 500 Hz (Vester et al. 2004, Verboom 2005). A verification process was conducted on all the ROIs classified as clicks and suspect clicks to assure the accuracy of the manual annotations. In this process, a verification software (see B.2, Appendix B) was used, in addition to the annotation software (see B.1, Appendix B). The timeseries revealed a shorter ICI than previously expected causing multiple clicks to appear in the same ROI. Therefore, the FFT-window in the annotation software was reduced to 200 in the click verification process (see Appendix A). The clicks which did not meet the definition were discarded at this stage, leaving a total of 1890 verified clicks. Lastly, to assure the accuracy of ICI and the duration of each click, a cutting software was used to cut all start and end times with precision (see B.3, Appendix B).

## ***2.5 Statistical analysis***

The sound characteristics of each click were extracted using a MATLAB (The MathWorks Inc 2022) script written by Rasmussen. Some clicks were above the limitation of the Nyquist frequency of the resampled files, meaning that information from the original sound files were required. Due to data management and logistics, this extraction was done by Rasmussen. Extreme values in sound characteristics were re-examined by plotting and visual inspection in the annotation software (see B.1, Appendix B) to verify whether they represented a click. This process reduced the total number of clicks to 1886. All statistical analyses were conducted in MATLAB (The MathWorks Inc 2022) and RStudio (Posit team 2023). Additionally, various

packages were used in RStudio. *ggpubr* was used to plot quantile-quantile (Q-Q) plots to check for normality (Kassambara 2023). *Tidyverse* was used for visualisation (Wickham et al. 2019) and *ggsignif* was used to mark significance in plots (Ahlmann-Eltze & Indrajeet 2021). The package *MASS* was used to run generalised linear models with a negative binomial distribution (Venables & Ripley 2002). Similarly, *lme4* was used to run generalised mixed effect models with negative binomial distribution (Bates et al. 2015). The *DHARMA* package was used to simulate residual diagnostics (Hartig 2022). Further the *car* package was used to test for outliers using the Bonferroni-corrected p-value (Fox & Weisberg 2019). The level of significance for all statistical analysis was set to  $\alpha = 0.05$ .

### 2.5.1 The response variables

The response variables (count, duration, peak-to-peak (P2P), and inter call interval (ICI)) were used to characterise variation in cod clicking. ICI was also used to attempt to identify previously reported classes of clicks by Vester et al. (2004) and Verboom (2005) (see Appendix C).

The count variable was defined as the number of clicks occurring in each period (before/after) for each feeding day ( $n = 36$ ). All clicks were analysed with a fixed time interval of 30 min before and after feeding. One period (before) of one feeding day had a limit to the recording of 24 min. However, the mean time-interval for the whole period was  $29.83 \pm 0.99$  min. The low variation justified using count as a proxy to describe the frequency of clicks instead of click rate over time.

The duration of each click was calculated using a script in MATLAB (The MathWorks Inc 2022). The start and end times for each click were determined in the cutting software (see B.3, Appendix B). The duration in ms was then calculated by the following formula:

$$Duration (ms) = Endtimes - Starttimes * 1000$$

There are multiple ways to characterise the amplitude of a signal. However, two common ways are by root-mean-square (RMS) and P2P. RMS is often used to describe simple sinusoid waves. However, it is dependent on a fixed duration to calculate the average pressure on. It is therefore less suitable for describing transient signals such as clicks which can also have

reverberations. P2P is the pressure difference between the lowest and the highest pressure of a signal. P2P is better suited for measuring short impulsive signals such as clicks (Madsen 2005) and hence it was chosen for the clicks examined here.

ICI was defined as the time from the end of the first click to the start of the second click within the  $\pm 30$  min before and after feeding. The duration of each feeding varied, and annotation of clicks were not possible during feeding due to acoustical noise. Therefore, the ICI values between the periods before and after feeding (as well as between days) were excluded from the statistical analysis.

Mean, median and standard deviation were calculated for the four above mentioned variables as the high variation in the dataset were heavily affecting the mean. All variables were right-skewed and had an exponential distribution. All response variables were tested for normality using Shapiro-Wilks test and visual inspection through quantile-quantile (Q-Q) plots. The tests showed a significant ( $p < 0.05$ ) deviation from a normal distribution for all variables. Further, normal distribution was not achieved by log transformation. The variables were tested for correlation using non-parametric tests. There was a significant weak correlation between duration and ICI (Kendall rank correlation: tau: 0.1236551,  $n = 1886$ ,  $p\text{-value} = 1.664e-15$ ), and P2P and ICI (Kendall rank correlation: tau: 0.1058546,  $n = 1886$ ,  $p\text{-value} = 9.18e-12$ ). Further a moderate significant positive correlation was found between duration and P2P (Kendall rank correlation: tau: 0.38,  $n = 1886$ ,  $p\text{-value} < 2.2e-16$ ). Because no strong correlation was found, all variables were modelled separately.

### *2.5.2 Power spectrum*

To investigate the relative power distribution across frequencies, power spectrums were calculated in MATLAB (The MathWorks Inc 2022). Power spectrums describe the distribution of energy across frequency bins that combined constitutes a signal. The background noise in the study was constant, hence low amplitude clicks were dominated by the background noise. Therefore, a histogram of P2P was investigated to determine which clicks to include to get the best representation of the relative power distribution across frequencies. An amplitude of  $1e-3$  was chosen as a threshold for clicks to include in the power spectrogram calculations, as it included a significant part of the data (30.86%) while still excluding the lowest amplitude clicks highly influenced by background noise. The extraction

of values was done by Rasmussen. The mean minimum and maximum frequency were calculated from 10% and 90% of the energy level to better represent clicks and mitigate the influence of the background noise. Min, max and peak frequency were extracted using a script in MATLAB (The MathWorks Inc 2022). Peak frequency was defined as the frequency where the highest relative power of the call is located.

### *2.5.3 Model selection*

Information on extended statistical knowledge was gained from several sources (de Jong & Heller 2008, Zuur et al. 2009, Anderson et al. 2010, Dunn & Smyth 2018). The aim was to test for effects of feeding on click count and sound characteristics. However, as channel and date might influence clicks, they were included in the models.

Click count was tested with a poisson and quasi-poisson distribution (see D.1, Appendix D). Due to overdispersion and high residual deviance, a negative binomial distribution with a log-link function was used to model count. To assess if date should be included as a random effect, both a generalised linear model (GLM) and a generalised mixed effect model (GLMM) with date as a random effect was applied. The models were simplified using a backward stepwise approach where the full models were sequentially compared to simpler ones. Due to smaller samples, the goodness of fit was based on AICc criteria, where model with the lowest AICc was considered to be the best model for explaining the variance (Burnham & Anderson 2004). The residuals were examined through quantile-quantile (Q-Q) plots and residual vs. predicted-plots (Figure 20, Appendix D). Further, to test for outliers, the model was evaluated with only the datapoints within the quartiles (25% and 75%) (see D.1, Appendix D).

For the continuous response variables duration, P2P and ICI, the best model was assessed by applying assorted combinations of distributions and link functions and examining bias in the residual variance (see Appendix D). To assess if date should be included as a random effect or not, the following approach was conducted for both generalised linear models and generalised mixed effect models with date as a random effect. The models were first fitted with a gaussian distribution with both identity and log-link function. Due to overdispersion and the variables having a positively right-skewed distribution, the models were tested with both gamma and inverse gaussian distribution using both the default- and log-link function. All models were simplified through a backward stepwise approach where the full model was sequentially

compared to simpler ones, using Chi-square tests to select the minimum adequate model for each distribution and link function. The goodness of fit of each minimum adequate model were compared by AIC, where the lowest AIC value was considered to be the best model for explaining the variance for each response variable (Burnham & Anderson 2004).

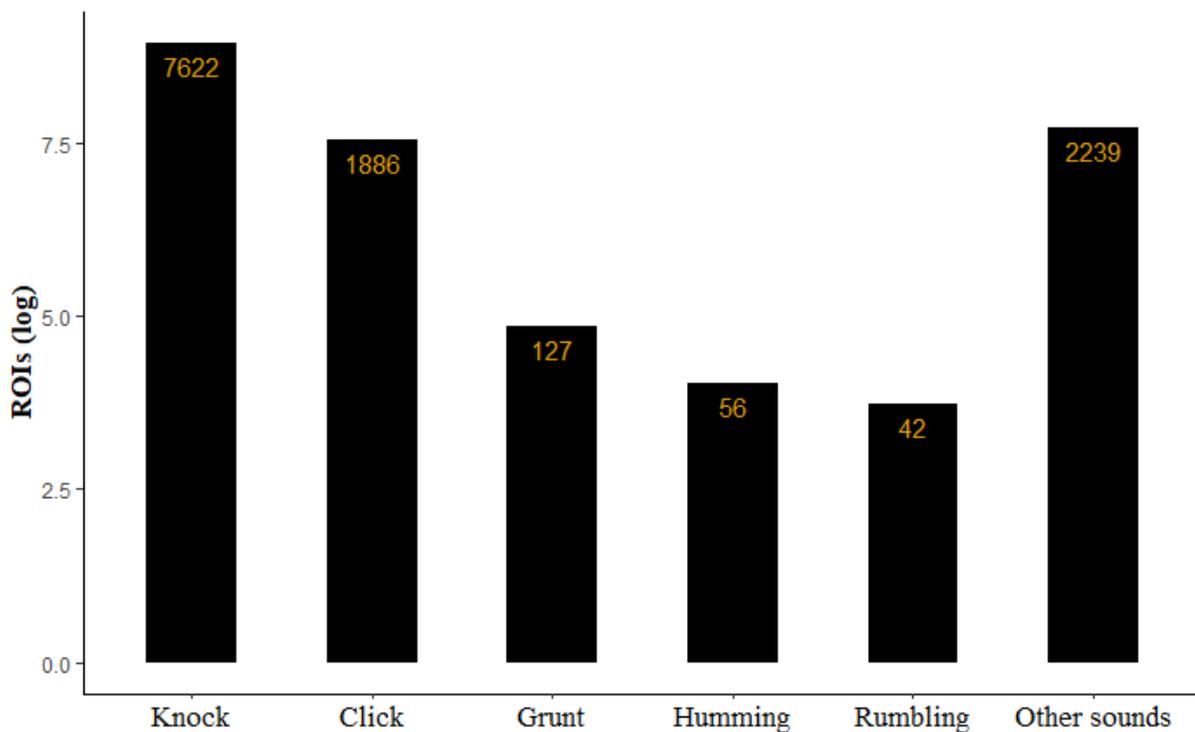
The best model for each response variable (see Appendix D) was examined using a forward stepwise approach and Chi-square test to acquire p-values. The residuals for each model were examined through quantile-quantile (Q-Q) plots and residual *vs.* predicted-plots. Further, to examine effects of extreme events, the models were tested without the most extreme values and with only the datapoints within the quartiles (25% and 75%) (see Appendix D). The explanatory power of GLM models were calculated by:

$$\textit{Explained deviance} = 100 \times \frac{\textit{Null deviance} - \textit{Residual deviance}}{\textit{Null deviance}}$$

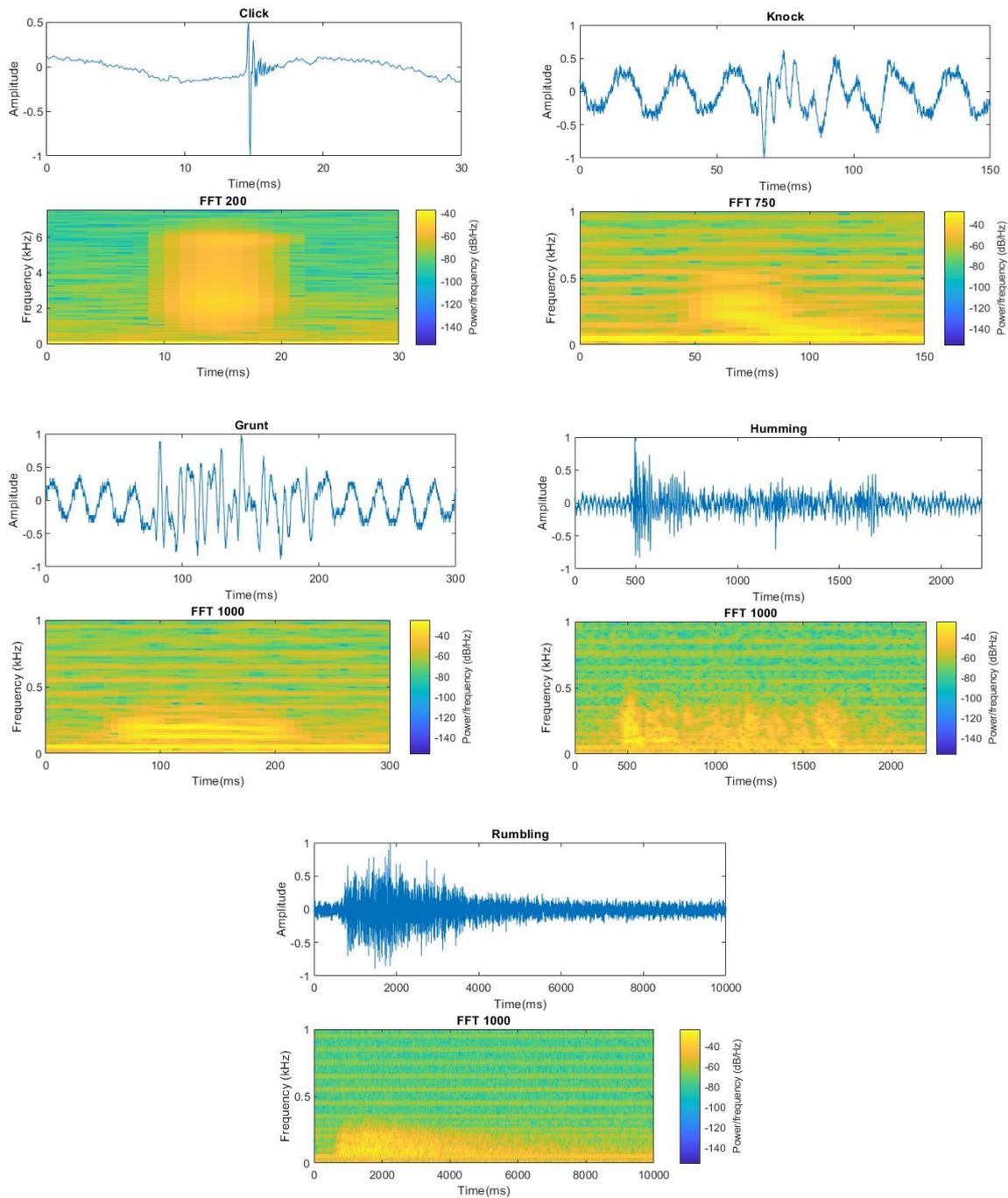
### 3 Results

#### 3.1 The complete repertoire of cod sounds

Of 36 hours of recorded material, a total of 11972 ROIs were manually classified through visual and auditory analysis. Excluding other sounds, 9733 ROIs were estimated to be sounds emerging from cod (Figure 3). There was a large variation in the occurrence of the different sounds from cod, where 78.3% were knocks. Further, 19.4 % were clicks and grunts (1.3%), hums (0.6%), and rumblings (0.4%) accounted for of less occurrence. The low frequency cod sounds had their main energy below 1 kHz and were differentiated by duration, energy, and frequency (Figure 4). Knocks had a mean duration of  $97.66 \pm 49.06$  ms, grunts  $224.52 \pm 167.48$  ms, hums  $887.97 \pm 607.22$  ms and rumblings  $8025.29 \pm 7536.72$  ms.



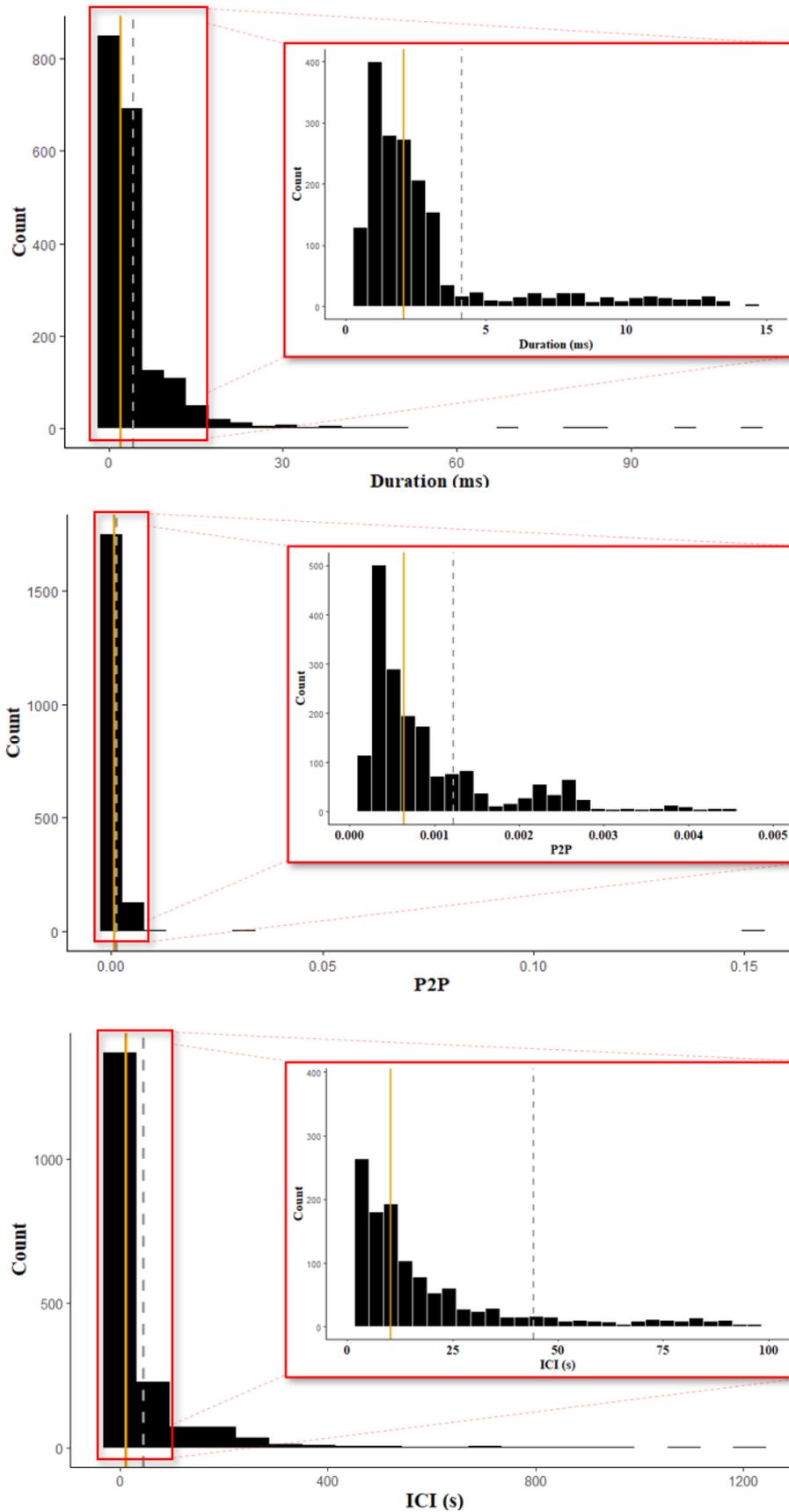
**Figure 3** All annotated regions of interests (ROIs) from the 36 hours of recorded material. The visualisation is in log and the yellow numbers refer to the absolute number.



**Figure 4** Timeseries and spectrogram of the five cod sound categories (click, knock, grunt, humming and rumbling) used during the sound analysis.

### 3.2 Acoustic variation in cod clicks

Click was the second-most occurring sound during the study (Figure 3). The 1886 clicks had high variation in sound characteristics (Table 1). The sensitivity analysis did not show statistically distinct classes of clicks (see Appendix C). The continuous variables duration, P2P and ICI were right-skewed and had an exponential distribution (Figure 5).



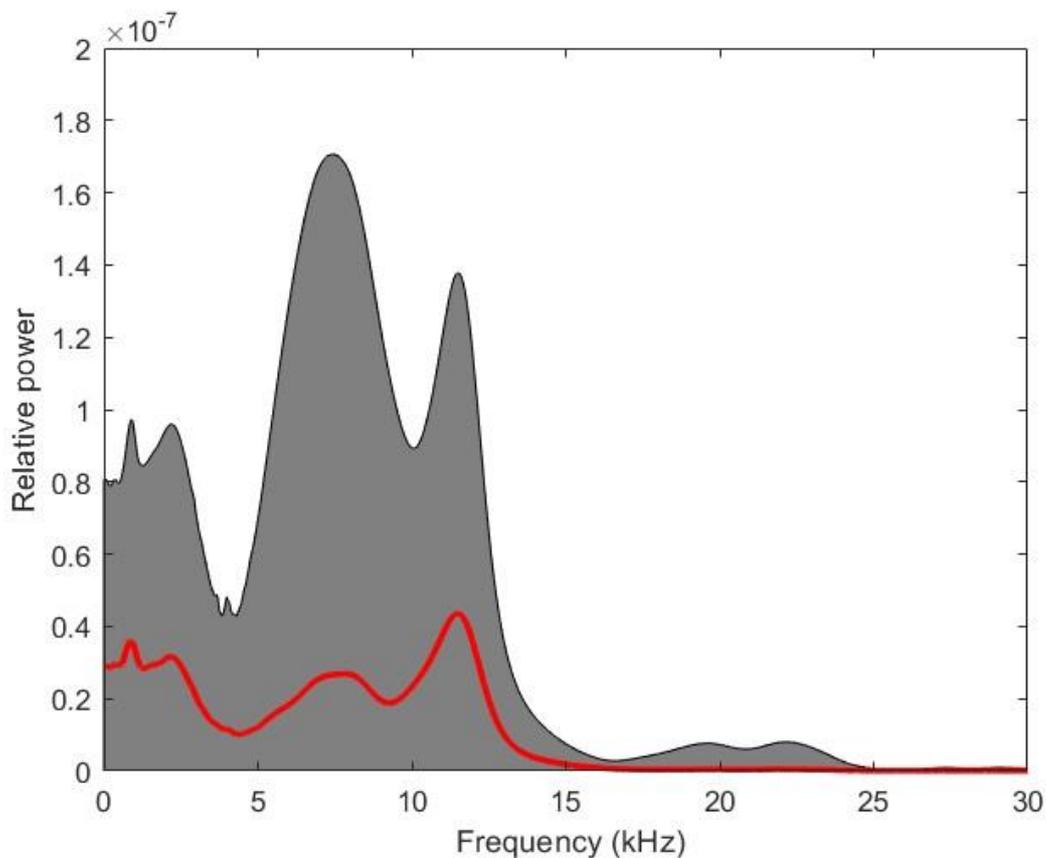
**Figure 5** The distribution of the response variables duration, peak-to-peak, and inter call interval were right skewed with some extreme values. Values on the lower scale is zoomed in. The grey dashed line presents the mean, while the yellow solid line presents the median.

**Table 1** Summary statistics for the response variables for the 1886 clicks analysed during the study.

Statistics	Count (n)	Duration (ms)	P2P (r. pressure)	ICI (s)
Mean	26.19	4.14	0.0012	44.19
SD	58.94	6.99	0.0051	104.63
Median	9	2.07	0.0064	10.32
Min	0	0.40	0.00012	0.0035
Max	340	111.35	0.15	1213.76

### 3.2.1 Power spectrum

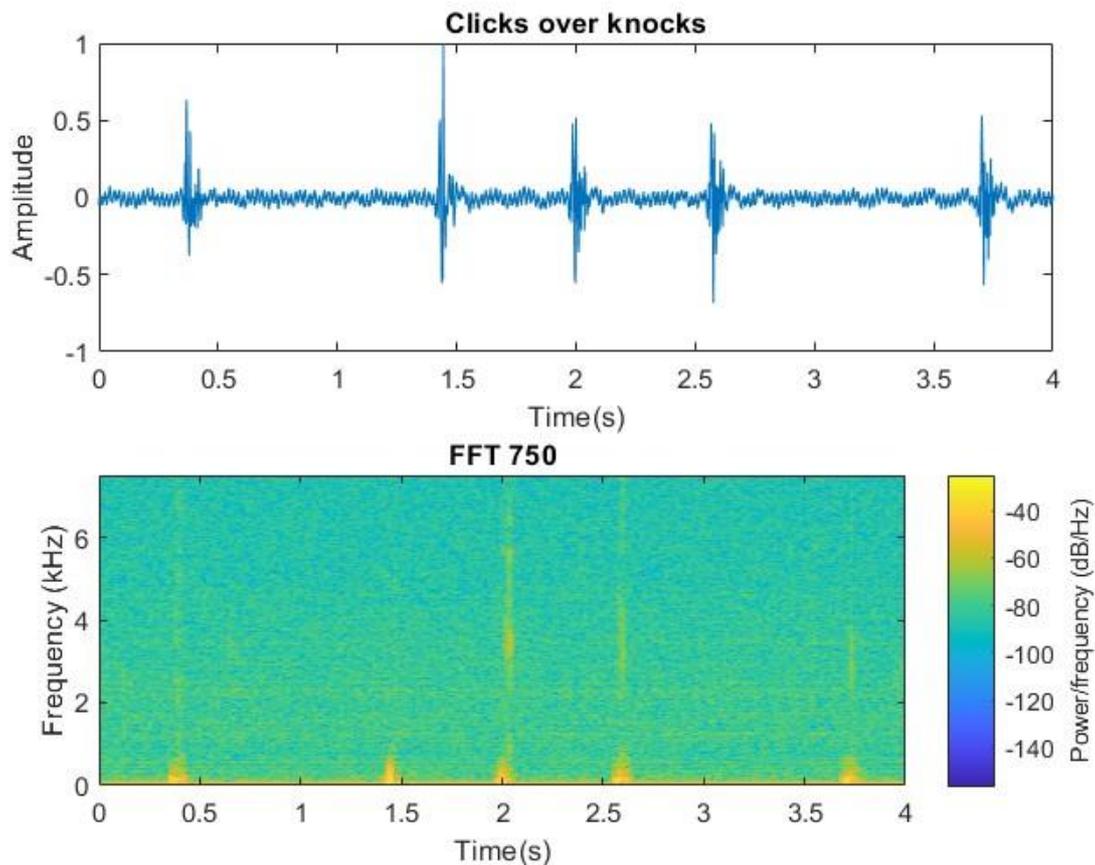
The power spectrum of the 582 (30.86 % of total) clicks with the highest relative power shows that the clicks were broadband (Figure 6). There was pass between 4-5 kHz where the frequencies had a lower relative power. The mean minimum frequency (10%) was  $1.24 \pm 1.55$  kHz and the mean maximum frequency (90%) was  $14.38 \pm 13.91$  kHz. Peak frequency was  $4.45 \pm 5.93$  kHz.



**Figure 6** Power spectrum of the 582 clicks with the highest relative power. The red line is the mean relative power. The grey colored area is the standard deviation.

### 3.2.2 Clicks and other sounds

On three feeding days (14/04/22, 25/04/22, 10/05/22), clicks occurred simultaneously as knocks (Figure 7). On the 14/04/22 (Figure 7) a series of knocks with clicks on top occurred, whereas on the 25/04/22 and 10/05/22 singular events occurred. Clicks did not co-occur with other cod sounds within the analysed recordings.



**Figure 7** Clicking occurring simultaneously as knocks in a series of five, observed on 14/04/22. The clicks had a low amplitude and are seen as the lines with a broadband frequency. The knocks were low frequency and are seen as the yellow markings in the lower end of the frequency-scale.

## 3.3 The effects of feeding on clicking

### 3.3.1 Click count

Clicks occurred both before and after feeding (Figure 8). There was a significant difference in number of clicks before and after feeding ( $X^2 = 68.717$ , d.f. =1, p-value < 0.001) (Table 2).

**Table 2** Summary statistics for clicks occurring before and after feeding.

Statistics	Before (n = 763)	After (n = 1123)
Mean	21.19	31.19
SD	58.01	60.25
Median	4.50	11.50
Min	0	0
Max	340	243

The backward selection process, AICc and residual deviance indicated that the generalised mixed-effect model with a negative binomial distribution and log-link function had the best fit. The model included period as a fixed effect and date as a random effect (Table 3 and D.1, Appendix D). The AICc difference for the two competing models, B2 and B3 was small. Considering the goodness of fit and the simplicity of the model, the most parsimonious model was found to be B3. Period had a significant effect on click count (Table 4), whereby the number of clicks increased after feeding (Table 5). The estimated variance of the random effect date was  $1.353 \pm 1.163$ . The variance was slightly deviating, however the residuals were fairly homogenous, and no emergent pattern was seen (Figure 20, Appendix D).

**Table 3** AICc based model selection for factors affecting click count, comparing generalised linear model and generalised mixed effect model with date as a random effect.

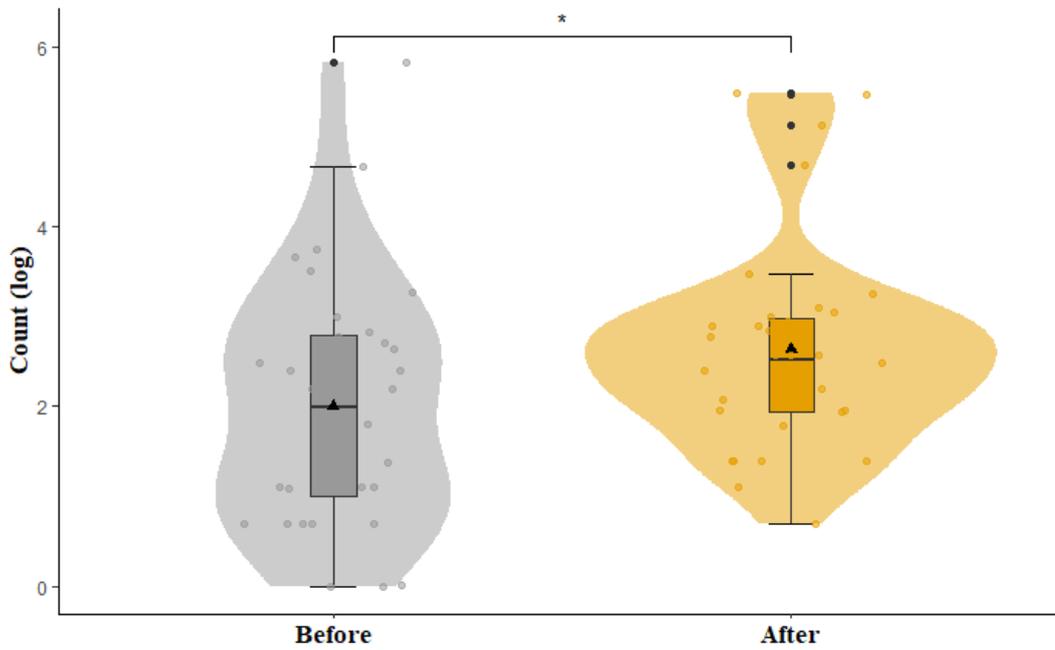
Model	Structure	AICc	Delta	d.f.
<b>A</b>	period x channel x date	567.95	8.44	9
<b>B</b>	period x channel + (1 date)	561.55	2.05	6
B2	period + channel + (1 date)	559.50	0.00	5
B3	period + (1 date)	560.44	0.93	4

**Table 4** Results from the generalised mixed effect model for the effect of feeding period on click count (B3). The p-value is calculated from the F-value. Asterisks indicate a significance level of  $\alpha = 0.05$ .

Model term	d.f.	Sum of squares	Mean of squares	F value	p-value
period	1	9.45	9.45	9.45	0.003*

**Table 5** Effects of the two feeding periods (before and after) on click count. The feeding period before is set as the reference in the model. Estimates are in log. Asterisks indicate a significance level of  $\alpha = 0.05$ .

Model term	Estimate	Std.Error	Z value	Pr(> z )
Intercept	1.995370	0.2488557	8.018179	< 0.001*
After	0.610239	0.2135393	2.857736	< 0.001*



**Figure 8** The effect of feeding period (before and after) on click count in log scale. Mean is presented by a black triangle.

### 3.3.2 Click duration

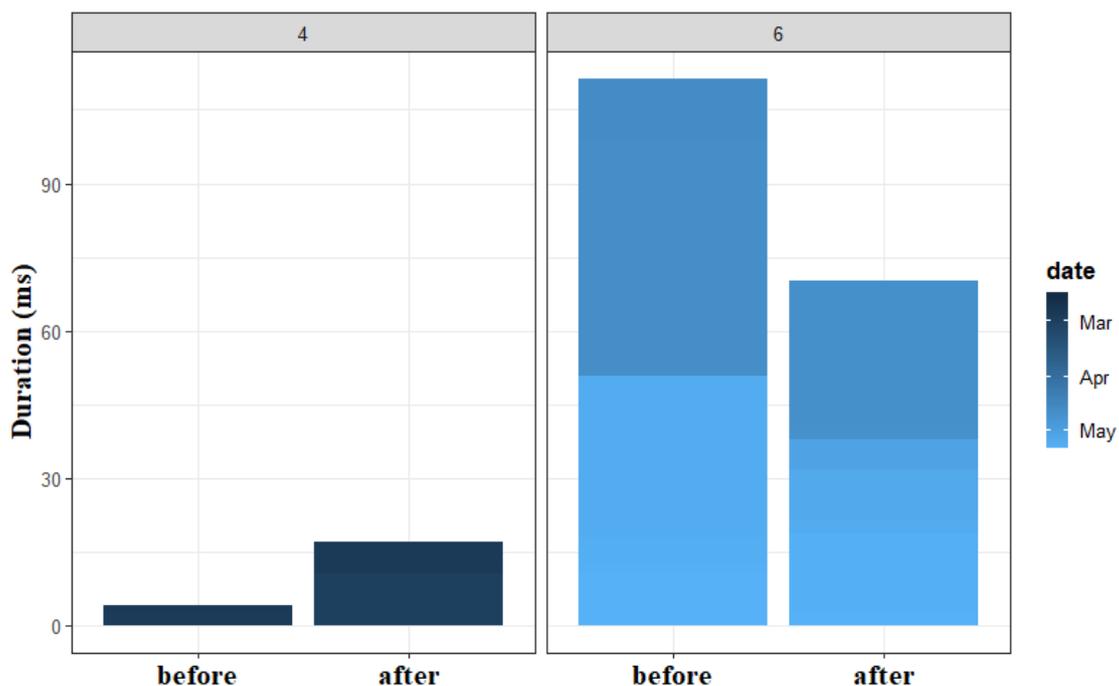
The backward selection process, AIC and residual deviance indicated that the generalised linear model with inverse gaussian distribution and log-link function had the best fit. The model included period, channel and date as fixed effects, and a three-way interaction between period, channel and date, and all lower-order interactions (see D.2, Appendix D). Period had no overall effect on duration (Table 6). However, channel, date and all interactions had a significant effect (Table 6). The effect of feeding on click duration was more negative at later dates on channel 6 than earlier dates on channel 4 (Table 7 and Figure 9). The model had an explanatory power of 46.1 %. The variance was slightly deviating, showing a tail in the quantile-quantile plot. However, the residuals were fairly homogenous, and no emergent pattern was seen (Figure 21, Appendix D). Further, approx. 98% of the data was explained by the model (see D.2, Appendix D)

**Table 6** Deviance table from the generalised linear model for the effects of period, channel and date on click duration. The P-values were obtained from  $X^2$ -tests by adding period, channel date and the interactions to the null model. Asterisks indicate a significance level of  $\alpha=0.05$ .

Model term	d.f.	Deviance	Residual d.f.	Resid. Dev	P-values
null			1885	641.06	
period	1	0.001	1884	641.06	0.972
channel	1	234.89	1883	406.17	< 0.001*
date	1	12.27	1882	393.90	< 0.001*
period x channel	1	3.09	1881	390.81	0.0015*
period x date	1	0.68	1880	390.13	< 0.001*
channel x date	1	36.70	1879	353.43	<0.001*
period x channel x date	1	7.90	1878	345.53	<0.001*

**Table 7** The parameter estimates and standard errors in log from the model describing the effects of period, channel and date on click duration. The feeding period before, channel 4 and first date is set as the intercept in the model. Asterisks indicate a significance level of  $\alpha=0.05$ .

Model term	Estimate	Std.Error	t value	Pr(> t )
(intercept)	-3.778e+02	6.227e+02	-0.61	0.544
after	-5.834e+03	7.743e+02	-7.54	<0.001*
channel	1.153e+02	1.109e+02	1.04	0.299
date	1.960e-02	3.268e-02	0.60	0.549
period x channel	1.150e+03	1.421e+02	8.09	<0.001*
period x date	3.060e-01	4.063e-02	7.53	<0.001*
channel x date	-5.986e-03	5.816e-03	-1.03	0.304
period x channel x date	-6.027e-02	7.452e-03	-8.09	<0.001*



**Figure 9** The effects of feeding period (before and after), channel (4 and 6), and date in absolute number.

### 3.3.3 Peak-to-peak of clicks

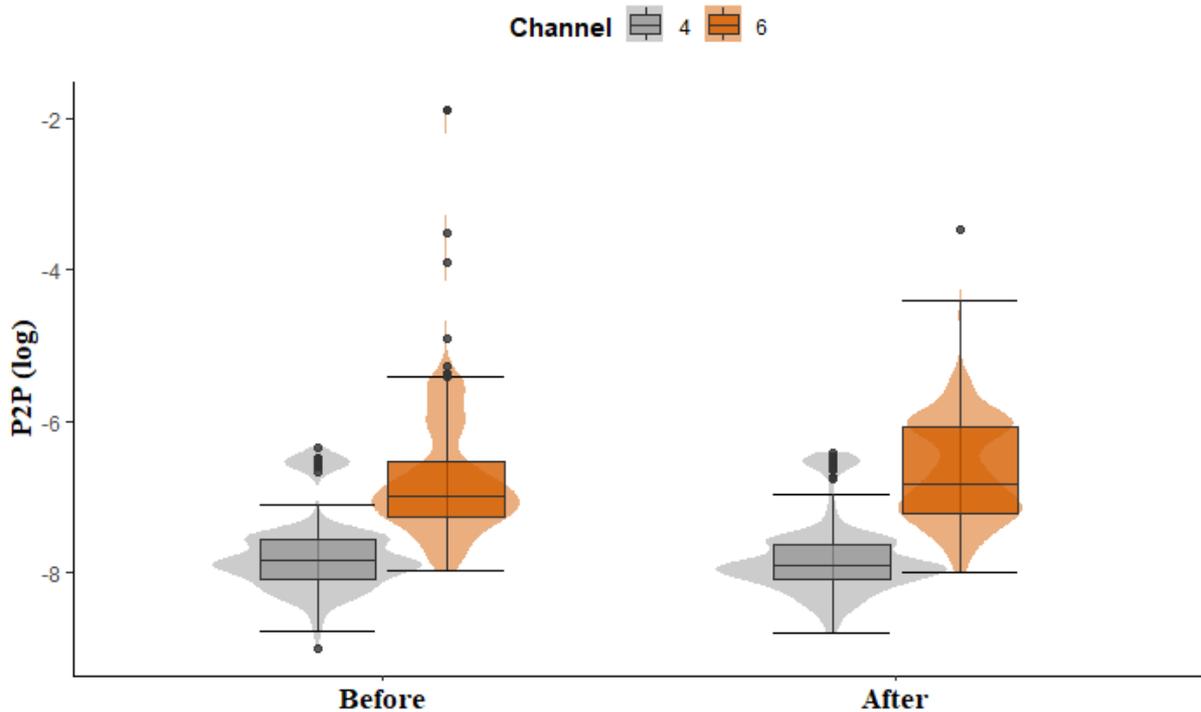
The backward selection process, AIC and residual deviance indicated that the generalised linear model with a gaussian distribution and identity-link function had the best fit. The model included period and channel as fixed effects (see D.3, Appendix D). Period had no significant effect on P2P of clicks (Table 8). However, channel had a significant effect (Table 8). The model had an explanatory power of 4 %. The estimates suggest that the P2P increased from channel 4 to channel 6 (Table 9 and Figure 10). The variance was slightly deviating, showing a few datapoints not well-explained by the model. Despite this, the residuals were fairly homogenous, and no emergent pattern was seen (Figure 22, Appendix D).

**Table 8** Deviance table from the generalised linear model for the effects of period and channel on the peak-to-peak of clicks. The P-values were obtained from  $X^2$ -tests by adding period and channel to the null model. Asterisks indicate a significance level of  $\alpha=0.05$ .

Model term	d.f.	Deviance	Residual d.f.	Resid. Dev	P-values
null			1885	0.49	
period	1	0.0000026	1884	0.49	0.750
channel	1	0.0010	1883	0.048	< 0.001*

**Table 9** The parameter estimates and standard errors in absolute numbers from the model describing the effects of period and channel on the peak-to-peak of clicks. The feeding period before and channel 4 is set as the intercept in the model. Asterisks indicate a significance level of  $\alpha=0.05$ .

Model term	Estimate	Std.Error	t value	Pr(> t )
(intercept)	-0.0024	0.00060	-3.94	< 0.001*
after	-0.00047	0.00025	-1.93	0.054
channel	0.00077	0.00012	6.36	< 0.001*



**Figure 10** The effect of feeding period (before and after) and channel (4 and 6) on peak-to-peak in log scale.

### 3.3.4 Inter call interval between clicks

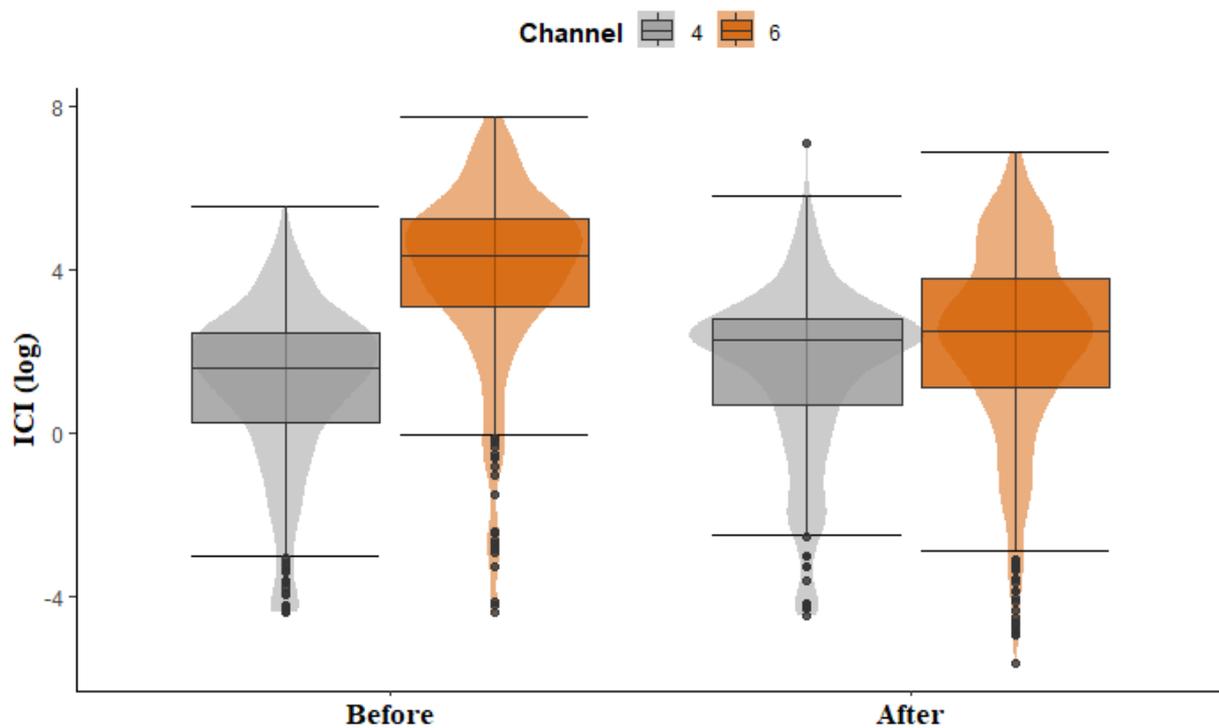
The backward selection process, AIC and residual deviance indicated that the generalised mixed effect model with a gamma distribution and log-link function had the best fit. The model included period and channel as fixed effects, and a two-way interaction between period and channel (see D.4, Appendix D). Period had no overall effect on ICI (Table 10). However, channel and the interaction between channel and period had a significant effect (Table 10). The estimated variance of the random effect date was  $1.12 \pm 1.06$ . The ICI increased from channel 4 to channel 6 (Table 11 and Figure 11). However, the effect of the interaction between period and channel had a negative effect on the ICI between clicks. The variance was slightly deviating, however the residuals were fairly homogenous, and no emergent pattern was seen (Figure 23, Appendix D).

**Table 10** Results from the generalised mixed effect model for the effects of period, channel and their interaction on the inter call interval between clicks. The p-value is calculated from the F-value. Asterisks indicate a significance level of  $\alpha=0.05$ .

Model term	d.f.	Sum. Sq	Mean sq	F value	p-value
period	1	4.13	4.13	2.90	0.089
channel	1	8.78	8.78	6.16	0.013*
period x channel	1	145.05	145.05	101.77	< 0.001*

**Table 11** Effects of the feeding period (before and after) and channel (4 and 6) on the inter call interval between clicks. The feeding period before and channel 4 is set as the intercept in the model. Estimates are in log. Asterisks indicate a significance level of  $\alpha= 0.05$ .

Model term	Estimate	Std.Error	t value	Pr(> t )
(intercept)	-1.005	1.25	-0.81	0.420
after	3.17	0.33	9.55	<0.001*
channel	1.07	0.22	4.88	<0.001*
period x channel	-0.67	0.65	-10.23	<0.001*



**Figure 11** The effect of feeding period (before and after) and channel (4 and 6) on the inter call interval between clicks in log scale.

## **4 Discussion**

### ***4.1 The complete repertoire of cod sounds***

The thesis aimed to expand the reference library for annotated cod sounds by developing a comprehensive catalogue of annotations of all known cod sounds. Within the span of 36 hours of recorded material, all previously reported cod sounds were observed (Figure 3). The annotations provided a comparative overview of the occurrence of each sound. Knocks were the most frequent sound, often in extended long knock series such as reported for wild cod (Midling et al. 2002). Further, the characteristics for grunts, hums and rumblings are consistent with previously reported cod sounds (Midling et al. 2002, Nilsson 2004, Rowe & Hutchings 2006) (Figure 4). Such baseline studies increase the access to annotated cod sounds, a major bottleneck in expanding bioacoustical studies today. The increased reference library provides essential information for development of automated analysis such as training deep learning algorithms (Waddell et al. 2021, Goodwin et al. 2022). Such technology increases the potential to analyse larger sets of data which might improve our understanding of cod sound production. To the best of my knowledge, this is the first study in which all sounds previously reported for cod are observed and described concurrently.

### ***4.2 Acoustic variation in cod clicks***

The thesis aimed to increase the understanding of cod clicks and how they relate to cod behaviour. Cod was the only fish present in the basin when the high frequency broadband clicks were observed which confirms previous studies in that clicking emerges from cod (Vester et al. 2004, Verboom 2005). Surprisingly, clicks were the second most occurring sound emerging from cod in the analysed period (Figure 3). This can indicate that clicking plays a bigger role in cod behaviour and sound production than previously estimated. The study was performed in an enclosed semi-natural basin using 45 wild-caught cod, while the two previous studies on clicks were performed in tanks both in- and outdoors. Further, both previous studies were conducted using captive bred cod. Verboom (2005) used 9 cod whereas Vester et al. (2004) had different amounts ranging from 7-65 cod depending on the experimental conditions. Studies conducted in tanks may restrict natural behaviour (Midling et al. 2002). The clicks had a wide variation in sound characteristics (Table 1) with some extreme values (Figure 5). The semi-natural environment and the wild-caught origin of the

cod might have allowed for a larger degree of natural behaviour which may explain the wide variation and extreme values. The present study also had a higher sample size which may have increased the chance of observing more variation and extreme events. All clicks were analysed through a three-step analysis by conducting manual examination through listening and visually through spectrograms and timeseries. Despite this, it might be that these extreme values are in fact not clicks, but a result of human error. Nevertheless, these clicks had similar characteristics to those described in previous studies and excluding these from the analysis could have led to the loss of vital information on variation in cod clicking. The variation and extreme values found in the sound characteristics might be confirmed by increasing the sample size in future studies.

The increased sample size in the present study did affect the ability to classify clicks. An extensive effort was put in to investigate classes of clicks reported by Vester et al. (2004) and Verboom (2005). However, the study found no support for a statistical or natural threshold for the previous classifications: single sonic clicks, pre-clicks, double-clicks and series of clicks (see Appendix C). This was not expected, as a high number of clicks seemingly occurred with the same ICI as previously reported for classes such as pre-clicks and double-clicks, during the sound analysis. This was also seen in the sensitivity analysis (see Appendix C). Additionally, the sensitivity analysis revealed clicks with a lower amplitude in front of others which is consistent with the class pre-clicks. However, the inter call interval within both ICI-thresholds of a 100 ms and 1 second (see Appendix C) indicated a continuous distribution. Further, the group of pre-clicks could not be statistically divided from the rest of the clicks. Lastly, no correlation was found between ICI and dB, which would have been expected based on previous descriptions of the class pre-clicks. Conclusively, describing all clicks as single events may be more informative than to divide them into categories when no evidence for discrete categorical classifications were found in the data. The initial approach was to determine classes of clicks before the sound analysis and classify them thereafter. These results emphasise the importance of objectivity when conducting baseline bioacoustical studies and investigating lesser-known sounds.

The three variables duration, P2P and ICI had right-skewed distributions. As the mean was influenced by the extreme values, the median is used to discuss the variables as it may be more representative of the majority of clicks. The median click duration was 2.1 ms, which is shorter than previously described. Verboom (2005) found a mean duration of  $150 \pm 51$  ms,

however, the mean duration in the present study was 4.14 ms. The duration is influenced by the length of reverberations which increase in smaller tanks (Hawkins & Amorim 2000). This suggests that the present study was less affected by acoustical issues such as reverberations. Additionally, Verboom (2005) and Vester et al. (2004) estimated that the click without reverberations was less than 1 ms. This is supported by the present study, as the minimum duration was 0.4 ms, and indicates that the study might have successfully recorded the click itself without reverberations. On the other hand, the longest click duration was 111.35 ms. The clicks with the longest duration also had the highest P2P, and duration and P2P had a moderate significant positive correlation. Most clicks had low relative amplitude, with the exception of a few clicks with a high duration and P2P. A higher P2P could have increased the potential to detect reverberations and thus increased the duration. The end of reverberations in clicks with low P2P could be difficult to detect as it fades out in background noise. The extreme values seen in P2P can be explained by the cod being really close to the hydrophone. Further, the P2P values exhibited large variation within short time intervals in which high and low P2P values occurred simultaneously. This could be caused by cod swimming towards and away from the hydrophone as well as the way the cod faced when the sound was produced. On the other hand, there were 45 cod in the basin and the variation may be caused by multiple cod emitting clicks at the same time. Lastly, the big basin allowed for free roaming cod, meaning that they were not necessarily close to the recording hydrophone at all times. Further studies including multiple hydrophones for triangulation of the location of the sound sources could give more in depth knowledge about source levels of the emitted sounds.

The median ICI of 10.31 s indicates that clicking occurred fairly often. As the ICI is the difference to the next click, periods with low clicking activity could have an ICI up to 1800 s if they only occurred one time in each feeding period. This influences the ICI median and mean. The ICI in previous papers on cod clicks (Vester et al. 2004, Verboom 2005) are only reported for classes and cannot be directly compared to this study. However, as previously discussed, there was a high number of clicks occurring close to each other. This might provide information on the sound producing mechanism. For example, assuming that the clicks with short ICI origin from the same cod, this may indicate that click emerge from a stridulatory and two-sided production mechanism such as movement of two fins simultaneously or side-to-side movement. However, this is speculation and further studies are needed to confirm the sound production mechanism.

During the click verification- and cutting stage (see B.2 and B.3, Appendix B), it was evident that clicks had a shorter duration and ICI than expected. This was revealed by the subsequent use of timeseries to precisely annotate the start and end times. The chosen FFT-window of 500 for the spectrogram was thought to be sufficient to discover and separate clicks. However, the minimum ICI was found to be 3.5 ms and such short intervals could not be separated by this window size in the spectrogram. Therefore, an FFT-window of 200 was necessary to separate clicks with low ICI and get a better time resolution of clicks with short duration in the spectrogram (see Appendix A). Future studies should take this into consideration when investigating cod clicks for a time-efficient analysis.

Clicks were broadband and had a high variation as seen on the power spectrum, where the spectra range from 0 to above 30 kHz. This is consistent with the previous studies on cod clicking (Vester et al. 2004, Verboom 2005). Similarly, the present study found a peak frequency of  $4.45 \pm 5.93$  kHz, whereas Vester et al. (2004) reported  $5.95 \pm 2.22$  kHz and Verboom (2005) reported  $4.75 \pm 0.72$  kHz. The study was performed in an open semi-natural environment, which implies less acoustical disturbance compared to indoor tanks. As previously discussed, most clicks in the study had a low relative amplitude, which increases the chance of the frequency values to be masked by background noise. The power spectrum was produced using only the 582 clicks with the highest relative power to minimise this influence from background noise. Despite this, the sample size was larger than what was previously used to report frequency values of clicks. The similar result among all studies may indicate that the frequency values did not change significantly with increased sample size. However, the large standard deviation indicate that more variation was captured by this study. The equivalent results suggest that the present study may be influenced by the same level of background noise as the previous studies. On the other hand, it may be that the peak frequency is underestimated and that the 582 clicks used in the power spectrum are still influenced by low relative amplitude. It is possible that the frequency distribution of clicks is truly as broadband as the power spectrum implies. However, the pass between 4-5 kHz where the frequencies had a lower relative power exhibits a separation of frequency values (Figure 6). This may indicate that the lower end of the frequencies (0-5 kHz) may be influenced more by background noise rather than reflect frequency values of clicks. This could explain why the mean minimum frequency was lower than previously reported. The mean minimum frequency calculated at 10% of the energy level was  $1.24 \pm 1.55$  kHz. This is small compared to the previously reported minimum frequency of  $3.26 \pm 2.05$  kHz (Vester et al. 2004). On the

other hand, the mean maximum frequency calculated at 90% was  $14.38 \pm 13.91$  kHz, whereas Vester et. al (2004) reported  $11.23 \pm 5.58$  kHz. This indicates that the study has captured a larger part of the higher frequencies of clicks. To conclude, clicks are high frequency broadband sounds with large variation in frequency values. The study was somewhat influenced by background noise. However, due to the larger sample size, it may be that more variation in click frequency has been captured by this study.

The sound producing mechanisms for clicks are out of the scope of this study. However, on three occasions clicks were found happening simultaneously as knocks (Figure 7). Low frequency sounds from cod are produced by the drumming muscle (Brawn 1961b, Rowe & Hutchings 2006). Despite these muscles being the fastest within vertebrates, they cannot contract fast enough to produce high frequency broadband clicks (Ladich 2015). Assuming that the knocks and clicks emerged from the same cod, this suggests that clicks are not produced by the drumming muscle, as they are unlikely to be able to produce these two sounds at the same time. Further, this supports the hypothesis that clicks originate from another production mechanism such as stridulation (Kaatz 2002, Vester et al. 2004, Verboom 2005). The simultaneous production of clicks and knocks could indicate that clicks are passively emitted sounds as a function of movement. Clicks have previously only been observed in relation to being threatened or approached by a predator-like figure. Vester et al. (2004) suggested that clicking was actively produced in certain threatening situations, as they did not observe clicks while cod were chased by a hoop net. Assuming that the simultaneous click and knock sounds were emitted from the same cod, the results suggest that the cod did not intentionally produce the click because the knock was the active vocalisation. Knocks from gadoids have been related to agonistic behaviour (Hawkins & Amorim 2000) and the clicks may be a side effect of an agonistic displays such as chases, lateral displays and circling movements (Rowe & Hutchings 2006). On the other hand, this rarity only occurred on three occasions and the sounds may also have been produced by different individuals.

Despite cod being a hearing specialist, the high frequency broadband clicks produced are outside of their hearing ability (Hawkins & Popper 2020). This indicates that clicks were not actively produced for intraspecific competition. However, it might be that cod could perceive the sound through particle motion, despite not hearing the sound (Hawkins 2022). The present study could not determine conclusively if the clicks were a result of passively emitted sound as a function of movements or related to agonistic behaviour. The sound producing

mechanism is still up for debate and further studies including video analysis are needed (Mouy et al. 2023). Video can support the acoustical information and might be the means to accurately determine where clicks emerge from, how many cod are present and vocalising, and in what behavioural contexts the sounds are emitted.

### ***4.3 The effects of feeding on clicking***

Cod clicks were observed both before and after feeding. Initially, feeding was thought to have potentially two outcomes. Either that cod perceived it as a positive experience due to reward, or a negative experience due to threat by human interaction or agonistic behaviour between cod competing for food. During the recording period, cod occasionally followed the feeding personnel along the basin edge when observed or heard (S. Stiansen, personal communication, 02.12.2022). This indicates that the feeding personnel acted a signal for a reward which cod recognised (Nilsson et al. 2008b). Additionally, cod has been found to show anticipatory behaviour and crowd together in the area where rewards are given (Nilsson et al. 2008). Combined, this indicates that the feeding was likely a positive experience for cod and that clicking has been studied in relation to a positive experience for the first time. There was a significantly higher amount of clicking after feeding. This may be caused by increased swimming activity while feeding if clicks are a passively emitted sound as previously discussed. However, sound production is found to increase in relation to competition for food for other fish (Amorim et al. 2004), which may explain the increased amount of clicking after feeding occurred. On the other hand, it may also be caused by cod being threatened. Verboom (2005) found that the clicking reduced when the cod no longer perceived the disturbance as a threat. Cod's habituation to threats has also been found in other studies (Walle 2020). If the feeding were perceived as a threat, habituation would have been expected throughout the season. This was not observed in this study, as date explained little variation in the occurrence of clicks. Independent of feeding being a positive or negative experience, the present study provides new evidence that clicking occurs independent of cod being disturbed.

Clicking sounds has only been observed from cod after being threatened or approached by a predator in previous studies (Vester et al. 2004, Verboom 2005). It was therefore inferred that clicks have a predator startling function. Clicks occurring both before and after feeding does not support that hypothesis. Although clicking occurs independent of disturbance, the study cannot rule out that clicking is a result of agonistic behaviour between the cod. Sounds with

similar characteristics in other fishes have been related to agonistic behaviour (Ladich 1997, Kasumyan 2009, Bussmann et al. 2021). Additionally, the study was conducted during the spawning period with increased activity and territorial behaviour (Brawn 1961a), which implies more agonistic behaviour. Further, cod sound production is found to increase during spawning (Midling et al. 2002, Finstad & Nordeide 2004), which means that the selection of time of study might have contributed positively to the ability to record cod sounds. If clicking was related to agonistic behaviour as a function of spawning, the number of clicks were expected to vary significantly during the spawning season. However, the different feeding days throughout the spawning season explained little variation in the number of clicks. This indicates that cod clicks occur randomly and that is it not a result of a specific agonistic behaviour. However, the results only cover one hour around the feeding time each day and thus capture a small part of the daily rhythm. Additionally, it may be that the recording period captured similar levels of spawning activity throughout. Cod sound production is found to increase at night (Rowe & Hutchings 2006), which may influence the number of clicks as well. The present study could not determine if clicks were related to spawning. Further studies should investigate the circadian rhythm of cod sound production and assess the seasonal temporal effects.

Surprisingly, no main effect of feeding was found for either duration, P2P, or ICI. This may substantiate that clicks occur randomly as a passively emitted sound. The aim of the study was to investigate clicking in relation to the effects of feeding. However, channel and date were included as they may have explained some of the variation. The estimates indicate that there was not sufficient data to explain the effect of channel and date on duration themselves. However, the effect of feeding on duration was more negative at later dates on channel 6 than earlier dates on channel 4. The effect might be random, as channel 4 was used for a shorter time and, therefore there was an increased chance of recording longer durations on channel 6. The explanatory power of the model was 46.1 % which is high for such a parsimonious model. This was not the case for P2P, as the model only explained 4% of the variation. Despite this, there was a significant increase in P2P between channel 4 and channel 6. The difference in P2P between the channels could have a technical rather than a biological explanation. The amplification was set higher on channel 6 to compensate for the loss of signal in the 50 m long hydrophone cable compared to the 25 m long cable at channel 4. Consequently, this might have amplified the clicks on channel 6 compared to channel 4. The effect may also be explained by the clicks being emitted far away from channel 4 and closer

to channel 6, as the cod were observed to spend more time in the area closer to the feeding (Figure 1). Clicks may have been appearing on both channel 4 and channel 6, however, as only one channel was analysed at a time during the season, direct comparisons were not possible in this study. Further studies in larger basins are advised to include multiple hydrophones to exclude such caveats. For both duration and P2P, the variance was slightly deviating, indicating that the most extreme values were not explained well by the models.

Similar to P2P, channel had a significant positive effect on the ICI between clicks, however this effect depended on the interaction between period and channel. The increase in ICI due to channel might be explained by the longer recording period on channel 6 and thus have a technical explanation. This is supported by that the fact that the ICI was expected to decrease at channel 6, as it was closer to the feeding area and, thus, higher activity levels. As with duration, the explanatory power of the parsimonious model was as high as 36.06%. However, as the ICI values only represent one hour each day, these results may vary by including temporal effects. The explanatory variables used in the present study were able to explain some of the variation, however, the results indicate that there is still much variance that is left unexplained. Temperature affects acoustic communication (Ladich 2018). Including temperature in the model may have increased the explanatory power. Further studies with more explanatory variables would achieve an even greater understanding of the variation in cod clicking.

#### ***4.4 Limitations and prospects***

Characterising lesser-known animal sounds requires extensive manual labour, such as listening, annotation, and analysis. Additionally, as an unexperienced bioacoustician, gaining experience was time consuming. This reduced the ability to include more hydrophones, as well as more variables which might have increased the explanatory power of the study. Further, the big size of the basin allowed good acoustic conditions. However, there was a constant low frequency background noise which might have been accumulated noise from the nearby research station. Although the sound was normalised in the annotation software (see B.1 Appendix B), the low frequency sounds from cod such as knocks, grunts, hums and rumblings may have occasionally been masked by this. The seminatural environment was exposed to wind and days with heavy rain which increased the level of noise. Water drops and bursting bubbles have similar frequency characteristics as clicks (Versluis et al. 2000).

Further, they sound and appear visually similar on the spectrogram, which made it difficult to separate clicks from water bubbles during periods with high disturbance. Additionally, two automatic water pumps regulated the saltwater in the basin. Occasionally, these ran for up to 40 s within the analysis time and limited the ability to observe and annotate clicks while they were running, due to acoustical noise. Therefore, the number of clicks in the study may be underestimated due to the potential masking from these pumps. The basin was occasionally visited by other people during the study, which may have influenced the results by creating a disturbance that was not accounted for. Despite the limitations, the study successfully observed all known cod sounds and was able to provide new insights into cod clicking. This represents a step forward in our understanding of the role of clicking in cod behaviour and ecology. Further, the extended repertoire of cod sounds could be applied to passive acoustic monitoring (PAM) systems *in-situ* to monitor our coastal ecosystems. This might contribute to locate important habitats during vulnerable stages of cod development and assist in rebuilding the depleted cod populations. Additionally, PAM may be applied to sustainable food production, such as the rising cod aquaculture industry, to monitor feeding, activity levels, agonistic interactions, and spawning.

## 5 Conclusion

There is still a lot we do not know about fish acoustics and there is a need for additional research in this domain. This is the first study which describes all known cod sounds simultaneously. Thereby serving as a reference library for annotated cod sounds, which is essential for the future development of automated analysis of cod sound production using deep learning. Clicks were the second most occurring sound from cod, which indicate that they are an important part of cod sound production, whether they occur intentionally or passively as a function of movement. Contrary to previous reports, the study observed continuous variation in click characteristics rather than discrete categories of click types. The study provides evidence that clicking occurs independent of disturbance and that feeding did not influence the sound characteristics, which suggests that clicks might have another function than previously reported. Despite its limitations, the present study advances our understanding of the role of clicking in cod behaviour and ecology and serves as a baseline for further investigation on cod clicking. However, subsequent studies are encouraged to increase the sample size and the number of hydrophones. Further, including diurnal and seasonal temporal effects and video analysis would provide further insight into the association of clicking with specific behaviours. Nonetheless, the present study expands our knowledge of the repertoire of cod acoustics and behaviour and assist in the development of new technology for acoustic monitoring. Combined, this research will contribute to effective conservation measures which can reverse the cod population decline, secure sustainable harvesting practices for the future, and assist in monitoring in cod aquaculture.

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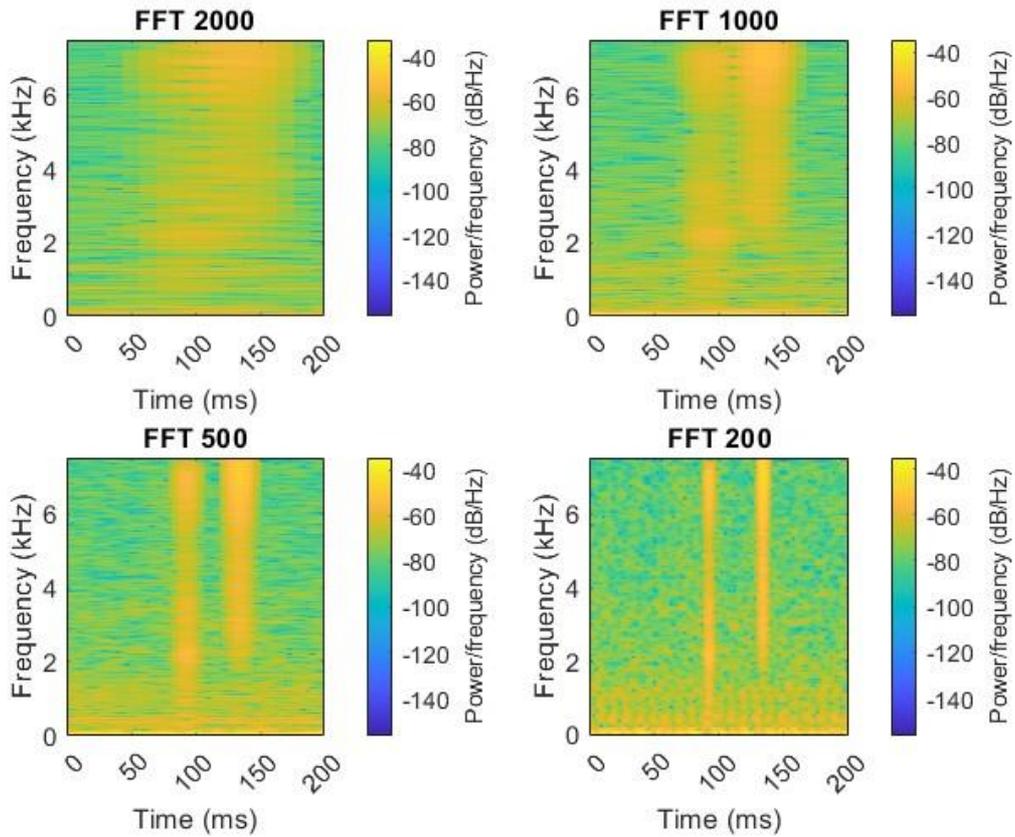
## **Appendix A** *Fast Fourier transformation*

Sound is longitudinal waves of vibrating particles. Transforming these waves into information that can be visually analysed requires algorithms. One can illustrate sound in two ways, either via a time domain (amplitude *vs.* time), which shows how a signal changes over time, or in the frequency domain, which show how much of the signal is present within each frequency band. A Fast Fourier Transform (FFT) algorithm converts sound waves into how much of each frequency is present in pre-determined time bins by computing a discrete Fourier transform and is usually illustrated as spectrograms.

Spectrograms are a graphical representation of the FFT. The sample window is determined based on the characteristics of the studied signal. The longer the sample window, the better determination of the frequencies in a signal. However, information on the time resolution is then lost. In contrast, shorter windows give better time resolution, but worse frequency resolution. Calculating the time resolution may be conducted using the following equation:

$$\textit{Time resolution} = \textit{window size} / \textit{sampling rate}$$

Cod clicks are short bursts with a broad frequency band and have no frequency modulation. Therefore, the frequency resolution is less informative, and clicks are better represented in a time domain with smaller windows (Figure 12).

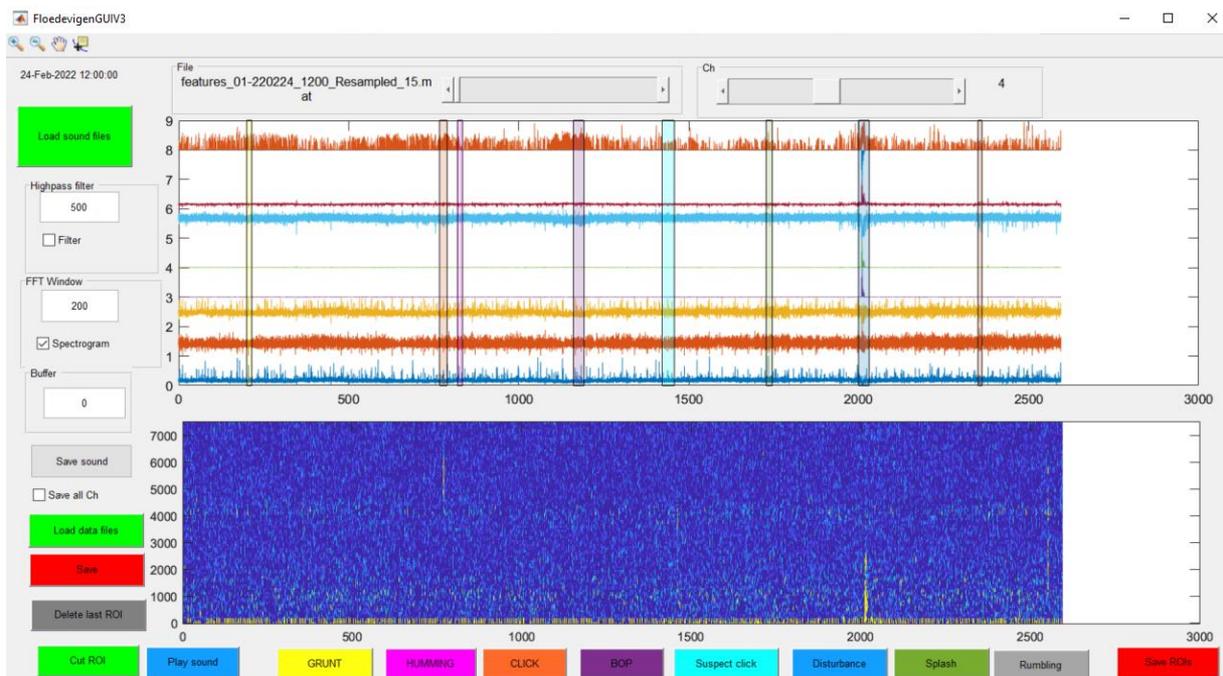


**Figure 12** Two clicks occurring with an ICI of approx. 40 ms, visualised with four different Fast Fourier transformation-windows with 90% overlap. An FFT-window of 500 was used during sound analysis, whereas an FFT-window of 200 was used during click verification.

## Appendix B Software

### B.1 Annotation software

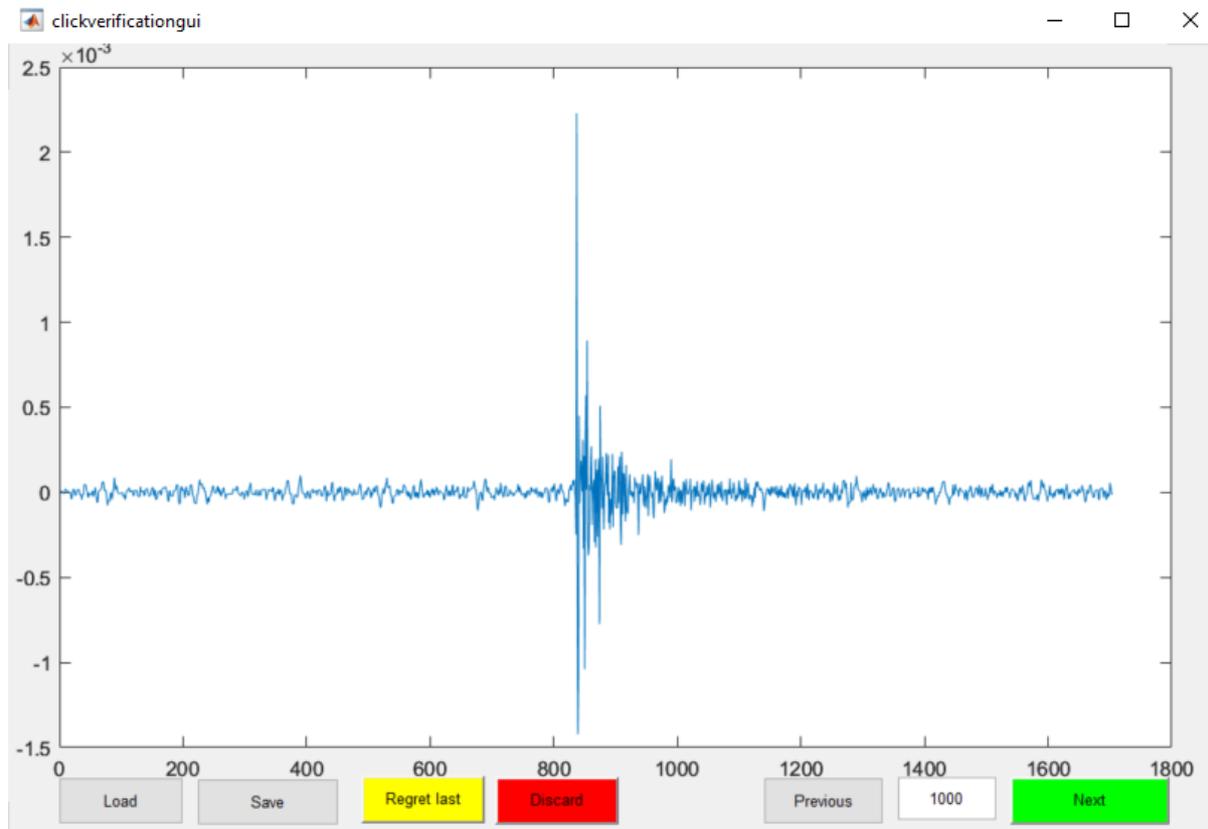
The software for manual annotation was developed as a user-friendly way to manually annotate and classify cod sounds by Jeppe Have Rasmussen in MATLAB (The MathWorks Inc 2022) (Figure 13). The software visualises nine spectral features extracted from the recordings which is efficient for vocal analysis in noisy environments (The MathWorks Inc 2023). Additionally, spectrograms are created for visual analysis of the sound. Wav and feature-files were loaded into the software and the sound was manually examined visually and aurally. ROIs were marked by “Cut ROI” and classified into categories based on sound characteristics. The ROIs for each sound file were saved for further analysis.



**Figure 13** A screenshot of the software used for manual annotation during the sound analysis. The different colours seen in the top right pane represent the corresponding categories of sounds. The visualisation is an example of how one sound file looked like after annotation.

## B.2 Verification software

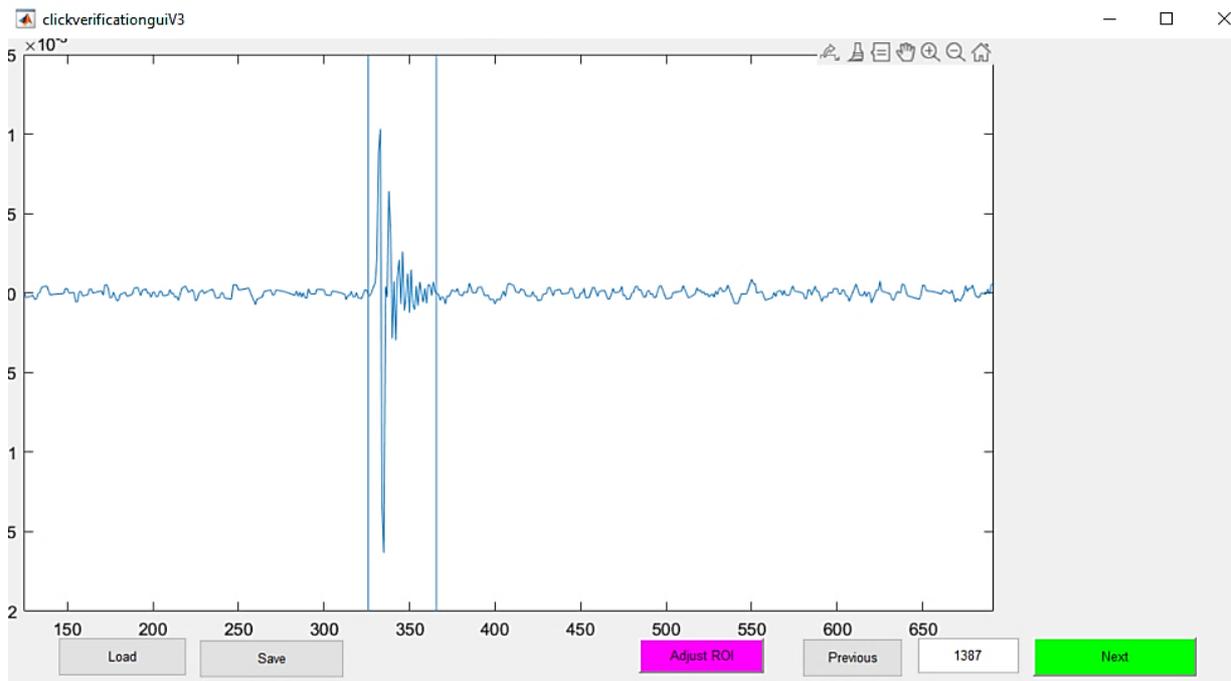
The software for click verification (Figure 14) was produced in MATLAB (The MathWorks Inc 2022) by Rasmussen. The software was developed to examine the accuracy of annotation of individual clicks and to further investigate the category “suspect clicks” (Figure 14). The annotated ROIs of clicks and suspect clicks were loaded into the software. The software displayed a timeseries of each click and the clicks that did not meet the definition of clicks were discarded in this process.



**Figure 14** A screen shot of the software used for click verification. The x-axis represents the relative power, and the y-axis represents the duration (ms) with a buffer on each side of the click.

### B.3 Cutting software

The software used to cut clicks were produced in MATLAB (The MathWorks Inc 2022) by Rasmussen. The software had the same visual structure as the software for verification and allowed for an easy approach to examine individual clicks and cut the start and end times with accuracy (Figure 15). The start and end times of a click were determined by the point where the amplitude showed a clear deviance from the rest of the soundwaves (Figure 15).



**Figure 15** A screen shot of the software for cutting clicks. The x-axis represents the relative power, and the y-axis represents the duration (ms). Here the click is marked with start and end times ready for cutting by “Adjust ROI”.

## Appendix C Investigating classes of clicks

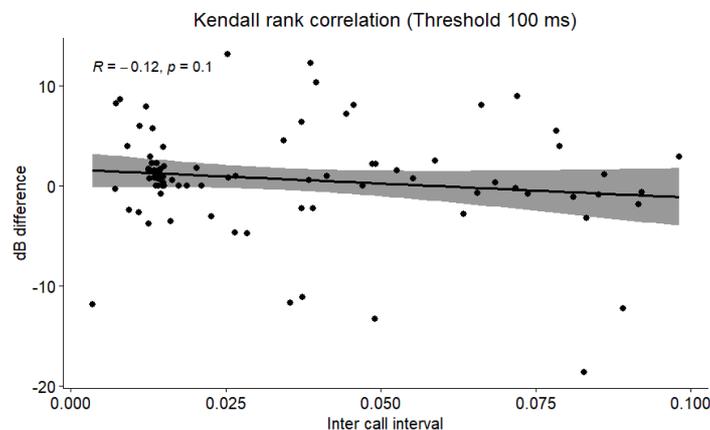
The interval between clicks (ICI) and the decibel (dB) has previously been used to categorise clicks in classes (Vester et al. 2004, Verboom 2005). *dB* is a common unit to describe sound intensity. It was not possible to determine the source level in dB for each click as the distance between the sound producing cod and the hydrophone was not measured during the study. However, by using the P2P, the dB difference between each click was calculated using the following equation:

$$dB = 20 * \log_{10}\left(\frac{P_{sound}}{P_{reference}}\right)$$

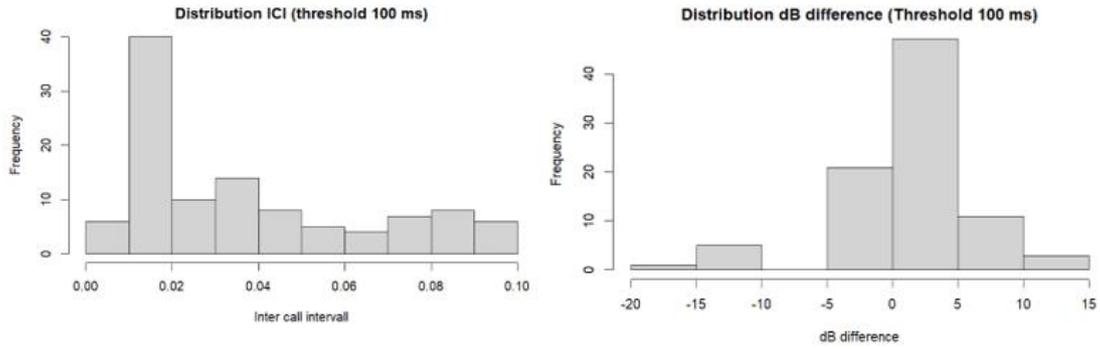
To investigate whether there was a quantifiable basis for the previously described classifications, a sensitivity analysis was performed. Two ICI thresholds were set based on previously reported ICIs for the various classes (Vester et al. 2004, Verboom 2005).

### Threshold 100 ms

A total of 88 clicks occurred within an ICI threshold of 100 ms. Although visual inspection suggested potential classes of clicks, no statistical correlation was found between the dB and ICI for clicks within the threshold of 100 ms (Kendall rank correlation: tau: - 0.1180773, n = 88, p-value = 0.1033) (Figure 16). The distributions of ICI indicated a higher occurrence of clicks around 10-20 ms, which was not statistically tested for (Figure 17). Further the distribution of dB visually indicated a potentially distinct group of clicks with a lower dB, however, the difference was not statistically significant (Hartigans dip test, D = 0.041954, p-value = 0.3085) (Figure 17). To confirm previous classifications of clicks, a more distinct separation of the clicks which fit the classes were expected.



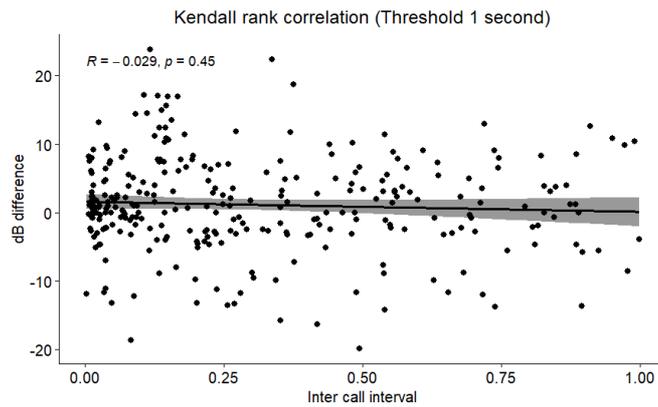
**Figure 16** Correlation test of decibel (dB) and inter call interval for clicks occurring within 100 ms of each other.



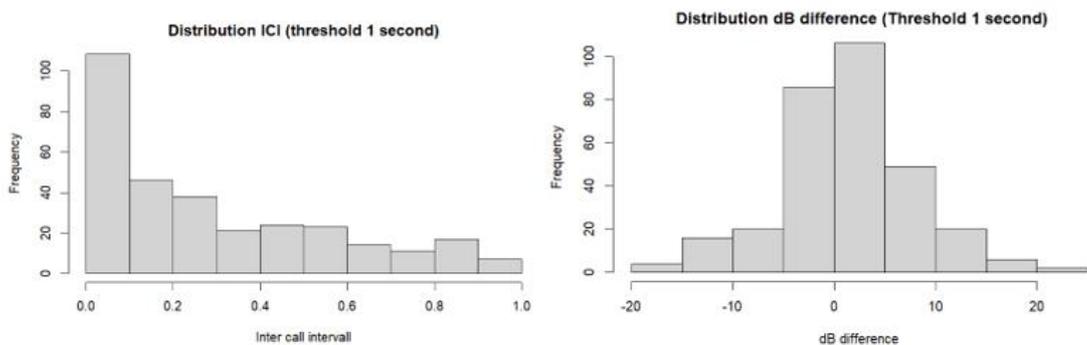
**Figure 17** Distribution of inter call interval and decibel (dB) for clicks within a threshold of 100 ms.

### **Threshold 1 second**

A total of 309 clicks occurred within an ICI threshold of 1 second. The visual inspection indicated clustering of clicks with a lower ICI. However, no correlation was found between dB and ICI (Kendall rank correlation: tau: -0.02870592, n = 309, p-value = 0.4517) (Figure 18). The distributions of both dB and ICI indicated were continuous (Figure 19).



**Figure 18** Correlation test of decibel (dB) and inter call interval for clicks occurring within 1 second of each other.



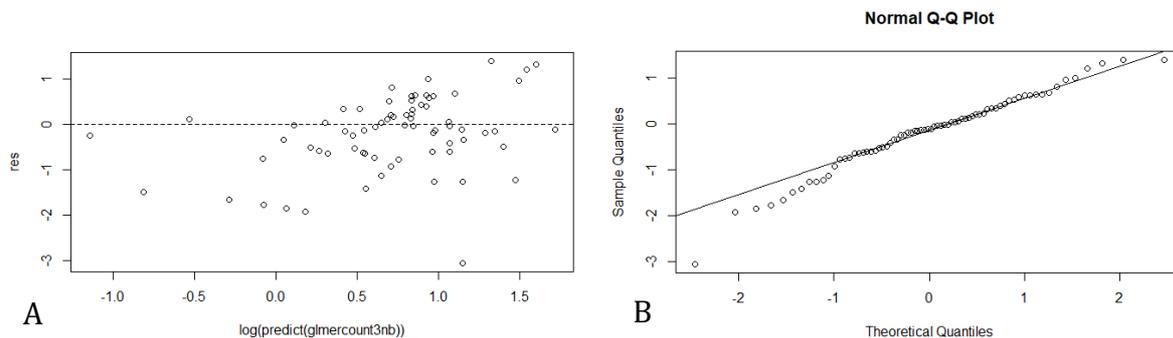
**Figure 19** Distribution of inter call interval and decibel (dB) for clicks within a threshold of 1 second.

## Appendix D Model fit

The following section provides supplementary information on the progress for finding the model with the best fit for each response variable, to investigate the effect of feeding. Additionally, it contains visual display of the residual variance for the models with the best fit.

### D.1 Click count

Distribution selection for click count (Table 12). To test for outliers, a model removing values outside the quartiles (25 % and 75%) was conducted. The AIC was lower (434.8). However, this excluded seven data points and residuals had the same distribution. Count was examined for zero-inflation. However, count had less zeroes than what was expected for the distribution of the models. The residuals from the model with the best fit were fairly homogenous and had no emergent pattern (Figure 20).



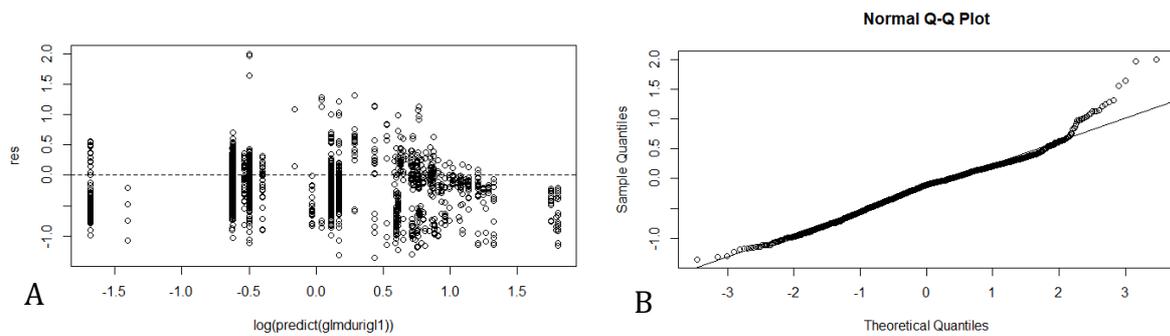
**Figure 20** Residuals from the generalised mixed effect model with negative binomial distribution and log-link function. The model included period as a fixed effect and date as a random effect. A) residual vs. fit-plot, B) quantile-quantile (Q-Q) plot.

**Table 12** Distribution selection for click count, testing the minimum adequate model for each family with a log-link function for generalised linear models (GLM) and generalised mixed effect models (GLMM).

Nr	Model	Link	Minimum adequate model	Dispersion	AIC
1	GLM, poisson	Log	~period*channel*date	45.79	2775
2	GLM, quasipoisson	Log	~channel + date	52.02	NA
3	GLM, negative binomial	Log	~period+channel+date	1.21	560.45
4	GLMM, poisson	Log	~period * channel + (1 date)	ratio (6.0)	848.1
5	GLMM, negative binomial	Log	~period + (1 date)	ratio (0.83)	559.8

## D.2 Click duration

Distribution selection for click duration (Table 13). All generalised mixed effect models (GLMM) failed to converge which suggests that GLMM may be too complex for the duration data. To test for outliers, a model without the two most extreme values indicated by a Bonferroni outlier test (Fox & Weisberg 2019) was applied. However, this model had higher AIC (-23742) and residuals had a greater variance. Another model removing values outside the quartiles (25% and 75%) was conducted. The AIC was lower (3718.5), however 337 data points were excluded, and the residuals had a greater variance. To assess the slightly deviating tail in the quantile-quantile (Q-Q) plot (Figure 21, B), the percentage of datapoint explained by the model was calculated by a conservative approach based on the residuals within the sampling quantiles (y-axis) < -1.0 to 0.65. According to this, approx. 98% of data is explained well by the model.



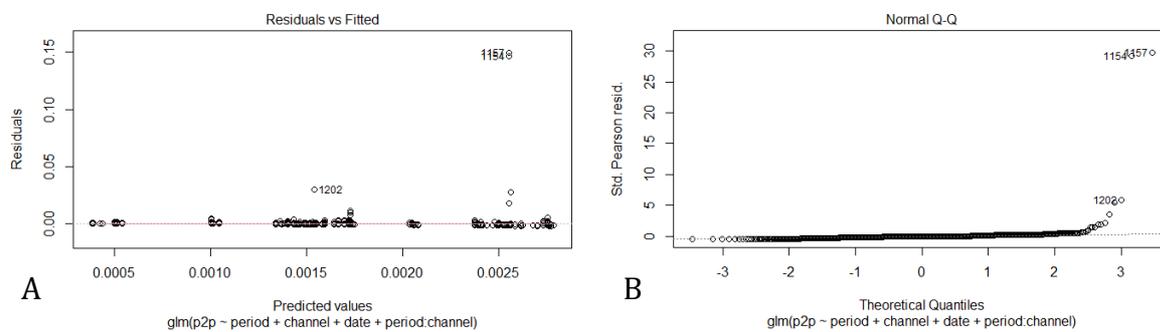
**Figure 21** Residuals from the generalised linear model with inverse gaussian distribution and log-link function. The model included period, channel and date as fixed effects, and a three-way interaction between period, channel and date, and all lower-order interactions. A) residual vs. fit-plot, B) quantile-quantile (Q-Q) plot.

**Table 13** Distribution selection for click duration, testing the minimum adequate model for each family and link function for generalised linear models (GLM) and generalised mixed effect models (GLMM).

Nr	Model	Link	Minimum adequate model	AIC
1	GLM, gaussian	Identity	~period*channel*date	12354
2	GLM, gaussian	Log	~period+channel+date + period x date	12381
3	GLM, Gamma	Inverse	~period*channel*date	7948.8
4	GLM, Gamma	Log	~period*channel*date	7858.9
5	GLM, inverse gaussian	Log	~period*channel*date	7081.4
6	GLM, inverse gaussian	1/mu <sup>2</sup>	Error	
7	GLMM, gaussian	Identity	Error	
8	GLMM, gaussian	Log	~period*channel + (1 date)	12194.3
9	GLMM, Gamma	Inverse	Failed to converge	
10	GLMM, Gamma	Log	Failed to converge	
11	GLMM, inverse gaussian	1/mu <sup>2</sup>	Error	
12	GLMM, inverse gaussian	Log	Failed to converge	

### D.3 Peak-to-peak of clicks

Distribution selection for P2P of clicks (Table 14). To test for outliers, a model without the four most extreme values indicated by a Bonferroni outlier test (Fox & Weisberg 2019) was applied. However, this model had higher AIC (-20700) and a greater residual variance. Another model removing values outside the quartiles (25 % and 75%) was conducted. This had a higher AIC (-21509), excluded 151 datapoints, and the residuals had a greater variance. The residuals from the model with the best fit had a few datapoints not well-explained by the model. Despite this, they were fairly homogenous and had no emergent pattern (Figure 22).



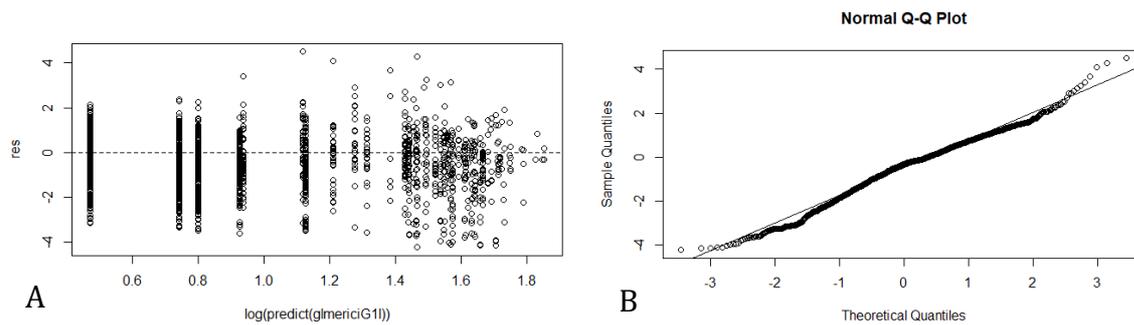
**Figure 22** Residuals from the generalised linear model with gaussian distribution and identity-link function. The model included period and channel as fixed effects. A) residual vs. fit-plot, B) quantile-quantile (Q-Q) plot.

**Table 14** Distribution selection for P2P, testing the minimum adequate model for each family and link function for generalised linear models (GLM) and generalised mixed effect models (GLMM).

Nr	Model	Link	Minimum adequate model	AIC
1	GLM, gaussian	Identity	~period+channel	-14592
2	GLM, gaussian	Log	~period+channel	-14596
3	GLM, Gamma	Inverse	Error	
4	GLM, Gamma	Log	~period*channel+date	-22740
5	GLM, inverse. gaussian	1/mu <sup>2</sup>	Error	
6	GLM, inverse. gaussian	Log	Algorithm did not converge	
7	GLMM gaussian	Identity	Error	
8	GLMM, gaussian	Log	Error	
9	GLMM, Gamma	Inverse	Error	
10	GLMM, Gamma	Log	Failed to converge	
11	GLMM, inverse. gaussian	1/mu <sup>2</sup>	Failed to converge	
12	GLMM, inverse. gaussian	Log	~period+channel + (1 date)	-23840.9

#### D.4 Inter call interval between clicks

Distribution selection for ICI between clicks (Table 15). To test for outliers, a model removing values outside the quartiles (25 % and 75%) was conducted. The AIC was lower (10169.4). However, this eluded 282 data points and the residuals had a greater variance. The residuals from the model with the best fit were fairly homogenous and had no emergent pattern (Figure 23).



**Figure 23** Residuals from the generalised mixed effect model (GLMM) with gamma distribution and log-link function. The model included period and channel as fixed effects, and a two-way interaction between period and channel. A) residual vs. fit-plot, B) quantile-quantile (Q-Q) plot.

**Table 15** Distribution selection for ICI, testing the minimum adequate model for each family and link function for generalised linear models (GLM) and generalised mixed effect models (GLMM).

Nr	Model	Link	Minimum adequate model	AIC
1	GLM, gaussian	Identity	~period+channel+date + period x date	21721
2	GLM, gaussian	Log	~period+channel*date	21782
3	GLM, Gamma	Inverse	~period*channel*date	14993
4	GLM, Gamma	Log	~period*channel*date	14893
5	GLM, inverse gaussian	1/mu <sup>2</sup>	Error	
6	GLM, inverse gaussian	Log	Error	
7	GLMM, gaussian	Identity	~period*channel + (1  date)	21505.9
8	GLMM, gaussian	Log	Error	
9	GLMM, Gamma	Inverse	Failed to converge	
10	GLMM, Gamma	Log	~period*channel + (1  date)	14311.2
11	GLMM, inverse gaussian	1/mu <sup>2</sup>	Error	
12	GLMM, inverse gaussian	Log	Failed to converge	