Sound investigation: effects of noise on marine animals across trophic levels
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Sound Investigation

Effects of noise on marine animals
across trophic levels

Jeroen Hubert

Sound Investigation

Effects of noise on marine animals across trophic levels

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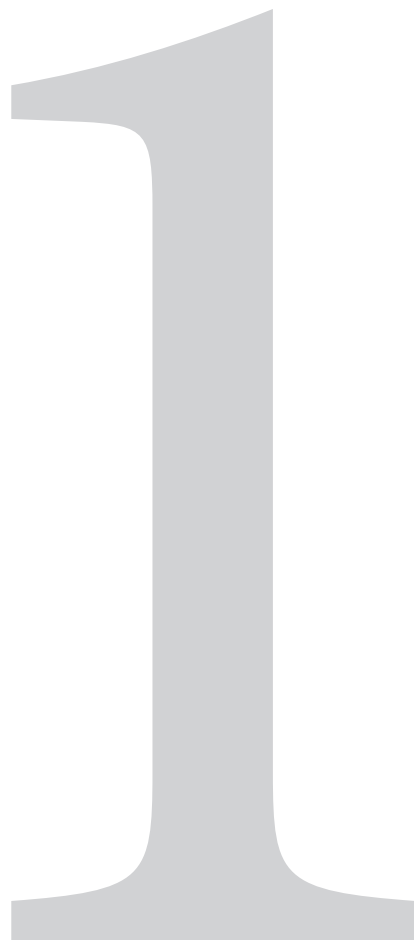Prof. dr. Martina G. Vijver (Leiden University)
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General introduction

The marine environment is filled with a cacophony of sound. Ambient sound originates from water surface roughness as a function of weather conditions and waves (Carey and Browning, 1988; Wenz, 1962), and many marine animals produce sounds to communicate (e.g. Vasconcelos et al., 2007; Wilson et al., 2014). Over the past decades, there are also increasing amounts of anthropogenic sound sources in the marine environment (Andrew et al., 2002; Hildebrand, 2009). In the Dutch part of the North Sea, shipping is the most prominent sound source (Sertlek et al., 2019). Shipping produces continuous low-frequency sound, and at larger distances, it is impossible to discriminate this from other ambient noise (Wright et al., 2007). Nevertheless, it does elevate the ambient noise levels in the marine environment, especially around harbours and shipping routes (Haver et al., 2018; Sertlek et al., 2016). Seismic surveys are another prominent sound source. Seismic surveys are explorations of the geological structure beneath the seafloor using airguns (Gisiner, 2016; Landrø and Amundsen, 2018). A third prominent source is pile driving for offshore constructions such as wind turbines (Matuschek and Betke, 2009). Both, seismic surveys and pile driving, produce high intensity, low-frequency impulsive sounds at a regular interval, and can persist for weeks to months (McCauley et al., 2000; Sertlek et al., 2019). Sounds from the above-mentioned sources are audible to most – if not all – marine animals, however, relatively little is known on the effects of anthropogenic sound on marine life, especially for fish and invertebrates (Hawkins et al., 2015; Slabbekoorn et al., 2010; Williams et al., 2015).

Fish use sound to orient, for example by finding suitable habitat and detecting prey or predators, and to communicate, for example to find a partner (Gordon et al., 2019; Popper and Hawkins, 2019; Wilson et al., 2014). Many fish species are known to produce sound (Amorim et al., 2015; Fine and Parmentier, 2015; Ladich, 2008), while all are expected to be able to hear sound (Popper and Hawkins, 2018). Fish can produce sound by a variety of mechanisms, such as by drumming the swim bladder with the sonic muscle, or rubbing bones or teeth together (stridulation) (Webb et al., 2008). Fish detect sound with their inner ear and lateral line system (Engelmann et al., 2000; Popper and Fay, 2011). The inner ear consists of three otoliths surrounded by sensory hair cells. Otoliths have a higher density than the fish and water. Hence, the otoliths move at a different amplitude and phase than the rest of the fish in response to the particle motion aspect of sound. This movement is subsequently detected by the hair cells. Many fish have a gas-filled swim bladder which fluctuates in volume in response to sound pressure waves, this contributes to sound perception if the swim bladder oscillation results in otolith motion. The role of the swim bladder in hearing depends on the proximity of the swim bladder to the inner ear and the presence of a connection between the swim bladder and inner ear, for example by the

Weberian ossicles. The lateral line system consists of hair cells in the skin of the fish which also detect low-frequency particle motion (Popper and Fay, 2011; Popper and Hawkins, 2019; Popper and Higgs, 2010).

Marine invertebrates can also detect and utilize sound. Many invertebrates (including crustaceans) have statocysts, just like with otoliths in the inner ear of fish. A statocyst consists of a mass, whose movements are detected by sensory hairs (Lovell et al., 2005). Just like the lateral line system in fish, crustaceans (e.g. crabs and shrimps) also have sensory hairs on their body that can detect low frequency sound (Derby, 1982). Both sensory systems detect the particle motion component of sound, and since crabs and shrimps do not have gas filled cavities, they are likely not sensitive to sound pressure (Edmonds et al., 2016). Crabs and shrimps in their pelagic life stage have been shown to orient to particular soundscapes to find suitable habitat (Jeffs et al., 2003; Simpson et al., 2011), and crabs in later life stages have been shown to alter foraging behaviour after playbacks of sounds from predatory fish (Hughes et al., 2014). Several bivalve families have a specialized hearing organ, the abdominal sense organ (ASO), a large mechanosensory organ which also detects particle motion. Bivalves that do not have the ASO possess structurally similar organs, possibly also for sound detection (Haszprunar, 1985; Zhadan, 2005). Pelagic larvae of eastern oyster (*Crassostrea virginica*) show increased settlement behaviour during the playback of sound from suitable habitat, and swash-riding clams (*Donax variabilis*) jump out of the sand during playback of wave sound to surf on the waves and maintain a position at the shoreline, along with the moving tides (Ellers, 1995; Lillis et al., 2013).

Since marine animals can hear sound and animals use sound for activities critical to their survival and reproduction, it is likely that they are affected by anthropogenic sound (Slabbekoorn et al., 2010). Close to a high-intensity sound source, fish may get physically injured, for example by swim bladder rupture and damaged capillaries, due to the high-amplitude pressure fluctuations around the swim bladder (Casper et al., 2013; Halvorsen et al., 2012). At larger distances from the sound source, anthropogenic sound may still mask biologically relevant sound, this may decrease the distance from which suitable habitat, potential mates, or predators and prey are detected (Codarin et al., 2009; Radford et al., 2014; Wysocki and Ladich, 2005). Anthropogenic sound may also affect behaviour and physiology. It has been shown to disrupt swimming patterns, change activity patterns, and elevate stress related physiological parameters (Filiciotto et al., 2016; Neo et al., 2014). Impact to fish close to a high-intensity sound source may seem most severe, but only applies to relatively small numbers of fish. Moderate effects at larger distances apply to many more individuals, and therefore have

more potential to be relevant for population level effects (Slabbekoorn et al., 2010; Soudijn et al., 2020). For this reason, it is especially important to study behavioural and physiological effects of sound on marine life.

There is a wide variety of factors that may influence the response of an animal to anthropogenic sound. Anthropogenic sound varies in amplitude, frequency, and temporal fluctuations in both domains, which may all influence the animals' response (Neo et al., 2014; Vetter et al., 2015; Wysocki et al., 2006). Not only the characteristics of the – potentially disturbing – stimulus may matter, but also the acoustic characteristics of the environment may modulate the nature and strength of a response. For example, higher ambient noise levels will decrease the signal to noise ratio of the stimulus and may therefore reduce the response (Wells, 2009). Other external factors, such as temperature, season, time of the day, may all influence behaviour and thereby also the behavioural response of animals (Bejder et al., 2009). Not only abiotic factors, but also biotic factors such as conspecific and heterospecific individuals, and their response to sound, may affect the response of an individual (Francis et al., 2009; Slabbekoorn and Halfwerk, 2009). Furthermore, the internal state of an individual will also determine its response to sound. A hungry, migrating or reproducing individual may face a different trade-off than when it has eaten well (Croy and Hughes, 1991; Schadegg and Herberholz, 2017). Previous experiences to sound may have led to habituation, desensitization or motor fatigue (Neo et al., 2018). Lastly, sound may not only affect animals through auditory masking or anxiety, but processing of the sound may also interfere with the processing of other relevant stimuli (Halfwerk and Slabbekoorn, 2015). Altogether, there is a great variety of factors and mechanisms that may determine the effects of sound on animals, knowledge about these mechanisms is critical to understand the impact of anthropogenic sound on animals (fig. 1).

Ideally, sound impact studies are conducted in the wild during anthropogenic sound exposure from a real source. Although proper replication in time and space is still required, both the possibility for animals to express natural behaviour and the acoustic exposure optimally resemble the conditions of real noisy human activities (Rogers et al., 2016; Slabbekoorn, 2016). However, fieldwork at sea also entails limitations and challenges. It is difficult to track individual animals for an extended period of time, and such experiments are expensive and logistically challenging, which often results in limited replication, making it hard to control for natural fluctuations in behaviour or confounding variables such as the weather. Hence, many sound impact studies are conducted in a captive environment, e.g. a tank, basin, or net pen. However, behaviour of animals, including their response to sound, likely differs from free-ranging

animals in situ (Wright et al., 2007). For example, captive fish are limited in their ability to swim away from a sound source and there is typically less inter- and intra-species interaction. Additionally, the animals' enclosure and sound source will also affect the sound field. Both sound pressure and particle motion are affected by the proximity of tank walls and water surface (Campbell et al., 2019; Parvulescu, 1967; Rogers et al., 2016). Additionally, speakers are often used to play back sound, even though speakers typically have frequency limitations and are point sources, in contrast to many anthropogenic sound sources – such as ships and pile driving – which are not (Lippert et al., 2018). The extent to which such limitations pose a problem for sound impact studies depends – among other things – on the research question, experimental design, and study species.

Despite the limitations of controlled experiments in tanks or net pens, there are also several benefits of such experiments. Controlled experiments typically enable replicated and independent trials and there is typically more control over environmental variables such as background noise, light intensity, temperature, and weather conditions. Additionally, the (recent) life history of the animals is sometimes known and can be standardized. Altogether, such experiments likely yield data with limited (unexplained) variance which makes it easier to compare treatment groups. Besides, it is also possible to manipulate environmental variables, previous experiences or the internal state of the animals. This allows to examine such characteristics without being confounded by other characteristics. This will increase our understanding of the mechanisms that underly the response of animals to sound, which will aid the interpretation of other experiments and the extrapolation of results from experiments to the wild. Finally, controlled experiments can be used as proof of concept to test and gain insight in particular methodology or (potential to change) behaviour, which can later be used or examined in situ. Altogether, both field and captive experiments can provide complementary insights into the effects of sound on animals. All studies thus have their own benefits and limitations, and we should interpret results accordingly. Despite all potential benefits and opportunities of controlled experiments, some experiments can only be conducted in the field, for example experiments aiming to quantify absolute response levels.

For this thesis, and together with colleagues and students, I conducted five experiments using captive animals and one experiment using free-ranging animals. I examined factors that modulate the effects of sound on animals and the quantification of behavioural effects that may ultimately be used to gain insight into fitness and population consequences. We collected data on Atlantic cod (*Gadus morhua*), European seabass (*Dicentrarchus labrax*), shore crabs (*Carcinus maenas*), common shrimps (*Crangon crangon*), and blue mussels

(*Mytilus edulis*). Both cod and seabass are demersal and shoaling fish (Frimodt and Dore, 1995). Cod and seabass hear best up to 400 and 1000 Hz respectively, and cod is also known to produce sound (Chapman and Hawkins, 1973; Lovell, 2003; Wilson et al., 2014). Cod and seabass forage – among other prey species – on crabs and shrimps (Reubens et al., 2014; Whitehead, 1984). Both shore crabs and common shrimps are epibenthic crustaceans and live in a wide range of shallow marine habitats (Campos et al., 2012; Carlton and Cohen, 2003). The hearing curves of shore crabs and shrimp are not known, but another crab and shrimp species hear up to at least 1600 and 3000 Hz respectively (Hughes et al., 2014; Lovell et al., 2005). The blue mussel is a major prey species of the shore crab (Matheson and Mckenzie, 2014). Mussels adopt a sessile lifestyle in intertidal areas and play an important role in ecosystems as filter-feeders and reef-builders (Borthagaray and Carranza, 2007; Jørgensen, 1990). Mussels hear up to at least 410 Hz (Roberts et al., 2015). Altogether, these species represent multiple trophic levels and many of them are of commercial interest (fig. 2).

Thesis outline

This thesis describes six sound exposure studies on marine animals from multiple trophic levels. In the first two data chapters, I aimed to quantify behavioural responses to sound relevant to fitness. In chapter 2, I examined the time expenditure in several behavioural states of Atlantic cod in a floating pen and changes in time budget due to the playback of seismic airgun sound. In chapter 3, I conducted a similar sound exposure experiment in a basin and using video observations to be able to quantify foraging behaviour, in addition to swimming and stationary behaviour, which are all relevant to energy budgets of Atlantic cod. In the next four chapters, I studied factors that may modulate the effects of sounds on different marine animals. In chapter 4, I examined the effects of the acoustic characteristics: pulse rate interval, signal to noise ratio, and elevated background level on the response of European seabass on sound exposures. In chapter 5, I studied species interactions during sound exposures by examining aggregation of shore crabs and common shrimps at a food item in the field. In chapter 6, I examined whether reduced aggregation at a food item by crabs can be explained cross-sensory interference. For this, I studied whether olfactory-mediated food finding by shore crabs was affected by sound exposures. Since short-term effects of sound may not be indicative of long-term effects, I also studied habituation. In chapter 7, I examined the habituation of blue mussels to repeated sound exposures, and their recovery to baseline levels during or after single pulses and pulse trains. In chapter 8, I summarized and discussed the results from the previous chapters and suggested follow-up steps.
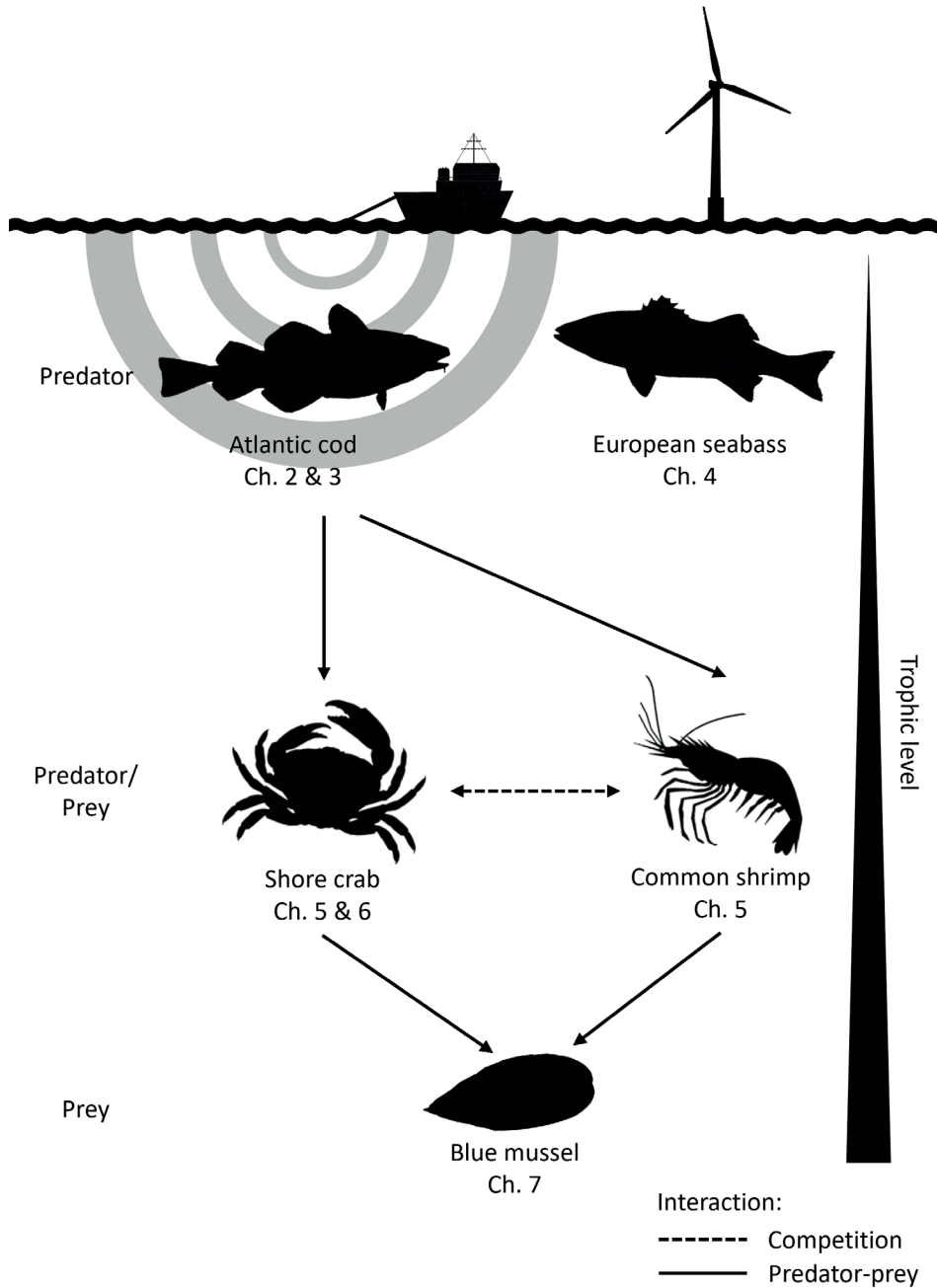
Fig. 1: Schematic of the study species that are used in the experiments described in this thesis. The effects of sound on marine animals from various trophic layers and the interaction between some of them were studied. The arrows indicate the interactions that were part of the studies. 'Ch. #' indicate the chapters in that describe experiments on this study. Note that crabs and shrimp only ate dead mussels in these experiments, common shrimps do not predate on live mussels.

References

Amorim, M. C. P., Vasconcelos, R. O. and Fonseca, P. J. (2015). Fish Sounds and Mate Choice.

Andrew, R. K., Howe, B. M., Mercer, J. A. and Dzieciuch, M. A. (2002). Ocean ambient sound: Comparing the 1960s with the 1990s for a receiver off the California coast. Acoust. Res. Lett. Online 3, 65–70.

Bejder, L., Samuels, A., Whitehead, H., Finn, H. and Allen, S. (2009). Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. Mar. Ecol. Prog. Ser. 395, 177–185.

Borthagaray, A. I. and Carranza, A. (2007). Mussels as ecosystem engineers: Their contribution to species richness in a rocky littoral community. Acta Oecologica 31, 243–250.
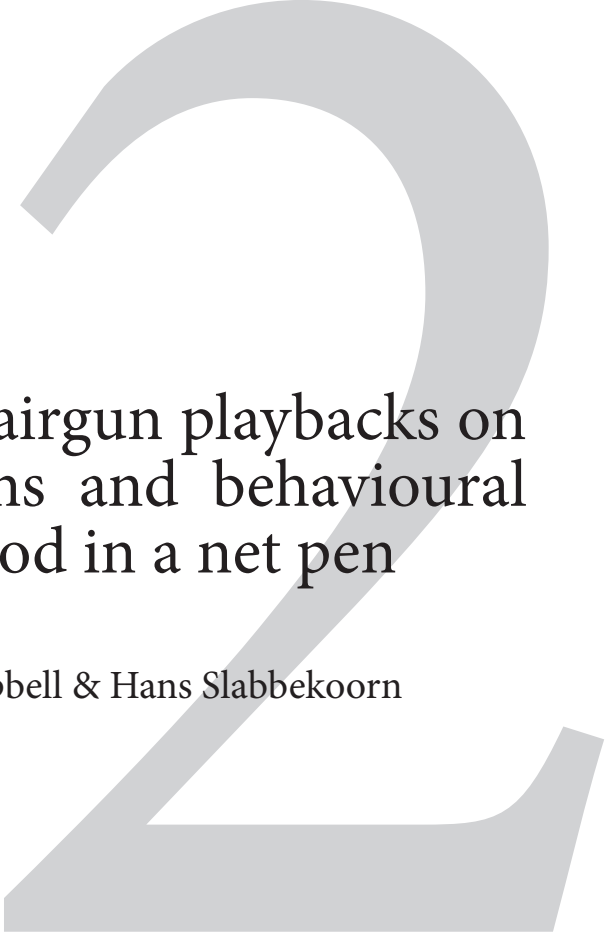
Campbell, J., Shafiei Sabet, S. and Slabbekoorn, H. (2019). Particle motion and sound pressure in fish tanks: A behavioural exploration of acoustic sensitivity in the zebrafish. Behav. Processes 164, 38–47.

Campos, J., Moreira, C., Freitas, F. and Van Der Veer, H. W. (2012). Short review of the eco-geography of *crangon*. J. Crustac. Biol. 32, 159–169.

Carey, W. M. and Browning, D. (1988). Low Frequency Ocean Ambient Noise: Measurements and Theory. In Sea Surface Sound, pp. 361–376. Dordrecht: Springer.

Carlton, J. T. and Cohen, A. N. (2003). Episodic global dispersal in shallow water marine organisms: The case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. J. Biogeogr. 30, 1809–1820.

Casper, B. M., Halvorsen, M. B., Matthews, F., Carlson, T. J. and Popper, A. N. (2013). Recovery of Barotrauma Injuries Resulting from Exposure to Pile Driving Sound in Two Sizes of Hybrid Striped Bass. PLoS One 8, e73844.

Chapman, C. J. and Hawkins, A. D. (1973). A field study of hearing in the cod, *Gadus morhua* L. J. Comp. Physiol. 85, 147–167.

Codarin, A., Wysocki, L. E., Ladich, F. and Picciulin, M. (2009). Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). Mar. Pollut. Bull. 58, 1880–7.

Croy, M. I. and Hughes, R. N. (1991). Effects of food supply, hunger, danger and competition on choice of foraging location by the fifteen-spined stickleback, *Spinachia spinachia* L . Anim. Behav. 42, 131–139.

Derby, C. D. (1982). Structure and Function of Cuticular Sensilla of the Lobster *Homarus americanus*. J. Crustac. Biol. 2, 1–21.

Edmonds, N. J., Firmin, C. J., Goldsmith, D., Faulkner, R. C. and Wood, D. T. (2016). A review of crustacean sensitivity to high amplitude underwater noise: Data needs for effective risk assessment in relation to UK commercial species. Mar. Pollut. Bull. 108, 5–11.

Ellers, O. (1995). Behavioral Control of Swash-Riding in the Clam *Donax variabilis*. Biol. Bull. 189, 120–127.

Engelmann, J., Hanke, W., Mogdans, J. and Bleckmann, H. (2000). Neurobiology: Hydrodynamic stimuli and the fish lateral line. Nature 408, 51–52.

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Arizza, V., de Vincenzi, G., Grammauta, R., Mazzola, S., et al. (2016). Underwater noise from boats: Measurement of its influence on the behaviour and biochemistry of the common prawn (*Palaemon serratus*, Pennant 1777). J. Exp. Mar. Bio. Ecol. 478, 24–33.

Fine, M. L. and Parmentier, E. (2015). Mechanisms of Fish Sound Production. In Sound Communication in Fishes (ed. Ladich, F.), pp. 77–126. Vienna: Springer Vienna.

Francis, C. D., Ortega, C. P. and Cruz, A. (2009). Noise Pollution Changes Avian Communities and Species Interactions. Curr. Biol. 19, 1415–1419.

Frimodt, C. and Dore, I. (1995). Multilingual Illustrated Guide to the World's Commercial Coldwater Fish. Wiley-Blackwell.

Gisiner, R. C. (2016). Sound and Marine Seismic Surveys. Acoust. Today 12, 10–18.

Gordon, T. A. C., Radford, A. N., Davidson, I. K., Barnes, K., McCloskey, K., Nedelec, S. L., Meekan, M. G., McCormick, M. I. and Simpson, S. D. (2019). Acoustic enrichment can enhance fish community development on degraded coral reef habitat. Nat. Commun. 10, 5414.

Halfwerk, W. and Slabbekoorn, H. (2015). Pollution going multimodal: The complex impact of the human-altered sensory environment on animal perception and performance. Biol. Lett. 11,.

Halvorsen, M. B., Casper, B. M., Woodley, C. M., Carlson, T. J. and Popper, A. N. (2012). Threshold for onset of injury in chinook salmon from exposure to impulsive pile driving sounds. PLoS One 7, 2–12.

Haszprunar, G. (1985). The Fine Structure of the Abdominal Sense Organs of Pteriomorpha (Mollusca, Bivalvia). J. Molluscan Stud. 51, 315–319.

Haver, S. M., Gedamke, J., Hatch, L. T., Dziak, R. P., Van Parijs, S., McKenna, M. F., Barlow, J., Berchok, C., DiDonato, E., Hanson, B., et al. (2018). Monitoring long-term soundscape trends in U.S. Waters: The NOAA/NPS Ocean Noise Reference Station Network. Mar. Policy 90, 6–13.

Hawkins, A. D., Pembroke, A. E. and Popper, A. N. (2015). Information gaps in understanding the effects of noise on fishes and invertebrates. Rev. Fish Biol. Fish. 25, 39–64.

Hildebrand, J. A. (2009). Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395, 5–20.

Hughes, A. R., Mann, D. A. and Kimbro, D. L. (2014). Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. Proc. Biol. Sci. 281, 1–8.

Jeffs, A., Tolimieri, N. and Montgomery, J. C. (2003). Crabs on cue for the coast: The use of underwater sound for orientation by pelagic crab stages. Mar. Freshw. Res. 54, 841–845.

Jørgensen, C. B. (1990). Bivalve filter feeding: hydrodynamics, bioenergetics, physiology and ecology. Fredensborg: Olsen & Olsen.

Ladich, F. (2008). Sound communication in fishes and the influence of ambient and anthropogenic noise. Bioacoustics 17, 34–38.

Landrø, M. and Amundsen, L. (2018). Introduction to Exploration Geophysics with Recent Advances. Bivrost.

Lillis, A., Eggleston, D. B. and Bohnenstiehl, D. R. (2013). Oyster larvae settle in response

to habitat-associated underwater sounds. PLoS One 8, 21–23.

Lippert, T., Ainslie, M. A. and von Estorff, O. (2018). Pile driving acoustics made simple: Damped cylindrical spreading model. J. Acoust. Soc. Am. 143, 310–317.

Lovell, J. M. (2003). The hearing abilities of the bass, *Dicentrarchus labrax*. Technical report commissioned by ARIA Marine Ltd. for the European Commission Fifth Framework Programme.

Lovell, J. M., Findlay, M. M., Moate, R. M. and Yan, H. Y. (2005). The hearing abilities of the prawn *Palaemon serratus*. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 140, 89–100.

Matheson, K. and Mckenzie, C. H. (2014). Predation of sea scallops and other indigenous bivalves by invasive green crab, *Carcinus maenas*, from Newfoundland, Canada. J. Shellfish Res. 33, 495–501.

Matuschek, R. and Betke, K. (2009). Measurements of Construction Noise During Pile Driving of Offshore Research Platforms and Wind Farms. In Proceedings of NAG/DAGA 2009, pp. 262–265.

McCauley, R. D., Fewtrell, J., Duncan, A. J., Jenner, C., Jenner, M., Penrose, J. D., Prince, R. I. T., Adhitya, A., Murdoch, J. and McCabe, K. (2000). Marine Seismic Surveys – A Study of Enviromental Implications. APPEA J. 40, 692–708.

Neo, Y. Y., Seitz, J., Kastelein, R. A., Winter, H. V., ten Cate, C. and Slabbekoorn, H. (2014). Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biol. Conserv. 178, 65–73.

Neo, Y. Y., Hubert, J., Bolle, L. J., Winter, H. V. and Slabbekoorn, H. (2018). European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environ. Pollut. 239, 367–374.

Parvulescu, A. (1967). The acoustics of small tanks. In Marine Bio-Acoustics Vol. 2 (ed. Tavolga, W. N.), pp. 7–13. London: Pergamon Press.

Popper, A. N. and Fay, R. R. (2011). Rethinking sound detection by fishes. Hear. Res. 273, 25–36.

Popper, A. N. and Hawkins, A. D. (2018). The importance of particle motion to fishes and invertebrates. J. Acoust. Soc. Am. 143, 470–488.

Popper, A. N. and Hawkins, A. D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. fish 94, 692–713.

Popper, A. N. and Higgs, D. M. (2010). Fish: Hearing, Lateral Lines (Mechanisms, Role in Behavior, Adaptations to Life Underwater). Encycl. Ocean Sci. 476–482.

Radford, A. N., Kerridge, E. and Simpson, S. D. (2014). Acoustic communication in a noisy world: Can fish compete with anthropogenic noise? Behav. Ecol. 25, 1022–1030.

Reubens, J., De Rijcke, M., Degraer, S. and Vincx, M. (2014). Diel variation in feeding and movement patterns of juvenile Atlantic cod at offshore wind farms. J. Sea Res. 85, 214–221.

Roberts, L., Cheesman, S., Breithaupt, T. and Elliott, M. (2015). Sensitivity of the mussel *Mytilus edulis* to substrate-borne vibration in relation to anthropogenically generated noise. Mar. Ecol. Prog. Ser. 538, 185–195.

Rogers, P. H., Hawkins, A. D., Popper, A. N., Fay, R. R. and Gray, M. D. (2016).

Parvulescu Revisited: Small Tank Acoustics for Bioacousticians. In The Effects of Noise on Aquatic Life II, pp. 933–941.

Schadegg, A. C. and Herberholz, J. (2017). Satiation level affects anti-predatory decisions in foraging juvenile crayfish. J. Comp. Physiol. A 203, 223–232.

Sertlek, H. Ö., Aarts, G., Brasseur, S., Slabbekoorn, H., ten Cate, C., von Benda-Beckmann, A. M. and Ainslie, M. A. (2016). Mapping Underwater Sound in the Dutch Part of the North Sea. In The Effects of Noise on Aquatic Life II, pp. 1001–1006.

Sertlek, H. Ö., Slabbekoorn, H., ten Cate, C. and Ainslie, M. A. (2019). Source specific sound mapping: Spatial, temporal and spectral distribution of sound in the Dutch North Sea. Environ. Pollut. 247, 1143–1157.

Simpson, S. D., Radford, A. N., Tickle, E. J., Meekan, M. G. and Jeffs, A. G. (2011). Adaptive avoidance of reef noise. PLoS One 6, 1–6.

Slabbekoorn, H. (2016). Aiming for Progress in Understanding Underwater Noise Impact on Fish: Complementary Need for Indoor and Outdoor Studies. In The Effects of Noise on Aquatic Life II (ed. Popper, N. A.) and Hawkins, A.), pp. 1057–1065. New York, NY: Springer New York.

Slabbekoorn, H. and Halfwerk, W. (2009). Behavioural Ecology: Noise Annoys at Community Level. Curr. Biol. 19, R693–R695.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C. and Popper, A. N. (2010). A noisy spring: The impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427.

Soudijn, F. H., van Kooten, T., Slabbekoorn, H. and de Roos, A. M. (2020). Population-level effects of acoustic disturbance in Atlantic cod: a size-structured analysis based on energy budgets. Proc. R. Soc. B Biol. Sci. 287, 20200490.

Vasconcelos, R. O., Amorim, M. C. P. and Ladich, F. (2007). Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish. J. Exp. Biol. 210, 2104–12.

Vetter, B. J., Cupp, A. R., Fredricks, K. T., Gaikowski, M. P. and Mensinger, A. F. (2015). Acoustical deterrence of Silver Carp (*Hypophthalmichthys molitrix*). Biol. Invasions 17, 3383–3392.

Webb, J. F., Popper, A. N. and Fay, R. R. (2008). Fish Bioacoustics. New York: Springer Scien-Business Media.

Wells, D. L. (2009). Sensory stimulation as environmental enrichment for captive animals: A review. Appl. Anim. Behav. Sci. 118, 1–11.

Wenz, G. M. (1962). Acoustic Ambient Noise in the Ocean: Spectra and Sources. J. Acoust. Soc. Am. 34, 1936–1956.

Whitehead, P. J. P. (1984). Fishes of the North-eastern Atlantic and the Mediterranean, Volume 2. Unesco.

Williams, R., Wright, a. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., Clark, C. W., Cullis-Suzuki, S., Dakin, D. T., Erbe, C., et al. (2015). Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24.

Wilson, L. J., Burrows, M. T., Hastie, G. D. and Wilson, B. (2014). Temporal variation

and characterization of grunt sounds produced by Atlantic cod *Gadus morhua* and pollack *Pollachius pollachius* during the spawning season. J. Fish Biol. 84, 1014–1030.

Wright, A. J., Soto, N. A., Baldwin, A. L., Bateson, M., Beale, C. M., Clark, C., Deak, T., Edwards, E. F., Fernández, A., Godinho, A., et al. (2007). Anthropogenic Noise as a Stressor in Animals: A Multidisciplinary Perspective. Int. J. Comp. Psychol. 20, 250–273.

Wysocki, L. E. and Ladich, F. (2005). Hearing in Fishes under Noise Conditions. J. Assoc. Res. Otolaryngol. 6, 28–36.

Wysocki, L. E., Dittami, J. P. and Ladich, F. (2006). Ship noise and cortisol secretion in European freshwater fishes. Biol. Conserv. 128, 501–508.

Zhadan, P. M. (2005). Directional Sensitivity of the Japanese Scallop *Mizuhopecten yessoensis* and Swift Scallop *Chlamys Swifti* to Water-Borne Vibrations. Psysiological Ecol. 31, 28–35.

Effects of seismic airgun playbacks on swimming patterns and behavioural states of Atlantic cod in a net pen

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Abstract

Anthropogenic sound can affect fish behaviour and physiology which may affect their well-being. However, it remains a major challenge to translate such effects to consequences for fitness at an individual and population level. For this, energy budget models have been developed, but suitable data to parametrize these models are lacking. A first step towards such parametrization concerns the objective quantification of behavioural states at high resolution. We experimentally exposed individual Atlantic cod (*Gadus morhua*) in a net pen to the playback of seismic airgun sounds. We demonstrated that individual cod in the net pen did not change their swimming patterns immediately at the onset of the sound exposure. However, several individuals changed their time spent in three different behavioural states during the 1 h exposure. This may be translated to changes in energy expenditure and provide suitable input for energy budget models that allow predictions about fitness and population consequences.

Introduction

Underwater sound pollution can impact fish and other marine life through a range of effects (Carroll et al., 2016; Popper and Hawkins, 2019). A high-amplitude sound source may have a potentially lethal effect through physical injury for nearby fish, while more moderate levels can still have a variety of non-lethal effects for fish over a large range of distances (Halvorsen et al., 2012; Slabbekoorn et al., 2010). Examples of non-lethal effects are acoustic masking, elevated stress levels, and disruption of swimming patterns (Sarà et al., 2007; Slotte et al., 2004; Wysocki et al., 2006). Although non-lethal effects may seem less dramatic, it is likely that more individuals are exposed to moderate sound levels, and therefore, potentially will have a stronger impact at the population level (Boudreau et al., 2018; Lima and Dill, 1989). While many studies have examined the effects of sounds on fish, extrapolating these results to individual fitness or population level effects remains a challenge (Carroll et al., 2016; Williams et al., 2015). Dynamic Energy Budget models (DEB) and the Population Consequence of (Acoustic) Disturbance (PCoD or PCAD) framework provide a tool and framework for this challenge (Leeuwen et al., 2013; Pirotta et al., 2018). Disturbance-induced changes in individual behaviour and physiology have to be translated into changes in vital rates (growth, reproduction and survival). These may subsequently be translated to changes in population dynamics (New et al., 2014; Pirotta et al., 2018). However, comprehensive assessments using these models are rare because of the lack of relevant data (Pirotta et al., 2018; Slabbekoorn et al., 2019).

So far, DEB and PCoD models have not been developed for fish in the context of acoustic disturbance (Hammar et al., 2014; Sivle et al., 2015). However, Soudijn et al. (2020) used a size-structured energy budget model for Atlantic cod to make a sensitivity analysis of potential effects of sound disturbance on population growth. The results indicated that additional energy expenditure and reduced food intake more easily lead to population-level effects than additional direct mortality and direct reproduction failure. In the model, additional energy expenditure and reduced food intake due to acoustic disturbances reduced growth, increased indirect mortality, delayed reproduction, and reduced reproductive output (Soudijn et al., 2020). Using the model, actual population-level effects of sound exposure could be estimated, but quantitative empirical data on behavioural and physiological effects of sound exposure are currently lacking (Pirotta et al., 2018; Slabbekoorn et al., 2019). The results of Soudijn et al. (2020) suggested that it is most relevant to study effect of sound exposures on energy expenditure and food intake.

A variety of studies has investigated behavioural changes of fishes to sound exposures and such non-lethal effects can also be found at larger distances from the sound source. Several studies have shown that sound exposures can affect swimming patterns by eliciting a startle or alarm response (Wardle et al., 2001), avoidance behaviour (Slotte et al., 2004), disrupting schools or groups (Sarà et al., 2007), and by changes in swimming depth (Hubert et al., 2020b). Responsiveness to sound can be lower in fish that live in high disturbance area's (Harding et al., 2018), or fade over sequential sound exposures (Nedelec et al., 2016; Neo et al., 2018), but long-term exposures may still have long-lasting effects (Becker et al., 2013; Payne et al., 2014). Several studies have also shown effects of sound exposure on foraging efficiency, with reduced feeding attempts and increased food handling errors in various captive species (Magnhagen et al., 2017; McLaughlin and Kunc, 2015; Purser and Radford, 2011; Shafiei Sabet et al., 2015; Voellmy et al., 2014) and one study on free ranging damselfish (*Chromis chromis*) (Bracciali et al., 2012). Payne et al. (2014) studied both swimming activity and foraging success in free ranging mulloways (*Argyrosomus japonicus*). Tagged fish in one estuary were less active and inhabited deeper waters during the weekend, and individuals in another estuary had less full stomachs and fewer fish in their diet over the weekend. These differences were likely due to higher boat activity in the weekends (Payne et al., 2014). Studies such as the last one may provide quantitative input for population models and allow parametrization of changes in energy expenditure and intake due to sound exposure.

So-called Hidden Markov Models (HMMs) provide a processing tool with high potential for swimming tracks from acoustic telemetry (Langrock et al., 2012; McClintock and Michelot, 2018). HMMs allow inference of behavioural states throughout the sampling period and they can be fitted to estimate the effect of external stressors on the time spent in the various behavioural states (see e.g. DeRuiter et al., 2017). The resultant time budget changes and step length (swimming speed) distributions can potentially be translated to changes in energy expenditure due to swimming and energy intake due to foraging behaviour, which is suitable input for energy budget calculations in PCoD frameworks (Hubert et al., 2020a; Leeuwen et al., 2013; Williams et al., 2006). HMMs have typically been used for data of relatively low temporal resolution on free ranging animals. For free ranging marine fish, it is especially challenging to track tagged fish at high-enough resolution through acoustic telemetry because of limited detection ranges of tag receivers, problems with relatively short battery life, and tag signal collisions. Furthermore, high resolution data may be necessary to distinguish among critical behavioural states (cf. Nams, 2013; Postlethwaite and Dennis, 2013).

Seismic surveys are a prominent contributor to the underwater soundscape and impact assessments are often part of the permit application (Ainslie et al., 2019; Gisiner, 2016; Sertlek et al., 2019). Marine seismic surveys are performed to explore the geological structure beneath the seafloor, often to search for oil and gas resources (Gisiner, 2016). They are conducted using a vessel towing one or two arrays of airguns and one up to more than ten streamers of hydrophones (Landrø and Amundsen, 2018). The airguns (seismic sources) produce high intensity, low-frequency impulsive sounds at regular intervals (5 – 15 s), potentially for several hours and repeated for several days to weeks and even months (McCauley et al., 2000; Slabbekoorn et al., 2019). Most energy of the sounds produced by airguns falls within the 10 – 300 Hz frequency range (Gisiner, 2016; Sertlek et al., 2019), which is within the hearing range of most – if not all – fish (Popper and Fay, 2011; Radford et al., 2012).

Atlantic cod is a popular model species to study the effects of noise pollution, because their hearing is well studied (Chapman and Hawkins, 1973; Sand and Karlsen, 1986) and because it is a commercially important species (Kurlansky, 1999). Catch rates of free ranging cod have been reported to be affected by actual seismic surveys (Engås et al., 1996; Løkkeborg and Soldai, 1993). Several experimental studies examined the effects of sound exposure on captive cod. Tagged cod in a floating pen exhibited increased swimming depth and heart rate during a single, unreplicated, experimental seismic airgun exposure (Davidsen et al., 2019). No startle or strong behavioural responses to exposures of pure tones were observed in a single group of cod in an indoor basis (Kastelein et al., 2008). Another study showed that short-term sound exposure did elicit startle responses in cod larvae, and also that long-term exposure led to initial reduction of growth rate, which disappeared at a later stage (Nedelec et al., 2015). Furthermore, cortisol levels increased during and after playback of sweep tones, and reproductive output of cod in a single treatment tank was lower compared to cod in a single control tank (Sierra-Flores et al., 2015). More studies like these – however, well-replicated – are needed. Especially data on sound impact on individual behaviour and physiology are required to gain insight into the potential effects of sound on cod populations.

In the current study, we examined the effects of sound exposure on cod behaviour in a net pen. We tagged cod with an acoustic tag and experimentally exposed them to playback of airgun sound pulses in a net pen in a cove. We examined the occurrence and magnitude of swimming pattern changes at the onset of the sound pulse series and analysed the time spent in various behavioural states during the exposure by exploiting current advances in processing of telemetry data. Fine-scale, high-resolution data on swimming patterns of captive cod

during sound stimulus playback provide insight into whether and how cod can respond to sound, which may help in interpreting more crude, lower-resolution data on free ranging cod in response to actual seismic survey sound. Our current study may therefore provide input for future experiments and individual energy budget models for the evaluation of sound impact on population dynamics.

Materials and methods

Study subjects

We used 20 wild-caught Atlantic cod (*Gadus morhua*) in this study, with body lengths ranging from 30 to 47 cm. Cod hear sound up to 470 Hz and they are most sensitive in the 60 – 310 Hz range. Below 50 Hz, cod are mostly sensitive to particle motion and above 50 Hz mostly to sound pressure (Chapman and Hawkins, 1973; Sand and Karlsen, 1986). The study subjects were caught by angling at ship wrecks in the Dutch North Sea from a recreational fishing boat during day trips on October 18th and November 15th 2017. During catching, the cod were kept in ~ 500 L transport boxes with continuous air supply and regular refreshment of sea water. After angling, the fish were kept in two cylindrical stock tanks (Ø 3.5 m, depth 1.2 m) at Stichting Zeeschelp, Kamperland, the Netherlands. The stock tanks were continuously refreshed with water from the nearby Oosterschelde marine inlet and the artificial dark-light cycle in the room followed the natural day-night cycle. The fish were fed with defrosted sprat (*Sprattus sprattus*) every other day and were kept in the stock basins for at least a week before being used in the experiment, the fish usually started eating the first or second day after capture.

Experimental arena

The study was conducted in the Jacobahaven, a cove in the Oosterschelde sea inlet of the North Sea, the Netherlands. The cove is about 200 m wide, 300 m long and 2-5 m deep at low and high tide, respectively. The location is relatively sheltered from wind and waves by surrounding dikes and piers and is isolated from external boat traffic, making it a suitable location for sound impact studies. The experiment was conducted using a floating study island (Candock, Canada), consisting of a working platform and an octagonal walkway that supported a net pen (Ø 11.5-12.5 m and a max. depth of 5 m, fig. 1) as arena for the study subjects. The equipment and underwater speaker were supported by the working platform. The study island was bottom-anchored in the centre of the cove with chains and stretchable mooring rope. Detailed sound field measurements in the current experimental setup during previous experiments have revealed a gradual decrease in sound level with increasing distance from the speaker and a ratio of

sound pressure and sound velocity level that is to be expected in the acoustic far field (Hubert et al., 2016).

Tagging and data acquisition

Each fish was tagged with an acoustic transmitter and accelerometer logger (resp. 2 and 6 gram in air). The fish were anaesthetized using 2-phenylethanol (0.6 ml/l seawater) or clove oil (0.05 ml/l). The tags were implanted in the intraperitoneal cavity by making an incision in the abdominal wall, inserting the tag, then suturing the incision. After tagging, the fish was allowed to recover for > 40 hours in a rectangular tank (1.20 m x 1.00 m x 0.65 m) with a continuous inflow of Oosterschelde water. The fish were not fed during this period. The acoustic tag (Model 795-LF, HTI, US) was used to determine the position of the fish. We set the tag to emit 0.5 ms pings of 307 kHz (inaudible to the fish) at a 1 s pulse repetition interval. Pings were recorded by four hydrophones (Model 590-series, HTI, US) that were attached to the outer edge of the octagonal walkway (two at the surface, two close to the bottom, fig. 1) and processed by an acoustic tag receiver (Model 291, HTI, US) which transferred detections to a laptop.

The accelerometer logger (MBLog Mini, Maritime bioLoggers, Canada) measured acceleration along three axes and was used to gain insight into the general activity levels. The logger was set to sample at 100 Hz at 16 bit and stored the data on a micro SD card in the tag. We aimed to time-synchronize the data from the accelerometer to the spatial data of the fish, as the spatial data and the
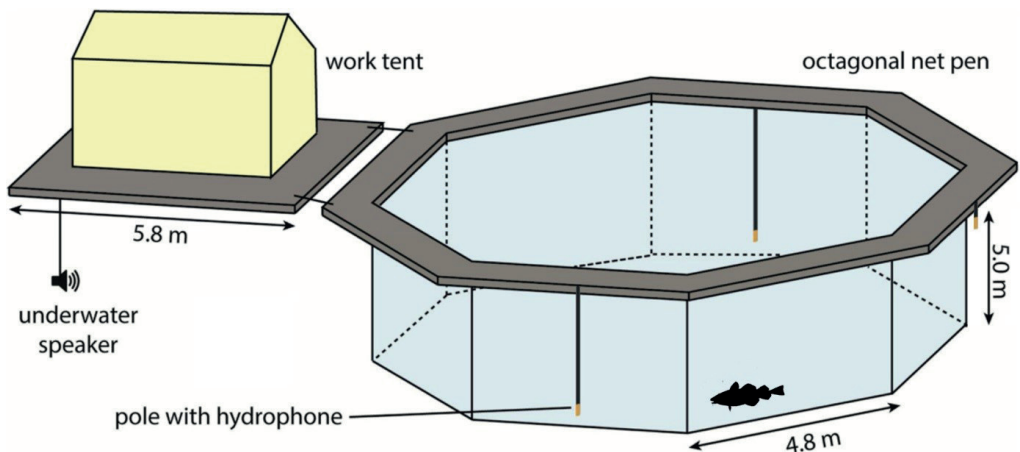

Fig. 1: A schematic overview of the study island (figure adapted from Neo et al., 2016). The square working platform held the telemetry and playback equipment and was connected to the octagonal walkway and net pen with ropes. The four hydrophones recorded the acoustic signals from the tags. The fish represents a single cod test individual, but is not drawn to scale.

sound exposure were linked to the UTC time. For this, we calculated the vector of the dynamic body acceleration (VeDBA) using the accelerometer data and the 2-dimensional swimming speed using the spatial positions. We used the optim function in R to find the strongest correlation between VeDBA and speed while allowing 4 Hz deviation from the set sampling period of the accelerometer and while allowing clock drift by using cross-correlations. Visual observation of the synchronized data streams revealed inconsistencies in the time-synchronisation, possibly due to inconsistent sampling rate of the accelerometer, so we decided to exclude the acceleration time series from further analyses.

Experimental design

We conducted 20 trials in which a single cod was exposed to one hour of airgun playback. The 1 h sound treatment was scheduled to start 18 – 22 hours after release in the pen and was followed by 30 min of silence (figure 2a). We scheduled the hour of airgun playback to start 1 hour before or at the peak of high tide to permit the propagation of lower sound frequencies. The shallow water cut-off frequency at 5 m depth was measured to be 150 Hz in the experimental setup. Playback schedules were alternated between 1 hour before and after absolute high tide to control for effects of flow or ebb tide. After the 30 min of silence,
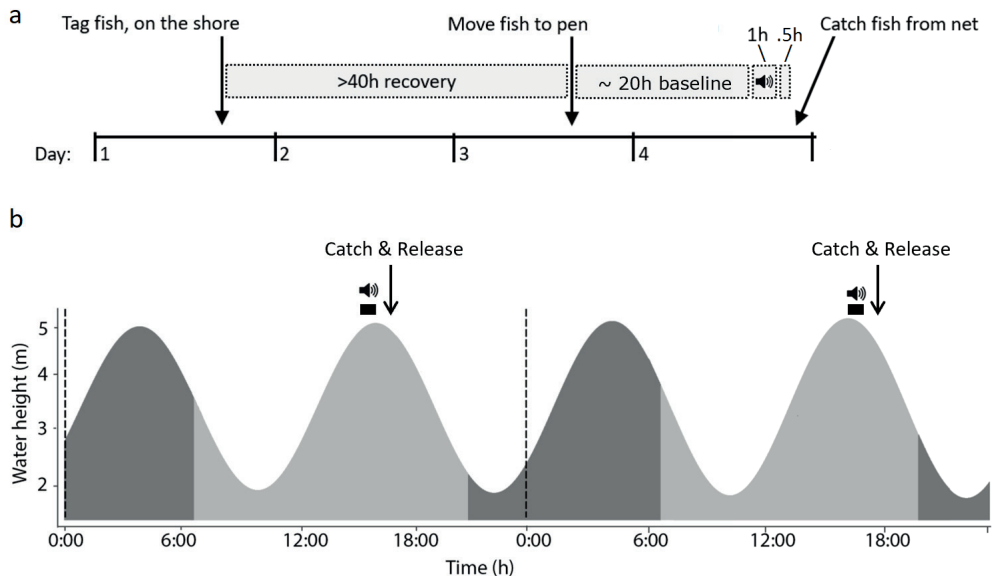
Fig. 2: (a) Timeline of a single trial: the horizontal grey bar represents recovery, baseline, exposure, and post-exposure periods, while the vertical arrows indicate fish tagging, moving, and catching events; (b) Water height levels related to the tidal fluctuation during night (dark) and day (light). Exposures took place just before or after high tide. After the exposure, the experimental fish was caught and a new tagged fish was released in the pen again.

the fish was retrieved from the net pen and replaced with the next experimental individual (figure 2b).

Sound exposure

We exposed the individual fish to one hour of playback of airgun sound pulses at a pulse rate interval (PRI) of 10 s, which is a realistic rate for seismic surveys (Gisiner, 2016; Slabbekoorn et al., 2019). The sound pulses were recordings of a down-scaled airgun (TNO, The Netherlands) with a volume of 10 in^3 and a pressure of 800 kPa (Reichmuth et al., 2016). The recordings were made, using a hydrophone (model 96-Min, HTI), from the study island during high tide (4.5 - 5.0 m water depth) at a distance of 5.8 m with both source and receiver suspended mid-water column. Playback tracks were generated by randomly selected airgun sound pulse recordings spaced by silent intervals (figure 3ab). The tracks were played back with an underwater transducer (LL-1424HP, Lubell Labs, US) from a recorder (DR-07, Tascam, US), via a power amplifier (DIGIT 3K6, SynQ, Belgium) and a transformer (AC1424HP, Lubell Labs). For 9 trials,
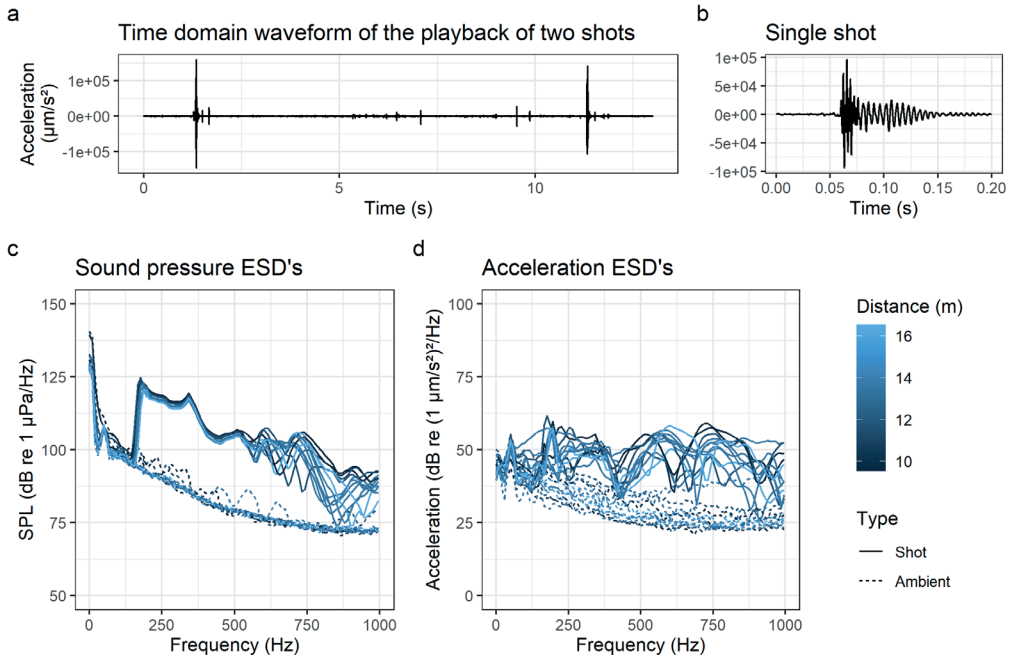
Fig. 3: (a) Sound pressure vs time recording from the net pen of two sequential airgun sound pulses with a 10 s inter-pulse interval and (b) a single sound pulse. (c & d) Energy spectral densities (ESD) of recordings of the playback of airgun sound pulses and the silent (ambient) intervals at various distances from the speaker at 2 m depth, provided in (c) sound pressure level (SPL) and (d) sound particle acceleration. The ESD's were generated using the Hann window type and a window length of 4096 and 50% overlap.

the speaker was suspended mid-water column (~ 2 m deep) at 2 m from the net pen, for 5 trials at 7.8 m from the net pen (~ 2 m deep), and for 6 trials, it was located on the sediment at about 20 m from the net pen (4.5 – 5 m deep). In this way we achieved variation in exposure level and the exposures of all trials were recorded in the pen using a calibrated hydrophone and the three set-ups resulted in mean zero-to-peak sound pressure levels (SPL_{z-p}) of 174, 169, and 152 dB re 1 µPa (100-600 Hz bandpass filter) with the speaker at 2, 7.8, and 20 m from the net pen respectively.

To determine the sound levels and spectra in the net pen, we measured sound pressure and particle velocity in the pen, at 9.7, 11.6, 13.5 and 16.4 m from the speaker, with the speaker at 7.8 m from the net pen. The measurements were done using a M20 particle velocity sensor (GeoSpectrum Technologies, Canada) and logged on a laptop using a differential oscilloscope (PicoScope 3425, Pico Technologies, UK). Recordings were analysed using the manufacturer provided receiver sensitivity data and a 100-600 Hz bandpass filter. The mean zero-to-peak sound pressure level (SPL_{z-p}) of the played back airgun shots at 9.7 m from the speaker was 164 dB re 1 µPa, and the sound particle acceleration (a_{z-p}) was 101 dB re 1 nm/s^2. At 16.4 m, this was 158 dB and 99 dB respectively. The mean SPL of the ambient conditions in the pen was 113 dB re 1 µPa and the mean sound particle acceleration was 61 dB re 1 nm/s^2 (fig. 3cd).

Telemetry positioning

We used YAPS (Yet Another Positioning Solver), a single-state continuous time animal movement model designed to account for positioning error in time of arrival localization, to estimate the positions of the fish (Baktoft et al., 2017). We extended the functionality of YAPS to apply a correlated walk model, implemented as an Ornstein–Uhlenbeck velocity process (Johnson et al., 2008), which should yield more suitable track estimates for highly autocorrelated velocity data. Due to memory limitations during telemetry processing and fitting issues for some trials, we subsampled the telemetry data resulting in pulse repetition intervals of 2 s (n = 17 trials), 3 s (n = 2) or 5 s (n = 1), depending on the detection rates. Across individuals, we obtained on average 91% (range: 45% – 100%) of all expected positions. We used the positions of the fish to determine the swimming speed, turning angle and depth.

Statistics

We applied three models to examine different aspects of changes in swimming behaviour: (1) A randomization test to examine the occurrence of short-term changes from baseline swimming behaviour in response to playback onset, (2) a

non-linear mixed effects model to examine the magnitude and decay of changes from baseline swimming behaviour in response to playback onset, and (3) a three-state discrete-time Hidden Markov Model (HMM) to examine if airgun playback resulted in changes in time spent in various behavioural states.

Randomization Test

Instantaneous changes in swimming behaviour throughout the trials were represented by Mahalanobis distance values, calculated as the mean number of standard deviations of 60 seconds of swimming behaviour (swimming depth, speed and turning angle) from the covariance matrix of the previous 60 seconds. This resulted in time series indicating the magnitude of short-term change in swimming behaviour (fig. 4). To account for potential clock drift, the maximum Mahalanobis distance within 30 seconds before and 30 seconds after the playback onset was taken as the observed response while a null hypothesis was generated by randomly reassigning the 60 second response period to any position in the period before exposure and measuring the maximum Mahalanobis distance within the randomized response period. We then examined whether the observed response exceeded the 95th percentile of 10,000 randomized responses (see Antunes et al., 2014; DeRuiter et al., 2013). This test was applied to each individual.

Non-linear Mixed Model

A mixed model was applied to examine the magnitude and decay of the response to playback onset over all trials. Here, Mahalanobis distance values were calculated with respect to the entire trial and deviations from baseline behaviour in response to the playback onset were assumed to decay exponentially. We only used the swimming speed and turning angle of the fish for this and not swimming depth because the tidal variation limited the maximum depth of the fish. This did not yield a bias for the previous analysis because that earlier analysis focussed on short-term behavioural changes when compared to short baselines. The inclusion of depth in the current analysis would possibly yield a problematic bias when the entire trial would be used as baseline (covariance matrix). The swimming speed and turning angle were converted into normal distributions by fitting, respectively, gamma and wrapped Cauchy distributions to the data streams before using them in Mahalanobis distance calculation. The model was formulated as (I) where the subscripts *a*, *b*, and *c* indicate design matrices and coefficients for the baseline, response magnitude, and response decay, respectively, and t is the time since exposure onset. φ is the latent auto-regressive (AR(1)) process where ρ indicates the strength of the process and ψ is the Gaussian distributed error term. The individual ID was treated as a random intercept effect applied to the baseline conditions (*a*). The model was implemented

using a maximum likelihood framework in R with TMB (Kristensen et al., 2016) and hypothesis testing was carried out using a parametric bootstrap.

$$\log(\mu) = X_a\beta_a + I(t) \cdot X_b\beta_b e^{X_c\beta_c \cdot (-t)} + \varphi$$

$$I(t) := \begin{cases} 1 & \text{if } t \geq 0, \\ 0 & \text{if } t < 0. \end{cases} \tag{I}$$

$$\varphi_t = \rho \cdot \varphi_{t-1} + \psi_t$$

$$\psi \sim \text{Gaussian}(0, \sigma^2)$$

Hidden Markov Model

HMMs for animal movement were used to examine if the sound exposure altered the time spent in various behavioural states. The R package MomenuHMM (McClintock and Michelot, 2018) was used to fit HMMs to the horizontal step length and turning angle of the fish. We fitted HMM null models with 1, 2 and 3 states to all individual fish and compared the AIC scores to determine the number of behavioural states that were best supported by the models. We do not report the fit of models with more than 3 states because the AIC tended to favour models with (much) higher number of states, whereas this is biologically less interpretable (cf. Pohle et al., 2017). Combinations of 'Tide height', 'Sun elevation', and 'Treatment period' (Before, During & After) were applied as state transition probability covariates during selection of the best fitting model. Comparison of AIC scores, broadly across all individuals, indicated that the inclusion of all covariates resulted in the best fitting models (Appendix 1, table 3).

When running the models, the resulting state transition probability p-values for the covariate 'Treatment period' appeared to over-attribute natural variation in swimming behaviour, commonly observed in the baseline period, to the effect of During and After in 'Treatment period'. This is likely due to the During and After period being too short, relative to the long baseline, to average out biases resulting from natural fluctuations in swimming behaviour, especially those related to tide (which we synchronized with the starting times of the exposure periods to guarantee sufficient water levels to allow propagation of relatively low frequencies). As a result, state transition coefficient p-values for 'Treatment period' were not used for hypothesis testing.

Instead, HMMs were used to simulate null hypothesis distributions for time spent in each behavioural state. Per each individual, the fitted HMMs were used

to simulate 10,000 realizations of the expected behavioural state during each trial in the absence of the sound exposure while preserving the remaining observed covariate values ('Tide height' and 'Sun elevation'). For each hour, the observed proportion of time in each behavioural state was compared to the null hypothesis distribution. If the deviation of the observed data from the null hypothesis was larger during or after the treatment period, we interpreted this as an effect of the sound treatment.

Results

All fish showed variable swimming patterns in time, but typically used the whole space available, horizontally and vertically. We found no strong overall pattern of
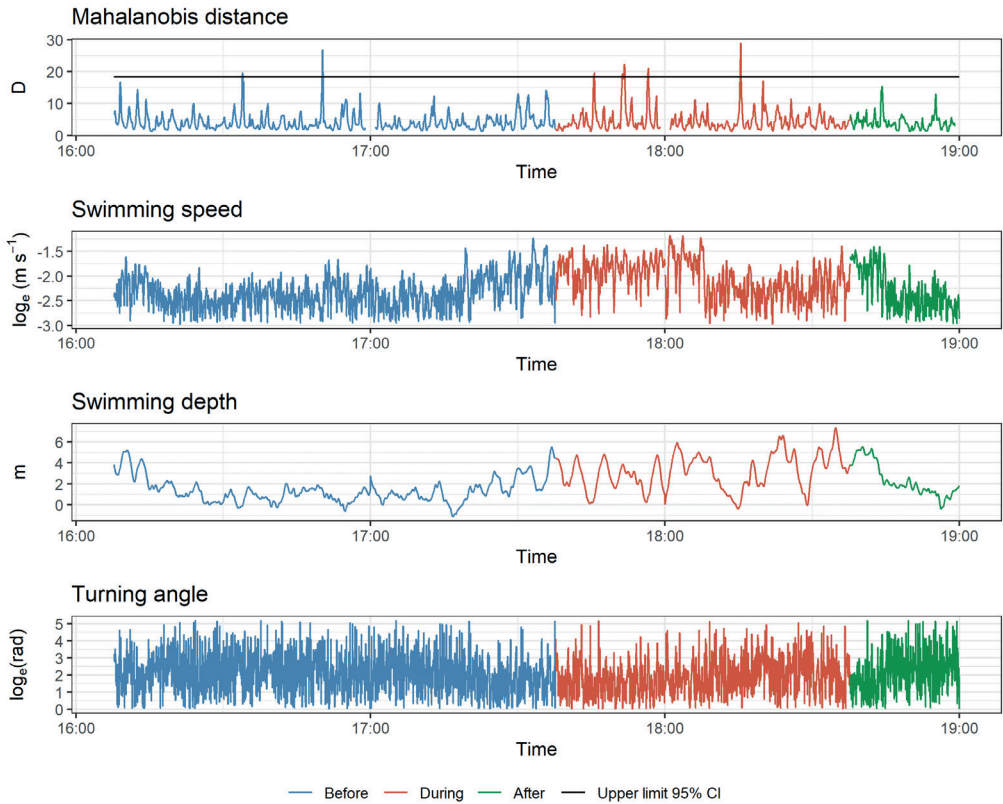
Fig. 4: Top panel shows the Mahalanobis distance over the course of 3 h of a single fish, with the median and upper limit of the 95th percentile of the Mahalanobis distances during the Before period, resp. pink and red line. The Mahalanobis distance at the start of the sound exposure does not exceed the 95th percentile, so the current fish did not immediately respond to the sound treatment. The panels below depict the swimming speed ($\log_e$(m s^{-1})), swimming depth (m, distance from the bottom of the grid) and turning angle ($\log_e$(radians)) that were used to construct the Mahalanobis distance (D).

change during the exposure, compared to the baseline period. The randomization test showed that only one individual exhibited a response magnitude that exceeded the 95th percentile of randomized responses (Appendix 1, table 1). This result is within the expected type 1 error rate for this test, thus there is no evidence for an immediate behavioural response at the onset of the sound exposure.

The results from the individual analyses were also reflected by our tests for significant changes at group level. With respect to the non-linear mixed effects model, no significant response was observed at the onset of the sound exposure (figure 5). In addition, there was no consistent significant change in swimming behaviour during the entire treatment period (Appendix 1, figure 1).

Using the HMMs, we identified support for three behavioural states in all fish based on AIC (Appendix 1, table 2): 1) high swimming speed and low turning angle (which we labelled 'transit'); 2) moderate speed and moderate turning angle ('locally active'); and 3) low speed and high turning angle ('inactive') (figure 6). Note that we tried to select relatively neutral labels, to not infer more interpretation than we can base on the kinetic description. When comparing the time spent in the behavioural states and mean step lengths from the observed data with the time spent in the states from the simulated data, there is a trend for larger deviations from the simulated baseline behaviour in the period during and after sound exposure (figure 7). Several individuals tended to spend
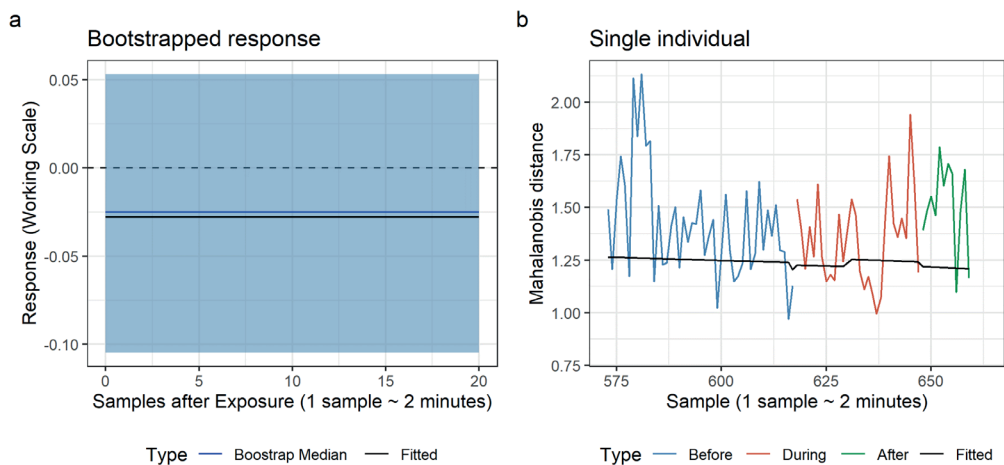

Fig. 5: (a) Bootstrapped response to exposure on the working scale ($\log(\mu)$, see formula I) of the model. The shaded area indicates the bootstrapped 95th percentile interval of the response. The response magnitude does not significantly differ from 0, indicating no immediate response to exposure onset. (b) Observed and fitted data of a single fish, the last 3 h of the trial.

relatively more time in the transit state and less time in locally active and inactive during and after the playback, which is supported by the higher step lengths. Due to experimental design limitations, we could not test the significance of these trends.
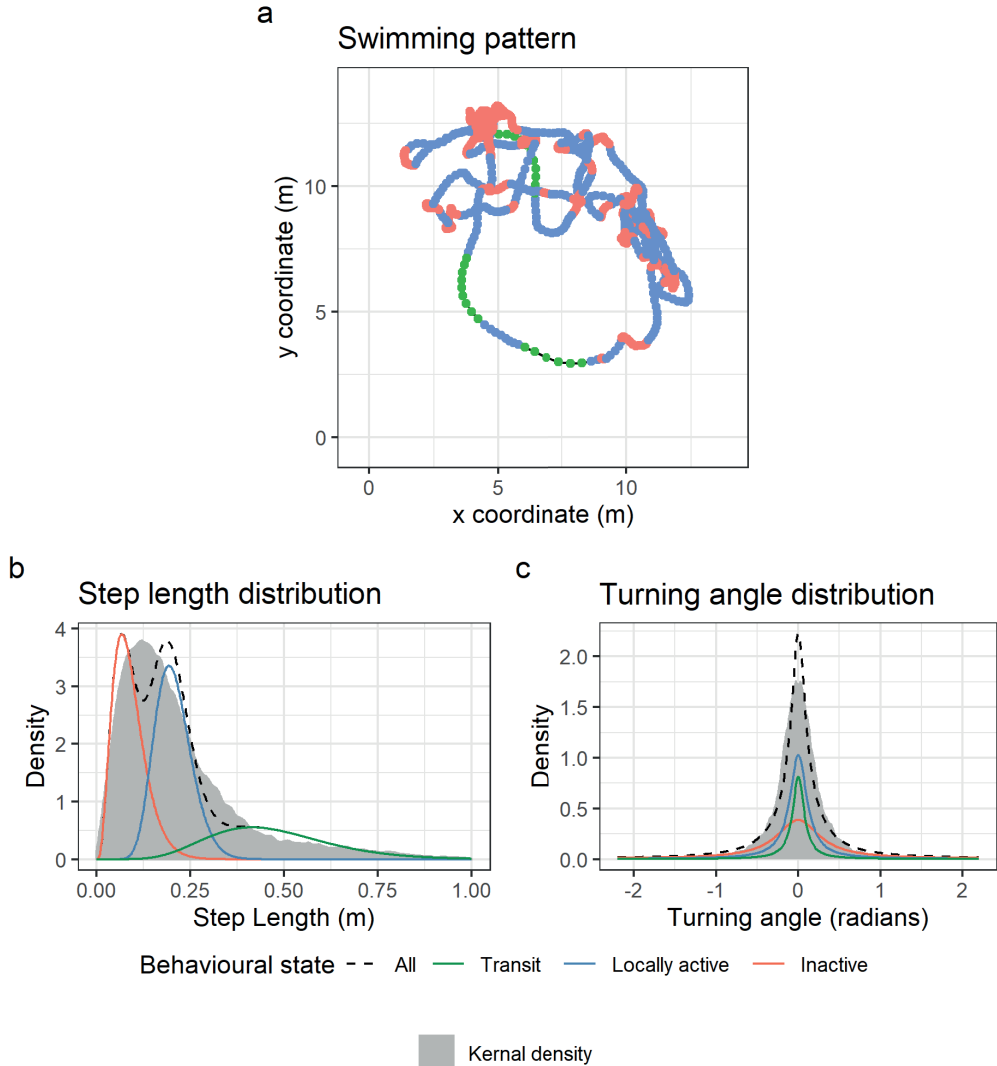

Fig. 6: (a) Top view swimming patterns (26 min) of a single individual with the behavioural state indicated by colour. (b & c) The step length (swimming speed) and turning angle distribution for each behavioural state of the same individual for the entire trial. The lines show the fitted distributions while the grey shaded area shows the kernel density plot of the observed distribution.
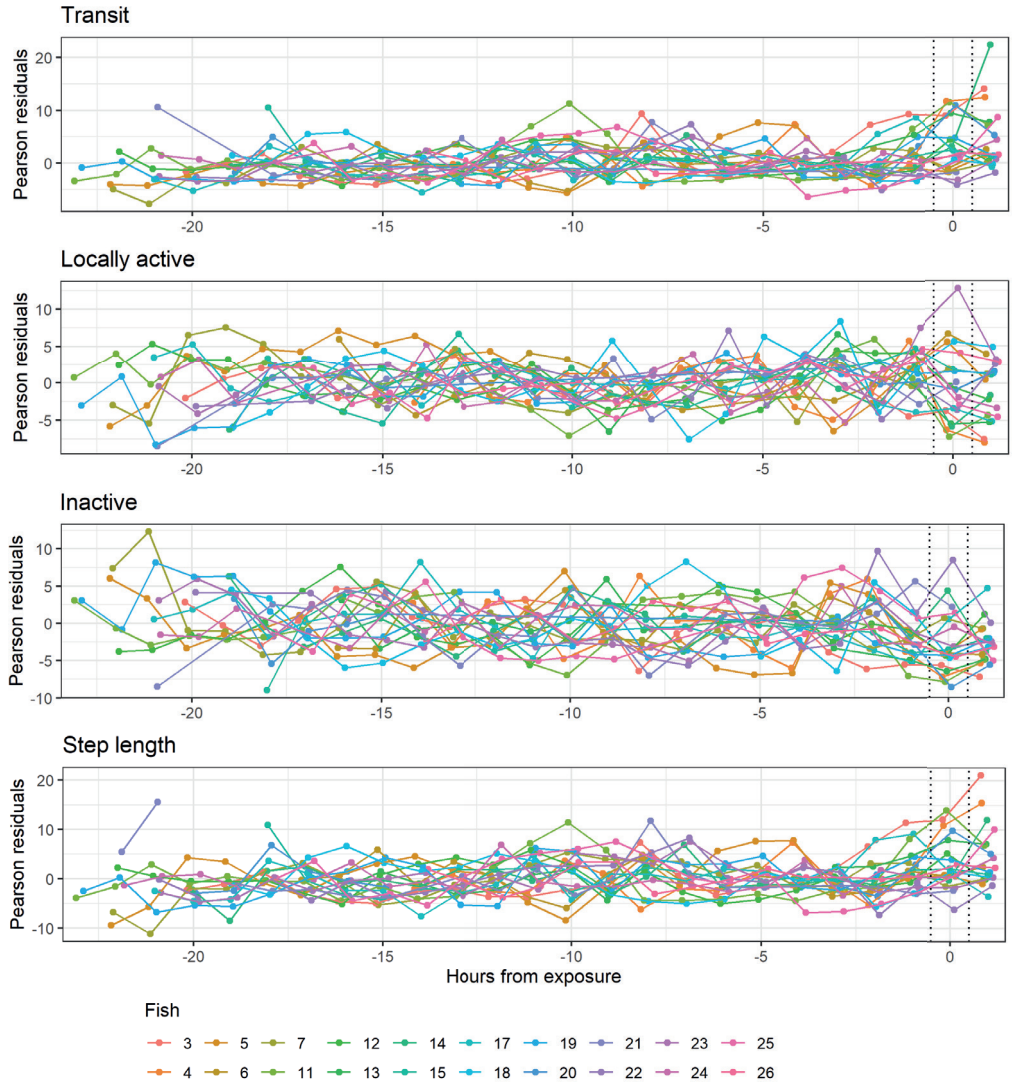
Fig. 7: For each of the three behavioural states and the step length distribution; the amount of standard deviations (Pearson residuals) of the observed time spent and step length from the mean time spent and step length of the simulated data (for absolute data, see Appendix I, figure 2). The vertical dashed lines indicate the hour of sound exposure. The colours indicate the different individuals, hourly datapoints are lacking if the individual had less than 25% of the expected positions that hour.

Discussion

In the current study, we experimentally exposed individual Atlantic cod in a net pen to the playback of seismic airgun sound pulses and examined changes in

swimming patterns of the fish. Our results demonstrate that only one individual altered its swimming pattern significantly at the onset of the sound and that altogether individual cod did not change their swimming patterns, neither immediately at the onset nor over the whole period of the sound exposure. However, several individuals seemed to change their distribution of time spent in three behavioural states during the 1 h exposure, compared to the baseline. The time spent in behavioural states may be translated to energy expenditure and in future experiments possibly also integrated with food intake, and thereby be used as input for Population Consequences of (Acoustic) Disturbance (PCoD or PCAD) models.

Short-term vs. long-term behavioural response

We did not find an immediate change in swimming patterns upon the start of the sound exposure, neither when using a short time window right before the sound exposure as baseline (Randomization test; individual analysis), nor with the entire trial as baseline (Non-linear mixed model; group analysis). In accordance with the current results, two other studies showed that a single group of captive cod did not exhibit an immediate short-term response to pure tones (cod of 42-46 cm, Kastelein et al., 2008) or to seismic airgun exposure (cod of 38-73 cm, Davidsen et al., 2019). Despite the lack of immediate, short-term behavioural responses in cod, several studies are indicative of more long-term behavioural changes in cod during sound exposures (Davidsen et al., 2019; Engås et al., 1996; Løkkeborg and Soldai, 1993).

We estimated the time spent in various behavioural states for all individuals using HMMs for animal movement. Several individuals seemed to have changed their time spent in various behavioural states due to the sound exposure; several spent more time transiting and less time being locally active or inactive. A change in time budget expenditure does not necessarily imply a sudden change at the onset of the sound, but can also mean staying in a particular state for longer or switching back to a state more quickly. It has been shown in zebrafish (*Danio rerio*), for example, that an immediate response does not necessarily correlate with a prolonged response (Shafiei Sabet et al., 2016). Nevertheless, most behavioural response studies on the effects of sound focus on immediate changes at the onset of the sound exposure, whereas long-term changes in activity patterns may be more relevant for exploring consequences at population level (Hubert et al., 2020a).

Individual energy budget

The various behavioural states and accompanying swimming speed distributions

may be indicative of different levels of energy expenditure and food intake (Lundquist et al., 2012; Williams et al., 2006), which in turn can be linked to growth, survival and reproductive output using PCoD/PCAD models (Pirotta et al., 2018; Soudijn et al., 2020). We labelled the states in the current study as 'transit', 'locally active' and 'inactive', representing respectively swimming patterns of high speed and low turning angle, moderate speed and moderate turning angle, and low speed and high turning angle. Swim tunnel experiments have shown that fish swimming at higher speed use more oxygen, which can be used as a proxy for energy use (Tudorache et al., 2008). For fish in swim tunnels that have been tagged with accelerometers, the vector of the dynamic body acceleration (VeDBA) could also be linked to oxygen use (Metcalfe et al., 2016; Wright et al., 2014). Such swim tunnel experiments with cod are necessary to translate our swimming speed data to energy use. It should be mentioned that additional energy expenditure due to potentially elevated stress levels is not covered by the current approach and requires additional experiments (Rabasa and Dickson, 2016; Sierra-Flores et al., 2015). The importance of the quantification of energy expenditure and intake in all behavioural states to quantify the impact of anthropogenic disturbance has been addressed in several marine mammal studies (Christiansen et al., 2010; Lundquist et al., 2012). Williams et al. (2006) actually quantified the consequence of boat traffic on killer wales (*Orcinus orca*) and estimated that the change in activity budget led to an increase in energy expenditure of 3-4% and a decrease in energy intake of 18% (based on reduced foraging time).

Foraging behaviour could serve as a proxy for energy intake. 18 out of 20 fish in the current experiment had food in their stomachs at the end of their trials, indicating that the majority of the fish exhibited foraging behaviour in the net pen. A basin experiment on Atlantic cod described foraging behaviour as relatively slow swimming close to the bottom and turning frequently (Hubert et al., 2020a), which was in line with reported behaviour of free ranging Atlantic cod (Rose, 2019). This reported foraging behaviour appears to resemble the swimming patterns from the locally active- and inactive-state most, so it may be that these states also included foraging behaviour. However, in the current study we cannot discriminate between foraging behaviour and other locally active and inactive behaviour. Such insight requires additional data from accelerometers and/or gyroscopes, validated by experiments with parallel video-tracking (Kawabata et al., 2014). Explicit confirmation of associations between foraging behaviour and behavioural states, enriched with accelerometer/gyroscope data, would likely yield critical entry data for PCoD/PCAD models. The actual energy intake for free ranging cod would also require data on success rate of foraging in the wild and about nutritional value of their prey items. Such data may come

from video experiments with captive fish (e.g. Shafiei Sabet et al., 2015) and stomach content data from free ranging fish (e.g. Payne et al., 2014).

Experimental design with respect to HMMs

We did not provide state transition probability p-values for the covariate 'Treatment period', whereas this is typically provided from analyses using HMMs (e.g. DeRuiter et al., 2017). In the current analysis, the state transition probabilities appeared to over-attribute natural variation in swimming behaviour to the effect of During and After in 'Treatment period', likely due to the During and After periods being relatively short (resp. 1 and 0.5 h) compared to the Before period (~ 20 h). So, for future experiments it should be considered to use longer and/or multiple exposures to reduce biases resulting from natural fluctuations in swimming behaviour. Despite the lack of reliable state transition probability p-values, we gained insight into the effect of the sound exposure by using the HMMs to simulate null hypothesis distributions for time spent in each behavioural state and to compare this to the observed time spent. However, we should be cautious while interpreting these results. For several individuals, the deviation of the observed from the simulated time spent in particular behavioural states during the sound exposure, seemed already initiated in the hour before the exposure. This may be due to the timing of the sound exposures, always ending or starting at absolute high tide to allow relatively low frequencies to propagate in the shallow cove. We aimed to prevent a bias from the tide by making sure that the baseline was long enough to contain one other high tide besides the high tide of the exposure (tidal period is ~ 12:25 hh:mm, baseline period was ~ 20 h) and by using the tide as covariate in the analysis. However, it may be that the behaviour in the first and second high tide was different (for some individuals) because of an interaction with acclimation to the pen over time. Such a bias can be avoided by longer trials and by exposing fish throughout the tidal period at a deeper test site.

Captive vs. free ranging fish

In studies on captive fish, the experimental control and data resolution is potentially relatively high (Slabbekoorn, 2016). This also enabled us to explore novel methods of data processing and analyses and may aid in the interpretation of lower resolution data of free ranging fish. It should be clear that it was not the goal of the current study to determine absolute response levels of cod to seismic surveys, because of the limited validity of behavioural responses and limited acoustic realism of the scaled sound exposure. The behaviour of the wild-caught individuals in the current study is likely not directly comparable to free ranging individuals (cf. Wright et al., 2007), for example, because they were not able to

swim away from the sound source. The location of the net pen was also relatively shallow, whereas cod typically live in deeper water, and only migrate closer to shore for spawning (Reubens et al., 2013; Righton et al., 2010). Furthermore, the acoustic stimulus differed from actual seismic survey sound at sea because the speaker did not produce low-frequency sound, was not moving, and both the soundscape and propagation in shallow water differ from deeper water, where cod are more likely to be exposed to seismic surveys.

Ultimately, data on the effects of sound on fish should be collected in the field with free ranging animals and actual anthropogenic sound sources (Popper and Hawkins, 2019). However, fish are difficult to observe in the field, they typically do not surface such as marine mammals and most are too small to carry the same sophisticated loggers as marine mammals. Free ranging fish can be observed with baited camera's, echosounders/Didsons, telemetry, and by diving researchers, but through all methods it is challenging to track individuals over an extended period of time (Bruce et al., 2018), to collect high resolution data, and to not affect fish behaviour by the observation method (Bracciali et al., 2012). Since both indoor and outdoor studies provide us with opportunities and limitations, it is good to be aware of them and to use a complementary approach to gain insight into the effects of sound on fish (Slabbekoorn, 2016).

Conclusions

In the current study, Atlantic cod seemed unresponsive to sound as they did not change their swimming patterns immediately at the onset of the sound exposure. However, several individuals changed their time spent in several behavioural states during the 1 h sound exposure. Several individuals spent more time transiting and less time being locally active or inactive, this may be indicative of changes in energy budgets and may ultimately affect their health and vital rates (growth, survival, and reproduction). Such data are suitable input for PCoD/PCAD models, but further validation of behavioural states and their link to energy budgets and health is needed. Nevertheless, we think that the current approach of data collection and processing is promising and could be applied in future studies on captive and free ranging fish. In future captive studies, video data combined with spatial data may increase insight into food intake and thereby aid in biological interpretation and the translation to bio-energetics of behavioural states.

Ethical statement

This experiment was conducted in accordance with the Dutch Experiments

on Animals Act and approved by the Dutch Central Commission Animal Experiments (CCD) under no. AVD1060020171085.

Supplementary information

Supplementary information associated with this article is available from the publication DOI:10.1016/j.marpolbul.2020.111680.

Data accessibility

All data used for the analyses reported in this article is available from the Zenodo Repository, DOI:10.5281/zenodo.4013336.

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References

Ainslie, M. A., Heaney, K. D. and Macgillivray, A. O. (2019). Guest Editorial Special Issue on Verification and Validation of Air Gun Source Signature and Sound Propagation Models. IEEE J. Ocean. Eng. 44, 551–559.

Antunes, R., Kvadsheim, P. H., Lam, F. P. A., Tyack, P. L., Thomas, L., Wensveen, P. J. and Miller, P. J. O. (2014). High thresholds for avoidance of sonar by free-ranging long-finned pilot whales (*Globicephala melas*). Mar. Pollut. Bull. 83, 165–180.

Baktoft, H., Gjelland, K. Ø., Økland, F. and Thygesen, U. H. (2017). Positioning of aquatic animals based on time-of-arrival and random walk models using YAPS (Yet Another Positioning Solver). Sci. Rep. 7, 1–10.

Becker, A., Whitfield, A. K., Cowley, P. D., Järnegren, J. and Næsje, T. F. (2013). Does boat traffic cause displacement of fish in estuaries? Mar. Pollut. Bull. 75, 168–73.

Boudreau, M. L., Scrosati, R. A. and Wong, M. C. (2018). Predator (*Carcinus maenas*) nonconsumptive limitation of prey (*Nucella lapillus*) feeding depends on prey density and predator cue type. J. Ethol. 36, 259–264.

Bracciali, C., Campobello, D., Giacoma, C. and Sarà, G. (2012). Effects of nautical traffic and noise on foraging patterns of mediterranean Damselfish (*Chromis chromis*).
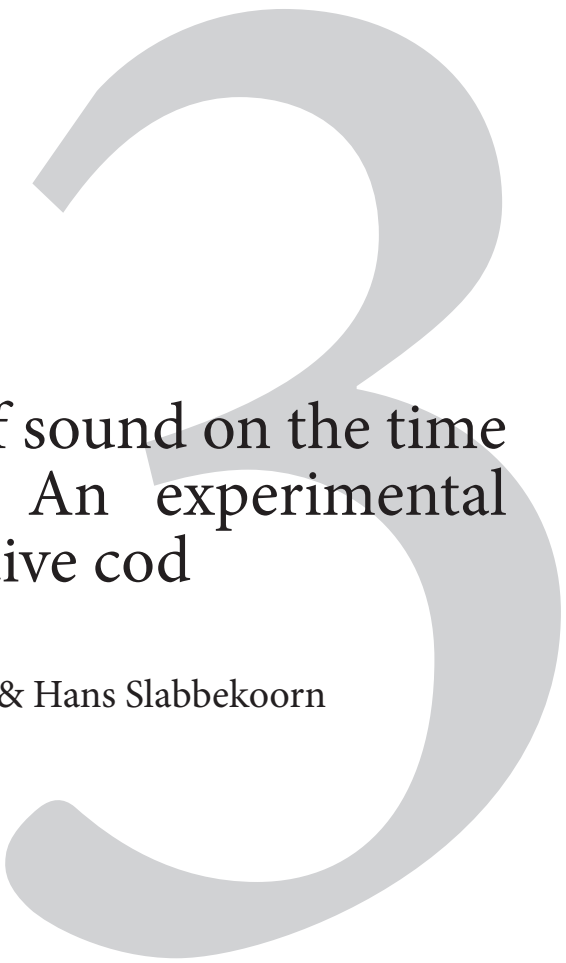
PLoS One 7,.

Bruce, B., Bradford, R., Foster, S., Lee, K., Lansdell, M., Cooper, S. and Przeslawski, R. (2018). Quantifying fish behaviour and commercial catch rates in relation to a marine seismic survey. Mar. Environ. Res. 140, 18–30.

Carroll, A. G., Przeslawski, R., Duncan, A., Gunning, M. and Bruce, B. (2016). A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pollut. Bull. 114, 9–24.

Chapman, C. J. and Hawkins, A. D. (1973). A field study of hearing in the cod, *Gadus morhua* L. J. Comp. Physiol. 85, 147–167.

Christiansen, F., Lusseau, D., Stensland, E. and Berggren, P. (2010). Effects of tourist boats on the behaviour of Indo-Pacific bottlenose dolphins off the south coast of Zanzibar. Endagered Species Res. 11, 91–99.

Davidsen, J. G., Dong, H., Linné, M., Andersson, M. H., Piper, A., Prystay, T. S., Hvam, E. B., Thorstad, E. B., Whoriskey, F., Cooke, S. J., et al. (2019). Effects of sound exposure from a seismic airgun on heart rate, acceleration and depth use in free-swimming Atlantic cod and saithe. Conserv. Physiol. 7, 1–19.

DeRuiter, S. L., Southall, B. L., Calambokidis, J., Zimmer, W. M. X., Sadykova, D., Falcone, E. A., Friedlaender, A. S., Joseph, J. E., Moretti, D., Schorr, G. S., et al. (2013). First direct measurements of behavioural responses by Cuvier's beaked whales to mid-frequency active sonar. Biol. Lett. 9, 0–4.

DeRuiter, S. L., Langrock, R., Skirbutas, T., Goldbogen, J. A., Calambokidis, J., Friedlaender, A. S. and Southall, B. L. (2017). A multivariate mixed hidden Markov model for blue whale behaviour and responses to sound exposure. Ann. Appl. Stat. 11, 362–392.

Engås, A., Løkkeborg, S., Ona, E. and Soldal, A. V. (1996). Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. 53, 2238–2249.

Gisiner, R. C. (2016). Sound and Marine Seismic Surveys. Acoust. Today 12, 10–18.

Halvorsen, M. B., Casper, B. M., Matthews, F., Carlson, T. J. and Popper, A. N. (2012). Effects of exposure to pile-driving sounds on the lake sturgeon, Nile tilapia and hogchoker. Proc. R. Soc. B Biol. Sci. 279, 4705–14.

Hammar, L., Wikström, A. and Molander, S. (2014). Assessing ecological risks of offshore wind power on Kattegat cod. Renew. Energy 66, 414–424.

Harding, H. R., Gordon, T. A. C., Hsuan, R. E., Mackaness, A. C. E., Radford, A. N. and Simpson, S. D. (2018). Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise. Biol. Lett. 14, 20180441.

Hubert, J., Neo, Y. Y., Campbell, J. A. and Slabbekoorn, H. (2016). Particle motion and pressure soundscape in outdoor vs indoor set-up. In Proceedings of Meetings on Acoustics, p. 070007.

Hubert, J., Wille, D. A. and Slabbekoorn, H. (2020a). Exploring effects of sound on the time budget of fishes: An experimental approach with captive cod. In Proceedings of Meetings on Acoustics, p. 010012.

Hubert, J., Neo, Y. Y., Winter, H. V. and Slabbekoorn, H. (2020b). The role of ambient sound levels, signal-to-noise ratio, and stimulus pulse rate on behavioural disturbance

of seabass in a net pen. Behav. Processes 170, 103992.

Johnson, D. S., London, J. M., Lea, M. A. and Durban, J. W. (2008). Continuous-time correlated random walk model for animal telemetry data. Ecology 89, 1208–1215.

Kastelein, R. A., Heul, S. Van Der, Verboom, W. C., Jennings, N., Veen, J. Van Der and de Haan, D. (2008). Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. Mar. Environ. Res. 65, 369–77.

Kawabata, Y., Noda, T., Nakashima, Y., Nanami, A., Sato, T., Takebe, T., Mitamura, H., Arai, N., Yamaguchi, T. and Soyano, K. (2014). Use of a gyroscope/accelerometer data logger to identify alternative feeding behaviours in fish. J. Exp. Biol. 217, 3204–3208.

Kristensen, K., Nielsen, A., Berg, C. W., Skaug, H. and Bell, B. M. (2016). TMB: Automatic Differentiation and Laplace Approximation. J. Stat. Softw. 70,.

Kurlansky, M. (1999). Cod: A Biography Of The Fish That Changed The World. Toronto: A.A. Knopf Canada.

Landrø, M. and Amundsen, L. (2018). Introduction to Exploration Geophysics with Recent Advances. Bivrost.

Langrock, R., King, R., Matthiopoulos, J., Thomas, L., Fortin, D. and Morales, J. M. (2012). Flexible and practical modeling of animal telemetry data: hidden Markov models and extensions. Ecology 93, 2336–2342.

Leeuwen, A. Van, Huss, M., Gårdmark, A., Casini, M., Vitale, F., Hjelm, J., Persson, L. and Roos, A. M. De (2013). Predators with Multiple Ontogenetic Niche Shifts Have Limited Potential for Population Growth and Top-Down. Am. Nat. 182, 53–66.

Lima, S. L. and Dill, L. M. (1989). Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68, 619–640.

Løkkeborg, S. and Soldai, A. V. (1993). The influence of seismic exploration with airguns on cod (*Gadus morhua*) behaviour and catch rates. ICES mar. Sci. Symp 196, 62–67.

Lundquist, D., Gemmell, N. J. and Würsig, B. (2012). Behavioural Responses of Dusky Dolphin Groups (*Lagenorhynchus obscurus*) to Tour Vessels off Kaikoura, New Zealand. PLoS One 7, e41969.

Magnhagen, C., Johansson, K. and Sigray, P. (2017). Effects of motorboat noise on foraging behaviour in Eurasian perch and roach: a field experiment. Mar. Ecol. Prog. Ser. 564, 115–125.

McCauley, R. D., Fewtrell, J., Duncan, A. J., Jenner, C., Jenner, M., Penrose, J. D., Prince, R. I. T., Adhitya, A., Murdoch, J. and McCabe, K. (2000). Marine Seismic Surveys – A Study of Enviromental Implications. APPEA J. 40, 692–708.

McClintock, B. T. and Michelot, T. (2018). momentuHMM: R package for generalized hidden Markov models of animal movement. Methods Ecol. Evol. 9, 1518–1530.

McLaughlin, K. E. and Kunc, H. P. (2015). Changes in the acoustic environment alter the foraging and sheltering behaviour of the cichlid *Amititlania nigrofasciata*. Behav. Processes 116, 75–79.

Metcalfe, J. D., Wright, S., Tudorache, C. and Wilson, R. P. (2016). Recent advances in telemetry for estimating the energy metabolism of wild fishes. J. Fish Biol. 88, 284–297.

Nams, V. O. (2013). Sampling Animal Movement Paths Causes Turn Autocorrelation.

Acta Biotheor. 61, 269–284.

Nedelec, S. L., Simpson, S. D., Morley, E. L., Nedelec, B. and Radford, A. N. (2015). Impacts of regular and random noise on the behaviour, growth and development of larval Atlantic cod (*Gadus morhua*). Proc. R. Soc. B Biol. Sci. 282, 1–7.

Nedelec, S. L., Mills, S. C., Lecchini, D., Nedelec, B., Simpson, S. D. and Radford, A. N. (2016). Repeated exposure to noise increases tolerance in a coral reef fish. Environ. Pollut. 216, 428–436.

Neo, Y. Y., Hubert, J., Bolle, L., Winter, H. V., Ten Cate, C. and Slabbekoorn, H. (2016). Sound exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure. Environ. Pollut. 214, 26–34.

Neo, Y. Y., Hubert, J., Bolle, L. J., Winter, H. V. and Slabbekoorn, H. (2018). European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environ. Pollut. 239, 367–374.

New, L. F., Clark, J. S., Costa, D. P., Fleishman, E., Hindell, M. A., Klanjšček, T., Lusseau, D., Kraus, S., McMahon, C. R., Robinson, P. W., et al. (2014). Using short-term measures of behaviour to estimate long-term fitness of southern elephant seals. Mar. Ecol. Prog. Ser. 496, 99–108.

Payne, N. L., van der Meulen, D. E., Suthers, I. M., Gray, C. A. and Taylor, M. D. (2014). Foraging intensity of wild mulloway *Argyrosomus japonicus* decreases with increasing anthropogenic disturbance. Mar. Biol. 162, 539–546.

Pirotta, E., Booth, C. G., Costa, D. P., Fleishman, E., Kraus, S. D., Lusseau, D., Moretti, D., New, L. F., Schick, R. S., Schwarz, L. K., et al. (2018). Understanding the population consequences of disturbance. Ecol. Evol. 8, 9934–9946.

Pohle, J., Langrock, R., van Beest, F. M. and Schmidt, N. M. (2017). Selecting the Number of States in Hidden Markov Models: Pragmatic Solutions Illustrated Using Animal Movement. J. Agric. Biol. Environ. Stat. 22, 270–293.

Popper, A. N. and Fay, R. R. (2011). Rethinking sound detection by fishes. Hear. Res. 273, 25–36.

Popper, A. N. and Hawkins, A. D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. fish 94, 692–713.

Postlethwaite, C. M. and Dennis, T. E. (2013). Effects of Temporal Resolution on an Inferential Model of Animal Movement. PLoS One 8, 1–11.

Purser, J. and Radford, A. N. (2011). Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). PLoS One 6, e17478.

Rabasa, C. and Dickson, S. L. (2016). Impact of stress on metabolism and energy balance. Curr. Opin. Behav. Sci. 9, 71–77.

Radford, C. A., Montgomery, J. C., Caiger, P. and Higgs, D. M. (2012). Pressure and particle motion detection thresholds in fish: a re-examination of salient auditory cues in teleosts. J. Exp. Biol. 215, 3429–35.

Reichmuth, C., Ghoul, A., Sills, J. M., Rouse, A. and Southall, B. L. (2016). Low-frequency temporary threshold shift not observed in spotted or ringed seals exposed to single air gun impulses. J. Acoust. Soc. Am. 140, 2646–2658.

Reubens, J. T., Braeckman, U., Vanaverbeke, J., Van Colen, C., Degraer, S. and Vincx, M. (2013). Aggregation at windmill artificial reefs: CPUE of Atlantic cod (*Gadus morhua*) and pouting (*Trisopterus luscus*) at different habitats in the Belgian part of the North Sea. Fish. Res. 139, 28–34.

Righton, D. a., Andersen, K. H., Neat, F., Thorsteinsson, V., Steingrund, P., Svedäng, H., Michalsen, K., Hinrichsen, H. H., Bendall, V., Neuenfeldt, S., et al. (2010). Thermal niche of Atlantic cod *Gadus morhua*: Limits, tolerance and optima. Mar. Ecol. Prog. Ser. 420, 1–13.

Rose, G. A. (2019). Atlantic Cod: Bio-Ecology of the Fish. John Wiley and Sons Ltd.

Sand, O. and Karlsen, H. E. (1986). Detection of infrasound by the Atlantic cod. J. Exp. Biol. 125, 197–204.

Sarà, G., Dean, J. M., Amato, D. D., Buscaino, G., Oliveri, A., Genovese, S., Ferro, S., Buffa, G., Martire, M. Lo and Mazzola, S. (2007). Effect of boat noise on the behaviour of bluefin tuna *Thunnus thynnus* in the Mediterranean Sea. Mar. Ecol. Prog. Ser. 331, 243–253.

Sertlek, H. Ö., Slabbekoorn, H., ten Cate, C. and Ainslie, M. A. (2019). Source specific sound mapping: Spatial, temporal and spectral distribution of sound in the Dutch North Sea. Environ. Pollut. 247, 1143–1157.

Shafiei Sabet, S., Neo, Y. Y. and Slabbekoorn, H. (2015). The effect of temporal variation in sound exposure on swimming and foraging behaviour of captive zebrafish. Anim. Behav. 107, 49–60.

Shafiei Sabet, S., Wesdorp, K., Campbell, J., Snelderwaard, P. and Slabbekoorn, H. (2016). Behavioural responses to sound exposure in captivity by two fish species with different hearing ability. Anim. Behav. 116, 1–11.

Sierra-Flores, R., Atack, T., Migaud, H. and Davie, A. (2015). Stress response to anthropogenic noise in Atlantic cod *Gadus morhua* L. Aquac. Eng. 67, 67–76.

Sivle, L. D., Kvadsheim, P. H. and Ainslie, M. A. (2015). Potential for population-level disturbance by active sonar in herring. ICES J. Mar. Sci. 72, 558–567.

Slabbekoorn, H. (2016). Aiming for Progress in Understanding Underwater Noise Impact on Fish: Complementary Need for Indoor and Outdoor Studies. In The Effects of Noise on Aquatic Life II (ed. Popper, N. A.) and Hawkins, A.), pp. 1057–1065. New York, NY: Springer New York.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C. and Popper, A. N. (2010). A noisy spring: The impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427.

Slabbekoorn, H., Dalen, J., de Haan, D., Winter, H. V., Radford, C., Ainslie, M. A., Heaney, K. D., van Kooten, T., Thomas, L. and Harwood, J. (2019). Population-level consequences of seismic surveys on fishes: An interdisciplinary challenge. Fish Fish. 20, 653–685.

Slotte, A., Hansen, K., Dalen, J. and Ona, E. (2004). Acoustic mapping of pelagic fish distribution and abundance in relation to a seismic shooting area off the Norwegian west coast. Fish. Res. 67, 143–150.

Soudijn, F. H., van Kooten, T., Slabbekoorn, H. and de Roos, A. M. (2020). Population-level effects of acoustic disturbance in Atlantic cod: a size-structured analysis based on energy budgets. Proc. R. Soc. B Biol. Sci. 287, 20200490.

Tudorache, C., Viaene, P., Blust, R., Vereecken, H. and De Boeck, G. (2008). A comparison of swimming capacity and energy use in seven European freshwater fish species. Ecol. Freshw. Fish 17, 284–291.

Voellmy, I. K., Purser, J., Flynn, D., Kennedy, P., Simpson, S. D. and Radford, A. N. (2014). Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Anim. Behav. 89, 191–198.

Wardle, C., Carter, T., Urquhart, G., Johnstone, A. D., Ziolkowski, A., Hampson, G. and Mackie, D. (2001). Effects of seismic air guns on marine fish. Cont. Shelf Res. 21, 1005–1027.

Williams, R., Lusseau, D. and Hammond, P. S. (2006). Estimating relative energetic costs of human disturbance to killer whales (*Orcinus orca*). Biol. Conserv. 133, 301–311.

Williams, R., Wright, A. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., Clark, C. W., Cullis-Suzuki, S., Dakin, D. T., Erbe, C., et al. (2015). Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24.

Wright, A. J., Soto, N. A., Baldwin, A. L., Bateson, M., Beale, C. M., Clark, C., Deak, T., Edwards, E. F., Fernández, A., Godinho, A., et al. (2007). Anthropogenic Noise as a Stressor in Animals: A Multidisciplinary Perspective. Int. J. Comp. Psychol. 20, 250–273.

Wright, S., Metcalfe, J., Hetherington, S. and Wilson, R. (2014). Estimating activity-specific energy expenditure in a teleost fish, using accelerometer loggers. Mar. Ecol. Prog. Ser. 496, 19–32.

Wysocki, L. E., Dittami, J. P. and Ladich, F. (2006). Ship noise and cortisol secretion in European freshwater fishes. Biol. Conserv. 128, 501–508.

Exploring effects of sound on the time budget of fishes: An experimental approach with captive cod

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Abstract

Energy intake and expenditure data are needed to estimate population level effects of anthropogenic sound on fish. We present an experimental design of a controlled behavioral experiment that allows to collect relatively long-term data (days) on Atlantic cod (*Gadus morhua*) during sound exposures. Data on the time spent foraging and swimming can be used as proxies for energy intake and expenditure. The wild-caught but captive Atlantic cod exhibited natural foraging behavior in the experimental basins and the design allowed for efficient scoring of the behavior throughout the 6-day trials. We conducted three pilot trials and share the experimental design to encourage other researchers to collect data on (proxies for) energy intake and expenditure to aid estimation of population level effects of sound exposures from noisy human activities such as seismic surveys.

Introduction

There is a growing interest in the effects of anthropogenic sound on marine life, including fish (Williams et al., 2015). While previous studies have found a range of effects on fish, one of the major challenges is to extrapolate results from controlled experiments to the population level (Carroll et al., 2016). For this purpose, population models that rely on Dynamic Energy Budgets (DEBs) can be used (Slabbekoorn et al., 2019). DEBs model the difference between acquired biomass and costs for maintenance, attributing the net energy to growth and reproduction (Leeuwen et al., 2013). To model the population level effects of anthropogenic sound, we need data on the effects of sound on energy intake and expenditure. Few studies have looked into the effects of sound on foraging behavior and most studies only examined short-term effects of a sound exposure, whereas long-term effects of repeated sound exposures are more relevant for models on population consequences.

Several tank studies, examining short-term effects (≤ 25 min) of sound exposure on foraging fishes, have found reduced feeding attempts and/or increased food handling errors (McLaughlin and Kunc, 2015; Purser and Radford, 2011; Shafiei Sabet et al., 2015a; Voellmy et al., 2014). Two studies, which did examine longer-term effects by exposing fish repeatedly (Magnhagen et al., 2017) and observing free-ranging fish (Bracciali et al., 2012), found reduced feeding events during sound exposure. However, these longer-term studies lack information on the energy expenditure during exposure. To our knowledge, only one sound impact study, on mulloways (*Argyrosomus japonicus*), examined both stomach content (food intake) and activity (a proxy for energy expenditure) of free-ranging fish. The authors found less stomach content on days with more boat traffic and activity levels were also lower on days with more boats (Payne et al., 2014).

Here, we present a design for a non-invasive, indoor mesocosm experiment which allows for high resolution quantification of both foraging (energy intake) and swimming behavior (expenditure) in a simulated shallow-water environment with acoustic disturbances at an ecologically realistic time-scale. Through personal observations, we noted that when provided with a sufficiently large basin and natural food items, Atlantic cod (*Gadus morhua*) exhibit discrete bouts of foraging behavior. This provided an opportunity to study the relatively long-term effects of seismic survey sound playback on foraging and swimming behavior of Atlantic cod in a closed system. Due to limited sample size and potential bias in the current study, we do not draw any conclusions from our results. Instead, our experimental design is presented as a promising approach to examine the effects of anthropogenic noise in a manner compatible with the parameterization of population level DEB models.

Materials and methods

Study subjects

We used three pairs of Atlantic cod of 30-50 cm body length in this study. The fish were caught from a charter fishing boat at shipwrecks in the Dutch North Sea on October 5th 2018. After catching, fish were kept in an indoor cylindrical holding tank (Ø 3.5 m, depth 1.1 m) at Stichting Zeeschelp (Kamperland, the Netherlands) for at least one week before being used in the experiment. The light-dark cycle followed the outdoor day-night cycle and the water in the holding tank was continuously filtered, cooled to 15 °C, and refreshed with seawater from the Oosterschelde, a sea inlet of the North Sea. The outlet of the cooling and filtering system caused a current against which the fish were swimming regularly. The bottom of the tanks was covered with sand and patches of live blue mussels (*Mytilus edulis*), Pacific oysters (*Magallana gigas*) and some rocks to provide shelter for the cod and small crabs. The cod were fed with dead European sprat (*Sprattus sprattus*) every other day and could feed ad-libitum on small live Asian shore crabs (*Hemigrapsus sanguineus* & *Hemigrapsus takanoi*) that were abundant in the tank. In the experimental tanks, we provided the cod with live shore crabs (*Carcinus maenas*) with a maximum carapace width of 1.5 cm. All live animals - other than the cod - were collected at the Jacobahaven, an adjacent cove of the Oosterschelde. Crustaceans, including crabs, form a dominant part of the natural diet of cod in the size class that we tested (Daan, 1973).

Experimental tanks

We used two experimental tanks that were identical to the holding tank. To film the behavior of the fish, five GoPro cameras were equally spaced along the tank wall, just below the water surface and pointed downwards at an angle of about 45°. The cameras were connected to a power grid to enable them to film for seven hours continuously. Several screens of white netting were attached along the tank wall to increase the visibility of the fish (Fig. 1a). The basins were covered with black plastic sheets and three TL light bars (36 W/840 lm) provided constant light conditions to ensure consistent video quality and to keep investigators out of sight from the fish (Fig. 1b). We used an amplifier (M033N, Kemo) and underwater speaker (UW30, Lubell), attached in the middle of the tank and middle of the water column, for the experimental sound exposures. We also placed three PVC pipes, vertically along the tank wall, to be able to release crabs into the experimental tank, while minimizing the chance they would be preyed upon already while on their way to the bottom of the basin.

Fig. 1: Pictures of a cylindrical experimental tank. (a) The tank before being filled with water, but with sand on the bottom and patches of mussels, oysters, and some rocks for shelter. Also visible are the frame holding the TL light bars and underwater speaker and the white netting to increase the visibility of the fish and the PVC pipes to release the crabs. (b) The same tank now filled with water and covered with black plastic sheet.

Experimental design

The evening before the start of a trial, a pair of individuals was introduced into an experimental tank. Paired individuals were selected to differ in body length, so they could be easily discriminated on video. Each trial consisted of six days; three consecutive days with two sound exposures of one hour each day, and three days without exposure. Trial 1 started with three days without exposure, followed by three with exposure. Trial 2 and 3 started with three days with sound exposures, followed by three days without. We made daily video recordings from approximately 10:00 to 17:00 CET. The two one-hour sound treatments started at 11:00 and 14:00. During the period without sound playback, we played back a silent track. Each day, just before the start of the video recordings, six small shore crabs were released in the tank as cod prey (Fig. 2). The crabs typically sought
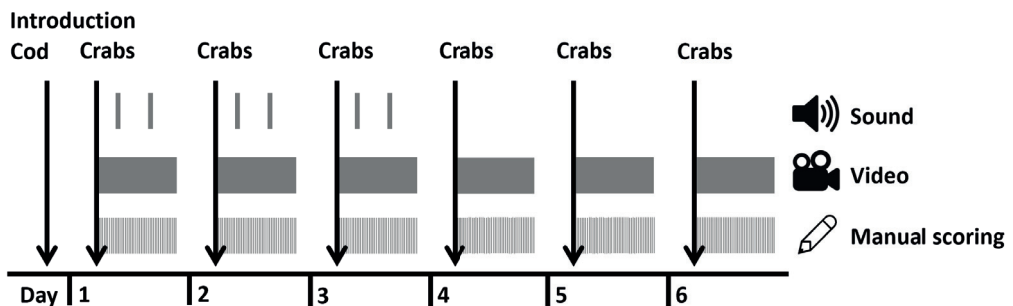

Fig. 2: Schematic of a single six-day trial: introduction events of the two cod and daily groups of six crabs are indicated by arrows, while the presence of grey bars reflect the time in which we played back sound (six traces on top) and in which we made video recordings (middle), which were later used for manual scoring of behavior (bottom).

refuge between or under the mussels and oysters. The cod were observed to scout the patches of mussels and oysters, lifting some shells and, when successful in finding a crab, gulping their target.

Experimental playbacks

We generated the exposure playbacks by randomly selecting airgun shots from recordings from a real seismic survey (Thompson et al., 2013). For each trial, we selected random shots that were recorded at either 10, 15 or 20 km from the survey. The recordings of the shots were band-passed between 150-1000 Hz and normalized with respect to peak amplitude. We determined the sound pressure levels (SPL) of the playback and ambient levels with an HTI-96-min hydrophone. The geometric mean zero-to-peak sound pressure level (SPL_{z-p}) of the shots was 150 dB re 1 μPa in the middle of the water column at 80 cm from the speaker. The geometric mean ambient SPL in the tank was 88 dB re 1 μPa (100 - 1000 Hz) (Fig. 3a).

To characterize spatial variation in the sound field across the basin during sound exposure, we recorded pure tones, that swept from 150 to 1000 Hz. We sampled at 20 cm radial distance steps ranging from 35 cm to 135 cm from the speaker (Fig. 3b). Up to approximately 600 Hz - the upper limit of the cod hearing (Chapman and Hawkins, 1973) - there is a monotonic decrease in power spectral density with increasing distance from the speaker, spanning a maximum of ~25 dB.
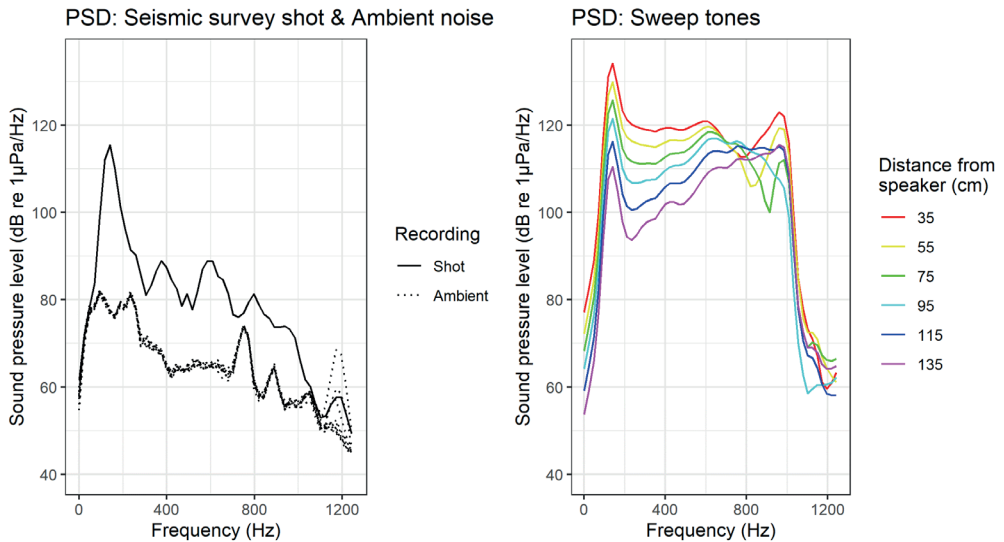

Fig. 3: (left) Power spectral density plot of a seismic airgun shot and ambient recordings in the basin. (right) Power spectral density plot of recordings from a sweep (150 to 1000 Hz) at various distances from the speaker (window length: 2048, window type: Hann).

Data scoring and analysis

We sampled the behavior of both individuals in each pair by scoring the most dominant behavior observed during 20 s video clips. We extracted video clips for observation at 5 min incremental steps throughout the daily seven-hour recording period. The behavioral states were: 'foraging', 'swimming' and 'stationary'. 'Foraging' indicated that an individual was swimming near the bottom, while turning frequently and occasionally lifting rocks or bivalves with its nose. 'Swimming' indicated swimming and turning infrequently, typically around the perimeter of the basin and in the mid or upper part of the water column. 'Stationary' indicated that the fish was not moving while being on the bottom of the tank. A custom-made Python script (utilizing FFmpeg) was used to extract and concatenate synchronized video clips from all cameras into one video (Fig. 4) to facilitate efficient scoring.

To test whether time budgets of cod behavioral states were different on days with two one-hour sound exposures, we determined daily proportions of video clips in which cod were in the foraging, swimming or stationary state. These proportions were fit to Generalized Linear Mixed-effect Models (GLMMs; R-package lme4 by Bates et al., 2015) with a binomial error distribution, 'day number' and 'treatment' as covariates, and 'fish ID' as random effect. We calculated the marginal (R2m) and conditional (R2c) R2 values of the models to show the proportion of variance of the response variable explained by the fixed effects (R2m) and the entire model (R2c) (Nakagawa and Schielzeth, 2013). For each model, a parametric bootstrap procedure (10,000 resamples) was performed, where the random effect intercepts from the fitted model were
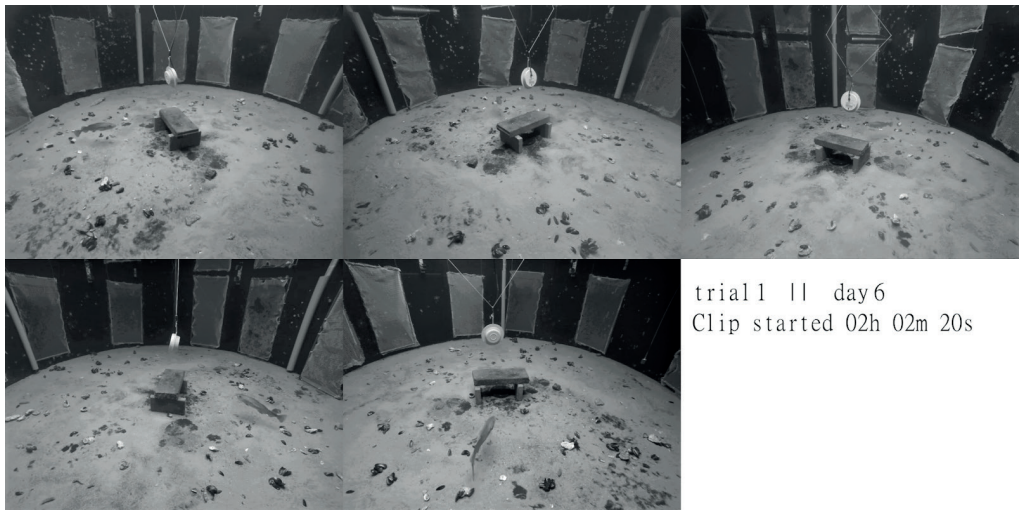

trial 1 || day 6
Clip started 02h 02m 20s

Fig. 4: A screenshot from a 20 s video clip with synchronized footage of all five cameras. The dominant behavior was scored for both individuals by an observer.

not resampled and where we used the fitted estimates instead. The bootstrap was used to determine the 95% confidence interval of the 'treatment' covariate estimate. If this confidence interval did not overlap with 0, we considered the sound exposure to have significantly changed the time expenditure in this behavioral state.

Results

Based on the behavioral scores from the video clips, we determined the daily proportion of time during which an individual was foraging, swimming, or remaining stationary (Fig. 5). Based on daily proportions of time spent in each behavioral state of all six individuals, we found that the subjects did not change the amount of time foraging (Intercept: -3.21, Sound Treatment 95% CI: -0.03 – 0.36, R2m: 0.00, R2c: 0.44). However, the subjects did increase the time swimming (Intercept: -1.07, Sound Treatment 95% CI: 0.06 – 0.40, R2m: 0.01, R2c: 0.33) and decreased the time being stationary (Intercept: 1.37, Sound Treatment 95% CI: -1.64 – -0.92, R2m: 0.07, R2c: 0.66).

Discussion

The current experimental design allowed us to expose Atlantic cod to repeated playback of seismic survey recordings over multiple days in a simulated shallow-
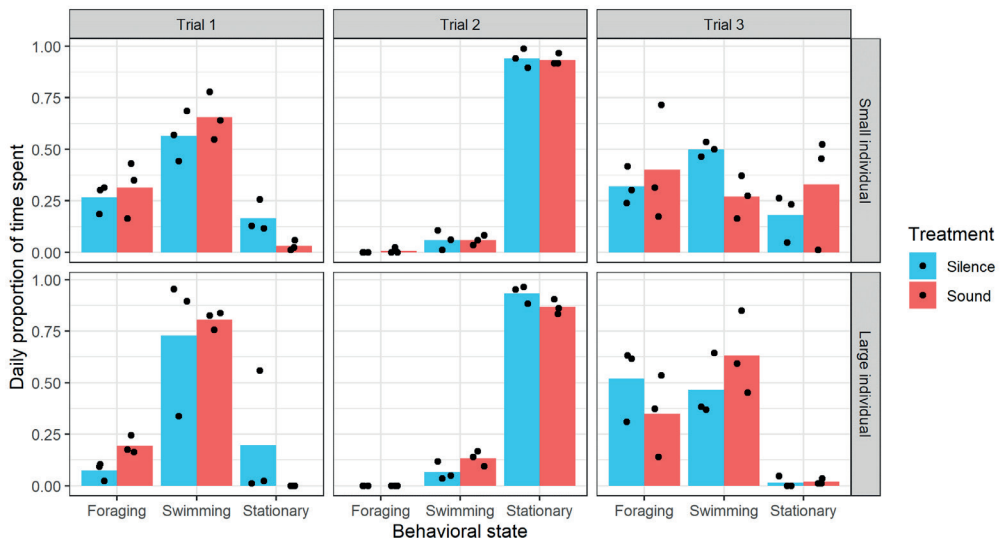
Fig. 5: Distribution of behavioral states as daily proportion of time spent Foraging, Swimming, and remaining Stationary. The black points indicate the proportions of individual days and the bars indicate the mean proportion over three sequential days during Silence playback or Sound exposure. The three facet columns depict the three trials, and the facet rows show the data for the smaller (above) and larger individual (below) of this trial.

water habitat. By scoring short video clips throughout six days, we were able to quantify time expenditure in the discrete behavioral states: foraging, swimming, and remaining stationary during days with sound exposures and days with silent controls. The current experiment was ceased after three trials due to concerns about the health of the study subjects, as their livers were discolored. This discoloration did likely not emerge during their short time in captivity as the discoloration was also reported in other non-experimental individuals that were caught and killed on the same day. Nevertheless, the fish in trial 1 and 3 regularly scouted the patches of mussels, oysters, and rocks; they sometimes lifted or pushed the patches with their nose; aiming to catch crabs that were hiding. Paired individuals were also observed to synchronize their foraging behavior. When one individual initiated foraging behavior, the other often immediately joined and they examined the same crab refuge patches together. These foraging patterns, including the social context, are in line with reports on free-ranging Atlantic cod (Rose, 2019).

The current study subjects, in the conditions of our experimental set-up, did increase the time swimming and decreased the time remaining stationary on days with sound exposures, but did not change the time spent foraging. Due to the limited sample size and the possibly biased sample because of the discolored livers, we do not make inferences to a healthy wild population from our results. However, we argue that this type of data, with adequate sample size and certainty about individual health status, can serve as proxies for energy intake and expenditure and could thereby be useful to parametrize DEB models (Slabbekoorn et al., 2019; Soudijn et al., 2020). In contrast to earlier studies (e.g. McLaughlin and Kunc, 2015; Shafiei Sabet et al., 2015b), we here examined both foraging and swimming activity at a relatively large time scale of days, which may be more relevant than minutes or an hour, as input to energy budget models that aim to investigate population effects (Soudijn et al., 2020).

It remains important to stress that fish behavior in captivity, and sound propagation in tanks, is different from outdoor conditions (Calisi and Bentley, 2009; Rogers et al., 2016). So, absolute response levels of fish to sound from captive and/or indoor experiments likely do not represent absolute response levels in the field (and also in the field, response levels will vary with life phase, season, experience, and weather, and with local water depth and propagation conditions). Ultimately, all experiments on the effects of sound are more realistic when conducted in situ, however, outdoor experiments on free-ranging animals are challenging and some research questions are currently impossible to address due to technical limitations (e.g. high-resolution telemetry to assess data on behavioral states at a resolution comparable to video observations). For

this reason, optimal progress in studies on response levels is likely achieved by complementary indoor and in situ observations and experiments.

Sound propagation in the experimental tanks is expected to differ from propagation in outdoor settings, largely due to the close proximity of tank walls, water surface and tank floor. Especially particle motion levels and directions are expected to differ from natural habitat (Rogers et al., 2016). In the current experimental tanks, we found a monotonic decrease in sound pressure levels with increasing distance from the speaker that was relatively consistent across frequencies up to 600 Hz. At ~750 Hz and above, complex acoustic artefacts were observed: The PSD was no longer monotonically decreasing with distance from the speaker at certain frequencies, indicative of a standing wave marking the shallow-water cut-off frequency (Akamatsu et al., 2002). In the current study particle motion measurements were unavailable. However, with detailed pressure measurements, it is feasible to make rough estimates of particle velocity in a geometrically simple basin setup or estimate particle motion by measuring pressure gradients with hydrophone pairs (Gray et al., 2016).

For the current dataset, we scored the behavioral state in short video segments, equally spaced throughout the daily seven-hour video recordings. This proved to be a quick and efficient method. For more detailed behavior measurements, the current camera set-up could facilitate the processing of videos with 3d tracking. Automated tracking would provide high resolution data on swimming behavior (energy expenditure) without requiring subsampling of videos. Additionally, swim tracks are suitable input for Hidden Markov Models (HMMs) for animal movement, allowing for automated quantification of behavioral states. While HMMs have been used before to study the behavior of large mammals over time and space at large scales by statistical ecologists (Langrock et al., 2012), recent advancements in the accessibility of these models to non-statisticians facilitate their use in broader applications (McClintock and Michelot, 2018). HMMs expect multivariate and temporally auto-correlated input and provide direct estimates of an animals' latent behavioral state, making them suitable for the analysis of swimming tracks. In addition, a fitted HMM can act as a foundation for simulating animal responses in the presence/absence of acoustic stressors, allowing one to extrapolate a fitted HMM to population level consequences (DeRuiter et al., 2017).

Conclusions

We here reported on an experimental design, that allowed us to collect data on foraging and swimming behavior of a demersal fish species during scaled

seismic survey sound exposures and a silent control in captive conditions. The current design allowed for efficient scoring of the behavioral states throughout the daily seven-hour video recordings. We found a shift in time spent swimming and remaining stationary, but not in the time spent foraging. However, the sample size was limited and possibly biased, so more data is required to confirm these results. These behavioral states from wild-caught but captive individuals can serve as proxies for energy intake and expenditure, and have high potential as complementary data to field studies on the same species. The proxies for energy intake (time spent foraging) and expenditure (time spent swimming and stationary) can be further specified by investigating catch success rates and prey energy content and swimming speed correlates to oxygen consumption from swim tunnel experiments. We believe these are feasible steps towards parametrizing Dynamic Energy Budget models which will allow estimates and predictions on population levels effects of acoustic disturbances such as during seismic surveys.

Ethical statement

This experiment was conducted in accordance with the Dutch Experiments on Animals Act and approved by the Dutch Central Commission Animal Experiments (CCD) under no. AVD1060020171085.

Data accessibility

All data used for the analyses reported in this article and some example 20 s video clips are available from the Zenodo Repository, DOI:10.5281/zenodo.3595208.
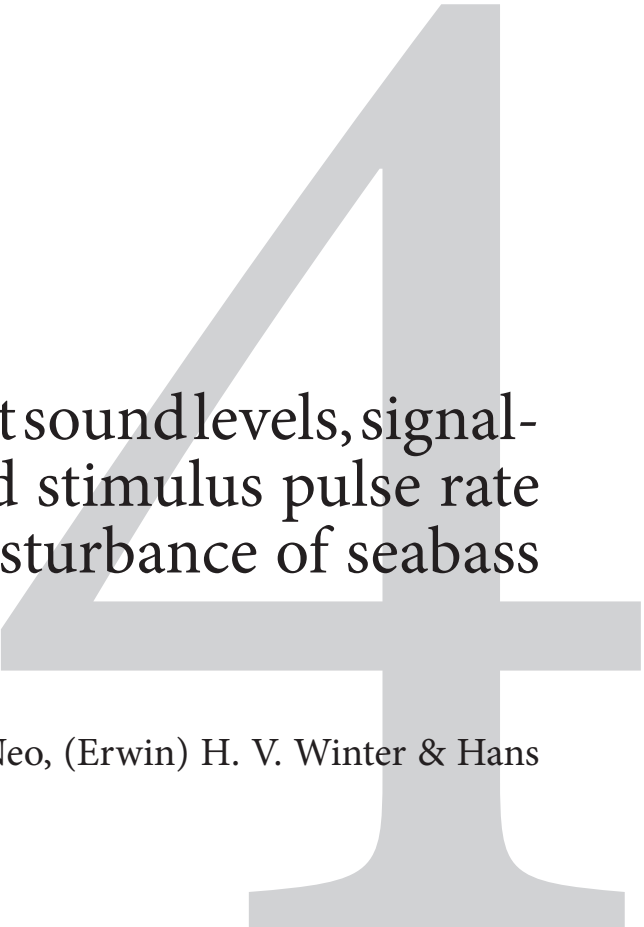
Acknowledgements

References

Akamatsu, T., Okumura, T., Novarini, N. and Yan, H. Y. (2002). Empirical refinements applicable to the recording of fish sounds in small tanks. J. Acoust. Soc. Am. 112, 3073.

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67, 1–48.

Bracciali, C., Campobello, D., Giacoma, C. and Sarà, G. (2012). Effects of nautical traffic and noise on foraging patterns of mediterranean Damselfish (*Chromis chromis*). PLoS One 7,.

Calisi, R. M. and Bentley, G. E. (2009). Lab and field experiments: are they the same animal? Horm. Behav. 56, 1–10.

Carroll, A. G., Przeslawski, R., Duncan, A., Gunning, M. and Bruce, B. (2016). A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pollut. Bull. 114, 9–24.

Chapman, C. J. and Hawkins, A. D. (1973). A field study of hearing in the cod, *Gadus morhua* L. J. Comp. Physiol. 85, 147–167.

Daan, N. (1973). A quantitative analysis of the food intake of North Sea cod, *Gadus Morhua*. Netherlands J. Sea Res. 6, 479–517.

DeRuiter, S. L., Langrock, R., Skirbutas, T., Goldbogen, J. A., Calambokidis, J., Friedlaender, A. S. and Southall, B. L. (2017). A multivariate mixed hidden Markov model for blue whale behaviour and responses to sound exposure. Ann. Appl. Stat. 11, 362–392.

Gray, M., Rogers, P. H. and Zeddies, D. G. (2016). Acoustic particle motion measurement for bioacousticians : principles and pitfalls. Proc. Meet. Acoust. 27,.

Langrock, R., King, R., Matthiopoulos, J., Thomas, L., Fortin, D. and Morales, J. M. (2012). Flexible and practical modeling of animal telemetry data: hidden Markov models and extensions. Ecology 93, 2336–2342.

Leeuwen, A. Van, Huss, M., Gårdmark, A., Casini, M., Vitale, F., Hjelm, J., Persson, L. and Roos, A. M. De (2013). Predators with Multiple Ontogenetic Niche Shifts Have Limited Potential for Population Growth and Top-Down. Am. Nat. 182, 53–66.

Magnhagen, C., Johansson, K. and Sigray, P. (2017). Effects of motorboat noise on foraging behaviour in Eurasian perch and roach: a field experiment. Mar. Ecol. Prog. Ser. 564, 115–125.

McClintock, B. T. and Michelot, T. (2018). momentuHMM: R package for generalized hidden Markov models of animal movement. Methods Ecol. Evol. 9, 1518–1530.

McLaughlin, K. E. and Kunc, H. P. (2015). Changes in the acoustic environment alter the foraging and sheltering behaviour of the cichlid *Amititlania nigrofasciata*. Behav. Processes 116, 75–79.

Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142.

Payne, N. L., van der Meulen, D. E., Suthers, I. M., Gray, C. A. and Taylor, M. D. (2014). Foraging intensity of wild mulloway *Argyrosomus japonicus* decreases with increasing anthropogenic disturbance. Mar. Biol. 162, 539–546.

Purser, J. and Radford, A. N. (2011). Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). PLoS One 6, e17478.

Rogers, P. H., Hawkins, A. D., Popper, A. N., Fay, R. R. and Gray, M. D. (2016). Parvulescu Revisited: Small Tank Acoustics for Bioacousticians. In The Effects of Noise on Aquatic Life II, pp. 933–941.

Rose, G. A. (2019). Atlantic Cod: Bio-Ecology of the Fish. John Wiley and Sons Ltd.

Shafiei Sabet, S., Neo, Y. Y. and Slabbekoorn, H. (2015a). The effect of temporal variation in sound exposure on swimming and foraging behaviour of captive zebrafish. Anim. Behav. 107, 49–60.

Shafiei Sabet, S., Neo, Y. Y. and Slabbekoorn, H. (2015b). The effect of temporal variation in experimental noise exposure on swimming and foraging behaviour of captive zebrafish. Anim. Behav. 49–60.

Slabbekoorn, H., Dalen, J., de Haan, D., Winter, H. V., Radford, C., Ainslie, M. A., Heaney, K. D., van Kooten, T., Thomas, L. and Harwood, J. (2019). Population-level consequences of seismic surveys on fishes: An interdisciplinary challenge. Fish Fish. 20, 653–685.

Soudijn, F. H., van Kooten, T., Slabbekoorn, H. and de Roos, A. M. (2020). Population-level effects of acoustic disturbance in Atlantic cod: a size-structured analysis based on energy budgets. Proc. R. Soc. B Biol. Sci. 287, 20200490.

Thompson, P. M., Brookes, K. L., Graham, I. M., Barton, T. R., Needham, K., Bradbury, G. and Merchant, N. D. (2013). Short-term disturbance by a commercial two-dimensional seismic survey does not lead to long-term displacement of harbour porpoises. Proc. R. Soc. B Biol. Sci. 280, 1–8.

Voellmy, I. K., Purser, J., Flynn, D., Kennedy, P., Simpson, S. D. and Radford, A. N. (2014). Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Anim. Behav. 89, 191–198.

Williams, R., Wright, A. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., Clark, C. W., Cullis-Suzuki, S., Dakin, D. T., Erbe, C., et al. (2015). Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24.

The role of ambient sound levels, signal-to-noise ratio, and stimulus pulse rate on behavioural disturbance of seabass in a net pen

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Abstract

Anthropogenic sources increasingly contribute to the underwater soundscape and this may negatively impact aquatic life, including fish. Anthropogenic sound may mask relevant sound, alter behaviour, physiology, and may lead to physical injury. Behavioural effect studies are often seen as critical to evaluate individual and population-level impact. However, behavioural responsiveness likely depends on context and characteristics of sound stimuli. We pose that ambient sound levels, signal-to-noise ratio (SNR), and pulse rate interval (PRI), could affect the behavioural response of fish. To study this, we experimentally exposed groups of tagged European seabass (*Dicentrarchus labrax*) to different impulsive sound treatments that varied in pulse level, elevated background level, SNR, and PRI. Upon sound exposure, the seabass increased their swimming depth. The variation in the increase in swimming depth could not be attributed to pulse level, background level, SNR or PRI. It may be that the current range of sound levels or PRIs was too narrow to find such effects.

Introduction

The aquatic world is filled with a large variety of sounds, which may affect aquatic animals. A lot of these sounds originate from natural sources like water movements and animal activities (Hildebrand, 2009). However, over the last century, anthropogenic sounds have become much more prominent in this cacophony of underwater sounds (Andrew et al., 2002; Slabbekoorn et al., 2010). Anthropogenic sound sources include shipping, offshore constructions, sonar exploration, seismic surveys and underwater explosions. The increasing numbers and source levels of these sound sources have led to an increased interest in the impact of anthropogenic sound on fish and other aquatic animals (Carroll et al., 2016; Williams et al., 2015). Anthropogenic sound can potentially cause physical injury, increase hearing thresholds, mask relevant sounds and change physiology and behaviour in fish (Popper and Hastings, 2009; Slabbekoorn et al., 2010). Insights into the impact at individual and population level likely depend on revealing and understanding behavioural effects. This is, however, a complex task as there are many environmental factors and anthropogenic sound characteristics that may modulate their impacts on animal behaviour (Bejder et al., 2009; Slabbekoorn, 2016).

An environmental factor that may be especially relevant for assessing sound impact is the ambient noise level (Ellison et al., 2012). At sea, ambient noise mainly originates from water surface roughness as a function of weather conditions and boat noise (Carey and Browning, 1988; Wenz, 1962). The boat noise mentioned here does not refer to a single nearby ship whose sound can easily be discriminated from background noise, but to chronic omnipresent low-frequency noise produced by ships (Wright et al., 2007). This means that exposed waterbodies around busy shipping routes have relatively high ambient sound levels (Haver et al., 2018; Sertlek et al., 2016), whereas areas that are away from boat traffic and sheltered from wind and waves have relatively low ambient levels (e.g. Merchant et al., 2016).

Elevated ambient noise levels have been shown to negatively affect auditory detection and recognition thresholds due to masking in a variety of species, such as humans (Beattie et al., 1994), birds (Noirot et al., 2011), and fishes (Hawkins and Chapman, 1975; Ladich and Schulz-Mirbach, 2013; Wysocki and Ladich, 2005). However, masking can also be utilized for positive effects. In humans, masking effects have been exploited in open-plan offices to reduce intelligibility of background speech by playing sound (Hongisto et al., 2017; Schlittmeier et al., 2008). Similarly, fountain sound has been shown to reduce perceived loudness of traffic noise (Coensel et al., 2011; Nilsson et al., 2010). For non-human animals,

it has also been suggested to elevate ambient noise levels in zoo exhibits in order to decrease the signal-to-noise ratio (SNR) of potentially disturbing sounds from visitors (Wells, 2009). Similarly, a harbour porpoise (*Phocoena phocoena*) has been shown to reduce its behavioural response to a sound signal when ambient levels were artificially increased (Kastelein et al., 2011). While SNR may affect behavioural responsiveness of animals to sound exposures, it has not been systematically studied as a factor in underwater sound impact assessments.

Not only the environment, but also the anthropogenic sounds themselves vary in several acoustic characteristics that are likely to affect the behavioural responsiveness of fish. Although this is often neglected in sound impact assessments and legislation, fish are known to be sensitive to temporal patterns in sound exposures (Neo et al., 2014). Fish detect high temporal resolution in sound pulses which could mediate species and individual recognition (Marvit and Crawford, 2000; Wysocki and Ladich, 2002). Anthropogenic sounds vary considerably in temporal pattern; ship traffic and wind farms produce relatively continuous sound, whereas pile driving and seismic surveys produce impulsive sound. The pulse rate interval (PRI) is usually 1 to 4 s for pile driving (Hall, 2013; Matuschek and Betke, 2009) and 5 to 15 s for seismic surveys (McCauley et al., 2000). Such variation in temporal patterns in sounds has been shown to influence behavioural responses in fish (Neo et al., 2014; Neo et al., 2015a).

Most studies on temporal patterns have been conducted using European seabass (*Dicentrarchus labrax*). Seabass is a demersal fish that commonly inhabits shallow waters, where juveniles form schools and adults may shoal loosely with fewer individuals (Frimodt and Dore, 1995). They hear best up to 1000 Hz and are known to show behavioural and physiological responses to sound exposures (Bruintjes et al., 2016; Kastelein et al., 2008; Lovell, 2003). They increased their swimming depth (swam deeper) during both continuous and impulsive sound exposures, but during the latter, the seabass took twice as long to return to their baseline swimming depth (Neo et al., 2014). When comparing the effects of different PRIs, Neo et al. (2015a) found that smaller PRIs - faster pulse rates - increased group cohesion, and that PRI affected post-exposure swimming depth. These findings, together with a later outdoor study using the same species (Neo et al., 2016), suggest that sound pressure level (SPL) and cumulative sound exposure level (SEL_{cum}) alone are not sufficient in scaling acoustic conditions for assessing behavioural impact.

In the current study, we exposed hatchery-reared European seabass in a net pen to impulsive sound treatments and examined their behavioural changes. We aimed at gaining more insight into the limitations of SPL and SEL_{cum} as measures for

behavioural thresholds by exploring variation in responsiveness depending on ambient sound levels, signal-to-noise ratios, and stimulus pulse rate. We created artificial sound treatments that varied in pulse level and background noise level, causing variation in signal-to-noise ratio (SNR), and pulse rate interval (PRI). We assessed experimental sound exposure levels in both sound pressure and sound velocity levels. By comparing the behavioural response among the different sound treatments, we aimed to determine if SNR and PRI modulate behavioural responses.

Materials and methods

Study subjects

We used 16 groups of four European seabass (*Dicentrarchus labrax*) of 35 to 40 cm in body length. The fish were acquired from a hatchery (FRESH Völklingen GmbH, Germany) and kept in two indoor holding tanks (Ø 3.5 m, depth 1.1 m) at Stichting Zeeschelp (The Netherlands) in a light-dark cycle following the outdoor day-night cycle. The water in the holding tanks was continuously refreshed with seawater from the Oosterschelde, a sea inlet of the North Sea. The fish were fed commercial pellets (Aller Blue Organic EX 8 mm, AllerAqua, Denmark), whose amount was determined by the water temperature according to the description of the manufacturer.

Experimental arena

The experiment was conducted using a study island in the Jacobahaven, a man-made cove in the Oosterschelde (Fig. 1). The Jacobahaven is about 200 m wide, 300 m long and depending on the tides 2-5 m deep. The Jacobahaven is situated near the Oosterscheldekering and no external boat traffic is allowed in its proximity. We used a modular floating system (Candock, Canada) to assemble the study island. It consisted of a working platform for the equipment and researchers, and an octagonal walkway that supported a net pen as experimental arena (Ø 11.5-12.5 m, > 3 m deep). The two parts were separated by a 0.5 m distance aiming to reduce direct sound transmission from the working platform to the net pen. One end of the working platform held the underwater speaker at a distance of 7.8 m from the net to avoid unwanted near-field effects of the speaker. The set-up has been used for previous sound exposure studies and detailed measurements of the underwater soundscape revealed gradually decreasing sound levels with increasing distance from the speaker (Hubert et al., 2016; Neo et al., 2016; Neo et al., 2018). The study island was located in the middle of the Jacobahaven and anchored with dead weights in combination with chains and stretchable bungee ropes that kept the island in place throughout the tides.
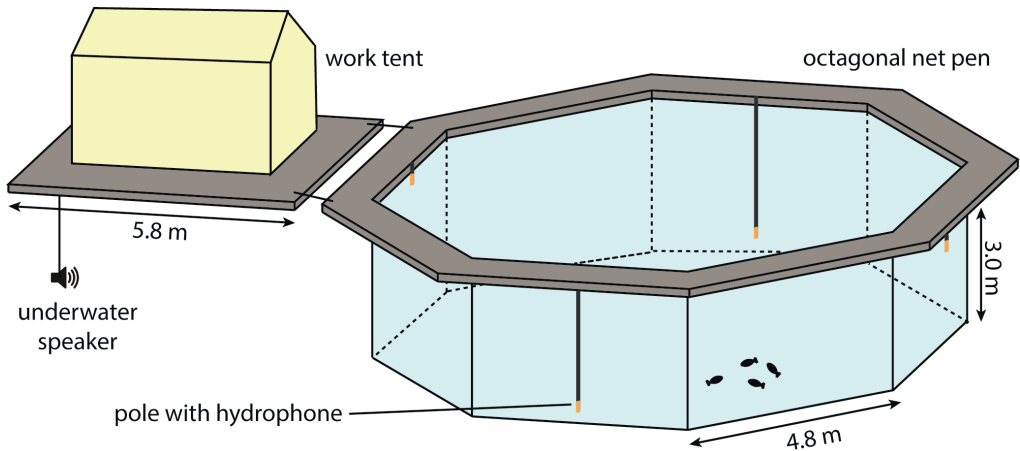
Fig. 1: A schematic overview of the net pen and research platform (figure from Neo et al., 2016). The square working platform was connected to the octagonal walkway used ropes, leaving a gap of approximately 0.5 m. The far end of the working platform held the underwater speaker at a depth of 2.2 m and a distance of 7.8 meter to the net. The four hydrophones recorded the acoustic signals from the tags. The distance between adjacent hydrophones was 8.7 m.

Tagging fish

We tracked the swimming patterns of the four fish in the net pen using acoustic tags (Model 795-LG, HTI, US) that emitted 0.5 ms 307 kHz signals at a fixed ~ 1 s interval. Fish could be identified and tracked individually because of small differences in the programmed signal interval of the different tags. At the net pen, the acoustic signals of the tags were received by four hydrophones (Model 590-series, HTI, US); two close to the surface and two close to the bottom. The received signals were logged on a laptop via a tag receiver (Model 291, HTI, US).

Before tagging, each fish was anaesthetised in a bath with 2-phenoxyethanol (0.5 ml/l seawater). Once anaesthetised, the fish was placed on its back in a v-shaped cradle to keep the fish's abdominal wall above water and its head submerged in seawater with half the amount of 2-phenoxyethanol (0.25 ml/l) to maintain anaesthesia. We then made an 1.5-cm incision in the abdominal wall, implanted a tag (volume: 1.4 cm3; in air weight: 4.6 g) in the intraperitoneal cavity and sutured the opening. After the tagging, the fish could recover in a rectangular tank (1.20 x 1.00 x 0.65 m) with continuously refreshed seawater for at least two days (> 40 h).

Treatment series

Using Audacity 2.0.5, we generated 24 different sound treatments, all consisting of 0.1 s pulses and elevated background noise. We used impulsive sound because

this elicited the strongest behavioural response in a previous study on European seabass (Neo et al., 2014) and continuous sound as elevated background noise because continuous sound has the most potential to mask other sound. All sound treatments followed the same structure and only differed in sound level of the pulses, sound level of the elevated background noise, and pulse rate intervals (PRI) (Fig. 2). To make the pulses, we generated a track of brown noise and applied a high-pass filter of 200 Hz and a low-pass filter of 1000 Hz. This frequency range was selected because it was not possible to playback sound below 200 Hz due to speaker limitations and physical limitations for propagation of low frequencies in shallow water and because seabass hear best up to 1000 Hz. The actual pulses were created by making silences in the track to obtain a fixed PRI of 0.5, 1.0, 2.0 or 4.0 s and we created tracks of three different sound levels for all PRIs. For the elevated background noise, we generated another track of brown noise with a fade-in of 5 minutes to smoothen the transition from the natural ambient noise to the elevated background noise. We created two different sound levels of this
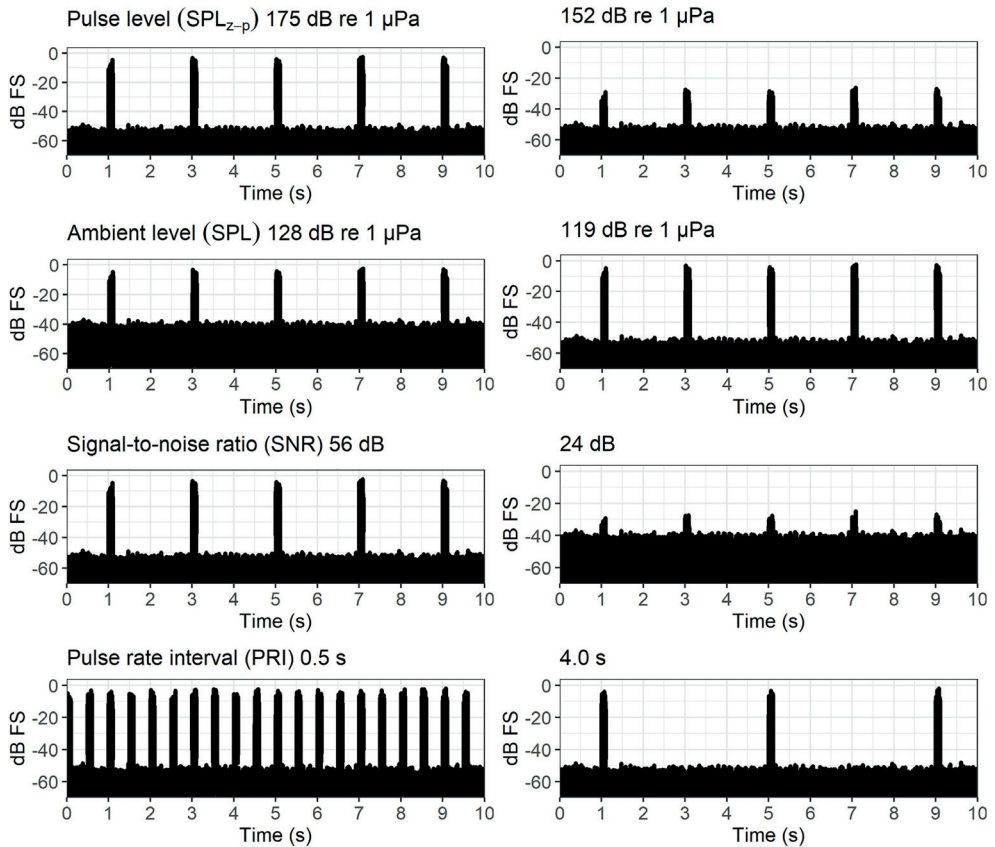

Fig. 2: Waveform in decibels relative to full scale (dB FS) to display the maximum variation in pulse level, ambient level, SNR, and PRI across the 24 sound treatments.

track. The elevated background noise started 20 minutes before the first pulse and ended 5 minutes after the last pulse. The impulsive sound series took 30 minutes, so the total playback lasted for 55 minutes. Altogether this resulted in 24 different sound treatments (four PRIs, three pulse levels and two background levels). The sounds were played back with an underwater transducer (LL-1424HP, Lubell Labs, US) using a laptop, a power amplifier (DIGIT 3K6, SynQ, Belgium) and a transformer (AC1424HP, Lubell Labs, US).

To examine the actual sound levels in the net pen, we measured sound pressure levels (SPL) and sound velocity levels (SVL) twice during flow, high and ebb tide and with both elevated background levels at 2 m deep at six distances from the speaker (every 2.1 m, from 8.3 to 18.8 m from the speaker). The measurements were conducted using the M20 particle velocity sensor (GeoSpectrum Technologies, Canada), which measures sound pressure using an omnidirectional hydrophone and 3D particle velocity using three orthogonal accelerometers. Calibration of the sensor was provided by the manufacturer. The signals were stored on a laptop at 40 kHz via a current-to-voltage convertor box (GeoSpectrum Technologies, Canada) and a differential oscilloscope (PicoScope 3425, Pico Technologies,
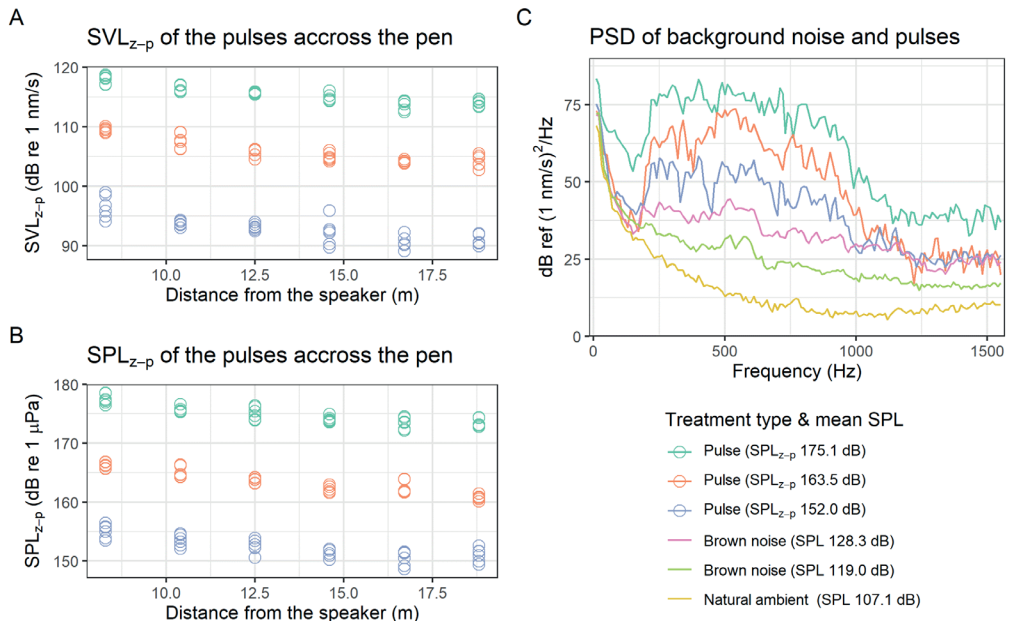
Fig. 3: (A) Zero to peak sound velocity levels (SVL) and (B) sound pressure levels (SPL) of the impulsive sound, across the net pen. This shows the variation of sound levels the fish have been exposed to. (C) Power spectral density (PSD) plots of the sound velocity of the elevated background noise and the pulses, both measured at 12.5 m from the speaker in the middle of the water column. The mean sound pressure levels are depicted in the legends.

UK). The recordings were later processed with Matlab application paPAM (c.f. Nedelec et al., 2016) using a 200-1000 Hz bandpass filter. Across measurement locations and replicate recordings, the mean zero to peak sound pressure levels (SPL_{z-p}) of the pulses in the 200-1000 Hz bandwidth range of the different playback levels were 175.1, 163.5 or 152.0 dB re 1 μPa (resp. 115.6, 106.3 or 93.3 dB re 1 nm/s; Fig. 3). The mean rms SPLs of the elevated background noise were 128.3 or 119.0 dB re 1 μPa (resp. 69.6 or 61.1 dB re 1 nm/s).

Experimental design

We exposed each of the 16 groups of four fish to six of the 24 sound treatments. The order of the treatments followed a counterbalanced design; each group was exposed to all PRIs at least once, all pulse sound levels twice and both background levels thrice. Each group of fish was tagged at least two days (> 40 h) before being transferred to the net pen (Fig. 4). The fish could acclimatize overnight, for at least 8 h. Each group was exposed to three sound treatments per day, for two days. We conducted one trial at flood tide (starting 2:45 h before

Fig. 4: Timetable for the five-day experimental processing of a single group of four fish (top panel). Tagging was done on day 1; moving to the net pen on day 3; and the six half-hour sound exposure trials took place on day 4 and 5. We conducted three trials per day, before, during and after absolute high tide (bottom panel).

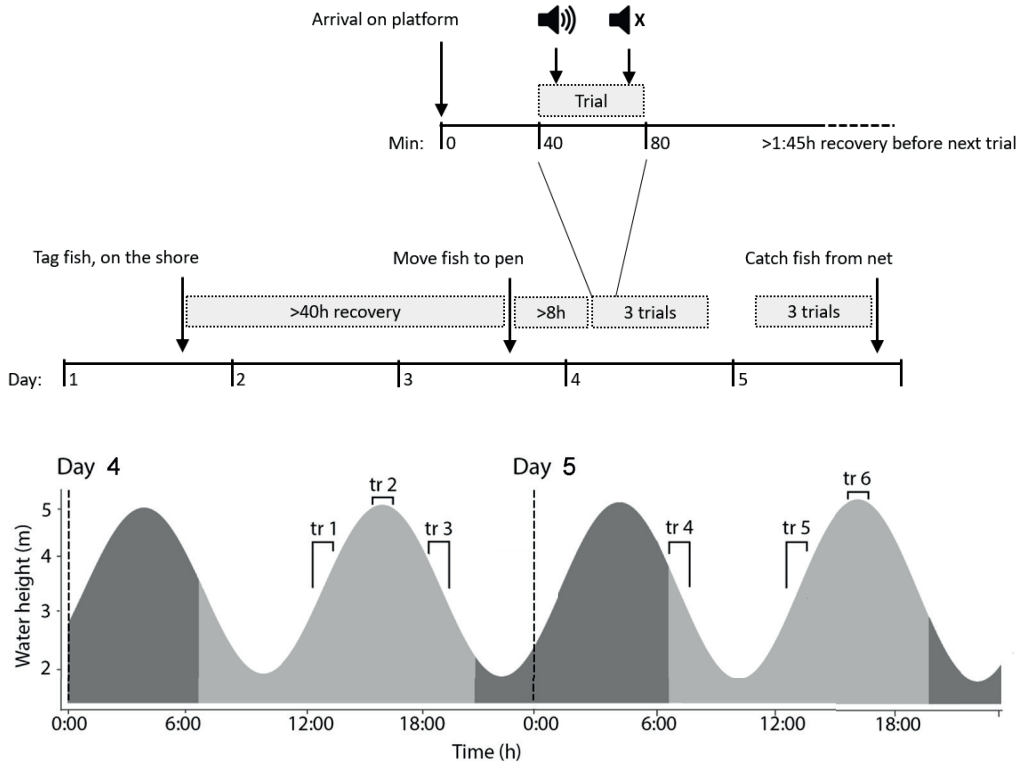absolute high tide), one at high tide (starting 0:20 h before absolute high tide) and one at ebb tide (ending 2:45 h after absolute high tide). This schedule was chosen to ensure that the water level in the Jacobahaven was deep enough (>3 m) during the trials to maintain a constant difference in depth between the tag receiving hydrophones and to allow most of the energy of the sound treatment to propagate (cut-off frequency <250 Hz).

The researchers arrived at the platform about 25 min before the start of the playback of the sound treatment. Upon arrival, all equipment was switched on. 15 minutes after the start of the sound treatment, the software to track the positions of the fish was started and ran till the end of the sound treatment. In this way, we tracked the fish 5 minutes before the start of the impulsive sound, during the 30 minutes of impulsive sound, and 5 minutes after the impulsive sound. After each group was exposed to six treatments over two days, the fish were caught and a new group was released into the pen.

Statistics

The received tag signals were processed on a computer using MarkTags v6.1 & AcousticTag v6.0 (HTI, US), generating the x-, y-, z-coordinates of the 3D swimming patterns of all fish. These coordinates were used to calculate swimming depth, distance from the speaker, swimming speed and average inter-individual distance (group cohesion). To test for behavioural responses to the impulsive sound, we used 5-minute-bin-group-averages of these parameters from before the impulsive sound ('before'), after the start of the impulsive sound ('during') (cf. Neo et al., 2014). To capture the transient speed change, we used 10-s-bin-averages for the parameter swimming speed. These four parameters were used as response variables in four Linear Mixed-effect models with Period ('before' or 'during') as a fixed effect and Group ID as a random effect. We compared these models with their corresponding null models using Akaike information criterion for small sample sizes (AICc). If the best model contained Period, we performed a parametric bootstrap procedure (10,000 resamples) where the random effect intercepts from the fitted model were not resampled and instead the fitted estimates were used. The bootstrap was used to determine the 95% confidence interval of the covariate estimate. If this confidence interval did not overlap with 0, we considered the sound exposure to have significantly changed this behavioural parameter.

The behavioural parameters that significantly changed during the sound exposure, were used to explore the effects of our acoustical parameters of interest: PRI, SPL_{z-p} of the pulses, rms SPL of the elevated background noise and the ratio between the latter two (signal-to-noise ratio; SNR), which was calculated as

follows (formula I):

$$\text{SNR}_{\text{dB}} = P_{\text{signal,dB (z-p)}} - P_{\text{noise,dB (rms)}} \qquad \text{(I)}$$

We used these acoustical parameters, together with other factors that may affect the change in swimming depth (tide, trial order, depth before the start of the sound), as fixed effects in a Linear Mixed-effect model to explain the variation in the change in a behavioural parameter. The best model was chosen by AICc using dredge model selection (package MuMIn) and a bootstrap procedure was used to determine significance of the covariates from the best model.

For all Linear Mixed-effect models, we calculated the marginal (R^2m) and conditional (R^2c) R^2 values to show the proportion of variance in the response variable that is explained by the fixed effects (R^2m) and the entire model (R^2c) (Nakagawa and Schielzeth, 2013). All statistics were done using RStudio (R Core Team, 2016) and the packages lme4 (Bates et al., 2015), MuMIn (Barton, 2016) and piecewiseSEM (Lefcheck, 2016).

Results

We first examined the overall effects of the sound exposures on the swimming patterns of the groups of seabass. The fish significantly increased their swimming depth (swam deeper) upon sound exposure according to model selection (Table 1b; Intercept: 2.28, During: -0.21; Fig. 5B) and bootstrap procedure (95% CI During: -0.34 to -0.08). Model selection of the other behavioural parameters did not show any change related to the sound exposure (Table 1a,c,d; Fig. 5A,C,D). Secondly, we examined the parameters that could explain the variation in the change in swimming depth. Model selection showed that this variation was best explained by the depth before the start of the impulsive sound (df = 4, R2m = 0.13, R2c = 0.44, Table 1e). Running this model showed that fish that were higher in the water column before the start of the sound, showed a larger increase in depth (Intercept: 0.29, slope Depth before: -0.22; Fig. 6E), the bootstrap procedure showed that this was a significant correlation (95% CI slope Depth before: -0.31 to -0.09). The model selection also showed that none of the treatment manipulations appeared to have an effect on the change in swimming depth (Fig. 6).

Discussion

In the current study, we experimentally exposed European seabass in a net pen to impulsive sound treatments, while varying the pulse rate intervals (PRI),
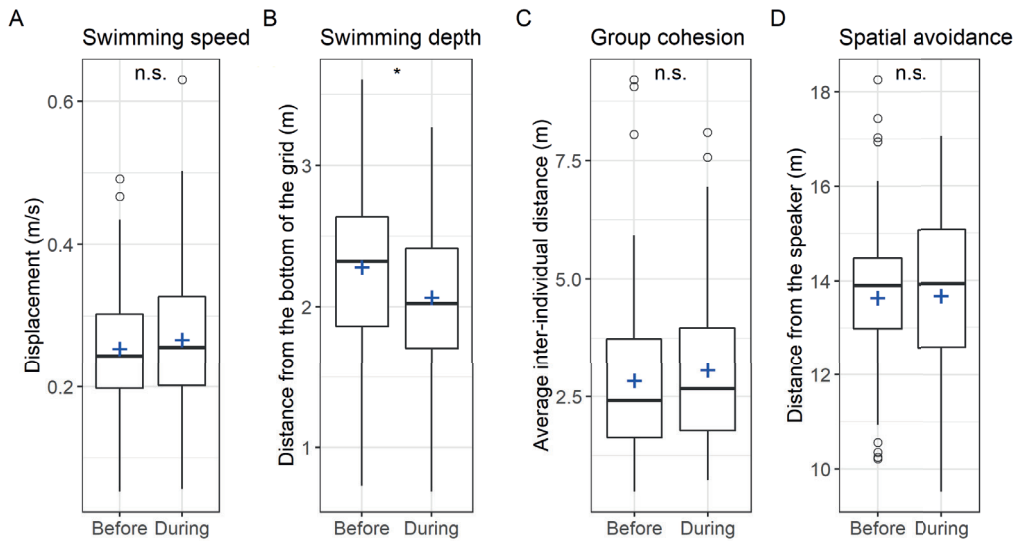
Fig. 5: Four behavioural parameters 5 minutes before and 5 minutes after the start of the impulsive sound, except for swimming speed, where we used 10-s-bins. A blue + indicates the mean and an * indicates a significant difference between the two time bins.

pulse levels, artificial background noise levels, and thereby also the signal-to-noise ratio (SNR). Our results demonstrated that seabass in outdoor conditions responded to the impulsive sound by increasing their swimming depth, but they did not change their swimming speed, group cohesion or distance from the speaker. Pulse level, elevated background level, SNR, or PRI were not significantly correlated to the increase in swimming depth. However, the baseline swimming depth was significantly correlated to the change in swimming depth.

Level dependent response

We did not find a significant effect of pulse level, ambient level or signal-to-noise ratio on the increase in swimming depth of the seabass. Several studies have found level-dependent reactions to sound exposures in a variety of species (e.g. Dunlop et al., 2018; Hawkins et al., 2014; Kok et al., 2018). Also in the current study species, a positive relationship has been found between pile driving pulse level and percentage of groups of four fish that showed a startle response (Kastelein et al., 2017). This contrast of the latter with the current study may be explained by the narrower range of sound levels in the current study. The different findings may also be explained by the type of behaviour that was analysed; startle responses are transient reflexes whereas the current analysis used 5-min-bins of swimming depth. These two measures were also shown not to correlate in a sound exposure study on zebrafish (*Danio rerio*) (Shafiei Sabet et al., 2016).

Table 1: Results of model selection (ranked by ΔAICc) for all four response variables (in front of ~). The marginal R² (R²m) shows the proportion of variance explained by the fixed effects, the conditional R² (R²c) shows the proportion of variance explained by the entire model and w$_i$ is the Akaike weight of the model. ΔAICc ≥ 2 indicates a significant difference between the models. An * indicates the best model.

#	Model	df	R²m	R²c	AICc	ΔAICc	w$_i$
A: Swimming speed ~ …							
null*	(1 | Group)	3		0.11	-380.5	-	0.98
1	Period + (1 | Group)	4	0.01	0.12	-372.6	7.96	0.02
B: Swimming depth ~ …							
1*	Period + (1 | Group)	4	0.03	0.48	278.5	-	0.91
null	(1 | Group)	3		0.45	283.2	4.74	0.09
C: Group cohesion ~ …							
null*	(1 | Group)	3		0.29	582.8	-	0.75
1	Period + (1 | Group)	4	0.00	0.30	585.0	2.24	0.25
D: Distance from the speaker ~ …							
null*	(1 | Group)	3		0.13	723.9	-	0.83
1	Period + (1 | Group)	4	0.00	0.13	727.1	3.19	0.17
E: Change in swimming depth ~ …							
1*	Depth before + (1 | Group)	4	0.13	0.44	61.6	-	0.83
null	(1 | Group)	3		0.29	67.7	6.07	0.04
3	Depth before + Tide + (1 | Group)	6	0.14	0.44	68.2	6.61	0.03
4	Depth before + Pulse SPL + (1 | Group)	5	0.16	0.47	68.9	7.33	0.02
5	Depth before + PRI + (1 | Group)	5	0.13	0.44	69.0	7.41	0.02

Signal-to-noise ratio has received limited attention in sound impact studies. Kastelein et al (2011) showed that a harbour porpoise increased its number of surfacings in response to signal sweeps, but less strongly during artificially elevated background levels. Similarly, several studies have shown that animals respond less strongly to experimental sounds in high disturbance areas (Bejder et al., 2006; Harding et al., 2018). This may be partly explained by SNR, but can only be shown by experiments – like the current – in which both background noise and signal levels are varied. The current study found no evidence for SNR as a covariate for behavioural response. However, given that auditory detection and
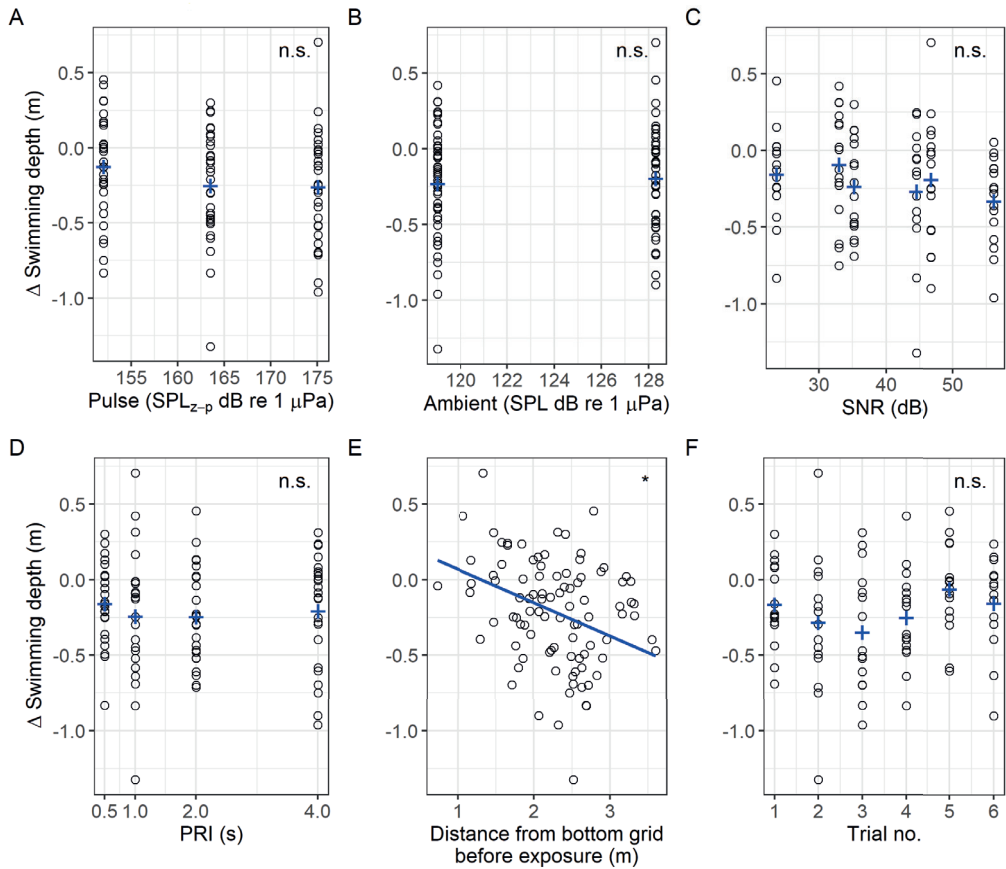
Fig. 6: The effects of pulse level, ambient level, SNR, PRI, depth before exposure and trial number on the change in swimming depth after the start of the impulsive sound. An * in the top-right corner of a plot indicates a significant correlation.

recognition thresholds in fish increase under higher background levels (Hawkins and Chapman, 1975; Wysocki and Ladich, 2005) and perceived loudness of traffic noise reduced with the playback of fountain sound in humans (Coensel et al., 2011; Nilsson et al., 2010), further studies to SNR using larger ranges of signal levels and SNRs are warranted. It may be most fruitful to compare the explanatory value of signal level and SNR in an experimental design and with a study species that has already revealed level dependent responses. Anyway, we strongly recommend to always report both ambient and signal levels in sound impact studies. For field studies that are conducted on different days or different locations, variation in ambient levels may be expected and may explain part of the variation in behavioural responses to sound stimuli.

Pulse rate interval

We did not find an effect of PRI on the change in depth of the seabass. Previous studies have found effects of PRI, however, across studies no clear patterns have emerged. In zebrafish, a significant increase in group cohesion and swimming speed in response to 1 s pulses with 1 s intervals has been found, but not to the same pulses with 9 s intervals (Neo et al., 2015b). However, another study on zebrafish found no differences in immediate response to 1 s pulses with either 1 or 4 s intervals (Shafiei Sabet et al., 2015). In a previous European seabass study with the same PRIs as the current study, faster PRIs were found to increase group cohesion whereas slower rates did not. Additionally, the difference between post-exposure swimming depth and baseline swimming depth was positively correlated with PRI (Neo et al., 2015a). Humans and rats (*Rattus norvegicus*) showed a faster decrease in startle-like responses to pulse trains with a lower PRI (humans: 20 vs. 100 s; rats: 2 vs. 16 s) (Davis, 1970; Gatchel, 1975), the tested range of PRI is however bigger than those described in the fish studies. Thus, it may be that the range of PRI in the current study was too small to find an effect, although, a bigger range would be beyond the range in which pile driving occurs. The PRI used for seismic surveys is usually 5 to 15 s (McCauley et al., 2000) and this range has not been tested for fish yet.

Due to the different PRIs, the sound treatments in the current study differed in cumulative sound exposure level (SEL_{cum}). The absence of an effect of SEL_{cum} on fish behavioural response is in line with other studies that compared the behavioural effect of different sound treatments (Neo et al., 2014; Neo et al., 2015a; Neo et al., 2015b; Neo et al., 2016; Shafiei Sabet et al., 2015). SEL_{cum} was originally proposed – along with zero-to-peak sound pressure level ($SPL_{z\text{-}p}$) – to assess the risk of physical injury to fish by pile driving (Stadler and Woodbury, 2009; Woordbury and Stadler, 2008). For physical injury, SEL_{cum} has been shown to be useful, but not as a single metric (Halvorsen et al., 2012). For behavioural impact assessments, new metrics are needed, and we may also require a combination of metrics. Kurtosis has been suggested as a good metric for continuous sounds (Hastings, 2008; Popper and Hawkins, 2019). However, behavioural response studies scaled to kurtosis are lacking and it is unsuitable as a metric for intermittent sounds, as pulse rate would dominate the metric.

Previous studies with European seabass

The current study followed a similar design as four earlier sound exposure studies on European seabass, this enabled us to qualitatively examine the consistency of seabass reaction to sound across years, seasons, experimental arenas, fish batches and sizes. The seabass in the current study swam to greater

depth upon the start of the sound exposure. Such a response to sound exposures has been shown before in the same species in very similar experimental designs (Neo et al., 2014; Neo et al., 2015a; Neo et al., 2016; Neo et al., 2018). However, we found no effects on group cohesion, swimming speed and spatial avoidance, while they have been found in some of the aforementioned studies (Table 2). In general, fewer behavioural changes have been found in the outdoor studies. In the outdoor studies, the fish were situated in a bigger experimental arena and experienced acoustic conditions that were more similar to those experienced in natural waters. These factors may offer fish more control over their environment (Koolhaas et al., 2011), as they may move around more freely and may perceive sound directionality better, which may reduce their stress levels caused by the sound exposure.

This comparison among well-replicated studies with different testing conditions nicely shows the value of captive studies and their complementary role to field studies. The overview above indicates that indoor studies with high resolution of measurements may be best to explore mechanistic processes and make progress in conceptual understanding. Once we have consensus about an optimal acoustic metric to scale responsiveness to anthropogenic sound of variable features and in variable conditions, we would need to follow up with tests in the field. Insight

Table 2: Overview of sound impact studies on European seabass with a similar design and testing the same behavioural parameters: depth (swimming depth), cohesion (inter-individual-distance), speed (swimming speed) and avoidance (distance from the speaker). The table provides an overview of the significant effects of the sound exposure, ignoring trends. Additional information on these experiments can be found in Supplementary material I.

Study	Arena	Changes in behavioural parameters, before vs. during sound exposure			
		Depth	Cohesion	Speed	Avoidance
Neo et al., 2014	Indoor	Deeper	Tighter	Faster	Not tested
Neo et al., 2015a	Indoor	Deeper	Tighter	Faster	Not tested
Neo et al., 2016	Outdoor	Deeper	No effect	Faster	Further
Neo et al., 2018	Outdoor	Deeper, only at night	No effect	Faster, only at night	No effect
Current study	Outdoor	Deeper	No effect	No effect	No effect

about absolute response thresholds that would be applicable to free-ranging fish in a natural context will not come from studies in captivity and will always require behavioural response studies in that natural context, replicated at the context of interest (i.e. with respect to habitat, species, season, etc.).

Conclusions

In the current study, we provided conceptual background to the potential importance of SNR and PRI in predicting the behavioural effects of sound exposures on animals and experimentally tested this in European seabass. The seabass increased their swimming depth upon sound exposure, however the variation in this increase could not be explained by any of the acoustic parameters of interest; PRI, pulse level, (elevated) background level or SNR. Several earlier studies are indicative of potential explanatory value of SNR, further testing with wider ranges of signal and background levels may yield different results.

Ethical statement

All experiments were conducted in accordance with the Dutch Experiments on Animals Act and approved by the Dutch Central Commission Animal Experiments (CCD) under no. AVD106002016610.

Supplementary information

Additional information on the studies from the overview in Table 2 is is available from the publication DOI: 10.1016/j.beproc.2019.103992.

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References

Andrew, R. K., Howe, B. M., Mercer, J. A. and Dzieciuch, M. A. (2002). Ocean ambient sound: Comparing the 1960s with the 1990s for a receiver off the California coast. Acoust. Res. Lett. Online 3, 65–70.

Barton, K. (2016). MuMIn: Multi-Model Inference.

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67, 1–48.

Beattie, R. C., Thielen, K. M. and Franzone, D. L. (1994). Effects of Signal-To-Noise Ratio on The Auditory Brainstem Response to Tone Bursts In Notch Noise and Broadband Noise. Scand. Audiol. 23, 47–56.

Bejder, L., Samuels, A., Whitehead, H. and Gales, N. (2006). Interpreting short-term behavioural responses to disturbance within a longitudinal perspective. Anim. Behav. 72, 1149–1158.

Bejder, L., Samuels, A., Whitehead, H., Finn, H. and Allen, S. (2009). Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. Mar. Ecol. Prog. Ser. 395, 177–185.

Bruintjes, R., Purser, J., Everley, K. A., Mangan, S., Simpson, S. D. and Radford, A. N. (2016). Rapid recovery following short-term acoustic disturbance in two fish species. R. Soc. Open Sci. 3,.

Carey, W. M. and Browning, D. (1988). Low Frequency Ocean Ambient Noise: Measurements and Theory. In Sea Surface Sound, pp. 361–376. Dordrecht: Springer.

Carroll, A. G., Przeslawski, R., Duncan, A., Gunning, M. and Bruce, B. (2016). A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pollut. Bull. 114, 9–24.

Coensel, B. De, Vanwetswinkel, S. and Botteldooren, D. (2011). Effects of natural sounds on the perception of road traffic noise. J. Acoust. Soc. Am. 129, EL148–EL153.

Davis, M. (1970). Effects of interstimulus interval length and variability on startle-response habituation in the rat. J. Comp. Physiol. Psychol. 72, 177–192.

Dunlop, R. A., Noad, M. J., McCauley, R. D., Kniest, E., Slade, R., Paton, D. and Cato, D. H. (2018). A behavioural dose-response model for migrating humpback whales and seismic air gun noise. Mar. Pollut. Bull. 133, 506–516.

Ellison, W. T., Southall, B. L., Clark, C. W. and Frankel, A. S. (2012). A New Context-Based Approach to Assess Marine Mammal Behavioral Responses to Anthropogenic Sounds. Conserv. Biol. 26, 21–28.

Frimodt, C. and Dore, I. (1995). Multilingual Illustrated Guide to the World's Commercial Coldwater Fish. Wiley-Blackwell.

Gatchel, R. J. (1975). Effects of interstimulus interval length on short-and long-term habituation of autonomic components of the orienting response. Physiol. Psychol. 3, 133–136.

Hall, M. V. (2013). A quasi-analytic model of the underwater sound signal from impact driving of an offshore semi-infinite pile. In Proceedings of Meetings on Acoustics, pp. 1–9.

Halvorsen, M. B., Casper, B. M., Woodley, C. M., Carlson, T. J. and Popper, A. N. (2012). Threshold for onset of injury in chinook salmon from exposure to impulsive pile driving sounds. PLoS One 7, 2–12.

Harding, H. R., Gordon, T. A. C., Hsuan, R. E., Mackaness, A. C. E., Radford, A. N. and Simpson, S. D. (2018). Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise. Biol. Lett. 14, 20180441.

Hastings, M. (2008). Sound-Exposure Metrics for Aquatic Animals. Bioacoustics 17, 118–120.

Haver, S. M., Gedamke, J., Hatch, L. T., Dziak, R. P., Van Parijs, S., McKenna, M. F.,

Barlow, J., Berchok, C., DiDonato, E., Hanson, B., et al. (2018). Monitoring long-term soundscape trends in U.S. Waters: The NOAA/NPS Ocean Noise Reference Station Network. Mar. Policy 90, 6–13.

Hawkins, A. D. and Chapman, C. J. (1975). Masked auditory thresholds in the cod, *Gadus morhua* L. J. Comp. Physiol. 103, 209–226.

Hawkins, A. D., Roberts, L. and Cheesman, S. (2014). Responses of free-living coastal pelagic fish to impulsive sounds. J. Acoust. Soc. Am. 135, 3101–3116.

Hildebrand, J. A. (2009). Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395, 5–20.

Hongisto, V., Varjo, J., Oliva, D., Haapakangas, A. and Benway, E. (2017). Perception of Water-Based Masking Sounds—Long-Term Experiment in an Open-Plan Office. Front. Psychol. 8, 1–14.

Hubert, J., Neo, Y. Y., Campbell, J. A. and Slabbekoorn, H. (2016). Particle motion and pressure soundscape in outdoor vs indoor set-up. In Proceedings of Meetings on Acoustics, p. 070007.

Kastelein, R. A., Heul, S. Van Der, Verboom, W. C., Jennings, N., Veen, J. Van Der and de Haan, D. (2008). Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. Mar. Environ. Res. 65, 369–77.

Kastelein, R. A., Steen, N., de Jong, C., Wensveen, P. J. and Verboom, W. C. (2011). Effect of broadband-noise masking on the behavioral response of a harbor porpoise (*Phocoena phocoena*) to 1-s duration 6–7 kHz sonar up-sweeps. J. Acoust. Soc. Am. 129, 2307–2315.

Kastelein, R. A., Jennings, N., Kommeren, A., Helder-Hoek, L. and Schop, J. (2017). Acoustic dose-behavioral response relationship in sea bass (*Dicentrarchus labrax*) exposed to playbacks of pile driving sounds. Mar. Environ. Res. 130, 315–324.

Kok, A. C. M., Engelberts, J. P., Kastelein, R. A., Helder-Hoek, L., van de Voorde, S., Visser, F. and Slabbekoorn, H. (2018). Spatial avoidance to experimental increase of intermittent and continuous sound in two captive harbour porpoises. Environ. Pollut. 233, 1024–1036.

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., Boer, S. F. De, Flügge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., et al. (2011). Neuroscience and Biobehavioral Reviews Stress revisited: A critical evaluation of the stress concept. Neurosci. Biobehav. Rev. 35, 1291–1301.

Ladich, F. and Schulz-Mirbach, T. (2013). Hearing in Cichlid Fishes under Noise Conditions. PLoS One 8, e57588.

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol. Evol. 7, 573–579.

Lovell, J. M. (2003). The hearing abilities of the bass, *Dicentrarchus labrax*. Technical report commissioned by ARIA Marine Ltd. for the European Commission Fifth Framework Programme.

Marvit, P. and Crawford, J. D. (2000). Auditory discrimination in a sound-producing electric fish (*Pollimyrus*): Tone frequency and click-rate difference detection. J. Acoust. Soc. Am. 108, 1819–1825.

Matuschek, R. and Betke, K. (2009). Measurements of Construction Noise During Pile

Driving of Offshore Research Platforms and Wind Farms. In Proceedings of NAG/DAGA 2009, pp. 262–265.

McCauley, R. D., Fewtrell, J., Duncan, A. J., Jenner, C., Jenner, M., Penrose, J. D., Prince, R. I. T., Adhitya, A., Murdoch, J. and McCabe, K. (2000). Marine Seismic Surveys – A Study of Enviromental Implications. APPEA J. 40, 692–708.

Merchant, N. D., Brookes, K. L., Faulkner, R. C., Bicknell, A. W. J., Godley, B. J. and Witt, M. J. (2016). Underwater noise levels in UK waters. Sci. Rep. 6, 36942.

Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142.

Nedelec, S. L., Campbell, J., Radford, A. N., Simpson, S. D. and Merchant, N. D. (2016). Particle motion: The missing link in underwater acoustic ecology. Methods Ecol. Evol. 7, 836–842.

Neo, Y. Y., Seitz, J., Kastelein, R. A., Winter, H. V., ten Cate, C. and Slabbekoorn, H. (2014). Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biol. Conserv. 178, 65–73.

Neo, Y. Y., Ufkes, E., Kastelein, R. A., Winter, H. V., ten Cate, C. and Slabbekoorn, H. (2015a). Impulsive sounds change European seabass swimming patterns: Influence of pulse repetition interval. Mar. Pollut. Bull. 97, 111–117.

Neo, Y. Y., Parie, L., Bakker, F., Snelderwaard, P., Tudorache, C., Schaaf, M. and Slabbekoorn, H. (2015b). Behavioral changes in response to sound exposure and no spatial avoidance of noisy conditions in captive zebrafish. Front. Behav. Neurosci. 9, 1–11.

Neo, Y. Y., Hubert, J., Bolle, L., Winter, H. V., Ten Cate, C. and Slabbekoorn, H. (2016). Sound exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure. Environ. Pollut. 214, 26–34.

Neo, Y. Y., Hubert, J., Bolle, L. J., Winter, H. V. and Slabbekoorn, H. (2018). European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environ. Pollut. 239, 367–374.

Nilsson, M. E., Alvarsson, J., Rådsten-ekman, M. and Bolin, K. (2010). Auditory masking of wanted and unwanted sounds in a city park. Noise Control Eng. 58, 524–531.

Noirot, I. C., Brittan-Powell, E. F. and Dooling, R. J. (2011). Masked auditory thresholds in three species of birds, as measured by the auditory brainstem response (L). J. Acoust. Soc. Am. 129, 3445–3448.

Popper, A. N. and Hastings, M. C. (2009). The effects of anthropogenic sources of sound on fishes. J. Fish Biol. 75, 455–89.

Popper, A. N. and Hawkins, A. D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. fish 94, 692–713.

R Core Team (2016). R: A Language and Environment for Statistical Computing.

Schlittmeier, S. J., Hellbrück, J., Thaden, R. and Vorländer, M. (2008). The impact of background speech varying in intelligibility: Effects on cognitive performance and perceived disturbance. Ergonomics 51, 719–736.

Sertlek, H. Ö., Aarts, G., Brasseur, S., Slabbekoorn, H., ten Cate, C., von Benda-Beckmann, A. M. and Ainslie, M. A. (2016). Mapping Underwater Sound in the

Dutch Part of the North Sea. In The Effects of Noise on Aquatic Life II, pp. 1001–1006.

Shafiei Sabet, S., Neo, Y. Y. and Slabbekoorn, H. (2015). The effect of temporal variation in sound exposure on swimming and foraging behaviour of captive zebrafish. Anim. Behav. 107, 49–60.

Shafiei Sabet, S., Wesdorp, K., Campbell, J., Snelderwaard, P. and Slabbekoorn, H. (2016). Behavioural responses to sound exposure in captivity by two fish species with different hearing ability. Anim. Behav. 116, 1–11.

Slabbekoorn, H. (2016). Aiming for Progress in Understanding Underwater Noise Impact on Fish: Complementary Need for Indoor and Outdoor Studies. In The Effects of Noise on Aquatic Life II (ed. Popper, N. A.) and Hawkins, A.), pp. 1057–1065. New York, NY: Springer New York.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C. and Popper, A. N. (2010). A noisy spring: The impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427.

Stadler, J. H. and Woodbury, D. P. (2009). Assessing the effects to fishes from pile driving: Application of new hydroacoustic criteria. In International Congress and Exposition on Noise Control Engineering 2009 (INTER-NOISE 2009) (ed. Bolton, J. S.), pp. 3580–3587. Ottawa, Canada: Curran Associates Inc.

Wells, D. L. (2009). Sensory stimulation as environmental enrichment for captive animals: A review. Appl. Anim. Behav. Sci. 118, 1–11.

Wenz, G. M. (1962). Acoustic Ambient Noise in the Ocean: Spectra and Sources. J. Acoust. Soc. Am. 34, 1936–1956.

Williams, R., Wright, a. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., Clark, C. W., Cullis-Suzuki, S., Dakin, D. T., Erbe, C., et al. (2015). Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24.

Woordbury, D. P. and Stadler, J. H. (2008). A proposed method to assess physical injury to fishes from underwater sound produced during pile driving. Bioacoustics 17, 289–291.

Wright, A. J., Soto, N., Aguilar, B. and Linda, A. (2007). UCLA International Journal of Comparative Psychology Title Anthropogenic Noise as a Stressor in Animals: A Multidisciplinary Perspective. Int. J. Comp. Psychol. 20,.

Wysocki, L. E. and Ladich, F. (2002). Can fishes resolve temporal characteristics of sounds? New insights using auditory brainstem responses. Hear. Res. 169, 36–46.

Wysocki, L. E. and Ladich, F. (2005). Hearing in Fishes under Noise Conditions. J. Assoc. Res. Otolaryngol. 6, 28–36.

Effects of broadband sound exposure on the interaction between foraging crab and shrimp – A field study

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Abstract

Aquatic animals live in an acoustic world in which they often rely on sound detection and recognition for various aspects of life that may affect survival and reproduction. Human exploitation of marine resources leads to increasing amounts of anthropogenic sound underwater, which may affect marine life negatively. Marine mammals and fishes are known to use sounds and to be affected by anthropogenic noise, but relatively little is known about invertebrates such as decapod crustaceans. We conducted experimental trials in the natural conditions of a quiet cove. We attracted shore crabs (*Carcinus maenas*) and common shrimps (*Crangon crangon*) with an experimentally fixed food item and compared trials in which we started playback of a broadband artificial sound to trials without exposure. During trials with sound exposure, the cumulative count of crabs that aggregated at the food item was lower, while variation in cumulative shrimp count could be explained by a negative correlation with crabs. These results suggest that crabs may be negatively affected by artificially elevated noise levels, but that shrimps may indirectly benefit by competitive release. Eating activity for the animals present was not affected by the sound treatment in either species. Our results show that moderate changes in acoustic conditions due to human activities can affect foraging interactions at the base of the marine food chain.

Introduction

Over the last century, anthropogenic sources have increasingly interfered with the natural cacophony of sounds in the aquatic environment (Andrew et al., 2002; Hildebrand, 2009). Many animals use sound for activities such as orientation, predator and prey detection, and communication, of which the latter can play a critical role in aggregation and reproduction (Slabbekoorn et al., 2010). Most energy of anthropogenic sounds is concentrated in the same frequency range as biologically relevant sounds and thereby has the potential to impact aquatic life (Kunc et al., 2016). This has led to an increased interest in the effects of anthropogenic sound sources on marine mammals and fish, but relatively little work has been done on invertebrates, including decapod crustaceans (Hawkins and Popper, 2016; Morley et al., 2013; Williams et al., 2015). Yet, invertebrates form the majority of the marine biomass and their abundance is critical for species in higher trophic levels (cf. Morley et al., 2013; Solan et al., 2016).

For decapod crustaceans, both the sensory mechanisms involved in hearing and their utilization of sound are not yet well understood. They are thought to be most sensitive to low-frequency particle motion as they lack gas-filled organs such as swim bladders (Edmonds et al., 2016). Hearing sensitivity curves of mud crabs (*Panopeus* spp.) and common prawn (*Palaemon serratus*) show highest sensitivity for the lowest tested frequencies (resp. 75 and 100 Hz) with decreasing sensitivity up to at least 1600 and 3000 Hz (Hughes et al., 2014; Lovell et al., 2005). There is also some evidence that decapod crustaceans use sound for orientation, experiments using light traps and binary choice chambers suggested that shrimps and coastal crabs species in their pelagic stages use coastal reef sound to orient on the coast (Jeffs et al., 2003; Radford et al., 2007; Simpson et al., 2011). Crabs in later life stages may also use acoustic cues to avoid predators. Mud crabs changed foraging behaviour during the playback of vocalisations of three predator fish species (Hughes et al., 2014). Furthermore, snapping shrimps do not only snap to stun prey items, but also snap during agonistic interactions; both the jet stream of water and the emitted sound possibly play a role in this potential case of multi-modal communication in an invertebrate (Au and Banks, 1998; Schein, 1975).

There are also some studies that indicate that elevated sound conditions may have physiological effects on decapod crustaceans. Studies in both common shrimps (*Crangon crangon*) and shore crabs (*Carcinus maenas*) show an increased oxygen consumption in elevated sound conditions (Regnault and Lagardère, 1983; Wale et al., 2013a). Lobsters (*Palinurus elephas*) and common prawn (*Palaemon serratus*) that were exposed to boat noise exhibited significant changes in stress-

related biochemistry (Filiciotto et al., 2014; Filiciotto et al., 2016). Furthermore, an early, long-term experiment with common shrimps under elevated sound conditions showed a reduced growth and delayed reproduction in comparison to the control (Lagardère, 1982).

The available studies investigating effects of elevated sound conditions on behaviour of decapod crustaceans are typically conducted in captivity. Terrestrial hermit crabs (*Coenobita clypeatus*), exposed to white noise in captivity, increased latency time to withdraw in their shell upon visual display of a predator (Chan et al., 2010) and marine hermit crabs (*Pagurus bernhardus*) took less time to approach, investigate, and enter a shell (Walsh et al., 2017). Filiciotto and colleagues (2016) found several noise-induced behavioural effects in captive common prawn: reduced locomotor activity, less encounters with conspecifics and differences in use of shelter. In contrast, lobsters increased locomotor behaviour during boat noise exposure (Filiciotto et al., 2014). Most relevant to the current study, Wale and colleagues (2013b) found no difference in food finding in captive crabs exposed to ambient noise or ship noise. But when they started the boat sound after the crabs began eating, the crabs were (temporary) disrupted in the first minute after the onset. It remains to be tested whether similar effects of noise on behaviour occur under natural conditions in the wild.

In the current study, we explored the effect of experimental playback of broadband noise on the foraging behaviour of shore crabs and common shrimps. We conducted this experiment in situ, in a cove without boat traffic, to ensure natural conditions in terms of sound field, animal behaviour, and species interactions. We aimed at answering three questions: (1) Do elevated sound levels affect the aggregation of crabs and shrimps at a food source? (2) Do elevated sound levels affect feeding rates in crabs and shrimps once they have arrived at a food source? (3) Are there any noise-dependent interactions among the two species?

Materials and methods

Study subjects and location

The experiment was performed in the Jacobahaven, an artificial cove in the Oosterschelde estuary in The Netherlands. The cove is about 200 m by 300 m in size and depending on the tide, 1.5 to 4.8 m deep. The cove is home to a large variety of marine life that is part of a natural food chain and typical of the region. Prominent plants are sea lettuce (*Ulva lactuca*) and sugar kelp (*Saccharina* sp.), prominent molluscs are blue mussels (*Mytilus edulis*) and Japanese oysters (*Magallana gigas*), and there is a variety of jellyfish and sea stars. Fish species include gobies (*Pomatoschistus* spp.) and European seabass (*Dicentrarchus*

labrax). Our study species, shore crab and common shrimp are very abundant. In the middle of the cove, we constructed a floating research platform from a plastic modular floating dock system (Candock, Canada). The platform consisted of a square platform with a tent for equipment connected to an octagonal walkway and has been used in previous experiments (cf. Neo et al., 2018). We used the 10 corners of the platforms as the locations for the trials and all locations were at least 5.5 m apart (fig. 1a). The position of the speaker was fixed and the distance from the trial-location to the speaker varied between 3 and 14 m. Trials were performed around low tide on May 9th-11th 2017.

Experimental procedure

We used two weighted crates as mooring device for an underwater camera (GoPro HERO4 Black and JVC Everio R GZ-R415) so we could perform paired trials at different locations. The cameras were positioned to film the sea floor around a cooked mussel (*Mytilus edulis*) that was connected to the crate using iron wire (fig. 1b). For each trial, we lowered both crates to the sea bottom from two of the 10 corners of the research platform. After 2 min of baseline data collection, we started a playback of either 5 min of silence (control) or 5 min of white noise (see Sound characteristics). The locations were allocated using an incomplete counterbalanced design, in which neighbouring locations during a single sound exposure and same locations in consecutive exposures were avoided. The time between sound exposures was at least 10 min.
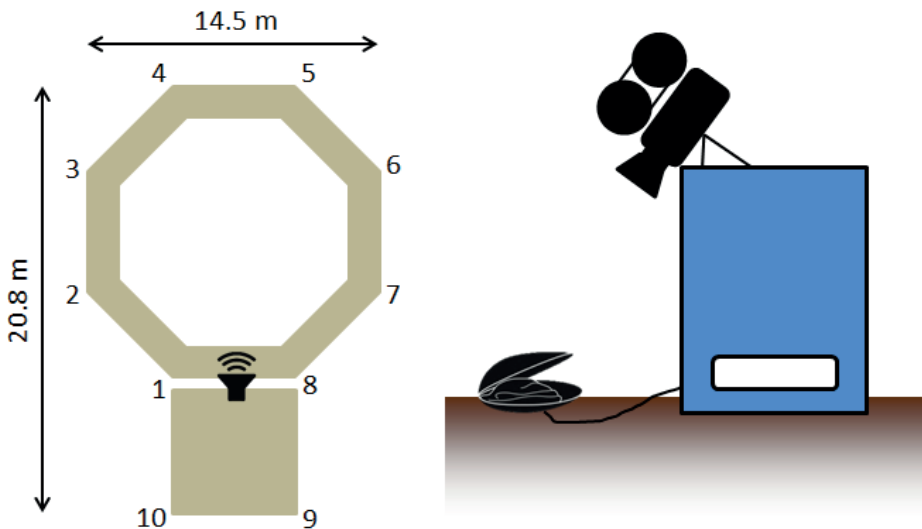

Fig. 1: (a) Top view schematic of the research platform; the numbers indicate the 10 different locations for the trials and the speaker symbol indicates the fixed location of the omnidirectional underwater speaker. (b) Side view schematic of a crate with camera and food item (mussel) to video and attract crabs and shrimps.

Behavioural measurements

We analysed 49 video recordings, 27 control trials and 22 white noise treatment trials. Due to variable visibility, not all videos could be analysed, typically caused by sea weed obstructing the camera view. We analysed the first 4 min of every video: 2 min immediately before the start of the treatment and 2 min immediately after. Every 10 s we scored the number of crabs and shrimps in view of the camera and the number of crabs and shrimps that were eating the mussel. We did not analyse video after 4 min as the crabs regularly finished the mussel soon after this mark or removed the food from view.

Sound characteristics

The Gaussian white noise sound treatment was created using Audacity v2.1.0 and played back using an underwater speaker (SynchroSound Aqua IIB). Standard spectra of white noise will have changed upon arrival at the animal depending on speaker characteristics and underwater propagation. We calibrated the microphone of the JVC Everio R GZ-R415 using a calibrated hydrophone to be able to use the audio track from the videos to determine the sound levels and spectra of the sound conditions. We analysed the audio tracks in Rstudio (R Core Team, 2016) using custom R scripts. The sound pressure levels (SPL) were calculated by summing the power spectral density (PSD) values within the 0 – 3000 Hz frequency range, which was assumed to be most representative of shrimps' hearing range (based on a single study: Lovell et al., 2005). The SPL of
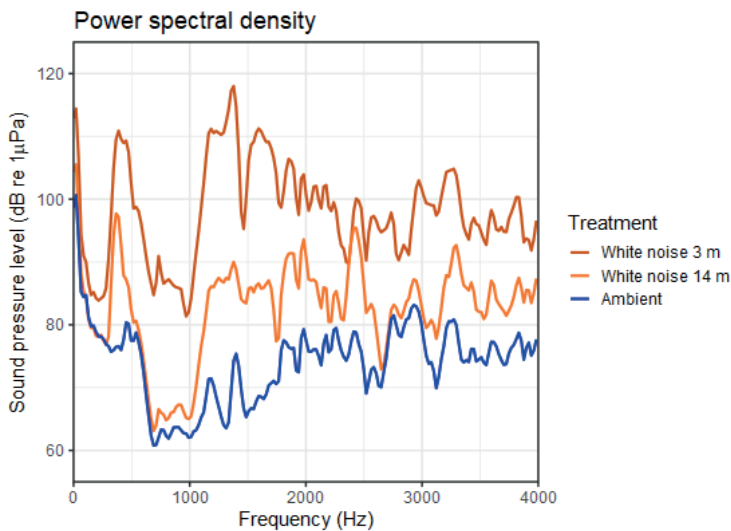

Fig. 2: Power spectral density (window length: 2048, window type: Hann) of the ambient (control) and white noise condition (spectrum altered by speaker and propagation) at the closest and furthest position from the speaker (resp. 3 & 14 m).

the ambient recordings was 119.5 dB re 1 μPa and during the playback of white noise this ranged from 129.5 to 142.0 dB re 1 μPa depending on the location (fig. 2).

Statistics

We calculated the cumulative counts of 'crabs present', 'shrimps present', 'crabs eating' and 'shrimps eating' within the 2 min period before sound exposure (t = 0-2 min) and after the start of the sound exposure (t = 2-4 min). All cumulative counts at t = 2-4 min were used as response variables in Poisson Generalized Linear Mixed-effect Models. All models included the treatment and cumulative count of the response variable at t = 0-2 min and the pair-ID of the trial as a fixed effect. For the response variables 'crabs present' and 'shrimps present', we also used the presence of the other species (shrimps or crabs) at t = 2-4 min as a fixed effect in the full model to gain insight into a possible interaction between species. For the response variables 'crabs eating' and 'shrimps eating', we also used the presence of the eating species (crabs or shrimps) at t = 2-4 min as a fixed effect in the full model. The location of the trial (1 thru 10) was included as a random effect.

The best model was chosen by AICc using dredge model selection (package MuMIn). Models differing in ΔAICc ≥ 2 are considered to have a significantly different fit. We calculated the marginal (R2m) and conditional (R2c) R2 values of the models to show the proportion of variance of the response variable explained by the fixed effects (R2m) and the entire model (R2c) (Nakagawa and Schielzeth, 2013). To further examine the potential interaction between crab and shrimp numbers, we applied a cross-correlation analysis to the time series of count data. As our dataset consisted of multiple small time series (25 time points per trial), we opted to analyse all our trials as a single time series to reduce the variation in the cross-correlation results and give a broad overview of the correlation between shrimp and crab presence over all trials. To apply the cross correlation analysis, we did the following: 1) Align the paired crab and shrimp counts and offset the shrimp with respect to a given lag value for all trials; 2) remove crab or shrimp time points at the beginning and end of each trial which do not have a paired sample; 3) append the paired time series across all trials, resulting in a single paired time series of crab and offset shrimp counts for the entire experiment; 4) calculate the Pearson's correlation coefficient between the paired series. This process was repeated for multiple lag values. All analyses were conducted in Rstudio (R Core Team, 2016) using the packages lme4 (Bates et al., 2015), MuMIn (Barton, 2016) and piecewiseSEM (Lefcheck, 2016).

Results

We consistently observed an increasing number of crabs and shrimps approaching the crates and accumulating at the cooked mussel during the 4-min trials (figure 3a-b). After the playback started in the white noise trials, the accumulation of crabs slowed down relative to the ambient control trials, while shrimp accumulation showed the opposite pattern. The relatively high and variable baseline counts of shrimps in the white noise trials can be attributed to a single trial that started off with the exceptionally numerous presence of seven shrimps (figure 3b).

Model selection showed that the cumulative crab count of the second half of the trial was best explained by the treatment, crab presence during the first half (baseline) of the trial and shrimp presence during the second half of the trial (df = 5, R2m = 0.55, R2c = 0.76, table 1). Running this model showed that significantly fewer crabs were counted during the white noise exposures than during the control trials (Intercept: 2.27, Treatment WN: -0.62; figure 4a) and fewer crabs were associated with more shrimps (Slope shrimp present: -0.01). The variance in cumulative shrimp count was best explained by the shrimp presence during the baseline and crab presence during the second half of the trial (df = 4, R2m = 0.41, R2c = 0.89, table 1). There was no significant effect of treatment for the shrimps (figure 4b), but running the model confirmed a negative correlation between shrimp and crab numbers (Intercept: 1.54, Slope crab present: -0.02).

The cumulative count of eating crabs was best explained by just crab presence (df = 3, R2m = 0.58, R2c = 0.76, table 1), so there was no significant effect of treatment (figure 4c). When more crabs were present, more were actively eating (Intercept: 0.63, Slope crab present: 0.07). Similarly, the cumulative count of eating shrimps was best explained by shrimp presence (df = 3, R2m = 0.23, R2c = 0.70, table 1), so there was also no significant effect of treatment (figure 4d). Also, when more shrimps were present, more were actively eating (Intercept: -2.60, Slope shrimp present: 0.09).

The first two models showed a negative correlation between crab and shrimp presence. To explore whether crab numbers followed shrimp numbers or vice versa, we applied a cross-correlation on the time series count data. The plot of the cross-correlation (figure 5) confirms that shrimp and crab numbers are negatively correlated. The strongest correlations are found in the lag range +10 to +50, suggesting that crab presence correlates best with shrimp presence 10-50 s later (i.e. crab changes precede shrimp changes).
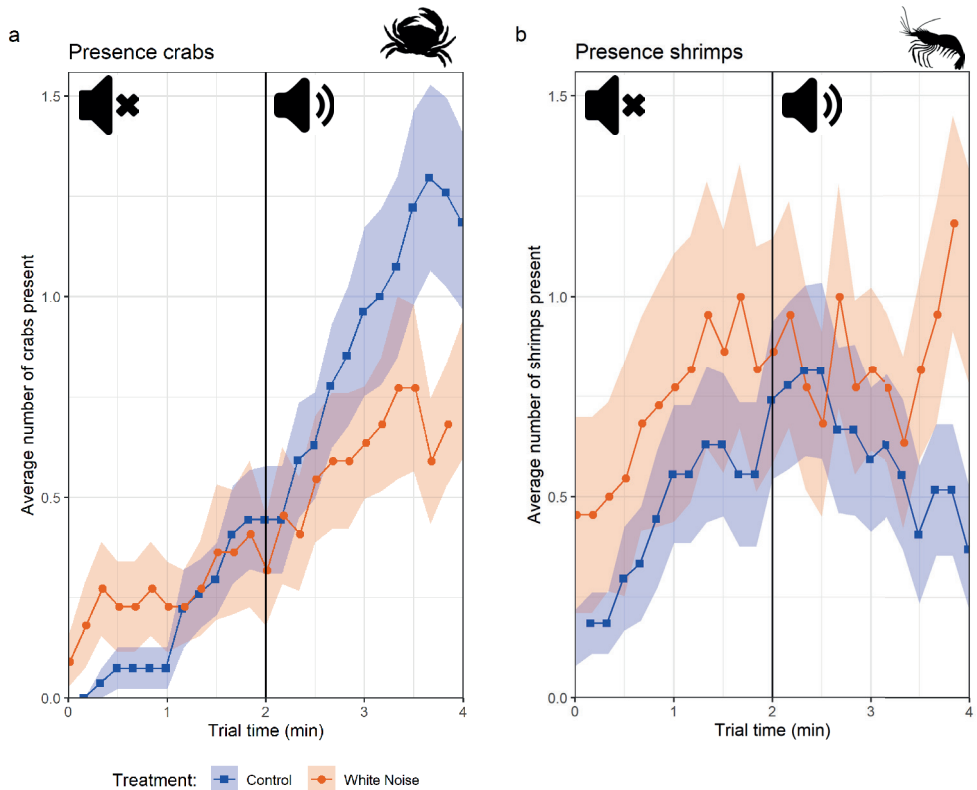
Fig. 3: The average number of crabs (left) and shrimps (right) counted from the videos of both treatments (Control (Co) n = 27 trials; White noise (WN) n = 22 trials). The shaded area indicates the standard error of the mean. The playback in the white noise trials started after 2 min, indicated with the vertical line and the speaker symbols.

Discussion

In the current study, we experimentally exposed shore crabs and common shrimps to elevated sound levels after offering a food item. This experiment was performed in situ, ensuring high acoustic and behavioural validity. Our results demonstrate that: (1) The current sound exposure reduced aggregation at a food item in shore crabs, but not in common shrimps. (2) The feeding rate, in both crabs and shrimps, was not directly affected by the sound exposures. (3) There was a negative correlation between crab and shrimp numbers that was likely driven by crabs. Even though the sound exposure did not affect shrimp aggregation directly, shrimps may have indirectly benefitted as lower numbers of crabs due to sound exposures released competition for shrimps.

Crab foraging behaviour

Our finding that sound exposure reduced food aggregation is in contrast with an

Fig. 4: Mean cumulative counts of the response variables during the second half of each trial. For the cumulative crab count, there was a significant effect of treatme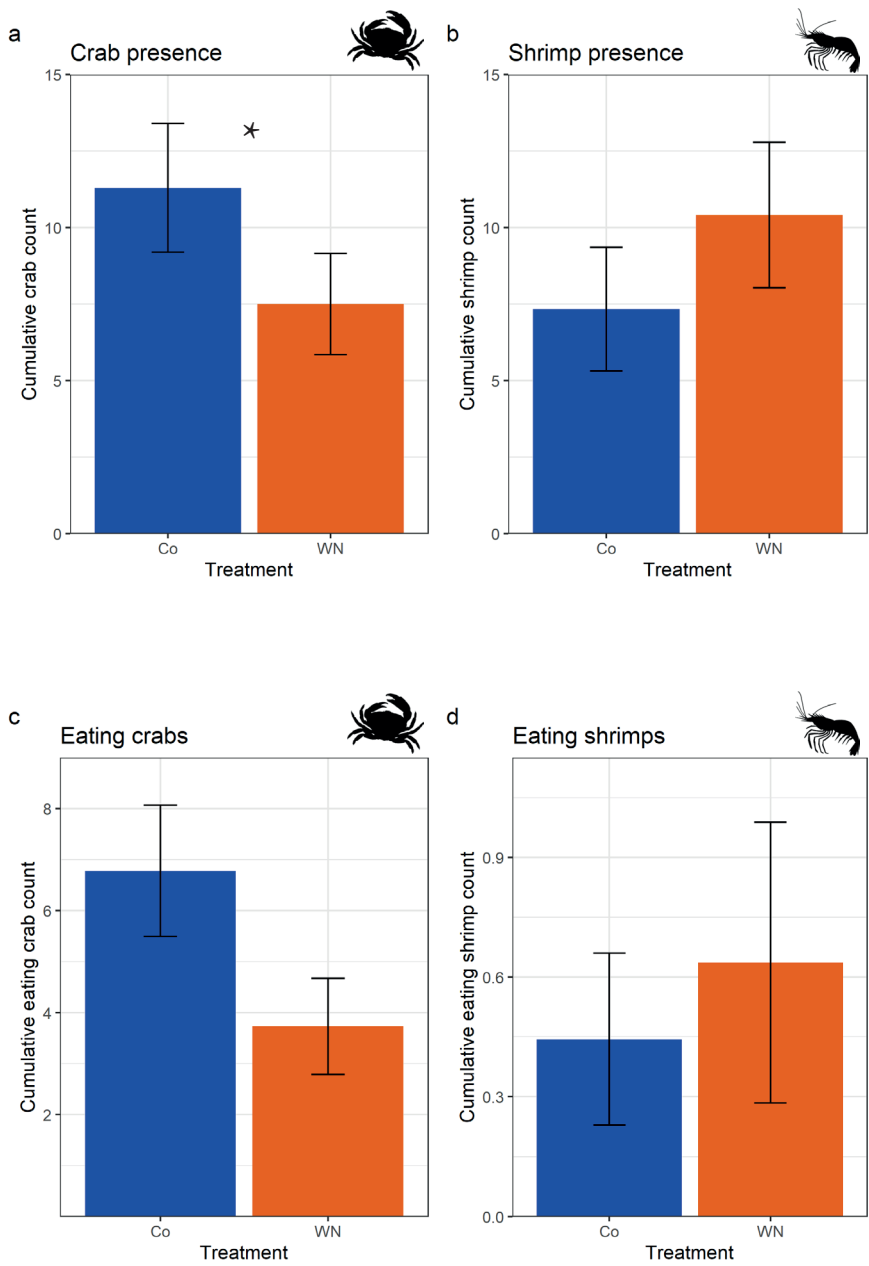nt, indicated by the *. 'Co' refers to the control (silence) treatment, 'WN' to the white noise treatment. The error bars represent the error of the mean.

Table 1: Best 3 results of model selection (ranked by AICc) and null models for all four response variables (in front of ~). The marginal R² (R²m) shows the proportion of variance explained by the fixed effects, the conditional R2 (R²c) shows the proportion of variance explained by the entire model. ΔAICc ≥ 2 indicates a significant difference between the models. * indicates best model.

| #	Model	df	R²m	R²c	AICc	ΔAICc
Cum crabs presence t = 2-4 min ~ …						
1*	Cum crabs presence t = 0-2 min + Cum shrimps presence t = 2-4 min + Treatment + (1 | Position)	5	0.55	0.76	484.7	-
2	Cum crabs presence t = 0-2 min + Treatment + (1 | Position)	4	0.54	0.74	489.5	4.81
3	Cum crabs presence t = 0-2 min + Cum shrimps presence t = 2-4 min + (1 | Position)	4	0.34	0.72	510.0	25.30
null	(1 | Position)	2		0.74	573.7	88.97
Cum shrimps presence t = 2-4 min ~ …						
1	Cum shrimps presence t = 0-2 min + Cum crabs presence t = 2-4 min + Treatment + (1 | Position)	5	0.40	0.90	472.1	-
2*	Cum shrimps presence t = 0-2 min + Cum crabs presence t = 2-4 min + (1 | Position)	4	0.41	0.89	473.9	1.79
3	Cum shrimps presence t = 0-2 min + Treatment + (1 | Position)	4	0.37	0.90	478.4	6.36
null	(1 | Position)	2		0.75	693.1	220.99
Cum crabs eating t = 2-4 min ~ …						
1*	Cum crabs presence t = 2-4 min + (1 | Position)	3	0.58	0.76	273.4	-
2	Cum crabs presence t = 2-4 min + Cum crabs eating t = 0-2 + (1 | Position)	4	0.57	0.76	274.6	1.16
3	Cum crabs presence t = 2-4 min + Treatment + (1 | Position)	4	0.58	0.76	275.8	2.35
null	(1 | Position)	2		0.59	416.1	142.73
Cum shrimps eating t = 2-4 min ~ …						
1*	Cum shrimps presence t = 2-4 min + (1 | Position)	3	0.23	0.70	102.7	-
2	Cum shrimps presence t = 2-4 min + Treatment + (1 | Position)	4	0.23	0.73	104.7	1.98
3	Cum shrimps presence t = 2-4 min + Cum shrimps eating t = 0-2 + (1 | Position)	4	0.23	0.69	104.8	2.10
null	(1 | Position)	2	-	0.44	121.9	19.15
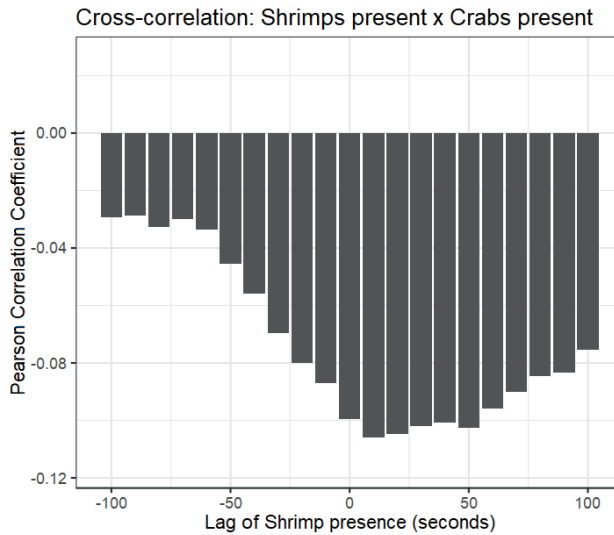
Fig. 5: Cross-correlation of 'shrimp present' and 'crab present' using the time series count data (25 time points per trial, 49 trials). The strongest correlation is found where the shrimp time series were delayed by 10 s relative to the crab time series (lag 10). Strongest correlations were found across positive lag values, suggesting that changes in shrimp presence follow changes in crab presence.

earlier study on shore crabs. Wale and colleagues (2013b) did not find an effect of ship noise on a food item being found by crabs and the time taken to find the food source. However, this experiment was conducted in a relatively small tank (0.12 m2) with a single crab whereas the current experiment was conducted in the wild where it is possibly much more challenging to find a food item. Also, the crabs in the indoor experiment were food deprived for 96 h before the foraging experiment, this might have led to a different trade-off in exploration and risk-taking behaviour than in the current experiment. The researchers did find increased disruption of feeding in the first minute after onset of the ship noise. This was defined as a ≥ 5 s interruption of feeding, freezing, or the animal moving away from the food. We did not find a drop in feeding rate. This might be because the sound that was played back in the current study was much softer (~ 12-32 dB re 1 µPa quieter than in Wale et al. 2013b). This might mean that crabs are only disturbed in their feeding activity above a certain sound level, from a louder or closer source.

There are several possible explanations for the reduced aggregation at a food item by crabs. It may be the case that crabs eating or interacting at a food item produce sound that attracts others (e.g. Coquereau et al., 2016). Such sounds could have been masked in our experiment during the playback of white noise. An alternative explanation of our results is that the playback sound disturbed

them (cf. Chan et al., 2010; Walsh et al., 2017). This might have resulted in reduced exploration and risk-taking behaviour in crabs due to potential masking of sounds from predators (Lima and Dill, 1990). In line with this, it might also be that crabs reduced their overall activity to increase readiness for escape responses (Edmonds et al., 2016). Confirmation of the latter hypothesis would require individual tracking instead of bait-targeted observations.

We did not find evidence that aggregation at a food item and feeding in shrimps were affected by the sound exposure. Shrimp presence (aggregation at a food item) could best be explained by crab presence. In contrast, Filiciotto and colleagues (2016) showed that captive common prawns in a controlled experiment reduced locomotor activity during the playback of boat recordings. Such direct effects might have been overshadowed by the interaction with crabs in the current study, thus highlighting the importance of looking beyond single species effects in sound impact studies (Francis et al., 2009; Shafiei Sabet et al., 2016).

Interaction between crabs and shrimps

We found a negative correlation between crab and shrimp presence. The cross-correlation showed that crab presence correlates best with later shrimp presence, this supported our expectation that crabs were deterring shrimps. Competition and interaction between species can be found throughout the animal kingdom. For example, Stahl and colleagues (2006) found that European brown hares (*Lepus europaeus*) naturally selected high biomass swards to forage on. However, after experimentally excluding geese from swards, hares foraged more on swards with both high plant quality and high biomass. Another prominent example by Estes and colleagues (1998) concerned killer whales (*Orcinus orca*) shifting prey choice towards sea otter (*Enhydra lutris*), which undermined the sea otters' control of the dominant herbivores, sea urchins (Echinoidea). As a consequence, the flourishing sea urchins overgrazed the kelp forest which dramatically changed the local ecosystem (Estes and Palmisano, 1974; Estes et al., 1998).

When interacting species respond differently to human influences, competitive balances between species may also shift (Tylianakis et al., 2008; Worm and Paine, 2016). Previous research has shown that anthropogenic sound can reduce species richness in avian communities, but may also indirectly facilitate breeding success of particular species because of lower abundance of a nest predator species (Francis et al., 2009; Slabbekoorn and Halfwerk, 2009). This avian example concerned a case of predator-release, while the current crustacean example concerns competitive release between two species competing over the same resources. The sound exposures released competition by the dominant species allowing the subordinate species to make use of the resource. Competitive

release is often shown in long term-studies by contrasting shifts in distribution (e.g. Anderson et al., 2002). We here provide evidence for a more short-term release in competition mediated by a species-specific behavioural response to sound exposures.

Revealing such interactions between species shows that single-species studies alone are not sufficient for determining impact of sound as there may be (local) community effects (Francis et al., 2009; Slabbekoorn and Halfwerk, 2009; Shafiei Sabet et al., 2016). Besides the importance of in situ studies, we also think that conducting controlled studies on captive animals can help in understanding processes that are important to free-ranging animals in the real world (Slabbekoorn, 2014). For example, it would be interesting to conduct a number of parallel exposure trials to study the effects of sound solely on crab food aggregation and eating, solely on shrimp food aggregation and eating, and on both species at the same time. In such a controlled study, it is likely possible to follow individual animals throughout entire trials, which should increase insights into the underlying mechanisms of our current results. In this way, synergy through studies in the lab and the wild will help in gaining understanding of biological processes and thereby increase the validity of sound impact assessments.

Conclusions

Our study provides evidence for the fact that artificial sound exposures can decrease the number of crabs aggregating at a food item and provide indirect benefits for shrimps via competitive release. This highlights the importance to study the potential impact of anthropogenic sound in situ and consider cross-species interactions. We believe it is especially important to study effects at and among lower trophic levels (e.g. invertebrates) as subtle effects here may accumulate at higher trophic levels (e.g. fish or marine mammals). We like to stress that our study provides a proof of concept and that our in situ approach strengthens behavioural and acoustic validity. However, our set-up does not provide insight into ecological relevance in absolute sense and more sound studies are needed for a better understanding of individual and population consequences of changes in multi-trophic interactions due to changes in underwater soundscapes.

Ethical statement

There are no legal requirements for studies involving decapod crustaceans and molluscs in The Netherlands. Our experiment likely only caused short periods

of mild discomfort in crabs and shrimps, as we observed free-ranging animals and only exposed them to short-lasting exposures with moderate sound levels. The sound exposure and food provisioning in our study are therefore unlikely to have caused any welfare problems to either species.

Data Accessibility

All data used for the analyses reported in this article is available from the Zenodo Repository, DOI:10.5281/zenodo.1403042.

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References
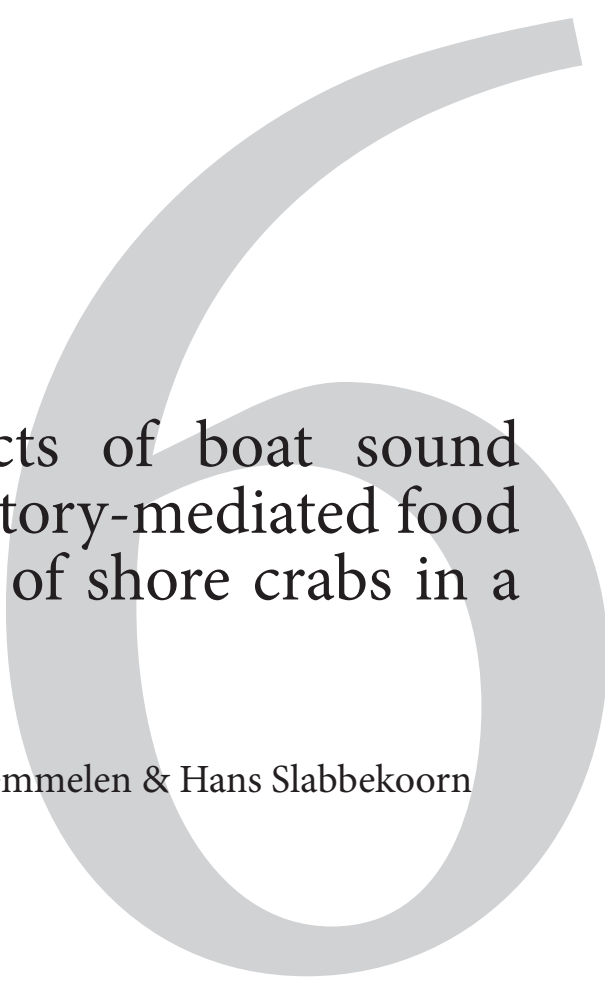
Anderson, R. P., Peterson, a. T. and Gómez-Laverde, M. (2002). Using niche-based GIS modeling to test geographic predictions of competitive exclusion and competitive release in South American pocket mice. Oikos 98, 3–16.

Andrew, R. K., Howe, B. M., Mercer, J. A. and Dzieciuch, M. A. (2002). Ocean ambient sound: Comparing the 1960s with the 1990s for a receiver off the California coast. Acoust. Res. Lett. Online 3, 65–70.

Au, W. W. L. and Banks, K. (1998). The acoustics of the snapping shrimp *Synalpheus parneomeris* in Kaneohe Bay. J. Acoust. Soc. Am. 103, 41–47.

Barton, K. (2016). MuMIn: Multi-Model Inference.

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67,.

Chan, A. A. Y. H., David Stahlman, W., Garlick, D., Fast, C. D., Blumstein, D. T. and Blaisdell, A. P. (2010). Increased amplitude and duration of acoustic stimuli enhance distraction. Anim. Behav. 80, 1075–1079.

Coquereau, L., Grall, J., Chauvaud, L., Gervaise, C., Clavier, J., Jolivet, A. and Di Iorio, L. (2016). Sound production and associated behaviours of benthic invertebrates from a coastal habitat in the north-east Atlantic. Mar. Biol. 163, 1–13.

Edmonds, N. J., Firmin, C. J., Goldsmith, D., Faulkner, R. C. and Wood, D. T. (2016). A review of crustacean sensitivity to high amplitude underwater noise: Data needs for effective risk assessment in relation to UK commercial species. Mar. Pollut. Bull.

108, 5–11.

Estes, J. A. and Palmisano, J. F. (1974). Sea Otters: Their Role in Structuring Nearshore Communities. Science (80-.). 185, 1058–1060.

Estes, J. A., Tinker, M. T., Williams, T. M. and Doak, D. F. (1998). Killer Whale Predation on Sea Otters Linking Oceanic and Nearshore Ecosystems. Science (80-.). 282, 473–476.

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Stefano, V. Di, Mazzola, S. and Buscaino, G. (2014). Behavioural and biochemical stress responses of *Palinurus elephas* after exposure to boat noise pollution in tank. Mar. Pollut. Bull. 84, 104–114.

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Arizza, V., de Vincenzi, G., Grammauta, R., Mazzola, S., et al. (2016). Underwater noise from boats: Measurement of its influence on the behaviour and biochemistry of the common prawn (*Palaemon serratus*, Pennant 1777). J. Exp. Mar. Bio. Ecol. 478, 24–33.

Francis, C. D., Ortega, C. P. and Cruz, A. (2009). Noise Pollution Changes Avian Communities and Species Interactions. Curr. Biol. 19, 1415–1419.

Hawkins, A. D. and Popper, A. N. (2016). A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES J. Mar. Sci. J. du Cons. fsw205.

Hildebrand, J. A. (2009). Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395, 5–20.

Hughes, A. R., Mann, D. A. and Kimbro, D. L. (2014). Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. Proc. Biol. Sci. 281, 20140715.

Jeffs, A., Tolimieri, N. and Montgomery, J. C. (2003). Crabs on cue for the coast: The use of underwater sound for orientation by pelagic crab stages. Mar. Freshw. Res. 54, 841–845.

Kunc, H. P., McLaughlin, K. E. and Schmidt, R. (2016). Aquatic noise pollution: Implications for individuals, populations, and ecosystems. Proc. R. Soc. B Biol. Sci. 283,.

Lagardère, J. P. (1982). Effects of noise on growth and reproduction of *Crangon crangon* in rearing tanks. Mar. Biol. 71, 177–185.

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. Methods Ecol. Evol. 7, 573–579.

Lima, S. L. and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68, 619–640.

Lovell, J. M., Findlay, M. M., Moate, R. M. and Yan, H. Y. (2005). The hearing abilities of the prawn *Palaemon serratus*. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 140, 89–100.

Morley, E. L., Jones, G. and Radford, a. N. (2013). The importance of invertebrates when considering the impacts of anthropogenic noise. Proc. R. Soc. B Biol. Sci. 281, 20132683–20132683.

Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142.

Neo, Y. Y., Hubert, J., Bolle, L. J., Winter, H. V. and Slabbekoorn, H. (2018). European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environ. Pollut. 239, 367–374.

R Core Team (2016). R: A Language and Environment for Statistical Computing.

Radford, C. A., Jeffs, A. G. and Montgomery, J. C. (2007). Directional swimming behavior by five species of crab postlarvae in response to reef sound. Bull. Mar. Sci. 80, 369–378.

Regnault, N. and Lagardère, J.-P. (1983). Effects of ambient noise on the metabolic level of *Crangon crangon* (Decapoda, Natantia). Mar. Ecol. Prog. Ser. 11, 71–78.

Schein, H. (1975). Aspects of the aggressive and sexual behaviour of *Alpheus heterochaelis* say. Mar. Behav. Physiol. 3, 83–96.

Shafiei Sabet, S., Neo, Y. Y. and Slabbekoorn, H. (2016). Impact of Anthropogenic Noise on Aquatic Animals: From Single Species to Community-Level Effects. In The Effects of Noise on Aquatic Life II (ed. Popper, A. N.) and Hawkins, A.), pp. 957–961.

Simpson, S. D., Radford, A. N., Tickle, E. J., Meekan, M. G. and Jeffs, A. G. (2011). Adaptive avoidance of reef noise. PLoS One 6, 2–6.

Slabbekoorn, H. (2014). Aiming for Progress in Understanding Underwater Noise Impact on Fish: Complementary Need for Indoor and Outdoor Studies. In The Effects of Noise on Aquatic Life II (ed. Popper, N. A.) and Hawkins, A.), pp. 1057–1065. New York, NY: Springer New York.

Slabbekoorn, H. and Halfwerk, W. (2009). Behavioural Ecology: Noise Annoys at Community Level. Curr. Biol. 19, R693–R695.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C. and Popper, A. N. (2010). A noisy spring: The impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427.

Solan, M., Hauton, C., Godbold, J. a., Wood, C. L., Leighton, T. G. and White, P. (2016). Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep. 6, 20540.

Stahl, J., Van Der Graaf, A. J., Drent, R. H. and Bakker, J. P. (2006). Subtle interplay of competition and facilitation among small herbivores in coastal grasslands. Funct. Ecol. 20, 908–915.

Tylianakis, J. M., Didham, R. K., Bascompte, J. and Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. Ecol. Lett. 11, 1351–1363.

Wale, M. a., Simpson, S. D. and Radford, A. N. (2013a). Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. Biol. Lett. 9, 20121194.

Wale, M. a., Simpson, S. D. and Radford, A. N. (2013b). Noise negatively affects foraging and antipredator behaviour in shore crabs. Anim. Behav. 86, 111–118.

Walsh, E. P., Arnott, G. and Kunc, H. P. (2017). Noise affects resource assessment in an invertebrate. Biol. Lett. 13,.

Williams, R., Wright, A. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., Clark, C. W., Cullis-Suzuki, S., Dakin, D. T., Erbe, C., et al. (2015). Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24.

Worm, B. and Paine, R. T. (2016). Humans as a Hyperkeystone Species. Trends Ecol. Evol. 31, 600–607.

No negative effects of boat sound playbacks on olfactory-mediated food finding behaviour of shore crabs in a T-maze

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Abstract

Anthropogenic noise underwater is increasingly recognized as a pollutant for marine ecology, as marine life often relies on sound for orientation and communication. However, noise may not only interfere with processes mediated through sound, but also have effects across sensory modalities. To understand the mechanisms of the impact of anthropogenic sound to its full extent, we also need to study cross-sensory interference. To study this, we examined the effect of boat sound playbacks on olfactory-mediated food finding behaviour of shore crabs (*Carcinus maenas*). We utilized opaque T-mazes with a consistent water flow from both ends towards the starting zone, while one end contained a dead food item. In this way, there were no visual or auditory cues and crabs could only find the food based on olfaction. We did not find an overall effect of boat sound on food finding success, foraging duration or walking distance. However, after excluding deviant data from one out of the six different boat stimuli, we found that crabs were faster to reach the food during boat sound playbacks. These results, with and without the deviant data, seem to contradict an earlier field study in which fewer crabs aggregated around a food source during elevated noise levels. We hypothesise that this difference could be explained by a difference in hunger level, with the current T-maze crabs being hungrier than the free-ranging crabs. Hunger level may affect the motivation to find food and the decision to avoid or take risks, but further research is needed to test this. In conclusion, we did not find unequivocal evidence for a negative impact of boat sound on the processing or use of olfactory cues. Nevertheless, the distinct pattern warrants follow up and calls for even larger replicate samples of acoustic stimuli for noise exposure experiments.

Introduction

Over the last decades, increasing numbers of anthropogenic sound sources have been contributing to the marine soundscape (Andrew et al., 2002; Hildebrand, 2009). This has led to concerns about the effects of anthropogenic sound on the environment as many marine animals rely on sound for orientation and communication (Carroll et al., 2017; Slabbekoorn et al., 2010). However, noise may not only hinder processes mediated through sound, but may also have effects across sensory modalities (Halfwerk and Slabbekoorn, 2015). Such effects may be salient in a crab species that relies on chemical cues for food-finding. Relatively few sound impact studies have been done on effects of sound on crabs and other invertebrates (Williams et al., 2015). Yet, the abundance of invertebrates is critical for higher trophic levels as food or through ecosystem services (Morley et al., 2014; Solan et al., 2016).

Auditory perception in crustaceans, including crabs, is still poorly understood, but is likely to be important for their survival and reproduction. They are thought to detect particle motion through mechanical stimulation of setae (hair-like) cells on the body-surface, chordotonal organs, and statocysts in contact with sensory hairs (Popper et al., 2001). Crustaceans lack gas-filled cavities such as a swim bladder and are assumed not to be sensitive to sound pressure, and they are therefore, most sensitive to low-frequency sound (Edmonds et al., 2016; Popper et al., 2001). Mud crabs (*Panopeus* spp.) have shown to be most sensitive to the lowest tested frequency (resp. 75 Hz) with decreasing sensitivity up to at least the highest tested frequency (1600 Hz; Hughes et al., 2014). There is evidence that natural sound is important to crabs: in their pelagic stages they were shown to use sound for orientation and navigation (Jeffs et al., 2003; Radford et al., 2007), and adult mud crabs changed foraging behaviour during the playback of predatory fish vocalisations (Hughes et al., 2014).

Elevated sound levels can have a range of effects on decapod crustaceans including crabs. Physiologically, sound exposures have been shown to increase oxygen consumption, exhibit changes in stress-related biochemistry, reduce growth and delay reproduction (Filiciotto et al., 2014; Filiciotto et al., 2016; Lagardère, 1982; Regnault and Lagardère, 1983; Wale et al., 2013a). Behaviourally, elevated sound levels have been shown to alter responses to a simulated predator, decrease resource assessment, change locomotor activity, decrease the amount of inter-individual encounters, change use of a shelter, disrupt feeding, and reduce aggregation at a food item (Chan et al., 2010a; Filiciotto et al., 2014; Filiciotto et al., 2016; Hubert et al., 2018; Wale et al., 2013b; Walsh et al., 2017). The underlying mechanism for most of the effects that have been found remain

however unknown.

Olfactory perception is considered to be an important sensory modality in decapod crustaceans (Krieger et al., 2012; Weissburg and Zimmer-Faust, 1994) and may be interfered by sound exposures. Crabs are shown to locate prey and conspecifics by sensing the direction of the water flow that contains olfactory cues of either prey or conspecifics (Weissburg and Zimmer-Faust, 1994; Zimmer-Faust, 1989). Even though there is no physical interference between the two modalities (e.g. sound and odour), simultaneous perception in an animal may hinder processing and interpretation of the stimuli through so-called cross-sensory interference (Halfwerk and Slabbekoorn, 2015). Studies across sensory modalities are therefore needed to truly understand the effects of noise pollution.

Cross-sensory interference has been found in a variety of species. The anti-predator response to a visual predator or a chemical alarm cue reduced during sound exposure in several fish species (Hasan et al., 2018; Simpson et al., 2014; Voellmy et al., 2014). Similarly, wild dwarf mongooses (*Helogale parvula*) responded less strong to predator faecal presentations during noise playbacks (Morris-Drake et al., 2016). Foraging behaviour has also shown to be affected across modalities. In great tits (*Parus major*), noise exposure increased approach and attack latencies of visually cryptic prey (Halfwerk and Van Oers, 2020). Several studies examined cross-sensory interference of sound exposures on hermit crabs, taxonomically more related to the current study species. Caribbean hermit crabs (*Coenobita clypeatus*) responded later to a silently approaching simulated predator during boat noise exposure (Chan et al., 2010b). Common hermit crabs (*Pagurus bernhardus*) invested less time in shell selection during white noise exposure (Walsh et al., 2017). Lastly, less Acadian hermit crabs (*Pagurus acadianus*) aggregated at the source of a chemical cue, indicative of new shells, during generation of impulsive sound (Roberts and Laidre, 2019). So, it may well be that the reduced aggregation at a food item by shore crabs during white noise exposures in our previous study (Hubert et al., 2018), can also be explained by cross-sensory interference due to the elevated noise levels.

Previous studies exploited the olfactory-mediated localization capability of crabs to assess their food and mate preferences using two-choice set-ups (Ekerholm, 2005; Hardege et al., 2011; Shelton and Mackie, 1971). A two-choice set-up, such as a T-maze, can be used to present a subject with two water flows with different chemical cues to assess a preference or capability to discriminate between cues. By visually blocking the source of the olfactory cues in the water flow, it can be assured that decision-making is based on olfactory perception alone (Zimmer-Faust, 1989). Such a set-up may prove to be useful in studying cross-sensory

interference of acoustic stressors on crustaceans.

In the current study, we explored the effect of experimental playback of boat sound on olfactory-mediated food finding behaviour of shore crabs. In the experiment, crabs were allowed to forage in a T-maze that contained a food item at the end of one of the arms. The food item was not visible from the intersection, but a water flow from the end of both arms towards the starting zone enabled the crabs to find food based on olfactory cues. We aimed at answering the following questions: (1) Is food finding success in crabs affected by the playback of boat sound? (2) Is food finding efficiency in crabs affected by the playback of boat sound?

Materials and methods

Study subjects

We used 239 wild-caught shore crabs (*Carcinus maenas*) with carapace widths ranging from 1.0 to 2.5 cm. The crabs were manually collected during low tides from rocks in the tidal area of the Jacobahaven, a cove in the Oosterschelde sea arm, the Netherlands. Before being used in the experiment, the crabs were group-housed in 1.7 L plastic boxes, stacked in a larger stock tank (1.2 x 1.0 x 0.6 m; L x W x H) that had a continuous inflow of fresh seawater and air supply. Each 1.7 L plastic box housed four crabs of similar size and also contained empty shells for shelter. Part of the walls of the plastic box was replaced by mesh to ensure fresh oxygen rich seawater to flow in. The crabs were housed in captivity for a maximum of 1.5 weeks and were fed with mussels (*Mytilus edulis*, without shell) on the day of catching and three days prior to the experiment. In this way, we standardized hunger-levels and made sure that each crab was familiar with the food source.

Experimental arena

The trials were performed using T-mazes with a continuous water flow from the ends of both arms to the starting zone. We created the two identical T-mazes from white acrylic sheets (0.2 cm thick) and submerged them partially (for 3.5 cm) in a tank that was identical to the stock tank. Holes at the ends of the arms allowed fresh seawater to flow in. Two tubes in the starting zone connected the water in the mazes to external boxes with a lower water level, allowing the water to flow out (fig. 1). The same principle applied to another pair of tubes connecting the water in the external boxes to a lower situated drain. We used the pumps in the external boxes to initialise the water flow. Once established, the water flow was maintained based on hydrostatic pressure, and the pumps

were turned off. The underwater speaker was placed between the two mazes on a 10 cm high frame at the tank floor. In this way, we maximized the distance between the speaker and the mazes to achieve a more homogeneous sound field within the mazes. We used a stationary hydrophone to record all trials to verify that sound exposure was played back correctly and no additional disturbance had taken place. All trials were recorded with a video camera (Everio GZ-R415, JVC, Japan) that was placed above the tank along with a LED tube. This set-up was covered up with black plastic sheets to ensure equal light conditions among trials and to prevent visual disturbance from the researcher or other objects in the direct vicinity.

Experimental design

During the experiment, individual crabs were allowed to forage on an opened mussel (*Mytilus edulis*) of 3.0 to 4.5 cm. At the start of each trial, we placed a mussel at the end of one of the two arms of a maze. After this, we introduced a crab in a maze by gently pouring the crab in the starting zone and closing the zone with a transparent lid to avoid escapes. This was done within a 1 min period, during which ambient sound was played back and thereafter linearly crossfaded (10 s) into the 10 min playback of a boat or a different ambient track. The hatches in front of the starting zones were lifted 5 min after the crossfade, allowing the crab to emerge and find the mussel during the remaining 5 min. The hatches
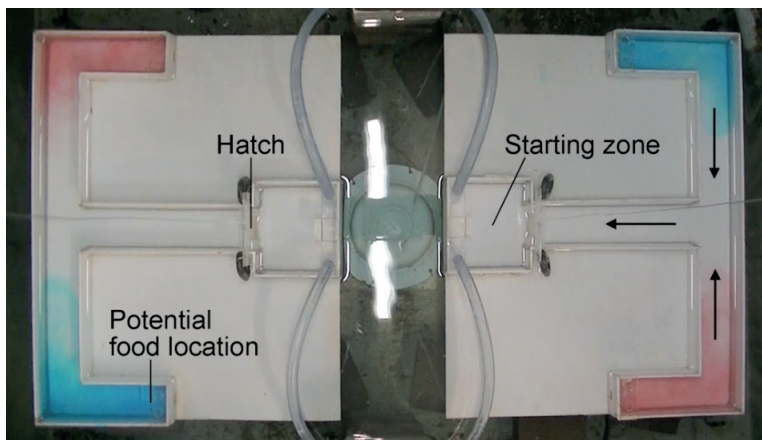
Fig. 1: A video still of the two T-mazes with the speaker in the middle. Crabs were introduced in the starting zone and after lifting the hatch, they could freely move through the T-maze. One of the ends contained a food item which could be found thanks to olfactory cues in the consistent water flow from both ends of the maze to the starting zone. The arrows indicate the direction of the water flow, we used colour dyes (blue and red) for demonstration purposes only (and not during the experiments with crabs). A movie of this can be found online (see Data accessibility). The dimensions of the mazes can be found in figure 3.

were opened from outside the plastic sheets around the experimental set-up by pulling wires. We tested two crabs simultaneously and each pair of trials was filmed for later analysis. After a pair of trials, we determined the crabs' sex and measured their carapace width to be able to examine potential differences sex and size differences in behaviour and a potential size-dependency in response to the sound. We also examined whether the crabs had a soft carapace due to recent moulting, these individuals were excluded from further analysis because of a potential difference in food-finding motivation. Each crab was used in a trial once. Between trials, we syphoned the mazes to clean them and placed new mussels in the mazes. We counterbalanced the order of the treatments and the position of the mussel.

Sound exposure

During the trials, we exposed the crabs to a boat or ambient sound treatment. Boat sound is mostly produced by the on board machinery, water displacement, and cavitation (formation and collapse of bubbles) at propeller blades (Popper and Hawkins, 2019; Ross, 1976). Ambient sound in marine waters and coastal crab habitat mainly originates from water surface roughness as a function of weather conditions and distant shipping (Carey and Browning, 1988; Wenz, 1962). The playback tracks were constructed using seven ambient recordings and six recordings of different boats (3-~30 m long), all at different locations across the IJ (river) and Oosterscheld, the Netherlands. From these calibrated recordings, we selected windows of 35 to 50 s with relatively consistent amplitude and looped them into longer tracks using 3 s linear crossfades. We bandpassed the tracks with 100-1600 Hz filters to anticipate on low-frequency speaker limitations and based on expected high-frequency hearing limits of our study subjects, based on data from another crab species (Hughes et al., 2014). For the actual playbacks, each track started with 1 min of ambient sound and linearly crossfaded in 10 s to a 10 min boat sound or ambient sound from a different location than the first min of ambient sound. Sound treatments were played back with an underwater speaker (UW30, Lubell labs, US), from a recorder (PMD620, Marantz, Japan), through an amplifier (M033N, Kemo, Germany).

We used playback tracks that were constructed using seven ambient recordings and six boat recordings to increase external validity and to reduce possible confounding effects from a single recording. If a single boat sound would have been used, results would only be relevant for this single recording of this particular boat, while results from multiple stimuli better resemble the possible acoustic variation of boat sound in general. The use of more than six boats reduces potential issues with the well-known methodological flaw of pseudoreplication (Hurlbert, 1984; Slabbekoorn and Bouton, 2008). For this

study, it was explicitly not the goal to explore the effect of different boat types or boat sound characteristics. In do this, multiple boat stimuli would have been needed for each boat type or sound characteristic of interest. For linear correlations between sound characteristics and crab response levels, much more than six boat stimuli or highly controlled stimuli (e.g. Hubert et al., 2020) would have been needed. Boat recordings vary in numerous ways in frequency distribution, amplitude, and temporal pattern. With limited playback tracks, it is not possible to disentangle different characteristics from one another.

We recorded all playbacks in the experimental tank using a calibrated hydrophone (96-min, HTI, US) to gain insight into the sound levels and spectra for the exposure conditions of the crabs. Shore crabs are likely sensitive to the particle motion component of sound, rather than sound pressure. The acoustic conditions, in terms of the ratio between particle motion and acoustic pressure, and the directionality of sound velocity in small tanks is not comparable to the natural environment of aquatic animals. This is caused by the proximity of the water surface, tank walls, and bottom (Rogers et al., 2016). We just measured sound pressure levels because we did not have a particle motion sensor that is small enough to be expected to reliably measure particle motion in our setup. We did not measure the sound pressure levels at the position of the crabs (3.5 cm below the surface) but at a 15 cm depth because the water surface is a pressure release boundary. This means that sound pressure levels (SPL) decrease close to the water surface whereas particle motion levels increase. Therefore, SPL measurements at 15 cm depth are a better indication for the sound levels the crabs were exposed to than when we would have measured them closer to the surface. Importantly, these measurements do not provide absolute data on exposure conditions, but rather make exposure conditions repeatable and comparable. The geometric mean rms SPL (75-1600 Hz bandpassed) of the measurements of boat playbacks was 123.4 dB re 1 µPa and 103.9 dB re 1 µPa of the ambient playbacks (figure 2). We made additional recordings of a sweep tone (100 – 4000 Hz) at different locations in the tank and with and without the maze to gain insight into the sound propagation in the tank (supplementary material I).

Behavioural observation

We analysed 239 trials: 117 trials with the ambient treatment, and 122 with the boat treatment. In blind scoring sessions (without audio), we manually scored whether the crab reached the food, the initial direction at the intersection, the time to emergence from the starting zone, and time from emergence till first physical contact with the mussel. Next, we used custom made video tracking software (depending on Python 3, FFmpeg and OpenCV 3.4.3; Bradski, 2000) to track the walking patterns of the crabs. We corrected the tracked positions

for camera angle, and only recognized new positions after a threshold of 2 cm was passed, in order to ignore movements of a single claw or leg (figure 3). We used positions from just after leaving the starting zone till the crab reached close
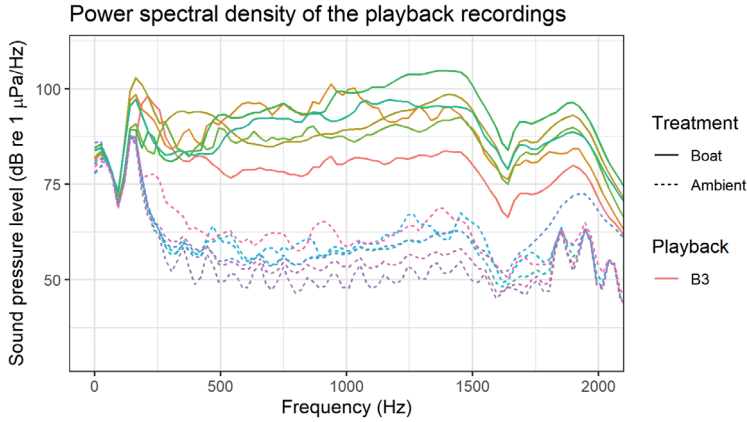
Fig. 2: Power spectral density (window length: 2048, window type: Hann) of all boat and ambient playbacks. The colours indicate the individual playbacks, the solid lines are the boat playbacks and the dotted lines are the ambient playbacks. We have highlighted boat playback B3 in the legend because this playback seemed to have yielded different results than the other boat playbacks.
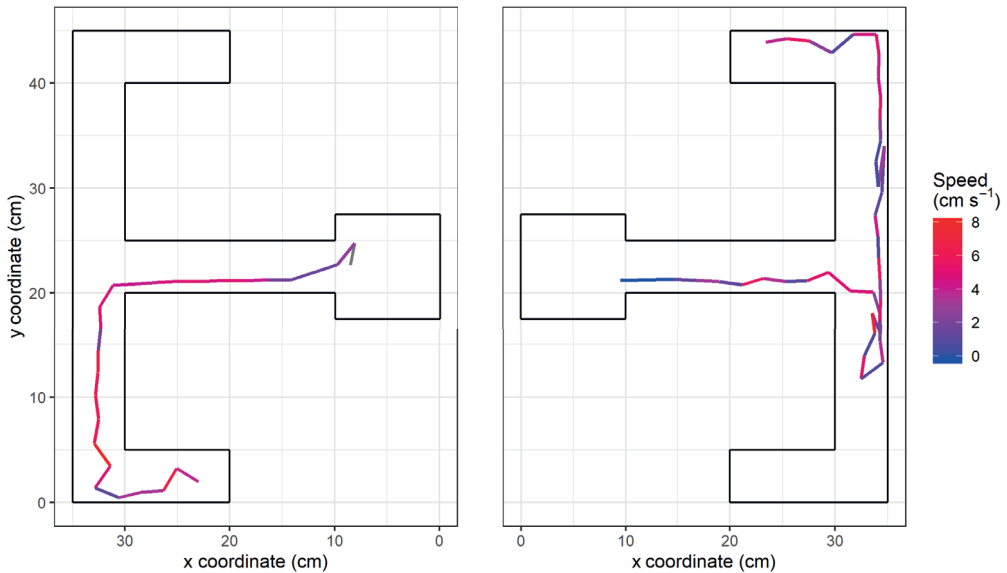
Fig. 3: Walking tracks of individuals in two simultaneous trials, from the opening of the hatches till the individuals reached the food. A new position was only recognized after a threshold of a 2 cm distance relative to the previous recognized position was passed. So, the speed – indicated by the colour – is the average speed over the previous ~ 2 cm.

proximity to the food (both with a 2.0 cm margin). Using the tracked walking paths, we determined the total walking distance.

Statistics

We used the observations from the videos as response variables in generalized linear models (GLMs) in R (R Core Team, 2016). For the response variables 'reached food' (Y/N) and 'Initial direction' (Towards food/Away from food/None), we used a binomial error distribution, and for 'Emergence time', 'Foraging time' and 'Walking distance', we used a negative binomial error distribution. We used 'Crab sex', 'Crab size', 'Treatment' (Ambient/Boat), and the interaction between the latter two as covariates in the full model. The best model was selected based on lowest AICc score (using the function Dredge, R package MuMIn; Barton, 2016), if 'Treatment' was not part of the best model, we kept it in the final model anyway because this was our variable of interest. To determine the effect and significance of the covariates, we ran the final models. Lastly, we determined the 95% confidence intervals of the intercept and slope (using the function confint, R package MASS; Venables and Ripley, 2002).

Results

225 of 239 crabs emerged from the starting zone and reached the food item, 5 did not emerge from the starting zone and 9 did emerge, but did not reach the food (fig. 4). There was no significant effect of sound treatment on the number of individuals that reached the food (Intercept: 2.75, CI: 2.07 – 3.62; Boat treatment: 0.04, CI: -1.06 – 1.15; p-value: 0.94; fig. 4a) or their initial direction at the intersection (Intercept: 2.75, CI: 2.07 – 3.62; Boat treatment: 0.21, CI: -0.92 – 1.37; p-value: 0.72; fig. 4b).

The size of the crabs was significantly negatively correlated with both emergence time (Intercept: 4.24, CI: 3.59 – 4.90; Crab size slope: -0.62, CI: -1.02 – -0.21; p-value: < 0.01; fig. 6a) and foraging time (Intercept: 4.31, CI: 3.90 – 4.73; Crab size slope: -0.37, CI: -0.62 – -0.11; p-value: < 0.01; fig. 6b). This means that larger crabs were faster than smaller crabs in emerging and reaching the food. However, there was no significant effect of the sound treatment on emergence time (Intercept: 4.24, CI: 3.59 – 4.90; Boat treatment: 0.18, CI: -0.08 – 0.43; p-value: 0.17; fig. 5a), foraging time (Intercept: 4.31, CI: 3.90 – 4.73; Boat treatment: -0.11, CI: -0.27 – 0.04; p-value: 0.15; fig. 5b), and walking distance (Intercept: 4.15, CI: 4.07 – 4.22; Boat treatment: -0.05, CI: -0.16 – 0.05; p-value: 0.33; fig. 5c).

Visual inspection of the foraging times per playback track indicated that the effect of a single boat playback track deviated from the other boat tracks (fig. 7).

We performed a parallel analysis, excluding the data from this particular boat track, and found that crabs exposed to the other five boat stimuli were affected by the sound and had a significantly lower foraging time (Intercept: 4.22, CI: 3.80 – 4.64; Boat treatment: -0.18, CI: -0.34 – -0.02; p-value: 0.03). The noisy conditions appeared not to interfere, but made the crabs reach the food more quickly. Excluding this boat stimulus did not yield different results for the walking distance of the crabs (Intercept: 4.13, CI: 4.06 – 4.21; Boat treatment: -0.07, CI: -0.18 – 0.04; p-value: 0.20).
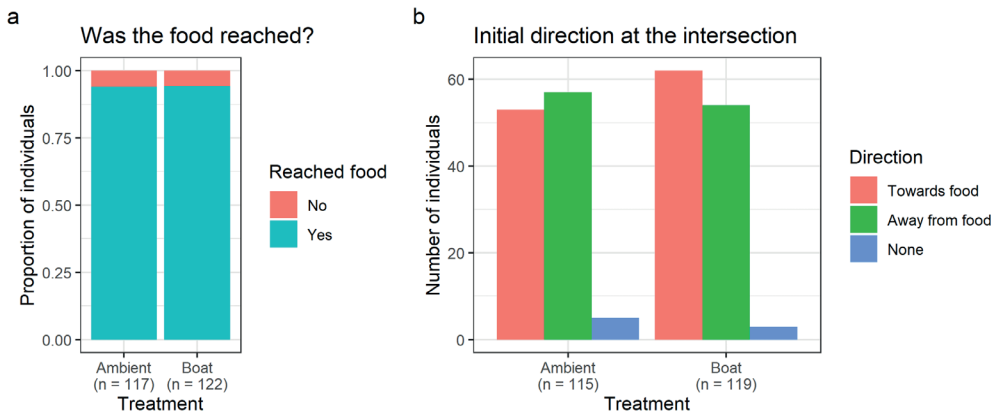
Fig. 4: (a) The proportion of individuals that reached the food item before the end of the trial, in both ambient as boat playback conditions. (b) The number of emerged individuals that initially moved towards, away from the food at the intersection, and did not reach the intersection.
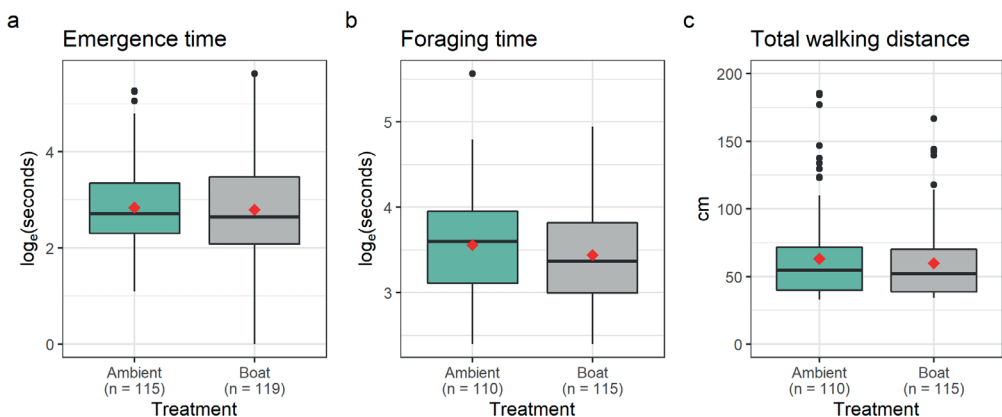
Fig. 5: (a) Emergence time, which is the time individuals took, from the opening of the hatch, to leave the starting zone. The red diamonds indicate the means. (b) Foraging time, which is the time the individuals took, from leaving the starting zone, to reach the food. (c) Total walking distance of the individuals, from leaving the starting zone till reaching the food.
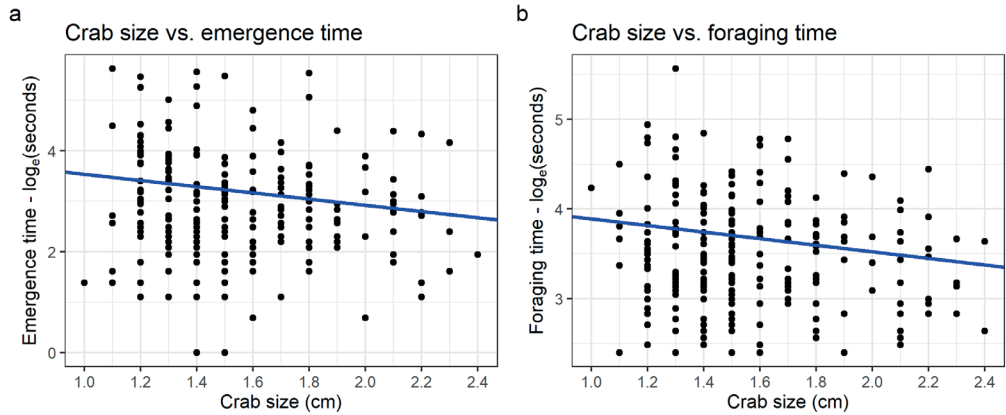
Fig. 6: (a) Correlation between crab size and emergence time. (b) Correlation between crab size and foraging time. The points indicate single individuals, and the blue trendline indicates the significant negative correlations between crab size and (a) emergence time and (b) foraging time. Note that the y-axes of both plots are not identical.
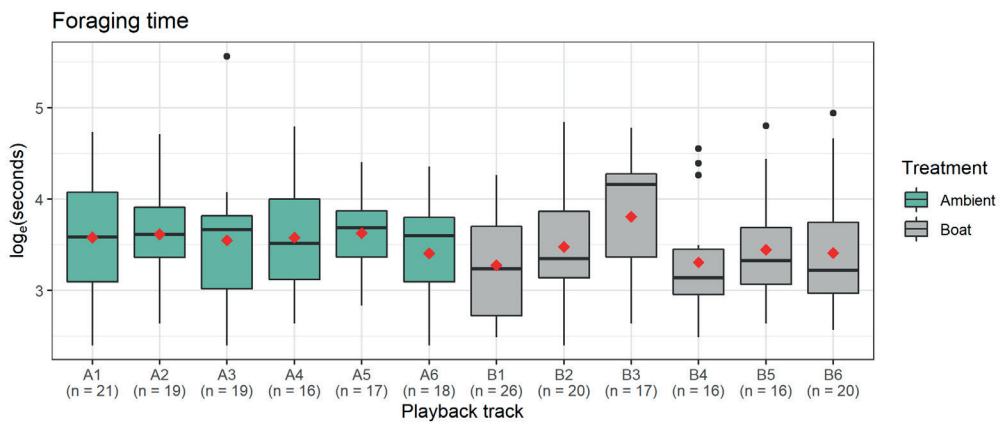
Fig. 7: Foraging duration for each of the playback tracks in box-and-whisker plots, indicating the median, first and third quartile, min and max excluding outliers, and outliers. The red diamonds indicate the means. There was no significant overall effect of sound treatment, although median values for all six ambient stimuli were higher than the median values of all but one boat sound stimulus. When excluding the data from the deviating boat sound stimulus B3, crabs under noisy boat conditions were not slower, but reached the food more quickly than under ambient conditions.

Discussion

In the current study, we allowed individual shore crabs in a T-maze to forage on an opened mussel, while exposing the crabs to a boat sound playback or an

ambient control. Our results demonstrated that food finding success and foraging efficiency of crabs in a T-maze was not affected by the boat sound playback. However, when we excluded deviant data from one out of the six boat playbacks, we found that crabs were faster to arrive at the food item during boat playbacks than crabs exposed to ambient playbacks. In any case, boat playbacks did not lead to increased foraging duration, which suggests that olfactory mediated food finding was not negatively affected by boat noise playback in this study. Additionally, we found that larger crabs were faster to emerge and to reach the food, but we found no size dependent response to sound.

Foraging behaviour during sound exposures

Overall, boat sound exposures did not affect food finding success, foraging duration, and walking distance during foraging. However, after exclusion of the results for the boat stimulus B3, the foraging duration of crabs that were exposed to boat sound appeared to be shorter. Two earlier studies examined the effect of sound exposures on the foraging behaviour of shore crabs. Wale et al. (2013b) also found no effect on food finding success and foraging time in a tank. Food finding might have been relatively easy in that study because the experimental tank was relatively small (30 x 30 cm; L x W), with the food item in the centre, and there were no visual or physical blockages between the crab and food. In the current study, the crabs had to walk at least ~ 45 cm and could only find the food based on olfactory cues.

However, the results of both studies (Wale et al., 2013b and the current study) are not in line with the results of Hubert et al. (2018), where we found reduced aggregation at a food item in the field. Differences between field and tank studies may be explained by the sound field, from which the particle motion component is typically not mapped. Crabs are thought to be sensitive to particle motion (Popper et al., 2001), for which levels and direction in tanks are influenced by the proximity of the water surface, tank walls, and bottom, which yields different conditions than in the natural environment (Rogers et al., 2016). For these foraging studies specifically, differences between the field and tank studies may also be explained by the hunger level of the study subjects. Although we do not know the hunger level of the free-ranging crabs, in both captive studies (Wale et al 2013b and our current study), crabs were food-deprived for three days before a trial, which may change the motivation to find food and take risks.

Various studies have shown that food-deprivation influences behaviour including foraging. Food-deprived individual three-spined sticklebacks (*Gasterosteus aculeatus*) were more likely to initiate predator inspection visits and had higher feeding rates than well-fed shoal mates (Godin and Crossman, 1994). Hungry

fifteen-spined sticklebacks (*Spinachia spinachia*) were more likely than partially satiated individuals to feed at a food source associated with predator threat (so hungry sticklebacks were shown to be less risk-averse) (Croy and Hughes, 1991). Well-fed crayfish (*Procambarus clarkii*) close to a food odour, were more likely to respond to a moving shadow by a tail-flip escape response, whereas food-deprived individual were more likely to freeze, potentially a riskier response (Schadegg and Herberholz, 2017). For the current study species, it has been shown that increased hunger levels can lead to decreased prey selectivity (Jubb et al., 1983) resulting in greater variation in prey size, including suboptimal sizes (Morris, 2008). So, it may well be that the lack of an overall negative effect of sound exposure on food finding success and foraging time in the current study and in Wale et al (2013b), in contrast to Hubert et al (2018), can be explained by the increased hunger levels, but future research has to confirm this hypothesis.

After exclusion of the data from boat stimulus B3, we found a significantly lower foraging duration during boat sound playbacks, but not a lower walking distance during foraging. This suggests that the crabs walked faster during boat sound, rather than navigating more efficiently. Crabs may have walked faster during boat sound playbacks to reduce predation risk or due to elevated stress levels, but they nevertheless had enough motivation to find the food, both in line with the previously stated hypothesis. Humans have also been shown to walk faster under traffic noise conditions, an effect that is possibly related to stress (Franěk et al., 2018). However, based on the current study, we cannot draw strong conclusions on the subjects' stress levels, anti-predator response, or a combination of both.

Cross-sensory interference

One of the major goals of this study was to examine whether boat sound playbacks interfered with olfactory-mediated food finding. This would be an indication of cross-sensory interference; meaning that increased capacity of the nervous system to process the acoustic stimulus interfered with the detection and information use of other sensory inputs (Halfwerk and Van Oers, 2020). If cross-sensory interference would have taken place, we expected a lower food finding success and/or a longer food-finding duration. Our results show that food finding success was not negatively affected by boat sound, in our set-up. Depending on including or excluding data from deviant response patterns to one of the six boat recording stimuli used, crabs were respectively either equally fast or even faster in reaching the food than crabs that were exposed to an ambient control. Thus, there is no evidence that cross-modal interference took place in the current study. Several other studies found cross-sensory interference, including effects on foraging behaviour in great tits (Halfwerk and Van Oers, 2020) and anti-predator behaviour, and aggregation and selection of a potential

new shell in hermit crabs (Chan et al., 2010b; Roberts and Laidre, 2019; Walsh et al., 2017). Not having found evidence for cross-sensory interference in shore crabs in the current study, does not exclude that this process may play a role in shore crabs under other conditions.

Boat stimuli

We provided the results of two parallel analyses: one for all our trials and one for a subset in which we excluded the trials of boat stimulus B3. The foraging times of the crabs that had been exposed to this particular boat seemed to deviate from the foraging times of the other boats. The analysis with all boats did not reveal an effect of boat playbacks on foraging duration, whereas the analysis without this particular boat showed a lower foraging duration for individuals that were exposed to boat sound. This provided some statistical support for the visually observed difference between B3 and the other boats.

We here speculate about some possible explanations for the deviant response patterns to the boat stimulus B3. The spectral profile of this playback was distinct from the other boats in being equally high in the low-frequency range, but the least loud above 500 Hz. Additionally, unlike the others, this boat was regularly used in the Jacobahaven, where all study subjects were caught. Future studies are needed to examine whether crabs respond differently to boat sounds of different spectral profiles or to familiar and unfamiliar boat sounds. However, the current results indicate that one out of six stimuli can still influence the results substantially. We therefore recommend the use of even more different playback stimuli for future noise impact studies, beyond our current set of six recordings, to further reduce problems of pseudoreplication (Hurlbert, 1984; Slabbekoorn and Bouton, 2008).

Conclusions

Our study examined the effect of boat sound playbacks on olfactory-mediated foraging behaviour in shore crabs. Our results do not provide evidence for a negative effect of boat sound playbacks on food finding success and foraging duration. However, after exclusion of the deviant data for one of the boat stimuli, crabs that were exposed to boat sound appeared to have reached the food faster than crabs that were exposed to ambient playbacks. These results add to what seems an emerging picture of anthropogenic noise exposure potentially affecting behaviour, but with contrasting results from captive and field studies on crabs (Hubert et al., 2018; Wale et al., 2013b; and the current study) We hypothesise that this contrast can be explained by a potential difference in hunger level, as crabs in both lab studies were food-deprived for three days, but future research

has to confirm this. Lastly, we call for sufficient replication of playback stimuli in sound exposure experiments, to reduce pseudoreplication and large single-stimulus impact (Hurlbert, 1984; Slabbekoorn and Bouton, 2008).

Ethical statement

We adhered to the Guidelines for the treatment of animals in behavioural research and teaching (ASAB, 2018). There are no legal requirements for studies involving crabs and bivalves in the Netherlands.

Supplementary information

Supplementary material associated with this article is available from the publication DOI:10.1016/j.envpol.2020.116184.

Data accessibility

All data used for the analyses reported in this article and some videos are available from the Zenodo Repository, DOI:10.5281/zenodo.4135946.

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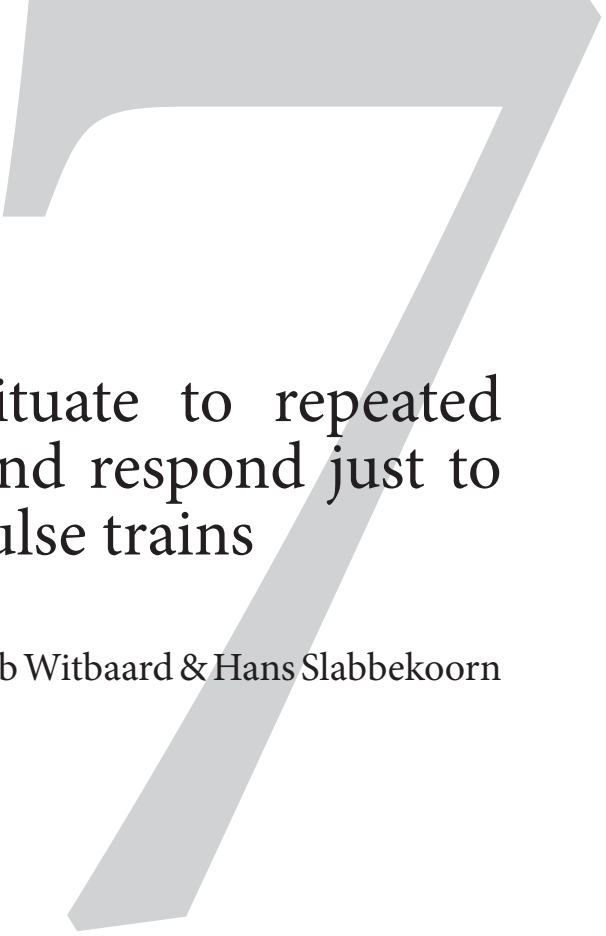
References

Andrew, R. K., Howe, B. M., Mercer, J. A. and Dzieciuch, M. A. (2002). Ocean ambient sound: Comparing the 1960s with the 1990s for a receiver off the California coast. Acoust. Res. Lett. Online 3, 65–70.

ASAB (2018). Guidelines for the treatment of animals in behavioural research and teaching. Anim. Behav. 135, I–X.

Barton, K. (2016). MuMIn: Multi-Model Inference.

Bradski, G. (2000). The OpenCV Library. Dr. Dobb's J. Softw. Tools 25, 120–125.

Carey, W. M. and Browning, D. (1988). Low Frequency Ocean Ambient Noise: Measurements and Theory. In Sea Surface Sound, pp. 361–376. Dordrecht: Springer.

Carroll, A. G., Przeslawski, R., Duncan, A., Gunning, M. and Bruce, B. (2017). A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pollut. Bull. 114, 9–24.

Chan, A. A. Y. H., David Stahlman, W., Garlick, D., Fast, C. D., Blumstein, D. T. and

Blaisdell, A. P. (2010a). Increased amplitude and duration of acoustic stimuli enhance distraction. Anim. Behav. 80, 1075–1079.

Chan, A. A. Y., Giraldo-Perez, P., Smith, S. and Blumstein, D. T. (2010b). Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biol. Lett. 6, 458–461.

Croy, M. I. and Hughes, R. N. (1991). Effects of food supply, hunger, danger and competition on choice of foraging location by the fifteen-spined stickleback, *Spinachia spinachia* L . Anim. Behav. 42, 131–139.

Edmonds, N. J., Firmin, C. J., Goldsmith, D., Faulkner, R. C. and Wood, D. T. (2016). A review of crustacean sensitivity to high amplitude underwater noise: Data needs for effective risk assessment in relation to UK commercial species. Mar. Pollut. Bull. 108, 5–11.

Ekerholm, M. (2005). Chemical communication in mating shore crabs *Carcinus maenas*.

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Stefano, V. Di, Mazzola, S. and Buscaino, G. (2014). Behavioural and biochemical stress responses of *Palinurus elephas* after exposure to boat noise pollution in tank. Mar. Pollut. Bull. 84, 104–114.

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Arizza, V., de Vincenzi, G., Grammauta, R., Mazzola, S., et al. (2016). Underwater noise from boats: Measurement of its influence on the behaviour and biochemistry of the common prawn (*Palaemon serratus*, Pennant 1777). J. Exp. Mar. Bio. Ecol. 478, 24–33.

Franěk, M., Režný, L., Šefara, D. and Cabal, J. (2018). Effect of Traffic Noise and Relaxations Sounds on Pedestrian Walking Speed. Int. J. Environ. Res. Public Health 15, 1–13.

Godin, J. J. and Crossman, S. L. (1994). Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (*Gasterosteus aculeatus*) under predation risk. Behav. Ecol. Sociobiol. 34, 359–366.

Halfwerk, W. and Slabbekoorn, H. (2015). Pollution going multimodal: The complex impact of the human-altered sensory environment on animal perception and performance. Biol. Lett. 11.

Halfwerk, W. and Van Oers, K. (2020). Anthropogenic noise impairs foraging for cryptic prey via cross-sensory interference. Proc. R. Soc. B Biol. Sci. 287.

Hardege, J., Bartels-Hardege, H., Fletcher, N., Terschak, J., Harley, M., Smith, M., Davidson, L., Hayden, D., Müller, C., Lorch, M., et al. (2011). Identification of a female sex pheromone in *Carcinus maenas*. Mar. Ecol. Prog. Ser. 436, 177–189.

Hasan, R., Crane, A. L., Ferrari, M. C. O. and Chivers, D. P. (2018). A cross-modal effect of noise: the disappearance of the alarm reaction of a freshwater fish. Anim. Cogn. 21, 419–424.

Hildebrand, J. A. (2009). Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395, 5–20.

Hubert, J., Campbell, J., van der Beek, J. G., den Haan, M. F., Verhave, R., Verkade, L. S. and Slabbekoorn, H. (2018). Effects of broadband sound exposure on the interaction between foraging crab and shrimp – A field study. Environ. Pollut. 243, 1923–1929.

Hubert, J., Neo, Y. Y., Winter, H. V. and Slabbekoorn, H. (2020). The role of ambient sound

levels, signal-to-noise ratio, and stimulus pulse rate on behavioural disturbance of seabass in a net pen. Behav. Processes 170, 103992.

Hughes, A. R., Mann, D. A. and Kimbro, D. L. (2014). Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. Proc. Biol. Sci. 281, 1–8.

Hurlbert, S. H. (1984). Pseudoreplication and the Design of Ecological Field Experiments. Ecol. Monogr. 54, 187–211.

Jeffs, A., Tolimieri, N. and Montgomery, J. C. (2003). Crabs on cue for the coast: The use of underwater sound for orientation by pelagic crab stages. Mar. Freshw. Res. 54, 841–845.

Jubb, C. A., Hughes, R. N. and ap Rheinallt, T. (1983). Behavioural mechanisms of size-selection by crabs, *Carcinus maenas* (L.) feeding on mussels, *Mytilus edulis* L. J. Exp. Mar. Bio. Ecol. 66, 81–87.

Krieger, J., Sombke, A., Seefluth, F., Kenning, M., Hansson, B. S. and Harzsch, S. (2012). Comparative brain architecture of the European shore crab *Carcinus maenas* (Brachyura) and the common hermit crab *Pagurus bernhardus* (Anomura) with notes on other marine hermit crabs. Cell Tissue Res. 348, 47–69.

Lagardère, J. P. (1982). Effects of noise on growth and reproduction of *Crangon crangon* in rearing tanks. Mar. Biol. 71, 177–185.

Morley, E. L., Jones, G. and Radford, A. N. (2014). The importance of invertebrates when considering the impacts of anthropogenic noise. Proc. R. Soc. B Biol. Sci. 281, 20132683.

Morris-Drake, A., Kern, J. M. and Radford, A. N. (2016). Cross-modal impacts of anthropogenic noise on information use. Curr. Biol. 26, R911–R912.

Morris, B. (2008). The effect of starvation on the size of mussels selected by the Green Shore Crab, *Carcinus maenas*. Plymouth Student Sci. 1, 4–18.

Popper, A. N. and Hawkins, A. D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. fish 94, 692–713.

Popper, A. N., Salmon, M. and Horch, K. W. (2001). Acoustic detection and communication by decapod crustaceans. J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol. 187, 83–89.

R Core Team (2016). R: A Language and Environment for Statistical Computing.

Radford, C. a., Jeffs, A. G. and Montgomery, J. C. (2007). Directional swimming behavior by five species of crab postlarvae in response to reef sound. Bull. Mar. Sci. 80, 369–378.

Regnault, N. and Lagardère, J.-P. (1983). Effects of ambient noise on the metabolic level of *Crangon crangon* (Decapoda, Natantia). Mar. Ecol. Prog. Ser. 11, 71–78.

Roberts, L. and Laidre, M. E. (2019). Finding a home in the noise: cross-modal impact of anthropogenic vibration on animal search behaviour. Biol. Open 8, bio041988.

Rogers, P. H., Hawkins, A. D., Popper, A. N., Fay, R. R. and Gray, M. D. (2016). Parvulescu Revisited: Small Tank Acoustics for Bioacousticians. In The Effects of Noise on Aquatic Life II, pp. 933–941.

Ross, D. (1976). Mechanics of Underwater Noise. New York: Pergamon Press.

Schadegg, A. C. and Herberholz, J. (2017). Satiation level affects anti-predatory decisions in foraging juvenile crayfish. J. Comp. Physiol. A 203, 223–232.

Shelton, R. G. J. and Mackie, A. M. (1971). Studies on the chemical preferences of the shore crab, *Carcinus maenas* (L.). J. Exp. Mar. Bio. Ecol. 7, 41–49.

Simpson, S. D., Purser, J. and Radford, A. N. (2014). Anthropogenic noise compromises antipredator behaviour in European eels. Glob. Chang. Biol. 586–593.

Slabbekoorn, H. and Bouton, N. (2008). Soundscape orientation: a new field in need of sound investigation. Anim. Behav. 76, e5–e8.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C. and Popper, A. N. (2010). A noisy spring: The impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427.

Solan, M., Hauton, C., Godbold, J. A., Wood, C. L., Leighton, T. G. and White, P. (2016). Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep. 6, 20540.

Venables, W. N. and Ripley, B. D. (2002). Modern Applied Statistics with S. Fourth. New York: Springer.

Voellmy, I. K., Purser, J., Simpson, S. D. and Radford, A. N. (2014). Increased noise levels have different impacts on the anti-predator behaviour of two sympatric fish species. PLoS One 9, e102946.

Wale, M. a., Simpson, S. D. and Radford, A. N. (2013a). Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. Biol. Lett. 9, 20121194.

Wale, M. a., Simpson, S. D. and Radford, A. N. (2013b). Noise negatively affects foraging and antipredator behaviour in shore crabs. Anim. Behav. 86, 111–118.

Walsh, E. P., Arnott, G. and Kunc, H. P. (2017). Noise affects resource assessment in an invertebrate. Biol. Lett. 13,.

Weissburg, M. J. and Zimmer-Faust, R. K. (1994). Odor plumes and how blue crabs use them in finding prey. J. Exp. Biol. 197, 349–375.

Wenz, G. M. (1962). Acoustic Ambient Noise in the Ocean: Spectra and Sources. J. Acoust. Soc. Am. 34, 1936–1956.

Williams, R., Wright, A. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., Clark, C. W., Cullis-Suzuki, S., Dakin, D. T., Erbe, C., et al. (2015). Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24.

Zimmer-Faust, R. K. (1989). The relationship between chemoreception and foraging behavior in crustaceans. Limnol. Oceanogr. 34, 1367–1374.

Blue mussels habituate to repeated sound exposures and respond just to the on-set of fast pulse trains

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Abstract

Anthropogenic sound has been shown to affect marine animals across taxa. However, bivalves and other invertebrates received limited attention and most studies across taxa focussed on immediate, rather than long-term, effects of sound. Most bivalves adopt a sessile or sedentary lifestyle and are therefore expected to be subject to frequent exposure to the same sounds for long periods or repeatedly. For this reason, bivalves are an especially relevant taxonomic group to study long-term effects of sound. In the current study, we examined whether blue mussels (*Mytilus edulis*) habituate to repeated sound exposures and whether they recover quicker from a single pulse exposure than from a pulse train. We equipped individual mussels with sensors to monitor their valve gape and exposed them to repeated sound playback. We found that mussels responded to sound by partially closing their valves. This response was consistent and repeatable, but decayed over sequential exposures to the same sound stimulus, and was stronger again with exposure to a different sound. This pattern is clear evidence for acoustic habituation in a bivalve. Additionally, we found no differences in the initial response and recovery (time to return to baseline levels) between mussels that were exposed to single pulses and pulse trains. Our results therefore show that mussels are able to habituate to sound and suggest that mussels mostly respond to the onset of a pulse train. Future research is needed to determine whether mussels also habituate in situ to actual anthropogenic sound and whether a lack of a behavioural response to repeated sound also implies that other negative effects are also absent.

Introduction

Anthropogenic sound is omnipresent in the marine environment and has the potential to affect marine animals across taxa (Carroll et al., 2017; Slabbekoorn et al., 2010). Animals use and produce sound for orientation and communication and these functions can be undermined by masking or disturbing noise (Gordon et al., 2019; Wilson et al., 2014). Anthropogenic noise has been shown to impact animals in various ways, including disruption of movement patterns, foraging behaviour, communication, and metabolism (Codarin et al., 2009; Harding et al., 2018; Hawkins et al., 2014; Hubert et al., 2018). Many anthropogenic activities that produce sound, such as shipping, pile driving, seismic surveys, and dredging occur continuously or repeatedly and can last for months (Popper and Hawkins, 2019; Slabbekoorn et al., 2019). The effects of sound on invertebrates received limited attention, yet they make-up the majority of the biomass and are important for higher trophic levels (Morley et al., 2014; Solan et al., 2016). Immediate and short-term effects of sound exposure have been examined most. However, many marine animals, and especially invertebrates with a sessile life style or small home ranges, are likely exposed to the same sound repeatedly or for extended periods of time.

Bivalves are known to hear sound. Bivalves lack gas-filled cavities and are, for this reason, not expected to be sensitive to sound pressure, but rather to the particle motion aspect of sound. Their hearing is not fully understood, but members of 19 bivalve families, including the current study species, possess a specialized hearing organ, the abdominal sense organ (ASO; Haszprunar, 1983). Bivalve lineages that lack the ASO possess structurally similar organs, possibly also for hearing (Haszprunar, 1985; Zhadan, 2005). Removal of the ASO in two scallop species resulted in a major decrease in acoustic sensitivity, the remaining sensitivity was thought to be attributable to cells with short cilia on the mantle and tentacles (Zhadan, 2005). Statocysts have also been suggested to be involved in hearing (Charifi et al., 2017; Roberts et al., 2015). The hearing range of blue mussels is not known, but they have been shown to respond to tones from 5 to 410 Hz (no tones outside this range used, Roberts et al., 2015). Another bivalve, the Pacific oyster (*Magallana gigas*), has been shown to respond to tones of up to 600 Hz (Charifi et al., 2017), however, both location and development of the ASO is different in these species (Haszprunar, 1983). Several bivalves have also been reported to incidentally produce sound, typically associated with valve movement and expulsion of water and other substances (de Melo Júnior et al., 2020; Di Iorio et al., 2012).

Bivalves may use sound for various reasons. Planktonic larvae of bivalves settle

and metamorphose at the seafloor, – in most species – to start their sedentary or sessile life stage. Larvae of the eastern oyster (*Crassostrea virginica*) showed increased settlement behaviour in response to playbacks of recordings from oyster reefs when compared to adjacent soft bottom habitats (Lillis et al., 2013). Swash-riding clams (*Donax variabilis*) jump out of the sand and ride waves to migrate shorewards during ebb and flood tides, specifically with the largest waves (Ellers, 1995a). In the lab, these clams have been shown to jump out of the sand during the playbacks of wave sound, they were most responsive to the loudest waves and around high tides (Ellers, 1995b). It is clear that bivalves can hear, use, and respond to sound, so they are potentially also affected by anthropogenic sound.

Various studies examined the effects of anthropogenic or artificial sound exposures on bivalves. Bivalve physiology has been shown to be affected by noisy conditions through an increase in several biochemical stress parameters, single-strand breaks in DNA, reduced oxygen consumption, oxidative stress, and adjusted metabolism and hemolymph (a blood analogue) biochemistry parameters (Day et al., 2017; Peng et al., 2016; Vazzana et al., 2016; Wale et al., 2019). Increased mortality was found in scallops (*Pecten fumatus*), 14 and 120 days after seismic survey passes (Day et al., 2017). Pelagic bivalve larvae showed delayed development and body abnormalities after seismic sound exposure (de Soto et al., 2013) and displayed increased and faster settlement behaviour in response to vessel noise (Jolivet et al., 2016; Wilkens et al., 2012). Bivalves in their benthic stage have been shown to respond by both immediate closure of their valves as well as an increased mean valve gape, both a higher and lower filtration rate, increased and deeper digging behaviour, a reduction in 'normal' behaviour (close movements, coughs, and locomotion), and by retraction of the velum (Charifi et al., 2017; Day et al., 2017; Mosher, 1972; Peng et al., 2016; Roberts et al., 2015; Spiga et al., 2016; Wale et al., 2019). In these experiments, subjects were typically exposed to sound once for a relatively short period and it was not examined whether responses reduced over time. However, bivalves in situ are likely exposed to anthropogenic sound throughout their lives and may habituate to sound.

Habituation is a wide-spread phenomenon and is critical to understand the long-term impact of behavioural changes due to noise pollution. The term habituation is often misused in impact studies to explain the absence or decrease in response, yet it follows a strict definition (Bejder et al., 2006): Habituation is a decrease in behavioural response to repeated stimulus presentations, when this decrease cannot be attributed to sensory adaptation, sensory fatigue, or motor fatigue. The latter, alternative explanations of a decrease in behavioural response,

can be excluded by showing dishabituation or stimulus specificity (Rankin et al., 2009). Several marine fish species and cephalopods have been shown to decrease their behavioural or physiological stress response over repeated exposures (Johansson et al., 2016; Mooney et al., 2016; Nedelec et al., 2016; Radford et al., 2016; Samson et al., 2014). However, only for European seabass (*Dicentrarchus labrax*), the decreased responses have actually been ascribed to habituation by showing stimulus specificity (Neo et al., 2015a). We are not aware of evidence that bivalves are able to habituate to sound, however, two giant clam species (*Tridacna derasa* & *Tridacna maxima*) have been shown to habituate to repeated visual and tactile stimuli (Dehaudt et al., 2019; Wilkens, 1986).

Blue mussels (*Mytilus edulis*) are both a relevant and practical species to use in studies on the impact of sound exposures. Mussels are filter-feeders, provide habitat as reef builders, and are a common prey item for many species, and hereby play an important role in ecosystems (Borthagaray and Carranza, 2007; Jørgensen, 1990; Kautsky, 1981). Furthermore, mussels are an important commercial species (Eurostat, 2019a; Eurostat, 2019b). Mussels are relatively easy to collect because of coastal abundance. Their semi-sessile lifestyle makes them also a suitable species to study in the lab, as this lifestyle makes them less affected by confinement compared to many other species. Mussels have already been shown to respond to sound physiologically and behaviourally, including immediate and clear valve closure upon sound exposure (Roberts et al., 2015), this provided us with the opportunity to test the effects of repeated sound exposures.

In the current study, we conducted two experiments to test whether blue mussels can habituate to sound. In experiment 1, we exposed mussels sequentially to identical tones, followed by a single different tone. We examined whether the mussels' response in valve gape diminished over sequential exposures and whether they responded more strongly again to the different sound exposure. In experiment 2, we exposed mussels to three sequential pulse trains or to three single pulses, with identical onset times, and examined whether mussels returned to baseline valve gape levels more quickly after a single pulse. We aimed to answer the following questions: (1) Do mussels change their valve gape after the onset of a sound exposure? (2) Does the change in valve gape diminish over sequential sound exposures and can this be attributed to habituation? (3) Do mussels return to baseline valve gape levels more quickly after a single pulse than during or after a pulse train?

Materials and methods

Study subjects

We used 180 wild-caught blue mussels (*Mytilus edulis*). The mussels were collected from the poles of the Scheveningen Pier in the inter-tidal area of the North Sea coast in Scheveningen, the Netherlands. The experiments were conducted in April and May 2020. Due to the COVID-19 pandemic, experiments were run at the home of EB. The mussels were kept in a salt water aquarium (120 x 44 x 43 m; L x W x H) at nearby restaurant 'Les Copains' in Delft, the Netherlands, for at least seven days before being used in an experiment. After being used in an experiment, we released the mussels back into the wild.

Experimental set-up

The trials were performed in a plastic container (53 x 39 x 35; L x W x H) with fresh water and an underwater speaker at the centre of the bottom. We hung four plastic one-litre bottles with 0.62 L of salt water at equal distances around the speaker. We cut the top ~ 6 cm of each bottle and hung them below bamboo sticks using wire, the bamboo sticks rested on the container edges (fig. 1). The bottles allowed us to test four mussels simultaneously while excluding chemical communication or physical contact between the individuals. For this reason, we also refreshed the water in the bottles with salt water from the stock tank before each trial and we added a few drops of phytoplankton (Reef phytoplankton, Seachem) to allow the mussels to feed.

We used a valve gape monitor to log the valve gape behaviour (Ballesta-Artero et al., 2017). The valve gape monitor consisted of multiple pairs of electromagnetic coils coated in epoxy and a plastic tube. The active coil of each pair, generated an electromagnetic field which resulted in a current in the responsive coil. The strength of the measured electromagnetic field was determined by the distance between the coils, and thus reflected gape opening at high resolution. We attached the coils of one pair on opposite valves of an individual mussel using a combination of hot glue and cyanoacrylate glue. Immediately after attaching the coils, the individual mussels were hung in the centre of one of the bottles and in the middle of the water column of the container. After we placed the last mussel in a bottle, we started the playback, which started with 25 min of silence before the first sound exposure (in the exposure conditions).

Sound exposure

During the experiments, we played back pure tones with silence intervals. For experiment 1, we used four different sound treatments with 150 and 300 Hz

tones and for experiment 2, we used two treatments with 150 Hz tones only. We chose these frequencies because mussels have been shown to respond to sound from 5 to 410 Hz (Roberts et al., 2015), and we aimed for two frequencies that were perceptually different enough to test stimulus specificity but still elicited a similar response. The sound treatments were created with Audacity (version 2.3.3) and played back with an underwater speaker (UW30, Lubell labs) from a recorder (DR-07, TASCAM), through an amplifier (M033N, Kemo). For both
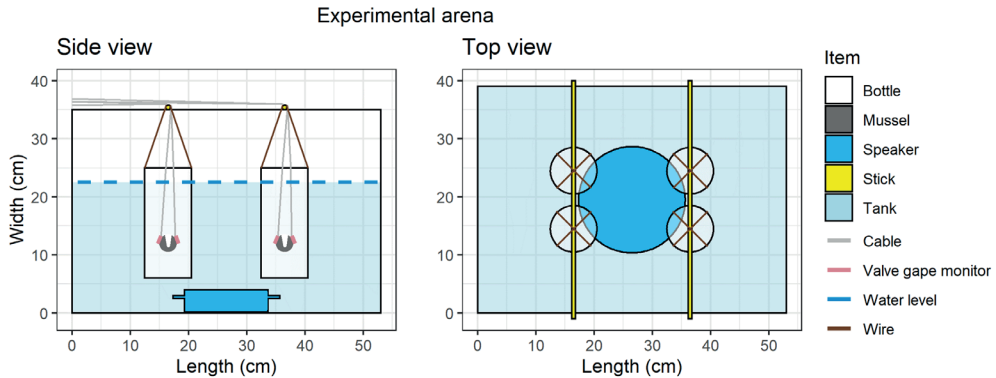
Fig. 1: Schematic views of the experimental arena (LxBxH = 54x48x35cm) from the front (Side view) and from above (Top view). During the experiments, the mussels were placed in plastic bottles hanging in the rectangular tank (see Side view). Four bottles hang at equal distances from the speaker (see Top view). The mussels were exposed to sound using a speaker on the bottom of the tank.
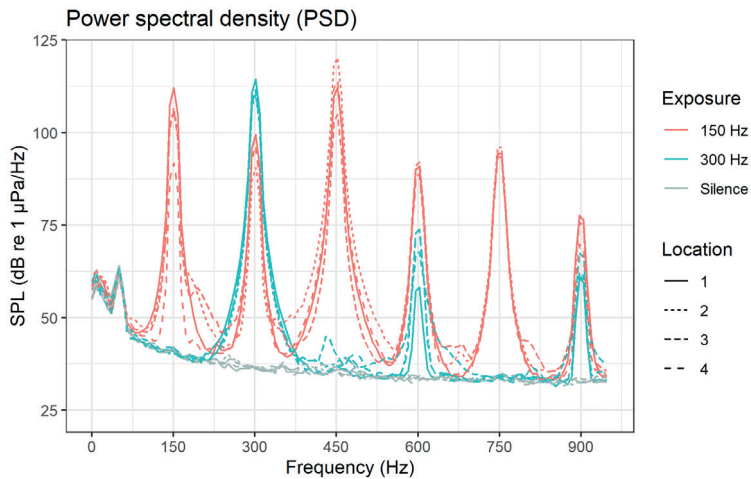
Fig. 2: Power spectral density (window length: 6144, window type: Hann) of recordings of the pure tones (red and blue) and silence playback (grey) at all mussel locations (line type). Higher harmonics of the fundamental frequency were present, probably due to speaker limitations.

experiments, we counterbalanced the order of the treatments. All trials were recorded with a calibrated hydrophone (96-min, HTI) and digital recorder (DR-100MKII, TASCAM) to confirm that all treatments had been played back correctly.

After the trials, we recorded both the pure tones that we used and the silent intervals in all four bottles, at the location of the mussel and generated power spectral density plots using a custom-made R-package (figure 2). The rms SPL (geometric mean of all locations in the 100-600 Hz bandwidth) was 138.4 dB re 1 μPa of the 150 Hz stimulus playback, 135.6 dB re 1 μPa of the 300 Hz stimulus, and 77.6 dB re 1 μPa for the silence playback. Both the sound levels across mussel locations and the harmonic structure (fundamental frequency plus higher harmonics at positive integer multiples) of both treatments were highly similar. Specifically, there was overlap between the higher harmonics of the 150 Hz treatment and the fundamental tone and higher harmonics of the 300 Hz treatment.

Sound propagation in tanks can be expected to differ substantially from sound propagation in the sea. The proximity of the tank walls and water surface affect the ratio between sound pressure and particle motion, and the directionality of particle motion (Campbell et al., 2019; Rogers et al., 2016). We placed the subjects as far as possible from the water surface and tank walls to minimize these effects, but the sound conditions are still expected to be substantially different from those in natural water bodies. This does not pose a problem for the current data, as our target was a proof of concept study into whether habituation to sound of any kind is possible in mussels and we did not aim to determine absolute response levels to a particular realistic anthropogenic sound.

Experiment 1

For experiment 1, we aimed to examine habituation of mussels to sequential sound exposures. To test this, we exposed individuals in the exposure conditions to 10 sequential pure tones of the same frequency, followed by a single pure tone of a different frequency. The first pure tone started after 25 min of playback of silence and each of the pure tones lasted for one minute and was followed by five min of silence, so, each trial lasted 91 min. We used a reciprocal design in which we aimed to expose half of the individuals in the exposure condition to 10 exposures of 150 Hz followed by a single 300 Hz exposure (figure 3A) and the other half to 10 exposures of 300 Hz followed by a single 150 Hz exposure (fig. 3C). We also ran control trials to examine whether the mussels in the treatment trials were responding to the sound exposures and to make sure that their responsiveness did not decrease over time in the experimental set-up

anyway, unrelated to the previous sound exposures. For the control condition, we replaced the first nine exposures of both exposure tracks with silence. In this way, we could expose half of the individuals to 79 min of silence followed by a single exposure of 150 Hz and a single sequential 300 Hz exposure (fig. 3B) and the other half first to 300 Hz and then a 150 Hz exposure (fig. 3D).

Experiment 2

For experiment 2, we aimed to examine the initial response and recovery time of mussels to single sound pulses and pulse trains. A pulse train consisted of 1 s pulses of 150 Hz separated with 9 s of silence (fig. 3E). In the single pulse
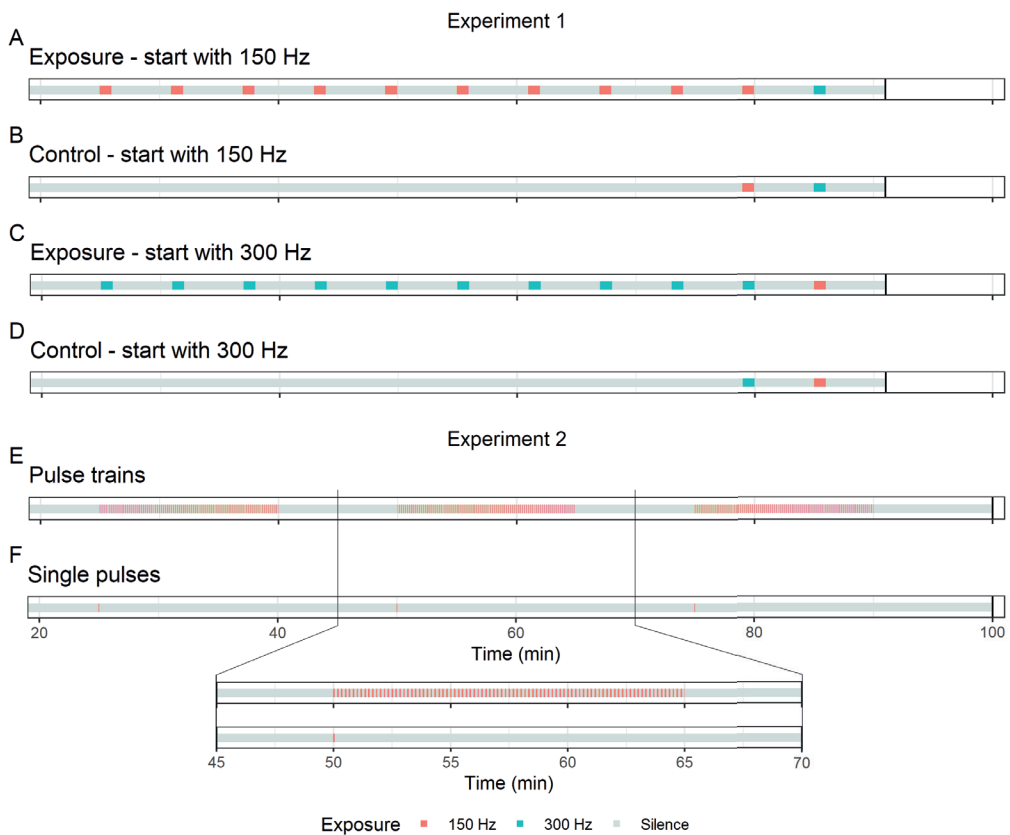
Fig. 3: Overview of the experimental playbacks in both experiments. In experiment 1, we exposed the mussels in the exposure condition to 11 sequential tones, this either started with 10 exposures of 150 Hz and ended with a single 300 Hz exposure (A) or the other way around (C). In the control conditions, we only exposed the mussels to the last two exposures (B & D). In experiment 2, we either exposed the mussels to three pulse trains (E) or three single pure tones (F). Note that this figure starts at 20 min, mussels in all conditions were only exposed to silence in the first 25 min. Duration of pulses is not to scale (E & F).

condition, we only exposed the individuals to the first pulse of a pulse train and replaced the others by silence (fig. 3f). We exposed individuals in the pulse train condition to three pulse trains of 15 min followed by 10 min of silence, the first pulse train started after 25 min of silence. In this way, both treatments lasted 100 min.

Behavioural observations

The valve gape monitor yielded on average 46 datapoints per minute of each individual. The raw data was the measured electromagnetic field strength that was converted to absolute distances using the calibration of the monitor. The size of the mussels and the location of the sensors on the mussels also influenced the absolute distance, therefore, we converted the absolute distance to the 'fraction open', with '0' being the minimal distance between the coils and '1' being the maximum distance between the coils during a complete trial of a single individual. We excluded individuals from the experiment if the absolute difference between the minimum and maximum opening distance during the entire trial was less than 1 mm (meaning the mussel barely opened, including before the start of the sound), if the mussels were not open for at least 25% at 23 min after the start of the trial (which is 2 minutes before the start of the first exposure in the exposure conditions in experiment 1 and both treatments of experiment 2), or when the coils got loose from the mussels. To examine the mussels' reaction to sound, we determined the mean fraction open the last 30 s before the onset of the sound and the first 30 s after the onset of the sound and subtracted fraction open before from the fraction open after; yielding our measure of Δ fraction open. For the second experiment, we also determined the time it took the mussels to return to at least 90% of the pre-exposure valve gape levels; the recovery time. For this, we used the fraction open before (30 s period) as pre-exposure level and used a moving average of 30 datapoints for the data after the onset of the sound to determine when the pre-exposure level was reached again (rounded to the nearest minute). If the mussel did not return to 90% of the pre-exposure levels within 25 min, we assigned 25 min as a recovery time for this individual.

For experiment 1, we analysed the data of 93 individuals; 49 in the exposure condition and 44 in the control condition. Another 15 individuals were excluded from the analysis because the valve gape sensors got detached from the mussel, the mussels did not open fast enough (< 25% at 23 min), or the mussel barely opened during the entire trial (difference between minimum and maximum distance < 1 mm). For experiment 2, we analysed the data of 61 individuals; 32 in the pulse train condition and 29 in the single pulse condition. Another 11 individuals were excluded from the analysis (reasons identical to experiment 1).

Statistics

We analysed the effect of the sound treatments on the change in valve gape (Δ fraction open) and the recovery time in R (R Core Team, 2016). For Δ fraction open, we used generalized linear models (GLMs) with a Gaussian error distribution and identity link-function. When treatment groups had unequal variance, we used a linear model using Generalized Least Squares instead (R-package nlme, Pinheiro et al., 2020). For recovery time (min), we used a zero-inflated regression (R-package pscl, Zeileis et al., 2008) with a binomial error distribution with logit link-function for the zero-inflation model and, a Poisson error distribution and log link-function for the count model. In experiment 1, we only used Δ fraction open as a response variable for all models and always used the fraction open before the sound exposure as an explanatory variable in the full model. Depending on the research question, we also used treatment type (exposure versus control) or both exposure number and treatment frequency (150 versus 300 Hz), and the interaction between them, in the full model. In experiment 2, we used Δ fraction open and recovery time as response variables in two separate models, both with the fraction open before the sound exposure, treatment type (pulse trains versus single pulses), exposure number, and the interaction between the latter two as explanatory variables in the full models. For each full model, we determined the AICc score of all possible explanatory variable combinations and selected the model with the lowest AICc as best model. If the explanatory variable of interest (relevant to the research question) was not part of the best model, we added it to the final model anyway to obtain an estimate and p-value. To determine the effect and significance of the covariates, we ran the final models.

Results

Experiment 1

Two example trials (fig. 4ab) show that these two mussels had closed their valves at the start of the trial, probably due to handling the individuals, and gradually opened during the first 25 min. Both individuals responded to the first few sound exposures by almost completely closing their valves. The magnitude of the response decreased over the first ten exposures in one of them (fig. 4a), whereas the other stopped responding altogether (fig. 4b). Both individuals responded again to the 11th tone of a different frequency than, but one of them did not respond as strongly as to the first exposure.

To test whether mussels changed their valve gape in response to the sound, we compared the Δ fraction open of the mussels in the exposure conditions at the

first exposure with the Δ fraction open at the same timestamp in the control condition (silence was played here). The mussels in the exposure condition significantly reduced their fraction open (they partially closed) when compared to the control mussels (Intercept: 0.32, Exposure: -0.35, p-value: < 0.01, fig. 5). Additionally, the more open their valves were before the onset of the sound, the more they closed (Intercept: 0.32, Before slope: -0.53, p-value < 0.01), this was the case in almost all models, so we do not mention it anymore hereafter. In the exposure conditions, their valve closure decayed (they closed less) over the first 10 sequential exposures (Intercept: -0.11, Exposure number slope: 0.03,
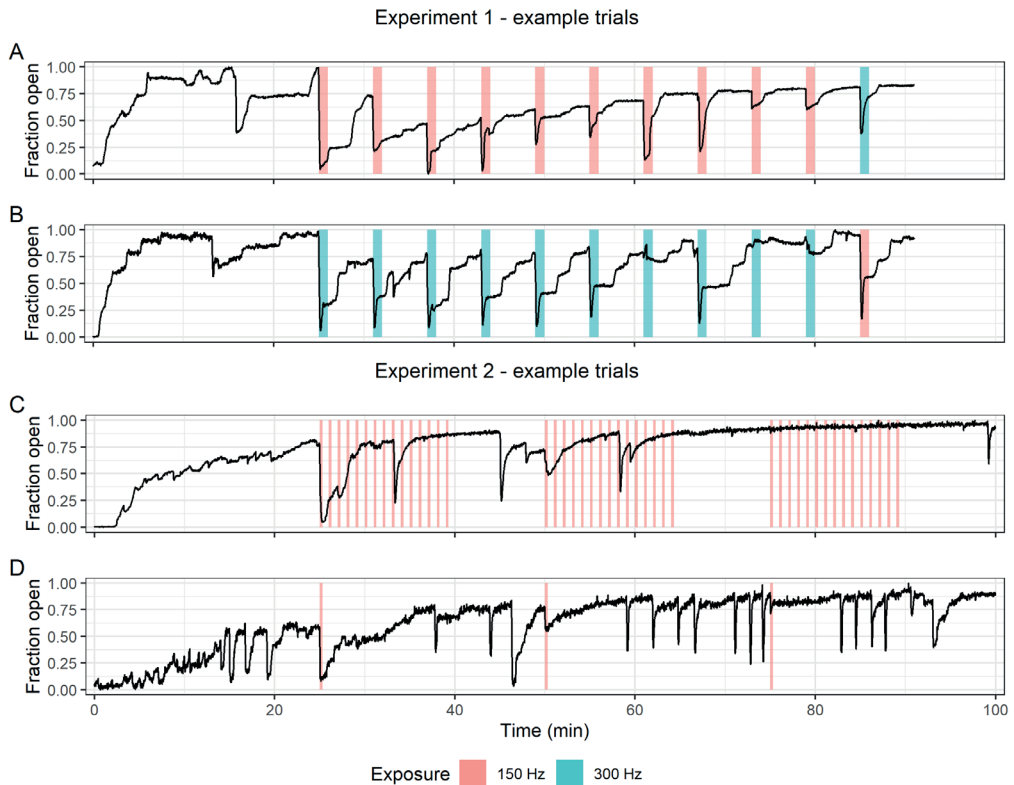
Fig. 4: Valve gape behaviour during an entire trial of four individuals. The individual in the top panel (A), responded to every single exposure, but the magnitude of the response decreased in the first 10 exposures and increased again at the 11th different exposure. The individual in the second panel (B) either responded quite strongly, or did not close at all (exposure number 7, 9 & 10), but responded strongly again to the last exposure with a pure tone of different frequency. The individual in the third panel (C) seems to have responded to the first pulse of the first and second pulse train and returned to pre-exposure levels before the end of the pulse trains. The individual in the bottom panel (D) seems to have responded to all three single pulses, but the magnitude of the response seems to have decreased. For display purposes, the pulse duration is not drawn to scale (C & D) and the amount of pulses is reduced (C).

p-value: < 0.01). The treatment frequency was not part of the best model, neither separately nor in interaction with exposure number. This meant that there were no differences in response to the pure tones nor a different pattern in the decay of the response. To test whether this decay in responsiveness can be attributed to habituation, we exposed the mussels in the exposure condition to an 11th tone of a different frequency and compared their response to this tone with their response to the 10th tone. Here, we found an interaction between the treatment frequency and exposure number; mussels that were first exposed to 150 Hz did not respond more strongly to a sequential 300 Hz tone (Intercept: 0.30, Exposure number slope: -0.03, p-value: 0.35). However, the mussels that were first exposed to 300 Hz tones, and subsequently to a 150 Hz tone, did close their valves more in response to the latter (Intercept: 0.30, Exposure number slope: -0.02, p-value:
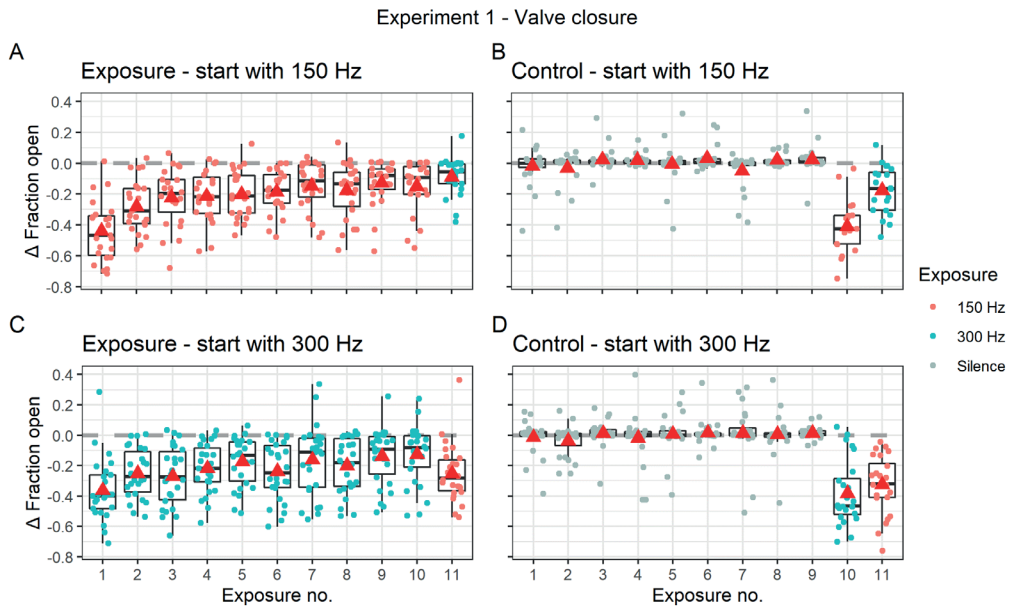
Fig. 5: The responses of all mussels that were exposed to any of the four treatments of experiment 1. A Δ fraction open below zero indicates (partial) closure of the valves. The coloured points indicate the individual responses to the sound exposures. The box-and-whisker plots indicate the median, first and third quartile and, minimum and maximum excluding outliers of all individuals per exposure, and the red triangles indicate the means. The dashed grey lines indicate 0, meaning no change in fraction open. The mussels significantly closed their valves in response to the first exposure (A & C) when compared to the same timestamp in the controls (B & D). There was a significant decay in response magnitude over the first 10 sequential exposures (A & C). Mussels that had been exposed to 10 sequential 300 Hz exposures responded more strongly again to the eleventh 150 Hz exposure (C), in the opposite order, this was not the case (A). There was no significant difference between the first exposure in the exposure treatments (A & C) and the first actual exposure (exposure number 10) in the control condition (B & D).

< 0.01). To verify that the reduced response of the mussels to sequential sound exposures cannot be explained by the increasing time in the experimental set-up, we compared the mussels' response to the first exposure in the exposure condition with the first exposure in the control condition (Exposure number 10). We found no difference between these responses (Intercept: 0.11, Treatment: -0.03, p-value: 0.34).

Experiment 2

Two example trials (fig. 4cd) show that these mussels responded to the sound exposure by almost completely closing their valves. Both mussels gradually returned to pre-exposure levels, the mussels that was exposed to the pulse train already during the sound exposure (fig. 4c). The response to the onset of the sound decreased over sequential exposures and one of them did not respond at all to the last exposure (fig. 4c).

Similar to the first experiment, there was a negative correlation between the mussels' response and the exposure number (Intercept: -0.04, Exposure number slope: 0.03, p-value: 0.02, fig. 6ab), the mussels reduced their valve closure with sequential sound exposures. The treatment type was not part of the best model, neither separately nor as interaction with exposure number, meaning that the response and decay in response was not significantly different for mussels that
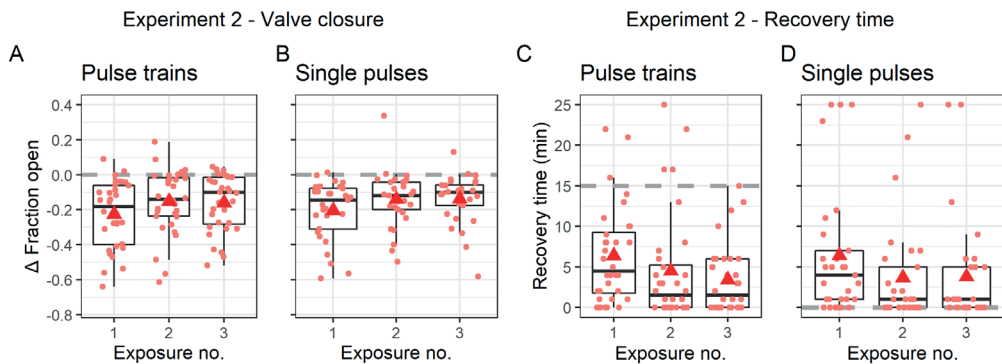
Fig. 6: The change in valve gape of all mussels that were exposed to one of the two playback treatments of experiment 2 (A & B). There was a significant reduction in response to the sound over the sequential sound exposures, but no differences between the reactions or decay in reaction in mussels that were exposed to pulse trains (A) or single pulses (B). Time it took the mussels in both treatments to return to 90% of the pre-exposure fraction open (C & D). There was a significant reduction in recovery time over the sequential sound exposures, but no differences between the recovery times or decay in recovery times in mussels that were exposed to pulse trains (C) or single pulses (D). The x-axes (Exposure no.) indicate the number of pulse trains (A & C) and the number of single pulses (B & D) in a trial.

were exposed to pulse trains or single pulses. Similarly, increasing numbers of mussels did not respond substantially (a Recovery time of 0 min) over sequential exposures (Intercept: 0.76, exposure number slope: 0.54, p-value: < 0.01, fig. 6cd). The mussels that responded (Recovery time ≥ 1 min), recovered quicker over sequential exposures (Intercept: 2.47, Exposure number slope: -0.12, p-value: < 0.01, fig. 6cd). Again, treatment type was not part of the best model, so, there were no differences between the recovery times or decay in recovery times to pulse trains and single pulses.

Discussion

In the current study, we exposed blue mussels to repeated sound exposures and examined their valve gape responses. Our results demonstrate that mussels responded to sound by partially closing their valve gape. Their response decreased over sequential sound exposures of 1 min, independent of stimulus frequency, and, in one of the exposure conditions, they responded stronger again to a different sound exposure. This result shows that mussels can habituate to sound. Their recovery time after a single pulse of 1 s, was not shorter than their recovery during and after a pulse train of 15 min. This result indicates that mussels mostly responded to the onset of the pulse train, with little effect of the rest of the pulses in the train.

Response to sound

The blue mussels in the current study responded to the sound exposures by (partially) closing their valves. Mussels have been shown to close their valves in response to a variety of environmental conditions, including fluctuations in temperature and salinity, air exposure, potentially poisonous chemicals and conspecific homogenate. So, valve closure seems to be a general defence response (Bayne et al., 1976; Curtis et al., 2000; Robson et al., 2010). The valve closure in the current study is in line with previous research in which mussels also responded to tonal sound by closing their valves (Roberts et al., 2015). In contrast, mussels that were exposed to ship noise for 1 h had a larger mean absolute valve opening than mussels in the control condition (Wale et al., 2019). However, only a limited number of individuals were tested in the latter study (n = 6 and 8, for noise and control condition respectively). Nevertheless, it may be that mussels respond differently to tonal and shipping sound, or initially respond by valve closure and later compensate for this.

The consequences of partial valve closure for shorter or longer periods due to sound exposure are not straightforward, as becomes clear from a brief review of the few relevant studies. Two earlier studies examined the filtration rate of

mussels during sound exposures. Mussels that were exposed to 50 min of pile driving were shown to increase their filtration rate (Spiga et al., 2016), whereas mussels exposed to 3 h of ship noise were observed to reduce filtration rate (Wale et al., 2019). These different results may be due to the use of different stimuli, but again, they may also be due to the limited number of individuals tested in the latter study (n = 5 for both treatment and control). It is, nevertheless, interesting that Wale et al. (2019) found both a larger valve gape and a reduced filtration rate during shipping sound, in the same study, but in different individuals. This suggests that valve gape is not necessarily positively correlated with filtration rate. In another study, without any particular stressor, Jørgensen et al. (1988) did find a positive correlation between valve gape and filtration rate. But large variation in the correlation between valve gape and both exhalant siphon area and pumping rate is apparently not uncommon (Maire et al., 2007). More research is obviously needed to understand the impact of sound on mussel behaviour and physiology. It would be revealing to test the same individuals to the different types of acoustic stimuli and by examining valve gape (and potentially also exhalant siphon area) and consequences for filtration rate simultaneously.

Habituation to sequential sound exposures

In experiment 1, the mussel valve closure in response to the sound exposures decayed over sequential exposures. The mussels in the control conditions were not exposed to the first nine exposures and responded as strongly to the 10th exposure as the mussels in the exposure conditions to the first exposure, this shows that the decrease in responsiveness is not caused by an increasing time in the experimental set-up. The mussels in the exposure condition that were first exposed to 10 sounds of 300 Hz followed by one of 150 Hz increased their response again to the 150 Hz. This test of stimulus specificity provided essential proof for that the decreased response can be attributed to habituation and not to sensory adaptation, sensory fatigue or motor fatigue (Rankin et al., 2009). Mussels that were first exposed to 10 sounds of 150 Hz did not show stimulus specificity, as they did not increase their response to the final test sound of 300 Hz. This result can probably be explained by the higher harmonic tones at 300 Hz and 600 Hz of the 150 Hz sound (fig. 2). As 300 Hz and 600 Hz tones were also present in the spectrum of the last 300 Hz exposure, this test sound was potentially not novel enough to elicit a stronger response again. The response and decay in response to the first 10 exposures was not different between 150 and 300 Hz, so the differences in response to the 11th tone cannot be explained by a higher sensitivity to either of the stimuli. In contrast, the presence of 150 Hz, besides shared energy at 300Hz and 600Hz, made the 150 Hz sound exposure apparently novel enough to elevate response strength, which reveals spectral discrimination at this resolution, which is as far as we know also a novelty for

this invertebrate taxon.

We were able to show that blue mussels are able to habituate to sound, which is important for understanding potential impact. The fact that mussels can habituate in general is in line with previous studies that have shown that oysters (other bivalves) habituate to visual and tactile stimuli (Dehaudt et al., 2019; Wilkens, 1986). Habituation may be advantageous to stimuli that are continuously or repeatedly present without being associated with harmful consequences (Bejder et al., 2009; Rankin et al., 2009). However, habituation of a particular behavioural response does not necessarily mean habituation in all behavioural responses (Neo et al., 2018). Also, behavioural habituation does not necessarily mean the absence of a negative effect of disturbance (Bejder et al., 2009). Sound may still cause physiological stress (Wale et al., 2019), masking (Wysocki and Ladich, 2005), and attention shifts (Chan et al., 2010; Walsh et al., 2017). Nevertheless, investigating the potential for mitigating impact through habituation, is critical to understand the effects of long-term and repeated sound exposures on marine life.

Response to pulse trains

No differences were found in the mussels' initial response and recovery time to a pulse trains and single pulses. This may indicate that the response and rate of recovery was mostly determined by the first pulse of the 15 min pulse train. While this may explain the disturbance potency of anthropogenic sounds that are more sudden and fluctuating in time (Vetter et al., 2015; Wysocki et al., 2006; Zhao et al., 2019), it may also open up possibilities to alternative mitigation strategies beyond just stopping noisy activities. We chose the current pulse rate (1 s pulse, 9 s silence) because it falls in the range of pulse rates of seismic surveys, with a pulse every 5 - 15 s depending on the type of survey (McCauley et al., 2000; Slabbekoorn et al., 2019). For pile driving, typically a faster pulse rate is used; a pulse every 1 - 4 s (Hall, 2013; Matuschek and Betke, 2009). Both seismic surveys as pile driving also have larger breaks in between separate pulse trains, to turn the seismic ship, to adjust a pile, start with a new pile, or because of marine mammal sightings or bad weather. Different pulse rates may elicit different behavioural responses (Neo et al., 2015b), but not necessarily (Hubert et al., 2020). In both rats (*Rattus norvegicus*) and humans, faster pulse rates resulted in a faster decrease of startle-like responses (Davis, 1970; Gatchel, 1975). It may be that a lower pulse rate than currently used would have delayed the recovery time. We believe that more studies are warranted into the importance of inter-pulse interval and inter-pulse train interval in determining the habituation rate and recovery time to explore mitigation potential.

Conclusions

Our study examined the behavioural response, habituation tendency, and recovery time of blue mussels to artificial sound exposures. We found that mussels responded to sound by partially closing their valves. This response decayed over sequential sound exposures and the mussels responded stronger again to a different sound stimulus. We thereby provide unambiguous evidence that the decay in response can be attributed to habituation. We did not find differences in the initial response and recovery between exposure to a pulse train and a single pulse, which revealed a strong bias in salience towards the on-set of pulse trains, and maybe to sound condition changes in general. Future studies are needed to examine the effects of variation in the sound stimulus and interval duration. It appears that the response and habituation of mussels to anthropogenic noise has potential for mitigating impact and this should also be investigated in their natural environment.

Ethical statement

Acknowledgements

Data accessibility

All data used for the analyses reported in this article are available from the Zenodo Repository, DOI:10.5281/zenodo.4068351.

References

ASAB (2018). Guidelines for the treatment of animals in behavioural research and teaching. Anim. Behav. 135, I–X.

Ballesta-Artero, I., Witbaard, R., Carroll, M. L. and van der Meer, J. (2017). Environmental factors regulating gaping activity of the bivalve *Arctica islandica* in Northern Norway. Mar. Biol. 164, 116.

Bayne, B. L., Thompson, R. J. and Widdows, J. (1976). Physiology I. In Marine Mussels:

Their Ecology and Physiology. (ed. Bayne, B. L.), pp. 121–206. London: Cambridge University Press.

Bejder, L., Samuels, A., Whitehead, H. and Gales, N. (2006). Interpreting short-term behavioural responses to disturbance within a longitudinal perspective. Anim. Behav. 72, 1149–1158.

Bejder, L., Samuels, A., Whitehead, H., Finn, H. and Allen, S. (2009). Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. Mar. Ecol. Prog. Ser. 395, 177–185.

Borthagaray, A. I. and Carranza, A. (2007). Mussels as ecosystem engineers: Their contribution to species richness in a rocky littoral community. Acta Oecologica 31, 243–250.

Campbell, J., Shafiei Sabet, S. and Slabbekoorn, H. (2019). Particle motion and sound pressure in fish tanks: A behavioural exploration of acoustic sensitivity in the zebrafish. Behav. Processes 164, 38–47.

Carroll, A. G., Przeslawski, R., Duncan, A., Gunning, M. and Bruce, B. (2017). A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pollut. Bull. 114, 9–24.

Chan, A. A. Y., Giraldo-Perez, P., Smith, S. and Blumstein, D. T. (2010). Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biol. Lett. 6, 458–461.

Charifi, M., Sow, M., Ciret, P., Benomar, S. and Massabuau, J. C. (2017). The sense of hearing in the Pacific oyster, *Magallana gigas*. PLoS One 12, 1–19.

Codarin, A., Wysocki, L. E., Ladich, F. and Picciulin, M. (2009). Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). Mar. Pollut. Bull. 58, 1880–7.

Curtis, T. M., Williamson, R. and Depledge, M. H. (2000). Simultaneous, long-term monitoring of valve and cardiac activity in the blue mussel *Mytilus edulis* exposed to copper. Mar. Biol. 136, 837–846.

Davis, M. (1970). Effects of interstimulus interval length and variability on startle-response habituation in the rat. J. Comp. Physiol. Psychol. 72, 177–192.

Day, R. D., McCauley, R. D., Fitzgibbon, Q. P., Hartmann, K. and Semmens, J. M. (2017). Exposure to seismic air gun signals causes physiological harm and alters behavior in the scallop *Pecten fumatus*. Proc. Natl. Acad. Sci. 114, E8537–E8546.

de Melo Júnior, U. G., Xavier, F. C., Campbell, D., Silveira, N., Versiani, L., Cumplido, R., Rodrigues, M. and Netto, E. B. F. (2020). Characterization of the acoustic activity of *Perna perna* (bivalve mollusc) under laboratory conditions. In Proceeding of Meetings in Acoustics, p. 010010.

de Soto, N. A., Delorme, N., Atkins, J., Howard, S., Williams, J. and Johnson, M. (2013). Anthropogenic noise causes body malformations and delays development in marine larvae. Sci. Rep. 3, 2831.

Dehaudt, B., Nguyen, M., Vadlamudi, A. and Blumstein, D. T. (2019). Giant clams discriminate threats along a risk gradient and display varying habituation rates to different stimuli. Ethology 125, 392–398.

Di Iorio, L., Gervaise, C., Jaud, V., Robson, A. A. and Chauvaud, L. (2012). Hydrophone

detects cracking sounds: Non-intrusive monitoring of bivalve movement. J. Exp. Mar. Bio. Ecol. 432–433, 9–16.

Ellers, O. (1995a). Behavioral Control of Swash-Riding in the Clam *Donax variabilis*. Biol. Bull. 189, 120–127.

Ellers, O. (1995b). Discrimination Among Wave-Generated Sounds by a Swash-Riding Clam. Biol. Bull. 189, 128–137.

Eurostat (2019a). Aquaculture statistics. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Archive:Aquaculture_statistics&direction=next&oldid=423407.

Eurostat (2019b). Fishery statistics. https://ec.europa.eu/eurostat/statistics-explained/index.php/Fishery_statistics.

Gatchel, R. J. (1975). Effects of interstimulus interval length on short-and long-term habituation of autonomic components of the orienting response. Physiol. Psychol. 3, 133–136.

Gordon, T. A. C., Radford, A. N., Davidson, I. K., Barnes, K., McCloskey, K., Nedelec, S. L., Meekan, M. G., McCormick, M. I. and Simpson, S. D. (2019). Acoustic enrichment can enhance fish community development on degraded coral reef habitat. Nat. Commun. 10, 5414.

Hall, M. V. (2013). A quasi-analytic model of the underwater sound signal from impact driving of an offshore semi-infinite pile. In Proceedings of Meetings on Acoustics, pp. 1–9.

Harding, H. R., Gordon, T. A. C., Hsuan, R. E., Mackaness, A. C. E., Radford, A. N. and Simpson, S. D. (2018). Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise. Biol. Lett. 14, 20180441.

Haszprunar, G. (1983). Comparative Analysis of the Abdominal Sense Organs of Pteriomorpha (Bivalvia). J. Molluscan Stud. 12A, 47–50.

Haszprunar, G. (1985). The Fine Structure of the Abdominal Sense Organs of Pteriomorpha (Mollusca, Bivalvia). J. Molluscan Stud. 51, 315–319.

Hawkins, A. D., Roberts, L. and Cheesman, S. (2014). Responses of free-living coastal pelagic fish to impulsive sounds. J. Acoust. Soc. Am. 135, 3101–3116.

Hubert, J., Campbell, J., van der Beek, J. G., den Haan, M. F., Verhave, R., Verkade, L. S. and Slabbekoorn, H. (2018). Effects of broadband sound exposure on the interaction between foraging crab and shrimp – A field study. Environ. Pollut. 243, 1923–1929.

Hubert, J., Neo, Y. Y., Winter, H. V. and Slabbekoorn, H. (2020). The role of ambient sound levels, signal-to-noise ratio, and stimulus pulse rate on behavioural disturbance of seabass in a net pen. Behav. Processes 170, 103992.

Johansson, K., Sigray, P., Backström, T. and Magnhagen, C. (2016). Stress Response and Habituation to Motorboat Noise in Two Coastal Fish Species in the Bothnian Sea. In (ed. Popper, A. N.) and Hawkins, A.), pp. 513–521. New York, NY: Springer New York.

Jolivet, A., Tremblay, R., Olivier, F., Gervaise, C., Sonier, R., Genard, B. and Chauvaud, L. (2016). Validation of trophic and anthropic underwater noise as settlement trigger in blue mussels. Sci. Rep. 6, 33829.

Jørgensen, C. B. (1990). Bivalve filter feeding: hydrodynamics, bioenergetics, physiology

and ecology. Fredensborg: Olsen & Olsen.

Jørgensen, C., Larsen, P., Møhlenberg, F. and Riisgård, H. (1988). The mussel pump: properties and modeling. Mar. Ecol. Prog. Ser. 45, 205–216.

Kautsky, N. (1981). On the trophic role of the blue mussel (*Mytilus edulis* L.) in the Baltic coastal ecosystem and the fate of the organic matter produced by the mussels. Kieler Meeresforsch 5, 454–461.

Lillis, A., Eggleston, D. B. and Bohnenstiehl, D. R. (2013). Oyster larvae settle in response to habitat-associated underwater sounds. PLoS One 8, 21–23.

Maire, O., Amouroux, J. M., Duchêne, J. C. and Grémare, A. (2007). Relationship between filtration activity and food availability in the Mediterranean mussel *Mytilus galloprovincialis*. Mar. Biol. 152, 1293–1307.

Matuschek, R. and Betke, K. (2009). Measurements of Construction Noise During Pile Driving of Offshore Research Platforms and Wind Farms. In Proceedings of NAG/DAGA 2009, pp. 262–265.

McCauley, R. D., Fewtrell, J., Duncan, A. J., Jenner, C., Jenner, M., Penrose, J. D., Prince, R. I. T., Adhitya, A., Murdoch, J. and McCabe, K. (2000). Marine Seismic Surveys – A Study of Enviromental Implications. APPEA J. 40, 692–708.

Mooney, T. A., Samson, J. E., Schlunk, A. D. and Zacarias, S. (2016). Loudness-dependent behavioral responses and habituation to sound by the longfin squid (*Doryteuthis pealeii*). J. Comp. Physiol. A 202, 489–501.

Morley, E. L., Jones, G. and Radford, A. N. (2014). The importance of invertebrates when considering the impacts of anthropogenic noise. Proc. R. Soc. B Biol. Sci. 281, 20132683.

Mosher, J. I. (1972). The Responses of *Macoma balthica* (Bivalvia) to Vibrations. J. Molluscan Stud. 40, 125–131.

Nedelec, S. L., Mills, S. C., Lecchini, D., Nedelec, B., Simpson, S. D. and Radford, A. N. (2016). Repeated exposure to noise increases tolerance in a coral reef fish. Environ. Pollut. 216, 428–436.

Neo, Y. Y., Ufkes, E., Kastelein, R. A., Winter, H. V., ten Cate, C. and Slabbekoorn, H. (2015a). Impulsive sounds change European seabass swimming patterns: Influence of pulse repetition interval. Mar. Pollut. Bull. 97, 111–117.

Neo, Y. Y., Parie, L., Bakker, F., Snelderwaard, P., Tudorache, C., Schaaf, M. and Slabbekoorn, H. (2015b). Behavioral changes in response to sound exposure and no spatial avoidance of noisy conditions in captive zebrafish. Front. Behav. Neurosci. 9, 1–11.

Neo, Y. Y., Hubert, J., Bolle, L. J., Winter, H. V. and Slabbekoorn, H. (2018). European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environ. Pollut. 239, 367–374.

Peng, C., Zhao, X., Liu, S., Shi, W., Han, Y., Guo, C., Jiang, J., Wan, H., Shen, T. and Liu, G. (2016). Effects of anthropogenic sound on digging behavior, metabolism, Ca2+/Mg2+ ATPase activity, and metabolism-related gene expression of the bivalve *Sinonovacula constricta*. Sci. Rep. 6, 24266.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Core Team (2020). nlme: Linear and Nonlinear Mixed Effects Models.

Popper, A. N. and Hawkins, A. D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. fish 94, 692–713.

R Core Team (2016). R: A Language and Environment for Statistical Computing.

Radford, A. N., Lèbre, L., Lecaillon, G., Nedelec, S. L. and Simpson, S. D. (2016). Repeated exposure reduces the response to impulsive noise in European seabass. Glob. Chang. Biol. 22, 3349–3360.

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., et al. (2009). Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. Neurobiol. Learn. Mem. 92, 135–138.

Roberts, L., Cheesman, S., Breithaupt, T. and Elliott, M. (2015). Sensitivity of the mussel *Mytilus edulis* to substrate-borne vibration in relation to anthropogenically generated noise. Mar. Ecol. Prog. Ser. 538, 185–195.

Robson, A. A., Garcia De Leaniz, C., Wilson, R. P. and Halsey, L. G. (2010). Behavioural adaptations of mussels to varying levels of food availability and predation risk. J. Molluscan Stud. 76, 348–353.

Rogers, P. H., Hawkins, A. D., Popper, A. N., Fay, R. R. and Gray, M. D. (2016). Parvulescu Revisited: Small Tank Acoustics for Bioacousticians. In The Effects of Noise on Aquatic Life II, pp. 933–941.

Samson, J. E., Mooney, T. A., Gussekloo, S. W. S. and Hanlon, R. T. (2014). Graded behavioral responses and habituation to sound in the common cuttlefish *Sepia officinalis*. J. Exp. Biol. 217, 4347–4355.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C. and Popper, A. N. (2010). A noisy spring: The impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427.

Slabbekoorn, H., Dalen, J., de Haan, D., Winter, H. V., Radford, C., Ainslie, M. A., Heaney, K. D., van Kooten, T., Thomas, L. and Harwood, J. (2019). Population-level consequences of seismic surveys on fishes: An interdisciplinary challenge. Fish Fish. 20, 653–685.

Solan, M., Hauton, C., Godbold, J. A., Wood, C. L., Leighton, T. G. and White, P. (2016). Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep. 6, 20540.

Spiga, I., Caldwell, G. S. and Bruintjes, R. (2016). Influence of pile driving on the clearance rate of the blue mussel, *Mytilus edulis* (L.). Proc. Meet. Acoust. 27,.

Vazzana, M., Celi, M., Maricchiolo, G., Genovese, L., Corrias, V., Quinci, E. M., de Vincenzi, G., Maccarrone, V., Cammilleri, G., Mazzola, S., et al. (2016). Are mussels able to distinguish underwater sounds? Assessment of the reactions of *Mytilus galloprovincialis* after exposure to lab-generated acoustic signals. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 201, 61–70.

Vetter, B. J., Cupp, A. R., Fredricks, K. T., Gaikowski, M. P. and Mensinger, A. F. (2015). Acoustical deterrence of Silver Carp (*Hypophthalmichthys molitrix*). Biol. Invasions 17, 3383–3392.

Wale, M. A., Briers, R. A., Hartl, M. G. J., Bryson, D. and Diele, K. (2019). From DNA to ecological performance: Effects of anthropogenic noise on a reef-building mussel. Sci. Total Environ. 689, 126–132.

Walsh, E. P., Arnott, G. and Kunc, H. P. (2017). Noise affects resource assessment in an invertebrate. Biol. Lett. 13,.

Wilkens, L. A. (1986). the Visual System of the Giant Clam *Tridacna*: Behavioral Adaptations. Biol. Bull. 170, 393–408.

Wilkens, S. L., Stanley, J. A. and Jeffs, A. G. (2012). Induction of settlement in mussel (*Perna canaliculus*) larvae by vessel noise. Biofouling 28, 65–72.

Wilson, L. J., Burrows, M. T., Hastie, G. D. and Wilson, B. (2014). Temporal variation and characterization of grunt sounds produced by Atlantic cod *Gadus morhua* and pollack *Pollachius pollachius* during the spawning season. J. Fish Biol. 84, 1014–1030.

Wysocki, L. E. and Ladich, F. (2005). Hearing in Fishes under Noise Conditions. J. Assoc. Res. Otolaryngol. 6, 28–36.

Wysocki, L. E., Dittami, J. P. and Ladich, F. (2006). Ship noise and cortisol secretion in European freshwater fishes. Biol. Conserv. 128, 501–508.

Zeileis, A., Kleiber, C. and Jackman, S. (2008). Regression Models for Count Data in R. J. Stat. Softw. 27, 1–25.

Zhadan, P. M. (2005). Directional Sensitivity of the Japanese Scallop *Mizuhopecten yessoensis* and Swift Scallop *Chlamys Swifti* to Water-Borne Vibrations. Psysiological Ecol. 31, 28–35.

Zhao, S., Yum, N. W., Benjamin, L., Benhamou, E., Yoneya, M., Furukawa, S., Dick, F., Slaney, M. and Chait, M. (2019). Rapid Ocular Responses Are Modulated by Bottom-up-Driven Auditory Salience. J. Neurosci. 39, 7703–7714.

Thesis summary & General discussion

Over the last decades, increasing amounts of studies on the effects of sound on marine life have been conducted (Erbe et al., 2019; Popper and Hawkins, 2019; Williams et al., 2015). Nevertheless, quantification of behavioural effects relevant to fitness consequences at individual and population level is still in its infancy (Slabbekoorn et al., 2019) and factors that modulate the effects of sound on animals are relatively unknown (Ellison et al., 2012). In this thesis, I addressed both knowledge gaps using captive and field studies on various marine animals from multiple trophic levels. For the quantification of behavioural responses relevant to fitness, I examined the changes in time budgets of Atlantic cod in a net pen in response to sound (chapter 2, Hubert et al., 2020), and conducted a similar experiment in a basin to be able to include quantification of foraging behaviour (chapter 3, Hubert et al., 2020b). To increase insight into factors that modulate sound impact, I examined the effect of variation in acoustic characteristics of the sound stimulus and the environment on European seabass in a net pen (chapter 4, Hubert et al., 2020c), the interspecific interaction between foraging shore crabs and common shrimps during sound exposure (chapter 6, Hubert et al., 2018), the cross-sensory interference by sound in foraging shore crabs (chapter 7, Hubert et al., 2021), and habituation to repeated sound exposures by blue mussels (chapter 8). Here, I summarize and discuss the main findings of all chapters and explore directions for future research.

Effects of sound on cod time budgets

A modelling study on Atlantic cod population growth indicated that reduced food intake and additional energy expenditure more easily lead to population-level effects than additional direct mortality and reproduction failure (Soudijn et al., 2020). So, to gain insight into population level consequences of acoustic disturbance, data on the energy budget of cod seems most relevant. Since it has not yet been possible to measure energy intake and expenditure directly in free-ranging cod that are exposed to sound, a first step may be to quantify the time spent in several behavioural states, which can later be linked to energy intake and expenditure. I quantified the time spent in various behavioural states by cod during and without sound exposure in two complementary experiments. In the net pen experiment (chapter 2), I used the swimming tracks of individual fish as input for Hidden Markov Models (HMMs), which allowed inference of behavioural states throughout the sampling period. The results indicated that some individuals tended to spend more time transiting and less time being locally active or inactive during sound exposure, which may indicate increased energy expenditure. The latter two states may include foraging behaviour, which is a proxy for energy intake and is critical to gain insight into population level effects. Future studies may be able to discriminate foraging behaviour as a

separate behavioural state by HMMs using additional data streams, for example from accelerometers.

Another way to quantify foraging behaviour is to record fish on video. I, therefore, designed a second experiment in basins in which the behavioural state of the fish could be manually scored from video footage (chapter 3). Despite several problems, resulting in a low sample size, time spent foraging, swimming and being stationary was scored in three pairs of cod. These fish spent more time swimming and less time being stationary on days with sound exposures, similar to the results from the net pen experiment, and no differences in time spent foraging were found. Qualitative observation of the videos showed that foraging typically involved low or intermediate swimming speed and a lot of turning. This confirmed that the behavioural states 'locally active' and 'inactive' might indeed include foraging behaviour. Future experiments are needed to examine how changes in time budget translate into changes in energy budget.

Time spent in various behavioural states can potentially be translated to energy expenditure and intake using swim tunnel experiments and various foraging experiments. The classification of behaviour into behavioural states in chapter 2 relied on swimming speed and turning angle. Swimming speed has been linked to oxygen use in fish, which is a proxy for energy use (Metcalfe et al., 2016; Tudorache et al., 2008). Such data can be used to translate swimming speed, derived from position data over time, to energy use. We still lack swim tunnel experiments that link oxygen use with swimming speed in Atlantic cod. Additionally, experiments are needed to also include additional energy use from potentially elevated stress levels and directional changes while swimming. Translating time spent foraging into energy intake requires data on the success rate of prey capture and on the energy content of the prey. The success rate can either be determined by an exposure experiment with video observations, such as in chapter 3, or by quantifying the fish' stomach content (e.g. Reubens et al., 2014). The energy content of the captured prey can be determined with a calorimeter (Benoit-Bird, 2004). Several earlier studies examined the effects of sound on swimming and foraging behaviour and found changes in swimming patterns including brief increases in swimming speed (e.g. Neo et al., 2014), reduced feeding attempts, and/or increased food handling errors (e.g. Bracciali et al., 2012; Shafiei Sabet et al., 2015). However, studies that examined both swimming patterns (or another proxy for energy use) and foraging behaviour simultaneously are scarce.

Ideally, behavioural and physiological effects of noise are quantified over a time window that resembles the actual duration of anthropogenic disturbance or over

the life time of the animal. Many sound impact studies examined behaviour over a relatively short time scale, at the onset of the sound or for a short period after the onset. However, anthropogenic sound is omnipresent, pile driving and seismic surveys can last for months and shipping is ongoing (Duarte et al., 2021; Slabbekoorn et al., 2019). Behavioural responses may change over time due to processes like habituation (chapter 7) and physiological stress levels may accumulate and only become problematic over time. Short-term responses are therefore likely not representative for long-term responses and short-term behavioural responses can be expected to be less likely than long-term responses to change individual fitness or population levels. So, to quantify effects of sound relevant to fitness and populations, more long-term experiments are needed. There seems only one study that examined the relatively long-term effects of increased vessel activity on both swimming activity and food intake in free-ranging fish. Tagged mulloways (Argyrosomus japonicus) were less active during the weekend, and other individuals had less full stomachs and fewer fish in their diet over the weekend. These differences were likely due to higher boat activity in the weekends (Payne et al., 2014). Such studies may aid to parametrization of changes in energy expenditure and intake due to sound exposure.

Effects of acoustic characteristics on seabass

Responsiveness of fish and other animals to sound may be partially modulated by acoustic characteristics of both the ambient noise and the sound stimulus. In efforts to mitigate the effects of anthropogenic sound, most attention is given to the amplitude of various sources. However, previous studies have shown that temporal patterns are modulating responses as well (Neo et al., 2014; Wysocki et al., 2006) and the same may apply to variation in the frequency distribution of exposure conditions (Marvit and Crawford, 2000; Slabbekoorn et al., 2010), and signal-to-noise levels associated with a particular disturbing sound (Kastelein et al., 2011; Wells, 2009). Ambient noise levels vary in amplitude due to weather conditions and distant boat noise (Carey and Browning, 1988; Wright et al., 2007). I tested the effect of experimentally elevated artificial background sound levels, various impulsive sound levels, and intervals between impulsive sound on European seabass in a net pen (chapter 4). The fish increased their swimming depth after the onset of the impulsive sound, but the magnitude of the change in depth could not be linked to any of the experimentally manipulated acoustic characteristics.

Previous studies have found differential effects of continuous versus impulsive sound, different pulse rates, and consistent versus fluctuating amplitude in fish behaviour and physiology (Neo et al., 2014; Neo et al., 2015a; Wysocki et al.,

2006). Additionally, increased background levels have been shown to increase hearing thresholds in cod (Hawkins and Chapman, 1975), and other vertebrates have also been shown to be sensitive to pulse intervals and elevated background levels (Davis, 1970; Gatchel, 1975; Schlittmeier et al., 2008). Since these other studies indicate that pulse interval and signal to noise ratio influence behaviour and sound detection (Davis, 1970; Gatchel, 1975; Hawkins and Chapman, 1975; Neo et al., 2015b), I call for further testing with wider ranges of all acoustic characteristics which may add to the current results. Complementary studies should be done using artificial sounds with specific ranges of acoustic variation, and realistic sounds that are relevant to occurrence in the field, for example pile driving sound with and without bubble screen, and different types of airguns or ships. Insights into the variable effects related to acoustic characteristics will aid in understanding and predicting behavioural responses, which can be used to mitigate and increase effects (for deterrence purposes).

Effects of sound on foraging crustaceans

Fish are relatively often subject of sound impact studies, probably mostly due to commercial interest. Invertebrates, including decapod crustaceans, still received limited attention, whereas their abundance is critical for higher trophic levels as food or through ecosystem services (Morley et al., 2014; Solan et al., 2016). Just like with fish, successful foraging behaviour in crustaceans is vital for growth, reproduction, and survival. So, impact of sound on foraging may negatively affect fitness at individual level and growth rate at population level. I examined the effects of sound on foraging crustaceans in two complementary studies: an in-situ experiment with free-ranging animals to allow interspecies interactions, and an indoor experiment to focus on a mechanism that might explain the results from the in-situ experiment. For the in-situ experiment, I used a baited camera to attract and film shore crabs and common shrimps (chapter 5). During sound exposure, fewer crabs aggregated around the food item than during a silent control. The increased shrimp numbers, however, could be explained by crab numbers rather than by the sound treatment. This means that shrimps could indirectly benefit from the sound exposure through competitive release (Slabbekoorn and Halfwerk, 2009). This shows that animals do not only respond directly to sound, but may also respond to the response to sound by other animals. Ultimately, we should be interested in the effects of sound on free-ranging animals in situ. So, more experiments that allow species interactions are needed on animals under natural conditions.

Since olfactory cues are important for foraging in crabs, I hypothesized that the reduced aggregation of crabs in the baited camera experiment could be

explained by cross-sensory interference. This entails interference of the sensory processing and interpretation of a stimulus, in this case most likely an olfactory cue, by simultaneous perception of a stimulus in another modality, in this case an auditory cue (Halfwerk and Slabbekoorn, 2015). To study this, I designed an experiment in which shore crabs were allowed to forage on a food item, but could only find it using olfactory cues (chapter 6). Food finding success and foraging efficiency were not negatively affected by the boat sound exposures, so no evidence to support the cross-sensory interference hypothesis was found.

Even though I found reduced aggregation at a food item during sound exposure by shore crabs in-situ, I did not find a lower food finding success rate or increased foraging duration during sound exposure in a T-maze. The seemingly different results in the two experiments may be explained in various ways including differences in sound exposures, experimental set ups, and study subject sizes. Another potential reason is the difference in food-deprivation. The individuals that were scored for the in-situ experiment were free-ranging animals and not tracked until they were in view of the camera, so their hunger levels were unknown. The individuals in the T-maze experiment were food-deprived for three days prior to their trial to standardize hunger levels across individuals in this experiment. Across experiments, it may however be that there were differences in motivation to go to the food item, resulting in different motivation levels to take risks. Various studies have experimentally shown that food-deprived individuals display more behaviour that the authors labelled as risky (Croy and Hughes, 1991; Godin and Crossman, 1994). Insight into such factors that modulate the effects of sound on animals will aid in understanding and the interpretation of sound impact studies, and will also help to extrapolate results from controlled experiments to the real-world and free-ranging conditions.

The aim of the T-maze study was not to determine absolute response levels to sound, but to examine cross-sensory interference as a potential mechanism for the reduced aggregation at a food item in-situ. Since we found no reduced food finding success rate or increased food finding duration, we found no evidence for cross-sensory interference of boat playbacks on olfactory mediated food finding. The seemingly different results across experiments again highlight that absolute response levels to sound can best be tested in-situ, whereas mechanisms underlying certain responses can best be studied in a controlled environment. Additionally, one of the six boat playback stimuli yielded deviating results: excluding this data showed that crabs were faster to reach the food during the remaining five boat playbacks than during the ambient control. This result did not affect our conclusion on cross-modal interference, but emphasised the need of sufficient replication of playback stimuli to prevent that a single stimulus

can affect the results substantially. This result also indicated that different boats may elicit different effects. Dedicated studies are needed to confirm and further examine this.

Habituation by mussels

Even sessile invertebrates are able to hear and respond to sound. Sessile animals, or other animals with a small home range, are likely to be exposed to anthropogenic sound repeatedly. Activities such as pile driving and seismic surveys can last for weeks to months in a particular area, and shipping intensity is continuously higher around harbours and shipping lanes (Haver et al., 2018; McCauley et al., 2000; Sertlek et al., 2019). During repeated or continuous exposures, habituation may mitigate part of the responses to sound. I examined whether blue mussels, a semi-sessile bivalve, can habituate to repeated sound exposures (chapter 7). Mussels were exposed to repeated sound exposures, followed by a single exposure to a different sound. After the onset of the first exposure, the mussels partially closed their valve gape. This response decreased in magnitude over repeated sound exposures, but was stronger again during the exposure to a different sound. This latter effect clearly showed that the decrease in response can be attributed to habituation (Bejder et al., 2009; Rankin et al., 2009). Habituation to sound does not necessarily mean the lack of any negative effects (Bejder et al., 2009), as sound may still cause physiological stress, mask relevant sounds, and result in shifting attention (Chan et al., 2010; Wale et al., 2019; Wysocki and Ladich, 2005). Nevertheless, studying the potential for a mitigating impact of habituation on the effects of anthropogenic noise is critical to understand the consequences of repeated sound exposures on animals.

Experimental set-ups and sound exposures

For this thesis, I conducted a variety of experiments that differed in both experimental set-up and sound exposures. The experiments were either conducted 1) indoors, using captive animals; 2) outdoors, using captive animals; or 3) outdoors using free-ranging animals. Indoors, it is easier to shield the experiment from external conditions (e.g., weather or unwanted sound) and typically also easier to perform standardized and high-resolution behavioural measurements. However, both the behaviour of the animals and the acoustic propagation is expected to be very different from the field (Rogers et al., 2016; Slabbekoorn, 2016). It is therefore critical to realize that some research questions can only be answered with the details and replication of measurements from the controlled conditions of an indoor experiment and that some research questions cannot be answered by just indoor studies. Outdoor experiments will

have a higher acoustic validity, meaning that the acoustic propagation in the experimental arena can be more easily translated to other outdoor locations. The behaviour of captive animals outdoors may also resemble that of free-ranging animals more. The best behavioural validity can obviously be achieved by using free-ranging individuals in their natural habitat (e.g. van der Knaap et al., 2021). The behaviour that can be measured outdoors is more limited and the resolution will likely be lower. However, again, it will depend on the goal of the experiment what set-up is best suited to answer a specific research question.

To expose the experimental animals to sound, I always used an underwater speaker and playbacks with either artificially generated sound or recordings of actual anthropogenic sound. I used artificially generated sound when highly controlled sound stimuli were required to answer the research questions, often related to the contribution of particular acoustic features to the response of the animals. Recordings of anthropogenic sound were used to increase the realism of the exposures. However, it should be noted that all exposure conditions tested in this thesis, including both indoor and outdoor experiments, were substantially different from actual exposure conditions in the outside world because of speaker limitations (lack of low frequencies: < 150 Hz), sound propagation complexity in tanks (different from outdoors), and a speaker being a point source, which is in contrast to most anthropogenic sources. The limitations in acoustic validity should make us refrain from any extrapolation from the results in terms of absolute sound or response levels. However, this was also not the target in this thesis, as I aimed to answer research questions which were fundamental in nature and did not aim to determine absolute threshold levels. Studies that do aim at absolute dose-response levels are best conducted in the field, using free-ranging animals, with actual anthropogenic sound sources, and taking a wide variety of response-modulating factors into account.

Ecological consequences

I found that animals at various trophic levels were affected by sound and that species interactions might also change due to sound exposures. When competitive or predator-prey balances between species shift, sound can have impact at a community and ecosystem level. There is already some evidence for this from other studies. Anthropogenic sound has been shown to reduce species richness in avian communities and to indirectly facilitate breeding success of particular species because of lower abundance of a nest predator species (Francis et al., 2009; Slabbekoorn and Halfwerk, 2009). Mulloways had an altered diet composition in the weekends, the days with most boating activity (Payne et al., 2014), from which we can infer shifts in predator-prey relationships. In chapter

5, I also showed that less crabs aggregated at a food item during sound, which created an opportunity for shrimps. Through such mechanisms, sound can change the environment beyond a single species. The current study species are all abundant species and play important roles in the ecosystem; as reef builder, water filterer, prey, or predator. If sound changes foraging, growth, reproduction or survival for one or more of these species, this may therefore change the ecosystem substantially. It should be noted, however, that all my experiments were relatively short-term and more without than with species interactions. More studies are therefore needed as also potential ecosystem effects have to be tested empirically. Again, this is best tested in the field, using free-ranging animals, but can be complementary to mechanistic studies in captivity.

Conclusion

The amount of anthropogenic activities at sea is not likely to decrease in the near future. Both the amount of shipping and the amount of offshore wind farms is expected to continue to increase (International Maritime Organization, 2015; Reed, 2020). The amount of seismic surveys is partly linked to the oil price and may be harder to predict. However, seismic surveys are also used to identify sites for carbon sequestration which may increase in popularity in an effort to mitigate global warming (Carroll et al., 2014). Mitigation measures to reduce sound levels of shipping and piling are being developed, and seismic airguns with less loud high frequency components are already available. Nevertheless, the amount of anthropogenic sound in the marine environment is expected to remain substantial (Duarte et al., 2021). So, it remains important to continue studying the effects of anthropogenic sound.

The variety in test conditions, model species, and test results in this thesis, indicates that this area of research remains a growing field of opportunity, for both fundamental studies and investigations of applied value. It is important to continue research that aims to quantify fitness effects for individuals and populations. This thesis may provide a first step, but similar research has to be conducted in-situ, complementary with experiments that will allow translation from changes in time budgets to changes in energy budgets, and consequently to changes in growth, reproduction, and survival. Additionally, studies into the factors that modulate the effects of sound are needed to fully understand the impact of sound. Such studies may be best conducted in captivity with a high level of experimental control and the ability to track individuals at a high resolution. The use of controlled indoor studies is occasionally debated, but in this way, both in-situ and controlled experiments are complementary and both valuable to increase insight into the effects of sound on animals while using the opportunities and being aware of the limitations of both types of experiments.

References

Bejder, L., Samuels, A., Whitehead, H., Finn, H. and Allen, S. (2009). Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. Mar. Ecol. Prog. Ser. 395, 177–185.

Benoit-Bird, K. J. (2004). Prey caloric value and predator energy needs: Foraging predictions for wild spinner dolphins. Mar. Biol. 145, 435–444.

Bracciali, C., Campobello, D., Giacoma, C. and Sarà, G. (2012). Effects of nautical traffic and noise on foraging patterns of mediterranean Damselfish (*Chromis chromis*). PLoS One 7,.

Carey, W. M. and Browning, D. (1988). Low Frequency Ocean Ambient Noise: Measurements and Theory. In Sea Surface Sound, pp. 361–376. Dordrecht: Springer.

Carroll, A. G., Przeslawski, R., Radke, L. C., Black, J. R., Picard, K., Moreau, J. W., Haese, R. R. and Nichol, S. (2014). Environmental considerations for subseabed geological storage of CO2: A review. Cont. Shelf Res. 83, 116–128.

Chan, A. A. Y., Giraldo-Perez, P., Smith, S. and Blumstein, D. T. (2010). Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biol. Lett. 6, 458–461.

Croy, M. I. and Hughes, R. N. (1991). Effects of food supply, hunger, danger and competition on choice of foraging location by the fifteen-spined stickleback, *Spinachia spinachia* L . Anim. Behav. 42, 131–139.

Davis, M. (1970). Effects of interstimulus interval length and variability on startle-response habituation in the rat. J. Comp. Physiol. Psychol. 72, 177–192.

Duarte, C. M., Chapuis, L., Collin, S. P., Costa, D. P., Devassy, R. P., Eguiluz, V. M., Erbe, C., Gordon, T. A. C., Halpern, B. S., Harding, H. R., et al. (2021). The soundscape of the Anthropocene ocean. Science (80-.). 371,.

Ellison, W. T., Southall, B. L., Clark, C. W. and Frankel, A. S. (2012). A New Context-Based Approach to Assess Marine Mammal Behavioral Responses to Anthropogenic Sounds. Conserv. Biol. 26, 21–28.

Erbe, C., Marley, S. A., Schoeman, R. P., Smith, J. N., Trigg, L. E. and Embling, C. B. (2019). The Effects of Ship Noise on Marine Mammals—A Review. Front. Mar. Sci. 6,.

Francis, C. D., Ortega, C. P. and Cruz, A. (2009). Noise Pollution Changes Avian Communities and Species Interactions. Curr. Biol. 19, 1415–1419.

Gatchel, R. J. (1975). Effects of interstimulus interval length on short-and long-term habituation of autonomic components of the orienting response. Physiol. Psychol. 3, 133–136.

Godin, J. J. and Crossman, S. L. (1994). Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (*Gasterosteus aculeatus*) under predation risk. Behav. Ecol. Sociobiol. 34, 359–366.

Halfwerk, W. and Slabbekoorn, H. (2015). Pollution going multimodal: The complex impact of the human-altered sensory environment on animal perception and performance. Biol. Lett. 11,.

Haver, S. M., Gedamke, J., Hatch, L. T., Dziak, R. P., Van Parijs, S., McKenna, M. F.,

Barlow, J., Berchok, C., DiDonato, E., Hanson, B., et al. (2018). Monitoring long-term soundscape trends in U.S. Waters: The NOAA/NPS Ocean Noise Reference Station Network. Mar. Policy 90, 6–13.

Hawkins, A. D. and Chapman, C. J. (1975). Masked auditory thresholds in the cod, *Gadus morhua* L. J. Comp. Physiol. 103, 209–226.

Hubert, J., Campbell, J., van der Beek, J. G., den Haan, M. F., Verhave, R., Verkade, L. S. and Slabbekoorn, H. (2018). Effects of broadband sound exposure on the interaction between foraging crab and shrimp – A field study. Environ. Pollut. 243, 1923–1929.

Hubert, J., Campbell, J. A. and Slabbekoorn, H. (2020a). Effects of seismic airgun playbacks on swimming patterns and behavioural states of Atlantic cod in a net pen. Mar. Pollut. Bull. 160, 111680.

Hubert, J., Wille, D. A. and Slabbekoorn, H. (2020b). Exploring effects of sound on the time budget of fishes: An experimental approach with captive cod. In Proceedings of Meetings on Acoustics, p. 010012.

Hubert, J., Neo, Y. Y., Winter, H. V. and Slabbekoorn, H. (2020c). The role of ambient sound levels, signal-to-noise ratio, and stimulus pulse rate on behavioural disturbance of seabass in a net pen. Behav. Processes 170, 103992.

Hubert, J., van Bemmelen, J. J. and Slabbekoorn, H. (2021). No negative effects of boat sound playbacks on olfactory-mediated food finding behaviour of shore crabs in a T-maze. Environ. Pollut. 270, 1–8.

International Maritime Organization (2015). Third IMO GHG Study 2014. Londen.

Kastelein, R. A., Steen, N., de Jong, C., Wensveen, P. J. and Verboom, W. C. (2011). Effect of broadband-noise masking on the behavioral response of a harbor porpoise (*Phocoena phocoena*) to 1-s duration 6–7 kHz sonar up-sweeps. J. Acoust. Soc. Am. 129, 2307–2315.

Marvit, P. and Crawford, J. D. (2000). Auditory discrimination in a sound-producing electric fish (Pollimyrus): Tone frequency and click-rate difference detection. J. Acoust. Soc. Am. 108, 1819–1825.

McCauley, R. D., Fewtrell, J., Duncan, A. J., Jenner, C., Jenner, M., Penrose, J. D., Prince, R. I. T., Adhitya, A., Murdoch, J. and McCabe, K. (2000). Marine Seismic Surveys – A Study of Enviromental Implications. APPEA J. 40, 692–708.

Metcalfe, J. D., Wright, S., Tudorache, C. and Wilson, R. P. (2016). Recent advances in telemetry for estimating the energy metabolism of wild fishes. J. Fish Biol. 88, 284–297.

Morley, E. L., Jones, G. and Radford, A. N. (2014). The importance of invertebrates when considering the impacts of anthropogenic noise. Proc. R. Soc. B Biol. Sci. 281, 20132683.

Neo, Y. Y., Seitz, J., Kastelein, R. A., Winter, H. V., ten Cate, C. and Slabbekoorn, H. (2014). Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biol. Conserv. 178, 65–73.

Neo, Y. Y., Parie, L., Bakker, F., Snelderwaard, P., Tudorache, C., Schaaf, M. and Slabbekoorn, H. (2015a). Behavioral changes in response to sound exposure and no spatial avoidance of noisy conditions in captive zebrafish. Front. Behav. Neurosci. 9, 1–11.

Neo, Y. Y., Ufkes, E., Kastelein, R. A., Winter, H. V., ten Cate, C. and Slabbekoorn, H. (2015b). Impulsive sounds change European seabass swimming patterns: Influence of pulse repetition interval. Mar. Pollut. Bull. 97, 111–117.

Payne, N. L., van der Meulen, D. E., Suthers, I. M., Gray, C. A. and Taylor, M. D. (2014). Foraging intensity of wild mulloway Argyrosomus japonicus decreases with increasing anthropogenic disturbance. Mar. Biol. 162, 539–546.

Popper, A. N. and Hawkins, A. D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. fish 94, 692–713.

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., et al. (2009). Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. Neurobiol. Learn. Mem. 92, 135–138.

Reed, S. (2020). A New Weapon Against Climate Change May Float. New York Times.

Reubens, J., De Rijcke, M., Degraer, S. and Vincx, M. (2014). Diel variation in feeding and movement patterns of juvenile Atlantic cod at offshore wind farms. J. Sea Res. 85, 214–221.

Rogers, P. H., Hawkins, A. D., Popper, A. N., Fay, R. R. and Gray, M. D. (2016). Parvulescu Revisited: Small Tank Acoustics for Bioacousticians. In The Effects of Noise on Aquatic Life II, pp. 933–941.

Schlittmeier, S. J., Hellbrück, J., Thaden, R. and Vorländer, M. (2008). The impact of background speech varying in intelligibility: Effects on cognitive performance and perceived disturbance. Ergonomics 51, 719–736.

Sertlek, H. Ö., Slabbekoorn, H., ten Cate, C. and Ainslie, M. A. (2019). Source specific sound mapping: Spatial, temporal and spectral distribution of sound in the Dutch North Sea. Environ. Pollut. 247, 1143–1157.

Shafiei Sabet, S., Neo, Y. Y. and Slabbekoorn, H. (2015). The effect of temporal variation in experimental noise exposure on swimming and foraging behaviour of captive zebrafish. Anim. Behav. 49–60.

Slabbekoorn, H. (2016). Aiming for Progress in Understanding Underwater Noise Impact on Fish: Complementary Need for Indoor and Outdoor Studies. In The Effects of Noise on Aquatic Life II (ed. Popper, N. A.) and Hawkins, A.), pp. 1057–1065. New York, NY: Springer New York.

Slabbekoorn, H. and Halfwerk, W. (2009). Behavioural Ecology: Noise Annoys at Community Level. Curr. Biol. 19, R693–R695.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C. and Popper, A. N. (2010). A noisy spring: The impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427.

Slabbekoorn, H., Dalen, J., de Haan, D., Winter, H. V., Radford, C., Ainslie, M. A., Heaney, K. D., van Kooten, T., Thomas, L. and Harwood, J. (2019). Population-level consequences of seismic surveys on fishes: An interdisciplinary challenge. Fish Fish. 20, 653–685.

Solan, M., Hauton, C., Godbold, J. A., Wood, C. L., Leighton, T. G. and White, P. (2016). Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep. 6, 20540.

Soudijn, F. H., van Kooten, T., Slabbekoorn, H. and de Roos, A. M. (2020). Population-level effects of acoustic disturbance in Atlantic cod: a size-structured analysis based on energy budgets. Proc. R. Soc. B Biol. Sci. 287, 20200490.

Tudorache, C., Viaene, P., Blust, R., Vereecken, H. and De Boeck, G. (2008). A comparison of swimming capacity and energy use in seven European freshwater fish species. Ecol. Freshw. Fish 17, 284–291.

van der Knaap, I., Reubens, J., Thomas, L., Ainslie, M. A., Winter, H. V, Hubert, J., Martin, B. and Slabbekoorn, H. (2021). Effects of a seismic survey on movement of free-ranging Atlantic cod. Curr. Biol. 1–8.

Wale, M. A., Briers, R. A., Hartl, M. G. J., Bryson, D. and Diele, K. (2019). From DNA to ecological performance: Effects of anthropogenic noise on a reef-building mussel. Sci. Total Environ. 689, 126–132.

Wells, D. L. (2009). Sensory stimulation as environmental enrichment for captive animals: A review. Appl. Anim. Behav. Sci. 118, 1–11.

Williams, R., Wright, a. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., Clark, C. W., Cullis-Suzuki, S., Dakin, D. T., Erbe, C., et al. (2015). Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24.

Wright, A. J., Soto, N. A., Baldwin, A. L., Bateson, M., Beale, C. M., Clark, C., Deak, T., Edwards, E. F., Fernández, A., Godinho, A., et al. (2007). Anthropogenic Noise as a Stressor in Animals: A Multidisciplinary Perspective. Int. J. Comp. Psychol. 20, 250–273.

Wysocki, L. E. and Ladich, F. (2005). Hearing in Fishes under Noise Conditions. J. Assoc. Res. Otolaryngol. 6, 28–36.

Wysocki, L. E., Dittami, J. P. and Ladich, F. (2006). Ship noise and cortisol secretion in European freshwater fishes. Biol. Conserv. 128, 501–508.

Nederlandse samenvatting

De mariene onderwaterwereld is gevuld met een kakofonie van geluid. Dit geluid wordt geproduceerd door de golven en het kolken van water en door de vele dieren die geluid gebruiken om te communiceren. Over de afgelopen tientallen jaren is er steeds meer menselijke activiteit op zee, waarbij ook veel geluid wordt geproduceerd. In dit proefschrift heb ik effecten van lawaai op verschillende zeedieren onderzocht, waaronder zowel prooien als predatoren. Ik begin hier met een toelichting op de belangrijkste lawaaibronnen, en hoe en waarom dieren horen, voordat ik de resultaten van mijn onderzoek zal bespreken.

In het Nederlandse deel van de Noordzee is scheepvaart de grootste geluidsbron. Andere prominente geluidsbronnen zijn de uitvoering van seismisch onderzoek, heiwerkzaamheden en het opruimen van opgeviste explosieven. Seismisch onderzoek wordt uitgevoerd om structuren in de zeebodem in kaart te brengen, onder andere voor de zoektocht naar olie en gas. Tijdens seismisch onderzoek vaart een schip met lage snelheid en trekt 'airguns' voort die elke 6-15 seconden een harde klap produceren. De weerkaatsing van dit geluid op de verschillende bodemlagen wordt opgenomen door de hydrofoons (onderwatermicrofoons) die aan lange kabels ook door het schip worden voortgetrokken. Heiwerkzaamheden worden onder andere uitgevoerd voor het plaatsen van windmolens, olieplatformen, en het bouwen van havens. Ten slotte liggen er nog vele explosieven uit de Tweede Wereldoorlog op de bodem van de Nederlandse Noordzee. Als er een explosief wordt gevonden kan het noodzakelijk blijken om het gecontroleerd tot ontploffing te laten brengen.

Het geluid van deze verschillende activiteiten is hoorbaar voor de meeste – of misschien wel alle – mariene dieren. Inzicht in wat dieren horen, hoe ze op geluid reageren, en de effecten van menselijk geluid op hun welzijn, kan helpen om mariene ecosystemen te beschermen. Dieren kunnen geluid gebruiken om zich te oriënteren, bijvoorbeeld om geschikt leefgebied te vinden of predatoren op te merken en om te communiceren, bijvoorbeeld om een partner aan te trekken. Van alle vissen wordt verwacht dat ze geluid kunnen horen, en veel soorten maken zelf geluid. Ze horen geluid met behulp van haarcellen rondom gehoorsteentjes (otolieten), vergelijkbaar met ons binnenoor. Vissen hebben ongeveer dezelfde dichtheid als water en bewegen mee met de waterdeeltjes die trillen door de geluidsgolf. Omdat de gehoorsteentjes een hogere dichtheid hebben, bewegen deze pas later mee (uit fase) en zo kan de beweging worden waargenomen door de trilharen van de haarcellen. De zwemblaas van vissen kan ook een rol spelen. Door de drukverschillen van een geluidsgolf fluctueert de zwemblaas in grootte, en deze beweging kan ook doorgegeven worden aan de haarcellen rondom de otolieten. Ten slotte hebben vissen ook haarcellen op of in hun huid, die ook trillingen van het geluid waar kunnen nemen. Veel zijn

geconcentreerd in een zijlijn op beide flanken van de vis en op de kop rondom de bek.

Ook ongewervelden kunnen geluid waarnemen, en de gehoororganen lijken vaak op die van vissen. In plaats van otolieten hebben veel ongewervelden statolieten; dat zijn ook door haarcellen omgeven steentjes die relatief zwaar zijn. Daarnaast hebben verschillende ongewervelden, zoals krabben en garnalen, ook haarcellen op de buitenkant van hun lichaam. Sommige ongewervelden hebben speciale organen om geluid te detecteren, bijvoorbeeld een deel van de tweekleppigen, waar mosselen onder vallen. Een belangrijk verschil met de meeste vissen is dat ongewervelden geen zwemblaas of andere met-gas-gevulde ruimte hebben. Dit houdt in dat ongewervelden alleen de trilling – die veroorzaakt wordt door geluid – kunnen waarnemen, en niet de geluidsdruk, terwijl vissen met een zwemblaas beide componenten kunnen detecteren.

Omdat mariene dieren gebruik maken van geluid voor activiteiten die belangrijk zijn voor hun overleving en voortplanting, is het aannemelijk dat dieren beïnvloed worden door menselijk geluid. Dichtbij een sterke geluidsbron, zoals heien, kunnen vissen zelfs fysiek gewond raken. Onder andere hun zwemblaas en haarvaten kunnen beschadigen door de hoge geluidsdruk. Tot een veel grotere afstand van de geluidsbron kan menselijk geluid ander biologisch relevant geluid maskeren. Dit verkleint de afstand waarop geschikt habitat, partners, predatoren en prooien kunnen worden waargenomen. Daarnaast kan ook het gedrag en de fysiologie van dieren beïnvloed worden. Het is onder andere aangetoond dat vissen hun zwempatronen aanpassen, minder efficiënt zijn bij het pakken van hun voedsel. Geluid kan ook hun stressniveau verhogen.

Deze voorbeelden laten zien dat er een grote variatie in mogelijke effecten van menselijk geluid is. Dit maakt het echter ook moeilijk om in te schatten hoe schadelijk de effecten van geluid op groei, overleving en voortplanting van dieren zijn. Fysieke verwondingen van vissen dichtbij een sterke geluidsbron lijken misschien het meest ernstig, maar dit is slechts van toepassing op relatief kleine aantallen dieren. De schijnbaar mildere effecten vinden echter plaats op veel grotere afstand van de geluidsbron en beïnvloeden veel meer dieren. Daarom hebben dit soort effecten de meeste potentie om tot populatieeffecten te leiden. Daarom is het extra belangrijk om de effecten van geluid op gedrag en fysiologie te onderzoeken.

Voor dit proefschrift heb ik diverse experimenten met geluidsblootstellingen uitgevoerd. Ik heb gedragseffecten die relevant zijn voor groei, overleving en voortplanting gekwantificeerd en een aantal factoren die effecten van

geluid mogelijk beïnvloeden onderzocht. Hiervoor heb ik zowel vissen als ongewervelden onderzocht: kabeljauwen, zeebaarzen, strandkrabben, grijze garnalen en gewone mosselen. Alle experimenten heb ik samen met studenten en collega's uitgevoerd (zie Acknowledgements).

Onderzoek naar de gedragseffecten van geluid op dieren kan helpen om de gevolgen op populatieniveau in te schatten. Een eerder onderzoek naar kabeljauw, met behulp van een computermodel, liet zien dat een toename in energieverbruik en een verminderde voedselinname door geluid meer potentie hebben om de populatiegroei af te remmen dan directe effecten op overleving of reproductie. Dit is te verklaren met indirecte effecten, die optellen over tijd, op overleving en reproductie, via groei, rijping en een goede conditie.

Gedrag dat kan leiden tot een verhoogd energieverbruik is bijvoorbeeld: vaker of sneller zwemmen. Gedrag dat leidt tot een verminderde voedselinname is bijvoorbeeld minder vaak forageren of minder succesvol forageergedrag. Voor hoofdstuk 2 en 3 heb ik op verschillende manieren de tijd vastgesteld die kabeljauw aan bepaald gedrag besteedt, met en zonder geluidsblootstelling aan opnames van airguns of seismisch onderzoek.

Voor hoofdstuk 2 heb ik individuele kabeljauwen losgelaten in een groot net in de Oosterschelde. Met behulp van een zender in de kabeljauw en ontvangers buiten het net kon ik nauwkeurig hun positie bepalen. Op basis van de zwempatronen heb ik hun gedrag geclassificeerd, hieruit bleek dat sommigen kabeljauwen tijdens geluid vaker sneller zwemmen en minder tijd niet bewegen en rustig zwemmen (en mogelijk voedsel zoeken). Het bleek echter niet mogelijk om forageergedrag als aparte categorie te classificeren, daarom heb ik nog een experiment met kabeljauw uitgevoerd.

Voor hoofdstuk 3 heb ik drie paar kabeljauwen in een groot binnenbassin blootgesteld aan geluid. Met behulp van videomateriaal werd bepaald hoeveel tijd de kabeljauwen zwommen, forageerden of stil zaten. In lijn met hoofdstuk 2 zwommen de kabeljauw vaker, en zaten ze minder vaak stil tijdens dagen met geluidsblootstelling. Er waren geen verschillen in tijdbesteding aan forageren. Vervolgstudies zijn nodig om dit soort experimenten ook in het wild uit te voeren, en om tijdsbestedingen aan de verschillende gedragingen te vertalen naar energieverbruik en -opname. Uiteindelijk kan dit type data gebruikt worden voor het inschatten van de gevolgen van geluid op kabeljauwpopulaties.

Behalve het bepalen van de gevolgen van geluid voor populaties, is het ook nuttig om te kijken welke factoren het effect van geluid kunnen beïnvloeden.

Het zou bijvoorbeeld kunnen dat het aanpassen van geluidskarakteristieken tot vermindering van effecten leidt. Daarnaast is inzage in de effecten van geluidskarakteristieken ook belangrijk om verschillen in effecten tussen menselijke geluidsbronnen te kunnen verklaren en effecten van nieuwe bronnen te kunnen inschatten.

Voor hoofdstuk 4 heb ik een experiment uitgevoerd om het inzicht in effecten van geluidkarakteristieken te vergroten. Ik heb groepjes van vier zeebaarzen in een net blootgesteld aan geluidspulsen en kunstmatig verhoogd achtergrondgeluid. Ik gebruikte verschillende geluidsniveaus van zowel het achtergrondgeluid als de pulsen, en verschillende tijdsintervallen tussen pulsen, om verschillen in reactie op geluidskarakteristieken aan te kunnen tonen. Wanneer de geluidspulsen startten, doken de zeebaarzen naar beneden. Echter, hoe ver ze naar beneden doken kon niet gelinkt worden aan het geluidspuls-interval, de geluidssterkte van de achtergrond en pulsen, of de verhouding tussen de geluidssterkte van de achtergrond en die van de pulsen (signaal-ruisverhouding). Het zou kunnen dat een groter bereik van deze geluidskarakteristieken wel tot verschillen zullen leiden, maar vervolgonderzoek moet dat uitwijzen.

Naast vissen, heb ik ook onderzoek gedaan naar ongewervelden om te kijken welke mechanismes bepalen of, en hoe sterk, dieren reageren op geluid. Het zou bijvoorbeeld kunnen dat individuen van bepaalde soorten elkaar beïnvloeden. Tot nu toe heeft dit echter weinig aandacht gekregen omdat veel experimenten in afgesloten omgevingen zijn uitgevoerd of omdat maar een enkele soort werd bestudeerd. Dit kan ertoe leiden dat resultaten uit gecontroleerde omgevingen niet overeenkomen met de reactie van dieren in het wild.

Voor hoofdstuk 5 heb ik dit onderzocht door meerdere keren camera's met aas op de bodem van de Oosterschelde te laten zakken. Tijdens een deel van de tests werd geluid afgespeeld. Na het plaatsen van de opstellingen kwamen er steeds meer krabben en garnalen op het voedsel af. Tijdens het afspelen van geluid kwamen er echter minder strandkrabben op het voedsel af en door de lagere hoeveelheid krabben kwamen er meer grijze garnalen op het voedsel af. De garnalen leken dus meer beïnvloed te worden door de krabben dan door het geluid. Er zijn meerdere verklaringen mogelijk waarom er minder krabben op het voedsel afkwamen. Geur is heel belangrijk voor krabben om voedsel te zoeken, en hoewel geluid geur niet beïnvloedt, zou het misschien wel kunnen dat krabben afgeleid raken en de geur daarom minder goed kunnen oppikken.

Voor hoofdstuk 6 heb ik dit getest door middel van een 'T-maze' experiment. Een T-maze is een opstelling in de vorm van de letter T waarbij een dier, in

dit geval een strandkrab, bij de T-splitsing kan kiezen om naar links of naar rechts te gaan. In dit geval bevatte één uiteinde een dode mossel, en het andere uiteinde niets, dit was vanaf de T-splitsing niet te zien voor de krab. Wel was er een continue waterstroom van beide uiteinden naar de beginplek, zodat de krab kon detecteren waar het voedsel lag. Een deel van de krabben werd blootgesteld aan opnames van natuurlijk achtergrondgeluid, en een deel aan bootgeluid. Er was geen negatief effect van het bootgeluid op de succeskans of de efficiëntie van het vinden van het voedsel door de krabben. Dit toont aan dat – in dit geval – geluid krabben niet afleidt bij het oppikken en verwerken van geuren.

Veel van de onderzoeken naar de effecten van geluid, inclusief de meeste onderzoeken in dit proefschrift, duren relatief kort terwijl dieren in het wild waarschijnlijk hun hele leven aan menselijk geluid worden blootgesteld. De reden voor kortdurende experimenten is vaak dat je veel individuen moet testen om betrouwbare conclusies te kunnen trekken. Echter kan het ook zijn dat dieren na herhaaldelijke blootstelling anders reageren op geluid, dus het is belangrijk om ook experimenten met herhaaldelijke blootstellingen uit te voeren. Dit heb ik voor mijn proefschrift gedaan met gewone mosselen.

Voor hoofdstuk 7 werden mosselen uitgerust met sensors zodat gemeten kon worden hoe ver ze hun schelpen openden terwijl ze herhaaldelijk aan geluid werden blootgesteld. De mosselen reageerden meestal op geluid door hun schelpen deels te sluiten, maar tijdens opeenvolgende geluidsblootstellingen werd deze reactie steeds minder sterk. Vervolgens werd er een nieuw geluid afgespeeld en – als dit genoeg verschilde van het voorgaande geluid – reageerden de mosselen weer net zo sterk als op de eerste geluidsblootstelling. Dit laat zien dat de mosselen niet moe of doof worden, maar dat ze aan het geluid wennen. Verder onderzoek moet aantonen of andere gedrags- of fysiologische reacties ook verminderen tijdens herhaalde blootstellingen en of dit betekent dat mosselen echt minder hinder van geluid ondervinden door gewenning.

De resultaten van dit proefschrift laten zien dat een grote variëteit van mariene dieren beïnvloed kan worden door geluidsverstoring. De methodologie en resultaten van dit proefschrift kunnen op termijn hopelijk bijdragen aan het inschatten van populatiegevolgen van geluid op kabeljauw of mariene dieren in het algemeen. Ze vergroten het inzicht in mechanismesn die het effect van geluid mede bepalen. Er zijn echter nog veel stappen te nemen om populatiegevolgen vast te kunnen stellen en te weten waarom sommige dieren wel op geluidsverstoring reageren en anderen schijnbaar niet.

Ook is er nog veel onderzoek nodig naar het voorkomen of verminderen van

lawaaioverlast. Er worden wel al pogingen gedaan om geluidsbronnen stiller te maken, maar de hoeveelheid menselijk geluid in de mariene wereld zal voorlopig substantieel blijven. Daarom blijft het noodzakelijk om effecten op dieren en de mogelijke gevolgen voor het mariene ecosysteem in kaart te brengen, te onderzoeken hoe de effecten verminderd kunnen worden en te testen of bestaande maatregelen ook echt werken.

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Curriculum Vitae

Jeroen Hubert was born in 1991 in Zoetermeer, the Netherlands. He attended high school at Stedelijk College Zoetermeer and finished a HAVO programme in 2008. He received a Bachelor's degree in Animal Husbandry at the University of Applied Sciences Hogeschool Inholland Delft in 2012. He continued his education at Leiden University and obtained his Master's degree in Animal Sciences and Health in 2015.

During his Bachelor, he did two internships, one at bird park Avifauna, during which he set up the European Studbook for white-headed vultures. For his graduation internship at De Dierenbescherming, he developed methodology to assess the welfare of birds of prey in captivity. During his Master, he also did two research projects. For his first project he investigated the effects of sound on European seabass, with Dr. Errol Neo and Dr. Hans Slabbekoorn. For his second project, he studied the perception of rhythmic patterns in birds and humans, with Dr. Michelle Spierings and Prof. dr. Carel ten Cate.

After his Master, he fulfilled two sequential positions as a research assistant at the Behavioural Biology lab of Leiden University. First, he studied the perception of iambic–trochaic grouping of acoustic patterns in zebra finches, with Dr. Michelle Spierings and Prof. dr. Carel ten Cate. For the second position, he studied acoustic deterrence of European seabass with Dr. Errol Neo and Dr. Hans Slabbekoorn. All Master research projects and research assistantships resulted in peer-reviewed scientific papers. In 2017, he started his PhD-project on the effects of anthropogenic sound on marine animals. During his PhD, he conducted several scientific studies in the field as well as in the lab. He worked on a variety of species including European seabass, Atlantic cod, shore crabs, spider conches, and several bivalves, and worked in the Netherlands, Belgium and Saudi Arabia. He also participated in teaching in courses on behavioural biology and supervised various internships of both Bachelor and Master students. After his PhD, he will continue his academic career as Postdoctoral researcher at Leiden University to study pelagic fish ecology and deterrence in offshore wind farms.
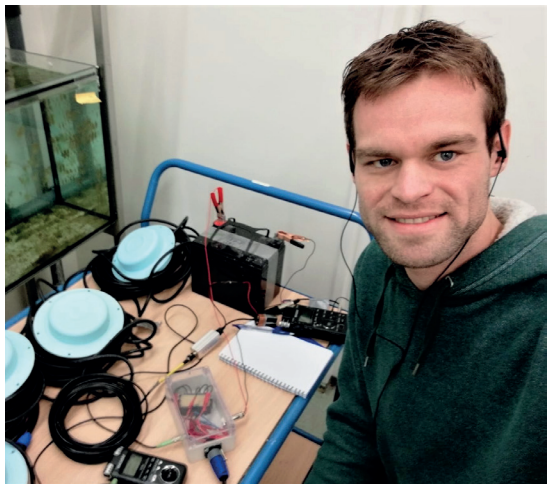
Publications

Under review

Hubert, J., Booms, E., Witbaard, R., & Slabbekoorn, H. Blue mussels habituate to repeated sound exposures and respond just to the on-set of fast pulse trains.

Peer-reviewed articles

van der Knaap, I., Reubens, J., Thomas, L., Ainslie, M., Winter, H. V., **Hubert, J.**, Martin, B., & Slabbekoorn, H. (2021) Effects of a seismic survey on movement of free- ranging Atlantic cod. Current Biology, 31, 1–8. DOI:10.1016/j. cub.2021.01.050.

Hubert, J., van Bemmelen, J. J., & Slabbekoorn, H. (2021). No negative effects of boat sound playbacks on olfactory-mediated food finding behaviour of shore crabs in a T-maze. Environmental Pollution, 270, 116184. DOI:10.1016/j. envpol.2020.116184

Hubert, J., Campbell, J. A., & Slabbekoorn, H. (2020). Effects of seismic airgun playbacks on swimming patterns and behavioural states of Atlantic cod in a net pen. Marine Pollution Bulletin, 160, 111680. DOI:10.1016/j. marpolbul.2020.111680

Hubert, J., Neo, Y. Y., Winter, H. V., & Slabbekoorn, H. (2020). The role of ambient sound levels, signal-to-noise ratio, and stimulus pulse rate on behavioural disturbance of seabass in a net pen. Behavioural Processes, 170, 103992. DOI:10.1016/j.beproc.2019.103992

Hubert, J., Campbell, J., van der Beek, J. G., den Haan, M. F., Verhave, R., Verkade, L. S., & Slabbekoorn, H. (2018). Effects of broadband sound exposure on the interaction between foraging crab and shrimp – A field study. Environmental Pollution, 243, 1923–1929. DOI:10.1016/j.envpol.2018.09.076

Neo, Y. Y., **Hubert, J.**, Bolle, L. J., Winter, H. V., & Slabbekoorn, H. (2018). European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environmental Pollution, 239, 367–374. DOI:10.1016/j.envpol.2018.04.018

Spierings, M., **Hubert, J.**, & ten Cate, C. (2017). Selective auditory grouping by zebra finches: testing the iambic–trochaic law. Animal Cognition, 20 (4), 665–675. DOI:10.1007/s10071-017-1089-3

Neo, Y. Y., **Hubert, J.**, Bolle, L., Winter, H. V., Ten Cate, C., & Slabbekoorn, H. (2016). Sound exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure. Environmental Pollution, 214, 26–34. DOI:10.1016/j.envpol.2016.03.075

ten Cate, C., Spierings, M., **Hubert, J.**, & Honing, H. (2016). Can birds perceive rhythmic patterns? A review and experiments on a songbird and a parrot species. Frontiers in Psychology, 7 (May). 1–14. DOI:10.3389/fpsyg.2016.00730

Conference proceedings

Hubert, J., Wille, D. A., & Slabbekoorn, H. (2020). Exploring effects of sound on the time budget of fishes: An experimental approach with captive cod. In Proceedings of Meetings on Acoustics (Vol. 37, p. 010012). DOI:10.1121/2.0001253

Hubert, J., Neo, Y. Y., Campbell, J. A., & Slabbekoorn, H. (2016). Particle motion and pressure soundscape in outdoor vs indoor set-up. In Proceedings of Meetings on Acoustics (Vol. 27, p. 070007). DOI:10.1121/2.0000273

Popular article

Soudijn, F., **Hubert, J.**, van der Knaap, I. (2020). Herrie onder water. Visionair, 57, 38-41.