On the effects of high intensity impulsive sound on young European sea bass *Dicentrarchus labrax,* with special attention to pile driving during offshore wind farm construction

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More than 25 years ago, the link was established between anthropogenic sound and negative effects on marine mammals. Since then, marine mammals have dominated the bioacoustics research, but recently the focus has widened to fish, and to a lesser extent, also invertebrates. The frequency range of anthropogenic sound often overlaps with the hearing range of the fish. Consequently, underwater sound has the potential to cause auditory injuries, physiological stress, and behavioural disturbance and to mask biological relevant sounds. In addition, sound pressure can influence the swim bladder volume which can result in (mortal) internal injuries. So, depending on the characteristics of the sound and the fish species, the impact of anthropogenic sound on fish can range from immediate death to no impact whatsoever. Since fish are a vital component in most ecosystem food webs, and since a lot of fish species have a high economic value, it is necessary to document sound effects and to define thresholds for different combinations of sound sources and fish species.

In this study, we addressed sound effects as a result of pile driving during offshore wind farm (OWF) construction, an increasingly important human activity throughout the North Sea. Pile driving effects were assessed for young individuals of European sea bass *Dicentrarchus labrax*, which is a fish species with a closed swim bladder (physoclists). The PhD study started from the assumption that there is a 100% mortality in fish eggs and larvae up to 1 km around a pile driving source, as formulated by Prins et al. (2009).

The multidisciplinary study aimed to disentangle the effects of impulsive sound (produced by pile driving) on young fish, focusing on the following research questions:
- Are young fish (larvae and juveniles) affected by impulsive sound, what are the effects, and at what level do they manifest, *e.g.* mortality, (sub)lethal injuries, stress responses or behavioural responses?
- Can the effects on young fish be linked to a specific sound-related metric or biological parameter? Can sound thresholds at which underwater sound negatively affects young fish be identified?
- What is the ecological significance of the observed effects?
- How will the results from this PhD add to management and policy regulations in Belgium (and Europe), *i.e.* in order to minimise the environmental impact of pile driving activities in future offshore wind farms, and to achieve Good Environmental Status (GES) for Marine Strategy Framework Directive (MSFD) descriptor 11?

The context of this multidisciplinary study on the impact of pile driving on European sea bass is outlined in Chapter 1. A general introduction to the underwater world of sound is given. In the North Sea, shipping, seismic surveys, underwater explosions and pile driving are identified as the main contributors of the anthropogenic sound energy. Sound plays an essential role in conveying environmental information to marine fauna (e.g. marine mammals, fish and invertebrates). Particularly in marine mammals, sound has a key role in social and foraging behaviour. But of all vertebrates, fish exhibit the greatest diversity of hearing sensitivity and structures. The frequency range of most anthropogenic sound overlaps with the hearing range of fish and in addition, the sound can cause (mortal) physical damage to the marine mammal and fish. The exact impact, the underlying mechanisms and the ecological consequences of anthropogenic sound on marine life are not yet understood, especially for fish. In Europe, anthropogenic underwater noise was labelled as a pollutant within the MSFD of the European Commission. Consequently, the impact of the underwater sound generated by various anthropogenic sound sources, need to be evaluated on marine life in order to take appropriate measures. In Belgium, a new anthropogenic sound source, pile driving, was recently introduced. It is the main method to install OWFs and will regularly be used in the next couple of year. Therefore, this PhD study took pile driving as the impulsive sound source to study its impact on marine fish.

The following four chapters (Chapter 2-5) present the assessment of the impact of high intensity pile driving sound on acute and delayed mortality, acute and chronic physiological stress responses and at low intensity impulsive sound the impact on behaviour of young European sea bass. Furthermore, the critical sound parameters of the physiological stress responses were studied in detail.

Chapter 2 presents the results of an *in situ* experiment on board of a pile driving vessel addressing acute and delayed mortality of juvenile (68 and 115 days old) European sea bass. It was the first field study to assess fish mortality as close as 45 m from the offshore pile driving source for a complete pile driving session. Fish were exposed to 1739 up to 3067 pile driving strikes with a single strike sound exposure level (SEL_{ss}) between 181 and 188 dB re 1 μPa².s, and a cumulative sound exposure level (SEL_{cum}) between 215 and 222 dB re 1 μPa².s. No increased acute mortality was observed when we compared European sea bass (of 68 and 115 days post hatching) exposed to pile driving with a control group exposed to ambient background sound levels in between the pile driving sessions. This study validates the results provided by previous laboratory studies inside acoustically controlled chambers. The surviving fish were transported back to the lab and their survival was further monitored for two weeks. At least under optimal laboratory conditions, we observed no delayed mortality caused by pile driving. This study rejected the 100 % mortality hypothesis, and if internal injuries were present, they were shown not to be mortal.

In Chapter 3 the physiological stress response of juvenile sea bass (68 and 115 days old) was investigated under pile driving. So far, this has never been studied. During the same *in situ* experiment as described in chapter 1, primary, secondary and tertiary stress responses were investigated during or after exposure to a complete pile driving session. As a primary stress response, whole-body cortisol seemed to be too sensitive to 'handling' bias. However, a strong secondary stress response to pile driving was detected as significant reductions in oxygen consumption rate (49 – 55%) and low whole-body lactate concentrations. Contrary to fish used on the first day of the experiment, the fish used on the second day were already indirectly exposed to pile driving. Fish in the control group of that experiment reduced their respiration by 34 – 40% compared to the control group on the first day. This may be indicative of a prolonged stress response or increased sensitivity towards new stressors. A tertiary stress response only manifests when homeostasis cannot be re-established. After 30 days in the laboratory, specific growth rate and fitness of the exposed fish were not affected compared to unhandled fish, so a tertiary stress response was absent. Still, a short term reduction in fitness was demonstrated. Hence, we can assume that repeated exposure to impulsive sound in the field will inevitably lead to less fit fish in the wild.

Chapter 4 explores the critical sound parameters responsible for the acoustic physiological stress response observed in the field experiment. The primary and secondary stress responses of larval and juvenile European sea bass to strong impulsive sound were compared between two lab experiments using different sound sources (SIG Sparker and Larvaebrator) and associated frequency spectra, and with the stress responses measured in a recently conducted field study (*in situ* pile driving). Both lab sound sources produced similar levels for the standard sound pressure metrics as the *in situ* pile driving, being zero-to-peak sound pressure level (L_{z-p}) of 208 dB re 1 μPa), SEL_{ss} of 181 dB re 1 μPa²·s and SEL_{cum} of 214 dB re 1 μPa²·s. However, the three sources differed in their sound frequency spectra. The whole-body cortisol results (a proxy for primary stress responses) confirmed the susceptibility of both juvenile and larval fish to handling stress. Still, based on the increased (or altered) whole-body cortisol levels, high intensity impulsive sound evoked an acoustic primary stress response. Common ground was found at the high energy levels (SEL_{ss}) produced between the 63 and 630 Hz 1/3 octave bands. This frequency range covers the hearing range of European sea bass, relating primary stress response in juvenile fish to hearing discomfort. Reduced oxygen consumption rates of ~50% were observed in the juveniles in the field experiment and larvae in the sparker experiment, and to a lesser extent in the juveniles of the sparker experiment. Consequently, the secondary stress response was most likely linked to high intensity sound produced at higher frequencies (>800 Hz). This secondary stress response may be related to pressure induced swim bladder discomfort.

Still, high intensity impulsive sound covering a broad frequency range (like a real *in situ* pile driving) is needed to evoke strong secondary stress responses (e.g. reduced oxygen consumption rate and whole-body lactate levels) in juvenile sea bass. This implies that lab results may not directly be translated to the real world, as some known or unknown parameters (like frequency content) may differ. Based on the experiments, the sound pressure level at which stress responses were evoked, seemed to be located between SEL_{ss} 170 and 180 dB re 1 μPa²·s. More studies on different life stages and the role of non-standard sound parameters such as particle motion are needed to confirm these values as real stress thresholds for fish.

Underwater sound has the potential to disturb the behaviour of fish even at lower sound pressure levels, resulting in a much wider impact range around the pile driving source than high sound pressure levels. Since functionally important behaviour, such as social interactions and foraging, can contribute significantly to the survival and reproduction of fish, any impact on functional traits can directly be translated into fitness consequences. However, so far only a couple of studies have tested the acute impact of anthropogenic sound exposure on fish behavior. Consequently, in Chapter 5, fish behaviour was studied in response to impulsive sound on three consecutive days in a laboratory set-up. In this indoor laboratory study, we tested the influence of pile driving sound on the swimming activity and aggression of young juvenile European sea bass *Dicentrarchus labrax* before, during and immediately after the 25 min sound exposure (1000 strikes, SEL_{ss} =156 dB re 1 μPa²·s, $L_{z\text{-}p}$ = 175 dB re 1 μPa; SEL_{cum} = 186 dB re 1 μPa²·s). We also tested the impact on feeding tendency and efficiency when fish were already 15 min exposed to the impulsive sound. The sea bass interrupted their swimming activities and ceased any aggressive actions to conspecifics at the onset of the impulsive sound exposure. The behavioural effects of sound exposure returned to the pre-exposure base line within the 25 minute exposure period. On the first day, the sound exposure caused an attention shift. Resulting in a delayed reappearance of the aggressive attacks to just after the sound exposure and a reduction in the number of food intake events during and immediately after the sound exposure compared to pre-exposure food intake events on the first day. This attention shift was no longer observed during day 2 or 3 of the experimental trials. These findings indicate that fish can habituate to the impulsive sound over a relatively short period of time, which may moderate the acute sound impacts on behaviour. It remains to be tested whether this also applies to wild-ranging fish and whether such habituation effects are also to be found in other species.

In Chapter 6, the technical and practical challenges to field and lab experiments in bioacoustics are discussed. Results of this PhD study are discussed in a broader perspective through comparison with literature on pile driving and other anthropogenic sources of impulsive sound. The results obtained during this study allowed describing the impact of pile driving sound on European sea bass. Exposure to a complete pile driving session as close as 45 m from the pile driving activity did not result in acute or delayed mortality of juvenile European sea bass. It did lead to a strong physiological stress response limited to a relative short period of time, which can be extended by multiple sound exposures. Based on the field and lab results, the physiological stress response found in larvae and juveniles could be related to the standard sound metrics (SEL_{ss}, SEL_{cum} and $L_{z\text{-}p}$) and the frequency range of the highest energy. Furthermore, hearing discomfort and swim bladder discomfort could explain respectively the primary and secondary stress response. The above mentioned studies involve high intensity underwater sound found at close range from the pile driving source. At larger distance from the pile driving source, the impulsive sounds contain less energy but can still induce a behavioural response in juvenile European sea bass at the onset of the sound exposure. During the sound exposure, European sea bass were able to recover from their initial response and repeated exposure had no effect on feeding. Combining these results with other data from literature reveals the interspecific variability of in their behavioural response to the same type of stressor. Therefore, more species varying in life history strategies need to be studied before the results can truthfully be generalised.

Integration of the study results with current knowledge allows us to make suggestions regarding sound thresholds for mortality, physiological stress and behavioural changes. Since mortality was absent in our field study, the mortality threshold must lie above the measure sound parameters (SEL_{ss} > 188 dB re 1 μPa².s; SEL_{cum} > 222 dB re 1 μPa².s.; $L_{z\text{-}p}$ = 210 dB re 1 μPa). This study was the first to propose a sound threshold range in which physiological stress could occur in fish which lied between SEL_{ss} of 170 - 181 dB re 1 μPa²·s at frequencies higher than 315 Hz. A threshold for behavioural disturbance linked to pile driving cannot yet be determined.

Additionally, consequences on an ecological level need to be evaluated. In other words, effects on an individual level need to be modelled into population effects since individual effects in fish are subordinate to population effects from an ecological point of view. In order to do so, data on the presence of sound sources, sound propagation, important impact on the individual, population size, distribution and affected (sub)population need to be estimated before the individual effect can be modelled into a population effect. This is not yet possible for fish, but given the results about the effects found on individual fish, we can expect that the ecological consequences of pile driving sound on fish health are subtle.

In Europe, the MSFD defined a GES in which underwater sound needs to be at levels that do not adversely affect the ecosystem. A Technical Subgroup Noise (TSG Noise) was commission to further develop the descriptor on underwater noise. They advised to set up a sound register, to log all sound producing human activities. In addition, they appointed 'considerable displacement' of marine organisms as the most relevant impact of impulsive sound. Finally, an inventory of the pulse-block days in the EU's regional seas can be modelled, based on the presence of anthropogenic sound sources in ¼ ICES rectangles that are producing sound levels above the threshold linked to the 'considerable displacement' impact. A GES contains all marine organisms while the TSG Noise mainly based its advice on marine mammals. 'Considerable displacement', may not be the most relevant impact of fish. Fish are also neglected in the national legislation of the Member States of Europe. Based on this PhD, the effects of pile driving sound on fish are more subtle than anticipated and no stringent measures are needed ad hoc in Belgium or other member states. Still, more research is needed to support or reject the decision to exclude fish from management in order to ensure a GES for all marine fauna.
Finally, future research targets are identified. These are needed to progress towards an acoustically sound approach. The lack of particle motion data remains the big gap and needs to be addressed by

future studies. The underlying critical sound parameters need to be further unravelled that induce the physiological stress and behavioural responses. Furthermore, data is needed on the long-term impact of the acoustic stressor in order to model the ecological consequences of pile driving at population level. Studying the fish in its natural environment with new technologies is the way to go. And finally, the impact of the continuous sound produced by the next 20 years of the operational OWF on fish health need to be addressed.

Reference

- Prins, T.C., Van Beek, J.K.L., Bolle, L.J., 2009. Modelschatting van de effecten van heien voor offshore windmolenparken op de aanvoer van vislarven naar Natura 2000. Deltares, Delft.