CHAPTER 11

THE EFFECTS OF HIGH INTENSITY IMPULSIVE SOUND ON YOUNG EUROPEAN SEA BASS *DICENTRARCHUS LABRAX*, WITH SPECIAL ATTENTION TO PILE DRIVING

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ABSTRACT

Throughout the North Sea, a new anthropogenic sound source, pile driving, was recently introduced. It is the main method to install offshore wind farms (OWFs) and will regularly be used during the next couple of years. Pile driving generates strong impulsive noise that can affect the health and wellbeing of marine life. However, the exact impact, the underlying mechanisms and the ecological consequences of anthropogenic sound on marine life are not yet understood, especially for fish. This study investigated the impact of pile driving on young sea bass *Dicentrarchus labrax*. More specifically, the acute and delayed mortality, acute and chronic physiological stress responses and the impact of lower intensity impulsive sound on the fish behaviour were assessed through field and laboratory experiments. A field experiment at 45 m from the pile driving activity revealed no acute or delayed mortality but the fish showed strong acute secondary stress responses, a 50% decrease in oxygen consumption rate. This result could not be completely reproduced by two laboratory studies, indicating the importance of the frequency content in addition to the standard sound metrics for the physiological stress responses. Furthermore, juvenile fish reduced their swimming activity and ceased all aggressive attacks on conspecifics at the onset of the impulsive sound exposure, but showed behavioural recovery within 25 minutes. The results also showed that the initial response can change under repeated exposure. Based on these acute short-term effects, the ecological consequences of pile driving sound on fish health are probably subtle. More research on multiple species and at population level are required as well as long-term data, especially on behavioural responses, in order to decide on the ecological relevance of pile driving on young fish.

11.1. INTRODUCTION

More than 25 years ago, a relation between man-made (anthropogenic) sound and its negative effects on marine mammals was established (Simmonds and Lopezjurado, 1991). Since then, marine mammals have dominated the bioacoustics research, although recently the focus has widened to fish, and to a lesser extent, also to invertebrates (Southall et al., 2007, Slabbekoorn et al., 2010, Williams et al., 2015). Sound plays an essential role in conveying environmental information to marine fauna. Particularly in marine mammals, sound is known to play a key role in social and foraging behaviour. But of all vertebrates, fish exhibit the greatest diversity in hearing sensitivity and hearing structures and are a vital component in most ecosystem food webs (Popper and Fay, 2011). The main contributors to the anthropogenic sound energy in the North Sea are shipping, seismic surveys, underwater explosions and pile driving (Ainslie et al., 2009). The frequency range of man-made 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 biologically relevant sounds (Slabbekoorn et al., 2010). In addition, sound pressure can influence the swim bladder volume which can result in (mortal) internal injuries (Halvorsen et al., 2012b). So, the impact of anthropogenic sound on fish can range from immediate death to no impact at all. However, 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 Marine Strategy Framework Directive of the European Commission (Directive 2008/56/EC). Consequently, the impact of underwater sound on marine life, generated by various anthropogenic sound sources, need to be evaluated in order to take appropriate measures. Throughout the North Sea, a new anthropogenic sound source, pile driving, was recently introduced. It is the main method to install offshore wind farms (OWFs) and will regularly be used during the next couple of years. As OWFs are one of the options EU member states choose in order to achieve the renewable targets set by the Europe (Renewables Directive 2009/28/EC). Therefore, this PhD study took pile driving as the source of high intensity impulsive sound to study its impact on marine fish. Pile driving effects were assessed for young individuals of European sea bass *Dicentrarchus labrax*, a fish species with a closed swim bladder, so-called physoclists (Debusschere, 2016). The PhD started from the assumption made by a Dutch report in 2009 (Prins et al., 2009), which hypothesized a 100% mortality in fish eggs and larvae up to 1 km around a pile driving source. This assumption was based on modelled fish larvae distributions, mortality rate due to underwater explosions and back-calculated energy levels of underwater sound related to pile driving activities in the Dutch part of the North Sea.

This study had a multidisciplinary approach, aiming to disentangle the effects of impulsive sound (produced by pile driving) on young fish, thereby focusing on the following research questions:

(I) Are young fish (larvae and juveniles) affected by impulsive sound, what are the effects, and at what level do they manifest, *e.g.* mortality, stress responses or behavioural responses?

(II) 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?

(III) What is the ecological significance of the observed effects?

(IV) 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 study was published as a doctoral thesis and this report corresponds to the executive summary (Debusschere, 2016). Within the PhD framework, field and lab experiments were carried out in order to assess the impact of high intensity pile driving sound on acute and delayed mortality, acute and chronic physiological stress responses and the impact of lower intensity impulsive sound on the behaviour of young European sea bass. In addition, the critical sound parameters of physiological stress responses were studied in detail.

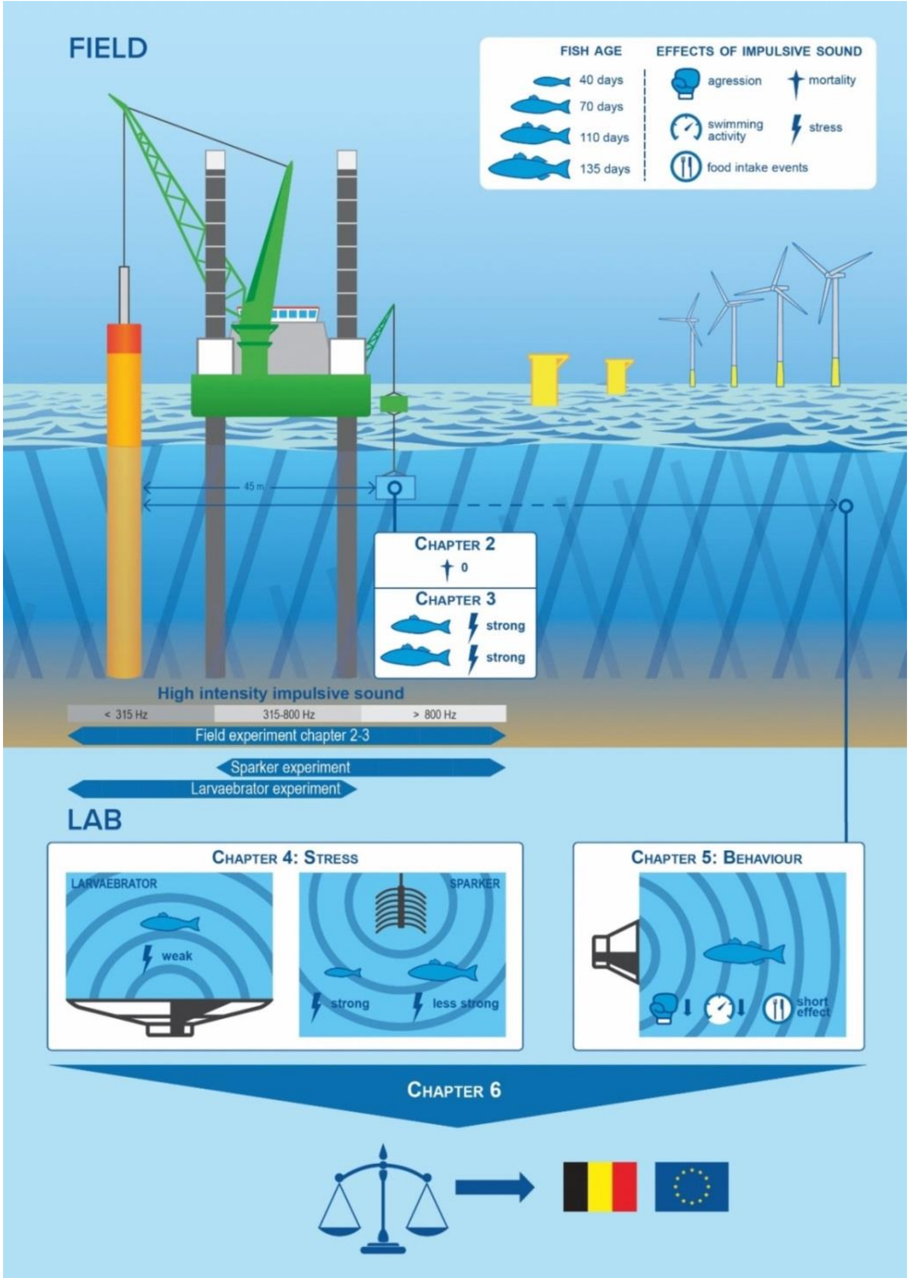

Figure 1. Schematic overview of the chapters of the PhD study comprising field and lab experiments with juvenile European sea bass, preceded by a general introduction (Chapter 1) and completed with a general discussion (Chapter 6) (Debusschere, 2016). Chapter 2 and 3 comprise the *in situ* experiment performed on board of the pile driving vessel assessing respectively, the impact on mortality and stress responses of young juvenile fish. Chapter 4 discussed the stress responses of the fish in two lab experiments with two high intensity sound sources whereas Chapter 5 used a lower intensity sound source in the lab to study fish behaviour. Figure taken from Debusschere (2016; availabe via www.vliz.be/nl/imis).

11.2. RESULTS

FIELD EXPERIMENTS: MORTALITY AND STRESS RESPONSES

An *in situ* experiment on board of a pile driving vessel was performed, addressing acute and delayed mortality of juvenile (68 and 115 days old) European sea bass (Debusschere et al., 2014). It was the first field study to assess fish mortality as close as 45 m from an offshore pile driving source over a complete pile driving session (Figure 1 - Chapter 2). 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 $\mu Pa^2 \cdot s$, and a cumulative sound exposure level (SEL_{cum}) between 215 and 222 dB re 1 $\mu Pa^2 \cdot s$. No increased acute mortality was observed when we compared European sea bass (68 and 115 days old) 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 other studies inside acoustically controlled chambers in the laboratory (Bolle et al., 2012, Halvorsen et al., 2012a, Casper et al., 2013a). Fish survival was further monitored in the lab 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 as stated by a Dutch report in 2009 (Prins et al., 2009). Moreover, if internal injuries were present, they were shown not to be mortal.

A second aim of the *in situ* experiment was to assess the physiological stress response of juvenile sea bass (68 and 115 days old) to high intensity sound produced by pile driving (Figure 1 - Chapter 3). So far, this was not yet studied. The primary, secondary and tertiary stress responses were investigated during and after exposure to a complete pile driving session (Debusschere et al., 2016). As a primary stress response proxy, whole-body cortisol seemed to be too sensitive to 'handling' bias (Figure 2). 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. In contrast to fish used on the first day of the trip (monopile 1), the fish used on the second day (monopile 2) had already been indirectly exposed to pile driving. Fish in the control group of that second day reduced their respiration by 34 to 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 condition of the exposed fish were not affected compared to unhandled fish, so a tertiary stress response was absent. Only a short-term reduction in metabolic rate was demonstrated while the long-term consequences of repeated impulsive sound exposure for fish in the field are yet to be determined.

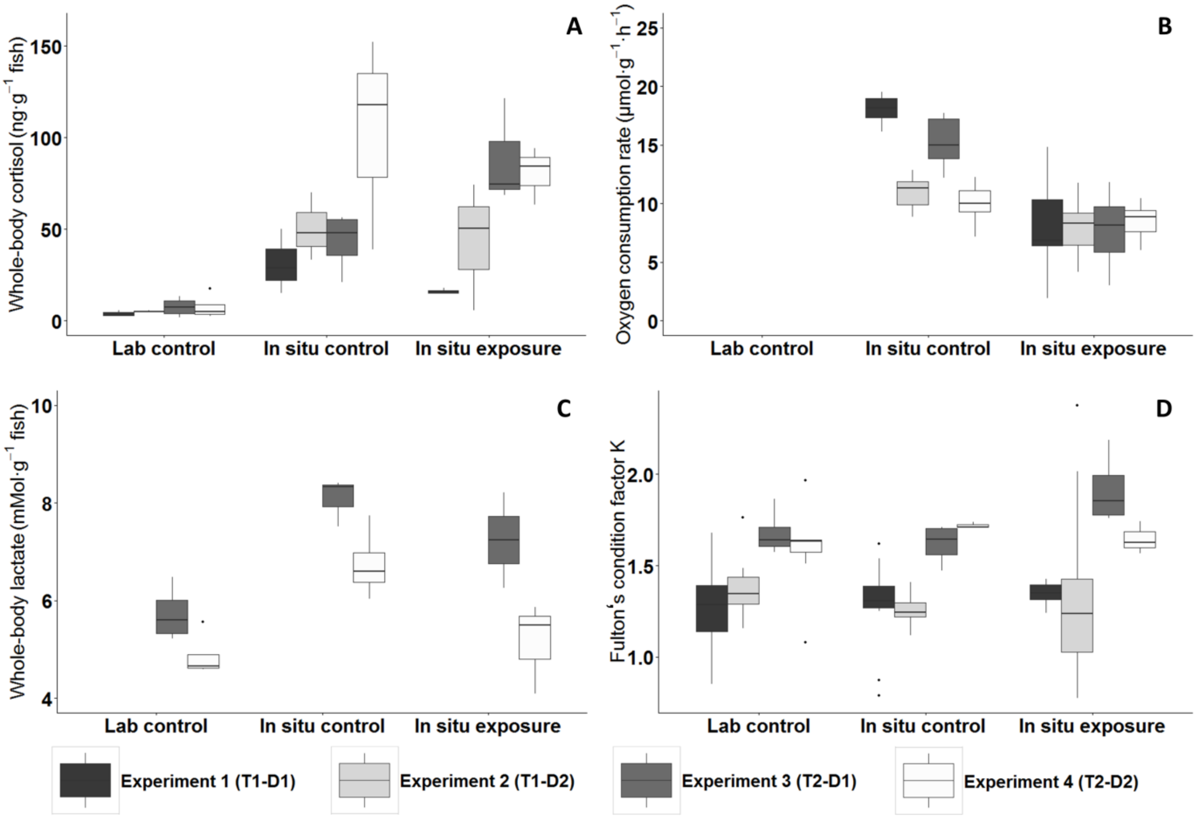

Figure 2. Stress responses of juvenile European sea bass *Dicentrarchus labrax*, based on four experiments (trip 1-2; day 1-2) and three treatments each: no handling/no exposure (lab control), exposed to ambient sound (*in situ* control), and exposed to impulsive sounds during a complete pile driving session at 45 m from the pile driving activity (*in situ* exposed). Fish were 68 days old (dph) at the start of experiments 1 and 2 and 115 dph in experiments 3 and 4. (A) Whole-body cortisol ($ng \cdot g^{-1}$ fish); (B) Oxygen consumption rate ($\mu mol \cdot g^{-1} \cdot h^{-1}$); (C) Whole-body lactate ($mMol \cdot g^{-1}$ fish, no data for experiments 1 and 2); (D) Fulton's condition factor K measured after 30 days. Figure taken from Debusschere et al. (2016).

LINKING LABORATORY TO FIELD OBSERVATIONS: STRESS RESPONSES

The critical sound parameters responsible for the acoustic physiological stress response observed in the field experiment needed to be explored further. Therefore, 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) (Debusschere et al., submitted). These results were then compared with the stress responses measured during an *in situ* pile driving study (Figure 1 - Chapter 4) (Debusschere et al., 2016). Both lab sound sources produced similar levels at maximum energy for the standard sound pressure metrics as the *in situ* pile driving, being zero-to-peak sound pressure level ($L_{z\text{-}p}$) 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 (Figure 3). The whole-body cortisol results (a proxy for primary stress responses) confirmed the susceptibility of both juvenile and larval fish to handling stress. Still, the increased (or altered) whole-body cortisol levels indicated that high intensity impulsive sound evoked an acoustic primary stress response (Figure 4A-C). Common ground between the field and two lab experiments was found at the high energy levels (SEL_{ss}) produced between the 315 and 630 Hz 1/3 octave bands (Figure 3). This frequency range covers the responsiveness range of European sea bass to sound, relating the primary stress response in juvenile sea bass to hearing. Reduced oxygen consumption rates of ~50% were observed in the juveniles in the field experiment and larvae in the sparker experiment (max. exposure), and to a lesser extent in the juveniles of the sparker experiment (Figure 4D-F). Consequently, the secondary stress response can most likely be linked to high intensity sound produced at higher frequencies (>800 Hz), above the responsiveness range of European sea bass. This secondary stress response may be associated with the pressure induced swim bladder oscillations. It may be clear that high intensity impulsive sound must cover a broad frequency range (similar to a real *in situ* pile driving) to evoke strong secondary stress responses, such as reduced oxygen consumption rate and reduced whole-body lactate levels in juvenile sea bass (Figure 4G-I). This implies that lab results can not directly be translated to the real world, as some known (like frequency content) and unknown parameters may not be comparable. More studies on different life stages and on the role of non-standard sound parameters - such as particle motion - are needed to further clarify the triggering parameters and sound thresholds of the stress response of fish.

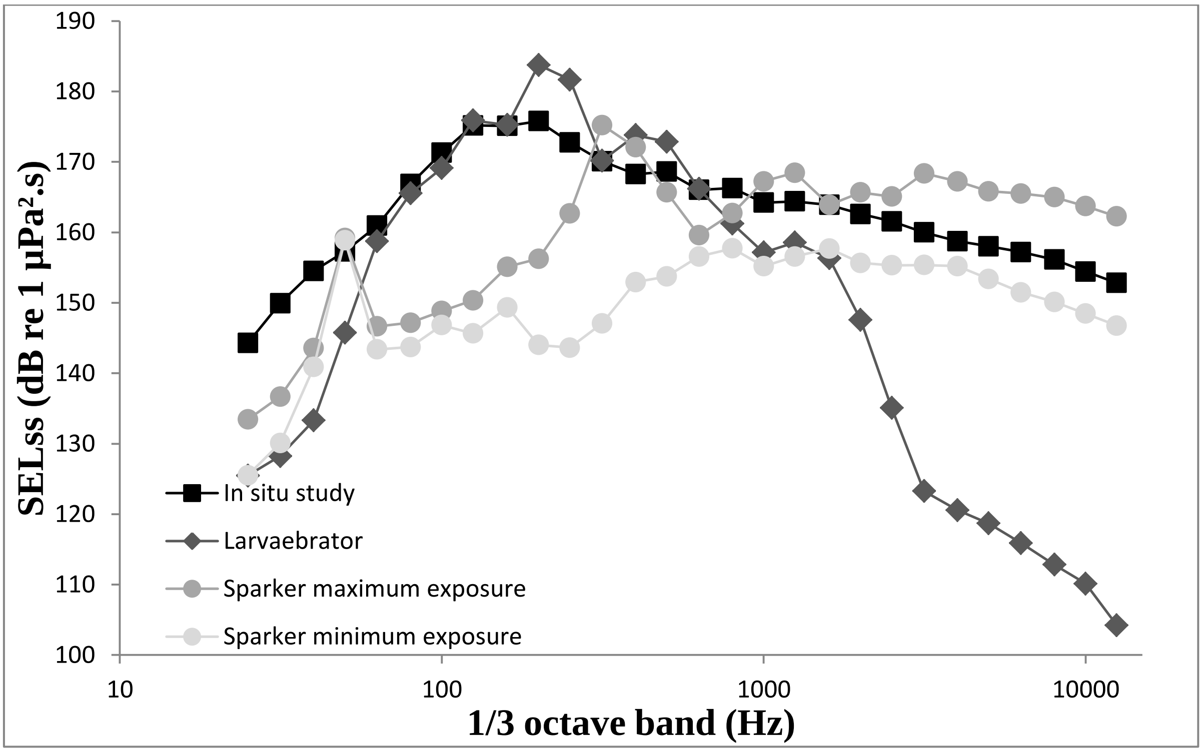

Figure 3. The measured frequency spectra of the sparker and larvaebrator experiments compared to the *in situ* experiment, showing the mean single strike sound exposure levels (SEL_{ss}) in the 1/3 octave bands (SD not shown for reasons of comparison). Figure taken from Debusschere et al. (submitted).

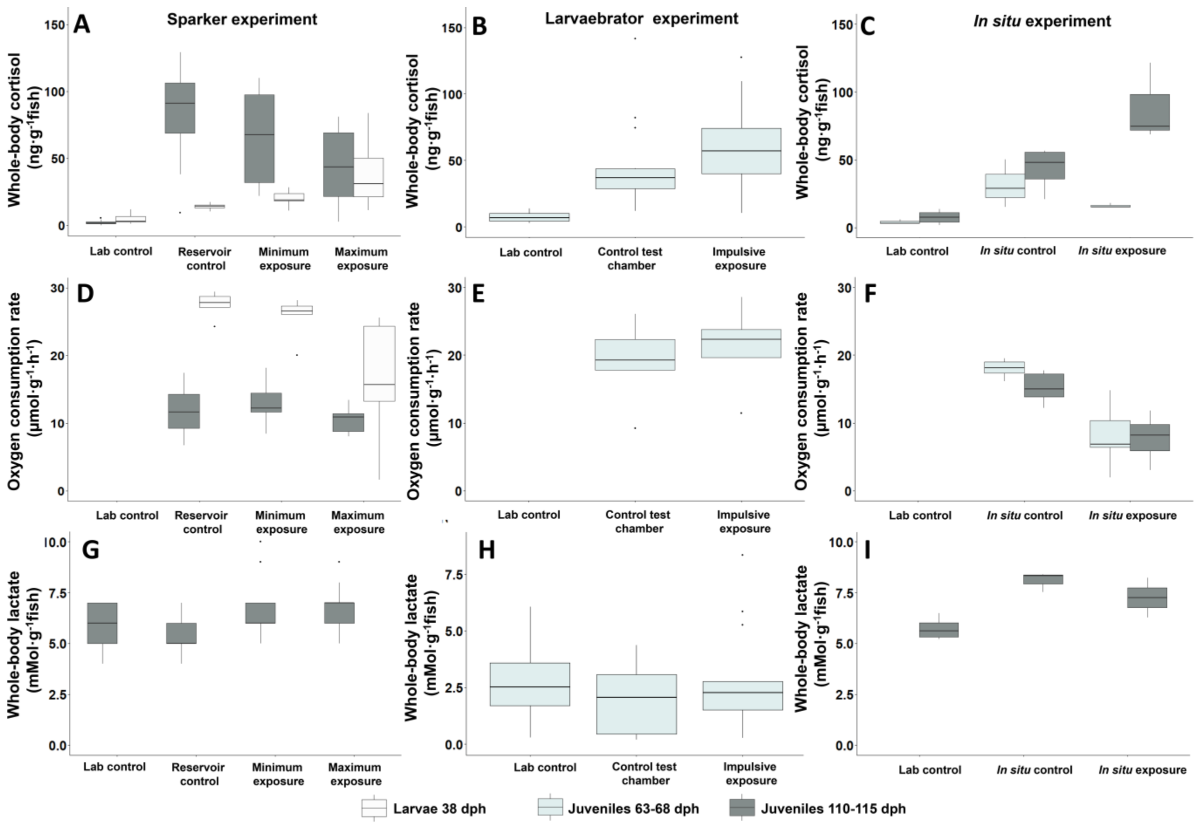

Figure 4. Biochemical and physiological stress responses of fish to high intensity impulsive sound in two lab experiments (sparker and larvaebrator experiment) and one field experiment (*in situ* experiment adapted from Debusschere et al. (2016). The experiments were performed with European sea bass larvae (38 dph, sparker experiment) and juveniles (110 dph in sparker experiment, 63 dph in larvaebrator experiment, 68 and 115 dph in *in situ* experiment). (A-C) whole-body cortisol levels, (D-F) Oxygen consumption rate, (G-I) whole-body lactate levels. The Box-and Whisker plots represent the median between the 25 and 75% percentiles of the box, outliers are plotted as individual points. Figure taken from Debusschere et al. (submitted).

BEHAVIOURAL RESPONSES

At a larger scale, 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 (Slabbekoorn et al., 2010). 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 behaviour (Purser and Radford, 2011, Voellmy, 2013, Voellmy et al., 2014a, Voellmy et al., 2014b, Shafei Sabet et al., 2015). Consequently, fish behaviour was studied in response to impulsive sound on three consecutive days in a laboratory set-up (Figure 1 - Chapter 5) (Debusschere et al., in prep.). In this laboratory study, we tested the influence of pile driving sound on the swimming activity and aggressive behaviour of young juvenile European sea bass *Dicentrarchus labrax* before, during and immediately after the 25 min sound exposure period (1000 strikes, SEL_{ss} =146 dB re 1 µPa²·s, $L_{z\text{-}p}$ = 165 dB re 1 µPa; SEL_{cum} = 176 dB re 1 µPa²·s). We also tested the impact on feeding tendency and efficiency of fish when they were already exposed to the impulsive sound for 15 minutes. Juvenile sea bass interrupted their swimming activities and ceased any aggressive actions to conspecifics at the onset of the impulsive sound exposure. These behavioural effects returned to the pre-exposure baseline within the 25 minute exposure period. On the first day, a slightly reduced number of food intake events were observed during and after the sound exposure, which can indicate an attention shift induced by the sound exposure (Figure 5). This attention shift was no longer clearly observed during the two following days of the experiment. Feeding efficiency was not affected by the sound exposure and illustrated that sea bass were alert to external stimuli under impulsive sound exposure. These findings indicate that the initial response does not persist but can progress over time or under repeated exposure. It remains to be tested whether this also applies to wild-ranging fish.

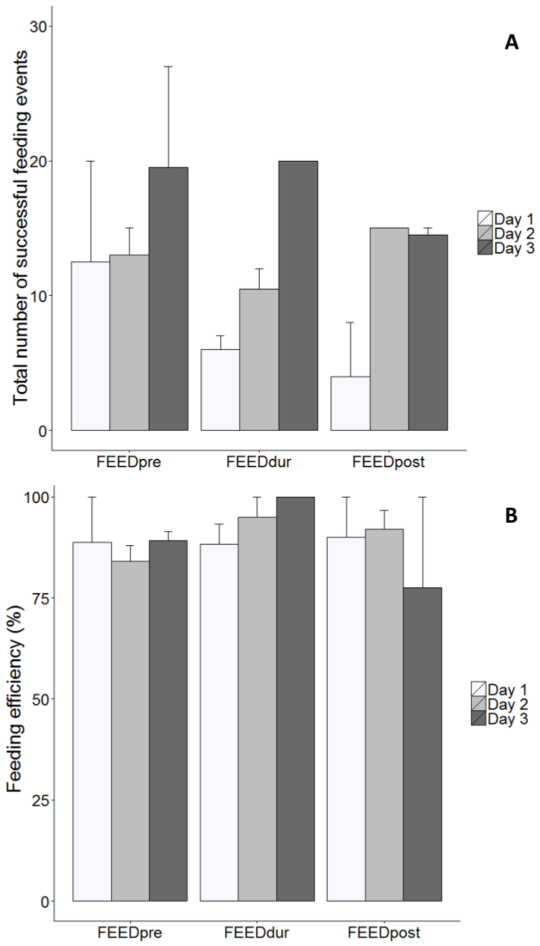

Figure 5. Feeding behaviour of the focal fish for each feeding moment, i.e. before (FEEDpre), during (FEEDdur) and after (FEEDpost) sound exposure on three consecutive days. (A) Total number (sum ± SE) of successful feeding events in 10 minutes after food was offered. (B) Feeding efficiency (%) during 10 minutes after food was offered (mean % ± SE). Figure taken from Debusschere (2016).

11.3. DISCUSSION

THE EFFECTS OF IMPULSIVE (PILE DRIVING) SOUND ON YOUNG FISH (LARVAE AND JUVENILES)

The results of both field and lab experiments allowed to answer research question I on the specific impact of high intensity or strong impulsive pile driving sound on European sea bass. Exposure to a complete pile driving session as close as 45 m from a pile driving activity did not result in acute or delayed mortality of juvenile European sea bass. Both our data and the laboratory results performed by other researchers in acoustically controlled chambers (*i.e.* the larvaebrator and the High Intensity Controlled Impedance Fluid filled wave Tube, HICI-ft) strongly contest the assumption of 100% mortality of fish larvae in a range of 1 km around the pile driving activity (Prins et al., 2009, Bolle et al., 2012, Casper et al., 2012, Halvorsen et al., 2012a, Halvorsen et al., 2012b, Casper et al., 2013a, Debusschere et al., 2014, Bolle et al., submitted). 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 (Debusschere et al., 2016). Based on the field and lab results, the physiological stress responses 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 in which the highest energy was found. Furthermore, the primary and secondary stress response could be related to hearing and swim bladder oscillations, respectively (Debusschere et al., submitted). The studies mentioned above involve high intensity underwater sound found at close range from the pile driving source. At larger distances 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 the initial stress response, and repeated exposure had no clear effect on feeding (Debusschere et al., submitted). Combining these results with other data from literature reveals the interspecific variability of fish in their behavioural response to the same type of stressor (Voellmy et al., 2014a, Shafei Sabet et al., 2015). More species with varying life history strategies need to be studied before the results can be generalised with confidence.

PROPOSING SOUND THRESHOLDS FOR THE EFFECTS ON YOUNG FISH

To provide an answer on **research question II**, the study results are integrated with current knowledge (Popper et al., 2014). This allows us to make suggestions regarding sound thresholds for mortality, physiological stress and behavioural changes of young physoclistous fish. Since mortality was absent in our field study, the mortality threshold must lie above the measured 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) (Debusschere et al., 2014). This study is the first to propose a sound threshold range at which physiological stress responses in juvenile fish are evoked: high-intensity impulsive sound need to have at least a SEL_{ss}

of 170 to 181 dB re 1 μPa²·s at frequencies higher than 315 Hz to evoke physiological stress (Debusschere et al., submitted). A threshold for behavioural disturbance linked to pile driving cannot yet be determined.

THE ECOLOGICAL SIGNIFICANCE OF THE OBSERVED EFFECTS

Additionally, consequences on an ecological level need to be evaluated (research question III). In other words, effects on an individual level need to be scaled up to population level, since individual effects in fish are subordinate to population effects from an ecological point of view (Bejder et al., 2009). In order to do so, data on the presence of sound sources, sound propagation, individual impact, population size, distribution, and affected (sub)population are needed 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, it can be assumed that the ecological consequences of pile driving sound on fish health are subtle (Bolle et al., 2012, Halvorsen et al., 2012a, Casper et al., 2013b).

REVIEWING EUROPEAN MANAGEMENT AND POLICY REGULATIONS WITH RESPECT TO THE STUDY RESULTS

Evaluating the European and national legislation on man-made underwater sound is necessary to provide adequate advice to minimise the impact of pile driving activities on the marine environment (research question IV). In Europe, the Marine Strategy Framework Directive (MSFD) defined a Good Environmental Status (GES) in which underwater sound needs to be at levels that do not adversely affect the ecosystem (Directive 2008/56/EC; Descriptor 11). A Technical Subgroup Noise (TSG Noise) has been commissioned to further develop the descriptor on underwater noise (Van der Graaf et al., 2012, Dekeling et al., 2014). This subgroup proposed the establishment of a sound register, to log all sound producing human activities. The subgroup also identified 'considerable displacement' of marine organisms as the most relevant impact of impulsive sound. Finally, an inventory of the pulse-block days in the EU regional seas can be modelled. This is based on the presence of anthropogenic sound sources that are producing sound levels above the threshold linked to the 'considerable displacement' in ¼ ICES rectangles, which are intervals of 30' (longitude) and 1° (latitude) over an area between 36°N and 85°30'N and 44°W and 68°30'E. A GES should be applicable to all marine organisms, while the TSG Noise mainly based its advice on marine mammals, whereas 'considerable displacement' may not be the most relevant impact on fish. Fish are also neglected in the national legislation of the EU Member States (JNCC, 2010, Betke, 2014, Dähne et al., 2014, Rumes et al., 2015, RWS, 2015). Based on this PhD, our management advice is that the effects of pile driving sound on fish are considered to be more subtle than anticipated and no stringent measures are needed ad hoc in Belgium or in other member states (Rumes et al., 2015). However, more research is needed to support or reject the decision to exclude fish from management, thereby still ensuring GES for all marine fauna.

FUTURE PERSPECTIVES

Finally, future research targets were identified to further unravel the impact of pile driving sound on fish which are needed to progress towards an acoustically sound approach. The lack of particle motion data remains a big gap and needs to be addressed by future studies. The underlying critical sound parameters that evoke physiological stress and behavioural responses in fish need to be unravelled further. Furthermore, data is needed on the long-term impact of acoustic stressors in order to model the ecological consequences of pile driving at population level. Studying the fish in their natural environment with new technologies is a promising strategy. Finally, the impact of continuous sound that will be produced for the next 20 years of the operational OWFs on fish health need to be addressed.

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