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Foraging is made up of three steps: first, to search and encounter a suitable foraging patch; next prey has to be found, and finally the prey has to be caught and eaten. Behavioral responses to anthropogenic noise may lead to a disruption of vital activities within this process, such as area avoidance, or reduced abilities to locate or catch prey. Gaining insight into these behavioral effects starts with a thorough understanding of within- and between-individual variation in the baseline behavior of experimental conditions. In this study, we analyzed control trials for two captive harbor porpoises that were tested for spatial behavior in a set-up for experimental sound exposure. Data from trials without any experimental sound exposure were used to investigate relationships between the response variable – time spent away from the preferred area – and two other behavioral metrics: swimming speed and surfacing rate. The results show that these metrics can be used as independent measures, the first being correlated to the response variable, the second uncorrelated. Combining the two makes a better and more complete judgment. Performing an adequate exploration of the variation in behavior during control trials is important, as it will aid scientists in revealing and interpreting effects of disturbance in sound exposure trials.

1. INTRODUCTION

Eating is important to growth, survival and reproduction for most animals and foraging is therefore a critical behavior contributing to individual fitness. Foraging is a process that can be made up of three steps: exploring the home-range to select a suitable foraging area; finding prey and approaching specific food items; and actually catching prey, including handling and swallowing of the food (Kamil and Roitblat, 1985). For carnivorous species, this involves hunting, an activity that can require quite a bit of effort, since it is in the best interest of the prey not to be caught (e.g. Aguilar Soto *et al.*, 2008). The efficiency with which an individual forages is directly correlated to the energy balance that will translate into fitness. Any disturbances that impede foraging can therefore have detrimental fitness consequences. Anthropogenic noise is such a disturbance that may affect foraging efficiency.

Evidence for a negative impact of environmental noise has been found in different taxonomic groups at all three levels of the foraging process. Daubenton's bats (*Myotis daubentonii*), for instance, avoided noisy parts of an arena, even though food could be found at those sites (Luo *et al.*, 2015). For finding and catching prey, several fish studies have reported lower performances due to anthropogenic noise. Shafiei Sabet *et al.* (2015) tested foraging zebrafish (*Danio rerio*) under relatively quiet ambient and experimentally elevated noisy conditions. They found that individuals delayed their initial acceleration in response to a feeding event and made more food handling errors during noisy compared to ambient conditions. Sticklebacks (*Gasterosteus aculeatus*) maintained an equally high foraging effort under noisy conditions, but also made more mistakes than under ambient conditions (Voellmy *et al.*, 2014). Foraging performance in these species appears not to rely on the use of sound, either actively or passively, implying that acoustic distraction seems to be the mechanism underlying the disturbance. Not all fish species show this same response pattern, as European minnows (*Phoxinus phoxinus*), for example, ceased foraging altogether and were overall more inactive during elevated sound levels (Voellmy *et al.*, 2014).

In harbor porpoises, effects of anthropogenic noise on foraging efficiency could have direct fitness consequences. As small marine mammals, their surface-to-volume ratio is relatively high, which means that they need relatively large amounts of food to survive (Feldman and McMahon, 1983). This has been corroborated recently by investigating click patterns and swimming behavior in tagged wild harbor porpoises (Wisniewska *et al.*, 2016). Individual harbor porpoises were found to consume up to 550 small prey items per day, with a 90% success rate per attack. The high prey number and low energetic content per prey suggest that these high feeding rates are necessary for survival of the porpoises. Even a small decrease in foraging efficiency could potentially have large fitness consequences.

The evidence for an effect of anthropogenic noise on foraging behavior of harbor porpoises, however, is still limited. The studies from other taxonomic groups reviewed above indicate that two main mechanisms can yield negative effects of anthropogenic noise on foraging efficiency: anxiety-related avoidance (reduced opportunity) and attention-related performance drop (missed and failed opportunity). Deterrence from potential food patches where noise levels were temporarily elevated, such as at wind farm construction sites, has been documented in the wild for harbor porpoises (*Phocoena phocoena*) (Carstensen *et al.*, 2006; Dähne *et al.*, 2013), although not in all cases (Scheidat et al., 2011; Thompson et al., 2013). Furthermore, causal relationships between anthropogenic noise and avoidance responses in harbor porpoises have received little attention to date.

Anxiety and attention are neurobiological states of mind that are slightly anthropomorphic and challenging to assess in an animal, respectively (e.g. (Egan *et al.*, 2009; Chan *et al.*, 2010)). Nevertheless, physiological measurements may provide more insights that help in the interpretation of behavioral measurements on performance and effects of environmental noise. For example, increased cortisol levels associated with a change in activity level have been interpreted as indications of acute stress with potentially detrimental consequences in wood frogs (*Lithobates sylvaticus)* and spiny lobsters (*Palinurus elephas)* (Filiciotto *et al.*, 2014; Tennessen *et al.*, 2014). Respiration rates have been shown to be a good proxy for cortisol levels in both terrestrial (e.g. great tits, *Parus major* (Carere and van Oers, 2004)) and aquatic animals (e.g. Nile tilapia, *Oreochromis niloticus* (Barreto and Volpato, 2011)) and also varied in response to sound exposure in a study on a harbor porpoise (Kastelein *et al.*, 2015). However, respiration rates and general activity levels may also vary independently, as O_2 uptake is not always maximal in odontocetes, especially not at moderate to low levels of arousal (Roos *et al.*, 2016).

We here introduce our work on foraging efficiency in two captive harbor porpoises and provide insight into the quantification of behavioral parameters and respiration rate. We performed a dual-tank experiment to investigate avoidance behavior in response to sound exposure. We used a large outdoor pool, where they preferred to stay for most of their time, while having an escape opportunity to swim away into a smaller indoor but more quiet pool. We here report on a pilot study in which we investigated the natural variation in our response metric – time spent in the quiet pool – and related it to general activity level and respiration rate during control trials. We hypothesized that time spent in the quiet pool would be positively correlated to activity level, because a higher swimming speed will cause the time between transitions to decrease and therefore the time spent in the quiet pool to increase. We did not expect that time spent in the quiet pool would be related to respiration rate, since we expected no increase in stress level during the control trials and we therefore also did not expect respiration rate and activity level to co-vary.

2. METHODS

A. EXPERIMENTAL SETUP

Two captive harbor porpoises, one male and one female, were observed during repeated periods of 15 min control conditions or 15 min of sound exposure to band-passed brown noise (400-2000 Hz). Two control trials had to be stopped prematurely (one after 11 min, the other after 14 min), due to technical problems. The sound could be either intermittent (50 ms pulse every 2 s) or continuous, and was presented at multiple SPLs. The animals were allowed to move freely between two connected pools, an indoor and an outdoor pool (Fig. 1). The outdoor pool was slightly larger than the indoor pool, 12x8 m vs. 8x7 m respectively. Sound was played in the outdoor pool, while the indoor pool stayed relatively quiet. All trials were recorded from above using cameras (outside: two cameras, one Go Pro Hero 3, one Conrad 750940; inside one Conrad 750940). We here report on behavior observed during a total of 17 control trials for both animals that were conducted over a period of one month in April-May 2016, between 10:30 am and 5:30 pm.

B. ANALYSES

Spatial behavior (time spent in either pool, number of transitions between pools) was measured per individual for every trial. A proxy for respiration rate was taken by counting the number of surfacing events per individual for every trial and dividing it by the trial duration in

minutes, leading to the surfacing rate per trial. Tracking software (Kinovea 0.8.15) was used to track both individuals when in the outdoor pool during the first two and last two minutes of the observation period. This provided a distance in number of pixels moved, which was divided by the number of frames to calculate swimming speed. The swimming speed was then averaged over both sampling periods per trial.

Behavioral patterns related to time spent in the indoor pool were investigated by testing for correlations between measurement variables. As none of the variables were normally distributed, a Spearman rank correlation was performed for all comparisons. A Wilcoxon rank sum test was used to see if male and female samples could be grouped together, or had to be tested separately. Furthermore, time spent in either pool was tested for skewedness (taking into account the different sizes of the pools) using a one-sample Wilcoxon test, to investigate a preference for either pool. Only data from control trials were used here, to prevent an influence of the sound exposure on these baseline analyses. All statistical analyses were performed using RStudio (www.r-project.org).

C. ETHICAL STATEMENT

All experiments were performed in accordance with National and European legislation on proceedings concerning animal testing. Animals were always able to avoid the sound and trials would be ceased if strong adverse effects were seen (the latter was, however, never the case).
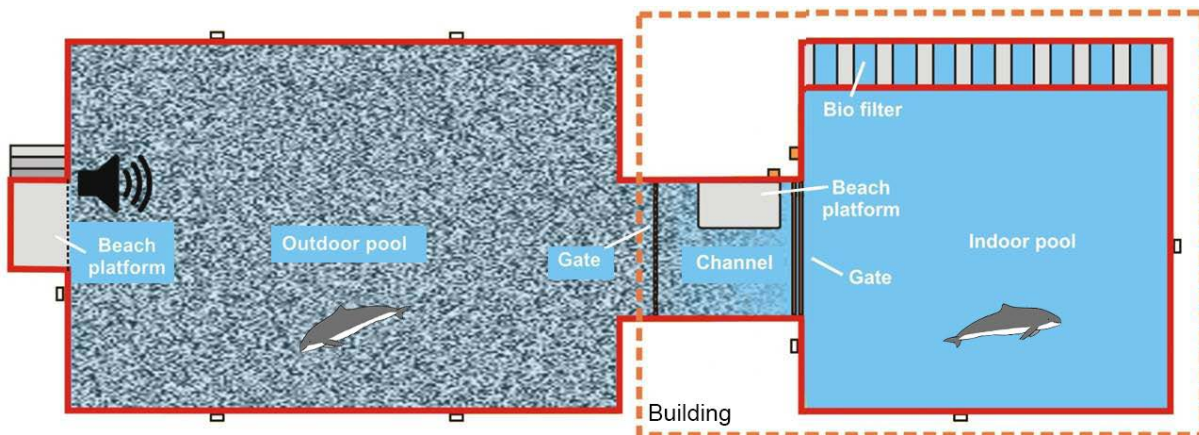

Figure 1. Schematic representation of the experimental set-up. The fill pattern of the outdoor pool on the left reflects the presence of sound during experimental exposure periods. The fill pattern on the right reflects the relatively quiet ambient conditions that were present in both pools during control trials. The speaker indicates the location of the sound source and gates were always open for both animals during the spatial avoidance test. Beach platforms were not used during our experiments.

Table 1: Spearman rank correlations for the behavioral variables measured. N = 17 for all tests; n.s. = not significant.

	Time inside		Surfacing rate		Swim speed		Transitions	
	Female	Male	Female	Male	Female	Male	Female	Male
Time inside	x	x	n.s.	n.s.	**p<0.0001** $\rho = 0.8$	n.s.	**p<0.005** $\rho = 0.7$	**p<0.0001** $\rho = 0.9$
Surfacing rate			x	x	n.s.	n.s.	n.s.	n.s.
Swim speed					x	x	**p<0.05** $\rho = 0.6$	n.s.
Transitions							x	x

3. RESULTS

For all variables except surfacing rate, male and female samples were significantly different and could therefore not be tested in a single group. For both individuals, the percentage of time spent in the indoor pool was significantly lower than chance (Wilcoxon signed rank test, female: V = 22, p<0.005; male: V = 0, p<0.0001, N = 21 for both). The percentage of time spent in the indoor pool was correlated to average swimming speed for the female, but not the male (Spearman rank correlation, Table 1, Fig. 2). Visual inspection of the plot indicated two outliers for the female. The correlation was rerun without these outliers, but the outcome was similar. Consequently, we decided to keep the outliers in the data set.

The percentage of time spent in the indoor pool and the surfacing rate were not correlated (Table 1, Fig. 3). Neither was the surfacing rate correlated to swimming speed or the number of transitions between pools (Table 1). However, the number of transitions between pools was correlated to the percentage of time spent in the indoor pool and for the female to the average swimming speed (Table 1).

4. DISCUSSION

We here reported analyses of baseline behavior during control trials for two harbor porpoises that were used to test for sound-dependent spatial avoidance and foraging efficiency. In the absence of any experimental sound exposure, both animals spent more time in the outdoor experimental pool than in the indoor escape pool. The time spent in the indoor pool was positively correlated to swimming speed, as expected, but only for the female and not for the male. The surfacing rate was not correlated to swimming speed or time spent in the indoor pool for either individual. We discuss these results below in the context of the experimental targets and related reports in the literature.
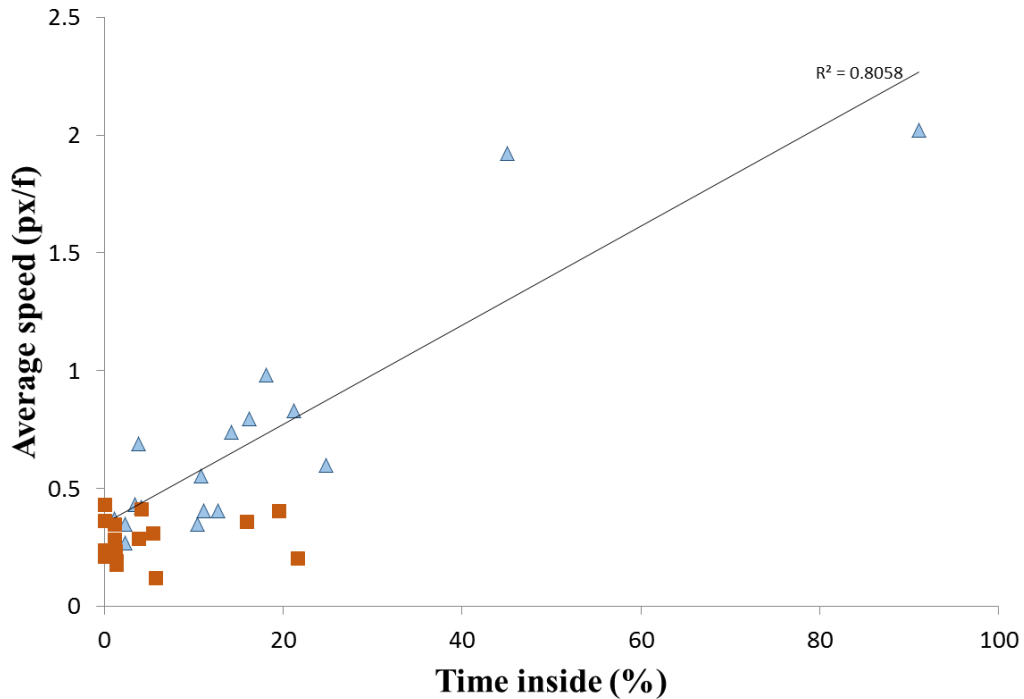
Figure 2. A positive correlation for the female between time spent in the indoor pool and the average speed in pixels per frame. Orange squares represent the male, blue triangles the female. The regression line is for the female data only.
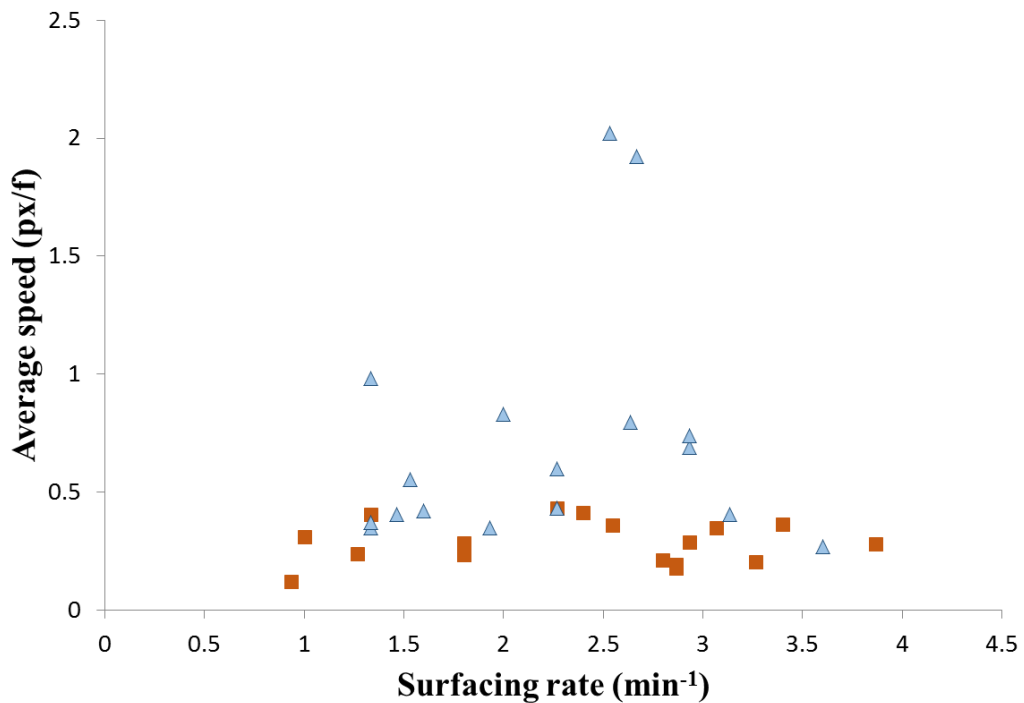

Figure 3. No correlation between surfacing rate (number of surfacing events per minute) and average swimming speed in pixels per frame. Orange squares represent the male, blue triangles the female.

A. POOL PREFERENCE

As both harbor porpoise individuals spent much more time in the outdoor than in the indoor pool (typically more than 75% of the time), we found a significant preference for the outdoor pool. Therefore, the time spent in the indoor pool can be seen as time spent away from the preferred area. We believe that this makes our set-up suitable to test whether anthropogenic sound could affect spatial avoidance in captivity in a way that could reflect to some extent free-ranging conditions. If elevated sound levels in our outdoor pool would make our animals shift to spend more time in the indoor pool, it would also be possible that free-ranging animals would spend less time in preferred areas for foraging due to their spatial response to anthropogenic noise. Furthermore, they spent so much more time in the outdoor than in the indoor pool that it is likely that even if they would shift away from the experimental sound exposure that they would still spend a considerable amount of time outside, sufficient to analyze changes in swimming patterns and foraging efficiency.

However, as hypothesized, we also found a positive correlation between time spent in the indoor pool and swimming speed for one of our individuals. This means that more time inside during a sound exposure trial could be caused by avoidance of the more noisy outdoor pool, but also by elevated swimming speed. We should therefore always take both of these measures into account when we want to interpret sound-dependent changes in behavior. Increased swimming speed could indicate level of arousal, which may or may not be related to anxiety. In free-ranging conditions, this could result in highly active individuals that stay in the area or individuals that flee away from the sound source. In our set-up this may be reflected in whether or not there is a correlation between swimming speed and time inside, although we already found such a discrepancy between the two different individuals exposed to the same control conditions.

The different results for our male and female harbor porpoise are due to stereotypic differences in individual behavior. As we only used two animals, it is not possible to pinpoint the cause underlying this difference. It could be due to a difference in sex, personality, previous experience or age, as these factors all differed between the two animals. What it does show, is that individual differences need to be taken into account when conducting this type of study. Individual variation in the context of sound impact should also be expected as studies on fish have already been reported for sex-dependent (Bruintjes and Radford, 2013) and body condition dependent responses to experimental sound exposure (Purser *et al.*, 2016).

B. SURFACING RATE

In line with our predictions, surfacing rate was uncorrelated to swimming speed or the time animals spent in the indoor pool. Surfacing rate was used as a proxy for respiration rate, which has been linked to increased stress hormone levels (Carere and van Oers, 2004; Barreto and Volpato, 2011), which in turn has been linked to behavioral response patterns and detrimental consequences of anthropogenic sound exposure (Filiciotto *et al.*, 2014). However, all these studies were in other taxa (birds, fish and frogs) and these correlations may be less clear for odontocetes. For one thing we know that it has been argued for killer whales that oxygen uptake is likely to vary per surfacing event and that they are typically not close to their limits in terms of oxygen consumption. As a consequence there was for example no link between killer whale surfacing rate and swimming speed (Roos *et al.*, 2016), like we found for both of our animals.

Nevertheless, we have to keep in mind that these analyses concern the baseline data for control trials without any experimental sound exposure. It may still be the case that respiration rate will reflect arousal level or anxiety under more stressful and energetically more demanding

circumstances. Kastelein *et al.* (2015), for example, found an increase in swimming speed combined with an increase in respiration rate in a harbor porpoise during experimental sound exposure. We will therefore certainly include the measure of surfacing rate in our analyses of sound dependent swimming behavior and foraging performance.

5. CONCLUSION

The results of this study show within- and between-individual variation in metrics suitable for measuring avoidance and arousal in sound exposure studies in harbor porpoise. Surfacing rate and swimming speed, metrics that are often correlated in other species, can be considered as two independent behavioral measures. Combining the two makes it possible to better judge the impact of sound exposure. We believe our current analyses indicate that performing an adequate exploration of the variation in baseline behavior during control trials is important, as it will aid scientists in revealing and interpreting effects of disturbance in sound exposure trials. Any indications of sound impact on spatial avoidance or foraging efficiency in captive studies such as ours can provide important insights and make people aware, but should always be considered as a proof of concept. This type of data should never be used to abstract quantitative effects on absolute intake rates for free-ranging animals.

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