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Group behavioral responses of juvenile common carp (*Cyprinus carpio*) to pulsed tonal stimuli in the presence of masking noise

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Acoustic behavioral deterrent systems are deployed to control range expansion of invasive species, and protect migratory fish from hazardous anthropogenic physical barriers. Installation sites (*e.g* . dams and weirs) are generally dominated by high-level background acoustic noise, a commonly neglected consideration when testing the effectiveness of acoustic behavioural devices. A laboratory study investigated the group behavioral responses of common carp (*Cyrpinus carpio*) to tonal stimuli under the presence or absence of a background masking noise. Preliminary data indicates a significant reduction in response under acoustically masked conditions. This has possible implications for future technological developments to ensure they are fit for purpose. Bespoke modifications may be required to ensure deterrents are effective under the background noise levels at proposed sites of interest.

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1. INTRODUCTION

In the aquatic environment, migratory freshwater fish species encounter many threats from human activity in the form of hydropower turbines, weirs, and water abstraction pumps. Traditionally, these have been mitigated using mesh screens. Physical screens, however, incur considerable maintenance and installation costs, and cause substantial mortality rates at higher water velocities (Schilt, 2007). As such, behavioral barriers and interest in the use of sound as a freshwater fisheries management tool has taken off in recent years (Popper and Carlson, 1998; Scruton *et al*., 2003; Taylor *et al*., 2005). Acoustical deterrents may be used alone, or in combination with other behavioural and/ or physical screens. For example, where a screen might be adequate for stronger swimming adult individuals, but not for other life-stages (with their weaker swimming ability and lower profile), an acoustic deterrent might be tuned to specifically deter them before reaching the screen (Deleau *et al.*, 2019; Piper *et al*., 2019).

Common carp (*Cyprinus carpio*) are of interest to fisheries managers both from a conservational and invasive perspective. In regions from which carp are native (*e.g.* Asia), overfishing and water pollution have led to population declines, and the species is categorised as vulnerable on the International Union for Conservation of Nature (IUCN) red list (Freyhof and Kottelat, 2008). Conversely, they are also deemed to be one of the most damaging aquatic invasive species across the globe (*e.g.* Australia, U.S.A.). The majority of continents have been subject to invasive carp detrimentally impacting water quality, native fish communities and aquatic macrophytes (Weber and Brown, 2009; 2011). The migratory nature and high fecundity of carp allow for a rapid establishment period, often reaching extreme levels of abundance, which is costly both from an ecosystem and economic damage standpoint (Pimentel *et al*., 2005). For instance, in

Australia, invasive carp management alone costs in excess of an estimated AUS$500 million annually (Australian Government, 2016).

A number of controlled laboratory studies have investigated the response of carp to sound within a fisheries management context, and have observed positive results to date (*e.g*. 95 % deflection rate to cyclic sound bursts of 0.02 – 2 kHz combined with air bubble curtain: Taylor *et al*., 2005; 58 % continued response rate to broadband stimuli: Vetter *et al*., 2017). However, experiments are typically conducted under relatively quiet ambient background noise conditions (*e.g*. up to 80 dB re 1 μPa: Zielinski *et al*., 2014). These scenarios do not reflect the high-level noise intensities encountered by fish in the wild (Amoser and Ladich, 2005; Wysocki and Ladich, 2005). Noise conditions at anthropogenic barriers (*e.g.* dams and weirs) act as a crucial constraint to the signal transmission of a deployed acoustic deterrent, and subsequent behavioral response of any targeted species (Wiley, 1994). Furthermore, development of acoustic deterrent systems are commonly based on our understanding of hearing thresholds, acoustic masking, signal to noise ratio and critical bands, which are mostly obtained through experiments investigating auditory sensitivity (*e.g.* ABR: Kojima *et al*., 2005; AEP: Amoser and Ladich, 2005). This may be problematic given that the capabilities of an individual to detect an acoustic signal, do not directly translate to the elicitation of a desirable behavioral response (Kemp *et al*., 2012).

In order to inform the development of more effective acoustic deterrent systems, we therefore looked to answer: how does masking background noise impact the group responses of common carp to a tonal acoustic stimulus? Here we present the preliminary results of a tank-based study. Groups of fish were exposed to a pulsed tonal acoustic stimulus (170 Hz) set a one of three signal-to-noise ratios, in either the presence or absence of a known broadband masking noise. Common carp belong to a group of fish with hearing specialisations, known as 'otophysines' (Popper and Fay, 2011). Accessory hearing structures allow for an enhanced auditory sensitivity to the sound pressure component, and carp were therefore chosen as the subject species based on their excellent hearing (evoked threshold responses: 0.1 – 4 kHz: Amoser and Ladich, 2005), and importance to fisheries management.

2. METHODS

A. EXPERIMENTAL SET-UP

C. carpio were sourced from an aquaculture facility, and housed in the International Centre for Ecohydraulics Research at the University of Southampton, at 9.3 °C. Fish were fed daily to satiation, and water quality maintained to adhere to optimum thresholds. Experiments were performed within a physically isolated section (86.0 x 30.6 x 30.2 cm^3; water depth: 27 cm) of a 3 m still-water acrylic flume (Fig. 1). In each trial, groups made up of five individual common carp were exposed to a pulsed 170 Hz tone (1 second ON: 2 seconds OFF), at one of three (low, medium, high) signal-to-noise ratios (S/N), either in the presence or absence of a masking broadband noise (120 – 3000 Hz; 110 dB re 1 μPa). Acoustic conditions in the pressure domain were mapped across three depths, allowing for a spatial description of the sound-field within the tank (Fig. 2). A control of no playback and a treatment consisting only of masking noise were deployed to gauge baseline behavior. Tones were switched on after a 30 minute acclimation period, and behavior recorded from above the flume using a camera (Logitech Pro, C920). We report preliminary analyses on behavior observed during a total of 80 trials involving 400 animals conducted in March 2018.

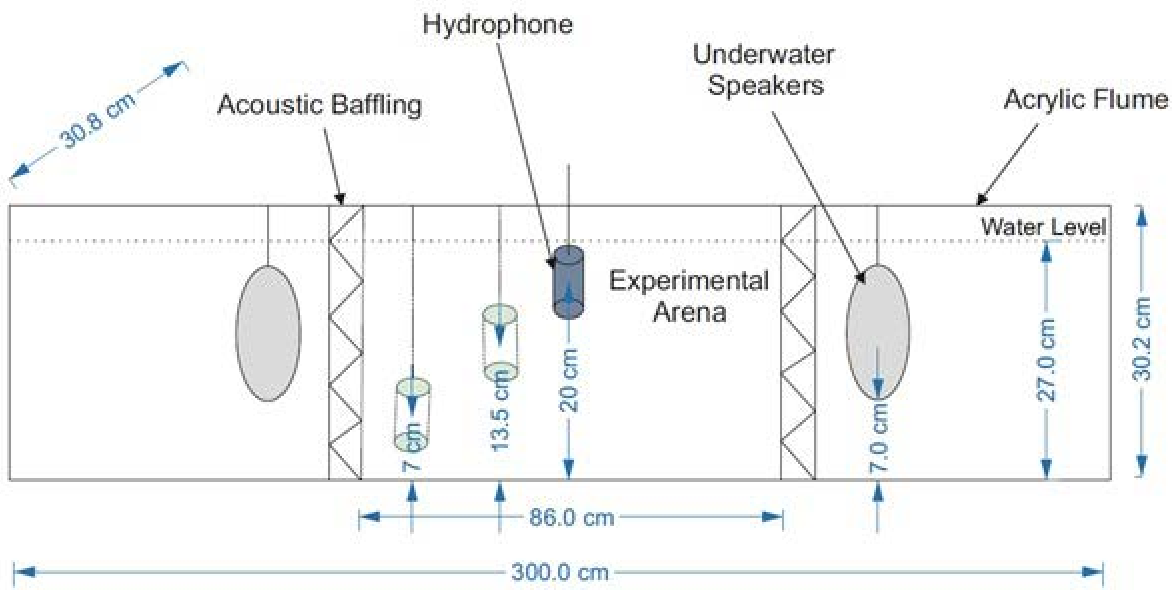

Figure 1. Schematic 2D section-view representation of flume experimental set-up.

B. ANALYSIS

Coarse-scale behavior was investigated by quantifying group startle response. At the onset of tonal stimulus presentation, the presence or absence of a c-start (a fast, coordinated contraction to one side of the body, in the shape of a "C", followed by a clear swimming burst: Bhandiwad *et al*., 2013), by at least one individual within a group was recorded. In trials where a group startle response was observed, the total number of individuals within a group exhibiting a c-start, were also counted (Table 1). Finally, the number of times at least one individual within a group exhibited a c-start to the presentation of each successive tonal pulse, without interruption, was also totaled. To assess for differences in continual startle response under ambient conditions, a Kruskal-Wallis test was performed across all three treatments and the control.

Fish movements were also tracked using custom-written MATLAB code. This allowed for the investigation of finer-scale group behavior, for example, group swimming speed (ms^{-1}), and inter-individual distance (m).

C. ETHICAL STATEMENT

All experiments were performed in accordance with national animal testing legislation and with the approval of the university ethics review board. Animals always had an area of lower acoustic intensity to retreat to, and experiments were remotely video monitored to ensure individuals were not under any adverse levels of stress (no trials had to be terminated).

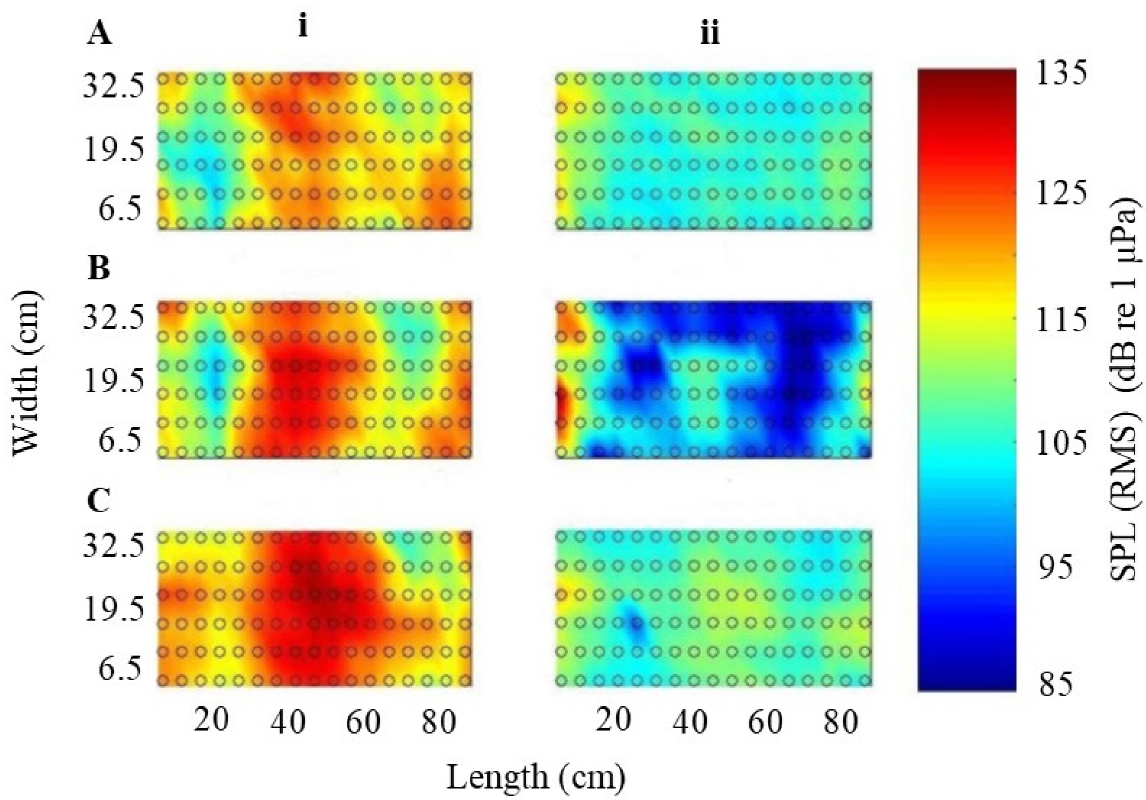

Figure 2. Sound Pressure Levels (RMS) (dB re 1μPa) with hydrophone matrix positioning indicated for (i) 170 Hz tone; and (ii) broadband noise (120-3000 Hz) recorded at (A) 7 cm; (B) 13.5 cm; and (C) 20 cm depth.

3. RESULTS AND DISCUSSION

We report preliminary analyses of the group responses of common carp to a pulsed tonal stimulus at one of three S/Ns, either in the presence or absence of a background masking noise. Fish were observed to startle at the onset of all tonal treatments under ambient noise conditions. The greater the S/N, the greater the magnitude of response (Table 1; Fig. 3; $p < 0.01$). Higher S/Ns also elicited an increase in the number of continuous c-starts, and greater numbers of individual fish within a group startled at the onset of tonal stimuli. Under masking noise conditions, however, startle responses significantly reduced (Table 1). Other than for one individual outlier within the medium S/N treatment, fish did not exhibit startle responses when tonal stimuli was switched on for masked treatments.

Table 1. Observed startle responses per treatment at onset of 170 Hz tone at varying S/N under presence or absence of masking noise.

Tone relative S/N	**Masked (M)/ Ambient (A)**	***n*-trials**	**Percentage (%) groups exhibiting startle response at onset**	**Average (mean) number of individuals startling (max 5) at onset**
Control (N/A)	A	10	0	0
Low		10	60	1.6
Medium		10	100	2.6
High		10	100	4.8
Control (N/A)	M	10	0	0
Low		10	0	0
Medium		10	10	0.02
High		10	0	0

Information available from ABR audiograms and psychoacoustic testing on carp suggested the incrementally increasing S/Ns selected for this experiment were within known critical ratio detection thresholds for common carp (Amoser and Ladich, 2005; Kojima *et al*., 2005). However, contrary to our expectations, no differences were observed between startle responses of carp to tonal stimuli of differing S/N under the acoustically masked conditions. Startle responses are an evolutionarily conserved behavior, and are typically mediated by one of a pair of large reticulospinal neurons, the Mauthner cells (Eaton, 2001). The behavior can be clearly observed, as it causes a fish to bend in a "C" shape away from a stimulus source during an escape (Bhandiwad, *et al*., 2013). As such, one would expect to observe the eliciting of a so-called "C-start" if an acoustic stimulus is above a desired sensitivity threshold. Conversely, the startle response is an extreme response to novel stimuli, therefore, an absence of this behavior does not necessarily indicate the absence of a signal detection, or behavioral change. Other internal non-locomotor processes, *for example*, motivation to escape, may determine the responsiveness of an individual to an acoustic stimulus (Kemp *et al*., 2012). Subsequently, completion of further refined scale analyses to detect more subtle changes in behavior is required before study conclusions can be determined.

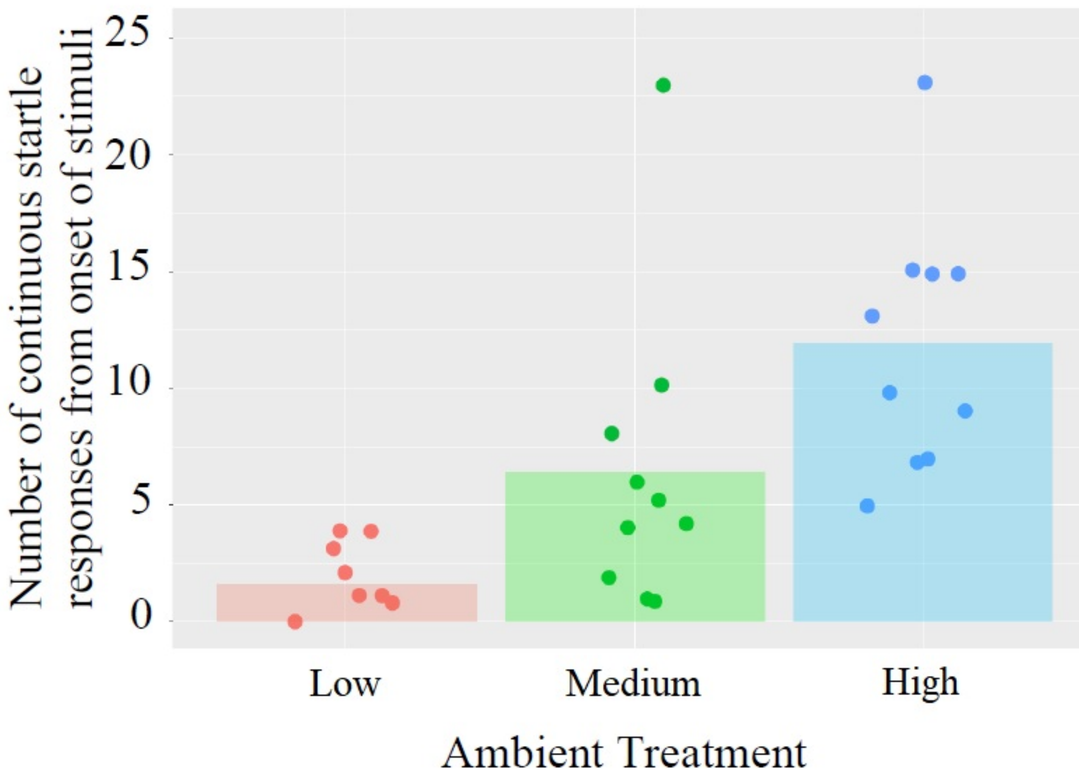

Figure 3. Number of continual startle responses to tonal stimuli under ambient noise conditions (Kruskal-Wallis test: $\chi^2_3 = 31.64$; p<0.01).

Fish movements will be tracked to look for commonly observed anti-predator escape tactics including: changes in group swimming speed, inter-individual distance, and orientation (Herbert-Read *et al*., 2017; Vetter *et al*., 2017). Investigation of these video tracked variables are ongoing, and therefore results are not reported. It is necessary to understand how masking noise impacts fish responses to a target acoustic stimulus. First, suppression of finer-scale behavior under background noise conditions, may reduce the effectiveness of acoustic deterrent systems. Second, it is vital that fish can extract important biological information from a local soundscape (Pijanowski *et al*., 2011). An inability to do so may have ecological implications regarding the conservation of a species.

In summary, a background broadband noise masks the startle responses of common carp to a tonal stimulus. Although results to date are preliminary, we suggest that more consideration be given to varying S/Ns when installing deterrent technologies, particularly on a site-by-site basis. The authors acknowledge the limitations of small tank acoustic studies (Parvelescu 1964; Rogers *et al*., 2016), and therefore, as always, we recommend validation of these results under real-world conditions, as results cannot directly be extrapolated to how animals will react in the wild.

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