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Stress response to anthropogenic noise in Atlantic cod *Gadus morhua* L.

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ABSTRACT

The potential effects of anthropogenic noise on the physiology of Atlantic cod have not been well described. The aim of the present study was to investigate the impact of anthropogenic noise on Atlantic cod stress response using cortisol as a biomarker as well as on broodstock spawning performance. Results showed that artificial noise consisting of a linear sweep from 100 to 1000 Hz can induce a transient and mild cortisol elevation with a clear noise intensity dose response. In all cases plasma levels returned to baseline levels <1 h post sound exposure. Daily exposure to a similar intensity and frequency noise range applied habitually to a broodstock population during the spawning window resulted in a significant reduction in total egg production and fertilisation rates thus reducing the total production of viable embryos by over 50%. In addition, a significant negative correlation between egg cortisol content and fertilisation rate was observed. These results confirm that cod can perceive noise generated within a frequency range of 100–1000 Hz and display a heightened cortisol plasma level. In addition, anthropogenic noise can have negative impacts on cod spawning performances.

1. Introduction

The acoustic environment plays an important role throughout the life cycle of most aquatic animals, however anthropogenic noise can act as a stressor, impacting negatively on animal behaviour and physiology (Wright et al., 2007). While most studies on the impact of noise in the aquatic environment have been performed in marine mammals (Thomsen et al., 2006; NRC, 2005), there is an increasing awareness of the potential negative effects on other marine organisms including invertebrates (Morley et al., 2014) and fish (Hawkins et al., 2014a; Popper and Hastings, 2009; Popper et al., 2014). With regard to fish, the literature is sparse and does show conflicting evidence with reports of fish being attracted as well as showing avoidance reactions depending on the sound source (Chapman et al., 1974; Hawkins et al., 2014b; Løkkeborg et al., 2012; Spiga et al., 2012). Equally some studies have found no effect (Wardle et al., 2001; Peña et al., 2013). In cases where an effect is reported it has also been suggested that migration patterns and reproductive behaviour may be disturbed by noise, forcing fish to find alternative routes or preventing them from settling in their usual spawning grounds (van Opzeeland and Slabbekoorn, 2012) and thus possibly impacting on larval settlement (Holles et al., 2013). The acoustic field in enclosed aquaculture systems is not exempt from noise pollution (Davidson et al., 2007; Wysocki et al., 2007) but its impact on fish stocks has been widely overlooked to date despite the drive towards increasing land-based facilities.

Most human activities in the aquatic environment generate noise in the frequency range below 1 kHz (Popper et al., 2014), which is well within the auditory range of most fish species (Ladich and Fay, 2013; Popper and Fay, 2011; Radford et al., 2012). When exposed to noise "challenges" in this audible range, a range of effects have been reported, in some cases fish audition can be altered with temporary threshold shifts (Scholik and Yan, 2001; Smith et al., 2004a), physical injuries occur that can reduce hearing capability (Hastings, 1995) and auditory tissue damage has been observed along with general physical barotraumas (Casper et al., 2013). Importantly, noise perturbations can also restrict or mask communication when it covers similar frequencies to the vocalizations emitted by aquatic animals (Hawkins and Chapman, 1975). Physiological response to stress varies widely between species (Barton, 2002) however it has been observed that noise can cause behavioural changes in fish (Kasumyan, 2008) as well as affect typical stress biomarkers including cortisol, glucose, lactate and haematocrit (Smith et al., 2004b; Buscaino et al., 2010).

Atlantic cod (*Gadus morhua*) is a species of great commercial fisheries and aquaculture interest. To optimise fisheries technology,

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cod auditory ability has been well studied. The optimal hearing bandwidth for cod ranges from 18 to 470 Hz although this range should not be considered absolute (Buerkle, 1967; Chapman and Hawkins, 1973). Importantly, Atlantic cod is a vocal species that has been reported to communicate at low frequencies during migration, aggression and escaping behaviours but mainly during courtship (Hawkins and Rasmussen, 1978; Engen and Folstad, 1999). One of the species main vocalisations, termed "grunts", are produced by repeatedly contracting a drumming muscle sending vibrations to the swim bladder (Brawn, 1961) generating repeated single pulses with frequencies ranging from 30 to 250 Hz that typically last for a duration of 60–200 ms. The volume of the drum muscle mass is correlated with the vigour and number of grunts and therefore to mating success (Rowe and Hutchings, 2006, 2008). To date, few studies have investigated the potential effects of anthropogenic noise on Atlantic cod behaviour, stress response and physiology (Engas et al., 1996). Behavioural responses including avoidance due to wind turbine noise, freezing to pile-driving noise (Mueller-Blenkle et al., 2010) and attraction to divers breathing (Chapman et al., 1974) have been reported. However, the possible physiological effects caused by noise have not been reported for Atlantic cod in either a wild "fisheries" or captive "aquaculture" context.

The aims of the present study were to (1) investigate whether sound can elicit a short-term stress response in Atlantic cod using plasma cortisol as a stress biomarker, and (2) study the potential effects of long-term sound disturbances on spawning performance.

2. Materials and methods

2.1. Facilities

All the experiments were carried out at the facilities of FAI Aquaculture, Ardtoe Marine Research Facility, Acharacle, Scotland (N56°46′, W05°53′). All working procedures complied with the United Kingdom Animals (Scientific Procedures) Act 1986 and were approved by the ethics committee of the University of Stirling.

2.2. Sound recording system

Acoustic measurements were obtained using an omnidirectional hydrophone (RESON TC4034, frequency range: 1 Hz to 470 kHz +3/−10 dB; receiving sensitivity −218 ± 3 dB (at 250 Hz), Avisoft Bioacoustics, Germany) connected to an UltraSoundGate Charge Amplifier with Hi-Pass filter (Avisoft Bioacoustics, Germany) to reduce low frequency noise (cut-off frequencies of 25 Hz for sound mapping of rearing facilities, 100 Hz cut-off for noise exposure trials to limit the noise analysis to the loudspeakers output). Acoustic data were pre-amplified (E-MU tracker pre 24-bit/192 kHz) and recorded using Avisoft SASLab Pro software (Avisofts Bioacoustics, Germany) to a computer hard disk at a sampling rate of 48 kHz with 16-bit resolution. The acoustics equipment was powered with the internal battery of the laptop to avoid noise from the AC power supply. Raven interactive acoustic analysis software (The Cornell Laboratory of Ornithology) was used for all sound recording analysis with sound pressure level (SPL) calculated over the frequency range 100–1000 Hz.

2.3. Noise mapping and vocalizations

Noise mapping analysis consisted of determining background sound levels for a range of tank systems (indoor and outdoor tanks with diameters ranging from 1 to 5 m). This mapping was followed by testing the impact of husbandry activities (hand feeding), disturbances (talking and walking next to the tank, simulated netting in the tank, knocks against the tank walls with increasing intensity) and equipment (aerator, water inflow, oxygenator) on the acoustic environment of the tank (SPL and frequencies only, particle motion was not measured). Sound recordings were repeated on several days and times of the day to obtain a representative sound profile with the hydrophone suspended on the side of the tank, 30 cm from the tank wall at a depth of 0.5–1 m depending on tank depth. In addition, vocalisations generated by Atlantic cod broodstock in captivity (circular holding tank, 5.3 m diameter, 88 m^3) were recorded (hydrophone placed 30 cm from tank wall at 1 m depth) during the spawning season. To do so, sound recordings were made during a 24 h period taking a 5 min sample at the start of every hour to record number of vocalisations and then perform further audio analysis (frequency, duration, SPL).

2.4. Experiment 1: sound as a short-term stressor in Atlantic cod

Prior to the experiment, Atlantic cod (total length 40.4 ± 2.8 cm, body weight 806.8 ± 173.5 g) were maintained in 2 m^3 (2 m diameter, conical bottom, 1 m depth) black circular fibreglass tanks under a constant 12L:12D artificial photoperiod with illumination provided by fluorescent lights distributed evenly across the experimental room. Tanks were on a flow through system with ambient water temperature of 6.9 ± 2.3 °C and constant salinity of 34.4 ± 0.4‰ over the course of the experiment. Fish were fed ad libitum everyday using commercial 4.5 mm marine dry pellets (Classic marine, Biomar Norway).

Naïve cod (not previously exposed to experimental noise stimulus) were randomly selected from the stock tanks and randomly allocated to seven identical experimental tanks (same size as stock tanks, 6 fish per tank) where they were acclimated for a week prior to the noise exposure. Each tank was equipped with a suspended omnidirectional underwater loudspeaker (UW30, frequency response 100–10,000 Hz; Impedance 8 Ω, EV, USA). The loudspeaker was suspended at the centre of the tank and submerged at mid water depth (0.5 m) with the hydrophone being suspended at the same depth equidistant from the speaker and the tank edge. The experimental noise exposure consisted in a linear sweep of 10 seconds (frequency range 100–1000 Hz) using a sweep function generator (FS502, Feedback Instruments Ltd., UK) and amplifier (RX-4105 2 channel stereo, Sherwood, UK). The linear sweep was repeated for 10 min simultaneously in all tanks. The voltage input used for the noise generation was 10, 15 and 20 V, that resulted in a final root mean square (RMS) SPL of 104.2 dB re 1 μPa (9.1 dB signal to noise ratio (SNR)); 108.7 dB re 1 μPa (13.3 dB SNR) and 110.3 dB re 1 μPa (15.3 dB SNR) within the 100–1000 Hz frequency range analysed (Fig. 1). Noise levels tested were intended to reflect the range in noise levels recorded during the sound mapping of the facilities (from feeding: 8 dB to hitting the tank wall: 26 dB SNR, see below for further details). For each noise level tested, the seven experimental tanks corresponded to a different sample point. The first tank was sampled prior to the 10 min exposure to determine basal levels, followed by the 10 min sound exposure. The remaining 6 tanks were sampled at 10, 20, 30, 40, 60 and 120 min post sound exposure. At each sampling point, 6 fish per tank were netted, anaesthetised using MS-222 (50 mg L^{-1}, Pharmaq, Fordingbridge, UK) and blood sampled from the caudal vein using pre-heparinised syringes. Sampling, from netting the fish to blood withdrawal of 6 individuals was performed in less than 5 min to minimise potential handling effects on cortisol release. Blood was kept on ice and haematocrit levels were determined within 10 min by centrifuging a whole blood aliquot at 14,000 × *g* for 5 min in a glass capillary tube and measuring the resulting proportion of packed red blood cells. Blood samples were then centrifuged at 1,200 × *g* for 10 min and plasma samples stored at −20 °C for later cortisol analysis.