Swimming Behavior of Roach (Rutilus rutilus) and Three-spined ...
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Ambio; Dec 2007; 36, 8; ProQuest
pg. 636

visual census technique is here put forward as a necessary complement to more wide-screening fish sampling methods (e.g., gill nets, echo-sounds, trawling).

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Synopsis

Swimming Behavior of Roach (*Rutilus rutilus*) and Three-spined Stickleback (*Gasterosteus aculeatus*) in Response to Wind Power Noise and Single-tone Frequencies

Human-induced underwater noise is drastically increasing as the result of offshore installations and human activities in the marine environment. Many of these structures and activities produce low-frequency noise that could potentially disturb or have harmful effects on several species of teleost fishes. Within the next decade, thousands of wind turbines will be in use in coastal and offshore waters and there is increasing concern on how they may influence marine life.

Low-frequency noise might have an immediate effect on fish inducing an instant behavioral response or, if chronic, influence fish fitness including physiology and reproduction. Knudsen and colleagues (1–2) found avoidance behaviour and subsequent physiological reactions when Atlantic salmon (*Salmo salar*) were exposed to two single-tone sounds at the frequencies 10 and 150 Hz. Maes and colleagues (3) noted a strong avoidance response from clupeids to an acoustic deterrent system that produced 174 dB re 1 μPa in the frequency interval 20–600 Hz. In addition, sound can have a negative impact on fish hearing ability (4–8). Further, noise may mask important information interfering with communication (particularly during spawning events) and predator detection (9).

The aims of this study were to examine how swimming behavior of roach (*Rutilus rutilus*) and three-spined stickleback (*Gasterosteus aculeatus*) were influenced by single-frequency sounds and noise generated by an offshore wind turbine, and the function of sound pressure level. These two species are common in Northern Europe in areas utilized for wind power developments including lakes, brackish

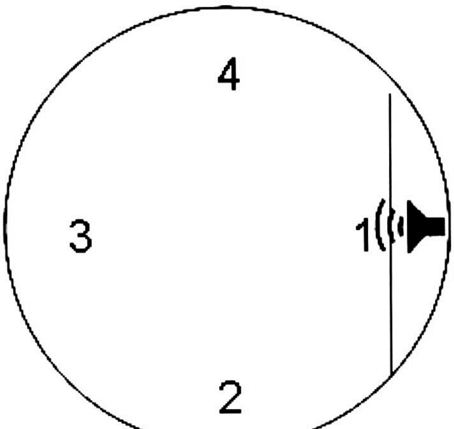

Figure 1. Test tank with numbers indicating where and in what order the reference measurements were noted using the hydrophone. The line in front of the speaker marks a sound transparent cloth that was placed in front of the loudspeaker to prevent fish from using it as a refuge.

waters such as the Baltic Sea and coastal areas (10).

The experiment was performed October–November 2004 under simulated daylight conditions in a room closed for other activities to minimize external disturbance. A circular plastic tank with a water depth of 15 cm containing 350l of water was used for the experiment. Beneath the test tank a 2 cm thick board of polystyrene was mounted in order to reduce acoustic disturbance from the floor (11). Efforts were made to keep sound to a minimum in the room, although air sound was mainly reflected by the air-water interface (11). To allow estimation of fish movements, squares of the size 10 × 10 cm were marked on the floor of the tank. The behaviour of the fish was recorded using a video camera placed on a tripod 180 cm above the water surface. For sound generation an underwater loudspeaker was positioned in the tank at the perimeter (Fig. 1).

Background noise was measured with a hydrophone (Burns Electronics), consequently placed at four locations in the test tank (Fig. 1). Both sound and noise were recorded and analysed using a Real Time Frequency Analyser (Agilent model HP3569A). Preparatory tests confirmed that the sound levels were homogenous in the tank. A slight increase of 2–5 dB (depending on frequency) was noticed at position 1, as expected, due to being closer to the source.

The roach, from the family Cyprinidae, has a swim bladder that is connected to the hearing organs (the saccule, lagena, and utricle) by the Weberian ossicles, a chain of three or four small bones. This physiognomy gives it an enhanced hearing which is why roach is generally classified as a hearing specialist. The stickleback (*Gasterosteidae*) is classified as a hearing generalist as it has a swim bladder but no connection between the hearing organs and the swim bladder. Due to their different physical hearing ability, it can be assumed that roaches and sticklebacks will show different responses to sound.

To determine the frequency interval to be used in the experiment, reference data from species with similar hearing abilities were applied. For the roaches the hearing frequency interval for another cyprinid, the goldfish (*Carassius auratus*), was used: 10–5000 Hz with a maximum sensitivity around 500 Hz (12–13). Hence, roaches were exposed to 25 Hz, 80 Hz, 500 Hz, and 1000 Hz. For the stickleback the hearing frequency interval of cod (*Gadus morhua*), 30–500 Hz with maximum sensitivity around 100 Hz (11, 14), was applied. Sticklebacks were then exposed to 25 Hz, 160 Hz, 200 Hz, and 500 Hz.

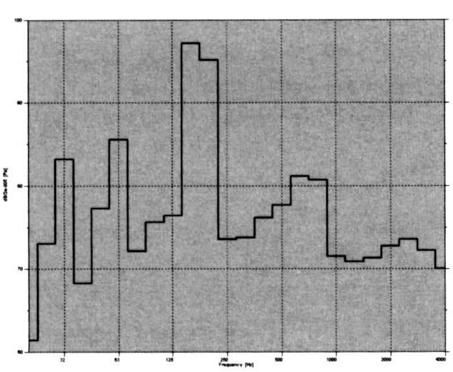

Figure 2. Frequency spectra of the recorded noise, WPN, at Utgrunden. To transform the sound pressure to levels relative 1 μPa, 26 dB has to be added.

The wind power noise (WPN) was originally recorded at the wind farm Utgrunden in the southern Baltic Sea (Kalmarsund) at a depth of 12.9 m. The distance to the windmill, which had a monopile foundation made of steel, was 83 m. The wind speed during recording was 14 $m \cdot s^{-1}$ with generator revolving at 1780 rpm. The recording contained a frequency interval varying between 0–4 kHz with four strong tones (35 Hz, 60 Hz, 178 Hz, and 750 Hz) (Fig. 2). The strongest tone (178 Hz) was used as a reference frequency in the sound control measurements. To test the fishes' response to changes in sound pressure levels (SPL) 80 and 100 dB re 1 μPa SPL were applied (denoted with low and high) for both single-tone frequencies and WPN. An additional experiment was performed with WPN where SPL was raised to 115 and 120 dB re 1 μPa (denoted WPN-H low and WPN-H high) to investigate the response of fish to higher SPL. A control group was kept in the test tank for the same period of time as the other test groups.

Sticklebacks used in the experiment were caught in the Baltic Sea and roaches were caught in a lake. They were kept in holding tanks (200 L) with a water temperature of 14°C, a salinity of 5 psu, and a diurnal light cycle of 08.00–16.00 and were fed daily with frozen midge larvae. The total length (L_T) and weight of the roaches were 11.2 ± 0.9 cm and 17.4 ± 4.9 g, respectively, while the sticklebacks had a length of 5.8 ± 0.4 cm and weighed 2.4 ± 0.6 g. Sixty sticklebacks were placed in the holding aquarium, of which 10 were used as controls (distinguished by clipping off the second dorsal spine). As roaches were more difficult to mark five control fish where separated from the remaining 45 and placed into two separate aquaria.

Five fish were moved from the aquaria to the testing tank and kept there for 30 minutes without disturbance. After the habituation period, the observer entered the room and waited an additional 7 minutes before starting the experiment (15, 16). The fishes were exposed to sequences of sounds in a random order including both the single tone frequencies and WPN. Every sequence was played for 10 seconds followed by 2 minute silence before the next sequence was started (17).

To analyse fish response to the acoustic disturbance quantitatively, the difference between mean horizontal movements in cm was compared using a two-way analysis of variance (ANOVA) with frequency and SPL as statistical factors. The non-parametric Kruskal-Wallis test was applied when the data did not meet the requirements of an ANOVA. A post-hoc test (Tukey-HSD test) was used to determine the specific differences between test variables. The group effect was ignored in the analysis as each set of five fish was randomized and unique.

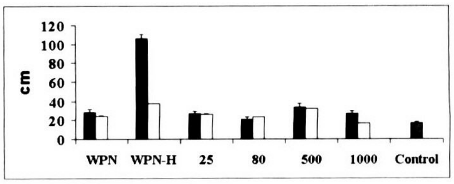

Figure. 3. Movements for roach (*Rutilus rutilus*) when exposed to the recorded offshore wind power noise WPN, WPN-H, and single-tone frequencies at the two different sound pressure levels: black bars denote high sound pressure level, white bars denote low sound pressure level, and grey denotes the control group exposed only to background noise. Mean values and standard error.

Both roach and stickleback responded to wind power noise and single-tone frequencies at sound pressure level 80–120 dB re 1 μPa, although in different ways. The roach displayed an escape behavior by swimming in sudden bursts. The swimming distance varied between different sounds both in response to frequency and SPL, and for the combination of both variables, ($p < 0.001$). Usually roach moved between 20 and 40 cm when exposed to any sound with a tendency to move less in the controls (see fig. 3). The post-hoc test showed a difference ($p < 0.001$) between WPN-H and the other test variables at high SPL where roaches exposed to the WPN-H (120 dB re 1 μPa) moved an average of 106 cm with a standard error (SE) of 4.7. The noticed escape behavior in the roaches is a natural reaction in fish to avoid potential predators, superior competitors, or materials in motion (11). Changes in sound pressure level were not the only influencing factor. It was observed that the 500 Hz frequency generated a slightly larger response than the other single tones which agrees with results for other Cyprinids (12, 13). Experiments with the related goldfish (*Carassius auratus*) showed that they were sensitive to small changes in SPL as they were observed to be able to distinguish between pulses of 3–6 dB with the greatest sensitivity between 300–400 Hz (11).

For sticklebacks, a statistical difference ($p < 0.05$) among tests was noted for swimming distance, both for low and high SPL. Although in contrast to roaches, fish in the control group of sticklebacks were more inclined to alter their positions (average 31.5 cm, SE 1.3) than the fish exposed to sound. Fish exposed to WPN-H of high SPL responded the most, 3.9 cm

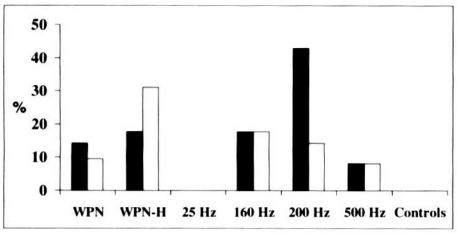

Figure. 4. Percentage of twitching three-spined sticklebacks (*Gasterosteus aculeatus*) when exposed to recorded offshore wind power noise WPN, WPN-H, and single-tone frequencies at the two different sound pressure levels: black bars denote high sound pressure level and white bars denote low sound pressure level. No sticklebacks in the control group twitched.

(SE 0.1). Same patterns were observed for the low SPL. Even though we found significant results in swimming distance, this may not be the most suitable method to test stickleback's reactions to sound when only short scale horizontal movements were noticed (i.e., less than 5 cm).

In comparison to the roaches, a different set of behavioural reactions were observed for the sticklebacks. In addition to forward swimming, four different movements were seen: "twitching," a sudden twitchy movement of the entire body; "backing," backward swimming; "vertical movement," the sudden change of depth (mostly upward) without any obvious fin movements and; "freezing," the fish froze in one position. Notably, when sticklebacks where exposed to 200 Hz at the high SPL, 43% showed twitching behaviour (Fig. 4). In contrast to high SPL, the higher level of wind power noise (WPN-H) generated greater numbers of twitching fish (31%) at a low then high SPL (18%). The behavioral responses in sticklebacks are most likely natural reactions to avoid predation (16, 18, 19). When threatened, sticklebacks usually remain still and in cover for a long period of time (15). The twitching behaviour has also been noted in other species that are startled by a predator or other disturbances such as juvenile saithe (*Pollachius virens*), adult pollack (*Pollachius pollachius*), juvenile cod (*Gadus morhua*) and adult mackerel (*Scomber scombrus*) (14). In such situations the fish veers suddenly to one side due to the involuntary contralateral contraction of its whole lateral muscle, a Mauthner reflex or C-start, followed by a voluntary movement bringing it back to a normal condition.

There is an environmental concern of how fish may be influenced by the developments of wind power offshore installations (20–23). In this study, two different species of fish were exposed to single-tone frequencies and sound generated by an offshore wind power plant. Both species reacted to the wind power noise which indicate that the noise may cause stress. However, fish have been noticed to habituate to sound (9) and to associate with windmills at sea (20).

This study was a small scale experiment. For a comprehensive understanding on how fish respond to wind power noise, additional studies are needed involving more species and large scale laboratory and field experiments based on detailed measurements of the noise generated from wind power plants.

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