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MULTIPLE EXPOSURE TO THUNDERSTORM SOUND IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*): PHYSIOLOGICAL RESPONSE AND STRESS RECOVERY

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Abstract

The present study investigated the impacts of multiple thunderstorm-sound exposures on growth and respiratory parameters in Nile tilapia (*Oreochromis niloticus*) in order to evaluate the acoustic stress response. Thunderstorm-sound exposure for 3 hours triggered respiration speed with an alarm reflex and rapid elevation of opercula beat rate (OBR) and pectoral wing rate (PWR), which increased two-fold over the control with no sound treatment, and peaked (OBR, 71.33±5.86 beat/min; PWR, 75.00±3.61 beat/min) in 10 hours after initiation of sound. Thereafter, respiration rates declined over the following days and returned to near-initial levels (45.33±4.04 beat/min OBR and 43.00±1.00 beat/min PWR) by day 3, an indication that fish recovered from thunderstorm-sound stress after 3 days of exposure. However, the same reaction course was observed each time of multiple sound exposures, repeated 20 times in a row with 4-day intervals, underlining that fish could not attune to repeated thunderstorm sound. Reduced voluntary feed intake as a result of anxiety and appetite loss was recorded in fish exposed to multiple thunderstorm sound, resulting in 50% less growth compared to those without sound treatment by the end of the 80-day experimentation. Therefore, it is advisable to monitor fish behavior during the 3-day stress period after a thunderstorm event in order to prevent waste from excess feeding, that in turn may contribute environment-friendly aquaculture for the future and sustainability of the oceans.

Key words: acoustic noise, growth, respiratory rate, stress response, thunderstorm, tilapia

The oceans experience several types of storms characterized by strong winds such as hurricanes and thunderstorms which are marked by the occurrence of thunder and lightning. As a consequence of climate change, the number of thunderstorm events has increased with fluctuations between 90 and 310 records from 2010 to 2019 in the Mediterranean, and doubled from around 110 to 220 severe storms over the last decade (MGM, 2020). While thunder and lightning usually occur at the same time, light travels faster than sound, hence lightning is seen before thunder can be heard (Emelda, 2011). Rather than penetrating the water, lightning disperses in all directions favoring the surface, since water is a reasonably good conductor, and a good conductor keeps most of the current on the surface, however, a lightning strike during thunderstorm may generate a sound up to 260 dB (decibels) in 1 m distance at water surface, which is around twice the level of a gunshot or firecracker (CMCCLAIN, 2008). Smith et al. (2004) reported a substantial hearing loss in goldfish (*Carassius auratus*), but not in Nile tilapia (*O. niloticus*), after 20 days of exposure to a broadband noise of 170 dB, which shows that impacts of anthropogenic noise can be species specific, and different animals may respond in different ways against various types of stimuli, length of sound exposure, repeated or continued sounds, etc. (Bejder et al., 2009). Most of the earlier studies so far, reported behavioral responses (Beale and Monaghan, 2004; Simpson et al., 2015; Spiga et al., 2017), and physiological consequences (Smith et al., 2004; Buscaino et al., 2010; Filiciotto et al., 2017; de Jong et al., 2020) in fish exposed to anthropogenic (human generated) acoustic sounds which has already been drastically increased in the oceans over the last decade. However, there are still substantial gaps in the knowledge regarding impacts of sounds on aquatic animals (Popper et al., 2014; Southall et al., 2019; Hawkins et al., 2020; Popper et al., 2020; Thomsen et al., 2020), and the impacts of natural sounds such as thunderstorm and lightning sound have been ignored, which might influence physiological response and behavior as a potential stress factor for fish in capture conditions. When fish is exposed to unfamiliar sounds, decrescent impacts on appetite loss and voluntary feeding as a sign of anxiety and physiological response can occur (Metcalfe et al., 1987; Wendelaar Bonga, 1997; Kusku et al., 2018 a, 2020). Eventually, fish exposed to stress may react physiologically, and the dispersal of unconsumed feeds due to loss of appetite may cause deterioration of water and also economic loss for farmers, due to reduced feed efficiency. Considering the climate change and the increased impacts of thunderstorms over the last decade, the question "how natural sounds like thunderstorm or lightning may affect aquatic animals" is still open for the understandings of fish welfare in capture conditions. Tilapia was used in the present study, as it is the second most farmed fish after carps in the world, with 4-fold production increase over recent years (Wang et al., 2016). Different than other fish species, tilapias are extremely strong and highly tolerant to stress conditions, and can be grown under wide range of culture systems such as ponds, cage culture systems, raceways and super-intensive culture systems under a wide range of environmental conditions (Siddik et al., 2014; Prabu et al., 2019).

Indeed, fish in the nature may tend to swim deeper and typically may not be influenced by thunderstorm impacts occurring at water surface. In contrast, farmed fish in capture conditions such as cage systems with limited depth of nettings, or ponds with less water depth, swim in a relatively narrow environment close to water surface. Irregular and abnormal swimming behavior accompanied with less appetite have been observed in rainbow trout (*Oncorhynchus mykiss*), gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) for 4 to 5 days after the hit of a thunderstorm (personal communications: Mr. Melih Geçgil, Operation and Logistic Coordinator, and Mr. Emre Şen, Farm Manager of Kilic Deniz Company, Turkey, 4 January 2021). Similarly, remarkable appetite loss has also been reported in rainbow trout (*O. mykiss*), and high mortalities were observed in maegre (*Argyrosomus regius*) when thunderstorm hit the cage farm facilities (personal communications: Mr. Hamza Firat, Farm Manager of Penta Seafood Fishark Company, Turkey, 24 May 2021). Hence, it is likely that farmed fish are affected to a certain extent by hits of thunderstorms.

In recent years, reports provided clear evidence that anthropogenic noise can significantly impact mammals and marine fishes, however much less is known about these effects in fresh water environment, that interest fishery managers due to rising levels of this background noise. The influence of anthropogenic noise on freshwater fish can be quantified using the same methods as with marine species via behavioral and physiological responses (Mickle and Higgs, 2018). So far, there is no clear published evidence yet regarding the impacts of thunderstorm-sound on physiological response and stress-recovery time of fish either in marine or freshwater capture conditions. Therefore, this study aimed to evaluate impacts of multiple thunderstorm-sound exposures on physiological response and stress recovery-time in fish with the assessment of respiration speed of opercula beat rate (OBR) and pectoral wing rate (PWR) in order to secure best feeding practice and effective farm management for the prevention of feed losses in fish farms, which is considered as one of the main sources of environmental impacts from aquaculture facilities.

Material and methods

Transmission of natural thunderstorm sound and monitoring of physiological response

A repeated sound of thunderstorm was used as a stress factor to monitor physiological response of fish. Underwater transmission of the thunderstorm sound tested in the present study was retrieved from https://www.youtube.com/watch?v=jsASzgzV71o. All experimental containments were set with waterproof hydrophones (Mini Speaker w/Wires–8 Ohm, 1.5 W Stw-c, 8.24 × 3.1 cm), deployed at the opposite side of water inlet, 6 cm below surface. Magic Voice brand Mp3 amplifier with three outputs was used to distribute experimental thunderstorm sound to each of the test containments with triplicate groups. A Sound Meter model device (Sound Meter Version: 1352-EN-00) was used for recording underwater noise levels in decibel (dB re 1 µPa SPL). Schematic diagram for the underwater transmission of experimental sound and hydrophone output recordings in the experimental containment is given in Figure 1.

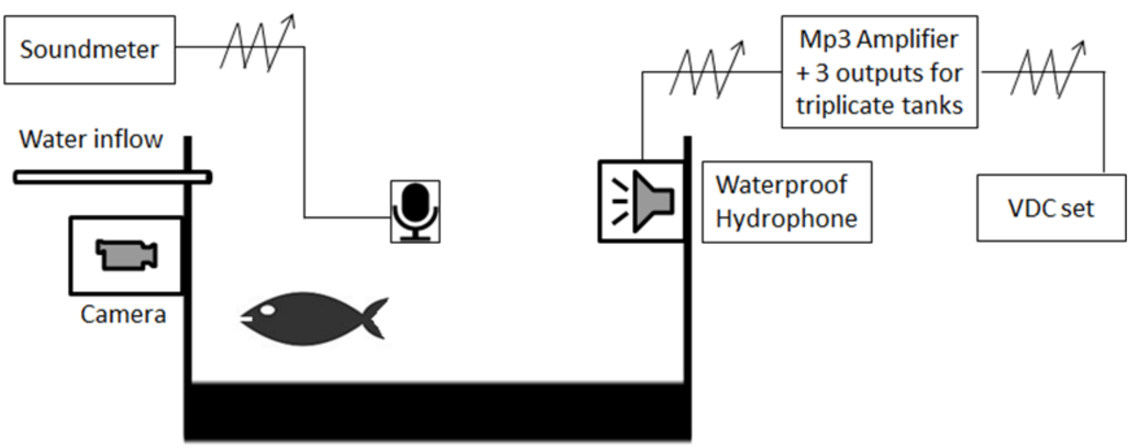

Figure 1. Schematic diagram of thunderstorm-sound transmission and hydrophone output recording

The even distribution of fish in the experimental tanks and external appearance was a sign of non-stress environment and healthy individuals. Playbacks of repeated thunderstorm sound in each test containment was simultaneously initiated at 09:00 a.m. and terminated at 12:00 p.m., lasting for 3 hours in total. Time and exposure duration of daily sound transmission was adjusted using an automatic timer (TS-814 AB Tak-TS-816 AU model). Special care was given to avoid contacts between vibrating pipes and tank surface in order to buffer any possible ambient sound in the experimental system, and to receive same levels of ambient sounds in all test containments following the method reported by Davidson et al. (2007), and Davidson et al. (2009). The control group received no additional sound treatment, and ambient mean sound pressure level (SPL) was measured as 50.25±2.13 dB re 1 µPa. Once the thunderstorm-sound transmission was initiated, mean SPLs increased from the ambient sound level to 130.18±17.09 (range: 70–138) dB re 1 µPa. The thunderstorm sound tested in the experiment masked and overlapped the ambient noise level of 50.25±2.13 dB re 1 µPa.

The influence of underwater sound transmission on fish were evaluated using physiological tests because behavioral changes may not always be sensitive enough for a timely assessment of a reaction to stimuli as earlier reported by Beale and Monaghan (2004). Hence, respiration rates measured as opercula beat rate (OBR) and pectoral wing rate (PWR) were performed as physiological stress indicators following the reports of Barton (2002), Gibson and Mathis (2006), Tantarpale et al. (2012), Nedelec et al. (2016), Radford et al. (2016), Spiga et al. (2017), Kusku (2020), and Kusku et al. (2020).

The OBRs and PWRs in fish exposed to thunderstorm sound were monitored with an I-Phone 6S Mobile Cam, set to the aquaria glass from outside and positioned in a way to observe all fish in the containment. Video recording was initiated simultaneously with the start of sound transmission at 9:00 am. While the thunderstorm sound was switched off at 12:00 a.m., the video recording continued until 7:00 p.m., and no recording was performed during night time, since the study followed the natural photoperiod course and low visibility made it difficult to follow fish ventilation rates over the dark hours. The counts of opercular beats and pectoral wings were noted by video image tracking after termination of the experiments.

The thunderstorm-sound transmission program was scheduled as "3 hours sound exposure" followed by "4 days monitoring by video recording" of OBRs and PWRs as a result of physiological stress response. This was repeated for 20 times with multiple exposures with 4-day repetition intervals over a period of 80 days. Based on preliminary testing prior to start of the experiment, it was observed that peaks of OBRs and PWRs returned to near-initial levels (similar to the control) after 3 days of sound exposure, which was in accordance with the information received from personal communications as mentioned earlier. Hence in the present study, a 3-hour sound exposure was followed by a 4-day monitoring in order to ensure a full recovery from thunderstorm-sound stress before the initiation of a new sound exposure. The entire process with 20-times repetition of multiple exposures with 4-day intervals conducted over 80 days in total has been illustrated in Figure 2.

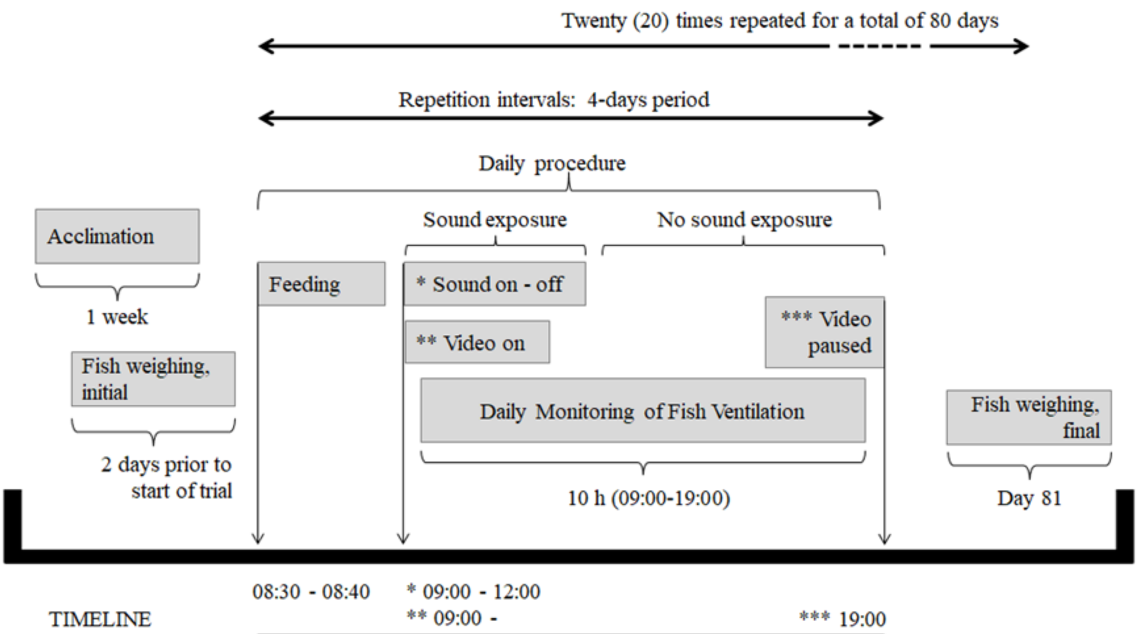

Figure 2. Flow diagram of the experimental protocol; vertical direction arrows show each stage of the sound treatment and monitoring throughout the timeline in daily procedure

Experimental fish and rearing conditions

One hundred and eighty specimens of scaled Nile tilapia (*O. niloticus*) with a mean body weight of 10 g, were previously acclimated to the laboratory conditions in the research facility of Canakkale Onsekiz Mart University (COMU), Department of Aquaculture Industry Engineering, Faculty of Marine Science and Technology (Turkey). A total of 90 fish of mean initial body weight 9.98±0.30 g were randomly withdrawn out of the total batch of 180 fish, and evenly distributed into 6 identical glass aquariums with 100 L volume (15 fish per tank, 45 fish per treatment). Fish were acclimated to the test conditions for 1 week prior to the start of sound transmission. Two experimental groups, one control with no sound and the second with thunderstorm-sound treatment were assigned according to a 2 × 3 factorial design in triplicates. The recirculating aquaculture system (RAS) used at the experimental research facility was a convertible system that can use either freshwater or seawater. In this study, freshwater was used and all test containments were supplied with continuous aeration using air stones. Water flow rate was equalized and maintained with an average inflow rate of 31.5±0.4 L/min in all experimental tanks, and photoperiod followed the natural course throughout the study. Weekly measurements of water temperature, dissolved O_2, ammonia, nitrite, nitrate, and pH values were performed using YSI brand automatic water quality measurements device, and recorded as 21.08±1.05°C, 7.3±1.2 mg/L, 0.01±0.001 mg/L NH_3, 0.029±0.01 mg/L NO_2, 2.23±0.37 mg/L NO_3, and 7.6, respectively. A commercial diet with 21% crude protein and 12.7% lipid was offered daily at 08:30 a.m. by hand-feeding for 10 minutes prior to start of sound transmission throughout the study period of 80 days. Feeding was withheld when fish were reluctant to feed, which was accepted as a sign of satiety.

Statistical analyses

In the present study measured variables have been recorded as means ± SD. Respiration rates (OBRs and PWRs) were evaluated with Tukey multiple range test when homogeneity and normally distributed data was seen. Kruskal–Wallis test was performed when homogeneity without normal distribution was recorded. The Tamhane test was conducted when data showed no homogeneity in terms of distribution using SPSS 19 statistical software (IBMM SPSS Statistics 19), and critical limits for significance were set at P<0.05 level. Correlations between OBRs and PWRs were evaluated using Microsoft Excel for Mac based on the following equation:

$$Correl\ (X, Y) = \frac{\sum (x-\overline{x})(y-\overline{y})}{\sqrt{\sum (x-\overline{x})^2 \sum (y-\overline{y})^2}}$$

where $\overline{x}$ and $\overline{y}$ indicate sample mean values for series 1 and series 2, respectively.

Results

Based on the findings of the present study, it was observed that fish exposed to thunderstorm sound for 3 hours, recovered from the stress conditions after 3 days of exposure. With the initiation of sound transmission, fish showed an immediate startle response as an alarm reflex and both OBRs and PWRs increased significantly (P<0.05) to nearly two-fold over the control group with no-sound-treatment at all, and peaked in 10 hours after initiation of sound for the first 4-day interval period, with average levels of 71.33±5.86 and 75.00±3.61 beat/min for OBR and PWR, respectively (day 1). Considering the entire study period of 80 days with 20 times repetition of 4-day intervals, the average beats of opercular and pectoral movements showed similar results with a mean peak value of 73.95±4.22 beat/min and 75.75±4.27 beat/min, respectively, at 10 hours post-sound exposure for 3 hours.

Elevated OBRs and PWRs showed a declining trend from 71.33±5.86 and 75.00±3.61 beat/min to 57.00±4.00 and 58.00±4.00 beat/min by the end of day 2; to 45.33±4.04 and 43.00±1.00 beat/min by the end of day 3; and to 44.00±1.73 and 42.00±1.00 beat/min, respectively by the end of day 4 within the first 4-day interval period (Figures 3, 4).

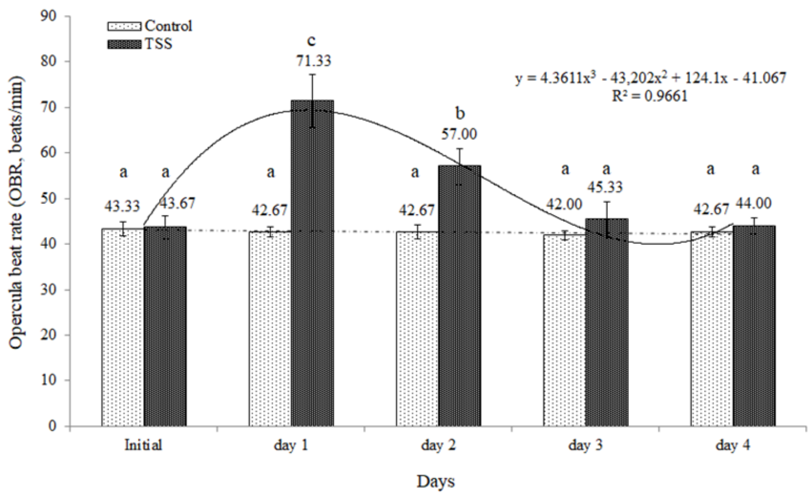

Figure 3. Opercula beat rates (OBR, beats/min) and recovery time from stress of fish exposed to thunderstorm-sound stress for 3 hours. n∑=90 split evenly among treatment groups (n=15 in each of triplicate treatment; control , sound exposure). Different letters above bars indicate significant difference at P<0.05 level

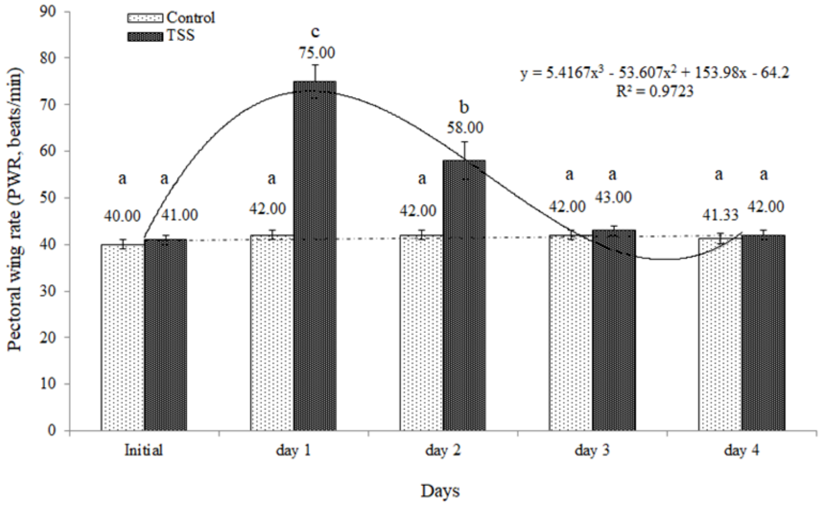

Figure 4. Pectoral wing rates (PWR, beats/min) and recovery time from stress of fish exposed to thunderstorm-sound stress for 3 hours. n∑=90 split evenly among treatment groups (n=15 in each of triplicate treatment; control , sound exposure). Different letters above bars indicate significant difference at P<0.05 level

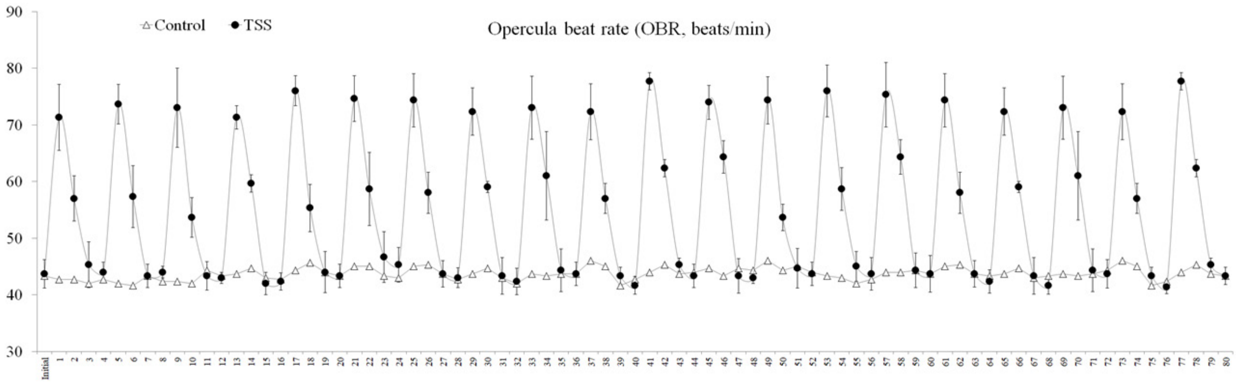

Figure 5. Variations in opercula beat rates (OBR, beats/min) in fish exposed to multiple thunderstorm-sound stress (TSS) with 20 times repetition in 4-day intervals over a period of 80 days. n∑=90 split evenly between treatment groups (n=15 in each of the triplicate treatment; control, sound exposure TSS)

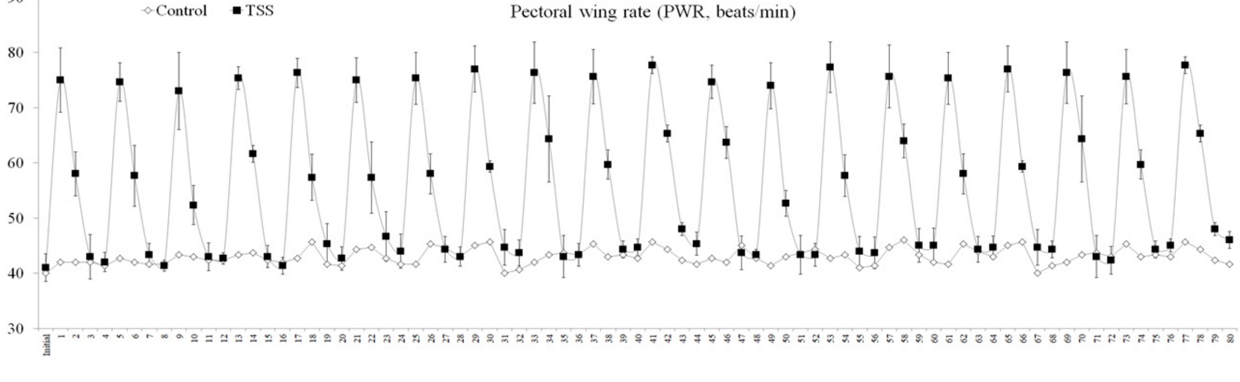

Figure 6. Variations in pectoral wing rates (PWR, beats/min) in fish exposed to multiple thunderstorm-sound stress (TSS) with 20 times repetition in 4-day intervals over a period of 80 days. n∑=90 split evenly between treatment groups (n=15 in each of the triplicate treatment; control, sound exposure TSS)

Table 1. Growth performance and feed utilization of fish exposed to multiple thunderstorm stress with 20 times repetition in 4-day intervals over 80-day period. Data (mean ± standard deviation) with different superscript letters in the same line differ significantly among test groups (P<0.05)

	Control	Thunderstorm sound
Initial body weight (g)	9.98±0.30 a	10.13±0.31 a
Final body weight (g)	18.0±0.13 b	14.29±0.25 a
Wet weight gain (WWG, g)	8.02±0.25 b	4.16±0.33 a
Relative growth rate (RGR, %)	80.5±4.81 b	41.08±4.24 a
Specific growth rate (SGR, % / day)	8.02±0.25 b	4.16±0.33 a
Voluntary feed intake (VFI, g)	0.19±0.002 b	0.17±0.02 a
Feed conversion rate (FCR)	1.31±0.06 a	2.02±0.05 b
Relative biomass gain (RBG, % of control)	100	51.80±3.15
Relative specific growth rate (RSGR, % of control)	100	58.22±3.61
Mortality (%)	0	0
Survival rate (%)	100	100

WWG (g) = final wet weight (g) – initial wet weight (g).
RGR (%) = [(final weight – initial weight) / initial weight] × 100.
SGR (%/day) = [(ln final weight – ln initial weight) / days] × 100.
VFI (g) = dry feed intake (g) / [(initial weight (g) + final weight (g)] / 2).
FCR = feed consumption (g) / wet weight gain (g).
RBG (% of control) = (biomass of treatment group × 100) / biomass of control group.
RSGR (% of control) = (SGR of treatment group × 100) / SGR of control group.

These lowered respiration rates were close to the initial beats (43.67±2.52 beat/min OBR; 41.00±1.00 beat/min PWR), and also similar to the respiration rates of the control group with no sound treatment (43.33±1.53 beat/min OBR; 40.00±1.00 beat/min PWR). The blank remained almost stable within a range of 42.00±1.00 and 43.33±1.53 beat/min for OBRs and 40.00±1.00 and 41.33±1.15 beat/min for the PWRs over the first 4-day interval period. When the same protocol was repeated for 20 times, similar trends were noted for both OBR (Figure 5) and PWR (Figure 6), each of the 4 days intervals over the course of 80 days in total, and the correlation between OPRs and PWRs was found as R = 0.9923, showing a strong positive correlation between both physiological responses to the stressor. Fish showed same response in terms of sudden alarm reflex followed by rapid increase of respiration at day 1 and a smooth decline afterwards over the "4-day monitoring intervals". This responsive cycle was repeated each time of multiple sound exposures of 20 times repetition in 4-day intervals throughout the 80-day experimentation.

By the end of the study conducted for a period of 80 days, the control group without sound treatment demonstrated significantly better growth performance (P<0.05) compared to the sound-treatment group, with a wet weight of 8.02±0.25 g for the control and 4.16±0.33 g for the sound-treatment group (Table 1). Relative growth rate (RGR) and specific growth rate (SGR) followed the same trend with highest rates in the control (RGR, 80.5±4.81%; SGR, 8.02±0.25%/day), and significantly lower values in the thunderstorm-sound induced groups (RGR, 41.08±4.24%; SGR, 4.16±0.33%/day) (P>0.05). No mortality was observed during the course of the study and survival was 100% by the end of the 80-day period.

Relative biomass gain in fish exposed to thunderstorm sound (RBG, 51.8±3.15%) was about 50% lower than the control group (RBG, 100%). A similar trend was observed for relative SGRs in the thunderstorm-sound induced group (RSGR, 58.22±3.61%) with significant lower rates (P<0.05) over the control (RSGR, 100%). Significantly lower voluntary feed intake (VFI) was observed in fish exposed to thunderstorm sound (0.17±0.02 g) compared to the control group without sound treatment (0.19±0.02 g), with a difference of 12% between test groups. Significantly better feed conversion rate (FCR) was recorded in the control (1.31±0.06) over the thunderstorm exposed group (2.02±0.05).

Discussion

In intensive aquaculture conditions, common stressors have been listed with highest occurrence rates for chemical stressors such as metabolic waste (accumulation of ammonia or nitrite; 35%), poor water quality (low dissolved oxygen, improper pH, etc.; 20%), dietary imbalance (10%), while lower occurrence rates of 3–5% were reported for procedural stressors of handling, grading and transportation, and 1% occurrence rate was given for physical stressors such as temperature, light, sounds, etc. (Gabriel and Akinrotimi, 2011). Despite the 1% occurrence rate estimated for sounds, marine industrial developments and rapid urbanization at coastal areas brought new interests to researchers focusing on aquaculture and environmental interactions (Kusku et al., 2018 a). The increasing concern of underwater noise has been an increasing problem influencing fish, mammals, invertebrates and all living organisms in the aquatic ecosystem (Andrew et al., 2002; Southall et al., 2007; André et al., 2011), as well as fish under controlled farm conditions (Bejder et al., 2009; McLaughlin and Kunc, 2013; Nichols et al., 2015; Simpson et al., 2015; Nedelec et al., 2016; Radford et al., 2016; Spiga et al., 2017; Kusku et al., 2018 b, 2020; Kusku, 2020). However, besides anthropogenic sounds, natural sounds like thunderstorm lightning are also unfamiliar to aquatic world and likely to have influence especially on fish swimming near surface in capture conditions. Any scientific information supporting this hypothesis would provide useful tools for best management efforts in aquaculture operations.

Bacheler et al. (2019) measured the movements of gray triggerfish, *Balistes capriscus* (a demersal oceanic fish species) before, during, and after two hurricanes using fine-scale acoustic telemetry at a depth of 37 m in North Carolina (USA). Movement and emigration rates of gray triggerfish were 100% and 2550% higher, respectively, during storms compared to days without storm.

Bacheler et al. (2019) also found that increased movement rates were much more strongly correlated with wave orbital velocity (wave-generated oscillatory flow at the sea bottom) than either barometric pressure or) than either barometric pressure or bottom water temperature, two covariates that have been demonstrated to be important for organisms in shallower water. The authors addressed that higher movement rates during storms were due to increased mobility at night, and emigrations typically occurred at night in the direction of deeper water. The findings of Bacheler et al. (2019) provide strong evidence that extreme weather conditions strongly influence marine, freshwater, and estuarine ecosystems in different ways, and overall, it was found that heavy storm events significantly affect movement behavior of fish species in open ocean environments.

Among several relevant physiological measures involved in the assessment of fish stress, blood, glucose, and lactate are commonly used indicators (Wendelaar Bonga, 1997; Yavuzcan-Yıldız and Kırkağaç-Uzbilek, 2001; Vazzana et al., 2002; Kuo and Hsieh, 2006; Martínez-Porchas et al., 2009; McLean et al., 2016). Blood parameters were also used for the assessment of impacts of acoustic stimuli in European sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.) (Buscaino et al., 2010). However, measuring cortisol, glucose or lactate as stress indicator needs plasma collecting by a syringe, which is another source of stress on animals (Laidley and Leatherland, 1988; Marino et al., 2001), and capturing fish from the experimental tank at each time of sampling may repeatedly disturb and influence the level of blood parameters (Pickering et al., 1982). Unlike all these methods representing off-topic stressors, quantification of fish respiration by opercula beats and pectoral fin movement can be considered as a physiological assessment for the “target-stress-source” only, along with a wide range of responsive behavior such as oxygen consumption, swimming, opercula movements, or motility rates have been investigated in a number of studies, providing strong evidence in terms of correlation between physiological status and behavioral response in fish exposed to stressors (Barton, 2002; Dalla Valle et al., 2003; Gibson and Mathis, 2006; Tantarpale et al., 2012; Wale et al., 2013; Nedelec et al., 2016; Radford et al., 2016; Spiga et al., 2017; Kusku, 2020; Kusku et al., 2020). For instance, strong relations have been found between oxygen consumption and respiration rate, as well as plasma cortisol concentration and heart beats (Wendelaar Bonga, 1997; Barton, 2002; Kammerer et al., 2010). Further, reliable results have been demonstrated with special indications on acute ammonia-stress-induced behavioral response without disturbance in tilapia (*O. niloticus*) as a result of increased level of unionized ammonia in the water environment (Xu et al., 2005). This was confirmed by Kayali et al. (2011) who measured ammonia nitrogen excretion rates directly from the water containment for the assessment of stress response of European seabass (*D. labrax*) without disturbance.

Different than earlier quantification efforts for the physiological stress challenge in fish, the present study used a stress quantifying method through measurements of respiration rate via OBRs and PWRs as a physiological response following earlier reports of Barton (2002), Gibson and Mathis (2006), Tantarpale et al. (2012), Nedelec et al. (2016), Radford et al. (2016), Spiga et al. (2017), Kusku (2020), and Kusku et al. (2020).

Impacts of several stressors in farm conditions have been evaluated in European seabass (*D. labrax*, Caruso et al., 2005; Gornati et al., 2004; Guerriero et al., 2002; Simontacchi et al., 2008), gilthead seabream (*S. aurata*, Caruso et al., 2005), rainbow trout (*O. mykiss*, Kubilay and Uluköy, 2002), and Atlantic salmon (*S. salar*, Skjervold et al., 2001); however, no published information is yet available concerning the timely response and stress recovery time after exposure to thunderstorm sound in fish under capture conditions. Hence, the present study is the first attempt to investigate the timely response and stress recovery of fish in capture from stress conditions and return to normal status with renewed appetite after repeated exposures to thunderstorm sound.

Due to lack of publications related to thunderstorm-sound effects on physiological response or growth performance of fish, findings in the present study have been compared with some earlier reports explaining impacts of several types of underwater sounds on fish respiration and growth. In the present study, growth performance of fish exposed to thunderstorm sound was lower than the no-sound-treated control group after repeated exposures for 80 days, which was in line with earlier reports in Atlantic salmon (*Salmo salar*, Terhune et al., 1990), in carp (*Cyprinus carpio*, Sun et al., 2001), and European seabass (*D. labrax*, Radford et al., 2016), Nile tilapia (*O. niloticus*, Kusku, 2020; Kusku et al., 2020), with damaging effects of underwater sounds unfamiliar to the aquatic animal. Atlantic salmon (*S. salar*) exposed to predator-risk-effect showed a behavioral disturbance in voluntary feeding activity (Metcalfe et al., 1987). Similarly, growth performance of European seabass (*D. labrax*), northern pike (*Esox lucius*), and lake-chub (*Couesius plumbeus*) were influenced by underwater seismic air gun shootings (Santulli et al., 1999; Popper et al., 2005). Sounds of piling and drilling generated during bridge construction also affected farmed fish such as chinook salmon (*Oncorhynchus tshawytscha*), steelhead (*O. mykiss*) (PIDP, 2001), and tilapia (*O. niloticus*, Kusku et al., 2020).

Based on voluntary feed intake in fish exposed to thunderstorm sound in this study, it was evident that fish showed appetite loss and reduced feed utilization as a result of stress. Similar to the findings in the present study, reduced appetite and disturbance in voluntary feeding as a sign of anxiety and physiological response has been reported in Atlantic salmon (*Salmo salar*, Metcalfe et al., 1987), Coho salmon (*Oncorhynchus kisutch*, Wedemeyer, 1976), European seabass (*Dicentrarchus labrax*, Kayali et al., 2011), and Nile tilapia (*O. niloticus*, Kusku et al., 2018 a, 2020) after exposure to several sources of stressors. In line with voluntary feed intake, the biomass gain of experimental fish was also negatively affected by multiple thunderstorm sound consecutively transmitted for 3 hours over 4-day intervals and 20 times repetition throughout the course of 80 days in total. Despite 50% reduced growth performance in Nile tilapia (*O. niloticus*) exposed to thunderstorm sound, no mortality was observed in either sound treated or no-sound treated groups in the present study. Reduced growth performance was also reported in Nile tilapia (*O. niloticus*) earlier when fish was exposed to continuous noise of urban and shipping sounds (Kusku et al., 2020) however, fish acclimated to continuous sound exposure after 45 days. Acclimatization to increased noise conditions was also reported by Wysocki et al. (2007), Davidson et al. (2009), Lee et al. (2013), Bruintjes and Radford (2014), Nedelec et al. (2016), Radford et al. (2016), Kusku et al. (2018 b).

In nature, noise levels ranging between 5 and 50 dB have been reported earlier with relatively higher sound pressure frequencies (between 50–95 dB) in nearshore shallow waters (Wenz, 1962), meaning that the sound level in the aquatic ecosystem varies according to environmental conditions. In the present study, ambient sound level in the control group without sound treatment was measured as 50.25±2.13 dB re 1 μPa, which is in full agreement with Wenz (1962). After initiation of thunderstorm sound, mean SPLs increased over 2-fold to 130.18±17.09 (range: 70–138) dB re 1 μPa, which was twice higher than the noise level of a natural marine environment (Wenz, 1962). Davidson et al. (2009) and Wysocki et al. (2007) reported that survival, growth and health status of rainbow trout (*O. mykiss*) did not change in a noisy indoor facility using loud machineries and fish could attune to sound levels up to 149 dB re 1 μPa or 150 dB re 1 μPa SPL, respectively by long-term treatment. No reactions were also reported in sockeye salmon (*Oncorhynchus nerka*), sturgeon (*Acipenser* sp.), or herring (*Clupea harengus*) when fish were exposed to pinger sound (Culik et al., 2001). Radford et al. (2016) noted a rapid increase of ventilation in European seabass (*D. labrax*) exposed to impulsive noises of piling and seismic survey, however, fish gradually reduced ventilation rate, a sign of adaptation to the unfamiliar sound source. A rapid increase in heartbeat and respiration rate has been reported in fish exposed to several stressors (Moberg, 1985; Brown, 1993). Also, Kusku (2020) and Kusku et al. (2020) observed an alarm reflex in Nile tilapia (*O. niloticus*) exposed to anthropogenic sounds, however, fish showed acclimation to the sound after 12 weeks, an indication that fish can develop tolerance to repeated acoustic sounds over a certain time. In the present study, a stress recovery of fish exposed to "3-hour thunderstorm sound" was reached after 3 days (72 hours). However, the same alarm reflex and physiological response was observed every time of disturbance from multiple exposures repeated 20 times with "4-day exposure intervals". This finding indicated that Nile tilapia (*O. niloticus*) under capture conditions could not attune to multiple sounds of thunderstorm.

Overall, the result of this study in terms of "3-day stress-recovery-time" is comparable with the findings of Wells et al. (1984) who reported stress-recovery time of 24–70 hours, when evaluating blood parameters for the assessment of capture stress levels in Arctic fish. In contrast, coral-reef fish exposed to a motorboat noise for a week showed initial response, but developed tolerance to repeated noise exposures, with reduced physiological response after a certain time (Nedelec et al., 2016). Attuning to continuous underwater sounds was also reported by Nichols et al. (2015), who stressed evoked physiological response in giant kelp fish (*Heterostichus rostratus*) when treated with acute stress of intermittent noise, however, no physiological response was recorded when fish was exposed to continuous underwater sound. Pickering et al. (1982) addressed strong relation between the level of stressors and the time of exposure. Stress-responsive behavior and the recovery time from stress condition may vary according to several factors such as environmental conditions, species, age, or the combination of all these factors. For example, young Coho salmon (*O. kisutch*) started feeding 4 to 7 days after the exposure to handling and netting stress, whereas rainbow trout returned to normal conditions and started voluntary feeding only one day after transfer (Wedemeyer, 1976). Further, Kayali et al. (2011) reported a stress recovery time of 24 hours for European seabass exposed to handling and transport stress. Casper et al. (2013) reported that hybrid striped bass recovered from swim bladder injuries within 10 days of exposure to loud sounds of piling generated by underwater constructions. All these earlier studies provide evidence that different types of acoustic sounds may impact fish behavioral response in different ways and manners. It is likely that thunderstorm sound generated by lightning during a severe storm can manipulate fish welfare in farm conditions. However, the answer to the question "how far can thunderstorm sound affect the swim bladder or health condition of fish", and "what is the level of decibels to rupture a swim bladder or interfere with other organs coping with health threat?" is a question that needs further investigations.

Despite the fact that increased respiration rates of OBRs and PWRs in this study present evidence for the increased fish stress after exposure to thunderstorm sound, we may encourage the incorporation of serum biochemical parameters along with quantification of OBR and PWRs in future studies for the understanding of mechanisms lying under the lowered appetite, reduced feed utilization and the overall health status of fish during and after thunderstorm-sound exposure.

Conclusion

This study provides evidence that Nile tilapia has a capability to recover from thunderstorm stress after

3 days of exposure. However, fish could not attune to multiple exposures to thunderstorm sound. Based on the results of this study, it is advisable to consider thunderstorm-induced stress in fish under capture conditions in order to avoid feed loss in fish farms that in turn may help to reduce environmental impacts of uneaten feed through deterioration of water quality, and also support to reduce economic consequences as a result of saving feed expenses with improved feed efficiency. Therefore, especially in production facilities using automatic feeding systems, deactivating the automation and monitoring fish behavior through underwater image tracking might reduce feed loss during the stress-recovery period of 3 days after exposure to thunderstorm. The results in this study may not be indicative of what happens to fishes in nature, but demonstrates the capability and length of recovery from thunderstorm-sound induced stress, providing important indications for good farm management for a sustainable and environment friendly aquaculture practice.

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Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

We acknowledge that all authors have contributed significantly, and confirm that all authors are in agreement with the content of the manuscript.

Ethical Statement

Regulations of "Animal Behavior Society Guidelines" have been followed during the experimental procedures and all procedures applied in this study have been approved by the Commission of Ethics at Canakkale Onsekiz Mart University (Turkey) with the Approval Number of 2021/03-16.

Conflicts of interests

The authors declare no conflict of interest or competing interests.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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