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ANIMAL SCIENCE

Physiological stress response in Nile tilapia (*Oreochromis niloticus*) exposed to firework noise

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Abstract: In the present study, impacts of firework noise on physiological response and stress recovery were investigated in Nile tilapia (*Oreochromis niloticus*). Two treatment groups, one with firework noise exposure, and a control with no noise exposure at all was used in the study. After a disturbance challenge with firework noise for 2-hour playback, fish behavior in terms of opercula beats and pectoral wing flaps as physiological response were monitored for 96-hours via video image tracking, which was then consecutively repeated with 4-days intervals and 15 repetitions over a period of 60 days. Elevated fish respiration rates after noise exposure, peaked in 24 hours, and declining to initial levels after 72 hours, an indication of stress recovery. In the long-term challenge with multiple exposures to firework noise, fish showed no attunuation to firework noise and presented the same alarm reflex every time of noise exposure with 4-days intervals. It has been evidenced that fireworks noise influenced fish stress and welfare, hence the findings in this study may support ecology-based management of coastal developments. Further, these results raise the question of whether short-term investigations are sufficient for understanding anthropogenic disturbance on aquatic animals.

Key words: Anthropogenic noise, firework, respiration, sound exposure, stress recovery, tilapia.

INTRODUCTION

Urban and industrial developments at coastal areas cause severe human pressure of increasing population growth, exerting impacts on coastal management plans (Veloso-Gomes & Taveira-Pinto 2003), especially when coupled with irregular migration waves from areas with political instability and protracted conflicts (McAuliffe & Khadria 2020). The estimated increase of world population towards 9 billion in 2037 (Worldometer 2024), may potentially increase its pressure on anthropogenic (human-generated) sound pollution by rapid urbanization at waterfronts. Impacts of acoustic noise, induced by marine industrial works of bridge construction, seismic surveys, motorboat or shipping, have been widely studied in aquatic livings so far (Codarin et al. 2009, Buscaino et al. 2010). The level of acoustic noise in the oceans between 1994 and 2001 were around 10 dB higher than in the years from 1963 to 1965, which is closely linked to the distant shipping industry worldwide, that has been increased by 2-fold from 1965 to 2003 (Andrew et al. 2002). Acoustic ambient noise in the oceans were reported to range between 5-50 dB five decades ago (Wenz 1962). In years 2003-2004, ambient noise levels in the oceans were reported as 10–12 dB higher than 30 to 40 years ago between the years 1964-1966 (McDonald et al. 2006), underlining an average noise increase by 2.5–3 dB per decade. Different characteristics of environmental conditions in various regions, as well as the level of industrialization and human activities may influence aquatic livings in different manners (Chahouri et al. 2022). Therefore, the control of chronic underwater pollution is advised with noise-specific investigations for the protection of environmental health with interactions of human being (Chahouri et al. 2022), otherwise, the increasing underwater noise pollution may impact the aquatic ecosystem and livings in long run (Tsouvalas & Metrikine 2016). It has been reported that noise-generating human activities may cause irregular behaviors, species recognition in response to predator attacks or behavioral disorders in mating or foraging, and eventually growth performance in aquatic animals (Radford et al. 2016, Spiga et al. 2017). Short term acoustics have been reported to affect aquatic animals (McLaughlin & Kunc 2013, Simpson et al. 2015), and sound exposures for long-term influenced fish behavior (Nedelec et al. 2016, Kusku 2020, Kusku et al. 2020). It has been noted that sound disturbance can be occasional and aquatic livings can develop tolerance to sound sources in long-term (Bejder et al. 2006, Nichols et al. 2015, Nedelec et al. 2016, Radford et al. 2016), possibly because they experience human generated sounds not only once but several times during their life. Unfamiliar sound sources caused reduce foraging in fish (Purse & Radford 2011) and crabs (Wale et al. 2013a), and despite the adaptation to a short-term sound-induced stress, fish did not demonstrate acclimation to multiple exposures of thunderstorm sound over long-term (Kusku et al. 2023). The harmony of biodiversity with the industry is a value for sustainable development of humanity. Therefore, monitoring the impacts of anthropogenic sounds which are unfamiliar to the aquatic environment is important for coastal zone management and ecosystem planning. Despite the well-documented review study on underwater sound pollution by Chahouri et al. (2022), no information has been given in respect to firework noise. Further, firework noise-induced stress effects have been reported for a wide range of land animals (Animal Ethics 2024), however no published studies regarding potential impacts of firework noise on aquatic animals are available so far. Hence, the intense of entertainment venues in waterfronts need attention in coastal zone management with efficient regulatory protocols.

The aim of this study was to investigate impacts of firework noise on physiological response and stress recovery in tilapia with reference to respiration rates of opercula beats and pectoral wings for wider understanding of anthropogenic disturbance of firework noise on aquatic animals.

MATERIALS AND METHODS

Sound transmission and experimental protocol

Initially, firework noise was air-recorded in the waterfront area of Canakkale city (Türkiye) during the New-Year's celebrations of 2022 (01.01.2022). Sound recording in air conditions were performed for 20 minutes using a sound measuring device (Soundmeter, product no: 1352 EN 00), withing 5 cm above water surface and 15 m in distance from the fireworks launch area (40°09'14.05"N-26°24'18.93"E; Canakkale). Thereafter, the sound meter device was submerged 5 cm below water surface, and again sound recorded was held for another 20 minutes. The estimated loss of firework noise penetrating the water body, showed an attenuation by around 20 dB in the water ambience beneath air-water boundary. Air-recorded firework noise was then transmitted into experimental tanks in laboratory conditions by waterproof hydrophones (w/Wires–8 Ohm, 1.5 W speaker), set 6 cm below water surface. An mp3 amplifier (Magic Voice) with three distributors was used for each test aquarium in a triplicate experimental design.

A control group with no noise exposure at all (ambient sound) was accompanied by a noise exposure treatment group, which received disturbance for 2 hours playback of firework noise, initiated at 09:00 a.m., ten minutes after termination of feeding. Then, fish behavior was monitored for 96-hours (4 days). Opercula beat rates (OBR) and pectoral wing rates (PWR) were visually counted through eye observation, following earlier reports of Spiga et al. (2017) and Kusku et al. (2020). Manual counts of OBRs and PWRs were conducted and noted by 2 persons at the same time for 9 hours a day, due to low visibility and difficulty in observation of fish ventilation rates over dark hours. This was consecutively repeated for 15 times with 4-days intervals over a period of 60 days.

Also, a digital camera (SONY DCR-DVD105E Digital Video Camera, Carl Zeiss Lens 800x digital zoom Nightshot Plus, JAPAN) was positioned outside the glass aquarium for prompt capture of all fish individuals. The Video recorder was switched on simultaneously with noise playback at 09:00 a.m. and switched off at 18:00 p.m., following the same course of the visual counts protocol. Opercula beats and pectoral wing pulses were noted by video image tracking after termination of the experiments. The OBRs and PWRs determined in the initial counting made by eye observation were compared with the pulse numbers determined by 3-repeat video image tracking afterwards, and their similarities were confirmed in order to capture the most accurate average rates of physiological response. It has been noted that both values from visual and camera counts were close in all comparisons. The experiment was completed when the OBRs and PWRs returned to a level close to the initial values or rates similar to the control group. It is worth noting that only one camera system was used in the experimental setup, hence random comparison of manual counts and video image tracking was conducted.

The determination of respiration levels through OBR and PWR as an indicator of physiological response, followed earlier reports of Gibson & Mathis (2006), Tantarpale et al. (2012), Nedelec et al. (2016), Radford et al. (2016), Spiga et al. (2017), and Kusku et al. (2020).

Time was adjusted for equal exposure durations in all test aquariums by a timer (model number: TS 814, TS-816 AU), and 60-sec spectrum analyses for firework noise and the ambient without noise were performed using a Spectrum Octave Band Real-Time Analyzer program (Figure 1).

Experimental fish and test containments

Initially, scaled fish of Nile tilapia (*O. niloticus*) were previously acclimated for a week to experimental conditions in the Freshwater research facility of Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology (Çanakkale - Türkiye). A total of 120 fish with initial mean weight of 9.83±0.34 g was randomly withdrawn out of a batch with 480 fish and distributed to 6 aquariums, each with 100-L volume capacity (20 fish per tank, 60 fish per treatment). Two experimental groups, one control with no noise exposure at all (ambient sound), and the treatment group with firework noise were set according to a 2 x 3 factorial design in triplicates. Three aquariums served as the control group without noise treatment at all. After weighing and allocation, experimental fish were remained untreated for four days in order to acclimate to new test conditions and to recover from possible handling stress, according to Kayali et al. (2011).

The experimental freshwater facility was a recirculating system, and all test aquariums were supplied with aeration by air stones, and

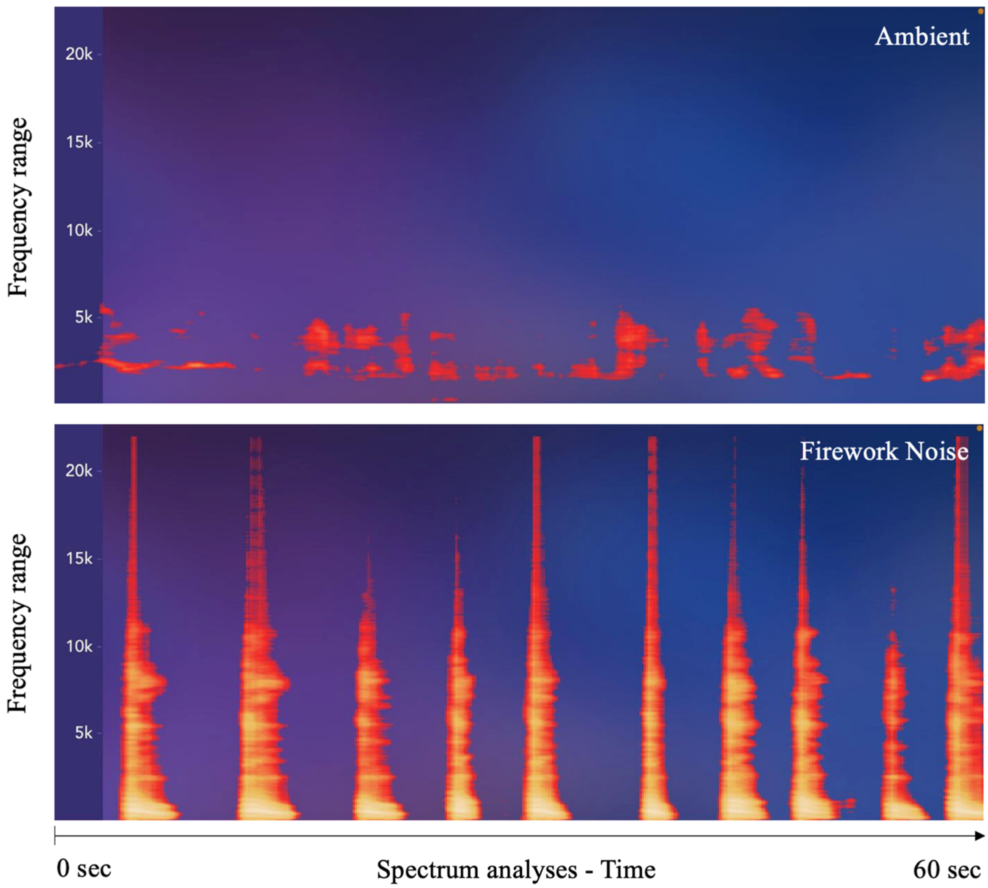

Figure 1. **Real-time spectrum range in 60-sec for firework noise penetrating water body. The "Ambient" diagram presents background noise without firework in the control.**

water inflow in each aquarium was 30.8±0.6 L/min. A natural photoperiod course was followed, and water quality was measured weekly using "YSI" brand automatic water quality analyzer. Throughout the study, water quality indices were measured as 20.26±1.14 °C temperature, 7.2±1.5 mg l^{-1} dissolved oxygen, 0.01±0.001 mg l^{-1} ammonia (NH_3-N), 0.027±0.01 mg l^{-1} nitrite (NO_2-N), 2.18±0.29 mg l^{-1} nitrate (NO_3-N), and 7.5 pH. Once-a-day feeding for 10 minutes (08:40 - 08:50 a.m.) was performed by using a commercial carp diet (SERA Co., Germany; crude protein: 21.0%, crude lipid: 2.7%, crude fiber: 0.5%, crude ash: 4.1%, and moisture: 6.7%).

Statistical analyses

Data presented in this study are given as means ± SD. Tukey Multiple Range Test was used for the statistical evaluation of data with homogeneity and even distribution. When data met homogeneity but no normally distributed, Kruskal-Wallis Test was used. For data comprising no homogeneity, the Tamhane Test was applied using SPSS 19 (IBMM SPSS Statistics 19) program. Significant limits were critically set at P<0.05. Any possible correlation between the respiration rates of opercula beats and pectoral wing flaps were calculated by Microsoft Excel (MacBook Pro, macOS Bic Sur 11.7.3) following the Correl equation:

$$Correl\,(x, y) = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2 \Sigma(y - \bar{y})^2}}$$

where, $\bar{x}$ and $\bar{y}$ are mean values of sample for group series 1 and group series 2, respectively.

The present study followed the Regulations of "Animal Behavior Society Guidelines", and all experimental procedures applied were approved by the Ethical Committee of Canakkale Onsekiz Mart University (approval number: 2020/10-05, dated 27.11.2020).

RESULTS

Ambient noise level in the glass aquariums prior to noise playback was recorded as 51.03±2.32 dB re 1 µPa. No noise treatment was applied in the control group throughout the course of the study. When playback of firework noise was initiated, the noise level increased 2.5-fold relative to the control, and reached 108.22±16.35 (range: 70-114) dB re 1 µPa. A clear masking and overlapping of the background noise level was noted.

Based on the findings, fish exposed to a single disturbance of noise playback, recovered from firework noise stress after 72-h. Fish demonstrated an immediate alarm reflex when noise playback was initiated and both OBR and PWR increased nearly 2-fold (P<0.05) over the control without noise exposure. After 96-h monitoring, following the 2-h noise exposure, both OBRs and PWRs were highest at time “24-h”, with average peaks of 72.0±4.58 and 79.0±4.36 beat/min, respectively. Thereafter, OBRs and PWRs showed a declining trend, returning to near-initial levels (44.67±3.06 and 42.67±2.52 beat/min, respectively) after 72-h. The following 24 hours thereafter, fish respiration rates (OBR and PWR) remained almost stable (Figure 2, 3).

Superimposed illustration of OBRs and PWRs over a 96-h circle of noise-induced stress trend after 2 hours exposure to firework playback is given in Figure 4. The correlation between opercula beats and pectoral wing flaps was calculated as 96.08% (R=0.9608), presenting a strong positive correlation between the physiological response indicators and the noise-induced stress level.

After the challenge of firework noise disturbance for 2 hours, elevated respiration rates declined after 72 hours. When fish was challenged with multiple exposures to firework noise with 4-days intervals, fish showed stress recovery at time “72-h” as well, however it was interesting to see that fish showed the same

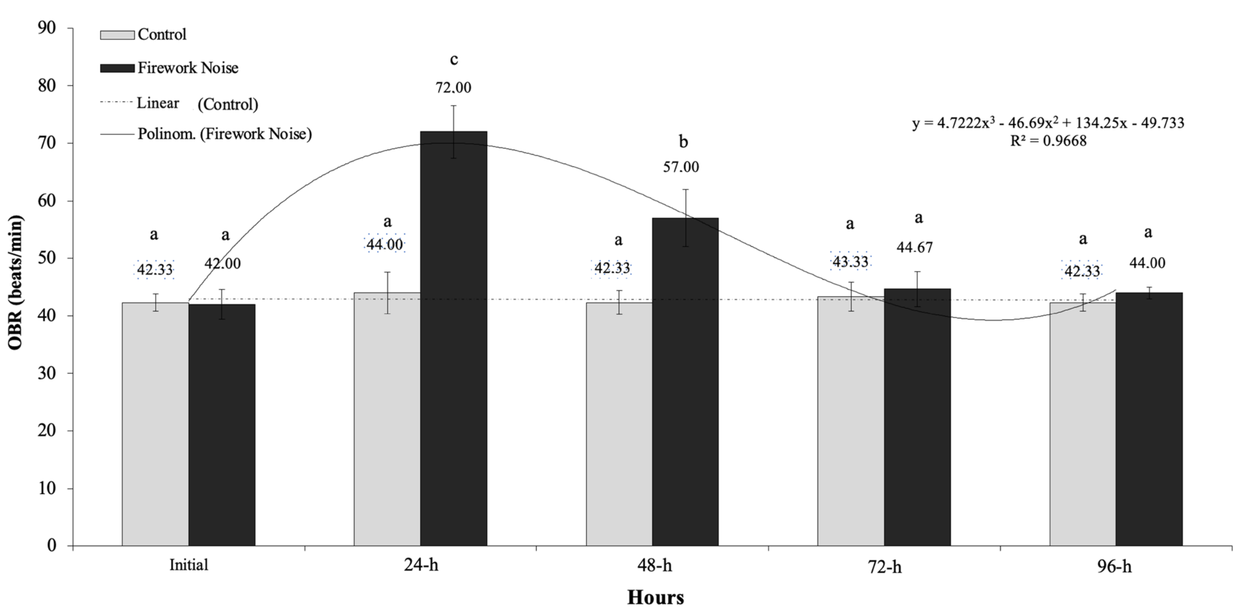

Figure 2. Monitoring of opercula beat rates (OBR, beats/min) in fish for 96 hours after 2-h exposure to firework noise. Different superscript letters show significant differences (P<0.05).

alarm reflex every time when firework noise playbacks were initiated every 4 days with multiple stimuli of 15-times repetition over the 60-days experiment. The OBRs and PWRs peaked 24 hours after noise initiation (73.04±4.83 beat/min and 76.24±4.27 beat/min in average, respectively). Thereafter, average beat rates from multiple exposures with 15-times repetition, declined gradually to 59.11±3.33 and 60.40±3.54 beat/min for OBRs and PWRs by the end of 24-h; to 46.16±3.39 and 45.89±2.26 beat/min by 72-h; and to 43.44±2.21 and 43.00±1.91 beat/min by 96-h, respectively (Figure 5, 6).

DISCUSSION

Several stress indicators such as glucose, hematocrit, plasma cortisol (Simontacchi et al. 2008, Yavuzcan-Yıldız et al. 2001) or ammonia nitrogen excretion rates (Xu et al. 2005, Kayali et al. 2011) are parameters applied so far for the evaluation of fish stress. However, the use of a syringe in blood sampling may insert an additional stress source (Laidley & Leatherland 1988, Marino et al. 2001), or the handling during blood sampling is another disturbance effect (Pickering et al. 1982) that in turns can influence blood parameters in fish. Other indicators of stress response such as swimming speed, or oxygen consumption have been used in various studies in fish with strong relation between physiological response and behavior in stressful conditions (Dalla Valle et al. 2003, Wale et al. 2013b). Further, it has been reported that changes in fish behavior may not be sensitive enough to measure the stress response of fish against sound stimuli (Beale & Monaghan 2004). Therefore, opercula beats and pectoral wing flaps have been used indicators of respiration level and stress response in earlier studies (Gibson & Mathis 2006, Tantarpale et al. 2012, Nedelec et al. 2016). Gibson & Mathis (2006) reported that increased opercula beats are useful indicators for behavioral response of fish to predator stress, and Kusku (2020) and Kusku et al. (2020, 2023) also underlined strong relation among

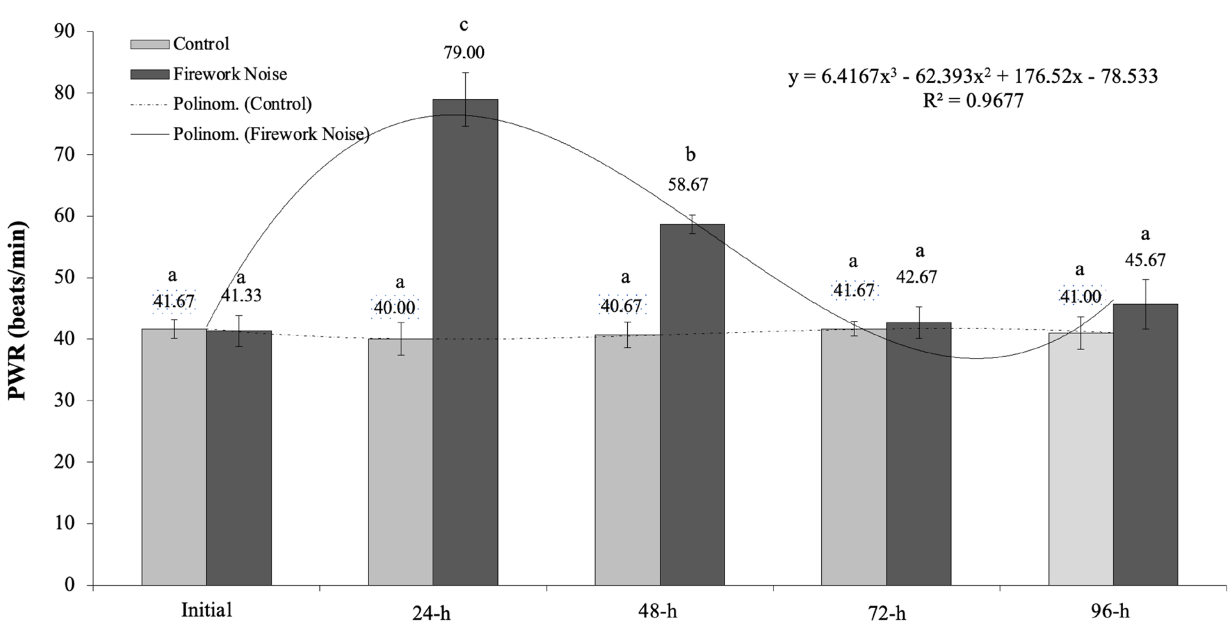

Figure 3. Monitoring of pectoral wing rates (PWR, beats/min) in fish for 96 hours after 2-h exposure to firework noise. Different superscript letters show significant differences (P<0.05).

sound-induced stress and opercula beats or pectoral wing flaps, which are in line with the findings of the present study, presenting 96.08% (R=0.9608) positive correlation of between opercula beat rates and pectoral wing rates.

The level of underwater noise in the nature were reported in a range between 5-50 dB sixty years ago (Wenz 1962), however much higher median sound pressure levels from 81.5–95.5 dB have been reported fifty years later in UK waters by Merchant et al. (2016), showing a severe increase of sound level in the oceans over the decades. In their observations in the Northeast Pacific, Merchant et al. (2016) underlined that minimum three decades of observation might be necessary to figure the trends of the increasing underwater sound levels. Further, in the Baltic Sea, median sound levels in dB re 1 µPa have been reported between 65-115 dB (Mustonen et al. 2019), who noted that maritime traffic increased the ambient sound levels in the Baltic Sea, but added that the ambient sound presented a combination of natural sounds, mainly by waves, whereas human-made acoustic noise was mostly generated by the shipping industry. Hence, it is likely that underwater sound levels in the nature may differ with the conditions of weather, wave and currents, as well as a combined effect of these all.

In the present study, the control group without any noise treatment presented ambient noise level of 51.03±2.32 dB re 1 µPa, likely representing silent underwater background noise without anthropogenic disturbance as reported for a natural ambience (5-50 dB) by Wenz (1962). The onset of firework noise playback raised the background noise by 2.5-fold (108.22±16.35 dB re 1 µPa), overlapping and masking the background noise that was 51.03±2.32 dB re 1 µPa. Considering the air-recorded noise level of firework with median sound pressure level of 118.13±17.28 dB re 1 µPa, an attenuation of around 10 dB occurred at air-water boundary.

Severe influences of firework noise have been reported for terrestrial animals in different

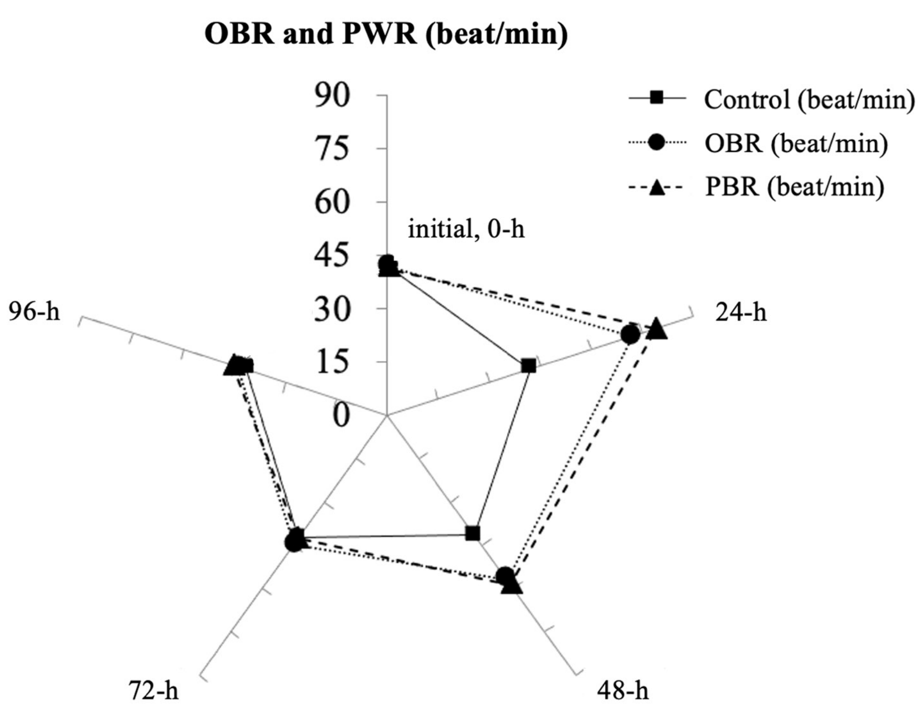

Figure 4. Superimposed illustration covering 96-h circle of firework-induced stress trend for opercula beat rates (OBR) and pectoral wing rates (PWR) in fish after 2 hours noise playback.

manners (Animal Ethics 2024), however to our knowledge so far, firework noise induced stress response in fish is still unknown, and the present study provides the first data on firework noise impacts on physiological response of fish. Due to the lack of data on firework noise effects on aquatic animals, findings in this paper have been compared and extrapolated with studies reporting other sound sources generated by human activities in respect to physiological stress response in fish.

Disturbance effects of human-made acoustic sounds were found in salmon exposed to piling noise generated by bridge construction (PIDP 2001). Underwater seismic air gun shootings influenced European seabass (*Dicentrarchus labrax*) (Santulli et al. 1999), with increased physiological response through ventilation and startle response. This was in line with the report of Spiga et al. (2017), who noted reflexive alarm response in European seabass exposed to impulsive noise of piling noise. This was supported later by Kusku (2020), and Kusku et al. (2020), who also noted a startle response in tilapia exposed to underwater drilling and piling noise. A responsive startle reflex in tilapia was also reported later by Kusku et al. (2023), when fish was challenged with thunderstorm sound stress, which might be attributed to the involvement of hindbrain mauthner neurons, controlling unwanted reflex behavior in fish (Szabo et al. 2006). Despite the sudden startle response to the stimuli, Radford et al. (2016) evidenced that respiratory response reduced in long-term exposure to piling and seismic air gun noise, and fish did not show any reaction to the sound exposures after a 12-weeks period. This was in line with Kusku et al. (2020), who underlined that fish developed tolerance to the underwater piling and drilling noise from marine industrial activities and the ventilation rates went into a declining period after 4 weeks of exposure. Further, Kusku (2020) indicated that Nile tilapia (*O. niloticus*) did not respond any longer to external sound of urban noise and shipping after 12-weeks. The coral-reef

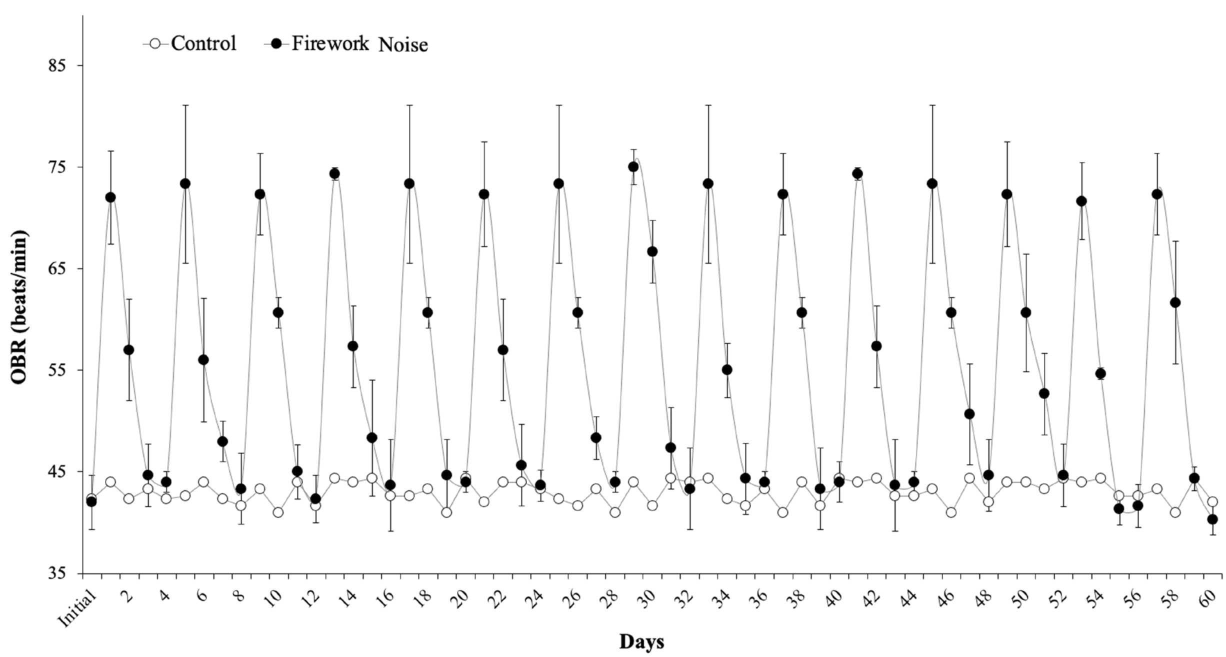

Figure 5. Opercula beat rates (OBR, beats/min) of fish treated with multiple exposures to firework noise following 15-times repetition with 4-days intervals, and 60 days in total.

fish increased respiration when exposed to motorboat noise, but no physiological response was seen after seven days following motorboat sound exposure (Nedelec et al. 2016). Hence, invesigations show evidence that that fish likely can develop attunuation to single or short term exposures against stimuli. The findings in the present study overlaps with previous works in terms of sudden response to the stimuli, followed by a stress recovery period thereafter, when fish was exposed to a single short-term treatment with underwater firework noise transmission. However, despite a stress recovery of Nile tilapia after three days of thunderstorm sound exposure, fish did not show acclimation to multiple exposures to thunderstorm sound over a long-term exposure period of 80 days (Kusku et al. 2023), which was in close agreement with the present study. When fish was challenged with stimuli of firework launch for multiple times with 15 repetitions, fish encountered the same state of stress every time of exposure period, an indication that even if the fish recovered from stress by 72-h, no habituation was developed against the multiple exposure of firework noise in the present study, which arises the question wheter studies with short-term behavioral responses provide reliable information for explicating human-made acoustic disturbance on fish (Bejder et al. 2006). When fish was exposed to broadband noise of at a level of 170 dB re 1 µPa (rms) for 20 days, hearing loss was noted in goldfish (*Carassius auratus*), whereas no hearing loss was noted in Nile tilapia (*O. niloticus*) (Smith et al. 2004a, b), which might be an explanation for the no-habituation response in Nile tilapia after multiple exposure to firework noise.

In regards to the stress-recovery time of 72-h observed in the present study, similar findings were reported by Wells et al. (1984) who underlined stress recovery in 24-70 hours for Arctic fish challenged with captive stress, whereas Coho salmon (*O. kisutch*) recovered from handling and netting stress in 96-168 hours, and rainbow (*O. mykiss*) only in 24 hours (Wedemeyer 1976). Kayali et al. (2011) found a stress recovery for European seabass in 24 hours after handling and transport stress. Based on findings of earlier investigations, it can be

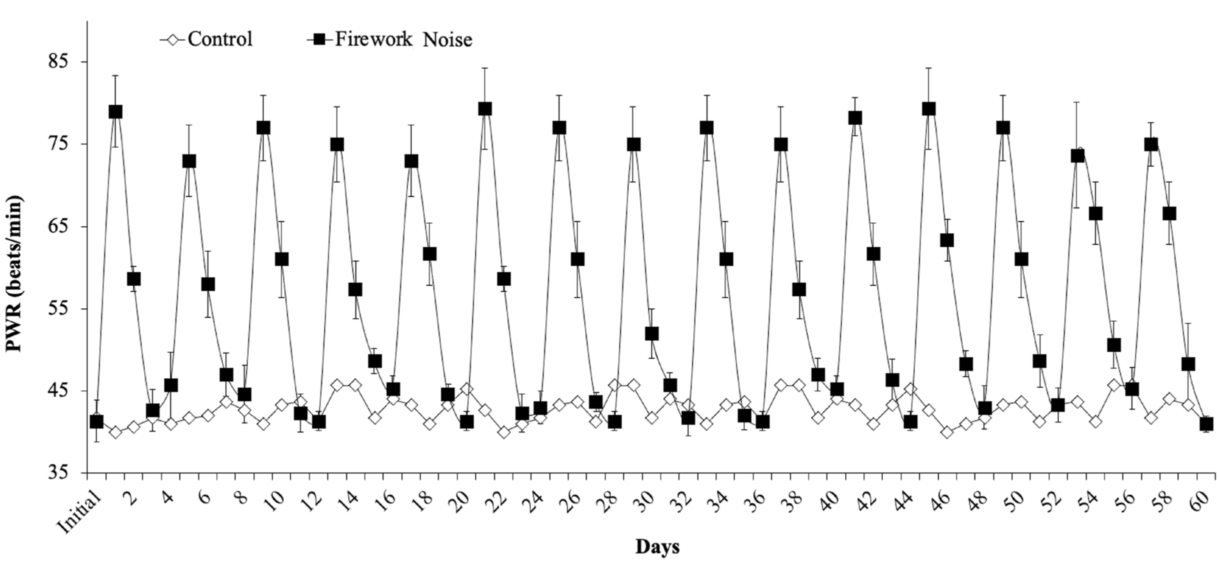

Figure 6. Pectoral beat rate (PBR, beats/min) of fish treated with multiple exposures to firework noise following 15-times repetition with 4-days intervals, and 60 days in total.

outlined that different fish species demonstrate different responsive behaviors against different stimuli and stress sources.

CONCLUSIONS

Present findings provide evidence that fish showed stress-recovery by 72 hours after short-time exposure to firework noise playback. However, when fish was challenged with firework noise multiple times for long-term, fish did not show attenuation to firework noise, which was 2.5-fold above the background level measured beneath water/air boundary. Opercula beats and pectoral wing flaps as a measure of respiratory response against noise-induced stress, increased 2-fold in regards to the no-noise exposed fish in the control group. The present study provides evidence that firework noise launched from waterfront venues need attention for coastal zone management in order to reduce anthropogenic sound pollution towards sustainable and healthy aquatic ecosystem. In particular, the use of low-noise fireworks might be encouraged in order to reduce their impacts on aquatic animals as well as other terrestrials, which however still needs further investigations.

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