

Impacts of Urban Noise and Musical Stimuli on Growth Performance and Feed Utilization of Koi fish (*Cyprinus carpio*) in Recirculating Water Conditions

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Abstract

In the present study, effects of underwater sound transmission on growth, feed utilization and behavior of Koi fish (*Cyprinus carpio*, initial weight 3.67±0.18 g) was investigated via exposure to Urban Noise, Silk Road, Sufi Ney, and a Quran performance. Underwater sound transmissions were performed daily with playbacks between 08:00-08.30, 12:30-13:00, and 17:00-17:30 hours, throughout the feeding trial for a period of 90 days in a recirculating aquaculture system. An experimental group without any sound served as control. Results showed that musical stimuli tested in this study positively influenced fish growth and feed efficiency. Experimental fish presented slow growth performance during the first period however the disturbed swimming behavior of fish scattering in the tanks changed to a more regular swimming and improved growth thereafter, an indication of lower stress condition or acclimatization of fish to sounds. As a result, fish growths and feed efficiencies were influenced by musical stimuli with remarkably higher rates in the Quran performance and instrumental Sufi Ney treatments, compared to the Silk Road or the control group. Urban noise presented adverse effect on fish growth and feed efficiency. Hence, musical stimuli could be considered as a growth promoting factor ensuring fish welfare in intensive aquaculture facilities.

Keywords:

Introduction

Finfish species in aquaculture facilities are subject to stressful conditions affecting growth performance, feed efficiency (Rowland, Mifsud, Nixon, & Boyd, 2006), reproduction (Campbell, Pottinger, & Sumpter, 1994), or immune responses (Vazzana, Cammarata, Cooper, &

Parrinello, 2002). Stress is an important criterion for the suitability assessment of the culture conditions and husbandry in the aquaculture facility. Several stress conditions can be listed as fish handling and transport (Kayali, Yigit, & Bulut, 2011); temperature fluctuations (Hsieh, Chen, & Kuo, 2003); exposure to medicinal

compounds (Yildiz & Pulatsü, 1999), stocking and dominance hierarchy (Clement, Parikh, Schrupf, & Fernald, 2005; Gilmour, DiBattista, & Thomas, 2005), tank color (Cotter *et al.*, 2005; Kesbiç, Yiğit, & Acar, 2016) or long photoperiod regimes (Ergün, Yigit, & Turker, 2003). In general, the intensive production system itself is likely to affect fish welfare due to a variety of stressors (Galhardo & Oliveira, 2009; Wysocki *et al.*, 2007).

The acoustic sense of fish is proposed as a sensory modality, a pathway for acoustic stressors to affect fish in containment (Anderson, Berzins, Fogarty, Hamlin, & Guillette Jr, 2011). Earlier investigations regarding the effects of anthropogenic noise on fish and marine life are increasing concerns (Popper, 2003). Increased sound levels in the environment might affect habitat selection, and fish behavior (Pearson, Skalski, & Malme, 1992; Popper, 2003; Tolimieri, Haine, Montgomery, & Jeffs, 2002). Additionally, elevated levels of sound in the acoustic environment may damage hearing in fish (Amoser & Ladich, 2003; McCauley, Fewtrell, & Popper, 2003; Scholik & Yan, 2001; Smith, Kane, & Popper, 2004), reduce growth rates (Sun *et al.*, 2001) and stress responses (Smith *et al.*, 2004; Wysocki *et al.*, 2006).

Besides, earlier reports showed that music stimuli produces a specific response in fish brain functions that enables a certain relaxation in fish (Bass & Ladich, 2008; Papoutsoglou, Karakatsouli, Papoutsoglou, & Vasilikos, 2010), while simultaneously achieving homeostasis. This is a very similar response to that in human beings, even though the anatomical structure complex between fish and human brains are significantly different (Braithwaite & Boulcott, 2008; Fay & Edds-Walton, 2008; Rodríguez, Broglio, Durán, Gómez, & Salas, 2006). The transmission of underwater music has been reported to influence fish growth performance or welfare in a positive manner. Common carp (*Cyprinus carpio*) performed better growth and physiological response in an environment where Mozart K525 was transmitted underwater

compared to the individuals exposed to Romanza music, whereas both music transmitted groups performed better than the fish populations treated without musical stimuli (Papoutsoglou *et al.*, 2007; Papoutsoglou *et al.*, 2010). Improved growth performance and enhanced fish welfare were reported in gilthead sea bream (*Sparus aurata*) exposed to underwater transmission of Mozart K525 (Papoutsoglou, Karakatsouli, Batzina, Papoutsoglou, & Tsopelakos, 2008). In the present study, we investigated growth performance and feed utilization of Koi fish (*Cyprinus carpio*) exposed to underwater transmission of urban noise in comparison to musical stimuli of three different sounds, two of them composing music pieces of instrumental Silk Road and Sufi Ney, and the other composing Quran performance.

Materials and Methods

Ethical Note

All procedures and applications in the present study were approved by the Ethical Commission of Canakkale Onsekiz Mart University (Ethical Commission Approval Number: 2017/10-04) and followed regulations of Animal Behavior Society Guidelines.

Musical Stimuli and Underwater Transmission of Sounds

Underwater transmission of four different sounds was tested. Types of sounds were chosen as Urban Noise (<https://www.youtube.com/watch?v=gRjJvml0Xs>), two instrumental music pieces of Silk Road (performed by Kitaro - Aqua) and Sufi Ney (performed by Engin Agar, Neyzenbashi Flute Maestro, Bursa - Turkey), while the third sound was designated as Quran performance (Surah-55, Ar-Rahman retrieved from <http://kuran.diyagnet.gov.tr/mushaf/meal-2/rahman-suresi-55/ayet-17/diyagnet-vakfi-meali-4>). Prior to the initiation of sound transmissions, it was ensured that fish in experimental tanks were in well condition and not under stress due to any external effects. The even

distribution of fish in the tank environment was considered as a sign of non-stress condition before the start of underwater sound transmission.

The methodology of the present study followed Popper & Hastings (2009), Papoutsoglou *et al.* (2008, 2010) and Catli *et al.* (2015), with slight modifications. Each of the test aquariums were set with underwater speakers (hydrophone) (8.24 x 3.1 cm, Mini Speaker w/Wires–8 ohm, 1.5 W Stw-c) located at the upper corner opposite to water inflow 5 cm below surface. For the dispersion and adjustment of the sounds in the aquariums, Mp3 amplifiers with three outputs (Magic Voice brand) were provided separately for each of the triplicated four test groups. The underwater sound frequency was measured and recorded by a Sound Meter-X program adaptable to Samsung G7 model mobile phone. Mean ambient noise levels were determined by Xyses decibel (db) Sound Recording Program adaptable to I-Phone 5s Series 16 GB model mobile phone.

Fish in all tanks were exposed to sound transmissions 3 times a day for 30 minutes and playback between 08:00-08:30, 12:30-13:00, and 17:00-17:30 hours, over a period of 90 days. An automatic timer (TS-814 AB Tak-TS-816 AU model) was used for the setting of daily transmission time and durations of different sounds.

All experimental glass aquariums were designed to buffer ambient sound by reducing contact between vibrating pipes and the glass surfaces of the aquariums as described by (Davidson *et al.*, 2007; Davidson, Bebak, & Mazik, 2009). The experimental tanks were design to present same levels of ambient noise. In the control group without underwater sound transmission, a mean sound level of 57.06±0.43 dB re 1 µPa SPL (sound pressure level) was recorded. When musical stimuli were initiated in the sound treatment groups, the mean sound levels increased to 66.92±0.16 dB re 1 µPa SPL. Hence, any other effect then musical stimuli treatment that could influence fish performance was

eliminated. SPLs were measured weekly in order to ensure that the sound levels were consistent throughout the growth trial.

Experimental Fish, Rearing Conditions and Husbandry

A total of 225 Koi fish (*C. carpio* L., 1758), purchased from a commercial aquarium fish facility (Ensar Aquarium Co.) in Istanbul, Turkey, were transported in 100 Lt plastic bags to the experimental facilities of Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology in Canakkale, Turkey. After the transport for 6 hours, fish in plastic bags were gradually transferred into the aquariums after equalization of water temperature in the plastic transport bags and the aquarium ambience with a temperature difference of maximum 0.5 °C. After an acclimatization period of 30 days to the experimental conditions, fish were randomly allotted in 15 glass aquariums (15 fish per tank, 3 test groups in triplicates) with 100 Lt water-volume and dimensions of 70x40x40 cm filled with fresh water to 5-6 cm water level below surface. The experimental unit consisted of a recirculating water system where three aquariums were connected to a separate filtration system. Continues aeration was supplied by air pump and all fish were cultured under a natural photoperiod regime at 24.75 ± 1.34 °C. Dissolved Oxygen, ammonia, nitrite, nitrate, and pH were measured daily using an automatic water quality measurements device (YSI), and recorded as 7.2±2.1 mg/L O₂, 0.01±0.001 mg/L NH₃, 0.033±0.012 mg/L NO₂, 2.15±0.58 mg/L NO₃, and 7.3, respectively. Equal flow rates of inflow water with a mean rate of 32.8±0.2 L/min were maintained in all treatment groups.

The growth experiment was conducted over a three month period. Koi fish with an initial mean weight of 3.67±0.18 g were fed once a day at 13:00 for 10 minutes with commercial flake diets for koi fishes (21% crude protein, 2.7 % lipid) throughout the study. Feeding was carefully monitored to assure an even distribution of diets among all fish in the aquarium and

avoid feed settlements on the tank bottom. Fish refusing the diets was accepted as a sign of satiety and feeding was stopped.

Growth performance and feed utilization was tested in fish exposed to different musical stimuli and sounds, namely: (a) "Control" - no musical stimuli or sound transmission; (b) transmission of "Urban noise" (UN); (c) musical stimuli with instrumental "Silk Road" from Kitaro - Aqua (SR); (d) musical stimuli with instrumental "Sufi Ney" performed by Engin Agar, Neyzenbashi Flute Maestro, Bursa-Turkey (SN); and (e) "Quran performance", Surah-55 Ar-Rahman (QP).

Statistical analyses

Values of all measured variables are expressed as means \pm SEM. Data obtained from growth parameters were examined by Tukey Multiple Range Test when homogeneity and normal distribution of data was observed. For data not normally distributed but in homogeneity Kruskal-Wallis test was applied, while Tamhane tests were used for the data without homogeneity using SPSS 19 (IBMM SPSS Statistics 19) Statistical Software. Levels of significance were determined with critical limits set at $P < 0.05$.

Results

Water quality parameters were recorded within safe limits recommended by Bregnballe (2015) for recirculating aquaculture systems and similar water quality parameters were observed in all treatment groups, therefore no effect of the water quality in terms growth performance or feed utilization was expected.

When the musical stimuli were turned on, the fish in all treatment groups responded with an initial reaction of stimulation, swimming irregularly and scattering in the tank. This erratically swimming behavior was observed every time when the sound transmission was initiated. Recovery to normal swimming with non-disturbed distribution patterns was observed after 2-3 hours

following the initiation of the sounds. Hence, all fish recovered from the alarm reflex to normal behavior before the onset of the next sound transmission.

Fish exposed to different underwater sounds grew slower during the first 35 days in all treatment groups however fish adapted soon to the sounds thereafter with significant differences among treatments 90 days after start of the experiment. Fish growth was highest ($P < 0.05$) in the experimental tanks exposed to Quran performance (QP) (10.73 ± 0.07 g), followed by musical stimuli groups of Sufi Ney (SN) (10.13 ± 0.13 g) and Silk Road (SR) (9.22 ± 0.19 g), respectively. Significantly lower fish growths ($P < 0.05$) were recorded in the control (8.80 ± 0.07 g) and the Urban Noise (UN) (8.60 ± 0.07 g) groups compared to the QP, SN, and SR treatments (Figure 1). Similar trend was also observed in the cumulative wet weight gain (WWG) with lower values in the first 35 days, and improved values at day-70 and day-90 (Figure 2).

The RGRs followed the same trend with highest levels of 209.6%, 181.8%, and 146.0% in the QP, SN, and SR treatments, compared to the UN and control groups with RGRs of 138.8% and 123.9%, respectively. The lowest growth performance was recorded in the UN treatment group, while the best was achieved in fish exposed to QP with significant higher rate ($P < 0.05$) over the other groups. The FCRs at the end of the 90-days growth experiment demonstrated significant differences among experimental treatments ($P < 0.05$), with the best FCR in the QP (1.58), followed by the SN, SR, control and the UN treatments with rates of 1.73, 2.07, 2.20, and 2.35, respectively (Table 1).

During the course of the study, fish exposed SN and QP presented significantly higher ($P < 0.05$) individual wet weight gain over the other experimental groups. At day 90, no significance was found in WWG of fish in the control and the SR groups ($P > 0.05$), but both were significantly higher than the control, however these two groups reached significantly lower WWGs

compared to the SF and QP treatment. One month after start of the experiment, relative WWGs (RWWG) in the UN and SR groups were 77.27% and 22.73% lower compared to the control group, whereas the SN and the QP groups reached 36.36% and 159.09% higher RWWGs than the control. At days 70 and 90, the UN group remained lower than the control, while all other experimental fish exposed to musical stimuli reached higher RWWGs over the control and the UN groups. At the end of the 90 days growth study, the QP group presented highest RWWG of 142.17%, followed by the SF and SR groups with levels of 127.82% and 106.95%, respectively, whereas the fish exposed to UN resulted in -6.96% lower RWWG compared to the control group (Figure 3).

SGRs followed the same trend with WWGs, resulting in lower rates ($P < 0.05$) in fish exposed to UN but higher levels in the SN and QP treatments compared to the control group. The SR treatment remained similar ($P > 0.05$) to the control during the course of the study. When fish were exposed to underwater sound transmission for the first time, the relative SGRs (RSGR) of fish exposed to UN and SR during the first 35 days were 77.78% and 25.00% lower than the control group, whereas the fish exposed to musical stimuli of SN and QR treatments presented 33.33% and 147.22% higher RSGRs over the control, respectively. At the end of the 90-days growth experiment, fish exposed to QP presented highest RSGR of 129.90%, followed by the SF and SR groups with rates of 118.50% and 103.09%, respectively, while fish exposed to UN remained -7.22% lower in terms of RSGR compared to the control (Figure 4).

Discussion

Teleost fishes such as carp and catfish are capable to increase hearing ability by the Weberian ossicles, which are bony connections combining the swim bladder to the inner ear (Popper, Fay, Platt, & Sand, 2003; Smith

et al., 2004). Carp, goldfish and catfish known as hearing specialists (Fay & Popper, 1974; Fay & Wilber, 1988; Papoutsoglou *et al.*, 2007) can detect low sound pressures at levels of 50–75 dB re 1 μ Pa (Popper, 2003; Popper *et al.*, 2003). Thus, *Cyprinus carpio* was chosen as a model fish characterized as a hearing specialist for the exposure of 67 dB re 1 μ Pa SPL in our study.

The onset of underwater sound transmission induced an alarm reflex in Koi fish (*C. carpio*), and none of the fish in the experimental groups displayed stationary behavior once the sounds were turned on. Fish in all test groups showed a tendency to move athwart, swimming in the opposite direction of the sound source and presenting association with companions near the tank wall at first exposure to underwater sounds. In tanks exposed to urban noise, this tendency was more distinctively observed throughout the study period every time when the sound was turned on. Exposure of fish to musical stimuli of Silk Road or Sufi Ney induced a remarkable lower startle response (sudden movement at high speed) in fish. Conversely, the exposure to Quran performance did not evoke any immediate response, except the first time acquaintance of fish stimuli.

Common carp (*C. carpio*) performed better growth and physiological conditions when fish was stimulated with musical pieces of Mozart K525 or Romanza compared to the individuals in rearing tanks without music transmission (Papoutsoglou *et al.*, 2007; Papoutsoglou *et al.*, 2010). Musical stimuli on gilthead sea bream (*S. aurata*) by Mozart K525 also provided increased growth, welfare and production quality (Papoutsoglou *et al.*, 2008), which are in agreement with the findings in the present study on Koi fish (*C. carpio*). Similar results were also found in marine fish species by Catli *et al.* (2015), who recorded best growth performance in turbot (*Psetta maotica*) exposed to slow tempo music transmission compared to medium or high tempo sounds. The authors also stated that turbot exposed to

high tempo music with greater beats showed a decline in feed intake levels as a sign of stress, which was in close agreement with our results in terms of negative effects on feed conversion rate in fish exposed to urban noise that might be addressed as a sign of stress.

The discrepancy in responses to the sound treatments may be attributed to the acoustic characteristics such as rise time of the applied stimuli as reported by Spiga, Aldred, & Caldwell (2017). The urban noise was composed by a sound with a rapid rise time reaching the peak of 67 dB re 1 μ Pa SPL, and fish were unprepared thus utterly bewildered. In contrast to the UN conditions, a smooth and gradual increase of the stimuli levels in the SN and QP groups did not drastically trigger the alarm reflex in fish. It is reported that SPLs ranging from 70 to 160 dB are produced in aquaculture facilities (Clark, Young, Bart, & Zohar, 1996), possibly above threshold levels of many finfish species. Hence, the underwater sound transmissions applied in the present study represented much lower level of sound than those reported in earlier studies in recirculating aquaculture production facilities. Information regarding the effects of different rise times of underwater sound pressures on responses in marine animals is scarce with a review in fish and invertebrates (Hawkins, Pembroke, & Popper, 2015) and only one report in seabass (*Dicentrarchus labrax*) (Spiga *et al.*, 2017).

The exposure to increased levels of unusual noise might lead to a diminishing effect on foraging behavior in terms of triggering a fear-related response due to distraction by unexpected acoustic sounds. The disturbed foraging activity and the increased startle responses in fish are related to a typical defense mechanism that interrupts an ongoing activity such as feeding. This is a classic response associated with stress factors or anxiety-related stimuli triggered under risky conditions such as predator attacks (Metcalf, Huntingford, & Thorpe, 1987). In the present study, the

swimming tendency in the opposite direction of the sound source and association with companions in fish at first exposure to sounds might be explained by the induced stress or fear under conditions of additional noise, since social affinity implements defensive nature (Magurran, 1990). Furthermore, stress can defect cognitive behavior (Mendl, 1999) and disturbance of focusing on a special task (de Kloet, Oitzl, & Joëls, 1999). Similarly, in the present study the significant decline in feed efficiency in fish exposed to urban noise compared to the control with no sound or even to the musical stimuli treated individuals might be explained with the reduction in appetite expected as a typical constituent of a physiological stress response (Wendelaar Bonga, 1997), linked to induced startle responses and interruptions of foraging activity observed in fish exposed to sound transmissions in the present study, with remarkable stronger effects in urban noise treated fish.

It seems possible that the acoustic characteristics of the urban noise applied in the present study might have induced the Mauthner cells, which initiated the alarm reflex as also reported in seabass juveniles by Spiga *et al.* (2017). Unintentional alarm reflex of fish are reported to be mediated by a pair of hindbrain Mauthner neurons (Szabo, Weiss, Faber, & Preuss, 2006) and the musical stimuli sounds pressures provided less or similar levels to the ambient background acoustic conditions of the tank environment, probably not enough to generate a high level of sound sufficient to trigger the alarm reflex as also reported by Spiga *et al.* (2017). In a natural sea environment the ambient noise level may range between 5 and 50 dB (Wenz, 1962), while sound pressure frequencies in a shallow depth of 1 m close to the sediment were measured in a range between 50 and 95 dB (Lagardère, 1982), which are subject to differ according to sea conditions such as weather state, waves, tidal and anthropogenic influences. On the

contrary, sound levels in rearing conditions in aquaculture facilities have been reported to be 20–50dB higher than in the natural water environment, with levels of 153 dB re 1 μ Pa in a recirculating system using in fiberglass tanks, which is within the frequency range of fish hearing ability (Bart, Clark, Young, & Zohar, 2001). The highest levels of underwater sounds in aquaculture production facilities were recorded as 160 dB re 1 μ Pa (Clark *et al.*, 1996). Spiga *et al.* (2017) stated that the involuntary response of fish to sound might be affected when the average sound pressure level significantly increased over the background sound level, which was also supported by Neo *et al.* (2014) indicating that a consistent acoustic noise of 165 dB re 1 μ Pa SPL were high enough in level to initiate the startle response in fish. Information on fish species with lower SPL thresholds are scares and need further investigation in intensive aquaculture conditions.

Based on earlier reports, it is evidence that fish may attune to environmental conditions of highest levels of underwater sounds pressure limits (160 dB re 1 μ Pa) in aquaculture production systems (Clark *et al.*, 1996), and in long-term finfish species growth and survival are unlikely to be influenced by noise levels occurring in intensive aquaculture systems, providing information that fish in aquaculture facilities may acclimate to the noise level of 149 dB re 1 μ Pa in the culture environment (Davidson *et al.*, 2009). Similarly, Wysocki *et al.* (2007) underlined that in rainbow trout (*Oncorhynchus mykiss*), commonly cultured in noisy aquaculture facilities, the hearing, growth performance, survival rate, and disease resistance were not negatively influenced by a long-term exposure to intensive aquaculture production noise at SPL levels of 115, 130, and 150 dB re 1 μ Pa. Further, Nedelec *et al.* (2016) stated that repeated exposure to noise may increase tolerance in fish and found that behavioral and physiological responses decreased after a week of motorboat-noise exposure.

Quite a few numbers of studies investigated the effects of underwater sounds in aquaculture conditions on fish growth or reproduction performance, egg survival and stress responses and reported that aquaculture species might be affected by high levels of ambient sound with a potential of detrimental effects such as reduced growth rate (Papoutsoglou *et al.*, 2007; Papoutsoglou *et al.*, 2010; Papoutsoglou *et al.*, 2013), reproductive performance, and reduced egg survival rates (Banner & Hyatt, 1973; Lagardère, 1982). Findings in our study also underlined the hypothesis of these earlier studies with reduced growth performance and feed efficiency when fish was exposed to urban noise at a level of 67 dB re 1 μ Pa SPL. It is important to note that this level of urban noise was only 10 dB re 1 μ Pa SPL higher than the non-sound treated control tanks which exhibited a water environment of 57 dB re 1 μ Pa SPL. However, when fish was stimulated with instrumental musical pieces or Quran performance in the present study, growth and feed efficiency in Koi fish was positively influenced with highest RGRs and SGRs in Sufi Ney and Quran performance compared to the other sound treatments and the control group without musical stimuli. In contrast to our results however, Imanpoor, Enayat Gholampour, & Zolfaghari (2011) did not find any growth effect of musical stimuli in goldfish (*Carassius auratus*). Additionally, underwater sound transmissions other than musical stimuli such as human generated sounds, random acoustic noise or aquaculture facility noise was tested in other studies where no effects or even negative effects on fish performance were reported (Davidson *et al.*, 2007; Nedelec, Simpson, Morley, Nedelec, & Radford, 2015; Popper & Hastings, 2009; Wysocki *et al.*, 2007). It is advisable that these studies need to be extended to different fish species as the sound effects and musical stimuli appear to be species specific (Smith *et al.*, 2004; Voellmy *et al.*, 2014), and species-specific effects of noise types and sound levels in aquaculture production

systems need more definitions (Davidson *et al.*, 2009). Results from our study show that fish growth and feed efficiency were influenced by musical stimuli with remarkably higher rates in the instrumental Sufi Ney and Quran performance treatments at SPLs of 67 dB re 1 μ Pa, compared to the control group without musical stimuli (57 dB re 1 μ Pa SPL), while converse effect of urban noise at 67 dB re 1 μ Pa SPL was recorded in fish with lower growth performance and feed efficiency over the fish with no underwater sound transmission. As a conclusion, musical stimuli seems to affect growth and welfare in several finfish species with possible differences caused by the acoustic properties such as the rise time of the musical stimuli, but knowledge on the influence of different rise times of sounds on the startle response of fish is lacking with only two reports published recently (Hawkins *et al.*, 2015; Spiga *et al.*, 2017). Hence, more insight into the organismal physiology is encouraged for understanding the complex behavioral effects of anxiety and alarm reflex in different finfish species induced by underwater sound exposures in future studies.

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Table 1. Growth performance and feed efficiency (mean \pm SEM) of Koi fish (*Cyprinus carpio*) exposed to different underwater sound transmissions for 90 days

	Musical Stimuli				
	Control	Urban noise	Silk Road	Sufi Ney	Quran
IW (g)	3.69 \pm 0.14	3.84 \pm 0.14	3.76 \pm 0.17	3.60 \pm 0.18	3.47 \pm 0.00
FW (g)	8.80 \pm 0.07 ^b	8.60 \pm 0.07 ^a	9.22 \pm 0.19 ^b	10.13 \pm 0.13 ^c	10.73 \pm 0.07 ^d
RGR (%)	138.77 \pm 7.4 ^a	123.90 \pm 8.6 ^a	146.02 \pm 15.6 ^a	181.81 \pm 10.1 ^b	209.62 \pm 1.9 ^c
FCR	2.20 \pm 0.04 ^d	2.35 \pm 0.09 ^e	2.07 \pm 0.10 ^c	1.73 \pm 0.03 ^b	1.58 \pm 0.02 ^a
Survival (%)	100	100	100	100	100

Means \pm SEM with different superscript letters within the same row are significantly different (P<0.05).

Relatively growth rate (RGR, %)= [(final wet weight-initial wet weight)/initial wet weight] \times 100

Feed conversion ratio (FCR) = feed consumed / weight gain

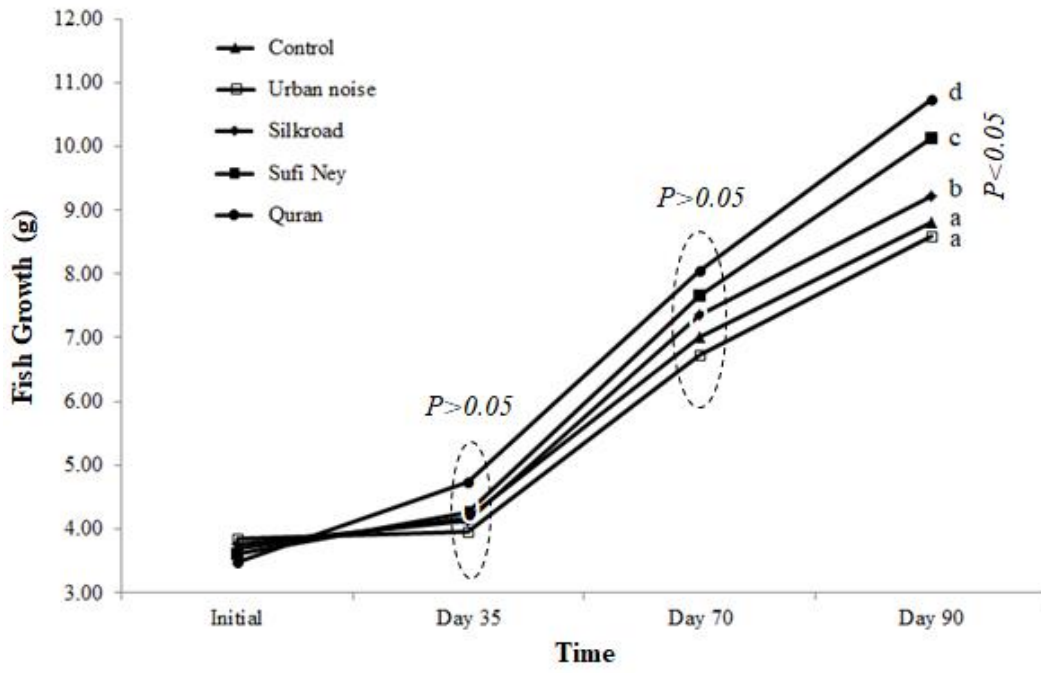


Figure 1. Growth trend of Koi fish exposed to different sounds. Different letters show significant difference at 0.05 levels

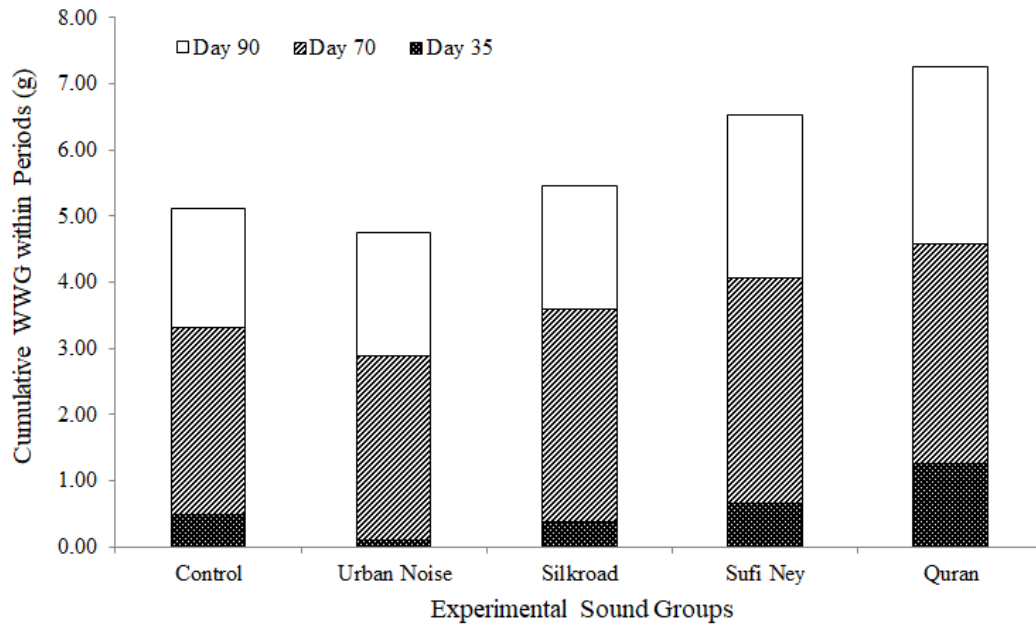


Figure 2. Cumulative individual wet weight gain (WWG, g) of Koi fish exposed to different sounds within periods

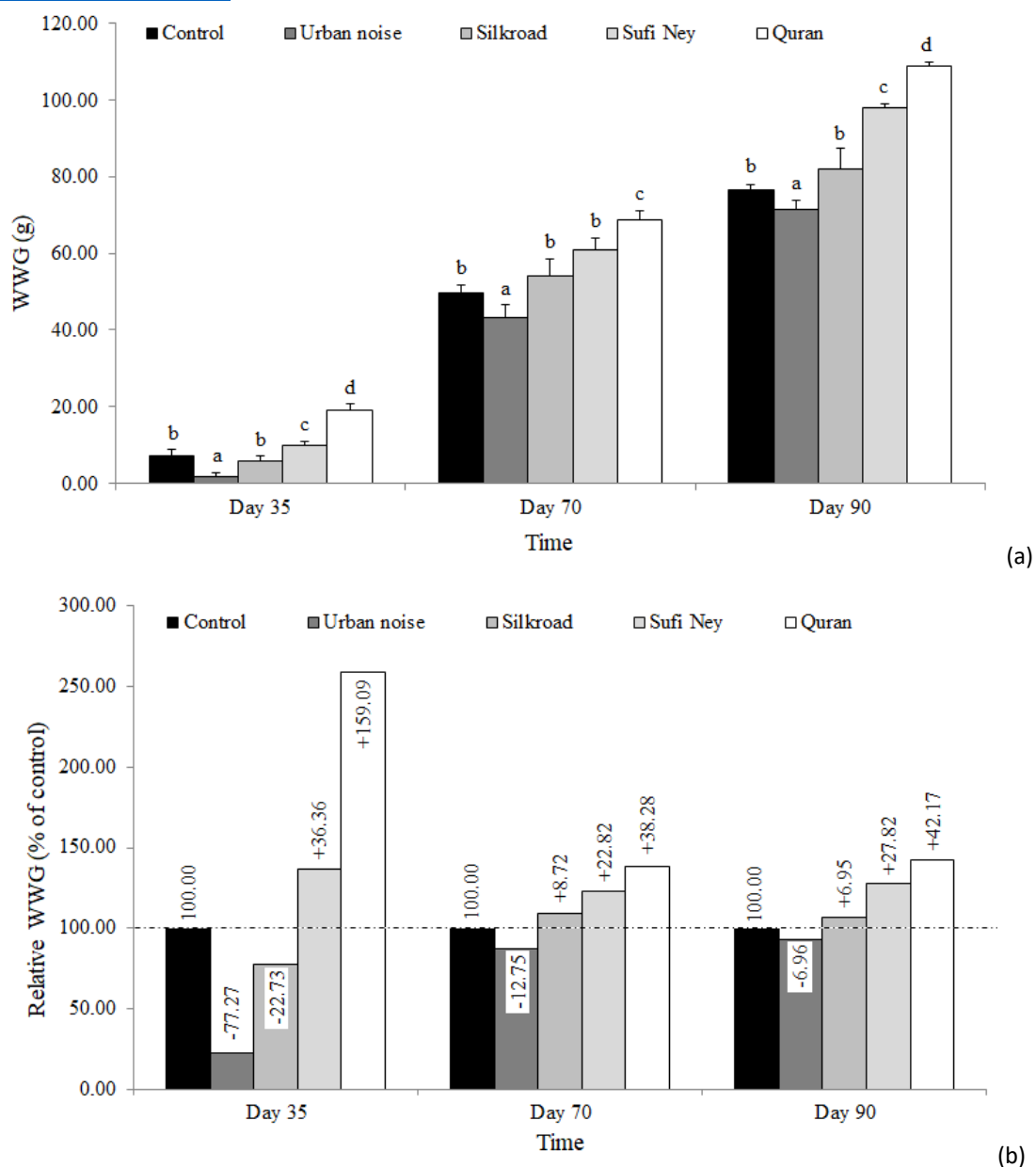


Figure 3. Wet weight gain (WWG) (a) and WWG relative to the control group of Koi fish exposed to different sounds for 90 days (b). Data are means \pm SEM. Bars with different letters are significantly different within groups ($P < 0.05$). WWG was calculated from difference of final and initial wet weight of individual fish. Relative WWG was calculated as $RWWG = WWG \times 100 / WWG \text{ of the control}$

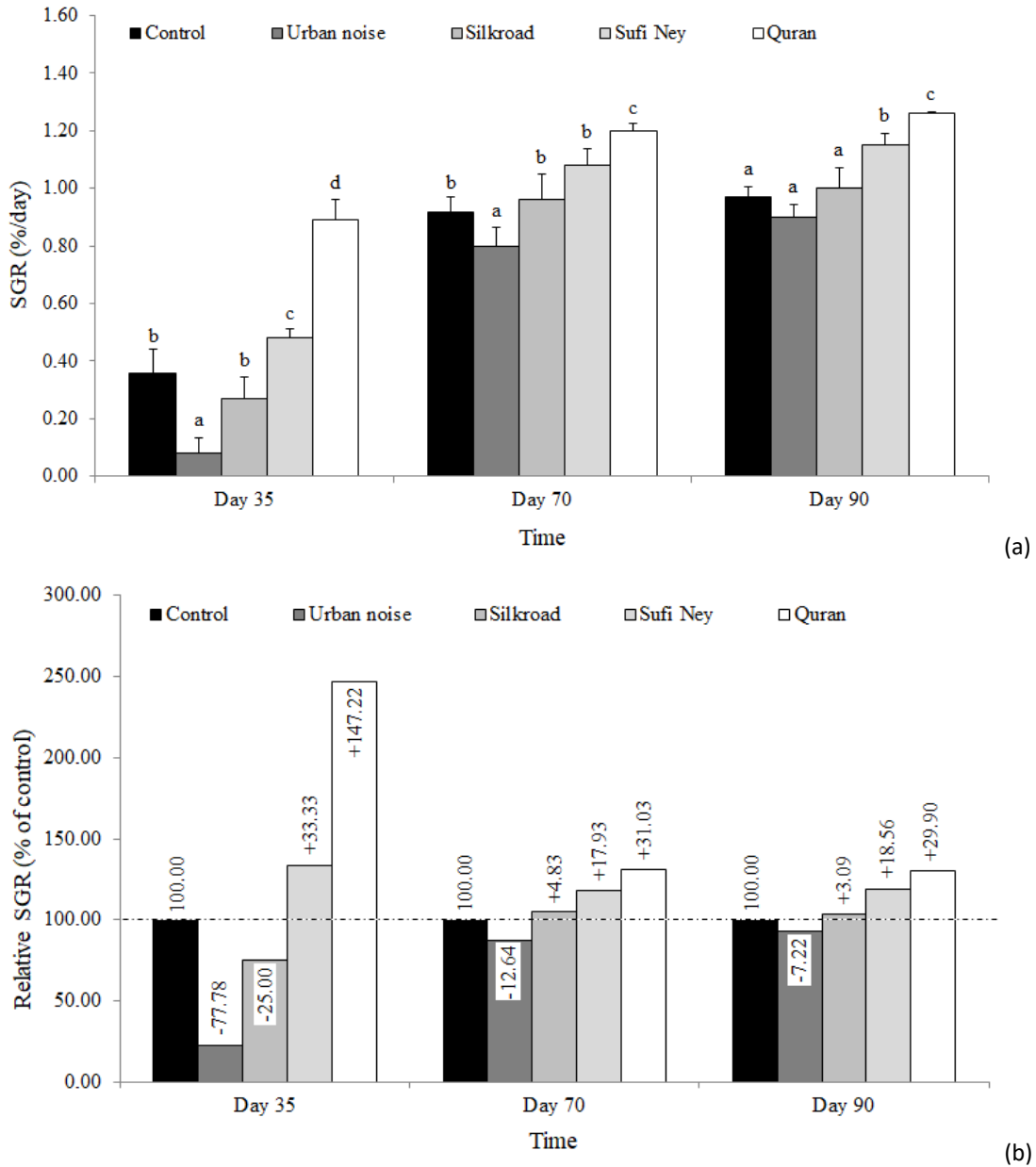


Figure 4. Specific growth rate (SGR) (a) and SGRs relative to the control group of Koi fish exposed to different sounds for 90 days (b). Data are means±SEM. Bars with different letters are significantly different within groups (P<0.05). Specific growth rate was calculated as $SGR = 100 \times (\ln \text{ final weight} - \ln \text{ initial weight})/\text{days}$. Relative SGR was calculated as $RSGR = SGR \times 100/SGR \text{ of the control}$

