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# Binaural beat stimulation - a non-invasive method for inducing zebrafish growth

**Doğankaya L.[1*]; Gültekin T.[1]; Coşkun T.[1]; Alptekin E.[1]**

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**Abstract**

An initial experimental study was conducted to evaluate the effects of binaural beats on fish growth performance. A 90-day trial with four triplicate groups was conducted with 240 zebrafish, *Danio rerio,* under aquarium conditions. Binaural beat file complexes were played for each aquarium using computer controlled directional speakers for 0 min $d^{-1}$(Control), 90 min $d^{-1}$(Group 1), 180 min $d^{-1}$ (Group 2), and 270 min $d^{-1}$ (Group 3). Fish with an average initial weight of 0.26±0.001g were fed twice a day on standard commercial feeds meant for ornamental fish. Body weight gain improved significantly and the highest final body weight was observed in Group I, followed by the other two treatment groups, and finally the Control. Specific growth rate data similarly resulted in significant differences ($p<0.05$). The highest Average Daily Growth (ADG: 0.26±0.038) and Specific Growth Rate (SGR: 0.31±0.034) values were found for Group I, while other treatments were found statistically equal and all represented significantly higher values than the Control. Improved Feed Efficiency (FE) was also observed in Group I. These results support the enhancing effects of binaural beats on growth performance and feed utilization in *D. rerio*. The system and the procedure followed in this study hold the promise of being regarded as eco-friendly and non-invasive growth promoters for fish.

**Keywords**: Binaural beats, *Danio rerio*, Feed utilization, Fish growth, Growth promoter

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1-Department of Fisheries and Aquaculture, Faculty of Agriculture, Ankara University, 06110 Ankara, Turkey
*Corresponding author's Email: dogankaya@ankara.edu.tr

## Introduction

Music has been used from ancient times as a therapeutic agent; many contemporary studies have focused on the curative effects of music for human epileptiform activity (Hughes *et al.*, 1998; Hughes, 2002), hearing disorders (Tomatis, 1996), autism (Jaušovec *et al.*, 2006) and neurophysiology (Auzou *et al.*, 1995; Sarnthein *et al.*, 1997; Arikan *et al.*, 1999). Moreover, sonic and ultrasonic sound waves are used in the healing of fractures and for influencing visual brain activity (Rubin *et al.*, 2001; Jaušovec and Habe, 2004). The fish auditory system detects sounds with a combined function of the lateral line, swim bladder and otolith organs (Sand, 1981; Popper *et al.*, 2003). Fishes are known to use sound waves basically when hunting for food organisms, for communication and as a warning for danger. However, there is a more complex influence of environmental artificial sounds on the neural and physiological mechanism of fishes.

While their hearing systems differ from other higher vertebrates, aquatic species are known to be affected positively or negatively by noise and other sources of sound. Previous studies revealed many findings on the effects of sound on fish brain activity and physiology (Vasantha *et al.*, 2003; Wysocki *et al.*, 2007; Codarin *et al.*, 2009; Davidson *et al.*, 2009; Papoutsoglou *et al.*, 2008, 2014).

The greater part of the research investigating the effects of sound on animals is focused on the negative effects of human-generated noise and stress. Most of these articles on aquatic species mainly inquire about the effects of environmental noise generated by shipping, pile driving, sonars, seismic activity, off-shore wind farms, coastal activities and aquaculture facilities (Hastings and Popper, 2005; Davidson *et al.*, 2007; Wysocki *et al.*, 2007; Codarin *et al.*, 2009; Bailey *et al.*, 2010; Mueller-Blenkle *et al.*, 2010). The beneficial use of sound on animals was also studied by researchers in a previous study to observe milking behavior of cows (Uetake *et al.*, 1997), but only a few research addressed the possible positive effects of various sounds on fish health and growth. There are only a few but remarkable reports on the effects of music on fish growth, where fish such as carp *Cyprinus carpio*, sea bream *Sparus aurata* and koi positively responded to different kinds of music (Vasantha *et al.*, 2003; Papoutsoglou *et al.*, 2007; 2008; 2010; 2014).

Binaural beats (BB) were discovered in 1839 by Heinrich Wilhelm Dove (Oster, 1973). A binaural beat is an auditory brainstem response that originates in the superior olivary nucleus where auditory signals from each ear are integrated and precipitate electrical impulses along neural pathways through the reticular formation up the midbrain to the thalamus, auditory cortex, and other cortical regions. When two different pure-tone sound (both with frequencies lower than 1500 Hz, with less than a 35 Hz difference between them) are presented to a listener separately, the listener hears a beat at a frequency equal to the difference between the applied tone (Licklider *et al.*, 1950; Wahbeh *et al.*, 2007). Most fishes are known to have a hearing ability in a narrow frequency band less than 1000 Hz, however, hearing specialists as cyprinoids and siluroids have an extended hearing frequency range up to 8000 Hz (Yan *et al*., 2010).

However, music and other audio tracks have been used since ancient times for therapeutic effects, and to maximize the positive features in the foreground on plants, vertebrates and especially on humans, no present data is available on the effects of binaural beats on fish growth. The question is whether the positive effects of music transmission is useful for farmed animals under stressful captivity environments. To play different kinds of music in and around aquaculture ponds has been reported, but these types of applications would prove noisy for the staff and it would be difficult to ensure that all sound waves are received by fish. The directional speaker system used in this study is more like a laser beam with the sound focused at a high density into a small area; this focused broadcast enables targeting any area of the pond and avoids the spreading of the broadcasted audio. Hereby the effect of audio waves in the water body can be verified and disturbing noises in a working environment (subjective) can be prevented.

In aquaculture, as in all animal rearing operations, the main objective of the producers is to obtain a healthy produce in a short time at low cost. For this purpose, in addition to a variety of fish feed ingredients, feed additives (vitamins, hormones, antimicrobials, pro/prebiotics, etc.), biotechnological applications (chromosome/gene manipulations, hybridization) and alternative procedures which would improve feed utilization are currently being studied. Even if these applications are expensive, regionally or globally prohibited by regulations, and/or require high labor and technical knowledge, growth promoters and new procedures that enable reducing feed costs are of great interest to aquaculture producers. Furthermore, the current trend in sustainable aquaculture is to discover eco-friendly products besides realizing low costs.

This initial experimental study was conducted to evaluate whether binaural beats have any potential to accelerate fish growth and increase survival. The experiment was conducted under separately aerated, filtered and heated aquarium conditions under a standard photoperiod and feeding regime to assess the influence of treatment duration as well as the positive effects of binaural beats. Zebrafish is preferred for experiments both for being a hearing specialist species and a model organism (Whitfield, 2002).

## Materials and methods

### *Experimental design*

A 90-day trial was designed in triplicate groups and directional speaker systems were placed over each aquarium, 15 cm above the water surface. A directional speaker is more like a laser beam which can focus high-intensity sound into a small area. The sound is released with a number of small loudspeakers at small wavelengths, similar to a high-frequency ultrasound (Fig. 1). When the sound travels through the air and is incident on any surface, the frequency rises and becomes audible. All speakers were connected to a computer which was programmed to play the files for iterations at different durations between 0 min/day (Control) to 270 min/day. Since the former experiments in literature with cyprinids indicated effective stimuli durations between 1.5 h – 4 h, the current experiment was designed at a time range of 1.5 – 4.5h (Vasantha *et al*., 2003; Papoutsoglou *et al*., 2007). Binaural beat file complexes (BB) were prepared by integrating three dose files downloadable from the web (designed for appetizing and bulk) with I-Doser software (I-Doser Labs, Binaural Brainwave). BB were played daily for 0 min, 90 min, 180 min and 270 min, respectively for the experimental groups (Control, GI, GII, GIII) during the dark period of the day, starting at 18:00 hours. The sound stimulation was given during the non-feeding period so as not to cause conditioning in fish. Isolation has been achieved with 2.0 cm thick polystyrene plates to prevent the interference between each aquarium (Chen *et al*., 2015). The treatments were randomly assigned to 12 tanks (72 L volume/each).

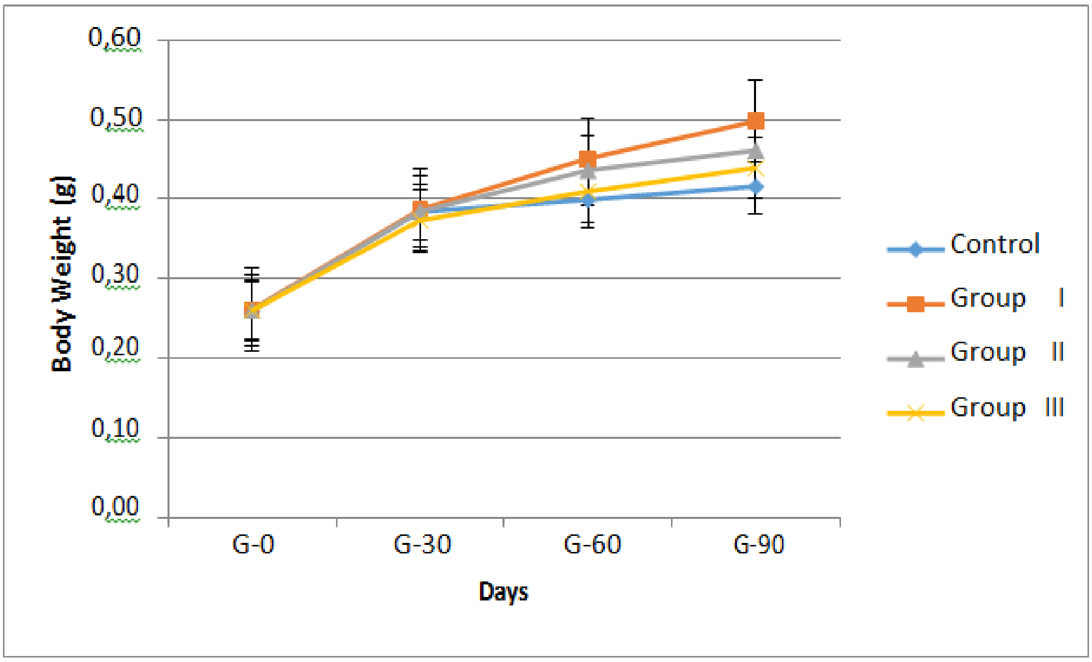


**Figure 1: Mean body weight of *Danio rerio* stimulated with binaural beats for 0 min d$^{-1}$(Control), 90 min d$^{-1}$, 180 min d$^{-1}$ and 270 min d$^{-1}$ for 90 days.**

*Fish management and feeding*

The zebrafish, *Danio rerio* used in this experiment were purchased from a local pet shop. A total number of 240 fish (average initial weight $W_i$: 0.26±0.001g and initial standard body length $L_i$: 2.46±0.006cm) were randomly assigned to 12 glass aquaria with 20 fish in each replicate. Because the fish were small in age, they were assigned to iterations in a random sex ratio. Fish were acclimatized for two weeks before the trial period and fed on the same feed used during the experiment. Each aquarium was individually aerated and filtered during the experiment. Moreover, one-third of the water was refreshed weekly. Water temperature was controlled with digital thermostat heaters. Water quality parameters were measured daily by YSI Pro Plus. Photoperiod was set at 14L:10D (light period 04:00 – 18:00 h) (Brand *et al.*, 2002; Matthews *et al.*, 2002). Fish were fed twice a day (08:00/17:00 h) *ad libitum* on commercial feeds (Table 1). Feed consumption was calculated by weighing the remaining feeds on a monthly basis.

**Table 1: Commercial granule feed composition*.**

| Analytical constituents | (%) | Additives | Dietary level | Trace elements | Dietary level |
|---|---|---|---|---|---|
| Crude Protein | 47.5 | Vitamin A | 29 770 IU $kg^{-1}$ | E5 Manganese | 67 mg $kg^{-1}$ |
| Crude oils and fats | 6.5 | Vitamin D | 1 860 IU $kg^{-1}$ | E6 Zinc | 40 mg $kg^{-1}$ |
| Crude fibre | 2.0 | Colourants | NA | E1 Iron | 26 mg $kg^{-1}$ |
| Ash | 7.0 | Preservatives | NA | E3 Cobalt | 0.5 mg $kg^{-1}$ |
| Moisture | 6.0 | Antioxidants | NA | | |

*Declared by the producer on the package label

*Fish growth assessment*

At the beginning of the experiment, all fish were weighed and measured for standard body length. Following measurements were taken every 30 days because fish were small and fragile. Prior to sampling, fish were anesthetized in 25 ppm eugenol (Hikasa *et al.*, 1986). All fish in groups were weighted to the nearest 0.001 g and measured for standard length ($L_s$) to the nearest 1 mm. The following formulae were used for calculation: Relative growth in length (RGL)=$(L_t$-$L_i)/L_i)$x100 (Bekcan *et al.*, 2006); percent weight gain (PWG)=$[(W_t$-$W_i)/W_i)]$x100 (Llanes and Toledo, 2012); average daily growth (ADG, %)=$[((W_t$-$W_i)/W_i)$x$T]$x100 (Bekcan *et al.*, 2006); specific growth rate (SGR,%$day^{-1}$)= $[(\ln W_t$-$\ln W_i)/T]$x100 (Mohseni *et al.*, 2008); daily growth index (DGI)=100x $[(W_t)^{1/3}$-$(W_i)^{1/3}]/T$ (Kaushik *et al.*, 2004); feed efficiency (FE)=$(W_t$-$W_i)$/feed intake (Korkut *et al.*, 2007); condition factor (K)=$W$x$L^{-3}$x100 (Ricker, 1975); survival (%)=$(n_t/n_i)$x 100 (Keskin and Erdem, 2005). Where $W_i$: Initial body weight (g), $W_t$: Final body weight (g), $L_i$: Initial standard length (cm), $L_t$: Final standard length (cm), $n_t$: Final number of fish in each group, $n_i$: Initial number of fish in each group, and $T$: Experimental period in days.

*Statistical analysis*

Data were analyzed using IBM-SPSS v.22.0 for Windows and presented as means±standard error. One-way analysis of variance (ANOVA) was performed and Duncan's multiple-range test was used to compare differences among mean values. The level of significant differences was set at $p<0.05$.

## Results

During the 90-day trial, mean water quality parameters were measured at a water temperature of 24.0±1.0 °C, dissolved oxygen 6.55±0.02 mg $L^{-1}$ (saturation 78.5%), pH 7.45±0.2 and conductivity 240.7±0.005 µS. Survival rates varied between 93.3% and 98.3% at the end of 90 days, representing the best result in Group I although no significant difference was observed. All groups stimulated with binaural beats showed better weight gain against the Control. The highest final body weight with 0.49±0.033g was observed in Group I and found to be significantly different ($p<0.05$). Final mean body weights in treatment groups I, II, and III were 20%, 12% and 5%, respectively higher than the Control, (Fig. 2). A reduction in body weight gain ratio was noted as the exposure time to BB extended.

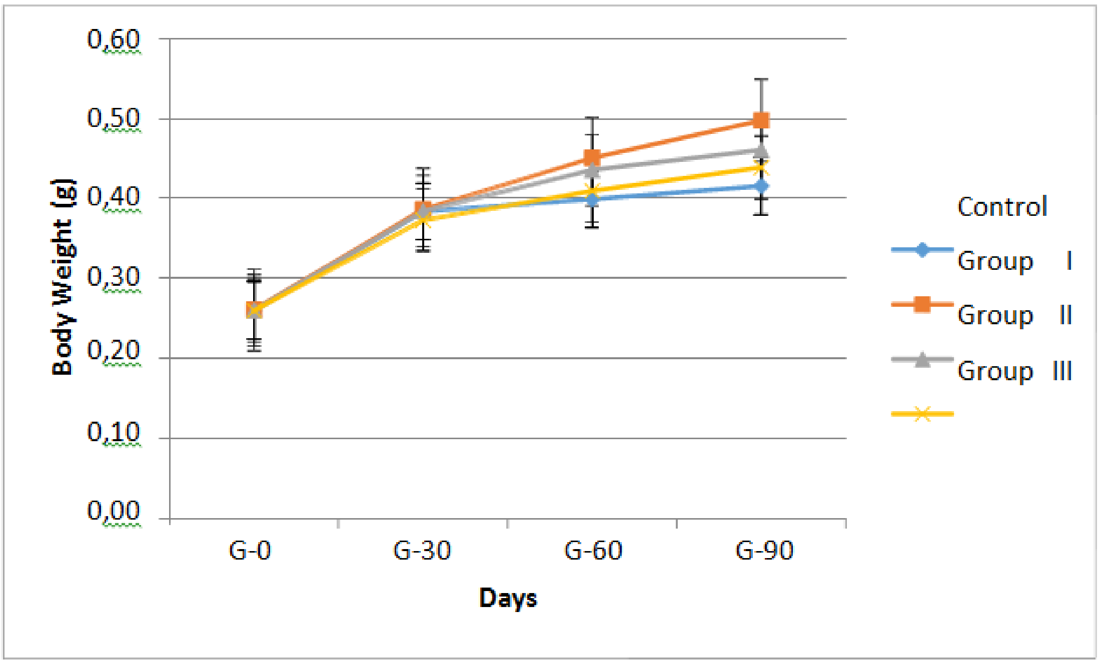


**Figure 2: Mean body weight of *Danio rerio* stimulated with binaural beats for 0 min $d^{-1}$(Control), 90 min $d^{-1}$,180 min $d^{-1}$ and 270 min $d^{-1}$ for 90 days.**

PWG showed a significant difference after 90 days. While Group I had the highest PWG value, Group II and Group III were slightly different but statistically identical. ADG, DGI and SGR data showed significant differences similarly ($p<0.05$). The highest ADG (0.26±0.038), DGI

(0.09±0.012) and SGR (0.31±0.034) values were calculated for Group I. The other treatments were found statistically equal and all represented significantly higher values of ADG, DGI and SGR in comparison with the Control. Fish subjected to BB for 90 min day$^{-1}$ had the highest FE value, whereas a reduction in this value was noted with increasing exposure time.

Final standard lengths of treatment groups I, II, and III were 7.6%, 7.9% and 3.8%, respectively higher than the Control. With the highest values, Group I and Group II were found to be the same in final $L_s$ and significantly different from other groups ($p$<0.05). Even so, although Group II had the highest value for relative growth in length, no significant difference was found within a 95% confidence interval. Moreover, there was no significant difference in K between groups and condition factors were not affected by binaural beats ($p$>0.05). All results are summarized in Table 2.

**Table 2: Growth and feed utilization parameters of *Danio rerio* under different exposure times to binaural beats (means ± S.E., *n=3*).**

| | Experimental groups | | | |
|---|---|---|---|---|
| | Control<br>0 min d$^{-1}$ | Group I<br>90 min d$^{-1}$ | Group II<br>180 min d$^{-1}$ | Group III<br>270 min d$^{-1}$ |
| Initial Body Weight (g) | 0.26±0.003 $^{a}$ * | 0.26±0.002 $^{a}$ | 0.26±0.001 $^{a}$ | 0.26±0.002 $^{a}$ |
| Final Body Weight (g) | 0.41±0.017 $^{b}$ | 0.49±0.033 $^{a}$ | 0.46±0.018 $^{ab}$ | 0.43±0.009 $^{ab}$ |
| PWG (%) 0-90 days | 59.87±5.775 $^{b}$ | 90.81±13.133 $^{a}$ | 77.04±6.765 $^{ab}$ | 68.81±2.420 $^{ab}$ |
| SGR (% body weight d$^{-1}$) | 0.23±0.018$^{b}$ | 0.31±0.034$^{a}$ | 0.28±0.019$^{ab}$ | 0.25±0.007$^{ab}$ |
| Final $L_S$ (cm) | 2.90±0.012$^{b}$ | 3.12±0.032$^{a}$ | 3.13±0.122$^{a}$ | 3.01±0.026 $^{ab}$ |
| RGL (%) 0-90 days | 18.41±0.763$^{a}$ | 26.61±2.083$^{a}$ | 27.14±4.486$^{a}$ | 21.86±0.822$^{a}$ |
| FE | 0.14±0.0 13$^{b}$ | 0.21±0.025$^{a}$ | 0.17±0.015$^{ab}$ | 0.15±0.004$^{ab}$ |
| Survival (%) | 95.00±0.629$^{a}$ | 98.33±0.479$^{a}$ | 96.67±0.500$^{a}$ | 93.33±1.000$^{a}$ |
| Condition Factor K | 1.70±0.075$^{a}$ | 1.63±0.090$^{a}$ | 1.54±0.231$^{a}$ | 1.61±0.036$^{a}$ |

*Different superscripts in the same row denote statistically significant differences ($p$<0.05)

## Discussion

Effects of different sources of sound on fish have long been investigated (Fish, 1972; Hawkins, 1986; Wysocki *et al.*, 2007; Davidson *et al.*, 2009; Popper and Hastings, 2009; Sabet *et al.*, 2016), but limited data are available regarding the effects of sound on fish growth. The present study has demonstrated that a significant increase in weight gain was achievable through 90 min day$^{-1}$ binaural beat treatment. Moreover, the duration of treatment also has an influence on growth performance. As there are no available data regarding the effects of BB on fish, it is difficult to make comparisons, yet the results of stimuli music on fish from previous studies may at least provide a perspective. Papoutsoglou *et al.* (2007) regarded music as a stress relieving or inducing factor for *C. carpio* under different light conditions but not a promoter for growth. Imanpoor *et al.* (2011) noted that music in a rearing environment had no positive or negative effect on growth performance of goldfish *Carassius auratus* under different light and music, because goldfish could distinguish music from stressful sounds. The fish *D. rerio* in the present study are in the same family, Cyprinidae, which is referred to as a "hearing specialist"; however, as the hearing abilities of fish rely on a very complex mechanism, the differences in species/subspecies levels and environmental conditions should be considered for comparison. These findings on *C. carpio* were interpreted to suit brain neurotransmitters well, indicating increased levels where reduced growth was observed. The music alone in addition to environmental noise would be a relative source of stress for fish depending on its species, size, age, hearing ability and living ethology.

Vasantha *et al.* (2003) concluded music (violin) as an environmental factor like temperature and light which have enhanced growth in koi carp. However, the pathway for enhanced growth performance was not elaborated. Papoutsoglou *et al.* (2008) declared the initial evidence on the enhancing effects of music on *S. aurata* (hearing generalist) growth performance. When fish were reared in a recirculating system with 200 lx and 4 h music transmission (140 dB re 1µPa), brain neurotransmitter levels were reduced and growth was enhanced in the first 89 days. At the end of day 117, they found body mass equal in all groups including the Control, but music stimulated fish were more homogenous. These results were in agreement with the findings of the present study which showed a significantly enhanced growth in BB stimulated groups. However, they reported that music treatment time did not alter the growth performance. Contradictory findings in the present study showed a significant difference between 90 min $d^{-1}$, 180 min $d^{-1}$ and 270 min $d^{-1}$ BB exposure.

A further study on *C. carpio,* where fish were stimulated with two pieces of music, resulted in positive findings demonstrating increased growth performance and feed efficiency (Papoutsoglou *et al.*, 2010). If evaluated independently from the light regime, the type of music (Romanza or Mozart) was effective for growth. In the present study, the same binaural file complex was used in all experimental groups at different periods under a standard photoperiod (14L:10D); thus treatment time stands alone as the only factor affecting growth acceleration rate but considering the experimental results as a whole, the current findings coincide.

Catli *et al.* (2015) investigated the effects of three different tempos of music (slow, medium and fast) on black sea turbot *Psetta maeotica* in a recirculating system for 8 weeks and reported that while fast tempo had negative effects in relation to signs of stress, slow tempo music improved fish growth. They also reported the highest fat and the lowest ash content in the group of fish under slow tempo music while no difference in protein content of flesh was observed. Their findings regarding growth enhancement with music effect were consistent with the present study.

The basis for concern about the potential effects of anthropogenic sounds is the negative effects of such sounds on hearing ability, behavior and physiology. Many experimental studies were cited by Popper (2008) as grey literature often lacking appropriate controls, statistics or analysis. Even so, conflicting data were reported in different species under different environments exposed to a variety of sounds; some of the findings indicated no adverse effects of noise in the long term. Wysocki *et al.* (2006) reported increased cortisol levels following exposure to boat noise but no significant change in response to continuous Gaussian noise in *C. carpio*, gudgeon *Gobio gobio* and European perch *Perca fluviatilis*. Even so, it should not be generalized for all species; Wysocki *et al.* (2007) observed no negative impact on rainbow trout *Oncorhynchus mykiss* hearing sensitivity, growth and survival after an 8-month rearing period in a recirculating aquaculture system. Similarly, Davidson *et al.* (2009) expressed no negative impact of noise on *O. mykiss* reared in the recirculating system for 5 months and suggested that experimental fish were acclimated to noise and were unlikely to be affected in the long term.

When the data were analyzed in 30-day intervals, it was clear that the relative difference in body weight gain gradually decreased after the 60$^{th}$ day of the trial. BB transmitted fish had enhanced growth in all groups after 90 days but the boost in growth performance was greater between the days 30 and 60; then the difference gradually decreased through day 90. This can be explained with regard to the tendency of fish to acclimate to the presence of any environmental sound transmission as described above in Wysocki *et al.* (2006), Wysocki *et al.* (2007) and Davidson *et al.* (2009).

Standard body length showed a significant difference by the end of day 60, and after 90 days Group I and Group II had about 8% higher values in $L_s$ compared with the Control. Relative growths in length (RGL) were significantly different at the end of day 60, with the highest value in Group II; however, at the end of the trial (90 days), even the final $L_s$ values were different; no statistical difference was found in RGL (%). Furthermore, condition factor was unaffected by binaural beat treatment.

Within the scope of the data from this initial study, an overall evaluation of growth performance parameters of *D. rerio* reared under BB treatments revealed a significant improvement in weight gain and FCR. Because no primer data exists on the effects of BB on fish, the experiment was conducted to see whether there would be any enhancement in growth, and the results were evaluated in accordance with available literature which discussed the effects of different sound sources on fish.

Finally, it can be concluded that the BB treatment system combination used in this study has a great potential to be referred to as an eco-friendly, economic and novel growth promoter for aquaculture. In addition, the authors suggest future detailed research under different conditions with different species to ensure that present results are precisely attributed to BB treatment.

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