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Effect of Mozart's music (Romanze-Andante of ''Eine Kleine Nacht Musik'', sol major, K525) stimulus on common carp (*Cyprinus carpio* L.) physiology under different light conditions

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Abstract

It is well known that music could have relaxing and antidepressant effects on humans, other mammals and birds. The present study aimed to evaluate music effects on common carp *Cyprinus carpio* growth and physiology, under different light conditions. Therefore, common carp (130.9 ± 0.67 g) was reared, in recirculating water system, under constant darkness (D) or normally illuminated conditions (L) for 12 weeks. Classical music was transmitted underwater and music treatments consisted of no music at all (control groups, C) and three music transmissions either of 30 min duration at 1.5 h intervals (M30) or of 60 min duration at 1 h intervals (M60). Obtained results showed that light conditions had a negative effect on fish growth (L–C versus D–C, $P < 0.01$), but when 30 min music was transmitted (L-M30) growth was improved and equal to D–C. Fish groups presenting reduced growth exhibited significantly increased brain neurotransmitters levels, indicating the occurrence of stressful conditions. Also, music transmission significantly affected carcass and liver fatty acid composition. Present results showed that music could be regarded as a stress relieving or inducing factor. Possible use of music as a growth and product quality promoter, as well as a means to ensure fish welfare under intensive fish farming is discussed.

Keywords: Music; *Cyprinus carpio*; Light conditions; Growth; Physiology; Brain neurotransmitters

''Music and rhythm find their way into the secret places of the soul'' (Plato, 387–388 BC, ''The Republic'')

1. Introduction

Soothing effects of music on human psychology and behaviour are well known (Snyder and Chan, 1999). The question arising is whether the same can be the case for animals, and especially farmed animals that are obliged to live in captivity and, quite often, in stressful environments. Related research results are not yet clear

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and are difficult to interpret due to differences in experimental conditions and limited knowledge on bioacoustics (Newberry, 1995). Nevertheless, among music transmission effects, it has been reported that cows were more willing to approach the automatic milking system (Uetake et al., 1997), broiler chicks were less fearful and grew better (Gvaryahu et al., 1989), while increased distress was observed for piglets after weaning (Cloutier et al., 2000) or laying hens (Campo et al., 2005). Generally, the whole concept falls in the section of ''environmental enrichment'' that has been defined as an improvement in the biological functioning of captive animals resulting from modifications to their environment (Newberry, 1995).

What about fish that lack brain cortex? Could underwater music transmission, as an environmental enrichment attempt, help fish to better cope with the frequently stressful rearing conditions that arise in fish farming of intensive and, especially, recirculating water systems, and thus contribute to fish welfare?

Fish auditory sense is based on detection of sound pressure and involves the combined function of otolith organs, lateral line and swimbladder (Fay and Popper, 2000; Popper et al., 2003). Fish are able to detect, respond to and even produce a wide range of sounds and, more importantly, to discriminate between sounds of different frequencies and magnitudes, to determine the direction of a sound source (sound source localization) and to detect a biologically relevant sound in the presence of other signals (Fay, 1998; Popper et al., 2003). In general, at the opposite ends of fish hearing capabilities stand fish that detect sounds up to 1000 Hz (the majority of fishes, e.g. Nile tilapia *Oreochromis niloticus* L., Smith et al., 2004a; Japanese sand lace *Ammodytes personatus* (Girard), Suga et al., 2005) and fish that have special adaptations enhancing hearing bandwidth and sensitivity for sounds even up to 3000–4000 Hz (e.g. catfish *Ictalurus punctatus* (Rafinesque), Fay and Popper, 1975; goldfish *Carassius auratus* L., Smith et al., 2004a; common carp *Cyprinus carpio* L., Popper, 1972; Kojima et al., 2005). In addition, there are species that can detect ultrasounds to over 180 kHz (e.g. American shad *Alosa sapidissima* (Wilson), Higgs et al., 2004).

Up to now, concern of sound effects on fish has been focused mainly on the impact of human-generated (anthropogenic) sounds (Popper, 2003). Ships and fishing vessels, seismic air guns, sonar, as well as machinery in aquaculture facilities (Bart et al., 2001) are some of the sources that increase underwater noise far above natural ambient levels but within fish hearing thresholds. High levels of ambient sound may prevent fish from hearing biologically important sounds and alter fish normal behaviour (Popper, 2003). In addition, temporary hearing loss and deterioration of auditory sensitivity have been reported in studies of laboratory-induced noise exposure (Smith et al., 2004a,b). Also, sound emissions have been used to attract fish for feeding (Okamoto, 1982), to make fish move to a specific area (Willis et al., 2002) or to deter fish from approaching power plants water inlet (Maes et al., 2004).

Furthermore, it has been widely accepted that the first two (commonly called subcortical brain centers including hypothalamus-amygdala) out of the three neurohormonal steps involved in emotional behavior existing in human brain (sense neuroaxons, sensorial reaction and emotional expression), are also present in fish brain (Davis, 1992; Verheijen and Flight, 1997; Blood et al., 1999; Peretz, 2001; Puschina and Varaksin, 2002; Levysohn and Madsen, 2005). Thus, it could be pointed out that fish might not be too far away from being, relaxed or not, or even ''happy'' or not by specific musical stimuli (Darwin, 1872; Papez, 1937). An example of such a stimulus could be the music of Mozart, which is characterized by pure and single sounds, rhythms and melodies of relatively high frequencies and exerts a calming and almost clear anti-stress effect on humans (Rauscher et al., 1995; Sloboda and Juslin, 2001).

To our knowledge, there are not scientifically documented literature data concerning music influence on fish. The aim of the present study was to evaluate classical music effects on common carp *C. carpio* growth performance and physiological status (blood and plasma parameters, brain neurotransmitters, tissue fatty acid composition, etc.). Common carp has been chosen due to its increased hearing sensitivity (Popper, 1972; Kojima et al., 2005), and also because, as it has been recently demonstrated, koi carp *C. carpio* is able to discriminate between musical stimuli that humans define as blues or classical recordings (Chase, 2001). In addition, in order to investigate the possibility of using musical auditory stimuli as a means of environmental enrichment in aquaculture facilities, the experiment was carried out under two different light conditions since it remains still unclear whether rearing in darkness is stressful for carp or not (Meske, 1985; Ruchin, 2001).

2. Materials and methods

Sixty specimens of scaled common carp *C. carpio*, previously acclimated to laboratory conditions, of mean initial body weight (±S.E.) 130.9 g (±0.67) and mean initial total length 20.7 cm (±0.07) were randomly distributed (in groups of five), in six, duplicated treatments according to a 3 × 2 factorial design. Music

treatments consisted of no music at all (control groups, C), three music transmissions of 30 min duration at 1.5 h intervals (M30) and three music transmissions of 60 min duration at 1 h intervals (M60). Music transmission took place daily at 9:30, 11:30 and 13:30 from Monday to Friday, while no music was played back during weekends. On the other hand, light conditions on a daily basis were either constant darkness (D) 0L–24D (by covering all tank sides with opaque black bags) or photoperiod (L) 12L–12D (room ambient light, fluorescence lamps, 150 lx at water surface).

The piece of music chosen was ''Romanze-Andante'' from W.A. Mozart ''Eine Kleine Nacht Musik'' (sol major, K525) performed by Holland Symphonic Orchestra (Orbish Publishing Ltd., 1993). Music from a computer controlled compact disk player was wire fed to a transmitter unit (developed and designed by ICS DUELL Ltd., Athens, Greece) to be modulated and transmitted. The track had 6.43 min duration and the computer was adjusted to repeatedly play it back. The signal was received, demodulated, processed, amplified, calibrated and driven to underwater cylindrical transducers (Fig. 1a and

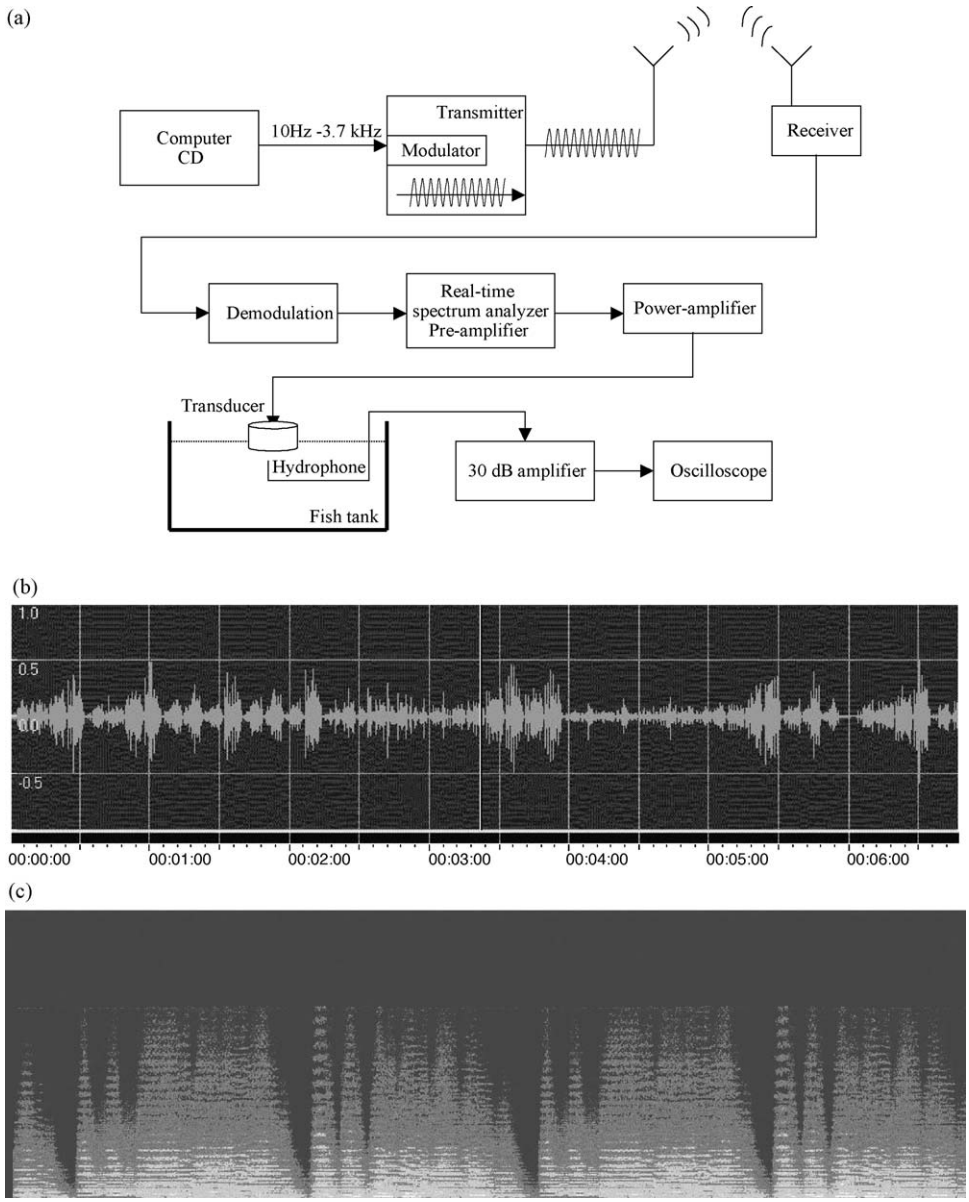

Fig. 1. Schematic diagram of music transmission to fish tank and hydrophone output recording (a), waveform analysis (b) of music track (total duration: 6.43 min) and detail of spectrum analysis of music track from 00:03:56 to 00:04:13 (c).

b). Frequencies that were of interest to be reproduced ranged from 10 Hz to 3.7 kHz (higher frequencies were removed by a software parametric equalizer).

Transducers (15 cm diameter, 10 cm height, developed and designed by ICS DUELL Ltd., Athens, Greece) contained 10 cm diameter speakers (100 W rms each), encapsulated by a very sensitive and flexible plastic material, providing high propagation of transmitted frequencies and successful waterproof packing. Each transducer was immersed 8 cm below water surface level, at equal distances from all top corners of the tank and secured in place with appropriate fasteners. A real-time spectrum analyzer/pre-amplifier (ALTO RSA 27, Taichung, Taiwan, Fig. 1a) was used to pre-amplify and form the final shape of the signal prior to the power amplifier (SKYTEC 6200, 2 × 100 W rms, Germany), which was used to adjust the sound volume that was transmitted by the transducers into the mass of water. Transmitted sound levels, as well as ambient noise, were measured by a hydrophone (receiving sensitivity at 250 Hz: −201 dB re: 1V/μPa ± 2 dB, 2 Hz to 160 kHz, omnidirectional, TC4043, RESON, Denmark). The output of the hydrophone was passed through a 30 dB amplifier (developed and designed by ICS DUELL Ltd., Athens, Greece) and analyzed by a digital oscilloscope (Digital PC Scope, Velleman Instruments, Belgium). Ambient noise levels were recorded for each tank (without fish but with water and air flow set at the experimental levels) before the beginning of the experiment for a duration corresponding to the scheduled daily music transmission period. Ambient noise level was found to be approximately 70 dB re 1 μPa in all experimental tanks (controls included). Signal calibration was based on a 1 kHz sinusoidal and set at 122 dB re 1 μPa, using an audio generator (AG-7001C, EZ Digital Co. Ltd., Korea). Music transmission level fluctuated up to 122 dB re 1 μPa (Fig. 1b and c) and was within the hearing range of common carp (Popper, 1972; Kojima et al., 2005). Hydrophone measurements for music level control were made at a 10-cm distance from the transducers. The functionality of equipment used was tested daily, before the onset of transmission. The presence of the transducers did not obstruct fish swimming activity.

Experimental tanks (glass rectangular tanks of 147 L water capacity, height 36.5 × width 40 × length 100.5 cm) were part of an indoor recirculating fresh water system provided with mechanical and biological filters, compressed air supply and temperature control apparatus. Water flow rate was 2.1 L/min (complete water turnover 20.6 times/day). Water physicochemical characteristics were monitored daily (before the first meal) and water quality was maintained as follows: temperature, 27.2 ± 0.07 °C; dissolved oxygen, 6.0 ± 0.05 mg/L (76.3 ± 0.62% saturation); pH, 7.66 ± 0.014; NH_4^+ and NO_2^- never exceeded 1.0 mg/L. Fish populations were fed, by hand, a commercial pelleted diet (moisture, 9.0%; crude protein, 44.0%; crude lipid, 11.0%; ash, 9.5%; nitrogen-free extract, 23.5%) to 30-min satiation, twice daily from Monday to Friday at 9:00 and 14:30 that is before and after music treatments. No food was offered on weekends. While feeding, the front side of the tanks under darkness was uncovered in order to ascertain about the food consumed by experimental fish populations. Food consumption was recorded daily. In order not to disturb fish during the week, thorough cleaning of the tanks was carried out only on Saturdays, when no music or food was applied. Fish were acclimated to experimental tanks for 1 week and then lighting treatments were applied. Fish remained under these conditions for another 3 weeks (a total pre-experimental period of 4 weeks) before the beginning of music transmission. At the end of the pre-experimental period, fish mean body weight (±S.E.) had reached 190.3 ± 5.11 g and no statistically significant differences were detected among all twelve experimental populations ($P = 0.9974$). The main experimental period lasted 8 weeks (music transmission).

At the end of the experimental period all fish were sacrificed by a blow to the head and subjected to individual weighing (precision 0.2 g) and body measurements (precision 0.1 mm), blood sampling and brain removal, within about 3.5 min. After decapitation, the whole brain was isolated, weighed (precision 0.1 mg), frozen in dry ice and stored at −80 °C till analyzed for brain neurotransmitters. Blood sampling took place from the ventral aorta by means of heparinized syringes. Blood was immediately used for haematocrit analysis and then centrifuged (12,000 × g for 10 min) for plasma separation. Plasma was used for the determination of glucose (enzymatic colorimetric methods, Elitech diagnostics, Sees, France) and cortisol, which was measured by radioimmunoassay, using a commercially available kit (Coat-A-Count Cortisol, DPC, Los Angeles, CA, USA) that has been previously validated for fish (Ainsworth et al., 1985). In the present study, the sensitivity of the assay was 0.2 μg/dL and intra- and inter-assay coefficient of variation was 3.2 and 6.5%, respectively. Afterwards, liver was removed from each fish and used for the determination of moisture, total lipids (Folch et al., 1957), as well as for the calculation of hepatosomatic index (HSI). Spleen was also isolated, weighed and expressed as percent body weight. All fish of each population were minced (without viscera) and lyophilized for carcass proximate composition determination

according to Kjeldahl and Soxhlet methods (AOAC, 1984).

Specific growth rate [SGR = (ln W_{fn} − ln W_{in}) × 100 /t, W_{fn} is the mean final body weight (g), W_{in} the mean initial body weight (g), t is the days of rearing] and food conversion ratio [FCR = (food consumed, g)/(weight gain, g)] were calculated for the whole fish population in each tank. Condition factor [100 × (body weight, g)/(total length, cm)^3] was calculated for each fish.

Liver and carcass lipids were extracted according to Folch et al. (1957) and analyzed for fatty acid composition. Liver lipid samples, for each duplicated treatment, were pooled in order to obtain sufficient sample for the analysis. Fatty acid methyl esters (FAMEs) were prepared by acid-catalyzed transesterification as described by Christie (1990), and were separated by gas chromatography (Agilent Technologies 6890N Network GC System (Wilmington, DE, USA) with flame ionization detector, Omegawax 320 fused silica capillary column (Supelco, Bellefonte, PA, USA): 30 m × 0.32 mm i.d. × 0.25 μm d.f., split/splitless injector, with helium as carrier gas). Individual FAMEs were identified by comparison to known standards (Larodan Fine Chemicals, Malmö, Sweden).

Frozen brains were homogenized and deproteinized in 500 μL of 0.2N perchloric acid solution containing 7.9 mM $Na_2S_2O_5$ and 1.3 mM Na_2EDTA. The homogenate was centrifuged at 37,000 × g for 30 min in 4 °C and the supernatant was again stored at −80 °C until analysis of neurotransmitters was performed by high-performance liquid chromatography (HPLC) with an electrochemical detector (ECD), as described by Sharp et al. (1987) and Papadopoulou-Daifotis et al. (1995) with some minor modifications. A reverse-phase ion pair chromatography was used in all analyses of noradrenaline (NA), dopamine (DA) and its metabolites 3,4 dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). The mobile phase consisted of an acetonitrile-50 mM phosphate buffer (10.5:89.5) pH 3.0, containing 300 mg/L 5-octylsulfate sodium salt as the ion-pair reagent and 20 mg/L Na_2EDTA. Reference standards were prepared in 0.2N perchloric acid solution containing 7.9 mM $Na_2S_2O_5$ and 1.3 mM Na_2EDTA. The sensitivity of the assays was always tested using external standards and a HPLC system BAS-LC4B with an amperometric detector. The working electrode was glassy carbon; the columns were Thermo Hypersil-Keystone, 150 mm × 2.1 mm 5 μm Hypersil, Elite C18 and the HPLC system (Thermo Electron, Cheshire, UK) was connected to a computer. Samples were quantified by comparison of the area under peaks with those of reference standards by using HPLC software (Chromatography Station for Windows). The limit of detection was 1 pg/27 μL (injection volume). Additionally, the ratios of DOPAC:DA, HVA:DA, (DOPAC + HVA):DA and 5-HIAA:5-HT were calculated as an index of dopamine and serotonin turnover rate, in order to have a better evaluation with respect to the serotonergic and dopaminergic activity.

Data were analyzed by two-way analysis of variance (ANOVA). There was no significant difference ($P > 0.05$) between duplicated tanks, so data concerning replicate treatments were pooled. Where P values were significant ($P < 0.05$) multiple comparisons were carried out using the Duncan test. Wherever necessary, data were transformed (logarithm or square root) in order to obtain normal distribution and/or homogeneity of variance. All values presented in the text and tables are untransformed means ± S.E. (Sokal and Rohlf, 1995).

3. Results

Control fish reared under constant darkness (D–C) and fish of M30 under light conditions (L-M30) showed similar growth and the highest final weight significantly different from that obtained from L–C and D-M30 fish groups (interaction term $P < 0.01$, Table 1). For fish subjected to the M60 treatment, intermediate growth performance was observed. D–C and L-M30 fish also presented the highest SGR (interaction term $P < 0.05$) and condition factor ($P > 0.05$) values (Table 1). Under light conditions food consumption was increased and not differentiated among music treatments, while under constant darkness both music treatments led to reduced food consumption, an effect that was more pronounced in D-M30 (interaction term $P < 0.001$, Table 1). However, food utilization, as assessed by FCR, was not affected by the experimental treatments (Table 1). The same was observed for carcass proximate composition and spleen-somatic index, as well as for hepatosomatic index, liver moisture and liver lipids (Table 1).

Haematocrit and plasma cortisol were not significantly affected by music transmission or lighting conditions, although it should be mentioned that in the case of cortisol a great variability among individuals of the same treatment was present. Plasma glucose was reduced in all fish populations kept in constant darkness (Table 2).

Significant interactions between music transmission and lighting conditions were observed for all brain neurotransmitters studied, apart from dopamine metabolite DOPAC (Table 2). Fish of L-M30 treatment

Table 1
Growth performance, carcass proximate composition, spleen-somatic index and liver parameters in common carp reared under continuous darkness (D) or photoperiod 12L–12D (L) and exposed to no music (C), 30 min (M30) or 60 min (M60) music transmission for 8 weeks

	Continuous darkness (D)			Photoperiod 12L:12D (L)			Two-way ANOVA[a]		
	C	M30	M60	C	M30	M60	Lighting	Music	Lighting x Music
Final weight (g)[b]	545.3 ± 25.27 b	444.5 ± 17.02 a	471.5 ± 32.48 a,b	444.5 ± 31.59 a	542.7 ± 29.60 b	497.4 ± 23.43 a,b	NS	NS	**
SGR[c]	1.62 ± 0.04 b	1.39 ± 0.01 a,b	1.45 ± 0.00 a,b	1.58 ± 0.18 a	1.62 ± 0.06 b	1.52 ± 0.01 a,b	NS	NS	*
Final total length (cm)[b]	32.7 ± 0.4 b	30.9 ± 0.4 a	31.4 ± 0.6 a,b	30.9 ± 0.6 a	32.1 ± 0.5 a,b	32.2 ± 0.5 a,b	NS	NS	**
Condition factor[b]	1.564 ± 0.074	1.507 ± 0.026	1.504 ± 0.034	1.497 ± 0.042	1.565 ± 0.048	1.486 ± 0.034	NS	NS	NS
Daily food consumption (g/fish)	15.4 ± 0.32 c	12.5 ± 0.24 a	13.9 ± 0.26 b	15.2 ± 0.27 c	15.8 ± 0.27 c	15.6 ± 0.26 c	D < L***	M30 < C = M60***	***
FCR[c]	1.85 ± 0.01	2.00 ± 0.01	2.07 ± 0.10	1.94 ± 0.11	1.93 ± 0.15	2.19 ± 0.03	NS	NS	NS
Carcass composition (% wet weight)[c]									
Moisture	71.1 ± 1.11	71.7 ± 0.10	71.5 ± 0.20	71.2 ± 0.33	70.7 ± 0.11	71.7 ± 0.13	NS	NS	NS
Protein	16.8 ± 0.56	16.7 ± 0.58	16.6 ± 0.32	16.9 ± 0.16	16.7 ± 0.14	16.4 ± 0.34	NS	NS	NS
Fat	8.5 ± 0.75	7.5 ± 0.31	7.9 ± 0.51	8.7 ± 0.55	8.2 ± 1.07	7.5 ± 0.32	NS	NS	NS
Ash	2.4 ± 0.29	2.2 ± 0.01	2.3 ± 0.10	2.2 ± 0.10	2.5 ± 0.09	2.2 ± 0.01	NS	NS	NS
Spleen and liver[b]									
Spleen-somatic index	0.42 ± 0.03	0.39 ± 0.01	0.45 ± 0.09	0.40 ± 0.03	0.40 ± 0.05	0.34 ± 0.04	NS	NS	NS
Hepatosomatic index	1.72 ± 0.09	1.73 ± 0.09	1.74 ± 0.08	1.70 ± 0.12	1.98 ± 0.15	1.94 ± 0.23	NS	NS	NS
Liver moisture (%)	62.37 ± 0.43	63.28 ± 0.83	63.92 ± 1.36	62.42 ± 1.33	63.07 ± 1.61	65.23 ± 1.25	NS	NS	NS
Liver lipids (% wet weight)	18.85 ± 0.66	16.47 ± 1.12	17.39 ± 1.51	17.55 ± 2.11	16.74 ± 1.92	18.98 ± 1.64	NS	NS	NS

[a] Significant treatment effects and the interaction are shown as letters; means with the same letters are not significantly different ($P > 0.05$, two-way ANOVA); $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. NS: non-significant; SGR: specific growth rate; FCR: food conversion ratio.
[b] Values are means ± S.E. of 10 fish.
[c] Values are means ± S.E. of duplicated tanks.

Table 2
Haematocrit, plasma parameters and brain neurotransmitters in common carp reared under continuous darkness (D) or photoperiod 12L–12D (L) and exposed to no music (C), 30 min (M30) or 60 min (M60) music transmission for 8 weeks

	Continuous darkness (D)			Photoperiod 12L:12D (L)			Two-way ANOVA[a]		
	C	M30	M60	C	M30	M60	Lighting	Music	Lighting x music
Haematocrit	25.1 ± 0.57	25.4 ± 0.78	25.6 ± 0.97	27.6 ± 0.97	25.2 ± 2.51	27.8 ± 1.22	NS	NS	NS
Glucose (mg/100 ml)	50.56 ± 3.28	60.30 ± 2.54	54.94 ± 4.65	60.64 ± 3.48	68.25 ± 6.88	64.20 ± 5.57	D < L*	NS	NS
Cortisol (ng/ml)	48.10 ± 20.32	100.90 ± 33.21	127.30 ± 40.43	65.40 ± 15.40	134.25 ± 29.00	26.86 ± 5.55	NS	NS	NS
5-HT (ng/g)	178.1 ± 10.35 a,b	201.7 ± 9.29 b	205.0 ± 12.64 b	200.8 ± 13.13 b	168.8 ± 14.54 a	207.3 ± 11.87 b	NS	NS	*
5-HIAA (ng/g)	25.3 ± 1.21 a,b	29.2 ± 1.49 b	26.9 ± 1.99 a,b	26.9 ± 2.34 a,b	22.4 ± 2.67 a	31.7 ± 2.48 b	NS	NS	*
HIAA:HAT	0.144 ± 0.006	0.147 ± 0.010	0.133 ± 0.009	0.137 ± 0.012	0.134 ± 0.014	0.152 ± 0.006	NS	NS	NS
NA (ng/g)	248.6 ± 20.13 a	295.4 ± 14.83 b	268.7 ± 9.42 a,b	280.5 ± 19.92 a,b	249.3 ± 18.83 a	297.4 ± 15.81 b	NS	NS	*
DA (ng/g)	58.4 ± 1.54 a,b	69.3 ± 4.40 c	64.7 ± 2.68 b,c	70.0 ± 3.25 c	50.3 ± 4.20 a	70.4 ± 3.52 c	NS	NS	***
DOPAC (ng/g)	3.70 ± 0.31	4.76 ± 0.83	5.60 ± 1.00	4.43 ± 0.65	4.30 ± 0.68	4.64 ± 0.70	NS	NS	NS
HVA (ng/g)	6.87 ± 0.30 a,b	7.54 ± 0.35 b,c	9.09 ± 1.17 c	6.74 ± 0.33 a,b	5.37 ± 0.56 a	7.52 ± 0.61 b,c	D > L**	M30 ≤ C ≤ M60** M30 < M60**	*
DOPAC:DA	0.061 ± 0.006	0.069 ± 0.011	0.087 ± 0.016	0.067 ± 0.013	0.096 ± 0.024	0.068 ± 0.013	NS	NS	NS
HVA:DA	0.112 ± 0.007	0.117 ± 0.010	0.144 ± 0.021	0.099 ± 0.008	0.107 ± 0.007	0.108 ± 0.008	D > L*	NS	NS
(DOPAC + HVA):DA	0.173 ± 0.012	0.187 ± 0.014	0.231 ± 0.031	0.162 ± 0.021	0.204 ± 0.029	0.176 ± 0.018	NS	NS	NS

Values are means ± S.E. of 10 fish.

[a] Significant treatment effects and the interaction are shown as letters; means with the same letters are not significantly different ($P > 0.05$, two-way ANOVA); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. NS: non-significant; 5-HT: serotonin; 5-HIAA: 5-hydroxyindoleacetic acid; NA: noradrenaline; DA: dopamine; HVA: homovanillic acid; DOPAC: 3,4 dihydroxyphenylacetic acid.

Table 3
Carcass fatty acids composition (% of total fatty acids) in common carp reared under continuous darkness (D) or photoperiod 12L–12D (L) and exposed to no music (C), 30 min (M30) or 60 min (M60) music transmission for 8 weeks

	Photoperiod 12L:12D (L)			Photoperiod 12L:12D (L)			Two-way ANOVA[a]		
	C	M30	M60	C	M30	M60	Lighting	Music	Lighting x music
14:0	3.69 ± 0.04	3.81 ± 0.06	3.89 ± 0.03	3.67 ± 0.03	3.64 ± 0.09	3.74 ± 0.04	D > L*	NS	NS
16:0	19.04 ± 0.11	19.43 ± 0.17	18.86 ± 0.22	19.48 ± 0.22	19.48 ± 0.18	19.02 ± 0.17	NS	NS	NS
16:1 $n-7$	6.98 ± 0.04	6.88 ± 0.05	6.92 ± 0.11	6.94 ± 0.04	7.00 ± 0.01	7.02 ± 0.04	NS	NS	NS
18:0	3.47 ± 0.11	3.59 ± 0.00	3.45 ± 0.14	3.62 ± 0.01	3.73 ± 0.12	3.45 ± 0.00	NS	NS	NS
18:1 $n-9$	25.27 ± 0.70	24.67 ± 0.41	24.38 ± 0.33	25.22 ± 0.06	26.58 ± 0.46	25.63 ± 0.21	D < L*	NS	NS
18:1 $n-7$	2.30 ± 0.04	2.42 ± 0.07	2.50 ± 0.06	2.47 ± 0.03	2.33 ± 0.07	2.43 ± 0.06	NS	NS	NS
18:2 $n-6$	8.87 ± 0.27	9.08 ± 0.12	9.58 ± 0.09	8.88 ± 0.03	8.61 ± 0.01	9.19 ± 0.08	D > L*	M30 = C < M60**	NS
18:3 $n-3$	1.30 ± 0.02	1.30 ± 0.02	1.42 ± 0.01	1.29 ± 0.01	1.26 ± 0.05	1.36 ± 0.03	NS	M30 = C < M60**	NS
18:4 $n-3$	0.90 ± 0.02	0.88 ± 0.01	0.92 ± 0.01	0.88 ± 0.02	0.87 ± 0.03	0.91 ± 0.02	NS	NS	NS
20:0	0.13 ± 0.02	0.23 ± 0.02	0.18 ± 0.03	0.14 ± 0.01	0.19 ± 0.02	0.14 ± 0.02	NS	M30 > C = M60*	NS
20:1 $n-9+n-11$	3.47 ± 0.09 a	3.69 ± 0.05 b	3.72 ± 0.01 b	3.56 ± 0.09 a,b	3.47 ± 0.05 a	3.60 ± 0.02 a,b	NS	NS	*
20:2 $n-6$	0.31 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.32 ± 0.01	0.30 ± 0.00	0.32 ± 0.01	D > L*	NS	NS
20:4 $n-3$	0.89 ± 0.00	0.84 ± 0.01	0.88 ± 0.02	0.86 ± 0.02	0.78 ± 0.02	0.85 ± 0.02	D > L*	M30 < C = M60*	NS
20:4 $n-6$	0.58 ± 0.00 a,b	0.61 ± 0.01 b	0.61 ± 0.00 b	0.60 ± 0.03 a,b	0.55 ± 0.02 a	0.55 ± 0.00 a	D > L*	NS	*
20:5 $n-3$ (EPA)	4.65 ± 0.06	4.40 ± 0.00	4.56 ± 0.04	4.66 ± 0.10	4.27 ± 0.14	4.56 ± 0.01	NS	M30 < C = M60*	NS
22:1 $n-11$	1.54 ± 0.09	1.68 ± 0.01	1.71 ± 0.03	1.60 ± 0.04	1.52 ± 0.05	1.58 ± 0.02	NS	NS	NS
22:5 $n-3$	1.41 ± 0.03	1.40 ± 0.04	1.43 ± 0.02	1.39 ± 0.02	1.32 ± 0.04	1.38 ± 0.01	D > L*	NS	NS
22:6 $n-3$ (DHA)	9.39 ± 0.14	8.99 ± 0.08	8.99 ± 0.07	9.39 ± 0.16	8.48 ± 0.43	8.83 ± 0.02	NS	M30 ≤ M60 ≤ C*, M30 < C*	NS
SFA	26.66 ± 0.15	27.40 ± 0.28	26.69 ± 0.43	27.22 ± 0.25	27.34 ± 0.36	26.65 ± 0.16	NS	NS	NS
MUFA	39.67 ± 0.51	39.46 ± 0.24	39.36 ± 0.48	39.91 ± 0.05	41.02 ± 0.51	40.39 ± 0.20	D < L*	NS	NS
PUFA	28.29 ± 0.54	27.82 ± 0.29	28.71 ± 0.24	28.25 ± 0.31	26.41 ± 0.68	27.92 ± 0.09	NS	M30 < C = M60*	NS
HUFA	15.45 ± 0.23	14.79 ± 0.12	14.98 ± 0.13	15.43 ± 0.27	14.06 ± 0.60	14.76 ± 0.03	NS	M30 ≤ M60 ≤ C*, M30 < C*	NS
$n-3/n-6$	1.90 ± 0.03	1.78 ± 0.01	1.73 ± 0.00	1.89 ± 0.03	1.79 ± 0.06	1.78 ± 0.01	NS	M30 = M60 < C*	NS
DHA/EPA	2.02 ± 0.00	2.04 ± 0.02	1.97 ± 0.00	2.02 ± 0.01	1.99 ± 0.04	1.94 ± 0.00	NS	M30 = C > M60*	NS

Results represent means ± S.E. of duplicated tanks.

[a] Significant treatment effects and the interaction are shown as letters; means with the same letters are not significantly different ($P > 0.05$, two-way ANOVA); $^{*}P < 0.05$; $^{**}P < 0.01$. NS: non-significant; SFA: sum of saturated fatty acids; MUFA: sum of Monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; HUFA: sum of highly-unsaturated fatty acids.

Table 4
Liver fatty acids composition (% of total fatty acids) in common carp reared under continuous darkness (D) or photoperiod 12L–12D (L) and exposed to no music (C), 30 min (M30) or 60 min (M60) music transmission for 8 weeks

	Photoperiod 12L:12D (L)			Photoperiod 12L:12D (L)			Two-way ANOVA[a]		
	C	M30	M60	C	M30	M60	Lighting	Music	Lighting x music
14:0	1.76 ± 0.05	1.94 ± 0.24	1.88 ± 0.13	1.85 ± 0.07	2.07 ± 0.10	1.92 ± 0.01	NS	NS	NS
16:0	21.84 ± 0.23 b,c	21.86 ± 0.45 b,c	21.71 ± 0.16 b	22.92 ± 0.53 c	21.84 ± 0.11 b,c	20.07 ± 0.19 a	NS	M30 = C > M60**	*
16:1 $n-7$	5.99 ± 0.07	6.05 ± 0.27	5.64 ± 0.36	6.02 ± 0.19	5.90 ± 0.27	5.70 ± 0.25	NS	NS	NS
18:0	7.18 ± 0.27	7.31 ± 0.33	6.88 ± 0.26	7.11 ± 0.31	7.24 ± 0.02	6.72 ± 0.57	NS	NS	NS
18:1 $n-9$	32.76 ± 0.76	32.72 ± 1.65	31.62 ± 0.78	32.37 ± 1.21	32.66 ± 0.91	32.22 ± 1.61	NS	NS	NS
18:1 $n-7$	1.79 ± 0.05	1.81 ± 0.13	1.94 ± 0.02	1.98 ± 0.05	1.99 ± 0.11	1.85 ± 0.18	NS	NS	NS
18:2 $n-6$	3.94 ± 0.05	4.29 ± 0.58	4.45 ± 0.24	3.94 ± 0.25	4.42 ± 0.30	4.37 ± 0.10	NS	NS	NS
18:3 $n-3$	0.64 ± 0.01	0.65 ± 0.10	0.69 ± 0.04	0.61 ± 0.04	0.70 ± 0.06	0.68 ± 0.00	NS	NS	NS
18:4 $n-3$	0.29 ± 0.02	0.33 ± 0.08	0.32 ± 0.03	0.28 ± 0.01	0.37 ± 0.04	0.37 ± 0.00	NS	NS	NS
20:0	0.16 ± 0.01	0.26 ± 0.02	0.19 ± 0.02	0.16 ± 0.00	0.20 ± 0.03	0.15 ± 0.04	NS	M30 > C = M60*	NS
20:1 $n-9+n-11$	3.70 ± 0.04 a	3.75 ± 0.06 a	4.37 ± 0.15 b	3.90 ± 0.12 a	3.70 ± 0.08 a	3.75 ± 0.19 a	NS	NS	*
20:2 $n-6$	0.30 ± 0.01 a	0.30 ± 0.01 a	0.36 ± 0.01 b	0.32 ± 0.03 a	0.30 ± 0.02 a	0.30 ± 0.01 a	NS	NS	*
20:4 $n-3$	0.51 ± 0.01	0.46 ± 0.03	0.55 ± 0.01	0.46 ± 0.04	0.49 ± 0.01	0.46 ± 0.04	NS	NS	NS
20:4 $n-6$	0.80 ± 0.03	0.83 ± 0.06	0.86 ± 0.02	0.70 ± 0.03	0.81 ± 0.04	0.77 ± 0.06	NS	NS	NS
20:5 $n-3$ (EPA)	3.43 ± 0.16	3.08 ± 0.24	3.55 ± 0.07	3.23 ± 0.37	3.20 ± 0.18	3.09 ± 0.06	NS	NS	NS
22:1 $n-11$	1.33 ± 0.04	1.43 ± 0.04	1.59 ± 0.08	1.34 ± 0.06	1.18 ± 0.02	1.31 ± 0.21	NS	NS	NS
22:5 $n-3$	0.92 ± 0.05	0.81 ± 0.06	0.93 ± 0.02	0.87 ± 0.10	0.91 ± 0.04	0.84 ± 0.06	NS	NS	NS
22:6 $n-3$ (DHA)	8.62 ± 0.64	7.43 ± 0.50	8.18 ± 0.08	7.53 ± 0.79	7.89 ± 0.50	7.67 ± 0.52	NS	NS	NS
SFA	31.26 ± 0.11	31.69 ± 0.55	30.99 ± 0.52	32.42 ± 0.78	31.68 ± 0.04	29.18 ± 0.80	NS	M30 = C > M60*	NS
MUFA	45.71 ± 0.72	45.91 ± 1.37	45.34 ± 0.68	45.76 ± 0.92	45.55 ± 0.96	44.96 ± 1.12	NS	NS	NS
PUFA	19.43 ± 0.63	18.17 ± 1.53	19.86 ± 0.27	17.92 ± 1.64	19.06 ± 0.81	18.53 ± 0.33	NS	NS	NS
HUFA	12.97 ± 0.53	11.32 ± 0.80	12.66 ± 0.02	11.63 ± 1.26	11.99 ± 0.36	11.59 ± 0.40	NS	NS	NS
$n-3/n-6$	2.86 ± 0.07	2.36 ± 0.04	2.51 ± 0.09	2.61 ± 0.11	2.50 ± 0.08	2.42 ± 0.08	NS	C > M30 = M60*	NS
DHA/EPA	2.53 ± 0.30	2.42 ± 0.02	2.30 ± 0.07	2.33 ± 0.02	2.49 ± 0.29	2.48 ± 0.21	NS	NS	NS

Results represent means ± S.E. of duplicated tanks.

[a] Significant treatment effects and the interaction are shown as letters; means with the same letters are not significantly different ($P > 0.05$, two-way ANOVA); $^{*}P < 0.05$; $^{**}P < 0.01$. NS: non-significant; SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; HUFA: sum of highly-unsaturated fatty acids.

exhibited the lowest levels of brain 5-HT, its metabolite 5-HIAA, dopamine DA, HVA and NA. These neurotransmitters levels were not statistically different from those observed for D–C groups, while they were significantly lower from fish groups of D-M30 and L-M60. Also, when L-M30 groups were compared with their respective control (L–C), significantly increased levels of 5-HT and DA were observed in L–C. Despite the above-mentioned differences, the ratio 5-HIAA:5-HT was not differentiated among treatments, while increased dopaminergic activity (HVA:DA ratio) was observed for fish populations kept under darkness (Table 2).

Music transmission, as well as lighting conditions had significant effects on both carcass and liver fatty acid composition (Tables 3–4). For instance, rearing under constant darkness led to reduction of carcass oleic acid (18:1 $n-9$), as well as to small increases in linoleic (18:2 $n-6$), 20:2 $n-6$, 20:4 $n-3$, arachidonic (20:4 $n-6$) and DPA (22:5 $n-3$) acids (Table 3), but no effect was detected for liver fatty acids (Table 4). On the other hand, for fish of the M30 treatment, increased levels of carcass and liver arachidic acid (20:0) and reduced levels of carcass 20:4 $n-3$, EPA (20:5 $n-3$) and DHA compared with controls (no music) were observed, while for fish of the M60 treatment increased carcass linoleic and linolenic (18:3 $n-3$) acids and reduced levels of palmitic acid (16:0), especially under light conditions were detected (Tables 3–4).

4. Discussion

In the present study rearing of common carp under constant darkness proved to positively affect fish growth, when music was not transmitted (D–C versus L–C). This is in contrast to previously reported data of either reduced growth (Ruchin, 2001) or no effect (Meske, 1985) when compared with growth under normally illuminated conditions. Nevertheless, improved growth in the absence of light has been reported for other fish species (e.g. silver catfish *Rhamdia quelen* (Quoy and Gaimard), Piaia et al., 1999; African catfish *Clarias gariepinus* (Burchell), Appelbaum and Kamler, 2000) and could be related to carp ethology as a species that is well acclimated to turbid (and thus almost dark) environments (Chow-Fraser, 1998). On the other hand, music transmission under illuminated rearing conditions (L-M30) seemed to alleviate the negative effect light has exerted, resulting in equal growth performance as observed in D–C fish groups, while under constant darkness growth was reduced (D-M30).

Since there are no available data concerning music effects on fish, it is not easy to make comparisons. Nevertheless, the observed music effect on carp growth seems to suit well enough with brain neurotransmitters results, as increased levels were detected in experimental treatments where reduced growth was observed. Elevated levels of fish brain serotonin, dopamine and noradrenaline or their metabolites (5-HIAA, DOPAC, HVA) have been associated with stress induced increases in brain serotonergic and dopaminergic activity when several kind of stressors are imposed (Winberg and Nilsson, 1993; Zhou et al., 1999; Lepage et al., 2000; Øverli et al., 2001; Amcoff et al., 2002; Papoutsoglou et al., 2006). Thus, in the present study, the occurrence of stressful conditions is indicated for treatments D-M30, L-M60, D-M60 and L–C. Besides, the simultaneous increase in the concentration of monoamines and their metabolites (5-HT and 5-HIAA, DA and HVA) suggests that both synthesis and metabolism of these transmitters were elevated, as it has been reported in rainbow trout *Oncorhynchus mykiss* (Walbaum) showing high stress responsiveness (Øverli et al., 2001). The increase in dopaminergic activity (HVA:DA ratio) observed in fish under darkness is probably related to observed changes in DA and HVA levels, while the involvement of melatonin on dopaminergic system function can not be excluded (Behrens et al., 2000).

Although increasing literature data support the hypothesis that brain serotonergic and catecholaminergic activity are involved in cortisol secretion and in the control of hypothalamic-pituitary-interrenal axis (HPI) in fish (Winberg and Nilsson, 1993; Winberg et al., 1997; Øverli et al., 2001; Höglund et al., 2001; Lepage et al., 2000), present plasma cortisol results are not clear enough. The observed variability of cortisol values among specimens even of the same treatment is probably due to the lack of anaesthetization before blood sampling. This procedure was followed in order to obtain accurate brain neurotransmitters results, since commonly used anaesthetics (e.g. 2-phenoxyethanol) have been reported to interfere with monoaminergic neuronal activity (Loscher et al., 1993; Musshoff et al., 1999). However, it is well known that it is not the appropriate method for cortisol measurement.

Results obtained in the present study concerning plasma glucose and food consumption could be related to suppressed metabolic rhythm as it has been reported for carp reared under continuous darkness (Ruchin, 2001). Furthermore, although serotonin has been suggested to inhibit feeding (De Pedro et al., 1998), in the present study 5-HT levels are not consistent with observed food consumption, especially when music was transmitted.

Neither carcass nor liver lipid content was affected by the experimental treatments, while both carcass and liver fatty acid composition showed certain differentiations when music transmission took place with or without illuminated conditions. Even though, it was not possible to discriminate a certain pattern of music effect on fatty acid composition, the implication of adrenergic and/or serotonergic systems cannot be excluded (Alanärä et al., 1998; Van den Thillart et al., 2002).

Music is a complex auditory stimulus and its perception is known to involve neurohormonal mechanisms in mammals (Evers and Suhr, 2000; Koelsch and Siebel, 2005). Studying music effects on fish physiology status should be considered as a novel experimental field. For the time being, according to the results of the present study, it can be postulated that music could be regarded as a stress relieving or inducing factor. That is because of its obvious influence upon several fish metabolic and neurohormonal processes, which have been strongly suggested by present results. Also, it is indicated that further investigations on intensity, duration and sort of music, in relation to other rearing environment (tanks, water quality, etc.) and fish (species, life stage, feeding, rearing density, etc.) origin factors affecting farmed fish quality and welfare, would contribute to a very promising outcome in improving our understanding of fish physiology.

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