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DIPLOMARBEIT

Titel der Diplomarbeit

Der Einfluss der Temperatur auf das Hörvermögen des Karpfens *Cyprinus carpio* und des Welses *Silurus glanis*.

verfasst von

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Contents

Abstract

Background: Ectothermic animals, such as fish, are affected by ambient water temperature. Their body temperature depends on environmental heat sources, which influence the physiological and metabolic processes, including sensory systems such as the auditory system. In this study I investigated how the ambient water temperature affects the auditory system in two eurythermal otophysan fish species representing two different orders.
Methodology/Principal Findings: In order to investigate possible effects of temperature on the auditory sensitivity I utilized the auditory evoked potentials (AEP) recording technique. Auditory sensitivity and temporal resolution were measured in the common carp *Cyprinus carpio* (order Cypriniformes) and the Wels catfish *Silurus glanis* (order Siluriformes) after acclimating fish for at least three weeks to two different water temperatures (15°C, 25°C and again 15°C). Hearing sensitivity increased with temperature in both species. In *C. carpio* best hearing was detected at 1 kHz at both temperatures and the maximum increase was found at 0.8 kHz (7.8 dB). *S. glanis* showed highest sensitivity between 0.5 – 1 kHz and largest increase at 0.5 kHz (10.3 dB). The improvement in hearing abilities differed between species in particular at 4 kHz. The temporal resolution was measured by determining the latency in response to single clicks from the onset of the sound stimulus to the highest positive peak of the AEP. The latency decreased at the higher temperature in both species by 0.37 ms on average.
Conclusions/Significance: The current study showed that an increase in temperature results in an improvement of hearing (lower thresholds, shorter latencies) in eurythermal species representing different orders of otophysines. The increase in sensitivity seems to be more pronounced in eurythermal than stenothermal (tropical) species.

Key words: Otophysan Fish, *Silurus glanis, Cyprinus carpio,* Ambient Temperature, Hearing Sensitivity, Auditory Evoked Potentials (AEP), Latency

1. Introduction

Physiological and metabolic processes of ectothermic animals are affected in any environment that is characterized by major and rapid temperature changes. Many fish species occur in habitats in which the water temperature changes either quickly such as in shallow waters or slowly or not at all such as in oceans or deep lakes. Fishes experience rapid temperature changes when moving to different water depths or during seasonal changes, during which the acclimation time is longer (Wysocki et al., 2009).

Temperature has an important impact on the body conditions of ectothermic animals in general. These animals are dependent on environmental heat sources, which influence their body temperature. Rapid temperature changes of the water, either cold or warm adjustments, are stressors with a high physiological impact on fish (Crawshaw 1979), also because of the high rate of heat exchange between the animal and the circumfluent water. These temperature changes have relevance for fish in natural waters as well as in aquacultural conditions (Tanck et al. 2000). Ambient temperature affects the speed of metabolic- and other physiological processes, such as respiration (Sollid et al. 2005), the immune system (Le Morvan et al. 1998), metabolism (Moffitt and Crawshaw 1983), protein expression and binding (Huber and Guderley, 1993; Deane and Woo, 2005) and growth (David, 2006). Furthermore, temperature affects the behaviour including the locomotor activity (Friedlander et al. 1976; Cossins et al. 1977; Siegmund and Vogel, 1977, Zitek et al. 2004; Jones et al., 2008).

In addition, ambient temperature is also known to affect the sensitivity of sensory systems, such as the lateral line (Wiersinga-Post and Van Netten, 2000) and the auditory system. Influences of temperature on the auditory system have been studied in many ectothermal taxa such as in insects (Oldfield, 1988; Franz and Ronacher, 2002), in amphibians (Hubl and Schneider, 1978; Long et al., 1996; Egert and Lewis, 1995) and reptiles (Eatock and Manley, 1981; Smolder and Klinke, 1984). In general, a decrease in body temperature results in a decline in auditory sensitivity.

Fish rely on sound production and hearing for orientation, intraspecific communication or prey and predator detection (Fay and Popper, 2000; Ladich and Popper, 2004; Ladich and Myrberg 2006; Wysocki 2006; Fay, 2009). A few studies described influences of ambient temperature on sound characteristics and hearing sensitivity in fish. In general, the sound duration and the fundamental frequency increased with rising temperature,

while the pulse period decreased (Torricelli et al., 1990; Lugli et al., 1996; Connaughton et al., 2000; Amorim, 2005; Amorim et al., 2006; Papes and Ladich, 2011).

Within hearing, Dudok van Heel (1956), observed a broadening of the range of pitch detection with increasing temperature in the European minnow *Phoxinus phoxinus*. Fay and Ream (1992) showed that an increase in temperature results in an increase in spontaneous activity and sensitivity in the auditory neuron in the goldfish *Carassius auratus*. Mann et al. (2009) found a decrease in auditory sensitivity at lower temperature within hours in the walleye pollock *Theragra chalcogramma*. Among catfishes Wysocki et al. (2009) investigated the effects of temperature on hearing in the eurytherm channel catfish *Ictalurus punctatus* and a stenothermal pictus catfish *Pimelodus pictus* and Papes and Ladich (2011) in the Striped Raphael Catfish *Platydoras armatulus*. Interestingly, thresholds shifts were more pronounced in the eurythermal species than in both stenothermal catfish species from the Amazonian river system.

Temperatures affect hearing thresholds as well as the resolution of temporal patterns of acoustic information in ectothermic animals. The influence of ambient temperature on absolute latency was investigated by Carey and Zelick (1993) in amphibia. Interpeak latencies increased in three anuran species when the temperature dropped below 20 °C. Wysocki and Ladich (2002) showed that representatives of several fish families were able to detect pulse periods of less than 2 ms. In a subsequent study Wysocki and Ladich (2003) observed that fish were able to detect the temporal structure of conspecific sounds. The influence of ambient water temperature on temporal processing and latencies was also shown by Papes and Ladich (2011) in *P. armatulus*.

The goal of this study was to investigate the effect of ambient temperature on the auditory system of two eurythermal species representing two different otophysan orders. The Common carp *C. carpio* is a representative of the order Cypriniformes and the Wels catfish *S. glanis* a representative of the order Siluriformes. Both possess accessory hearing structures namely a Weberian apparatus which transmits swim bladder vibrations to the inner ear. Species with those accessory hearing structures are more likely affected by temperature changes (Wysocki et al., 2009). Both species inhabit freshwaters in Eurasia and are capable of surviving under a wide range of temperatures from 0°C to 30°C regimes (Hilge, 1985; Banarescu and Paepke, 2001; Itoi et al., 2003, Copp et al., 2009). Hence I asked if the hearing thresholds of eurythermal species belonging to different orders are similarly or differently

affected by identical changes in water temperature. Furthermore, I investigated the latencies in response to single click stimuli and thus temporal processing of acoustic signals at different temperatures in different fish orders.

2. Material and Methods

2.1. Animals

Nine common carps, *Cyprinus carpio* Linnaeus 1758 [11.3 – 12.8 cm standard length (SL) 40 – 64 g body mass (BM)] and eight Wels catfish, *Silurus glanis* Linnaeus 1758 [23.0 – 30.6 cm SL, 103 – 211 g BM] were used for his study. *S. glanis* were obtained from a fish hatchery (Fischzucht Pottenbrunn, Pottenbrunn, Austria) and *C. carpio* from a private fish pond near Vienna.

Fish were kept in glass tanks (110 x 55 x 30 cm or 100 x 50 x 50 cm) with a sand bottom equipped with plastic tubes, roots and artificial plants. External filters were used and a 12h : 12h L:D cycle was maintained. *S. glanis* were fed frozen food (chironomid larvae) and *C. carpio* were fed food sticks (Tetra Pond), as well as frozen food (chironomid larvae). The baseline temperature was 20 °C ± 1 °C.

Experiments were performed with permission of the Austrian Federal Ministry of Science and Research (GZ 66.006/0023-II/10b/2008).

2.2. Temperature changes

The fishes were acclimated to the baseline temperature (20 °C), for more then one month, before experiments started. The temperature in the holding tanks was controlled using a cooling system (Hailea HC-300A and HC-130A; Guangdong Heilea Group CO., Ltd.) and submersible heaters. Temperature was controlled daily. The temperature of the holding water was changed at a rate of 1 °C per day, until test temperature of 15 °C or 25 °C was reached. Fish had an acclimation time of at least three weeks to each experimental temperature before hearing measurements started. First, fish were acclimated and measured at 15 °C, followed by 25°C and finally again at 15°C for control purposes. Fish had more than three weeks rest after each hearing test.

At each water temperature the audiograms of eight *S. glanis* and nine *C. carpio* were measured. In *S. glanis* individuals were recognized by different body structures, fin shape and different colour patterns. Individuals of *C. carpio* were marked on their fins.

2.3. Auditory sensitivity measurements

Auditory sensitivity was measured using the auditory evoked potential (AEP) recording technique (Kenyon et al. 1998,Wysocki and Ladich 2005; for a review see Ladich and Fay 2013).

The test subjects were immobilized during the hearing test, using Flaxedil (gallamine triethiodide; Sigma-Aldrich, Vienna, Austria). The dosage used was 9 - 24 µg g^{-1} for *S. glanis* and 4 - 7 µg g^{-1} for *C. carpio* and allowed the fish to breath and do little movements during the experiment. A respiration pipette was inserted into the animals mouth. Respiration was achieved by a temperature-controlled gravity-fed circulation system.

The fish were fixed in a mesh, after it has been wrapped carefully in tissue paper. It was positioned in a plastic tub (45 x 35 x 18 cm), which was lined on the inside with air bubble film. The bottom was covered with fine sand. The temperature was maintained at either 15 ± 1 °C or 25 ± 1 °C using cooling packs or a submersible heater. The fish's head was positioned in the center of the tub below the water surface.

The plastic tub was positioned on an air table (TCM Micro-g 63-540), which rested on a vibration-isolated concrete plate. The entire setup was enclosed in a soundproof room, which was constructed as a Faraday cage (interior dimensions: 3.2 x 3.2 x 2.4 m).

For AEP recordings silver electrodes (0.32 mm diameter) were placed in the midline of the skull. The recording electrode was positioned over the region of the medulla and the reference electrode cranially between the nares; both were pressed firmly against the skin which was covered with a small piece of Kimwipes tissue paper to keep it moist, in order to ensure proper contact during experiments. Shielded electrodes leads were attached to the differential input of a preamplifier (Grass P-55, Grass Instruments, West Warwick, RI, USA; gain 10,000x, high-pass at 30 Hz, low-pass at 1 kHz). A ground electrode was placed in the water. Stimuli presentation and AEP-waveform recording were specified using a modular rackmount system (TDT System 3, Tucker-Davis Technologies, Gainesville, FL, USA) running TDT BioSig RP Software (Fig. 1).

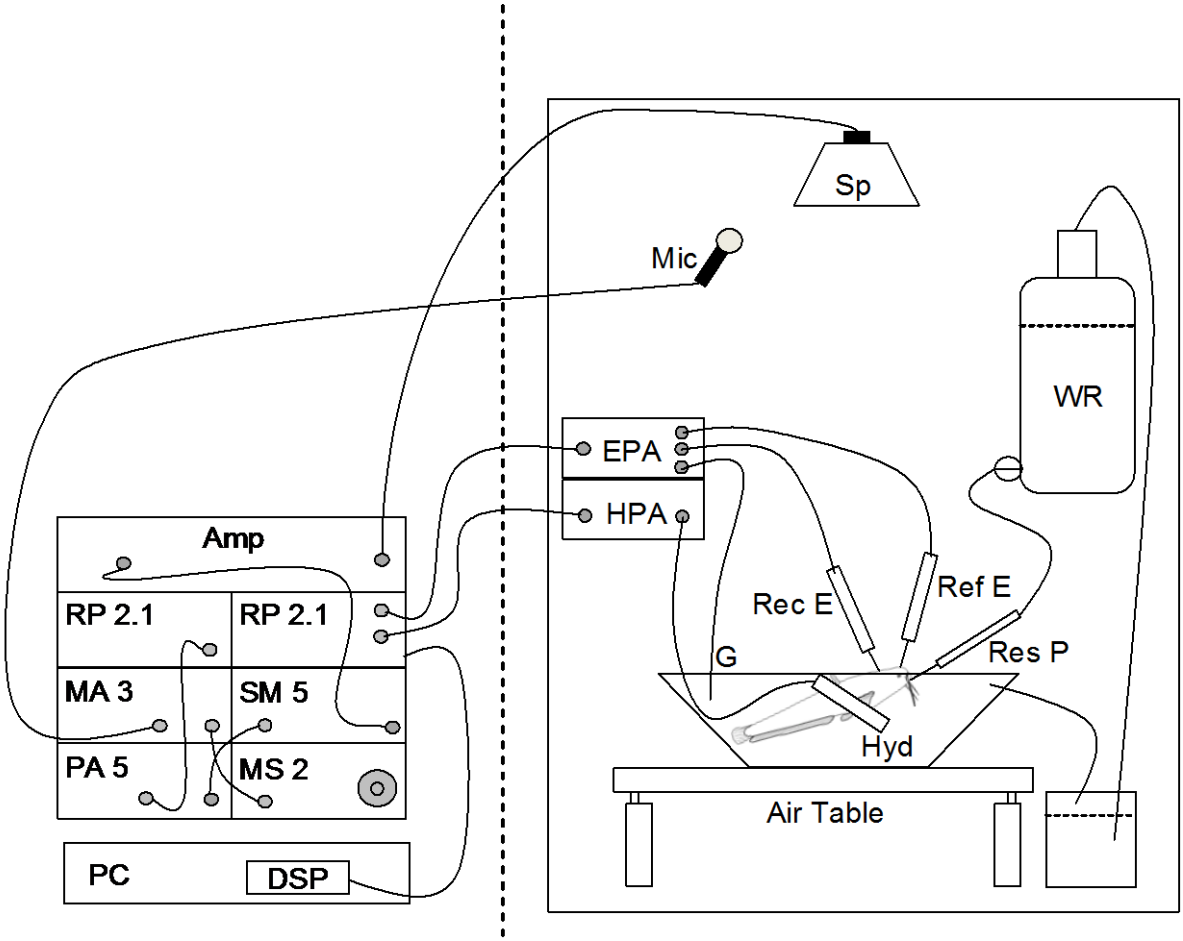

Fig. 1. Experimental setup for AEP (auditory evoked potential) measurements. Amp - Amplifier, DSP – Digital sound processing card, EPA - Electrode preamplifier, G - Grounding cable, HPA – Hydrophone preamplifier, Hyd - Hydrophone, MA 3 – Microphone amplifier, Mic - Microphone, MS2 – Microphone speaker, PA 5 - Programmable attenuator, PC - Personal computer, Rec E - Recording electrode, Ref E - Reference electrode, Res P - Respiration pipette, RP 2.1 – Realtime processor, SM 5 - Signal mixer, Sp – Speaker, WR - Water reservoir.

2.4. Sound stimuli

Sound stimuli were generated using TDT SigGen RP software and fed through a power amplifier (Alesis RA 300, Alesis Corporation, Los Angeles, CA, USA) to a dual-cone speaker (Tannoy System 600, frequency response 50 Hz to 15 kHz ± 3 dB), which was placed 1 m

above the tub. Sound stimuli were presented as tone bursts at a repetition rate of 21 per second.

Hearing thresholds were determined at frequencies of 0.1, 0.3, 0.5, 0.8, 1, 2, and 4 kHz, presented in random order. Rise and fall times were one cycle at 0.1 and 0.2 kHz and two cycles at all other frequencies. All bursts were gated using Blackman window. The stimuli were presented at opposite polarities (180° phase shifted) for each test condition and the corresponding AEPs were averaged by the BioSig RP software in order to eliminate stimulus artefacts. The sound pressure level (SPL) of tone-burst stimuli was reduced in 4 dB steps until the AEP waveform was no longer apparent. The lowest SPL for which a repeatable AEP trace could be obtained, which was determined by overlaying replicate traces, was considered the threshold (Kenyon et al., 1998; Ladich and Wysocki, 2009). A hydrophone (Brüel & Kjaer 8101) was positioned near the right side of each fish (2 cm apart) to determine absolute SPLs values underwater, close to the subjects.

2.5. Latency measurements

Latency measurements followed the method described by Wysocki and Ladich (2002) and Papes and Ladich (2011). AEPs in response to a single click consisted of a series of negative and positive deflections. The positive AEP peaks were denominated with P for positive peaks (directed upwards) by ascending numbers. The latency was defined as time between the onset of the click stimulus and the first constant prominent peak of the AEP (P2) recorded in responses to this click stimulus (Fig. 2).

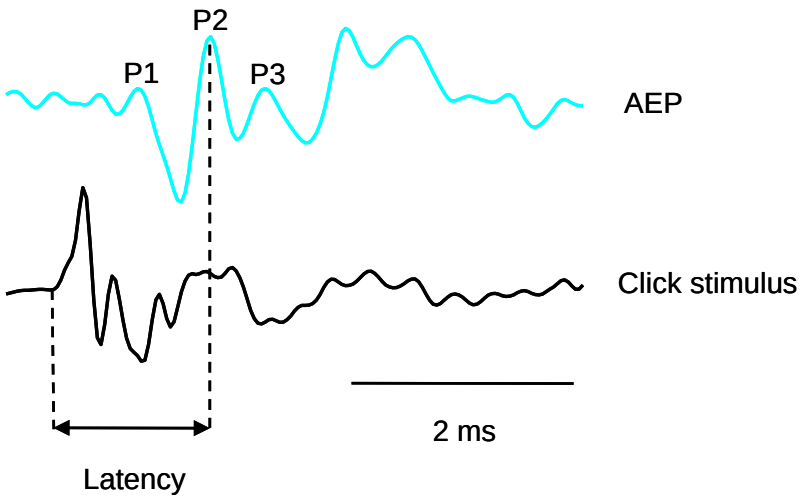

Fig. 2. AEP of one specimen of *S. glanis* in response to a single-click stimulus presented 28 dB above hearing threshold. Only positive peaks (P1, P2, P3) are shown in this figure. The double headed arrow indicates the latency measured from the onset of the click stimulus (left) to the second positive peak (P2).

The single click was presented 28 dB above hearing threshold. Clicks were generated and presented using the TDT SigGen RP software. They were fed through a RP 2.1 realtime processor, a PA5 programmable attenuator, and a power amplifier (Alesis RA 300) to the air speaker (Tannoy System 600). Single clicks were presented to the animals at a repetition rate of 35 per second.

2.6. Statistic Analyses

All data were tested for normal distribution using the Kolmogorov-Smirnov-test and when data were normally distributed, parametric statistical tests were applied.

Audiograms obtained at three temperatures (15° C, 25 ° C and 15 ° C repeated) were compared by a two-factorial analysis of variance (ANOVA) using a general linear model where one factor was temperature and the other was frequency. The temperature factor alone should indicate overall differences in sensitivity between temperatures and in combination with the frequency factor if different tendencies exist at different frequencies of the audiograms. A repeated measures ANOVA followed by a Bonferroni post hoc tests was

calculated to determine differences between thresholds at each frequency. Differences between latencies were calculated using a Friedman-test followed by a Wilcoxon test.

All statistical tests were run using SPSS 17.0. The significance level was set at $p \leq 0.05$.

3. Results

3.1. Auditory sensitivities

3.1.1 *Cyprinus carpio*

Hearing sensitivity increased between 0.1 and 1 kHz, on average by 5.3 dB and decreased rapidly between 1 and 4 kHz (Tab. 1, Fig. 3). Best hearing was detected at 1 kHz at both temperatures.

Table 1. Mean (± S.E.) hearing thresholds of *C. carpio* measured at 15° C, 25° C and 15° C repeated. N = 8.

Frequency (kHz)	15 °C	25 °C	15 °C repeated
0.1	73.4 ± 1.3	75.1 ± 1.1	78.7 ± 1.3
0.3	67.3 ± 1.0	62.3 ± 1.2	67.1 ± 1.1
0.5	65.9 ± 0.9	58.8 ± 1.1	65.8 ± 0.6
0.8	64.9 ± 1.1	57.7 ± 1.6	66 ± 1.2
1	62.8 ± 1.3	57.4 ± 0.6	64.7 ± 0.7
2	107 ± 2.0	101.4 ± 1.7	106 ± 1.1
4	121.2 ± 1.3	121.3 ± 0.7	121.2 ± 0.5

A two-factorial ANOVA revealed that the auditory sensitivity was significantly lower at 15 °C temperature ($F_{2,168} = 36.9$, $p \leq 0.001$) and that there was a significant interaction

between temperature and frequency ($F_{12,168} = 3.05$, $p \leq 0.001$). Thus changes in auditory sensitivity showed different trends at different frequencies. A Bonferroni Post-hoc test showed no significant difference between both 15 °C audiograms, but between both 15 °C and 25 °C (15 °C vs. 25°C: $p \leq 0.001$; 25°C vs. 15 °C repeated: $p \leq 0.001$; 15°C vs. 15°C repeated: n.s.).

Repeated measures ANOVA carried out at each frequency in *C. carpio* revealed that hearing thresholds differed between 0.3 and 2 kHz. No significant differences were found at 100 Hz and 4 kHz. The hearing sensitivity was higher at the higher temperature.

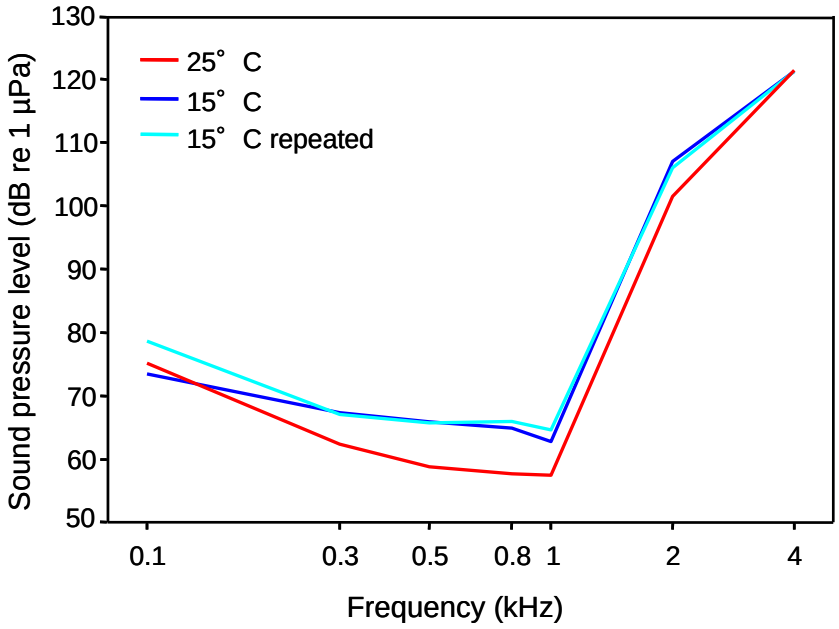

Fig. 3. Mean hearing thresholds of *C. carpio* measured at 15 °C, 25 °C and 15°C repeated. N = 9.

3.1.2 *Silurus glanis*

Hearing sensitivity increased between 100 Hz and 2 kHz, on average by 6.1 dB and decreased between 1 and 4 kHz (Tab. 2, Fig. 4). In the catfish the lowest sensitivity differed between temperatures was found between 0.5 kHz and 1 kHz.

Table 2. Mean (± S.E.) hearing thresholds of *S. glanis* measured at 15° C, 25° C and 15° C repeated. N = 8.

Frequency (kHz)	15 °C	25 °C	15 °C repeated
0.1	81.4 ± 1.8	83.9 ± 1.3	83 ± 1.6
0.3	67.9 ± 1.9	62.9 ± 0.8	71.8 ± 0.9
0.5	70.4 ± 2.3	60 ± 0.7	69 ± 0.6
0.8	65.9 ± 1.6	60.9 ± 1.3	65.5 ± 0.7
1	66.9 ± 1.7	62.5 ± 1.3	64.8 ± 0.5
2	80.9 ±1.6	71.4 ± 2.3	82.5 ± 1.2
4	104 ± 1.1	100.4 ± 1.7	111.5 ± 1.4

Auditory sensitivities were significantly lower at the lower temperatures as revealed by a two-factorial ANOVA ($F_{2,176} = 346.6$, $p < 0.001$) and that there was a significant interaction between temperature and frequency ($F_{7,176} = 4.313$, $p \leq 0.001$). Therefore, changes in auditory sensitivity showed different trends at different frequencies. Catfish had better hearing sensitivity at the higher temperature especially above 300 Hz (Fig. 4).

A Bonferroni Post-hoc test showed a significant difference between 25°C and both 15 °C audiograms but not difference between both 15°C audiograms (15 °C vs. 25°C: $p \leq 0.001$; 25°C vs. 15 °C repeated: $p \leq 0.001$; 15°C vs. 15°C repeated: n.s.).

Repeated measures ANOVA showed significant differences between hearing thresholds at almost all tested frequencies, except at 100 Hz.

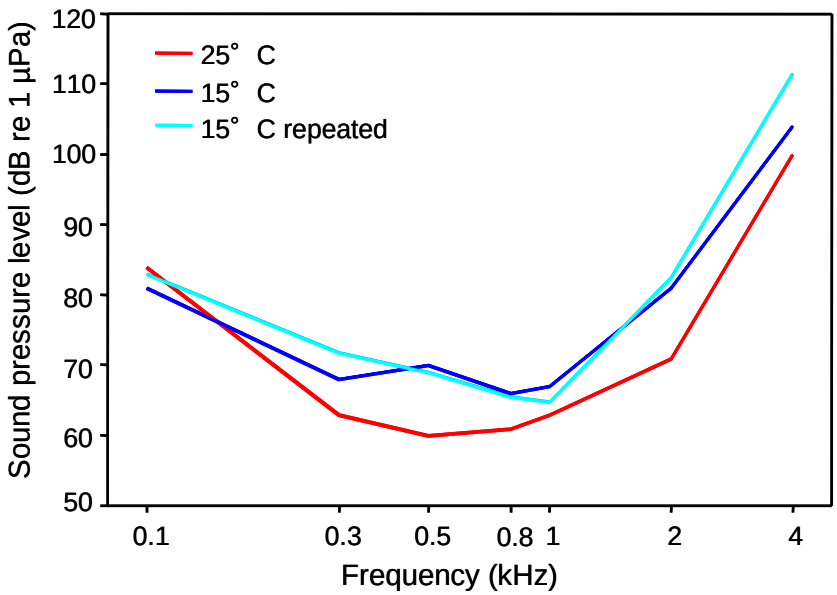

Fig. 4. Mean hearing thresholds of *S. glanis* measured at 15 °C, 25 °C and 15 °C repeated. N = 8.

3.1.3. Comparison between *C. carpio* and *S. glanis*

Both *C. carpio* and *S. glanis* showed no change in sensitivity when the temperature raised from 15 °C to 25 °C at the lowest frequency measured (0.1 kHz). A significant increase was found at higher frequencies except for 4 kHz in the carp. In *C. carpio* the main change in sensitivity was observed between 0.5 to 2 kHz whereas in the *S. glanis* changes of more than 5 dB were found up to 4 kHz (Tab. 5 and Fig. 9).

A two factorial ANOVA revealed that the improvement in hearing differed between both species ($F_{1,105} = 6.35$, $p < 0.05$) and that there was an interaction between the difference and the frequency. Therefore, improvement in hearing showed different trends at different frequencies ($F_{6,105} = 6.72$, $p < 0.001$). Hearing sensitivity improved to a higher degree in *C. carpio* at 1 and in *S. glanis* at 2 and 4 kHz (Table 5).

Table 5. Mean differences in hearing sensitivity (dB) of *C. carpio* and *S. glanis,* between the two tested temperatures (mean of 15°C and 15 °C repeated) and 25 °C. The last column gives the difference in threshold changes between species. Asterisks indicate significant differences between species.

Frequency (kHz)	*S. glanis*	*C. carpio*	Difference
0.1	1.7	0.9	0.8
0.3	6.9	4.9	2
0.5	9.7	7.1	2.6
0.8	4.8	7.8	- 3
1	3.4	6.3	- 2.9 *
2	10.3	5.1	5.2 *
4	7.4	0.1	7.3 *

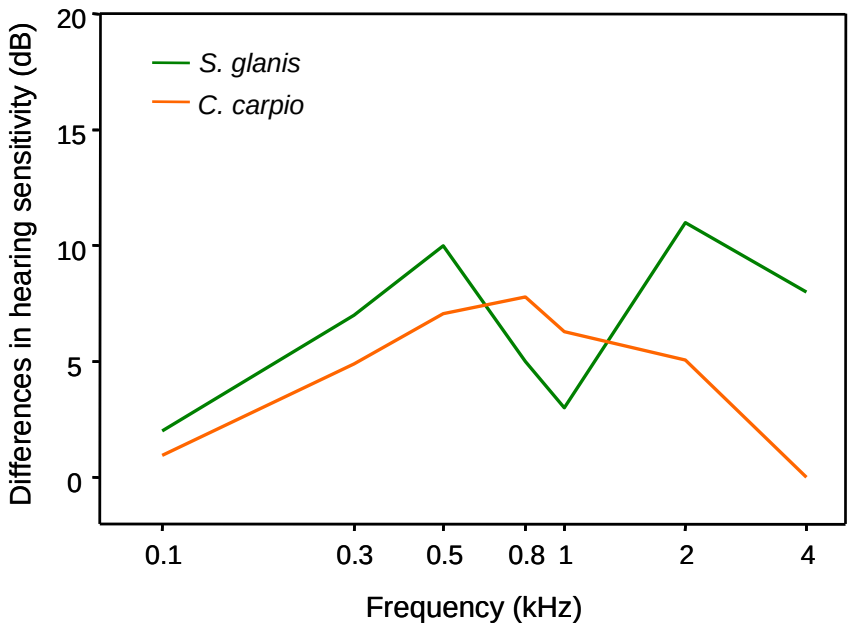

Fig. 5. Comparison of the change in hearing sensitivity in *C. carpio* and *S. glanis*. Differences are shown in both species after acclimation of at least three weeks to 15 °C and 25 °C.

3.2. Latencies in response to single click stimulus

3.2.1. *Cyprinus carpio*

AEPs waveforms of *C. carpio* in response to a single-click consisted of a series of negative and positive deflections. AEPs started with a positive peak, followed by a negative peak in all three tested temperatures (Fig. 6). In this study, the main constant positive peak (P2) of the AEPs was analyzed.

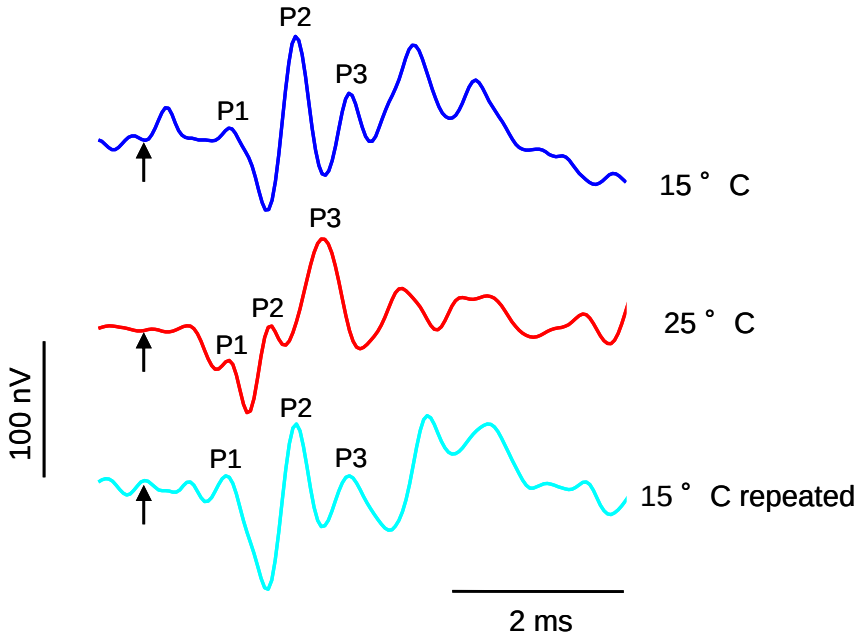

Fig. 6. AEPs of one individual of *C. carpio* in response to a single-click stimulus at different temperatures, presented 28 dB above hearing thresholds. Arrows indicate onset of the single-click stimulus. (P1 – first positive peak, P2 – second positive peak, P3 – third positive peak, as response to a single click stimulus).

The latency between the onset of the single click stimulus and P2 differed between temperatures in *C. carpio* (Friedman test: $\chi^2 = 10.34$, df = 2, $p \leq 0.01$). Wilcoxon tests showed that the delay in the onset of P2 was significantly longer at lower temperature and that there was no significant difference between latencies at both 15 °C tests (15 °C vs. 25°C: $p \leq 0.05$; 25°C vs. 15 °C repeated: $p \leq 0.05$; 15 °C vs. 15 °C repeated: n.s.)

Table 3. Mean (± S.E.) latency of the second positive peaks (P2) of *C. carpio* measured at 15 °C, 25 °C and 15 °C repeated calculated as the time period between the onset of a single click stimulus and the second positive peak.

Temperature	Latency (ms)
15 °C	1.59 ± 0.02
25 °C	1.22 ± 0.1
15 °C repeated	1.55 ± 0.02

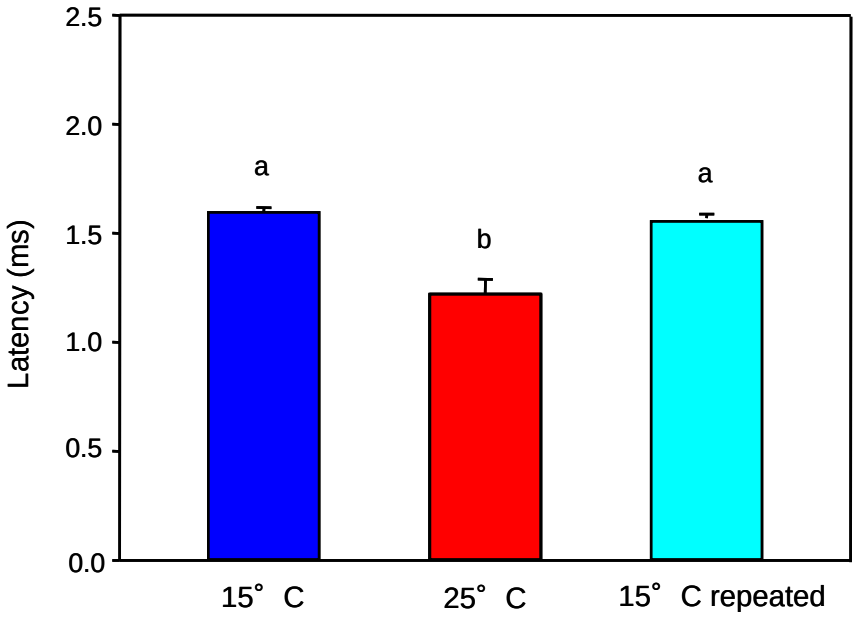

Fig. 7. Mean (+ S.E.) latency between the onset of the click stimulus and the second positive peak (P2) of *C. carpio* measured at 15 °C, 25 °C and 15 °C repeated. Different letters (a, b) indicate significant differences between temperatures ($p \leq 0.05$).

3.2.2 *Silurus glanis*

Similar to *C. carpio* the latency between the onset to the single-click stimulus and the second constant prominent positive peak (P2) was analyzed (Fig. 7). The delay between the onset of the single click stimulus and the first constant prominent peak (P2) was similar at both 15 °C tests and shorter at 25 °C (Friedman test: $\chi^2 = 7.45$, df = 2, $p \leq 0.05$) (Tab. 5 and Fig. 8, 9). A Wilcoxon test showed that the delay in the onset of P2 was significantly longer at lower temperature and that there was no significant difference between both 15 °C measurements (15 °C vs. 25°C: $p \leq 0.05$; 25°C vs. 15 °C repeated: $p \leq 0.05$; 15 °C vs. 15 °C repeated: n.s.).

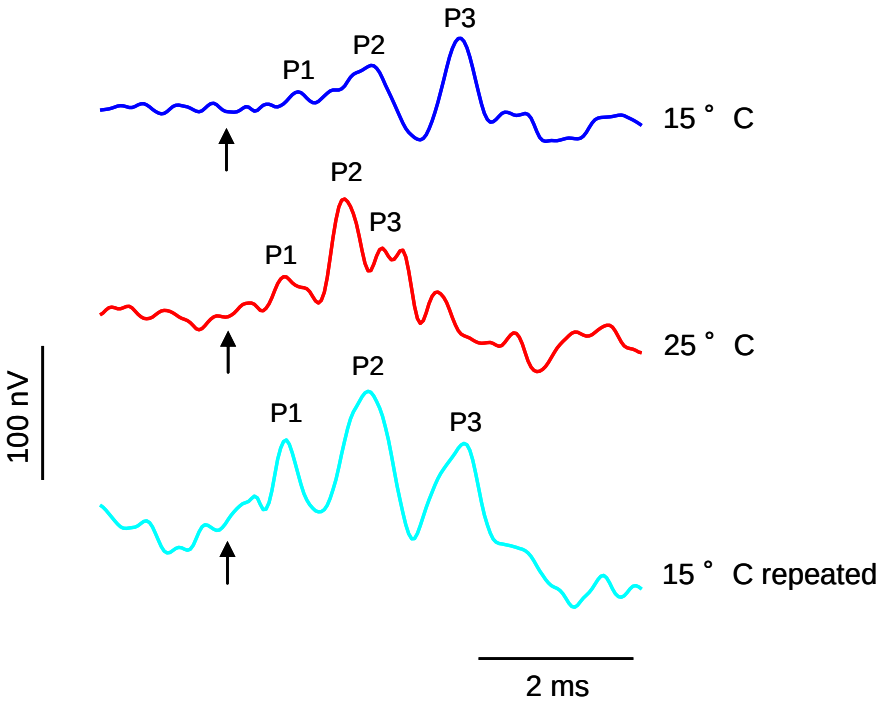

Fig. 8. AEPs of one individual of *S. glanis* in response to a single-click stimulus at different temperatures, presented 28 dB above hearing thresholds. Arrows indicate onset of the single-click stimulus. (P1 – first positive peak, P2 – second positive peak, P3 – third positive peak, as response to single click stimulus).

Table 5. Mean (± S.E.) latency of positive peaks (P2) of *S. glanis* measured at 15 °C, 25 °C and 15 °C repeated.

Temperature	Latency (ms)
15 °C	2.02 ± 0.2
25 °C	1.63 ± 0.03
15 °C repeated	2.02 ± 0.2

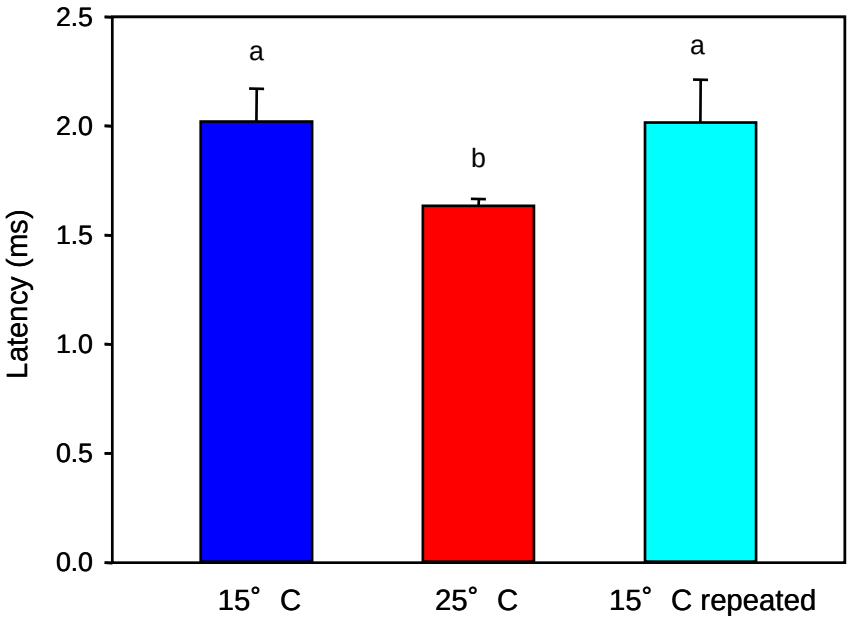

Fig. 9. Mean (+ S.E.) latency between the onset of the click stimulus and the second positive peak (P2) of *S. glanis* measured at 15 °C, 25 °C and 15 °C repeated. Different letters (a, b) indicate significant differences between temperatures ($p \leq 0.05$).

4. Discussion

Hearing sensitivity in *C. carpio*, as well as *S. glanis* is significantly higher at higher water temperatures but the change in sensitivity differs between species. Temperature dependence of hearing sensitivity has been described in ectothermal animals besides fish such as in insects, amphibians and reptiles. In insects an increase in the most sensitive hearing frequency, in spike rate and in sensitivity could be shown (Oldfield, 1988; Van Dijk et al., 1997; Fonseca and Correia, 2007). Similar increases in hearing capability with temperature were shown in amphibians (Hubl and Schneider, 1978; Walkowiak, 1980) and reptiles (Campbell, 1969).

Effects of ambient temperature on the auditory system of several fish species has been shown in a small number of species (Dudok van Heel, 1956; Mann et al., 2009; Wysocki et al., 2009; Papes and Ladich, 2011) but results of these studies vary. Dudok van Heel (1959) showed that the detectable frequency range became wider in the European minnow but he did not mention any change in absolute sensitivity. At higher temperature, the upper limit of frequency discrimination shifted in the minnow from 1200 Hz up to 1600 Hz. Mann et al. (2009) showed in a single specimen of the walleye pollock (*Theragra chalcogramma*) that the hearing thresholds decreased by 8 dB at 350 Hz when the temperature increased by 8 °. No acclimation periods were reported in the prior studies. Detailed studies including at least three weeks acclimation periods to different temperatures were only carried out in otophysines so far.

4.1. Temperature effects on hearing sensitivity in eurythermal fish

Temperature effects on auditory sensitivity have been studied in one eurythermal fish prior to this study in detail. The eurythermal species can bear major temperature changes and therefore have a larger tolerance to ambient water temperature. Wysocki et al. (2009) observed major shifts in hearing thresholds in the North American channel catfish after acclimation to different temperatures. Changes in hearing sensitivity were especially found at higher frequencies. Auditory sensitivity increased by 36 dB at 4 kHz when the temperature was raised from 10 °C to 26 °C. In the following changes in sensitivity observed between 18 °C and 26 °C in *I. punctatus* were compared to the changes found in the present study in *C. carpio* und *S. glanis* between 15 and 25 °C.

One explanation for better hearing sensitivity at higher frequencies, when racing water temperature, can be the use of the time domain for encoding acoustic stimuli, presumably then the frequency domain (Fay, 1982). Because of this suggestion higher frequencies, which need faster firing of neurons to synchronization with the shorter sound cycles, are more susceptible to change in transduction and refraction periods than lower frequencies with longer cycles, which would need more time (Wysocki et al. 2009).

Both eurythermal catfish species studied so far namely the channel catfish and the European wels showed a frequency-dependent increase in hearing sensitivity with increasing temperature. This trend was more pronounced in *I. punctatus* in which the sensitivity increased by 23 dB at 4 kHz but only by 7 dB in *S. glanis* (Fig. 10).

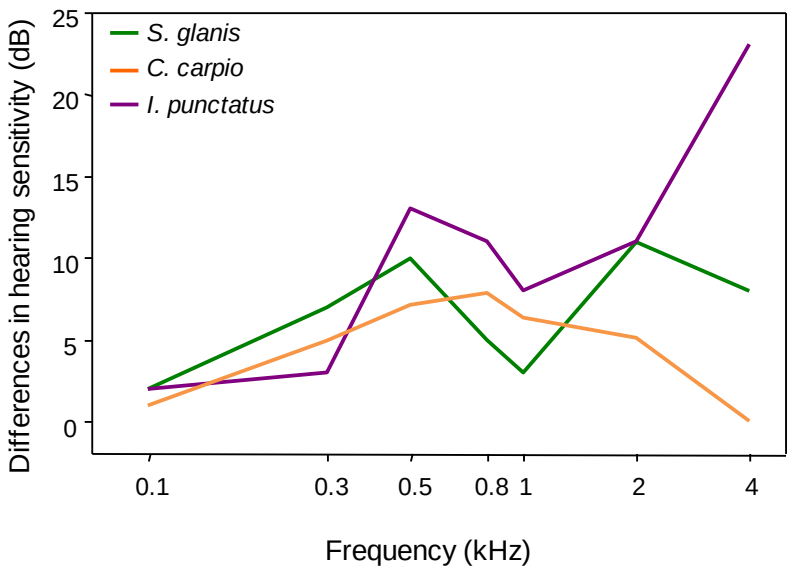

Fig. 10. Comparison of the change in hearing sensitivity in *C. carpio*, *S. glanis* (recent study, 15 °C vs. 25 °C) and *I. punctatus* (Wysocki et al., 2009, 18 °C vs. 26 °C). Differences are shown in all species after acclimation for at least 3 weeks to different temperatures.

Interestingly, the temperature dependent increase in sensitivity differed between both catfish species and the cypriniform in particular at 4 kHz. This may indicate major differences between otophysine families and orders. In fact the absolute hearing thresholds of the carp is higher at 4 kHz than that of both catfish species with large unpaired swimbladders (25 °C:

121 dB versus 100 in *S. glanis* and versus 81 dB in *I. punctatus*) (Wysocki et al. 2009). It seems that the low sensitivity of *C. carpio* at 4 kHz is not affected at all by temperature changes. In addition, the high sensitivity in *I. punctatus* at 4 kHz is affected by temperature changes much more than the lower sensitivity in *S. glanis* (23 dB change versus 7 dB). Thus the differences in thresholds shifts between eurythermal otophysines can mostly be explained by the difference in the absolute sensitivity at higher frequencies.

To what degree does acclimation time affect hearing sensitivity in fish and could this affect threshold shifts observed? Amoser (2007) acclimated *C. carpio* for 7 days to experimental temperatures (15° and 25°C) in contrast to the current study where carps were acclimated for at least 3 weeks. Both studies revealed a similar change in hearing sensitivity (Fig. 11). No difference was found at lower frequencies from 0.1 to 0.3 kHz. Differences between acclimation times were found at higher frequencies.

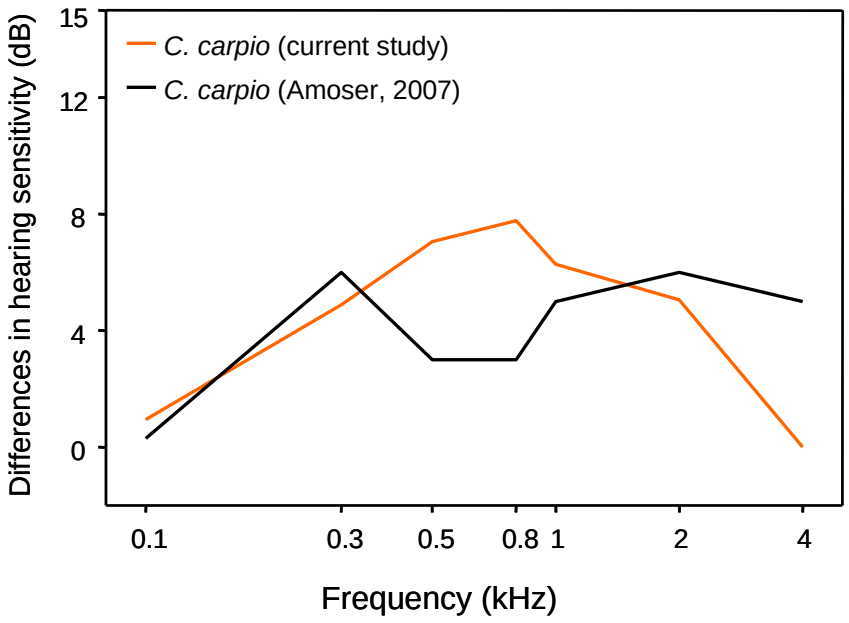

Fig. 11. Comparison of the change in hearing sensitivity in *C. carpio* measured in the present study, 15 °C vs. 25 °C) and in the study by Amoser (2007, 12 °C vs. 22 °C).

The differences in the change in hearing sensitivity between the prior study by Amoser (2007) and the current study are quite small (approx. 2 dB) and indicate that different acclimation

times (1 week versus 3 weeks) did not affect hearing in carps. This is partly in contrast to Wysocki et al. (2009) who showed in *I. punctatus*, that acclimation time affects hearing sensitivities especially at higher temperatures. Hearing thresholds of unacclimated *I. punctatus* (no acclimation time) were higher than of acclimated animals (4 weeks acclimation) especially at higher temperatures. No difference was found between acclimated and unacclimated animals at 10°C. However, when temperature was raised from18 °C and to 26 °C, acclimated animals showed on average 7 dB lower hearing thresholds then unacclimated ones, at all tested frequencies, except at 100 Hz. This difference between in carps and channel catfish is probably due to the fact that in channel catfish non-acclimated animals were compared to animals acclimated for 4 weeks whereas in carps both groups were acclimated for at least one week (1 week versus 3 weeks).

Carey and Zelick (1993) observed effects on the mechanism of acclimation-induced peripheral sensitivity reduction in three anuran amphibians. They exhibited differences in hearing sensitivity between anurans acclimated to 14 °C and 21 °C, measured with brainstem auditory evoked potentials (BAEPs). The three tested species were considerably less sensitive to acoustic signals, when acclimated to a lower temperature (14°C) than anuran acclimated to a warmer temperature (21°C). One reason for better hearing sensitivity found in species acclimated to warmer temperatures, is that nerve fibers adapted to cooler temperature have decreased action potential velocities. Cold- adapted membranes have a higher content of unsaturated fatty acids, which would mainly collaborate to acclimation effects. The time course for regulation of unsaturated fatty acids in the membrane is too long (days) to respond to temporarily changes in temperature (minute) (Carey and Zelick, 1993; Macdonald, 1981). Therefore acclimation time can play a role in affecting hearing sensitivity, because the whole body processes, be it physiological or metabolic, should get used to ambient situation during acclimation time.

Thermal acclimation of fish is a complex process, which also involves shifts in gene activities. These shifts may be another reason for variability in the sensitivity of the auditory system, observed with direct effect of temperature, especially for differences between unacclimated and acclimated animals at the same temperature (Wysocki et al., 2009).

Ambient water temperature affects the auditory system in all eurythermal species investigated so far although the degree of the sensitivity change differs between species. Wysocki et al. (2009) expected small changes in hearing in eurythermic fish species because

they are used to a wide range of temperature in their habitats, which should lead to more resistance to temperature changes. Even so differences were found in hearing ability between temperatures among all three species, especially at higher frequencies. One reason could be that lower temperatures affect the auditory system more than higher ones and that fish are able to gain heat tolerance more rapidly than cold tolerance (Davies 1973, Wysocki et al. 2009).

4.2. Comparison between stenothermal and eurythermal fish

Stenothermal species live in habitats characterized by only small fluctuations in ambient temperatures (tropical regions) and should tolerate only small changes in temperature. Wysocki et al. (2009) and Papes and Ladich (2011) observed that a temperature increase of 8°C resulted in rather small sensitivity improvement in two Amazonian catfish species from two different families. Both the pimelodid *Pimelodus pictus* and the doradid *Platydoras armatulus* showed a similar increase in hearing sensitivity of up to 5 dB (Fig. 12) despite the fact that the hearing curve is U-shaped in *P. armatulus* and ramp-like in *P. pictus*.

A comparison of the sensitivity change in the stenothermal and the eurythermal otophysines showed that the change in sensitivity is on average smaller in the stenothermal species than in the eurythermal species. This is in particular the case at 0.5, 2 and 4 kHz.

The cypriniform showed smaller changes in sensitivity, especially at higher frequencies, similar to the stenothermal species. This similarity is as discussed above most likely due to the low absolute thresholds of the carp at higher frequencies (threshold at 4 kHz at 22°C: *P. pictus*: 73 dB re 1 µPa, *P. armatulus:* 84 dB).

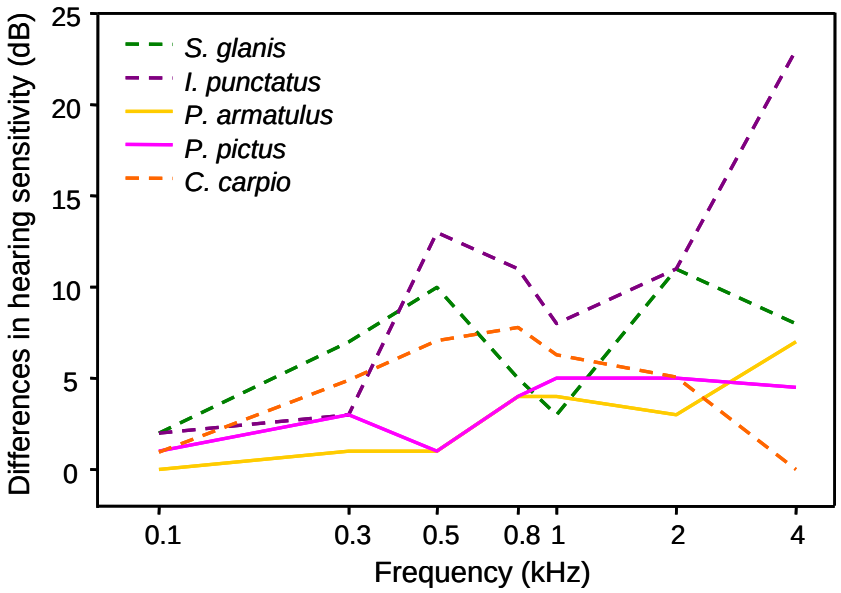

Fig. 12. Comparison of the changes in hearing sensitivity in eurythermal (dashed lines) and stenothermal (solid lines) otophysines. *C. carpio* and *S. glanis* (recent study, 15 °C vs. 25 °C), *I. punctatus* (Wysocki et al., 2009, 18 °C vs. 26 °C), Amazonian catfishes *P. pictus* (Wysocki et al., 2009, 22 °C vs. 30 °C) and the Lined Raphael catfish *P. armatulus* (Papes and Ladich, 2011, 22 °C vs. 30 °C).

The comparison between the eurythermal and stenothermal catfishes shows that the influence of temperature and therefore the temperature dependence of the auditory system can differ depending upon whether a species is physiologically adjusted to tolerate a wide or narrow temperature range (Wysocki et al., 2009). Eurythermal catfish species respond to temperature changes to a higher degree than stenothermal species.

In summary, all otophysines investigated so far, showed higher hearing sensitivity when temperature increased (Amoser 2007, Wysocki et al. 2009, Papes and Ladich 2011). This finding also agrees with results of other fish studies, showing that temperature changes affects the inner ear and the central auditory pathways. Fay and Ream (1992) already suggested that warming water temperatures increase the cell spontaneous activity, best frequency, as well as the cell's sensitivity and responsiveness in goldfish *Carassius auratus*.

4.3. Latencies in response to single click stimuli and temporal processing

The auditory system of fish species, particularly of otophysen fishes is well adapted for temporal processing of acoustic stimuli (Myrberg 1978).

In the current study, the latency between the onset of a single-click stimulus and the second positive peak of the AEP decreased similarly in *C. carpio* and *S. glanis* when the temperature increased. Latencies became shorter by approximately 0.35 ms in *C. carpio* and 0.39 ms in *S. glanis*. The cypriniform showed a 0.04 ms difference in latency between 15 °C and 15 °C repeated, whereas *S. glanis's* delay in the onset of P2 was equal at both 15 °C temperatures. A comparison of the P2 latencies between *C. carpio* and *S. glanis*, revealed that the carp's P2 showed up faster by approximately 0.45 ms at 15 °C and 0.41 ms at 25 °C. This faster response to click stimuli in carps could point to difference in temporal processing of short acoustic stimuli between otophysen families. The differences in latencies could furthermore be due to differences in the auditory pathway between both species indicating that P2's are generated in different brainstem nuclei (Ladich and Bass 2003). And finally the longer latencies in catfish could be due to fact that catfish were twice as long as carps which subsequently resulted in longer distances between the swimbladder and the inner ear and in longer auditory pathways in the catfish (standard length: 11-13 cm in carps versus 23-31 cm in catfish).

A similar trend toward shortening of the latencies at higher temperatures has been found in the stenothermal doradid *P. armatulus.* Papes and Ladich (2011) showed that the latency decreased in three out of four AEP peaks (P1, N2 and P2) at the higher temperature. However, a direct comparison of latencies between the silurid and the doradid catfish is not possible because waveform of AEPs differ considerably between species and it is not clear if P2's are generated by the same brainstem nuclei. Furthermore, specimen of *P. armatulus* were half as long as of *S. glanis* (SL: 11-12 cm versus 23-31 cm) which resulted in effects already mentioned above.

In addition, it had to be mentioned that different temperatures were used during tests (recent study, 15 °C vs. 25 °C; Papes and Ladich, 2011, 22 °C vs. 30 °C), which resulted in a 10 °C difference between highest and lowest temperature in the current study and in a 8°C difference in the study of Papes and Ladich (2011) .

Nevertheless, latencies decreased when increasing the temperature. Papes and Ladich (2011) already mentioned that this phenomenon could be an effect by temperature

dependence of spike conduction velocity, of spike shape or synaptic delay. These data indicate that the temporal resolution of the auditory system in otophysan fish is high and affected by changes in the ambient temperature.

Wysocki and Ladich (2002) showed that the minimum pulse period resolvable by the auditory system was below 1.5 ms which enables representatives of otophysines and osphronemids (labyrinth fishes or gouramis) to process each pulse within a series of intraspecific sounds. Shortening of the latency at higher temperatures shown in the current study and by Papes and Ladich (2011) indicates that the ability to resolve temporal patterns of acoustic stimuli is increasing.

4.4 Summary

Summing up, the data of the current study show clearly that temperature influences hearing in otophysen fish. Similar to prior fish studies, higher temperature leads to an increase in auditory sensitivity and an increase in temporal resolution of the auditory system.

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7. Zusammenfassung

Studien belegen den Einfluss der Umgebungstemperatur auf die physiologischen Prozesse im Körper von ektothermen Tieren. In der aktuellen Studie wurde der Einfluss der Außentemperatur auf das Hörvermögen von ektothermen Otophysen untersucht. Hierfür wurden der Karpfen *Cyprinus carpio* (Familie Cyprinidae) und der Welse *Silurus glanis* (Familie Siluridae) herangezogen. Die Versuchstiere wurden mindestens drei Wochen auf die zu testenden Temperaturen akklimatisiert. Zuerst wurden sie auf 15 °C akklimiert, dann auf 25 °C und anschließend wieder auf 15 °C zurückakklimiert. Mittels der Ableitung Auditorisch Evozierter Potentiale (AEP – Methode) wurde die Hörempfindlichkeit bei insgesamt sieben verschiedenen Frequenzen zwischen 100 bis 4000 Hz getestet. Weiters wurden die Latenzen der Antwort als Verzögerungen auf einen Klick-Stimulus gemessen. Die Latenz wurde als die Zeit definiert, welche zwischen dem Einsetzen des Klick-Stimulus und der ersten konstanten und höchsten Spitze des AEP (P2) registriert wurde. P2 zeigte sich als eindeutige Antworten auf den Klick-Stimulus in allen Temperaturen.

Bei der höheren Temperatur verbesserte sich das Hörvermögen bei den Karpfen zwischen 300 und 2000 Hz und bei den Welsen zwischen 300 und 4000 Hz. Die P2-Latenzen der AEPs nahmen bei 25°C im Vergleich zu 15°C bei beiden Arten um 0.37 ms ab. Diese Daten lassen erkennen, dass die Hörempfindlichkeit mit zunehmender Temperatur bei eurythermen Otophysen steigt.

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Ausbildung	2005 - 2013	Biologie an der Universität Wien
	1996 – 2004	Bundesrealgymnasium Viktring

Publikationen

I. Maiditsch, H.C. Liedtke, J.M. Ng'Wava, W. Hödl, (2011). Advertisement and close-range encounter call of Arthroleptis schubotzi NiedeN, 1911, with notes on phonotaxis and sexual dimorphism in the third manual digit (Anura: Arthroleptidae). Herpetozoa, 24_1_2: 23-31.

Beschäftigungsverhältnisse	2012 - 2013	Tutorin an der Universität Wien
	seit 08/2012	Tiergarten Schönbrunn, Mitarbeiterin der Zoopädagogischen Schule
	2011 - 2012	Wissenschaftliche Mitarbeiterin, FWF-Projekt der Universität Wien
	2002 – 2011	(Juli-August) Mitarbeiterin "Open Air Kino Klagenfurt", Volkskino
	2006-2008	Promotorin der Firma Babmusic Artist Management GmbH
	2002 – 2005	Mitarbeiterin Alternativ Kino, Volkskino Klagenfurt

10. Appendix

9.1. Data of hearing thresholds of *Silurus glansi* of each individual at a) 15 °C, b) 25 °C and c) 15 °C repeated.

a) 15 °C

Fish	Frequency (Hz)						
15 °C	**100**	**300**	**500**	**800**	**1000**	**2000**	**4000**
Ind. 1	82	74	80	63	68	86	102
Ind. 2	82	70	63	67	68	82	107
Ind. 3	70	63	65	62	61	71	107
Ind. 4	82	69	71	63	76	83	98
Ind. 5	85	64	66	67	67	84	105
Ind. 6	80	66	79	75	69	81	102
Ind. 7	86	61	66	61	62	82	105
Ind. 8	84	76	73	69	64	78	106

b) 25 °C

Fish	Frequency (Hz)						
25 °C	**100**	**300**	**500**	**800**	**1000**	**2000**	**4000**
Ind. 1	83	63	61	58	59	68	97
Ind. 2	86	64	60	63	66	75	94
Ind. 3	76	59	57	58	56	69	96
Ind. 4	84	63	60	58	63	74	100
Ind. 5	84	62	62	59	61	65	99
Ind. 6	88	67	59	61	67	65	104
Ind. 7	84	63	58	69	65	70	105
Ind. 8	86	62	63	61	63	85	108

c) 15 °C repeated

Fish	Frequency (Hz)						
15 °C (repeated)	100	300	500	800	1000	2000	4000
Ind. 1	84	74	69	68	65	87	112
Ind. 2	82	74	67	63	67	84	108
Ind. 3	80	72	68	63	64	76	115
Ind. 4	75	68	68	67	65	84	112
Ind. 5	87	73	69	66	64	80	116
Ind. 6	87	70	71	65	66	82	105
Ind. 7	81	69	68	65	65	83	115
Ind. 8	88	74	72	67	62	84	109

9.2. Data of hearing thresholds of *Cyprinus carpio* of each individual at a) 15 °C, b) 25 °C and c) 15 °C repeated.

a) 15 °C

Fish	Frequency (Hz)						
15 °C	100	300	500	800	1000	2000	4000
Ind. 1	69	68	67	62	59	94	115
Ind. 2	69	70	62	60	63	107	114
Ind. 3	71	67	65	64	62	104	123
Ind. 4	77	68	68	67	64	111	125
Ind. 5	81	73	70	71	67	110	124
Ind. 6	73	65	66	67	66	108	123
Ind. 7	73	64	63	64	55	109	124
Ind. 8	76	64	64	62	62	106	122
Ind. 9	72	67	68	67	67	114	121

b) 25 °C

Fish	Frequency (Hz)						
25 °C	100	300	500	800	1000	2000	4000
Ind. 1	79	66	59	60	55	102	123
Ind. 2	71	62	58	56	58	105	121
Ind. 3	72	61	61	59	56	100	121
Ind. 4	72	60	56	59	59	104	120
Ind. 5	76	62	59	52	57	97	121
Ind. 6	74	55	56	56	55	92	118
Ind. 7	74	63	58	53	58	100	125
Ind. 8	81	66	56	56	59	109	120
Ind. 9	77	66	66	68	60	104	123

c) 15 °C repeated

Fish	Frequency (Hz)						
15 °C (repeated)	100	300	500	800	1000	2000	4000
Ind. 1	81	66	64	61	64	107	120
Ind. 2	77	71	65	66	64	112	122
Ind. 3	85	70	68	66	67	104	121
Ind. 4	73	70	65	62	67	104	120
Ind. 5	78	63	69	72	65	102	120
Ind. 6	74	63	63	62	61	102	121
Ind. 7	81	70	67	68	62	108	121
Ind. 8	78	68	66	69	66	106	125
Ind. 9	81	63	65	68	66	109	121

9.3. Data of latencies measurement of *S. glanis* of each individual at each temperature at peak P2

Latencies (ms)			
Fish	**15 °C**	**25 °C**	**15 °C (repeated)**
Ind. 1	1,64	1,72	1,72
Ind. 2	2,05	1,56	1,72
Ind. 3	3,03	1,60	3,36
Ind. 4	1,92	1,64	1,80
Ind. 5	1,97	1,60	1,92
Ind. 6	1,92	1,64	1,72
Ind. 7	1,84	1,51	1,84
Ind. 8	1,80	1,80	2,05

9.4. Data of latencies measurement of *C. carpio* of each individual at each temperature at peak P2

Latencies (ms)			
Fish	**15 °C**	**25 °C**	**15 °C (repeated)**
Ind. 1	1,48	1,11	1,60
Ind. 2	1,65	1,15	1,56
Ind. 3	1,73	1,73	1,56
Ind. 4	1,56	1,15	1,60
Ind. 5	1,60	1,24	1,65
Ind. 6	1,56	1,07	1,52
Ind. 7	1,56	1,15	1,44
Ind. 8	1,56	1,11	1,52
Ind. 9	1,64	1,27	1,52