MASTERARBEIT / MASTER’S THESIS

Titel der Masterarbeit / Title of the Master‘s Thesis

„The effects of temperature and acclimation on hearing in fishes adapted to different temperature regimes”

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2021 / Vienna, 2021

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 878
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Verhaltens-, Neuro- und Kognitionsbiologie UG2002
Betreut von / Supervisor:	Ao. Univ.-Prof. Dr. Friedrich Ladich

Danksagung

„Es ist ein lobenswerter Brauch: Wer was Gutes bekommt, der bedankt sich auch!“, formulierte es einst Wilhelm Busch und in diesem Sinne möchte ich mich allen voran bei meinem Betreuer **Friedrich Ladich** bedanken, nicht nur für die Ermöglichung des Verfassens dieser Arbeit allein, sondern auch für seine Betreuung von der Versuchsplanung bis hin zur Auswertung und der Verschriftlichung all dessen, für all seine Hilfestellungen und Ratschläge sowie für die Zeit, die er sich dazu für mich genommen hat.

Besonders bedanken möchte ich mich auch bei **Isabelle Maiditsch**, sowohl in fachlicher Hinsicht, insbesondere die AEP-Messung sowie alles Aquaristische betreffend, als auch übers Fachliche hinaus hatte sie immer ein offenes Ohr für meine Fragen und Probleme und zeigte sich stets als großartige Hilfe beim Beantworten und Lösen dieser. Weiters gilt mein Dank **Noémie Liesch**, für so manch eine Ablenkung und erheiterndes Gespräch, wenn die Dinge mal nicht ganz so optimal und nach Plan verliefen. Es sei euch herzlich gedankt für die tolle Aufnahme in die Arbeitsgruppe, die mir den Start so erleichtert hat und in der ich auch später immer wieder die nötige Unterstützung und Aufmunterung finden konnte. Es war eine wunderschöne Zeit mit euch, die mir immer unvergesslich bleiben wird! Weiters Danke ans gesamte **Department der Verhaltensbiologie**, vornehmlich an **Dagmar Rotter**, die sich vor allem bei jeglichen administrativen Herausforderungen meiner mit größter Hilfsbereitschaft angenommen hatte und mir so dabei eine Ratgeberin unschätzbaren Wertes geworden ist.

Zu danken habe ich auch jenen, die ich im Laufe des Studiums als meine Freunde bezeichnen durfte, denn „von allen Geschenken, die uns das Schicksal gewährt, gibt es kein größeres Gut als die Freundschaft - keinen größeren Reichtum, keine größere Freude“ (Epikur von Samos). Drei von diesen möchte ich hier im Besonderen hervorheben. **Christopher Kahlich** sei mein Dank bekundet für all den nicht nur wissenschaftlichen Austausch seit unserer gemeinsamen Kölner Bachelor-Zeit. Für all die wundervollen Gespräche, ihre offene, direkte und ehrliche Art, sowie für ihre aufmunternden und bestärkenden Worte, möchte ich mich von Herzen bei **Aileen Ehrentraut** bedanken. Des Weiteren sei **Jessica Österreicher** mein tiefster Dank ausgesprochen, zum einen für ihre Hilfe beim Aufhellen so mancher sprachlicher Unklarheiten, vor allem das Englische, aber auch die Besonderheiten des Österreichischen, bzw. Wienerischen betreffend, zum anderen für die tiefgründigeren und besinnlicheren, wie auch für jene vielleicht belangloser erscheinenden Gespräche und Gedanken, die ich mit ihr teilen durfte. Danke euch, wie auch jenen hier nicht explizit genannten, ebenso für alles Weitere, all die vermeintlichen Kleinigkeiten, deren einzelnes Aufzählen hier sicher den

Rahmen sprengen würde, deren ich aber gewiss nie vergessen werde. Es war, ist und wird mir weiterhin immer eine Freude, verbunden mit größter Dankbarkeit, sein, Zeit mit euch verbringen zu dürfen und Gedanken mit euch auszutauschen, denn „das Einmalige an einer Freundschaft ist weder die Hand, die sich einem entgegenstreckt, noch das freundliche Lächeln oder die angenehme Gesellschaft. Das Einmalige an ihr ist die geistige Inspiration, die man erhält, wenn man merkt, dass jemand an einen glaubt“ (Ralph Waldo Emerson).

So unschätzbar und wichtig die Freundschaft auch ist, „das Erste, das der Mensch im Leben vorfindet, das Letzte, wonach er die Hand ausstreckt, das Kostbarste, was er im Leben besitzt, ist die Familie“ (Adolph Kolping). In diesem Sinne möchte ich mich zu guter Letzt, und damit den Höhepunkt meiner Danksagung erreichend, allerherzlichst bei meiner Familie bedanken, durch die mir dieses Studium überhaupt erst ermöglicht worden ist. Allerliebsten Dank meinen Eltern **Regina und Josef Schliwa** sowie meinen Brüdern **Andreas und Matthias Schliwa**. Dem Himmel sei Dank für all die Unterstützung, die ich durch euch erfahren habe. Ohne euch hätte ich dies alles nie geschafft! Darüber hinaus möchte ich an dieser Stelle auch meine Großeltern **Edeltraut und Günter Seidel** sowie **Ottilie und Georg Schliwa** nicht unerwähnt lassen. Auch wenn es ihnen leider nicht mehr vergönnt gewesen ist, meinen Abschluss mitzuerleben, haben sie ebenso einen erheblichen Teil zu meinem Werdegang beigetragen und werden mir stets in guter und liebevoller Erinnerung bleiben.

„Leider lässt sich eine wahrhafte Dankbarkeit mit Worten nicht ausdrücken,
und ebensowenig darf sie an eine unmittelbare Wiedervergeltung denken.“
Johann Wolfgang von Goethe

Contents

Abstract

Background: Temperature affects various metabolic and physiological processes in ectothermic animals, including auditory systems. The current study investigates the effects of temperature and acclimation time on hearing sensitivities in a eurythermal and a stenothermal fish possessing accessory hearing structures.

Methodology/ Principal Findings: Using the auditory evoked potential (AEP) recording technique thresholds from 0.1 to 4 kHz and peak latencies as well as peak-to-peak amplitudes of AEP waveforms in response to a click stimulus were determined in the goldfish *Carassius auratus* (eurythermal) and in the thorny catfish *Megalodoras uranoscopus* (stenothermal). Both species were tested at two different temperatures (*C. auratus*: 15 °C and 25 °C, *M. uranoscopus*: 22 °C and 30 °C) and acclimation stages (within 22 hours ('unacclimated') and within three to four weeks ('acclimated') after reaching the target temperature). A frequency-dependent increase in auditory sensitivity and a decrease of peak latencies was found in both species at higher temperatures independently of acclimation time. The change in hearing thresholds per degree Celsius was more pronounced in the stenothermal species. Peak-to-peak amplitudes showed different trends regarding temperature, acclimation and species.

Conclusions/ Significance: The data indicate that higher temperatures improved hearing (lower thresholds and shorter latencies), whereas acclimation did not affect hearing in both species. The latter data contradict previous findings in the eurythermal channel catfish *Ictalurus punctatus* in which acclimation improved hearing as temperatures increased. A comparison of changes in hearing sensitivity per degree Celsius of all seven species tested so far revealed no differences between eurythermal and stenothermal species.

Keywords: fish, *Carassius auratus*, *Megalodoras uranoscopus*, temperature, acclimation, eurythermal, stenothermal, hearing sensitivity, latency, amplitude, auditory evoked potentials (AEP)

1. Introduction

Depending on their habitat and geographic range, animals are confronted with different temperature regimes. In tropical regions they encounter constant high temperatures throughout the year whereas in temperate latitudes they have to deal with seasonal changes. While endothermic animals can maintain their body temperature at a constant level regardless of environmental temperature, ectothermic animals such as fish lack this ability, so their body temperature changes with the environmental temperature. Assuming they are adapted to different temperature regimes, eurythermal species can tolerate a wide temperature range, whereas stenothermal species have a narrower temperature tolerance range.

The goldfish *Carassius auratus* is a typical representative of eurythermal species, which can tolerate temperatures near the freezing point in winter, up to 30 °C in summer. Next to these seasonal changes, they also have to deal with daily temperature changes and temperature differences between different water depths. Depending on the size of the respective water system and other factors such as water flow, temperature may vary considerably. In contrast, the temperature of tropical waters is much more constant year-round mainly at about 25 °C. Thus, Amazonian species, such as the thorny catfish *Megalodoras uranoscopus*, are adjusted to a small temperature range.

However, temperature not only plays a role in determining the survivability of a species, but also affects diverse physiological and metabolic processes. Thus, in ectothermic taxa such as insects (Pires and Hoy, 1992; Franz and Ronacher, 2002), fish (Ladich, 2018), amphibians (Hubl et al., 1977; Gerhardt, 1978; Sun et al., 2019) and reptiles (Campbell, 1969; Martin and Bagby, 1972), temperature affects hearing as well as sound production.

In fishes, temperature affects hearing independently of their ability to vocalize and communicate acoustically (Ladich 2010; 2018). Therefore, hearing seems to be very important in other contexts besides communication, especially viewed from an evolutionary perspective (Popper and Fay, 1999; Ladich, 2000; 2014b). The reception of the “acoustic scene”, meaning the interception of sounds from predators, prey or conspecifics, or from abiotic origin, could be an important survival factor (Popper and Fay, 1999; Ladich, 2014a), which makes the study of temperature effects on hearing even more important during global warming.

Fishes possess inner ears to detect the particle motion component of sounds but lack outer or middle ears. The inner ears may be coupled to different accessory hearing structures, enabling fish to detect sound pressure which subsequently improves their hearing abilities (Ladich, 2014a; Ladich and Schulz-Mirbach, 2016). Otophysine fishes (minnows and carps, catfishes, tetras and piranhas), connect the anterior wall of their swim bladder, which oscillates in a sound pressure field, via a chain of auditory ossicles (Weberian ossicles) to the inner ear (Weber, 1819; 1820). Due to the enhanced hearing abilities all temperature studies (except one) were conducted in otophysines. An increase in auditory sensitivity with increasing temperature was found in eurythermal species (Channel catfish *Ictalurus punctatus*: Wysocki et al., 2009; Common carp *Cyprinus carpio* and Wels catfish *Silurus glanis*: Maiditsch and Ladich, 2014) as well as in stenothermal species (Pictus cat *Pimelodus pictus*: Wysocki et al., 2009; Southern striped raphael *Platydoras armatulus*: Papes and Ladich, 2011) and in one non-otophysine species (Alaska pollock *Gadus chalcogrammus*: Mann et al. 2009), after fishes had been acclimated to each experimental temperature for at least three or four weeks, except for the non-otophysine species, which was tested without any acclimation period. So far, only Wysocki et al. (2009) described that acclimation for a certain period improves auditory sensitivity. Temperature effects on latency in response to a single click stimulus were analyzed in two prior studies (Papes and Ladich, 2011; Maiditsch and Ladich 2014).

The aims of the current study are (1) to investigate effects of temperature on auditory sensitivities (thresholds, latency and amplitude), (2) whether it makes a difference how long fish are acclimated to particular temperatures (acclimation effect) and (3) if fishes adapted to different temperature regimes are affected differently. Otophysines were chosen due to their enhanced hearing abilities and to make comparisons to prior studies more meaningful. The eurythermal goldfish *C. auratus*, and the stenothermal species, the Amazonian catfish *M. uranoscopus*, were chosen. The AEP recording technique was applied to measure the auditory sensitivity of the experimental animals under different conditions. Hearing thresholds and peak latencies as well as peak-to-peak amplitudes of AEP waveforms in response to a click stimulus were measured. Furthermore, hearing was tested at two different temperatures and acclimation conditions, first directly after temperature was changed and second after an acclimation period of three to four weeks. This is the first study investigating temperature effects on peak-to-peak amplitudes of AEPs as well as acclimation effects on peak latencies. Finally, current data will be compared to species measured previously to find out, if changes in hearing thresholds per degree Celsius differ between species adapted to different temperature regimes.

2. Materials and Methods

2.1 Animals

For this study eight goldfish *C. auratus* (Linnaeus, 1758), and seven thorny catfish, *M. uranoscopus* (Eigenmann and Eigenmann, 1888), were used. All fish were obtained from a local pet store. *Carassius auratus* had a standard length of 70 - 86 mm and a body weight of 11.9 - 20.5 g. They were kept in a 100 × 50 × 50 cm glass tank at a baseline temperature of 20 ± 1 °C. The goldfish were divided into two groups in order to be able to distinguish them visually without any further markings. The two groups were separated from each other by a non-transparent plastic partition. *Megalodoras uranoscopus* had a standard length of 135 - 174 mm and a body weight of 38.7 - 95.6 g. They were kept in a 100 × 50 × 50 cm glass tank at a temperature of 26 ± 1 °C (baseline). The individual fish could be distinguished from one another by different markings on the dorsal spines. The sex of fishes was not determined because this was not possible without killing the animals.

2.2 Temperature regime

Before experiments started the fishes were acclimated to their baseline temperature (*C. auratus*: 20 ± 1 °C, *M. uranoscopus*: 26 ± 1 °C) for at least four weeks. The change of temperature took place in a smaller tank (70 × 50 × 40 cm) at a rate of 1 °C per day until the target temperature was reached (Fig. 1). The first hearing tests were carried out 16 to 22 hours after reaching the target temperature (‘unacclimated’). Afterwards the fish were acclimated to the target temperature for three to four weeks. After this time, the second hearing measurement was carried out at this temperature (‘acclimated’). A few days after resting, the temperature in the smaller tank was returned to the baseline temperature at 1 °C per day and the fish were again acclimated to their baseline temperature for at least four weeks. The same procedure was then repeated with the second target temperature, again in unacclimated and acclimated fish. In the first step, the temperatures were increased compared to the baseline (‘warm acclimation’) and in the second step they were decreased (‘cold acclimation’). The experimental temperatures were 25 °C and then 15 °C in *C. auratus* and 30 °C, followed by 22 °C in *M. uranoscopus* (Fig. 1). Each individual was tested at all four conditions.

All glass tanks were equipped with plastic tubes and artificial plants and had a bottom covered with sand. The glass tanks of *M. uranoscopus* were additionally equipped with roots and parts of clay pots to offer them additional hiding places. Only external filters were used. The water temperature was controlled by using cooling systems (Hailea HC-130A and Aqua

Medic Titan 500) and submersible heaters (Tetra HT100). A 12:12 hour light-dark cycle was maintained and fish were fed five days per week. *C. auratus* were fed flake food and *M. uranoscopus* were fed frozen chironomid larvae.

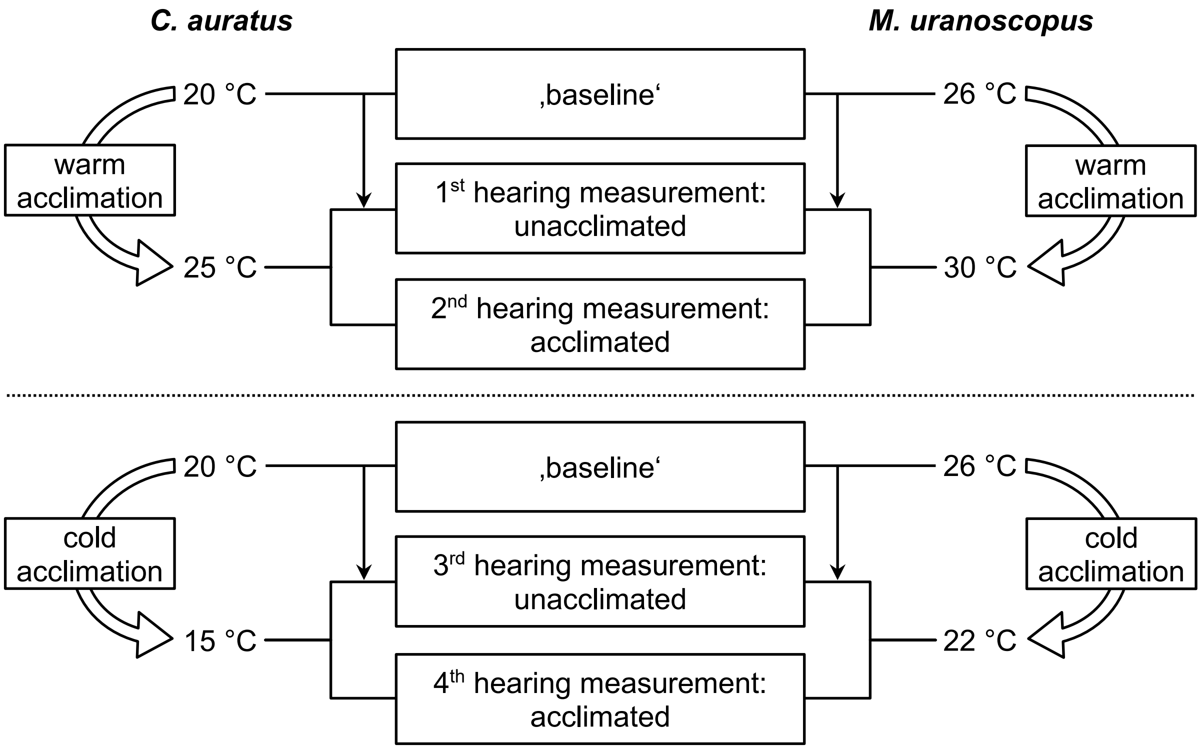

Fig. 1: Schematic diagram of the temperature regime used for *C. auratus* and *M. uranoscopus*. Temperatures were always changed by 1 °C per day. The periods of temperature changes are indicated by arrows. ‘Warm acclimation’ indicates an acclimation to a higher temperature, ‘cold acclimation’ to a lower temperature.

2.3 Auditory sensitivity measurements

The hearing thresholds, peak latencies to click stimuli and peak-to-peak amplitudes were measured using the auditory evoked potential (AEP) recording technique (Kenyon et al., 1998; Wysocki and Ladich, 2002). Test subjects were immobilized with an injection of Flaxedil (gallamine triethiodide; Sigma-Aldrich) before they were secured in a round plastic tub for the auditory sensitivity measurement. The used dosage of Flaxedil (*C. auratus*: 0.9 - 1.2 µg g^{-1} body mass, *M. uranoscopus*: 3.2 - 4.3 µg g^{-1} body mass) allowed the fish to continue breathing and also slight opercular movements during the measurement, but prevented an excessive myogenic noise level, which could interfere with the recordings. A respiration pipette coupled to a temperature-controlled gravity-fed circulation system was inserted into the fish´s mouth to aid breathing (Fig. 2). The round plastic tub (35 cm diameter, 15 cm height) was positioned on

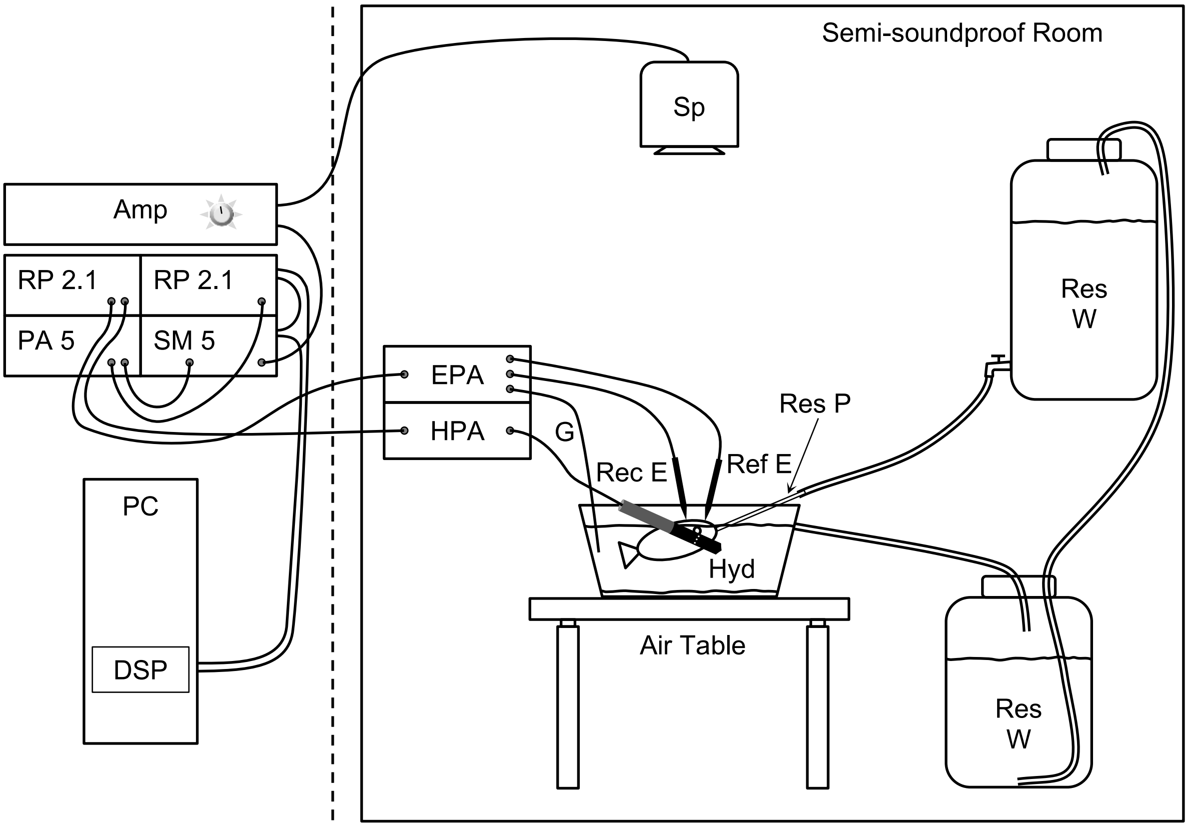

Fig. 2: Experimental setup for auditory sensitivity measurement using the auditory evoked potential (AEP) recording technique. Note that the fish and the hydrophone were not placed simultaneously in the tub as indicated here. Abbreviations: Amp - Amplifier, DSP - Digital sound processing card, EPA - Electrode preamplifier, G - Grounding cable, HPA - Hydrophone preamplifier, Hyd - Hydrophone, PA 5 - Programmable attenuator, PC - Personal computer, Rec E - Recording electrode, Ref E - Reference electrode, Res P - Respiration pipette, Res W - Respiration water reservoir, RP 2.1 - Realtime processor, SM 5 - Signal mixer, Sp - Speaker.

an air table (TCM Micro-g 63-540), which rested on a vibration-isolated concrete plate. It was lined on the inside with acoustically absorbent material and had a bottom covered with fine sand. The immobilized fish, which was attached to a tissue paper-lined mesh, was positioned in the tub so that the head of the fish was in the center of the tub, with the nape of the head at the water surface. The head was covered by a small piece of Kimwipes tissue paper to keep it moist, in order to ensure proper contact of electrodes during experiments. The electrodes consisted of silver wire (0.32 mm diameter) and were pressed firmly against the head and placed in the midline of the skull. The recording electrode was placed over the region of the medulla and the reference electrode cranially between the nares. Electrodes were connected to a preamplifier (Grass P-55, gain 10.000x, high-pass at 10 Hz, low-pass at 10 kHz). A ground electrode was placed in the water. The entire setup was enclosed in a walk-in semi-soundproof room which was constructed as a Faraday cage (interior dimensions: 3.2 × 3.2 × 2.4 m) (Fig. 2). Stimuli presentation and AEP-waveform recording were carried out using a modular rackmount system (TDT System 3) running TDT BioSig RP 4.4.11 Software.

2.3.1 Hearing threshold determination

Sound stimuli were generated using TDT SigGen RP software and fed through a power amplifier (Alesis RA 300) 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, 1, 2 and 4 kHz, presented in random order. All bursts were gated using a Blackman window. The stimuli were presented at opposite polarities (180° phase shifted) for each test condition and up to 1000 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 (Fig. 3).

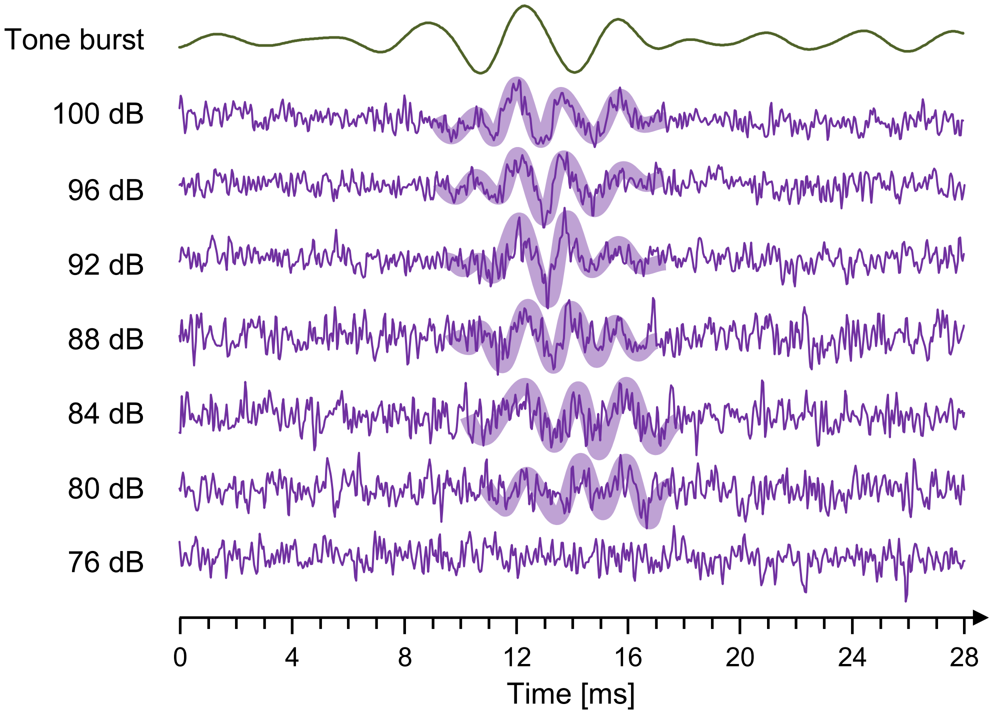

Fig. 3: AEPs of *M. uranoscopus* in response to a tone burst (300 Hz). Tone burst levels were reduced in 4 dB steps until the AEP waveform was no longer visible. The hearing threshold was found at 80 dB. All SPLs are given in dB re 1 µPa. AEPs are highlighted by transparent lines.

After the auditory sensitivity measurements (relative hearing thresholds at different frequencies and latency to click stimulus) a hydrophone (Brüel & Kjaer 8101, -184 dB re 1 V/µPa) was placed at the same position as the fish in order to determine absolute SPLs of hearing thresholds at different frequencies (Fig. 2). Using Bio-Sig RP, the RMS voltage of the largest (i.e., center) sinusoid of a particular tone-burst recording was determined. This RMS voltage was then used to calculate the absolute SPL re 1 µPa based on the sensitivity of the hydrophone and the amplification factor of the hydrophone amplifier (100x).

2.3.2 Peak latency and peak-to-peak amplitudes measurements

The latency to single click stimuli was measured as described in Wysocki and Ladich (2002). The auditory threshold for the click stimulus was determined using the method described above for tone bursts. Afterwards the stimulus was presented 28 dB above the hearing threshold to measure the peak latencies and amplitudes. In this case, the stimulus was presented three times each at opposite polarities (180° phase shifted) for each test condition and 1000 AEPs each were averaged by the BioSig RP software to eliminate stimulus artefacts and minimize further noise effects. The AEPs recorded in response to the click stimulus showed a particular waveform with six constant prominent peaks in both species. The upwards directed peaks were denominated with P for positive and the downwards directed peaks with N for negative, each followed by ascending numbers. Finally, the peak latency was determined as the time between the onset of the click stimulus and the particular constant peaks (Fig. 4A). Peak-to-peak amplitudes were defined as the voltage difference between P2 and N1 or N2 (depending on which of the two peaks was larger) (Fig. 4B).

2.4 Statistical Analyses

All data were tested for normal distribution using the Shapiro-Wilk test. If data were normally distributed parametric test were chosen otherwise nonparametric tests were carried out. Since fish were recognized individually dependent samples test could be calculated (paired t-test, Friedman-test, Repeated measures-(RM-)ANOVA). Auditory thresholds in each species were compared by a two-factorial ANOVA using a general linear model (GLM) with temperature and frequency as the required factors. The temperature or acclimation (unacclimated versus acclimated) factor alone indicates overall differences in sensitivity between temperatures or acclimation and in combination with the frequency factor if different tendencies exist at different frequencies of the audiograms. In order to reveal differences between thresholds at each frequency Friedman-tests were calculated followed by Dunn-

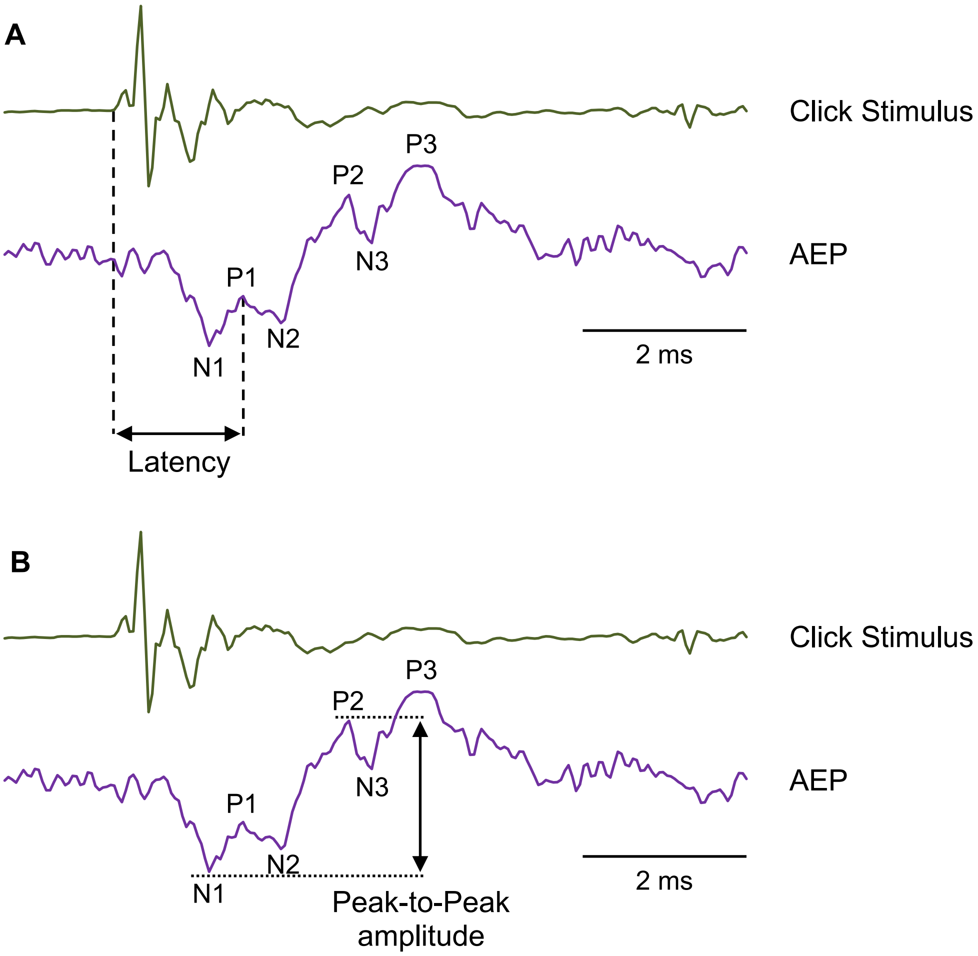

Fig. 4: Oscillograms of the click stimulus and the AEP waveform of one specimen of *M. uranoscopus* in response to the stimulus which was presented 28 dB above hearing threshold. All six peaks used for latency measurements are shown (P - positive, N - negative). (A) Peak latency was measured from the onset of the click stimulus to the particular peaks. (B) Peak-to-peak amplitude was measured between P2 and N1 or N2 (depending on which of the two peaks was larger).

Bonferroni post hoc tests. The extent of the temperature-induced change in auditory sensitivity per degree Celsius was compared between species (only after acclimation) by a two-factorial ANOVA, followed by a t-test to compare the thresholds at each frequency.

Differences between peak latencies and peak-to-peak amplitudes measured at different temperatures or acclimation conditions were calculated using a Friedman-test followed by a Dunn-Bonferroni post hoc test. Relationships between the temperature-induced decreases in latency in acclimated fish with the position of particular peaks were calculated using Spearman-Rho´s correlation coefficient. All statistical tests were run using IBM SPSS Statistics Version 26. The significance level was set at $p \leq 0.05$.

2.5 Ethical considerations

All applicable national and institutional guidelines for the care and use of animals were followed (permit numbers BMWFW-66.006/0035-WF/V/3b/2017 by the Austrian Federal Ministry of Science, Research and Economy; Animal Ethics and Experimental Board, Faculty of Life Science 2017-010). All animals were allowed to live in the animal care facilities of the Biocenter after the experiments. Experimental conditions had no lasting impacts on animals.

3. Results

The body weights of the individuals of the two species did not differ significantly over the experimental period of this study (*C. auratus*: RM-ANOVA: $F_{1,8} = 5.19$, n.s.; *M. uranoscopus*: RM-ANOVA: $F_{1,6} = 1.02$, n.s.).

3.1 Auditory sensitivities

3.1.1 *Carassius auratus*

Best hearing sensitivity was found between 0.3 and 1 kHz, with a rapid decrease at higher frequencies for all four test conditions (Fig. 5, Tab. 1). The hearing sensitivity was higher at the higher temperature in unacclimated and acclimated goldfish (two-factorial ANOVA: unacclimated fish: $F_{1,84} = 102.41$, $p < 0.001$; acclimated fish: $F_{1,84} = 76.57$, $p < 0.001$).

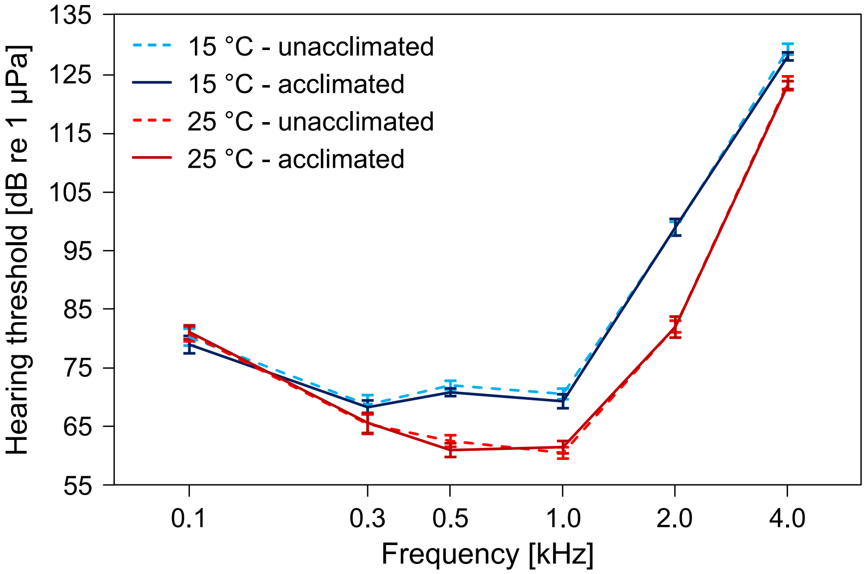

Fig. 5: Auditory evoked potential audiograms (mean ± S.E.) of unacclimated and acclimated *C. auratus* at 15 °C and 25 °C. N = 8.

Tab. 1: Mean (± S.E.) hearing thresholds of unacclimated and acclimated *C. auratus* at 15 °C and 25 °C. N = 8.

	0.1 kHz	**0.3 kHz**	**0.5 kHz**	**1 kHz**	**2 kHz**	**4 kHz**
15 °C unacclimated	80.5 ± 1.4	69.0 ± 1.6	72.3 ± 0.8	70.9 ± 0.9	99.0 ± 1.2	129.6 ± 0.9
15 °C acclimated	79.3 ± 1.5	68.6 ± 1.2	71.2 ± 0.7	69.6 ± 1.2	99.3 ± 1.4	128.4 ± 0.7
25 °C unacclimated	81.1 ± 1.3	65.7 ± 1.7	62.8 ± 1.0	60.8 ± 1.0	82.4 ± 1.0	123.8 ± 1.2
25 °C acclimated	81.4 ± 1.2	65.9 ± 1.7	61.3 ± 1.2	61.8 ± 1.1	82.3 ± 1.8	123.5 ± 0.7

Tab. 2: Comparison of hearing thresholds of *C. auratus* at the individual frequencies, tested under four different conditions, namely at 15 °C and 25 °C, both in unacclimated (unacc.) and acclimated (acc.) goldfish.

Frequency	**Friedman-test**	**Unacclimated 15 vs. 25 °C**	**Acclimated 15 vs. 25 °C**	**15 °C unacc. vs. acc.**	**25 °C unacc. vs. acc.**
0.1 kHz	$\chi^2 = 1.405$, df = 3, p = 0.704	---*	---*	---*	---*
0.3 kHz	$\chi^2 = 5.880$, df = 3, p = 0.118	---*	---*	---*	---*
0.5 kHz	$\chi^2 = 19.769$, df = 3, **p < 0.001**	**p < 0.05**	**p < 0.05**	n.s.	n.s.
1 kHz	$\chi^2 = 20.462$, df = 3, **p < 0.001**	**p < 0.01**	p = 0.071	n.s.	n.s.
2 kHz	$\chi^2 = 19.633$, df = 3, **p < 0.001**	**p < 0.01**	**p < 0.05**	n.s.	n.s.
4 kHz	$\chi^2 = 17.250$, df = 3, **p < 0.001**	**p < 0.05**	**p < 0.05**	n.s.	n.s.
*Multiple comparisons were not carried out because the overall test revealed that there are no differences between the four test conditions.					

Additionally, there was a significant interaction between temperature and frequency when comparing hearing curves in unacclimated as well as acclimated goldfish (15 °C versus 25 °C). The improvement in hearing sensitivity was more pronounced at higher frequencies (unacclimated fish: $F_{5,84} = 11.05$, $p < 0.001$; acclimated fish: $F_{5,84} = 12.24$, $p < 0.001$). Acclimation time did not affect hearing sensitivities in goldfish. At both temperatures there were no significant differences in hearing sensitivity between unacclimated and acclimated goldfish (two-factorial ANOVA: 15 °C: $F_{1, 84} = 1.41$, n.s.; 25 °C: $F_{1, 84} = 0.01$, n.s.). A subsequent Friedman-test, in which the hearing thresholds of all four test conditions were compared at each frequency, revealed that there were differences in hearing sensitivity at 0.5, 1, 2 and 4 kHz. A Dunn-Bonferroni post hoc test showed that differences exist between temperatures but not between acclimation conditions (for statistical details see Tab. 2).

3.1.2 *Megalodoras uranoscopus*

The best hearing sensitivity was found at 1 kHz at all four test conditions. Sensitivities decreased towards lower and higher frequencies at both temperatures (Fig. 6, Tab. 3). Auditory thresholds increased significantly with temperature in unacclimated (two-factorial ANOVA: $F_{1, 72} = 90.77$, $p < 0.001$) as well as acclimated catfish (two-factorial ANOVA: $F_{1, 72} = 152.29$, $p < 0.001$). Additionally, the temperature-induced changes in hearing sensitivity showed different trends at different frequencies and were more pronounced at higher frequencies (unacclimated: $F_{5, 72} = 9.08$, $p < 0.001$; acclimated: $F_{5, 72} = 8.90$, $p < 0.001$). There was no significant difference in hearing sensitivity between unacclimated and acclimated catfish when measured at the same experimental temperature (two-factorial ANOVA: 22 °C: $F_{1, 72} = 0.004$, n.s.; 30 °C: $F_{1, 72} = 1.12$, n.s.). A subsequent Friedman-test, in which the hearing thresholds of all four test conditions were compared at the individual frequencies, revealed that differences in hearing sensitivities were found at all frequencies except 0.1 kHz. A Dunn-Bonferroni post hoc test showed that differences generally exist between temperatures but not between acclimation conditions (for statistical details see Tab. 4).

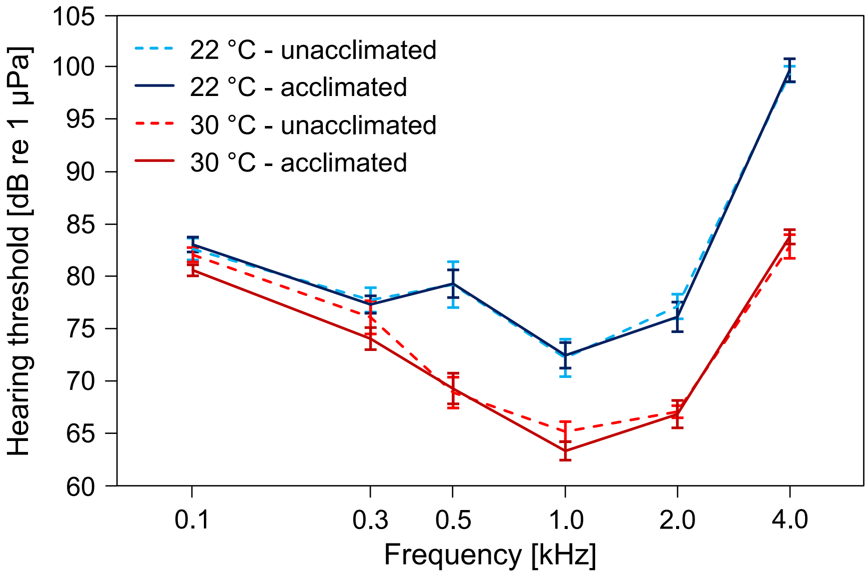

Fig. 6: Auditory evoked potential audiograms (mean ± S.E.) of unacclimated and acclimated *M. uranoscopus* at 22 °C and 30 °C. N = 7.

Tab. 3: Mean (± S.E.) hearing thresholds of unacclimated and acclimated *M. uranoscopus* at 22 °C and 30 °C. N = 7.

	0.1 kHz	**0.3 kHz**	**0.5 kHz**	**1 kHz**	**2 kHz**	**4 kHz**
22 °C unacclimated	82.6 ± 1.0	77.8 ± 1.2	79.2 ± 2.2	72.3 ± 1.8	77.1 ± 1.2	99.1 ± 0.7
22 °C acclimated	83.0 ± 0.7	77.3 ± 0.8	79.3 ± 1.3	72.5 ± 1.2	76.1 ± 1.4	99.5 ± 1.1
30 °C unacclimated	82.0 ± 0.7	76.1 ± 1.6	69.0 ± 1.5	65.3 ± 0.9	67.2 ± 0.6	82.8 ± 1.1
30 °C acclimated	80.6 ± 0.5	74.1 ± 1.0	69.4 ± 1.5	63.4 ± 0.9	66.9 ± 1.3	83.7 ± 0.7

Tab. 4: Comparison of hearing thresholds of *M. uranoscopus* at the individual frequencies, tested under four different conditions, namely at 22 °C and 30 °C, both in unacclimated (unacc.) and acclimated (acc.) catfish.

Frequency	**Friedman-test**	**Unacclimated 22 vs. 30 °C**	**Acclimated 22 vs. 30 °C**	**22 °C unacc. vs. acc.**	**30 °C unacc. vs. acc.**
0.1 kHz	$\chi^2 = 8.652$, df = 3, **$p < 0.05$**	n.s.	p = 0.058	n.s.	n.s.
0.3 kHz	$\chi^2 = 9.522$, df = 3, **$p < 0.05$**	n.s.	**$p < 0.05$**	n.s.	n.s.
0.5 kHz	$\chi^2 = 18.134$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.05$**	n.s.	n.s.
1 kHz	$\chi^2 = 19.174$, df = 3, **$p < 0.001$**	p = 0.178	**$p < 0.01$**	n.s.	n.s.
2 kHz	$\chi^2 = 18.304$, df = 3, **$p < 0.001$**	**$p < 0.01$**	p = 0.058	n.s.	n.s.
4 kHz	$\chi^2 = 17.261$, df = 3, **$p < 0.001$**	**$p < 0.01$**	p = 0.058	n.s.	n.s.

3.1.3 Comparison of hearing sensitivity between *C. auratus* and *M. uranoscopus*

Both species were measured at different temperature ranges (10 °C versus 8 °C). Therefore, both species were compared by calculating the change in threshold per degree Celsius in acclimated animals. The difference in hearing thresholds per degree Celsius between the two experimental temperatures was highest at 2 kHz (1.7 dB) in *C. auratus* and at 4 kHz (2 dB) in *M. uranoscopus* (Fig. 7). Species differed significantly in the change in auditory sensitivity per 1 °C (two-factorial ANOVA: $F_{1,78} = 11.596$, $p < 0.01$). The change was more pronounced in the stenothermal catfish, in particular at higher frequencies (two-factorial

ANOVA: $F_{5, 78} = 6.295$, $p < 0.001$). The extent of the threshold change differed between the two species significantly only at the lowest (0.1 kHz) and highest (4 kHz) frequency level (t-test: 0.1 kHz: $t = -2.391$, $df = 13$, $p < 0.05$; 4 kHz: $t = -7.610$, $df = 13$, $p < 0.001$).

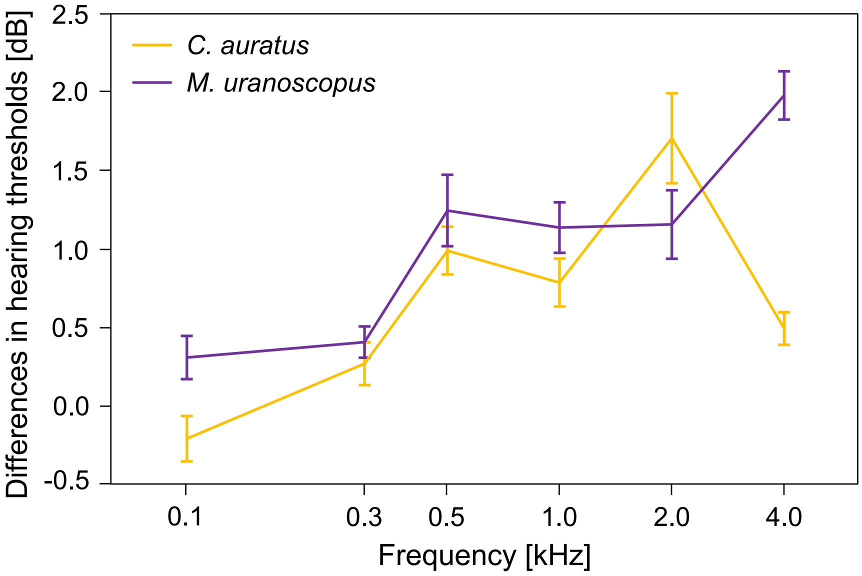

Fig. 7: Mean (± S.E.) differences in hearing thresholds per degree Celsius in acclimated *C. auratus* (15 °C vs. 25 °C) and acclimated *M. uranoscopus* (22 °C vs. 30 °C).

3.2 Peak latencies in response to single clicks

3.2.1 *Carassius auratus*

In all fish tested a similar AEP waveform with six constant prominent peaks could be found in response to the click stimulus at both temperatures tested. Due to the high similarity of individual AEPs, an average AEP was calculated (Fig. 8). Comparing the four test conditions, there were slight differences between peak latencies and amplitudes. In particular, the first negative and positive peaks (N1 and P1) were only faintly visible as deflections in individuals at 15 °C. While the first three deflections (N1, P1 and N2) were found below the baseline, the other three deflections (P2, N3 and P3) were located above the baseline.

The peak latency, namely the time between the onset of the click stimulus and the particular peaks of the AEP waveforms, differed significantly between the four testing conditions for all of the six peaks analyzed. In detail, the peak latencies decreased at the higher temperature whereas no differences were observed between unacclimated and acclimated goldfish (Fig. 8, Fig. 9, Tab. 5, for statistical details see Tab. 6). Furthermore, the decrease in peak latency at the higher temperature increased over time and was largest at the third positive peak (P3) ($r_s = 0.899$, $n = 6$, $p < 0.05$) (Fig. 10).

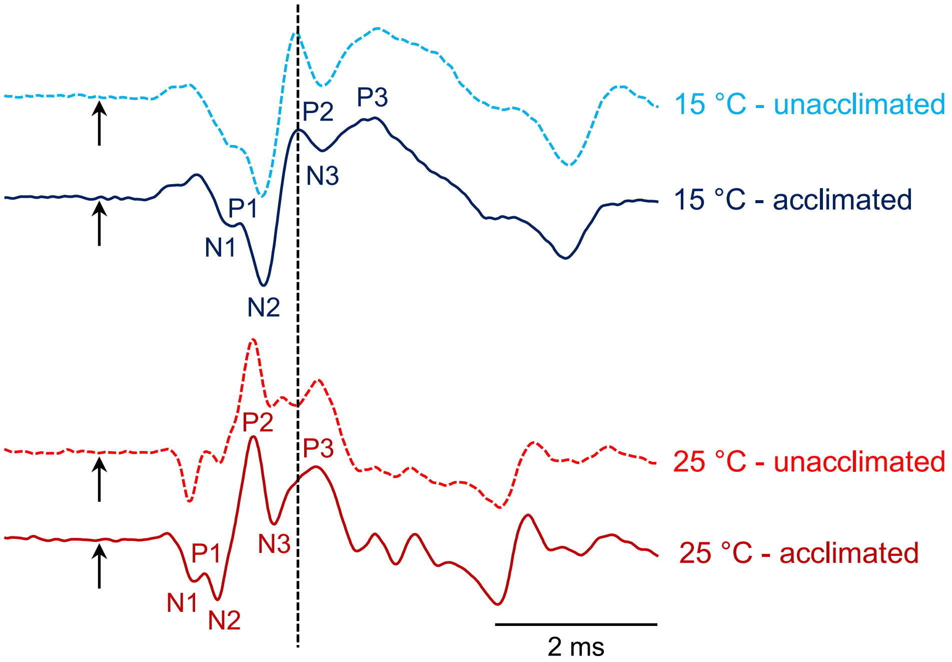

Fig. 8: Averaged AEP waveforms of eight unacclimated and acclimated *C. auratus* in response to a click stimulus at 15 °C and 25 °C. The click stimulus was presented 28 dB above hearing threshold. Only peaks used for the latency measurements were labelled. The arrows indicate the onset of the click stimulus. The vertical dashed line indicates the position of P2 at 15 °C (acclimated) compared to 25 °C. Note that the amplitudes of the individual test conditions are shown here not true to scale. See Fig. 14 for a comparison of amplitudes.

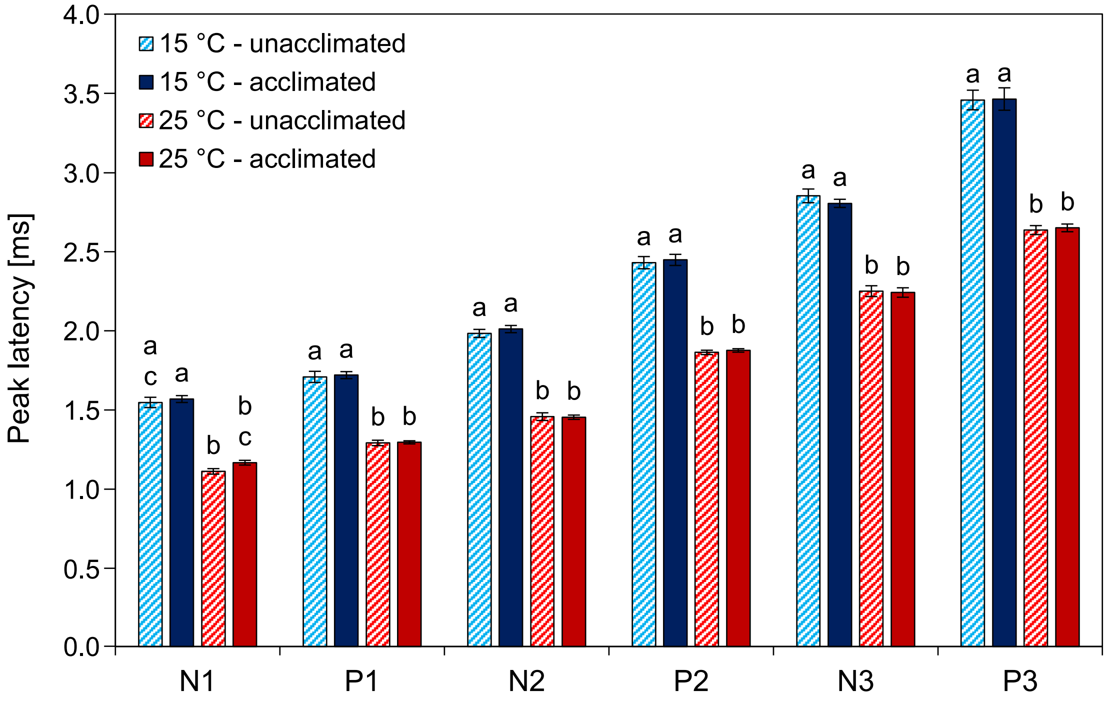

Fig. 9: Mean (± S.E.) peak latencies in response to the click stimulus of the AEP waveforms in *C. auratus*. N = 8. Different letters above bars indicate significant differences between peak latencies.

Tab. 5: Mean (± S.E.) peak latencies in response to single clicks of unacclimated and acclimated *C. auratus* at 15 °C and 25 °C, as well as mean (± S.E.) peak latency differences between both temperatures. The click stimulus was presented 28 dB above hearing threshold. N = 8. Peak latencies are given in ms.

Peak latencies	**N1**	**P1**	**N2**	**P2**	**N3**	**P3**
15 °C unacclimated	1.54 ± 0.03	1.71 ± 0.04	1.98 ± 0.03	2.43 ± 0.04	2.85 ± 0.04	3.46 ± 0.06
15 °C acclimated	1.57 ± 0.02	1.72 ± 0.02	2.01 ± 0.02	2.45 ± 0.04	2.80 ± 0.03	3.46 ± 0.07
25 °C unacclimated	1.11 ± 0.02	1.29 ± 0.02	1.45 ± 0.02	1.86 ± 0.01	2.25 ± 0.03	2.63 ± 0.03
25 °C acclimated	1.16 ± 0.01	1.29 ± 0.01	1.45 ± 0.01	1.87 ± 0.01	2.24 ± 0.03	2.65 ± 0.02
15 °C vs. 25 °C acclimated	0.40 ± 0.03	0.43 ± 0.03	0.56 ± 0.03	0.57 ± 0.04	0.56 ± 0.03	0.82 ± 0.09

Tab. 6: Comparison of peak latencies (N1-P3) in response to a click stimulus of *C. auratus* (see Fig. 8), measured under four different conditions, namely at 15 °C and 25 °C, in unacclimated (unacc.) and acclimated (acc.) *C. auratus*. Pair-wise comparisons were calculated using Dunn-Bonferroni post hoc tests.

Peak	**Friedman-test**	**Unacclimated 15 vs. 25 °C**	**Acclimated 15 vs. 25 °C**	**15 °C unacc. vs. acc.**	**25 °C unacc. vs. acc.**
N1	$\chi^2 = 21.545$, df = 3, **$p < 0.001$**	**$p < 0.01$**	**$p < 0.05$**	n.s.	n.s.
P1	$\chi^2 = 19.846$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.01$**	n.s.	n.s.
N2	$\chi^2 = 20.526$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.01$**	n.s.	n.s.
P2	$\chi^2 = 20.211$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.05$**	n.s.	n.s.
N3	$\chi^2 = 20.299$, df = 3, **$p < 0.001$**	**$p < 0.01$**	**$p < 0.05$**	n.s.	n.s.
P3	$\chi^2 = 19.500$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.05$**	n.s.	n.s.

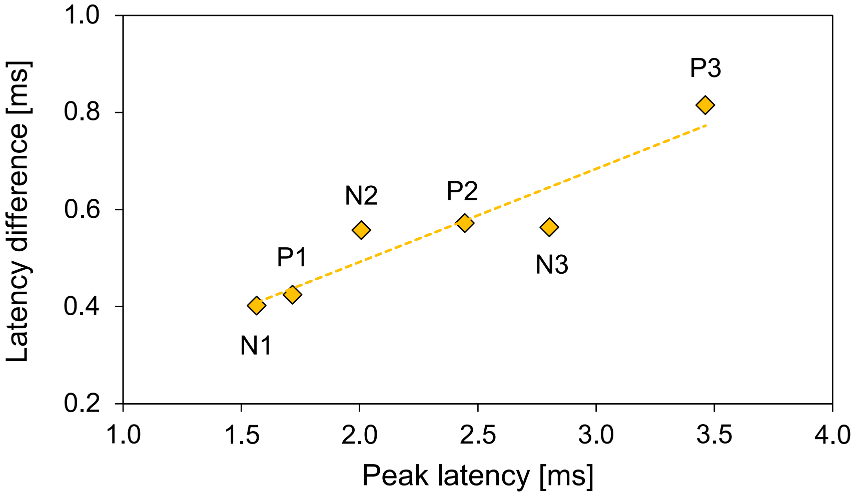

Fig. 10: Correlation between mean latencies of the individual peaks at 15 °C and mean differences in peak latencies between 15 °C and 25 °C in acclimated *C. auratus*. Latency difference = 0.192 * peak latency + 0.108; $r^2 = 0.881$.

3.2.2 *Megalodoras uranoscopus*

The AEPs of *M. uranoscopus* in response to a click stimulus showed a similar waveform as those of *C. auratus*, but there are some differences between the relative height and the distance between the individual peaks in the two species, but also between the different test conditions (temperatures). Because of the high similarity between individual AEPs an averaged AEP for each test condition could be obtained (Fig. 11). There were six constant prominent peaks, with the first three deflections (N1, P1 and N2) below and the following three (P2, N3 and P3) located above the baseline. In general, the individual peaks at the higher temperature were not quite as pronounced as at the lower temperature.

Latencies differed significantly between the four testing conditions for all of the six peaks analyzed. Similar to *C. auratus*, peak latencies decreased at the higher temperature (except in N1) but did not differ between unacclimated and acclimated catfish (Fig. 11, Fig. 12, Tab. 7, for statistical details see Tab. 8). Furthermore, the temperature-induced decrease in latency of different peaks was more pronounced at the latter peaks ($r_s = 1.000$, $n = 6$, $p < 0.01$) (Fig. 13).

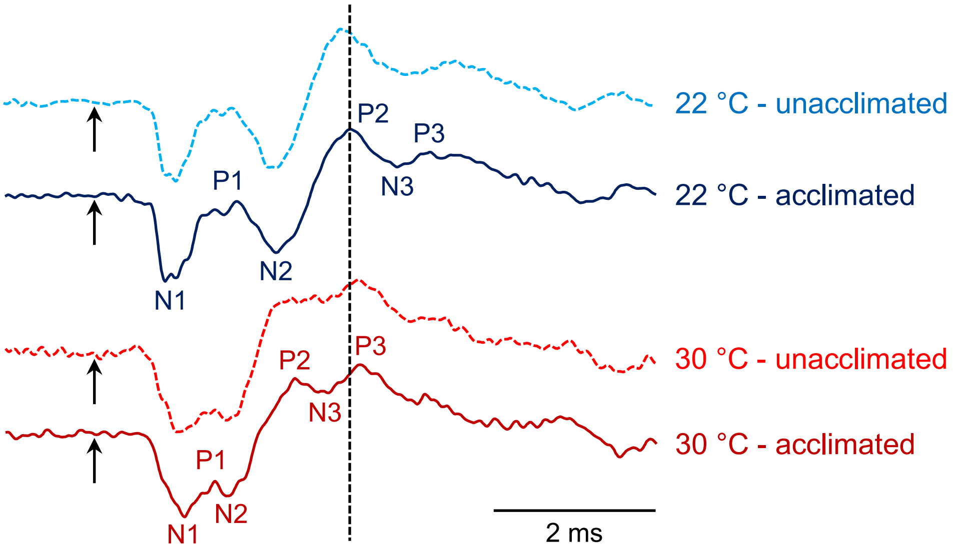

Fig. 11: Averaged AEP waveforms of seven unacclimated and acclimated *M. uranoscopus* in response to a click stimulus at 22 °C and 30 °C. The click stimulus was presented 28 dB above hearing threshold. Only peaks used for the latency measurements were labelled. The arrows indicate the onset of the click stimulus. The vertical dashed line indicates the position of P2 at 22 °C (acclimated) compared to 30 °C. Note that the amplitudes of the individual test conditions are shown here not true to scale. See Fig. 15 for a comparison of amplitudes.

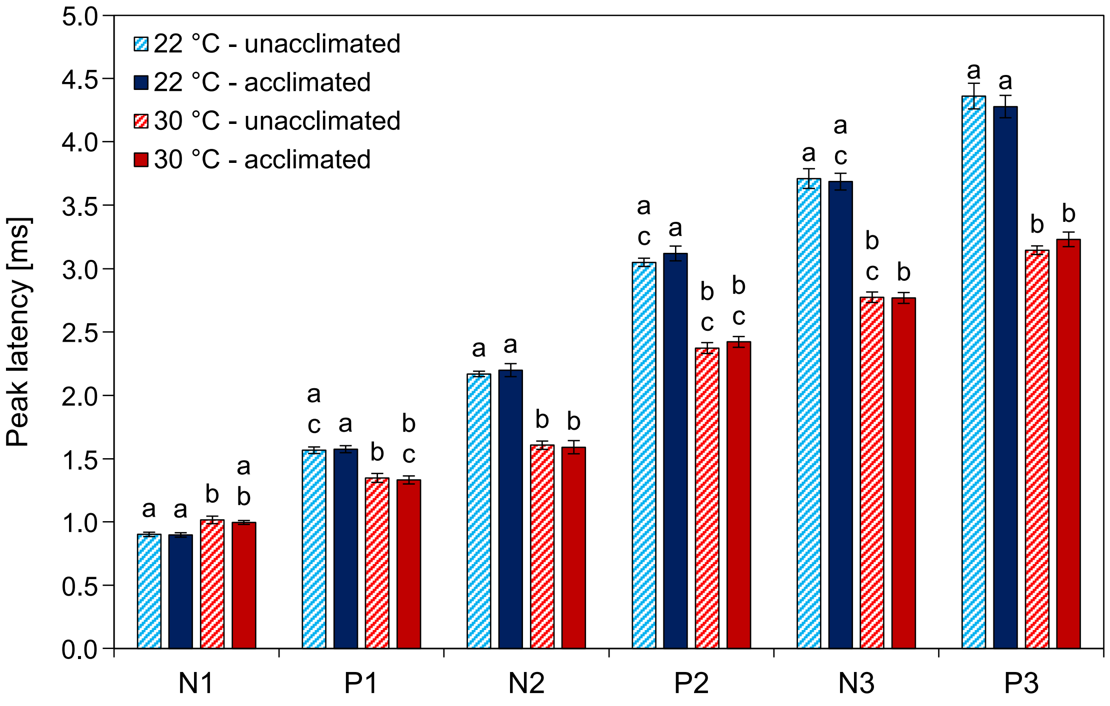

Fig. 12: Mean (± S.E.) peak latencies in response to the click stimulus of the AEP waveforms in *M. uranoscopus*. N = 7. Different letters above bars indicate significant differences between latencies.

Tab. 7: Mean (± S.E.) peak latencies in response to single clicks of unacclimated and acclimated *M. uranoscopus* at 22 °C and 30 °C, as well as mean (± S.E.) peak latency differences between both temperatures. The click stimulus was presented 28 dB above hearing threshold. N = 7. Peak latencies are given in ms.

Peak latencies	**N1**	**P1**	**N2**	**P2**	**N3**	**P3**
22 °C unacclimated	0.90 ± 0.02	1.57 ± 0.03	2.17 ± 0.02	3.05 ± 0.03	3.71 ± 0.08	4.36 ± 0.10
22 °C acclimated	0.90 ± 0.02	1.57 ± 0.03	2.20 ± 0.05	3.12 ± 0.06	3.69 ± 0.07	4.28 ± 0.09
30 °C unacclimated	1.02 ± 0.03	1.35 ± 0.04	1.61 ± 0.03	2.37 ± 0.04	2.77 ± 0.04	3.15 ± 0.03
30 °C acclimated	1.00 ± 0.02	1.33 ± 0.03	1.59 ± 0.05	2.42 ± 0.04	2.77 ± 0.04	3.23 ± 0.06
22 °C vs. 30 °C acclimated	-0.10 ± 0.02	0.24 ± 0.04	0.61 ± 0.06	0.70 ± 0.07	0.92 ± 0.08	1.05 ± 0.09

Tab. 8: Comparison of peak latencies (N1-P3) in response to a click stimulus of *M. uranoscopus* (see Fig. 11), measured under four different conditions, namely at 22 °C and 30 °C, in unacclimated (unacc.) and acclimated (acc.) *M. uranoscopus*. Pair-wise comparisons were calculated using Dunn-Bonferroni post hoc tests.

Peak	**Friedman-test**	**Unacclimated 22 vs. 30 °C**	**Acclimated 22 vs. 30 °C**	**22 °C unacc. vs. acc.**	**30 °C unacc. vs. acc.**
N1	$\chi^2 = 16.313$, df = 3, **$p < 0.001$**	**$p < 0.05$**	$p = 0.078$	n.s.	n.s.
P1	$\chi^2 = 18.134$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.05$**	n.s.	n.s.
N2	$\chi^2 = 17.294$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.05$**	n.s.	n.s.
P2	$\chi^2 = 17.435$, df = 3, **$p < 0.001$**	$p = 0.058$	**$p < 0.01$**	n.s.	n.s.
N3	$\chi^2 = 17.776$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.05$**	n.s.	n.s.
P3	$\chi^2 = 16.886$, df = 3, **$p < 0.001$**	**$p < 0.05$**	**$p < 0.05$**	n.s.	n.s.

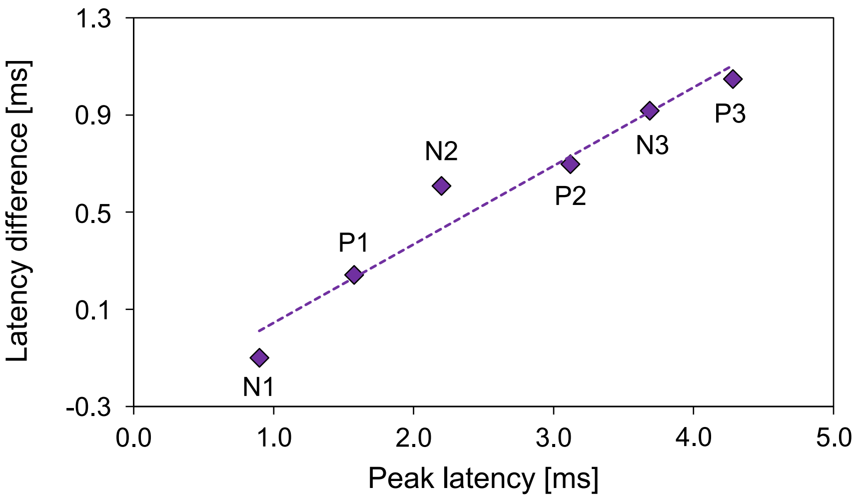

Fig. 13: Correlation between mean latencies of the individual peaks at 22 °C and mean differences in peak latencies between 22 °C and 30 °C in acclimated *M. uranoscopus*. Latency difference = 0.323 * peak latency - 0.279; $r^2 = 0.948$.

3.3 Peak-to-peak amplitudes in response to single clicks

3.3.1 *Carassius auratus*

Peak-to-peak amplitudes between P2 and N2 (N1) in the AEPs of goldfish differed between the four test conditions (Friedman-test: $\chi^2 = 8.550$, df = 3, $p < 0.05$). Therefore, the amplitude seems to be higher in unacclimated goldfish at 25 °C than in the three other test conditions (Fig.14), however, this could not be confirmed in subsequent pairwise comparisons (Dunn-Bonferroni post hoc test).

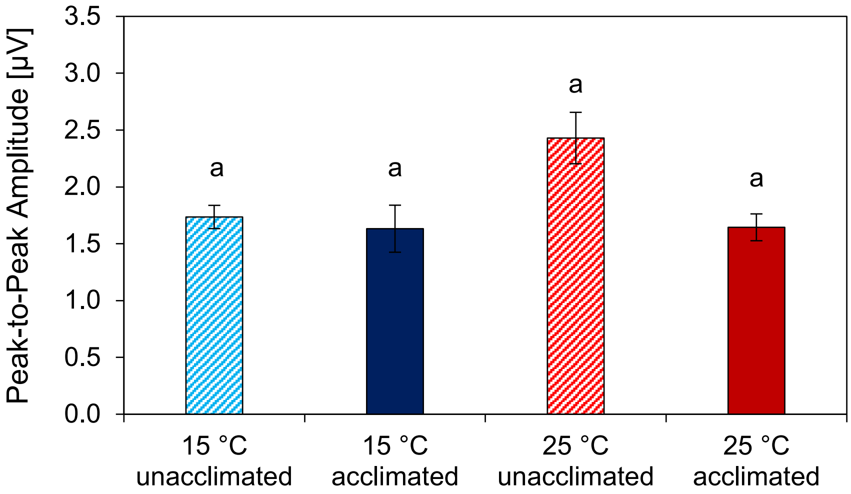

Fig. 14: Mean (± S.E.) peak-to-peak amplitudes of AEP waveforms in response to a click stimulus 28 dB above hearing threshold of unacclimated and acclimated *C. auratus* at 15 °C and 25 °C. N = 8. Different letters above bars indicate significant differences between amplitudes.

3.3.2 *Megalodoras uranoscopus*

In catfish, there was a difference in peak-to-peak amplitudes between P2 and N2 (N1) among the four test conditions (Friedman-test: $\chi^2 = 10.217$, df = 3, $p < 0.05$). Nevertheless, no effect of acclimation as well as no temperature effect was found (Fig. 15). The only significant difference was found between 15 °C unacclimated and 25 °C acclimated catfish (Dunn-Bonferroni post hoc test: $p < 0.05$).

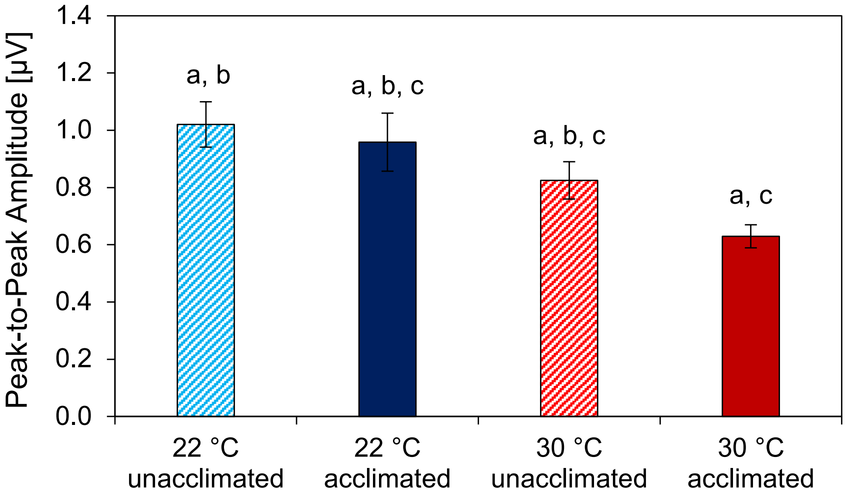

Fig. 15: Mean (± S.E.) peak-to-peak amplitudes of AEP waveforms in response to a click stimulus 28 dB above hearing threshold of unacclimated and acclimated *M. uranoscopus* at 22 °C and 30 °C. N = 7. Different letters above bars indicate significant differences between amplitudes.

4. Discussion

An increase in ambient temperature results in higher hearing sensitivity in *C. auratus* and *M. uranoscopus*. This is not only expressed in a lowered hearing threshold (especially at higher frequencies), but also in a shorter latency to a click stimulus. The latter constitutes a higher temporal resolution of pulsed sounds (Papes and Ladich, 2011).

Similar temperature effects on hearing were also found in other ectothermal animals such as insects, amphibians and reptiles. For example, locusts *Locusta migratoria* showed an improved temporal resolution of auditory receptors and local (second and third order) interneurons at higher temperatures (Franz and Ronacher, 2002). Frogs (*Rana ridibunda ridibunda* and *Bombina variegata variegata*) revealed a downshift of hearing thresholds and a shift in best heard frequency with increasing temperature (Hubl et al., 1977). Respectively in the little torrent frog *Amolops torrentis* lower thresholds and shorter latencies were found at warm compared to cold temperatures, but only for relatively low frequencies (1.8 - 2.6 kHz), while there was no significant temperature-induced hearing sensitivity change at higher frequencies (3.0 - 7.0 kHz) (Sun et al., 2019). Furthermore, several species of lizards (e.g. *Gekko gecko*, *Uma inornata*) also showed decreasing hearing thresholds (in particular at frequencies with highest hearing sensitivity) and shortened latencies with increasing temperature (Campbell, 1969).

4.1 Effects of temperature on hearing thresholds in steno- and eurythermal species

Several studies have shown an effect of temperature on hearing in fishes. Dudok van Heel (1956) found that in the Eurasian minnow *Phoxinus phoxinus* the detectable frequency range increased with increasing temperature. Measuring neural responses of auditory nerve fibers in the goldfish *C. auratus*, Fay and Ream (1992) were able to show that temperature also affected auditory sensitivity. Specifically, they described increased sensitivity and responsiveness, as well as a shift in best frequency with increasing temperature. Subsequent studies including the present one using the AEP recording technique found temperature effects on hearing sensitivity in all otophysine species investigated. The auditory sensitivity increased with temperature after fish were acclimated three to four weeks to testing temperatures in particular at frequencies above 0.5 kHz (Fig. 16A). The increase in sensitivity per degree Celsius differed between frequencies and species. For instance, change in sensitivity at 4 kHz ranged from almost 0 dB (*C. carpio*) to 2.8 dB per degree Celsius (*I. punctatus*). This might also be due to the already high thresholds at higher frequencies in *C. carpio* (Maiditsch and Ladich, 2014). Interestingly, Mann et al. (2009) observed a similar trend in *G. chalcogrammus*, the only non-otophysine species investigated so far.

In the current study, the stenothermal *M. uranoscopus* showed a more pronounced change in hearing threshold per degree Celsius than the eurythermal *C. auratus*. In contrast, Wysocki et al. (2009) found that the eurythermal catfish *I. punctatus* was more affected by a temperature change than the stenothermal *P. pictus* per degree Celsius, which raises the question if stenothermal fish differ from eurythermal species in adaptation to different temperatures. A comparison of all seven species tested so far, revealed that the shift in auditory sensitivity due to a change in temperature does not differ significantly between species adapted to different temperatures (two-factorial ANOVA: $F_{1,29} = 0.313$, $p = 0.580$) (Fig. 16B). This is in contrast to Wysocki et al. (2009) who assumed that eurythermal fish species which are exposed to a wider range of temperatures should show more resistance to temperature changes in their sensory system than stenothermal animals. Thus, the interspecific analysis does not support the

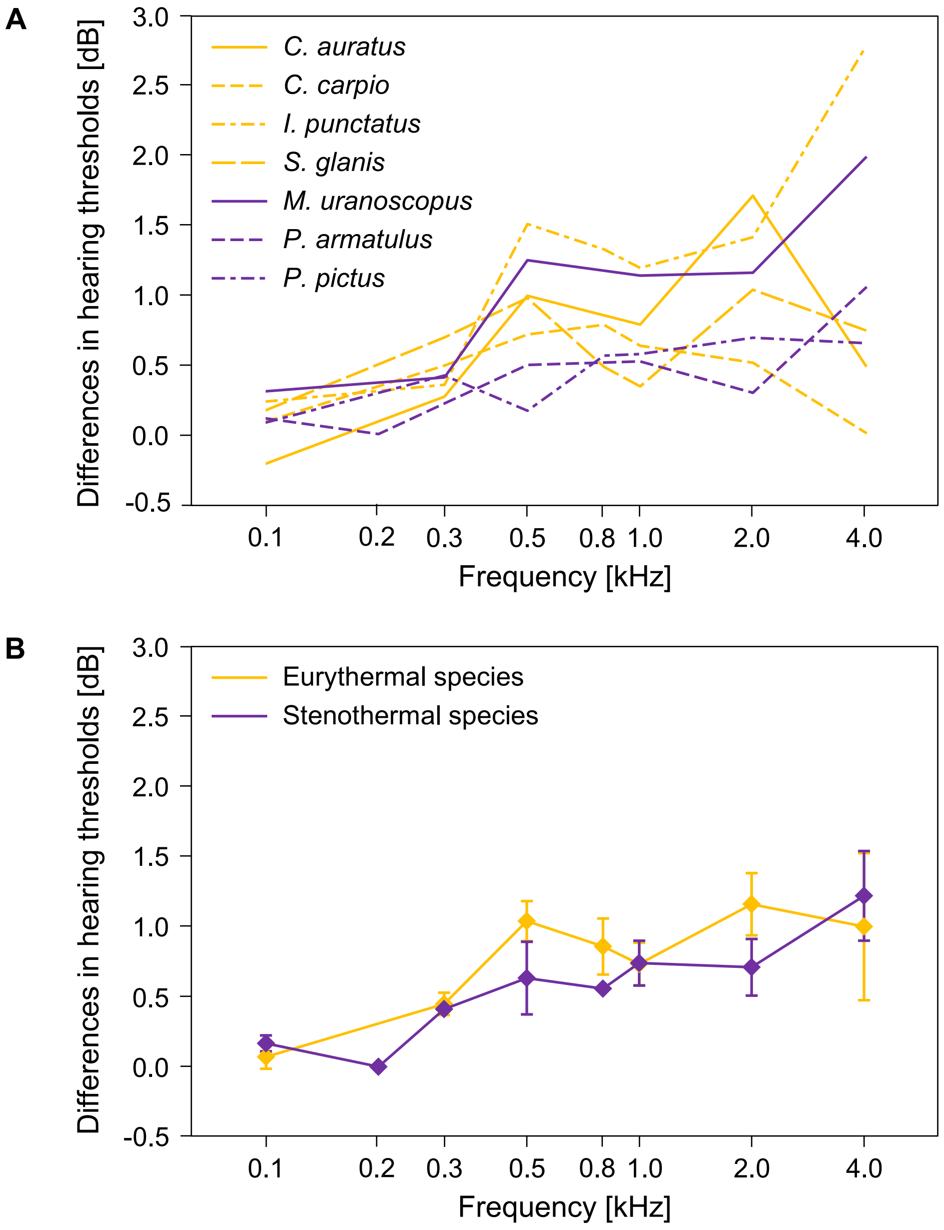

Fig. 16: Comparison of mean temperature-related changes in hearing thresholds per degree Celsius in eurythermal (orange) and stenothermal (purple) species after an acclimation period of three to four weeks. A positive difference between hearing thresholds indicates an improvement of hearing at the higher temperature. (A) *C. auratus*: 15 °C vs. 25 °C (recent study), *C. carpio*: 15 °C vs. 25 °C (Maiditsch and Ladich, 2014), *I. punctatus*: 18 °C vs. 26 °C (Wysocki et al., 2009), *S. glanis*: 15 °C vs. 25 °C (Maiditsch and Ladich, 2014), *M. uranoscopus*: 22 °C vs. 30 °C (recent study), *P. armatulus*: 22 °C vs. 30 °C (Papes and Ladich, 2011), *P. pictus*: 22 °C vs. 30 °C (Wysocki et al., 2009). Modified from Maiditsch and Ladich (2014). (B) Mean (± S.E) differences in hearing thresholds per degree Celsius of the eurythermal and stenothermal species shown in (A). Note that the means of the four eurythermal and three stenothermal species were averaged, and that there were not data at all frequencies for each species.

assumption that eurythermal species have a better ability to compensate temperature induced changes on auditory sensitivity than stenothermal species, even in a more changing environment.

Lower temperatures, to which eurythermal species are adapted, may have a different effect on hearing than higher temperatures. Additionally, the higher temperatures used for stenothermal species might be closer to the upper tolerance limit of these species and thus other factors that are caused by thermal stress are involved and counteract the hearing-improving temperature effect. For several species of lizards, it was shown that the temperature of the maximum auditory sensitivity varied as a function of the natural thermal preferences for each species (Campbell, 1969). If measurements are performed at only two temperatures, the effect of temperature on hearing may depend on the position of the two temperatures used relative to this temperature optimum, respectively the position of these relative to the tolerance limits of the species.

4.2 Effects of acclimation on hearing thresholds

Hearing thresholds of both species investigated were not affected by acclimation time in the present study (16 - 22 hours after target temperature was reached versus three to four weeks of acclimation). Consequently, there is also no difference in sensitivity when temperature was lowered from the baseline temperature (20 °C in goldfish and 26 °C in the catfish) to reach the target temperature (cold acclimation) or if temperature was increased (warm acclimation). In contrast, Wysocki et al. (2009) found acclimation effects in the eurythermal channel catfish when comparing unacclimated to acclimated animals (at least four weeks of acclimation). When the temperature was increased from 10 °C to 18 °C respectively from 18 °C to 26 °C (warm acclimation), hearing sensitivity increased in acclimated fish on average by 7 dB. However, no acclimation effects on the hearing thresholds were observed when *I. punctatus* was previously held at a higher temperature and acclimated to a lower temperature (cold acclimation). Wysocki et al. (2009) did not find a significant effect of acclimation on hearing thresholds in *P. pictus*, except at one frequency (0.8 kHz at 26 °C) after catfish have previously been acclimated to 22 °C (warm acclimation). Thus, it remains to be clarified if fish's hearing improves in general after periods of acclimation. So far, such an effect was only shown in one eurythermal species when temperature increased (Wysocki et al., 2009).

The differences between results may also be due to the smaller temperature ranges chosen in the current study (4 °C and 5 °C vs. 8 °C in *I. punctatus*) and acclimation time chosen (three to four weeks vs. at least four weeks in *I. punctatus*). The lack of acclimation effect in *P. pictus* may similarly be due to the smaller temperature range chosen (4 °C). Note that temperature was changed by 1 °C per day in both studies.

Current findings show that the fish's auditory system adapts quickly to new ambient temperatures, emphasizing the observation of Mann et al. (2009) that hearing changes simultaneously with temperature. Furthermore, this is also supported by observations in anurans. Carey and Zelick (1993) found a deterioration of thresholds due to a transient temperature change, especially below 20 °C, as well as frogs being previously acclimated to a higher temperature being less sensitive than frogs acclimated to a lower temperature, even when both were tested at a lower temperature. Note, however, that the frogs were tested here at the different temperatures without any acclimation time, but in the course of a temperature change from 6 °C to 26 °C, which was carried out much faster (0.2 °C per minute) than temperatures were changed in the current study (1 °C per day). Sun et al. (2019) further found differences in frogs hearing sensitivity between day and night. Although these studies show a rapid change in hearing with changing temperatures, a comparison of hearing after a longer acclimation period at those temperatures is lacking.

In general, ectothermal animals should adapt quickly to changes in ambient temperature to hear e.g. conspecific vocalizations optimally in particular when temperatures change in the course of a day or when fish swim in different water depths. McKibben and Bass (1998) showed that the auditory system of female midshipman *Porichthys notatus* is temperature dependent because they preferred different fundamental frequency of male advertisement calls at different temperatures.

4.3 Effects of temperature on peak latencies

The peak latency to a click stimulus decreased with increasing temperature in *C. auratus* as well as in *M. uranoscopus* which is in accordance with prior studies (Papes and Ladich, 2011; Maiditsch and Ladich, 2014). The AEP waveforms of *M. uranoscopus* are very similar to those of *P. armatulus*, both representatives of the family Doradidae (Papes and Ladich, 2011). Peak latencies are very similar (e.g. P1 at about 1.57 ms) or somewhat larger in *M. uranoscopus* (0.1 - 0.2 ms). Interestingly, at the first peak (N1) an opposite temperature effect is seen in both species, namely an increase in latency with increasing temperature. Differences in peak latencies between related species may be due to size differences. In larger species such as *M. uranoscopus* the distances between the swim bladder, the inner ear, the eight nerve and brain nuclei within the auditory pathways is larger than in smaller species (135 - 174 mm versus 109 - 121 mm in *P. armatulus*). In addition, peaks in response to click stimuli have not been standardized in any fish species, making a comparison between peak latencies in non-related taxa difficult. The same also applies to the click stimuli themselves, which is especially important since different stimulus parameters affect AEPs (Garabon and Higgs, 2017).

Fay and Ream (1992) found in auditory nerve fibers of *C. auratus* a reduction in spontaneous activity of the cells, sensitivity, best frequency (at a given signal level) and overall responsiveness to acoustic stimulation when temperature was lowered, and the opposite effects when the temperature was increased, with all effects being reversible. They suggest that, at least in part, changes in the release and replenishment of neurotransmitters at the synapses between hair cells and auditory nerve fibers could explain the revealed temperature effects. The study on single auditory nerve fibers or cells supports the effects of temperature on hearing sensitivity and on latency found in the current study. This results in a higher temporal resolution of the auditory system and subsequently of pulsed sounds used in acoustic communication in numerous fishes (Myrberg et al., 1978; Papes and Ladich, 2011).

4.4 Effects of acclimation on peak latencies

Acclimation periods did not affect peak latencies in response to a click stimulus in *C. auratus* and *M. uranoscopus*. As far as known, there are no other studies looking for an acclimation effect on latencies to click stimuli in fishes. Obviously, temperature changes directly affect peak latencies (as well as hearing thresholds) without a prolonged acclimation period. In this sense, physical and chemical effects of temperature with immediate effects might play a more important role than biological effects that affect metabolism or gene activity only after a certain time delay. These results are also consistent with findings in frogs, where, similar to hearing sensitivity, latency was also lower in cold-acclimated animals (14 °C) than in warm-acclimated ones (21 °C), even when both were tested at the same temperatures (15 °C - 25 °C) (Carey and Zelick, 1993).

4.5 Effects of temperature and acclimation on peak-to-peak amplitudes

Peak-to-peak amplitudes showed different trends in both species, but results were less conclusive here regarding effects of temperature and acclimation. In the goldfish, the amplitude tended to be higher in unacclimated measurements after an increase in temperature than in the other three test conditions, and in the catfish, there was a tendency for amplitudes to decrease with temperature increase and acclimation duration, the latter being particularly more pronounced at the higher temperature. Nevertheless, statistical significance of these effects is lacking. Since comparable data on this are lacking so far, it cannot be conclusively clarified at this point whether the sample size, especially in combination with larger individual differences in the measured amplitudes, plays a role here, or whether the amplitudes are actually more constant with temperature changes in contrast to the hearing thresholds and peak latencies. The different trends in the two species studied could be related to the different temperatures used in each case, both in an absolute sense and in relation to their tolerance range. Furthermore, comparing the two species, it should also be mentioned that the measured amplitudes were higher in goldfish than in thorny catfish. This can be explained by the dampening effect of the more developed cranial bones of the thorny catfish.

4.6 Summary and Conclusion

The current study demonstrates that an increase in ambient temperature results in an increase in auditory sensitivity (lower thresholds) which is similar to previous studies in fish and other ectothermal animals. The improvement is accompanied by shorter peak latencies in response to click sounds. However, current data do not support the notion that fishes adapted to a wide temperature regime differ from species adapted to a narrow regime (eurythermal versus stenothermal species).

It remains unclear if acclimation time affects hearing thresholds and latencies in fishes in addition to temperature. The current study in goldfish and thorny catfish contradicts one in the channel catfish, in which a small improvement of auditory sensitivity was observed after several weeks of warm acclimation. Further studies are needed to find out, if these differences depend on the extent of temperature changes used (or even the absolute temperatures themselves) or if it depends on other ecological constraints to which species are adapted. Species exposed to daily temperature fluctuations (or fluctuation due to swimming in different water depths) should exhibit hourly or diurnal changes in auditory sensitivity rather than only after weeks of acclimation. Similarly, temperature-dependent latencies of the auditory system in response to sound pulses should correlate with temporal patterns of sound pulses to be detectable during acoustic communication or orientation and subsequently to be adaptive for reproductive behaviour. Likewise, measurements at shorter intervals, in accordance with changes in ambient temperature under natural conditions should be considered.

Also, more measurements on representatives of non-otophysine taxa would be very important, since so far only data from a single specimen of Gadidae are available. Obviously, hearing sensitivity of fishes lacking accessory hearing structures are similarly affected by changes in ambient temperature.

Several studies showed that also sound production and sound characteristics depend on environmental temperature (Ladich, 2018). This subsequently results in the question whether both are coupled and temperature-related changes affect sound production as well as the auditory system. The existence of such a "temperature coupling" phenomenon was first described by Gerhardt (1978) for the gray treefrog *Hyla versicolor*, and later also in crickets (Doherty, 1985; Pires and Hoy, 1992). It was furthermore indicated in studies on fish by McKibben and Bass (1998) and Papes and Ladich (2011). This asks for more studies on the effects of temperature on the auditory system in fishes, particularly to find out how global warming affects acoustic orientation and communication in fishes, since fishes not only constitute a major source of protein in human nutrition and thus have a special importance in economic terms, but they are also very crucial and vital for the persistence of their respective ecosystems.

5. Acknowledgments

First of all, I would like to thank my supervisor, Friedrich Ladich, for the possibility of this work itself, and for all his help and guidance. In addition, I would also like to thank Isabelle Maiditsch for all of the technical instructions and explanations, as well as for her help with all problems that arose. This study was supported by the Austrian Science Fund (FWF, grant P31045 to F.L.).

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7. Attachment

Tab. A1: Anatomical data of unacclimated and acclimated *C. auratus* at 15 °C and 25 °C, as well as Flaxedil dosage used to immobilize them for the hearing measurement.

Fish-ID	**Test condition**	**body mass (g)**	**total length (cm)**	**standard length (cm)**	**Flaxedil (µg per g body mass)**
Ca1	15 °C unacclimated	16.4	9.9	7.9	0.916
Ca2		16.4	10.7	8.3	0.917
Ca3		18.5	10.7	8.3	0.899
Ca4		14.6	10.1	7.8	0.913
Ca5		18.0	10.7	8.6	0.925
Ca6		16.5	10.4	8.4	0.907
Ca7		12.3	9.2	7.2	0.947
Ca8		14.9	10.0	7.9	0.895
Ca1	15 °C acclimated	18.4	10.4	8.2	0.905
Ca2		17.7	10.9	8.4	0.943
Ca3		19.9	11.0	8.5	0.923
Ca4		15.4	10.3	8.1	0.972
Ca5		17.7	10.8	8.6	0.944
Ca6		18.1	10.8	8.6	0.922
Ca7		13.3	9.4	7.4	0.877
Ca8		16.1	10.2	8.1	0.934
Ca1	25 °C unacclimated	15.6	9.7	7.7	0.959
Ca2		14.9	10.1	8.0	1.008
Ca3		17.8	10.4	8.0	0.938
Ca4		13.9	9.8	7.8	0.958
Ca5		19.6	10.5	8.5	1.021
Ca6		15.3	10.2	8.1	0.981
Ca7		12.6	9.0	7.0	2.243
Ca8		14.6	9.9	7.8	1.714
Ca1	25 °C acclimated	15.4	9.9	7.8	0.975
Ca2		15.2	10.5	8.1	0.984
Ca3		17.8	10.4	8.0	0.937
Ca4		14.2	9.9	7.8	0.942
Ca5		20.5	10.7	8.6	0.978
Ca6		15.1	10.1	7.9	0.994
Ca7		11.9	9.1	7.2	1.118
Ca8		15.0	9.8	7.7	0.999

Tab. A2: Anatomical data of unacclimated and acclimated *M. uranoscopus* at 22 °C and 30 °C, as well as Flaxedil dosage used to immobilize them for the hearing measurement.

Fish-ID	**Test condition**	**body mass (g)**	**total length (cm)**	**standard length (cm)**	**Flaxedil (µg per g body mass)**
Mu1	22 °C unacclimated	39.5	16.7	13.7	3.379
Mu2		77.1	20.9	17.4	3.461
Mu3		48.4	17.7	14.4	3.447
Mu4		57.4	18.5	15.3	3.193
Mu5		88.1	20.5	16.6	3.217
Mu6		58.9	18.4	15.1	3.396
Mu7		50.5	18.5	15.1	3.303
Mu1	22 °C acclimated	39.5	16.7	13.5	3.377
Mu2		75.9	21.3	17.4	3.294
Mu3		47.4	17.6	14.3	3.519
Mu4		57.2	18.5	15.3	3.203
Mu5		95.6	20.5	16.7	3.661
Mu6		58.7	18.4	15.1	3.409
Mu7		50.1	18.5	15.2	3.328
Mu1	30 °C unacclimated	38.7	16.6	13.7	3.872
Mu2		78.7	20.9	17.2	4.023
Mu3		50.5	17.8	14.4	3.957
Mu4		54.5	18.4	15.1	3.974
Mu5		77.3	20.7	16.7	3.665
Mu6		58.1	18.4	15.0	4.302
Mu7		48.4	18.4	15.0	3.786
Mu1	30 °C acclimated	39.8	16.8	13.7	3.774
Mu2		79.7	21.0	17.2	3.764
Mu3		48.8	17.7	14.4	3.755
Mu4		53.9	18.4	15.1	3.401
Mu5		80.2	20.0	16.4	3.741
Mu6		57.6	18.3	14.8	3.764
Mu7		48.0	18.4	15.1	3.823

Tab. A3: Absolute hearing thresholds of unacclimated and acclimated *C. auratus*, determined at 15 °C and 25 °C. Hearing thresholds were measured using the auditory evoked potential (AEP) recording technique.

Fish-ID	**Test condition**	**Hearing threshold (dB re 1 µPa)**					
		0.1 kHz	**0.3 kHz**	**0.5 kHz**	**1 kHz**	**2 kHz**	**4 kHz**
Ca1	15 °C unacclimated	80.8	71.6	76.0	72.9	99.5	130.5
Ca2		80.8	68.6	72.6	70.0	100.5	130.1
Ca3		85.1	72.0	72.6	70.0	102.9	134.7
Ca4		77.1	72.6	68.6	69.6	100.1	130.6
Ca5		84.3	72.0	72.6	66.3	102.6	126.7
Ca6		77.6	59.6	72.9	74.1	92.9	126.5
Ca7		85.4	72.6	73.8	74.4	94.9	131.0
Ca8		73.2	63.1	69.6	69.6	98.9	126.8
Ca1	15 °C acclimated	76.7	72.0	72.9	67.5	94.1	126.5
Ca2		80.4	72.0	72.6	73.5	98.4	130.5
Ca3		85.0	71.2	73.2	73.8	102.3	130.4
Ca4		72.3	67.5	70.0	66.3	97.8	130.2
Ca5		85.4	70.8	69.6	70.4	106.8	126.9
Ca6		80.7	64.0	73.2	73.5	102.3	126.2
Ca7		76.5	68.1	68.6	65.6	98.0	126.2
Ca8		77.1	63.1	69.1	66.3	94.4	130.2
Ca1	25 °C unacclimated	84.2	63.1	58.0	62.1	84.3	129.6
Ca2		84.5	70.8	62.1	64.0	76.3	121.8
Ca3		84.3	70.8	62.6	61.1	80.7	121.7
Ca4		80.1	65.6	64.8	58.0	84.1	125.1
Ca5		84.5	70.4	65.6	64.8	80.8	125.6
Ca6		79.4	67.5	63.1	59.6	82.6	122.8
Ca7		75.4	59.6	59.6	56.0	84.5	117.8
Ca8		76.3	58.0	66.9	60.9	85.5	126.0
Ca1	25 °C acclimated	83.6	63.1	59.8	63.7	88.7	125.6
Ca2		83.6	71.2	64.8	64.8	76.7	121.7
Ca3		87.8	71.6	53.8	55.6	75.1	122.5
Ca4		80.1	66.3	64.0	59.6	84.3	125.8
Ca5		80.1	72.0	59.6	64.8	76.3	126.3
Ca6		79.7	59.6	63.1	64.0	88.0	121.5
Ca7		80.1	59.6	63.1	60.9	84.5	122.2
Ca8		76.0	64.0	62.1	60.9	84.4	122.3

Tab. A4: Absolute hearing thresholds of unacclimated and acclimated *M. uranoscopus*, determined at 22 °C and 30 °C. Hearing thresholds were measured using the auditory evoked potential (AEP) recording technique.

Fish-ID	**Test condition**	**Hearing threshold (dB re 1 µPa)**					
		0.1 kHz	**0.3 kHz**	**0.5 kHz**	**1 kHz**	**2 kHz**	**4 kHz**
Mu1	22 °C unacclimated	84.9	76.0	76.3	69.6	72.9	98.3
Mu2		85.0	75.8	80.0	69.6	72.9	102.2
Mu3		84.7	75.8	75.4	69.6	80.8	97.9
Mu4		84.3	84.4	87.2	68.1	78.8	97.4
Mu5		77.3	76.3	76.0	77.6	77.1	102.3
Mu6		81.0	76.0	71.6	70.0	76.5	98.0
Mu7		80.8	80.0	87.9	81.3	80.9	97.9
Mu1	22 °C acclimated	84.4	75.1	80.8	72.3	70.8	97.2
Mu2		80.7	74.9	80.5	68.6	71.6	101.3
Mu3		84.7	79.3	80.5	72.6	80.0	97.0
Mu4		84.7	75.6	80.7	68.1	75.1	97.4
Mu5		80.5	80.3	76.0	76.9	80.1	105.0
Mu6		81.4	76.3	72.6	72.3	75.4	97.3
Mu7		84.6	79.7	83.9	76.7	80.0	101.4
Mu1	30 °C unacclimated	83.6	78.5	68.6	68.6	67.5	84.8
Mu2		83.9	74.9	69.6	60.9	64.8	77.1
Mu3		80.8	79.0	65.6	68.6	64.8	81.3
Mu4		80.7	74.9	77.4	64.0	68.1	85.6
Mu5		84.7	79.2	66.0	64.4	68.2	80.7
Mu6		80.0	66.9	65.6	65.6	68.1	84.8
Mu7		80.5	79.3	70.0	64.8	68.6	85.4
Mu1	30 °C acclimated	80.4	74.9	70.0	63.1	69.1	80.7
Mu2		83.8	70.0	69.6	59.6	59.9	81.1
Mu3		79.4	74.4	65.6	68.1	64.8	84.7
Mu4		80.4	74.6	77.7	63.1	67.5	84.9
Mu5		80.4	79.0	70.0	63.1	67.5	85.1
Mu6		80.1	70.8	66.3	64.0	71.6	85.1
Mu7		79.4	74.9	66.3	63.1	68.1	84.5

Tab. A5: Peak latencies in response to a click stimulus of unacclimated and acclimated *C. auratus* at 15 °C and 25 °C. The click stimulus was presented 28 dB above hearing threshold.

Fish-ID	Test condition	Peak latency (ms)					
		N1	P1	N2	P2	N3	P3
Ca1	15 °C unacclimated	1.60	1.72	1.97	2.42	2.79	3.49
Ca2		1.56	1.64	1.93	2.38	2.96	3.78
Ca3		1.60	1.77	2.09	2.59	2.96	3.43
Ca4		1.31	1.50	1.93	2.38	2.71	3.53
Ca5		1.56	1.64	1.91	2.34	2.63	3.12
Ca6		1.56	1.79	1.93	2.32	2.83	3.49
Ca7		1.54	1.75	2.11	2.63	2.96	3.32
Ca8		1.62	1.83	1.97	2.36	2.96	3.49
Ca1	15 °C acclimated	1.64	1.79	2.01	2.55	2.79	3.28
Ca2		1.56	1.68	1.93	2.34	2.70	3.49
Ca3		1.44	1.58	2.03	2.48	2.83	3.49
Ca4		1.52	1.70	1.97	2.42	2.73	3.57
Ca5		1.60	1.77	2.13	2.61	2.96	3.32
Ca6		1.56	1.77	1.93	2.28	2.79	3.16
Ca7		1.56	1.72	1.99	2.42	2.79	3.53
Ca8		1.64	1.72	2.07	2.46	2.83	3.86
Ca1	25 °C unacclimated	1.07	1.27	1.44	1.89	2.30	2.71
Ca2		1.07	1.21	1.31	1.77	2.26	2.59
Ca3		1.19	1.35	1.52	1.89	2.18	2.69
Ca4		1.09	1.35	1.52	1.89	2.42	2.71
Ca5		1.11	1.31	1.48	1.89	2.34	2.71
Ca6		1.07	1.27	1.52	1.85	2.22	2.63
Ca7		1.19	1.31	1.44	1.85	2.13	2.53
Ca8		1.07	1.23	1.40	1.85	2.13	2.50
Ca1	25 °C acclimated	1.07	1.25	1.40	1.85	2.22	2.63
Ca2		1.15	1.31	1.48	1.93	2.26	2.65
Ca3		1.22	1.32	1.52	1.85	2.18	2.63
Ca4		1.19	1.31	1.48	1.91	2.22	2.63
Ca5		1.19	1.31	1.44	1.89	2.38	2.79
Ca6		1.15	1.27	1.40	1.85	2.34	2.71
Ca7		1.18	1.25	1.44	1.85	2.09	2.59
Ca8		1.15	1.31	1.44	1.85	2.22	2.55

Tab. A6: Peak latencies in response to a click stimulus of unacclimated and acclimated *M. uranoscopus* at 22 °C and 30 °C. The click stimulus was presented 28 dB above hearing threshold.

Fish-ID	**Test condition**	**Peak latency (ms)**					
		N1	**P1**	**N2**	**P2**	**N3**	**P3**
Mu1	22 °C unacclimated	0.86	1.64	2.11	2.94	3.61	4.23
Mu2		0.86	1.44	2.13	3.12	3.78	4.39
Mu3		0.86	1.60	2.09	2.96	3.41	3.94
Mu4		0.90	1.60	2.18	3.16	3.90	4.56
Mu5		0.90	1.52	2.22	3.12	3.69	4.56
Mu6		0.99	1.52	2.18	2.96	3.53	4.10
Mu7		0.94	1.64	2.26	3.08	4.06	4.76
Mu1	22 °C acclimated	0.94	1.52	2.18	2.96	3.57	4.06
Mu2		0.86	1.48	2.05	3.04	3.57	4.27
Mu3		0.94	1.68	2.34	3.32	3.61	3.98
Mu4		0.92	1.56	2.05	2.96	3.61	4.18
Mu5		0.86	1.58	2.42	3.24	3.94	4.60
Mu6		0.94	1.52	2.09	3.00	3.53	4.23
Mu7		0.82	1.68	2.26	3.32	3.98	4.64
Mu1	30 °C unacclimated	0.99	1.27	1.60	2.22	2.63	3.12
Mu2		0.94	1.23	1.48	2.38	2.79	3.20
Mu3		1.19	1.52	1.72	2.50	2.75	3.04
Mu4		0.94	1.31	1.56	2.34	2.79	3.04
Mu5		1.03	1.35	1.64	2.24	2.67	3.14
Mu6		1.03	1.31	1.52	2.55	3.00	3.32
Mu7		0.99	1.44	1.72	2.38	2.79	3.16
Mu1	30 °C acclimated	0.99	1.27	1.56	2.18	2.63	3.08
Mu2		0.94	1.27	1.60	2.42	2.79	3.28
Mu3		1.03	1.19	1.34	2.42	2.63	3.00
Mu4		1.05	1.40	1.54	2.55	2.96	3.41
Mu5		1.03	1.40	1.81	2.50	2.79	3.32
Mu6		0.99	1.35	1.56	2.50	2.87	3.41
Mu7		0.94	1.40	1.72	2.38	2.71	3.12

Tab. A7: Peak-to-peak amplitudes between N1/N2 and P2 of AEP waveforms in response to a click stimulus of unacclimated and acclimated *C. auratus* (15 °C and 25 °C) as well as *M. uranoscopus* (22 °C and 30 °C). The click stimulus was presented 28 dB above hearing threshold.

Fish-ID *C. auratus*	**Test condition**	**Peak-to-peak amplitude (µV)**		**Fish-ID** *M. uranoscopus*	**Test condition**	**Peak-to-peak amplitude (µV)**
Ca1	15 °C unacclimated	1.79		**Mu1**	22 °C unacclimated	1.32
Ca2		1.52		**Mu2**		1.34
Ca3		2.25		**Mu3**		0.89
Ca4		1.52		**Mu4**		0.86
Ca5		1.57		**Mu5**		0.90
Ca6		1.39		**Mu6**		0.90
Ca7		1.86		**Mu7**		0.94
Ca8		1.99		---	---	---
Ca1	15 °C acclimated	1.14		**Mu1**	22 °C acclimated	0.96
Ca2		1.53		**Mu2**		1.34
Ca3		2.91		**Mu3**		0.51
Ca4		1.40		**Mu4**		0.98
Ca5		1.39		**Mu5**		1.02
Ca6		1.37		**Mu6**		0.75
Ca7		1.26		**Mu7**		1.16
Ca8		2.07		---	---	---
Ca1	25 °C unacclimated	2.65		**Mu1**	30 °C unacclimated	1.00
Ca2		1.99		**Mu2**		0.89
Ca3		2.88		**Mu3**		0.78
Ca4		1.81		**Mu4**		0.80
Ca5		2.13		**Mu5**		1.06
Ca6		2.79		**Mu6**		0.68
Ca7		1.66		**Mu7**		0.57
Ca8		3.54		---	---	---
Ca1	25 °C acclimated	2.17		**Mu1**	30 °C acclimated	0.85
Ca2		1.38		**Mu2**		0.67
Ca3		1.93		**Mu3**		0.59
Ca4		1.09		**Mu4**		0.60
Ca5		1.64		**Mu5**		0.51
Ca6		1.68		**Mu6**		0.61
Ca7		1.78		**Mu7**		0.58
Ca8		1.49		---	---	---

Zusammenfassung

Hintergrund: Die Umgebungstemperatur beeinflusst verschiedene metabolische und physiologische Prozesse ektothermer Tiere, einschließlich des auditorischen Systems. Die aktuelle Studie untersucht den Effekt der Temperatur und der Akklimatisationszeit auf die Hörempfindlichkeit einer eurythermen und einer stenothermen Fischart, welche akzessorische Hörstrukturen besitzen.

Methode/ Hauptergebnisse: Mit Hilfe der Messung auditorisch evozierter Potentiale (AEP) wurden die Hörschwellen bei Frequenzen von 0,1 bis 4 kHz, als auch Spitzen-Latenzen sowie Spitzen-Spitzen-Amplituden von AEP-Oszillogrammen als Reaktion auf einen Klickreiz beim Goldfisch *Carassius auratus* (eurytherm) und dem tropischen Dornwels *Megalodoras uranoscopus* (stenotherm) bestimmt. Beide Arten wurden bei unterschiedlichen Temperaturen (*C. auratus*: 15 °C und 25 °C, *M. uranoscopus*: 22 °C und 30 °C) und Akklimatisationsstadien (innerhalb von 22 Stunden („unakklimatisiert") sowie drei bis vier Wochen nach Erreichen der Zieltemperatur („akklimatisiert")) getestet. Eine frequenzabhängige Zunahme der Hörempfindlichkeit und eine Abnahme der Spitzen-Latenzen wurden bei beiden Arten bei höheren Temperaturen, unabhängig von der Akklimatisationszeit, festgestellt. Die Veränderung der Hörempfindlichkeit pro Grad Celsius war beim stenothermen Wels ausgeprägter. Die Spitzen-Spitzen-Amplituden zeigten bei beiden Arten unterschiedliche Tendenzen und keinen klaren Bezug im Hinblick auf Temperaturänderungen oder die Dauer der Akklimatisation.

Schlussfolgerungen/ Bedeutung: Die Daten zeigen, dass höhere Temperaturen das Hörvermögen verbessern (niedrigere Hörschwellen und kürzere Latenzen), während die Akklimatisationszeit keinen Einfluss auf die Hörempfindlichkeit beider Arten hat. Dies widerspricht den Ergebnissen beim eurythermen Getüpfelten Gabelwels *Ictalurus punctatus*, bei dem die Akklimatisation das Hörvermögen nach einer Temperaturerhöhung verbesserte. Ein Vergleich der Veränderungen der Hörempfindlichkeit pro Grad Celsius aller sieben bisher getesteten Arten ergab keine Unterschiede zwischen eurythermen und stenothermen Arten.

Schlüsselwörter: Fische, *Carassius auratus*, *Megalodoras uranoscopus*, Temperatur, Akklimatisation, eurytherm, stenotherm, Hörschwellen, Latenz, Amplitude, auditorisch evozierte Potentiale (AEP)