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DIPLOMARBEIT

Titel der Diplomarbeit

Der Einfluss der Temperatur auf die Lautbildung und das Hörvermögen des Liniendornwelses *Platydoras armatulus* (Familie Doradidae)

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Contents

Abstract

Sound production and hearing sensitivity of ectothermic animals are affected by the ambient temperature. The present study investigates the influence of temperature on sound characteristics and the hearing ability in the neotropical Lined Raphael catfish *Platydoras armatulus*. Doradid catfishes produce stridulation sounds by rubbing the pectoral spines in the shoulder girdle and drumming sounds by an elastic spring mechanism which vibrates the swimbladder. Fish were acclimated for at least three weeks to 22°, then to 30° and again to 22° C. Sounds were recorded in distress situations when fish were hand-held. The stridulation sounds became shorter at the higher temperature, whereas the pulse number, the minimum and maximum pulse period, the sound pressure level and dominant frequency did not change with temperature. On the other hand, the fundamental frequency increased and the mean pulse period of drumming sounds decreased at the higher temperature in drumming sounds. Using the auditory evoked potential (AEP) recording technique, the hearing thresholds of eight specimens were tested at six different frequencies from 0.1 to 4 kHz. The temporal resolution was determined by analyzing the minimum resolvable click period (0.3 - 5 ms). Auditory sensitivity increased from 0.5 to 4 kHz with rising temperature, whereas temporal resolution in response to double-clicks did not change with temperature. Latencies of AEPs in response to single clicks decreased in three out of four peaks at higher temperature.

These data indicate that hearing sensitivity increases with temperature at higher frequencies. Constraints imposed on hearing sensitivity at different temperatures cannot be compensated even by longer acclimation periods. As sound characteristics also change with temperature, it is suggested that the ambient temperature directly affects acoustic orientation and communication in the neotropical catfish *P. armatulus*.

1. Introduction

Ectothermic animals are dependent on environmental heat sources and control their body temperature through external means. Compared to endothermic animals, they maintain relatively low metabolic rates. In general, the speed of all metabolic processes is influenced by the body temperature which depends on the ambient temperature (Cossins et al., 1977; Salem and Omura, 1998; Navarro et al., 2002; Andersson et al., 2003; Itoi et al., 2003). Therefore, ambient temperature affects various physiological processes such as neuronal and muscular activities including all sensory systems in ectothermic animals (Siegmund and Vogel, 1977; Johansen, 1984; Ratnasabapathi et al., 1992; Domingues et al., 2002; Jones et al., 2008; Bellgraph et al., 2010).

In temperate climates fish have to deal with pronounced seasonal and diurnal fluctuations in water temperature. Fish either cope with temperature fluctuations or they migrate. Thus, the thermal tolerance range of fish species differs to some degree. Certain physical constraints cannot be compensated for even when animals are acclimated (Bennet, 1985; Wysocki et al., 2009) suggesting that there exists an optimum temperature range.

Fish have evolved the largest diversity of sound producing mechanisms among vertebrates and sounds are emitted in numerous contexts: e.g. disturbance situations, during courtship, competitive feeding, territorial encounters (for reviews see Ladich and Myrberg, 2006; Ladich and Fine, 2006; Kasumyan, 2008). Representatives of some catfish families possess two different sound producing mechanisms (Ladich, 1997; Fine and Ladich, 2003). High-frequency stridulation sounds are emitted when pressing ridges of the dorsal process of the pectoral spine against the groove of the pectoral girdle while abducting or adducting pectoral spines (Tavolga, 1960; Pfeiffer and Eisenberg, 1965; Fine et al., 1997; Kaatz et al., 2010; Parmentier et al., 2010). On the other hand, vibrations of the swimbladder by sonic muscles result in the emission of low-frequency drumming sounds (Ladich, 1997; Ladich und Bass, 2003; Ladich and Fine, 2006). In the familiy Doradidae a thin round bony plate termed

elastic spring ('Springfeder'; Müller, 1842) vibrates the swimbladder. The elastic spring is rapidly pulled forward during contractions of sonic muscles which originate at the occipital bone and insert at the elastic spring (Ladich, 2001; Fine and Ladich, 2003).

Effects of temperature have not been studied in stridulation sounds so far, but in low-frequency sounds such as drumming sounds. In general, the sound duration and the fundamental frequency increased with rising ambient temperature, whereas the pulse period decreased (Torricelli et al., 1990; Lugli et al., 1996; Connaughton et al., 2000; Amorim, 2005; Maruska and Mensinger, 2009). The fundamental frequency increased due to the higher contraction rate of the drumming muscle at higher temperature. Similarly, the duration and fundamental frequency of boatwhistles increased in the Lusitanian toadfish, *Halobatrachus didactylus* (Amorim et al., 2006). Amorim (2005) found that acoustic features of knock and grunts of grey gurnards *Eutrigla gurnardus* were temperature-dependent (pulse periods decreased with rising temperature). The cod, *Gadus callarias*, emitted more sounds at higher temperatures (Brawn, 1960).

Fish depend on hearing for analyzing the acoustic scene, for orientation, prey and predator detection and for intraspecific communication (Fay and Popper, 2000; Ladich and Popper, 2004; Fay, 2009). Ambient temperature effects hearing in invertebrates and ectothermic vertebrates. Effects on hearing have been examined in insects (Fonseca and Correia, 2007; Franz and Ronacher, 2002; Oldfield, 1988), amphibians (Egert and Lewis, 1995; Long et al., 1996; Van Dijk et al., 1997) and reptiles (Eatock and Manley, 1981; Smolders and Klinke, 1984). In general, raising the temperature resulted in an increase of the most sensitive (best) frequency as well as of the absolute sensitivity (Hubl et al., 1977; Wallkowiak, 1980). The number of action potentials increased and the temporal tuning of auditory neurons shifted to higher rates of amplitude modulation (Brenowitz et al., 1985). Similar results have been found in the tuning of the auditory system in cicadas and locusts (Oldfield, 1988; Fonseca and Correia, 2007).

In fish, only a few studies investigated the effects of temperature changes. Dudok van Heel (1956) found that the European minnow, *Phoxinus phoxinus*, is able to discriminate between higher frequencies at higher ambient temperature. In goldfish, *Carassius auratus*, warming increased the spontaneous activity and sensivity of auditory neurons, the best frequency at a given signal level and the responsiveness to an acoustic stimulus (Fay and Ream, 1992). The walleye pollock, *Theragra chalcogramma*, showed a reduction in auditory sensitivity at lower ambient temperature within hours (Mann et al., 2009). Wysocki et al. (2009) showed that the eurytherm channel catfish, *Ictalurus punctatus*, and the stenotherm tropical catfish, *Pimelodus pictus*, exhibited higher hearing sensitivity at higher temperatures, especially at the highest frequency tested. Differences between temperatures were more pronounced in the eurytherm catfish species.

Sound characteristics are important for coding information in agonistic and reproductive contexts (conflict resolution, distress situations, courtship, establishment of territories). Fish often produce series of short broad-band pulses as in stridulation sounds of catfishes and gouramis (Ladich et al., 1992; Ladich, 1997) with distinct temporal patterns and variabel interpulse intervals (Myrberg et al., 1978; Ladich et al., 1992). Severals studies have suggested that temporal patterns are important carriers of information in fish (Myrberg et al., 1978; Wysocki and Ladich, 2002). Wysocki and Ladich (2002) showed that the auditory system of the catfish, *Platydoras armatulus* (formerly *P. costatus*), and the croaking gourami, *Trichopsis vittata*, were able to process each pulse within a stridulation sound.

The aims of the present study were to investigate the effects of temperature (1) on sound production and on sound characteristics, (2) on the absolute auditory sensitivity and (3) on the ability to resolve temporal patterns of sounds in the Lined Raphael catfish.

The neotropical catfish, *P. armatulus* (Piorski, 1999), was chosen because this group produces two different sound types (swimbladder and pectoral stridulatory sounds) and because it possesses accessory hearing structures (Weberian apparatus). Groups with

accessory hearing structures which couple air-filled cavities acoustically to the inner ear are most likely affected by temperature changes (Wysocki et al., 2009). *Platydoras armatulus* occurs in the Amazonian river system and is known to emit both types of sounds in distress situations (Ladich, 1997). This is the first study investigating the effects of temperature on vocalization, hearing and acoustic communication in a fish species.

2. Materials and Methods

2.1. Animals

Lined Raphael catfish were kept in a community tank (110 x 55 x 30 cm, 25 ± 1 °C) and a total of 8 adult specimens of *P. armatulus* was used in the present study. They were obtained from a local pet supplier. Four fish each were introduced into two experimental tanks (70 x 40 x 30 cm) which were equipped with half flower pots and the ground was covered with sand. The water was filtered by external filters and a 12:12 hour light-dark cycle was maintained. Fish were fed with frozen chironomid larvae and flake food five days per week. The size of fish was as follows: total length: 126.2 - 142.5 mm; standard length: 108.6 - 121.1 mm; body mass: 27.9 - 41.8 g. The sex of the fish was not determined because this was not possible without sacrificing animals.

Temperature in the experimental tanks was changed using submersible heaters by approximately one degree per day until final temperatures of 22 ± 1 °C and 30 ± 1 °C, respectively, were achieved. Fish were acclimated for at least three weeks to each experimental temperature, first to 22 °C, then to 30 °C and finally to 22 °C again. Auditory measurements were conducted prior to sound recordings. Experiments were performed with permission of the Austrian Federal Ministry of Science and Research (GZ 66.006/0023-II/10b/2008).

2.2. Sound and video recordings

Sound and video recordings were conducted in a sound proof room in a separate recording tank (50 x 27 x 30 cm) either at 22 ±1 °C or at 30 ±1 °C, depending on the acclimation temperature in the experimental tank. Fish were hand-held at a distance of 5 to 10 cm from the hydrophone which was positioned in the middle of the recording tank. In order to avoid overlap of stridulation sounds generated by both pectoral fins at the same time, one fin was fixed.

Sounds and fin movements were recorded using a hydrophone (Brüel & Kjaer 8101) connected to a power supply (Brüel & Kjaer 2804) and an amplifier (AKG B29L), and a video camera (Sony VX1). Both acoustic and video signals were recorded simultaneously on a harddisk video recorder (Panasonic DMR-EX95V). Videorecordings were necessary to determine which sounds were produced during abduction and adduction of pectoral fins. Sound pressure levels (RMS fast, L-weighting) were recorded using a sound level meter (Brüel & Kjaer Mediator 2238) which was connected to the power supply of the hydrophone. Three walls of the recording tank were lined on the inside by acoustically absorbent material (airfilled packing foil) and its bottom was covered with fine sand. The recording tank supporting table was placed on a vibration isolated plate (Fig. 1).
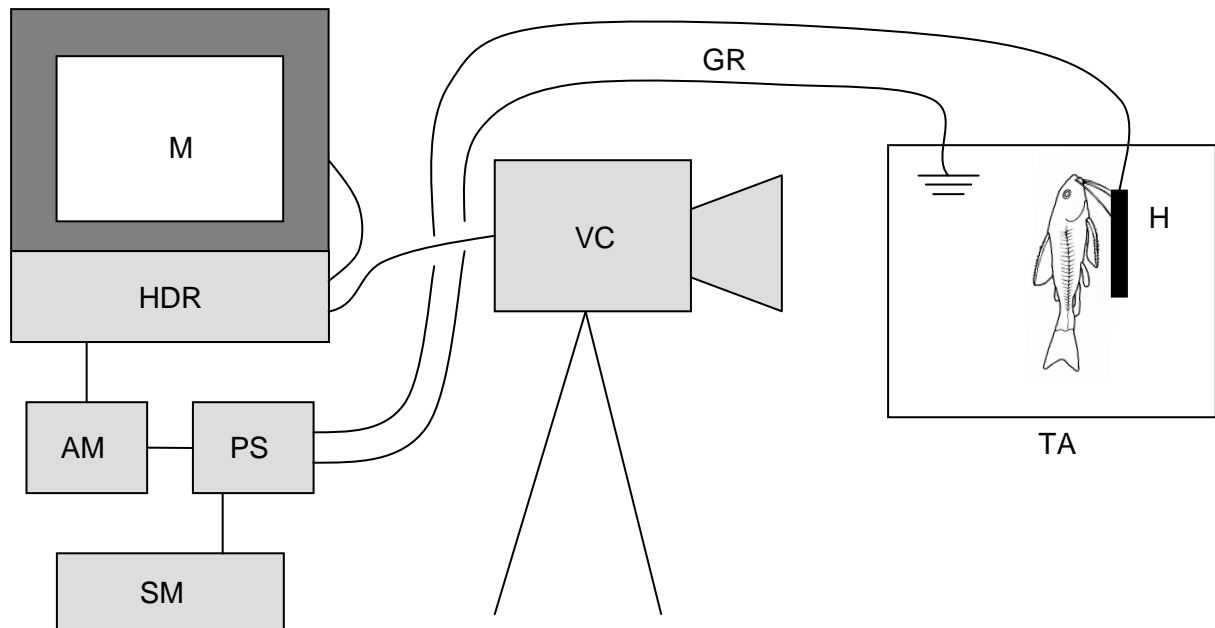
Fig. 1. Sound and video recording setup. Abbrevations: AM...Microphone amplifier, GR...Grounding, H...Hydrophone, HDR... harddisk videorecorder, M...Monitor, PS...Power supply, SM...Sound level meter, TA... Recording tank, VC...Video camera.

2.3. Sound analysis

Sounds were analysed using Cool Edit 2000 (Syntrillium Software Corporation, Phoenix, USA) and ST^x Soundtools 3.7.8. (Institute of Sound Research at the Austrian Academy of Sciences). *P. armatulus* produced sounds during the adduction (AD) and abduction (AB) of pectoral fins (Ladich, 1999). The following sound characteristics were determined in stridulatory sounds: the sound duration (ms), the number of pulses, the minimum and maximum pulse period (ms), the dominant frequency (Hz) and the sound pressure level (dB rel 1 µPa) (Fig. 2). In each individual five AD- and five AB-sounds (a total of 10 sounds) were examined. In the drumming sounds, the sound duration (ms), the number of pulses, the mean pulse period (ms) and the fundamental frequency (Hz) were determined. Sound pressure levels could not be determined for drumming sounds because fish produced stridulation sounds, which were much louder, at the same time.

The pulse period was defined as time between the peak amplitudes of two subsequent pulses within a sound. In stridulation sounds, only sounds consisting of at least four pulses were used for pulse period measurements. The average of the minimum and maximum pulse periods of stridulation sounds (each N = 3) were calculated separately for each fish instead of a total mean due to the large variabilty in these sound characteristics. For each individual, 60 pulse periods were measured and a total of 480 pulse periods at each temperature. The dominant frequencies of stridulation sounds were measured using cepstrum-smoothed power spectra determined from all stridulatory sounds emitted by one specimen including all AD- and AB-sounds, thus 10 sounds per fish. A sound file made up of stridulation sounds was created separately to calculate individual-specific dominant frequencies. A total of 80 sounds were examined at each temperature.

In drumming sounds, pulse periods were defined as the time between subsequent drumming muscle contractions. Pulse periods were analyzed in at least four drumming sounds per fish (10 pulse periods per fish). A total of 80 sounds were examined at each temperature. The mean pulse period was calculated for each fish. The fundamental frequency of drumming sounds was determined from sound power spectra calculated from 10 sounds per fish. Again, a sound file made up of drumming sounds emitted by one specimen was created separately to calculate the fundamental frequency of each individual.
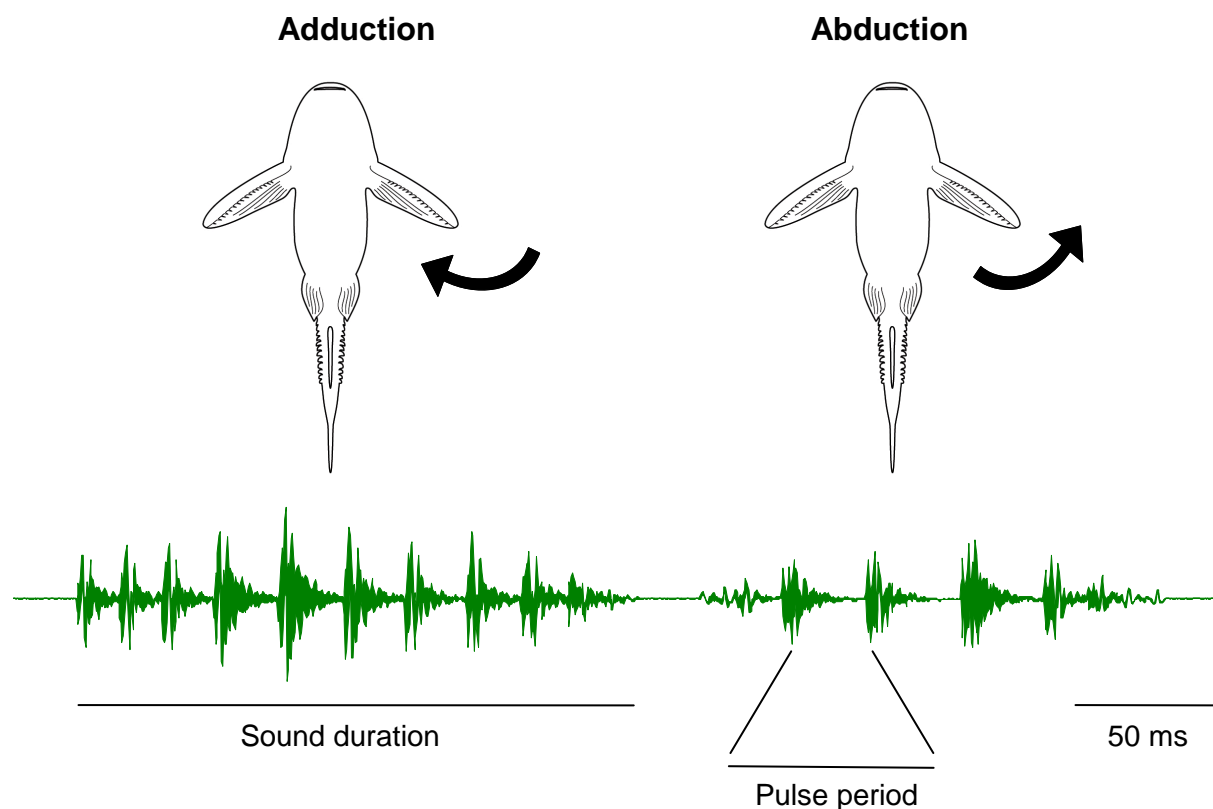
Fig. 2. Adduction and abduction movement of the pectoral fin and oscillogram of an AD-sound and AB-sound showing sound characteristics measured.

2.4. Auditory sensitivity measurements

Auditory sensitivity was measured using the auditory evoked potential (AEP) recording technique described by Kenyon et al. (1998) and modified by Wysocki and Ladich (Wysocki and Ladich, 2002; Wysocki and Ladich, 2005). Test subjects were secured in a round plastic tub (35 cm diameter, 15 cm height, lined on the inside by acoustically absorbent material, 1 cm layer of fine sand) filled with water and adjusted so that the nape of the head was just above the surface of the water, and a respiration pipette was inserted into the animal's mouth. The water temperature was either at 22 ±1 °C or 30 ±1 °C, depending on the temperature in the holding tanks.

Respiration was achieved by a temperature-controlled gravity-fed water circulation system. To immobilize animals and to reduce the myogenic noise level, they were injected

with a curariform agent (Flaxedil; gallamine triethiodide; Sigma-Aldrich, Vienna, Austria).

The dosage required was 1.5 - 2.8 µg g^{-1} and allowed the fish to perform opercular

movements during the experiment. 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 walk-in soundproof room which was constructed as a Faraday cage (interior

dimensions: 3.2 x 3.2 x 2.4 m).

The AEPs were recorded using silver wire electrodes (0.32 mm diameter) were

pressed firmly against the skin which was covered by small pieces of tissue paper to keep it

moist, in order to ensure proper contact during experiments (Fig. 3). The recording electrode

was placed in the midline of the skull over the region of the medulla and the reference

electrode cranially between the nares. Shielded electrodes leads were attached to the

differential input of an a.c. preamplifier (Grass P-55, Grass Instruments, West Warwick, RI,

USA; gain 100x, high-pass at 30 Hz, low-pass at 1 kHz). A ground electrode was placed in

the water near the subject. Both, stimuli presentation and AEP-waveform recording were

accomplished using a Tucker-Davis Technologies (TDT, Gainesville, FL, USA) modular

rackmount system (TDT System 3) controlled by a Pentium PC containing a TDT digital

processing board and running TDT BioSig RP Software.

Fig. 3. Experimental setup for AEP-measurement. Abbreviations: 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, Ref E...Reference Electrode, Rec E...Recording Electrode, Res P...Respiration Pipette, RP 2.1...Realtime-Processor, SM 5...Signal Mixer, WR...Water reservoir.

2.5. Sound stimuli

Sound stimuli waveforms were generated using TDT SigGen RP software and fed through a power amplifier (Alesis RA 300, Alesis Corporation, Los Angeles, CA, USA). A dual-cone speaker (Tannoy System 600, frequency response 50 Hz to 15 kHz $\pm$ 3 dB), mounted 1 m above test subjects in the air, was used to present the stimuli during testing (Fig. 3). Sound stimuli consisted of tone bursts presented at a repetition rate of 21 s^{-1}. Hearing thresholds were determined at frequencies of 0.1, 0.2, 0.5, 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.

2.6. Temporal resolution measurements

In order to analyze the temporal resolution ability at different temperature the tecchnique described by Wysocki and Ladich (2002) was applied. Clicks and double clicks were generated using TDT System II and TDT 'SigGen' Software and were fed through a DA1 digital-analog converter, a PA4 programmable attenuator, and a power amplifier (Denon PMA 715R) to the air speaker (Tannoy System 600). Each type of stimulus (single click and click pairs) were presented to the animals at a repetition rate of 35 s^{-1}. Double-click stimuli were presented at 28 dB above hearing threshold. Ten different click periods were presented, beginning with the shortest click period. Click periods tested were 0.3, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 5 ms.

The amplitudes of the responses to the second click of each pair of clicks were measured and compared to the response to a single click following the method used in Wysocki and Ladich (2002). The most consecutive peaks were used for analysis. The AEP components were denominated with P for positive peaks (directed upwards) and N for negative peaks (directed downwards) by ascending numbers. The main peaks for analysis

were N1, N2, P2 and P3. First, the hearing threshold in response to a single click was determined, followed by a presentation of double-clicks at 28 dB above hearing threshold.

For the purpose of isolating the response to the second click within a pair of clicks, a point-to-point subtraction operation was conducted (Wysocki and Ladich, 2002). The AEP in response to a single click was substracted from the response to a double click. The shortest click period at which a second respond was still detectable was classified as the minimum resolvable click period.

2.7. Latency measurements

The latency was defined as time between the onset of the single click stimulus and the first four constant peaks of the AEP recorded in responses to this click stimulus. The most constant peaks in the AEPs were N1, P1, N2 and P2 (see Fig. 2 in Wysocki and Ladich, 2002). The single click was presented 28 dB above hearing threshold.

2.8. Statistical analysis

All data were tested for normal distribution using the Kolmogorov-Smirnov-test and when data were normally distributed, parametric statistical tests were applied. Thus, data measured at three different experimental temperatures were compared using either a repeated measures ANOVA followed by Bonferroni post hoc tests (stridulation sound characteristics, minimum resolvable click period), or by a non-parametric test if the number of individuals which vocalized at all temperatures or where latency data collected were below the maximum number of fish investigated (eight individuals). A Kruskal-Wallis test was applied to calculate differences in drumming sound characteristics because only five individuals produced drumming sounds at all temperatures. A Friedman-test was used to compare group differences in latencies followed by a Wilcoxon-test because some data were limited to six individuals.

Audiograms obtained at three temperatures (22° C, 30° C and 22° 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. All statistical tests were run using SPSS 17.0. The significance level was set at $p \leq 0.05$.

3. Results

3.1. Stridulation sounds

All *P. armatulus* produced sounds during forward (abduction, AB) and backward movement (adduction, AD) of the fins, utilizing either one or both fins at the same time. Fish could also move fins without emitting sounds or lock spines in an abducted position. Subjects usually started producing sounds with an adduction movement due to the fact that they were spreading their pectoral fins in an adducted position during handling. Stridulation sounds consisted of series of broadband pulses with main energies ranging from 0.3 to 1.3 kHz (Fig. 4). All fish investigated emitted stridulation sounds when hand-held (but not all fish produced drumming sounds).
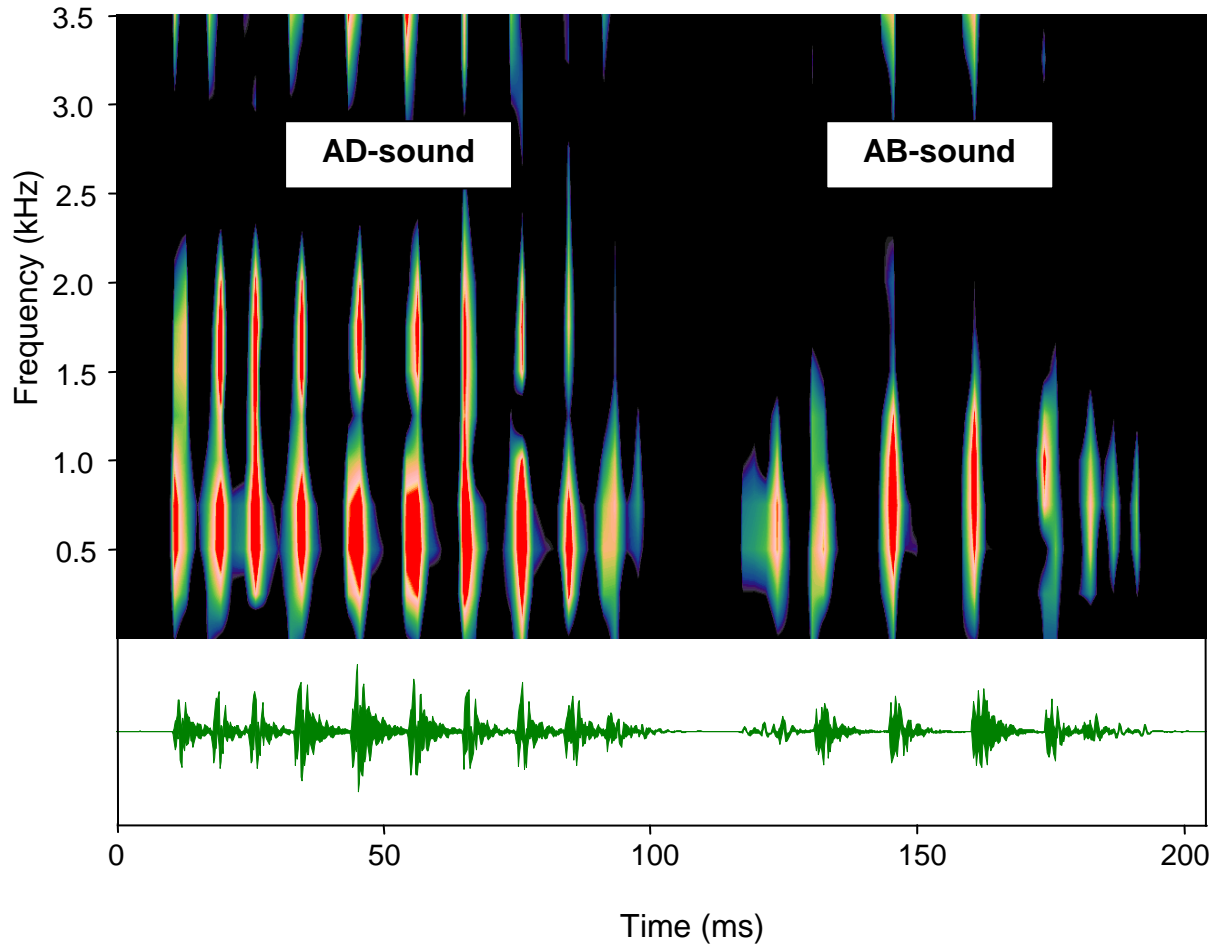
Fig. 4. Sonagram and oscillogram of stridulation sound produced during an adduction (AD) and abduction (AB) movement of pectoral fins. Sampling rate 44.1 kHz, filter bandwidth 320 Hz, hanning filter, overlap 30 %.

The duration in AD-sounds ranged from 62.8 to 160.2 ms at 22° C, from 48.8 to 100.4 ms at 30° C and from 85.6 to 163.8 ms at 22° C repeated. In AB-sounds, the duration ranged from 65.6 to 106.0 ms at 22° C, from 53.0 to 85.0 ms at 30° C and from 43.6 to 131.6 ms at 22° C repeated (Tab.1). A repeated measures ANOVA and a Bonferroni post hoc test revealed that AD- and AB-sounds were significantly shorter at 30° C as compared to 22° C ($F_{2, 14} = 15.63$, $p \leq 0.05$) (Fig. 5).

Tab. 1. Mean (± SE) sound duration, number of pulses, minumum and maximum pulse period and sound pressure level (SPL) in AD-sounds and AB-sounds in *P. armatulus*. N = 8.

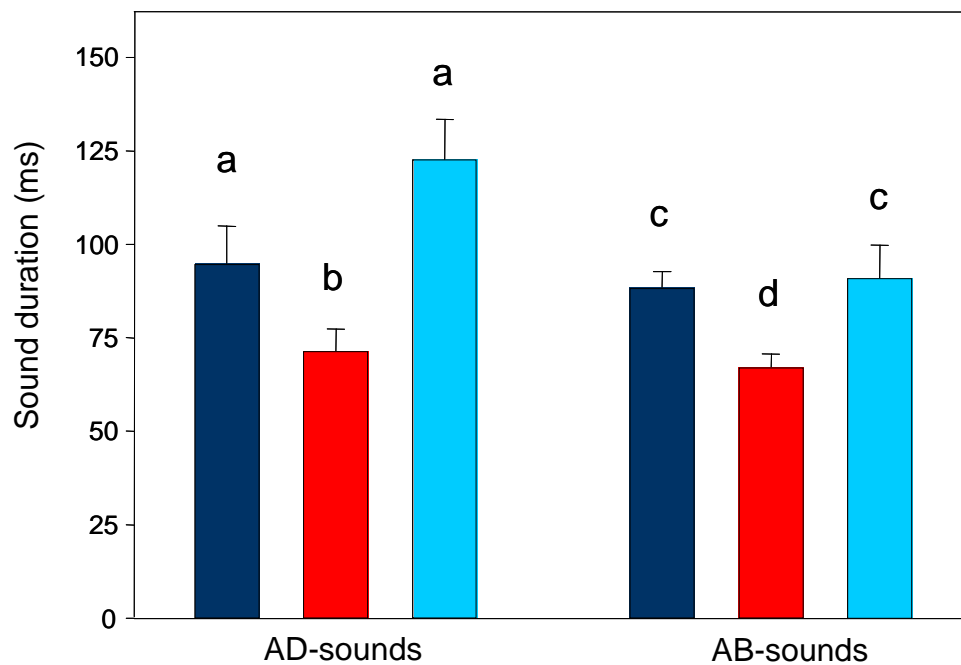| Temperature	22°C		30°C		22°C repeated	
	AD	AB	AD	AB	AD	AB
Duration (ms)	94.8 ± 10.0	88.4 ± 4.4	71.5 ± 5.9	67.1 ± 3.6	122.7 ± 10.8	91.0 ± 8.8
Number of pulses	7.9 ± 1.0	7.6 ± 0.3	6.0 ± 0.6	7.8 ± 1.1	7.7 ± 1.2	6.2 ± 0.9
Minimum pulse period (ms)	7.7 ± 1.3	5.8 ± 0.6	7.4 ± 1.2	5.1 ± 0.7	8.8 ± 1.3	7.7 ± 0.7
Maximum pulse period (ms)	23.2 ± 4.9	18.0 ± 1.2	20.7 ± 3.0	17.2 ± 2.3	29.2 ± 4.8	26.1 ± 4.4
SPL (dB re 1 µPa)	136.4 ± 0.7		137.9 ± 1.0		136.6 ± 1.1	
Dominant frequency (Hz)	601.6 ± 118.9		1271.9 ± 107.5		1203.0 ± 133.1	

Fig. 5. Mean (± S.E.) duration of AD-sounds and AB-sounds in *P. armatulus* kept at 22° C (■), 30° C (■) and 22° C repeated (■). N = 8 fish per temperature. Different letters indicate significant differences between temperatures (p ≤ 0.05).

Number of pulses in AD-sounds ranged from 3.4 to 12.2 at 22° C, from 3.4 to 8.6 at 30° C

and at 22° C repeated from 3.3 to 14.6. The number of pulses of AB-sounds ranged from 5.8

to 8.6 at 22° C, from 5.6 to 15.2 at 30° C and from 3.2 to 9.4 at 22° C repeated (Tab. 1). No

temperature-dependent differences were found in the number of pulses neither in AD- nor in

AB-sounds (Repeated measures ANOVA, $F_{2,14} = 2.366$, $p > 0.05$) (Fig. 6).
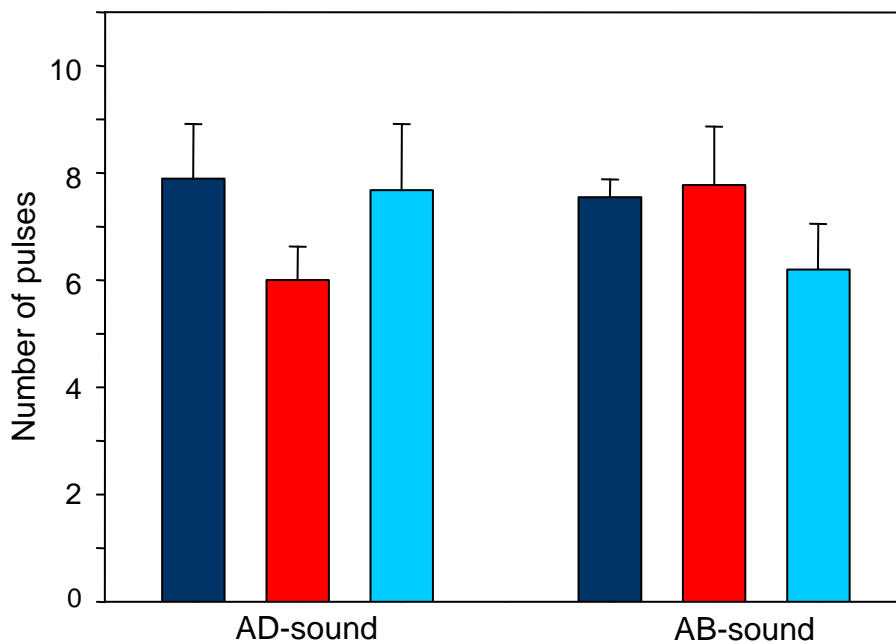

Fig. 6. Mean ($\pm$ S.E.) number of pulses of AD-sounds and of AB-sounds recorded at 22° C (■), 30° C

(■) and 22° C repeated (■). N = 8 fish per temperature.

The pulse period showed a large variability among and within individuals. In general, the

pulse periods were longest in the centre of the sounds and became shorter at the beginning

and at the end of the sound (Fig. 4). The mean minimum pulse period ranged from 7.4 - 8.8

ms in AD- and from 5.1 - 7.7 ms in AB-sounds (Tab. 1). Repeated measures ANOVA and

Bonferroni post hoc test revealed that the minimum pulse periods in AB-sounds were

significant shorter at 30° C than at 22° C repeated ($F_{2,14} = 7.987$, $p \leq 0.05$), but no other trend

could be observed (AD-sounds: $F_{2,14} = 2.174$, $p > 0.05$). The maximum pulse period was not

significantly shorter at the higher temperature in AD-sounds but showed such a trend in AB-sounds (AD-sounds: $F_{2,14} = 1.858$, $p > 0.05$; AB-sounds: $F_{2,14} = 3.625$, $p = 0.054$).

Sound pressure levels did not change significantly with temperature and remained almost constant at about 137 dB rel 1 µPa ($F_{2,14} = 0.948$, $p > 0.05$) (Tab. 1). Otherwise, the dominant frequency revealed significant differences between 22° C and 30° C and between 22° C and 22° C repeated. Dominant frequency doubled after fish were acclimated to 30° C from 601.6 Hz to 1271.9 Hz. However, dominant frequency did not decrease when repeating the low temperature measurements.

3.2. Drumming sounds

P. armatulus emitted two different types of drumming sounds: series of short drumming sounds and long drumming sounds. Series of short drumming sounds could be recorded in 6 out of 8 animals but not at all temperatures (22° C: N = 4; 30° C: N = 4; 22° C repeated: N = 1). Long drumming sounds, on the other hand, were recorded in every individual but again not at every temperature (22° C: N = 5; 30° C: N = 8; 22° C: repeated N = 5). The long drumming sounds revealed a harmonic structure with fundamental frequencies (drumming muscle contraction rate) between 100 and 150 Hz (Fig. 7).
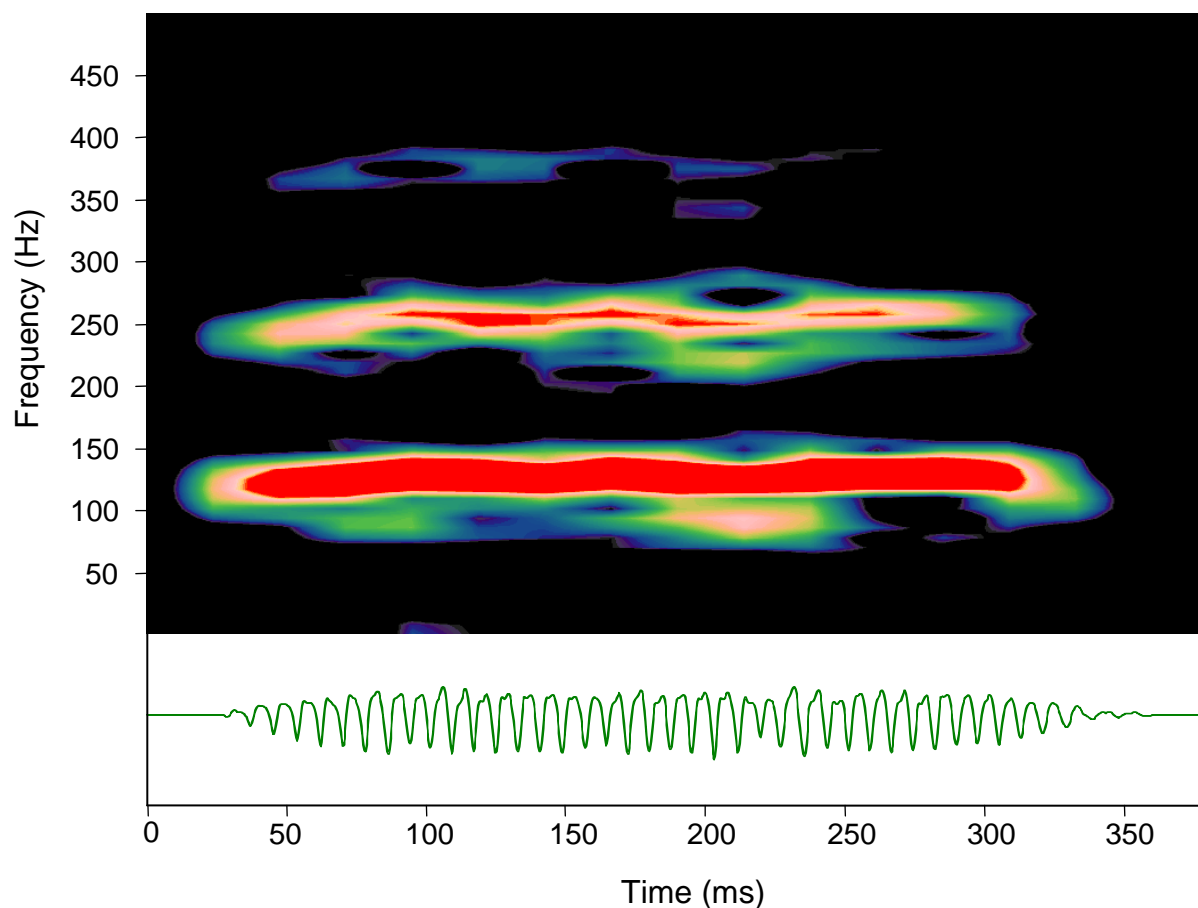
Fig. 7. Sonagram and oscillogram of a long drumming sound. The sonagram shows three harmonics with the main energy concentrated at the first harmonic (fundamental frequency). Sampling rate 44.1 kHz, Filter bandwidth 10 Hz, hanning filter, overlap 75 %.

P. armatulus produced more stridulation than drumming sounds. The vocalizing activity (the number of drumming sounds emitted) increased with temperature. Stridulation sounds and drumming sounds were often emitted at the same time. In general, drumming sounds were longer than stridulation sounds, in some cases over 300 ms. The mean duration of drumming sounds was 277.5 ms at 22° C, 277.2 ms at 30° C and 326.6 ms at 22° C repeated (Tab. 2). The mean number of pulses in drumming sounds were 16.7 at 22° C, 27.9 at 30° C and 25.2 at 22° C repeated.

Tab. 2. Mean (± SE) of sound duration, number of pulses, pulse period and fundamental frequency in drumming sounds in *P. armatulus*. N = 5 kept at 22° C and 22° C repeated; N = 8 at 30° C.

Temperature	22°C	30°C	22°C repeated
Duration (ms)	277.5 ± 100.7	277.2 ± 41.0	326.6 ± 65.4
Number of pulses	16.7 ± 5.7	27.9 ± 4.7	25.2 ± 5.1
Mean pulse period	14.4 ± 0.4	10.4 ± 0.8	12.8 ± 0.6
Fundamental frequency (Hz)	74.2 ± 2.4	99.1 ± 7.9	75.5 ± 1.5

The mean pulse periods in drumming sounds differed significantly between temperatures (Kruskal-Wallis test: $\chi^2 = 10,504$, df = 2, $p < 0.05$) (Fig. 8). The mean pulse period was significantly shorter at the higher temperatures (22° C and 30° C: U-test, U = 1.0, $N_1 = 5$, $N_2 = 8$, $p < 0.05$; 30° C and 22° C repeated: U = 6.5, $N_2 = 8$, $N_3 = 5$, $p \leq 0.05$) (Fig. 9). The fundamental frequency in drumming sounds differed significantly between temperatures (Kruskal-Wallis test: $\chi^2 = 10,719$, df = 2, $p < 0.05$ (Fig. 9). The fundamental frequency increased significantly with rising temperature (22° C and 30° C: U-test, U = 1.0, $N_1 = 5$, $N_2 = 8$, $p < 0.05$; 30° C and 22° C repeated: U = 6.5, $N_2 = 8$, $N_3 = 5$, $p \leq 0.05$). The mean fundamental frequency was approx. 75 Hz at 22° C and 22° C repeated and 99.1 Hz at 30° C (Tab. 2).

Fig. 8. Mean (± S.E.) pulse period of long drumming sounds in catfishes kept at 22° C (■), 30° C (■) and 22° C repeated (□).N = 5 at 22° C and 22° C repeated; N = 8 at 30° C. Different letters indicate significant differences between temperatures (p ≤ 0.05).
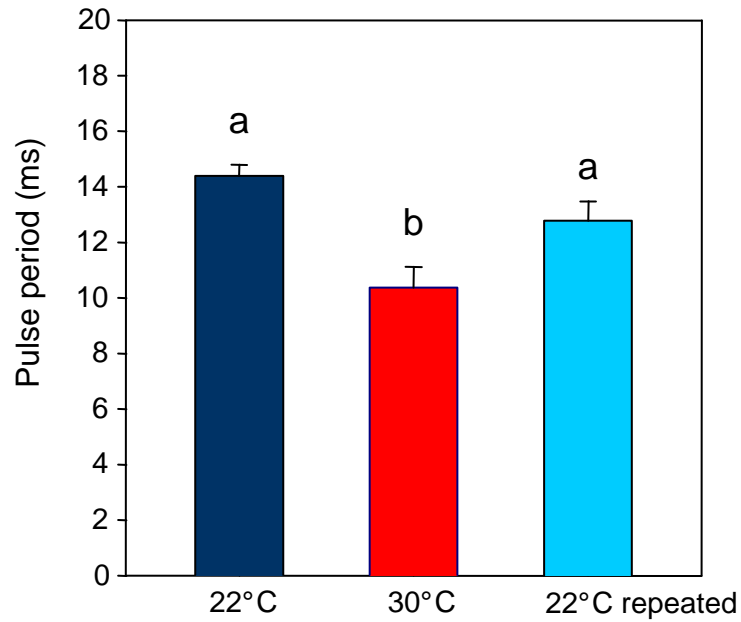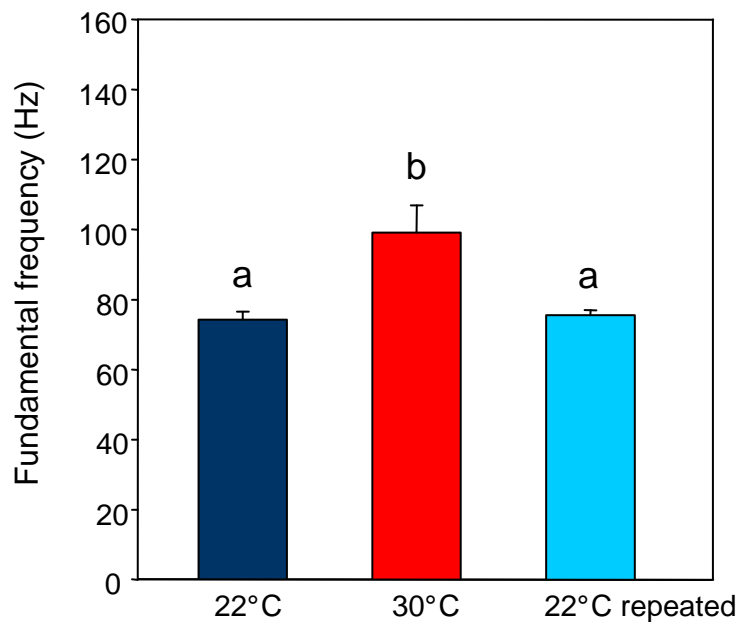

Fig. 9. Mean (± S.E.) fundamental frequency of long drumming sounds in catfishes kept at 22° C (■), 30° C (■) and 22° C repeated (□).N = 5 at 22° C and 22° C repeated; N = 8 at 30° C. Different letters indicate significant differences between temperatures (p ≤ 0.05).

3.3. Auditory sensitivity

Best hearing was found at 0.5 and 1 kHz at both temperatures. Hearing sensitivity increased at

higher frequencies on average by 4 dB when the temperature increased. At 4 kHz, hearing

threshold was on average by 8 dB lower (Tab. 3).

Tab. 3. Mean (± S.E.) hearing thresholds of *P. armatulus* kept at 22° C, 30° C and 22° C repeated. N

= 8.

Frequency (kHz)	22°C	30°C	22°C repeated
0.1	82.0 ± 0.8	82.1 ± 1.1	84.0 ± 1.1
0.2	76.1 ± 1.2	75.5 ± 1.6	74.9 ± 0.7
0.5	69.0 ± 1.5	65.3 ± 1.6	69.5 ± 0.8
1	68.1 ± 1.0	64.6 ± 1.5	69.4 ± 1.0
2	73.1 ± 1.5	70.0 ± 1.3	71.6 ± 1.1
4	82.6 ± 1.6	75.1 ± 1.6	84.3 ± 1.3

A two-factorial ANOVA revealed that the auditory sensitivity was significantly lower

at the lower temperature ($F_{2,126} = 13.46$, $p < 0.001$) and that there existed a significant

interaction between temperature and frequency ($F_{10,126} = 2.15$, $p \leq 0.05$). Thus, changes in

auditory sensitivity showed different trends at different frequencies. Repeated measures

ANOVA revealed significant differences between hearing thresholds at 1 and 4 kHz and no

differences at lower frequencies (Fig.10). Auditory sensitivity increased significantly with

frequency ($r = 0.842$, N = 6, $p \leq 0.05$).
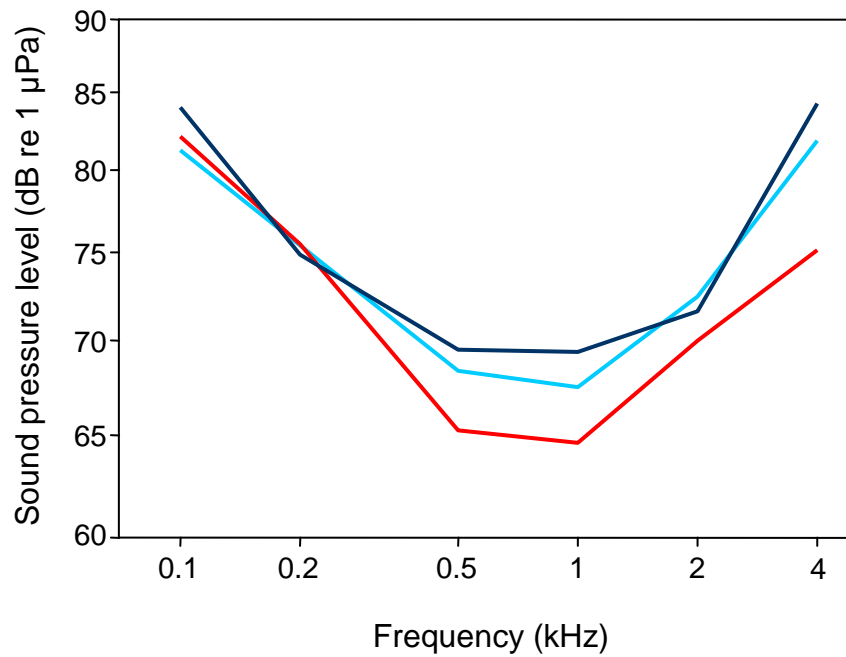
Fig. 10. Mean hearing thresholds of *P. armatulus* kept at 22° C (■), 30° C (■) and 22° C repeated (■). N = 8 per temperature.

Amplitudes of AEP waveforms were larger at the higher temperature (pers. obs.). Depended on the stimulus frequency, shapes of AEPs of *P. armatulus* differed between individuals, but showed no change with ambient temperature (Fig. 11). At 0.5 kHz, the AEPs response was larger at 30° C and consisted of more peaks than at the lower temperature. Interestingly, at 0.5, 1 and 2 kHz the onset of the response of AEPs at 22° C repeated showed a larger delay than at 22° C.

500 Hz

22°C

30°C

22°C repeated

1000 Hz

22°C

30°C

22°C repeated

2000 Hz

22°C

30°C

22°C repeated

5 ms

Fig. 11. Auditory evoked potentials (AEPs) of three different individuals of Lined Raphael catfish in response to stimuli of various frequencies recorded at 20 dB above hearing thresholds (500 and 2000 Hz) or 28 dB above hearing thresholds (1000 Hz). At each stimulus frequency, AEPs of the same indivuals were compared.

3.4. Waveforms and latencies in response to single clicks

AEPs of *P. armatulus* in response to clicks consisted of a series of negative and positive

deflections. In this study, the main negative and positive peaks of the AEPs were analyzed.
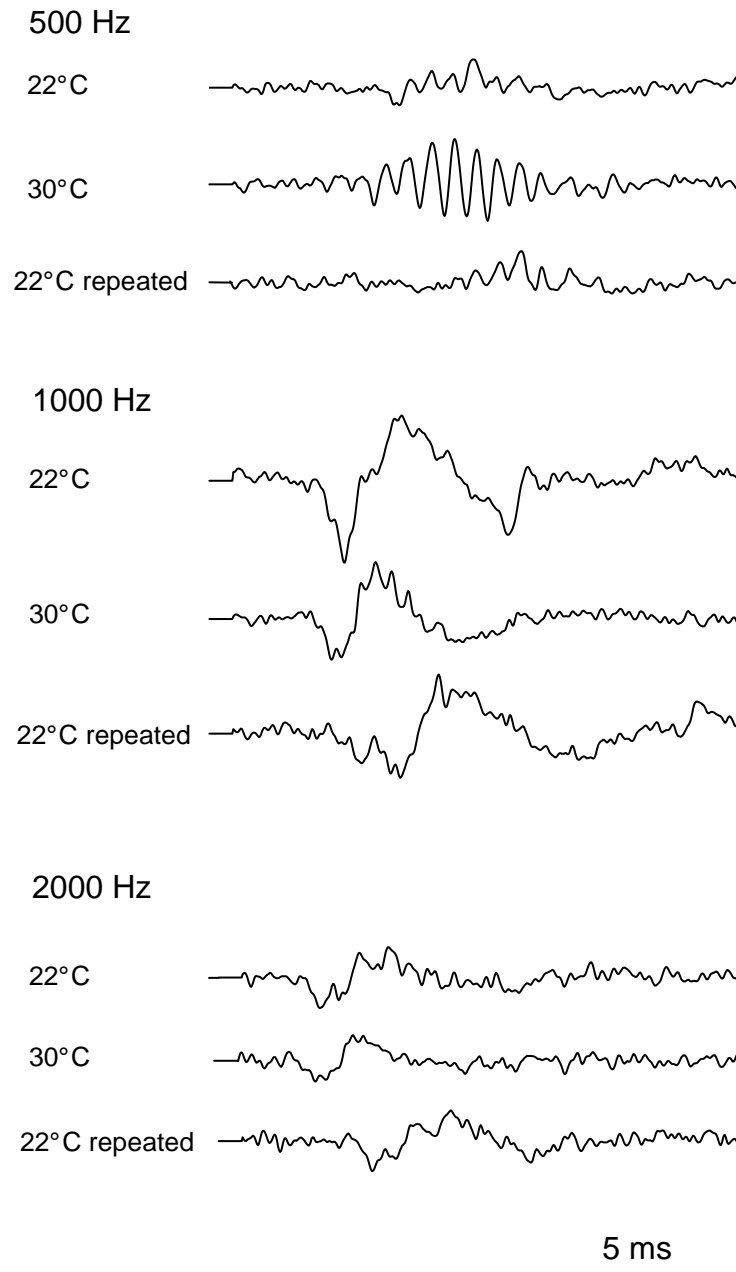
AEPs started with a negative peak (Fig. 12) and amplitude of AEPs decreased when lowering

SPLs. The most constant peaks N1, P1, N2 and P2 were found in the AEPs in response to a

single-click presentation at 22° C and 30° C. Significant differences in latencies of peaks P1,

N2 and P2 were found between temperatures (P1: Friedman-test, $\chi^2 = 12.000$, df = 2, p < 0.01;

N2: Friedman-test, $\chi^2 = 13.231$, df = 2, p < 0.01; P2: Friedman-test, $\chi^2 = 12.250$, df = 2, p <

0.01). The delay in the onset of P2 was significantly longer at lower temperature (Tab. 4) (22°

C and 30° C: Wilcoxon-test, N = 8, p ≤ 0.05; 30° C and 22° C repeated: Wilcoxon-test, N = 8,

p ≤ 0.05). The peak-to-peak amplitude between the first positive peak and the second positive

peak increased with rising temperature. N1 and N2 tended to fuse at higher temperature,

whereas P1 almost disappeared (Fig.12).
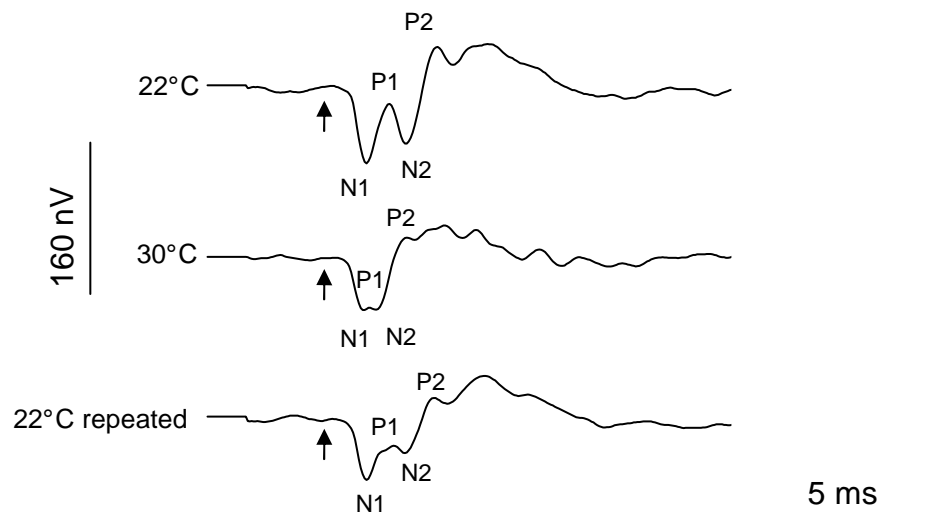
Fig. 12. AEPs of one specimen of *P. armatulus* in response to a single-click stimulus at different
temperatures, presented 28 dB above hearing thresholds. Arrows indicate onset of single-click
stimulus.

Tab. 4. Mean (± S.E.) latency of negative peaks (N1, N2) and positive peaks (P1, P2) of *P. armatulus* kept at 22° C, 30° C and 22° C repeated calculated as the time period between the onset of a single click stimulus 32 dB above hearing threshold and the peaks. N = 8, except N2 at 30° C N = 7 and P1 at 30° C N = 6.

Peak	22°C	30°C	22°C repeated
N1	0.99 ± 0.02	1.04 ± 0.08	1.03 ± 0.03
P1	1.49 ± 0.04	1.16 ± 0.02	1.66 ± 0.03
N2	2.03 ± 0.04	1.40 ± 0.03	2.14 ± 0.04
P2	2.81 ± 0.06	2.38 ± 0.10	2.96 ± 0.04

3.5. Temporal resolution measurements

Two distinct AEPs were clearly detectable in response to double-clicks at click periods of 5 ms down to 1.5 ms (Fig. 13). At shorter click periods, the responses to the first and to the second click were partly overlaid (Fig. 13). The minimum resolvable click period was 0.81 ms (Tab. 5). Near to the hearing threshold, N1 and N2 as well as P2 and P3 tended to merge until one negative and positive peak remained. AEPs varied within and between individuals in shape and latency. No significant difference was observed in the minimum resolvable click periods between temperatures (Friedman test: $\chi^2 = 3.5$, df = 2, p > 0.05).
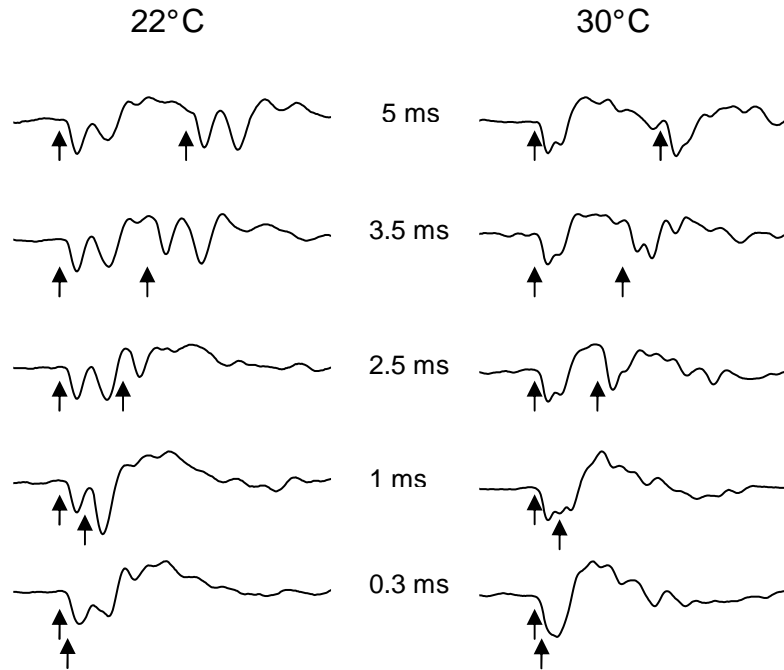
Fig. 13. AEPs of one specimen of *P. armatulus* in response to a double-click stimulus 28 dB above hearing threshold at different click periods (ms) and temperatures. The arrows indicate the onset of stimuli.

Tab. 5. Mean (± S.E.) minimum pulse period resolvable by the auditory system (ms) of *P. armatulus* kept at 22° C, 30° C and 22° C repeated. N = 8 per temperature.

Temperature	Mean minimum gap width (ms)
22°C	0.85 ± 0.10
30°C	0.81 ± 0.09
22°C repeated	1.00 ± 0.00

4. Discussion

Physiological processes depend the surrounding temperature in ectothermic animals. Subsequently, it has to be assumed that sound production (sound characteristics) as well as sound detection are affected by the temperature in fishes. A few investigators have studied these effects in a small number of fish species. They found that in several vocalizing fish species, temperature change induced changes in temporal characteristics of sounds such as in sound duration, in dominant/fundamental frequency, and/or sound pressure level (Kastberger, 1981; Torricelli et al., 1990; Lugli et al., 1996; Connaughton et al., 2000; Amorim, 2005; Amorim et al., 2006; Maruska and Mensinger, 2009). In general, sound duration and fundamental or dominant frequency increased, whereas pulse period and pulse duration decreased with rising ambient temperature. However, it has to be pointed out that not all sound characteristics are effected by temperature changes in species studied and that opposite trends have been observed in a few cases.

4.1. Temperature effects on sound characteristics

The duration of stridulation sounds in *P. armatulus* was affected significantly when elevating the ambient temperature. Both, AB- and AD-sounds became significantly shorter at the higher temperature. This is probably due to the fact that pectoral muscles contract faster and that it takes less time for a complete pectoral fin sweep (Fine and Ladich, 2003).

Stridulation sounds were influenced by temperature, whereas on the other hand duration of drumming sounds did not change with temperature in the current study. Similarly, in the sonic muscles of the searobin, *Prionotus carolinus*, Connaughton (2004) reported no relation between sound duration and temperature variation.

Temperature effects on drumming sounds are a well studied topic in fish biology. Drumming sounds in piranhas, *Serrasalmus nattereri*, in the oyster toadfish, *Opsanus tau*, and in the gobies, *Padogobius bonelli* and *P. nigricans*, became shorter at higher temperatures

(Kastberger, 1981; Torricelli et al., 1990; Lugli et al., 1996; Maruska and Mensinger, 2009). In contrast, drumming sound duration in the weakfish, *Cynoscion regalis*, and in the Lusitanian toadfish, *Halobatrachus didactylus*, increased with rising ambient temperature (Connaughton et al., 2000; Amorim, 2006). Thus, results on sound duration influenced by temperature showed different trends. For instance Amorim (2005) reported that 'knocks became shorter and 'grunts' became longer at higher temperature. So far sound characteristics are temperature-dependent though it could not be concluded which factors are responsible that sounds showed either an increase or a decrease in length with temperature change.

The maximum and minimum pulse periods of stridulation sounds showed temperature-dependence to some degree. The minimum and the maximum pulse period became shorter in AB-sounds at higher temperature, whereas in AD-sounds no such trend could be found. The shorter pulse periods at higher temperatures most likely resulted in shorter duration of AB-sounds because the number of pulses was constant. The lack of such a relationship in AD-sounds is probably due to the fact that the minimum and maximum pulse periods do not reflect the mean pulse period of sounds completely. Dominant frequency of stridulation sounds also did not change with temperature. No comparable studies focusing ambient temperature effects on stridulation sound parameters have been published so far.

On the other hand in drumming sounds of *P. armatulus*, the mean pulse period was temperature-dependent and subsequently resulted in a change of the fundamental frequency from approximately 75 Hz to 100 Hz. The pulses are produced by fast-contracting muscles consisting of an abundance of thin myofibrils encircled by layers of sarcotubules (Ladich, 2001). A change in temperature may affect the pulse pattern generator circuits and muscle contraction properties that causes changes in contraction rate of the drumming muscles. A warmer sarcoplasmic reticulum can cycle calcium more rapidly in the oyster toadfish, *Opsanus tau* (Feher et al., 1998). The muscle is therefore able to relax and to twitch again earlier at higher temperatures. This temperature-dependent process enhance the muscle

contration rate and decreases pulse period at higher temperature. Higher temperature results in higher contraction rate of drumming muscles due to shorter pulse periods and subsequently lead to higher dominant/fundamental frequencies (Walkowiak, 1980; Torricelli et al., 1990; Amorim et al., 2006). Studies on the Arno goby, *Padogobius nigricans*, the searobin, *Prionotus carolinus*, and the oyster toadfish, *Opsanus tau,* reported a rise in fundamental frequencies with higher temperature (Lugli et al., 1996; Connaughton, 2004; Maruska and Mensinger, 2009). These studies did not investigated if due to this outcome pulse periods decreased with elevated temperature. Interestingly, Connaughton et al. (2000) described shorter pulse duration but increasing pulse periods in the weakfish at higher temperature. Nevertheless, sound characteristics like pulse period and fundamental and/or dominant frequency showed overall a strong correlation with ambient temperature.

In *P. armatulus*, no temperature effect could be found in sound pressure level in stridulation sounds. Sound pressure levels in stridulation sounds ranged from 136.4 to 137.9 dB. Connaughton (2004) observed that the sound pressure level of the searobin, *Prionotus carolinus*, was not influenced by temperature as well. In contrast, lower sound pressure levels have been described in the piranha and the weakfish at lower temperatures (Kastberger, 1981; Connaughton et al., 2000).

4.2. Temperature effects on hearing

In several ectothermic animals, temperature-dependent effects on the auditory system have been reported. Amphibians showed in experiments that the hearing capability changed significantly by increasing the surrounding temperature (Hubl, 1977; Walkowiak, 1980). An increase in temperature resulted in lower hearing thresholds and thus in an enhancement of the auditory sensitivity. In insects, temperature changes showed similar influence on the auditory pathway. An increase in the characteristic hearing frequency or best frequency, in

spike rate and in sensitivity could be related to warming above ambient temperature (Oldfield, 1988; Fonseca and Correia, 2007).

Higher temperatures induced a frequency-dependent change in sensitivity in all fish species investigated so far (Mann et al, 2009; Wysocki et al., 2009). Dudok van Heel (1956), was the first describing temperature effects on the auditory funtion in fishes. He trained blinded European minnows (*Phoxinus phoxinus*) to react to different frequencies. At higher temperature, the upper limit of frequency discrimination shifted from 1200 Hz up to 1600 Hz. Subsequently, the detectable frequency range became wider. His suggestion was due to the volley theory by Wever (1949) that rising temperature resulted in shorter refractory period of nerve fibres and therefore, the auditory sensitivity increased in ectothermic animals.

Wysocki et al. (2009) were interested if ambient temperature influenced auditory sensitivity in an erythermal and stenothermal catfish differently. Hearing thresholds of the stenothermic tropical catfish, *Pimelodus pictus*, decreased when increasing the temperature from 22 to 30 °C (Wysocki et al., 2009). *Pimelodus pictus* and *P. armatulus*, showed a similar frequency-dependent increase in sensitivity when increasing the ambient temperature (Fig. 14). Temperature affected hearing sensitivity more at higher frequencies in these tropical catfish species.
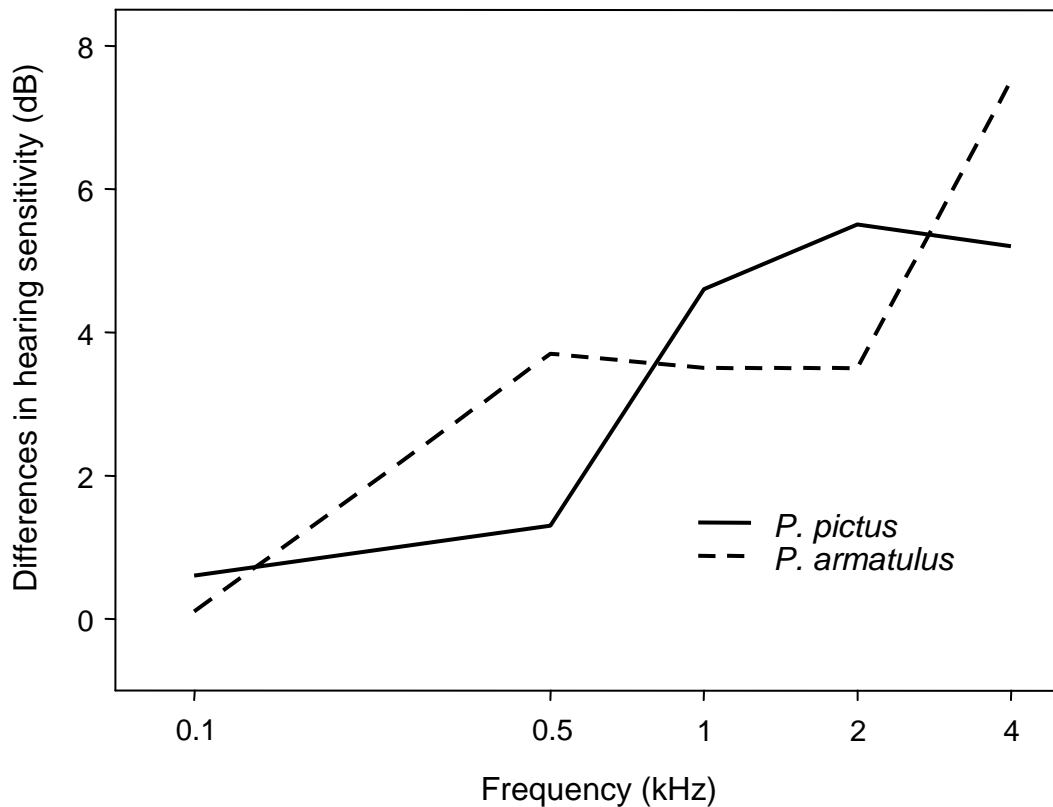
Fig. 14. Comparison of the change in hearing sensitivity in the Amazonian catfishes *Pimelodus pictus* (Wysocki et al., 2009) and *P. armatulus* (recent study). Differences are shown in both species after acclimation for at least 3 weeks to either 22° C and 30° C.

The eurythermal Northamerican channel catfish, *Ictalurus punctatus*, differed widely from the stenothermal tropical catfishes (*P. pictus* and *P. armatulus*) (Wysocki et al., 2009, and current study). The channel catfish exhibited higher changes in hearing sensitivity when the temperature changed, especially at the highest frequency tested. In *I. punctatus* hearing sensitivity at 4 kHz increased by 23 dB when temperature raised from 18 to 26°C. Hearing thresholds of the tropical catfishes, *P. pictus*, showed smaller differences (maximum change: 5 dB) at a similar temperature change of 8° C. Differences between temperatures were more pronounced in the eurythermal catfish species than in the stenothermal catfishes.

Similarly, at higher frequencies did the auditory sensitivity of *P. armatulus* increase to a larger extent. This effect was in particular found at 0.5 kHz and higher frequencies. The auditory sensitivity increased at 4 dB on average. It could be a hint that at least one process in

the auditory pathway might be temperature-dependent. Fay and Ream (1992) concluded that temperature-dependent effects on the nervous system in goldfish, *Carassius auratus*, may be due to changes in the release and reuptake of neurotransmitter at the synapses between hair cells and auditory nerve fibres. Elevating temperature resulted in an increase in cell's spontaneous activity, sensitivity, best frequency and responsiveness. In the process chain, there could be a another specific step for transducing high-frequent information.

There are quite reasonable explanation for the phenomenon that the hearing sensitivity at higher frequencies are more affected by temperature changes than at lower frequencies. High frequency hearing needs faster firing of action potentials due to synchronization with the shorter sound cycles (Wysocki et al., 2009). Maybe the refractory periods and transduction processes are more temperature-dependent than that of longer cycles of lower frequencies. This would be consistent with the frequency-dependent improvement of hearing in the present study.

Latencies decreased in three out of four peaks (P1, N2 and P2) at higher temperatures in *P. armatulus*. This result might be explained by temperature dependence of spike conduction velocity, of spike shape and perhaps of synaptic delay. Short latencies indicate better hearing cability at higher temperature (Amoser and Ladich, 2006). Besides, Wysocki and Popper (2006) observed also a difference in shape of AEPs at different temperatures. At higher temperature, peaks tended to fuse, especially the first and the second negative peak, and the amplitudes of AEPs increased.

In the locust, *Locusta migratoria*, was observed that higher temperatures resulted in a better resolution of gaps (Franz and Ronacher, 2002). No such change with temperature was found in the current study. Wysocki and Ladich (2002) reported that the mean minimum resolvable pulse period of the Lined Raphel catfish 0.52 ms, measured at 25° C. The current study found a mean minimum resolvable click period of 0.86 ($\pm$ 0.05) ms at 32 dB above hearing threshold at both temperatures investigated and two distinct AEPs were clearly

traceable at a click period larger than 3.5 ms (according to Wysocki and Ladich, 2002). The minimum pulse periods in the stridulation sounds (2 ms) and in the drumming sounds (6 ms) in *P. armatulus* - measured in the recent study - are longer than the mean pulse period. This indicates that these catfishes are able to encode the temporal information of sounds from conspecifics, independent from changes in ambient temperature

4.3. Temperature and acoustic communication

Many catfish species produce sounds in various behavioural contexts such as disturbance, agonistic behavior and male courtship display (Pruzsinszky and Ladich, 1998; Kaatz, 1999; Fine and Ladich, 2003). Thus, the detection of stridulation and drumming sounds are an important factor in catfish behavior. In disturbance situations catfish are likely to emit more stridulation sounds, whereas in intraspecific contexts more drumming sounds are produced (Kaatz, 1999). It can be concluded that stridulation sounds may have a warning or defense intention, while drumming sounds play an important role in intraspecific communication (Kaatz, 1999; Heyd and Pfeiffer, 2000).

Temperature affects sound characteristics in stridulation sounds (duration) as well as drumming sounds (pulse period, fundamental frequency). Both observations are in accordance with the fact that the muscle contraction rate increases with temperature. Higher contraction speed of the pectoral abductor and adductor muscle results in shorter AB- and AD-sounds. Similarly, higher drumming muscle contraction rate results in shorter pulse periods and higher fundamental frequency. Stridulation sounds tended to have higher dominant frequencies and shorter pulse periods. Sound frequencies of both sound types shift to higher frequencies with rising temperatures and hearing sensitivity increased at higher frequencies. Thus, low frequency (0.1 and 0.2 kHz) drumming sounds and in particular of high frequency stridulation sounds (above 500 Hz) will be better detectable at higher temperatures. The more sensitive hearing thresholds accompanied by the faster response of the auditory system at higher

temperatures (shorter latencies of AEP waves) leads to the assumption that acoustic communication is facilitated at higher temperatures in fishes. Future studies on vocal fish species may find out if this effect is more pronounced in eurythermic than stenothermic fish species.

5. Acknowledgements

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8. Appendix

8.1. Mean data of stridulation sounds per individual at a) 22° C, b) 30° C and c) 22° C repeated.

a) 22° C

Fish	Stridulation sounds					
	Sound duration (AD)	Sound duration (AB)	Number of pulses (AD)	number of pulses (AB)	minimum pulse period (AD)	minimum pulse period (AB)
Pa1	160	98	9	8	8	6
Pa2	92	66	12	7	4	4
Pa3	81	97	3	7	16	8
Pa4	63	82	6	7	7	6
Pa5	89	89	10	9	6	4
Pa6	92	85	10	9	5	4
Pa7	85	84	6	6	9	8
Pa8	96	106	8	8	7	7

Fish	Stridulation sounds			
	Maximum pulse period (AD)	Maximum pulse period (AB)	Sound pressure level (dB)	Dominant frequency (Hz)
Pa1	44	19	134.5	1226.6
Pa2	13	14	135.5	335.9
Pa3	45	18	140.0	625.0
Pa4	18	15	137.0	273.4
Pa5	12	14	137.5	546.9
Pa6	13	22	134.5	648.4
Pa7	21	23	137.0	906.3
Pa8	18	18	135.0	250.0

b) 30° C

Fish	Stridulation sounds					
	Sound duration (AD)	Sound duration (AB)	Number of pulses (AD)	Number of pulses (AB)	Minimum pulse period (AD)	Minimum pulse period (AB)
Pa1	100	56	8	15	8	2
Pa2	81	53	7	7	3	3
Pa3	75	70	4	7	14	5
Pa4	65	70	5	7	5	6
Pa5	61	63	7	6	5	7
Pa6	49	75	5	8	5	3
Pa7	57	67	3	6	9	6
Pa8	83	85	9	7	8	8

Fish	Stridulation sounds			
	Maximum pulse period (AD)	Maximum pulse period (AB)	Sound pressure level (dB)	Dominant frequency (Hz)
Pa1	22	5	134.0	1210.9
Pa2	26	23	141.6	1300.0
Pa3	28	14	141.0	1242.2
Pa4	16	21	136.0	992.2
Pa5	11	12	138.5	1132.8
Pa6	15	23	140.8	1976.6
Pa7	35	21	135.0	1054.7
Pa8	12	18	136.5	1265.6

c) 22° C repeated

Fish	Stridulation sounds					
	Sound duration (AD)	Sound duration (AB)	Number of pulses (AD)	Number of pulses (AB)	Minimum pulse period (AD)	Minimum pulse period (AB)
Pa1	164	86	8	9	6	6
Pa2	135	44	9	3	5	9
Pa3	86	96	4	8	15	7
Pa4	127	108	6	6	12	9
Pa5	96	84	8	6	7	7
Pa6	158	132	15	9	6	5
Pa7	87	83	3	3	10	10
Pa8	130	96	7	5	11	10

Fish	Stridulation sounds			
	Maximum pulse period (AD)	Maximum pulse period (AB)	Sound pressure level (dB)	Dominant frequency (Hz)
Pa1	51	14	133.5	1424.0
Pa2	17	12	135.3	894.0
Pa3	25	16	136.8	1691.0
Pa4	41	38	135.5	1590.0
Pa5	14	23	142.2	1464.0
Pa6	18	24	134.9	984.0
Pa7	43	47	134.6	784.0
Pa8	25	33	140.3	793.0

8.2. Mean data of drumming sounds per individual at a) 22° C, b) 30° C and c) 22° C

repeated.

a) 22° C

Fish	Drumming sounds			
	Sound duration (ms)	Number of pulses	Mean pulse period (ms)	Fundamental frequency (Hz)
Pa1	164	10	13	76.2
Pa2	677	39	15	68.4
Pa3	143	9	15	84.0
Pa4	184	12	14	68.4
Pa5	k.A.	k.A.	k.A.	74.2
Pa6	k.A.	k.A.	k.A.	k.A.
Pa7	k.A.	k.A.	k.A.	k.A.
Pa8	220	14	15	74.2

b) 30° C

Fish	Drumming sounds			
	Sound duration (ms)	Number of pulses	Mean pulse period (ms)	Fundamental frequency (Hz)
Pa1	359	35	10	87.9
Pa2	498	51	9	117.2
Pa3	181	15	13	80.1
Pa4	181	15	12	84.0
Pa5	261	36	7	134.8
Pa6	160	19	9	123.0
Pa7	345	36	10	87.9
Pa8	235	18	13	78.1

c) 22° C repeated

Fish	Drumming sounds			
	Sound duration (ms)	Number of pulses	Mean pulse period (ms)	Fundamental frequency (Hz)
Pa1	170	12	14	72.7
Pa2	547	42	13	80.1
Pa3	305	21	14	72.3
Pa4	k.A.	k.A.	k.A.	k.A.
Pa5	231	21	11	74.2
Pa6	380	31	12	78.1
Pa7	170	12	14	72.7
Pa8	547	42	13	80.1

8.3. Data of hearing thresholds of each individual at a) 22° C, b) 30° C and c) 22° C repeated.

a) 22° C

Fish	Frequency (Hz)					
	0.1	0.2	0.5	1k	2	4
Pa1	85	77	70	70	74	79
Pa2	80	72	64	70	73	83
Pa3	80	73	73	71	72	93
Pa4	84	80	77	67	77	84
Pa5	85	81	70	71	79	81
Pa6	80	72	66	66	68	79
Pa7	81	77	66	67	75	80
Pa8	81	77	66	63	67	82

b) 30° C

Fish	Frequency (kHz)					
	0.1	0.2	0.5	1	2	4
Pa1	85	78	64	66	72	85
Pa2	79	75	59	67	70	72
Pa3	83	75	65	73	76	70
Pa4	82	82	62	63	66	76
Pa5	79	79	73	65	72	73
Pa6	79	68	63	60	65	75
Pa7	83	71	71	62	72	76
Pa8	87	76	65	61	67	74

c) 22° C repeated

Fish	Frequency (kHz)					
	0.1	0.2	0.5	1	2	4
Pa1	82	75	72	72	70	80
Pa2	84	73	69	69	68	88
Pa3	88	77	71	73	76	91
Pa4	89	73	68	67	75	85
Pa5	82	74	72	70	74	84
Pa6	81	73	66	71	68	81
Pa7	85	77	70	69	70	82
Pa8	81	77	68	64	72	83

8.4. Data of latencies measurement of each individual at each temperature at peak a) N1, b) P1, c) N2 and d) P2.

a) Peak N1

Fish	Latencies (ms)		
	22°C	30°C	22°C repeated
Pa1	1.05	0.95	1.00
Pa2	1.05	1.00	1.00
Pa3	1.00	0.95	0.95
Pa4	0.90	0.85	1.00
Pa5	0.90	1.05	0.95
Pa6	1.00	0.95	1.10
Pa7	0.95	1.60	1.10
Pa8	1.05	1.00	1.20

b) Peak P1

Fish	Latencies (ms)		
	22°C	30°C	22°C repeated
Pa1	1.65	1.15	1.70
Pa2	1.60	1.15	1.75
Pa3	1.50	1.20	1.70
Pa4	1.35	1.10	1.45
Pa5	1.30	k.A.	1.70
Pa6	1.55	1.15	1.70
Pa7	1.45	k.A.	1.65
Pa8	1.50	1.20	1.65

c) Peak N2

Fish	Latencies (ms)		
	22°C	30°C	22°C repeated
Pa1	2.05	1.45	2.05
Pa2	2.05	1.50	2.05
Pa3	2.05	1.35	2.20
Pa4	1.85	1.25	2.15
Pa5	1.85	1.35	1.95
Pa6	2.15	1.40	2.30
Pa7	2.05	k.A.	2.15
Pa8	2.15	1.50	2.30

d) Peak P2

Fish	Latencies (ms)		
	22°C	30°C	22°C repeated
Pa1	2.90	2.10	3.05
Pa2	2.90	2.20	2.75
Pa3	2.85	2.15	2.90
Pa4	2.65	2.05	2.90
Pa5	2.60	2.55	2.95
Pa6	3.00	2.60	3.15
Pa7	2.60	2.80	2.95
Pa8	2.95	2.60	3.05

8.5. Data of temporal resolution measurement of each individual at each temperature.

Fish	Minimum resolvable click period (ms)		
	22°C	30°C	22°C repeated
Pa1	0.3	1	k.A.
Pa2	1	0.5	1
Pa3	1	0.5	1
Pa4	1	1	1
Pa5	0.5	1	1
Pa6	1	1	1
Pa7	1	0.5	1
Pa8	1	1	1

9. Zusammenfassung

Die Lauterzeugung und das Hörvermögen ektothermischer Tiere werden von der Umgebungstemperatur beeinflusst. Die aktuelle Studie hat den Einfluss der Außentemperatur auf die Lautparameter und das Hörvermögen des neotropischen Liniendornwelses *Platydoras armatulus* untersucht. Welse der Familie Doradidae (Dornwelse) produzieren einerseits Stridulationslaute durch das Reiben der Dornfortsätze der Brustflosse im Schultergürtel und andererseits Trommellaute mit Hilfe des Springapperates, der die Schwimmblase in Vibration versetzt. Die Versuchstiere wurden mindestens drei Wochen zuerst an 22° C akklimiert, dann an 30° C und anschließend wieder an 22° C akklimiert. Die Laute der Welse wurden in Stresssituationen, in denen sie in der Hand gehalten wurden, aufgenommen. Die Stridulationslaute wurden bei höherer Temperatur kürzer, während die Anzahl der Pulse, die kleinste und die größte Pulsperiode, der Schalldruckpegel und die Hauptfrequenz sich nicht mit der Temperatur veränderten. Auf der anderen Seite erhöhte sich die Grundfrequenz und die durchschnittliche Pulsperiode der Trommellaute verkürzte sich mit steigender Temperatur. Mittels der Auditory Evoked Potential (AEP) Recording Methode wurde die Hörempfindlichkeit bei insgesamt sechs verschiedenen Frequenzen zwischen 0.1 bis 4 kHz getestet. Das zeitliche Auflösungsvermögen des Hörvermögens wurde durch die minimale auflösbare Klickperiode (0.3 – 5 ms) bestimmt und die Latenzen der Antwort als Verzögerungen auf den präsentierten Klick gemessen. Das Hörvermögen verbesserte sich zwischen 0.5 und 4 kHz mit steigender Umgebungstemperatur, während das zeitliche Auflösungsvermögen als Antwort auf Doppelklicks nicht von der Temperatur beeinflusst wurde. Die Latenzen der AEPs verkürzte sich in drei von insgesamt vier Peaks bei höherer Temperatur.

Diese Daten lassen daraus schließen, dass die Hörempfindlichkeit mit zunehmender Temperatur steigt. Die biologischen Gegebenheiten, die sich auf das Hörvermögen bei unterschiedlichen Temperaturen anders auswirken, können selbst nach langer

Akklimierungsperiode nicht kompensiert werden. Da einige Lautparameter ebenfalls temperaturabhängig waren, lautet die Schlussfolgerung, dass die Umgebungstemperatur direkt die akustische Orientierung und die Kommunikation des neotropischen Liniendornwelses *P. armatulus* beeinflusst.