

The influence of ambient temperature and thermal acclimation on hearing in a eurythermal and a stenothermal otophysan fish

Lidia Eva Wysocki*, Karen Montey and Arthur N. Popper†

Department of Biology and Center for Comparative and Evolutionary Biology of Hearing, University of Maryland, College Park, MD 20742, USA

*Present address: Department of Behavioural Biology, Faculty Center of Zoology, University of Vienna, Vienna, Austria

†Author for correspondence (apopper@umd.edu)

Accepted 22 June 2009

SUMMARY

Being ectothermic, fish body temperature generally depends on ambient water temperature. Thus, ambient temperature might affect various sensory systems, including hearing, as a result of metabolic and physiological processes. However, the maintenance of sensory functions in a changing environment may be crucial for an animal's survival. Many fish species rely on hearing for acoustic orientation and communication. In order to investigate the influence of temperature on the auditory system, channel catfish *Ictalurus punctatus* was chosen as a model for a eurytherm species and the tropical catfish *Pimelodus pictus* as a model for a stenotherm fish. Hearing sensitivity was measured with animals acclimated or unacclimated to different water temperatures. Ambient water temperature significantly influenced hearing thresholds and the shape of auditory evoked potentials, especially at higher frequencies in *I. punctatus*. Hearing sensitivity of *I. punctatus* was lowest at 10°C and increased by up to 36 dB between 10°C and 26°C. Significant differences were also revealed between acclimated and unacclimated animals after an increase in water temperature but not a decrease. By contrast, differences in hearing thresholds were smaller in *P. pictus*, even if a similar temperature difference (8°C) was considered. However, *P. pictus* showed a similar trend as *I. punctatus* in exhibiting higher hearing sensitivity at the highest tested temperature, especially at the highest frequency tested. The results therefore suggest that the functional temperature dependence of sensory systems may differ depending upon whether a species is physiologically adapted to tolerate a wide or narrow temperature range.

Key words: auditory evoked potentials, catfish, hearing, thermal acclimation.

INTRODUCTION

Many fish species live in habitats where the water temperature undergoes considerable seasonal and diurnal fluctuations. Indeed, in some cases, such as in shallow South Texas tide pools (Bennett and Judd, 1992), seasonal water temperature fluctuations can vary by as much as 40°C. By contrast, water temperature shows only small fluctuations in other habitats such as in circumpolar regions, tropical waters or in the deep sea. Consequently, the thermal tolerance range of fishes varies greatly. Some Cyprinodontiforms, like the pupfish *Cyprinodon artifrons*, the mosquitofish *Gambusia yucatana* and the goldspotted killifish *Floridichthys carpio*, are able to cope with daily temperatures in excess of 40°C (Heath et al., 1993). By contrast, other species such as Antarctic icefishes (nototheniids) of the genus *Trematomus* complete their entire life cycles at temperatures just above –1.8°C and are unable to survive at warmer temperatures near 6°C (Somera and DeVries, 1967).

The body temperature of most fish species depends on the ambient water temperature, as in other ectothermic animals. Because body temperature influences the speed of all metabolic processes, the ambient temperature affects various physiological processes, such as protein expression and binding (Deane and Woo, 2005; Huber and Guderley, 1993), blood circulation (Aguilar et al., 2002; Lecklin et al., 1995), respiration (Sollid et al., 2005), hormonal activity (Fortune, 1958), the immune system (Le Morvan et al., 1998) and behavior (Cossins et al., 1977; Friedlander et al., 1976; McCormick et al., 2002; Zitek et al., 2004). In addition, ambient temperature is also known to affect several parameters directly related to reproductive fitness, such as growth (Dembski et al., 2006; Hall

and Johnston, 2003), egg size (Gillet and Quélin, 2006; Kokita, 2003) and life span (Dembski et al., 2006).

In addition to long-term effects, temperature has also been shown to influence the sensitivity of sensory systems, such as vision (Aho et al., 1993; Reilly and Thompson, 2007), the vestibulo-ocular reflex (McElligot et al., 1995), touch (French and Kuster, 1982) and olfaction (Kashiwayanagi et al., 1997; Nordin et al., 1998; Shoji et al., 1994). Thus, the ambient temperature has the potential to directly influence the detection of predators (Amo et al., 2004) and communication. In the latter case, the temperature-dependent variability is often paralleled by temperature-induced changes in signal production (Brenowitz et al., 1985; Connaughton et al., 2002; Lorenzo and Macadar, 2005).

Many fish species are very reliant on hearing for acoustic orientation, communication, detection of predators and prey, location of mates and for gathering information about the 'acoustic scene' (Fay and Popper, 2000; Hawkins and Myrberg, 1983; Ladich, 1999; Popper and Fay, 1993). The auditory system gains particular importance in murky habitats or at night when vision is limited. The dependence of auditory function on body temperature has been studied extensively in insects (Franz and Ronacher, 2002; Oldfield, 1988; Pires and Hoy, 1992), amphibians (Hubl and Schneider, 1978; Long et al., 1996; Mohneke and Schneider, 1979; Walkowiak, 1980), reptiles (Wilson et al., 1985), non-human mammals (Khvoles et al., 1998) and humans (e.g. Ferber-Viart et al., 1995; Kaga et al., 1979; O'Brien, 1994). Data on temperature effects on fish are very limited but provide some indication that temperature influences auditory function (Dudok van Heel, 1956; Fay and Ream, 1992).

The goal of the current study was to investigate the effects of ambient water temperatures on the functional auditory response of fish by examining changes in auditory evoked potentials (AEPs), with the main focus on a temperate species adapted to tolerate a wide temperature range in its natural environment (i.e. a eurythermal species). Furthermore, we were interested in whether potential changes in auditory function are also influenced by acclimation time to a specific water temperature (unacclimated *versus* acclimated fish). Finally, we compared a focal species with a taxonomically related species with similar hearing abilities but with a narrower temperature tolerance (i.e. a stenothermal species). We sought to determine if the stenothermal species differed from the eurythermal species in the temperature dependence of their auditory functions. More specifically, we asked whether fish that were well acclimated to a region of wide temperature ranges have specific adaptations to maintain their sensory abilities relative to species that only tolerate a narrow range of temperatures.

We selected two species of catfish, members of the taxonomic group Otophysi, for this study. Catfish, as other otophysans, possess a connection between the swim bladder and the inner ear (Weberian ossicles), which allows them to detect the pressure component of sound and enhances their hearing bandwidth and sensitivity (Poggendorf, 1952).

Channel catfish *Ictalurus punctatus* was chosen as a model for a eurythermal species with a large thermal tolerance scope because they are exposed in their area of occurrence to temperatures ranging from near freezing point to over 36°C (Currie et al., 1998; Ju et al., 2002). The tropical catfish *Pimelodus pictus* served as a model for a stenothermal fish with lower thermal tolerance. The mean temperatures in its area of occurrence range from 24°C to 29°C (Diaz-Sarmiento and Alvarez-León, 2004) and recommended holding temperatures in captivity are 22–25°C (Baensch and Riehl, 1985).

MATERIALS AND METHODS

Study animals

Test subjects were 17 channel catfish *Ictalurus punctatus* Rafinesque 1818 [91–125 mm standard length (SL), 9–11 g body mass (M_b)] and 14 *Pimelodus pictus* Steindachner 1876 (50–72 mm SL, 1.6–4.1 g M_b). *Ictalurus punctatus* were obtained from a fish hatchery (Zett's Tri-State Fish Farm & Hatchery, Inwood, WV, USA) and *P. pictus* from a commercial pet supplier (Scales Tropical Fish Warehouse, Cloverly, MD, USA). *Ictalurus punctatus* were kept in a cylindrical, re-circulating 890 l fiberglass tank, and *P. pictus* were kept in two 114 l all-glass aquaria. Holding tanks were biologically filtered and aerated. Temperature in the large re-circulating tank was maintained with either a Aqua Logic® Delta Star 5 Chiller (Aqua Logic, Lisle, IL, USA) or a 1000 W heater (Easy Plug EP10T, Apopka, FL, USA) connected to a digital controller. In the glass aquaria, temperature was maintained *via* commercial submersible aquarium heaters and monitored and controlled daily. Fish were fed daily and maintained at a 12 h:12 h L:D cycle. All experiments were approved by the University of Maryland Institutional Animal Care and Use Committee.

Temperature regime

Prior to experiments, fish were acclimated to the holding conditions at a stable baseline temperature for at least two months before initial measurement of hearing thresholds (baseline). Fig. 1 outlines the sequence of testing and acclimation. Fish were allowed to rest for at least a week after each hearing test and before each subsequent change of the holding water temperature. The temperature of the holding water was changed at a rate of 1°C per day. At each test

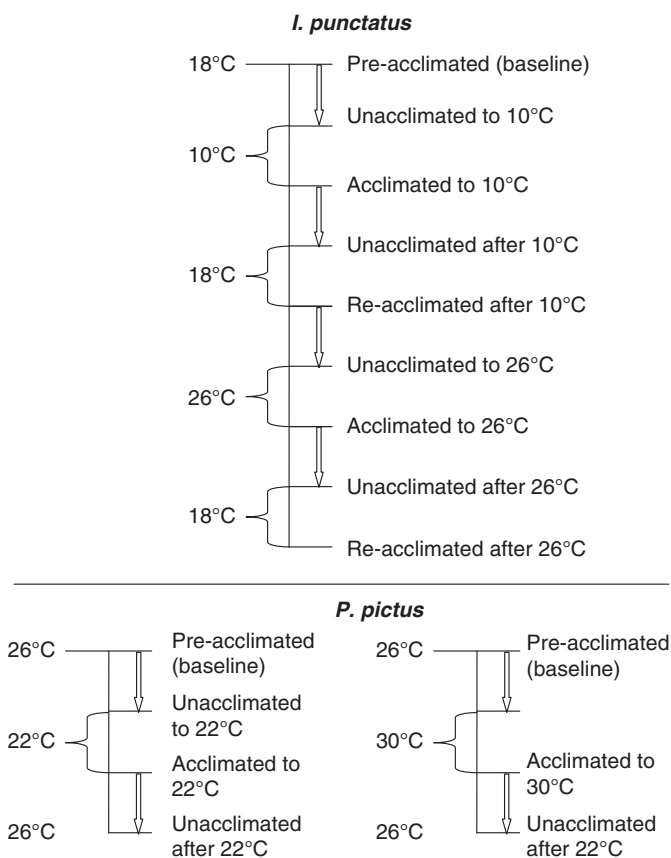


Fig. 1. Diagram of the different temperature protocols used. The arrows indicate periods when water temperature was changed by 1°C per day.

temperature (except for the original baseline measurements), fish were initially tested immediately after the holding tank reached the new test temperature (unacclimated) and again after at least four weeks of acclimation to this new temperature (acclimated). Measurements of pre-acclimated (baseline) *I. punctatus* at the initial test temperature of 18°C were performed three different times in order to test whether hearing changes observed at different temperatures were caused by temperature or, instead, reflected potential body mass variations of fish caused by different metabolic rates at the various temperatures or age effects (Fig. 1).

While all *I. punctatus* underwent the same temperature changes (over a total period of nine months), *P. pictus* underwent different temperature regimes in both aquaria, i.e. after the same original baseline temperature (26°C), the temperature in one tank was lowered to 22°C while the temperature in the other tank was elevated to 30°C. After auditory thresholds were measured in unacclimated (except for the 30°C unacclimated animals due to technical difficulties, it was not possible to maintain a sufficiently constant water temperature throughout the whole experiment at this stage) and acclimated fish for both temperatures, the temperature was set back to 26°C in both aquaria. Holding conditions other than temperature were identical for both *P. pictus* groups.

At each water temperature and acclimation stage, the audiograms of at least eight *I. punctatus* or five *P. pictus* individuals were measured. Individual fish could be recognized by the pattern of spots on either their body (*I. punctatus*) or their caudal fin (*P. pictus*) throughout the experimental period, and individuals were re-measured several times. However, not all measurements were

Table 1. Sequence of different temperature regimens in the holding tanks and in the experimental tank where auditory thresholds were determined

	Temperature (°C)	Acclimation stage	N	SL (mm)	M _b (g)
<i>Ictalurus punctatus</i>					
	18	Pre-acclimated (baseline)	9	92–125	10.2–22.3
	10	Unacclimated at 10°C	9	91–125	10.7–21.8
	10	Acclimated at 10°C	9	92–125	10.8–21.5
	18	Unacclimated after 10°C	8	91–116	10.0–20.3
	18	Re-acclimated after 10°C	8	91–116	9.0–18.7
	26	Unacclimated at 26°C	8	94–115	9.0–18.1
	26	Acclimated at 26°C	8	97–119	10.6–20.4
	18	Re-unacclimated after 26°C	8	96–119	9.9–18.5
	18	Acclimated after 26°C	8	96–118	9.8–16.9
<i>Pimelodus pictus</i>					
Baseline	26	Pre-acclimated (baseline)	7	52–66	1.9–3.7
Group1	22	Unacclimated at 22°C	5	50–66	1.7–4.1
	22	Acclimated at 22°C	6	50–60	1.9–4.0
	26	Unacclimated after 22°C	4	57–72	2.4–4.7
Group2	30	Unacclimated at 30°C	n.a.		
	30	Acclimated at 30°C	7	52–66	1.6–3.7
	26	Unacclimated after 30°C	5	56–69	2.2–4.1

M_b, body mass (g); N, number of individuals tested at each stage; SL, standard length (mm). Fig. 1 shows the testing scheme graphically.

performed on the same individuals for considerations of animal welfare. Thus, a maximum of five audiogram measurements were performed on one individual fish. The sequence of temperature changes and AEP measurements for both species including data on fish size is summarized in Table 1.

Auditory threshold determination

The AEP recording protocol closely followed the one described in Wysocki et al. (Wysocki et al., 2007) therefore only a brief description is given here. Fish were mildly immobilized during the hearing tests with an intra-muscular injection of Flaxedil (gallamine triethiodide, Sigma Chemical Co., St Louis, MO, USA) at doses of $1.5 \pm 0.05 \mu\text{g g}^{-1}$ body mass for *I. punctatus* and $2.0 \pm 0.12 \mu\text{g g}^{-1}$ body mass for *P. pictus*. This dosage allowed the fish to retain slight opercular movements during the experiments without creating significant myogenic noise to interfere with the recordings.

Test subjects were secured in the center of a rectangular plastic tub (51×41 cm; water depth: 25 cm) that had a 4 cm thick layer of fine gravel on the bottom. Fish were restrained in a mesh sling and suspended so that the top of the head was 6 cm below the water surface. A pipette was inserted into the mouth and provided water from a simple temperature-controlled, gravity-fed water circulation system. Water temperature during the experiments was controlled and maintained by using ice packs or heaters in the water supply tank and by thermal insulation of the experimental tub. The maximum deviation from the current temperature in the fish holding tanks was $\pm 1^\circ\text{C}$.

The AEPs were recorded using stainless steel electrodes (Rochester Electro-Medical, Tampa, FL, USA). The recording electrode was placed in the midline of the skull over the medulla region and the reference electrode was placed cranially between the nares. Both electrodes were inserted approximately 2 mm subdermally. All exposed surfaces of the electrode tips that were not in direct contact with the fish were insulated with fingernail polish. A ground electrode was placed in the water.

Sound stimuli presentation and AEP waveform recordings were performed with a modular rack-mount system (TDT System 3, Tucker-Davis Technologies, Gainesville, FL, USA) and TDT BioSig RP Software (Tucker-Davis Technologies). Sounds were

created using TDT SigGen RP software and fed through a power amplifier (Alesis RA 150, Cumberland, RI, USA) connected to an underwater speaker (UW-30, University Sound, Burnsville, MN, USA) that was placed centered on the bottom of the plastic tub. Sound stimuli were presented as repeated tone bursts at a rate of 20 per second.

Hearing thresholds were determined at frequencies of 100, 300, 500, 800, 1000, 2000 and 4000 Hz, presented in random order. The duration of sound stimuli was 15 ms for 100 and 300 Hz, 10 ms for 500–1000 Hz and 5 ms for 2000 and 4000 Hz. Rise and fall times were 2 ms for 100–1000 Hz and 1 ms for 2000 and 4000 Hz. All tone bursts were gated using a Blackman window. Absolute sound pressure levels were measured using a hydrophone (10 CT, frequency response: 30 Hz–100 kHz ± 3 dB; receiving sensitivity: $-211 \text{ dB} \pm 3 \text{ dB re. } 1 \text{ V}/\mu\text{Pa}$, G.R.A.S., Holte, Denmark) and a Kistler dual-mode amplifier (5010, Amherst, NY, USA) at the position where the fish was placed in the test tub. For each test condition, stimuli were presented at opposite polarities (180-phase shifted), and the corresponding AEP traces were averaged using the Bio-Sig RP software in order to eliminate stimulus artifacts. Up to 1000 responses were averaged for each stimulus level and polarity. Sound pressure levels of tone-burst stimuli were reduced in 4 dB steps until the AEP waveform was no longer apparent. The lowest sound pressure level for which a repeatable AEP trace could be obtained, as determined by overlaying replicate traces, was considered the threshold (Kenyon et al., 1998; Wysocki et al., 2007).

Sound stimuli were only measured in terms of sound pressure. As otophysan fishes are pressure sensitive (e.g. Poggendorf, 1952; Popper et al., 2003), we did not attempt to measure particle motion or whether the maximum stimulus to which the fish were exposed was pressure or particle motion. We did not focus on these issues because the question for the current study was a comparison of hearing thresholds at different water temperatures under exactly the same acoustic conditions for all determinations in order to investigate whether there are temperature-related differences (or a lack of such differences) in thresholds. As conditions (besides water temperature) were identical, the results are independent of the complexity of the sound field or of the question of whether AEP thresholds are comparable with behavioral thresholds.

Table 2. Hearing thresholds of *Ictalurus punctatus* recorded at different water temperatures and acclimation stages

	Temp (°C)	100 Hz	300 Hz	500 Hz	800 Hz	1000 Hz	2000 Hz	4000 Hz
Acc	18	96.44±1.53	89.11±0.92	88.22±0.68	89.11±0.51	87.78±1.01	90.00±1.55	104.00±1.38
Unacc	10	99.44±1.62	99.33±1.53	99.22±1.05	97.00±1.84	97.56±1.62	108.78±2.18	117.89±1.59
Acc	10	97.67±1.71	98.44±0.77	98.11±0.90	94.00±0.94	94.22±1.53	107.22±1.85	117.78±1.02
Unacc	18	99.00±1.93	93.50±1.79	92.13±1.06	92.63±0.96	92.63±1.03	98.13±1.01	117.38±1.39
Acc	18	94.38±1.25	87.13±0.69	86.88±0.95	87.25±0.65	85.88±1.32	89.75±1.26	104.63±1.59
Unacc	26	94.38±1.85	88.88±2.28	85.88±1.74	85.50±0.80	84.88±1.13	84.38±1.39	91.00±1.95
Acc	26	92.75±0.98	84.50±1.34	73.88±2.16	76.38±1.83	77.38±1.78	78.00±1.31	81.38±1.76
Unacc	18	97.50±1.39	86.88±1.71	83.25±2.03	85.25±1.49	84.88±0.88	90.25±1.18	101.00±1.55
Acc	18	93.00±0.76	85.69±1.40	82.50 ±1.76	84.44±0.64	86.94±0.52	88.00±1.00	101.88±1.46

Hearing threshold values (dB) are means ± s.e.m. Acc, acclimated; Unacc, unacclimated; Temp, water temperature.

Statistics

All data sets were tested for normal distribution and homogeneity of variances prior to tests. Because data sets of *I. punctatus* were not normally distributed and some data sets of *P. pictus* had small sample sizes, non-parametric tests were applied. Hearing thresholds at each frequency were compared using Kruskal–Wallis tests with the temperature regimen (e.g. pre-acclimated (baseline) at 18°C, unacclimated at 10°C, acclimated at 10°C, etc.) as the factor and hearing thresholds as variables to detect overall differences among temperature-regimen groups. Subsequently, Mann–Whitney *U*-tests were calculated to investigate which groups differed from each other in detail. All statistical tests were performed using SPSS 12.0 (SPSS, Chicago, IL, USA).

RESULTS

Ictalurus punctatus

Hearing thresholds differed significantly with temperature at each tested frequency among all tested temperatures and acclimation stages ($P<0.05$ at 100 Hz, $P<0.001$ at all other frequencies) (Table 2). Detailed comparisons at each frequency revealed that the pre-acclimated (baseline) thresholds to 18°C did not differ significantly from the thresholds of animals re-acclimated after 10°C. For most frequencies, except for 500 Hz ($U=3$, $P=0.001$) and 800 Hz ($U=0$, $P<0.001$) the pre-acclimated baseline thresholds did not differ significantly from the thresholds of animals re-acclimated after 26°C. Similarly, the thresholds of animals re-acclimated after 10°C did not differ significantly from the thresholds of animals re-acclimated after 26°C, except for 800 Hz ($U=10$, $P=0.021$).

The pre-acclimated (baseline) hearing thresholds differed significantly ($P<0.05$ in all cases) from the acclimated to 10°C as well as from the acclimated to 26°C thresholds at all frequencies, except 100 Hz ($P>0.05$ in both cases). Thresholds of animals acclimated to 10°C were on average 9 dB higher across the whole frequency range relative to the pre-acclimated (baseline) thresholds to 18°C with a maximum difference at 4000 Hz (Fig. 2), whereas the thresholds acclimated to 26°C were on average 12 dB lower than baselines, with again the maximum difference (23 dB) at 4000 Hz (Fig. 2). Similarly, thresholds acclimated to 10°C were significantly higher (mean: 18 dB, maximum difference 36 dB at 4000 Hz) than those acclimated at 26°C except, again, for 100 Hz where thresholds did not differ significantly.

When comparing thresholds at the same temperature, no significant difference was found between animals acclimated to 10°C and unacclimated to 10°C (Fig. 3A). However, when temperature was raised to 18°C, animals re-acclimated after 10°C showed on average 7 dB lower thresholds (maximum: 13 dB at 4000 Hz) than animals unacclimated after 10°C, which was statistically significant for all frequencies except for 100 Hz ($P<0.05$) (Fig. 3B). Similarly,

animals acclimated to 26°C had on average 7 dB (maximum: 12 dB at 4000 Hz) lower thresholds than animals unacclimated to 26°C (Fig. 3C). This was statistically significant for all frequencies ($P<0.05$) except 100 Hz and 300 Hz. When temperature was lowered back to 18°C, thresholds of animals re-acclimated after 26°C and unacclimated after 26°C did not statistically differ at any frequency, except 100 Hz ($P=0.021$) (Fig. 3D).

AEP waveform shapes were highly constant among individuals and were dependent on stimulus parameters (Fig. 4A). Additionally, the comparison of AEPs to a particular stimulus frequency at different water temperatures showed that the AEPs at 10°C (independent of whether animals were acclimated or not) showed fewer smaller peaks overlaid to the main deflections, were slightly longer and revealed an additional large deflection at some frequencies relative to AEPs at higher water temperatures (Fig. 5). This was consistently observed in all individuals.

Pimelodus pictus

Hearing thresholds measured in *P. pictus* significantly differed among all tested temperatures and acclimation stages at select frequencies 500, 800 and 1000 Hz ($P<0.05$) (Table 3). Thresholds at all other frequencies did not significantly ($P>0.05$) differ with water temperature. Detailed comparisons showed that the thresholds of animals pre-acclimated (baseline) to 26°C did not differ from animals acclimated at 22°C. However, the thresholds of animals pre-acclimated (baseline) to 26°C significantly differed ($P<0.05$) from animals acclimated to 30°C at only two frequencies, with a mean decrease in thresholds by 3 dB at 800 Hz and 7 dB at

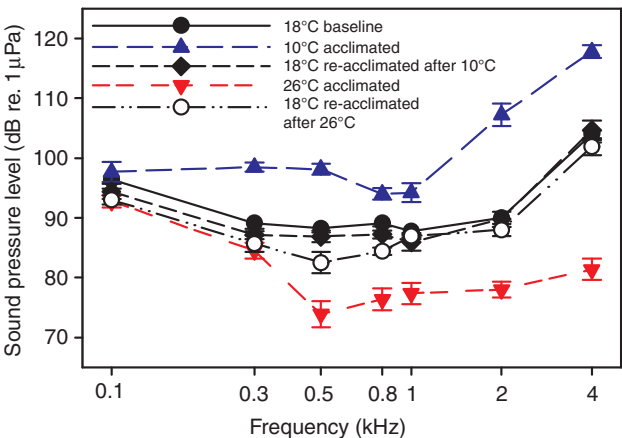


Fig. 2. Auditory evoked potential audiograms (means ± s.e.m.) of *Ictalurus punctatus* acclimated to three different water temperatures. (See Fig. 1 for explanation of different regimens.)

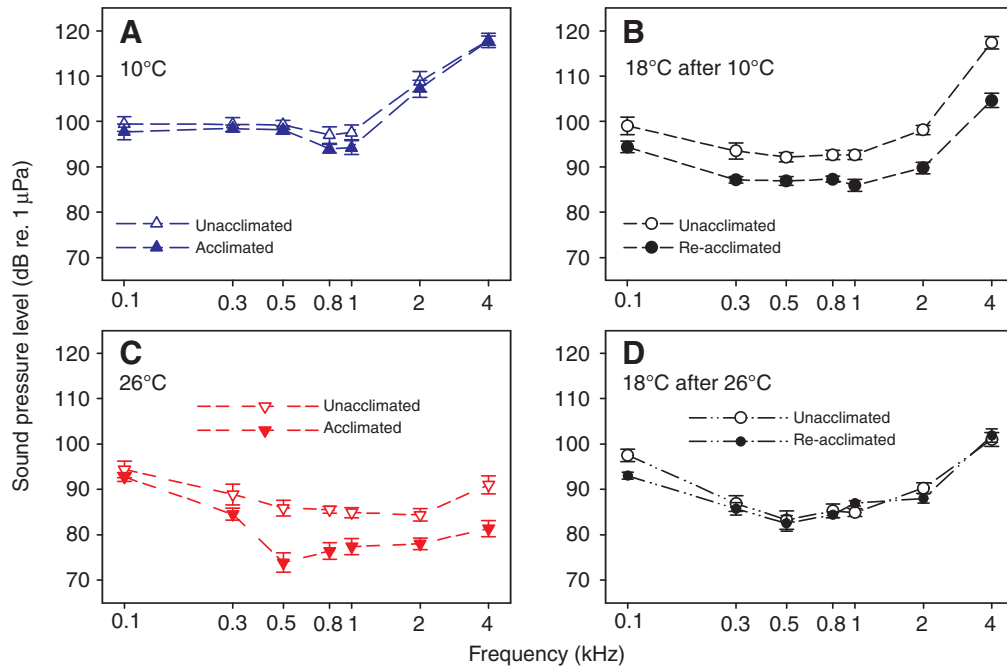


Fig. 3. Comparison between audiograms of unacclimated and acclimated *Ictalurus punctatus* at (A) 10°C, (B) 18°C re-acclimation process after 10°C, (C) 26°C and (D) 18°C re-acclimation process after 26°C.

4000 Hz in the animals acclimated to 30°C. Similarly, the thresholds at 4000 Hz of animals acclimated to 30°C were significantly lower (5 dB) than thresholds of animals acclimated to 22°C (Fig. 6).

Interestingly, the only significant difference between acclimated and unacclimated animals was at 800 Hz ($P < 0.05$) at 26°C in the group that had been previously acclimated to 22°C.

The shapes of AEPs of *P. pictus* again depended on the stimulus frequency and this was highly consistent between individuals and similar to the data for *I. punctatus* AEPs (Fig. 4B). No obvious change of waveform shape was observed as a result of ambient water temperature adjustments.

In summary, the ambient water temperature significantly influenced hearing thresholds and the shape of the AEPs in *I. punctatus*, especially at higher frequencies. Hearing sensitivity of *I. punctatus* was lowest at 10°C and increased by up to 36 dB between 10°C and 26°C. Significant differences were also revealed between acclimated and unacclimated animals after an increase in water temperature but not a decrease. By contrast, differences in hearing thresholds were smaller in *P. pictus*, even if a similar temperature difference (8°C) was considered. However, *P. pictus* showed a similar trend as *I. punctatus* in exhibiting higher hearing sensitivity at the highest tested temperature, especially at select high stimulus frequencies tested.

DISCUSSION

Effects of temperature

Water temperature significantly influenced hearing thresholds in *I. punctatus* and to a lesser degree and only at select high stimulus frequencies in *P. pictus*. Because growth rates within experimental groups over the course of the study were minimal (see Table 1) and the thresholds of the initial baseline acclimation temperature were repeatable after re-acclimation to the same temperature, size effects can be precluded as a cause for the threshold changes. Therefore, the threshold variations can be clearly attributed to the variations

in water temperature and acclimation effects. The current finding that thresholds increased (i.e. animals were less sensitive to the sound stimuli) with decreasing water and thus body temperature agrees

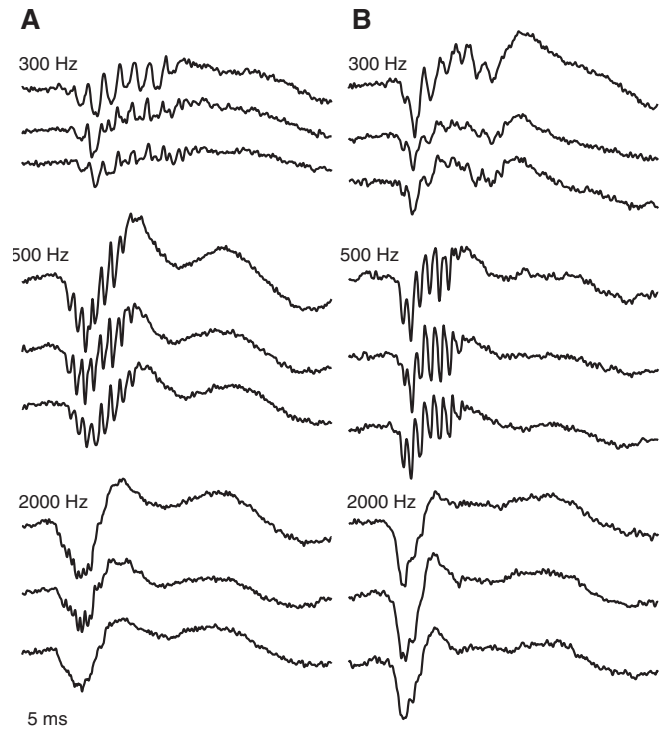


Fig. 4. Auditory evoked potentials (AEPs) of different individuals of (A) *Ictalurus punctatus* and (B) *Pimelodus pictus* to stimuli of various frequencies recorded at their respective baseline acclimation temperature 20 dB above hearing thresholds. Amplitudes of AEP waves are adjusted to fit the proportions of *P. punctatus* to those of *P. pictus* for the purpose of better visibility.

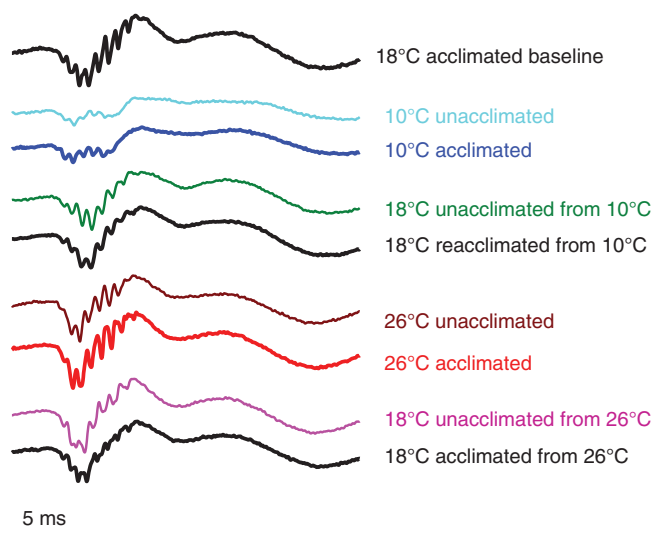


Fig. 5. Auditory evoked potentials of *Ictalurus punctatus* to a 500 Hz stimulus of the same level (20 dB above hearing threshold of the pre-acclimated baselines) but recorded at various water temperatures and acclimation stages.

with data for insects (Franz and Ronacher, 2002; Oldfield, 1988; Pires and Hoy, 1992), various other ectothermic vertebrates namely amphibians (Carey and Zelick, 1993; Hubl and Schneider, 1978; Long et al., 1996; Mohnke and Schneider, 1979; Walkowiak, 1980) and reptiles (Eatock and Manley, 1981; Wilson et al., 1985). The data are also similar to those for endothermic mammals when their body temperature was manipulated (Ferber-Viart et al., 1995; Kaga et al., 1979; Khvoles et al., 1998; O'Brien, 1994).

The only previous data available on the influence of temperature on hearing in fish are the study of Dudok van Heel (Dudok van Heel, 1956), who observed a broadening of the range of pitch detection with increasing temperature in the European minnow (*Phoxinus phoxinus*), and a study on goldfish, *Carassius auratus*, by Fay and Ream (Fay and Ream, 1992). In the latter study, the investigators observed increased responsiveness and lower thresholds of saccular nerve fibers, along with an upshift of the fiber's best frequency with increasing temperature (Fay and Ream, 1992). This finding of lower thresholds with increasing temperature matches with the current results.

In *I. punctatus*, the AEP waveforms were affected by the lowest water temperature. Similarly, Carey and Zelick (Carey and Zelick, 1993) found an influence of temperature on brainstem auditory evoked potentials (BAEPs) in three anuran species accompanying changes in auditory thresholds, with peripheral components of the BAEPs being most affected. The authors interpreted this latter finding as the sensory epithelium being the most-sensitive component of the auditory pathway.

Several studies have shown that temperature affects the physiology of the inner ear and the central auditory pathways (Campbell, 1969; Werner, 1976) as well as the latencies of neural responses (Carey and Zelick, 1993; Mohnke and Schneider, 1979) by influencing transduction processes at the sensory epithelium (Burrows, 1989; Fay and Ream, 1992), conduction velocities in nerve fibers (Macdonald, 1981) and the speed of the ionic flow through transduction channels in stereocilia of sensory hair cells (Corey and Hudspeth, 1983). This influence on transduction and conduction speed is particularly interesting with regard to the larger influence of temperature on thresholds at higher frequencies. Based

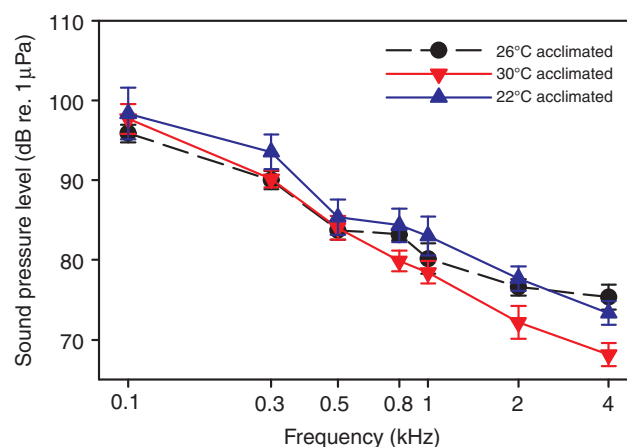


Fig. 6. Auditory evoked potential audiograms (means \pm s.e.m.) of *Pimelodus pictus* acclimated to three different water temperatures.

on various audiological studies on several fish species, it has been found that the auditory system, particularly of otophysan fishes (such as both catfish species), is particularly well adapted for temporal processing of acoustic stimuli. It has thus been suggested that fish may use the time rather than the frequency domain for encoding acoustic stimuli and also encode frequency information by temporal locking to the stimuli (Winn, 1967; Fay, 1982). As a consequence, the encoding of higher frequencies, which would require faster firing of neurons in order to synchronize with the shorter stimulus cycles, may be more susceptible to changes in transduction and refractory periods than that of relatively lower frequencies with longer cycles. This may therefore explain why, in our experiments, thresholds to the highest tested frequencies were more influenced by the water temperature than thresholds to the lower frequency stimuli.

Effects of acclimation

Audiograms of unacclimated and acclimated *I. punctatus* differed significantly at 26°C and 18°C when they had been previously held at a lower temperature. In both cases, the hearing thresholds of unacclimated animals were higher than after acclimation and showed intermediate values between the previous acclimated thresholds (to an 8°C lower water temperature) and the acclimated thresholds at the same temperature. Carey and Zelick (Carey and Zelick, 1993) described differences in hearing sensitivity between acclimated and unacclimated animals in three anuran species recording BAEPs: animals acclimated to a lower water temperature (14°C) were considerably less sensitive than animals acclimated to a higher temperature (20°C), even when both groups were tested at colder temperatures.

Thermal acclimation of fish is a complex process, accompanied by behavioral resistance adaptation (Cossins et al., 1977), changes in enzyme activity and protein synthesis, and differential gene expression (Airaksinen et al., 1998; Sidell et al., 1973). Ju et al. (Ju et al., 2002) systematically studied changes in gene expression in brain tissue from catfish in response to temperature shifts (12°C versus 24°C). Major categories of genes that were differentially expressed included chaperone genes and transcription factors as well as gene products involved in signal transduction pathways, lipid metabolism and translational machinery. Some genes were induced transiently, whereas others were induced in an enduring fashion. Such differences in gene activity may account for variability in the sensitivity of sensory systems observed besides direct 'physical'

Table 3. Hearing thresholds of *Pimelodus pictus* recorded at different water temperatures and acclimation stages

	Temp (°C)	100 Hz	300 Hz	500 Hz	800 Hz	1000 Hz	2000 Hz	4000 Hz
Acc	26	95.83±1.11	90.00±1.13	83.71±1.19	83.17±0.75	80.15±1.89	76.57±1.04	75.33±1.58
Unacc	22	101.20±2.42	93.80±1.36	86.20±2.25	82.80±0.97	81.40±2.29	79.80±3.18	73.40±2.40
Acc	22	98.33±3.21	93.50±2.17	85.33±2.20	84.33±2.12	83.00±2.42	77.67±1.52	73.33±1.48
Acc	30	97.67±1.87	90.15±1.06	84.00±1.50	79.86±1.28	78.43±1.43	72.17±2.09	68.14±1.47
Unacc 22°C	26	100.00±2.52	91.00±2.52	89.00±1.91	88.00±1.15	85.00±1.15	79.00±2.52	74.00±1.63
Unacc 30°C	26	94.80±1.16	88.80±2.42	79.20±1.59	79.40±1.50	74.80±2.20	74.60±1.86	69.40±2.11

Hearing threshold values (dB) are means \pm s.e.m. Acc, acclimated; unacc, unacclimated; unacc 22°C, unacclimated at 26°C after having been acclimated to 22°C; unacc 30°C, unacclimated at 26°C after having been acclimated to 30°C; Temp, water temperature.

effects of temperature, especially for differences between unacclimated and acclimated animals at the same temperature.

Interestingly, no significant differences between audiograms of unacclimated and acclimated fish were found at temperatures of 10°C and 18°C after previous acclimation to 26°C, i.e. in both cases after 'cold acclimation'. One possible explanation for this observation may be that the fish had not yet been fully acclimated to the colder temperature. Typical acclimation periods used in various studies on fish thermal acclimation are three weeks (Ali et al., 2003; Cossins et al., 1977; Currie et al., 1998; Hermesz et al., 2001; Viant et al., 2003) to one month (Currie et al., 1999; Sollid et al., 2005). Therefore, we selected an acclimation time of at least four weeks. It has, however, been shown that warm acclimation occurs in smaller time intervals than cold acclimation. Cossins et al. (Cossins et al., 1977) reported that the behavior of *C. auratus* transferred from 5°C to 25°C was behaviorally indistinguishable from 25°C acclimated fish after 10–15 days, while an acclimation time of 40–50 days was required for full behavioral recovery when the animals were transferred from 25°C to 5°C. It is, therefore, possible that still longer acclimation periods may have resulted in differences between acclimated and unacclimated animals at 10°C and 18°C cold acclimation. Further evidence for incomplete acclimation may be that the 18°C 'acclimated' hearing thresholds (after lowering the temperature from 26°C) differed at two frequencies from the initial 18°C baseline but also from the 18°C 'warm acclimation' (after raising the temperature from 10°C) thresholds. This, however, has no influence on the general picture that higher temperatures resulted in lower hearing thresholds and that hearing thresholds may have also depended on whether the fish had time to acclimate to a particular water temperature or not.

Species differences in temperature-dependent hearing sensitivity

Contrary to our expectation that a eurythermic fish species confronted with a wide range of temperatures in its natural habitat would show more resistance to temperature changes in its sensory systems than a stenothermic animal, we found far less temperature-dependent differences in the tropical *P. pictus* than in the temperate *I. punctatus*. One factor to account for this difference is the wider range of temperatures we could use in *I. punctatus*. However, the maximum temperature difference in *P. pictus* was 8°C, which is exactly the same difference we used between the three different test and acclimation temperatures in *I. punctatus* and for which the latter species showed significant differences in hearing.

Another argument may be that lower temperatures have a larger effect on the auditory system than higher temperatures and, as a consequence, the relatively 'high' temperatures at which *P. pictus* had to be tested did not have much influence on auditory processing mechanisms. Indeed, acclimation temperature has greater influence

on tolerance to low rather than high temperatures (Currie et al., 1998), and fish are able to gain heat tolerance more rapidly than cold tolerance (i.e. Davies, 1973).

Different effects of temperature on hearing have also been found in different anuran species (Hubl et al., 1977; Mohneke and Schneider, 1979; Walkowiak, 1980) and even between genders within the same species (Hubl and Schneider, 1978). These differences may be due to the relatively low temperature variability in tropical habitats as compared with temperate climate zones. Thus, it may be possible that tropical fish do not possess as elaborate and plastic adaptive mechanisms, including changes in gene expression and enzyme compositions, that temperate species require for survival. The small effects of temperature observed on audition may, therefore, be due to a direct effect of temperature on physical properties of auditory hair cells, such as stiffness or membrane attachment (Chen and Brownell, 1999), whereas in temperate species temperature-induced changes in metabolism may be the more important factor in influencing sensory capacities. Further comparative studies are needed to investigate whether these differences between species can be observed in a more general pattern between eurythermal and stenothermal fish species. Furthermore, the audiogram of *P. pictus* indicates that its hearing may expand to higher frequencies than those tested in the current study. Because the only influence of temperature on hearing in this species was found at the highest frequency, there may be more temperature-dependent changes to observe at higher frequencies. This was, however, not possible with our current setup. Interestingly, the audiogram of *P. pictus* closely resembles the audiograms obtained by Ladich (Ladich, 1999) at 28°C and Amoser and Ladich (Amoser and Ladich, 2003) at 24°C for the same species despite differences in experimental setup (air *versus* underwater speaker, recording depth and type electrodes used) except for the 4 kHz thresholds which were lowest in the current study and highest in the Ladich (Ladich, 1999) study. These differences may be related to the different size groups of animals used, with *P. pictus* being smallest in the current study and largest in the Ladich (Ladich, 1999) study.

In summary, the current data demonstrate clearly that temperature can affect hearing in fish similar to other ectothermic animals: lower temperatures lead to an increase in hearing thresholds, whereas thresholds are more sensitive at higher temperatures. Also, acclimation influences hearing at a given temperature. However, not all species of fish may be sensitive to temperature-induced changes in hearing. Therefore, caution should be taken in all audiometric tests on fishes to also consider the testing and holding temperatures as a potential source of variability.

LIST OF ABBREVIATIONS

AEP	auditory evoked potential
BAEP	brainstem auditory evoked potential

We thank Dr Andrew Kane, for valuable suggestions on catfish care, Nick Houde, Dr Michaela Meyer and Dr Jennifer Hill for help with animal care, and Dr E. Don Stevens for helpful comments on temperature acclimation procedures. The experimental protocols and methods described are in compliance with the Animal Welfare Act (9CFR) requirements and were approved by University of Maryland's Institutional Animal Care and Use Committees.

REFERENCES

- Aguilar, L. H., Kalinin, A. L. and Rantin, F. T. (2002). The effects of temperature on the cardio-respiratory function of the neotropical fish *Piaractus mesopotamicus*. *J. Therm. Biol.* **27**, 299-308.
- Aho, A. C., Donner, K. and Reuter, T. (1993). Retinal origins of the temperature effect on absolute visual sensitivity in frogs. *J. Physiol.* **463**, 501-521.
- Airaksinen, S., Rabergh, C. M. I., Sistonen, L. and Nikinmaa, M. (1998). Effects of heat shock and hypoxia on protein synthesis in rainbow trout (*Oncorhynchus mykiss*) cells. *J. Exp. Biol.* **201**, 2543-2551.
- Ali, K. S., Dorgai, L., Ábrám, M. and Hermesz, E. (2003). Tissue- and stressor-specific differential expression of two hsc70 genes in carp. *Bioch. Biophys. Res. Commun.* **307**, 503-509.
- Amo, L., López, P. and Martín, J. (2004). Thermal dependence of chemical assessment of predation risk affects the ability of wall lizards, *Podarcis muralis*, to avoid unsafe refuges. *Physiol. Behav.* **82**, 913-918.
- Amoser, S. and Ladich, F. (2003). Diversity in noise-induced temporary hearing loss in otophysine fishes. *J. Acoust. Soc. Am.* **113**, 2170-2179.
- Baensch, H. A. and Riehl, R. (1985). *Aquarien Atlas*. Melle: Mergus, Verlag für Natur- und Heimtierkunde GmbH.
- Bennett, W. A. and Judd, F. W. (1992). Factors affecting the low temperature tolerance of Texas pinfish. *Trans. Am. Fish. Soc.* **121**, 659-666.
- Brenowitz, E. A., Rose, G. and Capranica, R. R. (1985). Neural correlates of temperature coupling in the vocal communication system of grey treefrog (*Hyla versicolor*). *Brain Res.* **359**, 364-367.
- Burrows, M. (1989). Effects of temperature on a central synapse between identified motor neurons in the locust. *J. Comp. Physiol. A* **165**, 687-695.
- Campbell, H. W. (1969). The effects of temperature on the auditory sensitivity of lizards. *Physiol. Zool.* **42**, 183-210.
- Carey, M. B. and Zelik, R. (1993). The effect of sound level, temperature and dehydration on the brainstem auditory evoked potential in anuran amphibians. *Hear. Res.* **70**, 216-228.
- Chen, A. Y. and Brownell, W. E. (1999). Effect of temperature on lateral wall mechanics of the guinea pig outer hair cell. *Otolaryngol. Head Neck Surg.* **120**, 46-50.
- Connaughton, M. A., Fine, M. L. and Taylor, M. H. (2002). Weakfish sonic muscle: influence of size, temperature and season. *J. Exp. Biol.* **205**, 2183-2188.
- Corey, D. P. and Hudspeth, A. J. (1983). Kinetics of the receptor current in the bullfrog saccular hair cells. *J. Neurosci.* **3**, 962-976.
- Cossins, A. R., Friedlander, M. J. and Prosser, C. L. (1977). Correlations between behavioral temperature adaptations of goldfish and the viscosity and fatty acid composition of their synaptic membranes. *J. Comp. Physiol. A* **120**, 109-121.
- Currie, R. J., Bennett, W. A. and Bettinger, T. L. (1998). Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environ. Biol. Fishes* **51**, 187-200.
- Currie, S., Tufts, B. L. and Moyes, C. D. (1999). Influence of bioenergetic stress on heat shock protein gene expression in nucleated red blood cells of fish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **276**, 990-996.
- Davies, W. D. (1973). Rates of temperature acclimation for hatchery reared striped bass fry and fingerlings. *Prog. Fish-Cult.* **35**, 214-217.
- Deane, E. E. and Woo, N. Y. S. (2005). Cloning and characterization of the hsp70 multigene family from silver sea bream: modulated gene expression between warm and cold temperature acclimation. *Biochem. Biophys. Res. Commun.* **330**, 776-782.
- Dembski, S., Masson, G., Monnier, D., Wagner, P. and Pihan, J. C. (2006). Consequences of elevated temperatures on life-history traits of an introduced fish, pumpkinseed *Lepomis gibbosus*. *J. Fish Biol.* **69**, 331-346.
- Díaz-Sarmiento, J. A. and Alvarez-León, R. (2004). Migratory fishes of the Colombian Amazon. In *Migratory Fishes of South America: Biology, Fisheries and Conservation Status* (ed. J. Carolsfeld, B. Harvey, C. Ross and A. Baer), pp. 303-344. Victoria, BC: World Fisheries Trust/World Bank/IDRC.
- Dudok van Heel, W. H. (1956). Pitch discrimination in the minnow (*Phoxinus laevis*) at different temperatures. *Experientia* **12**, 75-77.
- Eatock, R. A. and Manley, G. A. (1981). Auditory nerve fibre activity in the tokay gecko: II. Temperature effect on tuning. *J. Comp. Physiol.* **142**, 219-226.
- Fay, R. R. (1982). Neural mechanism of an auditory temporal discrimination by the goldfish. *J. Comp. Physiol.* **147**, 201-216.
- Fay, R. R. and Popper, A. N. (2000). Evolution of hearing in vertebrates: the inner ears and processing. *Hear. Res.* **149**, 1-10.
- Fay, R. R. and Ream, T. J. (1992). The effects of temperature change and transient hypoxia on auditory nerve fiber response in the goldfish (*Carassius auratus*). *Hear. Res.* **58**, 9-18.
- Ferber-Viart, C., Savourey, G., Garcia, C., Duclaux, R., Bittel, J. and Collet, L. (1995). Influence of hyperthermia on cochlear micromechanical properties in humans. *Hear. Res.* **91**, 202-207.
- Fortune, P. Y. (1958). The effect of temperature changes on the thyroid-pituitary relationship in teleosts. *J. Exp. Biol.* **35**, 824-831.
- Franz, A. and Ronacher, B. (2002). Temperature dependence of temporal resolution in an insect nervous system. *J. Comp. Physiol. A* **188**, 261-271.
- French, A. S. and Kuster, J. E. (1982). The effects of temperature on mechanotransduction in the cockroach tactile spine. *J. Comp. Physiol. A* **147**, 251-258.
- Friedlander, M. J., Kotchabhakdi, N. and Prosser, C. L. (1976). Effects of cold and heat on behavior and cerebellar function in goldfish. *J. Comp. Physiol.* **112**, 19-45.
- Gillet, C. and Quélin, P. (2006). Effect of temperature changes on the reproductive cycle of roach in Lake Geneva from 1983 to 2001. *J. Fish Biol.* **69**, 518-534.
- Hall, T. E. and Johnston, I. A. (2003). Temperature and developmental plasticity during embryogenesis in the Atlantic cod *Gadus morhua* L. *Mar. Biol.* **142**, 833-840.
- Hawkins, A. D. and Myrberg, A. A., Jr (1983). Hearing and sound communication under water. In *Bioacoustics: A Comparative Approach* (ed. B. Lewis), pp. 347-405. London: Academic Press.
- Heath, A. G., Turner, B. J. and Davis, W. P. (1993). Temperature preferences and tolerances of three fish species inhabiting hyperthermal ponds on mangrove islands. *Hydrobiologia* **259**, 47-55.
- Hermesz, E., Abraham, M. and Nemcsok, J. (2001). Identification of two hsp90 genes in carp. *Comp. Biochem. Physiol. C* **129**, 397-407.
- Huber, M. and Guderley, H. (1993). The effect of thermal acclimation and exercise upon the binding of glycolytic enzymes in muscle of the goldfish *Carassius auratus*. *J. Exp. Biol.* **175**, 195-209.
- Hubl, L. and Schneider, H. (1979). Temperature and auditory thresholds: bioacoustic studies of the frogs *Rana r. ridibunda*, *Hyla a. arborea* and *Hyla a. savignyi* (Anura, Amphibia). *J. Comp. Physiol. A* **130**, 17-27.
- Hubl, L., Mohneke, R. and Schneider, H. (1977). Temperature dependence of auditory thresholds in two central European anurans, *Bombina variegata variegata* (L.) and *Rana ridibunda ridibunda* Pall. (Amphibia) and its relation to calling. *Behav. Processes* **2**, 305-314.
- Ju, Z., Dunham, R. A. and Liu, Z. (2002). Differential gene expression in the brain of channel catfish (*Ictalurus punctatus*) in response to cold acclimation. *Mol. Genet. Genomics* **268**, 87-95.
- Kaga, K., Takiguchi, T., Myokai, K. and Shiode, A. (1979). Effects of deep hypothermia and circulatory arrest on the auditory brain stem response. *Arch. Otorhinolaryngol.* **225**, 199-205.
- Kashiwayanagi, M., Sasaki, K., Iida, A., Saito, H. and Kurihara, K. (1997). Concentration and membrane fluidity dependence of odor discrimination in the turtle olfactory system. *Chem. Senses* **22**, 553-563.
- Kenyon, T. N., Ladich, F. and Yan, H. Y. (1998). A comparative study of hearing ability in fishes; the auditory brainstem response approach. *J. Comp. Physiol. A* **182**, 307-318.
- Khvoles, R., Freeman, S. and Sohmer, H. (1998). Effect of temperature on the transient evoked and distortion product otoacoustic emissions in rats. *Audiol. Neurotol.* **3**, 349-360.
- Kokita, T. (2003). Potential latitudinal variation in egg size and number of a geographically widespread reef fish, revealed by common-environment experiments. *Mar. Biol.* **143**, 593-601.
- Ladich, F. (1999). Did auditory sensitivity and vocalization evolve independently in otophysian fishes? *Brain Behav. Evol.* **53**, 288-304.
- Lecklin, T., Nash, G. B. and Eggington, S. (1995). Do fish acclimated to low temperature improve microcirculatory perfusion by adapting red cell rheology? *J. Exp. Biol.* **198**, 1801-1808.
- Le Morvan, C., Troutaud, D. and Deschaux, P. (1998). Differential effects of temperature on specific and nonspecific immune defences in fish. *J. Exp. Biol.* **201**, 165-168.
- Long, G. R., Van Dijk, P. and Wit, H. P. (1996). Temperature dependence of spontaneous otoacoustic emissions in the edible frog (*Rana esculenta*). *Hear. Res.* **98**, 22-28.
- Lorenzo, D. and Macadar, O. (2005). Influence of temperature and reproductive state upon the jamming avoidance response in the pulse-type electric fish *Brachyhyopomus pinnicaudatus*. *J. Comp. Physiol. A* **191**, 85-94.
- Macdonald, J. A. (1981). Temperature compensation in the peripheral nervous system: Antarctic vs temperate poikilotherms. *J. Comp. Physiol.* **142**, 411-418.
- McCormick, S. D., Shrimpton, J. M., Moriama, S. and Björnsson, B. T. (2002). Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon. *J. Exp. Biol.* **205**, 3553-3560.
- McElligott, J. G., Weiser, M. and Baker, R. (1995). Effect of temperature on the normal and adapted vestibulo-ocular reflex in the goldfish. *J. Neurophysiol.* **74**, 1463-1472.
- Mohneke, R. and Schneider, H. (1979). Effect of temperature upon auditory thresholds in two anuran species, *Bombina v. variegata* and *Alytes o. obstetricans* (Amphibia, Discoglossidae). *J. Comp. Physiol. A* **130**, 9-16.
- Nordin, S., Lötsch, J., Kobal, G. and Murphy, C. (1998). Effects of nasal-airway volume and body temperature on intranasal chemosensitivity. *Physiol. Behav.* **63**, 463-466.
- O'Brien, A. J. (1994). Temperature dependency of the frequency and level of a spontaneous otoacoustic emission during fever. *Br. J. Audiol.* **28**, 281-290.
- Oldfield, B. P. (1988). The effect of temperature on the tuning and physiology of insect auditory receptors. *Hear. Res.* **35**, 151-158.
- Pires, A. and Hoy, R. R. (1992). Temperature coupling in cricket acoustic communication. I. Field and laboratory studies of temperature effects on calling song production and recognition in *Gryllus firmus*. *J. Comp. Physiol. A* **171**, 69-78.
- Poggendorf, D. (1952). Die absolute Hörschwelle des Zwerghwelses (*Amiurus nebulosus*) und Beiträge zur Physik des Weberschen Apparates der Ostariophysen. *Z. Vgl. Physiol.* **34**, 222-257.
- Popper, A. N. and Fay, R. R. (1993). Sound detection and processing by fish: critical review and major research questions. *Brain Behav. Evol.* **41**, 14-38.
- Popper, A. N., Fay, R. R., Platt, C. and Sand, O. (2003). Sound detection mechanisms and capabilities of teleost fishes. In *Sensory Processing in Aquatic Environments* (ed. S. P. Collin and N. J. Marshall), pp. 3-38. New York: Springer-Verlag.
- Reilly, C. R. and Thompson, S. H. (2007). Temperature effects on low-light vision in juvenile rockfish (genus *Sebastes*) and consequences for habitat utilization. *J. Comp. Physiol. A* **193**, 943-953.
- Shoji, T., Abe, Y., Furihata, E. and Kurihara, K. (1994). High sensitivity of the turtle olfactory system to nonvolatile substances: comparison of response properties with those in gustatory systems. *Brain Res.* **12**, 68-76.

- Sidell, B. D., Wilson, F. R., Hazel, J. and Prosser, C. L.** (1973). Time course of thermal acclimation in goldfish. *J. Comp. Physiol.* **84**, 119-127.
- Sollid, J., Weber, R. E. and Nilsson, G. E.** (2005). Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. *J. Exp. Biol.* **208**, 1109-1116.
- Somero, G. N. and DeVries, A. L.** (1967). Temperature tolerance of some Antarctic fishes. *Science* **156**, 257-258.
- Walkowiak, W.** (1980). Sensitivity, range and temperature dependence of hearing in the grass frog and fire-bellied toad. *Behav. Proc.* **5**, 363-372.
- Werner, Y. L.** (1976). Optimal temperatures for inner-ear performance in gekkonid lizards. *J. Exp. Zool.* **195**, 319-352.
- Wilson, J. P., Smolders, J. W. T. and Klinke, R.** (1985). Mechanics of the basilar membrane in *Caiman crocodiles*. *Hear. Res.* **18**, 1-14.
- Winn, H. E.** (1967). Vocal facilitation and the biological significance of toadfish sounds. In *Marine Bioacoustics* (ed. Tavolga, W. N.), pp. 283-304. Oxford: Pergamon Press.
- Wysocki, L. E., Davidson, J. W., Smith, M. E., Frankel, A. S., Ellison, W. T., Mazik, P. M., Popper, A. N. and Bebak, J.** (2007). Effects of aquaculture production noise on hearing, growth, and disease resistance of rainbow trout *Oncorhynchus mykiss*. *Aquaculture* **272**, 687-697.
- Zitek, A., Schmutz, S. and Ploner, A.** (2004). Fish drift in a Danube sidearm-system: II. Seasonal and diurnal patterns. *J. Fish Biol.* **65**, 1339-1357.