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The effects of temperature and pH change on the snapping sound characteristic of *Alpheus edwardsii*

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Abstract

Aim: The current study undertook manipulative experiments to observe changes in snapping shrimp sound signals in relation to temperature and pH changes.

Methodology: Sounds of intertidal snapping shrimp (*Alpheus edwardsii*) sequentially exposed to different temperature/pH treatments manipulation for a period of 2 week each, were recorded in the laboratory and analysed. The acoustic characteristics of snapping sound signal were examined to relate to the change in temperature, pH and combination of both parameters

Results: Our results showed that there was a significant reduction in the frequency of peak amplitude of snapping sound wave following a two week exposure to a combination of temperature and pH treatments. The frequency of snapping shrimp sound decreased by approximately 30% when exposed to a 2°C increase in temperature and a 0.7 unit decrease in pH, however, elevated temperature alone caused no significant effect on the peak frequency of snapping shrimp sound.

Interpretation: The finding suggests that following the prediction values of temperature and pH changes due to climate change in the coming century may implicate the ambient noise at habitats where snapping shrimps dominate.

Key words: Bioacoustics, Climate change, Snapping shrimp

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Introduction

The ambient noise of coastal marine habitat is dominated by the sounds of snapping shrimps. The loud sound (~210 dB re 1 μ Pa) is produced from the snapping action of its enlarged chelae that causes the formation and implosion of cavitation bubbles. Underwater snapping sounds characterized by their wide frequency range (2-100 kHz) and high amplitude can be detected using a hydrophone throughout the day in marine habitats where snapping shrimps dominate. Past studies show that snapping shrimp sounds in underwater reef noise could mediate larval settlement and swimming behaviour of many marine species. This indicates that the sound of snapping shrimps in nature plays an important role as navigational cue for marine organisms to find suitable habitat and food, hence, attraction for a wide range of marine species.

For many marine animals, temperature is one of the important factors that influence their distribution and abundance at certain habitats. Past studies have shown that poikilotherms, such as fish and aquatic invertebrates require a critical temperature to grow and reproduce where elevated temperature usually causes a range of negative effects. Changes in temperature may lead to hypoxia caused by anaerobic metabolism of body cells, especially in extreme temperatures. In addition, growth rate (Mann, 1979) and hormonal activities of marine invertebrates have been found to be vulnerable at elevated temperature. Moreover, extreme temperatures are lethal to marine animals and in many cases can decimate whole population, such as coral colonies. Several studies in recent years have examined the effects of environmental drivers on sound production by snapping shrimps in different regions have found that snap rate of snapping shrimps are positively correlated to elevated temperature (Bohnenstiehl et al., 2016; Kim et al., 2011; Lillis and Mooney, 2018). While these studies have focused on the relationship of environmental variables to snapping behaviour of snapping shrimp, however, studies on the effect of elevated temperature on the qualitative characteristics of snapping shrimp signals are meagre.

Other than temperature, pH of seawater has been found to be crucial to many marine taxa, especially to calcifying organisms. Acidification of seawater that affects the concentration of carbonate ions has been found impactful to exoskeleton and shell calcification rate. Varying effects of ocean acidification on Crustaceans have been broadly discussed in past studies, although its specific effect on bioacoustical aspects is rather vague. The mortality rate of northern shrimp, *Pandalus borealis* increased up to 90% after 30 week exposure to elevated carbon dioxide concentration (Branch et al., 2013). With regards to snapping shrimp, it was reported that its soniferous behaviour is altered in ocean acidification conditions. Suppression of sonic activity measured in terms of both overall sound levels and snapping frequency of snapping shrimp noise has been reported

(Rossi et al., 2016). Future changes in average seawater temperature and acidity are likely to impact snapping shrimp dominated coastal marine soundscapes. Consequently, such a big scale change in the soundscape can be deciphered from a better understanding at finer scale, qualitative changes of individual snapping sound as suggested in this study.

The changes in average temperature and pH of seawater have been linked to global climate change phenomenon. According to 2013 IPCC Fifth Assessment report, seawater temperature is predicted to increase by 2°C within the next century (Kirtman et al., 2013). In view of the above, this study aimed to identify the impact of pH and temperature in accordance to this global warming prediction scenario on the bioacoustics of snapping shrimp.

Materials and Methods

Snapping shrimps (*Alpheused wardsii*) were collected from an intertidal mudflat near Penyabong beach in Johor, Malaysia. The sampling area predominantly comprises muddy sand with scattered rocks and oyster shells. Snapping shrimps were collected under small rocks in tide pools by using scoop net or bare hands during low tide. Samples were then transported live to the marine bioacoustic laboratory at the Universiti Kebangsaan Malaysia in Bangi for subsequent experiments. In laboratory, the body length of each shrimp was measured and kept in six different holding tanks (50 cm × 25 cm × 15 cm) according to their body size (21-25 mm) with continuous supply of recirculating filtered seawater. Water temperature and salinity were maintained at 28°C and 33 psu respectively. The shrimps were fed with sinking pellets twice a day and their conditions were monitored on daily basis. Each shrimp was used only once throughout the experiment and were euthanized in 70% ethanol solution at the end of the experiment.

To examine the effect of temperature and pH on the snapping sound of snapping shrimp, two rectangular glass aquariums were set-up in a controlled environment. Each aquarium was 30 cm high, 60 cm long and 30 cm wide. The aquariums were filled up to 20 cm deep with filtered artificial seawater. In total, the experiment consisted of three treatments Effects of temperature, Effect of pH and Combined effects of pH and temperature. The temperature and pH treatment were partly based on the IPCC predictions of global warming scenario in 2100 and 2300 respectively. To investigate the effect of forecasted temperature and pH changes on the acoustic outputs (peak frequency) generated by snapping shrimp, three temperature and pH were used in this study, including combination of these two variables. The temperature was increased by 1°C for every temperature treatment, from 28°C (control) to 30°C. Meanwhile for pH treatments, pH was set at 8.1 (control), 7.7 and 7.4. Treatments for simultaneous effect of temperature and pH were the combination of readings mentioned

Fig. 1 : Example waveform of a snapping shrimp snap where $T=0.00028 \pm 0.000075$ s, is the period of first peak used to calculate peak frequency ($1/T$) of the snapping sound.

above. The temperature of seawater was manipulated by using an aquarium heating system (30 W) and consistently monitored the temperature using a glass thermometer. Meanwhile, pH of seawater was controlled and maintained by the continuous supply of carbon dioxide gas into the seawater. Carbon dioxide was bubbled into the seawater through an air stone at fixed rate and the pH level of seawater was regularly monitored using a calibrated pH meter (Extech, model PH100, USA) throughout the duration of the experiment. The assumption of this experiment was that the level of temperature and pH exposures were not lethal, and the resulting snapping sound was due to the adaptation of snapping shrimp to various exposures.

Individual snapping shrimp ($n=3$) was held separately in three small plastic rectangular cages (10 cm x 10 cm x 10 cm) inside a single glass aquarium. They were kept under controlled temperature (28°C) and pH (8.1) for a period of five days for the shrimps to acclimatise to laboratory conditions. The 'before treatment' snapping signals ($n=10$) of each snapping shrimp were recorded after five days. Following that, the same snapping shrimp was transferred to experimental tank maintained at various temperature (28°C, 29°C and 30°C) and pH (8.1, 7.7, 7.4) for treatments. The shrimp were then exposed to these experimental conditions for a period of two weeks each. The 'after treatment' snapping sounds of snapping shrimps were recorded again ($n=10$) at the end of two week exposure.

Snapping shrimp sounds were recorded in a rectangular polycarbonate tank (150 cm x 150 cm x 100 cm). The tank was filled with artificial seawater upto 80 cm. Underwater snapping

sounds were recorded using a hydrophone (Burns Electronics Aquaear model CR30-40 and HP-A1 amplifier, Australia) connected to a digital sound recorder (Edirol R09HR, Japan) and saved in a Secured Digital (SD) card as uncompressed format (.wav). Bit depth and sampling rate were set at 16 bits and 44.1 kHz respectively. Individual snapping shrimp was transferred from their plastic cages into a polyethylene bag and placed inside the recording tank. It was assumed that the polyethylene bag was acoustically invisible and did not affect the sound propagation due to relative thickness of bag in comparison to sound wavelength as well as small difference in acoustic impedance between polyethylene and seawater. The shrimp was positioned in the middle of the tank, 75 cm from the edge of the wall, 40 cm from the bottom of the tank and laterally 20 cm from the hydrophone. The shrimp was then stimulated for producing the snapping sound by occasionally approaching the bag with a small painting brush. Ten snapping sounds from each snapping shrimp were recorded during each recording session. The effect of tank resonance was calculated following Akamatsu *et al.* (2002), where the minimum resonance frequency was 1174 Hz and the cut-off frequency in lateral direction was 1062 Hz. Assuming that the expected peak frequency of snapping sound signal was higher (above 2000 Hz) than the minimum resonance frequency of the tank, it could be assumed that the effect of tank resonance was negligible. The frequency of spectrum was also higher than the cut-off frequency hence, the distance (20 cm) between the shrimp and hydrophone was acceptable.

Recorded sounds were analysed by software RAVEN Pro 1.4 (Bioacoustics Research Program, Cornell Laboratory of

Ornithology, Cornell, NY, USA) and statistical analysis was done by R 4.0.0 (R Core Team 2020) with ggplot2 (Wickham, 2016) and doBy (Højsgaard and Halekoh, 2020) packages. The peak frequency of snapping signal sound wave was calculated by the formula $1/T$, where T is the period of first cycle (signal with peak amplitude) of snapping sound signal waveform. The example of a single snap sound signal and the measurement of T in the waveform is shown in Fig. 1. The sound peak frequencies were analysed using paired t-test to examine the effect of exposure to temperature and pH treatments.

Results and Discussion

The temperature experiments showed that the peak frequency of snapping sound increased moderately after 2 week exposure to elevated temperature (Fig. 2). For the control experiment, the mean peak frequency increased from 4612 Hz to 4792 Hz. This revealed that there was a variation in the sound signals within the same individual of snapping shrimp. However, the paired t-test results proved that the differences were minor and statistically not significant. Similar trend was also observed in control experiment for pH treatments. At elevated temperature conditions, the mean peak frequency of snapping shrimp increased from 3440 Hz (29°C) and 3172 Hz (30°C) to 3678 Hz and 3187 Hz respectively. However, none of the treatments showed significant changes in respective sound peak frequencies. Consequently, this study showed that elevated temperature alone had minimum effect on the peak frequency of snapping shrimp snapping sounds. Previous studies which examined the snapping shrimp sound patterns in tropical habitats reported that small increase in temperature had no apparent influence on the high-frequency sound levels of snapping shrimp (Everest, 1948; Johnson *et al.*, 1947; Knowlton and Moulton, 1963). Nevertheless, more recent reports revealed that snap rates of snapping shrimps were positively correlated with environmental parameters, including temperature and light, both in field and controlled experiments (Bohnenstiehl *et al.*, 2016; Lillis and Mooney, 2018). Even so, none of the studies examined the effect of elevated temperature on the individual sound signals.

A modest rise in the snapping shrimp signals may be attributed to shorter pulse duration of first pressure peak at elevated temperature. Generally, it is thought that the frequency of sound underwater is independent of temperature although its propagation speed is directly proportional to temperature. One possible explanation is that the size of a single cavitation bubble expands rapidly at higher temperature, which leads to bubble collapse at shorter pulse duration with higher frequency. Kim *et al.* (2010) reported that the difference between sound spectra and pulse duration of snapping shrimp is related to the size of cavitation bubble and shape of chelae. In relation to this study, it appears that 1-2°C temperature increase did not affect the formation of cavitation bubbles.

In addition, the results of this study also suggest that prolonged temperature elevation of 1-2°C alone did not affect the mechanics of sound production by the enlarged chelae. Generally, shallow waters of tropical intertidal zones similar to the study site were subjected to large daily temperature range where daily temperature fluctuated by as much as 10°C above ambient temperature. Although most intertidal fauna are physiologically adapted to short daily temperature change, the non-lethal effect of longer exposure to temperature elevation that emulate global warming is less known. The physical property of the chelae is believed to play an important role in production of snapping sound due to high-energy mechanism of snaps. Studies focusing the structure and properties of snapping shrimp's chelae as functions of temperature are scarce Verma and Tomar (2014). Investigated the effect of temperature on the structural nanomechanical property of shrimp exoskeleton. The hardness values were found to reduced from around 1.6 GPa at 30°C to approximately 1.2 GPa at 80°C for the shrimp, *Pandalus platyceros*. This suggests that exposure at extreme temperature for a period of time could reduce the hardness of shrimp's exoskeleton unlike the relatively small increase in temperature in this experiment.

The peak frequency of snapping sound for control treatment at pH 8.1 was increased from 3298 Hz to 3455 Hz. Meanwhile, at pH 7.7, the peak frequency was decreased from 3042 Hz to 2815 Hz. However, both peak frequency changed for pH 8.1 (control) and 7.7 treatment were not significantly different. However, a significant difference in sound levels with a decreased of 2625 ± 558 Hz to 2504 ± 760 Hz after two weeks exposure at pH 7.4 (paired t-test: $t=2.4673$, $df=29$, $p=0.01976$) was noted our results indicate that the sound signals of snapping shrimp remain undisturbed at modest decrease in pH until a specific level of acidification was met i.e. pH 7.4 as found at in this study (Fig. 2). Seawater acidification has been reported to adversely affect marine animals. The calcification and growth rate of many marine decapods such as tiger prawn, shiba shrimp, and whiteleg shrimp have reported to be variably affected by low pH level or high concentration of dissolved carbon dioxide. This study indicates that the structural property of chelae such as hardness might play an important role in the snapping mechanism of snapping shrimps. Lower calcification and stunted growth rate due to acidification as might affect some of the chelae structural properties of snapping shrimp (Kurihara *et al.*, 2008). Rossi *et al.* (2016) reported that sound levels and the rate of snapping signals decreased after 3 months of elevated CO₂ exposure in controlled laboratory experiment but detected no significant differences. Further investigation is required to see if short period of exposure to a greater decrease in pH could potentially lead to physico-mechanical impairment of chelae due to a reduction in calcification rate.

The combined pH and temperature treatment experiment showed that the snapping sound peak frequency had decreased

Fig. 2 : Boxplots showing the changes in peak frequency of *Alpheus edwardsii* after 2wk exposure to different pH and temperature conditions. A. pH treatments (temperature fixed at 28°C); B. temperature treatments (pH fixed at pH 8.1). * indicates significant difference ($p < 0.05$) and mean is plotted with enlarged black point.

significantly from 2850 Hz to 2584 Hz after a 2 week exposure to 29°C and pH 7.7 (paired t-test: $t=3.0669$, $df=29$, $p=0.00465$) (Fig. 3A). Fig. 3B and 3C shows a significant reduction of peak frequency in both 29°C and pH 7.4 treatment (paired t-test: $t=3.433$, $df=29$, $p < 0.0021$) and 30°C and pH 7.7 treatment (paired t-test: $t=4.532$, $df=29$, $p < 0.0001$) by approximately 400 Hz and 700 Hz respectively. Treatment at 30°C and pH 7.4 experienced a significant drop in mean peak frequency from 3966 Hz to 2703 Hz, which is equivalent to approximately 30% of sound

level reduction (paired t-test: $t = 14.987$, $df = 29$, $p < 0.0001$) (Fig. 3D). This result showed that the mean peak frequency of snapping sounds were significantly reduced when low pH and temperature rise occurred simultaneously.

Our finding indicate a synergetic effect of environmental temperature and pH on the bioacoustics of intertidal snapping shrimps. As a consequence, such a change in snapping shrimp acoustics may implicate changes in the general characteristics of

Fig. 3 : Simultaneous effect of temperature and pH on snapping shrimp signals after 2 wk exposure. Paired boxplot showing the mean peak frequency recorded after the treatments at (A) 29°C; pH 7.7; (B) 29°C; pH 7.4; (C) 30°C; pH 7.7; (D) 30°C; pH 7.4. The symbol * and ** indicates significant differences ($p < 0.05$ and $p < 0.01$ respectively) after 2 wk of exposure. Mean of each treatment is plotted with enlarged black point in the boxplot.

underwater ambient noise emitted by tropical intertidal habitat. The *in-vivo* experiment exposed the snapping shrimps to increased temperature and decreased pH over a period of two weeks, which is longer than the daily fluctuation that they are usually exposed to in the wild. The results of this study showed that despite possible morphological or physiological effects as mentioned above, snapping shrimps are able to adapt to temperature and pH changes without significantly affecting their bioacoustics behaviour. Snapping sounds were still produced but with a change in acoustic characteristics *i.e.* sound peak frequency. A possible explanation could be the reduction of cavitation bubble strength due to increase in water temperature. Temperature rise increases the saturated vapour pressure of

water that weakens the formation and destabilizes the shape of cavitation bubbles. This is evident from the observed frequency characteristics of cavitation implosion sound.

Sounds of different frequencies have different propagation properties underwater. Generally, the propagation of low frequency sound in shallow waters is restricted by water depth. For snapping shrimp noise that originate from shallow water habitats, a reduction in sound frequency could affect propagation distance hence audibility. Considering the importance of snapping shrimp noise as an acoustic cue in the attraction and navigation of marine larvae, loss in acoustic quality may incur ecological implications. Recruitment and settlement to

habitats in some marine larvae such as crab and fish could be jeopardized due to reduction in the effective range of attraction cues as well as causing a mismatch between signal frequency and hearing sensitivity. It is also likely that due to the miniature size of marine larvae (in comparison to the wavelength of sound wave) they are more sensitive to higher frequency signal. In addition, coral fish larvae, particularly during settlement stage, has been found to prefer the higher frequency component of reef noise.

Long term changes in seawater temperature and pH can be attributed to global climate change. In previous studies efforts have been made to understand the effects of climate change on marine organisms under laboratory conditions that are comparable to the current study. Here, we report for the first time the potential effect of climate change on the bioacoustics of snapping shrimps. Accordingly, our findings provide an insight into how the natural ambient noise of marine habitats can be affected by climate change, thus providing a new understanding for assessing the overall impact of climate change on the underwater soundscape of marine habitats.

Add-on Information

Authors' contribution : K.S. Lai: Designed and performed experiments, collected and analysed data; co-wrote the paper; Z.Z. Goh: Performed experiments, collected and analysed data; revised the paper; S.M. Ghazali: Conceived and designed the experiments, analysed data and co-wrote the paper.

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