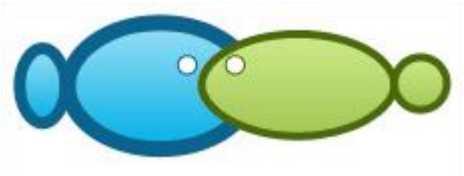

Effects of elevated temperature on the different life stages of tropical mollusk, donkey's ear abalone (*Haliotis asinina*)

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Abstract. The increase in sea surface temperature associated with climate change can cause tremendous impact on the different life stages of aquatic organisms, particularly on the tropical species. The present study investigates the effect of elevated temperature on hatching rate, growth and survival of larvae and breeders of tropical mollusk, donkey's ear abalone (*Haliotis asinina*). Different life stages of abalone were exposed to the following temperature treatments: ambient (29°C), +2°C (31°C), and +4°C (33°C). Hatching rate was significantly reduced when the fertilized eggs were incubated at temperature 4°C above ambient. Increase in temperature at 2°C above ambient can significantly influence the survival of larvae. Significant decline in the survival was observed when the larvae were exposed at temperatures 31°C and 33°C, however bigger larvae were observed in groups reared at 31°C, while those larvae that were reared in 33°C were significantly smaller. Furthermore, reduced growth, feeding rate and survival were also observed in breeders reared at elevated temperature. The result of the study suggests that early developmental stages and reproducing adult abalone were vulnerable to the impact of climate change.

Key Words: *Haliotis asinina*, climate change, elevated temperature, breeders, larvae.

Introduction. Increase in global atmospheric temperature has been progressing with high speed for the last 50 years. Modelling predicts that surface temperature will rise to 1.8-4.0°C globally by 2100 (IPCC 2007). It has been suggested that warming climate can cause tremendous impacts to terrestrial and aquatic organisms and their communities as well.

Aquatic invertebrates and fish are poikilotherms; their internal temperature varies directly with that of their environment. These organisms have limited thermal range which greatly affect their acclimation capacity (Barton et al 2002). Thus, this makes them very sensitive to changes in the temperature of their surrounding environment. Exposure to temperature beyond their physiological tolerance limit can be stressful or fatal. Few degrees change in habitat temperatures will significantly influence metabolic activity, and consequently affects the growth rate, survival, reproduction seasonality and possibly reproductive efficacy, and susceptibility to diseases and toxins (Wood & McDonald 1997; FAO 2008). Furthermore, aquatic organisms often have complex life-cycles with distinct life stages, each with different vulnerabilities to changing climate (Rijnsdorp et al 2009). Developing larvae and reproducing adults are more sensitive to changes in temperature since these stages have narrow thermal tolerance compared to juveniles (Portner & Farrel 2008). Elevated temperature has direct effect on the duration of embryonic and larval development and survival (Pankhurst & Munday 2011; Horwitz et al 2017). It can also cause timing-shift of spawning and adversely affect reproductive performance (Pankhurst & Munday 2011). Studies have also shown that increase in temperature above normal level can affect endocrine function, retard gametogenesis and gonadal maturation (Pankhurst & King 2010).

The physiological effect of climate change was well documented in temperate species (Urban & Silva 1998; Searle et al 2006; Pankhurst & King 2010; Talmage & Gobler 2011), however, information on tropical organism was comparatively few, however, models predict that tropical aquatic organisms will be more severely affected because they are constantly exposed to relatively stable thermal environment and experience less seasonal fluctuations in water temperature (Tewksbury et al 2008). Thus, they are more vulnerable to the impact of climate change. Here we investigated the response of the different life stages of tropical abalone, *Haliotis asinina* to the near-future warming effect. This species is found in the Indo-Pacific region and it is viable for tropical aquaculture. In the Philippines, it has shown promising economic potential. Its seed production has already been established in the country, however, the success of the production in the hatchery is reliant on the provision of optimum environmental condition for both spawners and larvae. Thus, considerable attention should be focused on the physiological response of the different developmental stages of this species to the effect of climate change to better understand the vulnerabilities of each stage to elevated temperature. The study could provide valuable information on the predicted impacts of climate change on abalone culture and this can help in the modification of the current farming practices to mitigate the effect of near-future increase in water temperature to ensure the sustainability of abalone culture industry in the country.

Material and Method

Exposure of fertilized eggs to elevated temperature. Newly fertilized abalone eggs were collected from spawning tanks. They were transferred to 2 L incubation containers provided with ultraviolet (UV) treated seawater and mild aeration. Stocking density of each container were 250 eggs L^{-1}. Plastic containers were placed in water basins provided with water heaters to achieve the desired temperature. Temperature was gradually increased at the rate of 1^oC per hour (hr) until the desired temperature was attained to allow the eggs to adjust to higher temperature. Temperature treatments were: ambient (29^oC) which serves as control, $+2^oC$ (31^oC), and $+4^oC$ (33^oC). Each treatment has 4 replicates. Hatching rates were assessed in 10 hours (hrs) after fertilization.

Exposure of larvae to elevated temperature. Pre-veliger abalone larvae were stocked in 60 L containers at a stocking density of 200 larvae L^{-1} at 4 replicates per treatment. Temperature treatments were: ambient (29^oC), $+2^oC$ (31^oC), and $+4^oC$ (33^oC). Culture containers were provided with water heaters to maintain the desired temperature. For higher temperature treatment, the water temperature was gradually increased so that the larvae can adjust to the elevated temperature treatments. The larvae were reared until they metamorphose into postlarvae (settlement stage) at day 7. Each culture container was provided with mixed diatoms grown in polyvinyl plates with crustose coralline algae. Preparation of the diatom plates and feeding management of the larvae were done following de la Peña et al (2010). Minimal water change was done to maintain the temperature. Growth and survival of postlarvae were assessed at the end of the experiment.

Exposure of breeders to elevated temperature. Prior to the start of the experiment breeders were acclimated to the experimental temperature for 2 weeks. Breeders (size: 49-70 mm) were transferred to 250 L fiberglass tanks at stocking density of thirty individuals per tank at 3 replicates per treatment. Separate tanks were used for male and female breeders. Temperature treatments were: ambient (29^oC), $+2^oC$ (31^oC), and $+4^oC$ (33^oC). Broodstocks were fed with with *Gracillaria* sp. ad libitum for the entire duration of the experiment. Feed consumption was determined every 3 days by getting the weight of the feed before feeding and the remaining feed after three days. The duration of the experiment was 3 months. Monthly sampling was done to determine the growth and survival of breeders.

The experiments performed were conducted in Abalone Hatchery of Southeast Asian Fisheries and Development Center – Aquaculture Department, Tigbauan, Iloilo, Philippines, between the months of February to May 2014.

Statistical analyses. Statistical analyses were performed using SPSS 24 software package. Normality of data and homogeneity of variances were checked using Levene's test. Values using percentages were transformed to square root prior to analysis. One-way ANOVA was used to analyze the data. Tukey-Kramer post-hoc test was conducted to determine differences between the treatment means ($p < 0.05$).

Results and Discussion. The present study determines the effect of elevated temperature on the different life stages of tropical mollusk, donkey's ear abalone, *Haliotis asinina*. The temperature treatments used in the present study was based on the predicted global mean temperature increase by IPCC (Intergovernmental Panel on Climate Change). The first experiment conducted was to evaluate the effect of elevated temperature on the hatching rate of abalone. The result showed that hatching rate was highest in ambient temperature (28-29^{o}C) which was 84.33%, while hatching rate in 31^{o}C was 74.5% and lowest was observed in 33^{o}C which was 20.5% (Figure 1). There was no significant difference in the hatching rate between ambient and 31^{o}C, however when the eggs were incubated at 4^{o}C above ambient, egg viability and development were negatively affected which results in very low hatching rate. This indicates that incubation at 33°C is the upper temperature threshold for *H. asinina* embryos. In studies done in early stages of development of other species, it was observed that increase in temperature beyond their tolerance limit could hinder development or cause deformities during egg incubation and eventually results in mortalities (Kumlu et al 2000; Paula et al 2001; Soundarapandian et al 2014).

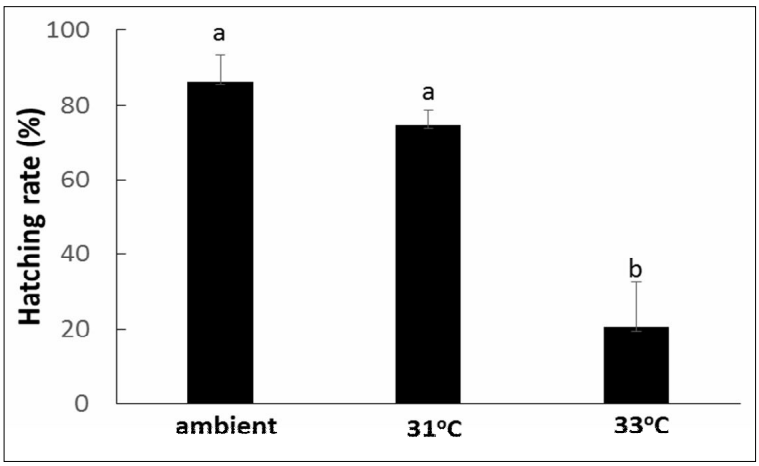

Figure 1. Mean hatching rate of abalone eggs incubated at different temperature treatments. Values with different labels are significantly different ($p < 0.05$).

Another experiment was done to determine the influence of warming on larval growth and survival. Increase in temperature significantly affects survival of larvae, the data on Figure 2 shows that as the temperature increases, survival rate decreases. Larvae exposed at 31^{o}C and 33^{o}C has significantly lower survival compared to control. The result of the present study showed that 2^{o}C increase in ambient temperature can already cause adverse effect on the survival of the early larval stage of *H. asinina*. This conforms to the observations of Kurihara (2008) and Byrne (2011) that early life history of marine invertebrates have narrow aerobic thermal window thus they are particularly sensitive to climate change drivers such as ocean warming. Moreover at this stage, the larvae undergo abrupt and dramatic changes in their morphological and physiological development (Portner & Farrell 2008). It was also observed that larvae reared at 31^{o}C were largest, however the larval size of those groups reared in 33^{o}C were lowest in value (Figure 3). This result indicates that increase in water temperature may increase the

metabolic rate at a level that it can still support increase in growth but only until a certain threshold limit. However, more extreme (≥ 4°C) ocean warming may already cause detrimental effect on aquatic organism which can significantly affect the growth and survival of larvae (Byrne 2011).

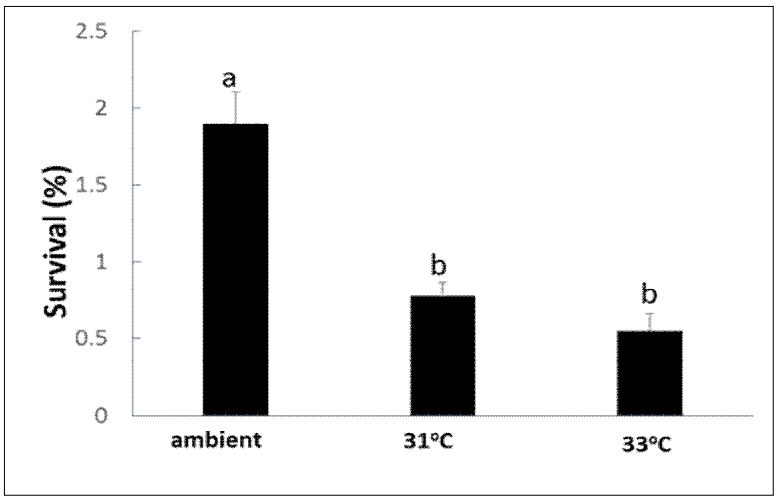

Figure 2. Mean survival of abalone larvae exposed to three temperature treatments. Values with different labels are significantly different ($p < 0.05$).

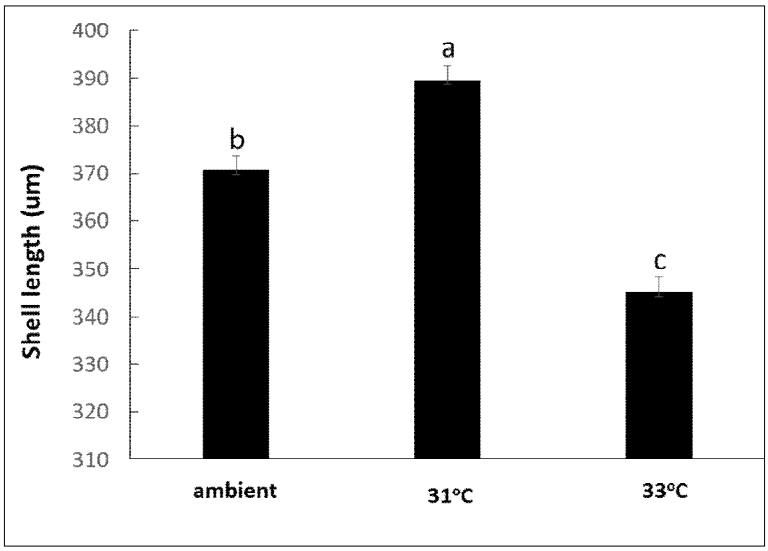

Figure 3. Mean shell length of larvae exposed to three temperature treatments. Values with different labels are significantly different ($p < 0.05$).

An experiment was also conducted to assess the impact of elevated temperature on reproducing adult abalone. This study determines the sensitivity of male and female breeders when exposed to higher temperature for three months. The growth of breeders was negatively affected by elevated temperature. Reduction in the specific growth rate (g month^{-1}) of groups reared at 31°C and 33°C was very evident as shown in Table 1 caused by significant reduction in their feed consumption (Table 2). This observation is in agreement with the findings of Britz et al (1997) on *Haliotis midae*, which a significant decline in the growth of abalone reared at higher temperature was reported. Survival was also affected by elevated temperature, a significant reduction in the survival of breeders exposed at 31°C and 33°C was also observed. It was very evident in the data presented in Figure 4 that female breeders were more sensitive compared to males.

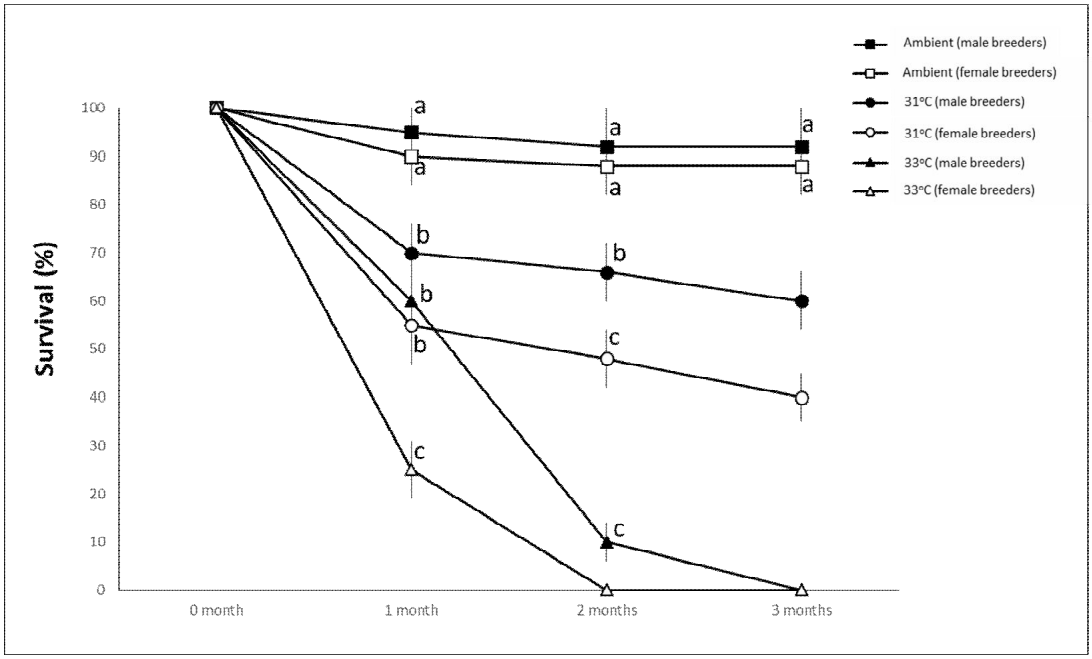

Figure 4. Mean (±SE) survival of abalone breeders exposed at different temperature treatments. Values with different labels are significantly different ($p < 0.05$).

Higher mortality rates were observed in reproducing female adults. After two months, a 100% mortality was recorded in female breeders reared at 33°C. It was also noted that breeders exposed at 33°C has spent or regressed gonads. Loss and reduction of germ cell was also reported in other aquatic species exposed at elevated temperature (Strussmann et al 1998; Strussmann & Patino 1999). Thus, prolong exposure of breeders to higher temperature can result to gonadal atrophy and inhibition of gametogenesis (Strussman & Patino 1999). The results of the present study showed that reproducing adults were as sensitive as the early developmental stages. Reproducing adults exposed at increasing temperature may require more oxygen to meet higher metabolic demand (Portner & Farrell 2008).

Table 1

Growth performance of male and female abalone breeders reared at different temperature treatments

Experimental treatments	*Specific growth rate (g month^{-1}) Month 1*	*Specific growth rate (g month-1) Month 2*	*Specific growth rate (g month^{-1}) Month 3*
Ambient (male breeders)	4.50 ± 0.75^{a}	4.50 ± 0.75^{a}	6.5 ± 0.90^{b}
Ambient (female breeders)	5.05 ± 1.2^{a}	4.95 ± 0.98^{a}	11.34 ± 1.40^{a}
31°C (male breeders)	-10.9 ± 1.5^{b}	-10.41 ± 2.2^{b}	-5.41 ± 0.76^{c}
31°C (female breeders)	-7.31 ± 0.9^{b}	-7.32 ± 1.3^{b}	-1.71 ± 0.21^{c}
33°C (male breeders)	-21.82 ± 2.1^{c}	-29.2 ± 0.4^{c}	-
33°C (female breeders)	-19.75 ± 1.9^{c}	-	-

Note: Values in the same column with different superscript differ significantly ($p < 0.05$).

Table 2

Mean daily feed consumption rates of male and female breeders reared at different temperature treatments

Experimental treatments	*Daily consumption (% BW day^{-1}) Month 1*	*Daily consumption (% BW day^{-1}) Month 2*	*Daily consumption (% BW day^{-1}) Month 3*
Ambient (male breeders)	6.4±0.31^{a}	7.6±0.29^{a}	11.5±0.43^{a}
Ambient (female breeders)	7.0±0.26^{a}	8.2±0.34^{a}	10.6±0.55^{a}
31^{o}C (male breeders)	4.6±0.21^{b}	5.7±0.45^{b}	9.5±0.75^{a}
31^{o}C (female breeders)	4.0±0.33^{b}	4.5±0.39^{b}	7.7±0.32^{b}
33^{o}C (male breeders)	2.8±0.41^{c}	2.2±0.19^{c}	0
33^{o}C (female breeders)	2.3±0.35^{c}	0.5±0.08^{d}	0

Note: Values in the same column with different superscript differ significantly ($p < 0.05$); BW - body weight.

Conclusions. This study provides beneficial information on the response of the different life stages of tropical abalone to elevated temperature. The results suggest that elevated seawater temperature can bring adverse effect on the developing embryo and larvae as well as in reproducing adults. Understanding the sensitivity of different developmental stages of tropical organism is an important factor to consider in the assessment of the impacts of predicted temperature increases associated with climate change.

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