Philippine Journal of Science
144 (1): 21-29, June 2015
ISSN 0031 - 7683

Effects of Reduced pH on Larval Settlement and Survival of the Donkey's Ear Abalone, *Haliotis asinina* (Linnaeus 1758)

Abduraji S. Tahil[1] and **Danilo T. Dy**

Department of Biology, University of San Carlos,
Talamban Campus, Cebu City
[1]Mindanao State University Tawi-Tawi, Sanga-Sanga, Bongao, Tawi-Tawi

The potential effects of reduced pH as a result of an increased CO_2 concentration on settlement and survival of *Haliotis asinina* larvae were investigated. The settlement frequency (%F) was significantly different with respect to pH levels. On day 5, 100% of the settlement plates contained postlarvae at ambient conditions (pH 7.98) and pH 7.76. Lower %F was obtained at pH 7.41 (12.5% - 37.5%) and pH 7.57 (25% - 62.5%). Hence, significantly higher number of larvae attached to plates at ambient conditions (16 postlarvae plate^{-1}) and at pH 7.76 (10 postlarvae plate^{-1}). On the other hand, the concentration of carbonate ion was lowest in the high-CO_2 or reduced pH treatment and the larval settlement was also lower. Fewer larvae settled on plates exposed to pH 7.41 (3 postlarvae plate^{-1}) and pH 7.57 (5 postlarvae plate^{-1}). Post settlement survival (10 and 15 days after exposure) was significantly lower at reduced pH levels compared to ambient conditions. Settlement rate was also affected by the reduction in % cover of crustose coralline algae (CCA) of the plates and delayed morphological development of larvae at reduced pH. This study confirmed that reduction in pH of seawater to the levels predicted by the end of this century will have negative effect on the settlement and survival of *H. asinina* larvae, and by extension, the future economy of the abalone industry of the Philippines.

Key Words: abalone seed production, larval abundance, ocean acidification, pH, settlement success

INTRODUCTION

The planktonic larval phase in the life cycle of abalone ends with settlement onto the substratum and metamorphosis into the benthic adult form. Upon settlement, the larvae continue to creep for sometime before adhering firmly to a favorable substratum. After settlement, the larvae secrete mucus from the foot sole, adhere firmly to the substratum and start feeding on benthic diatoms (Singhagraiwan & Doi 1993) until they metamorphose into plantigrade juveniles.

Poor larval settlement and survival, and abnormal metamorphosis are among the major problems in seed production of *H. asinina*. This may be related to insufficient amount of effective settlement cue (i.e. crustose coralline algae) and morphological deformities under unfavorable conditions of the culture system (i.e. reduced pH level of seawater) (De Vicose et al. 2010; Li et al. 2013). Thus, cultivation of this species requires reliable and sustainable production of seeds in large quantities. Hatchery production depends largely on the success of settlement and metamorphosis of postlarvae. Prior to settlement the larvae will undergo a series of developmental stages such as shell secretion, development of velum with long cilia, operculum, eye spot, propodium and cephalic tentacles (Singhagraiwan and Doi 1993;

*Corresponding author: rajstahil@yahoo.com

Najmudeen and Victor 2004), among others. During this transformation, the abalone larvae are sensitive and may be affected negatively by any changes in the chemical composition of seawater such as reduced pH. The first deposition of $CaCO_3$ occurs during the larval stage or during the settlement stage of the shell-forming organisms (Kurihara 2008).

The concentration of CO_2 in the ocean is expected to rise to 750 ppm by 2100, which is about twice the present 385–390 ppm (Feely et al. 2004; Raven et al. 2005). With increasing partial pressure of CO_2 (pCO_2), the equilibrium of the carbonate system will shift to higher CO_2 and HCO_3^- levels, while CO_3^{2-} concentration and pH will decrease (Cigliano et al. 2010). By the year 2100, the pH of the ocean's surface is predicted to decrease by 0.3 – 0.5 units (Caldeira & Wickett 2003, 2005). This predicted decrease in pH would likely affect the sensitive and vulnerable early larval development and settlement of abalone and other calcifying molluscs since these life stages have specific environmental needs. The impact of reduced pH is expected to be more significant in larvae than adults (Kurihara 2008; Ross et al. 2011). Adult organisms exposed to hypercapnia (high CO_2 concentration in the blood) suffer from physiological stress and reduced calcification (Michaelidis et al. 2005; Miles et al. 2007; Spicer et al. 2007). In *Haliotis coccoradiata*, exposure of ectodermal calcifying system to reduced pH levels (7.6 – 7.8) resulted in unshelled larvae (Byrne et al. 2011). At present, there is no information on the direct effect of reduced pH on the settlement and survival of tropical haliotid species, particularly the Donkey's ear abalone, *H. asinina*. Since settlement success is very important in the production of postlarval and juvenile abalone in the hatchery, we present here the direct effects of reduced pH as a consequence of ocean acidification on settlement and survival of settled postlarvae of *H. asinina* under hatchery conditions. This is the first study in the Philippines to quantify the potential effect as planktonic abalone larvae try to settle onto the substratum under reduced pH condition.

MATERIALS AND METHODS

Experimental Design

The experiment used a randomized complete block design with trials as blocking variables. The experiment was conducted at the Tawi-Tawi Multi-Species Hatchery in Latu-Latu, Bongao, Tawi-Tawi, Philippines from May – July 2013. An epoxy-coated wooden tank with eight compartments (4 x 2 compartments) was used as the rearing vessel. Mild aeration using an air stone was provided on each tank compartment. Each compartment has a dimension of 60 x 60 x 60 cm. Each tank compartment was provided with filtered (sand-filter and 5 µm mesh filter bag) and UV-treated seawater during each trial. There were four pH levels used: ambient ranged from 7.95 – 8.03 (mean ± s.d.; 7.98±0.03), Treatment I 7.65 – 7.84 (7.76±0.05), Treatment II 7.46 –7.67 (7.57±0.04), and Treatment III 7.35 –7.48 (7.41±0.03). The basis for the selection of these pH levels was the predicted decrease in pH of the ocean's surface by 0.3 – 0.5 units by the year 2100 (Caldeira & Wickett 2003, 2005).

The experiment was done in four successive trials with two replicates for each pH treatment per trial, and a new batch of trochophore larvae was used during each trial. Immediately after larval stocking, the pH was gradually adjusted to the desired level for a particular treatment. The response variables for this experiment were % settlement frequency (i.e. number of plates where larvae settled divided by total number of plates inside each compartment), % survival and % settled postlarvae after exposure to different pH levels. Part of the water quality management was the siphoning of water near or at the bottom of the tank to get rid of dead larvae.

Manipulation and Maintenance of pH

The pH levels were adjusted and maintained through manual addition of a food-grade CO_2 (Pryce Gas Company) delivered via thin walled silicon tubings (8 mm dia.). Based on the preliminary simulation to determine the amount of CO_2 needed to maintain the pH within the range, CO_2 was added every 3 h for an average duration of 7 – 50 s depending on the desired pH level for each treatment. This procedure allowed the maintenance of desired pH treatments during the 15-day experiment. The pH and temperature of the water were measured with a calibrated pH meter (Orion Research, Inc., U.S.A.; Precision: 0.05 pH units).

Total alkalinity (TA) was measured every 5-day sampling period throughout the 15-day experiment using an alkalinity titration kit (Hanna Instruments, Woonsocket, USA). Concentrations of CO_2, carbonate (CO_3^{-2}) and bicarbonate (HCO_3^-), partial pressure of CO_2 (pCO_2), and saturation state of calcite (Ωcalc) and aragonite (Ωarag) were then calculated from measured TA and pH using the software CO_2SYS (Pierrot et al. 2006) and by using the dissociation constants of carbonic acid from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) (Moulin et al. 2011). This program, which performs calculations relating the parameters of CO_2 system in seawater and freshwater, uses two of the four measurable parameters of the CO_2 system [total alkalinity (TA), total inorganic carbon dioxide (TCO_2), pH, and either fugacity of carbon dioxide (fCO_2) or partial pressure of carbon dioxide (pCO_2)] to calculate the other two parameters at a set of

input conditions (temperature and pressure) and a set of output conditions chosen by the user (Pierrot et al. 2006). Salinity and dissolved oxygen were measured with a refractometer (ATAGO 100-S, S/Mill-E, Japan) and DO meter (Orion M810Aplus, Orion Research, Inc., U.S.A.), respectively. Water temperatures in all treatments ranged from 26.8 – 27.2°C, salinity from 33.9 – 34.2 $^{o}/_{oo}$ while dissolved oxygen varied between 7.94 and 8.04 mg L^{-1}.

Diatom Slurry and Settlement Plate Preparation

A pure stock culture of benthic diatoms (*Amphora* sp. and *Navicula ramosissima*) was acquired from SEAFDEC/AQD, Tigbauan, Iloilo and cultured at the larval food laboratory of the Tawi-Tawi Multi-Species Hatchery. The diatoms were harvested at their exponential growth phase and cultured to a larger scale (500 L) using the multi-step batch culture method (de la Peña et al. 2010). The culture was vigorously aerated from the bottom of the tank using a perforated PVC pipes (19 mm diameter) that served as the aeration line. The tank was installed with 120 pieces of 45 cm x 40 cm corrugated plastic sheet with grown crustose coralline algae (CCA) arranged vertically to induce the settlement and growth of benthic diatoms on the plates. The plates with CCA were old plates which have been placed for at least three months in the sea where population of *H. asinina* exists to allow the settlement of CCA. No actual identification of CCA species was made, although the common species identified by de la Peña et al. (2010) at SEAFDEC/AQD were *Mesophyllum* sp. on the top and *Hydrolliton samoense* at the bottom of the pink crust.

After four days when diatoms were expected to start attaching to the plates, flow through of seawater was initiated until the plates were covered with growth of diatoms. Following the procedures of de la Peña et al. (2010), diatoms were harvested using a soft paint brush and were concentrated to 25 μm net bag. The bigger size diatoms were removed by using a 60 μm net bag. Two-thirds of the diatom slurry was used for feeding the larvae of abalone while the remaining one-third was returned to the tank as a starter for the next batch of culture. One culture cycle lasted for three weeks and the diatom starter was replaced every cycle.

To determine the impact of CO_2 infusion or reduced pH on the CCA, the % cover of CCA was monitored during the day 5 sampling and at the end of the 15-day exposure period. A transparent plastic sheet of similar dimension with the settlement plate and gridded with 50 evenly-spaced small squares (2.5 x 2.5 cm) was overlaid on the plate and the presence of CCA in each square was noted. The procedure served as the basis for the calculation of the CCA's % cover.

Preparation of Settlement Tanks and Stocking of Larvae

Several hours before the transfer of the trochophore larvae into the experimental tanks, 12-pc settlement plates covered with CCA and diatom films were placed in each of the tank compartments. The trochophore larvae from the Tawi-Tawi Multi-Species Hatchery were obtained by siphoning the larvae into a 50-liter plastic bucket lined with 45 μm mesh size net to regulate the slow flow of UV-irradiated seawater for washing the larvae. The larval density was estimated by counting the larvae in 10 one-ml subsamples. The larvae were stocked and distributed to the experimental units at a density of 30 larvae liter-1 per experimental unit (100 L). Mild aeration using one air stone was provided on each tank compartment. A white fluorescent tube (30-watt, Toshiba) was placed on top of the settling tank to provide illumination during night time to reduce respiration of diatoms.

Monitoring of Settlement and Sample Collection

Monitoring of the settled postlarvae was done every 5d for a period of 15 day. Larvae that have been seen attached (immobile) or crawling on the plates were considered settled postlarvae. Unsettled larvae may have suffered from delayed or abnormal morphological development or succumbed to death under reduced pH levels. The settled postlarvae were dislodged from 3 replicate plates per sampling using a soft paint brush and placed in a small white basin. The collected larvae were concentrated in a 45 μm net and placed in a sampling bottle with 50 ml filtered seawater. The larvae were then examined under a monocular 40x microscope (Cole Parmer, Vernon Hills, Illinois) for counting. Dead larvae were excluded from the counting.

The % settlement frequency (%F) referred to in this experiment was expressed as the number of corrugated plastic plates on which postlarvae settled (not the number of settled larvae per plate) divided by the total number of plates (i.e. 12 pcs) placed in the tank compartment x 100. This was calculated to determine how many of the total number of settlement plates exposed to a particular pH level were settled by the postlarvae, and to identify factors (i.e. inducer of settlement) that may contribute to such variations in %F relative to pH treatments.

Statistical Analysis

A Kruskal–Wallis one-way analysis of variance (ANOVA) was used to test the null hypothesis that the % settlement frequency (%F) onto the plates is independent of the pH levels to which the settlement plates were assigned. Data on % settled postlarvae and survival was log transformed before using repeated measures ANOVA [experimental units (tank compartments) were repeatedly sampled – day

5, day 10, and day 15] to determine differences between pH treatments and across exposure period. Homoscedasticity of the transformed data was obtained with a Levene's test. Differences between means were tested for significance ($p < 0.05$) using the post hoc Tukey HSD test.

RESULTS AND DISCUSSION

The carbonate chemistry of experimental seawater is shown in Table 1. Carbonate ion concentration was lowest in the high-CO_2 or reduced pH treatment (57.6 µmol kg^{-1} compared to 192.7 µmol kg^{-1} at ambient conditions) where the % larval settlement was also lower. The ambient pCO_2 (453.5 µatm) in this experiment is closely similar to the studies of other workers for mollusks from the Pacific area [e.g., Crim et al. (2011) – 400 µatm; Timmins-Schiffman & O'Donnell (2012) – 468 µatm; Andersen et al. (2013) – 469 µatm], suggesting comparable ambient pCO_2 conditions. The % settlement frequency (%F) on plate samples was significantly different with respect to pH levels [Kruskal-Wallis ANOVA: H (3, $n = 32$) = 24.51; $p = 0.00$] (Table 2). All the settlement plates under ambient conditions contained postlarvae during the entire 15 days rearing (100%). At pH 7.76, 100% was also observed from day 5 to day 10 but it reduced to 75.0% on day 15. When exposed to pH 7.57, the %F was reduced significantly to 62.5% and 25% on day 10 and day 15, respectively, while in a much lower pH level (7.41) %F ranged from 12.5% (day 15) to 37.5% (day 5). These observations may also indicate that under ambient pH, natural inducers of settlement (i.e. CCA) on each plate may be available in sufficient cover, in contrast to CO_2-treated (reduced pH) water where attachment selectivity with respect to plates was observed. This means that larvae failed to settle on plates with minimal amount of natural inducers of settlement at reduced pH levels (e.g., 34% CCA cover at pH 7.57 and 19% at pH 7.41 – Fig. 2). This is suggestive of a negative, possibly additive effect of reduced pH on CCA recruitment rate and % cover, and the competence of the larvae to settle onto the substratum.

The postlarval settlement on day 5, day 10 and day 15 is shown in Figure 1. Highly significant differences ($p < 0.001$) in the number of attached postlarvae were observed among reduced pH levels. Under ambient conditions, larval attachment on day 5 (6.5% of the larvae stocked $tank^{-1}$) was significantly higher than in plates exposed to reduced pH 7.76 (4.0%), pH 7.57 (2.1%) and pH 7.41 (1.0%). Post settlement survival was significantly lower at reduced pH levels compared to the ambient

Table 1. Carbonate chemistry of seawater in ambient (control) and at reduced pH levels. Data are means ± SD ($n = 8$).

	pH Treatment			
Parameter	7.95 – 8.03	7.63 – 7.89	7.46 – 7.70	7.24 – 7.52
	(Ambient)	(T – I)	(T – II)	(T – III)
Temperature (°C)	27.2±0.6	27.0±0.5	26.9±0.6	26.8±0.4
Salinity (psu)	33.9±0.4	34.1±0.3	34.0±0.3	34.2±0.3
pH	7.98±0.03	7.76±0.05	7.57±0.04	7.41±0.03
Total alkalinity (µmol kg^{-1})	2193.6±38.5	2108.3±35.3	2093.7±37.2	2078.0±26.4
CO_2 (µmol kg^{-1})	11.9±01.0	20.9±2.5	34.0±3.5	50.4±4.6
$CO3^{-2}$ (µmol kg^{-1})	192.7±11.4	121.5±9.8	81.7±6.6	57.6±4.0
HCO_3- (µmol kg^{-1})	1718.2±38.5	1806.7±39.9	1890.7±31.7	1934.8±29.4
pCO_2 (µatm)	453.5±34.7	800.2±98.8	1302.4±141.7	1925.1±171.5
Calcite saturation (Ωcalc)	4.7±0.3	3.0±0.2	2.0±0.2	1.4±0.1
Aragonite saturation (Ωarag)	3.1±0.2	2.0±0.2	1.3±0.1	0.9±0.1

Table 2. The percent settlement frequency (%F) of plates where *Haliotis asinina* postlarvae settled after 5, 10, and 15 days at ambient conditions and at reduced pH levels. Values are means ± SEM. Kruskal-Wallis test: H (3, $n = 32$) = 24.51, $p = 0.00$. Means with the same supercripts are not significantly different.

pH Treatment	Day 5 (%F)	Day 10 (%F)	Day 15 (%F)
7.95 - 8.03 (Ambient)	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
7.63 - 7.89 (T – I)	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	75.0 ± 22.0^{b}
7.46 - 7.70 (T – II)	87.5 ± 16.1^{a}	62.5 ± 33.0^{b}	25.0 ± 15.4^{c}
7.24 - 7.52 (T – III)	37.5 ± 26.0^{b}	25.0 ± 15.4^{c}	12.5 ± 17.3^{d}

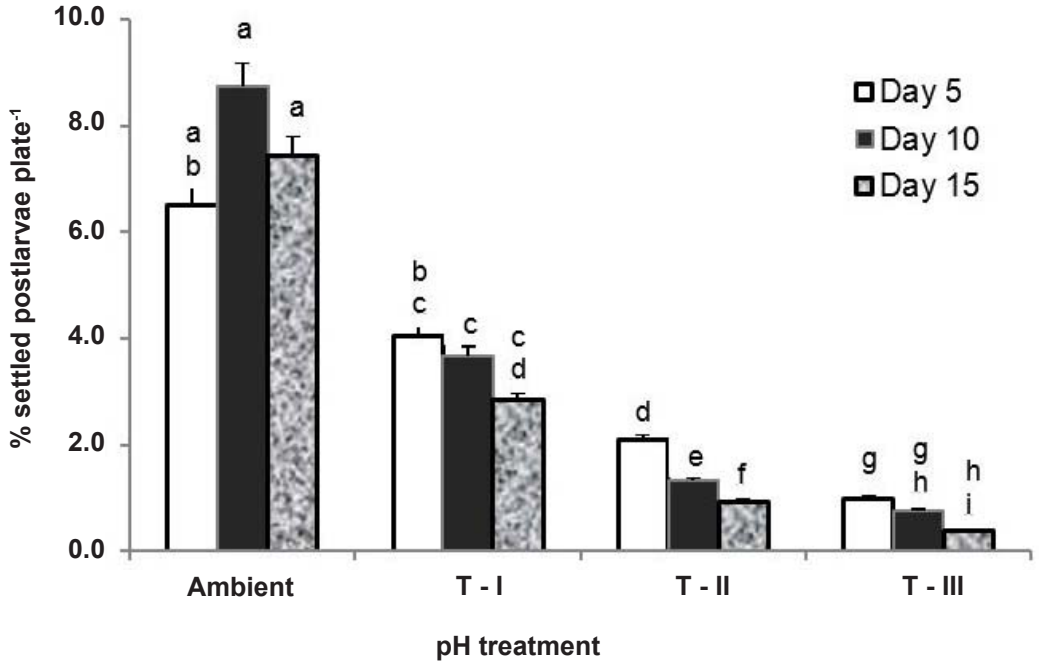

Figure 1. Percent settled larvae of *Haliotis asinina* exposed to different pH levels at days 5, 10, and 15. The different letters above the bar indicate significant difference between pH treatments and across exposure period (T: $p = 0.000$; Day: $p = 0.000$ and T * Day: $p = 0.000$). Error bars are sd, $n = 8$.

conditions (see Figure 1). At pH 7.3, larval settlement was only 2.7% after 48 h for another Pacific abalone, *Haliotis discus hannai* (Li et al. 2013), corroborating our results. Low survival in reduced pH levels was attributed to the lower settlement rate during the early period of exposure, particularly at high CO_2 (elevated pCO_2) and low carbonate ion concentrations.

The relatively higher number of settled postlarvae at ambient conditions may be attributed to the CCA of the settlement plates which remained intact on which biofilms (diatoms, bacteria and other microorganisms) are embedded. In contrast, the lower number of settled postlarvae from CO_2-treated seawater is likely attributed to the observed removal or reduction in the amount of CCA on the settlement plates as a consequence of reduced pH levels. Coralline algae (i.e. *Mesophyllum* sp. and *Hydrolithon samoense*) excrete macromolecules and chemical inducers that favored settlement of abalone larvae (de la Peña et al. 2010; de Vicose et al. 2010). A study on settlement responses of *H. asinina* larvae to various species of coralline algae demonstrated that settlement specificity with respect to algae species was likely driven by chemical, rather than physical, properties of the algae (Williams et al. 2009). Doropoulos et al. (2012) observed a reduction in settlement of another calcifying organism (coral) with a profound decline in the CCA cover of the settlement substrata when exposed to elevated CO_2 treatments (= lower pH). Crustose coralline algae have been reported to be an effective settlement cues (de Vicose et al. 2010) and *Amphiroa* spp. are considered highly effective natural inducers of settlement in the Donkey's ear abalone *H. asinina* (Williams et al. 2008). De la Peña et al. (2010) reported a significantly higher number of *H. asinina* larvae (15 – 26 postlarvae plate^{-1}) settled on plates with CCA compared to plates without CCA (6 – 19 postlarvae plate^{-1}). Similar observation was also reported for other abalone species, *H. iris*, where > 88% of larvae attached to a CCA-covered settlement substrata (Roberts et al. 2004). There is now clear evidence that reduced pH resulted to lesser cover of CCA and consequently lower number of larvae settling onto the plates.

CCA are sensitive to changes in the carbonate system of seawater and will tend to reduce at a lower pH level. We observed significant reduction or removal ($p < 0.001$) in % cover of CCA (crust became less pink) on the settlement plates exposed to CO_2-treated waters (Figure 2). After 15 days of exposure to pH 7.57 and pH 7.41, CCA cover of the plates was reduced to 33.6% and 19.4%, respectively, compared to the ambient conditions (66.8% cover). Crustose coralline algae accrete carbonate containing greater proportion of magnesium-calcite (Webster et al. 2013) which makes them susceptible to reduced carbonate saturation state as pH decreases, and many studies have documented this vulnerability (i.e. Martin & Gattuso 2009; Diaz-Pulido et al. 2012). Other studies showed that exposure to low pH had significant effect on the structure

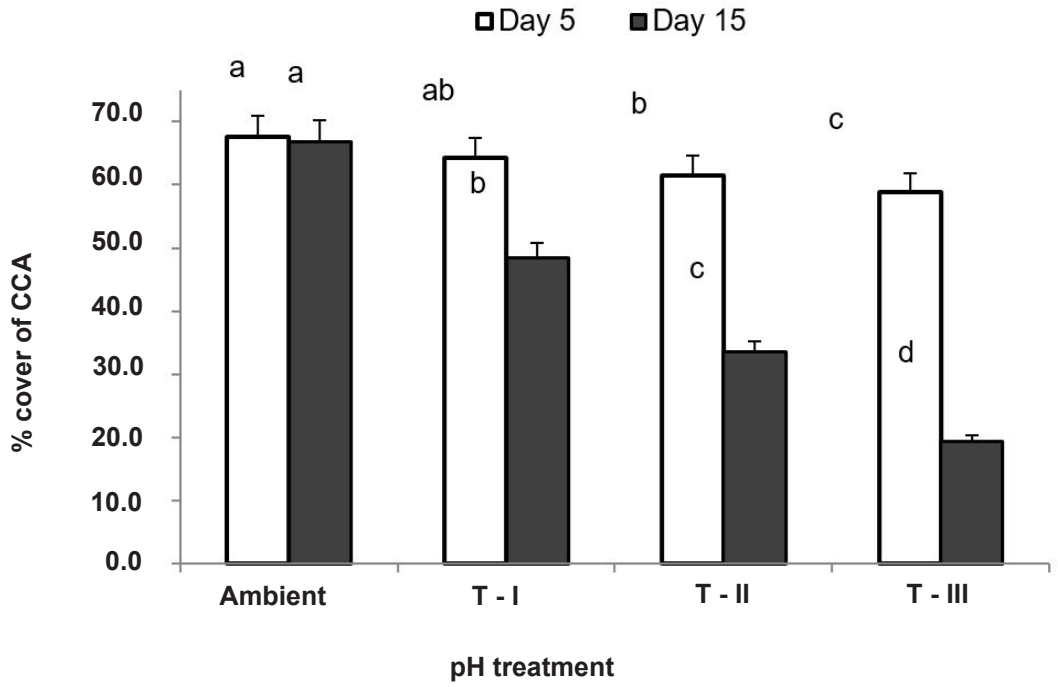

Figure 2. Percentage cover of the crustose coralline algae (CCA) on the settlement plates recorded after 5 days and 15 days of exposure to different pH levels. The different letters above the bar indicate significant difference between pH treatments ($p = 0.001$). Errors bars are sd, $n = 8$.

of the CCA-derived microbial communities that favor larval settlement (Webster et al. 2013).

Low settlement rate at reduced pH levels can also be attributed to the delayed development or abnormal larvae under such conditions. We observed that about 68% of the larvae at pH 7.57 and 97% at pH 7.41 were morphologically deformed compared to the attached postlarvae under ambient conditions with well developed major organs such as shell and epipodial tentacles (Figure 3). Apparently, morphological deformities are caused by reduced pH which consequently affects larval competence to settle. This negative effect has been reported for other larvae of mollusks exposed to reduced pH levels (e.g. Ellis et al. 2009; Talmage & Gobler 2009; Watson et al. 2009). Larval development was delayed and abnormal larvae were observed due to reduced pH levels which resulted to low settlement rate – indicating that abnormal development of larval morphology is a common response among species of calcifying mollusks. However, the severity of abnormalities at a given pH decrease differs greatly among species of abalone. Shell abnormalities in *H. kamtschatkana* larvae manifested with relatively minor pH reduction such that 40% of larvae developed abnormal shells at pH 7.8 (Crim et al. 2011). Veliger larvae of *H. coccoradiata* also appeared particularly sensitive to reduced pH (0.4 - 0.6 units decrease) with deleterious effects evident at pH 7.8 and below in which 23% unshelled and abnormal phenotypes were observed (Wong et al. 2010; Byrne et al. 2011). These morphological abnormalities will have a negative effect on the competence of the larvae to settle as they transform from the planktonic phase to benthic form in their life cycle and metamorphose into juvenile and adult stage.

It is, however, interesting to note that even at reduced pH levels some of the larvae were able to settle and survive, and some CCA also thrived under such conditions favoring some larvae to settle. This suggests that variation exists within the population of abalone and among species of CCA to cope with reduced pH, and this variation can be acted upon by natural selection to sustain and increase the number of survivors in the face of future decreases in ocean pH. Although the current results – focusing on mean responses – are directly applicable to required conditions in present-day aquaculture setting, they are not directly applicable to effects on natural populations of future scenarios. Of course under ambient pH, we expect that larvae would do better compared to reduced pH levels because the animals we used for this experiment were already adapted to the ambient conditions. Much more important to future scenarios in nature is the fact that there were some individuals that survived, and it is these survivors that provide the fuel for future evolution, possibly allowing for adaptation to changing conditions of their natural habitats.

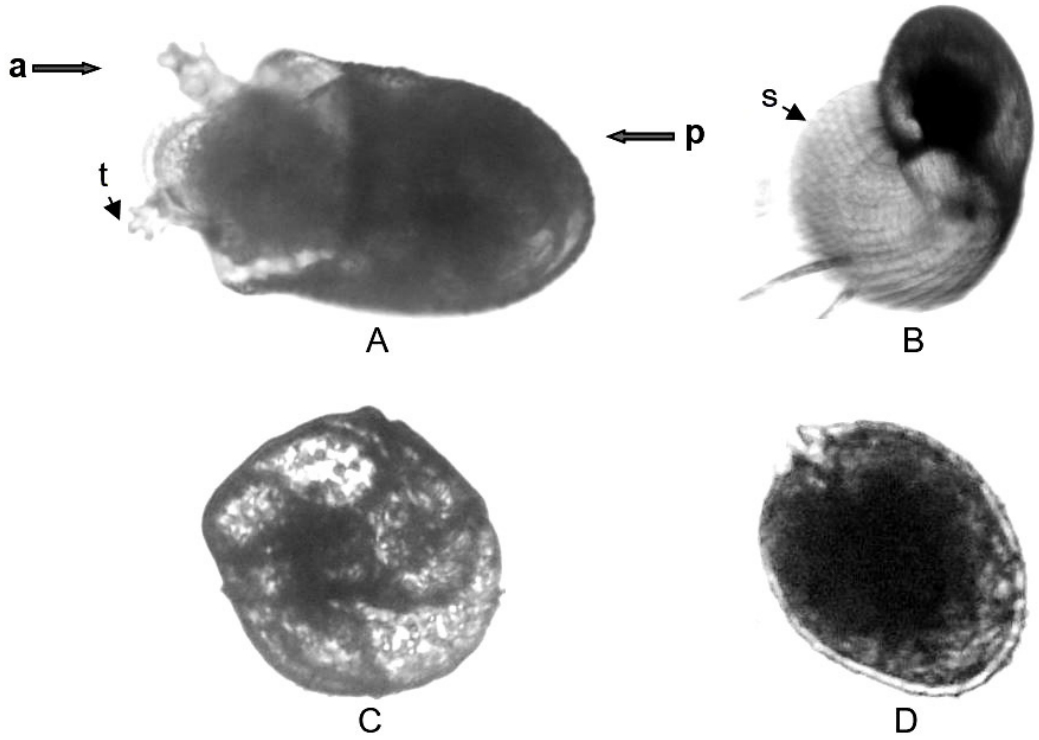

Figure 3. Normal postlarvae after settlement with shell (s) and tentacles (t) well developed under ambient conditions (pH 7.98) (A and B). Larvae with underdeveloped and undefined morphological characters exposed to pH 7.57 (67.9% abnormal) and pH 7.41 (97.0% abnormal), respectively (C and D). a = anterior and p = posterior end.

CONCLUSIONS

This study showed that larval settlement and survival of the Donkey's ear abalone *H. asinina* was adversely affected by reduced pH of seawater – suggesting that pH levels must be within the range favorable to the early developmental stages of abalone. Crustose coralline algae (CCA), which is considered as effective natural inducers of abalone settlement, are sensitive to changes in the carbonate system of the seawater – their % cover decreased at lower pH levels. This study confirmed that reduction of pH in seawater to the levels predicted by the end of this century will have a negative effect on the settlement and survival of *H. asinina* larvae.

ACKNOWLEGMENTS

We thank the management of the Tawi-Tawi Multi-Species Hatchery, Lato-Lato, Bongao, Tawi-Tawi, Philippines through its manager Ms. Arlyn J. Carroz for permission to conduct the experiment in the hatchery complex. Many thanks also to Dr. Greg Pelletier, Department of Ecology, P.O. Box 47600 Olympia, Washington, U.S.A. for his assistance in the calculations of the carbonate system of experimental seawater using the CO_2SYS software, Ms. Juliet F. Francisco for helping in the measurement of total alkalinity of the experimental seawater, and Mr. Japrin L. Hadji-Amin and Mr. Abdurasul Sarail for their assistance in data gathering. We also thank Prof. Regimar Rashid, Mindanao State University Tawi-Tawi, Bongao, Tawi-Tawi for his assistance in statistical analysis. This is a joint marine science contribution of the University of San Carlos and the Mindanao State University Tawi-Tawi, Philippines.

REFERENCES

ANDERSEN S, GREFSRUD ES, HARBOE T. 2013. Effect of increased *p*CO2 level on early shell development in great scallop (*Pecten maximus* Lamarck) larvae. Biogeosciences 10: 6161-6184.

BYRNE M, HO M, WONG E, SOARS NA, SELVAKUMASARASWAMY P, SHEPAHRD-BRENNAND H, DWORJANYN SA, DAVIS, AR. 2011. Unshelled abalone and corrupted urchins: Development of marine calcifiers in a changing ocean. Proceedings of the Royal Society of Biological Sciences 278: 2376-2383.

CALDEIRA K, WICKETT ME. 2003. Oceanography: Anthropogenic carbon and ocean pH. Nature 425: 365-372.

CALDEIRA K, WICKETT ME. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J Geophys Res 110: 1-12.

CAPINPIN EC. 1995. Spawning and larval development of a tropical abalone *Haliotis asinina* (Linne). Philipp J Sci 124: 215-232.

CIGLIANO M, GAMBI MC, RODOLFO-METALPA R, PATTI FP, HALL-SPENCER JM. 2010. Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. Mar Biol 157: 2489-2502.

CRIM RN, SUNDAY JM, HARLEY CDG. 2011. Elevated seawater CO_2 concentrations impair larval development and reduce larval survival in endangered northern abalone (*Haliotis kamtschatkana*). J Exp Mar Biol Ecol 400: 272-277.

DE LA PENA MR, BAUTISTA JI, BUEN-URSUA SM, BAYONA N, TITULAR VST. 2010. Settlement, growth and survival of the donkey's ear abalone *Haliotis asinina* (Linne) in response to diatom diets and attachment substrate. Philipp J Sci 139: 27-34.

DE VICOSE GC, VIERA M, BILBAO A, IZQUIERDO M. 2010. Larval settlement of *Haliotis coccinea* in response to different inductive cues and the effect of larval density on settlement, early growth, and survival. J Shelfish Res 29: 587-591.

DIAZ-PULIDO G, ANTHONY KRN, KLINE DI. 2012. Interactions between ocean acidification and warming on the mortality and dissolution of coralline algae. J Phycol 48: 32–39.

DICKSON AG, MILLERO FJ. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceano Res Papers 34: 1733-1743.

DOROPOULOS C, WARD S, DIAZ-PULLIDO G, HOEGH-GULDBERG O, MUMBY PJ. 2012. Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecol Letters 1-9. doi 10.1111/j.1461-0248.2012.01743.x.

ELLIS RP, BERSEY J, RUNDLE SD, HALL-SPENCER JM, SPICER JI. 2009. Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*. Aquatic Biol 5: 41-48.

FEELY RA, SABINE CL, LEE K, BERELSON W, KLEYPAS J, FRABRY VJ, MILLERO FJ. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305: 362-366.

KURIHARA H. 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar Ecol Prog Ser 373: 275-284.

LI J, JIANG Z, ZHANG J, QIU JW, DU M, BIAN D, FANG J. 2013. Detrimental effects of reduced seawater pH on the early development of the Pacific abalone. Mar Pollu Bull 74: 320-324. 2013.

MARTIN S, GATTUSO S. 2009. Response of Mediterranean coralline algae to ocean acidification and elevated temperature. Glob Change Biol 15: 2089–2100.

MEHRBACH C, CULBERSO CH, HAWLEY JE, PYTKOWIC RM. 1973. Measurement of apparent dissociation-constants of carbonic-acid in seawater at atmospheric pressure. Limnol and Oceanol 18: 897-907.

MICHAELIDIS B, OUZOUNIS C, PALERAS A, PÖRTNER HO. 2005. Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. Mar Ecol Prog Ser 293: 109–118.

MILES H, WIDDICOMBE S, SPICER JI, HALL-SPENCER J. 2007. Effects of anthropogenic seawater acidification on acid–base balance in the sea urchin *Psammechinus miliaris*. Mar Pollut Bull 54: 89–96.

MOULIN L, CATARINO AI, CLAESSENS T, DUBOIS P. 2011. Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck 1816). Mar Pollu Bull 62: 48-54.

NAJMUDEEN TM, VICTOR ACC. 2004. Seed production and juvenile rearing of the tropical abalone *Haliotis varia* Linnaeus 1758. Aquaculture 234: 277–292.

PIERROT D, LEWIS E AND WALLACE DWR. 2006. MS Excel program developed for CO2 system calculations, ORNL/CDIAC-105a.Carbon dioxide information analysis center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, USA. DOI: 10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a.

RAVEN J, CALDEIRA K, ELDERFIELD H, HOEGH-GULBERG O, LISS P, RIEBESELL U, SHEPHERD J, TURLEY C, TWATSON A. 2005. Ocean acidification due to increasing atmospheric carbon dioxide. The Royal Society Policy Document, UK: London. 68p.

RENAUD SM, PARRY DL, LUONG-VAN T, KUO C, PADOVAN A, SAMMY N. 1991. Effect of light intensity in the proximate biochemical and fatty acid composition of *Isochrysis* sp. and *Nannochloropsis oculata* for use in tropical aquaculture. J Appl Phycol 3: 43-53.

ROBERTS RD, KAWAMURA T, HANDLEY CM. 2004. Factors affecting settlement of abalone (*Haliotis iris*) larvae on benthic diatom films. J Shellfish Res 26: 323-334

ROSS PM, PARKER L, O'CONNOR WA, BAILEY EA. 2011. The impact of ocean acidification on reproduction, early development and settlement of marine organisms. Water 3: 1005-1030.

SINGHAGRAIWAN T, DOI M. 1993. Seed production and culture of a tropical abalone, *Haliotis asinina* Linne. Thailand: The Eastern Marine Fisheries Development Center, Department of Fisheries, Ministry of Agriculture and Cooperatives. p. 32.

SPICER JI, RAFFO A, WIDDICOMBE S. 2007. Influence of CO_2- related seawater acidification on extracellular acid–base balance in the velvet swimming crab *Necora puber*. Mar Biol 151: 1117–1125.

TALMAGE SC, GOBLER CJ. 2009. The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*). Limnol and Oceanogr 54: 2072-2080.

TIMMINS-SCHIFFMAN E, O'DONNELL MJ. 2012. Elevated *p*CO2 causes developmental delay in early larval Pacific oysters, *Crassostrea gigas*. Mar Bio DOI 10.1007/s00227-012-2055-x.

WATSON SA, SOUTHGATE PC, TYLER PA, PECK LS. 2009. Early larval development of the Sydney rock oyster *Saccostrea glomerata* under near-future predictions of CO_2-driven ocean acidification. J Shellfish Res 28: 431-437.

WEBSTER NS, UTHICKE S, BOTTE ES, FLORES F, NEGRI AP. 2013. Ocean acidification reduces induction of coral settlement by crustose coralline algae. Glob Change Biol 19: 303-315.

WILLIAMS EA, CRAIGIE A, YEATES A, DEGNAN SM. 2008. Articulated coralline algae of the genus *Amphiroa* are highly effective natural inducers of settlement in the tropical abalone, *Haliotis asinina*. Biol Bull 215: 98-107.

WONG E, DAVIS AR, BYRNE M. 2010. Reproduction and early development in *Haliotis coccoradiata* (Vetigastropoda: Haliotidae). Invert Rep Dev 54: 77–87.