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CO_2-induced pH Reduction Hinders Shell Development of Early Larvae Donkey's Ear Abalone *Haliotis asinina* (Linnaeus 1758)

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Abstract

This study elucidated the effects of CO_2-induced pH reduction on the early larval development of ecologically and economically important donkey's ear abalone *Haliotis asinina* (Linnaeus 1758). Results showed significant decrease in shell length with decreased pH (pH 8.15 > 7.85 > 7.65). More than 50 % of shell malformations were evident from as early as 2 h exposure at pH 7.85 and pH 7.65. At pH 7.65, no normal shell formation was observed at post 2 h. Induced pCO_2 reduced pH in this study resulted to saturation levels of calcite and aragonite at 2.65 – 2.74 Xcalc; 1.76 – 1.83 Xarag (pH 7.65) and 3.84 – 4.00 Xcalc; 2.57 – 2.67 Xarag (pH 7.85). These values were lower than the control (pH 8.15) at 6.66 – 6.85 Xcalc; 4.43 – 4.57 Xarag, which may explain the observed impaired development of *H. asinina* at reduced pH.

Keywords: early larval development, donkey's ear abalone, pCO_2 ocean acidification, *Haliotis asinina*

Introduction

The burning of fossil fuels and other anthropogenic activities such as deforestation and industrial emissions release excessive CO_2 resulting in increasing global temperature. The rapid air-sea interaction warms the ocean and increases the partial pressure of CO_2 (pCO_2) forming carbonic acid which decreases seawater pH. This results to the reduction of carbonate ion concentration of the seawater due to shifting oceanic-carbonate equilibrium (Feely et al. 2004). Assuming the IS92A scenario, pCO_2 will increase at 750 µatm that will result to a drop of seawater carbonate ion concentration at 50 % and pH of 0.4 unit by the year 2100 (Orr et al. 2005).

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This rapid increase in the atmospheric carbon dioxide concentration which leads to a lower water pH is called ocean acidification. Ocean acidification is known to impair early life history stages, including larval development, which are the most sensitive stages to environmental perturbations (Kurihara 2008; Crim et al. 2011). Generally, larvae are more fragile and show fewer species-specific adaptations to environmental variation (Kimura et al. 2011). Also, increased pCO_2 may have a complex effect on the physiology, growth and reproductive success of marine calcifiers (Kurihara 2008).

Ecologically relevant CO_2 perturbation experiments revealed substantial effects of ocean acidification on a wide range of marine organisms (reviewed in Crim et al. 2011). Decreased shell mineralization of the early developmental stage of oyster *Crassostrea* gigas (Thunberg 1793) (Kurihara 2008), reduced calcification and growth of corals (Hoegh-Guldberg et al. 2007), smaller size of sea urchin *Tripneustes gratilla* (Linnaeus 1758) larvae (Sheppard et al. 2010); and dissolution of pteropod (*Clio pyramidata*) shell surface (Orr et al. 2005) were among these studies. *Haliotis asinina* (Linnaeus 1758) commonly known as donkey's ear abalone, a marine calcifying organism, may also face a serious threat from ocean acidification. Ezo abalone *Haliotis discus* Reeve, 1846, larvae showed malformations and decreased shell length in pCO_2-acidified waters (Kimura et al. 2011). Crim et al. (2011) revealed that elevated CO_2 concentrations in seawater impair larval development and reduce survival of endangered northern abalone *Haliotis kamtschatkana* Jonas 1845. Tahil and Dy (2015a, 2015b) revealed the negative effects of reduced pH on larval and post-larval settlement and survivorship of *H. asinina* through tank experiments. However, the early larval development of *H. asinina* on reduced pH is still unknown. *Haliotis asinina* in the Philippines are over-harvested in the wild due to its high-value (Maliao et al. 2004). It is now cultured to supply food demands and to restock the dwindling natural population (Fermin et al. 2008). The response of this commercially important species to pH reduction in the oceans is vital knowledge to manage its future population properly. The objective of this study was to determine the effects of reduced pH on *H. asinina's* early larval development through shell length, malformation, and development stages within a 24 h period using a microcosm experiment. Based on the previous review of Byrne (2011) and to date, studies on the effects of ocean acidification on early larval stages are still limited and most are still focused on the calcifying larval stage.

Materials and Methods

Spawning and egg collection

Eggs of abalone, produced through natural spawning, were provided by the Southeast Asian Fisheries Development Center (SEAFDEC/AQD). These were collected from the spawning tank using a 1 cm diameter siphon and were washed by passing it through a 250 µm filter to separate the dirt particles before transferring to an incubation tank containing UV-filtered seawater (Fermin et al. 2008).

CO_2 treatment on abalone larvae

Five litres of filtered seawater with abalone larvae at a density of 3–4 larvae per mL was transferred into a clear cylindrical glass container (30.5 cm height, 15 cm diameter) and bubbled with CO_2 to desired pH levels of 7.85 and 7.65, for around 1 min and 2 min, respectively. The pH treatments were based on the Intergovernmental Panel on Climate Change (IPCC) IS92A "business-as-usual" pH scenario by the year 2100 (Orr et al. 2005). Ambient pH 8.13 was used as a control for target pH 8.15.

Abalone larvae were transferred immediately after CO_2 treatment using a siphon into 120 mL glass vials with rubber cover sealed with parafilm to avoid leakage and diffusion of CO_2. While transferring the larvae into the vials, a pH probe (Horiba, pH meter D-14, Horiba Ltd., Kyoto, Japan) was set in the glass container to monitor the pH of the treatment and gradual, constant stirring was done with a siphon set at the center of container to collect the larvae at constant density possible. Ambient temperature (27.6–28.6 °C) was used since *H. asinina* was found to have a wide range of optimum temperature at 28–35 °C (Sawatpeera et al. 2001). Every two hours (2 h) within the 24 h experiment, the samples were fixed using 5 mL of 10 % buffered formalin for larval development analysis. pH and dissolved oxygen (DO) were measured prior to addition of formalin for monitoring purposes. Forty-five vials were prepared for each treatment to allow triplicate samples for each 2 h sampling and other water analysis.

Total alkalinity

Three vials from each treatment (pH 8.15, 7.8 and 7.6) were sampled at 0 h, 12 h, and 24 h. pH was measured using a pH probe with auto temperature check (Horiba, pH meter D-14, Horiba Ltd., Kyoto, Japan) and DO using a DO meter (YSI, DO-200, YSI Incorporated, Yellow Springs Ohio, USA). Salinity was checked using a refractometer (Atago Co. Ltd., Japan). Triplicate samples from each treatment were then pooled, preserved with 240 µL of saturated HgCl, and analysed for total alkalinity using the Kimoto total alkalinity analyser (Kimura et al. 2011). Total alkalinity, pH, temperature, and salinity data were used to calculate for the concentrations of CO_2, carbonate (CO_3^{-2}) and bicarbonates (HCO_3^-); partial pressure of CO_2 (pCO_2), total inorganic carbon; saturation state of calcite (X_{calc}) and aragonite (X_{arag}) using the software CO_2SYS (Pierrot and Wallace 2006). Dissociation constants of carbonic acid were based from Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

Analysis of larvae

Two mL of treated water with larvae from the 120 mL vial was sub-sampled using a pipette. The sample was taken from the middle part in the bottom of the vial and set in a petri dish for analysis using a dissecting microscope (Stereo, Olympus dP 21, Model IX70, Olympus Corporation, Japan). Observation of the larval development stages of *H. asinina* was done following the protocol described by Sawatpeera et al. (2001).

Ten larvae were chosen for length measurements. Shell length of larvae was measured from the anterior end to the posterior end of the shell. Ten larvae from the pooled sample were also checked for malformation. Malformation was observed as those with shell abnormalities, undeveloped shell, and burst bodies.

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to check the significant effect of pH on the average (0-24 h) shell length of abalone larvae. To check the significant effects and interaction of both factors pH and time on shell length Two-way ANOVA was applied. P-values at < 0.05 were considered significant. Tukey HSD Post-Hoc Test was used to check which treatment contributed to the significant differences.

Results

Reduced pH in abalone larvae significantly decreased mean shell length ($P < 0.05$) (Fig. 1). Mean shell length was highest at ambient pH 8.15 (150.99 ± 4.70 µm) followed by pH 7.85 (117.16 ± 6.60 µm) and lowest at pH 7.65 (16.03 ± 3.41 µm) (pH 8.15 > pH 7.85 > pH 7.65, Tukey's HSD Test) (Fig. 1).

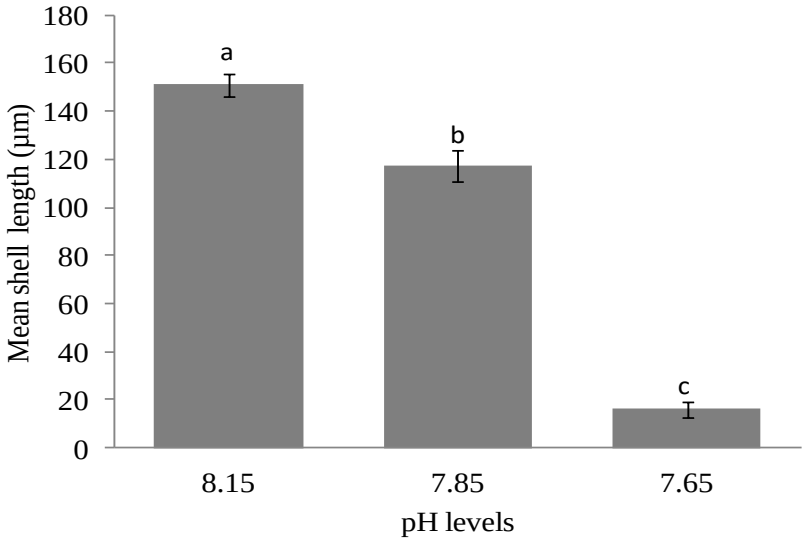

Fig. 1. Mean shell length (µm) of *Haliotis asinina* larvae from 0 h to 24 h at different pH levels. Letters above the bars denote significant difference among treatments (Tukey's HSD Test, $P < 0.05$). Data are mean ± standard error of means (SEM) with n = 130.

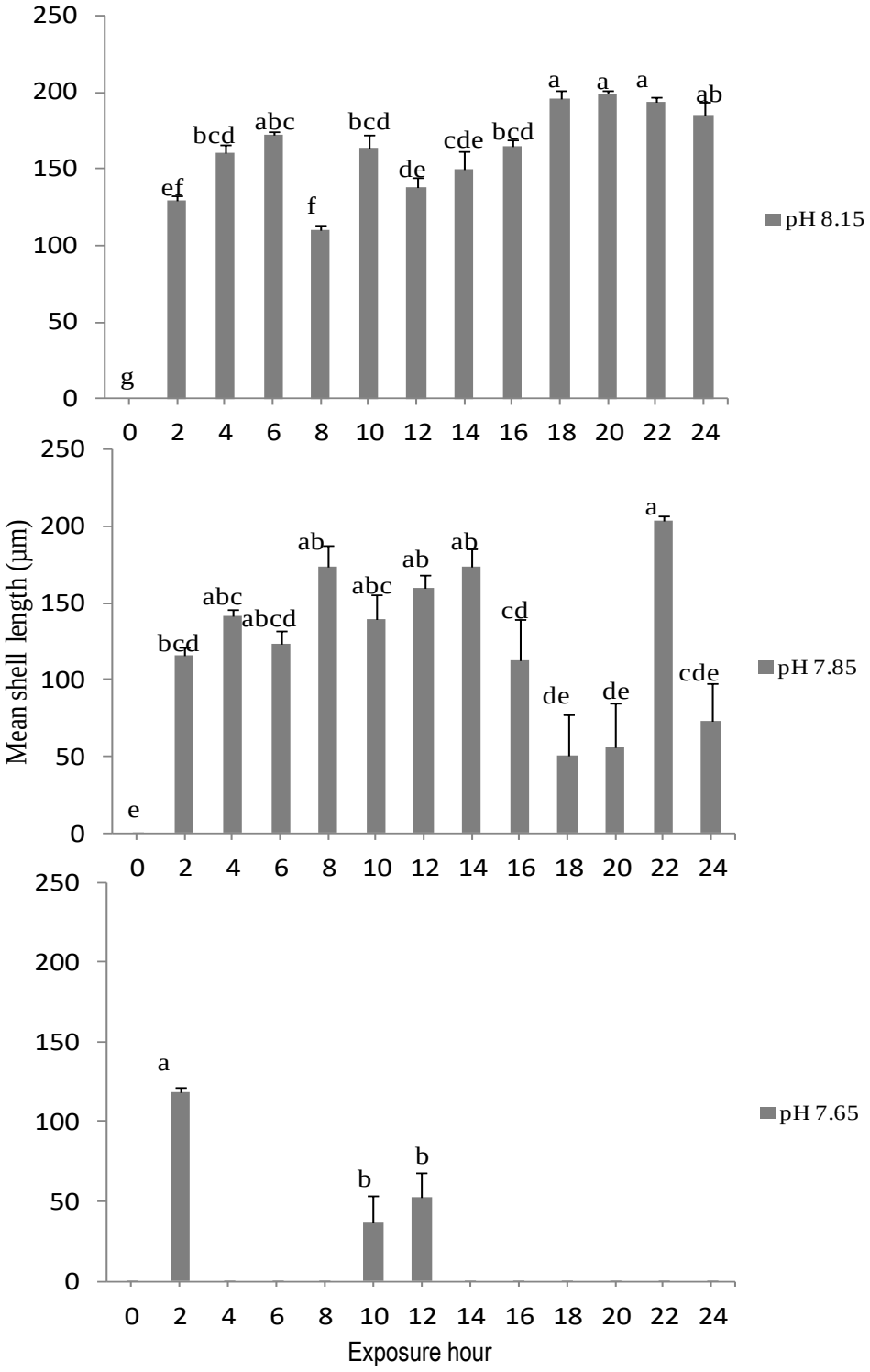

Fig. 2. Mean shell length (µm) of donkey's ear abalone, *Haliotis asinina* observed every two hours at each pH level (pH 8.15, 7.85, and 7.65). Letters above the bars denote significant difference (Tukey's HSD Test, $P < 0.05$). Error bars are SEM with n = 10. Note: Data with no shell length measurements means larvae had too small shells to measure or no shells were observed at all.

Two-way ANOVA showed a significant interaction between pH and time ($P < 0.05$). At pH 8.15, shell length was highest at 20 h (198.83 µm) (Fig. 2). At pH 7.85, shell length (203.54 µm) peaked at 22 h but decline at 24 h similar to 18 h (50.78 µm) and 20 h (55.96 µm). At pH 7.65, shell formation stopped after 2 h except at 10 h and 12 h where some samples were observed with the shell.

Malformation started early at pH 7.65 and pH 7.85 at 2 h at ≥50 % (Fig. 3). Malformations occurred at 4 h in pH 8.15 at lower than 10 %. At post 2 h, larvae at pH 7.65 in most samples did not develop a shell, except for 10 h and 12 h, and exhibited burst bodies which were recorded as a malformation in this experiment.

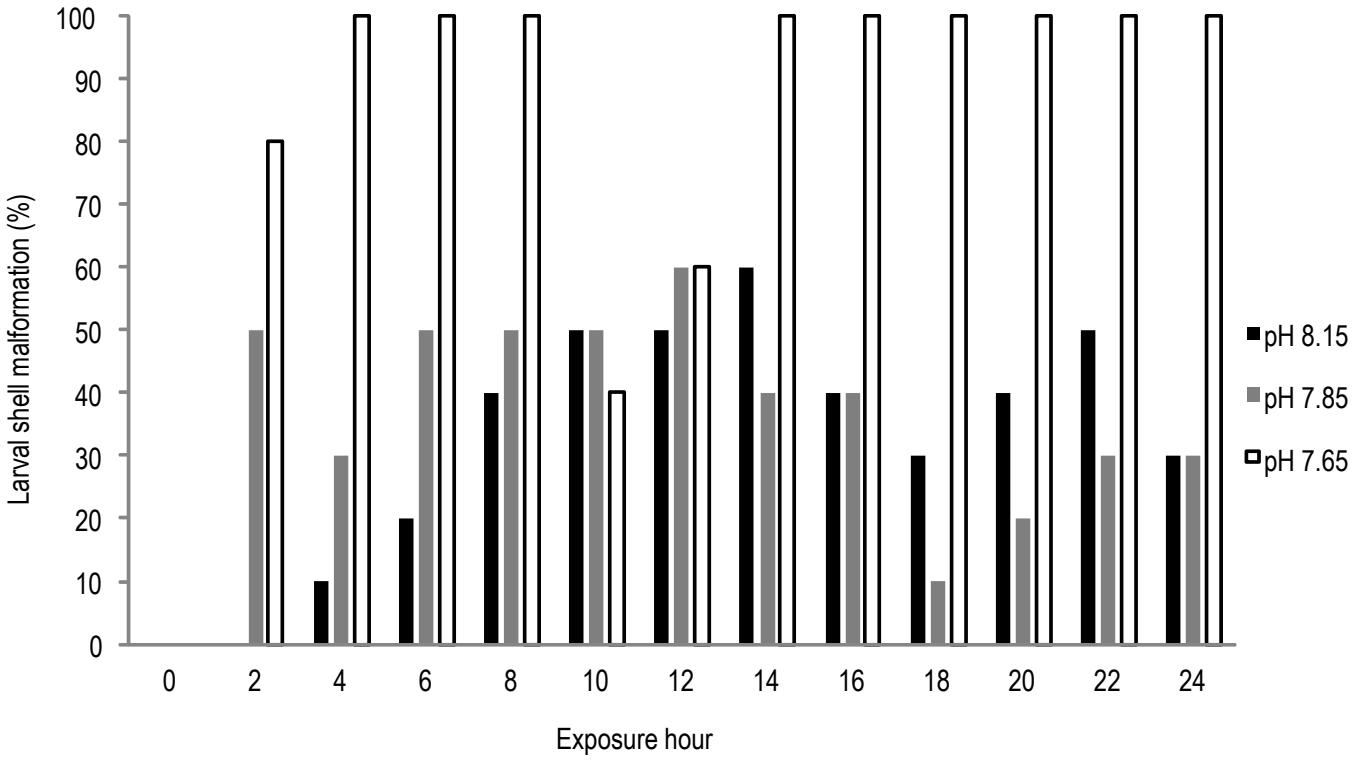

Fig. 3. Shell malformation observed in *Haliotis asinina* larvae at different pH within 24 h. n = 10.

At 0 h most larvae at all pH levels were observed to be in hatch-out stage (Stage 15) (Table 1). At 2 h, the larval shell was already developed and larvae were already at Stage 17/18 wherein the apical region becomes flat and the velum was completely developed with long cilia present and in some larvae, larval retractor muscle started to appear. At 6 h, larvae reached the complete larval shell stage (Stage 21) in both pH 7.85 and control, but not in pH 7.65. Although shell malformations were visible within 10 h exposure in pH 7.65 and pH 7.85 larval parts were still identifiable but not beyond 10 h when almost all larvae showed burst bodies.

Table 1. Early larval development stages of *Haliotis asinina* at different pH treatments. Legend for larval stages (Sawatpeera et al. 2001): Stage 15: hatch-out, Stage 16: beginning of larval shell, Stage 17: the apical region becomes flat and the velum is completely developed with long cilia present, Stage 18: appearance of larval retractor muscle, Stage 19: appearance of integumental attachment, Stage 20: development of foot mass, Stage 21: completion of larval shell. Post 10 h almost all larvae showed burst bodies.

	Larval stages		
Time	pH 8.15	pH 7.85	pH 7.65
0	15	15	15/17
2	17/18	17/18	17/18
4	19–20	17, 19, 20	18
6	19, 20, 21	18–20/21	18–20
8	18–20	18–20	18–20
10	18–20	21/18–20	18–20

Monitoring of pH showed maintained conditions during the 24 h experiment (Fig. 4) using the one-step CO_2 bubbling of seawater, kept in a tightly-sealed microcosm bottle.

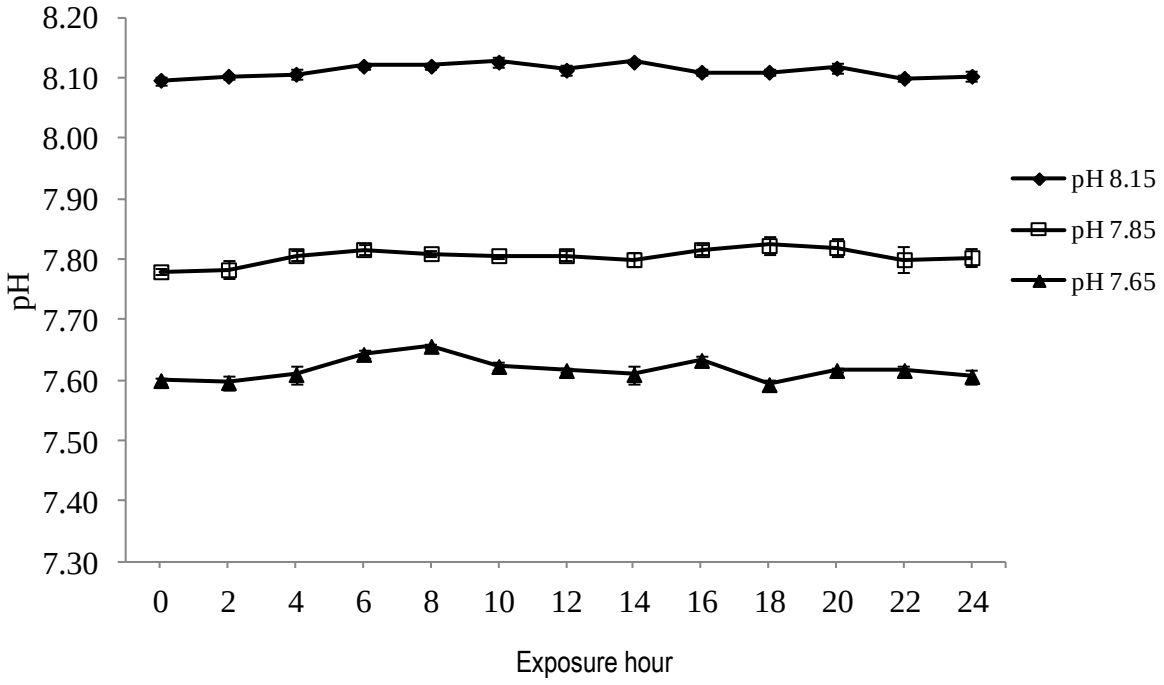

Fig. 4. Monitoring of pH level on treatments from 0 h through 24 h experiment. Error bars are standard error of means (SEM) with n = 3.

Discussion

This study investigated the effect of reduced pH on the early larval development of *H. asinina.* The first 24 h in the life cycle of *H. asinina* is crucial for the development of its shell that may hugely influence its settlement success. It is also in this period where fertilised eggs develop into shelled planktonic larvae (Sawatpeera et al. 2001).

Larval shell length

In general, normal shell development of early stage larvae at pH 7.65 was not observed in this study. At pH 7.85, although shell was visible and developed, shell length was significantly lower than control pH 8.15. Shell reduction compared to control was at 37 % and 94 % in pH 7.85 and 7.65, respectively. Relative to time, the absence of shell development among larvae at pH 7.65 after 2 h suggests that a short exposure time in low pH may inhibit larval development in abalone. Although some larvae developed shell at 10 h (37.61 µm) and 12 h (52.51 µm) (Fig. 2) these were a magnitude of 3.1–3.6 shorter than that at pH 7.85 and pH 8.15; and all larvae were observed with malformations (Fig. 3). This result may indicate high vulnerability to environmental conditions and implies physiological effects. At pH 7.85, the up and down trend of shell length throughout exposure time may indicate varied responses of and resilience in some *H. asinina* population at the early larval stage.

Early larval stage of *Haliotis discus hannai* exhibited significantly shorter shell length in increased pCO_2 concentrations in pH 7.68–7.43 (Kimura et al. 2011). These were not only observed in abalone but to most invertebrates which have sensitive and short early larval development (Kurihara 2008). Sea urchin *Tripneustes gratilla* larvae exposed to pH 7.6 and pH 7.8, were observed with smaller postoral arms (Sheppard et al. 2010). Larval and arm sizes of sea urchins *Hemicentrotus pulcherrimus* (Mortensen 1942) and *Echinometra mathaei* (Blainville 1825) at embryo stage were significantly smaller and abnormal when exposed to increased pCO_2 and reduced pH (Kurihara and Shirayama 2004a). Tahil and Dy (2015a, b) studied the post-larvae of *H. asinina* and observed it every 5 days within 15- and 20-day periods. Results showed high shell length reduction (20–28 %) and significantly lower post-larval settlement in reduced pH.

Malformation

Malformation or deformation observations were apparent in developing larvae of invertebrates exposed to CO_2-induced pH reduced conditions (Orr et al. 2005, Kurihara 2008, Sheppard et al. 2010). Malformations may impede development and decrease survivorship of the organism. The evident shell malformation from 2 h in both pH 7.65 and pH 7.85 at $\geq$50 % were one of the most notable effects in this study. Various studies have shown similar findings. Percentage of normal cleaving embryo of *Haliotis coccoradiata* Reeve 1846 was lowest at pH 7.6 leading to abnormal veligers (Byrne 2011).

Ezo abalone *Haliotis discus hannai* showed significant shell malformation at pH 7.68 as compared to pH 7.94 (Kimura et al. 2011). Malformations were observed to further increase to 20 % at pH 7.54 and pH 7.43. Shell abnormalities were also exhibited by northern abalone *Haliotis kamtschatkana* subjected to pH 8.07 (80 %) and 7.81 (100 %) (Crim et al. 2011). These abnormalities or malformations were commonly described as irregular edges of the shell, deformed shape of the shell, burst bodies, and lacking or complete absence of shell, which were similar to the findings of this study.

Larval stages

Across ontogenetic stages, the early larval stages were proven to be most sensitive but least studied related to pCO_2 induced reduced pH. This study revealed that malformations observed in *H. asinina* at reduced pH could hamper further development. Although the appearance of retractor muscle and foot mass were observed, most larvae had completely no shell specifically at pH 7.65. These malformations and reduction or complete lack of shell may be linked with low seawater carbonate concentrations induced by increased pCO_2 (Table 2). Calcite and aragonite saturation measurements were 2.65 – 2.74 $X_{calc;}$ 1.76 – 1.83 X_{arag} at pH 7.65; 3.84 – 4.00 $X_{calc;}$ 2.57 – 2.67 X_{arag} at pH 7.85; and 6.66 – 6.85 $X_{calc;}$ 4.43 – 4.57 X_{arag} at control. At these carbonate saturation levels, pCO_2 levels were at 1365.83 – 1449.04 μatm at pH 7.65; 833.01 – 902.98 μatm at pH 7.85; and 359.35 – 369.95 μatm at control.

Numerous studies (Sheppard et al. 2010; Kimura et al. 2011; Tahil and Dy 2015b) likewise associated shorter shell length and shell abnormalities to low carbonate concentration (aragonite and calcite). The decline in ocean carbonates (CO_3^{-2}) comes with a decrease in calcification rates (Orr et al. 2005). Most marine calcifying organisms will be affected at lower than 1.0 aragonite saturation. However, in this study aragonite saturation of 1.76 – 1.83 at pH 7.65 and 2.57 – 2.67 at pH 7.85 already exhibited adverse effects to *H. asinina*. Similar observations on Ezo abalone were noted by Kimura et al. 2011 at above 1.0 aragonite saturation. Studies on the mechanisms of these impairments may still be limited to explain the phenomena. Aside from impede aragonite and calcite synthesis to make up the prismatic layer and nacreous shell layers, some studies suggest hypercapnic suppression to metabolic pathways required for calcification (Byrne 2011), low protein synthesis (Grainger et al. 1979), and changes in larval feeding rates (Dupont and Thorndyke 2009) as contributing factors to low calcification or decalcification. These factors may also explain the effects of pCO_2 induced reduced pH to *H. asinina* revealed in this study.

Table 2. Carbonate chemistry at pH treatments. Measured parameters were temperature, salinity, dissolved oxygen, pH (n = 3) and total alkalinity (pooled samples). Error bars are standard error of means (SEM). Total inorganic carbon, CO_2, carbonate (CO_3^{-2}), bicarbonate (HCO_3^-) concentrations, the partial pressure of CO_2 (pCO_2) and saturation state of calcite (X_{calc}) and aragonite (X_{arag}) were measured using CO_2-SYS.

Parameter	pH 8.15 treatment			pH 7.85 treatment			pH 7.65 treatment		
	0h	12h	24h	0h	12h	24h	0h	12h	24h
Temperature (°C)	28.63±0.06	27.97±0.06	27.63±0.06	28.6 ±0.00	28.07±0.06	27.63±0.06	28.67±0.06	28.13±0.06	27.73±0.06
Salinity (psu)	36±0	36±0	36±0	36±0	36±0	36±0	36±0	36±0	36±0
Dissolved Oxygen (mg. L^{-1})	4.85±0.03	5.28±0.00	5.20±0.06	4.8±0.00	5.18±0.00	4.62±0.06	5.61±0.03	5.22±0.03	4.63±0.07
pH	8.10±0.01	8.11±0.02	8.10±0.01	7.78±0.01	7.81±0.02	7.80±0.03	7.60±0.01	7.62±0.01	7.61±0.02
Total Alkalinity ($\mu mol.kg^{-1}$)	2521.39	2525.31	2520.55	2533.82	2530.64	2528.91	2546.45	2527.17	2528.35
Total Inorganic Carbon ($\mu mol.kg^{-1}$)	2259.5	2279.7	2299.4	2364.8	2374.3	2384.3	2416.9	2539.4	2468.1
CO_2 ($\mu mol.kg^{-1}$)	9.49	9.41	9.76	23.29	21.76	22.57	37.32	35.62	36.90
CO_3^{-2} ($\mu mol.kg^{-1}$)	285.19	285.55	277.79	160.08	166.78	161.32	112.38	114.21	110.53
HCO_3^- ($\mu mol.kg^{-1}$)	1828.42	1831.52	1845.44	2146.57	2126.89	2138.29	2275.17	2250.98	2261.04
pCO_2 (μatm)	368.18	359.35	369.95	902.98	833.01	855.28	1449.04	1365.83	1401.35
Calcite saturation (X_{calc})	6.85	6.85	6.66	3.84	4.00	3.87	2.70	2.74	2.65
Aragonite saturation (X_{arag})	4.57	4.56	4.43	2.57	2.67	2.57	1.80	1.83	1.76

Conclusion

The results revealed that reduction in pH influenced the development of early larval stage of *H. asinina*. At pH 7.65 and 7.85, larvae had shorter shell length and early (2 h exposure) shell malformations as compared to ambient pH 8.15. Larval shell completion at 6 h exposure was only achieved by larvae in pH 7.85 and ambient pH 8.15. These impediments in development in reduced pH appear to be linked with the low saturation of calcite and aragonite in water introduced with CO_2. This study revealed lack of resilience of early larval stage of *H. asinina* to pH reduction which may deter successful settlement and further development.

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References

Byrne M. 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages: Vulnerabilities and potential for persistence in a changing ocean. Oceanography and Marine Biology 49:1–42.

Crim R.N., J.M. Sunday and C.D.G. Harley. 2011. Elevated seawater CO_2 concentrations impair larval development and reduce larval survival in endangered northern abalone (*Haliotis kamtschatkana*). Journal of Experimental Marine Biology and Ecology 400:272–277.

Dickson A.G. and F.J. Millero. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research A 34:1733–1743.

Dupont S. and M.C. Thorndyke. 2009. Impact of CO_2- driven ocean acidification on invertebrates early life-history - what we know, what we need to know and what we can do. Biogeosciences Discussions 6:3109–3131.

Feely R.A., C.L. Sabine, K. Lee, W. Berelson, J. Kleypas, V.J. Fabry and F.J. Millero. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305:362–366.

Fermin A.C., M.R. dela Peña, R.S.J. Gapasin, M.B. Teruel, S.M.B. Ursua, V.C. Encena II and N.C. Bayona. 2008. Abalone hatchery. SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines. 31 pp.

Grainger J.L., M.M. Winkler and R.A. Steinhardt. 1979. Intracellular pH controls protein synthesis rate in the sea urchin egg and early embryo. Developmental Biology 68:396–406.

Hoegh-Guldberg O., P.J. Mumby, A. J. Hooten, R.S. Steneck, P. Greenfield, E. Gomez, C.D. Harvell, P.F. Sale, A.J. Edwards, K. Caldeira, N. Knowlton, C.M. Eakin, R. Iglesias-Prieto, N. Muthiga, R.H. Bradbury, A. Dubi and M.E. Hatziolos. 2007. Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742.

Kimura R, T.O. Hidekitakami, O. Toshihiro and N. Yukihiro. 2011. Effects of elevated pCO_2 on the early development of the commercially important gastropod, Ezo abalone *Haliotis discus hannai*. Fisheries Oceanography 20:357–366.

Kurihara H. 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Marine Ecology Progress Series 373:275–284.

Kurihara H. and Y. Shirayama. 2004a. Effects of increased atmospheric CO_2 on sea urchin early development. Marine Ecology Progress Series 274:161–169.

Maliao R.J., E.L. Webb and K.R. Jensen. 2004. A survey of stock of the donkey's ear abalone, *Haliotis asinina* L. in the Sagay Marine Reserve, Philippines: evaluating the effectiveness of marine protected area enforcement. Fisheries Research 66:343–353.

Mehrbach C., C.H. Culberson, J.E. Hawley and R.M. Pytkowicz. 1973. Measurement of apparent dissociation-constants of carbonic-acid in seawater at atmospheric pressure. Limnology and Oceanography 18:897–907.

Orr J.C., V.J. Fabry, O. Aumont, L. Bopp, S.C. Doney, R.A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R.M. Key, K. Lindsay, E. Maier-Reimer, E. Matear, P. Monfray, A. Mouchet, R.G. Najjar, G.K. Plattner, K.B. Rodgers, C.L. Sabine, J.L. Sarmiento, R. Schlitzer, R.D. Slater, I.J. Totterdell, M. Weirig, Y. Yamanaka and A. Yool. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on the calcifying organisms. Nature 437:681–686.

Pierrot D, E. Lewis and DWR Wallace. 2006. MS Excel Program developed for CO_2 system calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory. US Department of Energy, Oak Ridge, Tennessee. doi:10.3334/CDIAC/otg.

Sawatpeera S, E.S. Upatham, M. Kruatrachue, Y.P. Chitramvon, P. Sonchaeng, T. Pumthong and J. Nugranad. 2001. Larval development in *Haliotis asinina* Linnaeus. Journal of Shellfish Research 20:593–601.

Sheppard Brennand H., N. Soars, S.A. Dworjanyn, A.R. Davis and M. Byrne. 2010. Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. PLoS ONE 5:e11372.

Tahil A.S. and D.T. Dy. 2015a. Effects of reduced pH on the growth and survival of postlarvae of the donkey's ear abalone, *Haliotis asinina* (L.). Aquaculture International 23:141–153.

Tahil, A.S. and D.T. Dy. 2015b. Effects of reduced pH on larval settlement and survival of the donkey's ear abalone, *Haliotis asinina* (Linnaeus 1758). Philippine Journal of Science144:21–29.

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