INTRODUCTION

Global temperatures are predicted to increase by 3–4°C by the end of this century according to the Intergovernmental Panel on Climate Change scenario (IPCC, 2014). As a result, ocean temperatures are expected to increase accordingly (Belkin, 2009; Keeling, Kortzinger, & Gruber, 2010). Ocean warming is not the only stressor of marine organisms because oxygen solubility will decrease as temperatures increase (Keeling & Garcia, 2002; Keeling et al., 2010). The resulting deoxygenation will cause problems for marine animals. Higher temperatures will also increase the metabolisms of marine organisms and facilitate phytoplankton blooms that will increase hypoxia during subsequent decomposition (Brewer & Peltzer, 2016; Praetorius et al., 2015). Therefore, temperature increases in water cause oxygen depletion, and in combination, these two stressors influence the behaviors and physiologies of marine animals (Portner, 2012; Rosa & Seibel, 2008).

At the tipping point: Differential influences of warming and deoxygenation on the survival, emergence, and respiration of cosmopolitan clams

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Abstract
Although warming and low dissolved oxygen (DO) levels are co-occurring significant climatic stressors in the ocean, the combined effects of these stressors on marine benthic animals have not been well established. Here, we tested the effects of elevated temperatures and low dissolved oxygen levels on the survival, emerging behavior from sediment, and the respiration of juvenile cosmopolitan Manila clams (Venerupis philippinarum) by exposing them to two temperatures (20 and 23.5°C) and DO levels (3.5 and 6–7 mg/L). Although within previously described tolerable ranges of temperature and DO, this 3.5°C increase in temperature combined with a 50% decrease in DO had a devastating effect on the survival of clams (85% mortality after 8 days). The mortality of clams under normoxia at 23.5°C appeared to be higher than under the low DO condition at 20°C. On the other hand, more clams emerged from sediment under the low DO condition at 20°C than under any other conditions. Oxygen consumption rates were not significantly affected by different conditions. Our results suggest temperature elevation combined with low oxygen additively increases stress on Manila clams and that warming is at least as stressful as low DO in terms of mortality. However, low DO poses another threat as it may induce emergence from sediment, and, thus increase predation risk. This is the first evidence that a combination of warming and deoxygenation stressors should reduce population survival of clams much more so than changes in a single stressor.

KEYWORDS
dissolved oxygen, emergence, Manila clams, mortality, respiration, warming

1 INTRODUCTION

Global temperatures are predicted to increase by 3–4°C by the end of this century according to the Intergovernmental Panel on Climate Change scenario (IPCC, 2014). As a result, ocean temperatures are expected to increase accordingly (Belkin, 2009; Keeling, Kortzinger, & Gruber, 2010). Ocean warming is not the only stressor of marine organisms because oxygen solubility will decrease as temperatures increase (Keeling & Garcia, 2002; Keeling et al., 2010). The resulting deoxygenation will cause problems for marine animals. Higher temperatures will also increase the metabolisms of marine organisms and facilitate phytoplankton blooms that will increase hypoxia during subsequent decomposition (Brewer & Peltzer, 2016; Praetorius et al., 2015). Therefore, temperature increases in water cause oxygen depletion, and in combination, these two stressors influence the behaviors and physiologies of marine animals (Portner, 2012; Rosa & Seibel, 2008).
A number of studies have been conducted on the effect of increases in water temperature on marine animals (e.g., Kim & Micheli, 2013; Moline, Claustre, Frazer, Schofield, & Vernet, 2004; Portner, 2002). Notably, the physiological processes of ectothermic animals are dependent on temperatures remaining within optimal ranges (Newell, 1966; Portner, 2012). When temperatures increase beyond optimal ranges, animals fail to adapt or acclimate and may eventually become extinct (Portner, 2002; Rummer et al., 2014). Likewise, the effects of low dissolved oxygen (DO) levels on marine animals have been well investigated (Conley, Carstensen, Vaquer-Sunyer, & Duarte, 2009; Diaz & Rosenberg, 1995; Kim, Barry, & Micheli, 2013; Wu, 2002). Generally, many benthic animals do not cope well with hypoxia (<2 mg/L DO) and eventually die (Vaquer-Sunyer & Duarte, 2008). As compared with the number of studies conducted on the influences of temperature increases or hypoxia, the combined effects of increases in temperature and low DO levels in a controlled environment have been far less studied despite their relevance to marine ecosystems (Cheng et al., 2015; Vaquer-Sunyer & Duarte, 2011).

Here, we explored the effects of an increase in temperature and a reduction in DO level on a major fishery and aquaculture resource, the Manila clam Venerupis philippinarum. This species originated in Asia and was then maricultured in America and Europe and is now one of the most popular marine food sources worldwide (Becker, Barringer, & Marelli, 2008; Cordero, Delgado, Liu, Ruesink, & Saavedra, 2017). V. philippinarum lives in intertidal or subtidal areas that experience considerable fluctuations in abiotic factors, including temperature and oxygen. We exposed juvenile Manila clams to combinations of two temperatures (20 and 23.5°C) and two DO levels (3.5 and 6–7 mg/L), which model conditions frequently experienced (Kozuki et al., 2013; Shin, Kim, Chung, & Hur, 2000; Usaki, Kai, Aoyama, & Suzuki, 2003), to determine the effects of combined temperature and DO changes on mortality, emerging behavior from sediment, and oxygen consumption rates. Because clams generally burrow into sediment to escape predation, emerging behavior is a behavioral response to stressors, such as hypoxia and hypercapnia (Lee & Kim, 2017; Lee, Lee, & Chin, 2012; Saloom & Duncan, 2005).

We predicted clam survival would not be seriously influenced by temperature and DO changes within their tolerable ranges, but that emerging response from sediment and oxygen consumption rates would be influenced by both stressors. In addition, we predicted oxygen consumption rate, an indicator of metabolic activity, would be influenced by temperature and DO changes.

2 MATERIALS AND METHODS

2.1 Mortality and emergence

For the experiment, approximately 600 Manila clams were collected from an intertidal mudflat in Jugyo-ri, Jugyo-myeon, Boryeong-si, Chungcheongnam-do, Republic of Korea, on 3 August 2015. They were immediately placed in an ice box without seawater and transferred to a well-aerated acclimation aquarium (500 L, 150 cm × 70 cm × 80 cm, W × L × H) in a laboratory at the Korea Institute of Ocean Science and Technology (KIOST) (Ansan, Korea). Water temperature and salinity were maintained at 20°C and at 33 psu, respectively. The mean annual temperature of seawater at Jugyo-ri is approximately 21°C, and the optimal temperature range for Manila clams is known to be 18 to 23°C, as determined by clearance rates (Kang et al., 2016). Experimental temperatures were regulated using a chiller and/or heater connected to a thermostat. Salinity was controlled by adding unrefined sea salt or distilled water to seawater in accord with saltmeter (YSI-30) readings. Clams were fed sufficiently once every 2 days at 50 ml of 1:10 diluted Sellfish Diet 1800® (Reed Mariculture Inc., USA, two billion cells per 10 ml) per 80 individuals. The photoperiod used matched the natural light: dark (L:D) cycle outside the laboratory. The clams were acclimated to laboratory condition for 7 days.

For the experiment, we selected 400 healthy juvenile manila clams of similar size (15.99 ± 1.28 mm shell length). Ten clams were allocated randomly to each of forty 500-ml transparent glass jars containing 3 cm of sediment collected at the same Jugyo-ri intertidal mudflat where the clams were collected. Clams in each jar were marked from 1 to 10 on both sides of the shell with a Monami® permanent marker pen for individual identification. Ten randomly selected jars were assigned to each of four acrylic tanks (80 cm × 75 cm × 20 cm) maintained under the same conditions for acclimation. The water in each tank was maintained at 20°C and 33 psu and was passed through two filters and a cooler (DAEIL, DBA-075 or JEIO TECH, RW-3025G Jeio Tech®) before being delivered independently to jars at 75 ml/min using a 5-mm-diameter hose. Water in each jar overflowed separately into a holding tank. Room temperature was maintained at ~21.5°C using an air conditioner. The photoperiod followed a 12/12-hr light/dark cycle.

The experiment was conducted using a 2 × 2 matrix design at temperatures of 23.5°C (high) or 20°C (normal) and DO levels of 6–7 mg/L (85%-100%; normoxia) or 3.5 mg/L (40%-50%; low oxygen). Clams were gradually exposed to the higher temperature by increasing water temperature from 20 to 23.5°C at a rate of 0.5°C daily using a RW-3025 Jeio Tech® heater. The low target DO level (3.5 mg/L) was achieved at water temperatures of 20 and 23.5°C by mixing N₂ gas with air at an approximate volume ratio of 4:1 (N₂ gas = 400 cc/s, air = 100 cc/s) using flow meters. An ambient salinity of 33 psu was maintained throughout during all four experimental conditions.

Clams were maintained under the four experimental conditions for 8 days. Because over 80% succumbed to the high temperature/low DO treatment after 8 days, experimental exposure was terminated at this time. Temperature and DO were measured at 9:00 a.m. and 6:00 p.m. daily using a YSI 30 or a YSI 5000, respectively. The higher temperature was maintained at 23.60 ± 0.21°C under normoxia and at 23.46 ± 0.18°C under low DO conditions, and low temperature was maintained at 20.31 ± 0.19°C under normoxia and 20.25 ± 0.28°C under low DO conditions. Normoxia was maintained at 7.32 ± 0.87 mg/L at high temperature and at 7.90 ± 0.96 mg/L at low temperature, and low DO was maintained...
at 3.65 ± 0.31 mg/L at high temperature and at 3.91 ± 0.63 mg/L at low temperature.

Animal status (live/dead) was checked in each jar at 10:00 a.m. daily during the experimental period. Individuals that did not close in response to touch or were too light were considered dead, and removed from jars. Mortality (%) was calculated by expressing the number of dead individuals as a percentage of initial individuals. The locations of each clam in each jar (in/out of sediment) were also checked daily. Emergence from sediment was interpreted as a significant response to stressors (Lee & Kim, 2017), and emergence rates (%) were calculated by expressing the number of clams that emerged from sediment as a percentage of live individuals.

2.2 | Respiration

In this experiment, we used approximately 300 Manila clams collected from an intertidal mudflat at Namdang-ri, Hongseong-gun, Chungcheongnam-do, Republic of Korea, in 19 August 2016. They were immediately placed into an ice box and transferred to 2 well-aerated acclimation water baths (575 mm × 415 mm × 255 mm, W × L × H) in an incubator at KIOST. The incubator temperature was set at 20°C, and a salinity of 33 psu was maintained by adding unrechecked daily. Emergence from sediment was interpreted as a significant response to stressors (Lee & Kim, 2017), and emergence rates (%) were calculated by expressing the number of clams that emerged from sediment as a percentage of live individuals.

After acclimation for 5 days, 196 healthy juvenile clams (17.45 ± 1.14 mm in shell length, 7.94 ± 0.66 mm in shell height, and 1.08 ± 0.23 g in wet weight including shells) were selected randomly for the experiment. These clams were numbered 1–196 with Monami® permanent marker pen, and seven clams were allocated randomly to each of 28 transparent glass jars (1,200 ml). Seven randomly selected jars were then assigned to each of four aquariums (= seven clams × seven jars × four treatments). Experimental treatment water was supplied as mentioned above in the previous experiment. Each jar was supplied with a stream of water (200 ml/min). All experimental aquariums were aerated continuously, and water environments were kept clean.

In the high temperature group, the temperature was gradually increased in situ to 23.5°C at rate of 2°C over the first 24 hr. A heater (RW-3025 Jeio Tech®) was used to increase water temperature and chillers (DAEIL®, DBA-075) were used to reduce water temperature. After the targeted water temperature had been reached, the low oxygen level of 3.5 mg/L was obtained by mixing N₂ (800 cc/min) using gas flow meters (MFC Korea®) and Witrox control software (Loligo® system, USA). Ambient salinity was maintained at 33 psu.

Experiments under different temperature and dissolved oxygen level conditions were conducted over 4 days. Dissolved oxygen and temperature were monitored every second using Witrox control software (Loligo® system, USA). During this experiment, high temperature was maintained at 23.40 ± 0.21°C under normoxia and at 23.49 ± 0.11°C under low DO, and low temperature was maintained at 20.05 ± 0.29°C under normoxia and at 19.70 ± 0.73°C under low DO. Normoxia was maintained at 6.72 ± 0.22 mg/L at high temperature and at 7.27 ± 0.25 mg/L at low temperature, and low DO was maintained at 3.57 ± 0.27 mg/L at high temperature and at 3.52 ± 0.30 mg/L at low temperature. Salinity and pH were measured using a salt meter (YSI-30, YSI Inc., USA) and a pH meter (Mettler Toledo® SevenMulti) each morning during the entire experimental period. Jars were checked daily for dead clams.

Oxygen consumption rates were measured three times: 3 days before, 1 day after, and 4 days after exposure to the different treatments. Three clams were selected randomly from each jar and transported to a chamber (500 ml) connected to an optical dissolved oxygen sensor and filled with water that matched the conditions clams had been subjected to. For respirometry, an autorespirometry system (Loligo® systems) was used and the oxygen consumption rates of three randomly selected clams were measured for 30 min using the closed respirometry method. Oxygen consumption rate was defined as the amount of oxygen consumed per kg of wet weight per hour (mg O₂ kg⁻¹ hr⁻¹). After completing the experiments, all soft tissues were scraped from shells and dried at 80°C for 24 hr in an oven, and oxygen consumption rates were then recalculated on a dry weight basis. Measurements were conducted in the dark and feeding was stopped 2 days before measurements to prevent metabolic activity induced by light or digestion.

2.3 | Statistical analysis

All proportional data (cumulative mortalities and emergence rates) were normalized by arcsine-root-square transformation. Two-way ANOVA was used to determine whether temperature and/or DO influenced mortality. Repeated-measures two-way ANOVA was performed to determine whether temperature, DO, or/and exposure time (days) influenced emergence rates. When the assumption for equal between-group correlations and group variances (“sphericity”) was violated (Mauchly’s test, p < .05), Huynh–Feldt corrections were applied. Oxygen consumption rate data were only used when the coefficient of determination r² was >.25. Because oxygen consumption rate might have been dependent on time of measurement, endogenous respiration rhythm (circadian and/or circatidal rhythms) they were compared by two-way ANOVA at each measurement time. The statistical analysis was conducted using SPSS ver. 24.0.

3 | RESULTS

3.1 | Mortality and emergence

Both temperature increase and oxygen depletion significantly increased clam mortality (Two-way ANOVA, temperature: F₁,36 = 157.892, p < .001, DO: F₁,36 = 72.456, p < .001, Figure 1). However, the interaction between temperature and DO (F₁,36 = 2.209, p = .146) did not have a significant influence on mortality.
Although emergence rate appeared to increase under low DO conditions, DO ($F_{1,34} = 3.732$, $p = .062$) and temperature ($F_{1,34} = 1.547$, $p = .222$) had no significant effect on emergence rate (Figure 2). However, time ($F_{4.968,168.9111} = 8.074$, $p < .001$) and the interaction between time and DO ($F_{4.968,168.9111} = 2.204$, $p = .05$) did significantly influence emergence rate. On the other hand, interactions between time and other factors had no significant effect on emergence rate (Time × Temperature: $F_{4.968,168.9111} = 1.277$, $p = .276$, Time × Temperature × DO: $F_{4.968,168.9111} = 0.583$, $p = .712$).

3.2 | Respiration

Temperature increase and/or decrease in DO had no significant effect on respiration before ($F_{3,18} = 0.712$, $p = .558$) or after exposure (1st measurement: $F_{3,18} = 1.113$, $p = .370$, 2nd measurement: $F_{3,16} = 0.493$, $p = .692$) (Figure 3).

4 | DISCUSSION

Increasing temperature and decreasing DO level within known tolerable ranges (Kang et al., 2016; Shin, Kim, Chung, & Hur, 2001; Shin et al., 2000) had a surprisingly devastating effect on Manila clams. In the present study, clam mortality was significantly higher under the high temperature plus low DO condition than under any other condition, and more than 80% of clams died after 8 days of exposure. Although not significantly different, mortality under the high temperature plus high DO condition appeared to be higher than under the low temperature plus low DO treatment. Furthermore, low DO significantly increased emergence rate in a time-dependent manner. Unexpectedly, respiration was not found to be significantly affected by the different treatments. Our results suggest that temperature increase and DO decrease act as independent significant stressors in terms of mortality and emerging behavior.
sediment to breath more efficiently, but emergence from burrows solve oxygen deficiency issues in sea water, clams may emerge from sediment is a response to low oxygen and high CO2 levels. To re-

in a previous study (Lee & Kim, 2017), it appears emergence from mortality was highest under the high temperature/low DO con-

significant effect on the mortality of Manila clams. However, given emergence rates when CO2 levels increased or pH levels decreased ably due to high mortality. Given that Manila clams showed higher tal period under high temperature/normoxia conditions was prob-

emergence rate observed during the latter part of the experimen-
ture increase from 20°C appeared to be more stressful than a 50% decrease in DO in terms of mortality. Probably, juvenile clams are more sensitive to temperature increases than adults, which have been shown to be tolerant to temperature increases (Kang et al., 2016; Shin et al., 2000), a 3.5°C temperature increase from 20°C appeared to be more stressful than a 50% decrease in DO. It is generally believed that increase in temperature decreases hypoxia thresholds for many species, and therefore, high temperature and hypoxia act as detrimental stressors (Portner, 2010; Vaquer-Sunyer & Duarte, 2011). However, in a previous study, a temperature increase was found to enhance Olympia oyster growth, whereas hypoxia had a detrimental effect (Cheng et al., 2015). Our results also suggest that although high temperature and low DO in combination have a detrimental effect on Manila clams in terms of mortality and that these two stressors affect clam physiology in different ways. Taken together, our findings suggest concomitant increases in temperature and decreases in DO may differentially influence benthic animal physiologies in a species-dependent manner.

In conclusion, both ocean warming and low oxygenation may seriously influence the survival of Manila clams, but each of these cli
matic stressors differentially influences this species. A combination of warming and hypoxic stressors is expected to reduce the likelihood of population survival much more so than changes in a single stressor. Global warming is not an independent stressor as it also causes oxygen deficiencies in local environments, and thus, these stressors should be considered in combination.

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CONFLICT OF INTEREST

None declared.
AUTHOR CONTRIBUTIONS

TWK designed and conducted the experiment, and wrote the manuscript. SP conducted the experiment, analyzed the result, and wrote the manuscript. ES conducted the experiment, analyzed the result, and wrote the manuscript.

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REFERENCES

Becker, P., Barringer, C., & Marelli, D. C. (2008). Thirty years of sea ranching Manila clams (Venerupis philippinarum): Successful techniques and lessons learned. Reviews in Fisheries Science, 16(1–3), 44–50. https://doi.org/10.1080/10641260701790259

Belkin, I. M. (2009). Rapid warming of large marine ecosystems. Progress in Oceanography, 81(1–4), 207–213. https://doi.org/10.1016/j.pocean.2009.04.011

Brewer, P. G., & Peltzer, E. T. (2016). Ocean chemistry, ocean warming, and emerging hypoxia: Commentary. Journal of Geophysical Research: Oceans, 121(5), 3659–3667. https://doi.org/10.1002/2016JC011651

Cheng, B. S., Bible, J. M., Chang, A. L., Ferner, M. C., Wasson, K., Zabin, C. J., & Grosholz, E. D. (2015). Testing local and global stressor impacts on a coastal foundation species using an ecologically realistic framework. Global Change Biology, 21(7), 2488–2499. https://doi.org/10.1111/gcb.12895

Clark, M. S., Husmann, G., Thorne, M. A. S., Burns, G., Truebano, M., Peck, L. S., & Philipp, E. E. R. (2013). Hypoxia impacts large adults first: Consequences in a warming world. Global Change Biology, 19(7), 2251–2263. https://doi.org/10.1111/gcb.121197

Conley, D. J., Carstensen, J., Vaquer-Sunyer, R., & Duarte, C. M. (2009). Ecosystem thresholds with hypoxia. Hydrobiologia, 629(1–2), 21–29. https://doi.org/10.1007/s10750-009-09764-2

Cordero, D., Delgado, M., Liu, B. Z., Ruesink, J., & Saavedra, C. (2017). Population genetics of the Manila clam (Ruditapes philippinarum) introduced in North America and Europe. Scientific Reports, 7, ARTN 39745. https://doi.org/10.1038/srep39745

Diaz, R. J., & Rosenberg, R. (1995). Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and Marine Biology. An Annual Review, 33, 245–303.

Howard, A. C., Poirrier, M. A., & Caputo, C. E. (2017). Exposure of rangia clams to hypoxia enhances blue crab predation. Journal of Experimental Marine Biology and Ecology, 489, 32–35. https://doi.org/10.1016/j.jembe.2017.01.012

IPCC. (2014). Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland: IPCC.

Kang, H. Y., Lee, Y. J., Choi, K. S., Park, H. J., Yun, S. G., & Kang, C. K. (2016). Combined effects of temperature and Seston concentration on the physiological energetics of the Manila clam Ruditapes philippinarum. PloS ONE, 11(3), e0152427. https://doi.org/10.1371/journal.pone.0152427

Keeling, R. F., & Garcia, H. E. (2002). The change in oceanic O2 inventory associated with recent global warming. Proceedings of the National Academy of Sciences of the United States of America, 99(12), 7848–7853. https://doi.org/10.1073/pnas.122154899

Keeling, R. F., Kortzinger, A., & Gruber, N. (2010). Ocean deoxygenation in a warming world. Annual Review of Marine Science, 2, 199–229. https://doi.org/10.1146/annurev.marine.010908.163855

Kim, T. W., Barry, J. P., & Micheli, F. (2013). The effects of intermittent exposure to low-pH and low-oxygen conditions on survival and growth of juvenile red abalone. Biogosciences, 10(11), 7255–7262. https://doi.org/10.5194/bg-10-7255-2013

Kim, T. W., & Micheli, F. (2013). Decreased solar radiation and increased temperature combine to facilitate fouling by marine non-indigenous species. Biofouling, 29(5), 501–512. https://doi.org/10.1080/089270 14.2013.784964

Kozuki, Y., Yamanaka, R., Matsushige, M., Saitoh, A., Otani, S., & Ishida, T. (2013). The after-effects of hypoxia exposure on the clam Ruditapes philippinarum in Omaehama beach, Japan. Estuarine Coastal and Shelf Science, 116, 50–56. https://doi.org/10.1016/j.ecss.2012.08.026

Lee, J. A., & Kim, T. W. (2017). Effects of potential future CO2 levels in seawater on emerging behaviour and respiration of Manila clams, Venerupis philippinarum. Ices Journal of Marine Science, 74(4), 1013–1020. https://doi.org/10.1093/icesjms/fsx124

Lee, A. C., Lee, Y. C., & Chin, T. S. (2012). Effects of low dissolved oxygen on the digging behaviour and metabolism of the hard clam (Mercenaria mercenaria). Aquaculture Research, 43(1), 1–13. https://doi.org/10.1111/j.1365-2109.2010.02785.x

Moline, M. A., Claustrte, H., Frazer, T. K., Schofield, O., & Vernet, M. (2004). Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend. Global Change Biology, 10(12), 1973–1980. https://doi.org/10.1111/j.1365-2486.2004.00825.x

Newell, R. C. (1966). Effect of temperature on the metabolism of poikilotherms. Nature, 212, 426–428. https://doi.org/10.1038/212426a0

Portner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology, 132(4), 739–761. https://doi.org/10.1016/S1095-6433(02)00045-4

Portner, H. O. (2010). Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology, 213(6), 881–893. https://doi.org/10.1242/jeb.037523

Portner, H. O. (2012). Integrating climate-related stressor effects on marine organisms: Unifying principles linking molecule to ecosystem-level changes. Marine Ecology Progress Series, 470, 273–290. https://doi.org/10.3354/meps10123

Prætorius, S. K., Mix, A. C., Walczak, M. H., Wolhowe, M. D., Addison, J. A., & Prahi, F. G. (2015). North Pacific deglacial hypoxic events linked to abrupt ocean warming. Nature, 527(7578), 362–366. https://doi.org/10.1038/nature15753

Rosa, R., & Seibel, B. A. (2008). Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proceedings of the National Academy of Sciences of the United States of America, 105(52), 20776–20780. https://doi.org/10.1073/pnas.0806886105

Rummer, J. L., Couturier, C. S., Stecyk, J. A. W., Gardiner, N. M., Kinch, J. P., Nilsson, G. E., & Munday, P. L. (2014). Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. Global Change Biology, 20(4), 1055–1066. https://doi.org/10.1111/gcb.12455

Salomo, M. E., & Duncan, D. S. (2005). Low dissolved oxygen levels reduce anti-predation behaviours of the freshwater clam Corbicula fluminea. Freshwater Biology, 50(7), 1233–1238. https://doi.org/10.1111/j.1365-2427.2005.01396.x

Seitz, R. D., Marshall, L. Jr, Hines, A., & Clark, K. (2003). Effects of hypoxia on predator-prey dynamics of the blue crab Callinectes sapidus and the Baltic clam Macoma baltica in Chesapeake Bay. Marine Ecology Progress Series, 257, 179–188. https://doi.org/10.3354/meps257179

Shin, Y.-K., Kim, Y., Chung, E.-Y., & Hur, S.-B. (2000). Temperature and salinity tolerance of the Manila clam, Ruditapes philippinarum. Korean Journal of Fisheries and Aquatic Sciences, 33(3), 213–218.
Shin, Y.-K., Kim, Y., Chung, E.-Y., & Hur, S.-B. (2001). Effects of the dissolved oxygen concentration on the physiology of the Manila clam, *Ruditapes philippinarum*. *Korean Journal of Fisheries and Aquatic Sciences, 34*(3), 190–193.

Taylor, D. L., & Eggleston, D. B. (2000). Effects of hypoxia on an estuarine predator-prey interaction: Foraging behavior and mutual interference in the blue crab *Callinectes sapidus* and the infaunal clam prey *Mya arenaria*. *Marine Ecology Progress Series, 196*, 221–237. https://doi.org/10.3354/meps196221

Uzaki, N., Kai, M., Aoyama, H., & Suzuki, T. (2003). Changes in mortality rate and glycogen content of the Manila clam *Ruditapes philippinarum* during the development of oxygen-deficient waters. *Fisheries Science, 69*(5), 936–943. https://doi.org/10.1046/j.1444-2906.2003.00710.x

Vaquer-Sunyer, R., & Duarte, C. M. (2008). Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences of the United States of America, 105*(40), 15452–15457. https://doi.org/10.1073/pnas.0803833105

Vaquer-Sunyer, R., & Duarte, C. M. (2011). Temperature effects on oxygen thresholds for hypoxia in marine benthic organisms. *Global Change Biology, 17*(5), 1788–1797. https://doi.org/10.1111/j.1365-2486.2010.02343.x

Wu, R. S. S. (2002). Hypoxia: From molecular responses to ecosystem responses. *Marine Pollution Bulletin, 45*(1–12), 35–45. https://doi.org/10.1016/S0025-326X(02)00061-9

Yin, X. W., Chen, P., Chen, H., Jin, W., & Yan, X. W. (2017). Physiological performance of the intertidal Manila clam (*Ruditapes philippinarum*) to long-term daily rhythms of air exposure. *Scientific Reports, 7*, https://doi.org/ARTN 4164810.1038/srep41648

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