Exposure of rangia clams to hypoxia enhances blue crab predation

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Abstract

This experimental study questions whether exposure of non-mobile prey to episodic hypoxia might enhance predation by a mobile normoxic predator, which moves into the former hypoxic area immediately after a shift back to normoxia. We used *Rangia cuneata* (common rangia clams) and *Callinectes sapidus* (blue crabs) from an oligohaline estuary where mature clams are rare in areas subject to episodic anoxia and hypoxia. Clams were exposed to severe laboratory hypoxia for 72 hours. One clam stressed by hypoxia and another clam maintained under aeration (normoxia) were placed in aerated aquaria containing a crab. Feeding choice of hypoxic *vs.* normoxic clams was then monitored for 12 hours. We conducted fourty trials that used twenty different crabs. To test for homogeneity of the feeding response, we used a 1-tail binomial test with 0.5 expected probabilities. Fourteen of the twenty crabs fed (70%), and nineteen out of twenty one hypoxia-stressed clams were eaten compared to two out of twenty one clams kept under normoxic conditions ($p < 0.001$). The significant choice of stressed clams indicates that in this experimental study, exposure of clams to hypoxia enhanced crab predation.

Key Words: *Callinectes sapidus*, *Rangia cuneata*, feeding, dissolved oxygen, Lake Pontchartrain.

1. Introduction

Hypoxia (DO < 2 mg l^{-1}) is known to cause stress and mortality in benthic organisms (Diaz and Rosenberg, 1995) and after hypoxic and anoxic episodes, mobile predators can take advantage of weakened, sedentary prey (Pihl et al., 1992, Long and Seitz, 2008, Powers et al., 2005, Riedel et al., 2014). *Rangia cuneata* (Sowerby, 1831), the common rangia (also called Atlantic rangia or wedge clam), occurs in Atlantic and Gulf of Mexico estuaries at salinities below 19 (La Salle and de la Cruz, 1985) and dominates the benthos of Lake Pontchartrain (Darnell, 1961). This study was prompted by long-term field studies of the decline in *R. cuneata* density in Lake Pontchartrain, a large shallow, oligohaline estuary located north of New Orleans, Louisiana.

Through predation, *Callinectes sapidus* (Rathbun, 1895), the blue crab, can influence the community structure of bivalves (Laughlin, 1982) and other biota (Micheli, 1995). *Callinectes sapidus* feeds on small *R. cuneata* by crushing shells, but larger clams can withstand cheliped crushing power (Blundon and Kennedy, 1982) so crabs use a combination of chipping and wedging to open large clams (Linton et al., 2007). Since *C. sapidus* is sensitive to hypoxia (Hines, 2007), and *R. cuneata* can withstand persistent levels of moderate hypoxia, under some conditions hypoxia might provide a refuge from predation similar to that described for the quahog, *Mercenaria mercenaria* (Altieri, 2008). Although *C. sapidus* avoid hypoxic areas (Bell et al., 2003), they are abundant and active foragers (Clark et al., 1999). Therefore, blue crabs may move into areas after severe episodic hypoxia or anoxia to take advantage of stressed clams as easy prey before the clams have a chance to recover from hypoxia. This is similar to the hypoxia-enhanced foraging described by Long and Seitz (2008) for several prey species.

In 1998 large (>20 mm) *R. cuneata* were abundant in most of Lake Pontchartrain except for a 250 km^2 episodic, anoxic/hypoxic zone caused by stratification from higher salinity bottom water entering from the Gulf of Mexico through navigation canals (Poirrier, 1978, Abadie and Poirrier, 2000, 2001). The overall density of large clams declined from 2001 to 2014, which appeared to have been caused by an El Niño Southern Oscillation (ENSO) shift that produced a local drought and was then followed by a period of increased hurricane frequency and intensity, which introduced high salinity water into the Lake (Poirrier and Caputo, 2015). Patchy episodes of severe hypoxia were associated with storm surges (Poirrier et al., 2008) which appeared to be increasing due to relative sea level rise, coastal erosion and wetland loss (Poirrier and Caputo, 2015). If hypoxia enhanced crab predation, this interaction may have been a contributing factor in the reduction of the density of large clams.

Salinity shifts can increase the stress of hypoxia on *R. cuneata*. Henry et al. (1980) found 50% mortality in rangia clams after 5–7 days of hypoxia, and clams that survived did not recover after being returned to normoxic conditions. They also found that exposure to a hypo or hypersaline shock during hypoxia produced 25% mortality at 2 days and 100% at 3 days.

This experimental study was conducted to determine if predation by *C. sapidus* on *R. cuneata* is enhanced by clam exposure to hypoxic stress. The specific goal was to determine whether *C. sapidus* would have a significant feeding preference for *R. cuneata* exposed to hypoxia over clams kept under normoxic conditions.

2. Materials and methods

2.1. Experimental Design

Choice experiments were devised to determine if crabs fed on hypoxia-stressed over non-hypoxic stressed clams. Each experiment had five trials (Table 1) using five aerated 42 l glass aquaria containing one crab and one hypoxic and one normoxic clam that were added at the beginning of each trial. A replicate experiment was performed with the same five crabs used in the first experiment after 6 or 7 days (Table 1) giving a total of eight experiments based on four experiments and four crab replicate experiments designated *a* for the initial experiment and *b* for the crab replicate experiment in Table 1. This resulted in eight experiments (four + four crab replicates with five trials) with forty trials using twenty crabs (each used twice), forty hypoxia-stressed clams, and forty clams kept under normoxic conditions (Table 1). Crabs were not used more than twice to avoid possible pseudoreplication from learning after numerous trials, changes in crab condition and feeding performance. Only crabs that fed are presented in Table 1.

Clam hypoxia exposures and crab choice experiments were conducted at room temperature (22-25ºC). Hypoxic stress was produced by exposing clams to DO between 0.3 to 0.8 mg l^{-1} for 72 hours by continuous nitrogen sparging in a covered 42 l aquarium. Longer exposure periods were not used, because in other unpublished studies, some mortality occurred after 96 hours. Hypoxic clams were distinguished from normoxic clams by etching two horizontal lines or vertical lines in the periostraca depending on the treatment. Markings were alternated among experiments to eliminate any potential effect of marking on feeding. Feeding experiments were run for 12 hours to determine whether a hypoxic or a normoxic clam was eaten and if both were eaten, which one was eaten first.

2.2. Study Organisms and Collection Sites

Experiments were conducted from October 2007 through April 2008 (Table 1). Clams (30–35 mm long) were collected by hand while wading in southeastern Lake Pontchartrain and replaced after each experiment. Crabs were captured using crab traps from Pirate's Bayou, a canal connected to Lake Pontchartrain south of Slidell, LA. Male crabs (130–160 mm carapace width) with all appendages including cutter and crusher chelae were used.

2.3. Maintenance of Study Organisms

Crabs were held separately in the five 42 l experimental aquaria connected to a 189 l closed recirculating, biological filtration tank. Ammonia levels were monitored twice a week to ensure safe levels of biological filtration. Clams were kept in aerated 42 l aquaria and held for a week to a month before each experiment. Both crabs and clams were held in de-chlorinated, aged New Orleans tap water with synthetic sea salts added to maintain the salinity at 5, the mean salinity of eastern Lake Pontchartrain. Between experiments, crabs were fed Wardley shrimp pellets supplemented with shucked rangia clams. Crabs were not fed for 48 hours prior to each experiment.

2.4. Statistical analysis

To test for homogeneity of feeding response, we employed a 1-tail binomial test with 0.5 expected probabilities (Moore DS, McCabe GP, 1998). We used the twenty one trials (Table 1) in which crabs fed. All trials were analyzed together, but we also conducted separate tests on initial and replicate experiments conducted a week later to help evaluate if the outcome of the second experiment was affected by the first. A paired samples t-test was conducted to determine if there was a significant change in the number of crabs that fed between the first and second set of experiments.

3. Results

In the eight experiments, fourteen crabs fed (70%) and six did not feed (Table 1). Out of the twenty one clams that were eaten, nineteen were hypoxia stressed and two were not stressed (Table 1). The results of the feeding choice test showed crabs significantly preferred hypoxic clams over normoxic clams ($p < 0.001$). A separate analysis of results of the initial experiment using the twelve crabs (labeled *a* in Table 1) that ate eleven hypoxic and one normoxic clam produced significant results ($p = 0.006$). The result of the set of replicate experiments where the same crabs were used a week later (labeled *b* in Table 1) had eight of nine clams eaten by nine crabs being hypoxic and was also significant ($p = 0.039$). There was no significant change (12 *vs* 9) in the number of crabs that fed ($p = 0.426$) between the initial and replicate experiments.

The methods that crabs used to open clams were consistent with feeding methods described by Ebersole and Kennedy (1995) and Linton et al. (2007) for large *R. cuneata*. The clams were too large to be crushed by the size class crabs used. Instead crabs chipped the posterior edge of the shell with mandibles to create a gap that allowed chelipeds to enter and widen the gap to cut adductor muscles.

4. Discussion

The use of the same crabs in replicate experiments the week after the initial experiment did not appear to affect the independence of choice in the replicate experiments. We regard the high significance of the combined data as a valid interpretation of the results. Even if this was not the case, the separate analyses of the two experimental data sets both independently support a significant choice for hypoxic clams. Crabs recognized and fed on hypoxia stressed clams because they are easier to open. This makes them a more profitable food item than normoxic clams which are more difficult to open.

In nature, crab predation on large hypoxia stressed clams is at least as significant as our experimental results because periods of hypoxia are often longer (Poirrier et al., 2009), crabs larger than those used in our experiments are present, and salinity shifts often coupled with hypoxia may contribute to total clam stress (Henry et al., 1980). In general, study results should also apply to similar predator-prey outcomes in diverse aquatic systems.

An interesting result was 30% of the crabs did not feed. Our experiments were limited to 12 hours because when hypoxic clams are not handled by crabs they open their valves, extend their siphons and ventilate their gills and possibly recover from hypoxia. In other studies, clams were exposed to crab predation for several days (Ebersole and Kennedy, 1995, Linton et al., 2007). The short duration of our experiments, individual variation in the degree of clam hypoxic stress and recovery, small test aquaria, the biological condition of the crabs, and prior feeding attempts could have contributed to 30% of the crabs not feeding. Overall, these results support field studies which indicate that a legacy of hypoxia can enhance predation by mobile predators on sedentary prey (Pihl et al., 1992, Long and Seitz, 2008, Powers et al., 2005, Riedel et al., 2014).

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References

Abadie, S.W., Poirrier, M.A., 2000. Increased density of large *Rangia* clams in Lake Pontchartrain after the cessation of shell dredging. J. Shellfish Res. 19, 481–485.

Abadie, S.W., Poirrier, M.A., 2001. *Rangia* clams as indicators of hypoxia in Lake Pontchartrain. In: Penland S, Beall A, Waters J (eds) Environmental Atlas of the Lake Pontchartrain Basin. United States Geological Survey, pp. 166.

Altieri, A.H., 2008. Dead zones enhance key fisheries species by providing predation refuge. Ecology 89, 2808–2818. doi:10.1890/07-0994.1

Bell, G.W., Eggleston, D.B., Wolcott, T.G., 2003. Behavioral responses of free-ranging blue crabs to episodic hypoxia. I. Movement. Mar. Ecol. Prog. Ser. 259, 215–225. doi:10.3354/meps259227

Blundon, J.A., Kennedy, V.S., 1982. Mechanical and behavioral aspects of blue crab, *Callinectes sapidus* (Rathbun), predation on Chesapeake Bay bivalves. J. Exp. Mar. Bio. Ecol. 65, 47–65. doi:10.1016/0022-0981(82)90175-7

Clark, M.E., Wolcott, T.G., Wolcott, D.L., Hines, A.H., 1999. Intraspecific interference among foraging blue crabs *Callinectes sapidus*: Interactive effects of predator density and prey patch distribution. Mar. Ecol. Prog. Ser. 178, 69–78. doi:10.3354/meps178069

Darnell, R.M., 1961. Trophic spectrum of an estuarine community, based on studies of Lake Pontchartrain, Louisiana. Ecology 42(3), 553-568. doi:10.2307/1932242

Diaz, R.J., Rosenberg, R., 1995. Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr. Mar. Biol. - an Annu. Rev. 33, 245–303.

Ebersole, E.L., Kennedy, V.S., 1995. Prey preferences of blue crabs *Callinectes sapidus* feeding on three bivalve species. Mar. Ecol. Prog. Ser. 118, 167–178. doi:10.3354/meps118167

Henry, R.P., Mangum, C.P., Webb, K.L., 1980. Salt and water balance in the oligohaline clam, *Rangia cuneata*: accumulation of intracellular free amino acids during high salinity adaptation. J Exp. Zoolog. A Comp. Exp. Biol. 211, 11-24. doi:10.1002/jez.1402110103

Hines, A.H., 2003. Ecology of juvenile and adult blue crabs, in: Kennedy, V.S., Cronin, L.E. (Eds.), The Blue Crab, *Callinectes sapidus*. Maryland Sea Grant College, Maryland, pp. 565-654.

LaSalle, M.W., de la Cruz, A.A., 1985. Species profile: life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico) – common rangia. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.31). U.S. Army Corps of Engineers, TR EL-82-4. 16 pp.

Laughlin, R.A., 1982. Feeding habits of the blue crab, *Callinectes sapidus* Rathbun, in the Apalachicola Estuary, Florida. Bull. Mar. Sci. 32, 807–822.

Linton, C.M., Rebach, S., Kennedy, V.S., 2007. Notes on the behavior of blue crabs, *Callinectes sapidus* Rathbun, 1896 feeding on two morphologically dissimilar clams. Crustaceana 80, 779–792. doi:10.1163/156854007781363088

Long, W.C., Seitz, R.D., 2008. Trophic interactions under stress: Hypoxia enhances foraging in an estuarine food web. Mar. Ecol. Prog. Ser. 362, 59–68. doi:10.3354/meps07395

Micheli, F., 1995. Behavioural plasticity in prey-size selectivity of the blue crab *Callinectes sapidus* feeding on bivalve prey. J. Anim. Ecol. 64, 63–74. doi:10.2307/5827

Moore, D.S., McCabe, G.P., 1998. Introduction to Practice of Statistics. WH Freeman and Company, New York.

Pihl, L., Baden, S.P., Diaz, R.J., Schaffner, L.C., 1992. Hypoxia-induced structural changes in the diet of bottom feeding fish and Crustacea. Mar. Biol. 112, 349-361.

Poirrier, M.A., 1978. Studies of salinity stratification in southern Lake Pontchartrain near the Inner Harbor Navigation Canal. Louisiana Acad. Sci. 41, 26-35.

Poirrier, M.A., Rodriguez del Rey, Z., Spalding, E.A., 2008. Acute disturbance of Lake Pontchartrain benthic communities by Hurricane Katrina. Estuar Coasts 31, 1221–1228.

Poirrier, M.A., Spalding, E.A., Franze, C.D., 2009. Lessons learned from a decade of assessment and restoration studies of benthic invertebrates and submersed aquatic vegetation in Lake Pontchartrain. J. Coast. Res. 10054, 88–100. doi:10.2112/SI54-005.1

Poirrier, M.A., Caputo, C.E., 2015. *Rangia cuneata* clam decline in Lake Pontchartrain from 2001 to 2014 due to an El Niño Southern Oscillation shift coupled with a period of high hurricane intensity and frequency. Gulf Caribb. Res. 26, 9–20. doi:10.18785/gcr.2601.04

Powers, S.P., Peterson, C.H., Christian, R.R., Sullivan, E., Powers, M.J., Bishop, M.J., Buzzelli, C.P., 2005. Effects of eutrophication on bottom habitat and prey resources of demersal fishes. Mar. Ecol. Prog. Ser. 302, 233–243. doi:10.3354/meps302233

Riedel, B., Pados, T., Pretterebner, K., Schiemer, L., Steckbauer, A., Haselmair, A., Zuschin, M., Stachowitsch, M., 2014. Effect of hypoxia and anoxia on invertebrate behaviour: ecological perspectives from species to community level. Biogeosciences 11, 1491-1518. doi: 10.5194/bg-11-1491-2014

Experiment (Date run)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Total Hypoxic	Total Normoxic
1 (10/09/07)	(1*a*) H	(2*a*) N	--	--	--	1	1
2 (10/16/07)	--	(2*b*) H	--	--	--	1	0
3 (10/29/07)	(3*a*) H	(4*a*) H	--	(5*a*) H	(6*a*) H	4	0
4 (11/06/07)	(3*b*) H	(4*b*) H	--	(5*b*) N	(6*b*) H	3	1
5 (02/05/08)	--	(7*a*) H	--	(9*a*) H	(10*a*) H	3	0
6 (02/21/08)	--	(7*b*) H	(8*b*) H	--	(10*b*) H	3	0
7 (04/14/08)	--	(11*b*) H	--	--	--	1	0
8 (04/21/08)	--	(12*a*) H	--	(13*a*) H	(14*a*) H	3	0
TOTALS						**19**	**2**

Table 1: Results of the choice experiments: 8 experiments with 5 trials were run at different times. Numbers (1) to (14) refer to the individual crab that fed. The crab number with *a*, or *b* indicate experiments in which the same crab was used twice. Results were: N = normoxic clam eaten, H = hypoxic clam eaten, -- = no clam eaten. Total of hypoxic or normoxic clams eaten are for the 5 trials in each experiment.