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Physiological responses of postlarval and juvenile blue crabs *Callinectes sapidus* to hypoxia and anoxia

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ABSTRACT: The influence of hypoxia and anoxia on the oxygen consumption, survivorship and rate of metamorphosis of field-caught postlarvae (megalopae) and first-instar juveniles of the blue crab *Callinectes sapidus* was observed under laboratory conditions. Rates of oxygen uptake by megalopae were independent of P_{O_2} at oxygen tensions above 8.88 kPa. However, P_c for juvenile crabs was significantly higher than for megalopae (89.6 vs 43.2% saturation). Tolerance of blue crab megalopae of hypoxic conditions below 20% saturation (~4.12 kPa) was greater than for newly metamorphosed juveniles. Juvenile crabs also succumbed to the effects of anoxia more rapidly than megalopae, but neither group survived exposure of >5 h. Megalopae and crabs that became immobile in anoxic water quickly recovered when returned to normoxic conditions. Metamorphosis of megalopae to the first-juvenile stage was delayed when they were exposed to P_{O_2} values of 8.21 and 12.32 kPa (40 and 60% saturation). Similarly, time to metamorphosis increased significantly when megalopae were temporarily exposed to hypoxic conditions (P_{O_2} = 4.12 kPa) for 4 h each day. However, there was no significant difference between the time to metamorphosis for megalopae exposed to hypoxic conditions for 2 h each day and those maintained in oxygen-saturated water. These results suggest that the presence of hypoxic and anoxic water in deep water layers and shallow near-shore habitats of estuaries during the summer months may influence the onshore migration, settlement and survival of blue crab megalopae and newly metamorphosed juvenile crabs.

KEY WORDS: *Callinectes sapidus* · Blue crab · Respiration · Hypoxia · Anoxia · Megalopae · Juveniles · Metamorphosis · Survivorship

INTRODUCTION

Many benthic marine invertebrates live in areas that regularly experience reductions in dissolved oxygen concentrations. In estuarine and shallow coastal habitats, the occurrence of hypoxic and anoxic water masses is a seasonal phenomenon resulting from the combined effects of eutrophication, increased organic loading and water column stratification (e.g. Falkowski et al. 1980, Officer et al. 1984, Kuo & Neilson 1987, Breitburg 1990, Sanford et al. 1990). Bottom water oxygen depletion in the summer may last for several weeks, and concentrations often reach levels that are physiologically stressful or lethal to animals (see Diaz & Rosenberg 1995 for review), resulting in massive faunal mortality (e.g. Steimle & Sinderman 1978, Rosenberg & Loo 1988, Rosenberg et al. 1990) and emigration from affected habitats (Baden et al. 1990, Pihl et al. 1991). Reduced oxygen concentrations are also common in shallow near-shore habitats, such as seagrass beds and salt marshes. These areas frequently experience short-term diel fluxes in dissolved oxygen with hypoxic periods occurring during late night/early morning hours as a result of plant and animal respiration in the absence of photosynthesis (Johnson & Welsh 1985, Kenney et al. 1988, Fitt & Coon 1992, Asmus et al. 1994, Cochran & Burnett 1996). Since shallow near-shore habitats often serve as important settlement and

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refuge sites for commercially and ecologically important estuarine-dependent species, such as the blue crab *Callinectes sapidus* (Rathbun) (e.g. Orth & van Montfrans 1987, 1990, Sogard & Able 1991, Ruiz et al. 1993, and references therein), periodic or intermittent hypoxia may reduce their suitability as nursery areas by affecting the composition and abundance of benthic macrofauna (e.g. Santos & Simon 1980, Harper et al. 1981, Officer et al. 1984, Gaston 1985, Llansó 1992, Diaz & Rosenberg 1995), altering trophic interactions (Pihl et al. 1992, Breitburg et al. 1997), or increasing the susceptibility of resident species to predation (Poulin et al. 1987, Rahel & Kolar 1990, Pihl et al. 1992, Breitburg et al. 1994, 1997).

Numerous studies have examined the behavioral and physiological responses of benthic invertebrates, including *Callinectes sapidus*, to hypoxia and anoxia (see reviews by Taylor 1982, Cameron 1989, Grieshaber et al. 1994, Diaz & Rosenberg 1995). Field observations indicate that adult and juvenile blue crabs escape mortality by avoiding hypoxic bottom water and migrating to shallower areas (Pihl et al. 1991). However, in laboratory assays juvenile *C. sapidus* failed to exhibit any significant avoidance behaviors in response to hypoxic or anoxic water (Das & Stickle 1994). Physiological tolerance of blue crabs to low oxygen conditions is also limited. Adult crabs are more tolerant of moderate hypoxia ($P_{O_2} \geq 6.7$ kPa) than juveniles, but neither group is able to survive even short periods of severe hypoxia (Carpenter & Cargo 1957, Stickle et al. 1989, deFur et al. 1990, Das & Stickle 1993).

Although the effect of hypoxia on the physiology and behavior of adult crustaceans is well documented (for reviews see Wovlekamp & Waterman 1960, Herreid 1980, Diaz & Rosenberg 1995), little information is available on the physiological responses and tolerances of decapod larvae and early juvenile instars to reduced oxygen (but see Belman & Childress 1974, Johnson & Welsh 1985, Nakanishi 1987, Eriksson & Baden 1997). For species possessing complex life histories, the probability of encountering hypoxic conditions may vary significantly between pelagic larval phases and post-settlement juvenile and adult (benthic) phases, which often occupy different habitats. Likewise, tolerance of low environmental oxygen may also vary among developmental stages, especially between larvae and newly metamorphosed benthic juveniles which may have different oxygen demands, sites of gas exchange, and compensatory mechanisms for responding to low oxygen conditions.

The blue crab *Callinectes sapidus* is a common inhabitant of estuaries and coastal areas along the Atlantic and Gulf of Mexico coasts of the USA (Williams 1984). While adult crabs occur primarily within estuaries, larvae are released near the entrance and are transported offshore to coastal waters where they undergo zoeal development before reinvading estuaries as postlarvae (megalopae) and migrating upstream toward nursery areas (see Epifanio 1995 for review). Up-estuary movement against strong net seaward flow is accomplished by means of selective tidal-stream transport, in which postlarval crabs are in the water column during rising tides at night and remain on or near the bottom at all other times (see Forward et al. 1995 for review). Megalopae then settle, metamorphose to the first-juvenile crab stage (J1), and undergo early juvenile development in beds of submerged aquatic vegetation and marsh-creek habitats (Orth & van Montfrans 1987, Wilson et al. 1987, 1990, Lipcius et al. 1990, van Montfrans et al. 1991). Although it is unlikely that zoeal stages encounter hypoxic conditions in coastal waters, the ingress, settlement and early development of blue crab postlarvae and juveniles in estuarine habitats along the Atlantic and Gulf coasts coincides with seasonal hypoxic and anoxic events during the late summer and early fall (e.g. Officer et al. 1984, Pihl et al. 1991, Diaz et al. 1992, Howell & Simpson 1994, Rabalais et al. 1994). Consequently, megalopae entering estuaries may encounter hypoxic or anoxic conditions (1) while migrating vertically toward bottom waters at the end of flood tide, (2) while located on or near the bottom during ebb tides, or (3) upon settlement and metamorphosis in shallow near-shore habitats, especially seagrass beds and tidal marshes. Thus, the physiological responses and tolerances of postlarval and early juvenile crabs to reduced oxygen conditions may have a significant impact on their migratory success, survivorship and growth, and may ultimately affect the structure and abundance of adult populations.

The objectives of the present study were (1) to compare the rates of oxygen consumption (V_{O_2}) of postlarval and newly metamorphosed juvenile crabs under declining oxygen tensions, (2) to determine their sensitivity to short-term hypoxia and anoxia, (3) to correlate ontogenetic shifts in oxyregulation with changes in tolerance of hypoxia and anoxia, and (4) to determine the effect of exposure to hypoxia on the metamorphosis of megalopae to the first-juvenile crab (J1) stage. Results indicate that megalopae and J1 crabs are extremely sensitive to oxygen deficiency, with juveniles having a lower resistance to episodic hypoxia and anoxia than megalopae. Ontogenetic changes in the tolerance of crabs to low oxygen conditions were consistent with their respiratory responses to progressive hypoxia and suggest that low oxygen availability may be an important density-independent factor affecting the recruitment and survival of *Callinectes sapidus* megalopae and post-settlement juveniles.

MATERIALS AND METHODS

Collection and maintenance. We collected *Callinectes sapidus* megalopae from July to October 1996 and 1997 in plankton tows (0.67 m diameter nets; 500 µm mesh) conducted during nocturnal flood tides near the entrance to the Newport River estuary (Beaufort, North Carolina, USA; 34° 41' N; 76° 40' W). Following collection, postlarvae were sorted by molt stage (Aiken 1973, Anger 1983, Stevenson 1985) and transferred to circular glass culture dishes (9 cm diam.) containing filtered (<5 µm) estuarine water (32 to 33 psu). To avoid the potential confounding effects of metamorphosis or molting on survivorship and oxygen consumption (Lewis & Haefner 1976, Mangum et al. 1985), only megalopae determined to be in intermolt were used. Because early stage juvenile crabs are difficult to collect in the field, we maintained premolt postlarvae in circular glass culture dishes (50 to 100 crabs per dish) until metamorphosis (~2 to 10 d). First-instar (J1) crabs were allowed to 'harden' for at least 48 h before they were used in experiments. Both megalopae and juvenile crabs were maintained in an environmental chamber (Sherer Model CEL4-4) at temperatures (24 to 25°C) and light:dark cycles that were similar to ambient at the time of collection. Crabs were transferred daily to new water and fed newly hatched brine shrimp (*Artemia* spp.) nauplii.

Oxygen consumption. Oxygen consumption of postlarvae and newly metamorphosed juveniles was measured using a closed-system respirometer consisting of two 28 ml glass chambers submerged in a refrigerated water bath. The top of each chamber was sealed with a rubber stopper, and the oxygen concentration of the chamber water was measured by inserting a polarographic oxygen sensor (Diamond General Mini-Oxygen Electrode Model No. 733) connected to a digital oxygen meter (Cameron Instrument Co.) through a small hole in the stopper. Because of their small size relative to the volume of the respirometery chamber, it was necessary to measure oxygen uptake for groups of animals. For each trial, 20 megalopae or 10 first-instar juvenile crabs were placed in 1 of the 2 chambers filled with filtered (<0.45 µm) estuarine water (32 psu). After a 1 h acclimation period, the chamber was sealed and the oxygen tension (P_{O_2}) of the water was recorded at 5 min intervals using an A/D converter (ComputerBoards CIO-DAS 16) and data acquisition software (Labtech Notebook Pro) attached to the output port of the meter. The second (control) chamber was filled with only filtered estuarine water and monitored simultaneously with an identical oxygen electrode and meter to control for sensor drift. During each trial, temperature was maintained at 25 ± 0.1°C. Trials continued until the oxygen tension in the experimental chamber dropped below 5% saturation (~1 kPa). Trials were repeated 10 times using separate groups of newly collected megalopae and J1 crabs. Oxygen consumption rates (V_{O_2}; µl O_2 h^{-1} $ind.^{-1}$) were calculated from the rate of change in the P_{O_2} of the chamber water at intervals of 2 kPa (~10% air saturation). Since crabs remained undisturbed throughout the trial, rates of oxygen consumption were assumed to be equivalent to 'routine' levels.

Rates of oxygen consumption for megalopae and juveniles were compared using repeated measures analysis of variance (ANOVAR; Potvin et al. 1990). The relative degree of oxyconformity (oxygen consumption declines linearly with decreasing P_{O_2}) or oxyregulation (oxygen consumption is maintained at a constant rate over a range of P_{O_2} values) was evaluated by fitting the quadratic polynomial model to the data using the procedures outlined by Van Winkle & Mangum (1975). The method uses the coefficient for the quadratic term (β_2) as an index of the shape of the response curve. Oxyregulation is indicated by β_2 values significantly less than 0, while coefficents not significantly different from 0 indicate oxyconformity (Van Winkle & Mangum 1975). Similarly, the oxygen tension at which postlarval and juvenile crabs switch from oxygen-independent to oxygen-dependent respiration (i.e. critical oxygen tension, P_c; Hill 1976) was determined by fitting a segmented quadratic model (with plateau) to the response curve using nonlinear (least squares) regression. The procedure estimates P_c by fitting 2 models to the data that must meet at P_c. At values above P_c, the equation is constant (i.e. a horizontal line at the plateau; $V_{O_2} = V_{O_{2max}}$), but below P_c the quadratic model [$V_{O_2} = \beta_0 + \beta_1 P_{O_2} + \beta_2 (P_{O_2})^2$] is fitted to the data. All model parameters, including $V_{O_{2max}}$ and P_c, were estimated iteratively using a modified Gauss-Newton method (SAS NLIN Procedure; SAS Institute 1990).

Tolerance of hypoxia and anoxia. We examined the effects of hypoxia and anoxia on the survival of postlarvae and J1 crabs by subjecting crabs to P_{O_2} values of 0.2 kPa (<1% air saturation; considered anoxia for the purpose of this study), 2.05 kPa (10% saturation), 4.12 kPa (20% saturation) and 20.53 kPa (normoxic control). Treatment levels were selected based upon preliminary experiments that indicated megalopae and newly metamorphosed juvenile blue crabs are able to tolerate exposure to moderate hypoxia (≥6.16 kPa; ~30% saturation) for several days. During each 24 h exposure period, crabs were placed individually in glass vials (5.9 cm × 2.7 cm diam.) containing 20 ml of filtered (<1 µm) estuarine water (32 psu). The tops of the vials were sealed with rubber stoppers, and target P_{O_2} values were obtained by equilibrating the water in the chambers with a mixture of N_2 and compressed air delivered by stainless steel needles (18 gauge) inserted

through small holes in the stopper. This method had no effect on the pH of the water in the vials during the exposure period. Oxygen saturation levels within the chambers were periodically checked using a YSI oxygen electrode and meter (Yellow Spring Instruments Model 57). Oxygen levels were consistently within ±2% of target values in the hypoxia treatments and remained below the detection limit of the sensor in the anoxia treatment (i.e. 0.2 kPa).

At each oxygen tension (100, 20, 10 and 0% saturation), 60 megalopae and 54 J1 crabs were tested, and mortality was recorded at hourly intervals for the first 18 h and then 6 h later (total exposure time = 24 h). Crabs were considered dead when their carapace turned opaque and they were inactive (legs retracted) and unresponsive to mechanical stimulation, even when transferred to normoxic conditions. Survival curves for postlarval and juvenile crabs were constructed using the product-limit method (i.e. Kaplan-Meier method; Kleinbaum 1996) and survival functions for each treatment group (i.e. oxygen tension) were compared using the Mantel log-rank test (χ^2 approximation; SYSTAT 7.0, SPSS Inc.). Since all megalopae and juvenile crabs subjected to normoxic conditions survived the 24 h exposure period (i.e. mortality was 0%), control groups were not included in the analysis. Postlarval and juvenile crabs in the hypoxia and anoxia treatments that were alive at the end of the 24 h exposure period were treated as right-censored observations. Median survival times (i.e. time to 50% mortality; MST) for each treatment group were calculated from the survivorship functions using probit analysis (SAS PROBIT Procedure, SAS Institute 1990).

Recovery from short-term exposure to anoxia. Since anoxic conditions in deep water and shallow nearshore nursery habitats are often episodic and ephemeral, we examined the ability of megalopae and J1 crabs to survive and recover from short-term (≤3 h) exposure to anoxic conditions. Postlarvae and newly metamorphosed crabs were placed separately in glass vials (5.9 cm × 2.7 cm diam.) containing 20 ml of deoxygenated water and exposed to anoxic conditions for 0 (normoxic controls), 1, 2, or 3 h. At each treatment (exposure) level, 50 megalopae and J1 crabs were tested. Anoxic conditions were maintained during the exposure period by continuously bubbling N_2 into chambers via a needle inserted through a rubber stopper sealing the top of the glass vial. The condition of crabs and postlarvae was determined at 20 min intervals. Crabs were scored as being (1) alive (i.e. actively swimming or walking and responsive to mechanical simulation); (2) immobile (i.e. inactive and unable to swim or walk but occasionally responsive to mechanical simulation); or (3) dead (i.e. legs retracted, carapace opaque and unresponsive to mechanical simulation). At the end of the exposure period, active and immobile crabs were transferred to air-saturated water and their condition and recovery from anoxia was monitored at 20 min intervals for 4 h.

Effect of hypoxia on metamorphosis. Two experiments were conducted to examine the effects of hypoxia on the time to metamorphosis (molting) of crabs from megalopae to the J1 stage. In the first, a cohort of megalopae was collected at the same time and transferred to glass vials containing 20 ml of either hypoxic (P_{O_2} = 8.21 or 12.32 kPa [40 or 60% saturation]) or normoxic (100% saturation) water and monitored every 6 h for metamorphosis. As in the previous experiments, oxygen tensions within the chambers were maintained at target P_{O_2} values by gently bubbling the water in the vials with mixtures of N_2/air delivered through needles inserted through the rubber stoppers sealing the tops of the vials. Megalopae were maintained at 24 to 25°C and transferred to new water and fed brine shrimp (*Artemia* spp.) nauplii daily.

Since oxygen levels in shallow areas often fluctuate throughout the day, the second experiment examined the possible effects of brief, daily exposure to hypoxic conditions on metamorphosis. The protocol was the same as for the previous experiment except megalopae were exposed to hypoxic conditions (20% saturation) for only 2 or 4 h each day. Hypoxic exposure occurred at the same time each day (14:00 to 18:00 h) and was initiated by transferring megalopae to new vials containing hypoxic (P_{O_2} = 4.12 kPa) water. At the end of each exposure period, postlarvae were returned to vials containing normoxic water. To avoid the potential confounding effects of disturbance and handling on metamorphosis, control animals were transferred to new vials containing normoxic water.

At each oxygen tension (Experiment 1: 40, 60 and 100% saturation) or exposure period (Experiment 2: 0, 2 and 4 h), 18 megalopae were tested, and each experiment was repeated 3 times with different cohorts (18 animals/treatment/trial × 3 trials = 54 animals/treatment). Time to metamorphosis was determined to be the time interval (to the nearest 6 h) between placement in the vials and molting. Comparisons of time to metamorphosis among treatments were made using failure-time analysis (Cox Proportional Hazards Model, SAS PHREG Procedure, SAS Institute 1990). Time to metamorphosis was the response of interest and was substituted for 'time until an event occurs' in the analysis (Muenchow 1986, Kleinbaum 1996). Thus, the 'hazard function' for each treatment group was the probability that a crab would metamorphose during the next time interval (Δt), given that it had not metamorphosed since the experiment began (Muenchow 1986, Kleinbaum 1996). Experiments continued for up to 10 d and megalopae that died (<1%) or failed to metamorphose

by the end of the experiment were treated as right-censored observations in the analysis. Since time to metamorphosis often varies significantly between megalopae collected at different times (Forward et al. 1996), cohort group was included as a covariate in the analysis. Thus, the Cox Model was used to calculate 'metamorphosis curves' (cumulative percent metamorphosis vs time) adjusted for the effects of cohort group for each treatment, and comparisons between hypoxia treatments and normoxic controls were made using a Wald (χ^2) test (Allison 1995).

RESULTS

Oxygen consumption

Oxygen consumption (V_{O_2}) of J1 crabs was significantly greater than that of megalopae at the same P_{O_2} (Fig. 1, Table 1). Although V_{O_2} for both groups declined significantly with progressive hypoxia (i.e. significant within subject effects; Table 1), the pattern of the response differed significantly between postlarval and newly metamorphosed crabs (i.e. significant P_{O_2} × Stage interaction; Table 1). Examination of the first order polynomial contrasts indicated the linear trend accounted for most (83.9%) of the variability across oxygen tensions (Table 1). Oxygen uptake rates for megalopae remained relatively constant ($V_{O_{2max}}$ = 175.53 µl O_2 h^{-1} $ind.^{-1}$) and independent of P_{O_2} at tensions above ~43% saturation (P_c = 8.88 kPa; Fig. 1A). In contrast, juvenile crabs were only able to maintain maximum oxygen consumption ($V_{O_{2max}}$ = 561.0 µl O_2 h^{-1} $ind.^{-1}$) at P_{O_2} > 18.4 kPa (89.6% saturation; Fig. 1B). The observed increase (3.1-fold) in $V_{O_{2max}}$ following metamorphosis cannot be attributed fully to size differences since J1 crabs are only ~60% larger than megalopae (based on dry wt; Tankersley unpubl. data). Both J1 crabs and megalopae continued to consume oxygen at P_{O_2} = 1 kPa (~5% saturation).

Table 1. *Callinectes sapidus*. Results of repeated measures analysis of variance (ANOVAR) on the effect of developmental stage (Stage; megalopae vs first-instar crab) on the oxygen consumption (V_{O_2}). Results of first degree polynomial contrasts are presented to test for significant within-subjects (i.e. declining P_{O_2}) effects

Source	df	MS × 10^4	*F*	p
Between subjects				
Stage	1	377.18	99.30	<0.0001
Error	18	3.80		
Within subjects				
P_{O_2}	9	13.77	39.78	<0.0001
Linear	1	104.10	49.11	<0.0001
P_{O_2} × Stage interaction	9	6.11	17.65	<0.0001
Error	162	0.35		

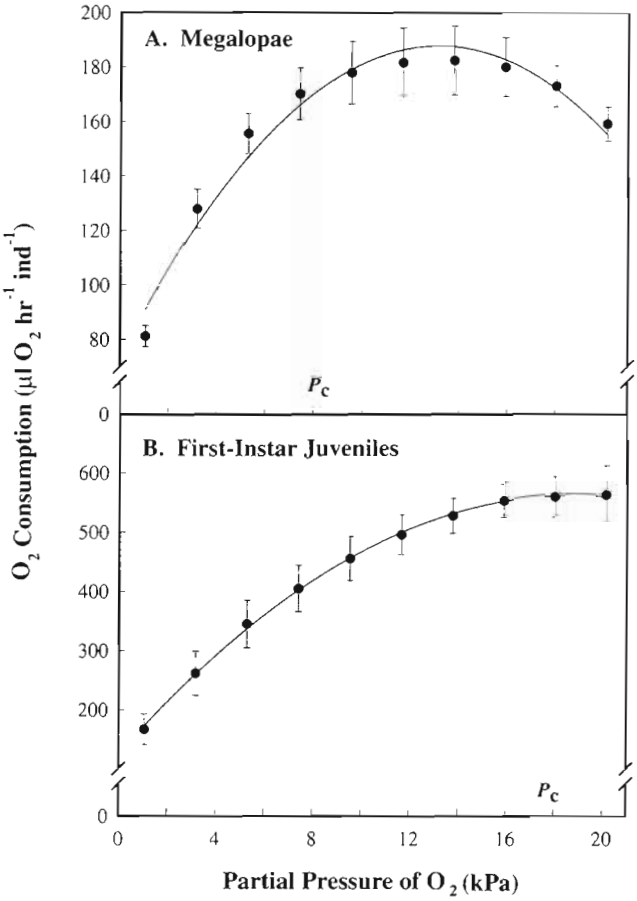

Fig. 1 *Callinectes sapidus*. Mean (±SE) rates of oxygen consumption (V_{O_2}) of (A) megalopae and (B) first-instar juvenile crabs as a function of water P_{O_2} (N = 10). Oxygen uptake was measured at intervals of 2 kPa. Fit of the quadratic polynomial model is represented by the solid line. Vertical dashed line indicates the position of the critical oxygen tension (P_c)

Analysis of the V_{O_2} response curves using the quadratic (i.e. second-degree polynomial) model technique described by Van Winkle & Mangum (1975) revealed the same ontogenetic shift in the ability of newly metamorphosed J1 crabs to regulate aerobic metabolism. The quadratic model provided a good fit to the data for both groups (Fig. 1; megalopae: R^2 = 0.97; juveniles: R^2 = 0.99), but the value of the coefficient (β_2) for the quadratic term was significantly less for megalopae than for juveniles (-6.47×10^{-5} vs -3.92×10^{-5}, respectively), indicating a greater degree of oxyregulation by megalopae at high oxygen tensions (Mangum & Van Winkle 1973, Van Winkle & Mangum 1975). Nevertheless, β_2 values for both groups were significantly less than 0 (megalopae: $t = -10.60$, df = 7, $p < 0.0001$; juveniles: $t = -27.66$, df = 7, $p < 0.0001$).

Tolerance of hypoxia and anoxia

Tolerance of anoxic and hypoxic conditions by postlarval and juvenile *Callinectes sapidus* was dependent upon developmental state, with J1 crabs experiencing greater mortality than megalopae at the same P_{O_2} level and exposure time (Fig. 2). No mortality occurred in megalopae and juveniles maintained in normoxic (control) conditions during the 24 h exposure period. Comparison of the survivorship curves for the hypoxia and anoxia treatments using a Mantel log-rank test indicated that mortality for both developmental stages increased significantly with decreasing P_{O_2} levels (Fig. 2: megalopae: $\chi^2 = 230.4$, df = 2, $p < 0.0001$; J1 crabs: $\chi^2 = 144.7$, df = 2, $p < 0.0001$). Although median survival times (MST) were consistently higher for megalopae than for juveniles (Table 2), neither group was able to tolerate anoxia for more than a few hours (Fig. 2, Table 2).

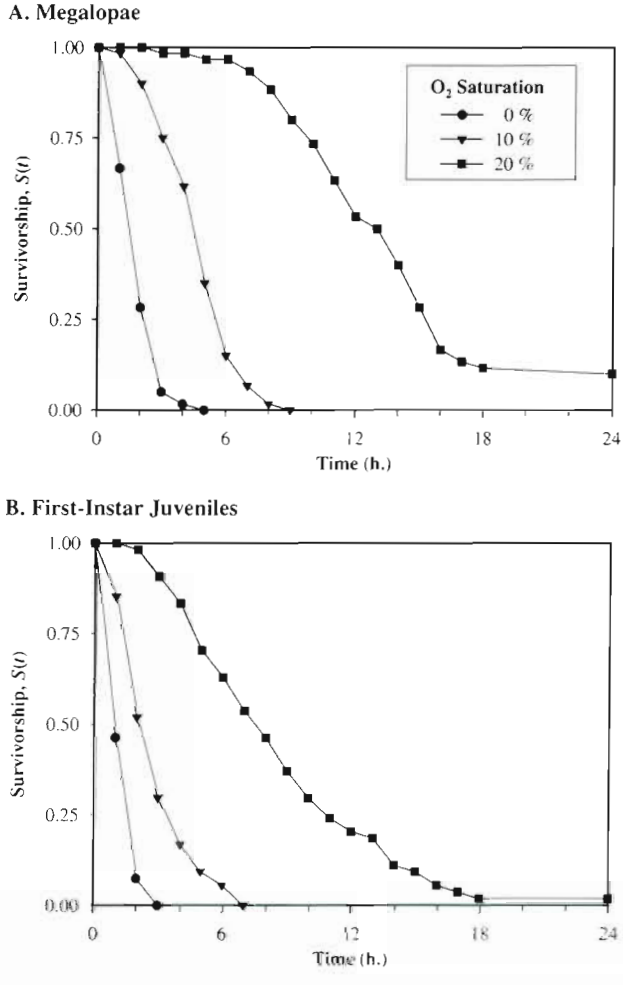

Fig. 2. *Callinectes sapidus*. Kaplan-Meier survivorship [$S(t)$] functions for (A) megalopae and (B) first-instar juvenile crabs subjected to continuous hypoxia (10 or 20% saturation) or anoxia (<1% saturation) for 24 h (N = 60 for megalopae and 54 for juveniles)

Table 2. *Callinectes sapidus*. Median survival times (h) for postlarvae and first-instar juveniles exposed to anoxic (<1% saturation) and hypoxic (10 and 20% saturation) conditions for 24 h

	Oxygen tension		
	<0.2 kPa (0% saturation)	2 kPa (10% saturation)	4 kPa (20% saturation)
Megalopae	1.31	4.00	12.29
Juveniles (J1)	0.99	2.07	7.17

Recovery from short-term exposure to anoxia

As in the previous experiment, both postlarvae and first-instar crabs were unable to withstand short periods of anoxia, but mortality rates for juvenile crabs were consistently higher than for postlarvae. Nearly all (98%) megalopae survived 1 h of anoxia (Fig. 3A), while only 48% of the first-stage crabs were able to tolerate the same period of exposure (Fig. 3D). After 2 h of anoxia, megalopae were either dead (44%) or immobile (56%) (Fig. 3B), while mortality for J1 crabs was nearly 100% (Fig. 3E). None of the first-instar crabs and only 7.5% of the postlarvae survived 3 h of anoxia (Fig. 3C & F). Crabs which became immobile were moribund and typically died within 1 h if they were not transferred to normoxic water. However, all megalopae and J1 crabs that survived the exposure period quickly recovered and survived for at least 4 h upon return to normoxia (Fig. 3).

Effect of hypoxia on metamorphosis

Both continuous and periodic (daily) exposure to hypoxia had a significant effect on the time to metamorphosis of crabs from megalopae to the first-instar stage. Under continuous hypoxia, time to metamorphosis increased significantly (Wald $\chi^2 = 17.77$, df = 1, $p < 0.0001$; Fig. 4), but there was no significant difference between the times to metamorphosis of megalopae subjected to oxygen tensions of 40 and 60% saturation (Wald $\chi^2 = 3.15$, df = 1, $p > 0.05$; Fig. 4). Comparisons of the hazard ratios (risk ratios) revealed that megalopae maintained in normoxic conditions were 2.5 and 1.7 times more likely to metamorphose at any given time than those in the 40 and 60% oxygen saturation treatments, respectively.

Periodic (daily) exposure to hypoxia also had a significant effect on time to metamorphosis (Fig. 5). Metamorphosis was delayed for megalopae exposed to 20% oxygen-saturated sea water for 4 h each day compared to megalopae maintained under constant normoxia

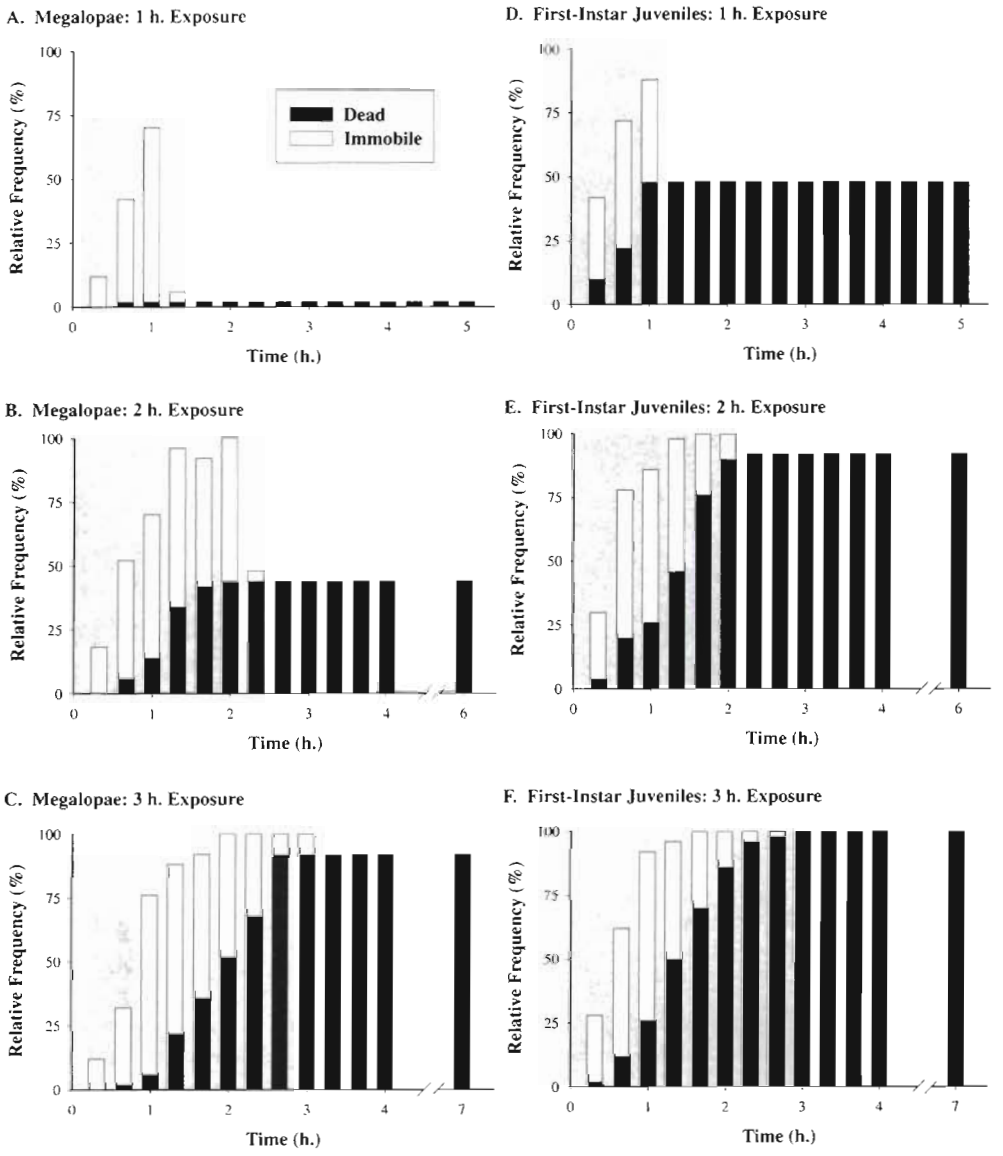

Fig. 3. *Callinectes sapidus*. Relative frequency (%) of immobile (white bars) and dead (dark bars) megalopae (A to C) and newly metamorphosed juvenile crabs (D to F) following exposure to anoxic conditions for 1, 2, and 3 h. Exposure period is indicated by the shaded region. Following exposure, megalopae and juveniles were returned to normoxia and their condition monitored for 4 h (N = 50)

(Wald $\chi^2 = 4.71$, df = 1, $p < 0.05$). Similarly, comparison of the hazard functions for the 2 treatments indicated that postlarvae in the normoxic controls were 1.54 times more likely to metamorphose at any given time than those in the 4 h hypoxia treatment. Although time to metamorphosis increased slightly in crabs exposed to hypoxic conditions for 2 h each day compared to the normoxic controls (Fig. 5), the difference was not statistically significant (Wald $\chi^2 = 1.74$, df = 1, $p > 0.05$).

DISCUSSION

Postlarval and juvenile *Callinectes sapidus* differed significantly in their tolerance of reduced oxygen and their ability to regulate oxygen-uptake under progressive hypoxia. Although megalopae were able to maintain aerobic metabolism at oxygen levels above 8.88 kPa (~43 % saturation; Fig. 1A), oxyregulation was greatly reduced following metamorphosis, with J1 crabs becoming oxydependent at tensions near saturation (P_c = 18.4 kPa; ~90 % saturation; Fig. 1B). Although ontogenetic shifts in oxyregulatory ability have been reported for other decapods, the general trend is toward increased regulation with age. Zoeal and postlarval stages of the king crab *Paralithodes camtschaticus* remain oxyconformers throughout development, but early benthic juveniles show partial regulation following metamorphosis (Nakanishi 1987). Likewise, adult Norwegian lobster, *Nephrops norvegicus* have been reported to be better oxyregulators than the larvae (Spicer 1995). The oxyconformity exhibited by first-instar blue crabs (Fig. 1B) is not retained in older juvenile and adult crabs which possess a pattern that is remarkably similar to the one obtained for megalopae (Fig. 1A; Cameron 1989, Das & Stickle 1993). There-

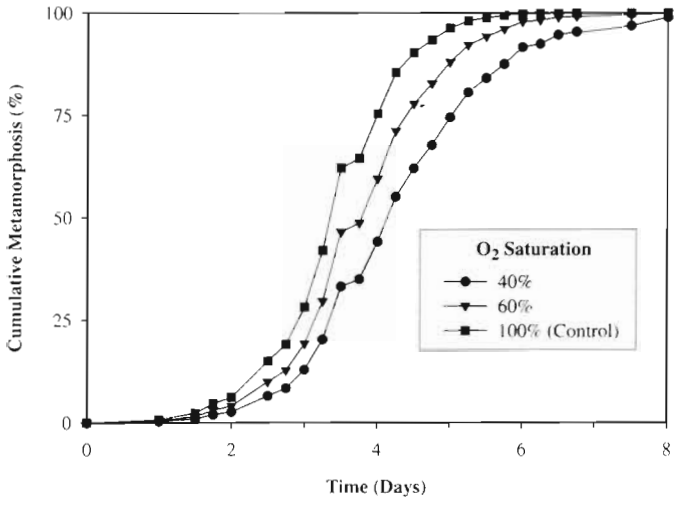

Fig. 4. *Callinectes sapidus*. Plot of cumulative percentage of metamorphosis of megalopae in estuarine water under continuous hypoxia (40 or 60% saturation) and normoxia (air-saturated control). Metamorphosis curves were calculated using the Cox Proportional Hazards Model (N = 54)

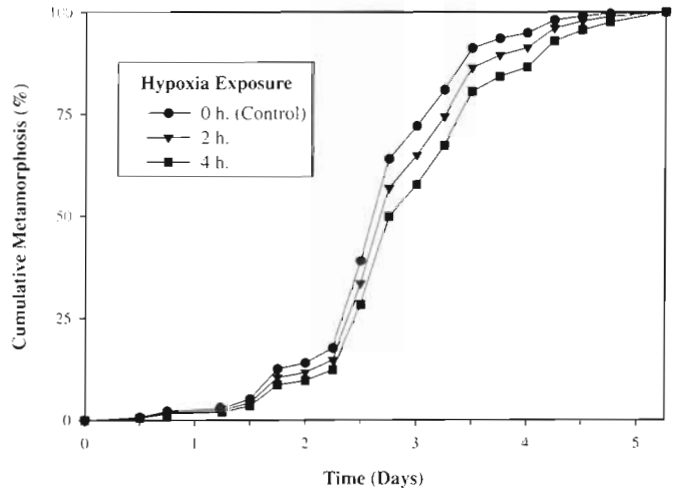

Fig. 5. *Callinectes sapidus*. Plot of cumulative percentage of metamorphosis of megalopae exposed to hypoxic conditions (20% saturation) for 0 (continuous normoxia; control), 2 or 4 h each day. Following each exposure period, postlarvae were returned to normoxic (100% air saturation) conditions. Metamorphosis curves were calculated using the Cox Proportional Hazards Model (N = 54)

fore, respiratory dependence is not a common physiological response to environmental hypoxia for most *C. sapidus* life-history stages and does not appear to be merely a consequence of the transition from a planktonic to benthic existence that accompanies metamorphosis. Additional observations of the aerobic metabolism of older instar juveniles during progressive hypoxia are needed to identify the stage at which oxyregulation improves during development.

Observed differences in the tolerances of megalopae and early benthic juveniles to hypoxic and anoxic conditions were consistent with the V_{O_2} data. Megalopae survived and metamorphosed under chronic exposure to oxygen tensions near or greater than their P_c (≥40% saturation; Fig. 4), but died within a few hours when subjected to P_{O_2} values ≤ 4.12 kPa (≤20% saturation; Table 2, Figs. 2 & 3). First-instar crabs succumbed to the effects of anoxia faster than megalopae (Figs. 2 & 3) and had shorter MSTs at all oxygen tensions (Table 2). However, these results conflict with previous studies of other invertebrate larvae and juveniles which indicate they become more tolerant of hypoxia as they get older (e.g. Widdows et al. 1989, Baker & Mann 1992, Spicer 1995, Eriksson & Baden 1997). Nevertheless, comparisons of the sensitivity of blue crab megalopae and J1 crabs with previous reports of hypoxia tolerance in older crabs indicate there is a general trend towards increased tolerance with age. Das & Stickle (1993) reported juvenile *Callinectes sapidus* are very sensitive to chronic (28 d) hypoxia, with a LC_{50} value of 69.4% saturation (14.12 kPa). Crabs exposed to P_{O_2} values of 0 and 3.33 kPa (0 and 16% saturation, respectively) died within 6 d of exposure, but most crabs survived at least 7 d at P_{O_2} ≥ 6.67 kPa (32% saturation). Comparative laboratory studies conducted by Stickle et al. (1989) yielded similar results but indicated that *C. sapidus* is less tolerant of hypoxic conditions than other estuarine crustaceans including the xanthid crabs *Eurypanopeus depresus* and *Rhithropanopeus harrisii* and the grass shrimp *Palaemonetes pugio*. Collectively these results suggest that (1) blue crabs, in general, are very sensitive to hypoxia; (2) tolerance increases with development from megalopa to adult; but (3) first-instar juveniles deviate from this pattern and are more vulnerable to hypoxia, most likely as a result of rapid anatomical or physiological changes that occur during metamorphosis.

In the current study, tolerance of hypoxia and anoxia was measured using intermolt (Stage C) megalopae and J1 crabs. Consequently, our results most likely underestimate the sensitivity of newly metamorphosed crabs to reduced oxygen conditions since hypoxia tolerance is expected to decrease during metamorphosis. In adult blue crabs, molting is a stressful, energy-demanding process that is accompanied by changes in the performance of the cardiovascular and ventilatory systems, including blood gas tensions, acid-base balance and oxygen-transport properties (deFur et al. 1985, Mangum 1985, Mangum et al. 1985, Cameron 1989). Decreased net synthesis of the oxygen-carrying protein hemocyanin (Hc) and water uptake prior to ecdysis causes Hc levels to decline more than 4-fold in postmolt crabs (Mangum et al. 1985). Ventilation also decreases briefly during and immediately following

exuviation as the scaphognathite hardens (deFur et al. 1985). Although it is unknown whether the respiratory and cardiovascular systems of megalopae undergo similar changes during metamorphosis, it is safe to assume that the mechanisms for oxygen transport are perturbed during ecdysis and the chances of surviving hypoxia stress and other adverse environmental conditions are severely reduced.

Metabolic regulation and P_c have been reported to vary with environmental conditions and physiological state of the animal, including temperature, salinity, activity, molt cycle, time of day, circulation and availability of respiratory structures (for reviews see Herreid 1980, McMahon & Wilkens 1983). Since the experimental procedures and conditions used to quantify respiratory response and sensitivity to hypoxia and anoxia were identical for postlarvae and juveniles, the observed differences in the ability of post-settlement crabs to regulate oxygen-uptake and tolerate hypoxia and anoxia are most likely the result of ontogenetic changes in the respiratory and cardiovascular systems and the compensatory mechanisms available for coping with reduced oxygen. In adult blue crabs, oxygen supply is maintained under moderate hypoxia by increasing ventilation through increased scaphognathite activity, while stroke volume and extraction efficiency remain constant (Batterton & Cameron 1978). Prolonged hypoxic (sublethal) exposure results in structural changes in blue crab Hc, which increases its oxygen affinity, thereby enhancing blood oxygenation at the gill (deFur et al. 1990, Mangum 1997). The structure of crustacean Hc also often changes with ontogeny (see Terwilliger 1998 for review) and larvae of several decapod species have been shown to possess an Hc with a lower oxygen-affinity than adult Hc (Terwilliger et al. 1986, Olsen et al. 1990, Terwilliger & Brown 1993, Spicer 1995, Brown & Terwilliger 1998). Similar developmental changes in the site of gas exchange and the concentration, structure and function of Hc may impose constraints on oxygen transport in J1 crabs and may explain, at least in part, the observed shift in the respiratory responses of megalopae and first-instar crabs to hypoxia.

Metamorphosis of blue crab megalopae to the J1 crab stage occurs within estuaries following development in offshore coastal/oceanic areas. Recent studies indicate that the duration of the megalopal stage, and therefore the rate of development, is influenced by the presence of chemical cues from estuaries and known settlement habitats. Metamorphosis is delayed in offshore water (Forward et al. 1994, 1996, 1997, Wolcott & DeVries 1994) but is accelerated by exposure to estuarine (Forward et al. 1994, 1996) and lower salinity water (Forward et al. 1994), and odors from seagrasses (Forward et al. 1994, 1996), marsh cord grass (Forward et al. 1996), humic acids (Forward et al. 1997) and some macroalgae (Brumbaugh & McConaugha 1995, Forward et al. 1996). Similar cues from settlement habitats, including estuarine sediment and adult odor, accelerate metamorphosis in other brachyuran crabs including *Uca* spp. (Christy 1989, O'Connor 1991, O'Connor & Judge 1997), *Panopeus herbstii* (Weber & Epifanio 1996) and *Rhithropanopeus harrisii* (Fitzgerald et al. 1998). At normal temperature and salinities, the only chemical cue known to reverse the effects of stimulatory cues and increase the time to metamorphosis is ammonium (Forward et al. 1997). Our results indicate that the metamorphosis-accelerating effects of estuarine water are also reversed by exposure to chronic and intermittent hypoxia (Figs. 4 & 5). Constant sublethal hypoxia has previously been found to have a similar effect on the molting rate of later-stage juvenile *Callinectes sapidus* (Das & Stickle 1993). Since ammonium levels in the field are inversely related to oxygen levels (Fitt & Coon 1992), their combined effects may further inhibit metamorphosis. Thus, the effects of hypoxia on metamorphosis are consistent with the hypothesis that conditions in suboptimal settlement habitats inhibit estuarine cues which accelerate metamorphosis, presumably delaying settlement so that megalopae have time to locate other more suitable substrates.

It is well known that marine planktonic larvae use physical and chemical cues in settlement site recognition and settlement (for reviews see Crisp 1984, Pawlik 1992, Rittschof et al. 1998). Recent field studies indicate that blue crab megalopae can distinguish among possible settlement sites using chemical cues (Welch et al. 1997). Thus, high densities of megalopae and early-instar blue crabs in vegetative habitats, such as seagrass beds and tidal marshes (Orth & van Montfrans 1987, Mense & Wenner 1989, Lipcius et al. 1990, Wilson et al. 1990, van Montfrans et al. 1991, Morgan et al. 1996), are probably the result of differences in post-settlement mortality and emigration among habitats as well as active selection or rejection of potential vegetative or unvegetative habitats based upon chemical cues (Welch et al. 1997). Since habitats experiencing both long-term and periodic hypoxia would be physiologically stressful or lethal to megalopae and post-metamorphic crabs, differences in their distribution among habitats may reflect active avoidance of oxygen-depleted water in shallow areas as well as increased mortality in areas prone to seasonal or diel occurrences of hypoxia. Likewise, habitat avoidance and post-settlement mortality may depend upon multiple negative cues or abiotic factors that typically co-occur in areas of reduced oxygen, such as salinity, hypercapnia and the presence of sulfide, which may be more toxic or effective negative cues when combined with hypoxia than hypoxia alone (Diaz & Rosenberg 1995).

In addition to affecting habitat selection, behavioral responses for avoiding hypoxic bottom water (e.g. increased activity or negative geotaxis) may also interfere with the flood-tide transport behavior of crab megalopae during shoreward migration to settlement habitats by (1) serving as a physical barrier to vertical migration and preventing crabs from reaching the substrate and exiting the water column at the end of flood tide or (2) inducing crabs to reenter the water column at the incorrect phase of the tide. Both scenarios would reduce recruitment success by increasing the likelihood that crabs are transported seaward by ebb currents. Behavioral avoidance of lethal and sublethal bottom water has been documented for a variety of benthic invertebrates and fish (e.g. May 1973, Harper et al. 1981, Baden et al. 1990, Petersen & Petersen 1990, Pihl et al. 1991, Breitburg 1994, Howell & Simpson 1994, Nilsson & Rosenberg 1994). Field studies examining the effect of reduced oxygen conditions on the vertical distribution of adult *Callinectes sapidus* indicate they avoid hypoxic bottom water by migrating to shallower areas (Pihl et al. 1991, Diaz et al. 1992). However, laboratory assays suggest the ability of juvenile blue crabs to detect and avoid such conditions may be limited (Das & Stickle 1994). Experiments are currently underway to evaluate the behavioral responses of postlarvae and early-instar juvenile blue crabs to hypoxia in order to determine its potential influence on up-estuary transport, habitat selection and settlement.

Previous studies of the effects of hypoxia on the physiology, behavior and population dynamics of *Callinectes sapidus* have not included postlarval and early juvenile stages. Moreover, studies examining the population dynamics of early benthic stage crabs have focused on factors influencing the availability of postlarvae among habitats (Olmi et al. 1990, Morgan et al. 1996) and the contribution of density-dependent processes to observed patterns in habitat use (Wilson et al. 1987, 1990, Mense & Wenner 1989, Heck & Coen 1995, Morgan et al. 1996, Pile et al. 1996, Moksnes et al. 1997). The present study indicates that reduced oxygen conditions may be an important density-independent (abiotic) factor regulating the supply of postlarvae and survivorship of post-settlement crabs. This suggests, together with the ability of megalopae to select among potential habitats, that the recruitment of megalopae may be significantly impacted by variation in the distribution and severity of hypoxic water in vegetative areas. Since blue crab megalopae and juveniles depend upon habitat refugia, additional field studies are needed to determine the extent to which hypoxia limits the availability or relative value of nursery and refuge habitats, and its impact, either direct or indirect, on important post-settlement processes such as predation, competition and emigration.

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