Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean

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The most fragile skeletons produced by benthic marine calcifiers are those that larvae and juveniles make to support their bodies. Ocean warming, acidification, decreased carbonate saturation and their interactive effects are likely to impair skeletogenesis. Failure to produce skeleton in a changing ocean has negative implications for a diversity of marine species. We examined the interactive effects of warming and acidification on an abalone (Haliotis coccoradiata) and a sea urchin (Heliocidaris erythrogramma) reared from fertilization in temperature and pH/pCO2 treatments in a climatically and regionally relevant setting. Exposure of ectodermal (abalone) and mesodermal (echinoid) calcifying systems to warming (+2 °C to 4 °C) and acidification (pH 7.6–7.8) resulted in unshelled larvae and abnormal juveniles. Haliotis development was most sensitive with no interaction between stressors. For Heliocidaris, the percentage of normal juveniles decreased in response to both stressors, although a +2 °C warming diminished the negative effect of low pH. The number of spines produced decreased with increasing acidification/pCO2, and the interactive effect between stressors indicated that a +2 °C warming reduced the negative effects of low pH. At +4 °C, the developmental thermal tolerance was breached. Our results show that projected near-future climate change will have deleterious effects on development with differences in vulnerability in the two species.

Keywords: climate change; ocean warming; ocean acidification; calcification; sea urchin; abalone

1. INTRODUCTION

The gametes of marine invertebrates are being spawned into an ocean simultaneously warming, decreasing in pH and increasing in pCO2 [1–4]. These stressors exert negative effects on marine biota [5–7] and may have deleterious interactive effects [8,9]. Marine calcifiers appear particularly vulnerable to climate change because ocean acidification reduces the availability of the carbonate ions required to construct their skeletons [10], although this differs among species and life stages [6–8,11–20]. The most fragile skeletons produced by benthic marine invertebrates are those that larvae and early juveniles make to support their bodies [13]. Failure to produce these skeletons in a changing ocean would have negative consequences for a great diversity of marine species. For marine invertebrates, future prospects for the early life-history stages are of particular concern because their sensitivity to climate change stressors may be the bottleneck for persistence of some species in a changing ocean [5–7].

Ocean acidification increases physiological pCO2 and so is linked to increased organism hypercapnia (acidosis), a stressor that suppresses metabolism [21–23]. By contrast, increased temperature has a stimulatory effect on metabolism, potentially countering the negative effects of acidification and hypercapnia on calcification and other physiological processes. Small increases in temperature (approx. 2 °C) can diminish the negative effects of ocean acidification on calcification in sea urchin larvae and coral growth [9,24]. For some species, ocean acidification has a positive effect on growth, although in a juvenile sea star this was not because of an increase in skeleton [12,17]. The responses of organisms and marine ecosystems to multiple climate change stressors appear to be species-specific and so are difficult to predict [5–7,25–27].

The Mollusca and Echinodermata are significant calcifying marine phyla and have different calcifying systems [28,29]. In mollusc development an aragonite skeleton is produced in the ectoderm tissue layer, while in echinoderm development a magnesium calcite skeleton is produced within mesoderm. Here we investigated the response of skeleton development in a mollusc (abalone Haliotis coccoradiata) and an echinoderm (sea urchin Heliocidaris erythrogramma) to near-future ocean warming and acidification. These species co-occur in southeast...
Australia, a climate change hotspot, where the ocean is warming several times faster than the global average [30]. They represent ecologically and commercially important marine invertebrates because they function as grazers that structure habitats and are targeted by fisheries [31,32]. We investigated the interactive effects of near-future (ca 2100 [4]) ocean warming, acidification/hypercapnia, and decreased aragonite and calcite saturation states on development in these two species. Owing to uptake of CO$_2$, surface ocean waters are projected to decrease in pH by 0.4 U by 2100 and 0.7 U by 2300 [1]. In southeast Australia, climate-driven changes to the East Australian Current has resulted in significant warming of coastal waters, with a projected increase of 1–2°C by 2030 and 2–3°C by 2100 [30,33].

Fertilization in _H. coccordiata_ and _H. erythrogramma_ is robust to climate change stressors and early development is sensitive to warming [15,34–37]. These species have large eggs (_H. coccordiata_, 175 μm diameter eggs; _H. erythrogramma_, 440 μm diameter eggs), and their rapid development through lecithotrophic larvae facilitates access to the first calcified stage [37,38]. Owing to their developmental mode, we did not have to feed the larvae, avoiding the potentially confounding influence of the variable responses of feeding larvae to nutrient regime [39]. This mode of development is characteristic of _Haliotis_ [37]. The endpoint we used was the 21 h shelled veliger stage. Lecithotrophic development, as seen in _H. erythrogramma_, is reported in approximately 45 per cent of Echinoidea that have been investigated [40]. This species lacks a calcified larva [38] and so we focused on the early juvenile (5 day), one of the least studied stages of marine invertebrate development with respect to changing ocean conditions.

Concerns about future prospects for marine calcifiers have prompted numerous studies on the impacts of climate change on development with a focus on acidification (reviews in [5–7,13]). The most common approach has involved the transfer of larvae or juveniles from present-day to experimental conditions (e.g. [19,41]). A recent study, however, has shown that oyster larvae reared in ocean change conditions from the outset of development (fertilization) were more severely impacted by stressors than those placed in treatments as later-stage embryos [8]. Developmental success requires that all ontogenetic stages be completed successfully, and it is likely that stressor effects are integrated across developmental stages. To identify potential developmental bottlenecks, we reared abalone and urchin embryos from the onset of development (fertilization) in multifactorial experimental treatments. To our knowledge, this is the first study of the interactive effects of ocean warming and CO$_2$-driven acidification on marine life histories from the beginning of development to the benthic juvenile. We predicted that ocean warming and acidification would have a negative impact on development (e.g. decreased calcification), with the possibility that warming might diminish the negative effects of decreased carbonate availability on growth and calcification. The morphology of the skeleton and shell was used as an indicator of calcification, as in previous studies [5–7,13]. We also predicted that because of its location close to the surface in ectoderm, the _H. coccordiata_ shell might be more vulnerable to stressors than the internal skeleton of _H. erythrogramma_.

2. METHODS

(a) _Study species and collection site_

_Haliotis coccordiata_ and _H. erythrogramma_ were collected during their peak reproductive season (October–November and December–January, respectively) [37,42], from Little Bay (33°58’S, 151°14’E), an open ocean site near Sydney where they co-occur in shallow-water boulder habitat. The size range used (_H. coccordiata_ shell length 25–35 mm; _H. erythrogramma_, test diameter 65–85 mm) was kept as small as possible to reduce interindividual variability in gamete quality. Animals were transported in ambient sea water in a cool box and placed in aquaria at ambient sea surface temperature (SST). They were used for experiments within days of collection. Sea water for experiments was collected at high tide near the collection site. During the collection period, SST averaged 20°C for abalone and 22°C for urchin as determined from the Physical Oceanography DAAC Ocean ESIP Tool (POET) website (http://poet.jpl.nasa.gov) and a local reference station (www.mhl.nsw.gov.au). These temperatures were used as the control treatments.

Freshly collected filtered sea water (FSW; 1.0 μm) was used for all experiments. Sea water parameters—pH$_{NBS}$, dissolved oxygen (DO), temperature—were measured using a WTW multimeter (Multiline P4) and total alkalinity (TA) was determined by potentiometric titration (CSIRO Laboratories, Hobart). Sea water was collected twice for the abalone experiments and three times for the sea urchin experiments. Parameters for these water sources (electronic supplementary material, tables S1 and S2) were similar across the five water collections (x salinity 36.9 ppt, s.e. = 0.2; x pH = 8.18, s.e. = 0.03; x TA = 2368, s.e. = 15).

The experiments were designed within the context of the ‘business-as-usual’ emissions scenario for an estimated increase in surface ocean pCO$_2$ from present levels of around 380 to 700–1000 ppm by 2100 and greater than 2000 ppm by 2300, levels modelled to drop in surface ocean pH by 0.14–0.41 and 0.30–0.70 U, respectively [2,4,10]. Local waters are projected to increase in SST by 2–3°C by 2100 (CSIRO Model 3.5 in [30,33]). While we do not know the details of future local SST/pH/pCO$_2$ conditions in the habitat where the study organisms live, we used these modelled estimates to guide our experimental design to reflect projections for 2100 and beyond. The experiments involved three temperature (ambient control, +2°C, +4°C) and three pH (ambient control, pH 7.8, pH 7.6) treatments used in all combinations.

Experimental pH$_{NBS}$ and DO greater than 90 per cent were achieved by bubbling CO$_2$ gas and air into the FSW until the desired pH was reached. Large volumes (20–30 l) of sea water were equilibrated to each temperature/pH treatment to ensure that embryos from experimental replicates were placed in the same conditions. This water was placed in the 500 ml beakers for fertilization and into the rearing chambers (140 ml glass jars), sealed with minimal headspace. These were placed in water baths set to the experimental temperature. Data from temperature loggers (iBTag model iBCod G, Thermodata) placed in the baths confirmed that the temperature conditions were stable. Experimental pCO$_2$ and aragonite (_I$_{50}$_) and calcite (_I$_{90}$_) saturation values for each temperature–pH combination (electronic supplementary material, tables S1 and S2) were determined from TA, pH$_{NBS}$ and salinity data using CO2SYS [43]. For abalone, we used _I$_{50}$_ to assess mineral availability. For urchin, we consulted data for _I$_{50}$ _ and _I$_{90}$_ because the...
saturation state of echinoderm magnesium calcite is not known, although is likely to be closest to aragonite [44]. The saturation dynamics of the echinoderm skeleton depends on its Mg\(^{2+}\) content, and this is variable [28,45]. The mean \(\Omega_{sa}\) ranged from 1.47 (20\(^\circ\)C/pH 7.6) to 5.11 (26\(^\circ\)C/pH 8.2), while that for \(\Omega_{sa}\) ranged from 0.95 (20\(^\circ\)C/ pH 7.6) to 3.76 (26\(^\circ\)C/pH 8.2; electronic supplementary material, tables S1 and S2).

**(b) Fertilization and rearing conditions**

For each experimental replicate, a different population of embryos derived from multiple parents (greater than or equal to two to three of each sex) were used. For sea urchin, we used six embryo populations across the multifactorial treatments. For abalone, we used 10 embryo populations. The urchins were induced to spawn by injection of 1–3 ml of 0.5 M KCl and the gametes were collected using pipettes. To induce spawning in abalone, males and females were separately placed in 500 ml of FSW at control temperature with addition of 1.5 ml of 6 per cent hydrogen peroxide. The pooled eggs were transferred into beakers (500 ml) of fresh FSW at control temperature. Urchin sperm were collected dry and abalone sperm were collected on release through the shell pore. Pooled sperm from multiple males were mixed in a small dish, covered and kept cool (4\(^\circ\)C until use. The eggs were checked microscopically for shape and integrity, and sperm were checked for motility before use. Baths were switched between runs to avoid a potential bath effect. The experiments were conducted over a four-week period for *H. erythrogramma* and a six-week period for *H. coccoradiata*.

Prior to fertilization, the eggs (approx. 3 ml\(^{-1}\)) were placed in 500 ml of experimental FSW for 15 min. The required amount of sperm to have a final concentration of 10\(^6\) sperm ml\(^{-1}\) was determined in haemocytometer counts of the semen samples. Sperm were briefly (1–2 s) activated in experimental water and determined in haemocytometer counts of the semen samples. Embryos derived from multiple parents (greater than or equal to two to three of each sex) were used. For sea urchin, we performed using NCSS software (v. 17.0).

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3. EFFECTS OF OCEAN WARMING AND ACIDIFICATION ON THE PERCENTAGE OF CALCIFIED H. COCCORADIATA VELIGERS

In H. coccoradiata reared in control conditions, the shell gland is evident by 11 h in trochophore larvae [37]. This is followed by hatching (18.5 h) and formation of the larval shell at 19.5 h. By 21 h, the veligers have a well-developed shell (figure 1a).

With increased temperature (+2°C, +4°C) and acidification (−0.4 pH units, −0.6 pH units), abnormal embryos and developmental failure were observed (figure 1b–d and electronic supplementary material, table S1). There was a significant reduction in the percentage of calcified veligers (temperature: \( p < 0.005 \); pH: \( p < 0.001 \)), and Neuman–Keul’s tests indicated that the percentage of calcified larvae was significantly lower in the pH 7.6–7.8 treatments and in the +2 to +4°C treatments compared with controls (figure 2 and table 1). There was no significant interaction between factors with a consistent pattern across temperature at each pH (figure 2; temperature \( \times \) pH: \( p = 0.552 \)).

A 4°C warming breached the thermotolerance of H. coccoradiata embryos, with only 20% per cent normal larvae at control pH (figure 2 and table 1). Most of the larvae reared in all pH 7.6 treatments were dead or severely abnormal at 21 h (electronic supplementary material, figure S1). The sharp drop in \( \Omega_P \) at pH 7.8 (electronic supplementary material, table S1) may have influenced the prevalence of unshelled veligers in these treatments (figure 1b–d and electronic supplementary material, table S1). Some veligers reared in 20°C/pH 7.8 appeared anatomically normal except for the absence of a shell (figure 1c). However, they did not form a shell after transfer to control sea water for 3 days.

4. EFFECTS OF OCEAN WARMING AND ACIDIFICATION ON DEVELOPMENT OF THE JUVENILE H. ERYTHRORAGNUMA

In H. erythrogramma, addition of the algal cue on day 4 induced rapid settlement in most larvae and by day 5 most specimens in the control pH treatments reared at 22°C and 24°C had metamorphosed into juveniles (figure 1e–h).

Increased temperature (4°C) and lower pH (pH 7.6–7.8) had a significant effect on the percentage of normal juveniles (figure 3 and table 2). Abnormal phenotypes were observed in the more extreme temperature and pH treatments (figure 1g,h and electronic supplementary material, table S1). Both factors exerted a significant negative effect on development (temperature: \( p < 0.001 \); pH: \( p < 0.001 \)). There was a significant interaction between factors (temperature \( \times \) pH: \( p < 0.005 \)). Neuman–Keul’s test indicated that the percentage of normal juveniles was higher in the 22°C and 24°C control pH treatments compared with all reduced pH treatments and the +4°C control pH treatment (figures 1 and 3a, table 2 and electronic supplementary material, table S1). At +4°C (26°C), development failed (less than 20% normal juveniles) across all treatments (figure 3a). This temperature increase breached the thermal tolerance for development in most larvae.

Temperature and pH had a significant effect on spine number (temperature: \( p < 0.001 \); pH: \( p < 0.001 \), with
Warming and acidification both exerted deleterious effects, the magnitude of which differed between the species. Development in the abalone _H. coccoradiata_ was vulnerable to near-future levels of warming and acidification (+2°C/pH ∼0.4 U). Limited thermotolerance appears to be a feature of abalone development [46]. Only a small percentage of embryos formed normal larvae in warm (+2°C to 4°C) and low-pH (pH ∼0.4 to 0.6 U) conditions. Calcification was inhibited in the low-pH treatments as aragonite became undersaturated, potentially exacerbated by hypercapnic suppression of metabolism. Experimental conditions exceeded the ability of the larvae to control the calcification environment in the shell gland in favour of aragonite accretion. The abalone shell is secreted on the larval surface, so its integrity is likely to be sensitive to water chemistry. Calcification did not recover when unshelled larvae were placed in control sea water. This differs from what has been reported for corals where skeleton-less polyps grown in ocean acidification conditions were able to secrete skeleton on transfer to ambient sea water [47]. Near-future ocean conditions resulted in unshelled _H. coccoradiata_ larvae, a condition that prevents survival to the juvenile stage in abalone [48].

The only other molluscs veligers that have been investigated with regard to climate change stressors are those of bivalves. In _Crosostrea gigas_, _Crosostrea ariakensis_ and _Mytilus galloprovincialis_, deleterious effects in single-stressor acidification studies are reported at more extreme pH levels (pH ≤ 7.4/Ωar ≤ 0.8) than used here [13,18,49]. However, this varies even among congeners, with the veligers of _C. virginica_ being more sensitive, producing smaller larval shells (but not unshelled veligers) at pH 7.8 [18]. Calcification in bivalve veligers is less sensitive to acidification than seen here for _H. coccoradiata_. If larvae of other _Haliotis_ species are similarly sensitive, this does not bode well for abalone populations, which are of significant economic value and are already in trouble owing to global warming, increasing risk of disease [32]. More data are required to determine whether high sensitivity to climate change stressors is specific to _Haliotis_ or also a feature of other gastropod larvae.

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**Table 1. Analysis of variance results for percentage of calcified _H. coccoradiata_ veliger larvae from 10 independent populations of embryos. Temperature and pH were fixed factors. NK, Neuman–Keul’s post hoc test.**

<table>
<thead>
<tr>
<th>factor</th>
<th>sum of squares</th>
<th>mean square</th>
<th>d.f.</th>
<th>F-value</th>
<th>p-value</th>
<th>NK</th>
</tr>
</thead>
<tbody>
<tr>
<td>temperature</td>
<td>1.705</td>
<td>1.705</td>
<td>1</td>
<td>10.96</td>
<td>&lt;0.005</td>
<td>22 &lt; 20</td>
</tr>
<tr>
<td>pH</td>
<td>5.096</td>
<td>2.547</td>
<td>2</td>
<td>16.39</td>
<td>&lt;0.001</td>
<td>(7.6, 7.8) &lt; 8.2</td>
</tr>
<tr>
<td>temperature × pH</td>
<td>0.186</td>
<td>0.093</td>
<td>2</td>
<td>0.60</td>
<td>0.552</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>13.06</td>
<td>0.155</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>89</td>
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</tr>
<tr>
<td>total</td>
<td>90</td>
<td></td>
<td></td>
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</tr>
</tbody>
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**Figure 3. (a) Mean percentage (± s.e.) of normal _H. erythrogramma_ juveniles in nine treatments (three pH × three temperature). (b) Mean number of spines (± s.e.) produced by juveniles in treatments. Values for pCO2 and aragonite and calcite saturation are provided in electronic supplementary material, table S2. Letters above the bars indicate treatments that did not differ significantly. n = 6. White bars, pH 8.2; grey bars, pH 7.8; black bars, pH 7.6.**

A significant interaction between factors (temperature × pH; p < 0.001; figure 3b and table 2). The number of spines produced at +2°C in pH 7.8 and 7.6 did not differ from the number produced at ambient temperature and pH (figure 3b and electronic supplementary material, table S1). Newman–Keul’s tests indicated that juveniles reared at +2°C and pH 7.6 and 7.8 had more spines than juveniles reared in control temperature at pH 7.6 and 7.8 (figure 3b, table 2 and electronic supplementary material, table S1). Thus, a 2°C warming diminished the negative effects of acidification on spine development.

Although the percentage of normal juveniles was lower in the pH 7.6 and 7.8 treatments and reduced Ωar and Ωca (electronic supplementary material, table S2), most juveniles possessed skeletal elements, indicating that they had managed to calcify (figures 1 and 3, and electronic supplementary material, table S1). The equivalent of a naked juvenile was not seen for the echi- noid. A substantial proportion of larvae (approx. 25%) reared at +2°C produced normal juveniles at pH 7.6 and 7.8 (figures 1 and 3, and electronic supplementary material, table S1). These offspring tolerated near-future warming and acidification.

**5. DISCUSSION**

Our results show that the early life-history stages of two species from two major benthic calcifying taxa (abalone and sea urchins) reared from fertilization in experimental treatments will be impacted by near-future ocean change. Warming and acidification both exerted deleterious effects, the magnitude of which differed between the species.
Development and calcification in the sea urchin

H. erythrogramma were significantly affected by experimental treatments, but less so than seen for H. coccoradiata. A 2°C warming promoted spine development across all pH levels tested. This level of warming also promotes early metamorphosis and settlement in H. erythrogramma, reducing the planktonic larval duration by 25% per cent with the suggested benefits of reduced planktonic mortality [36]. A further 2°C warming breached the thermostolerance of development (approx. 80% dead or abnormal). The decrease in calcification as seen in spine production in the control-temperature (22°C) and low-pH (7.6, 7.8) treatments is similar to that found in single-stressor studies of echinopluteus larvae, where less skeleton is produced at low pH in larvae reared at ambient temperature [9,16,20,41]. Our multi-stressor study showed, however, that interactive effects of these stressors influenced the outcome for development in a warmer and higher pCO2 ocean. A 2°C warming diminished the negative influence of lowered pH on juvenile calcification in H. erythrogramma, similar to that seen for echinopluteus of Tripneustes gratilla reared in similar multifactorial experiments [9]. Owing to the interdependence between temperature, mineral saturation state and pH, the mechanism of this facilitation is likely to be complex. The increase in development rate and calcification in H. erythrogramma and T. gratilla with increased temperature (+2°C) is typical of echinoderm development [50]. However, the decrease in the percentage of normal juveniles at 20–22°C and pH 7.6–7.8, and the more extreme treatments projected by 2100 (approx. 26°C/pH 7.8), will be detrimental to the fitness of H. erythrogramma.

A substantial proportion (approx. 20–25%) of H. erythrogramma progeny formed normal juveniles in the +2°C/pH 7.6, +2°C/pH 7.8 and +4°C/pH 7.8 treatments. These juveniles were thus able to maintain a high calcium carbonate saturation state at the calcification site to make the test and spines. Owing to enhanced maternal provisioning and deletion of the echinopluteus larva, a developmental stage negatively affected by ocean acidification [9,16,20], H. erythrogramma is independent of the need to produce a functional larval skeleton [38]. Development of the lecithotrophic larvae of the sea star Crossaster papposus is also robust to low pH conditions [12]. Abbreviated lecithotrophic development may make echinoderms with this life-history mode comparatively more robust to ocean change, a trend evident in the evolutionary history of the phylum through past climate change [40].

Despite the well-known controlling influence of temperature on marine invertebrate development [36,50], recent studies on impacts of climate change on life histories have focused on ocean acidification as a single stressor (reviews in [5–7,13]). Marine propagules of shallow-water species, however, are experiencing simultaneous warming and acidification from the outset of development. It is thus crucial to consider tolerance to multiple stressors and potential interactive effects across ontogenetic stages as this more appropriately reflects the real-world scenario predicted for the oceans in the coming decades. Our experiments provided an estimate of the tolerance of abalone and urchin development to warming and acidification, and for H. erythrogramma revealed the interactive effects of stressors.

Ocean warming projected for southeastern Australia [30,33] will breach developmental thermostolerance in the species investigated here with respect to their current tolerance. A significant proportion of H. coccoradiata and H. erythrogramma embryos may not reach the calcifying stage in the conditions projected for local waters. This will probably also be the case for a suite of co-occurring benthic species. However, unlike the experimental conditions in this study, ocean change will be gradual over the coming decades, creating the possibility that adaptation may occur to facilitate survival of planktonic and benthic stages.

In the short-term (ca 2030, 1–2°C increase SST [30]), maintenance of H. coccoradiata and H. erythrogramma populations may be facilitated by southerly migration of warm-adapted propagules in strong boundary current flow with contraction at northern range limits [36]. Owing to poleward invasion of the sea urchin
Centrostephanus rodgersii along new larval migratory corridors, major changes are already under way in the marine ecosystems of southeastern Australia [31]. In the longer term, however, calcifying species may be caught between the need to move south as the waters warm and the need to avoid acidified water expanding northward in the Southern Ocean [51].

Development can fail at any stage, and it is important to identify where vulnerabilities lie. It is clear that mortality bottlenecks in development and impaired calcification will compromise persistence of H. coccoradiata and H. erythrogramma in the face of ocean change. Calcification was inhibited (abalone larvae) or impaired (sea urchin juveniles) in conditions projected for 2100 and beyond [4]. These negative impacts will have significant downstream effects on the survival of populations of these ecologically and economically important organisms. Our results underscore the deleterious effects of projected ocean change on these species based on the business-as-usual scenario, and reveal differences in species vulnerability.

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