Present review discusses about the effects of ocean acidification on key marine invertebrates groups which are major players of ecosystem processes across different coastal environments ranging from mangroves to coral reefs. Effects of ocean acidification on important calcifying marine invertebrate groups such as echinoderms, molluscs and crustaceans have been thoroughly discussed based on observations rendered from laboratory CO
_2_ manipulation experiments as well as from the field. Majority of the experiments have shown sub-lethal effects on larval development and size which could ultimately affect adult population recruitment in coastal ecosystems. Particularly studies which have used ‘omics’ based approaches to investigate effects of ocean acidification on marine invertebrates have been thoroughly discussed in this review.

[Keywords: ocean acidification, carbonate chemistry, marine invertebrates, CO
_2_ experiments]

Introduction

Acidity of the world’s oceans is increasing by absorbing atmospheric carbon dioxide (CO
_2_). There has been a significant rise in atmospheric CO
_2_ concentration due to continuous burning of fossil fuels and projected to rise reaching up to 970 ppm by the end of this century and 1900 ppm by the year 2300
_1_.
_2_. It has been estimated that 50% of the anthropogenic CO
_2_ released in atmosphere has been absorbed by world’s oceans over the last two centuries and approximately 30% of more recent emissions has been taken up by oceans
_3_.
_4_. As a result the average pH of ocean surface have thought to decline by 0.1 unit from pre-industrial level and projected to decrease by 0.3 to 0.46 units by the end of this century, concurrent with the present CO
_2_ emission scenario
_2_.
_5_. Resulting decline in ocean pH is referred to as ocean acidification (OA)
_5_. Predictions are mainly focused on surface ocean waters but estuarine and coastal environments are not well represented due to lack of reliable data, complexity of circulation processes in coastal environments and resulting unreliable models
_6_.

The economic value of coastal ecosystems including mangroves, estuaries, seagrass beds, coral reefs and continental shelf has been estimated in trillions of US dollars annually and accounts for 38% of the global total
_7_.
_8_. Given the economic importance of coastal ecosystems, it is extremely important to understand how organisms in these environments may respond to ocean acidification resulting from ever increasing anthropogenic CO
_2_ concentrations.

Over the last decade scientific research on ocean acidification has been mainly focused towards understanding the carbonate chemistry and resulting responses on calcification of major calcifying organisms to altered pH seawater as per projections of year 2100 and beyond
_4_.
_9_. The diffusion of CO
_2_ in seawater immediately results in its reaction with water and formation of carbonic acid. Subsequently, carbonic acid dissociates rapidly into bicarbonate (HCO
_3_\(^-)\) and a proton and can further dissociate to form carbonate ion CO
_3_\(^2-\) and a proton as evident from Fig 1. Under abiotic conditions of temperature, salinity and alkalinity, nearly 90% of the CO
_2_ is present in the form of HCO
_3_\(^-)\) and almost 10% in the form of CO
_3_\(^2-\) while less than 1% is dissolved CO
_2_ and carbonic acid. These forms altogether are also known as dissolved inorganic carbon (DIC) and if the surface ocean continues to be a sink of anthropogenic released atmospheric CO
_2_, it can lead to an increase in DIC pool and resulting shift in carbonate speciation. This would result in an increase in HCO
_3_\(^-)\) and pCO
_2_ whereas decrease in CO
_2_ and a decrease in pH, also referred to as ocean acidification
_10_. Reduced availability of carbonate ions caused by increased CO
_2_ concentrations can result in reduction of
calcium carbonate saturation state (Ω) for the two common polymorphs, aragonite and calcite which are required by marine organisms for construction of their CaCO₃ shells or skeletons⁹,¹¹. Additionally, chemical changes associated with ocean acidification as mentioned above can have a number of impacts across variety of biological systems, biogeochemical cycling and associated processes¹¹.

Fig. 1—Diagrammatic representation of chemical processes for ocean acidification (modified from UK Ocean Acidification Research Programme website).

However, carbonate chemistry in coastal environments can differ from surface water of oceans due to interaction with river water, low pH seawater or acidic waste inputs from aquaculture farms. Variable pCO₂ values have been reported in the literature across different coastal environments. For example, pCO₂ values ranging from 500-9400 µatm has been reported in estuarine embayment from Europe¹². In the Pearl River estuary facing the South China Sea there are reports of pCO₂ of >4000 µatm¹³. As a result the effects on the coastal marine biota could be significantly altered. Nevertheless, in addition to calcification, other physiological processes including growth, development, metabolism, ion regulation and acid-base balance can get affected in coastal marine organisms due to increase in CO₂ (hypercapnia) or resulting acidosis⁶,¹⁴,¹⁵.

Marine invertebrates are functionally important in various coastal ecosystems including mangroves, estuaries and seagrass beds. They usually dominate in terms of abundance and diversity in different coastal environments and thereby mediate major ecological processes and form the basis of trophic levels. Coastal marine invertebrates, in particular echinoderms, molluses and crustaceans are thought to be more vulnerable from the effects of ocean acidification. These groups are mainly calcifiers, and therefore the formation of external shells and skeletons are controlled by calcification. It is well known that calcification process can be strongly influenced by changes in seawater pCO₂, pH and CO₃²⁻ associated with ocean acidification¹⁶,¹⁷,¹⁸.

The effects of ocean acidification on selected groups of coastal marine invertebrates as examples have been discussed in the present study. Emphasis has been laid in the review on the recent developments towards understanding the cellular and physiological responses of marine invertebrates to changing pH since mechanisms associated with response of groups such as echinoderms and molluses remain poorly understood.

Echinoderms
The Echinoderms are an ecologically important group on which numerous ocean acidification related experiments have been undertaken to date. Since the skeletons of these organisms are composed of magnesium calcite they are thought to be particularly susceptible to changes in ocean pH. Several studies have been conducted on sea urchins, one of the dominant groups and a major player in mediating coastal ecosystem processes. There are reports of decrease in fertilization success, developmental and cleavage rates and pluteus larval size in the sea urchins *Hemicentrotus pulcherrimum* and *Echinodetra mathaei* with elevated CO₂ concentration (pCO₂ level >5000 µatm) resulting in an increased dissolved inorganic carbon (DIC)¹⁹. In another study, involving the same species, significant reductions in weight gain was reported when exposed to CO₂ concentration of 550 µatm for six months²⁰. Authors explained that the reduction in weight gain could be partly explained by the larger surface area/body weight ratio of the sea urchins and their inability to regulate changes in their internal body condition, i.e., because of their direct connection with the ambient seawater through their madreporites. Since sea urchin tests are made of high magnesian calcite, it is substantially more soluble under lowered pH conditions²⁰. A study focused on the Australian sea urchin *Heliocidaris erythrogramma* has reported a decline in sperm swimming speed and % mortality when individuals were incubated at pCO₂ of 1000 µatm (seawater pH 7.7)²¹. The chemical nature of
seawater into which gametes of sea urchins such as *H. erythrogramma* are released, plays a major role in fertilization and larval development. The results from this study showed that under ocean acidification scenario there will be reduced fertilization success of a keystone coastal species *H. erythrogramma*.

But on the other hand, another study focused on the same Australian sea urchin species found no effect of elevated seawater CO₂ on fertilization or its early development (seawater pH 7.6)²². The authors found that temperature will be a serious embryonic teratogen for this species of sea urchin in Australia than acidification despite both stressors being applied simultaneously in the experiments²². This is because temperature increase can push inherent thermal adaptive capacity of successful reproduction in sea urchins and marine embryos such as those of *H. erythrogramma* may not ultimately reach calcification stages due to impaired early development mechanisms (e.g. cleavage, gastrulation)²². When the pluteus larvae of tropical sea urchin *Tripneustes gratilla*, two temperate sea urchins, *Pseudochinus huttoni* and *Evechinus chloroticus* and one polar sea urchin, *Sterechinus neumayeri* were exposed to pCO₂ of 1200-1400 µatm it was observed that the polar sea urchin was most tolerant in terms of calcification, skeletal structure and survival²³. Authors concluded that polar sea urchin species has greater capacity to acclimatise to lower seawater pH as these organisms have evolved in an environment with historically higher levels of CO₂ and a greater apparent solubility product for calcite²³.

Up-regulation of metabolism and calcification in the ophiuroid brittle star *Amphiura filiformis* kept in sediment cores maintained in CO₂ acidified seawater (pH 8.0, 7.7, 7.3 and 6.8, 40 days) has been also reported²⁴. The observation reported by the authors was in contrast to the results of most ocean acidification studies where reduced seawater pH lowered both metabolism and calcification⁶. Enhanced metabolism and calcification rate in brittle stars exposed to CO₂ resulted in muscle loss and have implications from the perspective of ecosystem functioning as arm movement is essential for the survival and function of these organisms. Impacts of acidification have been also documented on the early development of another brittle star species *Ophiothrix fragilis*²⁵. Ocean acidification (0.2 pH units) resulted in 100% larval mortality within 8 days in comparison to 70% found in controls. An apparent conflicting description of the results suggests either an inherent problem with control animals or error in reporting²⁵. Skeletogenesis was found to be severely impaired, with high proportion of abnormality and asymmetry was observed during this study.

Modern approaches at the cellular and physiological level have significantly aided our understanding of the responses of marine organisms including effects of ocean acidification on echinoderms. Acid-base responses of the sea urchin *Psammechinus miliaris* was examined based on exposure to pCO₂ of up to 52000 µatm for 8 days and no pH recovery was observed even under lowest pCO₂ (3000 µatm; seawater pH 7.44)²⁶. Authors found that magnesium concentration of coelomic fluid increased towards the end of the exposure suggesting dissolution of the test. A separate study on the sea urchin larvae (*Strongylocentrotus franciscanus*) has found increased CO₂ levels (up to 970 µatm) can alter expression of a heat shock protein (*hsp70*) when exposed to acute temperature stress²⁷. A microarray-based transcriptomic approach was applied to study the physiological response of *S. purpuratus* larvae to CO₂ -driven seawater acidification (pH 7.96 and 7.88)²⁸. Analysis involving 1000 genes showed broad scale decrease in gene expression of larvae represented by four major cellular processes namely biomineralization, cellular stress response, metabolism and apoptosis. Authors found that physiological processes beyond calcification were greatly impacted, suggesting that overall physiological capacity and not just a singular focus on biomineralization processes is essential for forecasting the impact of future CO₂ conditions on marine organisms including echinoderms. Growth, calcification and survival of pluteus larvae of *Strongylocentrotus purpuratus* in CO₂ acidified seawater with pH 7.7 (pCO₂ 1318 µatm) along with control experiments (pH 8.1, pCO₂ 399 µatm) was investigated by measuring gene expression of 26 representative genes important for metabolism, calcification and ion regulation²⁹. Authors found up regulation of metabolic genes (between 10%and 20% in ATP-synthase, citrate synthase, pyruvate kinase and thiolase at day 4) and down regulation of calcification related genes (between 23% and 36% in msp130, SM30B, and SM50 at day 4). There was up regulation of Na+/K+-ATPase at day 4 (15%) and down regulation of NHE3 at day 4 (45%). Authors concluded that it is crucial to employ experimental designs with a high time resolution in
order to rectify developmental artefacts based on genomic approaches\textsuperscript{29}. In another study, cell cycle checkpoints using biomarkers of cell cycle progression were investigated on the embryos of the purple sea urchin, Strongylocentrotus purpuratus, in seawater artificially buffered to a pH of \textasciitilde7.0, 7.5, and 8.0 by CO\textsubscript{2} infusion\textsuperscript{29}. Authors did not encounter significant differences in biomarkers assessed between pH treatments, indicating the embryos progress through the G\textsubscript{1}/S, G\textsubscript{2}/M and metaphase/anaphase transitions at relatively similar rates. They concluded that low pH environments may not impact developmental programs directly, but may act through secondary mechanisms such as cellular energetics\textsuperscript{30}.

Molluscs

Molluscs play major functional roles in coastal ecosystem processes including in bioturbation and also form the basis for commercially important marine products globally. Numerous studies have focused on the effect of ocean acidification on early development of molluscs and most of these studies have reported negative impacts of decreasing pH levels on the growth and development of these organisms\textsuperscript{31, 32, 33}. Some of the acidification studies involving molluscs have been outlined below.

It has been shown that reductions in seawater pH can lead to decreased calcification in the mussel Mytilus edulis and the Pacific oyster Crassostrea gigas even when seawater gets supersaturated with CaCO\textsubscript{3}\textsuperscript{18}. Another study on C. gigas revealed that only 5% of the C. gigas developed into normal veliger larvae when incubated at 2270 atm pCO\textsubscript{2} conditions, although no unusual effect was detected up to the trochophore stage\textsuperscript{34}. Same authors in a different study reported morphological aberrations in larvae of M. galloprovincialis when exposed to CO\textsubscript{2} under 2000 µatm conditions\textsuperscript{35}. A study by Green et al\textsuperscript{36} has shown that newly settled juveniles of the hard-shell clam Mercenaria mercenaria undergone substantial shell dissolution and increased mortality when they were introduced to surface sediments that were under-saturated with respect to aragonite (Ωarag\textsuperscript{18}0.3), a level that is typical of nearshore, organic-rich surface sediments. The same authors reported that CaCO\textsubscript{3} shells completely dissolved within two weeks of settlement, leaving only the organic matrix of the shell. It has been also shown that calcification rates in the mussel Mytilus edulis and the Pacific oyster Crassostrea gigas decreased by 25 and 10%, respectively, in response to an elevated pCO\textsubscript{2} level of 740 ppmv in 2100\textsuperscript{18}. Decreased shell growth in the edible snail Strombus luhuanus was observed when grown for more than six months in seawater bubbled with air containing 560 ppmv CO\textsubscript{2}\textsuperscript{20}. Impact of carbonate system perturbations on the growth of C. gigas larvae has been also investigated\textsuperscript{37}. Seawater with five different chemistries obtained by separately manipulating pH, total alkalinity and aragonite saturation state (calcium addition) were tested on the larvae of C. gigas. The authors showed that developmental success and growth rates were not directly affected by changes in pH or aragonite saturation state, but were highly correlated with the availability of carbonate ions. Observations indicate that the mechanisms used by these organisms to regulate calcification rates were not efficient enough to compensate for the low availability of carbonate ions under corrosive condition\textsuperscript{37}. In a very recent study the juveniles of C. virginica were exposed for eleven weeks to one of two environmentally relevant salinities (30 or 15 PSU) either at current atmospheric pCO\textsubscript{2} (~400 µatm, normocapnia) or pCO\textsubscript{2} projected by moderate IPCC scenarios for the year 2100 (~700-800 µatm, hypercapnia)\textsuperscript{38}. Authors found that juvenile oysters exposed to elevated pCO\textsubscript{2} and/or low salinity led to significant increase in mortality, reduction of tissue energy stores (glycogen and lipid) and negative soft tissue growth, indicating energy deficiency. Based on magnetic resonance spectroscopy, the authors reported lower acetate levels in hypercapnic than in normocapnic individuals at low salinity. The authors finally concluded that the combined effects of elevated pCO\textsubscript{2} and fluctuating salinity can significantly affect survival of oysters by weakening their shells and increasing energy consumption\textsuperscript{38}.

Other studies have also looked into the physiological responses of bivalves under experimental conditions mimicking ocean acidification. In acidified seawater, potential suppression of phagocytosis in M. edulis has been observed based on reductions of neutral red retention time of lysosomes\textsuperscript{39}. There are reports of inhibition of predator-induced shell thickening in snails belonging to Littorina littorea when exposed to CO\textsubscript{2}-acidified seawater of pH 6.63\textsuperscript{40}. Snails also exhibited reduced oxygen consumption only during predation under acidified conditions\textsuperscript{40}. Another study on L. obtusata has shown lowered viability
during embryonic development, slightly longer duration of development, changes in embryonic movement and significantly lower heart rate during development when incubated under pCO$_2$ of 1100 µatm$^{41}$. The veliger larvae of Crassostrea virginica showed 16% decrease in shell area and 42% reduction in calcium content following exposure to 800 µatm pCO$_2$.$^{42}$

Genomic and proteomics approaches have also aided towards an improved understanding of the response of molluscs from the effects of ocean acidification. Synergistic effects of elevated seawater temperature and declined seawater pH on gene expression patterns of aspein, calmodulin, nacrein, she-7-F10 and $hsp70$ in the pearl oyster Pinctada fucata has been recently investigated based on four experimental conditions: (1) ambient pH (8.10) and ambient temperature (27°C) (control condition), (2) ambient pH and elevated temperature (+3°C), (3) declined pH (7.70) and ambient temperature, (4) declined pH and elevated temperature.$^{43}$ The authors showed that under warming and acidic seawater conditions, expression of aspein and calmodulin showed no significant differences among different time point in condition 8.10 T. But the levels of aspein and calmodulin in conditions 8.10 T+3, 7.70 T and 7.70 T+3, and levels of nacrein, she-7-F10 in all the four treatments changed significantly. Low pH and pH×temperature interaction influenced the expression of aspein and calmodulin significantly after hours 48 and 96. Significant effects of low pH and pH×temperature interaction on the expression of nacrein were observed at hour 96. Expression level of she-7-F10 was affected significantly by pH after hours 48 and 96. The expression of $hsp70$ was significantly affected by temperature, pH, temperature x pH interaction at hour 6, and by temperature x pH interaction at hour 24. Study pointed that declined pH and pH x temperature interaction down regulated genes involved in calcification whereas the interaction between declined seawater pH and elevated temperature caused up regulation of $hsp70$ in P. fucata. The authors clearly demonstrated that decline in seawater pH and increase in temperature will have a serious impact on the physiological processes and potentially the adaptability of P. fucata to future warming and acidified ocean.$^{43}$

Hüning et al.$^{44}$ elucidated the impacts of an eight week acclimation period to four seawater pCO$_2$ treatments (39, 113, 243 and 405 Pa/385, 1120, 2400 and 4000 µatm) on mantle gene expression patterns in blue mussel Mytilus edulis from the Baltic Sea. Authors found that expression of genes involved in energy and protein metabolism such as F-ATPase, hexokinase and elongation factor alpha was strongly affected by acclimation to moderately elevated CO$_2$ partial pressures. The authors also encountered that expression of chitinase in blue mussel was strongly depressed resulting in decrease of shell growth in experimental animals. Additionally the authors encountered observed changes in physiology of blue mussel in response to ocean acidification based on correlation matrices and force directed layout network graph.$^{44}$

In another study physiological and metabolic responses of a key benthic bivalve, Laternula elliptica, was investigated at pCO$_2$ levels of their natural environment (430 µatm, pH 7.99; based on field measurements) with those predicted for 2100 (735 µatm, pH 7.78) and glacial levels (187 µatm, pH 8.32)$^{45}$. Authors found that the basal metabolism and heat shock protein ($hsp70$) gene expression levels in adult L. elliptica increased in response to lowering and elevation of pH. The expression of chitin synthase (CHS), an enzyme involved in synthesis of bivalve shells was up-regulated in individuals at pH 7.78, indicating L. elliptica were working harder to calcify in seawater under-saturated in aragonite ($\Omega_{ar} = 0.71$), the CaCO$_3$ polymorph of which their shells were comprised. Overall results indicated a negative effect of ocean acidification on whole-organism functioning of L. elliptica over relatively short terms (weeks-months) that may be energetically difficult to maintain over longer time periods and the CHS gene expression provided evidence for biological control over the shell formation process, which may enable some degree of adaptation or acclimation to future ocean acidification scenarios.$^{45}$

**Crustaceans**

Crustaceans are important members of the invertebrate communities in coastal environment; serve as food for higher trophic levels (e.g. fish) and constitute an important constituent of commercial aquaculture. Effects of ocean acidification on crustaceans are known to a lesser extent as these organisms have better acid-base regulation compared to echinoderms and mollusces.
The effects of ocean acidification scenarios have been studied on two marine copepods Acartia steueri and A. erythraea. It has been shown that the egg production rate and egg hatching rate decreased in both species with increasing pCO₂. Nauplius mortality rate was observed only in case of A. erythraea. However, one of the major drawbacks of the study was the level of pCO₂ which was much higher compared to predicted future atmospheric CO₂ concentrations and therefore the conclusion drawn had limited relevance. A separate study looked at the effects of 2380 μatm pCO₂ exposure on two subsequent generations in the copepod A. tsuensis. The authors did not detect significant effects for the parameters examined (survival, body size, egg production and hatching rates of the 1st and 2nd generation females) suggesting that A. tsuensis was tolerant to increased CO₂ compared to other marine organisms. A seawater manipulation experiment based on addition of HCl and increasing CO₂ concentration was set up to study the effects on two coastal harpacticoid copepods namely, Amphiascoides atopus and Schizopera knabeni. It was found that S. knabeni which lives in mudflats was less sensitive to projected ocean acidification scenarios.

The embryonic development of the amphipod Echinogammarus marinus was compared under 1900 μatm pCO₂ and three salinity regimes (10, 22 and 35 PSU). Salinity rather than CO₂ had major effects on number of hatchlings produced by a female and calcium content of hatchlings, significantly reduced at 22 PSU and no hatching occurred at 10 PSU. However, high CO₂ exposure significantly protracted embryonic development at 22 PSU indicating that the species was relatively tolerant to elevation of environmental CO₂. Effects of 1200 μatm pCO₂ exposure were tested on larval development of the European lobster, Homarus gammarus and it was found that the exposure level if prolonged could have detrimental impacts. Survival, zoeal progression and carapace length were not significantly affected by CO₂ exposure (Zoea I through IV, 28 days). But dry mass of carapace and calcium and magnesium contents of the same were both depressed at Zoea IV stage. The marine shrimp Palaemon pacificus when exposed to pCO₂ of 1000 μatm and 1900 μatm for 30 weeks and 15 weeks respectively, exhibited mortality after 18 and 7 weeks of exposure with a final survival rate of only 55% and 65%. Growth was found to be suppressed in 1900 μatm pCO₂ but the authors also found that body length of females in 1000 μatm was smaller compared to controls.

Studies have been also undertaken to examine the ability of crustaceans to adjust themselves to internal acid-base imbalances during medium-term exposure to projected pCO₂ levels. In the strong iono-regulating prawn species Palaemon elegans and P. serratus, complete compensation for a pCO₂ of 0.30 kPa was observed following 30 days of exposure although ion homeostasis was maintained at the expense of acid-base balance. On the other hand, two species of crabs namely Necora puber and Cancer magister which are relatively poor iono-regulators were able to compensate haemolymph acid-base disturbances within 24 hours when exposed to CO₂ at 0.10 to 0.20 kPa. When the exposure was continued at the same pCO₂ level in N. puber, detrimental effects were found as bicarbonate buffering started to fail after 16 days when HCO₃⁻ reached 27 mmol l⁻¹. But a separate study involving N. puber found the haemolymph (HCO₃⁻) to be much lower after 30 days at the same pCO₂ conditions of Spicer et al study.

Research has been undertaken to look at the effects of elevated pCO₂ and temperature on growth and survival of post-larvae from 2 species of barnacles. Both the barnacle species were collected from intertidal regions on the southwest coast of England but differences in growth and shell development were observed between the cold-water species Semibalanus balanoides and the warm-water species Elminius modestus. The exposure to pCO₂ levels of 0.04 and 0.10 kPa at two temperatures (14 and 18°C) had no effect on post-larval growth rates in S. balanoides, but higher pCO₂ and temperature treatment significantly reduced growth rates in E. modestus. The authors found that S. balanoides post-larvae were able to maintain growth, but at the expense of shell calcification. On the contrary, E. modestus post-larvae were able to maintain the integrity of their calcified shells, but at the expense of growth. Authors concluded that the ability to maintain mineralised shell plates during elevated pCO₂ and temperature exposure was attributed to differences in thermal tolerance brought about by sampling populations from different parts of their geographic distribution. Sub-arctic population of the cold-water species S. balanoides was more sensitive to CO₂ than the population in South Western England, at the southern limit of its distribution range.
Growth and development of post-larval *S. balanoides* from Kongsfjorden, Svalbard, was negatively impacted by elevated CO$_2$, but an increase in temperature of +4°C had no effect.$^{57}$ In contrast to the southern population, the northern population of *S. balanoides* also managed to maintain net calcification of their shells during elevated CO$_2$, suggesting that resources were reallocated from one energy-demanding process to another.$^{57}$ Larvae of two populations of the spider crab *Hyas araneus* raised at 710 and 3000 ppm pCO$_2$, displayed delayed development including reduced growth and fitness with zoeal and megalopa stages being most sensitive in the northernmost (79°N) and southernmost (54°N) populations respectively.$^{58}$

An investigation on the effects of increasing pCO$_2$ was undertaken to study growth and molecular physiology of the neritic amphipod *Gammarus locusta*, a cosmopolitan estuarine species.$^{59}$ There was no significant effect on the expression of hsp70 gene in amphipod individuals grown in acidified seawater. But there was a consistent and significant increase in the expression of the gapdh gene at a pH of 7.5 which indicated a possible disruption to oxidative metabolic processes. The authors concluded that future predicted changes in sea water pH may have subtle effects on the physiology and metabolism of coastal and marine species which may be overlooked in studies of whole organism response.$^{59}$ Herrroth *et al* have shown that under prolonged exposure to decreased pH mimicking ocean acidification scenario can lead to immune suppression in the crustacean *Nephrops norvegicus*.$^{60}$

**Other marine invertebrates**

Besides echinoderms, molluscs and crustaceans, effects of ocean acidification have been also studied in other marine invertebrates including corals. Studies have shown that different developmental stages and species of corals as well as coral reef communities exhibit similar and variable responses as observed in calcifiers.$^{61, 62, 63}$ Another group which play important role in benthic habitats are the foraminifer, the single celled protists. The foraminifer shell can be calcareous or agglutinated and therefore these organisms are thought to be more susceptible to subtle changes in pH of seawater. Studies have shown that foraminiferal assemblages along pH gradients in natural CO$_2$ vents undergoes changes in terms of distribution, diversity and nature of the fauna as pH decreases.$^{64}$ But on the other hand, a recent study undertaken by Pettit *et al* has shown that benthic foraminifer communities near CO$_2$ vents in Northern Gulf of California can cope up to adverse pH including at pH 7.5.$^{65}$ Durrant *et al*, in a recent study investigated the effects of increased temperature and lowered pH on the growth of an endemic bryozoan, *Celleporaria nodulosa* from Australia.$^{66}$ They found that lowered pH in winter significantly decreased growth in studied bryozoans, while elevated temperatures during summer significantly impeded their growth, possibly masking the effect of ocean acidification and resulted in an increased thermal tolerance. The authors concluded that effects of decreased pH and increased temperature on *C. nodulosa* colonies may be seasonally dependent and particularly acute during summer months while thermal stress is the initial stressor before ocean acidification negatively affected the organisms.$^{66}$ In non calcifying marine invertebrates such as nematodes and polychaetes the effects of OA has been studied to a limited extent. For example, a mesocosm experiment was conducted to quantify the effects of short- (2 wk) and long-term (20 wk) exposure to acidified seawater (pH 7.3) on the structure and diversity of macrofaunal and nematode assemblages in two different sediment types.$^{67}$ Authors found that sediments exposed to acidified seawater had significantly altered community structure and reduced diversity for macrofaunal and nematode assemblages. However, the impact on nematodes was less severe than that on macrofauna but the reason for such observation was not investigated. Using the embryos of marine annelid, *Hydroides elegans*, CO$_2$ perturbation experiments were undertaken to examine their pre and post settlement growth responses in seawater equilibrated to CO$_2$ values of about 480 (control), 980, 1480 and 2300 μatm resulting in pH values of around 8.1 (control), 7.9, 7.7, and 7.5, respectively.$^{68}$ The authors found that during the three decreased pH conditions did not affect either embryo or larval development but larval calcification at the time of metamorphosis and early juvenile growth were adversely affected. They concluded that decreased ability of these calcifying tubeworms to calcify at decreased pH were indicative that they will be one of the highly threatened species in future ocean.$^{68}$

More thorough studies has to be undertaken to study the cellular and physiological response of non calcifying coastal invertebrates to changing pCO$_2$.
conditions and the resulting impacts on ecosystem processes.

Conclusions

The impact of ocean acidification on the early development stages, particularly with reference to larval stages of calcifying marine invertebrates has shown reduction in the rate of larval development, reduction in larval size and alteration in shell integrity as discussed earlier. These could severely affect the survival of larval life forms and may ultimately alter higher trophic levels. Sub lethal effects of elevated pCO$_2$ as discussed earlier can also alter the composition and fitness of larvae and thereby alter settlement dynamics and ultimately affect recruitment of adult populations in coastal ecosystems.

It seems more likely that increasing anthropogenic CO$_2$ concentrations in atmosphere will result in the rise of surface ocean CO$_2$ as well as in coastal environments in combination with rising surface water temperatures. While efforts have been made towards understanding the responses of calcifying marine invertebrate communities but a knowledge gap exist on how the non calcifiers marine invertebrate might respond to such changes. Major questions still remain unanswered from the perspectives of biological consequences of acidification and warming of oceans and coastal ecosystems including effects on larval development and settlement of marine organisms.

At the same time, it has become increasingly important to study the impacts of ocean acidification (OA) from the perspective of whole organism effect. Majority of the studies as detailed throughout this review have focused on limited number of response variables and thus mostly likely have missed true impact of OA or might have over interpreted organismal responses. Increasingly it is being viewed that many of the negative impacts of OA are associated with the way in which organisms use, partition and gather metabolic energy. Previous studies assumed that all organisms which rely heavily on calcification would be negatively affected by seawater acidification primarily due to reduction in the saturation states of calcite or aragonite\cite{69,70}. On the other hand, recent research has shown that even within heavily calcifying taxa there are large differences between the responses of similar species within the same taxonomic groups and even between populations or individuals from within the same species\cite{70,71}. For example, blue mussel *Mytilus edulis* from North Sea showed a 25% drop in calcification rates after exposure to high atmospheric CO$_2$, whereas a Baltic population of the same species did not experience same set of problems\cite{72,73}. One of the explanations for such observation is that the response of individuals to elevated CO$_2$ is governed by the energy available to fuel the physiological responses needed to maintain acid-base balance and physiological function. The reallocation of energy will obviously have an impact on ecological processes such as trophic interactions and reproductive success that determine long-term survival of individuals and populations. In addition if organisms do not grow as big in a high CO$_2$ environment that could have an impact on socioeconomics including aquaculture and fisheries. Thus future studies on OA should incorporate aspects of whole organism effect while incorporating integrative approaches including genomics and proteomics.

While ocean acidification itself can affect marine invertebrates and their population structure, other factors induced by human intervention such as release of pollutants and warming in addition to natural factors (such as change in salinity, temperature, increased deoxygenation or hypoxia) can also affect marine invertebrate populations\cite{74}. It has been now clearly established that there are significant interactions between ocean acidification, temperature, salinity as well as pollutants within the marine environment\cite{74,75}. Interaction of these multiple stressors can lead to several physiological stresses in marine organisms including invertebrates that ultimately may result in their death\cite{75}.

In future long term experiments conducted under laboratory conditions should be reciprocated with field based approaches to study the response of whole organism as well as within and between populations while taking into account the interaction of OA with other stressors. It will be also important to particularly study the adaptability of marine invertebrates in coastal ecosystem and focusing on identifying underlying molecular mechanisms of such responses by integrating modern approaches such as ‘omics’ approach.

References


53. Pane, E.F. & Barry, J.P. Extracellular acid–base regulation during short-term hypercapnia is effective


