

Life Threatened in Acidic Coastal Waters

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Abstract—Increasing atmospheric CO₂ is not only increasing global temperature but also rapidly acidifying seawater through formation of carbonic acid (ocean acidification). Emerging evidence from laboratory research indicates that predicted changes in ocean environments could have profound implications for marine ecosystem, however, it is relatively unclear how marine biota will respond to ocean warming and acidification. Furthermore, most published papers have used future atmospheric CO₂ concentration in their experimental protocols, ignoring spatial heterogeneity of seawater carbonate chemistry, which is most manifested in coastal regions and deep sea. This paper first summarizes empirical evidence on effects of CO₂ on marine organisms, and then discusses the importance of considering local CO₂ conditions to improve our prediction ability on the fate of marine organisms in acidified oceans. Marine molluscs have been shown to be highly sensitive to elevations of ambient CO₂, in particular during larval shell formation. In addition, data have been accumulated on sublethal impacts on morphology, physiology and behavior. Early development of echinoderms are also significantly affected by elevations of seawater *p*CO₂, however, there seems to be a difference in inter-species CO₂ sensitivity from different latitudes and also a high intra-species sensitivity. Gonad development can be severely impacted by high CO₂. As compared with CO₂ sensitivity known for molluscs and echinoderms, some crustaceans, particularly copepods and amphipods, and fish appear to be less vulnerable to CO₂. Anthropogenic impacts on coastal environment are multifaceted and complex. Scientific endeavor is of utmost necessity to secure this most productive marine region. Investigations reflecting local biotic and abiotic conditions are needed to precisely predict how coastal ecosystem will shape in the face of changing environment.

Keywords: ocean acidification, CO₂, pH, coastal environment, estuary, early development

1. INTRODUCTION

The world oceans are increasing their acidity by absorbing atmospheric carbon dioxide (CO₂). The atmospheric CO₂ concentration has rapidly increased over past decades due largely to burning of fossil fuels and cement production, and is projected to continue to rise over present levels (380 ppm, IPCC, 2007) to reach between 540 ppm to 970 ppm by the end of this century, and thereafter up to 1900 ppm at around the year 2300 (Caldeira and Wickett, 2003; see also Caldeira and Wickett, 2005 for other projections). The oceans have absorbed about 50% of the anthro-

pogenic CO₂ emitted to the atmosphere between 1800 and 1994 (Sabine et al., 2004), and roughly 30% of recent emissions has been taken up by the ocean until today (Feely et al., 2004). Dissolution of CO₂ will lower seawater pH and affect its carbonate chemistry. It is generally held that average surface ocean pH has already declined by 0.1 from the pre-industrial level (Orr et al., 2005), and is projected to decrease 0.3 to 0.46 by the end of this century, depending on CO₂ emission scenarios (Caldeira and Wickett, 2005). These global-scale predictions deal with average surface ocean values, but coastal regions are not well-represented because of lack of data, complexities of nearshore circulation processes, and too-coarse model resolution (Fabry et al., 2008). However, given the profound importance of coastal areas to fisheries and other marine resources and services, it is urgently needed to understand how coastal ecosystems would respond to ocean acidification resulting from increasing CO₂ concentrations in coming centuries. Coastal marine habitats including estuaries, seagrass/algae beds, coral reefs and continental shelf are estimated to provide ca. US\$ 12.6 trillion worth of ecosystem goods and services per year, or 38% of the global total (Costanza et al., 1997).

Earlier research efforts of ocean acidification studies has centered on how and to what extent low pH seawater affects calcification of major calcifying organisms such as corals and coccolithophores. This is because dissolution of CO₂ not only increases hydrogen ion concentration of seawater and thereby reduces its pH, but also by shifting carbonate equilibrium reduces carbonate ion (CO₃²⁻) concentration, which determines saturation status of calcium carbonate in seawater and defined as:

$$\Omega = [\text{Ca}^{2+}][\text{CO}_3^{2-}]/K_{\text{sp}}^*$$

where K_{sp}^* is the stoichiometric solubility product for CaCO₃, and [Ca²⁺] and [CO₃²⁻] are the *in situ* calcium and carbonate ion concentrations, respectively. When Ω is greater than 1, seawater is supersaturated and CaCO₃ tends to form whereas when the value is below 1, seawater is undersaturated and CaCO₃ crystals tend to dissolve. Since seawater [Ca²⁺] is relatively stable for a given salinity, it is [CO₃²⁻] that usually determines Ω in changing pH conditions. Among dominant crystal forms of biogenic CaCO₃ of marine calcifiers (aragonite and calcite), aragonite has a lower Ω value than calcite, and is more soluble than calcite at a given temperature, salinity and pressure (Zeebe and Wolf-Gladrow, 2001). The present surface ocean water is supersaturated for both aragonite and calcite except for some regions (see below), but the global models predict that saturation status of the ocean surface will decrease overtly, and become undersaturated in the Southern Ocean by the year 2050 (Orr et al., 2005). Saturation status of much of the coastal regions is not predicted in these models and is left 'blank'.

Carbonate chemistry of coastal waters can be substantially different from that found in surface water of offshore regions. Some coastal ecosystems are subjected to persistent or episodic acid inputs as a result of interactions with river water, upwelling of deep CO₂-rich, low pH seawater, bottom sediments, atmospheric deposition of terrigenous materials, or acidic wastage of aquaculture. Salisbury et al. (2008)

estimated coastal acidification by major world rivers, and found a general trend of higher Ω -reducing effects for polar rivers because of lower temperature. They pointed out that even though shellfish (and other coastal) species inhabiting those estuarine waters are likely to have developed survival mechanisms, any lowering of coastal Ω caused by acid river discharge will be exacerbated as atmospheric CO_2 increases. Feely et al. (2008) demonstrated that some areas of the continental shelf near the coast along western North America has already been subjected to $p\text{CO}_2$ levels that are equivalent to the upper atmospheric limit projected for the end of the century (1,000 μatm , 1 μatm = 1 ppm for gaseous phase under barometric pressure of 1 atm. This relationship does not hold for aqueous phase. See Zeebe and Wolf-Gladrow, 2001) due to seasonal upwelling of high CO_2 bottom water, which is partly attributable to anthropogenic CO_2 . Even more extreme $p\text{CO}_2$ values have been reported for estuaries, which are generally thought to be CO_2 sources to the atmosphere (Borges et al., 2005). For instance, Frankignoulle et al. (1998) reported $p\text{CO}_2$ of 500–600 up to 9,400 μatm in estuarine embayment (inner estuaries) and up to 1330 μatm in river plumes at sea (outer estuaries) in Europe. Zhai et al. (2005) reported $p\text{CO}_2$ of >4,000 μatm in the Pearl River estuary, which drains into the South China Sea.

In this short review on the effects of ocean acidification due to increasing $p\text{CO}_2$, we mostly limit our discussion to coastal benthic invertebrates and fish, many of them of high commercial importance in coastal fisheries. Also, we touch upon CO_2 impacts on copepods and jellyfish, even though information is quite limited, because of their essential role in shaping coastal ecosystem. Jellyfish have been the focus of intensive research in the East China Sea area in these years, because of frequent occurrence of jellyfish blooming in recent years in the East China Sea, which resulted in substantial economical loss in coastal fisheries. Information on CO_2 effects on other taxa can be found elsewhere (see Kleypas et al., 2006, Atkinson and Cuet, 2008, and Logh, 2008 on corals, and Rost et al., 2008, and Balch and Fabry, 2008 on plankton);

2. EFFECTS ON MOLLUSCS

Bivalve molluscs play essential functional roles in coastal ecosystems as ecosystem engineers and also constitute commercially important marine products from coastal waters worldwide. For example, the Manila clam, *Ruditapes philippinarum*, is one of the major aquaculture species in the world, and particularly along the coast of the East China Sea, with a global aquaculture production of 2.36 million tons in 2002 (FAO, c2005–2008). Oysters and mussels are other important marine species with high commercial value.

Reductions in seawater pH have been shown to decrease calcification of the mussels *Mytilus edulis* and the Pacific oyster *Crassostrea gigas* even when seawater is supersaturated with regard to CaCO_3 (Gazeau et al., 2007). Negative impacts of elevated ambient CO_2 appear to be particularly strong during early development. We have shown that only 5% of *C. gigas* developed into normal veliger larvae when incubated under 2270 μatm $p\text{CO}_2$ conditions, even though no obvious effect was detected up to the trochophore stage (Kurihara et al., 2007, Fig. 1). Larval shell mineralization was completely inhibited in 45% of the CO_2 larvae in these conditions

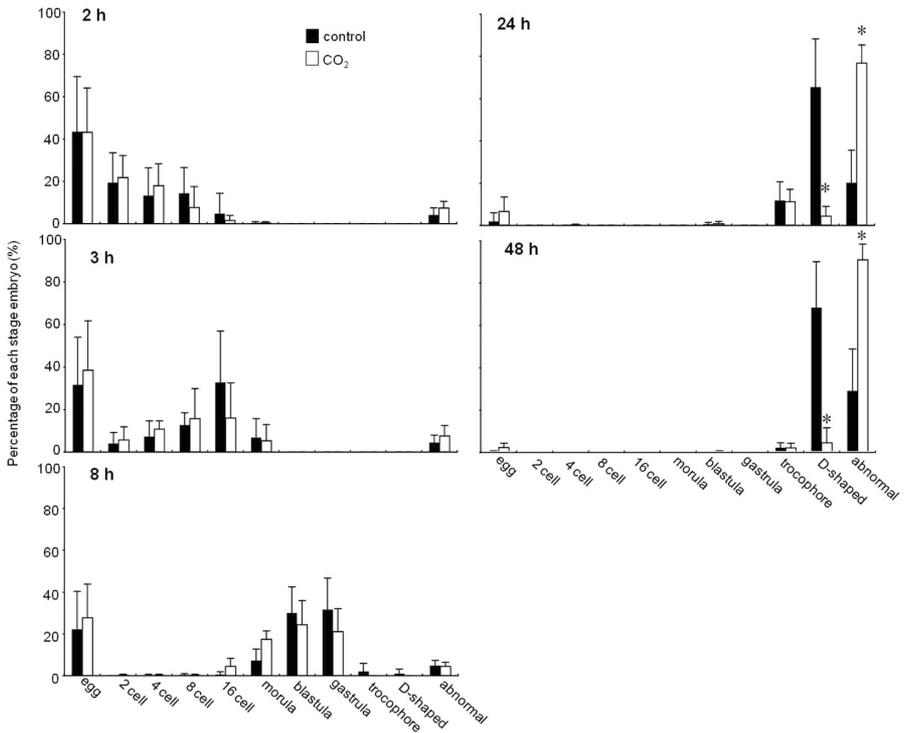


Fig. 1. Percentage distribution of each developmental stage of *Crassostrea gigas* embryos/larvae incubated in control (black bars) or CO₂ (white bars) seawater for 2, 3, 8, 24 and 48 h after fertilization (mean ± SD; n = 5). *Significant difference (paired t-test; p < 0.005). Reprinted from *Aquatic Biology*, 1, Kurihara et al., Effects of increased seawater pCO₂ on early development of the oyster *Crassostrea gigas*, 91–98, 2007, with permission from Inter-Research.

(Kurihara et al., 2007, Fig. 2). All CO₂-exposed larvae of *M. galloprovincialis* showed morphological aberrancies under 2000 μatm conditions (Kurihara et al., 2008a, Fig. 3. And also the larval morphology of this species is presented in a frontispiece of this book). CO₂-induced growth suppression has been reported for *M. edulis* (Berge et al., 2006), and *M. galloprovincialis* (Michaelidis et al., 2005). It should be pointed out, however, that pCO₂ levels used in these studies were higher than predicted in the next few centuries (5000 μatm in Michaelidis et al., 2005), and therefore could overestimate impacts of future acidification on these animals. Shirayama and Thornton (2005) exposed a marine snail *Strombus luhuanus* to pCO₂ of only 560 μatm for six month, and found a significant reduction in both shell height and wet weight from 8–14 weeks of exposure. The average seawater pH difference was reported to be only 0.03 in their experiment (control 7.936–7.945, 560 μatm 7.897–7.902). Michaelidis et al. (2005) also demonstrated reduced

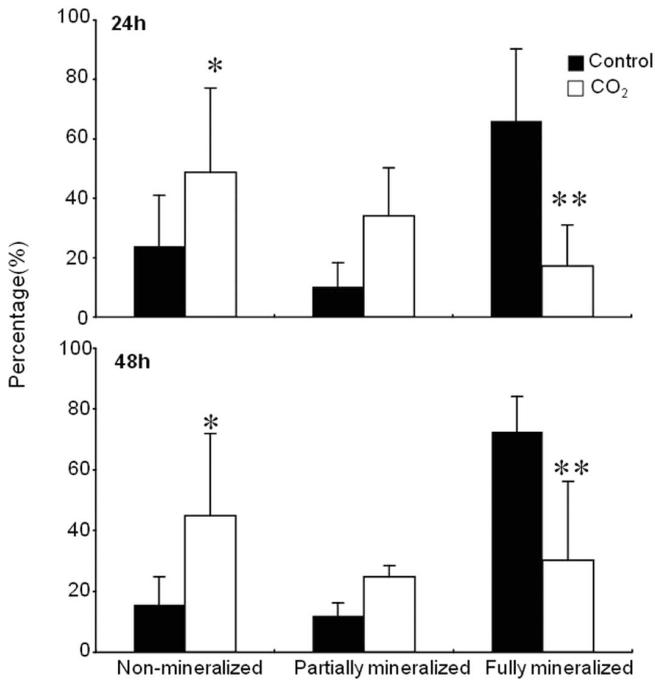


Fig. 2. Shell mineralization in *Crassostrea gigas* embryos incubated for 24 or 48 h in control or CO₂ conditions, determined by polarized light microscopy (mean \pm SD; $n = 5$). Significant difference between control and CO₂ groups by paired *t*-test: * $p < 0.05$, ** $p < 0.005$. Reprinted from *Aquatic Biology*, 1, Kurihara et al., Effects of increased seawater pCO₂ on early development of the oyster *Crassostrea gigas*, 91–98, 2007, with permission from Inter-Research.

oxygen consumption but increased ammonia excretion, indicating a lower metabolic rate and the net degradation of protein in *M. galloprovincialis* under their experimental conditions. Green et al. (2004) found that newly settled juveniles of the hard-shell clam *Mercenaria mercenaria* revealed substantial shell dissolution and increased mortality when they were introduced to surface sediments that were undersaturated with respect to aragonite ($\Omega_{\text{arag}} \approx 0.3$), a level that is typical of nearshore, organic-rich surficial sediments. Within two weeks of settlement, the CaCO₃ shells were completely dissolved, leaving only the organic matrix of the shell.

Other physiological responses elicited by acidified seawater in bivalves include a potential suppression of phagocytosis in *M. edulis* as determined from phagocytosed zymosan particles (Bibby et al., 2008). Even though their results showed a linear decrease in phagocytotic activity on day 32 of exposure, controls displayed an >8-fold increase in activity suggesting that the immune response was induced by confinement in their experimental setup, which somewhat clouds the implication of their results. Beesley et al. (2008) stated that ocean acidification could reduce the general health

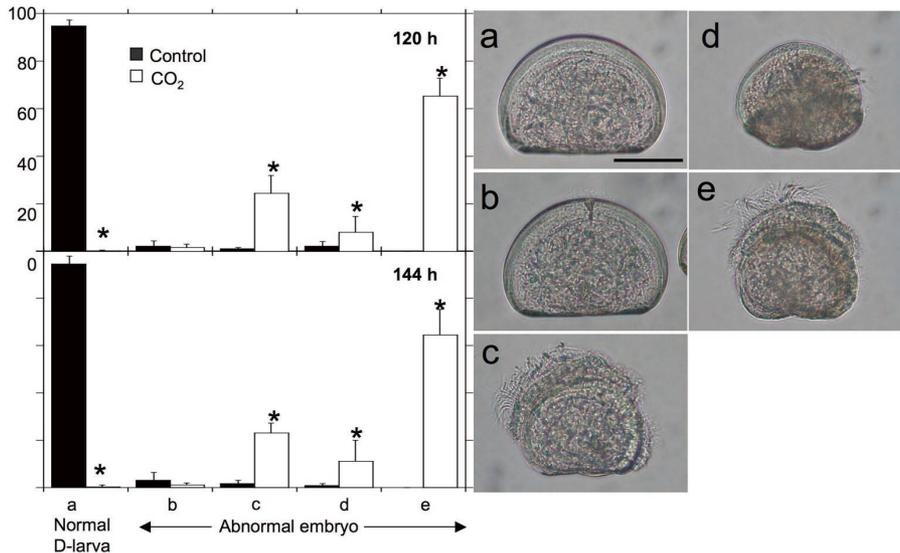


Fig. 3. Left: Percentage distribution of normal and different types of abnormal embryos of *Mytilus galloprovincialis* in the control (black bars) and in CO₂-seawater (white bars) at 120 and 144 h after fertilization. Each bar is the average of 5 replicate experiments. Error bar = SD, (*) significant difference between the control and high-CO₂ groups by paired *t*-test ($p < 0.005$). Right: Light micrographs of embryos/larvae: (a) normal, (b) abnormal embryos with indented shell margin, (c) protruding mantle, (d) convex hinge, and (e) both protruding mantle and convex hinge at 144 h after fertilization. Scale bar = 50 μ m. Reprinted from *Aquatic Biology*, **4**, Kurihara et al., Effects of elevated p CO₂ on early development in the mussel *Mytilus galloprovincialis*, 225–233, 2008, with permission from Inter-Research.

status of *M. edulis*, based on the observed reductions of neutral red retention time of lysosomes, as the method is generally perceived as a good indicator of the health status for a variety of invertebrates (Moore et al., 2004).

More subtle influences of high CO₂ exposure on morphology, physiology and behavior have been reported for two species of intertidal gastropods. Bibby et al. (2007) found inhibition of predator-induced shell thickening in *Littorina littorea* by exposure to CO₂-acidified seawater (pH 6.63, the p CO₂ estimated using CO₂SYS from the reported total CO₂ and pH is 1950 μ atm). The snails reared in acidified conditions showed reduced oxygen consumption only in the presence, but not in the absence, of predation. These snails spent significantly more time at or above water surface, presumably as a compensation for their inability to thicken the shell. Another snail, *L. obtusata*, showed lowered viability during embryonic development, slightly longer duration of development, changes in embryonic movement and significantly lower heart rate during development when incubated under p CO₂ of 1100 μ atm (Ellis et al., 2009).

The above studies were conducted all in full-strength seawater. Lower salinities could exacerbate CO₂ impacts by reducing CaCO₃ saturation. Thus, Miller et al.

(2009) demonstrated that veliger larvae of *C. virginica* experienced a 16% decrease in shell area and a 42% reduction in calcium content in response to exposure to 800 $\mu\text{atm } p\text{CO}_2$. The findings of Miller et al. (2009) are of particular relevance because the East China Sea receives discharge from several large rivers along its coast, the Changjiang River being by far the largest (see Section 7).

Bivalves themselves could be a significant generator of CO_2 , particularly when they occur in high density as a wild population or in an aquaculture ground. Chauvaud et al. (2003) estimated respiration and calcification by *Potamocorbula amurensis* on the basis of population density and size structure, and concluded that this species alone could be a major source of CO_2 to northern San Francisco Bay. The aquacultural implication is that high stocking density of these animals would deteriorate aquaculture ground environmental conditions, and may lead to poor productivity.

Cephalopods are another member of molluscs with high commercial value. Unlike bivalves and snails, this group of animals is characterized by high metabolic rate and active lifestyle. Formerly, squids were considered to be one of the most sensitive animals to CO_2 due to their high metabolic rate, large Bohr effects of their respiratory pigment (hemocyanin), low efficiency mode of swimming and low venous oxygen reserves (Pörtner et al., 2004). However, a recent study has demonstrated that the opposite is in fact true. Maintaining for 6 weeks under $p\text{CO}_2$ of 4000 to 6000 μatm , Gutowska et al. (2008) found that juveniles of *Sepia officinalis* grew and calcified their cuttlebone as in control animals. When plotted against seawater aragonite saturation (Ω_{arag}), calcification of *S. officinalis* was found to be insensitive to Ω_{arag} , in contrast to corals and bivalves whose calcification was strongly Ω_{arag} -dependent.

3. EFFECTS ON ECHINODERMS

Echinoderm skeletons are composed of magnesium calcite that is particularly susceptible to dissolution as ocean pH decreases (Shirayama and Thornton, 2005), rendering this group of animals as an important experimental material in ocean acidification research.

Sea urchins are harvested in high quantity and have high commercial value. They are often the dominant grazer in many communities (Lawrence, 2007) and can act as a keystone species. Shirayama and Thornton (2005) reported significant reductions in weight gain in *Hemicentrotus pulcherrimus* and *Echinometra mathaei* exposed to only 550 μatm for six months. As in the case of *S. luhuanus* (see above), the CO_2 effect on weight became significant only after 14 weeks of exposure. Kurihara and Shirayama (2004) examined effects of CO_2 on early development of the same two sea urchin species, and found that fertilization, cleavage and pluteus morphology were all negatively affected by increasing ambient $p\text{CO}_2$, even though the effects on the first two parameters were significant only at very high $p\text{CO}_2$ levels (>5000 μatm). In contrast, morphological impacts were already significant at 860 μatm , which became intensified with further elevations of $p\text{CO}_2$. More recently, Havenhand et al. (2008) reported a small but significant drop in sperm swimming speed and %motility in the Australian sea urchin *Helicoidaris erythrogramma* incubated at $p\text{CO}_2$ of 1000 μatm (seawater pH 7.7). This led to a 20% decrease of eggs that developed into cleaving

embryos, and a 26% drop of those that developed to swimming larvae. In sharp contrast, Byrne et al. (2009) found no effect of elevated seawater CO₂ on fertilization or early development (percentage of normal cleaving embryos and normal gastrulae) in the same species incubated down to the seawater pH of 7.6. The reason for this difference is not clear: Both studies used *H. erythrogramma* collected at or near Sydney, collected gametes by KCl injection, acidified experimental seawater by bubbling CO₂, and conducted at almost same temperature (19.5°C and 20°C) and salinity (36). Despite these unresolved uncertainties of experimental results, the data of Byrne et al. (2009) explicitly illustrated the importance of investigating synergistic impacts of environmental changes projected for the future ocean: even though early development was unaffected by the range of pCO₂ they used, increasing temperature dramatically suppressed cleavage and gastrulation of the embryos.

Clark et al. (2009) compared responses of pluteus larvae of one tropical *Tripneustes gratilla*, two temperate *Pseudechinus huttoni* and *Evechinus chloroticus*, and one polar *Sterechinus neumayeri* species exposed to pCO₂ of 1200 to 1400 µatm. They found that the polar sea urchin is the most tolerant in terms of calcification, skeletal structure, and survival. These authors pointed out that the species may have greater capacity to acclimate to lowered seawater pH since it has evolved in an environment with historically higher levels of CO₂ and a greater apparent solubility product for calcite.

Currently, there is a dearth of knowledge of possible CO₂ effects on gonad development and responses of early developmental stages to ocean acidification. We have recently examined gonad development of *Hemicentrotus pulcherrimus* reared under pCO₂ of 1000 µatm at ambient or elevated temperature (2°C) for 9 months. Our preliminary analysis demonstrated that high CO₂ delayed ovary maturation such that gonad-somatic index (GSI) peaked one month later but attained a comparable level as in control, however, when sea urchins were kept under high CO₂ and high temperature conditions, GSI not only peaked one month later but also was dramatically suppressed (Kurihara et al., in preparation).

Physiological, molecular and genetic responses to high CO₂ are relatively unknown for any marine organisms in the context of ocean acidification. Miles et al. (2007) examined acid-base responses of the sea urchin *Psammechinus miliaris* to 8-day exposure to pCO₂ of up to 52,000 µatm, and found no pH recovery even under the lowest pCO₂ they used (3,000 µatm, seawater pH 7.44). Magnesium concentration of the coelomic fluid increased towards the end of the exposure, suggesting dissolution of the test. O'Donnell et al. (2009) found increased CO₂ levels (up to 970 µatm) altered expression of a heat shock protein (hsp70) when sea urchin larvae (*Strongylocentrotus franciscanus*) were exposed to acute temperature stress. Hofmann et al. (2008) discussed the utility of gene expression profiling and its potential to provide deeper insight into mechanisms of biomineralization in marine calcifiers in ocean acidification research.

Another group of echinoderms, for which information is available regarding CO₂ effects, is the brittlestars. Though they are of no commercial value, these animals are still a key species in many seafloor communities. Wood et al. (2008) found up-regulation of metabolism and calcification in the ophiuroid brittlestar *Amphiura*

filiformis kept in sediment cores maintained in CO₂-acidified seawater (pH 8.0, 7.7, 7.3 and 6.8, 40 days). This is in contrast to the results of most ocean acidification studies where reduced seawater pH lowered both metabolism and calcification (see Fabry et al., 2008, for review). The apparent enhanced metabolism and calcification in CO₂-exposed brittlestars resulted in muscle loss, which may have implications for survival and ecosystem function since arm movement is necessary for feeding, burrow aeration and predator avoidance of these animals (see references cited by Wood et al., 2008 for these aspects). More drastic impacts have been documented on the early development of another brittlestar *Ophiothrix fragilis* by Dupont et al. (2008). Acidification (0.2 pH units) resulted in 100% larval mortality within 8 days in comparison to 70% found in the controls. An apparent conflicting description of the results suggests either an inherent problem with control animals (70% survival of controls reported in introduction, however, 30% mortality and 30% survival reported in the discussion and results sections, respectively) or error in reporting (see Dupont et al., 2008). Skeletogenesis was severely impaired, with high proportion of abnormality and asymmetry. These morphological aberrancies are supposed to be responsible for the observed marked mortality of the CO₂-exposed larvae

4. EFFECTS ON CRUSTACEANS

In comparison to what is known for molluscs and echinoderms on ocean acidification effects, much less is known for crustaceans. This is possibly due to the general supposition that this group of animals is equipped with better acid-base regulatory capacity than the other two groups of animals. Yet, crustaceans constitute an important member of coastal ecosystems as food for higher trophic levels (e.g. fish) and they themselves are a valuable target of commercial fisheries and aquaculture. These facts warrant careful examination of ocean acidification impacts on this group of animals.

Kurihara et al. (2004) examined effects of CO₂ on the two marine copepods, *Acartia steueri* and *A. erythraea*. Even though their data demonstrated that egg production rate (both species), egg hatching rate and nauplius mortality (only for *A. erythraea*) decreased with increasing *p*CO₂, the levels used were too high as compared with predicted future atmospheric CO₂ concentrations, and therefore have limited relevance to understanding of ocean acidification impacts. More recently, we studied the effects of 2380 µatm *p*CO₂ exposure from eggs to maturity and over two subsequent generations on another copepod *Acartia tsuensis* (Kurihara and Ishimatsu, 2008). Significant effects were not detected for any of the parameters examined (survival, body size, or development speed of the initial generation copepods, egg production and hatching rates of the 1st and 2nd generation females developed from eggs to maturity in high CO₂ conditions), which suggested that *A. tsuensis* was more tolerant to increased CO₂ than other marine organisms studied. Earlier, Mayor et al. (2007) reported CO₂ influence on hatching success of the copepod *Calanus finmarchicus*, although the *p*CO₂ used in this study (8000 µatm, seawater pH 6.95) is higher than predicted for the next few centuries and more relevant to risk assessment of possible leakage from ocean CO₂ storage/disposal. The

5-day CO₂ exposure did not affect carbon or nitrogen content of female adult *C. finmarchicus* or daily egg production rate, but did reduce the viability of eggs produced under the high CO₂ conditions. Egilsdottir et al. (2009) compared embryonic development of the amphipod *Echinogammarus marinus* under 1900 μatm pCO₂ conditions in three salinities (10, 22 and 35 PSU). Salinity, but not high CO₂, had strong effects on number of hatchlings produced by a female and calcium content of hatchlings, significantly reducing these parameters at 22 PSU and no hatching occurred at 10 PSU. High CO₂ exposure significantly protracted embryonic development at 22 PSU but not at 35 PSU. Thus, this species seems also relatively tolerant to elevation of environmental CO₂. Another species of the amphipod *Gammarus locusta* has also been observed to exhibit tolerance to elevated CO₂ levels. Hauton et al. (2009) observed no significant alteration to heat-shock protein (*hsp70*) as a surrogate indicator of acid-base homeostatic ability or impact on amphipod growth and survival when exposed to decreased seawater pH (7.8 and 7.6). Arnold et al. (2009) tested effects of 1200 μatm pCO₂ exposure on larval development of the European lobster, *Homarus gammarus*. Again, survival, zoeal progression and carapace length were not significantly affected by the CO₂ exposure (Zoea I through IV, 28 days). However, dry mass of carapace and its content of calcium and magnesium were both depressed at Zoea IV stage. Thus, exposure to this level of CO₂, if prolonged, could have detrimental impacts on crustaceans. In fact, we reported that when the shrimp *Palaemon pacificus* was exposed to pCO₂ of 1000 μatm and 1900 μatm for 30 weeks and 15 weeks respectively, mortality set in after 18 and 7 weeks of exposure with the final survival of 55% and 65% (Kurihara et al. 2008b, Fig. 4). Growth was suppressed in 1900 μatm but not in 1000 μatm . However, a closer inspection of data revealed that body length of females in the 1000 μatm group tended to be smaller than in controls. Possibly related to this suppressed growth, the percentage of females that bore eggs was smaller in high CO₂ shrimps, though needs to be reinvestigated due to their small sample size. Characteristically, the length of the second antennae were much shorter in 1000 μatm shrimps as determined at the end of 30 week exposure (54% of body length in 1000 μatm vs. 165% in the control; no measurement was conducted for 1900 μatm experiment). Recently, McDonald et al. (2009) reported the results of exposure of nauplius through reproductive adult of the barnacle, *Amphibalanus amphitrite* to CO₂-acidified seawater (pH 7.4; seawater pCO₂ is not given but calculated to be 2,700 μatm based on the reported pH and total alkalinity). No significant difference was detected for cyprid size, nauplius survival to attachment, growth from juveniles to adult (8 weeks) or egg production during the 9th to 11th week of exposure. There was an indication of enhanced calcification of the barnacle shell under acidic conditions as compared to controls.

5. EFFECTS ON FISH

Knowledge is limited on the possible impacts of ocean acidification on fish. In 2008, we surveyed 116 papers (published from 1969 through 2008) on the effects of high pCO₂ on fishes (Ishimatsu et al., 2008, Table 1). It revealed that the data from most of these studies are of limited value to predict the fate of fishes in the future acidified oceans for the following reasons: (1) the pCO₂ levels used were much higher

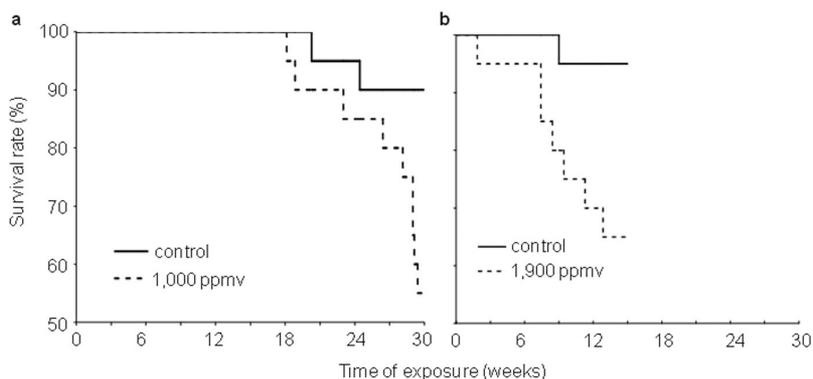


Fig. 4. Effects of long-term CO₂ exposure on the survival of the marine shrimp *Palaemon pacificus*. Survival was significantly suppressed (Log-rank test, $p < 0.05$) when shrimps were individually reared under (a) 1,000 ppmv and (b) 1,900 ppmv CO₂ conditions for 30 and 15 weeks, respectively. Reprinted from *Journal of Experimental Marine Biology and Ecology*, **367**, Kurihara et al., Long-term effects of predicted future seawater CO₂ condition on the survival and growth of the marine shrimp *Palaemon pacificus*, 41–46, 2008, with permission from Elsevier.

(above 50,000 μatm in 92% of the papers) than projected for the oceans in the next centuries, with only 2 studies covering the $p\text{CO}_2$ range below 2000 μatm (Jones et al., 1985; Ross et al., 2001); (2) CO₂ exposure periods were less than 4 days in 79% of the *in vivo* studies with only 8 experiments longer than 60 days; (3) marine species were used only in 25% of the studies; (4) research has focused largely on acid-base regulation and cardiorespiratory control (58% of the papers), and other aspects were little investigated; (5) effects on early development have been studied in only 2 papers (Kikkawa et al., 2003, this paper was counted under 'sequestration,' Sawada et al., 2008); and (6) all are laboratory experiments. Our earlier studies on CO₂ effects on fish were conducted to understand acute lethal impacts of CO₂ ocean sequestration. These used much higher CO₂ levels than those relevant to ocean acidification research and therefore are of limited value in considering how ocean acidification over the coming few centuries affects fish (see Ishimatsu et al., 2004, 2005 for review of these papers). In addition, Kikkawa et al. (2006) revealed that temporary varying CO₂ environment could have significantly higher negative impact on the survival of juvenile Japanese sillago *Sillago japonica* than static exposure.

Subsequent to the publication of our review paper in 2008, several new findings have been reported for fish under $p\text{CO}_2$ levels projected for the future ocean. Munday et al. (2009a) found that embryonic development of the clownfish *Amphiprion percula* was unaffected by exposure up to 1030 μatm , and thus apparently more tolerant than in invertebrates (see above and Kurihara, 2008 for review). Moreover, the larvae from some but not all parents were longer and heavier in acidified conditions than in controls. Critical swimming speed did not differ between the control and larvae reared under high CO₂ conditions. Thus, the levels of ocean

Table 1. Summary of the literature survey on the effects of CO₂ on fishes.

Habitat ^a	: Freshwater (88), Seawater (30: teleosts 22, elasmobranchs 8)
<i>p</i> CO ₂ (µatm) ^b	: <5,000 (9), 5,000–10,000 (52), 10,000–50,000 (44), >50,000 (8)
Duration (days) ^c	: <1 (52), 1–4 (27), 4–10 (3), >30 (18)
Purpose of study ^d	: Acid-base (38), Cardiorespiratory (29), Growth (10), Miscellaneous (39)

Numbers in parenthesis indicate the number of papers classified according to the fish habitat, *p*CO₂ level used, exposure duration, and purpose of study. Total number of papers we surveyed is 116. ^aTwo studies used both freshwater and seawater fish. ^bSum of the listed studies is 113 since several studies did not report *p*CO₂ values. For those studies in which several levels of *p*CO₂ were used, the lowest *p*CO₂ values were used for statistics. Original papers reported CO₂ levels as concentration (mg/L) or *p*CO₂ in mmHg, torr or kPa. We calculated *p*CO₂ in µatm using reported experimental temperature and CO₂ solubility values (Dejours, 1981). ^cSum of the listed studies is 100 since we excluded in vitro studies from counting. For those studies in which several exposure durations were tested, the longest duration was used for statistics. ^dFor those studies in which more than one purpose was stated, we chose major ones for statistics. “Miscellaneous” includes CO₂ anesthesia (6 studies), in vitro myocardium physiology (6), CO₂ sequestration (6), palatine CO₂ receptors (5), sperm motility (4), metabolism (2), behaviour (2), swimbladder gas (1), fillet attributes (1), Ca metabolism (1), ammonia (1), cataract (1), blood sugar (1), feed intake (1), and early development (1). Modified from Ishimatsu et al. (2008).

acidification that are projected by the end of this century appear not to have strong negative effects on early life-history of the species. Another aspect of possible larval response to high CO₂ conditions was studied using *A. percula* by Munday et al. (2009b). The results indicated that the ability of olfactory discrimination is disrupted by exposure to *p*CO₂ of 1000 µatm (pH 7.8). If this occurs in natural environment, it would impair the larval ability of locating suitable habitat and avoiding unsuitable settlement sites during transition from pelagic stage to demersal life in coral reefs.

Effects of increased seawater *p*CO₂ on swimming was tested by Munday et al. (2009c) and Melzner et al. (2009). Aerobic scope (difference between oxygen consumption rate at maximum swimming speed and resting) was significantly depressed by exposure to *p*CO₂ of 1000 µatm at 29–32°C in one coral reef fish *Ostorhincus cyanosoma*, but not in the other species, *O. doederleini* (Munday et al., 2009c). Resting oxygen uptake remained unaffected or increased by the CO₂ exposure, unlike most findings for invertebrates. In contrast, maximum oxygen consumption either decreased or remained stable in high CO₂ conditions, leading to the observed declined of aerobic scope. There was no interaction of temperature and high CO₂ on aerobic scope. Melzner et al. (2009) acclimated Atlantic cod *Gadus morhua* to 3000 µatm for 4 months and to 5800 µatm for 12 months, both at 5°C. Resting and active metabolic rates, critical swimming speed and aerobic scope were all unaffected by the long-term rearing under the elevated CO₂ conditions. Gill Na⁺/K⁺ ATPase activity and protein showed significantly higher values for the fish reared in 5800 µatm but not in 3000 µatm.

Fish otolith deposits aragonite and its formation could thus be affected by ocean acidification as calcification of shells and tests in most marine invertebrates (see

above). Therefore, the recent finding of stimulating effects of high environmental CO₂ (1000 μatm and 2500 μatm) on otolith size in larvae of the white sea bass *Atractoscion nobilis* is somewhat surprising (Checkley et al., 2009). Fish otolith is involved in both sound perception and the maintenance of postural equilibrium. Otoliths, as well as labyrinth, are formed before hatching (Noakes and Godin, 1988) when the capacity for acid–base regulation may not be fully developed (Alderdice, 1988). Thus, there is a need to further investigate effects of CO₂ on otolith growth, including asymmetry.

6. EFFECTS ON CNIDARIANS

Jellyfish are increasing in their abundance worldwide over the past decade, including the East China Sea and Yellow Sea. Frequency of Jellyfish blooms have been increasing, which can have substantial influence on plankton communities and fish populations and therefore fisheries (Hong et al., 2008; Xian et al., 2009). The possible link of jellyfish proliferation and ocean acidification was first pointed out by Attrill et al. (2007) on the basis of their analysis of records from the continuous plankton recorder and pH data from the central North Sea. They predicted that jellyfish frequency will increase over the next 100 years. The conclusion of Attrill et al. (2007) has been subsequently questioned by Richardson and Gibbons (2008) and Haddock (2008), and must therefore be viewed tentative at this time. Our experimental data on acute exposure of the scyphozoan jellyfish *Aurelia aurita* demonstrated that this species is exceptionally tolerant to CO₂, even though swimming activities are strongly inhibited by exposure to >50,000 μatm (Kikkawa et al., 2010). If this observation holds to other jellyfish species, then jellyfish might become more dominant than at present due to possible restructuring of marine ecosystems through differences in CO₂ tolerance among different animal groups.

7. THE EAST CHINA SEA, ITS STATUS QUO

The human population currently totals 6.63 billion and around 50% of the human population live within 200 km of the coasts. By 2025, the coastal human population is estimated to be 5 billion due to urbanization driving a movement in population towards the coast (Tibbetts, 2002; Creel, 2003; McGranahan et al., 2007; Diana, 2009). Consequently, the increase in the human population at major coastal cities around the globe has placed added anthropogenic pressure on these low elevated coastal areas (defined as areas less than 10 m above sea level) and has led to alteration of shorelines (see Fig 1 in Creel, 2003; McGranahan et al., 2007). Estuaries are also important coastal areas in ecological terms, as they may be deemed ‘nursery grounds’ where the most sensitive juvenile-stages of organisms are found (Haywood et al., 1998; Epifanio et al., 2003; Moksnes, 2004). Anthropogenic pressure resultant from population growth is exhibited in coastal waters such as shipping activities, resource exploitation, continual production, and disposal of chemicals into the environment leading to eutrophication, decreased biodiversity, habitat loss and alteration (reviewed in Kennish, 2002).

The East China Sea is the 3rd largest marginal sea in the world, of which 70% is the continental shelf zone shallower than 200 m depth, accounting for 3.2% of the

world continental shelf area ($27 \times 10^6 \text{ km}^2$). Riverine inputs into the East China Sea include the Changjiang (Yangtze) and Huanghe (Yellow) which are major sources of sediment loads. The Changjiang is the largest river in Asia (6300 km) and the 4th and 5th largest river in the world with respect to water ($944 \text{ km}^3 \text{ yr}^{-1}$) and sediment (0.5 Pg yr^{-1}) discharge into a large drainage basin ($1.8 \times 10^6 \text{ km}^2$) which is populated by 400 million people (see references in Bianchi and Allison, 2009). The Huanghe River was reported to discharge 1.1 Pg yr^{-1} sediment into the Yellow Sea. The East China Sea has been estimated to yield one of the highest biomass distributions of fish in the world's oceans and is one of the world's major fishing grounds (ca. $33\text{--}59 \text{ g wet weight m}^{-2}$, Wilson et al., 2009). High fish productivity is supported by high yearly primary production (ca. $145 \text{ g C m}^{-3} \text{ yr}^{-1}$) as found in the Changjiang River plume region (Gong et al., 2003; Chen et al., 2004). However, evaluation of the distribution and intensity of human impacts on coastal marine ecosystems reveals that the East China Sea is ranked as one of the most impacted ocean systems together with the North Sea (Europe) Norwegian Sea, South China Sea (Asia), Easter Caribbean, North America Eastern seaboard, Mediterranean, Persian Gulf, Bering Sea and waters around Sri Lanka (Halpern et al., 2008).

Even though the East China Sea acts as an annual CO_2 sink as a whole (Tsunogai et al., 1999), there appears to be areas showing high $p\text{CO}_2$ values, e.g., in the Changjiang Estuary, Hangzhou Bay, and their adjacent areas (Gao et al., 2008). The Changjiang River transports CO_2 -enriched freshwater in spring and summer where the excess CO_2 is deemed to be reduced by primary productivity. In contrast, in autumn the East China Sea acts as a CO_2 source as biological recycling of the products of primary production occurs. The integrated sea-air CO_2 flux of the Changjiang River was estimated at $-1.9 \pm 1.3 \text{ mol m}^{-2} \text{ yr}^{-1}$ (Zhai and Dai, 2009). Seawater $p\text{CO}_2$ has been recorded in the region as high as $621 \mu\text{atm}$ (Changjiang River, Zhai and Dai, 2009) and $950 \mu\text{atm}$ (Okinawa Reef, reviewed in Borges et al., 2005). Estuaries play a greater role in the global carbon cycle because the atmospheric-water flux in estuaries is far greater ($108.83 \text{ mol C m}^{-2} \text{ yr}^{-1}$) than in marginal seas ($-1.94 \text{ mol C m}^{-2} \text{ yr}^{-1}$) or in open oceans ($-0.43 \text{ mol C m}^{-2} \text{ yr}^{-1}$, Borges et al., 2005). Estuarine $p\text{CO}_2$ values have been recorded around the world averaging $531\text{--}3129 \mu\text{atm}$, but have also been recorded as high as $4900 \mu\text{atm}$ (Thames, UK), $5700 \mu\text{atm}$ (Sado, Portugal), $6653 \mu\text{atm}$ (Scheldt, Belgium) and $7800 \mu\text{atm}$ (Altamaha, USA) thereby demonstrating the elevated $p\text{CO}_2$ recorded in these important coastal water bodies (reviewed in Raymond et al., 2000). Moreover, coastal waters can be greatly impacted by anthropogenic nitrogen and sulfur deposition, which adds to the acidification by CO_2 . Coastal regions in East Asia including the East China Sea are thought to be one of the highest acid input regions in the world (Doney et al., 2007).

The East China Sea is prone to hypoxia ($<2\text{--}3 \text{ mg O}_2 \text{ L}^{-1}$), and an area currently estimated at greater than $12,000 \text{ km}^2$ (or 432 km^3 volume, Chen et al., 2007). The current threshold level of hypoxia may be underestimating the effects of hypoxia as a level above $2 \text{ mg O}_2 \text{ L}^{-1}$ results in the mass mortality of crustacean and fish species (Vaquer-Sunyer and Duarte, 2008). The number of hypoxic zones in the East China Sea is growing in size and is currently the largest area in Asian marine ecosystems (Diaz and Rosenberg, 2008). The hypoxic zone is also predicted to expand with depth

(300–700 m) with changes of 0.09 to 0.34 $\mu\text{mol kg}^{-1} \text{yr}^{-1}$ (Stramma et al., 2008). Due to the net effects of coastal currents (China Coastal, Taiwan Warm and Kurioshio Currents), much of the sediment and particulate organic matter (POC) exported into the East China Sea remains contained within this shallow water coastal zone (Bianchi and Allison, 2009). The accumulation of POC from riverine inputs and high primary productivity in this area coupled with the regional geomorphology and ocean circulation patterns may encourage microbial activity and enhanced consumption of dissolved oxygen in the benthos; this process may be the mechanism how hypoxic waters may arise in the East China Sea (Diaz and Rosenberg, 2008; Rabouille et al., 2008; Wang, 2009).

Coastal regions in East Asia, including the East China Sea are thought to be one of the highest acid input regions in the world (Doney et al., 2007). A $p\text{CO}_2$ of 450 μatm is thought to be the ‘tipping point’ of where aragonite undersaturation occurs (McNeil and Matear, 2006) and is currently found in coral ecosystems resulting in reduced coral reef calcification and thus increased reef dissolution (Manzello et al., 2008). Shallow-coastal waters (40 m) near the East China Sea have also been shown to become storage areas of low pH (7.8), CO_2 -rich hypoxic ($< 3 \text{ mg O}_2 \text{ L}^{-1}$) waters (Wei et al., 2007). CO_2 -enrichment of benthic water develops due to bacterial decomposition of organic matter (thus dissolved oxygen consumption) and accumulates due to the thermal stratification (Taguchi and Fujiwara, 2009).

Other anthropogenic inputs into coastal waters include organic contaminants such as polyaromatic hydrocarbons (PAHs) and organochlorines, and metals. Moderate to high levels have been recorded for both PAHs (17–187 ng g^{-1} , Bouloubassi et al., 2001) and metals (copper, 4.3–41.5 $\mu\text{g g}^{-1}$; manganese, 152–1152 $\mu\text{g g}^{-1}$; nickel, 8.2–48.6 $\mu\text{g g}^{-1}$; lead, 10.0–44.8 $\mu\text{g g}^{-1}$; and zinc, 18.2–114 $\mu\text{g g}^{-1}$) in both surface sediments (Fang et al., 2009) and in deep sea fish (50–900 ng g lipid^{-1} , Oshihoi et al., 2009), however, contaminant levels are predicted to increase due to the rapid expansion and urbanisation of China (Fang et al., 2009). The increased contamination of the East China Sea has been evaluated in terms of the contaminant load in seafood (bivalves) and the potential threat to humans. Results indicated that metals were of primary concern, as well as organic contamination indicating also that mercury could pose a health risk to the human population (Fung et al., 2004).

8. CONCLUSION

Despite that data are rapidly accumulating on the effects of ocean acidification due to increasing CO_2 on marine organisms, our ability of predicting potential consequences of this quietly progressing ocean environmental change is no more than infantile. Anthropogenic ocean acidification may reduce seawater pH to levels that are greater than any experienced in the past 300 million years, over the coming centuries, with the possible exception of those resulting from rare, catastrophic events in Earth’s history (Caldeira and Wickett, 2003). Moreover, the current rate of pH reduction is probably at least 100 times higher than the maximum rate during the past hundreds of thousands of years (Royal Society, 2005). Recent research has revealed that organisms could evolve on contemporary timescales (within few decades) in response to environmental changes (Stockwell et al., 2003). Generation length is an

important factor for the evolutionary potential of a species such that those with longer generation length are probably unable to adapt to the fast drop of seawater pH they are currently experiencing (Berteaux et al., 2004).

Compared with the research effort dedicated to understanding of warming impacts on marine life, much less work has been done about possible consequences of ocean acidification that would ensue if the current rapid rise of atmospheric CO₂ concentration is unabated. Ocean acidification has become recognized as a potentially devastating threat to ocean environments and ecosystems, which are already threatened by temperature and sea level rise. The first international expert symposium was held in 2004 (The Ocean in a High CO₂ World, UNESCO, Paris), and it was only after this symposium that research into ocean acidification has shown rapid growth as shown in the number of publications in the last couple of years.

Recent reviews have summarized future research themes of priority importance (Harley et al., 2006; Fabry et al., 2008; Vézina and Hoegh-Guldberg, 2008; Doney et al., 2009), and therefore we would not repeat that. What we would like to stress in this short review is that scientists of those countries surrounding the East China Sea need more collaboration not only in the field of ocean acidification but also on the issues of ocean warming, eutrophication, pollution and overfishing, to list a few.

Ocean acidification research has recently been exploding in European countries, US and Australia as attested by the large number of publications during the last few years from these countries. In contrast, far fewer research groups are active in Asian countries. There has been increasing awareness among scientists that impact assessment must pay attention to those conditions unique to coastal waters, taking local species and environmental conditions into experimental protocols to improve prediction accuracy of studies. Thus, it is important to predict how local environment will shape in the coming decades, and use such predictions in an experimental protocol to improve our prediction ability.

Acknowledgments—We are grateful for the help of Ms. Mizuri Murata during preparation of this manuscript.

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