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Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator

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By the end of this century, anthropogenic carbon dioxide (CO2) emissions are expected to decrease the surface ocean pH by as much as 0.3 unit. At the same time, the ocean is expected to warm with an associated expansion of the oxygen minimum layer (OML). Thus, there is a growing demand to understand the response of the marine biota to these global changes. We show that ocean acidification will substantially depress metabolic rates (31%) and activity levels (45%) in the jumbo squid, Dosidicus gigas, a top predator in the Eastern Pacific. This effect is exacerbated by high temperature. Reduced aerobic and locomotory scope in warm, high-CO2 surface waters will presumably impair predator–prey interactions with cascading consequences for growth, reproduction, and survival. Moreover, as the OML shoals, squids will have to retreat to these shallower, less hospitable, waters at night to feed and repay any oxygen debt that accumulates during their diel vertical migration into the OML. Thus, we demonstrate that, in the absence of adaptation or horizontal migration, the synergism between ocean acidification, global warming, and expanding hypoxia will compress the habitable depth range of the species. These interactions may ultimately define the long-term fate of this commercially and ecologically important predator.

Atmospheric carbon dioxide [CO2]atm has increased from preindustrial levels of 280 ppm to >380 ppm today (1) and is expected to rise to 730–1020 ppm by the year 2100 (2). Almost half of the anthropogenic CO2 released between 1800 and 1994 is now stored in the oceans (3), and ∼30% of recent emissions have been taken up by the ocean to date (4). Carbon dioxide reacts with seawater, resulting in a net increase in the concentration of H+ (lowered pH) and a decrease in the carbonate ion (CO32-) concentration. This process, termed ocean acidification (5), is projected to decrease the pH of surface waters between 0.14 and 0.35 unit by the end of the 21st century (2). These future changes in the ocean’s chemistry pose a serious problem for key marine organisms with CaCO3 skeletons, such as corals and some plankton (6, 7). However, elevated CO2 also has more broad detrimental effects on the survival, growth, and respiratory physiology of marine animals (8–10), although most of these experiments were not undertaken with ocean acidification in mind and used unrealistically high CO2 levels. Thus, the effects of environmentally relevant pH reductions on noncalcifying marine biota are still poorly understood (7). At the same time, global ocean temperature has risen over the past few decades by 0.1°C from the surface to a depth of 700 m (11), causing, among other things, an expansion of the oceanic oxygen minimum layer (12). Additional warming and encroaching hypoxia in the coming century will also influence physiological processes (13–15) and may drive, at the community level, profound changes to trophic interactions (16), diversity, and biogeography (13, 17).

The synergistic effects of elevated CO2, hypoxia and temperature, are, to date, completely unexplored.

The jumbo squid, Dosidicus gigas, is a large pelagic top predator endemic to the Eastern Tropical Pacific (ETP), where temperature and oxygen are already near the upper and lower extremes, respectively, found in the oceans and where climate changes are expected to be pronounced (2). D. gigas reaches >2 m in total length and 50 kg in mass. Over the last few years, it has greatly extended its tropical/subtropical range as far north as Canada and Alaska, where it is now exerting a significant top–down control on commercial fish stocks (18). Like other ommastrephid squids, D. gigas displays a high oxygen demand that reflects high activity levels dictated by the pelagic environment and low efficiency of jet propulsion relative to other forms of locomotion (19). The metabolic capacity of these muscular squids is surprising considering the limitations of their respiratory systems. Their blood has low oxygen-carrying capacity, relative to similarly active fishes, because of viscosity-related constraints associated with an extracellular respiratory protein. In fact, they use all of the oxygen carried in the blood on each cycle through the body, even at rest, leaving no venous oxygen reserve. Furthermore, blood–oxygen binding in most active squids is highly pH sensitive (supporting information (SI) Table S1), a property that facilitates oxygen release to demanding tissues, but presumably interferes with oxygen extraction from hypoxic or CO2-rich seawater. Consequently, these organisms are thought to live chronically “on the edge of oxygen limitation” (20) and are not well poised to adapt to future environmental changes that influence oxygen supply and demand. Jumbo squids are thus expected to be particularly vulnerable to ocean acidification, global warming, and hypoxia. Surprisingly, D. gigas undergoes diel vertical migrations into zones of pronounced hypoxia at mesopelagic depths (21), known as oxygen minimum layers (OML).

To evaluate the effects of rapidly changing environmental parameters on the metabolic physiology of D. gigas, we exposed the jumbo squids to short-term (<24 h) elevations in CO2 equivalent to pessimistic predictions for the year 2100 (0.1% CO2, ∼1000 ppm; A pH = 0.3), while varying temperature and oxygen levels to match the conditions currently found at their night and daytime distributions (20°-25°C, 21% O2 and 10°C, 1% O2, respectively).

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Results and Discussion

All animals displayed a distinct periodicity of oxygen consumption rates (Fig. 1). Video analysis confirmed that the cycles, referred to here as “active cycles,” correlated with activity levels as indicated by rates of mantle contraction for jet propulsion. The occurrence of such activity peaks enabled us to quantify what we define as the maximal (MaxMR), active (AMR), routine (RMR), and inactive metabolic rates (IMR) and the mean number of active cycles per hour. These designations are based on the highest recorded rate (MRM), the average of rates expressed during peaks in the active cycles that were 20% higher (AMR) and 15% lower (IMR) than the average for the entire run (RMR) (see Materials and Methods for details). The percentages chosen to designate AMR and IMR are based on levels that approximated continuous activity and apparent inactivity (modest ventilatory movements only) in the video observations. Thus, IMR is, we believe, a reasonable approximation of a standard metabolic rate.

The jumbo squids displayed oxygen consumption rates that are among the highest in the oceans (Fig. 2). The squid’s lowest rates of mass-specific metabolism are higher than those of top vertebrate predators of equivalent size, including sharks and tunas (Fig. 2B). However, under hypoxic conditions (Fig. 3B), IMR decreased by ~80% from an average of 7.0 (control) to 1.4 μmol O₂ g⁻¹ h⁻¹ (Fig. 3A). A concomitant enhancement of glycolysis, as indicated by an accumulation of octopine (Fig. S1), compensates for only a small fraction (~10%) of the energy deficit that resulted from the decline of aerobic ATP synthesis. In the absence of reduced oxygen demand, we calculate that the rate of ventilation that would be required to meet oxygen demand, assuming complete extraction of oxygen from the respiratory stream, increases dramatically with depth (Fig. 3C). Because ventilation and locomotion are tied via contraction of the large muscular mantle (23), ventilation is a very costly part of the energy budget in squids. Excess ventilation during hypoxic exposure would drive oxygen demand up in a positive feedback loop that would presumably prohibit the maintenance of routine aerobic metabolic rates in the oxygen minimum layer. Thus, whereas the OML restricts the depth distribution of competing vertebrate predators to the upper surface layers because of their more limited hypoxia tolerance (ref. 24, Fig. 3B), D. gigas circumvents similar restrictions via metabolic suppression and spends the daytime in deep, cold, and oxygen-depleted waters (Fig. 3).

The profound hypoxia-induced metabolic suppression (Figs. 2A and 3A) extends the squid’s survival time in the OML, by conserving the finite stores of fermentable substrate, minimizing cytotoxicity and limiting the oxygen debt that must be repaid upon return to oxygen-replete surface waters. Oxygen debt typically includes the costs of replenishing substrate stores and removing toxic end products of anaerobic metabolism. Payment of such debts entails a substantial increase in oxygen consumption (25). Although the oxygen debt incurred by D. gigas is minimized by the substantial suppression of ATP-consuming
activities, it is probably not negligible and will add to the already high metabolic costs associated with warm surface temperatures.

Under the elevated CO2 conditions predicted for the end of this century, the squid’s oxygen demand was significantly reduced (Figs. 1 and 4, and Fig. S2). At 25°C, MaxMR decreased, on average, from 70 to 48 $\mu$mol O$_2$ g$^{-1}$ h$^{-1}$ ($P < 0.05$; Fig. 4A), which represented a reduction of 31% (Fig. 4B). Similar CO2-induced decreases in MaxMR were attained at 10° and 20°C ($P < 0.05$; Fig. 4A and B). Significant reductions were also observed for all of the other measured rates and temperatures, with the

Fig. 3. Metabolic physiology and vertical ecology of Dosidicus gigas. (A) Projected inactive metabolic rates (10-g animal, $\mu$mol O$_2$ h$^{-1}$, black solid line) as a function of depth in the Gulf of California. The thick black dashed line reflects the expected metabolic depression after reaching the squid’s critical oxygen partial pressure, $P_c$ (21.5 $\mu$M, open circle) (21). The thin red dashed line shows the increase in metabolic rate because of expected ocean surface warming (2°C) in the Eastern Tropical Pacific (ETP) (2). Gray bars represent the main depth range occupied by $D.$ gigas during daytime (~250 m) and nighttime (~70 m) periods (21). (B) Depth-related changes in oxygen levels (ml liter$^{-1}$, black solid line) and hypoxic thresholds (critical PO$_2$, $P_c$, symbols) of several ETP top predators. Note that the partial pressure of oxygen (i.e., the actual driver of oxygen flux into the organism) at a given concentration will vary with temperature. Open circle, jumbo squid’s $P_c$, light gray circle, bigeye tuna ($Thunnus obesus$); deeper black circle, yellowfin tuna ($Thunnus albacares$); and shallower black circle, skipjack tuna ($Katsuwonus pelamis$). Sources for tuna data are available in Table S4. The thick blue dashed line represents the predicted oxygen levels in 1960. These estimates were based on a decrease in oxygen of 0.13 $\mu$mol kg$^{-1}$ year$^{-1}$ from 1960 to 2008 in the ETP pelagic ecosystem (12). The expansion of the oxygen-minimum layer (OML) drives the hypoxic threshold to shallower depths (illustrated by the change in the squid’s $P_c$, blue square and open circle), causing a more compressed epipelagic habitat. The bathymetric difference between the past and the present 21.5-$\mu$M (0.5 ml liter$^{-1}$) oxyline is 65 m. (C) Temperature (°C, thin blue dashed line) and projected ventilation rates in $D.$ gigas (10-g animal) as a function of depth. Ventilation rate ($l$ h$^{-1}$, black solid line) equals the water volume from which all O$_2$ must be extracted per hour to support standard metabolism. The thick black dashed line reflects the expected ventilation rate reduction after reaching the squid’s $P_c$. The thin red dashed line shows the increase in ventilation rate because of the ocean surface warming.

Fig. 4. Effect of hypercapnia in the metabolic physiology of jumbo squid, Dosidicus gigas. (A) Mean oxygen consumption rates ($\pm$ SE; $\mu$mol O$_2$ g$^{-1}$ h$^{-1}$) under control (black symbols) and hypercapnic treatments at inactive (IMR), routine (RMR), active (AMR), and maximum (MRM) levels (green, orange, red, and blue symbols, respectively). Oxygen consumption rates under control conditions are plotted against themselves and against rates of those same individuals under elevated (0.1%) CO$_2$. Open diamonds, 10°C; solid circles, 20°C; solid triangles, 25°C (plots with individual runs are available in supporting online material). (B) Oxygen consumption rates and activity levels (number of active cycles per hour) under high CO$_2$ expressed as a percentage of the control values at the three different temperatures. *, significant differences (paired t test, $P < 0.05$); x, nonsignificant differences (paired t test, $P > 0.05$).
exceptions of RMR and IMR at 10°C (crosses in Fig. 4). The number of activity cycles per hour was also significantly reduced between 55 and 84% (P < 0.05; Fig. 4B).

Although the OML environment is characterized by both hypoxia and high CO$_2$ levels (26), our results indicate that the effect of oxygen availability on the squid’s metabolic rate overshadows the more subtle CO$_2$ effect while at its cold, hypoxic daytime habitat depth. Low pH and high CO$_2$ are common triggers of metabolic depression (27), but they do not cause a substantial metabolic depression in $D. gigas$ while in the OML (Fig. 4B). However, at warmer temperatures, carbon dioxide becomes an important influence on $D. gigas$’ metabolic rate.

The effect of temperature on aerobic metabolism, expressed as a temperature coefficient ($Q_{10}$), changed dramatically across the temperature range studied (Fig. 2A). Coefficients of $2\approx$ and $7$ were measured for IMR between 10°–20°C and 20°–25°C, respectively. While the former is within the normal range of tolerances for the species (28), the unusually high $Q_{10}$ values over the 20°–25°C range may indicate that temperatures $>20°C$ are not optimal for these highly aerobic animals. Field data with satellite-linked pop-up archival transmitting tags in the Sea of Cortez support this contention. Gilly and collaborators (21) reported that $D. gigas$ spends <10% of its time at temperatures $>20°C$, while Davis and collaborators (29) reported that the cumulative probability of a squid making a deep dive was reduced to only a few minutes at temperatures $>22°C$. In other words, excursions into warm surface waters were often terminated by rapid deep dives to cooler waters that are thought to provide thermal relief.

By the end of the 21st century, the ETP is expected to warm, between 2° and 3°C because of weakening tropical circulations (2), and seawater pH will decline by as much as 0.3 unit. Warmer temperatures will elevate metabolism and require enhanced ocean transport is sufficient to meet very high rates of oxygen demand. Thus warming and acidification may cause ventilatory and circulatory stress that restricts the squid’s aerobic scope and impairs swimming activity. Together these variables may reduce the animal’s ability to respond to external stimuli, leaving it more vulnerable to predation and other threats. Furthermore, the temperature-dependent activity in the chambers. MaxMR were quantified as the highest rate achieved during a given treatment and were typically 35–100% of the criteria set for “standard” metabolic rates in mammalian and other model organisms, we feel that they are a reasonable approximation of a standard metabolic rate.

### Materials and Methods

Jumbo squid, $D. gigas$ (1.2–50.8 g of total weight), were collected in Santa Rosalía (27°N, 112°W) and Guaymas (27°N, 111°W) basins in the Gulf of California (aboard RV New Horizon, Scripps Institution) in May and June 2006 and June 2007 and in the Eastern Tropical Pacific (ETP, 13°N, 104°W; ET2, 8.5°N, 90°W; aboard RV Seward Johnson, Harbor Branch) in October and November 2007. Specimens were captured during the night, at the surface, by using a hand-held dip net (area $\approx$ 40 cm$^2$), and immediately transferred to 10–20°C seawater aquaria onboard the ship where they were maintained at about 12% oxygen before placement in a respiratory chamber. A total of 86 specimens were used. Specimens were placed in a flow-through respirometer (270 ml volume, Loligo Systems), where they were allowed to acclimate for 8–12 h before measurements of aerobic metabolism began. Respirometers were immersed in a large thermostated water bath. Filtered (0.2 mm) and treated (50 mg liter$^{-1}$ streptomycin) seawater was pumped from a water-jacketed gas-equilibration column through the respirometers at a constant flow rate of 140 ml min$^{-1}$. The water in the column was bubbled continuously with humidified certified mixtures of air to maintain incoming water at normal (21% O$_2$) or low PO$_2$ (certified gas mixture with 1% O$_2$) for the hypoxia experiments and high (certified gas mixture with 0.1% O$_2$) or normal PCO$_2$ (0.03% CO$_2$) for the CO$_2$ experiments. The pH of the afferent water was measured (Orion model 720A + meter, flow-thru pH, Microelectrodes Inc.) to ensure that a constant CO$_2$ level had been reached. CO$_2$ treatment resulted in a 0.3 unit pH decrease (normocapnia pH 7.93 ± 0.05; hypercapnia pH 7.2 ± 0.05). Oxygen concentrations were recorded at the entrance and the exit of each chamber with Clarke-type O$_2$ electrodes connected to a Strathkelvin Instruments 928 Oxygen Interface. The system was calibrated using air- and nitrogen-saturated seawater and checked for electrode drift and for microbial oxygen consumption before and after each trial. All experiments were carried out in darkness and at atmospheric pressure.

The animals showed a distinct periodicity to the oxygen consumption rates on time intervals of $\approx$20 min, and video analysis confirmed that the cycles correlated with activity levels as indicated by rates of mantle contraction for jet propulsion. During periods of activity, metabolic rates (MR) were $\approx$20% higher than the routine levels (RMR, average rate for the entire experiment). Thus, the AMR was quantified by averaging the rates for all peaks $\approx$20% higher than the RMR. IMR were quantified by averaging peaks of the experiment that were 15% lower than the routine rate. These rates correlated with hypercapnic pH (pH 7.2 ± 0.05), and were used for the data analysis. Oxygen consumption was quantified as the highest rate achieved during a given treatment and were typically 10–100% higher than the RMR.

Note that pelagic predators such as $D. gigas$ rarely stop swimming in nature and that we did not quantify locomotion continuously for any experiment in its entirety. Thus, while our inactive metabolic rates do not necessarily meet the criteria set for “standard” metabolic rates in mammalian and other model organisms, we feel that they are a reasonable approximation of a standard metabolic rate.

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