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Effects of Lethal Levels of Environmental Hypercapnia on Cardiovascular and Blood-Gas Status in Yellowtail, *Seriola quinqueradiata*

Kyoung-Seon Lee[1], Jun Kita[2] and Atsushi Ishimatsu[1]*

[1]*Marine Research Institute, Nagasaki University, Tairamachi, Nagasaki 851-2213, Japan*
[2]*Research Institute of Innovative Technology for the Earth (RITE), Kizucho, Kyoto 619-0209, Japan*

ABSTRACT—The cardiorespiratory responses were examined in yellowtail, *Seriola quinqueradiata* exposed to two levels of hypercapnia (seawater equilibrated with a gas mixture containing 1% CO_2 (water PCO_2 = 7 mmHg) or 5% CO_2 (38 mmHg)) for 72 hr at 20°C. Mortality was 100% within 8 hr at 5% CO_2, while no fish died at 1% CO_2. No cardiovascular variables (cardiac output, $\dot{Q}$; heart rate, HR; stroke volume, SV and arterial blood pressure, BP) significantly changed from pre-exposure values during exposure to 1% CO_2. Arterial CO_2 partial pressure ($PaCO_2$) significantly increased ($P < 0.05$), reaching a new steady-state level after 3 hr. Arterial blood pH (pHa) decreased initially ($P < 0.05$), but was subsequently restored by elevation of plasma bicarbonate ($[HCO_3^-]$). Arterial O_2 partial pressure (PaO_2), oxygen content (CaO_2), and hematocrit (Hct) were maintained throughout the exposure period. In contrast, exposure to 5% CO_2 dramatically reduced $\dot{Q}$ ($P < 0.05$) through decreasing SV ($P < 0.05$), although HR did not change. BP was transiently elevated ($P < 0.05$), followed by a precipitous fall before death. The pHa was restored incompletely despite a significant increase in $[HCO_3^-]$. PaO_2 decreased only shortly before death, whereas CaO_2 kept elevated due to a large increase in Hct ($P < 0.05$). We tentatively conclude that cardiac failure is a primary physiological disorder that would lead to death of fish subjected to high environmental CO_2 pressures.

Key words: yellowtail, hypercapnia, blood gas, cardiac output, CO_2

INTRODUCTION

The cumulative increase of atmospheric CO_2 of approximately 80 ppm has already taken place during the past 200 years (Oeschger, 1993), and a further increase is most likely to occur. To alleviate greenhouse effects through the ever-increasing emission of CO_2, technologies have been developing to capture CO_2 exhausted from large production sources and sequester it into the ocean (Haugan and Drange, 1992). However, when this happens, the CO_2 concentrations should significantly increase near a releasing site (Haugan and Drange, 1992), and the sudden changes in water CO_2 concentration will surely affect the physiology of marine organisms.

Although there is increasing awareness of the need for investigating biological impacts of ocean CO_2 sequestration (Seibel and Walsh, 2001), little information is currently available on this point. In fact, effects of elevated ambient CO_2 have been examined mostly on freshwater fishes, and information is critically lacking for marine animals (Ishimatsu and Kita, 1999). Fish exposed to sublethal levels of environmental hypercapnia usually show respiratory acidosis, subsequently compensated for by accumulation of bicarbonate ions largely through transepithelial ion transport (Heisler, 1986), hyperventilation (Gilmour, 2001), and lowered plasma Cl^- concentration (Cameron and Iwama, 1987). Not much is known about the cardiovascular responses to CO_2 exposure, and data available on this aspect only concern acute (commonly shorter than 30 min) responses under sublethal levels of ambient CO_2. Nothing is known about fish kill mechanism under more severe levels of hypercapnia.

In our previous study (Hayashi *et al.*, submitted), we studied acute acid-base responses to severe hypercapnia in three marine fishes (yellowtail *Seriola quinqueradiata*, Japanese flounder *Paralichthys olivaceus* and starspotted dogfish *Mustelus manazo*). Because mortality occurred after a complete pH recovery, we concluded that blood acidosis was not responsible for fish mortality, and speculated that fish kill was due to the failure of oxygen transport system.

* Corresponding author: Tel. +81-95-850-7312;
FAX. +81-95-840-1881.
E-mail: a-ishima@net.nagasaki-u.ac.jp

Therefore, the present study was conducted to assess the cardiovascular and blood-gas status in yellowtail during exposure to two (sublethal and lethal) levels of environmental hypercapnia.

MATERIALS AND METHODS

Experimental conditions and the treatment of animals were outlined in an earlier paper (Lee *et al.*, 2003). Yellowtail, *Seriola quinqueradiata*, with a mean mass of 1,414±70 (SEM) g (N=11), were acclimated to 20°C. Yellowtail was chronically cannulated in the dorsal aorta, and a Doppler flow probe was placed around the ventral aorta under anesthesia. Fish were then transferred to a Plexiglas box supplied with a continuous inflow of well-aerated seawater, and allowed to recover for about 24 hr.

Experimental protocol

After duplicate or triplicate control measurements, yellowtail was exposed to two levels of hypercapnia by bubbling seawater with a gas mixture of either 1% or 5% CO_2 in air (gas flow 6 l/min) delivered by a GF-3/MP gas-mixing pump (Cameron Instruments Company, Tex., USA). Within 1 hr after the onset of CO_2 bubbling, water pH had decreased from about 8.25 to a new steady-state level of ca. 7.00 (1%) or 6.26 (5%). Blood samples were drawn anaerobically for analysis of blood-gas and acid-base variables at 0.5, 1, 3, 8, 24, 48, and 72 hr.

Cardiac output ($\dot{Q}$) was continuously determined by connecting a Doppler flow probe (Model ES, diameter 2.0–2.8 mm, Iowa Doppler Products, USA) to a Directional Pulsed Doppler flowmeter (Model 545C-4, Bioengineering, Iowa University, USA). Arterial blood pressure (BP) was determined by connecting the cannula to a disposable pressure transducer (Kawasumi Lab. INC., Japan). The pressure signals were amplified with a polygraph (NEC-Sanei Model 366, Japan) whose outputs were digitized with a Power Lab data acquisition system (ADInstruments, USA). Flow and pressure data are presented as means of 5 min measurement at each sampling time. Heart rate (HR) was obtained by triggering an output rate-meter from $\dot{Q}$ or BP signal. Stroke volume (SV) was calculated by dividing $\dot{Q}$ by HR.

Arterial blood pH (pHa) and O_2 partial pressure (PaO_2) were measured at 20°C with a Cameron Blood Gas Analyzer (Cameron Instruments). Arterial blood O_2 content (CaO_2) was measured with an Oxycon (Cameron Instruments). Hematocrit value (Hct) was determined using a microcentrifuge (×11,000 rpm, 5 min). Plasma total CO_2 ($TaCO_2$) in dorsal aortic blood was measured with a Capnicon 5 (Cameron Instruments). Arterial PCO_2 ($PaCO_2$) and plasma bicarbonate concentration ($[HCO_3^-]$) were calculated from the measured values of pHa and $TaCO_2$, using the Henderson-Hasselbach equation. CO_2 solubility (αCO_2) and apparent pK' values for human plasma were used as described in Boutilier *et al.* (1984).

$$PCO_2 = TCO_2/(\alpha CO_2 \times (1+10^{pH-pK'}))$$
$$[HCO_3^-] = TCO_2 - \alpha CO_2 \times PCO_2$$

where,

$$\alpha CO_2 = 0.0869 - 0.0028(T) + 4.6143\times10^{-5}(T^2) - 2.8869^{-7}(T^3)$$
$$pK' = 6.125 - \log(1+10^{pH-8.7}) - 0.0026(T-37) + 0.00012(T-37)^2$$

T is the temperature in °C.

Water PCO_2 ($PwCO_2$) was indirectly monitored by measuring water pH after establishing the relationship between the two parameters using CO_2 solubility of seawater (Weiss, 1974) and the apparent dissociation constants of carbonic acid in seawater (Mehrbach *et al.*, 1973) at 20°C, assuming seawater alkalinity of 2.3 mEq/kg.

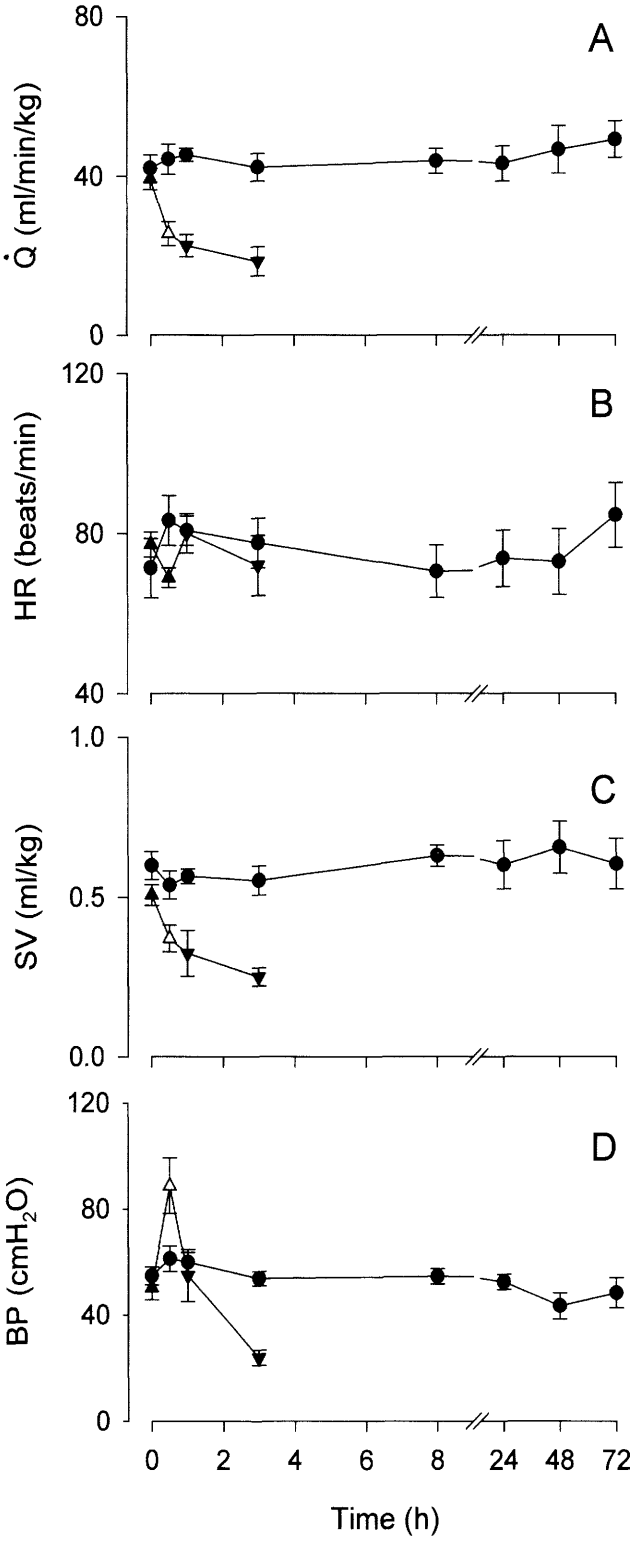

Fig. 1. Time-dependent changes in cardiac output ($\dot{Q}$: A), heart rate (HR: B), stroke volume (SV: C) and arterial blood pressure (BP: D) in yellowtail exposed to two levels of hypercapnia. Circles represent data at 1% CO_2 (water PCO_2 = 7 mmHg, N = 5), and triangles data at 5% CO_2 (water PCO_2 = 38 mmHg, N = 6). Open symbols indicate a significant difference from control values ($P < 0.05$). Down-triangles indicate that N was decreased due to fish death. No statistical analysis was applied to these points. Means±SEM.

Statistical analysis

Data are given as means±SEM wherever possible. Statistical comparison was made using one-way repeated-measures analysis of variance followed by Dunnett's test to identify data points that were significantly different from control values. The fiducial limit of significance was 5%.

RESULTS

During 72 hr exposure, no mortality occurred at 1% CO_2 ($PwCO_2$ = 7 mmHg, N = 5), while 100% mortality was recorded within 8 hr at 5% ($PwCO_2$ = 38 mmHg, N = 6); three fish died within 2 hr, the other three died between 3 and 8 hr.

The effects of hypercapnia on cardiovascular variables are shown in Fig.1. In fish exposed to 1% CO_2, none ($\dot{Q}$, Fig. 1A; HR, Fig. 1B; SV, Fig. 1C; or BP, Fig. 1D) changed significantly throughout the experiment. However, exposure to 5% CO_2 resulted in a significant fall in $\dot{Q}$ and SV, and a sig-

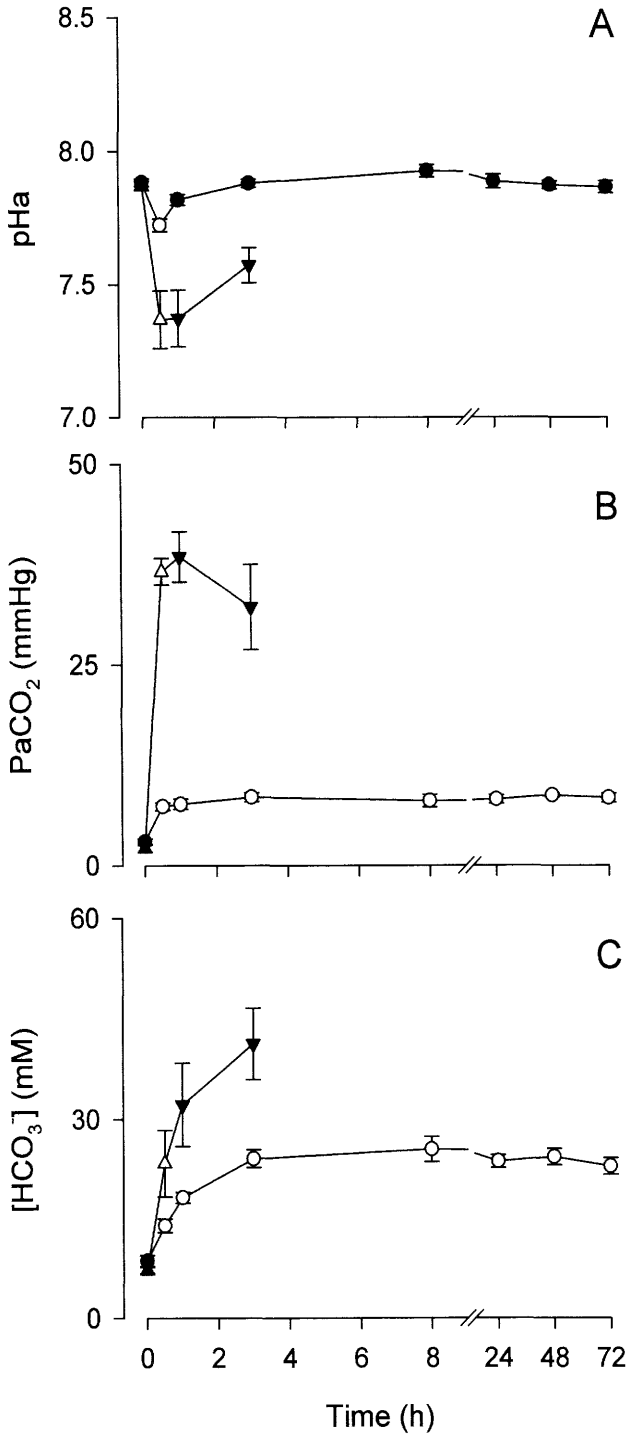

Fig. 2. Time-dependent changes in arterial blood pH (pHa: A), PCO_2 ($PaCO_2$: B) and plasma bicarbonate concentration (HCO_3^-: C) in dorsal aortic blood in yellowtail. Symbols are the same as in Fig. 1.

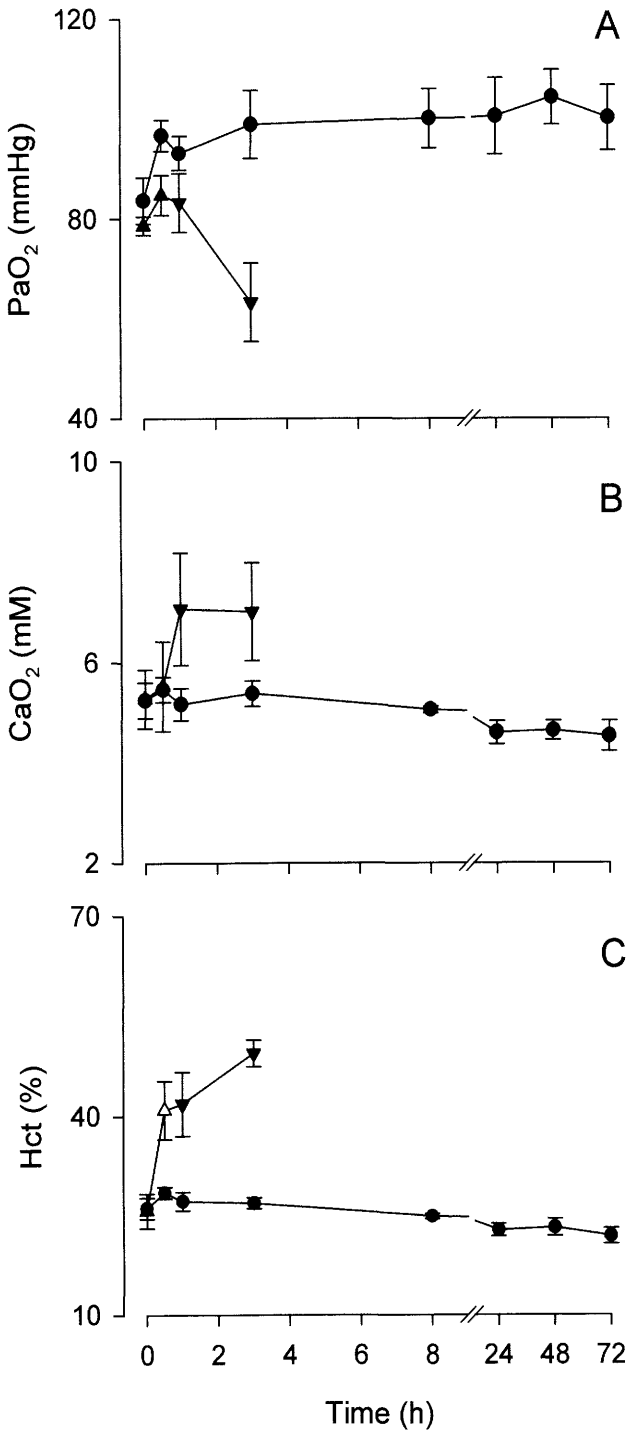

Fig. 3. Time-dependent changes in arterial PO_2 (PaO_2: A), arterial oxygen content (CaO_2: B) and hematocrit value (Hct: C) in yellowtail. Symbols are the same as in Fig. 1.

nificant rise in BP at 0.5 hr from the corresponding control normocapnic values, while HR was maintained throughout the experiment. Systemic vascular resistance, calculated as BP/$\dot{Q}$ assuming the central venous pressure to be zero, significantly increased at 30 min of 5% CO_2 exposure, whereas no significant change was detected for 1% (data not shown).

The pHa significantly fell from 7.885±0.014 to 7.725±0.024 in 0.5 hr at 1% CO_2, and then recovered completely, whereas pHa recovered only incompletely at 5% CO_2 (Fig. 2A). $PaCO_2$ increased significantly in 0.5 hr (from 3.1±0.4 mmHg to 7.4±0.5 mmHg at 1% CO_2, and from 2.2±0.4 mmHg to 36.4±1.7 mmHg at 5%; Fig. 2B). [HCO_3^-] increased significantly in 0.5 hr at both levels of hypercapnia (from 8.6±0.8 to 14.0±1.0 mM at 1% CO_2, and from 7.1±0.5 to 23.4±5.0 mM at 5%; Fig. 2C).

Neither PaO_2 (Fig. 3A) nor CaO_2 (Fig. 3B) showed significant changes during exposure to 1% CO_2. However, PaO_2 decreased shortly before death, and CaO_2 nearly doubled at 5% CO_2, although statistical comparison was not applied to these points because of partial mortality as stated above. Hct increased significantly only at 5% CO_2 (Fig. 3C).

DISCUSSION

The present data clearly demonstrated that high levels of ambient CO_2 markedly depressed cardiac functionality, followed by death within a short period. The cumulative mortality recorded in the present experiment is similar to that in our previous study employing less extensive surgery (only dorsal aorta cannulation) where 20% and 100% mortality recorded at 3 hr and 8 hr, respectively (Hayashi *et al.*, submitted). This indicates that the more invasive nature of surgical manipulation necessary for cardiac output measurement did not exert a significant negative influence upon CO_2 tolerance of yellowtail.

We think that fish mortality during exposure to 5% CO_2 is primarily a consequence of decreased blood flow to the tissues, as evidenced by the fall in $\dot{Q}$ (Fig. 1A). The delayed fall in PaO_2, as compared with the more rapid, significant drop in $\dot{Q}$, makes it unlikely that it is an immediate response to CO_2. More importantly, the higher CaO_2 suggests that the changes in blood oxygen levels are only of subsidiary importance in the fish death during exposure to the lethal levels of hypercapnia.

Yellowtail blood shows a rather large Bohr effect (Bohr factor – 0.74; Lee *et al.*, 2003), which would reduce O_2 affinity of hemoglobin and thereby decrease CaO_2 under the conditions of elevated CO_2. Thus, the only explanation for the maintained CaO_2 during exposure to 5% CO_2 is that a potential decline in CaO_2 due to both falling PaO_2 and respiratory acidosis was offset by increasing oxygen-carrying capacity of the blood through increases in blood hemoglobin concentration, as supported by the observed large increase in Hct under 5% CO_2 conditions (Fig. 3C). Hypercapnia is known to activate the autonomic adrenergic system in fish, stimulating sympathetic adrenergic nervous system (Perry *et al.*, 1999) and/or increasing concentrations of circulating catecholamines (Perry *et al.*, 1989). The contraction of fish spleen, the main storage organ for erythrocytes in fishes, is known to be under adrenergic nervous and/or humoral control, and stressed fish tend to deplete their splenic stores and have an elevated Hct (Gallaugher and Farrell, 1998).

Likewise, acidosis *per se* appears not to be the cause of CO_2 mortality because pHa was lower by only about 0.3 pH unit than the pre-exposure level before the fish death at 5% CO_2 (Fig. 2A). In addition, our previous study demonstrated that two thirds of the Japanese flounder (*Paralichthys olivaceus*) died between 24 and 48 hr of exposure to 5% CO_2, in spite of the fact that pHa had been completely restored to the normocapnic level by 24 hr following an initial pH drop of 0.8 unit (Hayashi *et al.*, submitted).

The cardiac failure during exposure to 5% CO_2 is solely attributable to decreasing SV (Fig. 1C) with no significant change in HR (Fig. 1B). Hypercapnic acidosis is known to have negative inotropic effects on fish myocardium *in vitro* (see Farrell and Jones, 1992 for review). The high solubility of CO_2 will quickly lower intracellular pH of the myocardium, depressing contractility of the myocardium through an antagonism between hydrogen ions and the inotropic effect of intracellular calcium ion (Gesser and Poupa, 1983). It is conceivable, therefore, that the reduced cardiac contractility of the fish subjected to high levels of CO_2 resulted in the observed lowering of SV. In our previous study, we found that CO_2 tolerance varied among fishes, i.e. starspotted dogfish (*Mustelus manazo*) being the most tolerant, followed in turn by Japanese flounder (*Paralichthys olivaceus*) and yellowtail (*Seriola quinqueradiata*; Hayashi *et al.*, submitted). In this context, it may be worth pointing out that flounder (*Platichthys* (= *Pleuronectes*) *flesus*) is exceptional among teleosts in that myocardial contractility restores under sustained hypercapnia as in mammals (Gesser and Poupa, 1983). To our knowledge, no data is available for CO_2 sensitivity of elasmobranch myocardium. However, one should be somewhat cautious about extrapolating these findings to *in vivo* conditions because these *in vitro* experiments compared myocardial forces at 2–3% and above 10%, the former 'low' CO_2 level already being far higher than *in vivo* CO_2 levels under normocapnic conditions.

In spite of the established negative inotropic effect of hypercapnia on fish myocardium, *in vivo* cardiac responses to hypercapnia varied among fishes (see Perry and Gilmour, 2002 for review). Perry *et al.* (1999) reported that rainbow trout exposed to $PwCO_2$ of 6 and 9 mmHg for 30 min elicited no change in $\dot{Q}$, a 15–26% increase in SV, but a significant drop in HR. In contrast, the white sturgeon (*Acipenser transmontanus*) exposed to $PwCO_2$ of 20 mmHg for 2 hr showed a 31% increase in $\dot{Q}$, a 41% increase in SV, and a smaller but significant (8%) increase in HR (Crocker *et al.*, 2000). McKendry *et al.* (2001) demonstrated that hypercapnia ($PwCO_2$ 6 mmHg for 20 min) elicited a 30% decrease in $\dot{Q}$, and a 64% reduction in HR in the Pacific spiny dogfish (*Squalus acanthias*), indicating that SV was increased.

McKenzie *et al.* (2002) reported acute cardiorespiratory responses of freshwater eel to graded levels of CO_2, and found no significant effect on $\dot{Q}$ of $PwCO_2$ up to as high as 80 mmHg; a significant rise in SV at PCO_2 higher than 40 mmHg accompanied by a corresponding fall in HR. Obviously, *in vivo* cardiovascular responses to hypercapnia varies with severity as well as duration of hypercapnia imposed on fish, let alone interspecific variability, and probably experimental temperature. Furthermore, the above studies all examined an acute response (commonly shorter than 30 min), and no information is available on effects of long-term exposure, during which respiratory acidosis is compensated for by transepithelial transfer of acid-base relevant ions. This may be particularly relevant in considering cardiac function under hypercapnia because the *in vitro* depression of myocardial contractility by hypercapnic acidosis depends on bicarbonate concentration in the bathing medium, high bicarbonate concentration (36 mM) abolishing effect of CO_2 on myocardium (Gesser and Poupa, 1983). Thus, it is possible that cardiac output depressed by hypercapnic exposure is restored as bicarbonate concentration is increased by the acid-base compensation unless hypercapnic stress is so severe that death would ensue in a short time.

In vivo blood pressure responses to hypercapnia are similarly variable. Trout showed a significant increase in the dorsal aortic pressure at $PwCO_2$ of above 3.5 mmHg accompanied by a $PwCO_2$-dependent increases in systemic vascular resistance (Perry *et al.*, 1999). Changes in dorsal aortic pressure in the white sturgeon were significant, but only marginal, i.e. from 21.9±0.7 mmHg during normocapnia to 22.5±0.8 in 2 hr of hypercapnia. Systemic resistance decreased significantly (20%; Crocker *et al.*, 2000). The dogfish showed a small, but significant decrease (11%) in dorsal aortic pressure with no change in systemic resistance (McKendry *et al.*, 2001). The dramatic increase in dorsal aortic pressure during exposure to 5% CO_2 (Fig. 4B) indicates a considerable hypertension of the ventral aorta, although no data is available for the latter. The very high pressure is likely beyond the range of homeometric regulation, with which SV is maintained in a range of output pressure (Farrell and Jones, 1992). But in fact, the drop in $\dot{Q}$ started only within a few minutes after the start of CO_2 bub-

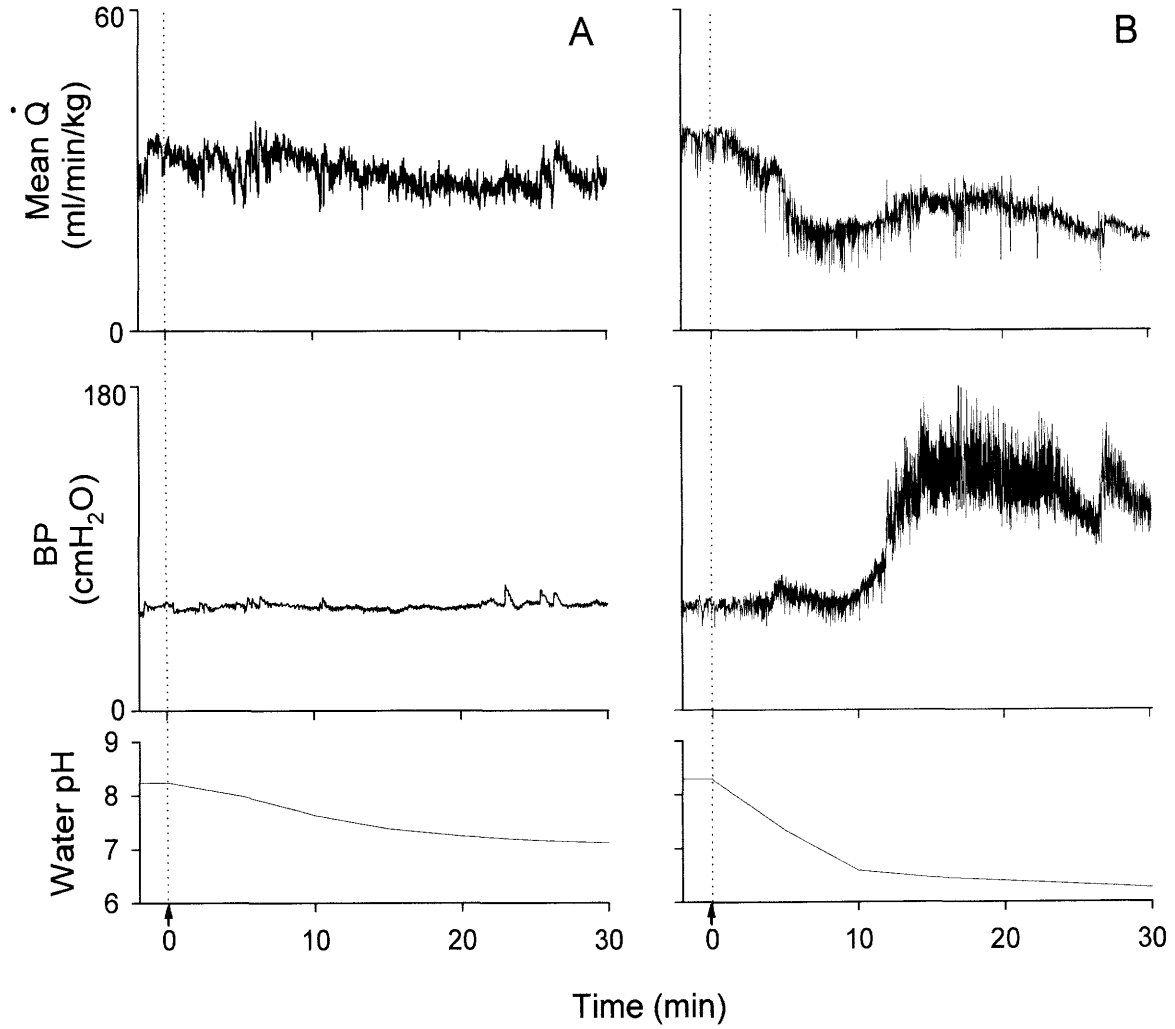

Fig. 4. Simultaneous records of mean cardiac output and arterial blood pressure for the initial 30 min after the onset of CO_2 exposure in yellowtail. (A) 1% CO_2: Fish survived 72 hr exposure. (B) 5% CO_2: Fish died in 3 hr 40 min. Arrows indicate start of hypercapnia. Water PCO_2 was recorded indirectly as water pH (see Materials and Methods).

bling when there was little increase in BP. This rapid onset of cardiac response suggests the involvement of external CO_2 receptors, which is in accord with the recent finding by Perry and Reid (2002) that cardiorespiratory adjustments such as bradycardia, systemic hypertension and hyperventilation during hypercapnia are initiated by external CO_2 receptors on the first gill arch in freshwater rainbow trout. The quick cardiac response also indicates that the hypercapnic hypertension cannot be the sole cause for the observed drop of SV at 5% CO_2. At 1% CO_2, neither $\dot{Q}$ nor BP showed significant changes except some transient response in a few fish (Fig. 4A). Certainly, more study is needed to elucidate neural and hormonal regulation of the cardiovascular function during hypercapnia.

We have shown that exposure to 5% CO_2 caused a rapid, large drop in $\dot{Q}$ through a reduction of SV without affecting HR. As a result, oxygen delivery to the tissues ($\dot{Q} \times CaO_2$) was severely limited in spite of the maintained oxygen concentration of the arterial blood. We therefore tentatively conclude that the cardiac inefficiency plays an important role in fish kill mechanisms by hypercapnia, though more comprehensive investigation is certainly needed before reaching a firm conclusion. For example, CO_2 effects on the fish nervous system need thorough scrutiny because of the known anesthetic effects of CO_2 (Bernier and Randall, 1998). In addition, the interaction between CO_2 and ambient pressure/temperature on deep-sea species should be clarified in the context of future ocean CO_2 disposal at proposed depths of 1,000 to 2,000 m.

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