

**EFFECTS OF CHRONIC HYPERCAPNIA
ON THE EUROPEAN EEL (ANGUILLA ANGUILLA)**

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Introduction

Eels reared intensively in systems with recirculating water may experience severe chronic hypercapnia, with water CO₂ partial pressures (pwCO₂) exceeding 30 mmHg (Steffensen and Lomholt, 1990). This is over 10 times normal ambient pwCO₂ in surface waters (1 to 3 mmHg) and would cause heavy mortalities in cultured salmonids (Fivelstad et al., 1998).

Hypercapnia is stressful for fish because it acidifies the blood and impairs blood oxygen transport through Bohr and Root effects. Freshwater fish correct acid-base imbalances through dynamic manipulation of Na⁺ uptake in exchange for H⁺ (either with a Na⁺/H⁺ exchanger or via the action of a proton pump and Na⁺ channel) or Cl⁻ uptake in exchange for HCO₃⁻ (a Cl⁻/HCO₃⁻ exchanger), these processes occurring almost exclusively at the gills (see Heisler, 1993; Lin and Randall, 1996; Claiborne, 1998, for reviews). Internal acidosis, such as that elicited by hypercapnia, stimulates Na⁺/H⁺ exchange or inhibits Cl⁻/HCO₃⁻ exchange, causing excretion of acid equivalents to the water, a net accumulation of HCO₃⁻, and compensation of blood pH (Heisler, 1993; Claiborne, 1998). Most freshwater teleosts rely on inhibition of Cl⁻/HCO₃⁻ exchange (Heisler,

1993; Claiborne, 1998). The eel is unusual in that it possesses very low or undetectable activity of the $\text{Cl}^-/\text{HCO}_3^-$ exchanger and seems to rely on manipulation of Na^+ influx to correct acidosis and passive Cl^- loss to correct alkalosis (Bornancin et al., 1977; Hyde and Perry, 1987; 1989; Goss and Perry, 1994). It is reported to have a rather limited ability to compensate for acidosis, if compared with Rainbow trout (Hyde and Perry, 1987; 1989).

The acid-base consequences of acute and chronic exposure to severe hypercapnia in the eel were, therefore, investigated. In recirculating eel farms, it is probable that the eels actually experience chronic diurnal fluctuations in pwCO_2 that are linked to daily feeding schedules, rather than chronic exposure to a fixed degree of hypercapnia. Thus, the effects of chronic diurnal fluctuations in hypercapnia were also investigated.

Acute Hypercapnia

Acute exposure to progressive hypercapnia (20 min at pwCO_2 's of 10, 20, 40, 60 then 80 mmHg) caused a linear increase in arterial pCO_2 (paCO_2) from 3.5 ± 0.4 mmHg in normocapnia to 44.9 ± 2.6 at $\text{pwCO}_2 = 80$ mmHg. As can be seen in the Davenport (fig 1), the increase in paCO_2 was coupled to a severe decline in arterial pH (pHa), a decline that paralleled the non-bicarbonate buffer line for eel plasma (Hyde et al., 1987). The acidosis was linked to a profound (~ 80%) reduction in arterial total O_2 content (caO_2), presumably as a consequence of Bohr and Root effects. Nonetheless, all of the eels survived the acute hypercapnia protocol, and exhibited no significant changes in oxygen uptake or cardiac output.

Chronic Hypercapnia at Fixed Levels

Eels were reared for 6 months at pwCO_2 's of ambient (control), 15, 30 and 45 mmHg, and the effects on plasma acid base status measured. As can be seen in the Davenport diagram in fig 1, paCO_2 equilibrated at approximately 2 to 3 mmHg above pwCO_2 in the eels in each group, and this was associated with a progressive decline in pHa, which was significantly lower than the control eels in all hypercapnic groups. However, as is clear from fig 1, the eels accumulated significant amounts of HCO_3^- in the plasma, reaching over 70 mmol l^{-1} in the animals acclimated to $\text{pwCO}_2 = 45$ mmHg, such that pHa deviated significantly from the non-bicarbonate buffer line and, for any given paCO_2 , pHa was significantly higher than under acute exposure conditions. This compensation of pHa was linked to a higher caO_2 for any given paCO_2 in the chronically versus

the acutely exposed animals, such that only animals at $\text{pwCO}_2 = 45$ mmHg exhibited a significant hypoxaemia relative to the control eels.

The accumulation of bicarbonate is higher than has hitherto been considered possible for teleost fish; Heisler (1993) suggested that any accumulation of HCO_3^- above 30 mmol l^{-1} would lead to too severe a reduction in plasma chloride levels. This because the accumulation of HCO_3^- is linked to the loss of a strong anion (Cl^-) in order to protect electroneutrality. As can be seen in fig 2, there was an almost equimolar loss of Cl^- for every HCO_3^- accumulated by the eels exposed to chronic hypercapnia, such that the eels exposed to $\text{pwCO}_2 = 45$ mmHg had plasma Cl^- levels that were 50% of control animals, at around 75 mmol l^{-1} . This profound decline in plasma Cl^- was not linked to any significant changes in plasma Na^+ levels or in plasma osmolarity. The ability of the eel to tolerate unusually low plasma Cl^- levels has been described previously (Farrell and Lutz, 1975).

Given the reported inability of the eel to manipulate $\text{Cl}^-/\text{HCO}_3^-$ exchange during extracellular acidosis (Bornancin et al., 1977; Hyde and Perry, 1987; 1989; Goss and Perry, 1994), the question arises as to how the eels in the current study were able to accumulate such large amounts of bicarbonate in the plasma. It has been suggested (Heisler, 1993) that the activity of a gill H^+ -ATPase alone could compensate an acidosis by removing protons and retaining bicarbonate, these two formed from the catalysed hydration of CO_2 in the gill epithelium. Indeed, Lin and Randall (1993) found that hypercapnia caused an increase in H^+ -ATPase activity in trout gills. However, chronic hypercapnia had no such effect on H^+ -ATPase activity in eel gills when assayed by the same technique, although it did cause an increase in Na^+, K^+ -ATPase activity. Goss and Perry (1994) found evidence that the eel may regulate passive efflux of Na^+ versus Cl^- to regulate strong ion difference. Thus, the hypercapnic eels may have let plasma Cl^- decline through loss to the water, and thereby accumulated HCO_3^- as a passive consequence of electroneutrality

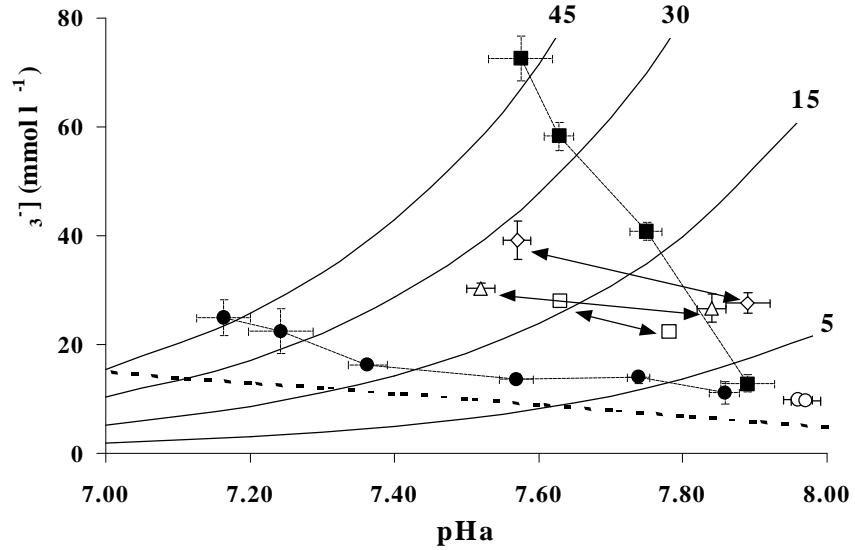


Figure 1. Davenport diagram showing the relationship between mean (\pm SE) arterial pH and bicarbonate concentration in eels exposed to acute hypercapnia (black circles), chronic fixed-level hypercapnia (black squares) and chronic diurnal fluctuations in hypercapnia (open symbols – squares = 5 to 15 mmHg pwCO₂; triangles = 5 to 25; diamonds = 5 to 35; circles = controls). See text for further details. n = between 4 and 7. Dotted line = non-bicarbonate buffer line for eel plasma (Hyde et al. 1987); curved solid lines are pCO₂ isopleths.

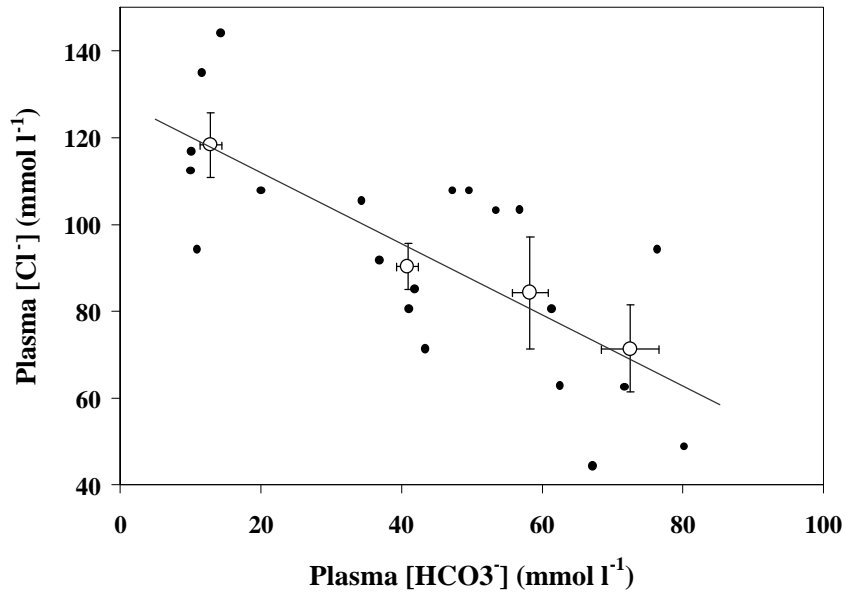


Figure 2. Relationship between plasma bicarbonate and chloride levels in eels exposed to chronic hypercapnia ($p\text{wCO}_2 = \text{ambient, 15; 30 or 45 mmHg}$ for 6 months). Black symbols are individual points; white symbols are mean (\pm SE) for each group ($n = 4$ to 6). Regression line describes linear relationship between individual data points, $y = -0.818x + 123$, $r^2 = 0.536$.

Despite the chronic extracellular acid-base disturbances, the eels regulated intracellular pH unchanged in muscle, liver and heart. As a consequence, calculation of tissue HCO_3^- levels indicated that they would not have risen above approximately 25 mmol l^{-1} , so that intracellular Cl^- levels would not have fallen to potentially dangerous levels (Heisler, 1993).

Chronic fluctuating hypercapnia

Eels were reared for 6 months with daily fluctuations in $p\text{wCO}_2$, of 5 to 15; 5 to 25, and 5 to 35, with the peak at 23:00 and the low at 11:00. Under these circumstances the animals exhibited diurnal fluctuations in acid-base status. As can be seen from figure 1, the diurnal increase in $p\text{wCO}_2$ and $p\text{aCO}_2$ was linked

to a reduction in pHa, which paralleled the non-bicarbonate buffer line for eel plasma (Hyde et al., 1987). This indicates that under fluctuating conditions the animals were not able to accumulate bicarbonate as effectively as when exposed to fixed hypercapnic conditions. Both at the low and high point, the eels were acidotic relative to control animals maintained at ambient pwCO₂. The acidosis was linked to chronic and quite severe hypoxaemia in all hypercapnic groups, relative to the control eels, although caO₂ did not fluctuate with the diurnal changes in pHa. It is a testament to the hardiness of the species that they were able to tolerate such severe diurnal acid-base disturbances and chronic hypoxaemia.

The diurnal fluctuations in pwCO₂ and plasma HCO₃⁻ were linked to fluctuations in plasma Cl⁻ levels, of up to 10 mmol⁻¹ in the eels exposed to the 5 to 35 mmHg pwCO₂ regime (figure 3). This diurnal rise in Cl⁻ as paCO₂ and plasma HCO₃⁻ fell is difficult to explain given the reported absence of branchial HCO₃⁻/Cl⁻ exchange in the eel (Bornancin et al., 1977; Hyde and Perry, 1987; 1989; Goss and Perry, 1994).

Indicators of stress during chronic hypercapnia

In the eels exposed to chronic fixed hypercapnia, there were no indicators of stress such as elevated plasma cortisol or catecholamine levels, increased metabolic rate, or impaired exercise performance. This, however, was not true of the animals exposed to fluctuating hypercapnia. These exhibited a diurnal catecholamine release associated with the high point of the pwCO₂ cycle, a clear primary stress indicator. Although there was no effect of fluctuating hypercapnia on metabolic rate, exercise performance was compromised in all hypercapnic groups relative to the control eels, when measured at the low point of the fluctuating cycle. Thus, fluctuating hypercapnia is significantly more stressful to the eel than is fixed hypercapnia. Nonetheless, the absence of any differences in standard metabolic rate amongst the hypercapnic groups may indicate that acid-base regulation has a very low metabolic cost for the eel.

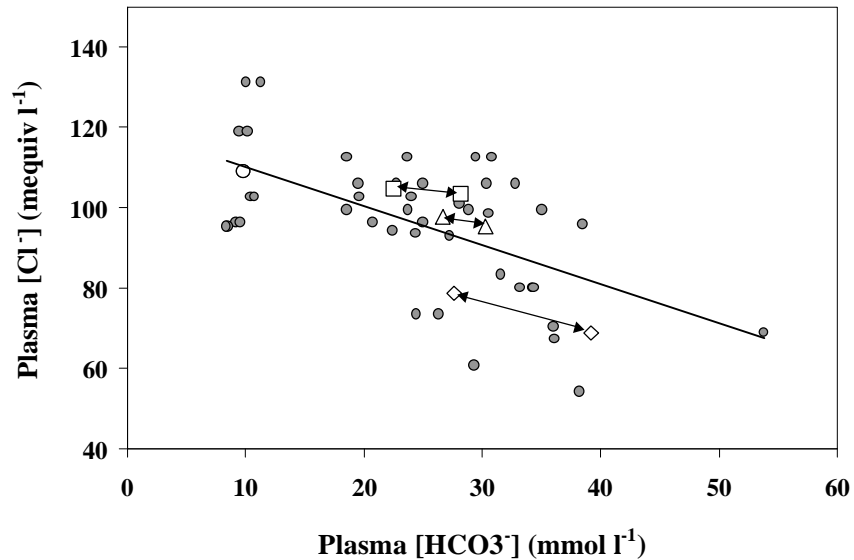


Figure 3. Relationship between plasma bicarbonate and chloride levels in eels exposed to chronic fluctuating hypercapnia ($p_w\text{CO}_2$ = ambient, 5 to 15; 5 to 25 and 5 to 35 mmHg, with peak at 23:00 and low at 11:00, for 6 months). Black symbols are individual points; white symbols are mean for each group ($n = 6$, squares = 5 to 15; triangles = 5 to 25; diamonds = 5 to 35 and circles = ambient). Regression line describes linear relationship between individual data points, $y = -0.971x + 120$, $r^2 = 0.340$.

Concluding Remarks

The results indicate that the eel is exceptionally tolerant of hypercapnia and the associated acidosis and hypoxaemia. The ability of the eel to tolerate such hypercapnic insults, including fluctuating hypercapnia, may be linked to their ability to survive (and exercise) in air when they migrate through wet vegetation in search of new habitats (Tesch, 1972; Berg and Steen, 1965; Hyde and Perry, 1987; Hyde et al., 1987). Hyde et al. (1987) showed that during air exposure the gills are no longer able to remove metabolic CO_2 and the animals exhibit a mixed respiratory (i.e. hypercapnic) and metabolic acidosis. These authors

noted the unusual tolerance of the animals for chronic hypoxaemia (Hyde et al., 1987). The evolution of the ability to survive what may be profound acid-base imbalances during air-exposure, particularly if associated with exercise, may, therefore, have pre-adapted the eel to the demanding conditions of intensive culture in recirculating systems.

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