OXYGEN-SENSITIVE CHEMORECEPTORS AND CARDIOVENTILATORY CONTROL IN CARP

by

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B.Sc., The University of Texas at Arlington, 1994

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE in THE FACULTY OF GRADUATE STUDIES Department of Zoology

We accept this thesis as conforming to the required standard

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Date Oct. 28, 1996
ABSTRACT

Carp responded to changes in partial pressure of oxygen in the water, both increases and decreases, in a manner similar to other teleost fish. Hypoxia resulted in significant increases in ventilation (both frequency and amplitude). A reflex hypoxic bradycardia, however, was not observed in the present study and, in fact, a tachycardia occurred at one hour and persisted through the remainder of the experiment. There was a significant decrease in ventilatory frequency during hyperoxia, while ventilatory amplitude, as well as heart rate, did not change from normoxic control values. NaCN (5 mg / ml) administered through a mouth cannulae in carp resulted in rapid, albeit transient, increases in frequency and amplitude of ventilation which were significantly different from control water injections. The response to NaCN was over within a minute following administration of NaCN (evidenced by the significant decrease in respiratory frequency). Heart rate was unaffected by NaCN and the absence of the bradycardia. Internal injections of NaCN (5-10 mg / ml) into the dorsal aorta of carp produced no cardioventilatory responses. Carbon monoxide (21.2 ± 9.0%) had no effect on ventilation in carp, however, it increased heart rate significantly. This indicates that carp possess O₂-sensitive chemoreceptors capable of monitoring changes in O₂ content, independent of changes in O₂ partial pressure, but they are not involved in the control of breathing. This refutes my hypothesis that changes in O₂ content of the blood in carp modulate cardioventilatory effects. Hyperoxic hypercapnia significantly increased the frequency of ventilation after the first five minutes of exposure. This increase in ventilation during hyperoxic hypercapnia indicates that in carp, CO₂ is acting directly on a receptor, as opposed to indirectly through Bohr and
Root effects in the blood. The changes in breathing pattern, with hypoxia, CO, and CO₂ occurred in the same manner for all carp (i.e. there was an increase in the number of breaths in an episode and a decrease in the number of episodes in a minute until a continuous breathing pattern was achieved).
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I would like to express my appreciation to my supervisor, Dr. W.K. Milsom, for allowing me the wonderful opportunity to work in his laboratory. His patience, support, and valuable lessons have made my two years at UBC an extraordinary educational experience. I would also like to extend my thanks to Dr. N.J. Smatresk for introducing me to the area of academic research. Without his guidance and encouragement I truly doubt I would have made it to Vancouver. Thanks to Mike Harris for always being there to lend a helping hand (he does indeed know "lots of stuff") and to Gillian Muir and Izzy for making me smile when things were rough. I am extremely fortunate to have spent my two years in the lab with Sarah Franks who has been my closest friend and confidant. I am indebted to Dr. Mark Burleson and Dr. Steve Reid for patience through my seemingly unending questions. Financial support from NSERC and Zoology Department Teaching Assistantships are gratefully acknowledged. I would like to thank Carmen Horton and Marnie Ward for their wonderful letters of encouragement which helped in my transition from Arlington to Vancouver and kept me going when I no longer felt I could. I would have never made it in Vancouver without the unconditional love of Maggie and Mason (who also kept me warm). I am especially grateful to my parents, Joe and Judy Lumsden, for their continued love, support, and encouragement and to David for his love, patience, and support.
INTRODUCTION

In order to effectively support their needs with appropriate cardiorespiratory responses, fish must be able to sense and respond to changes in environmental and / or blood oxygen and carbon dioxide levels. Given the low solubility of oxygen and high solubility of carbon dioxide in water, the major problem facing fish is obtaining oxygen, not excreting CO₂. In terrestrial vertebrates, the reverse is true, changes in partial pressure of CO₂ ($P_{CO_2}$) exert more influence over the cardiorespiratory system than changes in partial pressure of oxygen ($P_{O_2}$). Given this, it is not surprising that in fish, changes in oxygen tension stimulate cardiorespiratory reflexes more than changes in the tension of carbon dioxide (Randall and Jones, 1973; Burleson et al., 1992). Both O₂ and CO₂ do stimulate breathing, however, and the specific nature of each stimulus is the focus of this study.

I. Ventilatory Responses of Fish to Aquatic Hypoxia and Hyperoxia

Numerous studies characterize the responses of fish to changes in aquatic oxygen content. Hypoxia, or decreased partial pressure of oxygen, elicits profound respiratory effects in fish, which primarily consist of increases in ventilatory frequency and amplitude (van Dam, 1938; Saunders, 1962; Hughes and Shelton, 1963; Randall and Smith, 1963; Peyraud and Sefarty, 1964; Holeton and Randall, 1967; Saunders and Sutterlin, 1971; Cech and Wohlschlag, 1973; Hughes, 1973; Randall and Jones, 1973; Lomholt and Johansen, 1978; Smith and Jones, 1978; Hughes et al., 1982; Peyraud-Waitzenegger and Soutilier, 1989; Burleson and Smatresk, 1990b; Kinkead and Perry, 1992; Burleson and Smatresk, 1990c).
Kalinin et al., 1990; Nonnotte et al., 1993; Maxime et al., 1995). There have been a few
studies indicating that increases occur only in ventilatory frequency (Hall, 1931) or amplitude
(Gerald and Cech, 1970; Burggren and Cameron, 1980).

A variety of reflexogenic areas have been tested as putative oxygen-sensitive loci in fish
using a variety of indirect techniques. Based on these studies, the brain (Bamford, 1974), gills
(Powers and Clark, 1942), pseudobranch (Laurent and Rouzeau, 1972), venous vasculature (Barrett
and Taylor, 1984), arterial vasculature (Randall, 1982), and afferent branchial vasculature (Smatresk
et al., 1986) have all been proposed to be chemoreceptive sites. Recent studies provide fairly strong
evidence that the oxygen-sensitive chemoreceptors are located predominantly on the first gill arch
of most teleost fish innervated, by cranial nerves VII (facial), IX (glossopharyngeal), and X (vagus)
(see Burleson et al., 1992 for review).

Electrophysiological studies have indicated that $O_2$-sensitive chemoreceptors are indeed
present on the first gill arch of tuna (Milsom and Brill, 1986) and rainbow trout (Burleson and
Milsom, 1993) as well as in the pseudobranch of trout (Laurent and Rouzeau, 1972). These receptors
increase their firing rate in response to either decreasing perfusion rate, decreasing perfusion (blood)
$P_{O_2}$, and/or to decreasing external (water) $P_{O_2}$. Fibers responding to environmental hypoxia
exhibited an exponential increase in firing rate to decreasing external $P_{O_2}$ with a sensitivity similar
to that exhibited by mammalian carotid body chemoreceptors (Milsom and Brill, 1986; Burleson and
Milsom, 1993). Both of the studies on first gill arch chemoreceptors observed afferent activity
arising from fibers that responded only, or preferentially, to external $O_2$ stimulus levels and others
that responded only, or preferentially, to internal $O_2$ stimulus levels. These observations support
previous observations from reflex studies which suggest the presence of a population of internally
oriented O$_2$-sensitive chemoreceptors which elicit only ventilatory responses and a separate set of
externally oriented O$_2$-sensitive chemoreceptors which elicit both ventilatory and cardiovascular
reflex effects (Hall, 1931; van Dam, 1938; Saunders, 1962; Hughes and Shelton, 1962; Randall and
Saunders and Sutterlin, 1971; Cech and Wohlschlag, 1973; Randall and Jones, 1973; Lomholt and
Johansen, 1978; Smith and Jones, 1978; Burggren and Cameron, 1980; Hughes et al., 1982; Smith
and Davie, 1984; Peyraud-Waitzenegger and Soutilier, 1989; Burleson and Smatresk, 1990b;
Kinkead and Perry, 1990; Kalinin et al., 1993; Nonnette et al., 1993; Maxime et al., 1995)

Teleost fish generally respond to hyperoxia with a decrease in ventilation and little change
in heart rate. The decrease in ventilation may be accomplished by decreasing the frequency of gill
ventilation and/or ventilatory amplitude. In addition, rainbow trout (Randall and Jones, 1973) and
several other species (carp: Peyraud and Sefarty, 1964; Takeda, 1991; dogfish, striped mullet, bass,
gurnard, wrasse, and pollack: Dejours et al., 1977) breathe episodically under these conditions.

II. Cardiac Responses of Fish to Aquatic Hypoxia

In tench, Randall and Shelton (1963) noted that in addition to an increase in ventilation
during hypoxia, there was also an associated bradycardia. The injection of atropine into the
pericardial cavity abolished the hypoxic bradycardia indicating that the bradycardia arose from reflex
nervous control of the heart by receptors sensing oxygen. Phasic activity recorded from the cardiac
vagus in the tench increased when the fish were exposed to aquatic hypoxia, lending further support
to the hypothesis that the bradycardia arose from a neural reflex (Randall, 1966).

The pseudobranch, which is essentially a vestigial gill, was implicated as the possible site
for the oxygen-sensitive chemoreceptors eliciting bradycardia in fish (Laurent, 1967; Laurent and Rouzeau, 1972; Saunders and Sutterlin, 1971). Randall and Jones (1973), however, sectioned the branch of the glossopharyngeal nerve supplying the pseudobranch of rainbow trout and noted that exposure to hypoxic water \((P_{wO_2} = 75 \text{ torr})\) still resulted in increased ventilation along with a bradycardia. This led to the conclusion that the pseudobranch exerted little, or no role in the cardiorespiratory responses of rainbow trout to hypoxia.

Daxboeck and Holeton (1978) and Smith and Jones (1978) subsequently identified the first gill arch of rainbow trout as a peripheral oxygen-sensitive chemoreceptor site. Daxboeck and Holeton (1978) showed that hypoxic irrigation of the first gill arch alone produced a rapid bradycardia. Given the rapid onset of the bradycardia, it appeared that the location of the receptors mediating this response were peripheral; i.e. sensitive to changes in \(P_{wO_2}\). Smith and Jones (1978) showed that when the first gill arch on one side was irrigated with hypoxic water \((P_{wO_2} = 26 \text{ torr})\) and the other side with hyperoxic water \((P_{wO_2} = 300 \text{ torr})\), a bradycardia still occurred. The bradycardia was abolished, however, by replacing the hypoxic water flow with hyperoxic water. Removal of all gill arches on both sides, with the exception of the first gill arch, did not eliminate the hypoxic bradycardia. However, with all gill arches intact and the first gill arch denervated on each side, no hypoxic bradycardia occurred. Also, ligation of various sections of the first gill arch indicated that the receptors mediating the hypoxic bradycardia were located on the anterodorsal portion of the first gill arch.

Smith and Davie (1983) later found that receptors mediating the hypoxic bradycardia were also located solely on the first gill arch of coho salmon and were innervated by the glossopharyngeal nerve. In channel catfish, however, it appears that receptors innervated by the vagus nerve are
primarily responsible for modulating heart rate (Burleson and Smatresk, 1990a). In this species, denervation of the first gill arch produced only an attenuated bradycardia compared to sham operated fish exposed to NaCN (a potent stimulator of oxygen chemoreceptors that mimics the effects of hypoxia) in the water. Sectioning of the branchial branches of the vagus nerve innervating the first, second, and third gill arches while leaving the glossopharyngeal nerve intact, however, totally prevented the development of an hypoxic bradycardia. Thus, it appears that receptors mediating reflex bradycardia are not confined to the first gill arch and innervated by the glossopharyngeal nerve in all species (Burleson and Smatresk, 1990a).

The location of the receptors involved in producing the hypoxic bradycardia have not been as extensively studied in other species. Of the teleost species studied to date, all exhibit a hypoxic bradycardia. However, the level of hypoxia required to induce bradycardia, as well as the duration of the bradycardia, varies widely amongst species. Active species, such as trout and eel require that $P_{O_2}$ in the water fall below a critical level of approximately 75-80 torr to induce a bradycardia (Holeton and Randall, 1967; Randall and Smith, 1967; Smith and Jones, 1978). Sedentary bottom dwellers and less active species, such as carp, sturgeon, tench, and *Hoplias malabaricus*, do not exhibit a bradycardia until the partial pressure of oxygen in the water is lowered to 10-30 torr (Saunders, 1962; Randall and Shelton, 1963; Saunders and Sutterlin, 1971; Williams *et al.*, 1992; Nonnotte *et al.*, 1993; Rantin *et al.*, 1993; Maxime *et al.*, 1995). The mechanisms underlying these interspecific differences remain obscure and warrant further investigation.

The role of the hypoxic bradycardia has been a subject of speculation. Given the marked increase in ventilation that occurs with hypoxia, Randall and Shelton (1963) suggested that the role of the bradycardia was to slow blood flow through the gills increasing the time for gas exchange to
occur. It was found in later studies, however, that the bradycardia was compensated by an increase in stroke volume and cardiac output and, hence blood pressure (Holeton and Randall, 1967). Furthermore, a coupling between heart rate and ventilation was noted to occur with increasing hypoxia (Hughes, 1961; 1972). With increases in blood pressure, blood is more evenly distributed through the gills, a recruitment of previously poorly perfused secondary gill lamellae occurs which results in more surface area available for gas exchange (Randall, 1982). Also, there is a thinning of the gill water-blood barrier which reduces the diffusion barrier. Furthermore, the synchrony that occurs between blood flow and ventilation during hypoxia also appears to increase the effectiveness of gas transfer (Hughes, 1973). Increasing the cardiac output (i.e. blood pressure) during the mouth opening phase of ventilation will ensure an increased amount of blood available for gas transfer in the gills when freshly oxygenated water is flowing over the gills (see Randall, 1982 for review). Having said this, the relative importance of these factors that seemingly act to benefit the fish, remains elusive.

III. Cardioventilatory Responses of Fish to Aquatic Hypercapnia

There have been numerous studies of the effects of hypercapnia (increased $P_{w CO_2}$) on cardiorespiratory parameters in fish. Increases in ambient CO$_2$ have been shown to increase the frequency of breathing in the minnow and the perch as well as in the stargazer (Randall and Smith, 1967). The hypercapnic response in eel is quite variable, while trout respond by increasing ventilatory amplitude but not frequency (van Dam, 1938). Saunders (1962) exposed the white sucker, the bullhead catfish, and the carp to various levels of ambient CO$_2$. At moderate levels of hypercapnia ($P_{w CO_2} < 20$ torr) the effects on ventilatory frequency and amplitude were variable,
whereas at more severe levels of hypercapnia ($P_{wCO_2} = 25-90$ torr) the increase in frequency and amplitude of ventilation was more consistent. The effect of hypercapnia on heart rate was not investigated in these early studies.

Randall and Shelton (1963) were the first to measure the effects of increased environmental CO$_2$ in tench on heart rate in conjunction with ventilatory responses. Hypercapnia caused an increase in ventilation volume primarily by an increase in ventilatory amplitude, with a slight decrease in frequency, while heart rate increased with increasing levels of ambient CO$_2$. These effects were also observed under anaesthesia suggesting a specific role for CO$_2$ in stimulating these variables rather than a non-specific effect due to the increased activity often seen in hypercapnia. In rainbow trout, Janssen and Randall (1975) also reported a five-fold increase in ventilation volume and only slight increases in breathing frequency during hypercapnia. In addition, they observed that if ambient CO$_2$ remained elevated ($P_{wCO_2} = 5.2$ torr) for several days, ventilation volume gradually returned to pre-hypercapnic levels over the course of 48 hours. Subsequent studies of the effects of hypercapnia on fish indicate that in all fish ventilation eventually returns to pre-hypercapnic levels in a matter of hours or days (Randall et al., 1976; Eddy et al., 1977; Toews et al., 1983).

Several workers have observed that the hypercapnic response is attenuated under hyperoxic conditions (Dejours, 1973; Smith and Jones, 1982). The mechanism by which CO$_2$ stimulates breathing is controversial, and it has been suggested that it acts through its effect on O$_2$ transport in the blood. Once gas exchange has occurred at the gills, oxygen, bound to hemoglobin, is carried to the tissues. At the level of the tissues, oxygen diffuses from the blood into the tissues while CO$_2$ enters the red blood cell. Bicarbonate ($HCO_3^-$), the predominant form of CO$_2$ in the blood, and hydrogen ion ($H^+$) are formed upon hydration of CO$_2$ in the red blood cell. This process is expedited
by the enzyme carbonic anhydrase (see reaction equation below). The $H^+$ ion will then combine with the deoxygenated hemoglobin molecule and the $HCO_3^-$ molecule exits the red blood cell, in exchange for a $Cl^-$ ion (chloride shift), where it is carried in the plasma until the blood reaches the gills. At the gills, oxygen will displace the $H^+$ ion, and the above process is reversed with the bicarbonate entering the red blood cell and being dehydrated to form $CO_2$, which then diffuses across the red cell membrane and gill epithelia into the water. The reaction of oxygen with hemoglobin at the gills and in the tissues is as follows:

$$HHb + O_2 \rightleftharpoons HbO_2 + H^+$$

The hydration / dehydration of $CO_2$ which occurs in the tissues and at the gills is as follows:

$$H^+ + HCO_3^- \rightleftharpoons CO_2 + H_2O$$

Therefore, oxygenated blood carries less $CO_2$ than deoxygenated blood, and this is known as the Haldane effect (Perry and Wood, 1989).

The oxygen equilibrium curve describes the relationship between blood oxygen content and the partial pressure of oxygen of the blood. The percent saturation of the hemoglobin molecule is a rough indicator of the oxygen content in the blood since the oxygen capacity of the blood is equal to the amount of oxygen dissolved in plasma (small) and the amount of oxygen bound to hemoglobin (large). There are two mechanisms by which $CO_2$ can affect the oxygen equilibrium curve. These are the Bohr and Root effects and both are the result of pH altering either the hemoglobin-oxygen affinity or the oxygen carrying capacity of the hemoglobin molecule. The Bohr effect describes the influence of pH on the oxygen affinity of hemoglobin. The hyperventilation that occurs during hypoxia will cause a decrease in the arterial levels of $CO_2$ which, in turn, leads to an increase in pH (or a decrease in $H^+$ ion concentration). This causes a left Bohr shift of the oxygen dissociation
curve which increases the hemoglobin-oxygen affinity of blood and is manifested by the decrease in $P_{50}$ of the blood (i.e. the $P_{a,oxygen}$ at which 50% of the hemoglobin is bound to oxygen) (Weber and Jensen, 1988). Hyperoxia, on the other hand, results in hypoventilation of fish. This leads to an increase in $P_{a,CO_2}$ and a decrease of pH, consequently resulting in a right Bohr shift. The resulting decrease in oxygen-hemoglobin affinity is advantageous to oxygen unloading, and CO$_2$ loading is increased at the tissues. It is disadvantageous, however, to O$_2$ uptake at the gills (Black, 1940). An increase in arterial CO$_2$, resulting from exposure of fish to environmental hypercapnia, will also lead to a decrease in pH in the blood leading to a right Bohr shift of the oxygen dissociation curve. The Root effect describes the decrease in the oxygen carrying capacity of the blood. Low pH will reduce the ability of oxygen to bind to the hemoglobin, and, even at high partial pressures of oxygen in the blood, only some sites of the hemoglobin molecule will be oxygenated. The Root effect is unique to teleost fish and is largest at arterial $P_{CO_2}$ between 1-5 torr (Randall, 1970).

The mechanism through which hypercapnia acts remains elusive. Given the effects of CO$_2$ on $P_{O_2}$ due to the Bohr and Root effects, it has been suggested that CO$_2$ exerts its effect via oxygen chemoreceptors (Smith and Jones, 1982). Smith and Jones (1982) hypothesized that if ventilation was stimulated by hypercapnia indirectly through changes in oxygen content of the blood, then increases in $P_{O_2}$ in the blood would attenuate, if not eliminate, ventilatory responses to hypercapnia. Ventilatory responses were indeed attenuated under hyperoxic conditions (Smith and Jones, 1982; Randall and Perry, 1992). However, there have also been studies where ventilation remains elevated in response to CO$_2$ whether the hypercapnia is presented under normoxic or hyperoxic conditions (Thomas et al., 1983). After fish had been exposed to 72 hours of hyperoxia, a ventilatory response to hypercapnia was still observed as well as an increase in arterial $P_{O_2}$. In addition, goldfish have a
Root effect, yet this species shows no response to hypercapnia, while in dogfish hypercapnia does stimulate breathing but this species has no Root effect occurring in the blood (Dejours, 1973). Therefore, the precise mechanism, as well as the receptor site, through which CO$_2$ exerts its effect on ventilation in fish remain obscure.

IV. Catecholamines in Fish: Cardioventilatory Effects

Given that catecholamine (adrenaline and noradrenaline) levels are elevated in the plasma during periods of hyperventilation, it has been suggested that these hormones act to increase ventilation during respiratory disturbances. The catecholamine hormones are released into the circulation from chromaffin cells in response to stress. Chromaffin cells in teleost fish have been localized within the walls of the posterior cardinal vein and in close proximation to the lymphoid tissue in the region of the anterior, or head, kidney (Gallo et al., 1993; Hathaway and Epple, 1989; Mastriola et al., 1981, 1984; Nilsson, 1983, 1984; Reid and Perry, 1994; Reid et al., 1995). One of the primary actions of catecholamines is to diminish the adverse effects associated with environmental stressors in fish (see Reid et al., 1996 for review). Catecholamines released into the plasma are important in maintaining adequate levels of oxygen in the blood, hence to the tissues during times of stress. They allow optimization of cardiovascular and respiratory functions as well as mobilizing energy stores to provide for the increased demands that often accompany stress. Catecholamines are abruptly released into the plasma when arterial P$_{O_2}$ falls below a threshold level which generally corresponds to about 50% hemoglobin-oxygen saturation (Perry et al., 1992). The mechanism by which catecholamines are released from chromaffin cells appears to be through cholinergic fibers of the sympathetic nervous system via stimulation of the cells by acetylcholine.
Stressors do not result in release of catecholamines until a critical level is achieved (Boutilier et al., 1988; Ristori and Laurent, 1989; Kinkead and Perry, 1991). Once the stress to the fish has been removed, plasma catecholamine levels are rapidly returned to normal through combined effects of tissue accumulation and metabolic degradation (see Randall and Perry, 1992 for review). The function of catecholamine release on modulating ventilation has recently been debated (Randall and Taylor, 1992; Perry et al., 1992). Based on studies where intra-arterial injections of either adrenaline or noradrenaline into various species of fish resulted in hyperventilatory responses, some workers were led to believe that catecholamines were important in the control of ventilation (Peyraud-Waitzenegger, 1979; Peyraud-Waitzenegger et al., 1980; Randall and Daxboeck, 1984; Perry and Wood, 1989; Aota and Randall, 1989; Aota et al., 1990; Randall, 1990; Randall and Taylor, 1992). However, recent studies involving injections of catecholamines into the blood of fish exposed to various environmental stressors have indicated that catecholamines may not be as important in modulating ventilation as was originally thought (see Perry et al., 1992 for review; Perry and Gilmour, 1996). Elevations in circulating catecholamines do appear to act on the cardiovascular system through adrenergic stimulation which results in increases in heart rate (Wahlqvist and Nilsson, 1977; Smith and Jones, 1978).

V. A Unifying Concept: Changes in Oxygen Content

Smith and Jones (1982) have proposed that hypoxia and hypercapnia exert ventilatory effects through changing oxygen content in the blood, i.e. receptors respond to changes in arterial $O_2$ content rather than partial pressure.
Figure 1. The relationship between arterial oxygen content and ventilation for trout. Horizontal and vertical bars represent ± SEM. Note the linear increase in ventilation which corresponds directly to changes in oxygen content, whether it is through hypoxia or hypercapnia. Hyperoxic hypercapnia attenuates the ventilatory response indicating CO$_2$ exerts its effect through Bohr and Root shifts in the blood.
Hypoxia

Hypercapnia

Anaemia

Normoxia

Hyperoxic Hypercapnia

Arterial blood oxygen content (Vols. %)

Gill water flow (ml min⁻¹ kg⁻¹)
Fish do appear to respond to changes in $P_{w_{O_2}}$ evidenced by the rapid ventilatory response observed in some species (Holeton and Randall, 1967; Itazawa and Takeda, 1978; Burggren and Cameron, 1980). The relation between arterial $P_{O_2}$ and ventilation is more elusive. In fish with low blood oxygen affinities, $P_{a_{O_2}}$ falls to a greater extent than in those fish with a high blood-oxygen affinity in hypoxia (see Shelton et al., 1986 for review), and thus there is no good correlation between levels of ventilation and $P_{a_{O_2}}$ across species. As blood $P_{O_2}$ decreases with hypoxia, oxygen content decreases, which is also dependent on the blood-oxygen affinity, and ventilation increases linearly with changes in content (Figure 1, from Shelton et al., 1986). Furthermore, exposing fish to hypercapnia results in a decrease in oxygen content which is ameliorated under hyperoxic conditions (see Section III) (Smith and Jones, 1982; Shelton et al., 1986) suggesting that $CO_2$ exerts its effects on fish respiration through changes in oxygen content via Bohr and Root effects. Finally, administration of $CO_2$, which competes with oxygen for binding sites on hemoglobin, will reduce blood oxygen content independent of $O_2$ partial pressure, and this stimulates ventilation (Anthony, 1961; Holeton, 1971a, 1971b). Therefore, these authors concluded that all changes in ventilation could be explained by changes in oxygen content of the blood rather than partial pressure (see Figure 1).

**VI. Modulation of Ventilation in Carp—Partial Pressure or Oxygen Content?**

In carp, there have been recent studies suggesting that partial pressure of oxygen in the blood, not content, is the stimulus driving respiration under hypoxic conditions. Glass et al. (1990) exposed carp to two levels of hypoxia ($P_{w_{O_2}} = 110$ torr and 75 torr). Ventilation increased under these conditions although there appeared to be no change in arterial saturation (i.e. both levels of $P_{O_2}$ were
sufficient to fully saturate carp hemoglobin). There was, however, a marginal decrease in arterial oxygen partial pressure that led the investigators to speculate that, because there was no clear relationship between arterial oxygen content and ventilatory responses to moderate hypoxia, partial pressure of oxygen in the blood was the primary ventilatory stimulus. This conclusion was in stark contrast to previous theories suggesting that oxygen content, rather than partial pressure of oxygen in the blood was the primary mediator in respiratory responses (Graham et al., 1986). In a subsequent experiment, Williams et al. (1992) exposed carp to a 24 hour period of hypoxia and measured ventilation and blood gases. In addition to the hypoxia, they also elevated nitrite concentrations in the water. The purpose of the nitrite infusions was to reduce oxygen content independent of partial pressure in order to differentiate between the effects of partial pressure and oxygen content of the blood. These workers contended that if oxygen content of the blood was significant in stimulating gill ventilation, then the decline in content from nitrite infusion would cause a gradual increase in ventilation and hence in the arterial partial pressure of oxygen, and a further reduction in partial pressure of CO$_2$ in the blood. As in an earlier study (Jensen et al., 1987), the final conclusion was that hypoxia was acting through changes in partial pressure of oxygen in the blood.

VII. Goals for the Present Study

Through reflex studies, carp have been shown to possess oxygen-sensitive chemoreceptors. Hypoxia results in large increases in gill ventilation (both amplitude and frequency) and an associated bradycardia (Peyraud and Sefarty, 1964; Glass et al., 1990; Glass et al., 1991; Williams et al., 1992). Carp exposed to hyperoxia decrease ventilation (Peyraud and Sefarty, 1964). From
the aforementioned studies (Glass et al., 1990; Williams et al., 1992) suggesting carp regulate
ventilation primarily through changes in $P_{O_2}$, it appears that carp may be quite unique from other
teleosts regarding control of ventilation. Therefore, the first goal of my study was to attempt to
verify that changes in ventilation and heart rate associated with hypoxia and hyperoxia could not be
produced by changes in oxygen content of the blood independent of changes in oxygen partial
pressure. My first hypothesis was that changes in ventilation and heart rate in response to hypoxia
and hyperoxia are due to changes in oxygen content, not changes in oxygen partial pressure. The
second goal of my study was to determine the effects of hypercapnia on ventilation and heart rate
in carp. To my knowledge there are no reports of the effects of exposure to hypercapnia in carp.
The mechanism through which hypercapnia acts remains elusive as well, but it has been suggested
to act indirectly through changes in oxygen content via Bohr and Root shifts (Smith and Jones, 1982;
Perry et al., 1992), through the release of catecholamines (Aota et al., 1989; Randall and Taylor,
1991), or through a direct stimulus of $CO_2$ in the blood (Thomas et al., 1983). My second hypothesis
was that hypercapnia stimulates ventilation in carp indirectly through changes in oxygen content via
Bohr and Root effects.
MATERIALS AND METHODS

Experimental Animals

Adult carp (*Cyprinus carpio*), weighing 100-350g, were obtained from the Naitobi Gardens, University of British Columbia and were transported to the zoology department under aerated conditions. Animals were maintained in a large circular fiberglass tank supplied with a continual flow of aerated dechlorinated Vancouver tap water (temperature of 8-15°C). The fish were kept on a natural photoperiod and were fed commercial fish pellets every two-three days *ad libitum*. Animals were transferred to a separate holding tank a few days prior to surgery and feeding was discontinued. Before being chosen for experiments, the fish were visually checked to ensure they were in good health.

Animal Preparation

Fish were anaesthetized in a 0.1g/L solution of MS-222 (tricaine methanesulphonate) in dechlorinated water, buffered to pH 7.5 with NaHCO$_3$. When the fish could no longer right themselves, they were weighed and placed on a surgical table where the gills were artificially ventilated with an aerated solution of the anaesthetic.

Indwelling cannulae (Clay Adams PE-50 polyethylene tubing, Intramedic) were implanted percutaneously into the dorsal aorta of two fish to permit periodic blood sampling. The cannula was secured to the roof of the mouth with silk suture (000, Ethicon) and exited the buccal cavity via a hole containing a flared piece of polyethylene tubing (PE-160, Intramedic) slightly rostral to the
nares. Once secured, the cannulae were filled with heparinized Cortland saline (Wolf, 1963) and plugged with a 23 gauge needle. Due to the steep angle required to puncture the DA percutaneously, it proved problematic to bend the catheter and advance it further into the vessel. Attempts to do so generally resulted in puncture of the vessel and subsequent death of the animal. As such, cannula placement was successful in only two fish (C1 & C2) as judged by the fact they appeared to have been healthy with normal haematocrits.

Attempts to cannulate both the coeliac artery and caudal artery were also unsuccessful given the size and/or inaccessibility of these vessels. Thus blood samples were only available from the two successfully cannulated fish (in the dorsal aorta).

A tripolar electrocardiogram (EKG) array was constructed in order to measure heart rate. To insert the EKG electrodes, a small incision (1.3 cm) was made ventrally along the midline just beneath the pectoral girdle. Pole A of the EKG was positioned proximal to the heart within the pericardial cavity and pole B was positioned distal to the heart within the peritoneal cavity. The incision was sewn up using a blanket stitch technique (000 silk suture, Ethicon) and pole C floated freely in the water and served as a ground.

A small hole was made in the operculum with a hypodermic needle (12-gauge) for placement of an opercular cannula. A flared piece of polyethylene tubing (PE-200, Intramedic) was placed through the hole in the operculum and served to anchor the cannula, which was connected to a pressure transducer via polyethylene tubing (PE-90, Intramedic). The cannula was secured with silk suture (000, Ethicon).

A second hole was made just anterolateral to the nares, and a flared piece of polyethylene tubing (PE-200, Intramedic) was positioned through the hole along with a cannula (PE-90,
Intramedic). This cannula provided a means for administering bolus injections of NaCN into the ventilatory water flow via the mouth. On the day of the NaCN treatment, dechlorinated tap water was continuously dripped through the mouth cannula to acclimate the fish to intrabuccal infusions. Following surgery, fish were recovered on the surgery table by irrigating the gills with aerated dechlorinated tap water. When the fish began to ventilate on their own, they were transferred to a black perspex chamber and were allowed to recover overnight with a continuous flow of aerated tap water ($P_{\text{W}O_2} = 140-175$ torr). Fish were kept in the darkened chamber for the duration of the experiment which did not exceed three days.

**Experimental Setup**

The schematic drawing presented in Figure 2 illustrates the basic setup of the experiment. The black perspex chamber, in which fish resided for the duration of the experiment, was equipped with inflow and outflow ports to provide a continuous flow of dechlorinated Vancouver tap water ($10^\circ\text{C}$) through the chamber. An equilibration column prior to the inflow port allowed manipulation of gas levels within the water. The top of the perspex chamber had two small holes to allow for passage of the opercular cannula, the mouth cannula, and the EKG electrodes.

**Analytical Techniques**

The partial pressure of $O_2$ and $CO_2$ in the water ($P_{\text{WO}_2}$ and $P_{\text{W}CO_2}$, respectively) were monitored continuously using Radiometer electrodes (model #'s E5046 and E5036, respectively) housed in thermostatted cuvettes kept at ambient temperature. Values were displayed on a Radiometer PHM73 acid-base analyzer. Electrodes were calibrated and corrected for barometric
Figure 2. Basic experimental setup utilized for experiments on carp. Gas mixtures (O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub>, air, CO) were flowed into an equilibration column before reaching the inlet valve of the perspex chamber containing the fish. In addition to the inlet valve, there was also an outlet valve to provide a continuous flow of dechlorinated water. The carp had an opercular cannula connected to a pressure transducer to measure ventilation and an EKG electrode to measure heart rate. These two variables were then collected on a chart recorder for analysis.
Experimental Setup

- Dechlorinated Water In
- Water Column
- Opercular Pressure Catheter
- Heart Rate (EKG) Lead
- Chart Recorder and Amplifiers
- Gas Mixer
- H₂O In
- Fish in tank
- H₂O Out
pressure and temperature. A two-point calibration was employed for the \( O_2 \) electrode using both nitrogen (0% \( O_2 \)) and air (20.9% \( O_2 \)) equilibrated water. A three-point calibration was used for the \( CO_2 \) electrode. A gas mixture containing 0% \( CO_2 \) was produced by equilibrating water with nitrogen, while the other levels were set using water equilibrated with 0.4% \( CO_2 \) and 1.5% \( CO_2 \) in air (Praxair, analyzed), respectively. Once the electrodes were calibrated, water from the perspex chamber was continually siphoned across the electrodes with the aid of negative pressure to provide a constant measure of \( Pw_{O2} \) and \( Pw_{CO2} \).

For the measurement of blood gases, the electrodes within the thermostatted cuvettes were maintained at 10°C with a water chiller (Lauda model RM6), and calibration of the electrodes was achieved using the same concentrations of gases mentioned previously, but humidified rather than equilibrated with water. Water samples, taken before each blood sample, and blood samples were gently injected across the \( O_2 \) and \( CO_2 \) electrodes, and measurements were recorded from the Radiometer PHM73 meter after two minutes. Blood pH was measured using a Radiometer Copenhagen pH capillary electrode (model # G297 / G2) which was also thermostatted to 10°C. The pH electrode was calibrated prior to each blood sample using Radiometer Copenhagen precision buffer solutions (standards S1510 and S1500). Three separate pH measurements per sample were taken, and these values were averaged.

Gill ventilation rate (\( f_g \)) and opercular pressure amplitude (Pop) were measured from the opercular cannula using a pressure transducer (Narco model #320-1000E) connected to one channel of a pen recorder (Gould 2200S or 2400S) via a transducer amplifier (Gould model #13-4615-50). In some cases this signal was further amplified by an integrating amplifier in direct mode (Gould model #13-4615-70) which was displayed on another channel of the recorder. Heart rate (\( f_{hr} \)) was
measured either from pressure pulses in the DA cannula (n= 2) using a pressure transducer (Narco model #320-1000E) or from the EKG electrodes (n= 8) connected to a Universal amplifier (Gould model #13-4615-58) with output to another channel on the pen recorder. The transducer amplifier was calibrated on each day of an experiment using the level of the perspex chamber for zero pressure and 40 cmH$_2$O for the high pressure calibration.

Protocol

Following overnight recovery from surgery, the opercular cannula was flushed with water and connected to the pressure transducer. The DA cannula (where appropriate) was flushed with heparinized saline and attached to a pressure transducer. Fish with EKG electrodes were connected to an amplifier at all times. The overall condition of the fish was determined primarily by observing the ventilatory trace, and, to a lesser extent, the EKG trace. If there was excessive struggling or an abnormal breathing pattern, the experiment was aborted, and the fish were given more time to recover. If these conditions persisted on the second day post surgery, the fish was discarded from the study.

Fish were exposed to one hour of normoxic normocapnia prior to each condition to allow each fish to serve as its own control. Each condition was introduced randomly, and ventilation and heart rate were recorded for up to one hour after steady state was achieved, which generally occurred 30-45 minutes after the condition was initiated. The CO$_2$ response in some fish can be rapid and transient, therefore, the one hour time period for fish exposed to hypercapnia commenced immediately at the onset of hypercapnia. Animals were exposed to the following environmental conditions: normoxic normocapnia (n= 10), hypoxic normocapnia (n= 11),
Table I. The mean (± 1 SEM) partial pressures of O₂ and CO₂ in the water for each of the experimental conditions utilized on carp.
<table>
<thead>
<tr>
<th>Condition</th>
<th>$P_{\text{WO}_2}$ (torr)</th>
<th>$P_{\text{WCO}_2}$ (torr)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic Normocapnia</td>
<td>161.0 ± 15.8</td>
<td>1.1 ± 0.5</td>
<td>55</td>
</tr>
<tr>
<td>Hypoxic Normocapnia</td>
<td>40.0 ± 2.7</td>
<td>0.9 ± 0.3</td>
<td>10</td>
</tr>
<tr>
<td>Hyperoxic Normocapnia</td>
<td>515.0 ± 75.0</td>
<td>1.0 ± 0.5</td>
<td>11</td>
</tr>
<tr>
<td>Hyperoxic Hypercapnia</td>
<td>570.0 ± 16.0</td>
<td>13.5 ± 0.8</td>
<td>6</td>
</tr>
<tr>
<td>Normoxic 21.2% CO</td>
<td>152.0 ± 15.0</td>
<td>1.1 ± 0.1</td>
<td>6</td>
</tr>
</tbody>
</table>
hyperoxic hypercapnia (n= 6), and normoxic carbon monoxide (n=6) Gas mixtures were produced by mixing air, nitrogen, oxygen, carbon dioxide and / or carbon monoxide with flow meters (Union Carbide Corp. model #’s 4333 and 4334) and were bubbled into the equilibration column. Table I lists the partial pressures of oxygen and carbon dioxide in each of the above conditions. In fish with a DA cannula, a maximum of four blood samples (0.7 to 1.0 ml) were taken during a one hour experiment. Following the measurements, the remaining blood was returned to the fish. Due to the inability to measure CO content in the water and the lack of blood samples in this study, CO was bubbled into the aeration column until a behavioral response was observed, and then the level was either maintained or decreased slightly. Sodium cyanide (NaCN) was administered externally (n= 7) through the mouth cannula. NaCN (2.5-5 mg / ml, dissolved in dechlorinated tap water) was administered as a 0.3 ml bolus into the mouth cannula, and the response was recorded for two to five minutes post-injection. A control injection of 0.3 ml of dechlorinated water was also administered prior to the NaCN injection. If ventilation and heart rate remained constant for two minutes following control injection, the bolus of NaCN was then administered in fish with a DA cannula. Internal injections of NaCN (5-10 mg / ml, dissolved in heparinized saline) were given in 0.5 ml bolus injections via the DA cannula. The control for internal injections was a 0.5 ml bolus of heparinized saline. At the end of an experiment, fish were euthanized with an overdose of MS-222, and the EKG electrode was removed.

Data Analysis and Statistics

Cardiovascular and ventilatory variables were measured for 1 min time intervals at 5, 10, 15, 30, 45, 60, 75, and 90 minutes after either the onset of a condition (hypercapnia) or steady state was
achieved (hypoxia, hyperoxia, CO). Heart rate was calculated as beats / min from the EKG or dorsal aorta blood pressure trace. Breathing frequency (fg), episodes of breathing per minute, and breaths per episode were obtained from the opercular cannula pressure trace. Integrated opercular pressure (I_{Op}) and opercular pressure amplitude (P_{Op}) were also determined from the opercular cannula pressure trace. The values for both I_{Op} and P_{Op}, for fish 1-5, were acquired from digitizing the image into SigmaScan and correcting the value to the proper units (cmH_{2}O·s / min and cmH_{2}O, respectively). For the remaining fish (n= 6), data were collected using a data acquisition program (CODAS) connected to a 486 desktop computer. The same variables were measured and imported into a QuattroPro spreadsheet where they were converted to their appropriate units. For each of the conditions, a time course plot was generated (SigmaPlot) for the mean data of all fish at each time point in order to demonstrate the time course of the response of the fish during the exposure to the condition. The overall means were also calculated under conditions where steady state was reached to illustrate the mean effect of the condition on each of the variables measured.

The data are presented as the mean percent change ± the standard error of the mean (SEM). All statistical analysis was performed with a statistical software package (SigmaStat, Jandel Scientific). Where appropriate the data have been analyzed with a one way analysis of variance (ANOVA). If parametric test assumptions were invalid, the data were analyzed with a Kruskal-Wallis ANOVA. Data were deemed significant at P ≤ 0.05.
RESULTS

I. Cardioventilatory Effects of Hypoxia and Hyperoxia under Normocapnic Conditions

Representative traces illustrating the breathing pattern and EKG of carp under normoxic, hypoxic, and hyperoxic conditions are presented in Figure 3. During normoxia, breaths are clustered into episodes, and entrainment occurs between heart rate and individual breaths. Hypoxia eliminates the breathing episodes, and the fish switch to a continuous breathing pattern. Episodic breathing was converted to a continuous pattern by progressively increasing the number of breaths in an episode and decreasing the number of episodes in a minute. In hyperoxia, there were fewer breaths per episode as well as longer pauses between episodes (Figures 3 & 4). Entrainment of heart rate and breathing during hyperoxia was not as evident as during normoxia.

After exposure to hypoxia, heart rate ($f_h$) remained constant for the first 60 minutes (Figure 5), but then heart rate slowly increased to levels which became significantly different from normoxic values at 75 minutes. Breathing frequency ($f_g$), integrated opercular pressure (IOP), and opercular pressure amplitude (POp) all increased slowly over the first 30 minutes and, at 30 minutes, became significantly different from normoxic values. Animals were considered to have reached a new steady state after one hour of exposure to hypoxia when levels of $f_g$, IOP, and POp reached a plateau (Figure 5). In steady state, the ventilatory responses as well as the cardiovascular responses were significantly increased from normoxia (Figure 7). The data for hypoxia are presented as percent change and were calculated relative to normoxia as follows:

$$\text{percent change} = \frac{(\text{normoxia} - \text{hypoxia})}{\text{normoxia}} \times 100.$$
Figure 3. Representative traces of ventilation (as measured by deflections in pressure from an opercular cannula, top trace) and heart rate (as measured by an EKG electrode, bottom trace) under conditions of normoxic normocapnia, hypoxic normocapnia, and hyperoxic normocapnia. Downward deflection represents inspiration. Note the clustering of breaths into episodes during normoxia. Clustering of breaths also occurred in hyperoxia although the breaths per episode were less than those in normoxia. Unlike normoxia and hyperoxia, hypoxia produced a continuous, rather than an episodic pattern of breathing.
Figure 4. The breathing pattern (episodes per minute and breaths per episode) of carp under conditions of normoxia (n= 11) and hyperoxia (n= 11), and hypoxia (n= 10). The data are presented as the mean ± 1 SEM. The breathing pattern became continuous during hypoxia.
Figure 5. Changes in heart rate (f_{hr} beats / min), breathing frequency (f_{bre} breaths / min), integrated opercular pressure (I_{Op}, cmH$_2$O-s / min), and opercular pressure amplitude (P_{Op}, cmH$_2$O) in carp (n= 10) exposed to 95 minutes of hypoxic normocapnia. The data are presented as percent change from normoxia ± 1 SEM. The dashed line represents the control normoxic value. An asterisk (*) denotes a significant difference from the normoxic (time zero) value.
The absolute values used in calculation of percent change from normoxia for hypoxia are listed in Table II.

Exposure to hyperoxia had minimal effects on all variables measured over time (Figure 6). With the exception of breathing frequency ($f_g$), which decreased significantly during hyperoxia (~-20%), all other variables ($f_{hr}$, IOP, $P_{OP}$) did not differ from the normoxic control values. Steady state (30, 45, 60, 75, and 90 minutes) responses during hyperoxia were consistent with the time course data, with breathing frequency being the only variable which decreased significantly from normoxic control values (Figure 7). Hyperoxia data are also plotted as percent change calculated relative to normoxia (see Table II for absolute values).

Blood gas data are presented in Table III for the two carp having functional DA cannulae (carp #1 & 2) under normoxic, hypoxic, and hyperoxic conditions. Due to the small sample size ($n=2$), statistics were not performed on blood gas values, and any reference to "increases or decreases" in these values refer to the trends in the data. Blood gas values obtained during normoxia preceding the hypoxic and hyperoxic conditions are reported as well. During normoxia, arterial $P_{O_2}$ ranged from 52-65 torr in carp #1 and 129-134 torr in carp #2, while arterial $P_{CO_2}$ ranged from 2.3-2.5 torr and 1.5-2.1 torr, respectively. Hypoxic exposure resulted in a fall of $P_{aO_2}$ as well as $P_{aCO_2}$. $pH_a$ was not measured in carp #1, however, carp #2 exhibited an increase in $pH_a$ under hypoxic conditions (7.9 during normoxia to 8.03 during hypoxia). Hematocrit increased for both fish during hypoxia. Conversely, during hyperoxia, arterial $P_{O_2}$ increased (~300 torr) with increasing partial pressure of $O_2$ in the water, while arterial $P_{CO_2}$, $pH_a$ (for carp #2), and hematocrit remained relatively unaffected.
Figure 6. Changes in heart rate ($f_{hr}$, beats / min), breathing frequency ($f_{bg}$, breaths / min), integrated opercular pressure ($I_{Op}$, cmH$_2$O·s / min), and opercular pressure amplitude ($P_{Op}$, cmH$_2$O) in carp ($n=11$) exposed to 95 minutes of hyperoxic normocapnia. The data are presented as percent change from normoxia ± 1 SEM. The dashed line represents the control normoxic value. An asterisk (*) denotes a significant difference from the normoxic (time zero) value.
Figure 7. Steady state changes in heart rate ($f_h$, beats / min), breathing frequency ($f_g$, breaths / min), integrated opercular pressure ($I_{op}$, cmH$_2$O·s / min), and opercular pressure amplitude ($P_{op}$, cmH$_2$O) in carp exposed to hypoxia (n= 10) and hyperoxia (n= 11) compared to levels measured under normoxic conditions. The data are presented as mean percent change from normoxia ± 1 SEM. The dashed line represents the control normoxic value. An asterisk (*) denotes a significant difference from the normoxic (time zero) value.
Table II. Absolute values for heart rate ($f_h$), breathing frequency ($f_g$), integrated opercular pressure ($I_{Op}$), and opercular pressure amplitude ($Pop$) measured under normoxic, hypoxic, and hyperoxic conditions. Time zero represents the average of normoxia preceding hypoxia and hyperoxia.
### A. Hypoxia

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>75 min</th>
<th>90 min</th>
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<tbody>
<tr>
<td>$f_{hr}$</td>
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<td>$21.6 \pm 2.1$</td>
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<tr>
<td>$f_{g}$</td>
<td>$18.7 \pm 2.5$</td>
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<td>$I_{Op}$</td>
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<td>$P_{Op}$</td>
<td>$0.28 \pm 0.14$</td>
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<td>$0.81 \pm 0.38$</td>
<td>$0.70 \pm 0.31$</td>
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</tr>
</tbody>
</table>

### B. Hyperoxia

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>75 min</th>
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<td>$f_{hr}$</td>
<td>$21.2 \pm 1.2$</td>
<td>$19.9 \pm 1.2$</td>
<td>$18.9 \pm 1.1$</td>
<td>$20.8 \pm 1.4$</td>
<td>$20.0 \pm 1.3$</td>
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<tr>
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<td>$0.13 \pm 0.04$</td>
<td>$0.14 \pm 0.04$</td>
<td>$0.16 \pm 0.05$</td>
<td>$0.18 \pm 0.08$</td>
</tr>
</tbody>
</table>
Table III. Average blood gas measurements for two carp during normoxic normocapnia, hypoxic normocapnia, and hyperoxic normocapnia. Blood was drawn from the dorsal aorta cannula and arterial $P_{O_2}$, $P_{CO_2}$, and pH were measured with Radiometer electrodes. Values for hematocrit are also presented. The partial pressures of $O_2$ and $CO_2$ in the water ($P_{wO_2}$ and $P_{wCO_2}$ respectively) were measured prior to each blood sample.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Normoxic</th>
<th>Hypoxic</th>
<th>Normoxic</th>
<th>Hyperoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 140 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 40 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 159 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 393 torr</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 134 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 13.7 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 129 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 323 torr</td>
</tr>
<tr>
<td>Normocapnia</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 0.7 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 0.6 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 1.2 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 0.6 torr</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 2.5 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 1.2 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 2.3 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 2.1 torr</td>
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<td></td>
<td>Hct = 7.1%</td>
<td>Hct = 8.4%</td>
<td>Hct = 9.5%</td>
<td>Hct = 9.5%</td>
</tr>
<tr>
<td>Carp #1</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 135 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 43 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 129 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 357 torr</td>
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<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 52 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 15 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 65 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 300 torr</td>
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<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 0.6 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 0.8 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 0.4 torr</td>
<td>P&lt;sub&gt;W&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 0.3 torr</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 2.1 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 1.7 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 1.5 torr</td>
<td>P&lt;sub&gt;A&lt;/sub&gt;&lt;sub&gt;C&lt;/sub&gt;&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; = 0.9 torr</td>
</tr>
<tr>
<td></td>
<td>pH&lt;sub&gt;a&lt;/sub&gt; = 7.90</td>
<td>pH&lt;sub&gt;a&lt;/sub&gt; = 8.03</td>
<td>pH&lt;sub&gt;a&lt;/sub&gt; = 8.00</td>
<td>pH&lt;sub&gt;a&lt;/sub&gt; = 7.90</td>
</tr>
<tr>
<td></td>
<td>Hct = 8.6%</td>
<td>Hct = 12.3%</td>
<td>Hct = 9.5%</td>
<td>Hct = 11.6%</td>
</tr>
</tbody>
</table>
Figure 8. Representative traces demonstrating cardioventilatory effects of administration of 0.2 ml dechlorinated water and 0.2 ml NaCN (5 mg/ml) into the mouth cannula of carp (n=7). Note the change in scale between ventilation traces shown for injections of water and cyanide, indicating huge ventilatory responses. The ventilatory response to NaCN was of rapid onset and was transient, with ventilation returning to a normal level within one minute. A slight increase in the intensity of heart contraction occurred, but there was no effect of NaCN on heart rate.
Table IV. Cardioventilatory effects of bolus injections of NaCN (0.2 ml, 5 mg / ml) into the mouth cannula of carp (n= 7). Instantaneous breathing frequency (fg) and heart rate (fhr) were calculated, and integrated opercular pressure (IoP) and opercular pressure amplitude (Pop) were measured for control injections (0.2 ml dechlorinated water), cyanide injections, and one minute following cyanide injections. A one-way ANOVA indicated there was a significant difference between control values for ventilation and values obtained for NaCN which are indicated by an asterisk (●). There was also a significant difference between NaCN and post-NaCN values for ventilation (●). Control values and post-NaCN values were not significantly different indicating, ventilation had returned to normal levels. There were no significant changes in heart rate.
<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>NaCN</th>
<th>post-NaCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instantaneous $f_g$</td>
<td>15.5 ± 3.1</td>
<td>56.6 ± 7.6*</td>
<td>12.9 ± 2.4*</td>
</tr>
<tr>
<td>(breaths / min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instantaneous $f_{hr}$</td>
<td>17.0 ± 2.7</td>
<td>15.2 ± 3.0</td>
<td>12.0 ± 0.9</td>
</tr>
<tr>
<td>(beats / min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IOP</td>
<td>0.2 ± .06</td>
<td>1.5 ± 0.40*</td>
<td></td>
</tr>
<tr>
<td>(cmH₂O·s / min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POP</td>
<td>0.1 ± .02</td>
<td>2.0 ± 0.04*</td>
<td></td>
</tr>
<tr>
<td>(cmH₂O)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
External NaCN (5 mg / ml) was administered in seven fish (see Figure 8 and Table IV). Bolus injections (0.2 ml) of NaCN were given through the mouth cannula of each carp. Prior to the NaCN injection, a 0.2 ml injection of dechlorinated water was administered as a control. There were no changes in any variable associated with the water injections. With NaCN injections, however, while heart rate ($f_h$) did not differ from the control, immediate increases in breathing frequency ($f_g$) and amplitude ($I_{op}$ & $P_{op}$) were observed which lasted ~5-10 seconds. The complete response was over within a minute (Figure 8). The increases in ventilation associated with administration of NaCN were significant (Table IV). Ventilatory measurements made one minute after administration of NaCN were no longer significantly different from the control values (Table IV).

II. Cardioventilatory Effects of Carbon Monoxide

Five of the six carp exposed to carbon monoxide went from an episodic breathing pattern to a continuous breathing pattern (Figures 9 & 10). The transition was similar to that found during hypoxia in that the number of breaths per episode increasing progressively while the number of episodes occurring in a minute decreased until breathing became continuous. In the normoxic control period preceding the administration of CO, many of the carp had an elevated breathing frequency and were already very close to breathing continuously at the beginning of the CO experiment.

Carbon monoxide introduced through the water equilibration column (21.2 ± 9.0%) resulted primarily in significant increases of heart rate (Figure 11). Breathing frequency ($f_g$) was slightly, though not significantly elevated throughout the exposure period to CO. Integrated opercular pressure ($I_{op}$) and opercular pressure amplitude ($P_{op}$) also increased slightly, but not significantly,
Figure 9. Representative traces illustrating the effects of 21.2 ± 9.0% CO on ventilation (as measured by deflections in pressure from an opercular cannula, top trace) and heart rate (as measured by an EKG electrode, bottom trace). Downward deflection represents inspiration. Note the change from an episodic breathing pattern during normoxia to a continuous breathing pattern during exposure of this carp to carbon monoxide.
Normoxia

Opercular Pressure

EKG

0.2 cmH₂O

Normoxic CO

Opercular Pressure

EKG

0.33 cmH₂O

10 sec
Figure 10. The effects of carbon monoxide (n= 6) on breathing pattern (episodes per minute and breaths per episode) in carp. The data are presented as the mean ± 1 SEM. Five of the six fish switched from an episodic breathing pattern to a continuous breathing pattern.
The graph shows the comparison of episodes and breaths per episode between normoxia and normoxic conditions.

- **Continuous**: $n=5$
- **Normoxia**: $n=6$
- **Normoxic 20% CO**: $n=6$

**Episodes per Minute**
- Normoxia: 4 episodes per minute
- Normoxic 20% CO: 6 episodes per minute

**Breaths per Episode**
- Normoxia: 5 breaths per episode
- Normoxic 20% CO: 3 breaths per episode
Figure 11. Changes in heart rate ($f_h$, beats/min), breathing frequency ($f_g$, breaths/min), integrated opercular pressure ($I_{Op}$, cmH$_2$O·s/min), and opercular pressure amplitude ($P_{Op}$, cmH$_2$O) in carp (n= 6) exposed to 95 minutes of carbon monoxide (21.2 ± 9.0% bubbled into the water equilibration column). The data are presented as mean percent change from normoxia ± 1 SEM. The dashed line represents the control normoxic value. An asterisk (*) denotes a significant difference from the normoxic (time zero) value.
Table V. Absolute values for heart rate ($f_h$), breathing frequency ($f_g$), integrated opercular pressure ($I_Op$), and opercular pressure amplitude ($P_{Op}$) measured during CO exposure. The value at time zero is the average for normoxia preceding CO.
<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>75 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>f_{hr}</td>
<td>19.7 ± 1.5</td>
<td>27.4 ± 4.1</td>
<td>28.2 ± 3.8</td>
<td>28.6 ± 4.1</td>
<td>28.6 ± 3.5</td>
<td>29.2 ± 3.8</td>
</tr>
<tr>
<td>f_g</td>
<td>19.4 ± 5.2</td>
<td>24.8 ± 5.0</td>
<td>25.5 ± 5.0</td>
<td>25.7 ± 5.0</td>
<td>24.8 ± 6.3</td>
<td>26.2 ± 6.3</td>
</tr>
<tr>
<td>I_{op}</td>
<td>0.27 ± 0.15</td>
<td>0.36 ± 0.18</td>
<td>0.30 ± 0.12</td>
<td>0.16 ± 0.04</td>
<td>0.18 ± 0.04</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>P_{op}</td>
<td>0.23 ± 0.07</td>
<td>0.31 ± 0.07</td>
<td>0.31 ± 0.08</td>
<td>0.33 ± 0.08</td>
<td>0.35 ± 0.09</td>
<td>0.30 ± 0.08</td>
</tr>
</tbody>
</table>
with exposure to CO. Data were calculated as percent change from normoxia and absolute values are listed in Table V.

III. Cardioventilatory Effects of Hyperoxic Hypercapnia

Hyperoxic hypercapnia (~2.5%) produced an initial decrease in \( f_{hr} \) and an initial increase in \( I_{op} \) and \( P_{op} \) which were all transient and non-significant (Figures 12 & 13). Heart rate (\( f_{hr} \)), integrated opercular pressure (\( I_{op} \)), and opercular pressure amplitude (\( P_{op} \)) returned to control values after 10 minutes (Figures 12 & 13). However, breathing frequency (\( f_b \)) increased significantly and remained elevated for the remainder of the experiment (Figure 13). The percent change values for hyperoxic hypercapnia were calculated as follows:

\[
\left\{ \left( \text{normoxia-hyperoxia} \right)^\text{normox} \times 100 \right\} - \left\{ \left( \text{normoxia-hyperoxic hypercap} \right)^\text{normox} \times 100 \right\}
\]

Table VI lists the absolute values for fish exposed to hyperoxic hypercapnia (absolute values for hyperoxia are presented in Table II).

Hyperoxic Hypercapnia exerted a minimal effect on breathing pattern (Figure 14). There was a trend for the number of episodes of breathing per minute to decrease in steady state (i.e. beginning at 10 minutes which was after the transient response), and for the number of breaths in an episode to increase; however, these changes were not significant.
Figure 12. Representative traces illustrating the effects of hyperoxic hypercapnia on ventilation (as measured by deflections in pressure from an opercular cannula, top trace) two minutes after the beginning of hyperoxic hypercapnia and 60 minutes after the beginning of hyperoxic hypercapnia. Downward deflection represents inspiration. Note the slight change in breathing pattern from hyperoxia, which consists of a slight increase in breaths per episode and in the time between episodes.
Hyperoxic Hypercapnia

Opercular Pressure

0.3 cmH₂O

Hydroxic Hypercapnia, 2 min

Opercular Pressure

0.6 cmH₂O

Hyperoxic Hypercapnia, 60 min

Opercular Pressure

0.3 cmH₂O

10 sec
Figure 13. Effects of 1 hour exposure of carp to hyperoxic hypercapnia (n= 6) on heart rate (f hr, beats / min), breathing frequency (f g, breaths / min), integrated opercular pressure (lOp, cmH2O·s / min), and opercular pressure amplitude (PoP, cmH2O). The data are presented as mean percent change from hyperoxia ± 1 SEM. The dashed line represents the control normoxic value. An asterisk (*) denotes a significant difference from the normoxic (time zero) value.
Figure 14. The effects of hyperoxic hypercapnia on breathing pattern (episodes per minute and breaths per episode) in carp (n= 6). A slight, but non-significant, increase in breaths per episode occurred along with a slight decrease in the number of episodes in a minute.
Table VI. Absolute values for heart rate ($f_h$), breathing frequency ($f_b$), integrated opercular pressure ($IOP$), and opercular pressure amplitude ($POP$) measured during hyperoxic hypercapnia.
<table>
<thead>
<tr>
<th></th>
<th>2 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_{hr} )</td>
<td>15.5 ± 1.5</td>
<td>20.8 ± 2.7</td>
<td>18.8 ± 1.9</td>
<td>17.5 ± 1.7</td>
<td>18.2 ± 1.9</td>
<td>17.5 ± 2.1</td>
<td>16.2 ± 1.4</td>
</tr>
<tr>
<td>( f_g )</td>
<td>9.2 ± 1.5</td>
<td>13.2 ± 2.1</td>
<td>16.3 ± 2.4</td>
<td>15.0 ± 3.0</td>
<td>16.3 ± 3.4</td>
<td>18.7 ± 4.4</td>
<td>19.2 ± 3.8</td>
</tr>
<tr>
<td>( I_{OP} )</td>
<td>0.22 ± 0.13</td>
<td>0.25 ± 0.12</td>
<td>0.18 ± 0.11</td>
<td>0.14 ± 0.07</td>
<td>0.10 ± 0.04</td>
<td>0.15 ± 0.07</td>
<td>0.16 ± 0.09</td>
</tr>
<tr>
<td>( P_{OP} )</td>
<td>0.60 ± 0.25</td>
<td>0.43 ± 0.18</td>
<td>0.34 ± 0.16</td>
<td>0.26 ± 0.12</td>
<td>0.20 ± 0.06</td>
<td>0.27 ± 0.13</td>
<td>0.30 ± 0.14</td>
</tr>
</tbody>
</table>
DISCUSSION

Carp responded to changes in partial pressure of oxygen in the water, both increases and decreases, in a manner similar to other teleost fish. Hypoxia resulted in significant increases in ventilation (both frequency and amplitude). A reflex hypoxic bradycardia, however, was not observed in the present study, and, in fact, a tachycardia occurred at one hour and persisted through the remainder of the experiment. There was a significant decrease in ventilatory frequency during hyperoxia, while ventilatory amplitude as well as heart rate did not change from normoxic control values. NaCN (5 mg / ml) administered through a mouth cannula in carp resulted in rapid, albeit transient, increases in frequency and amplitude of ventilation which were significantly different from control water injections. The response to NaCN was over within a minute following administration of NaCN (evidenced by the significant decrease in respiratory frequency). Heart rate was unaffected by NaCN. Internal injections of NaCN (5-10 mg / ml) into the dorsal aorta of carp produced no cardioventilatory responses. Carbon monoxide (21.2 ± 9.0%) had no effect on ventilation in carp, however, it increased heart rate significantly. This indicates that carp possess O₂-sensitive chemoreceptors capable of monitoring changes in O₂ content, independent of changes in O₂ partial pressure, but they are not involved in the control of breathing. Hyperoxic hypercapnia significantly increased the frequency of ventilation after the first five minutes of exposure. This increase in ventilation during hyperoxic hypercapnia indicates that in carp, CO₂ is acting directly on a receptor, as opposed to indirectly through Bohr and Root effects in the blood. The changes in breathing pattern with hypoxia and CO occurred in the same manner for all carp (i.e. there was an increase in the number of breaths in an episode and a decrease in the number of episodes in a minute.
until a continuous breathing pattern was achieved).

**I. Effects of Changes in $O_2$ Partial Pressure and/or Content on Cardioventilatory Responses in Carp**

**A. Hypoxia**

Previous studies (Peyraud and Sefarty, 1964; Itazawa and Takeda, 1978; Glass *et al.*, 1990; Williams *et al.*, 1992) have described hypoxia as a potent respiratory stimulant in carp (*Cyprinus carpio*). In the current study, the ventilatory response of carp to environmental hypoxia consisted of a significant increase in ventilatory frequency and amplitude (see Figures 5 & 7). These responses are consistent with some previous observations in this species (Peyraud and Sefarty, 1964; Lomholt and Johansen, 1978). Other investigators (Itazawa and Takeda, 1978; Glass *et al.*, 1990; Williams *et al.*, 1992), however, have reported increases in frequency alone. The reasons for this discrepancy are not clear, but preliminary experiments in the present study indicated that the exact location of cannula placement in the opercular cavity greatly affected the amplitude of the pressure traces. In the present study it was found that placement of the opercular cannula on the mid-dorsal region of the operculum provided the best measurement of amplitude of ventilation. It is possible that the absence of any increase in ventilatory amplitude by some investigators was due to a less than optimal placement of the opercular catheter.

In other species of fish, the ventilatory response to hypoxia is similar to that found for carp in the present study, with increases occurring both in ventilatory frequency and amplitude (rainbow trout: Randall and Smith, 1967; Holeton and Randall, 1967; Randall and Jones, 1973; Smith and Jones, 1978; Randall, 1982; Thomas and Hughes, 1982; tench: Randall and Shelton, 1963; white
sucker: Saunders, 1962; eel: van Dam, 1938; Peyraud-Waitzenegger and Soutilier, 1989; sea raven: Saunders and Sutterlin, 1971; striped mullet: Cech and Wohlschlag, 1973; catfish: Burleson and Smatresk, 1990a; sturgeon: Nonnotte et al., 1993; Maxime et al., 1995; Hoplias malabaricus: Kalinin et al., 1993). Additionally, several studies exist where the primary ventilatory response to hypoxia is through increases in ventilatory amplitude (catfish: Gerald and Cech, 1970; Burggren and Cameron, 1980), and there are also studies indicating increases in frequency of ventilation as the primary response upon hypoxic exposure (pufferfish: Hall, 1931). The purpose of hyperventilation under hypoxic conditions is to maintain adequate oxygen exchange at the gills, and whether frequency, amplitude or both increase is probably a function of the mechanics of breathing, which are different for each species, as well as the level of hypoxia (i.e. the amount of oxygen available in the water).

The reflex bradycardia typically associated with hypoxia in teleost fish was not observed in this study. Indeed, following one hour of hypoxic exposure, heart rate actually increased significantly. Carp have been reported to exhibit a reflex hypoxic bradycardia in some previous studies (Peyraud and Sefarty, 1964; Itazawa and Takeda, 1978; Glass et al., 1990; Williams et al., 1992). Williams et al. (1992) suggested that the hypoxic bradycardia in carp did not occur until the partial pressure of oxygen in the water approached 30 torr. Furthermore, these workers noted at more moderate levels of hypoxia ($P_{w,02} = 50$ torr) that carp actually exhibited a slight increase in heart rate. This observation was consistent with the present study and indicates the level of hypoxia utilized in the present study ($P_{w,02} = 40$ torr) may not have been severe enough to induce a reflex bradycardia. In other species of bottom-dwelling fish, the level of hypoxia required to evoke a bradycardia is also low. Sturgeon do not exhibit changes in heart rate until partial pressures of
oxygen in the water approach 10 torr (Maxime et al., 1995), while Hoplias malabaricus do not show a reflex bradycardia until \( P_{wO2} = 20 \) torr (Rantin et al., 1993). Tench appear to be more similar to carp than sturgeon or Hoplias malabaricus, with hypoxic bradycardia occurring around 35 torr (Randall and Shelton, 1963). In more active species of fish, such as rainbow trout, reflex bradycardia is associated with hypoxia at a \( P_{wO2} \) of 75-80 torr (Randall and Smith, 1967; Holeton and Randall, 1967; Smith and Jones, 1978). The reflex bradycardia has been proposed to increase the transit time of blood through the gills thereby enhancing gas exchange across the gills. Stroke volume increases in order to offset the reduction in heart rate, and cardiac output actually increases (Randall and Smith, 1963; Randall, 1966). This increase in cardiac output may also lead to an increase in blood pressure which will recruit additional gill lamellae not perfused at normal systemic pressure, thereby increasing the surface area available for gas exchange (Soivio and Tuurala, 1981).

Cyprinids, such as carp, have a greater hemoglobin-oxygen affinity than salmonids (i.e. trout) evidenced by the low \( P_{50} \) value for carp compared with trout (~7 torr and 14 torr, respectively). Thus, although the reflex bradycardia associated with hypoxia in carp occurs at a much lower oxygen level than in rainbow trout, it appears well correlated with the increased ability of carp hemoglobin to bind oxygen.

Blood gases were measured on only two carp in this study due to the difficulties in cannulating the dorsal aorta. The trends observed from these measurements, however, are worth mentioning. The partial pressures of oxygen in the blood \( (P_{aO2}) \) of carp measured under normoxic conditions in the current study (~100 torr; Table III) were similar to those reported by Hughes et al. (1983) and greater than values reported by other workers (Garey, 1967; Glass et al., 1990). In normoxic water \( (P_{wO2} = 108 \) torr and 150 torr, respectively), Garey and Glass et al. reported a \( P_{aO2} \)
of 33 torr and 15 torr, respectively, which were similar to the hypoxic values measured in the current study \( (P_{W_{O_2}} = 40 \text{ torr}, \ P_{A_{O_2}} = 13.7 \text{ and 15 torr}) \). The hypoxic conditions used by Glass \textit{et al.} (1990) were quite moderate \( (P_{W_{O_2}} = 75 \text{ torr}) \) and did not change arterial \( P_{O_2} \). Itazawa and Takeda (1978) also reported low arterial \( P_{O_2} \) associated with normoxia \( (P_{W_{O_2}} = 141 \text{ torr}, \ P_{A_{O_2}} = 24.8 \text{ torr}) \), and exposure to hypoxia only caused arterial \( P_{O_2} \) to decrease slightly \( (P_{W_{O_2}} = 51.6 \text{ torr}, \ P_{A_{O_2}} = 18.7 \text{ torr}) \).

It is difficult to reconcile the differences observed in arterial \( P_{O_2} \) in these latter studies with those in the current study given the small sample size, but the data suggest that gill function or cardiac output may have been impaired in the studies of these other workers. Partial pressures of carbon dioxide in the blood decreased upon exposure to hypoxia in the present study as well as in the studies mentioned previously. A fall in \( P_{A_{CO_2}} \) during hypoxia would be expected since hyperventilation results in increased elimination of \( CO_2 \) at the gills.

Under normoxic conditions, carp are episodic breathers (Peyraud and Sefarty, 1964; Eclancher and Dejours, 1975; Lomholt and Johansen, 1978; Glass \textit{et al.}, 1990; Williams \textit{et al.}, 1992). In the current study, during breathing episodes, the carp also exhibited a synchrony between heart rate and ventilation (Peyraud and Sefarty, 1964). This entrainment, however, was not present in all animals (see Figure 3). The purpose of entrainment between heart rate and breathing has been suggested to ensure that periods of peak blood flow through the gills and peak water flow (hence maximum oxygen availability) coincide in order to provide optimal gas exchange at the gills (Randall and Shelton, 1963). The absence of entrainment at other times suggests that this is not terribly important.

Hypoxic exposure eliminated breathing episodes and produced a continuous breathing pattern which was achieved by progressively increasing the number of breaths in an episode and decreasing
the length of non-ventilatory periods. This change in breathing pattern during hypoxia was consistent with previous studies in carp (Lomholt and Johansen, 1978; Glass et al., 1990). Sturgeon are also episodic breathers, and upon exposure to hypoxia, they shift to continuous breathing in a manner similar to carp (Nonnotte et al., 1993). Other species that exhibit a periodic breathing pattern include tench (Randall and Shelton, 1963) and eel (Forster, 1981; Hipkins and Smith, 1983). However, the transition from an episodic to continuous breathing pattern during hypoxia has not been documented in these species.

B. Hyperoxia

Carp exposed to environmental hyperoxia ($P_{O_2} = 515$ torr) significantly decreased ventilatory frequency compared with normoxia; however, integrated opercular pressure and opercular pressure amplitude did not change over time. Heart rate also did not differ from control values over time or in steady state (see Figures 5 & 6). Takeda (1991) observed significant decreases in frequency of ventilation when carp were exposed to two levels of hyperoxia ($P_{O_2} = 240-330$ torr & 430-490 torr), yet, there were no significant decrease in breathing amplitude. Thus, the trends observed in the present study are consistent with those of Takeda (1991). Peyraud and Sefarty (1964) also found significant decreases in ventilation associated with hyperoxia, but whether the decrease was due to frequency and / or amplitude was not reported. Dejours et al. (1977) exposed a number of teleost and elasmobranch species to hyperoxia ($P_{O_2} = 430-680$ torr). In all instances, whether the species was active or a sluggish bottom dweller, a decrease in frequency of gill ventilation occurred. Although these authors made no direct measurements of changes in ventilatory amplitude, they concluded that a lowering of ventilatory requirement in hyperoxia would
imply a decrease in the overall ventilatory flow rate, which may not be the case as evidenced in the current study. Trout also respond to hyperoxia by decreasing both frequency and amplitude of ventilation (Randall and Jones, 1973; Kinkead and Perry, 1991; Perry and Gilmour, 1996).

It is not surprising that $P_{\text{a}O_2}$ levels increase moderately due to environmental hyperoxia in carp and that $P_{\text{a}CO_2}$ levels increase to a greater extent due to decreased elimination of CO$_2$ associated with the hyperoxia-induced hypoventilation (Takeda, 1991). In the present study, however, although $P_{\text{a}O_2}$ levels increased substantially more than those reported by Takeda (1991), $P_{\text{a}CO_2}$ levels decreased from normoxic values (see Table III). A possible explanation for this paradox is that, in the present study, although ventilatory frequency decreased significantly, ventilatory amplitude and gill blood flow (as indicated by heart rate) may have been adequate to offset any buildup of CO$_2$ in the blood. Whatever the case, there is a definite need for more experiments measuring blood gases before any further conclusions can be made.

The effect of hyperoxia on breathing pattern was modest (see Figure 6) with a marginal decrease in the number of episodes per minute as well as the number of breaths in each episode. Peyraud and Sefarty (1964) also observed that exposing carp to hyperoxia lengthened periods between episodes and reduced the number of breaths in an episode. However, the data did not indicate whether the change in breathing pattern was significantly reduced from normoxia. Takeda (1991) did not characterize the breathing pattern of carp nor did Dejours et al. (1977) for marine species, and the change in breathing patterns for other episodically breathing teleosts have not been documented. However, a normally continuous breather, the rainbow trout, has been demonstrated to go from a continuous breathing pattern to an episodic breathing pattern under hyperoxic conditions (Randall and Jones, 1973).


C. NaCN

External NaCN (5 mg / ml) produced rapid transient effects on cardiorespiratory variables. Instantaneous frequency of ventilation as well as both integrated opercular pressure and opercular pressure amplitude increased significantly (see Table IV). The response began almost immediately upon application of NaCN into the mouth cannula and was complete within one minute. As with hypoxia, no bradycardia was observed with administration of cyanide in this study. Due to the transient response of carp to cyanide, instantaneous, rather than mean, frequency and heart rate were calculated in order to give a more accurate indication of the magnitude of the response of carp to NaCN. The concentration of the bolus injection of cyanide used in this study was greater than that used in other studies (10 µg / kg, Eclancher and Dejours, 1975; 0.02 mg / l, Sawyer and Heath, 1988; 1 mg / ml, Burleson and Smatresk, 1990b). However, concentrations lower than 2 mg / ml elicited no response in the present study. Eclancher and Dejours (1975) reported that carp did not respond to addition of external cyanide into the water flowing over the gills. The concentration of NaCN (10 µg / kg) utilized by Eclancher and Dejours (1975), however, did not produce any response in the present study either. The absence of a bradycardia, although consistent with the hypoxia results, is confounding given the magnitude of the ventilatory response and indicates that strong stimulation of O₂-sensitive chemoreceptors by severe hypoxia does produce a bradycardia.

Trout and bullhead catfish respond to external cyanide by increasing ventilation and decreasing heart rate (Sawyer and Heath, 1988). Burleson and Smatresk (1990b) described large increases in ventilatory amplitude and frequency in catfish exposed to NaCN similar to those observed in carp in the present study. The ventilatory effects of cyanide were less transient in catfish
compared with carp despite the fact that lower concentrations of cyanide (1 mg / ml) were used in the catfish study. This suggests a greater sensitivity of oxygen-sensitive chemoreceptors in catfish than in carp. Catfish also exhibited a transient bradycardia as well as a decrease in dorsal aortic blood pressure (also transient) with waterborne cyanide. Once again, it appears that carp chemoreceptors are less sensitive than those of catfish.

Internal injections of NaCN (0.2 ml of 5 or 10 mg / ml) were performed in the carp possessing indwelling dorsal aortic cannulae. Absolutely no ventilatory or cardiac responses were observed. In contrast to the present study, Eclancher and Dejours (1975) observed that NaCN (5 μl) injected into the ventral aorta of carp produced increases in ventilation and decreases in heart rate similar to those produced by hypoxia. The difference between results of the present study and those of Eclancher and Dejours (1975) could be the result of the transit time required for the blood to circulate back to the gills from the dorsal aorta in the present study. This may have been sufficient time for dilution of the NaCN to occur. An injection of NaCN into the ventral aorta would have been more appropriate in the present study since the blood would have gone directly to the gills where the proposed receptors involved in eliciting bradycardia reside. Injection of much lower concentrations of NaCN into the dorsal aorta of catfish, however, did result in ventilatory responses only (Burleson and Smatresk, 1990b).

D. Carbon Monoxide

Carbon monoxide affects the affinity of hemoglobin for oxygen by competing with O₂ binding sites on the hemoglobin molecule. Therefore, it provides a means for changing O₂ content of the blood without affecting PaO₂. The effects of carbon monoxide on ventilation and heart rate in
fish has only been examined in one other study. CO is relatively insoluble in water. Since the concentration of CO in water cannot be measured easily, the consequences of adding CO to water are usually quantified by their effect on the oxygen content of the blood of the fish. Since blood samples were not available in the present study, CO was bubbled into the water equilibration column until a behavioral response was observed in the fish, and then the level of CO was either maintained or decreased slightly. In the present study, all carp responded to carbon monoxide in a similar manner; all increased ventilation. In five of six animals, breathing became continuous. Despite this visually obvious change, however, due to the variability in ventilation during normoxia (i.e. some carp were breathing episodically and some continuously, probably due to increased stress levels), no significant changes from normoxia were evident (see Figure 10). In trout exposed to 5% CO (Holeton, 1971a), ventilation did increase significantly.

CO did produce significant increases in heart rate in carp. Holeton (1971b) has also observed increases in heart rate in trout larvae exposed to CO. He found that trout larvae could tolerate three hours of 5% CO and that older fish (18 days), had a more pronounced increase in heart rate with exposure to CO.

Trout have a lower $O_2$-hemoglobin affinity than carp (as evidenced by a higher $P_{50}$ in trout); therefore, theoretically CO will outcompete $O_2$ for more binding sites on the hemoglobin molecule in trout than in carp. This may explain why trout showed a significant ventilatory response to 5% CO while carp did not, even with 20% CO. That carp showed a tachycardia similar to that shown by trout, however, suggested that carp do possess receptors either sensitive to CO or to CO-induced changes in oxygen content, independent of changes in partial pressure, but that stimulation of these receptors only elicits a cardiac response.
E. Does \( Pa_{02} \) or \( Ca_{02} \) modulate cardioventilatory responses of carp?

When carp are made hypoxic, they respond with an increase in ventilation frequency and amplitude. Under these conditions, both the oxygen content and partial pressure of the water are reduced. The partial pressure of arterial blood will also be reduced, as will the oxygen content if the partial pressure falls sufficiently that hemoglobin fails to saturate completely. To separate out whether it is the change in partial pressure or content of oxygen that was producing the increase in ventilation, carbon monoxide was administered. This gas competes for binding sites on the hemoglobin molecule and reduces oxygen content of the blood at a constant partial pressure. Although the degree of saturation of carp hemoglobin and the oxygen content of the blood could not be directly evaluated in the present study, behavioral responses, as well as significant increases in heart rate, indicate that the levels of CO used were producing reflex effects by activation of some receptor group. Because CO did not cause a ventilatory response, yet hypoxia sufficient to cause a ventilatory response did not produce a cardiac response until after one hour, the data indicate that changes in the partial pressure of oxygen must be the primary stimulus for receptors involved in respiratory reflexes and not changes in oxygen content. This result is consistent with the work of Glass et al. (1990) and Williams et al. (1992) and refutes the first hypothesis proposed in this thesis.

Earlier studies suggest that all fish possess externally oriented \( O_2 \)-sensitive chemoreceptors in the gills and that stimulation of these receptors produces an increase in ventilation and a fall in heart rate. These studies also suggest that fish possess internally oriented \( O_2 \)-sensitive chemoreceptors as well, and the stimulation of these receptors only elicits a ventilatory response (see Burleson et al., 1992 for review). Finally, adrenal chromaffin cells in the head kidney of teleost fish are also sensitive to hypoxia and release catecholamines that will, amongst other things, stimulate...
heart rate and possibly ventilation (see Randall and Perry, 1992 for review). While similar data are not available for carp, the data collected in the present study can be interpreted by such a scenario. The moderate level of hypoxia ($P_{w_2} = 40$ torr) utilized in the present study produced an increase in ventilation and heart rate as seen by others (Williams et al., 1992). Others have also shown that more severe hypoxia produces a further increase in ventilation and a fall in heart rate (Williams et al., 1992). This could be explained if moderate hypoxia stimulated the internally oriented chemoreceptors only (increased ventilation but no change in heart rate) and the chromaffin cells of the head kidney (small release of catecholamines and increase in heart rate) (see Figure 15). With more severe hypoxia, the externally oriented chemoreceptors are also stimulated. This further increases ventilation, and produces an inhibition of heart rate. Heart rate would only fall at this time if the inhibitory effect of stimulation of the externally oriented chemoreceptors more than offset the effect of the increase in circulating catecholamines (Figure 16). The rise in heart rate without any significant rise in ventilation with administration of CO would be explained if the CO was acting only on the chromaffin cells of the head kidney (Figure 17). The only drawback to this interpretation is that it implies that NaCN, which only stimulated ventilation, must have its site of action on internally oriented chemoreceptors on the gills. The rapid nature of the response to NaCN places this in some doubt, but given the high concentration of the bolus injected through the water in the mouth, it is possible.
Figure 15. A hypothetical model for the effects of mild hypoxia on ventilation. Stimulation of and internal $O_2$-sensitive chemoreceptors will result in increases in ventilation and the release of catecholamines will exert excitatory effects on the heart.
Mild Hypoxia

- External Gill Receptors
  - Increase Ventilation

- Internal Gill Receptors

- Adrenal Gland
  - Circulating Catecholamines
    - Heart
      - Tachycardia
Figure 16. A hypothetical model of the cardioventilatory responses of carp to severe hypoxia. External and internal chemoreceptors will stimulate breathing and, provided the hypoxic stimulus is sufficient, a bradycardia will also be induced. Stress imposed on the fish from exposure to hypoxia will result in release of catecholamines from the adrenal gland in the head kidney which will act on the heart to produce a tachycardia. The stimulation of the external receptors must be greater than the effects exerted by circulating catecholamines in order for an evident bradycardia to occur.
Severe Hypoxia

External Gill Receptors

Bradycardia

Internal Gill Receptors

Increase Ventilation

Adrenal Gland

Circulating Catecholamines

Heart

Tachycardia
Figure 17. A hypothetical model of the cardioventilatory effects of carp in response to carbon monoxide. The potential depression of oxygen content by CO may cause a secretion of catecholamines which may, in turn, cause an increase in heart rate.
Carbon Monoxide

External Gill Receptors

Internal Gill Receptors

Adrenal Gland

Circulating Catecholamines

Heart

Tachycardia

Increase Ventilation
II. Effects of Hyperoxic Hypercapnia

Although the alteration in breathing pattern was slight (decrease in the episodes per minute, increase in breaths per episode, Figure 14) in carp exposed to hyperoxic hypercapnia, there was a significant increase in ventilatory frequency (Figure 13). This is consistent with studies showing that trout (Thomas et al., 1983) and dogfish (Truchot et al., 1980) respond vigorously to hypercapnia even in the presence of hyperoxia.

Are the effects of hypercapnia on ventilation consistent with the hypothesis that \( CO_2 \) acts indirectly through changes in oxygen content of the blood?

While \( CO_2 \) will act to reduce the \( O_2 \) content of the blood through Bohr and Root effects on oxygen-hemoglobin binding, the Root effect is diminished at levels above 5 torr \( P_{CO_2} \). Furthermore, hyperoxia will progressively diminish both Bohr and Root effects and, if severe enough, it will eliminate them completely. Thus, the persistent response of carp to hypercapnia under hyperoxic conditions in the present study refutes the hypothesis that hypercapnia exerts its effect indirectly through changes in oxygen content of the blood. The data suggest there are specific \( CO_2 \) receptors although their location remains obscure. Since there were no blood gas measurements to substantiate the assumption that oxygen content was not changing in the present study, the possibility that \( CO_2 \) exerts its effects on ventilation indirectly through Bohr and Root effects can not be ruled out entirely. Experiments which have measured blood gases in carp indicate that arterial \( P_{CO_2} \) increases linearly with increases in ambient \( CO_2 \); there is also an associated decrease in \( p\text{H}a \) (Takeda, 1991). This author found that despite the decrease in \( p\text{H}a \) (from 7.9 to 7.64 as \( P_{acO_2} \) rose from 3.9 to 11.3 torr), oxygen saturation of hemoglobin in arterial blood remained constant. Weber and Lykkeboe (1978)
found that although CO₂ in itself depresses the oxygen affinity of hemoglobin in carp, the effect was absent below pHa 8.3 due to increases in ATP and GTP. These two studies indicate that Bohr and Root effects are not likely to alter arterial O₂ content under the conditions of the present study.

In conclusion, the unifying hypothesis proposed by Smith and Jones (1982) is not supported by the data collected on carp in the present study. While the data do show that changes in O₂ content independent of changes in O₂ partial pressure will alter heart rate, they also show that hypoxia and hypercapnia produce their ventilatory responses through changes in P₀₂ and P_CO₂ respectively.
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