The role of hypoxia in a fresh water environment: The ecological implications in a piscine predator-prey relationship

By
Tonia Robb

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfilment of the requirements of the degree of

Master of Science

Department of Zoology
University of Manitoba
Winnipeg, Manitoba

The role of hypoxia in a fresh water environment: The ecological implications in a piscine predator-prey relationship

BY

Tonia Robb

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of Master of Science

Abstract

Various factors including environmental variables, metabolic rate, activity levels and recently body size have been found to determine tolerance of hypoxia in teleosts. This study focused on the influence of body size on tolerance of hypoxia and the implications in a predator and prey relationship. Body size limitations are evident in many predator and prey relationships and as a result there is the potential for variation in tolerance to hypoxia. It was predicted that prey would have a greater tolerance of hypoxia than its piscine predator. I suggested the difference in body size would account for this difference as some physiological evidence was found to supports this. Three physiological parameters, expected to increase in response to a reduction on dissolved oxygen, were measured in response to hypoxia and were used to determine tolerance. All of the physiological variables measured suggested a size sensitive relationship in which the smaller prey (fathead minnow, *Pimephales promelas*) was better able to withstand hypoxic conditions than the predatory yellow perch (*Perca flavescens*). Based on this size-sensitive relationship of tolerance to hypoxia, I developed a theoretical model based on the ideal free distribution to determine the distribution of a predator and prey population in response to fluctuating dissolved oxygen levels. I predicted that a greater tolerance of hypoxia by prey would allow them to take advantage of hypoxic habitats intolerable to piscine predators. I also determined the distribution of a predator and prey community found in a marsh of fluctuating dissolve oxygen concentrations to test the assumptions of the model. Some similarities were found between the model and field studies suggesting that limited dissolved oxygen played a role in habitat selection by a predator and prey. The model predicted and the actual distribution suggested that predators were restricted to habitats of greater dissolved oxygen concentrations while prey were found to use moderately hypoxic habitats with a small proportion using hypoxic habitats. I also predicted that physiological exclusion of predators from hypoxic habitats may not be necessary as in mildly hypoxic habitats predators may exhibit behaviours not conducive to feeding such as the search for more normoxic waters. Therefore prey should integrate this into habitat selection and use these areas of moderate hypoxia without the risk of predation. To test this I used the ideal free distribution continuous input model of habitat matching to predict the distribution of prey at two

feeders (habitats) providing equal amounts of food. Feeders were placed at high- and low-risk locations and the response to the predator was measured under normoxic and moderate hypoxic conditions. In moderately hypoxic waters, stressful to predators but not to prey, minnows had a reduced response to the risk of predation when compared to normoxic conditions. The ecological implications of the findings of this thesis suggest that smaller size classes of fish will have an advantage in an environment where dissolved oxygen levels are fluctuating. The creation of temporary hypoxic habitats may provide refuges, intolerable to piscine predators, in which populations of smaller can establish.

Acknowledgements

Thank-you first to my advisor, Dr. M.V. Abrahams, as he has made my work for this thesis most enjoyable. Without his support and encouragement, this thesis and Master's degree would not have been possible. Also thank-you to the rest of my committee members Dr. N. Kenkel, Dr. R.A. MacArthur and K.W. Stewart for their comments and input into this thesis.

I am also grateful to my family for supporting me and putting up with 'science' at the dinner table.

I would like to thank all of my fellow graduate students in the zoology department. In particular I would like to thank my lab-mates throughout my degree, E. Hartman, Dr. M. Koops, and W. Vandersteen, for your input on the model as well as providing all those great suggestions and letting me 'talk ideas' with you.

I would like to especially thank C. Abraham for getting me through the first year of my thesis and K. Ellison for getting me through the second. Both of you brightened my days with great conversation and kept me sane through it all.

I would like to thank the University of Manitoba Field Station at Delta Marsh for their support, accommodations and of course, the great food.

Finally I would like to thank Dr. M. Reid for her encouragement and mentoring, as without it I don't think I would have attempted this degree.

This thesis was funded by the Lubinsky Award to myself and a research grant to M.V. Abrahams from the National Science and Engineering Research Council.

Table of Contents

List of Figures

List of Tables

CHAPTER I

GENERAL INTRODUCTION: THE ROLE OF LIMITED DISSOLVED OXYGEN IN A FRESH WATER ENVIRONMENT

Teleost Response to Hypoxia

In many freshwater environments, fluctuations in dissolved oxygen are known to develop from an increase in the amount of photosynthetic plant material, as well as variations in temperature and pollution (Alabaster & Robertson 1961; Suthers & Gee 1986; Holeton 1980). These changes will often create distinct habitats of limited dissolved oxygen (hypoxia) in an environment that is normally air-saturated (normoxic). Teleosts have adapted to these environmental fluctuations with a variety of responses that enable them to endure the low oxygen environment. Physiological mechanisms that can increase an obligate water breather's tolerance to hypoxia include variations in anaerobic capacity (Holeton 1980; Blažka 1958), oxygen extracting efficiency (Lomholt & Johansen 1979; Galis & Smit 1979), hemoglobin structure and concentration (Fänge 1992; Powers 1980), adjustment of metabolic rate or a combination of these factors (Saint-Paul 1984). The point at which physiological responses no longer compensate for the increased costs of extracting dissolved oxygen occur when activity levels, and hence oxygen requirement, increases (Davenport & Sayer 1993; Claireaux & Dutil 1992; Peterson 1990). This initial increase in activity, as well as vertical migration to the surface, is a response to seek more oxygenated habitats (Gee *et al.* 1978; Petrosky & Magnuson 1973). Subsequent to a further decrease in environmental oxygen, individuals will make use of aquatic surface respiration (ASR) and decrease activity levels to conserve energy for life sustaining processes (Gee *et al.* 1978). As a result of the costs of living in a hypoxic environment, teleost distribution should reflect critical oxygen levels along with other parameters that affect habitat quality (Brazner & Beals 1997; Breitburg *et al.* 1997; Breitburg 1992; Coutant 1985; Meffe 1984)

Physiological Compensation of Hypoxia

As a respiratory medium, water has a low oxygen solubility, high density and high viscosity. These properties of water make it difficult for fish to acquire oxygen even from an oxygen rich environment (Holeton 1980). In response, they have developed a countercurrent gas exchange system at the gill lamellae (Fig. 1.1). The maximum efficiency of oxygen uptake or a complete overlap of the partial pressures of water and blood along the contact length will depend on the ratio of diffusion conductance (G_{diff})

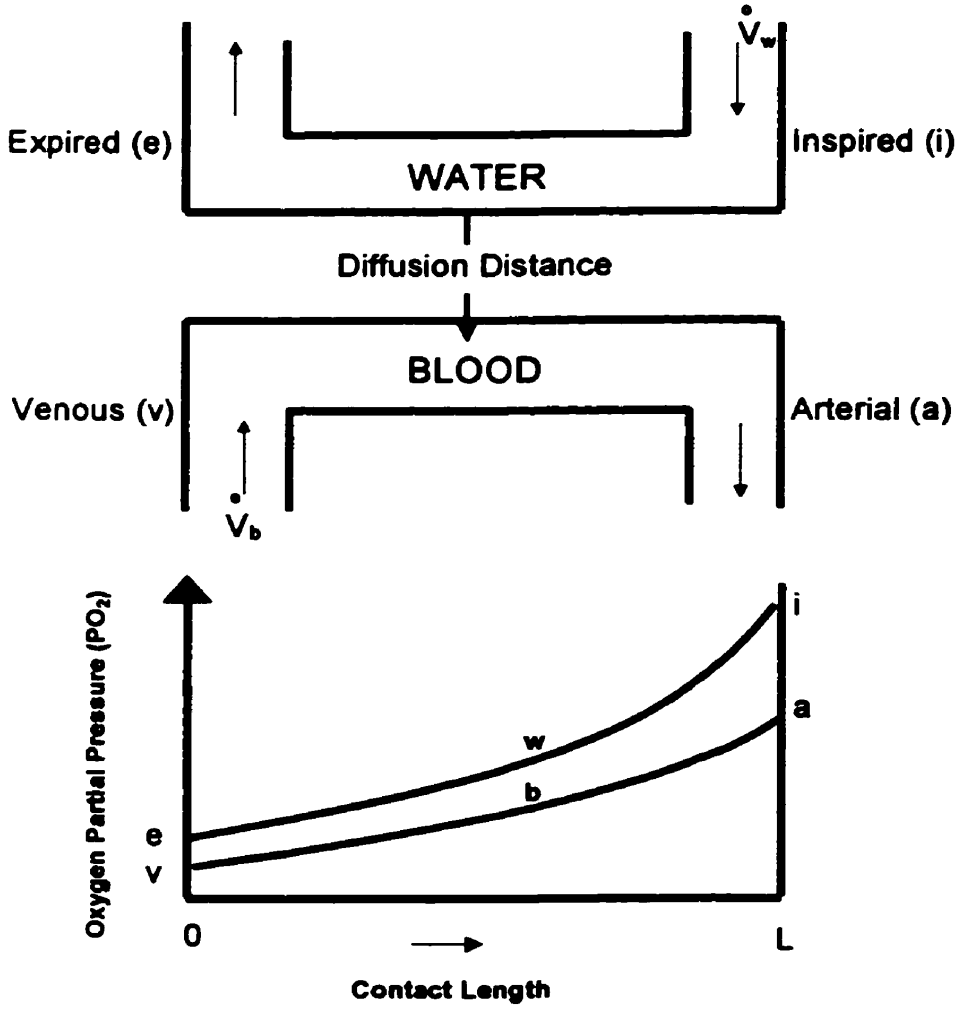


Figure 1.1. Diagram to represent countercurrent gas exchange at the gill and the relationship of oxygen partial pressures along the contact length of water (w) and blood (b) at the gill surface. Oxygen at the gill/blood surface diffuses down its partial pressure gradient into the gill capillaries (large arrow) over the length of the gill/blood interface (L). Flow rates of blood and water are defined by $\dot{V}_b$ and $\dot{V}_w$ respectively. (Adapted from Piiper & Scheid 1984)

and ventilation conductance (G_{vent}), where conductance is defined as the transfer rate per partial pressure (Piiper & Scheid 1984).

Three component processes are involved in gas exchange and can be modified to maintain adequate oxygen extraction (and arterial partial pressures) at low dissolved oxygen levels (Nikinmaa & Salama 1998; Piiper & Scheid 1984; Johansen 1982; Lomholt & Johansen 1979). First, the ventilation frequency and depth (amplitude) will together determine the minute ventilation (ml/min) or rate of water flow ($\dot{V}_w$) and consequently the amount of oxygen flowing over the gills. A second component of gas exchange is the rate of oxygen diffusion, which is defined primarily by Fick's Law. The transfer rate is the product of Krogh's diffusion constant, the ratio of the respiratory surface area to the water-blood barrier thickness, and the water/blood PO_2 gradient. Perfusion or gill blood flow, a third fundamental process, is a function of the rate of blood flow ($\dot{V}_b$). Hemoglobin-oxygen affinity and concentration of hemoglobin is also regulated in response to varying environmental dissolved oxygen levels as well as the relative demand of the individual (Nikinmaa & Salama 1998). For the purpose of this study, the physiological parameters involved in oxygen extraction from the environment as well as blood oxygen affinity and oxygen-carrying capacity will be used as measures of hypoxia tolerance.

Although gas exchange occurs mainly at the gill interface, skin may also play a role as an organ of gas exchange (Feder & Burggren 1985). The uptake of oxygen by the skin varies considerably for teleosts, where under normoxic conditions marine teleosts are able to extract as much as 30-40% of the total oxygen consumed via the skin (Feder & Burggren 1985). However, in six fresh water species studied, this percentage was between 10 and 20% (Nonnotte 1981). Under normoxic conditions, cutaneous gas exchange accounted only for a small portion of the total oxygen extraction, and this amount is most likely accounted for by skin oxygen consumption (Nonnotte & Kirsch 1978). For some species, such as the black bullhead, *Ictalurus melas*, however, cutaneous gas exchange greatly exceeds cutaneous oxygen consumption indicating this excess is added to the total gas exchange for other tissues (Nonnotte 1981). The measurement of cutaneous gas exchange would add an interesting component to teleost

response to hypoxia since it's scaling effects would be obvious (see Steffensen *et al.* 1981).

1. Gill Ventilation

As one of the key components in gas exchange, gill ventilation rates of fish are altered to maintain the amount of oxygen at the gills needed to saturate the blood to equilibrium (Randall & Shelton 1963). This is a receptor-mediated process which increases gill ventilation frequency in response to a decrease in arterial blood PO_2 (Randall 1982). The increase in breathing rate and depth of each breath will be associated with an increase in ventilation volume (Holeton & Randall 1967; Randall & Shelton 1963). However an increase in ventilation rate does not always preclude an increase in depth of breath and a subsequent rise in ventilation volume (Smith & Jones 1982). Maintaining a high ventilation frequency during acute hypoxic events increases the erythrocytic and the plasma pH, which will in turn increase the hemoglobin-oxygen affinity (Nikinmaa & Salama 1998). Therefore the elevation of ventilation volume in response to hypoxia has two advantages. It provides a greater amount of oxygen flowing over the gill and maintains the oxygen in the tissues by means of increasing the amount of oxygen dissolved in the plasma through the preservation of oxygenated hemoglobin (Nikinmaa & Salama 1998; Smith & Jones 1982; Johansen 1982).

2. Gill Surface Area and Water-Blood Barrier Thickness

As previously mentioned the rate of gas exchange will be determined in part by the diffusion distance as well as the total gill surface area. Therefore a large gill surface area and relatively small diffusive distance will serve to increase the overall oxygen transfer (Hughes 1984; Randall *et al.* 1967). To determine total gill surface area many studies have demonstrated that the gill secondary lamellae surface area correlates well with the total gill surface area (Palzenberger & Pohla 1992; Hughes & Morgan 1973). In comparing the gill surface areas among teleosts there is a large degree of phenotypic variation. The environmental oxygen concentration a teleost inhabits has been found to account for most of the variation, with larger gill surface areas occurring in individuals subject to chronic hypoxia (Chapman *et al.* 1999; Chapman & Liem 1995; Palzenberger & Pohla 1992). In addition to environment, activity levels play a role in determining the gill surface area in freshwater fish (Palzenberger & Pohla 1992; Hughes & Morgan

1973). More active fish have closer spacing of gill filaments and a larger total secondary lamellae area (Hughes 1984). Total gill surface area has also been found to correlate well with gill ventilation abilities, permeability of the secondary lamellae, and oxygen capacity of the blood (Palzenberger & Pohla 1992).

3. Gill perfusion

At rest the gills are not fully perfused in normoxic waters and increasing perfusion of the gills will enhance oxygen extraction by increasing the functional gill surface area (Johansen 1982; Booth 1979). Hypoxia stimulates a myogenic vasoconstriction response thereby increasing afferent lamellar pressure causing perfusion of any unperfused gill secondary lamellae (Johansen 1982). The importance of the ratio of ventilation conductance (G_{vent}) to perfusion conductance (G_{perf}) becomes apparent when fish are subjected to stress such as hypoxia (Fig. 1; Johansen 1982). As this ratio increases the oxygen extracting efficiency will decrease as the difference between P_a and P_e becomes negative (Johansen 1982). Subsequently blood flow to the gills must coincide with the rate of ventilation during hypoxia to obtain an optimal range or efficiency in gill function (Johansen 1982; Randall 1982).

4. Blood Properties

The number of erythrocytes in the blood, as well as the concentration and structure of hemoglobin within the erythrocyte will influence the oxygen-carrying capacity and the blood oxygen affinity (Nikinmaa & Salama 1998; Randall 1982). The concentration of erythrocytes in the blood is defined by the hematocrit (the percentage of packed red blood cells in the blood) (Gallaugher & Farrell 1998; Fänge 1992). Under normoxic conditions, the hematocrit of a teleost ranges from 20 to 40% (Fänge 1992). The initial response to hypoxia is to increase both the hematocrit and hemoglobin concentration in an attempt to increase the total oxygen carrying capacity of the blood (Claireaux & Dutil 1992; Peterson 1990; Powers 1980). Under normal conditions, erythrocytes are continuously entering the circulatory system and worn out ones are destroyed at the same rate by macrophages (Fänge 1992). However, under stress conditions such as environmental hypoxia, two main processes can elevate the number of red blood cells. Hemopoiesis, production of cells and fluid of the blood, is primarily a function of the spleen and is stimulated by factors such as blood oxygen and carbon dioxide levels, nutritional

substances, photoperiod, temperature and metabolites (Fänge 1992; Tun & Houston 1986; Yamamoto *et al.* 1980). The production and release of red blood cells usually occurs over a period of several days, with the first increase seen at one to two days following chronic exposure to a reduction in dissolved oxygen (Nikinmaa 1990). However, the spleen can also be stimulated for immediate release of red blood cells in response to short term stress, such as exercise and oxygen depletion (Stevens 1968). Alternatively, the concentration of red blood cells can be increased from a water shift or plasma skimming in the gill vasculature (Yamamoto *et al.* 1980). This is also a more immediate response and is most likely to occur during the initial responses to hypoxia (Nikinmaa 1990).

Interspecific differences in hematocrit, hemoglobin concentration and oxygen affinity have often been linked to differences in the environment and mode of life (Powers 1980; Larsson *et al.* 1976). Typically more active species as well as those species found in environments of chronic hypoxia exhibit the highest hematological values (Gallaugher & Farrell 1998; Powers 1980; Larsson *et al.* 1976). In a low oxygen environment, species characteristically have higher oxygen affinities and within a changing environment species possess a wide spectrum of blood parameters (Powers 1980).

Behavioural Response to Hypoxia

Four main stages of response to progressive hypoxia have been identified in fresh water obligate water breathing teleosts (Gee *et al.* 1978). First, when dissolved oxygen levels are too low for physiological mechanisms to compensate, there is an initial increase in activity levels (Suthers & Gee 1986; Weber & Kramer 1983; Gee *et al.* 1978; Petrosky & Magnuson 1973; Doudoroff & Shumway 1970). This response most likely serves to locate more normoxic environments (Gee *et al.* 1978). This habitat displacement can be horizontal to areas of greater oxygen supply as well upward in the water column to avoid benthic depleted oxygen areas (Klinger *et al.* 1982; Petrosky & Magnuson 1973). Despite the advantages in habitat shift there are many costs associated with this behaviour. Physiological costs of hypoxia, as described above, will reduce the amount of energy available for locomotion as well as growth and reproduction (van Dam & Pauly 1995; Petersen & Petersen 1990; Kramer & Mehegan 1981; Brett & Groves 1979). In

addition, feeding rates are decreased, associated with the reduced food availability as well as the reduction in energy available for foraging (Weber & Kramer 1983; Petit 1973; Doudoroff & Shumway 1970). Increased movement will often increase encounter rates or detection by visual predators thus increasing the risk of predation (Werner & Anholt 1993). In addition vertical migration to more oxygenated surface waters will also increase the risk of aerial predation (Kramer *et al.* 1983). Habitat displacement, as a result of the need to breathe, often results in fish moving to open water habitats where they are more likely to be captured (Suthers & Gee 1986; Werner *et al.* 1983; Wolf & Kramer 1987).

Access to surface waters is also an important aspect in behavioural compensation to lowered dissolved oxygen levels. The use of aquatic surface respiration (ASR), a second stage in response to hypoxia, will usually occur near critical dissolved oxygen levels (Klinger *et al.* 1982; Kramer & Mehegan 1981; Gee *et al.* 1978). This behaviour involves the use of the oxygen rich surface film to provide additional energy for the increased activity levels as well as other activities such as foraging (Kramer & Mehegan 1981). Thirdly, at near lethal dissolved oxygen levels, individuals decrease their activity levels (Gee *et al.* 1978; Petit 1973). This behaviour will increase energy allocation to ventilation and other life sustaining activities (Boese 1988). Finally, when physiological mechanisms can no longer support life, the fish will lose equilibrium and at this dissolved oxygen concentration death will eventually occur (Gee *et al.* 1978).

The critical dissolved oxygen levels at which we see habitat displacement and lethal levels both correspond to the physiological tolerance of the fish (Petersen & Petersen 1990). Therefore it would be beneficial to have a more efficient oxygen extracting mechanisms in order to exploit hypoxic areas lethal to other individuals. Use of more stressful habitats may be an important aspect of a predator-prey relationship in which prey can avoid predators that are unable to tolerate those conditions (Poulin *et al.* 1987). Under moderately stressful conditions limited dissolved oxygen will also influence predators, and avoidance or escape may not be necessary (Rahel & Nutzman 1994; Pihl *et al.* 1992; Poulin *et al.* 1987).

Small Versus Large: The Role of Body Size in a Fluctuating Environment

Characteristics of an individual such as physiological, morphological and life history traits, as well as ecological factors such as distribution, are often related to the body size of an individual (Schmidt-Nielsen 1984; Calder 1984). The relationship of most of these traits is determined by the equation $Y=aw^b$, where w is the body size, y is the characteristic or variable predicted to change with body size and b is the allometric exponent or slope. The size of the slope is the important factor in determining the costs and benefits of size and possibly abilities to adapt to an environment. In applying this relationship to feeding energetics, survival and reproduction, there are often greater costs associated with being a smaller individual (Peters 1983; Brown & Maurer 1986). Smaller individuals are also more likely to be preyed upon, adding costs of predation risk to decision-making (Werner & Gilliam 1984). Despite these disadvantages, smaller individuals have been able to exploit resources not available to larger individuals. This is accomplished through the use of a large number of small food organisms as well as the use of spatially complex or smaller enclosed habitats as areas of refuge. Alternatively, these refuges could also be areas where smaller prey might be better adapted, in physiology or morphology, to the environment (Wright & Shapiro 1990). A greater tolerance to changes in environmental dissolved oxygen may provide smaller prey a potential refuge from larger piscine predators.

Physiological Parameters- The Role of Body Size

Numerous studies have subjected various species of teleosts to varying levels of hypoxia in order to measure both short- and long-term physiological responses (see reviews by Gallaugher & Farrell 1998; Holeton 1980; Powers 1980; Doudoroff & Shumway 1970). An alternative explanation of variability in tolerance to dissolved oxygen to the already established explanation of mode of life, metabolic requirement and environment is based on body size considerations. In terms of physiological mechanisms that will increase the oxygen extraction efficiency in oxygen poor environments, there is some evidence to support size dependence. First, the size of the individual will play a role in the cost of increasing the ventilation in response to hypoxia (Jones 1971). Increasing the gill ventilation frequency is an expensive measure and this expense increases exponentially as an individual gets larger (Boutilier *et al.* 1988; Saint-Paul 1984; Smith & Jones 1982;

Jones 1971). For example, two species of freshwater fish, Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*), exhibit decreases in ventilation volume with increasing body mass under resting normoxic conditions (Yamamoto 1992; Yamamoto 1991). Within each species, mass dependent variation in ventilation volume enabled these animals to achieve similar rates of gill oxygen consumption. This suggests that, for at least these species, an increase in ventilation volume for larger individuals would have offset oxygen use (by increasing gill oxygen consumption) indicating the greater expense. There are some examples of the influence of body size in intraspecific variation of hematological (blood) parameters. Under normal conditions the cellular blood parameters of rainbow trout, *Oncorhynchus mykiss*, are influenced by body size (Lowe-Jinde & Niimi 1983). Zanuy & Carrillo (1985) also found a lower hematocrit in larger and older sea bass, *Dicentrarchus labrax*. To make interspecific comparisons, Fish (1956) examined hypoxia tolerance in species from a fresh water lake and found that one of the largest fresh water fish, the Nile perch (*Lates niloticus*), showed the lowest tolerance (measure of hemoglobin-oxygen affinity) to decreased oxygen levels. Within a species, body size is the major determinant of gill surface area (Palzenberger & Pohla 1992; Santos *et al.* 1994; Hughes *et al.* 1986; Saint-Paul 1984; Muir 1969). This well-established allometric scaling relationship illustrates gill surface area increases allometrically with body mass and the exponent is approximately 0.8 (Palzenberger & Pohla 1992; Santos *et al.* 1994; Hughes *et al.* 1986; Saint-Paul 1984; Muir 1969).

However, there are some physiological mechanisms that do not appear to be related to body size. The blood-water barrier thickness does not vary with body mass as activity best defines this trait with the shortest diffusion distance being found in more active fish (Hughes *et al.* 1986; Hughes & Morgan 1973). Cardiac output, related to perfusion, increases with body weight (Jones 1971). However, the costs associated with cardiac work are proportional to that of smaller individuals. Therefore, we would not expect perfusion to play a large role in size related differences in oxygen extracting abilities.

Influence of Limited Dissolved Oxygen on a Predator-Prey Model

Body size limitations are most evident in many predator and prey relationships where predators must be larger than their prey. As a result of this large difference in size, there

is a potential for variation in tolerance to a hypoxic environment. In an environment of limited dissolved oxygen, when oxygen supply to the tissue is compromised a series of physiological compensations are initiated (Gallaugher & Farrell 1998; Johansen 1982; Randall 1982; Powers 1980). It has been suggested, and indeed much evidence supports the contention, that larger piscivorous teleosts must initiate physiological compensation at a greater dissolved oxygen concentration than the smaller teleosts they prey upon (Fox & Keast 1990; Tonn & Paszkowski 1987; Tonn & Paszkowski 1986; Zanuy & Carrillo 1985; Lowe-Jinde & Niimi 1983; Klinger *et al.* 1982). In maintaining sufficient oxygen flow to the tissue for life-sustaining activities, as well as an adequate activity level to search for more favourable areas, there are several physiological costs (see physiological responses of hypoxia in this chapter). Figure 1.2 depicts this relationship of cost associated with maintaining equilibrium for a piscine predator and prey with the reduction in environmental dissolved oxygen concentration. The dissolved oxygen concentration at which physiological mechanisms are inadequate to cope with the stress or costs have reached a maximum (dotted line), define the critical dissolved oxygen concentrations for predator ($pred_c$) and prey ($prey_c$). For the purpose of this study these costs are assumed to be proportional for predator and prey (represented by the parallel lines).

I have predicted that piscine predators will, as a result of their size, initiate a response to the reduction in environmental dissolved oxygen at greater levels than their prey thus reaching a critical dissolved oxygen level before their prey (Fig. 1.2). A subsequent decrease in dissolved oxygen will eventually result in death of the predator (Davenport & Sayer 1993; Gee *et al.* 1978). Therefore it is assumed that predators will avoid habitats where dissolved oxygen concentrations are lethal. Assuming prey have critical levels lower than their predators, a refuge will be created (Fig. 1.2). Under ideal conditions the region between critical levels for prey and predator, would most likely define this refuge. However, brief visits or hypoxic dives have been documented by predators making trips into hypoxic habitats thought to be well below critical levels

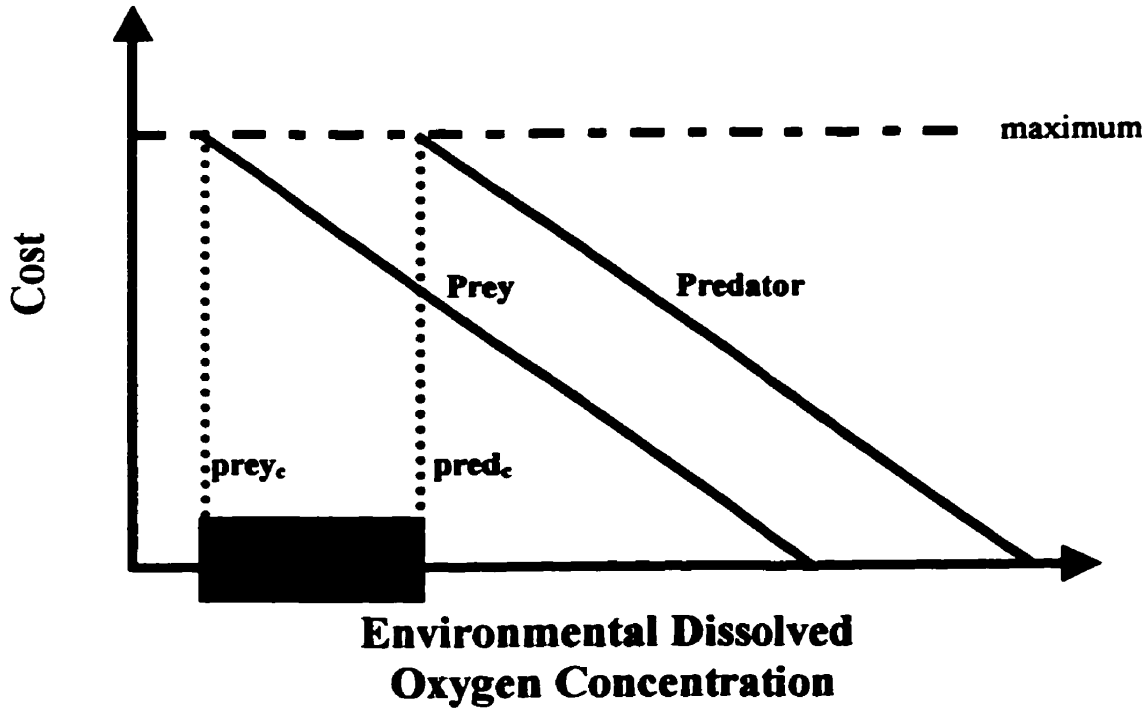


Figure 1.2. The predicted relationship of a piscine predator and prey costs (associated with maintaining equilibrium) and the environmental dissolved oxygen concentration. At low dissolved oxygen concentrations, critical levels (prey$_c$ and pred$_c$) will be reached based on the assumption of an allometric or size-sensitive relationship. This relationship suggests the maximum (dotted line) cost is reached at greater dissolved oxygen levels for predators than that for the smaller prey. As a result, a predicted temporary refuge (the shaded region) for prey exists in which dissolved oxygen levels are lethal or have a greater cost for the predator.

(Rahel & Nutzman 1994; Pihl *et al.* 1992). In addition, the upper limit of the refuge may be greater than the predator critical dissolved oxygen level. Prey choice of habitat will depend on the maximum net benefits or where fitness outweighs the costs (Fretwell & Lucas 1970). A major cost for prey is the risk of predation as even if this does not end in death, it often initiates a series of antipredator behaviours such as habitat shift (see Sih 1987 for review; Werner *et al.* 1983). However, the reduction of feeding is a common behavioural response to stressful levels of hypoxia (Weber & Kramer 1983; Petit 1973; Doudoroff & Shumway 1970). Therefore at critical dissolved oxygen levels for the predator, interest in prey may be overlooked in the search for oxygen rich waters. In addition there is a reduction in energy available for locomotion other than the search for more favourable habitats (van Dam & Pauly 1995; Brett & Groves 1979). Thus the efficiency of the predator in obtaining prey may be compromised when inhabiting hypoxic habitats. At near critical levels for the predator, risk of predation will likely be low providing prey with a greater net benefit.

Objectives of the Study

To determine if there is difference in tolerance of hypoxia between a predator and prey, chapter two of this thesis focused on two species of fish, the fathead minnow, *Pimephales promelas*, the prey species and yellow perch, *Perca flavescens*, the predator. The perch, much larger than the fathead minnow, was predicted to have a lower tolerance to limited dissolved oxygen. As indices of physiological tolerance I used two blood parameters, hemoglobin concentration and hematocrit, as well as ventilation frequency. Although I focus on the physiological mechanism to measure tolerance, the importance of this chapter was to determine the difference between these two species and its ecological relevance in a predator and prey relationship. I have predicted that a greater tolerance of hypoxia by the minnow would allow them to take advantage of habitats subject to hypoxic events that are intolerable or costly to predators.

To test this prediction I first developed a computer model based on the ideal free distribution (IFD) with the main assumption being size-sensitive tolerance to hypoxia. The model predicted predator and prey habitat selection in a simulated environment of changing dissolved oxygen levels, predation pressure and food availability. The model output and a subsequent field study described in chapter three of this thesis illustrate not

only the actual distributions of a predator and prey populations in an environment with fluctuating dissolved oxygen levels but predict underlying mechanisms driving this distribution. The field study comprised mapping out the spatial and temporal distribution of a predator and prey community in an environment of changing environmental dissolved oxygen. The predator population was represented by yellow perch. The fathead minnow and spottail shiner (*Notropis hudsonius*) represented the prey population found at my study site in Delta marsh, Manitoba.

In addition to predators being restricted from hypoxic areas, I also predicted that moderate hypoxia will inhibit the effectiveness of a predator to capture prey. In chapter four I presented the comparison of the perceived risk of predation by the minnows under hypoxic and normoxic conditions. Again I used the IFD model of maximising net benefits to predict the distribution of prey at two feeders (habitats) providing equal amounts of food with one of the habitats being risky or adjacent to a predator (yellow perch). It was expected, under normoxic conditions, the fathead minnow would avoid the risky feeder in favour of the low risk habitat. However with the reduction of dissolved oxygen to levels stressful for the perch but not the minnows, avoidance of the risky habitat would not be as prominent. This would require the minnows to detect the perch indifference towards the minnows as a result of the costs associated with maintaining perch equilibrium under hypoxic conditions.

Literature Cited

Alabaster, J.S. & Robertson, K.G. 1961. The effect of diurnal changes in temperature, dissolved oxygen and illumination on the behaviour of Roach (*Rutilis rutilis* (L.)), Bream (*Abramis brama* (L.)), and Perch (*Perca fluviatilis* (L.)). Anim. Behav. **9**:187-192.

Blažka, P. 1958. The anaerobic metabolism of fish. Physiol. Zool. **31**: 117-128.

Boese, B.L. 1988. Hypoxia-induced respiratory changes in English sole (*Parophrys vetulus* Girard). Comp. Biochem. Physiol. **89A**: 257-260.

Booth, J.H. 1979. The effect of oxygen supply, epinephrine and acetycholine on the distribution of blood flow in trout gills. J. Exp. Biol. **83**: 31-39.

Boutilier, R.G., Dobson, G., Hoeger, U. & Randall, D.J. 1988. Acute exposure to graded levels of hypoxia in rainbow trout (*Salmo gairdneri*): metabolic and respiratory adaptations. Resp. Physiol. **71**: 69-82.

Brazner, J.C. & Beals, E.W. 1997. Patterns in fish assemblages from coastal wetland and beach habitats in Green Bay, Lake Michigan: a multivariate analysis of abiotic and biotic forcing factors. Can. J. Fish Aquat. Sci. **54**: 1743-1761.

Breitburg, D.L. 1992. Episodic hypoxia in Chesapeake Bay: Interacting effects of recruitment, behavior, and physical disturbance. Ecol. Mono. **62(4)**: 525-546.

Breitburg, D.L., Loher, T., Pacey, C.A. & Gerstein, A. 1997. Varying effects of low dissolved oxygen on trophic interactions in an estuarine food web. Ecol. Mono. **67(4)**: 489-507.

Brett, J.R. & Groves, T.D.D. 1979. Physiological energetics. IN Fish Physiology Volume 8: Bioenergetics and Growth. eds W.S. Hoar, D.J. Randall & J.R. Brett. Academic Press, New York. pp. 279-352.

Brown, J.H. & Maurer, B.A. 1986. Body size, ecological dominance and Cope's rule. Nature **324**: 248-250.

Calder, W.A. 1984. Size, function, and life history. Harvard University Press, Cambridge, Mass. 431p

Chapman, L.J., Chapman, C.A., Brazeau, D.A., McLaughlin, B., & Jordan, M. 1999. Papyrus swamps, hypoxia and faunal diversification: variation among populations of *Barbus neumayeri*. J. Fish Biol. **54**: 310-327.

Chapman, L.J. & Liem, K.F. 1995. Papyrus swamps and the respiratory ecology of *Barbus neumayeri*. Env. Biol. Fishes **44**:193-197.

Claireaux, G. & Dutil, J-D. 1992. Physiological response of the Atlantic cod (*Gadus Morhua*) to hypoxia at various environmental salinities. J. Exp. Biol. **163**: 97-118.

Coutant, C.C. 1985. Striped bass, temperature and dissolved oxygen: A speculative hypothesis for environmental risk. Trans. Am. Fish. Soc. **114**:31-61.

Davenport, J. & Sayer, M.D.J. 1993. Physiological determinants of distribution in fish. J. Fish Biol. **43(Supplement A)**: 121-145.

Doudoroff, P. & Shumway, D.L. 1970. Dissolved oxygen requirements of freshwater fishes. Food and Agriculture Organization of the United Nations. Technical Paper 86. 291p.

Feder, M.E. & Burggren, W.W. 1985. Cutaneous gas exchange in vertebrates: Design, patterns, control and implications. Biol. Rev. **60**:1-45.

Fänge, R. 1992. Fish blood cells. IN Fish Physiology Volume 7B. eds W.S. Hoar, D.J. Randall and J.R. Brett. Academic Press, New York. pp. 1-53.

Fish, G.R. 1956. Some aspects of the respiration of six species of fish from Uganda. J Exp. Biol. **33**: 186-195.

Fox, M.G. & Keast, A. 1990. Effects of winterkill on population structure, body size and prey consumption patterns of pumpkinseed in isolated beaver ponds. Can. J. Zool. **68**: 2489-2498.

Fretwell, S.D. & Lucas Jr., H.L. 1970. On territorial behavior and other factors influencing habitat distribution in birds. Acta. Biotheor. **19**: 16-36.

Gallaugher, P. & Farrell, A.P.1998. Hematocrit and blood oxygen-carrying capacity. IN Fish Physiology Volume 17: Fish Respiration. eds S.F. Perry & B.L. Tufts. Academic Press. pp 185-227.

Galis, F. & Smit, H. 1979. Hypoxia tolerance of two closely related Haplochromis species (Pisces: Cichlidae): *Haplochromis elegans* Trewavas, 1933 and *H. angustifrons* Boulenger, 1914. Comp. Biochem. Physiol. **64A**: 137-139.

Gee, J.H., Tallman, R.F. & Smart, H.J. 1978. Reactions of some great plains fishes to progressive hypoxia. Can. J. Zool. **56**:1962-1966.

Holeton, G.F. 1980. Oxygen as an environmental factor of fishes. IN Environmental Physiology of Fishes. eds M.A. Ali. Plenum, New York. pp. 7-32.

Holeton, G.F. & Randall, D.J. 1967. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gill of rainbow trout. J. Exp. Biol. **46**: 317-327.

Hughes, G.M. 1984. Scaling of respiratory areas in relation to oxygen consumption of vertebrates. Experientia **40(6)**: 519-652.

Hughes, G.M. & Morgan, M. 1973. The structure of fish gills in relation to their respiratory function. Biol. Rev. **48**: 419-475.

Hughes, G.M., Perry, S.F. & Piiper, J. 1986. Morphometry of the gills of the elasmobranch *Scyliorhinus stellaris* in relation to body size. J. Exp. Biol. **121**: 27-42.

Johansen, K. 1982. Respiratory gas exchange of vertebrate gills. IN Gills. eds. D.F. Houlihan, J.C. Rankin & T.J. Shuttleworth. Cambridge University Press, Cambridge. pp 99-128.

Jones, D.R. 1971. Theoretical analysis of factors which may limit the maximum oxygen uptake of fish: The oxygen cost of the cardiac and branchial pumps. J. Theor. Biol. **32**: 341-349.

Klinger, S.A., Magnuson, J.J., & Gallepp, G.W. 1982. Survival mechanisms of the central mudminnow (*Umbra limi*), fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) for low oxygen in winter. Env. Biol. Fish **7(2)**: 113-120.

Kramer, D.L., Manley, D. & Bourgeois, R. 1983. The effect of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. Can. J. Zool. **61**: 653-665.

Kramer, D.L. & Mehegan, J.P. 1981. Aquatic surface respiration, an adaptive response to hypoxia in the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). Env. Biol. Fish **6(3/4)**: 299-313.

Larsson, Å., Johansson-Sjöbeck, M. & Fänge, R. 1976. Comparative study of some haematological and biochemical blood parameters in fishes from the Skagerrak. J. Fish. Biol. **9**: 425-440.

Lomholt J.P. & Johansen, K. 1979. Hypoxia acclimation in carp-How it affects oxygen uptake, ventilation, and oxygen extraction from water. Physiol. Zool. **52**: 38-49.

Lowe-Jinde, L. & Niimi, A.J. 1983. Influence of sampling on the interpretation of haematological measurements of rainbow trout, *Salmo gairdneri*. Can. J. Zool. **61**: 396-402.

Meffe, G.K. 1984. Effects of abiotic disturbance on coexistence of predator-prey fish species. Ecology **65(5)**: 1525-1534.

Muir, B.S. 1969. Gill dimensions as a function of fish size. J. Fish. Res. Bd. Canada **26**: 165-170.

Nikinmaa, M. 1990. Vertebrate Red Blood Cells: adaptations of function to respiratory requirements. Zoolphysiology Volume 28. pp 262.

Nikinmaa, M. & Salama, A. 1998. Oxygen transport in fish. IN Fish Physiology Volume 17: Fish Respiration. eds S.F. Perry & B.L. Tufts. Academic Press. pp.141-184.

Nonnotte, G. 1981. Cutaneous respiration in six freshwater teleosts. Comp. Biochem. Physiol. **70A**: 541-543.

Nonnotte, G. & Kirsch, R. 1978. Cutaneous respiration in seven sea-water teleosts. Resp. Physiol. **35**:111-118.

Palzenberger, M. & Pohla, H. 1992. Gill surface area of water-breathing freshwater fish. Rev. Fish Biol. Fish. **2**:187-216.

Peters, R.H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge. 329p.

Petersen, J.K. & Petersen, G.I. 1990. Tolerance, behaviour and oxygen consumption in the sand goby, *Pomatoschistus minutus* (Pallas), exposed to hypoxia. J. Fish Biol. **37**: 921-933.

Peterson, M.S. 1990. Hypoxia-induced physiological changes in two mangrove swamp fishes: Sheepshead minnow, *Cyprinodon variegatus* Lacepede and sailfin molly, *Poecilia Latipinna* (Lesueur). Comp. Biochem. Physiol. **97A**: 17-21.

Petit, G.D. 1973. Effects of dissolved oxygen and behavior of selected fishes of western lake Erie. Bull. Ohio. Biol. Surv. **4(4)**: 1-77.

Petrosky, B.R. & Magnuson, J.J. 1973. Behavioral responses Of Northern Pike, Yellow Perch and Bluegill to oxygen concentrations under simulated winterkill conditions. Copeia **1**:124-133.

Pihl, L., S.P. Baden, Diaz, R.J., & Schaffner, L.C. 1992. Hypoxia-induced structural changes in the diet of bottom-feeding fish and Crustacea. Marine Biology **112**: 349-361.

Piiper, J. & Scheid, P. 1984. Model analysis of gas transfer in fish gills. IN Fish Physiology Vol. 10A: Anatomy, Gas Transfer and Acid-Base Regulation. eds W.S. Hoar & D.J. Randall. Academic Press, New York. pp. 229-262.

Piiper, J. & Scheid, P. 1982. Physical principles of respiratory gas exchange in fish gills. IN Gills. eds. D.F. Houlihan, J.C. Rankin & T.J. Shuttleworth. Cambridge University Press, Cambridge. pp 45-61.

Poulin, R., Wolf, N.G., & Kramer, D.L. 1987. The effect of hypoxia on the vulnerability of guppies (*Poecilia reticulata*, Poeciliidae) to an aquatic predator (*Astronotus ocellatus*, Cichlidae). Env. Biol. Fish **20(4)**: 285-292.

Powers, D.A. 1980. Molecular ecology of teleost fish hemoglobins: Strategies for adapting to changing environments. Amer. Zool. **20**: 139-162.

Rahel, F.J. & Nutzman, J.W. 1994. Foraging in a lethal environment: Fish predation in hypoxic waters of a stratified lake. Ecology **75(5)**: 1246-1253.

Randall, D. 1982. The control of respiration and circulation in fish during exercise and hypoxia. J. Exp. Biol. **100**: 275-288.

Randall, D.J. & Shelton, G. 1963. The effects of changes in environmental gas concentrations on the breathing and heart rate of a teleost fish. Comp. Biochem. Physiol. **9**: 229-239.

Randall, D.J., Holeton, G.F. & Stevens, E.D. 1967. The exchange of oxygen and carbon dioxide across the gills of rainbow trout. J. Exp. Biol. **6**: 339-348.

Saint-Paul, U. 1984. Physiological adaptation to hypoxia of a neotropical characoid fish *Colossoma macropomum*, Serrasalmidae. Env. Biol. Fish. **11(1)**: 53-62.

Santos, C.T.C., Fernandes, M.N. & Severi, W. 1994. Respiratory gill surface area of a facultative air-breathing loricariid fish, *Rhinelepis strigosa*. Can. J. Zool. **72**: 2009-2015.

Schmidt-Nielsen, K. 1984. Scaling, why is animal size so important. Cambridge University Press, Cambridge. 241p

Sih, A. 1987. Predators and prey lifestyles: An evolutionary and ecological overview. IN Predation: direct and indirect impacts on aquatic communities. eds. W.C. Kerfoot & A. Sih. University Press of New England, Hanover, New Hampshire. pp. 203-224.

Smith, F.M. & Jones, D.R. 1982. The effect of changes in blood oxygen-carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). J. Exp. Biol. **97**: 325-334.

Steffensen, J. F., Lomholt, J.P. & Johansen, K. 1981. The relative importance of skin oxygen uptake in the naturally buried plaice, *Pleuronectes platessa*, exposed to graded hypoxia. Resp. Physiol. **44**: 269-272.

Stevens, E.D. 1968. The effect of exercise on the distribution of blood to various organs in Rainbow Trout. Comp. Biochem. Physiol. **25**: 615-625.

Suthers, I.M. & Gee, J.H. 1986. Role of hypoxia in limiting diel spring and summer distribution of juvenile Yellow Perch (*Perca flavescens*) in a prairie marsh. Can. J. Fish. Aquat. Sci. **43**: 1562-1570.

Tonn, W.M. & Paszkowski, C.A. 1987. Habitat use of the central mudminnow (*Umbra limi*) and yellow perch (*Perca flavescens*) in *Umbra-Perca* assemblages: the roles of competition, predation, and the abiotic environment. Can. J. Zool. **65**: 862-870.

Tonn, W.M. & Paszkowski, C.A. 1986. Size-limited predation, winterkill, and the organization of *Umbra-Perca* fish assemblages. Can. J. Fish Aquat. Sci. **43**: 194-202.

Tun, N. & Houston, A.H. 1986. Temperature, oxygen, photoperiod and the hemoglobin system of the rainbow trout, *Salmo gairdneri*. Can. J. Zool. **64**: 1883-1888.

van Dam, A.A. & Pauly, D. 1995. Simulation of the effects of oxygen on food consumption and growth of Nile tilapia, *Oreochromis niloticus* (L.). Aqua. Res. **26**: 427-440.

Weber, J.M. & Kramer, D.L. 1983. Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, *Poecilia reticulata*. Can. J. Fish Aquat. Sci. **40**: 1583-1588.

Werner, E.E. & Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am. Nat. **142**: 242-272.

Werner, E.E. & Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size-structured populations. Ann. Rev. Ecol. Syst. **15**: 393-425.

Werner, E.E., Gilliam, J.F., Hall, D.J. and Mittelbach, G.G. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology **64(6)**: 1540-1548.

Wolf, N.G. & Kramer, D.L. 1987. Use of cover and the need to breathe: the effects of hypoxia on the vulnerability of dwarf gouramis to predatory snakeheads. Oecologia **73**:127-132.

Wright, D. & Shapiro, J. 1990. Refuge availability: a key to understanding the summer disappearance of *Daphnia*. Fresh. Biol. **24**: 43-62.

Yamamoto, K. 1991. Relationship of respiration to body weight in the carp *Cyprinus carpio* under resting and normoxic conditions. Comp. Biochem. Physiol. **100A**: 113-116.

Yamamoto, K. 1992. Relationship of respiration to body weight in the tilapia *Oreochromis niloticus* under resting and normoxic conditions. Comp. Biochem. Physiol. **103A**: 81-83.

Yamamoto, K., Itazawa, Y. & Kobayahi, H. 1980. Supply of erythrocytes into the circulating blood from the spleen of exercised fish. Comp. Biochem. Physiol. **65A**: 5-11.

Zanuy, S. & Carrillo, M. 1985. Annual cycles of growth, feeding rate, gross conversion efficiency and hematocrit levels of Sea Bass (*Dicentrarchus labrax* L.) adapted to different osmotic media. Aquaculture **44**: 11-25.

CHAPTER II

VARIATION IN TOLERANCE OF HYPOXIA BY AN AQUATIC PREDATOR AND PREY: AN ECOLOGICAL ADVANTAGE OF BEING SMALL?

Introduction

A survey of the literature values for tolerance of limited dissolved oxygen (hypoxia) or critical dissolved oxygen levels of teleosts depicts a large degree of variation among and sometimes within species (Smale & Rabeni 1995; Gee *et al.*1978; see review by Doudoroff & Shumway 1970). Physiological mechanisms that can enhance a water breathers tolerance to hypoxia include an increase in minute ventilation (Randall 1982; Smith & Jones 1982; Johansen 1982), increase in gill perfusion with subsequent increase in functional gill surface area (Johansen 1982; Booth 1979) and an increase in both the blood oxygen carrying capacity and blood oxygen affinity (Powers 1980). In addition to these responses, many teleosts are able to withstand hypoxia as a result of an enlarged gill surface area (Palzenberger & Pohla 1992; Hughes & Morgan 1973) and/or the use of anaerobic metabolism (Hochachka 1986; Holeton 1980; Blažka 1958). As a result of these physiological responses and adaptations the oxygen extracting efficiency are in turn positively correlated with environmental variables, metabolic requirement, and activity level of the individual (Palzenberger & Pohla 1992; Powers 1980; Johansen 1982; Holeton 1980). For example, in environments characterised by chronic hypoxia, individuals often exhibit enlarged gill surface areas (Palzenberger & Pohla 1992) or higher blood oxygen affinities (Powers 1980; Larsson *et al.* 1976). Fast swimming teleosts have also been found to have greater oxygen extracting capacities than less active fish (Holeton 1980). However, body size has also been found to play a role in the limitations of the physiological responses to an oxygen poor environment (Fox & Keast 1990; Tonn & Paszkowski 1986; Zanuy & Carrillo 1985; Doudoroff & Shumway 1970).

The findings of several studies support a size-sensitive tolerance to hypoxia with smaller individuals being more tolerant of low oxygen environments. For the most part the comparisons were within a single species of fish and amongst fish of various ages (Smale & Rabeni 1995; Zanuy & Carrillo 1985; Lowe-Jinde & Niimi 1983; and a review by Doudoroff & Shumway 1970). Of the physiological mechanisms proposed to increase oxygen extracting efficiency, both ventilation frequency and the oxygen carrying capacity have been found to be influenced by body size (Zanuy & Carrillo 1985; Lowe-Jinde & Niimi 1983; Jones 1971). Increasing the minute ventilation, or the amount of oxygen flowing over the gills per unit time, is an expensive measure for all fish and this

expense increases with an increase in body mass (Yamamoto 1992; Yamamoto 1991; Boutilier *et al.* 1988; Jones 1971). The oxygen carrying capacity of the blood determined by both the number of red blood cells and hemoglobin concentration, will also affect the ability of a teleost to tolerate hypoxic conditions (Gallaugher & Farrell 1998; Powers 1980). Although Schmidt-Nielsen (1984) states that blood parameters are not scaleable, several studies have found some effect of body size (Doudoroff & Shumway, 1970) and two even demonstrate an inverse relation between body size and hematocrit within a species (Lowe-Jinde & Niimi 1983; Zanuy & Carrillo 1985). Winterkill provides additional evidence of a scaling effect on tolerance. The reduction in dissolved oxygen in benthic regions of ice covered lakes often results in a differential mortality of larger individuals within a species (Fox & Keast 1990; Klinger *et al.* 1982). The death of predominantly larger individuals subjected to low temperatures and reduced oxygen levels also occurs in lakes with multiple-species fish communities, such as the Umbra-Perca assemblages in the lakes of Wisconsin (Tonn & Paszkowski 1987; Tonn & Paszkowski 1986).

Not all findings suggest an advantage for smaller individuals. A general relationship for birds, mammals, fish and plants suggests that only larger individuals can disproportionately monopolise resources, especially in response to fluctuations in the environment. This provides the selective pressure for the evolutionary trend of all species to become larger over time in order to dominate resource use and as a result leave more offspring (Brown & Maurer 1986; Stanley 1973). In addition, larger fish have lower weight specific metabolic rates and therefore require less oxygen per gram of body weight (van Dam & Pauly 1995; Holeton 1980). For this reason it has been suggested that larger individuals will have an advantage during short-term fluctuations of dissolved oxygen (Holeton 1980). Furthermore, Doudoroff & Shumway (1970) reviewed many of the early studies of size differences in tolerance to limiting dissolved oxygen levels during early development and concluded that younger, smaller individuals were less resistant to hypoxic conditions.

Two possible mechanisms that might explain the limitations of body size include an allometric and/or fractal scaling relationship with body size. Both of these relationships predict that smaller individuals will have a greater tolerance of hypoxic

conditions although for different reasons. An allometric relationship is predicted due to the negative allometric relationship of mass specific gill surface area, a key component in the rate of gas exchange, and body mass (Hughes 1984; Muir 1969). The fractal scaling model predicts that, independent of body size, fish are limited by the fixed size of cells (West *et al.* 1997). Therefore, larger fish would require greater branching of blood vessels and subsequently a greater time and effort for red blood cells to get to the tissue. Thus this model illustrates that rather than species or size differences in ability to produce red blood cells their transport is limited.

The ecological implications of a size effect would be most obvious between a predator and its prey. Body size has already been established as an important parameter in a predator-prey relationship as predators are limited by gape size and therefore prey consumption. As a result smaller individuals are often more susceptible to predation (Werner & Gilliam 1984; Mittelbach 1981). Alternatively, in an environment of changing physiochemical parameters such as dissolved oxygen, refuges can be created by eliminating larger piscine predators that are unable to tolerate conditions that smaller piscine prey can (Chapman *et al.* 1996a; Chapman *et al.* 1996b; Kolar & Rahel 1993; Wright & Shapiro 1990). I predicted that smaller prey teleosts will have an advantage in hypoxic habitats as a result of a greater mass specific gill surface area, small costs (compared to larger individuals) in increasing gill ventilation rates, and blood parameters such as the red blood cell concentration and hemoglobin concentration.

Methods

Study Animals

To test the hypothesis of size-sensitive variation in tolerance to dissolved oxygen, the fathead minnow, *Pimephales promelas*, and its predator the yellow perch, *Perca flavescens*, were selected for study. The fathead minnow, with an average length of 51mm, is commonly found in many ponds, streams and fresh water lakes of western and central North America (Scott & Crossman 1973). Yellow perch have an average length of 102-254mm and are also commonly found in freshwater lakes across western Canada (Scott & Crossman 1973). Both species are likely to encounter abrupt diurnal changes in dissolved oxygen, however it is unlikely this will be chronic hypoxia during the summer months. The fathead minnows were collected using minnow traps from the University of

Manitoba Field Station, Delta Marsh at the southern tip of Lake Manitoba ($50^0$11 'N, $98^0$23 'W) in early and late September of 1998. They were held in 200-litre tanks at room temperature (approximately 22°C), fed Nutrafin flakes and kept at a photoperiod of 12 hours light:12 hours dark. Yellow perch were angled using barbless hooks, from Stephenfield Lake in Stephenfield Provincial Recreation Park, Manitoba ($50^0$23 'N, $98^0$10 'W) in April of 1999. The perch were held in groups of ten in 200-litre tanks, fed trout pellets and worms and held at the same photoperiod and water temperature as the minnows. Approximately 24 hours prior to a trial a group of minnows or single perch was placed in an isolated tank without food.

Environmental Conditions

The apparatus consisted of a 50 litre aquarium (80cm x 12cm x14cm), but fish were restricted to an experimental area (9cm x 12cm x 8cm) by a transparent plexiglass partition, allowing the fish to be continuously monitored with a video camera. The tank provided a large enough volume of water to maintain a consistent dissolved oxygen concentration for the duration of the experiment as determined by pre-experiment trials. The video camera was used to monitor fish for signs of stress (loss of equilibrium) and to measure their ventilation frequency. To lower the dissolved oxygen concentration, nitrogen gas was bubbled through five air stones evenly spaced throughout the tank. For control trials, air rather than nitrogen was bubbled at a similar rate. The dissolved oxygen was measured using a YSI model 33 dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA) calibrated daily with air saturated tank water. The dissolved oxygen probe was placed in the tank within a perforated 10cm long tube over a magnetic stirrer to facilitate water movement over the probe. After the pre-specified dissolved oxygen level was reached the air stones were removed and plastic was placed over the water to prevent diffusion of atmospheric oxygen into the water. The dissolved oxygen was recorded for the duration of the trial with a Linear model 2030 chart recorder (Linear, Reno, Nevada, USA).

Each minnow was identified by a tag for the measurement of ventilation frequency over the duration of the trial. Tags were surgically placed on either side of the minnow allowing them to be identified independent of their orientation with respect to the video camera (see Abrahams & Sutterlin 1999 for details of the tagging procedure).

The numbered tags were comprised of two square pieces of yellow painted acetate (the paint side faced the body of the minnow to prevent removal). These tags allowed fish to move freely without any observed behavioural changes (personal observation). All minnows were allowed at least two days for recovery and any individual showing signs of lethargy or hematoma was excluded from the experiment.

Experimental Protocol

A group of five to six minnows or a single perch was placed into the apparatus at approximately 17:00h the day before the trial to allow for acclimation. The oxygen consumption of each perch to be used in the experiment was calculated and the total oxygen consumption of the minnows placed in a group corresponded to the average perch value. Thirty minutes prior to the trial the dissolved oxygen concentration was lowered. Once the desired level was reached the trial started and ventilation rates were measured. Individuals exhibiting any signs of stress were removed from the apparatus and actual time spent in the chamber was noted. Upon completion of a trial the tank water was drained and the apparatus was cleaned to prevent build up of nitrogen. A group of minnows or a single perch were subjected to one of a total of four dissolved oxygen ranges, including three hypoxic levels [extreme (1.57-1.96 mg/L), moderate (2.35-2.74 mg/L), and mild (3.13-3.92 mg/L)] and one normoxic (control) level (6.27- 8.22 mg/L). Six replicates of each of the experimental dissolved oxygen ranges were conducted on a total of 24 groups of minnows and on 24 perch. All experiments were performed between 10:00 and 16:00h with the water temperature maintained at 22°C in all cases. For the minnows the experiments were conducted daily from 14 May through 16 June 1999 and the perch trials were conducted during the periods 16-26 June, 17-20 August and 16-18 September 1999.

Measurements

For each trial, opercular movements were recorded for 28 seconds at 30-minute intervals. The time intervals were reduced to 5 minutes for the perch at extreme hypoxic levels due to the short length of these trials (see below). Mean ventilation frequency (opercular beats per second) was calculated for each time period for each perch and three randomly selected minnows of each trial. At the end of each trial, fish were anaesthetised with an

Table 2.1. Summary of mean wet weights (+/- 1 SE) of the fathead minnows and yellow perch used for analysis at each dissolved oxygen range used in the study.

Species	Dissolved Oxygen Range	Mean Weight (g)	N
Fathead minnow	Normoxic (6.27-8.22 mg/L)	2.7 +/- 0.16	30
	Mild Hypoxia (3.13-3.92 mg/L)	2.73 +/- 0.13	32
	Moderate Hypoxia (2.35-2.74 mg/L)	2.71 +/- 0.12	30
	Extreme Hypoxia (1.57-1.96 mg/L)	2.82 +/- 0.14	32
Yellow perch	Normoxic (6.27-8.22 mg/L)	34.11 +/- 3.55	6
	Mild Hypoxia (3.13-3.92 mg/L)	31.52 +/- 1.96	6
	Moderate Hypoxia (2.35-2.74 mg/L)	37.81 +/- 2.54	6
	Extreme Hypoxia (1.57-1.96 mg/L)	32.83 +/- 1.65	6

approximate 0.125 ml/L dose of 2-phenoxyethanol. The weights of all fish were measured (Table 2.1) and blood samples obtained. Due to size limitations, blood sampling required sacrificing all of the minnows with an overdose of 2-phenoxyethanol and combining blood samples of each group for analysis. For both species, blood was taken from a puncture of a caudal vessel using heparinized 27 gauge 1-3 cm long needles. At least 10ul was used to determine the hemoglobin concentration using Sigma Kit 525 and the remaining blood volume was drawn into a heparinized microcapillary tube for hematocrit determination. Microcapillary tubes were centrifuged for approximately 5 minutes and the percentage of red blood cells was calculated (hematocrit). From these parameters the mean cellular hemoglobin content (MCHC) was determined, as the concentration of hemoglobin divided by the hematocrit times 100. In a small portion of trials (no more than one for each dissolved oxygen range) blood hemolysis was apparent in blood samples and for these individuals blood hematological variables were not used in the analysis. However all individuals tested were used in determining the ventilation frequency.

Means were reported with +/- one standard error (SE) and statistical analyses used the GLM procedure of SPSS unless otherwise noted.

Results

The total time spent in the chamber (maximum possible time was 360 minutes) was significantly different between perch and minnows at both the extreme and moderate hypoxic trials (Table 2.2; ANOVA extreme $F_{1,37} = 108.06$, $p < 0.001$; moderate $F_{1,34} = 21.65$, $p < 0.001$). Prior to the end of the 6-hour time period, all of the perch lost equilibrium and were removed from the apparatus during all of the extreme hypoxic trials. During the course of the moderate hypoxic trials, three of the six perch tested were removed before the end of the trial. No perch lost equilibrium during the mild hypoxic and normoxic trials. A total of four minnows lost equilibrium while subjected to the extreme hypoxic range and a single minnow at each of the moderate and mild hypoxic levels were removed before the end of the trial. Due to the small proportion (2 of 62) of minnows that lost equilibrium prior to the end of both moderate and mild hypoxic levels, this was most likely due to stresses other than limited dissolved oxygen. At the extreme hypoxic trial, those minnows that lost equilibrium were relatively larger than the

Table 2.2. Mean time spent in chamber (+/- 1 SE) at all dissolved oxygen ranges (times less than 360 minutes indicate individuals lost equilibrium prior to end of experiment). The number in brackets indicates the number of individuals.

Dissolved Oxygen Range	Mean Time in Chamber (minutes)	
	Fathead Minnow	Yellow Perch
Normoxic	360 (30)	360 (6)
Mild Hypoxia	358.5 +/- 1.4 (32)	360 (6)
Moderate Hypoxia*	358.5 +/- 1.5 (30)	286.7 +/-35.3 (6)
Extreme Hypoxia*	336.8 +/- 12.7 (32)	27.3 +/- 8.3 (6)

* significantly different between species $p < 0.001$

minnows able to remain in the chamber for the full trial and the largest individuals had to be removed the earliest (Fig. 2.1). The mean weight of minnows removed before the end of the extreme hypoxic trials was 3.83g +/- 0.44 while the remaining 28 fatheads that were able to withstand the length of hypoxia had a mean weight of 2.68 +/- 0.13. Minnows removed from the apparatus prior to the end of the trial were not included in blood or ventilation analyses.

Blood Analysis

To determine the degree of the response of the minnow and perch to hypoxic conditions, the blood parameters measured were compared to the mean values observed at normoxia. In response to reduction in dissolved oxygen levels the minnows significantly increased the number of red blood cells (Table 2.3). Mean values at extreme and moderate hypoxic ranges were significantly different from the mean normoxic hematocrit (LSD Comparison of means test $p< 0.01$). The mean normoxic hematocrit value for the minnow was 26.32% +/- 1.12. At the extreme hypoxic range minnows increased hemoglobin concentrations to nearly 1.5 times that of the mean normoxic value (normoxic 5.79 g/dl +/- 1.29, extreme hypoxia 7.76 g/dl +/- 1.00). However, limited dissolved oxygen concentrations did not have a significant influence on the hemoglobin concentration and MCHC values of the minnow (Table 2.3). Body mass of the minnow did not influence either the hematocrit or hemoglobin concentration but did have a minor effect on the MCHC (Table 2.3). The mean hematocrit and hemoglobin values for the perch at normoxic levels were 28.67% +/- 1.81 and 6.00 g/dl +/- 0.54 respectively. None of the hematological variables measured in the perch were affected by a change in dissolved oxygen level (Table 2.3). Nor did the body weight of the perch influence changes in the values of the hematological variables (Table 2.3). For the minnows the hematocrit and hemoglobin concentration from all dissolved oxygen ranges tested were positively correlated however there was no correlation found for the perch (Pearson's product correlation- minnow $P=0.497$, $p= 0.004$; perch $P=0.406$, $p= 0.061$).

The mean difference from the mean normoxic hematological value at each of the three hypoxic ranges was used to compare species response (Fig. 2.2a and b). Although there was a time difference spent in the experimental chamber for perch and minnow, blood variables at the extreme and moderate hypoxic ranges were still compared. The

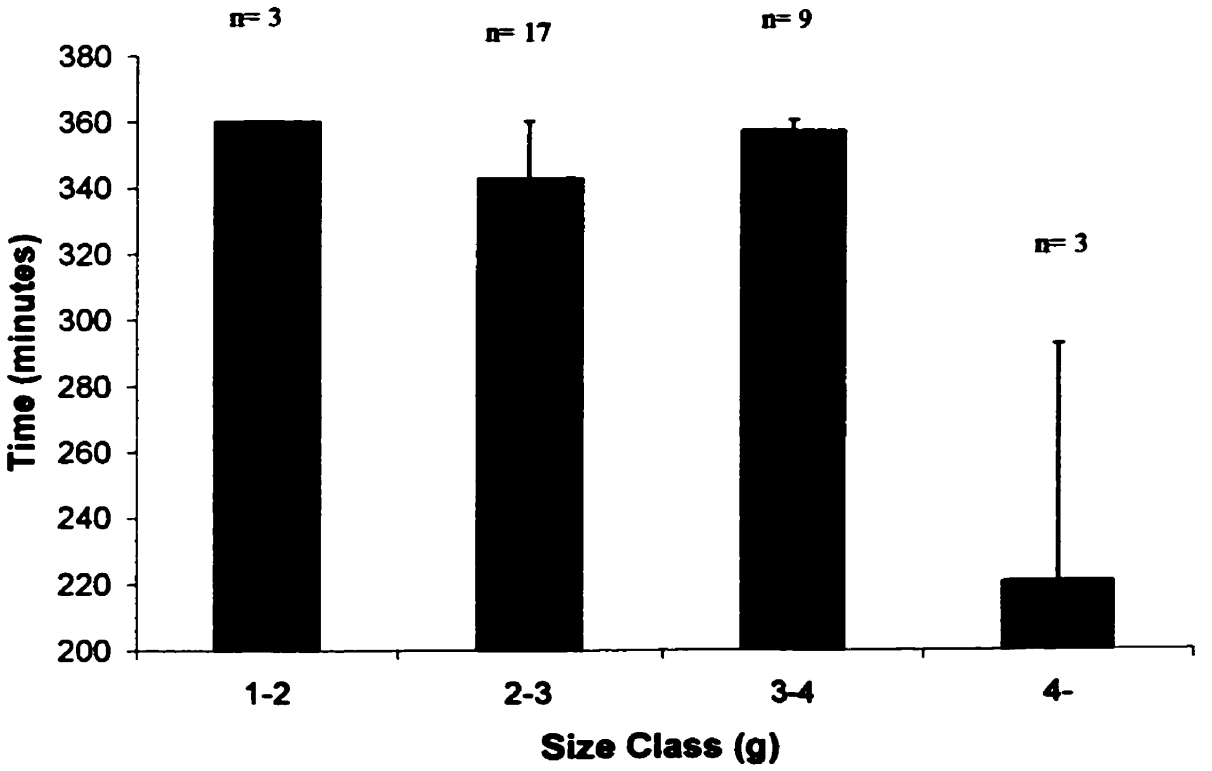


Figure 2.1. Mean times (+ 1 SE) spent in chamber for the minnows at the extreme hypoxic treatment. Times less than 360 minutes indicate individuals were removed when they lost equilibrium. An ANOVA using computer intensive statistics (Approximate randomisation) demonstrated significant differences between the size classes (Actual statistic $F_{3,29}= 3.78$, $p= 0.038$).

Table 2.3. Summary of ANCOVA results with all hematological parameters and ventilation frequency. In this analysis body weight is the covariate and dissolved oxygen (DO) range is a factor.

Species	Variable	Source	df	F	p
Fathead minnow	Hematocrit	Body Weight	1	1.19	0.291
		DO	3	6.43	**0.005**
		Error	16		
	Hemoglobin concentration	Body Weight	1	0.41	0.502
		DO	3	2.80	0.069
		Error	18		
	MCHC	Body Weight	1	5.21	**0.037**
		DO	3	3.07	0.060
		Error	15		
	Ventilation frequency	Body Weight	1	0.05	0.823
		DO	13	22.55	**< 0.001**
		Error	57		
Yellow perch	Hematocrit	Body Weight	1	0.00	0.996
		DO	3	0.05	0.984
		Error	17		
	Hemoglobin concentration	Body Weight	1	0.01	0.942
		DO	3	0.43	0.732
		Error	18		
	MCHC	Body Weight	1	0.03	0.871
		DO	3	0.47	0.707
		Error	17		
	Ventilation frequency	Body Weight	1	0.72	0.406
		DO	3	7.89	**0.001**
		Error	20		

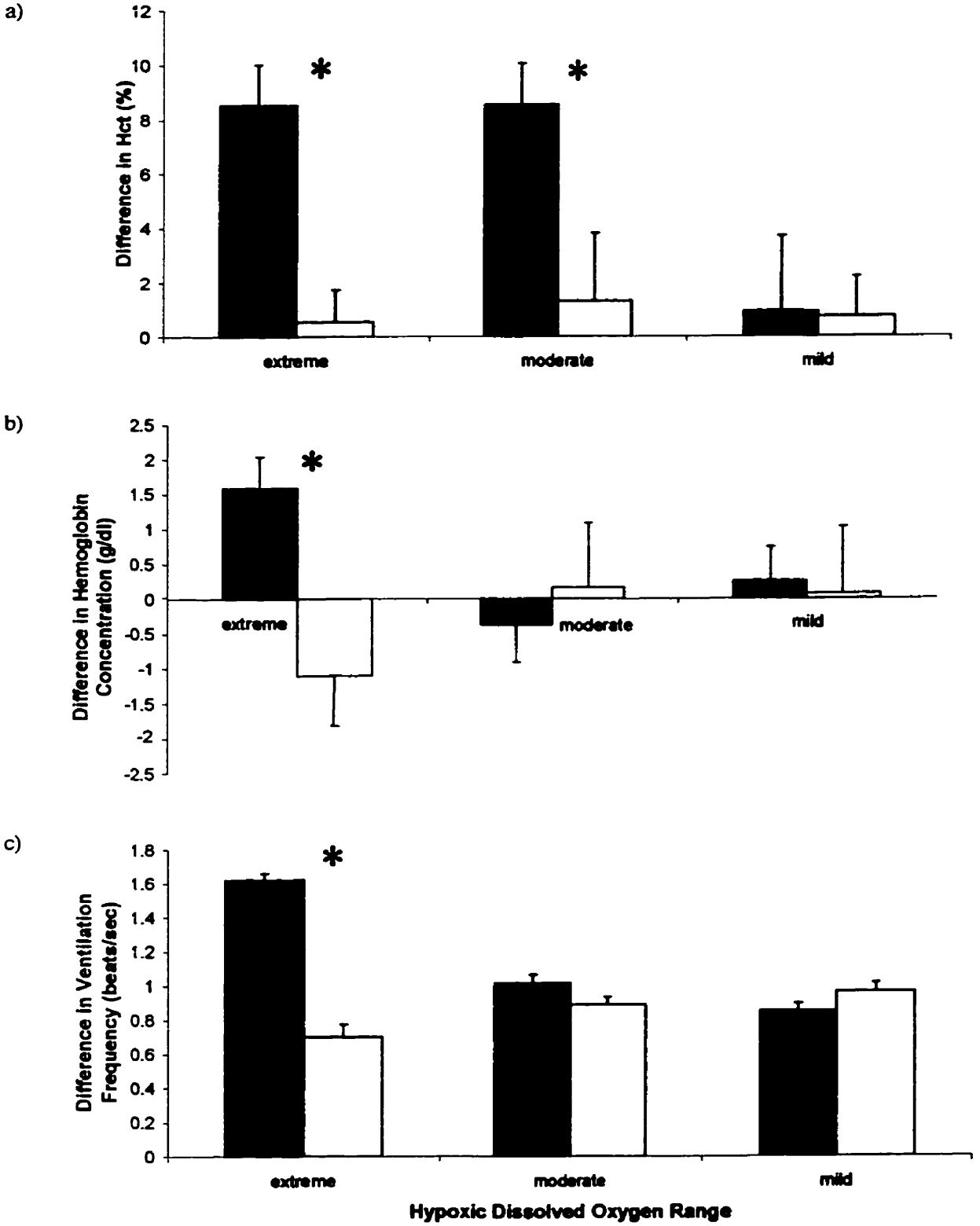


Figure 2.2. Mean difference (+ 1 SE) from mean normoxic values of a) hematocrit (Hct) b) hemoglobin concentration and c) ventilation frequency for minnow (black bar) and perch (open bar) at hypoxic dissolved oxygen ranges (see text for values).
(*significantly different between species at $p < 0.05$)

response by the minnows was greater than the perch at the extreme hypoxic range for hematocrit (Fig. 2.2a; ANOVA $F_{1,9} = 16.501$ $p = 0.003$) and hemoglobin concentration (Fig. 2.2b; ANOVA $F_{1,9} = 10.073$ $p = 0.013$) but not for MCHC (ANOVA $F_{1,9} = 1.304$ $p = 0.286$). The minnow mean difference from the normoxic mean hematocrit values were significantly greater than perch at moderate hypoxic levels (ANOVA $F_{1,9} = 6.106$ $p = 0.039$). However, the minnow response to moderate hypoxic levels was no different from the perch when comparing hemoglobin concentration and MCHC (Fig. 2.2b; hemoglobin $F_{1,10} = 0.261$ $p = 0.620$; MCHC $F_{1,8} = 0.911$ $p = 0.368$). At mild hypoxic ranges there was no species difference in change of hematological variables from the mean normoxic value (ANOVA hematocrit $F_{1,9} = 0.003$ $p = 0.955$; hemoglobin $F_{1,9} = 0.029$ $p = 0.869$; MCHC $F_{1,9} = 0.126$ $p = 0.731$).

Ventilation Frequency Response

At all dissolved oxygen ranges measured, no significant change in ventilation frequency (number of opercular beats per second) was observed over the course of a single trial for either the perch or minnows (regression analyses revealed no slopes different from zero). Therefore the mean ventilation frequency for each perch and three minnows was used for all statistical analyses. The normoxic trial mean ventilation frequencies for the minnow and perch were 3.02 beats/sec +/- 0.05 and 0.79 beats/sec +/- 0.04 respectively. A negative linear relationship described the minnow ventilation response to an increase in the environmental dissolved oxygen (Fig. 2.3). The perch, in response to hypoxia, also significantly increased ventilation frequency when compared to the ventilation response at normoxia (Table 2.3). However, the opercular frequency at the extreme, moderate and mild hypoxic ranges were not significantly different from each other (LSD $p > 0.05$). Only at mild and moderate hypoxic ranges were perch able to maintain a ventilation increase similar to the minnow response (Fig. 2.2c; ANOVA mild $F_{(1,22)} = 0.063$, $p = 0.84$; moderate $F_{1,22} = 2.786$, $p = 0.109$). At the extreme hypoxic range the difference from normoxic value was significantly greater for the minnow than perch (Fig. 2.2c; ANOVA $F_{1,22} = 21.704$, $p < 0.001$). Within each species body weight did not influence the ventilation frequency (Table 2.3).

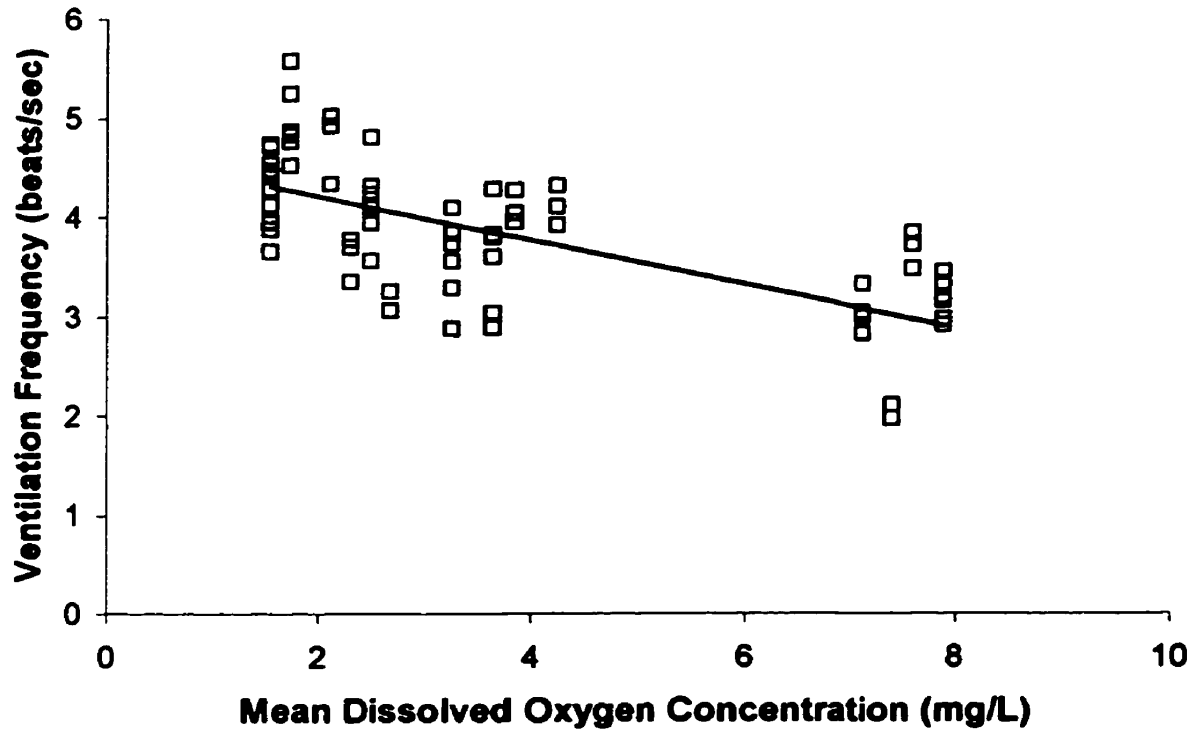


Figure 2.3. Influence of mean dissolved oxygen on the mean ventilation frequency for each fathead minnow observed (n= 72). Y= -0.2215x +4.6546, r^2=0.4734 (Regression $F_{1,71} = 62.94$, $p < 0.05$)

Discussion

Reduction in environmental dissolved oxygen influenced both the hematological variables and the ventilation responses of the fathead minnow and yellow perch. At extreme and moderate hypoxic ranges these responses varied between species such that only the perch were exhibiting signs of respiratory distress. Fathead minnows were able to survive more extreme hypoxic conditions and had a greater oxygen carrying capacity than the perch in response to the reduction of environmental dissolved oxygen levels. The minnows were most likely able to obtain greater amounts of dissolved oxygen from the environment as a result of an increase in ventilation frequency that was much greater than the perch at extreme hypoxic ranges. The few minnows that were unable to endure the hypoxic conditions for the duration of the trial at extreme hypoxic conditions were on average relatively larger than the remaining minnows. The perch response to hypoxia in this study was limited with no significant increase in the blood variables measured at all hypoxic ranges and a small increase in ventilation frequency relative to the minnow at the extreme hypoxic range. Despite the lower weight specific metabolic rate, for the perch, the increase in ventilation frequency was not sufficient to compensate for the environmental change in dissolved oxygen at extreme hypoxic levels and for some individuals at moderate hypoxic conditions.

As with many teleosts subjected to hypoxic conditions the fathead minnow responded with an increase in hematocrit and hemoglobin concentration (Peterson 1990; Nikinmaa *et al.* 1984; Garcia *et al.* 1992; Lowe-Jinde & Niimi 1983; Larsson *et al.* 1976). In a previous study fathead minnows subjected to various levels of pulp fibres (and subsequent drop in dissolved oxygen levels) increased hematocrit by about 1.4 fold for each one ppm drop of dissolved oxygen (MacLeod & Smith 1966). Despite the increase in both hematocrit and hemoglobin concentration there was no change in the calculated MCHC when minnows were subjected to hypoxic conditions. The stable MCHC indicates that the increase in hematocrit was not entirely due to red blood cell swelling and therefore represents an increase in the number of red blood cells (Gallaugher & Farrell 1998). This is further supported by the positive correlation of hematocrit and hemoglobin concentration among all dissolved oxygen levels. Perch response to lower dissolved oxygen levels was limited and in fact there was no detectable

increase in hematological variables measured in response to hypoxia in this study. At extreme and moderate dissolved oxygen trials this had severe effects on several perch in which they lost equilibrium prior to the end of the trial. The inability of the perch to increase hematocrit and hemoglobin concentration in response to hypoxia may be attributed to the limitations of one or a combination of physiological mechanisms including splenic red blood cell production, cardiac work, or ventilation abilities (Wells & Weber 1991). Previous studies have found perch tolerance of limited dissolved oxygen to be much greater than observed in this study (Suthers & Gee 1986; Petit, 1973; Petrosky & Magnuson 1973). However the perch in these previous studies were juveniles (Suthers & Gee 1986), acclimated to lower dissolved oxygen levels prior to being used in experiments (Petit 1973) or subjected to a concurrent decrease in temperature with the reduction in dissolved oxygen levels (Petrosky & Magnuson 1973). The influence of temperature and maturity along with season, nutrition, handling time and sampling methods have been found to alter hematological values (Zanuy & Carrillo 1985; Gallaugher & Farrell 1998; Lowe-Jinde & Niimi 1983; Larsson *et al.* 1976; Saint-Paul 1984). One or more of these factors may explain the diversity of the intraspecific tolerance values observed in comparing these values to previous studies.

Three possible mechanisms or a combination of these likely accounted for the increase in hematocrit of minnows in response to hypoxic conditions. First, plasma skimming (movement of plasma from the primary to secondary circulation) within the gill vasculature will increase the hematocrit although the number of red blood cells does not actually change (Nikinmaa 1990; Gallaugher & Farrell 1998). In addition, the release of erythrocytes from splenic reserves often occurs in response to stress such as oxygen depletion. This usually occurs between one and five minutes after the onset of the stress factor (Pearson & Stevens 1991; Nikinmaa 1990). For this reason we would expect to see the increase in hematocrit in a short term study such as this. Splenic release of red blood cells was found to also occur in rainbow trout, *Oncorhynchus mykiss*, (Wells & Weber 1991) and Atlantic cod, *Gadus morhua*, (Gallaugher & Farrell 1998) in response to acute hypoxia. Thirdly, erythrocyte swelling also results in an increase in hematocrit (Gallaugher & Farrell 1998). The swelling results from fluid moving from the plasma into the erythrocyte due to adrenergic stimulation (Gallaugher & Farrell 1998). This

effect is also beneficial to the fish as swelling increases blood oxygen affinity from the increase in erythrocyte pH (Nikinmaa 1983).

Both the minnows and perch increased ventilation frequency to higher rates than at normoxia in response to lowering dissolved oxygen. This mechanism was similar to previous studies where both the fathead minnow and yellow perch increased their gill ventilation rates in response to depleted oxygen levels at low temperatures (Klinger *et al.* 1982; Petrosky & Magnuson 1973). Claireaux & Dutil (1992) also found an initial increase in the number of opercular beats in Atlantic cod in response to both severe and mild hypoxia. Increases in ventilation frequency maintains a steep PO_2 gradient between blood and water at the gill to promote gas diffusion in addition to the maintenance of oxygen at the tissue (Smith & Jones 1982; Johansen 1982; Nikinmaa & Salama 1998). However there are costs associated with the increase in frequency, as individual must provide enough oxygen for normal life processes in addition to the muscular activity required in moving the opercular. The limited ability of the perch to increase ventilation frequency in response to the extreme and moderate hypoxic levels suggested that these costs were greater than the benefits. Other findings have found the rate of ventilation increase not to be a linear function as it slows down at critical oxygen tensions (Saint-Paul 1984; Larsson *et al.* 1976; Rantin *et al.* 1992). At these critical oxygen tensions the increase in ventilation frequency may be counter-productive as too much actually reduces the net amount of oxygen extracted (Jones & Randall 1978).

A negative relationship of body size and hypoxia tolerance has been suggested as the key mechanism in the variation of response to hypoxic conditions by the fathead minnow and yellow perch. The basis for this explanation being the negative allometric relationship of gill surface area and body size. With a few exceptions this relationship can be applied to many freshwater fish (Palzenberger & Pohla 1992). Additional evidence of intraspecific size sensitive variation in tolerance to hypoxic conditions included ventilation frequencies, blood oxygen carrying capacity, gill uptake rates and winterkill (Sijm *et al.* 1995; Tonn & Paszkowski 1986; Zanuy & Carrillo 1985; Lowe-Jinde & Niimi 1983; Jones 1971). However as previously suggested alternative explanations that may account for the observed difference in tolerance to hypoxia include the possibility of fractal scaling which would show the same results (West *et al.* 1997) or

a species effect (Palzenberger & Pohla 1992; Johansen 1982; Holeton 1980; Powers 1980). Indeed species differences are evident in the literature with a diversity of morphological and physiological adaptations to the environment in which they live (Chapman *et al.* 1999; Kramer 1987; Gee *et al.* 1978; Doudoroff & Shumway 1970).

Critical dissolved oxygen levels were found to be greater for yellow perch than for the fathead minnow. An important aspect of these findings is the ecological implications this variation in tolerance will have in a predator-prey relationship. Despite the different explanations for the observed differences in tolerance to hypoxia, the consequences of prey being more tolerant than their predator will be the same. Minnows will have an advantage in an environment in which dissolved oxygen can fluctuate from normoxic to hypoxic in short time periods. The creation of temporary hypoxic habitats both spatially and temporally will in turn form refuges in that smaller minnows are able to use as a temporary evasion of predators at little cost or as a long-term habitat choice in response to predation pressures.

Measurement of critical environmental dissolved oxygen concentrations not only provides correlation for fish habitat use (Smale & Rabeni 1995; Cech *et al.* 1990) but also as this study suggests, plays a key role in predicting piscine predator-prey relationships. Two large scale studies have also found a large degree of variation among species in critical dissolved oxygen levels (Smale & Rabeni 1995; Gee *et al.* 1978). Upon closer look of the data a pattern emerges amongst known predator and prey species, where generally smaller prey are more tolerant of hypoxia. For example the piscivorous predators rock bass (*Ambloplites rupestris*), yellow perch, and walleye (*Stizostedion vitreum*) had estimated limiting (critical) dissolved oxygen levels that were on average larger than many of the smaller cyprinids (a primary prey item of these predators) such as the fathead minnow and spottail shiner, *Notropis hudsonius* (Gee *et al.* 1978). Similarly the central stoneroller, *Campostoma anomalum*, a common prey item of the small mouth bass, *Micropterus dolomieui*, was significantly more tolerant of hypoxia than the bass (Smale & Rabeni 1995). A majority of the larger species tested by Smale & Rabeni (1995) had the lowest critical dissolved oxygen concentrations suggesting larger individuals are more tolerant. However, these larger species including the bluegill, *Lepomis macrochirus*, and green sunfish, *L. cyanellus*, are relatively larger than a

majority of common prey items such as the minnows, are primarily non-piscivorous and are often prey items of larger piscivorous predators such as the largemouth bass, *M. salmoides*. The large mouth bass did not differ in tolerance from these larger insect and plant eating fish species, however the body sizes tested between these groups also did not differ.

The ecological and evolutionary advantages of being small are rather limited as small individuals are often out competed through domination of resource use by larger individuals (Brown & Maurer 1986). Being small also implies greater costs related to feeding energetics, survival and reproduction (Peters 1983; Brown & Maurer 1986). In addition, smaller individuals are often subject to a high risk of predation (Werner & Gilliam 1984). However, the use of hypoxic (piscine predator free) refuges can provide prey with an advantage in these short term and possibly long-term situations. The recent findings of Chapman *et al.* (1996a) and Chapman *et al.* (1996b) suggested that hypoxic refuges exist and the findings of this study further supports their conclusions by providing body size as a possible mechanism that makes this relationship possible. Being small or more tolerant to hypoxia is potentially an ecologically important advantage when body size is relevant in a relationship such as that between a predator and prey.

Literature Cited

Abrahams, M.V. & Sutterlin, A. 1999. The foraging and antipredator behaviour of growth-enhanced transgenic Atlantic salmon. Anim. Behav. **58**: 933-942.

Blažka, P. 1958. The anaerobic metabolism of fish. Physiol. Zool. **31**: 117-128.

Booth, J.H. 1979. The effect of oxygen supply, epinephrine and acetycholine on the distribution of blood flow in trout gills. J. Exp. Biol. **83**: 31-39.

Boutilier, R.G., Dobson, G., Hoeger, U. & Randall, D.J. 1988. Acute exposure to graded levels of hypoxia in rainbow trout (*Salmo gairdneri*): metabolic and respiratory adaptations. Resp. Physiol. **71**: 69-82.

Brown, J.H. & Maurer, B.A. 1986. Body size, ecological dominance and Cope's rule. Nature **324**: 248-250.

Chapman, L.J., Chapman, C.A., Brazeau, D.A., McLaughlin, B., & Jordan, M. 1999. Papyrus swamps, hypoxia and faunal diversification: variation among populations of *Barbus neumayeri*. J. Fish Biol. **54**: 310-327.

Chapman, L.J., Chapman, C.A., & Chandler, M. 1996a. Wetland ecotones as refugia for endangered fishes. Biol. Conserv. **78**:263-270.

Chapman, L.J., Chapman, C.A., Ogutu-Ohwayo, R., Chandler, M., Kaufman, L. & Keiter, A.E. 1996b. Refugia for endangered fishes from an in introduced predator in Lake Nabugabo, Uganda. Conserv. Biol. **10**: 554-561.

Cech, Jr., J.J., Mitchell, S.J., Castleberry, D.T. & McEnroe, M. 1990. Distribution of California stream fishes: influence of environmental temperature and hypoxia. Env. Biol. Fish **29**: 95-105.

Claireaux, G. & Dutil, J-D. 1992. Physiological response of the Atlantic cod (*Gadus Morhua*) to hypoxia at various environmental salinities. J. Exp. Biol. **163**: 97-118.

Doudoroff, P. & Shumway, D.L. 1970. Dissolved oxygen requirements of freshwater fishes. Food and Agriculture Organization of the United Nations. Technical Paper 86. 291p.

Fox, M.G. & Keast, A. 1990. Effects of winterkill on population structure, body size and prey consumption patterns of pumpkinseed in isolated beaver ponds. Can. J. Zool. **68**: 2489-2498.

Gallaugher, P. & Farrell, A.P.1998. Hematocrit and blood oxygen-carrying capacity. IN Fish Physiology Volume 17: Fish Respiration. eds S.F. Perry and B.L. Tufts. Academic Press. pp 185-227.

García, M.P., Echevarría, G., Martínez, F.J. & Zamora, S. 1992. Influence of blood sample collection on the haematocrit value of two teleosts: Rainbow Trout (*Oncorhynchus mykiss*) and European Sea Bass (*Dicentrarchus labrax* L.). Comp. Biochem. Physiol. **101A(4)**: 733-736.

Gee, J.H., Tallman, R.F. & Smart, H.J. 1978. Reactions of some great plains fishes to progressive hypoxia. Can. J. Zool. **56**:1962-1966.

Hughes, G.M. 1984. Scaling of respiratory areas in relation to oxygen consumption of vertebrates. Experientia **40(6)**: 519-652.

Hughes, G.M. & Morgan, M. 1973. The structure of fish gills in relation to their respiratory function. Biol. Rev. **48**: 419-475.

Hochachka, P.W. 1986. Defense strategies against hypoxia and hypothermia. Science **231**: 234-241.

Holeton, G.F. 1980. Oxygen as an environmental factor of fishes. IN Environmental Physiology of Fishes. eds M.A. Ali. Plenum, New York. pp. 7-32.

Johansen, K. 1982. Respiratory gas exchange of vertebrate gills. IN Gills. eds. D.F. Houlihan, J.C. Rankin & T.J. Shuttleworth. Cambridge University Press, Cambridge. pp 99-128.

Jones, D.R. 1971. Theoretical analysis of factors which may limit the maximum oxygen uptake of fish: The oxygen cost of the cardiac and branchial pumps. J. Theor. Biol. **32**: 341-349.

Jones, D.R. & Randall, D.J. 1978. The respiratory and circulatory systems during exercise. IN Fish Physiology, Volume 7 (eds W.S. Hoar & D.J. Randall), Academic Press, New York. pp 425-501.

Kramer, D.L. 1987. Dissolved oxygen and fish behavior. Env. Biol. Fish. **18(2)**: 81-92.

Klinger, S.A., Magnuson, J.J., & Gallepp, G.W. 1982. Survival mechanisms of the central mudminnow (*Umbra limi*), fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) for low oxygen in winter. Env. Biol. Fish **7(2)**: 113-120.

Kolar, C.S. & Rahel, F.J. 1993. Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia **95**: 210-219.

Larsson, Å., Johansson-Sjöbeck, M. & Fänge, R. 1976. Comparative study of some haematological and biochemical blood parameters in fishes from the Skagerrak. J. Fish. Biol. **9**: 425-440.

Lowe-Jinde, L. & Niimi, A.J. 1983. Influence of sampling on the interpretation of haematological measurements of rainbow trout, *Salmo gairdneri*. Can. J. Zool. **61**: 396-402.

MacLeod, J.C. & Smith Jr., L.L. 1966. Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow, *Pimephales promelas*. Trans. Am. Fish. Soc. **95**: 71-84.

Mittelbach, G.G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology **62(5)**: 1370-1386.

Muir, B.S. 1969. Gill dimensions as a function of fish size. J. Fish. Res. Bd. Canada **26**: 165-170.

Nikinmaa, M. 1990. Vertebrate Red Blood Cells: adaptations of function to respiratory requirements. Zoolphysiology Volume 28. pp 262.

Nikinmaa, M. 1983. Adrenergic regulation of haemoglobin oxygen affinity in rainbow trout red cells. J. Comp. Physiol. **152**: 67-72.

Nikinmaa, M. & Salama, A. 1998. Oxygen transport in fish. IN Fish Physiology Volume 17: Fish Respiration. eds S.F. Perry & B.L. Tufts. Academic Press. pp.141-184.

Nikinmaa, M., Cech Jr., J.J. & McEnroe, M. 1984. Blood oxygen transport in stressed striped bass (*Morone saxatilis*): role of beta-adrenergic responses. J. Comp. Physiol. **154B**: 365-369.

Palzenberger, M. & Pohla, H. 1992. Gill surface area of water-breathing freshwater fish. Rev. Fish Biol. Fish. **2**:187-216.

Pearson, M.P. & Stevens, E.D. 1991. Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. **9(1)**: 39-50.

Peters, R.H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge. 329p.

Peterson, M.S. 1990. Hypoxia-induced physiological changes in two mangrove swamp fishes: Sheepshead minnow, *Cyprinodon variegatus* Lacepede and sailfin molly, *Poecilia Latipinna* (Lesueur). Comp. Biochem. Physiol. **97A**: 17-21.

Petit, G.D. 1973. Effects of dissolved oxygen and behavior of selected fishes of western lake Erie. Bull. Ohio. Biol. Surv. **4(4)**: 1-77.

Petrosky, B.R. & Magnuson, J.J. 1973. Behavioral responses Of Northern Pike, Yellow Perch and Bluegill to oxygen concentrations under simulated winterkill conditions. Copeia 1:124-133.

Powers, D.A. 1980. Molecular ecology of teleost fish hemoglobins: Strategies for adapting to changing environments. Amer. Zool. **20**: 139-162.

Randall, D. 1982. The control of respiration and circulation in fish during exercise and hypoxia. J. Exp. Biol. **100**: 275-288.

Rantin, F.T., Kalinin, A.L., Glass, M.L. & Fernandes, M.N. 1992. Respiratory responses to hypoxia in relation to mode of life of two erythrinid species (*Hoplias malabaricus* and *Hoplias lacerdae*). J. Fish Biol. **41**: 805-812.

Saint-Paul, U. 1984. Physiological adaptation to hypoxia of a neotropical characoid fish *Colossoma macropomum*, Serrasalmidae. Env. Biol. Fish. **11(1)**: 53-62.

Scott, W.B. & Crossman, E.J. 1973. Freshwater fishes of Canada. Bull 184. Fisheries Research Board of Canada Ottawa.

Sijm, D.T.H.M., Verberne, M.E., DeJonge, W.J., Pärt, P. & Opperhuizen, A. 1995. Allometry in the uptake of hydrophobic chemicals determined in vivo and in isolated perfused gills. Toxicol. Appl. Pharmacol. **131**: 130-135.

Smale, M.A. & Rabeni, C.F. 1995. Hypoxia and hypothermia tolerances of headwater stream fishes. Trans. Am. Fish. Soc. **124**: 698-710.

Schmidt-Nielsen, K. 1984. Scaling,, why is animal size so important. Cambridge University Press, Cambridge. 241p

Smith, F.M. & Jones, D.R. 1982. The effect of changes in blood oxygen-carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). J. Exp. Biol. **97**: 325-334.

Stanley, S.M. 1973. An explanation for Cope's rule. Evolution **27**: 1-26.

Suthers, I.M. & Gee, J.H. 1986. Role of hypoxia in limiting diel spring and summer distribution of juvenile Yellow Perch (*Perca flavescens*) in a prairie marsh. Can. J. Fish. Aquat. Sci. **43**: 1562-1570.

Tonn, W.M. & Paszkowski, C.A. 1987. Habitat use of the central mudminnow (*Umbra limi*) and yellow perch (*Perca flavescens*) in *Umbra-Perca* assemblages: the roles of competition, predation, and the abiotic environment. Can. J. Zool. **65**: 862-870.

Tonn, W.M. & Paszkowski, C.A. 1986. Size-limited predation, winterkill, and the organization of *Umbra-Perca* fish assemblages. Can. J. Fish Aquat. Sci. **43**: 194-202.

van Dam, A.A. & Pauly, D. 1995. Simulation of the effects of oxygen on food consumption and growth of Nile tilapia, *Oreochromis niloticus* (L.). Aqua. Res. **26**: 427-440.

Wells, R.M.G. & Weber, R.E. 1991. Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. J. Fish Biol. **38**: 53-65.

Werner, E.E. & Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size-structured populations. Ann. Rev. Ecol. Syst. **15**: 393-425.

West, G.B., Brown, J.H. & Enquist, B.J. 1997. A general model for the origin of allometric scaling laws in biology. Science **276**: 122-126.

Wright, D. & Shapiro, J. 1990. Refuge availability: a key to understanding the summer disappearance of *Daphnia*. Fresh. Biol. **24**: 43-62.

Yamamoto, K. 1992. Relationship of respiration to body weight in the tilapia *Oreochromis niloticus* under resting and normoxic conditions. Comp. Biochem. Physiol. **103A**: 81-83.

Yamamoto, K. 1991. Relationship of respiration to body weight in the carp *Cyprinus carpio* under resting and normoxic conditions. Comp. Biochem. Physiol. **100A**: 113-116.

Zanuy, S. & Carrillo, M. 1985. Annual cycles of growth, feeding rate, gross conversion efficiency and hematocrit levels of Sea Bass (*Dicentrarchus labrax* L.) adapted to different osmotic media. Aquaculture **44**: 11-25.

CHAPTER III

HABITAT SELECTION IN RESPONSE TO FLUCTUATING DISSOLVED OXYGEN CONCENTRATIONS AND PREDATION PRESSURES: DO PREY CHOOSE HYPOXIC HABITATS?

Introduction

Environmental parameters such as dissolved oxygen, temperature, pH and water levels as well as predation, competition and food availability will alone or in combination influence the distribution of freshwater fish (Brazner & Beals 1997; Tonn & Paszkowski 1987; Eadie & Keast 1984; Werner *et al.* 1977). Of particular interest is the influence of fluctuations in environmental dissolved oxygen concentrations as fish physiology can be severely compromised under these sometimes abrupt and stressful conditions (Breitburg *et al.* 1997; Chapman *et al.* 1996a; Tonn & Paszkowski 1986; Coutant 1985; Clady 1976). Thus distribution of fish will be limited by their physiology and these limits have been found to vary with environmental conditions, metabolic rate and activity levels of individuals (Davenport & Sayer 1993; Palzenberger & Pohla 1992; Holeton 1980; Powers, 1980). Habitat selection is further complicated by the risk of predation, as movement from stressful environments to oxygen rich areas will likely increase encounter rates with predators or detection by predators relying on vision (Werner & Anholt 1993). These combined effects of predation risk and reduction of dissolved oxygen will influence habitat choice by the prey based on the costs and benefits of each. Therefore, prey that are more tolerant to limited dissolved oxygen (hypoxia) will reduce the risk of predation as well as competition by remaining in stressful habitats longer (Kolar & Rahel 1993; Rahel & Kolar 1990; Poulin *et al.* 1987).

Many freshwater teleosts exhibit a graded response to decreasing levels of dissolved oxygen that progresses from increasing activity, followed by a decrease in activity, the use of aquatic surface respiration (ASR) and, finally, the loss of equilibrium at lethal levels (Gee *et al.* 1978). Raising the level of activity, although physiologically expensive, most likely serves to aid in avoidance of hypoxic habitats in the search for more normoxic waters (Petersen & Petersen 1990; Kramer 1987; Suthers & Gee 1986; Petrosky & Magnuson 1973). If an individual is unable to escape from extreme hypoxia, death is imminent, and reduced activity will delay certain death (Boese 1988; Gee *et al.* 1978). Alternatively, fish may respond to hypoxia with an increased use of the oxygen rich surface film where oxygen is in greater concentration due to atmospheric exchange (Klinger *et al.* 1982; Kramer & Mehegan 1981; Gee *et al.* 1978). The use of ASR will normally occur at critical dissolved oxygen levels, however all fish do not use this

behavioural modification and morphological adaptations, such as an upturned mouth, will likely increase its efficiency (Klinger *et al.* 1982; Gee *et al.* 1978). These observed changes in behaviour due to hypoxia will have implications not only in a predator-prey relationship but will also result in changes in food availability (and possibly less energy expended to search for food), as well as reduced growth rates, reproduction and often survival rates (see Kramer 1987).

When given a choice, the typical response to hypoxia by fresh water fishes is to avoid the area, shifting into areas of greater oxygen supply but where predation and competition is often greater (Kramer 1987; Suthers & Gee 1986; Coutant 1985; Werner *et al.* 1983; Wolf & Kramer 1987). The recent findings that suggest size-dependent tolerance exists where smaller fish are more tolerant of hypoxia in fish assemblages, has new implications on prey habitat selection (Chapter two of this thesis). In response to predation pressures when environments are subjected to spatial and episodic fluctuations in dissolved oxygen, smaller fish could use a hypoxic refuge that is intolerable to larger piscine predatory fish. Therefore the refuge can be defined by the critical dissolved oxygen concentrations of a prey and predator.

Recent examples exist where smaller prey use hypoxic habitats as refuges. The introduction of the piscivorous predator, the Nile perch (*Lates niloticus*), into Lake Victoria caused many native haplochromine species to use hypoxic refuges at the edges of the lake (Chapman *et al.* 1995). These spatially complex refuges provided hypoxic conditions preventing exploitation by predators unable to tolerate the adverse conditions (Chapman *et al.* 1996a; Chapman *et al.* 1996b; Chapman & Liem 1995). The effectiveness of a piscine predator may also be limited by reduced environmental dissolved oxygen concentrations. For example, the cichlid, *Astronotus ocellatus*, was found to be less effective in preying upon the guppy, *Poecilia reticulata*, in hypoxic waters (Poulin *et al.* 1987). The movement of prey to more hypoxic areas further influenced this reduction in efficiency. Invertebrates more tolerant of low dissolved oxygen have been found to use hypoxic regions as a refuge when in the presence of predatory fish (Kolar & Rahel 1993; Rahel & Kolar 1990; Wright & Shapiro 1990). For example, the use of a predator-free refuge defined by temperature, dissolved oxygen and

light by large bodied *Daphnia* prevented population decreases during the summer months (Wright & Shapiro 1990).

In order to predict habitat selection of a predator and prey population in response to fluctuations of dissolved oxygen, I developed a model, based on the ideal free distribution (IFD). This relationship, developed by Fretwell & Lucas (1970), describes when individuals are presented with a patchy environment they will choose habitats in order to maximise their net benefit. The underlying assumptions of the IFD are that each individual has equal competitive ability, a complete knowledge of each of the patches and their benefits and individuals are free to move to any patch that will provide the greatest net benefit (equally competitive). Thus when food is the only source of benefit in a patch and is always available at a constant rate, the distribution of individuals will be equal to the distribution of food. However, I've incorporated costs into the quality of each patch so that not only is there competition for a food source but the added costs of both predation risk and physiological tolerance of hypoxia.

The physiological costs of hypoxia were assumed to be less for prey under hypoxic conditions when compared to predator's costs based on the predicted size-sensitive tolerance of hypoxia (Chapter two of this thesis). Thus prey selected a habitat primarily on the basis of food supply, however predation risk and hypoxia influenced their decision. Predation risk was not only determined by the ratio of prey to predators (dilution effect) but was also reduced under hypoxic conditions. The costs of hypoxia and the abundance of prey influenced the habitat selection by predators where predators were much more limited in choice of habitat due to greater physiological costs. By comparing the distribution of individuals in a continuous input IFD (distribute according to food allocation) to the distributions in which I have incorporated costs one can assess the differences in fitness costs associated with each habitat (Abrahams & Dill 1989). Thus the level of predation risk and costs of dissolved oxygen that influences habitat selection by prey and predators can be determined. In addition, the comparison to an actual distribution will provide possible mechanisms such as hypoxia that drives the actual distribution in environments of fluctuating dissolved oxygen.

To test the predictions of the model the relative distribution of a real predator and prey population found in an environment of fluctuating dissolved oxygen concentrations

was determined. Delta Marsh, situated on the southern edge of Lake Manitoba, is subject to many hypoxic events both spatially and temporally. The abundant emergent and submerged macrophytes create distinct habitats defined by dissolved oxygen concentrations (Suthers & Gee 1986). As a result of photosynthesis exceeding respiration during the day and respiration occurring overnight there is also a diurnal fluctuation in dissolved oxygen. During the months of July and August, the majority of the minnow population in the marsh is juveniles (personal observation) and these in turn are abundant prey items for piscine predators such as the yellow perch (*Perca flavescens*). It was predicted that juveniles, because of their small size, use hypoxic habitats of the emerged macrophytes in response to the interaction of predation pressure, food availability, and fluctuation of dissolved oxygen levels in the marsh.

Methods

The Model

For the purpose of this study a three-patch model was developed using Excel Visual Basic 7.0 (Appendix 1) to describe the relationship of predator and prey when dissolved oxygen levels are changing both temporally and spatially. Within the computer environment, three patches or habitats of different costs and benefits were defined and predator and prey were able to choose between them so as to maximise their net benefits. The suitability of each patch was based on the combined effects of the food availability, predation risk (for prey only) and the costs associated with a particular dissolved oxygen level. The latter costs reflected the physiological expenditure attributed to maintaining the normal amount of oxygen supplied to the tissue in an oxygen-depleted environment.

In addition to the assumptions of an IFD described above, I have assumed that each individual has the ability to integrate the costs associated with varying levels of dissolved oxygen into their habitat decision. The ability of a teleost to sense variation in dissolved oxygen concentration has been challenged (Tonn & Paszkowski 1987). However, the observation that fish avoid hypoxic regions has been used to justify the premise that fish do sense the changes in dissolved oxygen that are compromising to the physiology of the individual (Suthers & Gee 1986; Doudoroff & Shumway 1970). The main assumption of this model, however, is the size-sensitive relationship of tolerance to dissolved oxygen levels. This relationship was further assumed to be a negative

allometric relationship in which smaller teleosts are able to tolerate lower dissolved oxygen levels than a piscine predator (see Chapter two of this thesis). This relationship suggests that in hypoxic conditions a small reduction in dissolved oxygen concentration results in a large increase in the physiological costs associated with maintaining equilibrium. However, for predators the costs are substantially higher than for prey as initiation of physiological mechanisms (and therefore costs) in response to hypoxia for predators most likely occurs at greater dissolved oxygen levels than prey (Fig. 3.1; see Chapter two of this thesis).

Program- The Main Subroutine

The main subroutine of the program first initialised the manipulative variables as the total number of prey and predators in the system, total food available to the prey and the first and last sample times with desired sampling interval (this was set at 5:00h to 17:00h with four hour intervals to replicate the samples taken in the field). The dissolved oxygen levels of the three patches were based on data collected from my study site at Delta Marsh (Table 3.1). The dissolved oxygen levels increased throughout the day with patch one being representative of an open water habitat and therefore the one with the greatest environmental dissolved oxygen levels. Intermediate dissolved oxygen levels were characteristic of patch two, which represented a habitat adjacent to emergent vegetation. Patch three portrayed a habitat within the emergent macrophytes and therefore experienced the lowest environmental dissolved oxygen levels throughout the day. Food available at each station was based on previous data gathered from Delta Marsh (Abrahams unpublished data). Half of the total food was distributed along the edge of the macrophytes, patch two, and the other half was evenly distributed between patches one and three (Table 3.1). To determine the sensitivity of food availability on prey choice of habitat, the food proportions were varied in a separate analysis. All other parameters remained the same. The number of prey and predators were set at 200 and 20, respectively.

Distribute Predators

Once initial patch conditions were set, the predators were distributed one at a time until all were entered into the patches (DistributePredators). For this initial distribution the number of prey in each patch was the same as their food allocation. Switching after the

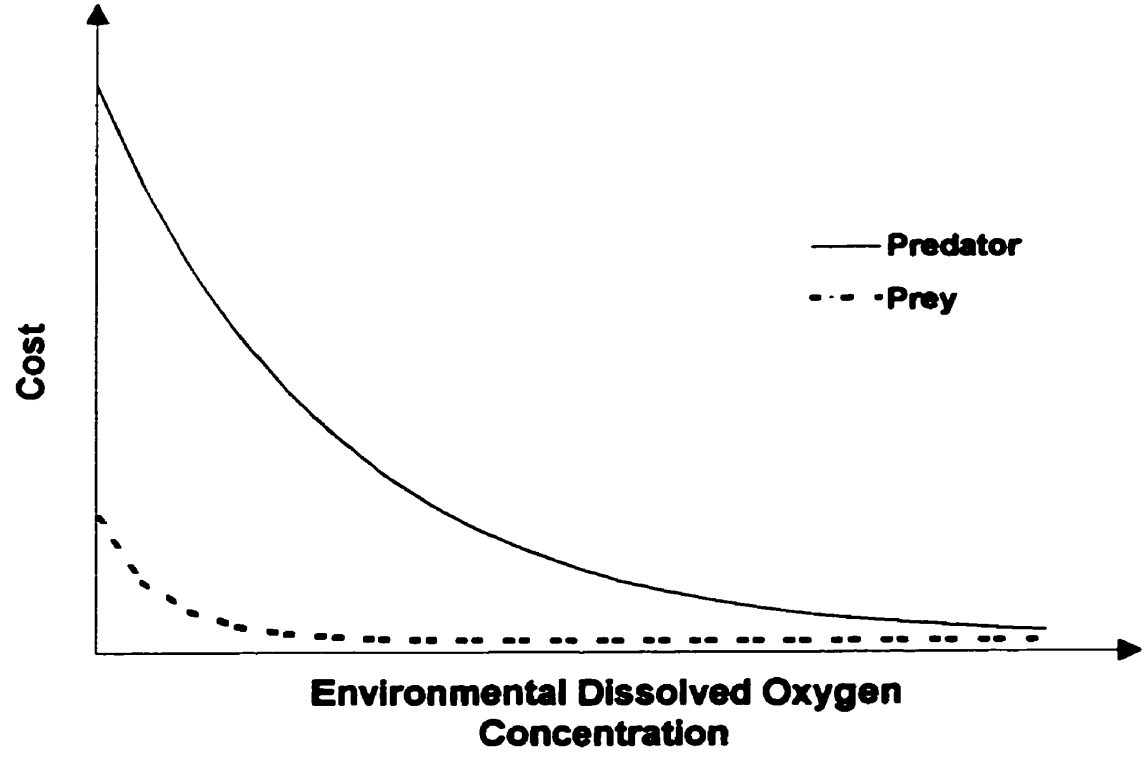


Figure 3.1. Predicted allometric cost functions (based on the assumptions outlined in the text) for predator and prey associated with maintaining equilibrium at variable environmental dissolved oxygen concentrations.

Table 3.1. Parameter values for each of the patches available in the model.

Patch identification	Dissolved Oxygen Concentration	Proportion of Food Available to Prey
1. Open water	normoxic (6.74-10.60)	0.25
2. Edge of Macrophytes	hypoxic/ normoxic (4.71-9.74)	0.5
3. Within Macrophytes	hypoxic (1.88-5.00)	0.25

initial distribution of prey (see below) allowed predators to re-select habitats based on both the new number of prey and costs. Predators will maximise their net energy gain (choose a habitat based on the benefits minus the costs), thus the quality of the patches were defined as follows:

$$\text{Quality(i)} = \left[\frac{\text{preyhab(i)}}{\text{predhab(i)} + 1}\right] - \text{DOpredcost(i)} \qquad (1)$$

where

$$\text{DOpredcost(i)} = \frac{1}{ae^{bDO(i)}} \qquad (2)$$

Table 3.2 provides a list of definitions of the commonly used symbols for the above and following equations. The first term of the quality equation (Eq. 1) is simply the amount of prey available to the predator when it enters the habitat or the benefits of entering the habitat. This perceived benefit or individual intake rate was determined by the total number of prey in the habitat divided by the number of predators in the habitat plus the individual entering (Abrahams 1986). The quality was offset by the costs associated with being in the particular dissolved oxygen level in the habitat (Eq. 2; Fig. 3.1). The costs were based on the negative allometric relationship of mass specific gill surface area and mass. However, the costs were not dependent on the number of individuals in the habitat as I assumed that the number of individuals did not influence the dissolved oxygen concentration. Lethal levels of dissolved oxygen, the point at which an individual can no longer maintain co-ordinated movement (Gee *et al.* 1978), was set for all predators when dissolved concentration was at 1.0. This was an arbitrary value set where costs of dissolved oxygen were much higher than any benefit of prey consumption. If the habitat is lethal, the predator will select between the remaining patches that are non-lethal and provide the greatest net benefit.

Distribute Prey

After initial distribution of predators was established, prey were removed from their initial condition (distributed in the same proportions as food availability) and then each

Table 3.2. List of commonly used symbols for equations used to calculate the costs and benefits of a patch.

Symbol	Definition
i	patch number (1, 2 or 3)
preyhab(i)	number of prey in patch i
predhab(i)	number of predators in patch i
DO(i)	dissolved oxygen concentration in patch i
DOcostprey(i)	cost of dissolved oxygen for prey in patch i
DOcostpred(i)	cost of dissolved oxygen for predators in patch i
costdiff(i)	difference in cost in patch i
a, b, c	constants

were entered consecutively into the environment. Non-reproducing foragers should choose a habitat that will maximise the ratio of food intake (energetic gain) in each potential habitat to mortality rate (predation rate) (for example Gilliam & Fraser 1987; Moody *et al.* 1996). This relationship was incorporated into the decision rule for prey as the quality of each habitat was defined as:

$$\text{Quality(i)} = \left(\frac{\text{f(i)}}{\mu\text{(i)}}\right) - \text{DOpreycost(i)} \qquad (3)$$

Food intake (f), similar to predators will be based on the amount of food in the patch divided by the individuals in the habitat plus the individual entering (Abrahams 1986). This assumes that food is not depleted in the habitat over time.

The risk of mortality (μ) due to the presence of a predator was calculated as a product of two mortality functions (μ1 and μ2). The first of which was a function of the difference in the costs of dissolved oxygen to predators and prey (Eq. 4).

$$\mu 1\text{(i)} = \text{a(costdiff(i))} - \text{b(costdiff(i))}^2 + \text{c} \qquad (4)$$

I predicted that at extreme levels of hypoxia, predators would be less effective in capturing prey (see Chapter 4 of this thesis). As dissolved oxygen is reduced, the effectiveness of the predator would be more pronounced due to the large physiological costs associated with hypoxia. Energy available for activities such as swimming are often reduced in conditions of hypoxia and subsequently feeding will likely be reduced (Boese 1988; Weber & Kramer 1983). Thus the perceived risk of predation will decrease when the costs of hypoxia are much greater for predator than for prey. To measure this I've calculated the differences between predators and prey costs associated with each dissolved oxygen concentration (costdiff). This difference was incorporated into a probability of death between 0 and 1 so that at extreme hypoxia the difference is large and predation risk is small (Eq. 4). The second mortality function (Eq. 5) accounted for the risk of predation simply due to the number of predators as a probability of being eaten between 0 and 1.

$$\mu 2(i) = 1 - e^{a\left(\frac{predhab(i)}{preyhab(i)+1}\right)} \quad (5)$$

This incorporates the risk dilution effect of an ideal free distribution where the probability of being killed by a predator is inversely proportional to the number of animals feeding in the habitat (Moody *et al.* 1996). I have incorporated this into a relationship where as the ratio of predators to prey approaches one so does the predation risk. In the above mortality rate (Eq. 5) I have assumed that prey are able to assess the number of predators and risk will be a function of the ratio of predator to prey already in the habitat plus the individual entering. A background predation risk (0.001) was arbitrarily chosen for the prey if there were no predators present or if the probability function was less than 0.001.

The costs associated with extracting sufficient oxygen from an environment of varying dissolved oxygen concentrations was defined by the prey cost function (Eq. 6) where only a small relative increase in cost (compared to the predators) occurs at levels of environmental hypoxia (Fig. 3.1).

$$DOpreycost(i) = \frac{1}{ae^{bDO(i)}} \quad (6)$$

Prey lethal dissolved oxygen levels have been incorporated so that an individual will always choose between the remaining non-lethal habitats when dissolved oxygen is equal to or less than 0.5 (this value was also arbitrarily chosen and in accordance to the predicted influence of body size).

Predators and Prey Re-choose

Following the initial distribution of both predator and prey, individuals were allowed to redistribute in order to represent the dynamics of changing distributions under natural conditions or switching (Moody et al. 1996; Abrahams 1986). This was accomplished by randomly removing a single predator and allowing that individual to re-enter the environment again using the above predator decision rule (DistributePredators) to choose a new patch. A single prey was redistributed in the same manner using the prey decision rule (DistributePrey), so that for a single redistribution only one predator and one prey

were removed and allowed to select a new patch. The total number of redistributions was set at 1000; this produced a stable equilibrium (Fig. 3.2).

Output

The mean proportion of individuals and standard error was calculated for each patch (station) number and time interval.

Field Study

To test the model predictions of the distributions of a predator and prey population according to fluctuation dissolved oxygen concentrations a field study was conducted in a marsh environment. Dissolved oxygen levels as well as the distribution of prey and predators were mapped out both spatially and temporally in Blind Channel of the University of Manitoba Field Station, Delta Marsh at the southern tip of Lake Manitoba (Fig. 3.3; $50^0$11 'N, $98^0$23 'W). Five transects were positioned in the channel, two on the north edge and three on the south edge. Along each transect three stations were marked at points at least 2-3 meters from the emergent vegetation in open water (station one), at the edge of the emergent vegetation (station two) and two meters within the emergent vegetation (station three). Within the emergent macrophytes of station three, a circular area of approximately one metre diameter was cut to allow the use of a lift net. Two randomly chosen transects were sampled each day and traps were pulled in a random order for each sample period. Each transect was sampled ten times over a 22 day sampling period (21-24 July, 27 July-6 August, and 8-14 August). Sampling began at approximately 5:00h and continued at 4 hour time intervals with the last sampling period at 17:00h. Exact sampling times were also recorded. The first and last sampling times represent approximate midpoints of lowest and greatest dissolved oxygen concentrations, respectively, within Delta Marsh (Suthers & Gee 1986).

A lift net was designed to obtain samples of fish at each particular time interval. The net itself consisted of a blind ended sac of 3mm mesh, approximately one meter in diameter, and a heavy pipe frame around the lip of the sac to maintain the opening as it was pulled through the water column. A pulley system was used to raise the nets from a distance of 3-4m from the trap. This ensured that fish were not disturbed prior to the sampling period. Small juveniles and larger predators were caught in this type of trap, however minnow traps were also placed at station one and two of each transect at mid-

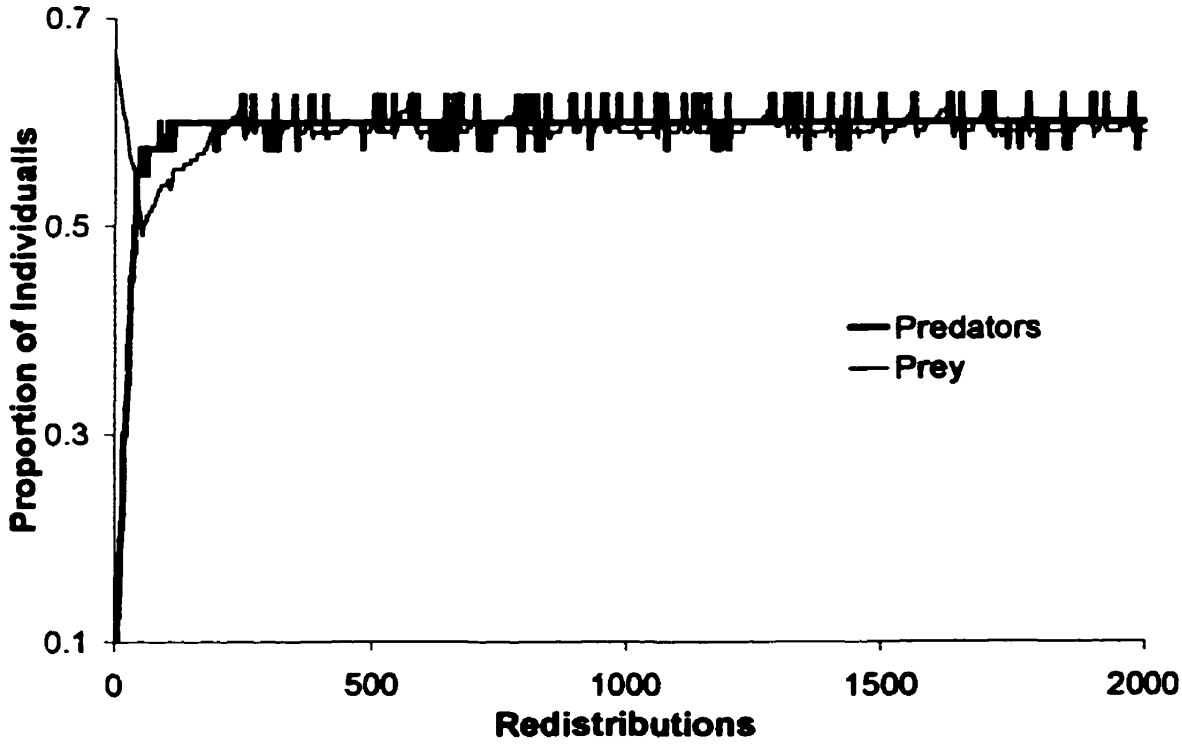


Figure 3.2. The time to equilibrium for the proportion of individuals entering patch two when dissolved oxygen concentrations are most variable between patches (5:00h). Redistributions refers to the number of times animals were allowed to switch (see text for details).

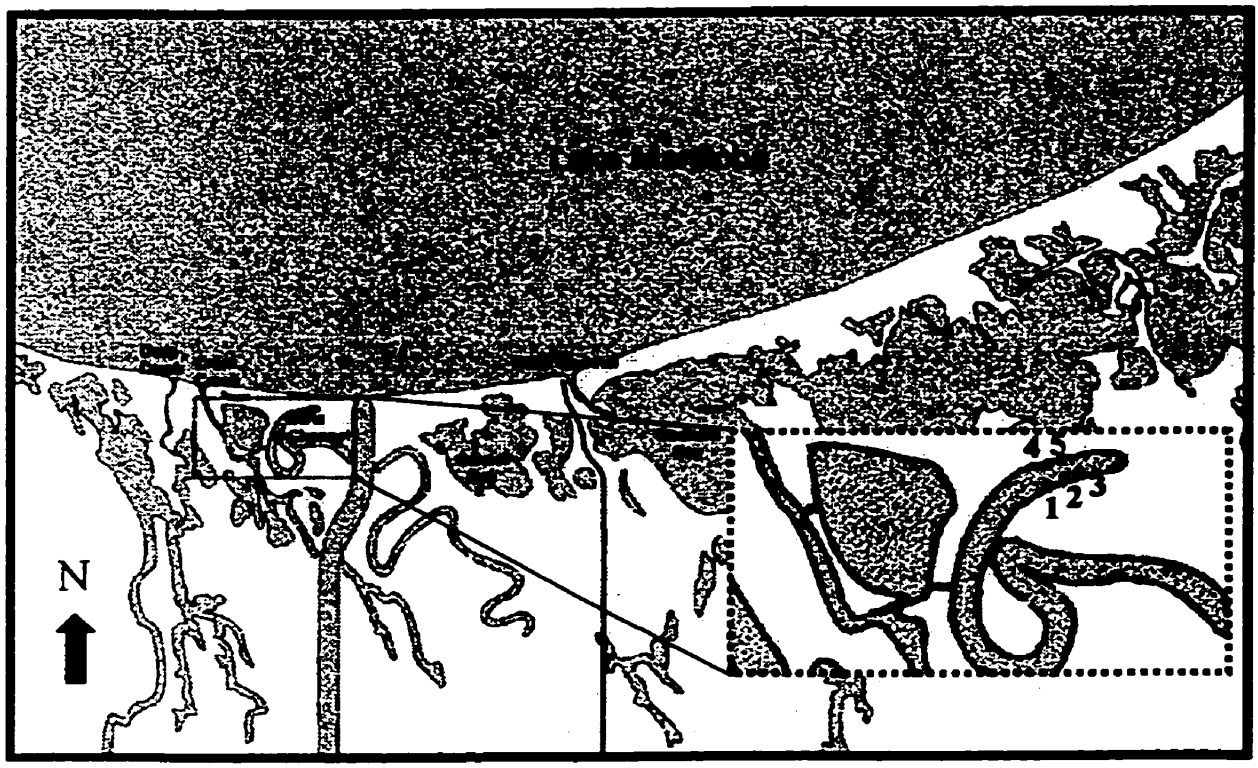


Figure 3.3. Location of field study site. Inset shows the approximate locations of the transects within Blind Channel, Delta Marsh.

water depths to collect larger predators. These traps were checked daily. No minnow traps were placed at station three (within the macrophytes) due to the shallow water. In addition to the minnow traps, gill nets of 3.83cm and 5.08cm mesh size were also placed in the channel to assess predator presence (Courtesy of D. Wrubleski). All individuals collected were first fixed in a 10% Formalin solution and then preserved in 50% Isopropyl alcohol. Length and weight measurements as well as species identification were made on the preserved specimens.

Measurements of dissolved oxygen using a YSI model 55 dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA) were made at each station during each sampling time. Measurements were made near the bottom (approximately 5cm above the substrate), the middle of the water column, and near the surface (approximately 5cm from the water surface). For analysis these measurements were averaged since I assumed the fish moved throughout the water column. The total water depth and water temperature at each station was also measured during each sample period (Appendix 2).

Analysis

To determine the spatial distribution of juvenile fish in relation to station location a three-way ANOVA using computer intensive statistics (CIS) was used. The three factors included the five transects sampled, the four sampling times and station location with corresponding dissolved oxygen level. This method was used because the large number of zero catches created a non-normal distribution. This method of approximate randomisation used the F values from a three way ANOVA as the actual statistic. Data were then randomised (shuffled) a number of times and a new F value was calculated for each new random data set and compared to the actual statistic in order to determine if the actual data was a random event. The number of shuffles (10000) used reflected p (probability of being random) values with a low variation. All means were +/- one standard error unless otherwise noted.

Results

Predicted Distributions

Stabilisation of the predator and prey distributions occurred at approximately 300 redistributions with relatively little fluctuation around the mean subsequent to this (Fig.

3.2). Small changes the proportion of food available to the prey in each of the habitats produced prey distributions that did not change drastically (Table 3.3). However, when drastic changes in food allocation are made (ie. all of the food is in habitat one or two) prey preferentially selected the habitat with the greatest food abundance. The effects of hypoxia were evident in the prey selection of patches, as even when all food was allocated to patch three (hypoxic habitat) prey abundance was less than those found in patch one (Table 3.3)

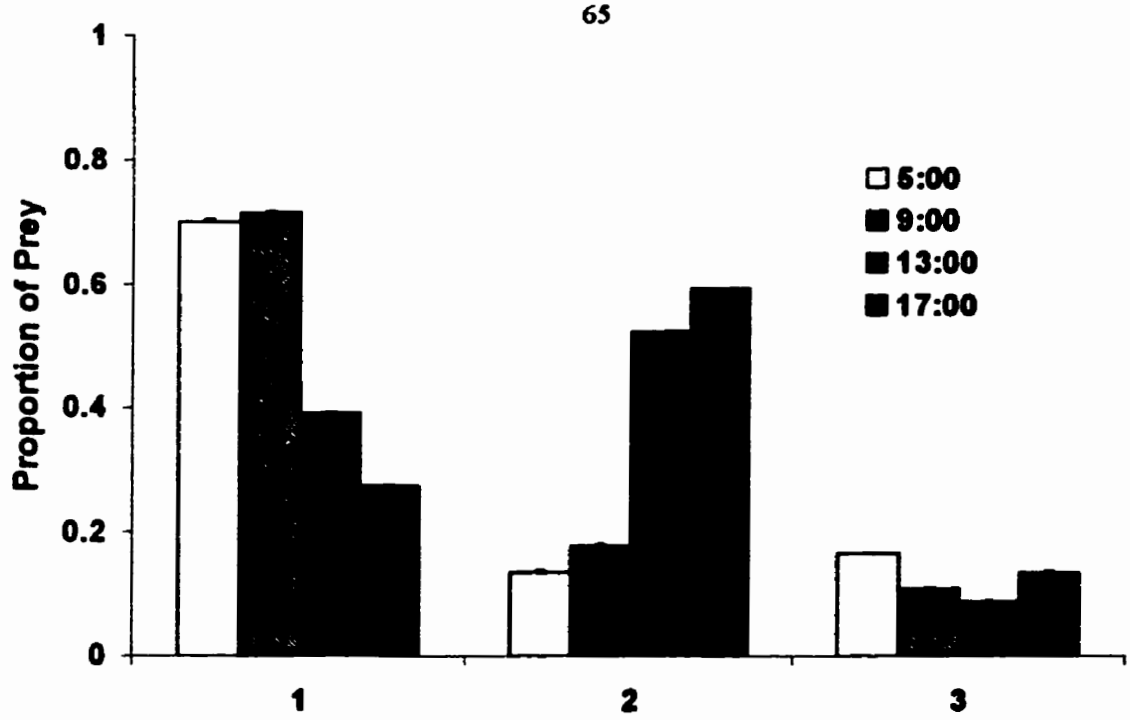
The model prey distribution differed in many respects from an IFD, where it is expected prey to be distributed according to food allocation (Fig. 3.4a). Although habitat two had the greatest food resources prey still selected patch one where dissolved oxygen levels were normoxic at 5:00h and 9:00h. However at later samples (13:00h and 17:00h) more prey than predicted by the IFD continuous input model selected patch two. At these time periods dissolved oxygen levels were higher than earlier and almost equal to patch one (Table 3.1). Prey use of patch three was consistently under the IFD expected proportion of 0.25. These results indicate habitat choice by the prey was influenced by the costs of low dissolved oxygen, despite costs to the prey being much lower than predators.

There were many similarities of the model predator and prey distributions in response to variation in environmental hypoxia despite the apparent cost differential of inhabiting even mildly hypoxic habitats (Fig. 3.4). The predicted prey proportions in habitat three were not found in great abundance but use of this habitat was continuous through all sampling periods with the greatest number found at the lowest dissolved oxygen levels of the day. The influence of time of day and therefore dissolved oxygen levels was most evident in patches one and two where there was a gradual decrease in the number of individuals over time in patch one with a subsequent rise in the number of individuals using patch two (Fig. 3.4). Predators chose habitats where prey were in abundance and a small proportion of predators chose habitats where prey were in smaller numbers. However, as predicted by the size-sensitive relationship, the predator models were restricted from within the hypoxic 'emergent macrophytes' patch at 5:00h and subsequent sample times predicted very few predators inhabiting these costly areas (less than one percent of the total for each sampling period).

Table 3.3. The mean proportion of prey (+/- 1 SE) in each patch in response to variation of food allocation. All other parameters were the same as those selected for the model. Patch one refers to the open water habitat and patch two and three are representative of the edge of and within the macrophytes respectively. (bolded text refers to the model food proportions)

Ratio of Food	**Mean Proportion of Prey**		
patch 1:patch 2:patch 3	**patch 1**	**patch 2**	**patch 3**
0.33 : 0.33 : 0.33	0.64 +/- 0.09	0.24 +/- 0.10	0.12 +/- 0.04
0.5 : 0.25 : 0.25	0.73 +/- 0.06	0.16 +/- 0.07	0.11 +/- 0.02
0.25 : 0.5 : 0.25	**0.52 +/- 0.11**	**0.36 +/- 0.12**	**0.12 +/- 0.02**
0.25 : 0.25 : 0.5	0.60 +/- 0.07	0.21 +/- 0.09	0.19 +/- 0.05
1 : 0 : 0	1	0	0
0 : 1 : 0	0.39 +/- 0.23	0.61 +/- 0.23	0
0 : 0 : 1	0.63 +/- 0.11	0	0.37 +/- 0.11

a)



b)

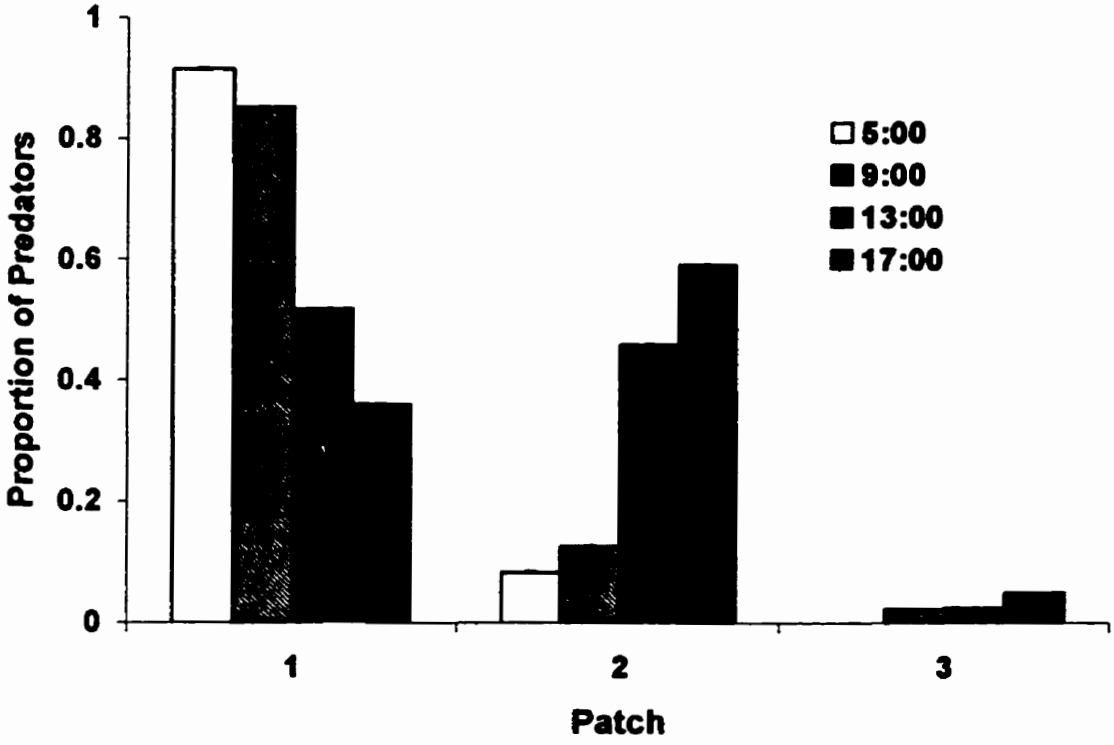


Figure 3.4. Predicted mean (+ 1 SE) proportion of a) prey and b) predators at each patch(1= open water, 2= edge of macrophytes and 3= within macrophytes) and at each time interval.

Field Study

During the course of sampling there were a total of ten species collected, the central mudminnow (*Umbra limi*), carp (*Cyprinus carpio*), white sucker (*Catostomus commersoni*), fresh water drum (*Aplodinotus grunniens*), black bullhead (*Ameiurus melas*), brook stickleback (*Culaea inconstans*), Iowa darter (*Etheostoma exile*), fathead minnow (*P. promelas*), spottail shiner (*N. hudsonius*), and yellow perch (*P. flavescens*). For analysis, spottail shiners and fathead minnows were used as they represented the majority of the prey juvenile species. Yellow perch were used for analysis of a predator population, as they were the most abundant predator species. Over the 22-day sampling period, 325 fathead minnows, 126 spottail shiners and 20 yellow perch were obtained using the lift net style of trap as well as the minnow traps (Table 3.4). Perch are known to become piscivorous when they are as small as 40mm, will consume prey that are most abundant and are often cannibalistic (Craig 1987). Thus the perch collected likely created a significant predation risk to the juvenile minnows. In addition, the number of perch collected within the open water habitat of the marsh using gill nets indicates a significant risk of predation to both the minnows and possibly the smaller perch caught in the lift nets.

Dissolved oxygen varied in the marsh over 12 hours and was greatest overall at station one in the open water (Table 3.5). The dissolved oxygen concentration did not vary greatly throughout the water column although the top measurements were consistently greater than bottom measurements (Table 3.5). As expected the emergent macrophytes provided a hypoxic habitat throughout the sampling period with the lowest dissolved oxygen levels at 5:00h (Table 3.5).

Several qualitative similarities exist between the predictions of the model and the observed distributions of predator and prey that may provide some insight to the underlying principles in determining a predator-prey relationship in this type of an environment. Similar to the predicted distributions, the station location significantly affected the spatial distribution of fathead minnows and spottail shiners (Table 3.6a; Fig. 3.5a). Minnows used the edge of the macrophytes (station two) consistently, however there was some use of the predicted hypoxic habitat within the emergent macrophytes (Fig. 3.5a). In addition, the mean proportion of minnows decreased over time at station

Table 3.4. Summary of mean weights (+/- 1 SE) of three focus species caught in Blind Channel and used for analysis.

Species	Trap type	Mean weight (g)	Mean length (mm)	N
Fathead minnow	lift net	0.15 +/- .03	16.04 +/- 0.56	325
Spottail shiner	lift net	0.1 +/- 0.02	17.25 +/- 1.54	126
Yellow perch	lift net/ minnow trap	4.16 +/- 1.44	61.33 +/- 5.75	20
Yellow perch	1.5in Gill net	69.29 +/- 8.5	*	17
Yellow perch	2.0in Gill net	102.23 +/- 4.61	*	22

* data not available

Table 3.5. Variation in measured dissolved oxygen concentrations (+/- 1 SE) within the water column (+/- 1 SE) at each of the stations (see text for exact locations of measurements).

Station	Time	Dissolved Oxygen Concentration (mg/L)			
		bottom	middle	top	mean
Open Water	5:00	5.06 +/- 0.30	6.09 +/- 0.17	6.20 +/- 0.18	5.74 +/- 0.20
	9:00	5.84 +/- 0.32	6.86 +/- 0.21	7.03 +/- 0.03	6.54 +/- 0.23
	13:00	8.21 +/- 0.42	9.41 +/-0.28	9.92 +/- 0.20	9.10 +/- 0.29
	17:00	10.38 +/- 0.27	11.15 +/-0.24	11.51 +/- 0.25	10.98 +/- 0.24
Edge of Macrophytes	5:00	4.45 +/- 0.29	5.63 +/- 0.16	5.74 +/- 0.16	5.23 +/- 0.19
	9:00	5.39 +/- 0.32	6.47 +/- 0.20	6.68 +/- 0.20	6.13 +/- 0.23
	13:00	7.57 +/- 0.42	9.08 +/-0.19	9.38 +/- 0.20	8.60 +/- 0.26
	17:00	9.42 +/- 0.30	10.47 +/- 0.25	10.79 +/- 0.26	10.17 +/- 0.27
Within Macrophytes	5:00	1.76 +/- 0.19	2.40 +/- 0.22	2.70 +/- 0.24	2.27 +/- 0.21
	9:00	2.45 +/- 0.22	3.08 +/- 0.26	3.34 +/- 0.27	2.94 +/- 0.25
	13:00	3.29 +/- 0.35	4.53 +/- 0.38	4.88 +/- 0.38	4.20 +/- 0.35
	17:00	4.18 +/- 0.38	5.01 +/- 0.40	5.50 +/- 0.39	4.88 +/- 0.38

Table 3.6. Summary of 3-way ANOVA using CIS (10000 shuffles) for a) fathead minnows and spottail shiners and b) yellow perch.

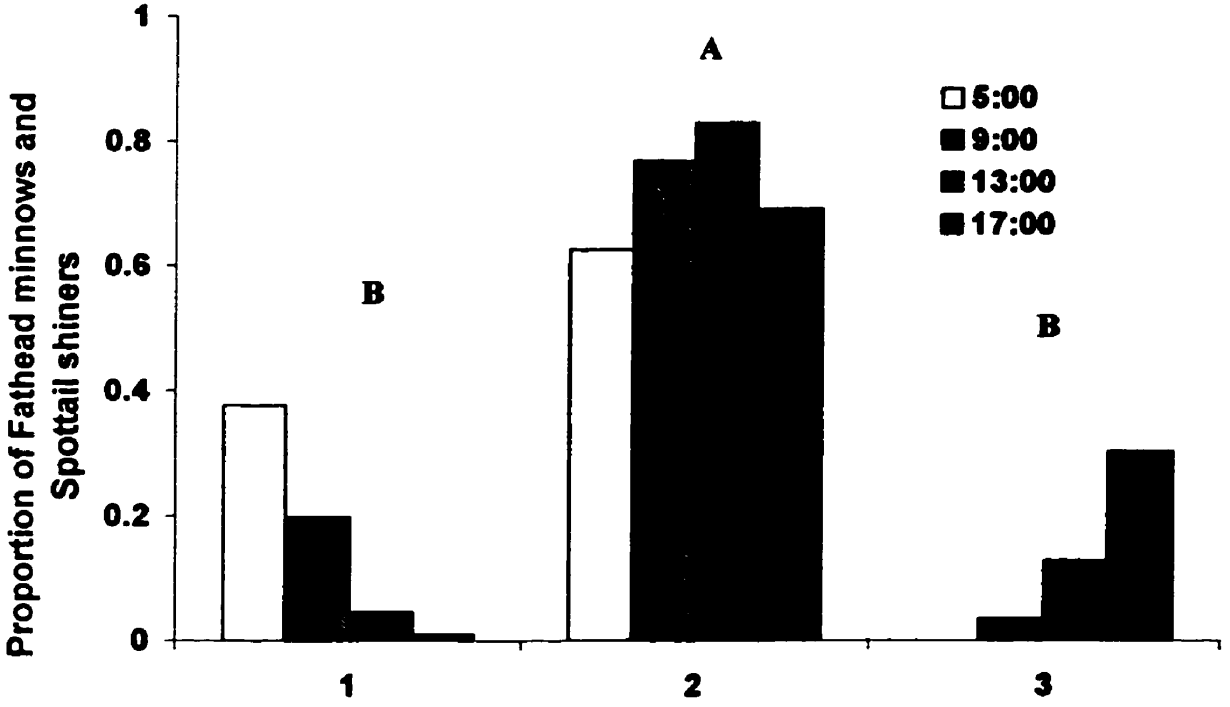
a)

Source of variation	df	Actual F value	p
Transect	4	0	1
Time	3	0	1
Station	2	39.678	**0.001**
Transect*Time	12	0	1
Transect*Station	8	1.054	0.433
Time*Station	5	4.163	**0.012**

b)

Source of variation	df	Actual F value	p
Transect	4	0.483	0.723
Time	3	2.835	**0.045**
Station	2	6.3	**0.007**
Transect*Time	12	0.741	0.689
Transect*Station	8	2.385	**0.049**
Time*Station	5	1.403	0.232

a)



b)

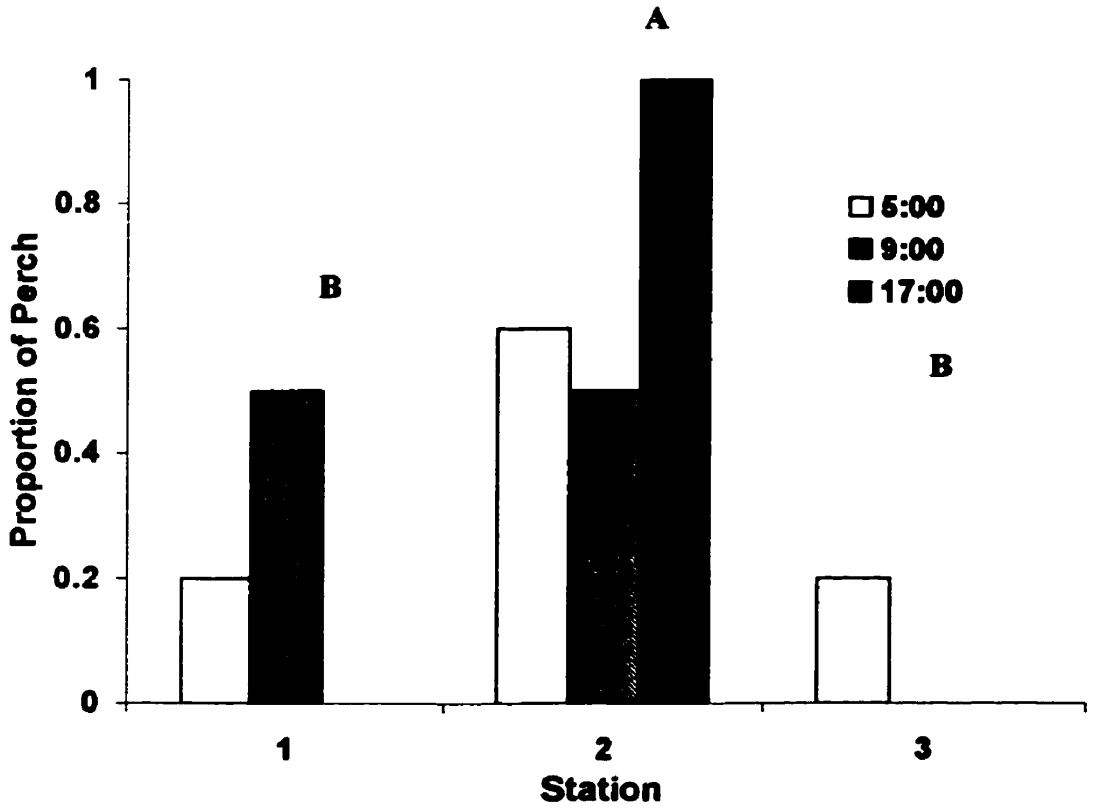


Figure 3.5. a) Combined proportion of fathead minnows and spottail shiners and the b) the proportion of perch caught at each station (1= open water, 2= edge of macrophytes and 3= within macrophytes) at each time interval sampled. (A significantly different from B at $p < 0.05$)

one while they increased at station three indicative of the strong time interaction (Table 3.6a). The proportion of minnows caught at station three increased through time with the greatest proportion of prey found at 17:00h, unlike the predicted distribution where minnows were expected to use the hypoxic habitat consistently over time. The most notable difference between predicted and actual distributions is the lack of time effect in station two of the actual distribution as well as the lack of minnows caught at station three at 5:00h (Fig. 3.4a and 3.5a). In addition the model predicted a larger proportion of predators and prey in the open water habitat than that observed in the field (Fig. 3.4 and 3.5).

The mean proportion of perch caught was also influenced by the station location (Table 3.6b; Fig. 3.5b). This observed distribution was similar to the model predictions in the small proportion of predators within the hypoxic emergent macrophytes (Fig. 3.4b and 3.5b). However the distribution of perch did not gradually decrease from habitat one and increase in the edge habitat as predicted by the model. Actual perch distributions matched that of the minnow distribution with the greatest proportion of perch caught at station two where dissolved oxygen levels ranged from hypoxic to normoxic. Perch were caught at station three on only one occasion and this was during the early morning sample when no minnows were collected at this time. Time of day also influenced the number of perch collected, as there were no perch caught at 13:00h. As well there was a significant interaction between transect and time (Table 3.6b).

The distribution of all species (fathead minnow, spottail shiner and yellow perch) was found to be dependent on the mean dissolved oxygen concentration (Fig. 3.6). Both fathead minnow and spottail shiner were caught from habitats in which environmental dissolved oxygen concentrations ranged between 0 mg/L and 14 mg/L. This distribution was significantly different from the range of dissolved oxygen concentration from which the perch were caught ($G= 20.42$, $df= 6$, $p< 0.05$). The distribution of perch was restricted from dissolved oxygen concentrations less than 2 mg/L (Fig. 3.6).

The weight of the minnows collected from the marsh was independent of the mean dissolved oxygen level (minnow $G=11.58$, $df= 6$, $p> 0.05$; shiner $G= 4.35$, $df= 6$, $p> 0.05$). However a majority of the minnows (315 of 325) and shiners (125 of 126) were in size classes less than one gram. Perch size classes ranged from less than 1 to 29g

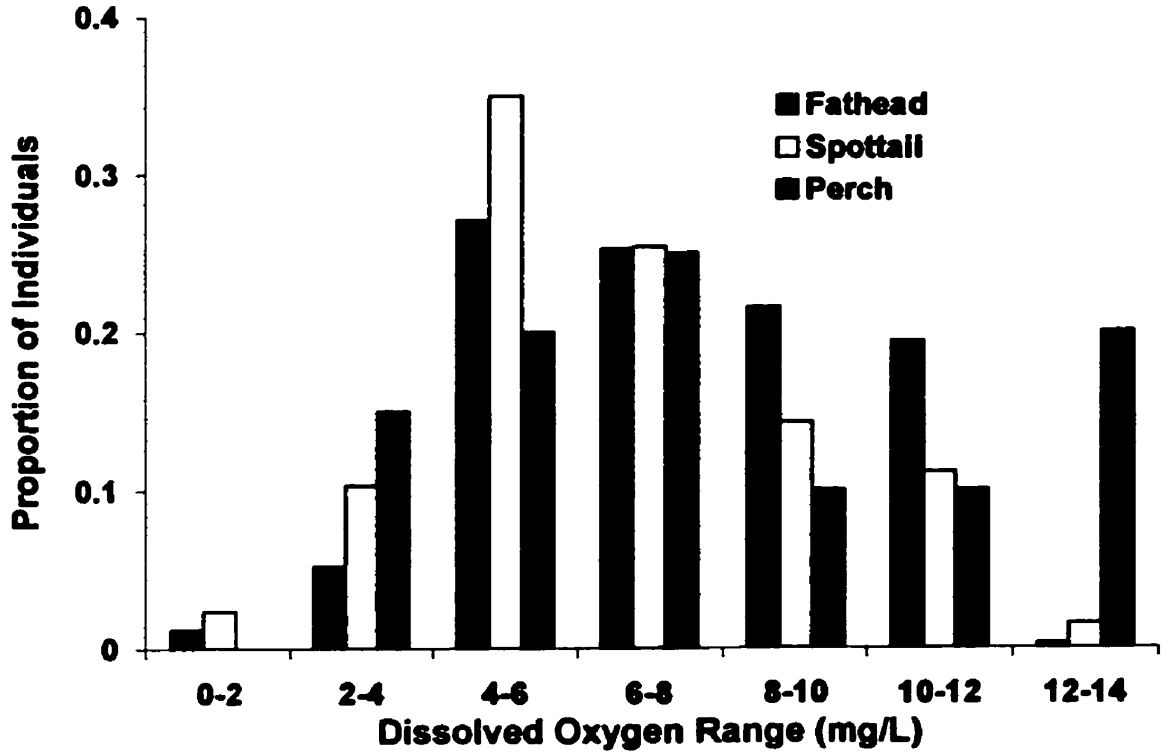


Figure 3.6. Proportion of individuals caught according to dissolved oxygen levels in the marsh. A G-test of independence found distributions to be dependent on the dissolved oxygen range ($G = 34.35$, $df = 12$, $p < 0.01$)

with a majority of individuals weighing greater than 2g (13 of 20). Dissolved oxygen did not influence the distribution of perch size classes sampled ($G= 1.90$, $df= 6$, $p> 0.05$).

Discussion

Predation pressure as well as the effects of limiting dissolved oxygen and food availability in Delta Marsh appeared to influence the distribution of both predator and prey populations studied. The costs associated with a low dissolved oxygen environment were evident in the minnow and perch distribution as only a small proportion of either species were found within the macrophytes as predicted by the model. However, the consistent use of this habitat by the minnows with increasing use over time also suggested predation risk limited choice of alternative higher dissolved oxygen habitats. Although food availability was not measured in the field study, it was likely a major determinant of habitat choice by the juvenile minnows. As predicted by the high costs associated with hypoxia, predators avoided the extremely low dissolved oxygen levels. In addition the benefits of greater proportions of prey in the 'open water' habitat predicted that the largest proportion of predators would occur in this habitat.

The combined effects of predation pressure and fluctuations in dissolved oxygen appeared to influence the distribution of fathead minnows and spottail shiners in the Delta marsh. This was also apparent in the predicted distribution whereby, despite the costs of low dissolved oxygen, prey consistently used the hypoxic habitat. The gradual shift of minnows from the open water environment to the emergent macrophytes indicated a preference for the higher cost hypoxic but relatively predator-free habitat over the normoxic habitat. The ability of prey to tolerate this more stressful environment that predators cannot endure will create temporal and spatial refuges from predators (Meffe 1984).

During the winter, ice covered lakes result in a reduction of dissolved oxygen levels which has been found to influence the distribution of over wintering fish populations (Fox & Keast 1990; Tonn & Paszkowski 1987; Tonn & Paszkowski 1986). Tonn & Paszkowski (1987) found perch to be located in offshore regions where dissolved oxygen levels are often the greatest during the winter ice cover. However, their primary prey item in this particular lake, the central mudminnow (*Umbra limi*), were concentrated inshore as a result of the predation pressure and because of their ability to tolerate much

lower dissolved oxygen concentrations (Tonn & Paszkowski 1987; Klinger *et al.* 1982). Invertebrates also have varying tolerances to low dissolved oxygen, which influence the community structure during episodic and seasonal hypoxic events (Kolar & Rahel 1993). The influence of both predation and low dissolved oxygen caused invertebrates vulnerable to these stresses to remain longer in hypoxic waters that are absent of predatory fish (Kolar & Rahel 1993; Rahel & Kolar 1990). The length of time spent in these hypoxic habitats was attributed to their tolerance to hypoxia as well as the prey's vulnerability to predation under normoxic conditions. The hypoxic habitat not only provides a predator free refuge but also piscine predators are more likely to be less effective at prey capture in this costly environment (Poulin *et al.* 1987).

The use of emergent macrophytes by the prey may have also been due to the complexity of the environment. The structural complexity of the emergent vegetation can also provided a refuge, in which larger predators are unable to manoeuvre (Chapman *et al.* 1996a; Eadie & Keast 1984; Heck & Thoman 1981). Heck & Thoman (1981) found predation rates on seagrass invertebrates to be much lower in dense stands of vegetation. A positive relationship between the structural complexity and species richness was also found in Lake Nabugabo (Chapman *et al.* 1996a). This would also explain the discrepancy between the model and field proportions of predator and prey in the open water habitat. The model did not incorporate the benefits of cover thus predicting greater use of the open water habitat.

The large proportions of the minnows found and predicted to be at the edge of the macrophytes suggest that this habitat provided sufficient food availability and low costs associated with dissolved oxygen. Although the structurally complex habitat of the cattails may provide an attractive habitat for small juvenile fish (Eadie & Keast 1984) the high densities of the emergent macrophytes may also limit the swimming capabilities of prey and therefore their use of it (Crowder & Cooper 1982). The sometimes extreme hypoxia (less than 2 mg/L) experienced in this habitat will also likely deter its use. This was further exemplified by prey use of habitat three below that predicted by the IFD in the model and field study. In addition, food availability may have been more abundant in habitat two as zooplankton is often found to be in greater abundance at the edge of complex habitats and shorelines compared to open water habitats (Abrahams unpublished

data; Price *et al.* 1991; Elgmork 1964). However to avoid the predation pressures minnows may have made trips into the emergent vegetation. In determining feeding sites the ability to escape risk into protective cover has been implicated as a major determinant in habitat selection by prey (see Lima & Dill 1990). When prey are relatively mobile, they will avoid predators via a habitat shift to an obvious refuge where capture success is reduced (Sih 1984). The food abundance and high survival are all characteristics of a high quality habitat and indeed the emergent vegetation would provide this when the risk of predation increases in other habitats (Werner *et al.* 1977). The use of the edge may therefore be more beneficial as dissolved oxygen levels are not as low, and the ability to search for food may not be compromised by the high structural complexity.

The large proportion of minnows using the edge habitat with dissolved oxygen ranging from mildly hypoxic to normoxic also suggests minnows were limited by hypoxia. This was further supported by distinct avoidance of extreme hypoxia during the early morning hours by the juvenile minnows, although this was not observed in the predicted distributions. Suthers & Gee (1986) found similar results with seasonal and diurnal movements of juvenile perch. They found these changes in distribution were as a result of severe hypoxia as well as growth of alternative cover in the open water habitat providing a complex habitat with little cost. The reduction in energy available for growth and development also may limit the distribution of fish species when there is a reduction in environmental dissolved oxygen (van Dam & Pauly 1995; Brett & Groves 1979). Juvenile guppies prevented from reaching surface oxygen to perform ASR during hypoxic events were limited in growth, apparently due to reduced feeding rates (Weber & Kramer 1983).

Predators were found in similar proportions to the minnows in both open water and edge habitats as predicted by net benefits. However they were not found in any extreme hypoxic (less than 2 mg/L) environments and only once was a single perch found within the macrophytes. It is quite possible food availability was sufficient at the edge and open water, however it is more likely that perch were unable to inhabit these hypoxic waters for any length of time (Chapter two of this thesis). Adult striped bass, *Morone saxatilis*, were commonly found in habitats characterised by high dissolved oxygen and low temperatures and appeared to be restricted from areas of higher temperatures (and

subsequent reduced dissolved oxygen) even when prey densities were abundant in these latter habitats (Coutant 1985). Variation in tolerance to low dissolved oxygen will influence not only predator-prey relationships through habitat shift but also the swimming and feeding behaviour of the predators. The lower tolerance of hypoxia by the striped bass, *Morone saxatilis*, than its predatory counterpart the sea nettle, *Chrysaora quinquecirrha*, influenced the variation of predation rates on fish larvae and eggs (Breitburg *et al.* 1997). The reduced attack rate by the sea bass was attributed to its reduction of activity levels at low dissolved oxygen levels.

The single event of perch being caught within the emergent macrophytes, a costly hypoxic habitat, could be best explained by the use of hypoxic dives. A low tolerance to dissolved oxygen does not always exclude individuals from hypoxic habitats as predatory fish and invertebrates have been found to make use of these potentially lethal environments (Rahel & Nutzman 1994; Pihl *et al.* 1992). Brief feeding forays into hypoxic habitats or hypoxic dives have been documented by predatory fish such as the hogchoker, *Trinectes maculatus* (Pihl *et al.* 1992). These predatory behaviours are characterised by individuals spending most of their time in normoxic conditions but occasionally making trips into lethal hypoxic regions for feeding on invertebrates (Pihl *et al.* 1992). Rahel & Nutzman (1994) also documented the use of hypoxic dives by the central mudminnow foraging in severely hypoxic bottom habitats on the phantom midge larvae (Diptera: *Chaoborus*). The time spent diving and the number of dives will vary on the ability to tolerate hypoxic conditions as well as the energy rewards. If prey abundance is low in areas of normoxia more time may be spent foraging in oxygen depleted areas (Rahel & Nutzman 1994).

Changing habitat preference in response to increase risk of predation is a common strategy for many freshwater fish (Brown & Moyle 1991; Werner *et al.* 1983; Werner *et al.* 1977). However, this habitat shift often has costs associated with it such as increase competition or reduction of food availability. Variation in dissolved oxygen concentrations can further influence this relationship by creating distinct physiologically stressful hypoxic habitats. By virtue of their small size, the findings of this study support the suggestion that prey species are able to take advantage of these stressful habitats in which larger predators are unable to tolerate. These relatively low cost and short term

refuges will allow juveniles such as the fathead minnow and spottail shiner to evade predation during their development. Thus the influence of a marsh habitat or any area with sufficient plant material to lower dissolved oxygen concentrations significantly becomes important in maintaining fish community structure. This is further exemplified by the findings of several prey species of fish in wetland ecotones characterised by hypoxia and high structural complexity, thought to be extinct after the introduction of the hypoxia intolerant predatory Nile perch (Chapman *et al* 1996a; Chapman *et al* 1996b).

Literature Cited

Abrahams, M.V. 1986. Patch choice under perceptual constraints: a cause for departures from an ideal free distribution. Behav. Ecol. Sociobiol. **19**: 409-415.

Abrahams, M.V. & Dill, L.M. 1989. A determination of the energetic equivalence of the risk of predation. Ecology **70(4)**: 999-1007.

Boese, B.L. 1988. Hypoxia-induced respiratory changes in English sole (*Parophrys vetulus* Girard). Comp. Biochem. Physiol. **89A**: 257-260.

Brazner, J.C. & Beals, E.W. 1997. Patterns in fish assemblages from coastal wetland and beach habitats in Green Bay, Lake Michigan: a multivariate analysis of abiotic and biotic forcing factors. Can. J. Fish Aquat. Sci. **54**: 1743-1761.

Breitburg, D.L., Loher, T., Pacey, C.A. & Gerstein, A. 1997. Varying effects of low dissolved oxygen on trophic interaction in an estuarine food web. Ecol. Mono. **67(4)**: 489-507.

Brett, J.R. & Groves, T.D.D. 1979. Physiological energetics. IN Fish Physiology Volume 8: Bioenergetics and Growth. eds W.S. Hoar, D.J. Randall & J.R. Brett. Academic Press, New York. pp. 229-352.

Brown, L.R. & Moyle, P.B. 1991. Changes in habitat and microhabitat partitioning within an assemblage of stream fishes in response to predation by Sacramento Squawfish (*Ptychocheilus grandis*). Can. J. Fish. Aquat. Sci. **48**:849-856.

Chapman, L.J. & Liem, K.F. 1995. Papyrus swamps and the respiratory ecology of *Barbus neumayeri*. Env. Biol. Fishes **44**:193-197.

Chapman, L.J., Chapman, C.A., & Chandler, M. 1996a. Wetland ecotones as refugia for endangered fishes. Biol. Conserv. **78**:263-270.

Chapman, L.J., Kaufman, L.S., Chapman, C.A., & McKenzie, F.E. 1995. Hypoxia tolerance in twelve species of East African Cichlids: Potential for low oxygen refugia in Lake Victoria. Conserv. Biol. **5**:1274-1288.

Chapman, L.J., Chapman, C.A., Ogutu-Ohwayo, R., Chandler, M., Kaufman, L.S., & Keiter, A.E. 1996b. Refugia for endangered fishes from an introduced predator in Lake Nabugabo, Uganda. Conserv. Biol. **10**: 554-561.

Clady, M.D. 1976.Decline in the abundance and survival of three benthic fishes in relation to reduced oxygen levels in a eutrophic lake. Am. Mid. Nat. **97(2)**: 419-432.

Coutant, C.C. 1985. Striped bass, temperature, and dissolved oxygen: A speculative hypothesis for environmental risk. Trans. Amer. Fish. Soc. **114**: 31-61.

Craig, J.F. 1987. The Biology of Perch and Related Fish. Beckenham, Croom Helm.

Crowder, L.B. & Cooper, W.E. 1982. Habitat structural complexity and the interaction between bluegills and their prey. Ecology **63**: 1802-1813.

Davenport, J. & Sayer, M.D.J. 1993. Physiological determinants of distribution in fish. J. Fish Biol. **43(Supplement A)**: 121-145.

Doudoroff, P. & Shumway, D.L. 1970. Dissolved oxygen requirements of freshwater fishes. Food and Agriculture Organization of the United Nations. Technical Paper 86. 291p.

Eadie, J. McA.& Keast, A. 1984. Resource heterogeneity and fish species diversity in lakes. Can. J. Zool. **62**: 1689-1695.

Elgmork, K. 1964. Dynamics of zooplankton communities in some small inundated ponds. IN Folia Limnologica Scandinavica. eds K. Berg, H. Järnefelt, K. Strom & S. Thonmark. Norwegian Research Council for Scienc and the Humanities, Norway. no. 12.

Fox, M.G. & Keast, A. 1990. Effects of winterkill on population structure, body size and prey consumption patterns of pumpkinseed in isolated beaver ponds. Can. J. Zool. **68**: 2489-2498.

Fretwell, S.D. & Lucas Jr., H.L. 1970. On territorial behavior and other factors influencing habitat distribution in birds. Acta. Biotheor. **19**: 16-36.

Gee, J.H., Tallman, R.F. & Smart, H.J. 1978. Reactions of some great plains fishes to progressive hypoxia. Can. J. Zool. **56**:1962-1966.

Gilliam, J.F. & Fraser, D.F. 1987. Habitat selection under predation hazard: Test of a model with foraging minnows. Ecology **68(6)**: 1856-1862.

Heck, Jr. K.L., & Thoman, T.A. 1981. Experiments on predator-prey interactions in vegetated aquatic habitats. J. Exp. Mar. Biol. Ecol. **53**:125-134.

Holeton, G.F. 1980. Oxygen as an environmental factor of fishes. IN Environmental Physiology of Fishes. eds M.A. Ali. Plenum, New York. pp. 7-32.

Klinger, S.A., Magnuson, J.J., & Gallepp, G.W. 1982. Survival mechanisms of the central mudminnow (*Umbra limi*), fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) for low oxygen in winter. Env. Biol. Fish **7(2)**: 113-120.

Kolar, C.S. & Rahel, F.J. 1993. Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia **95**: 210-219.

Kramer, D.L. 1987. Dissolved oxygen and fish behavior. Env. Biol. Fish. **18(2)**: 81-92.

Kramer, D.L. & Mehegan, J.P. 1981. Aquatic surface respiration, an adaptive response to hypoxia in the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). Env. Biol. Fish **6(3/4)**: 299-313.

Lima, S.L. & Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68**: 619-640.

Meffe, G.K. 1984. Effects of abiotic disturbance on coexistence of predator-prey fish species. Ecology **65(5)**: 1525-1534.

Moody, A.A., Houston, A.I. & McNamara, J.M. 1996. Ideal free distributions under predation risk. Behav. Ecol. Sociobiol. **38**: 131-143.

Palzenberger, M. & Pohla, H. 1992. Gill surface area of water-breathing freshwater fish. Rev. Fish Biol. Fish. **2**:187-216.

Petersen, J.K. & Petersen, G.I. 1990. Tolerance, behaviour and oxygen consumption in the sand goby, *Pomatoschistus minutus* (Pallas), exposed to hypoxia. J. Fish Biol. **37**: 921-933.

Petrosky, B.R. & Magnuson, J.J. 1973. Behavioral responses Of Northern Pike, Yellow Perch and Bluegill to oxygen concentrations under simulated winterkill conditions. Copeia **1**:124-133.

Pihl, L., Baden, S.P., Diaz, R.J., & Schaffner, L.C. 1992. Hypoxia-induced structural changes in the diet of bottom-feeding fish and Crustacea. Marine Biology **112**: 349-361.

Poulin, R., Wolf, N.G., & Kramer, D.L. 1987. The effect of hypoxia on the vulnerability of guppies (*Poecilia reticulata*, Poeciliidae) to an aquatic predator (*Astronotus ocellatus*, Cichlidae). Env. Biol. Fish **20(4)**: 285-292.

Powers, D.A. 1980. Molecular ecology of teleost fish hemoglobins: Strategies for adapting to changing environments. Amer. Zool. **20**: 139-162.

Price, C.J., Tonn, W.M., & Paszkowski, C.A. 1991. Intraspecific patterns of resource use by fathead minnows in a small boreal lake. Can. J. Zool. **69**: 2109-2115.

Rahel, F.J. & Nutzman, J.W. 1994. Foraging in a lethal environment: Fish predation in hypoxic waters of a stratified lake. Ecology **75(5)**: 1246-1253.

Rahel, F.J. & Kolar, C.S. 1990. Trade-offs in the response of mayflies to low oxygen and fish predation. Oecologia **84**: 39-44.

Sih, A. 1984. The behavioral response race between predator and prey. Am. Nat **123**: 143-150.

Suthers, I.M. & Gee, J.H. 1986. Role of hypoxia in limiting diel spring and summer distribution of juvenile Yellow Perch (*Perca flavescens*) in a prairie marsh. Can. J. Fish. Aquat. Sci. **43**: 1562-1570.

Tonn, W.M. & Paszkowski, C.A. 1987. Habitat use of the central mudminnow (*Umbra limi*) and yellow perch (*Perca flavescens*) in *Umbra-Perca* assemblages: the roles of competition, predation, and the abiotic environment. Can. J. Zool. **65**: 862-870.

Tonn, W.M. & Paszkowski, C.A. 1986. Size-limited predation, winterkill, and the organization of *Umbra-Perca* fish assemblages. Can. J. Fish Aquat. Sci. **43**: 194-202.

van Dam, A.A. & Pauly, D. 1995. Simulation of the effects of oxygen on food consumption and growth of Nile tilapia, *Oreochromis niloticus* (L.). Aqua. Res. **26**: 427-440.

Weber, J.M. & Kramer, D.L. 1983. Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, *Poecilia reticulata*. Can. J. Fish Aquat. Sci. **40**: 1583-1588.

Werner, E.E. & Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am. Nat. **142(2)**: 242-272.

Werner, E.E., Gilliam, J.F., Hall, D.J. & Mittelbach, G.G. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology **64(6)**: 1540-1548.

Werner, E.E., Hall, D.J., Laughlin, D.R., Wagner, D.J., Wilsmann, L.A. & Funk, F.C. 1977. Habitat partitioning in a freshwater community. J. Fish. Res. Board Can. **34**: 360-370

Wolf, N.G. & Kramer, D.L. 1987. Use of cover and the need to breathe: the effects of hypoxia on the vulnerability of dwarf gouramis to predatory snakeheads. Oecologia **73**:127-132.

Wright, D. & Shapiro, J. 1990. Refuge availability: a key to understanding the summer disappearance of *Daphnia*. Fresh. Biol. **24**: 43-62.

CHAPTER IV

THE INFLUENCE OF HYPOXIA AND RISK OF PREDATION ON HABITAT SELECTION BY THE FATHEAD MINNOW, *Pimephales promelas*

Introduction

The role of predators in an ecosystem can be direct through the consumption of prey and therefore reducing prey densities. However, the presence of a predator has been found to have far greater indirect effects by altering the behaviour of the prey which subsequently plays an important role in the patterns of habitat use (Sih 1987; Mittelbach 1984; Werner *et al.* 1983; Sih 1982). Prey should choose a habitat that will maximise their net benefits, incorporating the costs and benefits of the presence of a predator (Lima & Dill 1990; Abrahams & Dill 1989; Gilliam & Fraser 1987). From the perspective of the prey choosing to stay in habitats of high predation risk, the primary cost will be an increased risk of mortality (Lima & Dill 1990). However, with the costs may come benefits such as increased net rate of energy intake (Abrahams & Dill 1989). Individuals unwilling to incur this risk must go to alternative patches that have less food or food of poorer quality (Mittelbach & Chesson 1987; Sih 1987).

Several characteristics of prey have been implicated in altering the magnitude of predation risk and thereby influencing the decision making process in response to a predator. These include hunger level (Godin & Crossman 1994; Dill & Fraser 1984), reproductive status (see Lima & Dill 1990), body size (Werner & Gilliam 1984), sex (Abrahams & Dill 1989) and possession of body amour (Abrahams 1995; McLean & Godin 1989). In an aquatic system, several environmental factors such as light level (see Lima & Dill 1990), turbidity (Abrahams & Kattenfeld 1997; Miner & Stein 1996) and dissolved oxygen (McIntyre & McCollum 2000; Poulin *et al.* 1987; Wolf & Kramer 1987; Kramer *et al.* 1983) will also influence prey habitat selection in the presence of a predator.

The influence of the reduction of environmental dissolved oxygen (hypoxia) on predator avoidance is unique, as teleosts often avoid the physiological costs of hypoxia in favour of more normoxic waters (Suthers & Gee 1986; Doudoroff & Shumway 1970). Several studies have found a large degree of variation in tolerance of dissolved oxygen that often results in segregation of fish communities (Smale & Rabeni 1995; Tonn & Paszkowski 1987; Gee *et al.* 1978; Doudoroff & Shumway 1970). Therefore, prey that possess a greater physiological tolerance of hypoxia than predators will have an ecological advantage in use of hypoxic habitats intolerable to predators. The refuge

would be defined by the difference between the predator and prey's lowest tolerable limits to dissolved oxygen. Within this refuge prey would presumably be free to choose habitats of greatest net benefits (greatest energy intake or mating attempts) without the added costs of predation risk. Recently it has been demonstrated that the fathead minnow, *Pimephales promelas*, has a greater tolerance of hypoxia than the predatory yellow perch, *Perca flavescens* (Chapter two of this thesis). Based on these findings it was suggested that in an environment of fluctuating dissolved oxygen, prey would be able to use a hypoxic habitat as a perch-free refuge.

In addition to physiological exclusion, at or near the critical dissolved oxygen level, fish exhibit behaviours that are not conducive to feeding (Weber & Kramer 1983; Petit 1973; Doudoroff & Shumway 1970). Behaviours in response to a reduction of dissolved oxygen include an increase in activity in order to find more favourable waters as well as increased use of oxygen rich surface waters (aquatic surface respiration, ASR) (Kramer 1983; Gee *et al.* 1978). In addition, at critical levels, activity is often decreased in order to reduce oxygen demand. Therefore, predators less tolerant of hypoxia will exhibit behavioural modifications at greater dissolved oxygen concentrations than the more tolerant prey. Subsequently prey should be able to take further advantage of moderately hypoxic environments stressful to predators in order to reduce the magnitude of the predation risk.

It is known that prey are able to assess risk of predation based on the behaviour of their predators through predator inspection behaviour (see Dugatkin & Godin 1992). Although this is a costly behaviour due to increased risk of mortality, prey can obtain valuable information about the predator such as satiation (Pitcher *et al.* 1986). Upon assessing risk, prey can subsequently make a decision based on the perceived risk of predation. If the threat is judged to be real then prey can react with antipredator tactics to reduce the risk or, if judged as not dangerous, then prey can resume normal activities (Dugatkin & Godin 1992). Predators performing one or more of the above behavioural responses to hypoxia would probably be judged to be an unlikely threat to prey. Therefore, the physiological exclusion from a habitat may not be necessary to generate a refuge free of predation risk. We would expect prey to sense predators that are stressed due to hypoxic conditions and integrate this into a decision making process of habitat

choice. To test this prediction, it was determined if the role of predators in the habitat selection by prey declines in waters in which environmental dissolved oxygen is stressful to the predators but not the prey.

Methods

Study Animals

The fathead minnows and yellow perch used as prey and predator respectively in this study were collected using minnow traps in September of 1997 and 1998. Both species were collected from Delta Marsh at the University of Manitoba Field Station at the southern tip of Lake Manitoba ($50^0$11 'N, $98^0$23 'W). Minnows were held in a 200 litre aquarium, fed Nutrafin flakes and kept at room temperature (approximately 22^0C) and at a photoperiod of 12 hours light:12 hours dark. Four yellow perch of similar size (Table 4.1) were held individually in 50 litre aquaria and fed maintenance rations of trout pellets and live minnows. They were held at the same water temperature and photoperiod as the minnows. Three days prior to experiment, four groups of six similar sized minnow (Table 4.1) were randomly chosen. Groups were housed separately for the duration of the experiment in 20 litre aquaria. During this time the minnows were fed maintenance rations of frozen brine shrimp (*Artemia* sp) using the same feeding protocol used in the trials.

Experimental Protocol

Trials were set up to test the relative risk taken by minnows at hypoxic and normoxic conditions. Under normoxic conditions and continuous food input, feeding is expected to follow an ideal free distribution (IFD) as described by Fretwell & Lucas (1970). Under these conditions individuals will distribute according to the food available in the habitat as determined by net benefits. This assumes that each individual has an ideal knowledge of the food allocation and that each individual is free to enter any habitat. Therefore any deviations from the IFD when a predator was present should illustrate the response of the minnows to the predator presence (see Abrahams & Dill (1989) for a description of this technique).

The experimental apparatus consisted of a 40 litre aquarium that was divided into two sections by placing a transparent plexiglass divider 23 cm from the edge of the tank (Fig. 4.1). In the smaller section one yellow perch was placed behind the plexiglass to

Table 4.1. Mean wet weights (g) (+/- 1 SE) of the fathead minnow groups (n= 6 for each group) and wet weights (g) of the yellow perch used in the study.

Group	Fathead Minnow	Yellow Perch
1	1.95 +/- 0.28	99.78
2	1.77 +/- 0.30	86.54
3	2.17 +/- 0.14	89.76
4	1.80 +/- 0.35	92.85

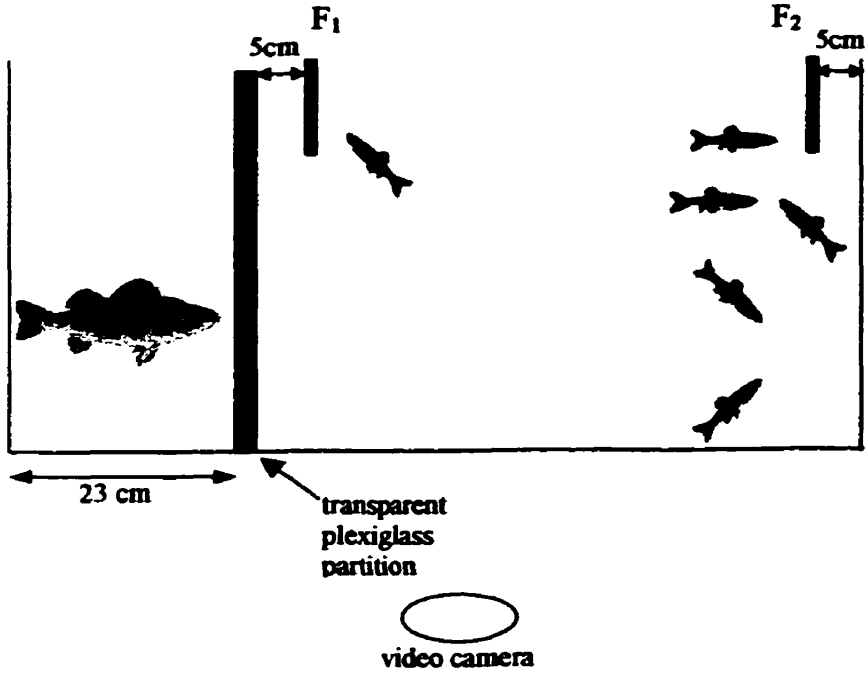


Figure 4.1. Diagram of the experimental apparatus used to determine the effects of hypoxia on response of minnow to a predator presence. F_1 represents the high risk feeder, F_2 represents the low risk feeder; The transparent plexiglass partition allowed minnows to view the perch for the duration of the trial while preventing the perch from capturing the minnows. (Note this would be the set-up for the predator location on the left side.)

prevent contact with the minnows yet minnows were able to view the predator for the duration of the trial. Predators were positioned behind the divider either on the right or left side of the tank so as to control for a side effect. For control treatments (trials with no predators) the plexiglass divider was randomly placed on either side of the tank. In the larger section of the apparatus two automated feeders were set-up to provide equal amounts of food to a group of minnows and placed at high and low risk locations. One feeder was placed 5cm from the edge of the plexiglass divider (high risk) and the other 5cm from the opposite edge of the tank (low risk). Each feeder provided 0.5g of frozen brine shrimp over a 25 minute time period (see Abrahams (1989) for a description of the feeders).

Approximately 3 hours prior to the trial one group of minnows and, for predator trials, one randomly selected predator (identity was recorded) were placed in the apparatus to acclimate to their new surroundings. To prevent the minnows from becoming habituated to the presence of a predator, a temporary opaque divider was placed in front of the transparent divider. Fifteen minutes prior to the start of trials using hypoxic conditions the dissolved oxygen was lowered in the tank by bubbling nitrogen gas through perforated Tygon® tubing placed at the bottom of the aquarium. For trials using normoxic conditions, air was used. The dissolved oxygen was measured using a YSI 53 dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA) at the beginning and end of each trial. Pre-experiment trials found no fluctuation in dissolved oxygen during this short time period, therefore levels were not monitored for the trial duration. The dissolved oxygen was lowered to 2.74 mg/L for all hypoxic trials as this provided an environment in which perch were compromised but the minnows behaved normally (moderate hypoxia in Chapter two of this thesis). For the normoxic trials, dissolved oxygen was maintained at 8.22 mg/L. For all trials water temperature was maintained at 22°C. Once the desired dissolved oxygen levels were attained the opaque plexiglass divider was removed and plastic was placed over the water for the duration of the trial to prevent atmospheric oxygen from dissolving into the tank water. The trial began when feeders and the video camera (for analysis) were turned on. Each trial lasted 25 minutes and upon completion water was changed and all aquaria were cleaned.

At each dissolved oxygen concentration, six replicates of each predator location (right and left side) and no predator (controls) were completed for a total of 36 trials. All trials were completed in nine days with trials being performed twice a day in two aquaria. The first trial began at 11:00h and the second at 15:00h. For the duration of the experiment the feeders were the only source of food supply for the minnows. In addition to the proportion of minnows using the feeders, the spatial distribution of the feeding minnows was observed every 30 seconds at each of the feeders. The mean proportion of minnows was calculated for the high risk habitat or that adjacent to the plexiglass. A feeding minnow was considered to be any individual that had or was consuming brine shrimp within 5cm on either side of the feeder. The mean response to predation risk was calculated as the difference of minnows using feeders in control situations (IFD input matching) and the proportion of minnows using the same feeder in the presence of a predator (means were calculated for predators located on both the right and left side).

Each group of minnows was used as one experimental unit and all means were reported +/- one standard error (SE). All statistical analyses used the GLM procedure of SPSS and experiments were blocked by predator.

Results

There was a visible difference in the behaviour of the perch between the normoxic and hypoxic treatments. Under normoxic conditions, perch were usually positioned against the partition and oriented themselves to minnows that were in the vicinity. Such behaviour was seldom observed in the hypoxic treatment.

In conformity with the IFD, the minnows were distributed equally between the two feeders in both normoxic and hypoxic conditions in the absence of predation risk (Table 4.2a). In the presence of a predator the proportion of minnows using the feeder adjacent to the plexiglass (high risk) was altered (Table 4.2a and 4.3a). Under normoxic conditions and in the presence of a predator there was a smaller proportion of minnows using the high risk feeder than when the predator was absent (Table 4.2a and 4.3a). The reduction in dissolved oxygen, however, altered the response of the minnows when a predator was present (Table 4.2a). During the hypoxia trials the minnow response to the predator was reduced when compared to their response to predators under normoxic

Table 4.2. Summary of a) mean proportion of minnows at the high risk feeder (adjacent to the plexiglass) and b) the mean number of minnows using the feeders at each dissolved oxygen concentration and for each predator location or absence of the predator (control).

a)

Dissolved Oxygen Concetration	Predator Location	Mean Proportion
Hypoxic	Left	0.49 +/- 0.05
	Right	0.55 +/- 0.02
	No Predator	0.51 +/- 0.05
Normoxic	Left	0.29 +/- 0.03
	Right	0.21 +/- 0.04
	No Predator	0.56 +/- 0.01

b)

Dissolved Oxygen Concetration	Predator Location	Mean Number Feeding
Hypoxic	Left	2.95 +/-0.45
	Right	3.78 +/- 0.56
	No Predator	2.97 +/- 0.55
Normoxic	Left	3.44 +/- 0.39
	Right	3.25 +/- 0.49
	No Predator	3.40 +/- 0.23

Table 4.3. Summary of ANOVA results of the influence of dissolved oxygen (hypoxic or normoxic), prey group number, predator location (right, left or no predator) and predator identity on a) the proportion of minnows using the high risk feeder or adjacent to the plexiglass and b) the mean number of minnows feeding.

a)

Source of Variation	df	F	p
Dissolved Oxygen (DO)	1	0.20	0.67
Prey Group # (G)	3	1.57	0.25
Predator Location (L)	2	16.93	**<0.001**
Predator Identification	3	2.61	0.10
DO * G	3	0.87	0.48
DO * L	2	17.91	**< 0.001**
G * L	5	0.91	0.50
DO * G * L	3	0.93	0.45

b)

Source of Variation	df	F	p
Dissolved Oxygen (DO)	1	0.25	0.63
Prey Group # (G)	3	1.37	0.30
Predator Location (L)	2	0.32	0.73
Predator Identification	3	0.98	0.43
DO * G	3	0.36	0.79
DO * L	2	0.18	0.84
G * L	5	0.62	0.68
DO * G * L	3	1.43	0.28

conditions (Fig. 4.2). For all treatments the predator identification and minnow group did not significantly affect the variation in response by the minnows (Table 4.2a).

The presence of a predator did not alter the mean number of individuals feeding for the duration of the trial (Table 4.2b and 4.3b). The reduction in dissolved oxygen levels also did not significantly alter the number of minnows feeding (Table 4.2b and 4.3b).

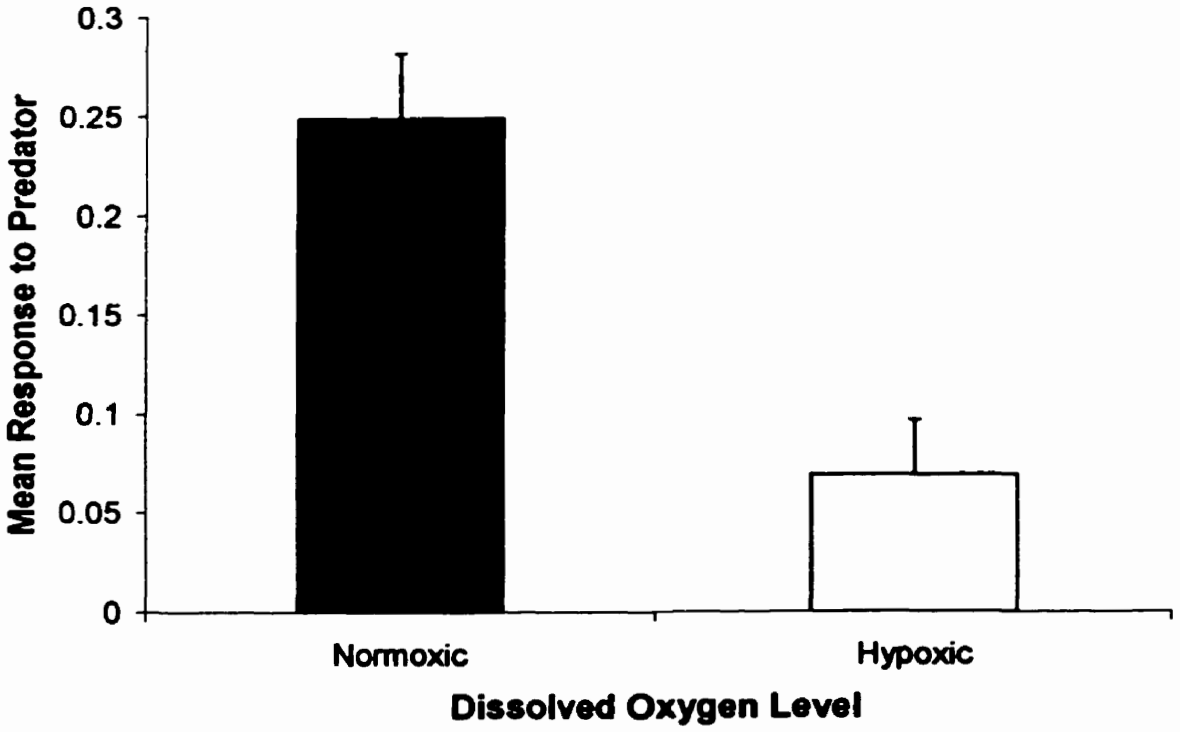


Figure 4.2. The mean response (+ 1 SE) of the fathead minnows to the presence of a predator. Mean response of minnows was calculated as the difference using a feeder with no predator and the proportion of minnows using the same feeder in the presence of a predator (large positive values indicate increased avoidance of high risk feeder).

Discussion

In the presence of a predator the minnows were less likely to choose the risky patch in favour of the safe habitat. Under hypoxic conditions, as was predicted, the role of the predator declined in the decision making process of the minnow. The minnows under hypoxic conditions were just as likely to feed at the high risk feeder as they were the low risk. This suggests that minnows will be able to take advantage of moderate hypoxic conditions and continue normal foraging behaviours with little impact of predation risk upon decision making. In addition, the limited dissolved oxygen and the presence of the predator did not influence the mean number of foraging minnows at each time interval measured. This suggests that costs associated with hypoxia and antipredator responses (predator avoidance) were not greater than the benefits of foraging. As well, under hypoxic conditions the similar feeding rates suggested that alteration in use of feeders was not simply due to the effects of hypoxia on the minnow.

In addition to the variation in tolerance to hypoxia, many studies have demonstrated that teleosts avoid hypoxic waters in favour of more oxygenated waters changing the distribution of teleosts in an aquatic community (Suthers & Gee 1986; Doudoroff & Shumway 1970). However, the role of predators in an aquatic environment will also influence community structure through their impact on the decision-making behaviours of piscine prey (Mittelbach 1984; Werner *et. al.* 1983). To avoid predators, prey often escape to habitats in which there is competition both among and between species (Mittelbach & Chesson 1987). However, to avoid predation pressures minnows in this study were able to take advantage of differences in physiological response to hypoxia. The use of hypoxic habitats intolerable or stressful to predators will not only reduce the effects of mortality on a minnow community but also allow minnows to carry on normal activities without the costs of predation pressures. Many haplochromine species thought to be extinct from Lake Victoria after the introduction of the Nile perch, *Lates niloticus*, were found in hypoxic wetland areas at the edges of the lake (Chapman *et al.* 1996a). The inability of the Nile perch to physiologically tolerate the adverse conditions (Fish, 1956) was implicated as the primary reason in preventing their infiltration into the wetlands subject to variable dissolve oxygen concentrations, allowing prey to take advantage of the absence of predation risk. However, this study implies the

behavioural modifications in response to critical dissolved oxygen levels also results in an advantage for prey by expanding the refuge to areas that are simply stressful and not necessary lethal to the predator. This would also provide refuges for prey more tolerant of hypoxic conditions without any adverse effects of the low dissolved oxygen.

The reduction in the use of the high risk feeder under normoxic conditions suggests that minnows were able to assess the risk associated with a predator. In addition, when dissolved oxygen was reduced minnows used the high risk habitat and fed at similar rates to when a predator was absent. Therefore the minnows likely gauged the predator to not be dangerous. Predator inspection is the most likely mechanism, in this study, that provides minnows with information on the predator to alter their behaviours in the presence of a predator (Dugatkin & Godin 1992). Godin & Crossman (1994) found that hungry threespine sticklebacks, *Gasterosteus aculeatus*, inspected predators more often and they did not significantly decrease their feeding rates under the predation hazard. In aquatic (normoxic) systems prey are able to detect a satiated or inactive predator and carry on with normal feeding regime (Appelberg *et al.* 1993; Christensen & Persson 1993). Eklöv and Persson (1996) found that even in the presence of a piscivorous pike, *Esox lucius*, both juvenile perch and roach, *Rutilus rutilus*, chose to stay in a vegetated habitat. The pike, an ambush predator, may have been ineffective at capturing prey in these structurally complex habitats thus predator inspection allowed the juveniles to use the riskier habitats. Under hypoxic conditions perch were observed to not be orientated towards minnows and their activity was limited to trips to the water surface. Thus prey most likely did not incorporate the presence of a predator suffering from the effects of hypoxia into its choice of habitat. In addition to the behaviours observed in response to hypoxia by perch, the minnows likely perceived the lack of intent to feed by the predators. At the level of hypoxia tested, perch in previous experiments were relatively stressed as compared to the fathead minnow under the same conditions and to those individuals held at normoxia (Chapter two of this thesis). In stressful dissolved oxygen levels in which physiological mechanisms must be altered to compensate for reduced environmental dissolved oxygen, individuals often have reduced feeding rates (Doudoroff & Shumway 1970; Petit 1973; Weber & Kramer 1983).

Poulin *et al.* (1987) suggested that the negative effects of hypoxia on a predator unable to use alternative air breathing mechanisms would present little risk to prey fish. They also found that the aquatic predatory cichlid, *Astronotus ocellatus*, was not as effective in obtaining prey guppies, *Poecilia reticulata*, in hypoxic waters. Similar to this study, the guppies altered antipredator behaviours in hypoxic conditions (Poulin *et al.* 1987). Rather than remaining motionless and avoiding surface waters, both antipredator behaviours under normoxic conditions, guppies increased surface time and time spent motionless decreased under hypoxic conditions. Although this was not suggested by Poulin *et al.* (1987), perhaps the guppies were able to assess the lack of intent by the predator cichlid in hypoxic conditions and carried out behaviours to obtain greater amounts of oxygen (Kramer 1983; Gee *et al.* 1978) regardless of the predator presence.

In the case of bimodal breathing (able to breathe air or water) piscine predator and prey species, hypoxia has very different effects (Wolf & Kramer 1987). When dissolved oxygen is reduced, both the predatory snakehead, *Channa micropeltes*, and their prey, dwarf gouramis, *Colisa lalia*, increase surface air breathing (Wolf & Kramer 1987). As a result, prey were more likely to be captured as they had to move away from areas of cover not conducive to air breathing. However, similar to this study, Wolf & Kramer (1987) suggested that individuals able to tolerate hypoxia at lower ranges of dissolved oxygen could reduce predation risk by staying in areas of cover longer.

In summary, this study suggests that variation in tolerance to hypoxia allow prey to choose habitats in which predation risk is perceived to be innocuous. Thus, the costly antipredator behaviours employed by prey are not necessary in an environment of reduced dissolved oxygen. By choosing waters with hypoxic conditions that are stressful (not necessarily lethal) to the predator but not to themselves, prey can carry on with normal foraging behaviours. This provides further support for the suggestion that prey can take advantage of hypoxic habitats in response to predation pressures (McIntyre & McCollum 2000; Chapman *et al.* 1996a; Chapman *et al.* 1996b).

Literature Cited

Abrahams, M. V. & Kattenfeld, M. 1997. The role of turbidity as a constraint on predator-prey interaction in aquatic environments. Behav. Ecol. Sociobiol. **40**: 169-174.

Abrahams, M.V. 1995. The interaction between antipredator behaviour and antipredator morphology: experiments with fathead minnows and brook sticklebacks. Can. J. Zool. **73**: 2209-2215.

Abrahams, M.V. 1989. Foraging guppies and the ideal free distribution: the influence of patch information of patch choice. Ethology **82**: 116-126.

Abrahams, M.V. & Dill, L.M. 1989. A determination of the energetic equivalence of the risk of predation. Ecology **70(4)**: 999-1007.

Appelberg, M., Söderbäck, B. & Odelström, T. 1993. Predator detection and perception of predation risk in the crayfish *Astacus astacus* L. Nordic J. Freshw. Res. **68**: 55-62.

Chapman, L.J., Chapman, C.A., & Chandler, M. 1996a. Wetland ecotones as refugia for endangered fishes. Biol. Conserv. **78**:263-270.

Chapman, L.J., Chapman, C.A., Ogutu-Ohwayo, R., Chandler, M., Kaufman, L.S., & Keiter, A.E. 1996b. Refugia for endangered fishes from an introduced predator in Lake Nabugabo, Uganda. Conserv. Biol. **10**: 554-561.

Christensen, B. & Persson, L. 1993. Species-specific antipredatory behaviours: effects on prey choice in different habitats. Behav. Ecol. Socio. **32**:1-9.

Dill, L.M. & Fraser, A.H.G. 1984. Risk of predation and the feeding behavior of juvenile coho salmon (*Oncorhynchus kisutch*). Behav. Ecol. Sociobiol. **16**: 65-71.

Dugatkin, L.A. & Godin, J.-G.J. 1992. Prey approaching predators: a cost-benefit perspective. Ann. Zool. Fennici. **29**: 233-252.

Doudoroff, P. & Shumway, D.L. 1970. Dissolved oxygen requirements of freshwater fishes. Food and Agriculture Organization of the United Nations. Technical Paper 86. 291p.

Eklöv, P. & Persson, L. 1996. The response of prey to the risk of predation: proximate cues for refuging juvenile fish. Anim. Behav. **51**: 105-115.

Fish, G.R. 1956. Some aspects of the respiration of six species of fish from Uganda. J Exp. Biol. **33**: 186-195.

Fretwell, S.D. & Lucas Jr., H.L. 1970. On territorial behavior and other factors influencing habitat distribution in birds. Acta. Biotheor. **19**: 16-36.

Gee, J.H., Tallman, R.F. & Smart, H.J. 1978. Reactions of some great plains fishes to progressive hypoxia. Can. J. Zool. **56**:1962-1966.

Gilliam, J.F. & Fraser, D.F. 1987. Habitat selection under predation hazard: Test of a model with foraging minnows. Ecology **68(6)**: 1856-1862.

Godin, J.-G.J. & Crossman, S.L. 1994. Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (*Gasterosteus aculeatus*) under predation risk. Behav. Ecol. Sociobiol. **34**: 359-366.

Kramer, D.L. 1983. The evolutionary ecology of respiratory mode in fishes: an analysis based on the costs of breathing. Env. Biol. Fish. **9(2)**: 145-158.

Kramer, D.L., Manley, D. & Bourgeois, R. 1983. The effect of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. Can. J. Zool. **61**: 653-665.

Lima, S.L. & Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68**: 619-640.

McIntyre, P.B. & McCollum, S.A. 2000. Responses of bullfrog tadpoles to hypoxia and predators. Oecologia [online] URL: http://link.springer.de/link/service/ journals/ 00442/contents/00/00451.

McLean, E.B. & Godin, J-G.J. 1989. Distance to cover and fleeing from predator in fish with different amounts of defensive armour. Oikos **55**: 261-290.

Miner, J.G. & Stein, R.A. 1996. Detection of predators and habitat choice by small bluegills: Effects of turbidity and alternative prey. Trans. Am. Fish. Soc. **125**: 97-103.

Mittelbach, G.G. 1984. Predation and resource partitioning in two sunfishes (Centrarchidae). Ecology **65**: 689-701.

Mittelbach, G.G. & Chesson, P.L. 1987. Predation risk: Indirect effects on fish populations. IN Predation: direct and indirect impacts on aquatic communities. eds. W.C. Kerfoot & A. Sih. University Press of New England, Hanover, New Hampshire. pp. 315-332.

Petit, G.D. 1973. Effects of dissolved oxygen and behavior of selected fishes of western lake Erie. Bull. Ohio. Biol. Surv. **4(4)**: 1-77.

Pitcher, T.J., Green, D.A. & Magurran, A.E. 1986. Dicing with death: predator inspection behaviour in minnow shoals. J. Fish Biol. **28**: 439-448.

Poulin, R., Wolf, N.G., and Kramer, D.L. 1987. The effect of hypoxia on the vulnerability of guppies (*Poecilia reticulata*, Poeciliidae) to an aquatic predator (*Astronotus ocellatus*, Cichlidae). Env. Biol. Fish **20(4)**: 285-292.

Sih, A. 1987. Predators and prey lifestyles: An evolutionary and ecological overview. IN Predation: direct and indirect impacts on aquatic communities. eds. W.C. Kerfoot & A. Sih. University Press of New England, Hanover, New Hampshire. pp. 203-224

Sih, A. 1982. Foraging strategies and the avoidance of predation by an aquatic insect, *Notonecta hoffmanni*. Ecology **63(3)**: 786-796.

Smale, M.A. & Rabeni, C.F. 1995. Hypoxia and hypothermia tolerances of headwater stream fishes. Trans. Am. Fish. Soc. **124**: 698-710.

Suthers, I.M. & Gee, J.H. 1986. Role of hypoxia in limiting diel spring and summer distribution of juvenile Yellow Perch (*Perca flavescens*) in a prairie marsh. Can. J. Fish. Aquat. Sci. **43**: 1562-1570.

Tonn, W.M. & Paszkowski, C.A. 1987. Habitat use of the central mudminnow (*Umbra limi*) and yellow perch (*Perca flavescens*) in *Umbra-Perca* assemblages: the roles of competition, predation, and the abiotic environment. Can. J. Zool. **65**: 862-870.

Weber, J.M. & Kramer, D.L. 1983. Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, *Poecilia reticulata*. Can. J. Fish Aquat. Sci. **40**: 1583-1588.

Werner, E.E. & Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size-structured populations. Ann. Rev. Ecol. Syst. **15**: 393-425.

Werner, E.E., Gilliam, J.F., Hall, D.J. and Mittelbach, G.G. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology **64(6)**: 1540-1548.

Wolf, N.G. and Kramer, D.L. 1987. Use of cover and the need to breathe: the effects of hypoxia on the vulnerability of dwarf gouramis to predatory snakeheads. Oecologia **73**:127-132.

CHAPTER V
GENERAL DISSCUSION

The results of this thesis suggest the importance of hypoxic environments on predator-prey interactions within an aquatic environment. In the first chapter, I've described the physiological and behavioural costs when a teleost is subjected to hypoxic waters and how variation in tolerance of hypoxia might affect a predator-prey relationship. The second chapter established a difference in tolerance of reduced dissolved oxygen concentrations between a predator, the yellow perch, and its prey, the fathead minnow. In addition to a species effect, I've suggested the difference in body size may account for the tendency of prey to have a greater tolerance of hypoxia than their predator. However, the exact mechanism is not relevant as the results suggest that prey can use hypoxic habitats as a piscine predator-free refuge. This becomes ecologically relevant in a predator-prey relationship as the use of refuges provide an advantage for smaller prey that are normally subjected to a high risk of predation.

Chapter three of this thesis provided support for the prediction of the use of hypoxic habitats by prey in response to predation pressures in environments of changing dissolved oxygen. To predict habitat selection by a piscine predator and prey, I developed a theoretical model based on the ideal free distribution with the predicted size-sensitive (allometric) relationship of tolerance of hypoxia as the main assumption. To test the assumptions of this model, a field study was conducted in a marsh habitat of fluctuating dissolved oxygen concentrations. The model predicted distributions were found to be qualitatively similar to those found in the field, suggesting a hypoxia limited predator distribution while prey were able to use hypoxic habitats free of predation pressures and competition.

In chapter four of this thesis, I predicted that the size of the hypoxic refuge, previously defined in chapter two as to simply exclude predators based on physiological tolerance, could be redefined. The refuge would also include habitats in which behavioural modifications of predators in response to critical levels of dissolved oxygen results in a reduced effectiveness of the predator to obtain prey but allow a prey, more tolerant of hypoxia, to behave normally. In moderately hypoxic waters, stressful to

predators and not prey, I found minnows to have a reduced response to the presence of a predator as compared to normoxic conditions. The predator's lack of interest in the minnows observed under hypoxic conditions suggested that the minnows were able to assess this behaviour as innocuous and carry on with a normal feeding regime.

The interaction of predator and prey in forming the dynamics of aquatic piscine communities is well established (see Mittelbach & Chesson 1987 for a review). However, the environmental impacts on this relationship have become increasingly important with the increase in human disturbance in many fresh water habitats (Chapman *et al.* 1996a; Chapman *et al.* 1996b; Shapiro 1990). Hypoxic environments have been traditionally assumed to be detrimental to teleosts as growth and development is often impeded by hypoxia (van Dam & Pauly 1995; Weber & Kramer 1983) as well as many teleosts avoid these stressful environments (Suthers and Gee 1986; Petit 1973; Doudoroff & Shumway 1970). However, recently the impact of variation in dissolved oxygen has become increasingly important in establishing refuges for prey use (McIntyre & McCollum 2000; Chapman *et al.* 1996a; Chapman *et al.* 1996b; Wright & Shapiro 1990).

As I have attempted to illustrate in each chapter, the results of this thesis provide evidence for the importance of wetlands or any body of water that produces fluctuations in dissolved oxygen creating hypoxic habitats. After the introduction of the Nile perch, *Lates niloticus*, into Lake Victoria many of the native haplochromine populations were thought to be reduced to extinction by this larger predator (Chapman *et al.* 1996a). However, subsequent studies established the importance of wetlands for smaller fish after finding resurgent haplochromine populations in wetland areas where abundant plant material resulted in the reduction of dissolved oxygen intolerable to the Nile perch (Chapman *et al.* 1996a; Chapman *et al.* 1996b). Prey with greater tolerance of hypoxia were subsequently able to exploit these structurally complex habitats without any adverse effects of the low dissolved oxygen or predation risk (Chapman *et al.* 1995).

The ecological advantage of prey use of a hypoxic predator free refuge becomes relevant not only in situations where prey populations have been compromised, such as in Lake Victoria, but also provides opportunity for prey to establish populations with reduced predation risk. The latter situation is the most likely case in this study. Thus the natural progression of wetlands in forming distinct hypoxic habitats can be beneficial to

fish communities of smaller size classes in the re-establishment from a natural population decline or due to human-induced population decrease. Maintenance of natural wetlands, therefore, should ensure the preservation of an aquatic ecosystem by providing prey habitats protected from the adverse effects of aquatic predators.

Literature Cited

Chapman, L.J., Chapman, C.A., & Chandler, M. 1996a. Wetland ecotones as refugia for endangered fishes. Biol. Conserv. **78**:263-270.

Chapman, L.J., Chapman, C.A., Ogutu-Ohwayo, R., Chandler, M., Kaufman, L.S., & Keiter, A.E. 1996b. Refugia for endangered fishes from an introduced predator in Lake Nabugabo, Uganda. Conserv. Biol. **10**: 554-561.

Chapman, L.J., Kaufman, L.S., Chapman, C.A., & McKenzie, F.E. 1995. Hypoxia tolerance in twelve species of East African Cichlids: Potential for low oxygen refugia in Lake Victoria. Conserv. Biol. 5:1274-1288.

Doudoroff, P. & Shumway, D.L. 1970. Dissolved oxygen requirements of freshwater fishes. Food and Agriculture Organization of the United Nations. Technical Paper 86. 291p.

McIntyre, P.B. & McCollum, S.A. 2000. Responses of bullfrog tadpoles to hypoxia and predators. Oecologia [online] URL: http://link.springer.de/link/service/ journals/ 00442/contents/00/00451.

Mittelbach, G.G. & Chesson, P.L. 1987. Predation risk: Indirect effects on fish populations. IN Predation: direct and indirect impacts on aquatic communities. eds. W.C. Kerfoot & A. Sih. University Press of New England, Hanover, New Hampshire. pp. 315-332.

Petit, G.D. 1973. Effects of dissolved oxygen and behavior of selected fishes of western lake Erie. Bull. Ohio. Biol. Surv. **4(4)**: 1-77.

Shapiro, J. 1990. Biomanipulation: the next phase- making it stable. Hydrobiologia **200/201**: 13-27.

Suthers, I.M. & Gee, J.H. 1986. Role of hypoxia in limiting diel spring and summer distribution of juvenile Yellow Perch (*Perca flavescens*) in a prairie marsh. Can. J. Fish. Aquat. Sci. **43**: 1562-1570.

van Dam, A.A. & Pauly, D. 1995. Simulation of the effects of oxygen on food consumption and growth of Nile tilapia, *Oreochromis niloticus* (L.). Aqua. Res. **26**: 427-440.

Weber, J.M. & Kramer, D.L. 1983. Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, *Poecilia reticulata*. Can. J. Fish Aquat. Sci. **40**: 1583-1588.

Wright, D. & Shapiro, J. 1990. Refuge availability: a key to understanding the summer disappearance of *Daphnia*. Fresh. Biol. **24**: 43-62.

Appendix 1. Visual Basic program for ideal free model predicting distribution of predator and prey according to dissolved oxygen levels.

```
'=============================================================
'THIS PROGRAM WILL CALCULATE THE DISTRIBUTION OF PREDATOR AND PREY
ACCORDING TO DISSOLVED OXYGEN LEVELS
'=============================================================
Option Explicit
'MAKE VARIABLES PUBLIC FOR USE IN ALL SUBROUTINES
Public predhab()
Public preyhab()
Public tpredhab()
Public tpreyhab()
Public pd()
Public fpd()
Public py()
Public fpy()
Public dissoxy() As Double
Public foodaval()
Dim npred As Double: Dim nprey As Double: Dim loopy As Integer:
Dim i As Integer: Dim k As Integer: Dim x As Integer:
Dim patches As Integer:  Dim q() As Double: Dim choices() As Double:
Dim rechoose() As Double: Dim u As Double: Dim totalfood As Integer:
Dim r As Integer: Dim redist As Double:
Dim n As Integer: Dim z As Integer: Dim t As Integer: Dim j As Integer:
Dim counter As Integer: Dim DOmax As Integer: Dim DOmin As Integer:
Dim DOcount As Integer: Dim y As Integer: Dim DevPred1 As Double:
Dim DevPrey1 As Double: Dim d As Double: Dim start As Double:
Dim ends As Double: Dim interval As Double: Dim writing As Integer:
Dim wcount As Integer: Dim timecount As Integer


'MAIN SUBROUTINE:
'**************************
Sub Redistribution()
'**************************
Worksheets("Output1").Select
Randomize

npred = Cells(2, 4) 'NUMBER OF PREDATORS IN THE ENVIRONMENT
nprey = Cells(3, 4) 'NUMBER OF PREY IN THE ENVIRONMENT
patches = Cells(4, 4) 'NUMBER OF PATCHES IN THE ENVIRONMENT
totalfood = Cells(8, 4) 'TOTAL FOOD AVAILABLE IN THE SYSTEM
start = Cells(5, 4)
ends = Cells(6, 4)
interval = Cells(7, 4)

ReDim preyhab(patches)
ReDim predhab(patches)
ReDim dissoxy(patches)
ReDim foodaval(patches)
ReDim tpredhab(patches)
ReDim tpreyhab(patches)
ReDim pd(patches)
```

```
ReDim fpd(patches)
ReDim py(patches)
ReDim fpy(patches)

Range("A18:L2000").ClearContents
Range("J1:K8").ClearContents
Range("c13:e14").ClearContents
Worksheets("Output2").Range("A3:f41").ClearContents
Worksheets("Output3").Range("b4:n7").ClearContents
Worksheets("Output3").Range("b11:n14").ClearContents
Worksheets("Output3").Range("b18:n21").ClearContents

'SET A LOOP WITH CHANGING DO LEVELS IN EACH OF THE THREE DIFFERENT HABITATS-
1= OPEN WATER, 2= EDGE OF MACROPHYTES, 3= WITHIN MACROPHYTES
DOcount = 1
counter = 0
timecount = 1
For d = start To ends Step interval
    dissoxy(1) = 22.5 * d - 0.1875 'REGRESSION LINES FROM FIELD DATA
    dissoxy(2) = 20.576 * d - 0.7739
    dissoxy(3) = 13.568 * d - 2.3462
  Cells(11, 7) = d

  foodaval(1) = totalfood / 4
  foodaval(2) = totalfood / 2
  foodaval(3) = totalfood / 4

'THIS JUST SETS THE NUMBER OF PREDATORS AND PREY IN EACH PATCH TO ZERO
    For x = 1 To patches
      preyhab(x) = 0
      predhab(x) = 0
    Next x

'THE PREDATOR ENTERS THE ENVIRONMENT
    If npred > 0 Then
      For k = 1 To npred
        Call DistributePredators(preyhab(), npred, patches)
        Cells(14, 3) = predhab(1)
        Cells(14, 4) = predhab(2)
        Cells(14, 5) = predhab(3)
      Next k
    Else
    End If

'THE PREY ENTERS THE ENVIRONMENT
    For r = 1 To nprey
        Call DistributePrey(predhab(), nprey, patches)
        For writing = 1 To patches Step 1
        Cells(12, 2 + writing) = foodaval(writing) / totalfood
        Cells(11, 2 + writing) = dissoxy(writing)
        Cells(13, 2 + writing) = preyhab(writing)
        Next writing
    Next r

'NOW REMOVE ONE PREDATOR THEN ALLOW PREDATORS TO REDISTRIBUTE AND THEN
REMOVE ONE PREY AND THEN ALLOW PREY TO REDISTRIBUTE
```

```
redist = Cells(9, 4) 'NUMBER OF REDISTRIBUTION'S
For i = 1 To patches
  tpredhab(i) = 0
  tpreyhab(i) = 0
  pd(i) = 0
  fpd(i) = 0
  py(i) = 0
  fpy(i) = 0
Next i
  For loopy = 1 To redist
    If npred > 0 Then Call predatorsrechoose(predhab(), npred, patches, tpredhab())
    Cells(14, 3) = predhab(1)
    Cells(14, 4) = predhab(2)
    Cells(14, 5) = predhab(3)
      Call preyrechoose(preyhab(), nprey, patches, tpreyhab())
      For writing = 1 To patches Step 1
        Cells(12, 2 + writing) = foodaval(writing) / totalfood
        Cells(11, 2 + writing) = dissoxy(writing)
        Cells(13, 2 + writing) = preyhab(writing)
      Next writing

      For n = 1 To patches
        Cells(17 + loopy, 1) = loopy
          If npred > 0 Then Cells(17 + loopy, 4 + n) = predhab(n) / npred
        Cells(17 + loopy, 7 + n) = preyhab(n) / nprey
        Cells(17 + loopy, 1 + n) = Cells(11, 2 + n)
      Next n
  Next loopy


'PRINTOUT ON SHEET 2 THE AVERAGE PREY AND PREDATOR DISTRIBUTIONS AFTER
REDISTRIBUTION'S AND FOR EACH FOOD PROPORTION

For k = 1 To patches
  Worksheets("Output2").Cells(2 + k + counter, 1) = d 'TIME
  Worksheets("Output2").Cells(2 + k + counter, 2) = k 'PATCH
  Worksheets("Output2").Cells(2 + k + counter, 3) = dissoxy(k) 'DISSOLVED OXYGEN
  If npred > 0 Then
    Worksheets("Output2").Cells(2 + k + counter, 4) = (tpredhab(k) / (redist)) / npred
  Else                                'MEAN PROPORTION OF PREDATORS
  End If
  Worksheets("Output2").Cells(2 + k + counter, 5) = (tpreyhab(k) / (redist)) / nprey
                                      'MEAN PROPORTION OF PREY
  Worksheets("Output2").Cells(2 + k + counter, 6) = foodaval(k) / totalfood
                                      'FOOD PROPORTION

  'OUTPUT OF DISTRIBUTION OF PREDATORS FOR EACH STATION THROUGH TIME
  Worksheets("Output3").Cells(4 + k, 1) = k
  If npred > 0 Then
  Worksheets("Output3").Cells(4 + k, 1 + timecount) = (tpredhab(k) / (redist)) / npred
  For i = 1 To redist
    pd(k) = (((Cells(17 + i, 4 + k)) - ((tpredhab(k) / (redist)) / npred)) ^ 2
    fpd(k) = pd(k) + fpd(k)
```

```
    Next i
    Else
    End If
    Worksheets("Output3").Cells(4 + k, 2 + timecount) = Sqr((fpd(k) / (redist - 1)) / redist)
    Worksheets("Output3").Cells(4, 1 + timecount) = d

    'OUTPUT OF DISTRIBUTION OF PREY FOR EACH STATION THROUGH TIME
    Worksheets("Output3").Cells(11 + k, 1 + timecount) = (tpreyhab(k) / (redist)) / nprey
    For i = 1 To redist
      py(k) = ((Cells(17 + i, 7 + k)) - ((tpreyhab(k) / (redist)) / nprey)) ^ 2
      fpy(k) = py(k) + fpy(k)
    Next i
    Worksheets("Output3").Cells(11 + k, 2 + timecount) = Sqr((fpy(k) / (redist - 1)) / redist)
    Worksheets("Output3").Cells(11 + k, 1) = k
    Worksheets("Output3").Cells(11, 1 + timecount) = d
    'OUTPUT OF DO THROUGH TIME
    Worksheets("Output3").Cells(18 + k, 1) = k
    Worksheets("Output3").Cells(18, 1 + timecount) = d
    Worksheets("Output3").Cells(18 + k, 1 + timecount) = dissoxy(k)
Next k

DOcount = DOcount + 2
counter = counter + 3
timecount = timecount + 2

Next d
End Sub
'*****************************************************
Sub DistributePredators(preyhab(), npred, patches)
'*****************************************************

ReDim q(patches)
ReDim choices(patches, 2)
ReDim pDOcost(patches)


'THIS WILL GIVE THE QUALITY OF THE PATCH ACCORDING TO THE NUMBER OF PREY
AVAILABLE MINUS THE COST ASSOCIATED WITH BEING AT THAT DISSOLVED OXYGEN
LEVEL

   For i = 1 To patches
     pDOcost(i) = (1 / (0.02 * Exp(0.2 * dissoxy(i))))

'IF THE DO LEVEL IS LESS THAN OR EQUAL TO THE CRITICAL DO THEN PREDATOR
CANNOT SURVIVE SO MUST CHOOSE BETWEEN REMAINING HABITATS
         If dissoxy(i) <= Cells(5, 5) Then
           pDOcost(i) = 10000000
         End If
     q(i) = (preyhab(i) / (predhab(i) + 1)) - pDOcost(i)
     Cells(1 + i, 7) = i
     Cells(1 + i, 8) = q(i)
   Next i

'SORT THE QUALITIES INTO DESCENDING ORDER

Range("G2:H4").Select
```

```
    Selection.Sort Key1:=Range("H2"), Order1:=xlDescending, Header:= _
      xlGuess, OrderCustom:=1, MatchCase:=False, Orientation:= _
      xlTopToBottom


'LOAD CELL VALUES INTO AN ARRAY

  For i = 1 To patches
    choices(i, 1) = Cells(i + 1, 7)
    choices(i, 2) = Cells(i + 1, 8)
  Next i

'USE LOOP VALUE TO PICK A PATCH

  For i = 2 To patches
    If choices(1, 2) - choices(i, 2) > 0 Then Exit For
  Next i

'THIS STATEMENT WILL THEN PICK THE CORRECT PATCH
  u = Rnd
  predhab(choices(Int(u * (i - 1) + 1), 1)) = (predhab(choices(Int(u * (i - 1) + 1), 1))) + 1


End Sub

'*********************************************
Sub DistributePrey(predhab(), nprey, patches)
'*********************************************
ReDim q(patches)
ReDim choices(patches, 2)
ReDim mortality1(patches)
ReDim mortality2(patches)
ReDim feeding(patches)
ReDim DOcost(patches)
ReDim DOcostprey(patches)
ReDim DOcostpred(patches)
ReDim costdiff(patches)
ReDim probdeath(patches)

  For i = 1 To patches
    DOcostprey(i) = (1 / (0.09 * Exp(0.7 * dissoxy(i))))
    DOcostpred(i) = (1 / (0.02 * Exp(0.2 * dissoxy(i))))
    costdiff(i) = DOcostpred(i) - DOcostprey(i)
    mortality1(i) = (0.00001 * costdiff(i)) - (0.001 * (costdiff(i) ^ 2)) + 0.8
    mortality2(i) = 1 - Exp(-5 * (predhab(i) / (preyhab(i) + 1)))

    probdeath(i) = mortality1(i) * mortality2(i)
      If probdeath(i) <= 0 Then
      probdeath(i) = 0.001
      End If
  feeding(i) = ((foodaval(i) / totalfood) / (preyhab(i) + 1))
  DOcost(i) = (1 / (0.09 * Exp(0.7 * dissoxy(i))))
  'IF THE DO LEVEL IS LESS THAN OR EQUAL TO THE CRITICAL DO THEN PREY CANNOT
SURVIVE SO MUST CHOOSE BETWEEN REMAINING HABITATS
      If dissoxy(i) <= Cells(3, 5) Then
        DOcost(i) = 10000000
```

```
        End If
      q(i) = (feeding(i) / probdeath(i)) - DOcost(i) '(mortality(i) / feeding(i)) + DOcost(i)

      Cells(5 + i, 7) = i
      Cells(5 + i, 8) = q(i)
   Next i

'SORT THE QUALITIES INTO DESCENDING ORDER:
Range("G6:H8").Select
      Selection.Sort Key1:=Range("H6"), Order1:=xlDescending, Header:= _
        xlGuess, OrderCustom:=1, MatchCase:=False, Orientation:= _
        xlTopToBottom
'Range("G6:H8").Select
'     Selection.Sort Key1:=Range("H6"), Order1:=xlAscending, Header:= _
'       xlGuess, OrderCustom:=1, MatchCase:=False, Orientation:= _
'       xlTopToBottom


'LOAD CELL VALUES INTO AN ARRAY

   For i = 1 To patches
      choices(i, 1) = Cells(i + 5, 7)
      choices(i, 2) = Cells(i + 5, 8)
   Next i

'USE LOOP VALUE TO PICK A PATCH

   For i = 2 To patches
      If choices(1, 2) - choices(i, 2) > 0 Then Exit For
   Next i

STATEMENT WILL THEN PICK THE CORRECT PATCH

   u = Rnd
   preyhab(choices(Int(u * (i - 1) + 1), 1)) = (preyhab(choices(Int(u * (i - 1) + 1), 1))) + 1


End Sub

'*****************************************************
Sub predatorsrechoose(predhab(), npred, patches, tpredhab())
'*****************************************************


ReDim rechoose(patches, 2)

   For t = 1 To patches
      If npred > 0 Then Cells(1 + t, 10) = predhab(t) / npred
   Next t

   For i = 2 To patches + 1
      Cells(i, 11) = Cells(i, 10) + Cells(i - 1, 11)
   Next i

   For j = 1 To patches
      rechoose(j, 1) = Cells(1 + j, 9)
```

```
      rechoose(j, 2) = Cells(1 + j, 11)
   Next j

u = Rnd
   For k = 1 To patches
      If u <= rechoose(k, 2) Then Exit For
   Next k

predhab(rechoose(k, 1)) = (predhab(rechoose(k, 1)) - 1)

Call DistributePredators(preyhab(), npred, patches)

For k = 1 To patches
   tpredhab(k) = predhab(k) + tpredhab(k)
Next k
End Sub

'*********************************************
Sub preyrechoose(preyhab(), nprey, patches, tpreyhab())
'*********************************************
ReDim rechoose(patches, 2)

For t = 1 To patches
      If nprey = 0 Then Exit Sub
      Cells(5 + t, 10) = preyhab(t) / nprey

   Next t

   For i = 2 To patches + 1
      Cells(4 + i, 11) = Cells(4 + i, 10) + Cells(3 + i, 11)
   Next i

   For j = 1 To patches
      rechoose(j, 1) = Cells(5 + j, 9)
      rechoose(j, 2) = Cells(5 + j, 11)
   Next j

u = Rnd
   For k = 1 To patches
      If u <= rechoose(k, 2) Then Exit For
   Next k

preyhab(rechoose(k, 1)) = (preyhab(rechoose(k, 1)) - 1)

Call DistributePrey(predhab(), nprey, patches)
For k = 1 To patches
   tpreyhab(k) = preyhab(k) + tpreyhab(k)
Next k
End Sub
```

Appendix 2. Variation in water depth and temperature at the stations sampled in Delta Marsh.

Station	Time	Temperature (°C)	Water Depth (cm)
1	5:00	20.96 +/- 0.32	74.61 +/- 0.79
	9:00	20.96 +/- 0.33	75.29 +/-0.75
	13:00	23.12 +/- 0.36	74.24 +/-0.79
	17:00	24.09 +/- 0.35	75.10 +/-0.63
2	5:00	21.05 +/- 0.32	69.81 +/- 1.06
	9:00	20.92 +/- 0.32	70.41 +/- 1.11
	13:00	23.15 +/- 0.36	69.37 +/- 1.51
	17:00	24.07 +/- 0.36	70.72 +/- 1.03
3	5:00	19.91 +/- 0.33	45.79 +/- 1.05
	9:00	19.97 +/- 0.29	47.10 +/- 0.97
	13:00	22.49 +/- 0.37	47.88 +/- 1.01
	17:00	22.96 +/- 0.31	47.54 +/- 0.93