GAS BUBBLE TRAUMA IN FISH

by

LARRY E. FIDLER

B.Sc., The Pennsylvania State University, 1960

M.Sc., The University of British Columbia, 1985

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Department of Zoology)

We accept this thesis as conforming

to the required standard.

THE UNIVERSITY OF BRITISH COLUMBIA

July 31, 1988

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of ZOOLOGY

The University of British Columbia
Vancouver, Canada

Date OCTOBER 13, 1988

ABSTRACT

Fish exposed to gas supersaturated water often experience a form of stress known as Gas Bubble Trauma (GBT). GBT is an acute condition involving various forms of bubble growth both internal and external to the animal. Theoretical models are developed which establish thresholds for bubble growth. These models apply to:

1.) Bubble growth in the vascular systems of fish.
2.) Bubble growth in the environmental water that can occur in the buccal cavity and between gill lamella.
4.) Overinflation of the swimbladder.
3.) Sub-dermal bubbles that occur on external skin surfaces such as the opercular flaps, between fin rays and in the lining of the mouth.

In order to develop the models for general use, it was necessary to establish the effective size of nucleation sites and other physiological parameters contained in the bubble growth threshold equations. This was accomplished through a review of data from the scientific literature and a two phase experimental program.

The literature review resulted in the compilation of a database containing over 1000 records of supersaturation data on salmonids. Various filters based on length, species, total gas pressure (TGP), partial pressure of oxygen (pO_2) and other criteria were applied to the database. The filtering operations established the existence of GBT mortality thresholds and identified relationships between other experimental parameters. The results of this analysis suggest that a lower threshold occurs at a water TGP of 1.10 Atms. and a higher threshold occurs at 1.15 to 1.18 Atms.

However, it was not established that the apparent mortality thresholds correspond to thresholds for bubble growth predicted by the theoretical models.

To make this correlation, a preliminary experimental study examined the physiological response of fish exposed to supersaturated water. It was found that arterial pO_2, hematocrit and blood pressure yield unique responses to bubble growth over specific ranges of water TGP. The results of these experiments also indicate that the lower mortality threshold of the database analyses is associated with a combination of sub-dermal bubble growth in the mouth and extracorporeal bubbles growing between gill lamella.

The second phase of experimental study included surveys of blood pO_2, hematocrit and pH along with microscopic studies of intravascular and extracorporeal bubble growth in gills. The results of these experiments confirm the source of mortality for the lower threshold at a water TGP of 1.1 Atms. In addition, the data demonstrate that the upper TGP threshold of 1.15 to 1.18 Atms. of the database analysis corresponds to the threshold for intravascular bubble growth. The results further confirm that, as predicted by the theoretical model, intravascular bubble growth thresholds are dependent on water pO_2 .

Combining the results of the database analysis and the experimental studies permitted the effective size of nucleation sites responsible for bubble growth to be back calculated from the theoretical equations. This completed the development of the bubble growth threshold equations. The equations can now be used to predict thresholds for the various forms of bubble growth and mortality that occur in fish

exposed to supersaturated water. The experimental results also provide valuable information regarding the physiological response of fish to gas supersaturated water.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to thank Dr. Dave Randall for his guidance in this work as well as for his stimulating conversations regarding matters scientific and otherwise. I also wish to acknowledge the many helpful and enlightening discussions with Dr. Don Alderdice and John Jensen of the Pacific Biological Station and Dr. Bob White of Montana State University.

The work described in this thesis was supported by a grant from the Canada Department of Fisheries and Oceans Pacific Biological Station, the NSERC grant of Dr. Dave Randall and a research assistantship at Montana State University sponsored by the U.S. Bureau of Land Management.

LIST OF ABBREVIATIONS

GBT = Gas Bubble Trauma.

TGP = Total Gas Pressure = $pO_2 + pN_2 + pH_2O$

pO_2 = partial pressure of dissolved oxygen in environmental water.

pN_2 = partial pressure of dissolved nitrogen in environmental water.

pH_2O = vapor pressure of water.

p_{Ob} = partial pressure of oxygen in bubble.

p_{Nb} = partial pressure of nitrogen in bubble.

p_{Op} = partial pressure of oxygen in plasma.

p_{Np} = partial pressure of nitrogen in plasma.

r_o = the radius of the critical nucleation sites from which bubble growth begins (environmental water or vascular system).

$K = H_O \cdot k_O / H_N \cdot k_N$ where,

H_O = Henrys constant for oxygen in fish blood.

H_N = Henrys constant for nitrogen in fish blood.

k_N = mass transfer coefficient for nitrogen.

k_O = mass transfer coefficient for oxygen.

D_O = coefficient of diffusion for oxygen in fish blood.

D_N = coefficient of diffusion for nitrogen in fish blood.

T = temperature in degrees C.

σ = surface tension of water or fish blood.

F = oxygen uptake ratio across gills = pO_2 (Arterial Blood)/pO_2 (Water).

$P = Patm. + \rho \cdot g \cdot h + Ps.$

Patm = atmospheric pressure.

Ps = system pressure where bubble is growing.

ρ = density of water.

h = depth of fish in water column.

k_o = mass transfer coefficient for oxygen.

k_n = mass transfer coefficient for nitrogen.

R' = gas constant.

C' = compliance.

Ro = effective nucleus radius.

r = radius of growing bubble.

V_B = volume of growing bubble.

N = number of bubbles growing in vascular system.

P_{OA} = total gas pressure of gas A in water.

g = gravitational constant.

1.0 INTRODUCTION

Gas Bubble Trauma (GBT) is a condition that arises when the water in which fish live becomes supersaturated with dissolved atmospheric gases. The condition, first described by Robert Boyle (1670), can produce a variety of physiological insults that are often fatal to fish and other aquatic organisms. The major symptoms that characterize Gas Bubble Trauma in fish include:

1.) Bubble formation in the cardiovascular system.
2.) Overinflation of the swim bladder, intestinal and peritoneal cavities.
3.) Sub-dermal emphysema on body surfaces including the lining of the mouth.
4.) Extracorporeal bubble formation in gill lamella.
5.) Emphysema in muscle, internal organs and the spinal cord.

In general, these symptoms involve the growth of gas bubbles in one form or another, both internal and external to the animal. Internally, bubbles can block the flow of blood, disrupt the function of organs and impair neural activity (Weitkamp and Katz, 1980; Stroud *et al.*, 1975). Externally, they can block the flow of respiratory water through the gills (Jensen, 1980 and Shirahata, 1966).

1.1 DISSOLVED GAS SUPERSATURATION

Natural bodies of water contain dissolved atmospheric gases that are usually in equilibrium with the atmosphere. That is, the partial pressures of the dissolved gas components (oxygen, nitrogen, water vapor, argon, etc.) are the same as the partial pressures of their respective atmospheric components. Occasionally, due to man made and natural causes, the dissolved gases are thrown into a state of disequilibrium with respect to atmospheric gases. When this disequilibrium involves a

dissolved gas partial pressure which exceeds that of the atmospheric component, water is supersaturated with that dissolved gas. This in itself may not create a problem for aquatic organisms. However, a unique condition arises when the sum of the partial pressures of all dissolved gases, (i.e. total dissolved gas pressure, henceforth referred to as Total Gas Pressure or TGP) exceeds atmospheric pressure. When this occurs, there is the potential for dissolved gases to diffuse into microscopic nucleation sites or hollow cavities and form bubbles (Harvey, 1951; Hills, 1977; Weitkamp and Katz, 1980; Fidler, 1985). These bubbles can form in both the environmental water and within the organisms that inhabit that water.

Expressed in a mathematical form, the necessary condition for bubble growth and overinflation of body cavities is:

$$TGP = pO_2 + pN_2 + pH_2O + pEtc. > P_{Atms.}$$

where pO_2 = the partial pressure of dissolved oxygen.

pN_2 = the partial pressure of dissolved nitrogen.

pH_2O = the partial pressure of water vapor.

pEtc. = the partial pressure of all other dissolved gases.

$P_{Atms.}$ = atmospheric pressure.

In general, the partial pressures of argon and other trace atmospheric gases are small in relation to those of oxygen, nitrogen and water vapor. Thus, most studies of Gas Bubble Trauma, include trace gases in the partial pressure of nitrogen (Colt, 1983 and 1984). Occasionally, high levels of dissolved CO_2 occur in the environmental waters. This usually involves high densities of fish in poorly aerated water such as

occurs in certain aquaculture operations (Steffensen, 1988 and Rosenthal, 1988: personal communications). In these situations, CO_2 must be included with the other major gas components as part of the Total Gas Pressure.

Equation 1.0 defines the necessary condition for bubble growth. However, this is not a sufficient condition. Physical constraints such as the surface tension of the medium in which the bubbles grow, water depth, atmospheric pressure and water temperature coupled with a variety of physiological constraints further restrict the conditions under which bubble growth and body cavity overinflation can occur (Fidler, 1985). The relationship between these parameters and bubble growth thresholds is the central theme of this thesis.

1.2 CAUSES OF SUPERSATURATION

Supersaturation of natural bodies of water occurs as a result of both man made and natural phenomena. Perhaps the most widely known form of supersaturation is that produced by hydroelectric dams. Water, spilling over dams, entrains air as it plunges into pools at the base of the dam. The air, in the form of bubbles, is forced into solution under hydrostatic pressure and increases water dissolved gas tensions. Coutant and Genoway (1968), Beiningen and Ebel (1970), Bouck *et al.* (1970), Boyer (1974), Dell (1975), Meekin and Turner (1974), Dawley *et al.* (1976), Ebel (1969, 1971 & 1979), Ebel *et al.* (1971), Ebel *et al.* (1973), Ebel *et al.* (1975), Ebel *et al.* (1979), Blahm *et al.* (1973), Blahm (1975), Stroud and Nebeker (1976) and Weitkamp (1974 & 1976) report the effects of this form of supersaturation on fish in the Columbia River system of the United States. Supersaturation resulting from other dams and hydroelectric installations are described by Colt (1984), Berg *et al.* (1984), White *et al.* (1986), Heggberget (1984) and Alderdice and Jensen (1985).

DeMont and Miller (1972), Becker (1973), Adair and Hains (1974), Miller (1974) Marcello and Fairbanks (1976), and Fairbanks and Lawton (1977), report that thermal waste water from steam or nuclear power generation can raise the temperature of receiving waters, reduce dissolved gas solubility, and produce supersaturation.

Natural supersaturation occurs in both the marine and fresh water environment. Harvey (1967) describes a fresh water lake in which solar radiation increased water temperatures to produce a Total Gas Pressure of 1.1 to 1.2 Atms. Similarly, Reintjes (1969), Westman and Nigrelli (1955), and Zaitsev (1971) report supersaturation caused by solar heating in ocean environments. Well water, commonly used in aquaculture, is often highly supersaturated with dissolve nitrogen (Marsh, 1910; Rucker and Tuttle, 1948; Matsue *et al.*, 1953). Jarnefelt (1948), Ebeling (1954), Holl (1955), Lindroth (1957), and Harvey and Cooper (1962) report high levels of dissolved gases below water falls and certain types of rapids. A combination of solar heating and phytoplankton blooms can also produce high levels of supersaturation. Woodbury (1941), Alikunhi *et al.* (1951), Schmassmann (1951), Rukavina and Varenika (1956), Renfro (1963), and Supplee and Lightner (1976) describe many of these occurrences. Renfro (1963) reports of massive fish mortality in Galveston Bay as a result of solar heating and oxygen production by phytoplankton. Dissolved oxygen concentrations of 250% of equilibrium were recorded in this incident. White *et al.* (1986) describe excessive total gas pressures produced by a combination of a dam and algae blooms on an inland river.

Water can also become supersaturated as a result of reducing ambient pressure. Hauck (1986) reports of mortalities incurred in pink salmon as a result of moving fry

by helicopter. A reduction in ambient pressure caused by a rapid increase in altitude quickly reduced gas solubility, thereby supersaturating the water.

1.3 SYMPTOMS OF GAS BUBBLE TRAUMA

The symptoms of GBT are surprisingly varied. The major ones are outlined in the initial introduction to this section. In virtually all cases, bubbles are responsible for the observed symptoms. The activation of symptoms, however, may not be an easily demonstrated cause and effect relationship. This is because internally bubbles can grow in all body compartments and produce disruptions of neurological, cardiovascular, respiratory, osmoregulatory and other physiological functions. Clearly, there are opportunities for both direct and indirect effects of bubble growth. GBT can also involve a combination of bubble induced physiological stress and bacterial, viral and fungal infections (Weitkamp, 1976; Nebeker *et al.*, 1976 and Meekin and Turner, 1974).

The definitive, although slightly dated, review of GBT in fish is that of Weitkamp and Katz (1980). Their review examines almost 200 papers on the subject and describes in great detail the many symptoms of GBT. In most cases, the conditions that produce the symptoms are also specify. The more recent review by Colt, Bouck and Fidler (1986) adds further information to the overall understanding of GBT, its symptoms, causes and treatment.

1.4 REPORTING OF DISSOLVED GAS TENSIONS

Early in the study of dissolved gas supersaturation, it was commonly believed that the symptoms of GBT and mortality were independent of water dissolved oxygen concentrations. As a result, many data in the literature are reported as a function of

pN_2 only. However, Fidler (1985), Dawley and Ebel (1975) and Rucker (1975) give evidence that the Total Gas Pressure as well as water pO_2 levels control bubble growth and time to mortality. Thus, data from the literature based only on dissolved nitrogen measurements are of little use in obtaining correlations between water dissolved gas levels, symptoms, time to mortality or bubble growth thresholds.

Because of the inconsistent manner in which many data on GBT and gas supersaturation are reported in the literature, Colt (1983 and 1984) derived the various equations for the reporting of Total Gas Pressure and other dissolved gas tensions. In more recent studies of GBT and supersaturation the standards proposed by Colt have been followed.

1.5 EXPERIMENTAL STUDIES

GBT research reported in the scientific literature focuses on the identification of symptoms and time to mortality as the primary response of fish to supersaturation (Blahm *et al.*, 1973; Blahm *et al.*, 1975; Dawley and Ebel, 1975; Dawley *et al.*, 1975; Knittel *et al.*, 1980; Meekin and Turner, 1974; Nebeker and Brett, 1976; Nebeker *et al.*, 1979a and 1979b; Nebeker *et al.*, 1978; Rucker, 1974; Jensen *et al.*, 1985; Stroud *et al.*, 1976; and Weitkamp, 1976). With few exceptions, there is no attempt to clearly identify the causes of mortality or thresholds that may be associated with mortality. Stroud and Nebeker (1976), Stroud *et al.* (1975) and Meekin and Turner (1974) provide a correlation of various symptoms of GBT with water TGP levels and time to mortality. Knittel *et al.* (1980), in studies of Steelhead trout, offer the only description of a threshold for mortality.

Other studies of GBT use decompression as a means of simulating supersaturation. Beyer (1976a and 1976b), Casillas *et al.* (1975), Casillas (1976a and 1976b), D'Aoust and Smith (1974) and Feathers and Knable (1983) show that upon decompression, fish exhibit internal symptoms similar to those seen in fish exposed to gas supersaturated water. That is, bubble formation is prevalent in virtually all body compartments. Unfortunately, there are no distinctions made of important differences between decompression and supersaturation in fish. For example, there is little attention given to the direction of dissolved gas movement. In dissolved gas supersaturation this movement is opposite to that in decompression. That is, during decompression, dissolved gases move from body compartments into the environmental respiratory medium. However, in supersaturation, dissolved gases move from the environmental respiratory medium into body compartments. Nor is the distinction made that, during decompression, respiration reduces dissolved gas tensions in the animal; whereas, in supersaturation, respiration increases dissolved gas tensions. Perhaps the most important point overlooked is that bubble growth during decompression must take place within minutes or hours. Otherwise, dissolved gas tensions decrease as a result of respiration to levels that prevent bubble growth (Hills, 1977). In many situations involving supersaturation, bubble growth has no time limit. Fish may spend months or their entire lifes in supersaturated water (Weitkamp and Katz, 1980 and White *et al.*, 1986).

In the literature, there is little reported in the way of physiological measurements for fish exposed to supersaturation. Casillas *et al.* (1975, 1976a and 1976b) describe changes in an array of blood parameters with particular emphasis on clotting mechanisms in fish undergoing decompression. Newcomb (1976) describes the changes in blood chemistry in Steelhead trout exposed to supersaturated water. For

the most part, histological studies have involved visual examination for bubbles in various body organs and external skin surfaces. Recently, Smith (1988) has obtained photomicrographs that show the presence of micro-nuclei and bubbles in heart and gill tissue.

A large volume of data were added to the literature as a result of many studies conducted on the Columbia River system in the United States. From this work, it is clear there is little in the way of a detailed understanding of the relationship between the physical causes of GBT and the physiological effects. For example, it is not known which symptoms lead to observed mortalities. Bubble growth in the vascular system is often cited as a cause (Weitkamp and Katz, 1980). Yet, other symptoms, such as extracorporeal bubbles in the gills and sub-dermal bubbles on the skin and in the mouth, are often present at the same time (Stroud and Nebeker, 1976; Stroud *et al.*, 1975 and Meekin and Turner, 1974). It is not clear whether the various symptoms act in concert or if different lethal symptoms are separated by water TGP, pO_2 levels or other parameters.

Furthermore, it is surprising there is no information on the overall cardiovascular or respiratory response of fish exposed to supersaturated water. It is often speculated that death is due to anoxia caused by the growth of intravascular bubbles (Stroud *et al.*, 1976; Bouck, 1980 and Newcomb, 1976). Yet, there are no measurements of arterial blood oxygen tensions under conditions of supersaturation. Other cardiovascular parameters (blood pH, blood pressure, heart rate, hematocrit) and respiratory parameters (ventilation frequency and volume) which would lead to an improved understanding of the response, are also absent from the literature.

Finally, there has been no attempt to determine if data from the literature exhibit differences in response to supersaturation in terms of time to mortality. That is, it is not known if mortality data show thresholds associated with specific levels of water TGP, pO_2, fish species or fish size.

1.6 ANALYTICAL MODELS

1.6.1 TIME TO 50% MORTALITY: The only significant attempt to relate mortality data to water parameters, species and fish size is that of Jensen, Schnute and Alderdice (1986a and 1986b). In this work, data records from the literature that include water TGP, pO_2, time to 50% mortality and other parameters (Jensen *et al.*, 1985), were incorporated into a generalized surface response analysis (Schnute and McKinnell, 1984). The various models produced by this analytical technique reflect the detail to which specific physical and physiological parameters are included in the models. In this work the authors assume that there is a single cause of mortality in fish exposed to supersaturated water. That is, the data used in the various models were not examined to determine if *multiple* thresholds for mortality were present. If more than one threshold is present, it is reasonable to suspect that mortality is caused by more than one factor. Furthermore, these factors may be separated by water TGP, pO_2, fish size, species, etc. This problem will be examined more fully in Sections 3 and 4 of this thesis.

1.62 BUBBLE GROWTH THRESHOLDS: In an earlier development by this author, equations were derived that describe thresholds for various forms of bubble growth in fish exposed to supersaturated water. The derivation involves a mass balance applied to the movement of dissolved gases from the environmental water, across the

gill membrane and into the blood and nucleation sites in the cardiovascular system. The resulting equations contain physical parameters related to the environmental water that include TGP, pO_2, temperature and depth. Other physical parameters in the equations account for barometric pressure, the solubilities and diffusivities of oxygen and nitrogen in water and blood, the vapor pressure of water, the surface tensions of water and fish blood and the mass transfer coefficients for the movement of dissolved gases into a growing bubble. The physiological parameters include the ratio of the partial pressure of oxygen in blood to the partial pressure of oxygen in the environmental water, the system pressure where bubble growth occurs and the size of nucleation sites from which bubble growth begins. The derivation of these equations is described in Appendix A of this thesis. Fidler (1985) gives a discussion of the role gaseous nucleation sites play in the growth of bubbles.

As described in Appendix A, if the size of a nucleation site is taken as very large, such that surface tension forces are small, the threshold equation can be applied to the problem of swimbladder overinflation in fish. Yet, a further simplification of the equation yields a description of thresholds for bubble growth in the environmental water.

The final results are three equations which describe the thresholds for bubble growth in the vascular system, the swimbladder and in the environmental water. Table I gives definitions of the terms appearing in the equations. From Appendix A, the threshold equations are:

THRESHOLD CRITERIA FOR BUBBLE GROWTH IN THE VASCULAR SYSTEM

$$TGP_{CV} \geq P_{Atm} + Ps + \rho \cdot g \cdot h + \frac{2 \cdot \sigma}{r_o} - pO_2 \cdot (K \cdot F - 1) - (1 - K) \cdot p_{Ob}$$

Equation 1

where the subscript CV refers to the vascular system.

THRESHOLD CRITERIA FOR OVERINFLATION OF THE SWIMBLADDER

$$TGP_{SB} \geq P_{Atm} + Ps + \rho \cdot g \cdot h - pO_2 \cdot (K \cdot F - 1) - (1 - K) \cdot p_{Ob}$$

Equation 2

where the subscript SB refers to the swimbladder.

THRESHOLD CRITERIA FOR BUBBLE GROWTH IN ENVIRONMENTAL WATER

$$TGP_{EW} \geq P_{Atm} + Ps + \rho \cdot g \cdot h + \frac{2 \cdot \sigma}{r_o} + (K - 1) \cdot (p_{Ob} - pO_2)$$

Equation 3

where the subscript EW refers to Environmental Water.

TABLE I NOMENCLATURE

$TGP = pO_2 + pN_2 + pH_2O$

pO_2 = partial pressure of dissolved oxygen in environmental water.

pN_2 = partial pressure of dissolved nitrogen in environmental water.

pH_2O = vapor pressure of water.

p_{Ob} = partial pressure of oxygen in nucleation site.

r_o = the radius of the critical nucleation sites from which bubble growth begins (environmental water or vascular system),

$K = H_O \cdot k_O / H_N \cdot k_N$ where,

H_O = Henrys constant for oxygen in fish blood,

H_N = Henrys constant for nitrogen in fish blood,

k_O = mass transfer coefficient for oxygen into nucleus,

k_N = mass transfer coefficient for nitrogen into nucleus,

T = temperature in degrees C..

σ = surface tension of water or fish blood.

F = oxygen uptake ratio across gills, = pO_2 (Arterial Blood)/pO_2 (Water),

$P = Patm. + \rho \cdot h + Ps$,

Patm = atmospheric pressure,

Ps = system pressure where bubble is growing ,

ρ = density of water,

h = depth of fish in water column.

g = gravitational constant.

Most of the physical parameters contained in the equations are well defined. However, when the equations were first derived, there was no information regarding the surface tension of fish blood. Later, Fidler (1985) found it to be essentially the same as water. In situations involving substantial depth, where fish are free to roam within that depth, it may be difficult to specify the depth term in the equations. However, in environments, where fish are confined to specific depths, all physical parameters contained in the equations are known or can be measured directly. The situation involving the physiological parameters is not as easily resolved. Although there is information in the literature that may allow the calculation of the gill oxygen uptake ratio (F) the remaining parameters, Ps and r_o are without definition. Thus, it is not possible to define thresholds or determine if the thresholds are distinctly different in terms of water TGP and pO_2. It is known that these forms of bubble growth are present in fish that have died as a result of exposure to supersaturated water. However, it is not known whether there are thresholds for mortality that may be directly correlated with thresholds for bubble growth.

2.0 STUDY DEFINITION

Based on the potential usefulness of the threshold equations, an initial goal of this study was to determine the unknown physiological parameters needed to complete the equations. Next, it was necessary to establish that these forms of bubble growth are in fact responsible for physiological stress and mortality. Without this correlation, the threshold equations are of limited value and of little practical use. Thus, a vital part of the study involved an examination of the physiological response of fish to supersaturation and bubble growth. This response was of fundamental importance in achieving a correlation between mortality and bubble growth thresholds. The work was performed in three phases as outlined below.

Phase 1.) A theoretical analysis expanded the threshold equations and added to their usefulness in relating mathematical parameters to measurable physiological parameters. Additional mathematical models were introduced that describe the bubble growth process more fully.

Phase 2.) GBT data from the literature were reviewed. A compilation of those data into a database aided in correlating observed thresholds for mortality to thresholds for bubble growth.

Phase 3.) An experimental program provided further definition of the physiological parameters contained in the threshold equations. The experimental data also allowed physiological symptoms to be related to bubble growth thresholds.

The results of the three phases of study were finally combined into a correlation of the bubble growth threshold equations with experimental data from this work and from the literature.

3.0 THEORETICAL STUDIES

3.1 INTRODUCTION

As outlined above, the purpose of this study was to extend and verify the GBT bubble growth threshold models described in Appendix A. In proceeding toward this goal, it became clear that various facets of the problem were amenable to additional mathematical analysis. First, it was necessary to modify the bubble growth threshold equations. The modifications improved their utility and allowed direct methods for evaluating physiological parameters. It was also useful to expand the equations to apply to thresholds for sub-dermal bubbles. As described in the introduction, these bubbles appear on external skin surfaces and in the lining of the buccal cavity. The importance of this threshold became apparent in the experimental studies described in Section 5 of this thesis. In addition, mathematical models were developed that provide approximate descriptions of bubble growth and the interaction of growing bubbles with cardiovascular system pressure. The solutions to these equations allowed an assessment of the time course for bubble growth and indicated experimental methods for determining the effective dimensions of vascular system nucleation sites. This section will begin with an examination of the threshold equations and the parameters contained in the equations.

3.2 THRESHOLDS FOR BUBBLE GROWTH

The bubble growth threshold equations were derived in the forms shown in Equations 1, 2 and 3. In general, the equations imply that thresholds for bubble growth increase with increasing water depth (h), system pressure (Ps), and barometric pressure (P_{Atms}). On the other hand, thresholds decrease as nucleation site radius (r_o), increases. The effect of water pO2 on bubble growth thresholds is dependent on the

relative magnitude of the transport parameters (i.e. mass transfer coefficients and Henrys constants). In Equations 1 and 2, the effect of water pO2 is also dependent on the oxygen uptake ratio (F) across the gill. In general, as F increases, the effect of pO_2 on threshold TGP diminishes. Finally, the partial pressure of oxygen in the initial nucleus plays a role in the TGP thresholds. The importance of bubble oxygen partial pressure is examined in more detail later in this section.

Physical parameters contained in the equations (water temperature, depth, pO_2 and transport parameters along with surface tension) are either definable, measurable or are controlled for many situations in which the equations can be applied. For example, mass transfer coefficients, diffusion coefficients and Henrys constant for most atmospheric gases in water and fish blood are known as a function of temperature (Epstein and Plesset, 1950; Plesset, 1964; Clift *et al.*, 1978; Altman and Dittmer, 1961, 1964 & 1971; Weiss, 1970 and Boutilier *et al.*, 1984). Also, the surface tension and vapor pressure of water are defined as a function of temperature (Perry, 1983 and Reid *et al.*, 1977). The surface tension of Rainbow trout blood, at a temperature of 7° C., was determined by Fidler (1985), and found to be close to that of water. Thus, the principal unknown parameters are physiological. These consist of the ratio of arterial pO_2 to water pO_2, the vascular system pressure where initial bubble growth begins and the size of critical nucleation sites. In order to examine these parameters in more detail, each threshold equation will be considered separately.

3.2.1 VASCULAR SYSTEM BUBBLE GROWTH: As described in the introduction, this symptom is commonly seen in fish exposed to dissolved gas supersaturation. It is considered to be the most lethal of all GBT symptoms (Stroud *et al.*, 1976; and

Bouck, 1980). Although not stated explicitly in the threshold equation, there is a strong coupling of the nucleation site location with system pressure and oxygen uptake ratio. The system pressure (Ps) and oxygen uptake ratio (F) are specifically those at the location of the nucleation site. Fidler (1985) has pointed out that the most likely location for initial intravascular bubble formation is the arterial side of the circulatory system. This is because venous levels of dissolved oxygen are on the order of 20 - 30 mmHg.; whereas, arterial values are 100 - 130 mmHg (Holeton and Randall, 1967; Randall, 1970; Davis and Cameron, 1971; Sovio *et al.*, 1981; Thomas and Hughes, 1982; Wood and Jackson, 1980 and Wood *et al.*, 1984). Because of the lower venous pO_2, the effective TGP for venous blood is significantly lower than that of arterial blood. This indicates that much higher levels of water TGP are required before the thresholds for bubble growth in venous blood are reached. Thus, the F term in Equation 1 applies primarily to arterial blood.

In general, the location of nucleation sites in the vascular system is unknown. Therefore, it is not possible to define Ps and F directly. Furthermore, the size of nucleation sites in physiological systems are unknown and this leaves the r_o term in the equation without definition.

As pointed out earlier, the threshold for intravascular bubble growth is dependent on water pO_2. This dependency is a result of differences between blood and water pO_2 caused by oxygen transport resistances at the gill (Randall, 1970; Randall, 1984 and Piiper and Scheid, 1984). The ratio of blood pO_2 to water pO_2 has not been determined explicitly. However, there may be sufficient data in the literature to make reasonable estimates of this ratio at the dorsal aorta. With F defined at the dorsal

aorta, there remains the problem of determining an appropriate value of F at nucleation sites.

3.2.2 SWIMBLADDER OVERINFLATION: The swimbladders of most fish are highly vascularized with arterial blood (Steen, 1970). Thus, there is the potential for dissolved gases to overinflate this organ (Fidler, 1985; Shrimpton *et al.*, 1988). Overinflation of the swimbladder and other body cavities accompanied by occasional mortality are reported by Shirahata, (1966); Kral, (1983); Bowser, (1983); Cornacchia and Colt, (1984); Johnson and Katavic, (1984); Kolbeinshavn and Wallace, (1985); Jensen, (1987) and Shrimpton *et al.*, (1988). Equation 2 was derived as a special case of Equation 1. As described in Appendix A, the swim bladder acts as an extremely large nucleation site. As such, surface tension effects are small which permits the $2\sigma/r_o$ term to be dropped from the general threshold equation. Equation 1 reduces to Equation 2 where the only physiological unknown is F, the ratio of arterial pO_2 to water pO_2. As pointed out above, there may be enough data in the literature to define this term for arterial blood at the dorsal aorta. However, it is still not known how the value of F at the swimbladder is related to F at the dorsal aorta.

3.2.3 WATER BUBBLES: Equation 3 is again a special case of Equation 1 where, for bubble growth in the environmental water, F is equal to 1.0. This reduces Equation 1 to Equation 3 where the only physiological unknown is the size of nuclei from which bubble growth begins. Again, this dimension is virtually unknown for physiological systems. Situations in which the equation can be applied include extracorporeal bubble growth in the buccal cavity or extracorporeal bubble growth between gill lamella. In the first case, it is observed that a single bubble in the buccal cavity of

larval fish can block respiratory water flow and cause death (Shirahata, 1966 and Jensen, 1980). Extracorporeal bubbles growing between gill lamella are observed in fish exposed to gas supersaturated water (Weitkamp and Katz, 1980). However, it has not been established that these bubbles are lethal to fish.

3.3 BACKGROUND AND METHODS

3.3.1 EQUATION MODIFICATION: The threshold equations can be simplified through an assumption regarding the initial partial pressure of oxygen in the nucleus. Before specifying this assumption, recall that the threshold equations are dependent on the partial pressure of oxygen in the nucleation sites (p_{Ob}). If, at time zero in the bubble growth process, this pressure is out of equilibrium with the dissolved gases in solution, there are a wide range of situations where oxygen and nitrogen could be undergoing countercurrent or cocurrent diffusion. For example, it is possible for bubble growth to occur when oxygen is diffusing outward from the nucleus while nitrogen is diffusing inward. Growth of the nucleus would imply that the net outward movement of oxygen is more than offset by the inward movement of nitrogen. The opposite situation can also occur. There are, of course, those situations where all gases are diffusing into the bubble. Thus, the equations involve the coupling of transport terms to initial conditions of bubble and water oxygen partial pressures. This accounts for the many possible directions and relative magnitudes of gas transport. The effect of transport parameters on nitrogen is implicit in the equations through the definition of Total Gas Pressure (Table I).

Upon exposure to supersaturated water, the development of supersaturation within fish occurs gradually. That is, at time zero in the bubble growth process, gases in

nucleation sites are in equilibrium with those in the surrounding medium. The subsequent disequilibrium is not instantaneous, but develops over a finite period. Thus, it will be assumed that all gases in nucleation sites are in equilibrium with the dissolved gases in the surrounding medium (i.e. water or plasma) before bubble growth begins. This allows p_{Ob} in Equations 1 and 2 to be replaced with F•pO2. With the same assumptions regarding bubble growth in the environmental water, p_{Ob} is replaced with pO2 in Equation 3. These substitutions allow the threshold equations to be written in the simpler forms given by Equations 4, 5 and 6. Again, Table I defines the terms appearing in the equations.

THRESHOLD CRITERIA FOR BUBBLE GROWTH IN THE VASCULAR SYSTEM

$$TGP_{CV} \geq P_{Atm} + Ps + \varrho \cdot g \cdot h + \frac{2 \cdot \sigma}{r_o} + pO_2 \cdot (1 - F)$$

Equation 4

THRESHOLD CRITERIA FOR OVERINFLATION OF THE SWIMBLADDER

$$TGP_{SB} \geq P_{Atm} + Ps + \varrho \cdot g \cdot h + pO_2 \cdot (1 - F)$$

Equation 5

THRESHOLD CRITERIA FOR BUBBLE GROWTH IN ENVIRONMENTAL WATER

$$TGP_{EW} \geq P_{Atm} + Ps + \varrho \cdot g \cdot h + \frac{2 \cdot \sigma}{r_o}$$

Equation 6

It will be noted that transport terms are absent in the new forms of the equations. The assumption of initial equilibrium between gas phases (i.e. in the bubble and in solution) assures that any increase in TGP above the threshold level will result in bubble growth. It is also noted that only Equations 4 and 5 retain a dependency on water pO2. Again, this is because plasma pO2 is reduced from that of the water by the factor F.

3.3.2 GILL OXYGEN UPTAKE RATIO: Throughout the literature there have been many reported measurements of arterial blood pO_2 from the dorsal aorta of fish. In most cases water pO_2 is also reported. In order to use this information for determining appropriate values of F, it was tabulated for Rainbow trout as shown in Appendix B. Included in the tabulation are data from the experimental phases of this investigation (Section 5). Table I of Appendix B defines the abbreviations used in the tabulation while Table II of the appendix identifies the data sources. Only data obtained for resting fish were selected for this tabulation. This was done in order to limit the variation in the data and to yield results that were applicable to the experimental phases of this study. The data were converted to F values and a mean along with standard deviations were calculated. This was done for data in the pO_2 range of 70 to 350 mmHg only. The selection of this range is explained more fully in the discussions that follow.

3.3.3 ARTERIAL BLOOD PRESSURE: The blood pressure in vertebrates varies considerably throughout the circulatory system. In general, the pressure at any point in the system is a function of both the local fluid velocity and all friction pressure losses occurring upstream of that point (Folkow and Neil, 1971 and Welty *et al.*, 1976). In fish, blood pressure is highest in the ventricle of the heart and decreases

continuously as blood moves along the arterial system toward tissue capillary beds (Randall, 1967a; Randall, 1983; and Kiceniuk and Jones, 1977). It continues to decrease in the venous system and reaches a minimum in the *sinus venosus* just upstream of the heart. Holeton and Randall (1967a) measured values of 60 to 70 mmHg. for blood pressure in the ventral aorta of Rainbow trout. Blood pressure in the dorsal aorta of the same species ranges from 20 to 50 mmHg. (Holeton and Randall, 1967a. also see Section 5 of this thesis). In Rainbow trout, the gills account for nearly 20% to 40% of the pressure loss between the ventral aorta and the venous return circulation (Holeton and Randall, 1967a; Stevens and Randall, 1967a). The next major drop in systemic pressure occurs in the arterioles just upstream of capillary beds (Feigl,1974). Although pressures have been measured at many locations in Rainbow trout vascular systems, there are no data for the arteriole or capillary levels. However, blood pressures have been measured on the venous side of the circulatory system of Rainbow trout by Kiceniuk and Jones (1977). These pressures are on the order of 5 to 7 mmHg. Based on these measurements for the dorsal aorta and venous system, capillary pressures are estimated to be on the order of 10 to 15 mmHg. or less (Farrell, 1988: personal communication).

Swimbladder Overinflation: The swimbladders of physostome fish, are highly vascularized with small arteries and capillaries (Fange, 1966; Steen, 1970; and Steen and Sund, 1977). In Equation 5, which applies to overinflation of the swimbladder, Ps should correspond to the pressure at the swimbladder capillaries. As with tissue arterioles and capillaries, there are no blood pressure measurements available for these regions in fish. However, as suggested above, these pressures should be low and perhaps on the order of 10 to 15 mmHg.

Vascular System Bubbles: For vascular system bubbles, Ps must decrease as Equation 4 is applied to arterial regions more and more distant from the heart. As a result, the thresholds for vascular system bubble growth also decline. However, there is a limit to this decline; for at the capillary beds, arterial pO_2 decreases as oxygen from the blood diffuses toward cells. This lowers the local TGP of the blood which raises the water TGP thresholds required to initiate bubble growth. This is equivalent to decreasing the F term in Equation 4 for locations in the capillary beds and beyond.

Based on this analysis, it appears that the most likely sites for bubble growth are the arterioles just upstream of the tissue capillary beds. Here system pressure is at its lowest before the decline in pO_2 begins at the capillaries. Although measurements of pressure at arteriole and capillary locations would be helpful, they are of limited value without confirmation of nucleation and bubble growth at these sites.

3.3.4 CRITICAL NUCLEI RADIUS: The r_o term in Equations 4 and 6 is the critical radius of nucleation sites needed to start bubble growth for specific levels of water TGP and pO_2. In studies of decompression in humans and animals, there have been many attempts to define the size of corporeal nuclei (Yount, 1979; Yount and Yeung, 1979; Yount, 1981; Philp *et al.*, 1972; Ackles, 1973 and Hemmingsen, 1986). Unfortunately, little quantitative information is available regarding their size. However, it is clear that size may vary with individual and with the number and frequency of previous decompression episodes (Hills, 1977). Using rats, Philp, Inwood and Warren (1972) have demonstrated that following decompression involving bubble formation, nuclei are free in the blood and are significantly larger than the original nuclei from which the bubbles formed. The residual nuclei appear to be bubbles that

have stabilized during collapse through the accumulation of blood protein components at the bubble surface (Philp, Inwood and Warren, 1972). This is an important finding from the standpoint of multiple decompression episodes. In fish, exposed to supersaturation, these larger nuclei would require lower TGP thresholds to initiate bubble growth during subsequent exposures to supersaturation. Knittel *et al.* (1980) have shown this effect in Steelhead trout.

This author (1985), pointed out that if vascular system nuclei are free in the blood, they could be no larger than erythrocytes. As it is, erythrocytes are just able to squeeze through sections of gill secondary lamella and tissue capillaries (Randall, 1970; Randall and Daxboeck, 1984 and Farrell *et al.*, 1980). In Rainbow trout, free nuclei would be on the order of 10 to 15 μM. in diameter; which is the characteristic dimension of erythrocytes in this species (Heming, 1984a and Mott, 1957 and Smith *et al.*, 1952).

However, there are indications that prior to the first episode of decompression, nucleation sites are not free in the blood but are associated with the walls of the vascular system. Harvey *et al.* (1944) could not produce bubble growth in blood from monkeys and rats, *in vitro*, at high levels of decompression. However, *in vivo* bubble growth was easily attained at comparatively low levels of decompression. Similarly, Hemmingsen *et al.* (1985) using *in vitro* studies could not produce bubbles in mammalian, avian or amphibian blood with decompression from 300 Atms. Thus, it appears that nuclei, rather than being free in blood, are in some way associated with the linings of the vascular system. This is consistent with the long held theory (Harvey, 1951) that nucleation sites are gas filled discontinuities in surfaces. Although this seems to conflict with the observations of Philp, Inwood and

Warren (1972), it should be recalled that their results were obtained following decompression. Thus, it is conceivable that during decompression, bubbles can grow to sizes that allow blood flow to remove them from their original sites. However, the dimensions of capillary bed vessels would be a restriction in their size and movement.

It will be recalled from a previous discussion that as blood moves from the heart to the capillaries, there is a progressive decline in blood pressure. Equation 4 implies that at a given water TGP and pO_2, the size of nucleation sites required to initiate bubble growth increases as blood pressure increases. This is illustrated by rewriting Equation 4 in the following form.

$$Ps = TGP - \frac{2 \cdot \sigma}{r_o} - (1 - F) \cdot pO_2$$

The solution of this equation is shown in Figure 1 where the nuclei radius required for bubble growth is plotted versus blood pressure. Other parameters used in the equation are as indicated in the figure notes. The levels of TGP and pO_2 specified in the figure are representative of those frequently reported in the literature where mortality and vascular system bubble growth are observed. As indicated earlier, absolute values of pressure are not known at all locations in the vascular systems of fish. However, it is known that for the arterial system in Rainbow trout, the range of pressures shown in Figure 2 are representative (Stevens and Randall, 1967a,b and Kiceniuk and Jones, 1977). It is surprising that for this range, the size requirements for nuclei vary only by a factor of three. If other levels of water TGP and pO_2 from the literature are examined, it is found that, over the range of these variables found in GBT, nuclei required for bubble growth range from 10 to 40 μM. in radius.

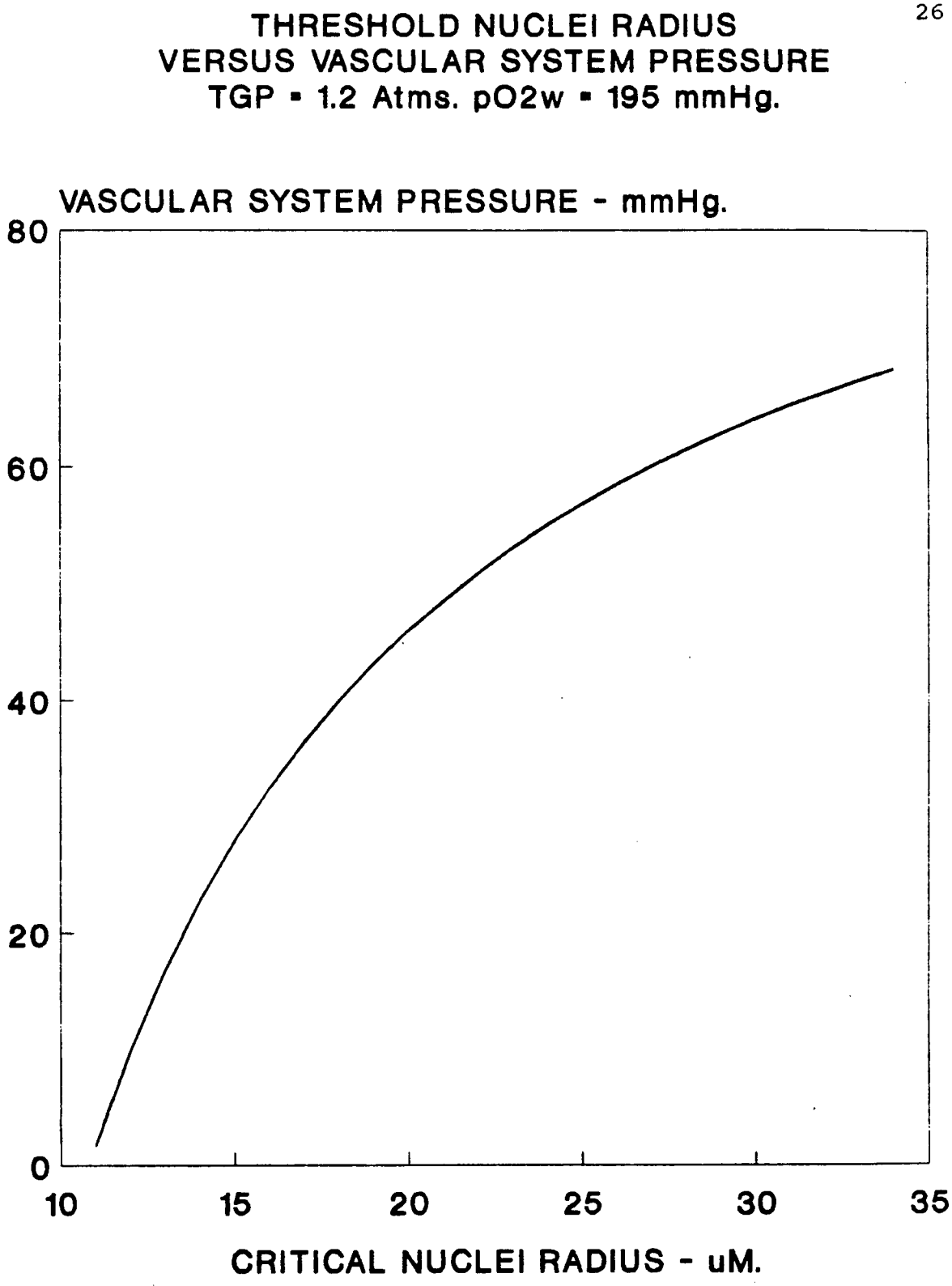


FIGURE 1 Threshold Nuclei Radius Versus Blood Pressure.

In situations involving bubble growth in the environmental water, nucleation sites can also be associated with the surfaces upon which bubble growth begins. This would include the external skin surfaces of the animal and the lining of the buccal cavity. It should be anticipated that extracorporeal bubble growth in the buccal cavity of small fish or in the gills of larger fish may involve nuclei carried to the site of growth by respiratory water flow. This is especially true if the environmental water carries bubbles, large silt or other particulate matter. However, nuclei radius, either associated with tissue surfaces or foreign matter, is again unknown and probably difficult to determine. Nevertheless, application of Equation 6 to a range of TGP levels typical of those found in problems of GBT, yields a radius for nucleation sites ranging from 5 to 20 μM. (Figure 2).

Although the above discussion has not provided absolute information regarding the size of nucleation sites or the system pressures where bubble growth begins, it provides some insight as to the range of these parameters. There remains the problem of evaluating these parameters in an absolute sense. In intravascular bubble growth, the problem can be simplified by re-examining the threshold equations.

3.3.5 EFFECTIVE NUCLEI RADIUS: In Figure 3 the equation for vascular system bubble growth is rewritten with the Ps and r_o terms highlighted (Equation 7). It will be noted that nucleation site radius, as contained in the $2\sigma/r_o$ portion of the equation, is a pressure term like Ps. Since both of these parameters are unknown and difficult to determine, the two can be combined into a single unknown parameter. In order to retain surface tension explicitly in the equation, the parameters are combined so that an effective radius, R_o, accounts for both Ps and r_o. This is shown as Equation 8.

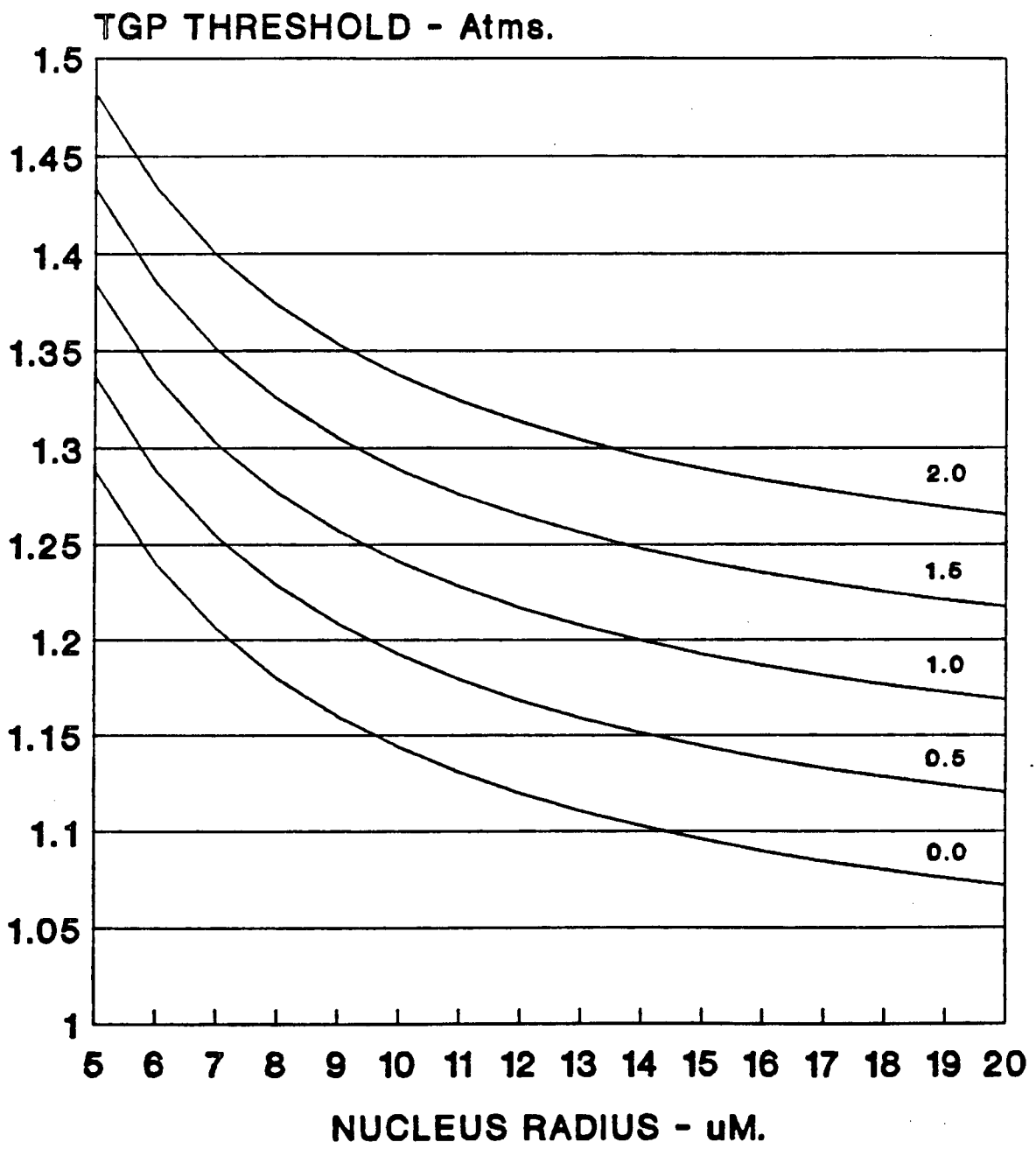


FIGURE 2 : Threshold Nuclei Radius Versus Water TGP.

THRESHOLD CRITERIA FOR VASCULAR SYSTEM BUBBLE GROWTH

$$TGP \geq P_{Atm.} + \varrho \cdot g \cdot h + \boxed{Ps} + \boxed{\frac{2 \cdot \sigma}{r_o}} + pO_2 \cdot (1 - F)$$

Equation 7.

$$\text{Letting} \quad Ps + \frac{2 \cdot \sigma}{r_o} = \frac{2 \cdot \sigma}{R_o}$$

$$TGP \geq P_{Atm.} + \varrho \cdot g \cdot h + \frac{2 \cdot \sigma}{R_o} + pO_2 \cdot (1 - F) \qquad \text{Equation 8.}$$

Figure 3: Effective Radius of Nucleation Sites in Vascular Bubble Growth Equation.

The result of this combination leaves Equation 8 with one unknown on the right side. Thus, if a threshold for bubble growth can be determined for known levels of water TGP and pO_2, or just TGP in the case of Equation 6, the R_o and r_o terms can be back-calculated from the equations. The equations can then be completed and used to predict thresholds of bubble growth for a wide range of conditions. Later in this section, experimental methods for determining bubble growth thresholds are examined. It should be noted that the use of an effective radius can compensate somewhat for uncertainties in the value of F at nucleation sites. That is, with F referenced to dorsal aorta blood pO_2, any variations between these values and those at the nucleation sites can be absorbed in the effective radius term.

3.3.6 SUB-DERMAL BUBBLE GROWTH: The literature offers many examples of sub-dermal bubbles or blisters growing on the external body surfaces of fish exposed to supersaturated water. These observations include bubbles on opercular flaps, between fin rays, and in the lining of the mouth (Weitkamp and Katz, 1980). It is hypothesized that the threshold equations apply to this form of bubble growth also. To select the appropriate equation, it is necessary to establish if dissolved gases are transported to these bubbles by blood flow through the circulatory system, or as a result of direct diffusion from the environmental water. Kirsch and Nonnote (1977) show that oxygen transport to the skin of Rainbow trout is primarily by direct diffusion from the environmental water. Furthermore, sub-dermal bubble growth appears to originate just below the epithelium tissue layer (Nebeker and Brett, 1976 and also Section 5, this thesis). Therefore, it is reasonable to assume that pO_2 and pN_2 levels are not significantly reduced from that of water. In this case, Equation 6 would be the appropriate form of the threshold equation.

Aside from the dimensions of nucleation sites, the other unknown parameter is the appropriate form of surface tension. Once bubble growth actually begins, the growth rate may be controlled by a combination of extracellular fluid surface tension forces and the tensile strength of tissue (Vann and Clark, 1975; Meisel *et al.*, 1981 and Yang and Liang, 1972). When bubbles become sufficiently large, such that surface tension forces are negligible, the tensile properties of the tissue should dominate the growth process. However, depending on the size and location of nucleation sites, the threshold situation may be controlled by water surface tension forces alone. With this assumption, Equation 6 is used as the combined water and sub-dermal bubble growth threshold equation in subsequent discussions and analyses. The validity of this assumption is examined further in Section 5 of this thesis.

3.3.7 BUBBLE GROWTH IN AN OPEN SYSTEM: It is of interest to examine the rate at which bubbles grow once threshold levels of TGP are exceeded. Time to mortality associated with GBT varies with water TGP and can range from several hundred hours at a TGP of 1.12 Atms. to just a few hours at TGP levels above 1.3 Atms. (Weitkamp and Katz. 1980). Part of this time must be associated with the period required for various body compartments of the fish to equilibrate with water dissolved gas tensions. Harvey (1963) and Beyer *et al.* (1976) give experimental evidence that shows this time is on the order of one to two hours. However, at high levels of TGP, bubble growth may occur before there is full equilibration between the water and all body compartments of the fish. In either case, it is expected that a significant portion of the time required to produce mortality would be that associated with bubble growth. Thus, depending on the water TGP, bubble growth periods on the order of several hundred hours to just a few hours would be expected.

Epstein and Plesset (1950) derive equations that describe the growth of a motionless, isothermal air bubble in still water. That is, a bubble that does not experience motion due to buoyant forces and grows as a result of diffusive gas transport. Although their derivation treated air as a single component gas (i.e. transport properties were weighted averages for oxygen and nitrogen), the resulting equations give a reasonable estimate of the time course for this type of bubble growth. For an initial nucleus radius of 10 μM., Epstein and Plesset calculate the time for a 10 fold increase in radius to be about 550 seconds at a water TGP of 1.25 Atms.

3.3.8 BUBBLE GROWTH IN A CLOSED SYSTEM: In the derivation of Epstein and Plesset (1950), it is assumed that as bubbles grow, the volume of gas added to the system does not effect system pressure. That is, ambient hydrostatic pressure is constant. This is appropriate for bubble growth in the environmental water; such as might occur in the buccal cavity or between gill lamella. However, this is not the case for intravascular bubbles where the system is somewhat closed. In this situation, the volume of growing bubbles will eventually affect system pressure and alter the bubble growth process. The actual relationship between volume and pressure will depend on many factors; however, the compliance of the vascular system will be central to the response. The coupling of volume to pressure can be modeled mathematically in an approximate form as follows.

To simplify the derivation and solution of the differential equations involved, this development will treat a single gas diffusing into bubbles forming in a closed system. The physical properties of this gas (i.e. diffusivity, Henrys constant, etc.) are taken as those of air. The derivation begins with the perfect gas relationship.

$$n_A = P_A \cdot V_B/(R' \cdot T) = (4/3) \cdot \pi \cdot r^3 \cdot P_A/(R' \cdot T)$$

where,

n_A = moles of gas A in bubble.

P_A = pressure of gas in bubble.

V_B = bubble volume = $(4/3) \cdot \pi \cdot r^3$

R' = gas constant.

r = bubble radius.

For ease of derivation, water vapor pressure is assumed to be small in relation to the gas pressure P_A and will be neglected.

Laplaces Equation relates bubble internal pressure (P_A) and external pressure (P_E) to surface tension and bubble radius as given by:

$$P_A = P_E + (2 \bullet \sigma / r)$$

where,

$P_E = P_{Atm.} + Ps + \rho \bullet g \bullet h$

$P_{Atm.}$ = atmospheric pressure.

P_S = vascular system pressure where bubble is growing.

ρ = density of water.

g = gravitational constant.

h = depth of fish in water column.

For convenience, it will be assumed that the pressure in the vascular system (Ps) is composed of a system pressure Ps' existing before bubble formation and a pressure Ps'' due to bubble growth. The component due to bubble growth will be a function of

the number of bubbles present, N, the volume of each bubble and the compliance of the vascular system, C. The component of system pressure due to bubble growth (Ps'') is C times the volume of the added bubbles. That is, it is assumed that the pressure - volume relationship is linear. Assuming a uniform radius for all bubbles at any given time and that Ps' is small, Ps takes the following form.

$$Ps = N \cdot C \cdot (4/3) \cdot \pi \cdot r^3 = N \cdot C' \cdot r^3$$

In the expression on the far right, the constants have been lumped into a single constant, C'. Substituting Laplaces Equation into the perfect gas equation, using the above definition of Ps and taking h as 0.0, gives:

$$n_A = (4/3) \cdot \pi \cdot (P_{Atms.} \cdot r^3 + N \cdot C' \cdot r^6 + 2 \cdot \sigma \cdot r^2) / (R' \cdot T)$$

Differentiating with respect to time, t, yields:

$$\frac{dn_A}{dt} = \frac{4 \cdot \pi}{3 \cdot R' \cdot T} \cdot \left[3 \cdot P_{Atms.} \cdot r^2 + 6 \cdot N \cdot C' \cdot r^5 + 4 \cdot \sigma \cdot r \right] \cdot \frac{dr}{dt}$$

Equation 9.

The rate of change in the number of moles of gas in a bubble must be balanced by the rate of gas added to the bubble by diffusive transport from the blood. Using the bubble mass transfer coefficient (D_A/r) of Epstein and Plesset (1950), the equation for gas transfer to or from the bubble is:

$$\frac{dn_A}{dt} = 4 \cdot \pi \cdot H_A \cdot r^2 \cdot \left[\frac{DA}{r} \right] \cdot \left[P_{OA} - P_A \right]$$

Equation 10.

where,

H_A = Henrys constant for gas A

D_A = Diffusivity of gas A in blood

P_{OA} = Total Gas Pressure of A in blood

For this derivation, it is assumed that P_{OA} of gas in the blood is the same as that in the water. Equating the right sides of Equations 9 and 10 yields an approximate expression for bubble growth in the vascular system of a fish exposed to supersaturated water. To arrange the equation in a form that can be integrated, variables are separated and the equation is written as:

$$t = \frac{1}{3 \cdot R' \cdot T \cdot D_A \cdot H_A} \cdot \int_{R_o}^{r} \frac{3 \cdot P_{Atms.} \cdot r + 6 \cdot N \cdot C' \cdot r^4 + 4 \cdot \sigma}{P_{OA} - P_{Atms.} - N \cdot C' \cdot r^3 - (2 \cdot \sigma / r)} \cdot dr$$

Equation 11.

If the compliance term C' is equal to zero, Equation 11 reduces to that for bubble growth in an open system.

The solution of Equation 11 is obtained numerically using the MathCad computer program. This involves substituting selected values of r into the equation and solving for t.

Because the solution is asymptotic, it is possible to select values of r which are too large; in which case the numerical solution will not converge. This problem can be avoided by using Equation 11, in its differential form, and setting dr/dt equal to zero. This yields:

$$P_{OA} - P_{Atms.} - N \cdot C' \cdot r^3 - (2\sigma / r) = 0 \qquad \text{Equation 12.}$$

One real root of this algebraic equation gives the asymptotic value of r which is the upper limit to be used in the solution of Equation 11. The other real root is the equilibrium radius corresponding to the threshold TGP. To calculate a threshold TGP (or P_{OA} in this case), Equation 12 is solved for P_{OA}. The result is:

$$P_{OA} = P_{Atms.} + N \cdot C' \cdot r^3 + (2\sigma / r) \qquad \text{Equation 13.}$$

Substituting r_o for the initial nucleus radius yields the threshold TGP.

3.4 RESULTS

3.4.1 THRESHOLD EQUATIONS: Equations 4 through 6 are plotted in Figure 4. The plots are based on an effective nuclei radius of 10 μM., a water depth of 0.0 M., a water temperature of 12° C., an F of 0.79 (see below) and sea level atmospheric pressure. The selection of R_o may appear somewhat arbitrary;

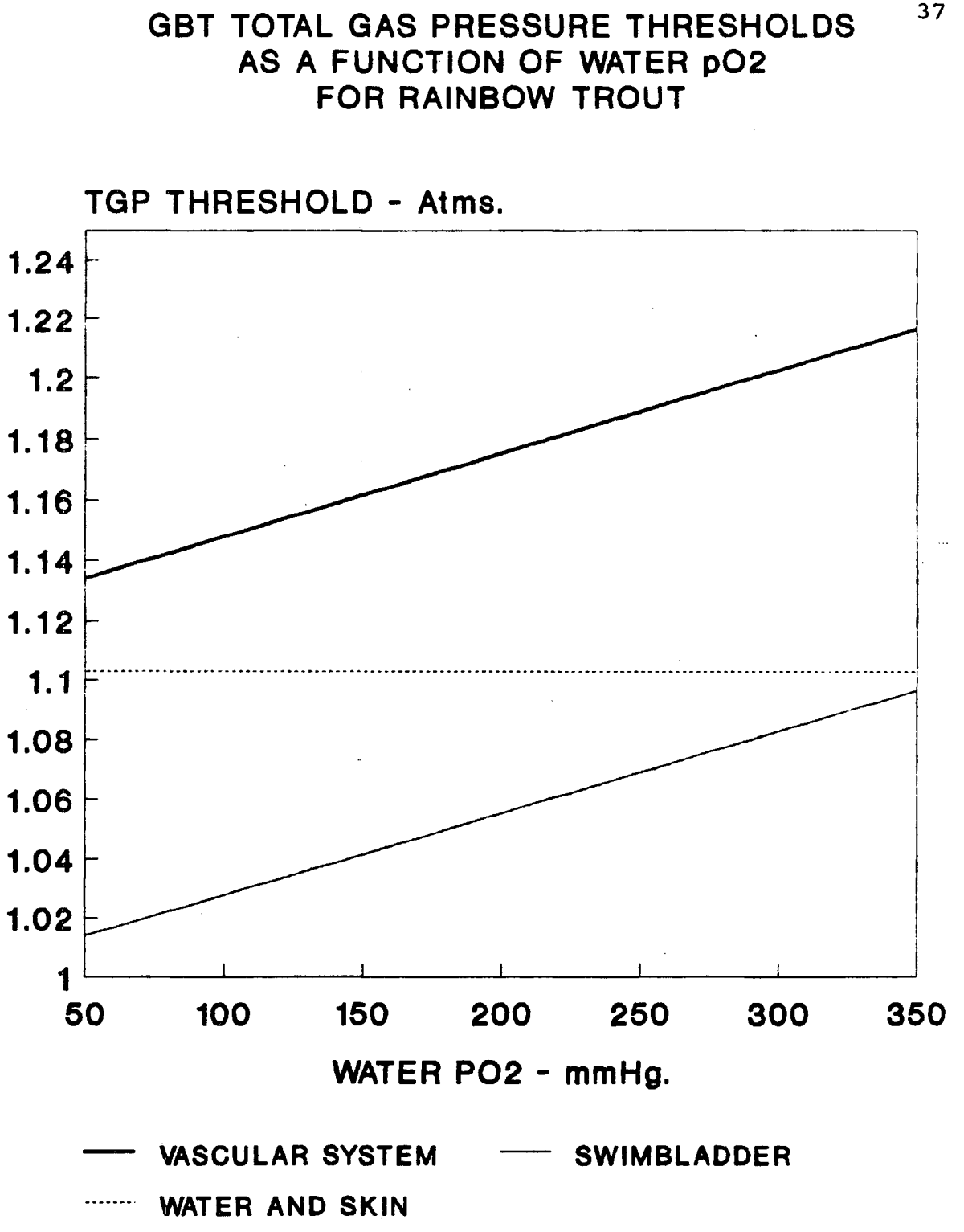


FIGURE 4: Theoretical Water Total Gas pressure Thresholds.

however, as will be shown in later sections, this value is representative. In the figure, it is seen that there is a clear separation of the various theoretical thresholds. The dependency of the thresholds for swimbladder overinflation and intravascular bubble growth on water pO_2 is also clearly shown. Interestingly, Equations 4 and 5 now contain water pO_2 as the only independent dissolved gas parameter. The other physical and physiological parameters are as in the original equations. Thus, the specification of water pO_2 and temperature is sufficient to define the TGP thresholds for swim bladder overinflation and intravascular bubble growth. Although this may appear odd at first, it should be remembered that TGP is made up of the vapor pressure of water plus the partial pressures of dissolved oxygen and dissolved nitrogen as defined in Table I. By specifying water pO_2 , water TGP is defined by the threshold equation, from which pN2 is determine by:

$$pN_2 = TGP - pO_2 - pH_2O \qquad \text{Equation 14}$$

With the specification of temperature, the vapor pressure of water is determined, and in turn pN_2. Thus, the definition of pN_2 is implicit in Equations 4 and 5.

3.4.2 OXYGEN UPTAKE RATIO: Figure 5 shows the gill oxygen uptake ratios (F) calculated from the data of Appendix B, and plotted as a function of water pO_2. From the figure, it is clear that F is quite variable with the highest variability occurring in the region where water pO2 levels are hypoxic for fish (Holeton and Randall, 1967a,b and Thomas and Hughes, 1982). Also, at the two extremes of water pO_2 (less than 70 mmHg. and greater than 350 mmHg.), the ratio of arterial pO_2 to water pO_2 appears to decline. Between these two values F is estimated to have a mean value of 0.79 with a standard deviation of 0.045. In most cases involving fish exposed to

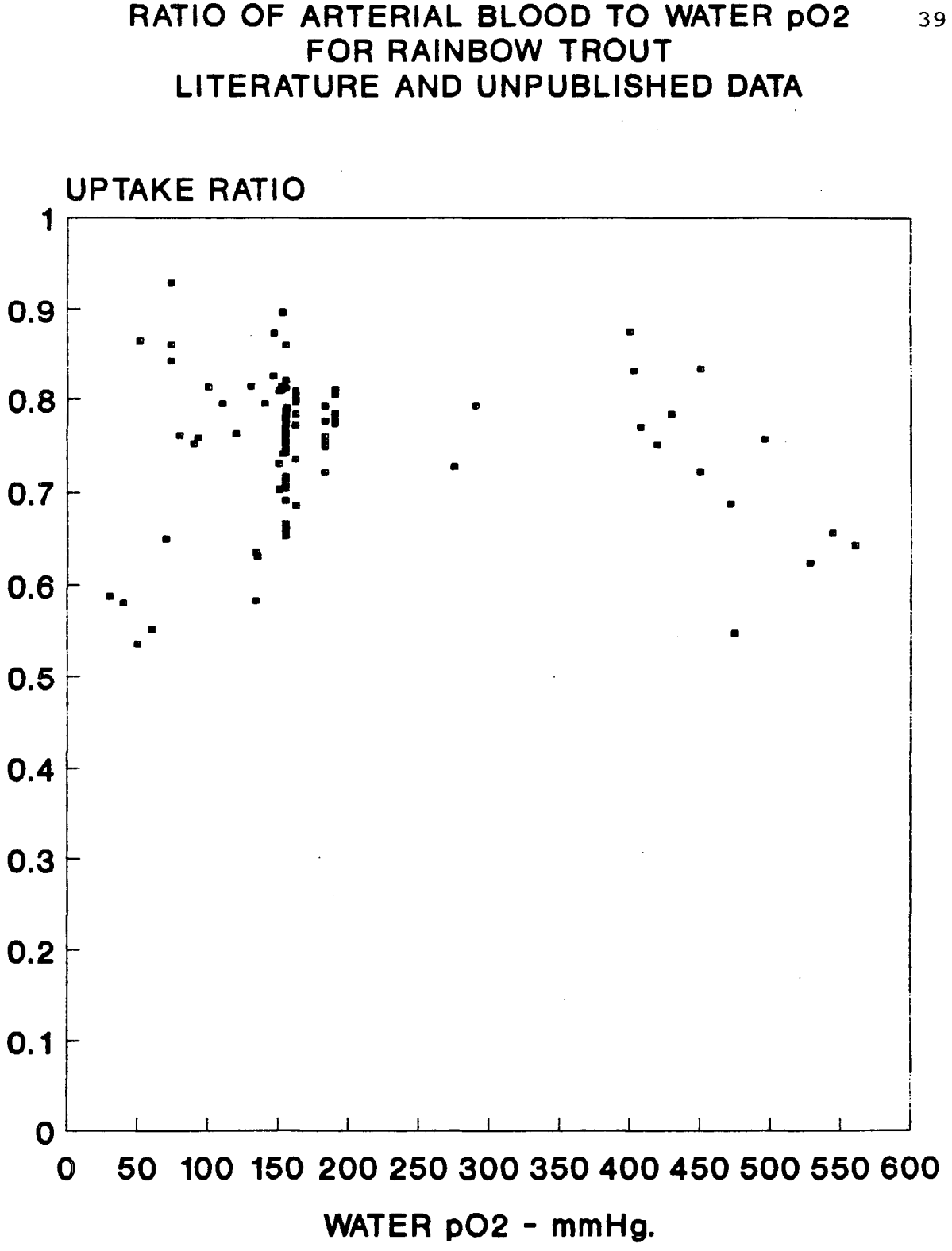


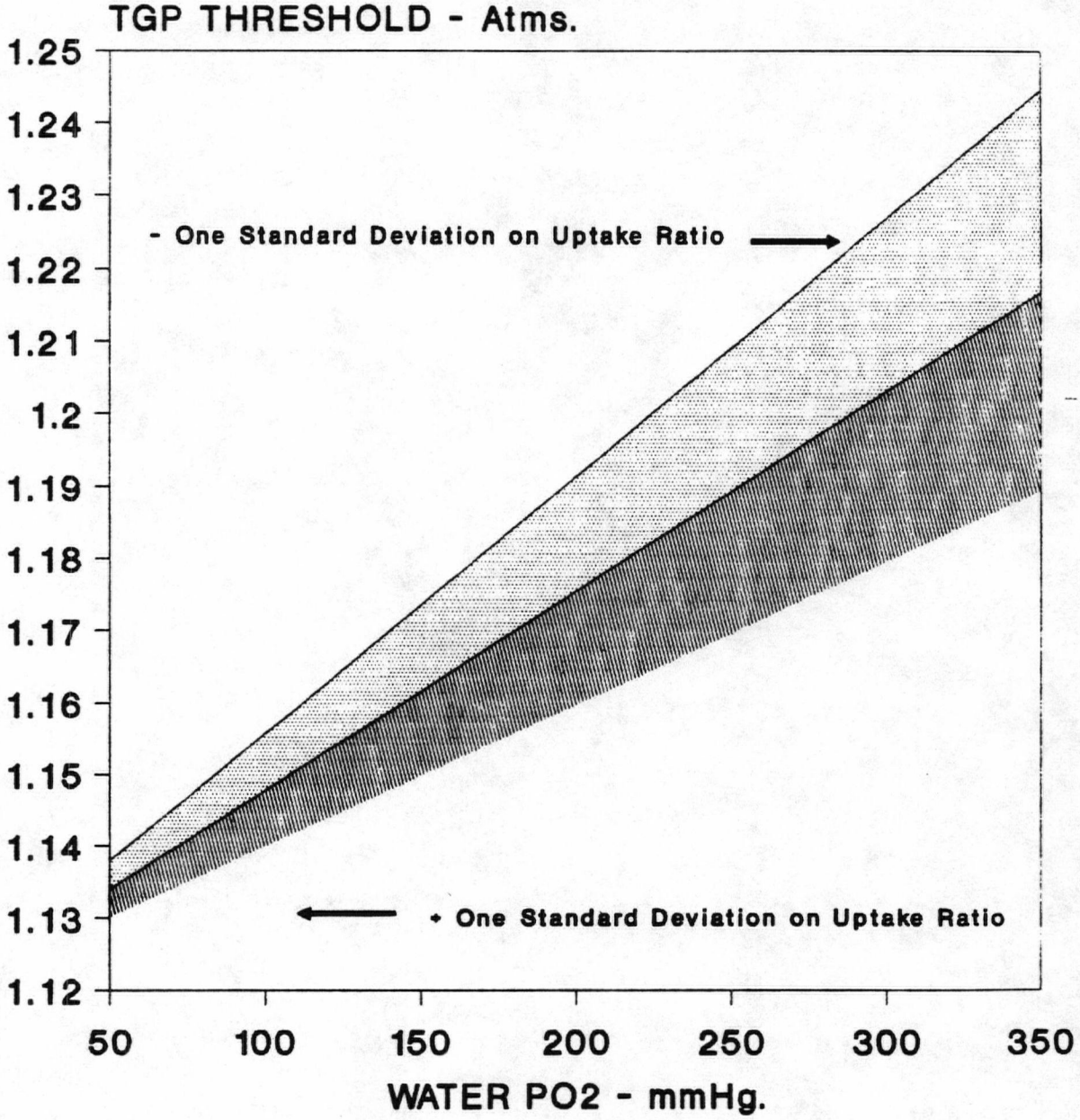
FIGURE 5: Ratio of Arterial to Water pO_2 for Rainbow Trout.

supersaturation, water pO_2 is usually elevated relative to atmospheric values (Weitkamp and Katz, 1980 and Colt, Bouck and Fidler, 1986). The maximum pO_2 reported in the literature is about 390 mmHg. (Renfro, 1963). Cases of supersaturation involving low levels of pO_2 usually involves well water. However, in these situations oxygen partial pressures less than 70 mmHg. have not been reported in the literature. Therefore, in subsequent analyses, the value of 0.79 for F will be assumed as representative of most situations involving supersaturation and GBT.

It order to determine the effect of variations in F on the intravascular bubble thresholds, Equation 4 was examined for water pO_2 values between 70 and 350 mmHg. Figure 6 shows the results of this analysis. In the figure, the mean value of 0.79 has been used in Equation 4 and plotted along with the standard deviations. Other parameters used in Equation 4 are as specified in the figure. From the three curves, it is clear that the effect of variations in F on threshold TGP is dependent on water pO_2. At a water pO_2 of 200 mmHg., the variation in threshold TGP is as much as 0.015 Atms.

3.4.3 BUBBLE GROWTH IN AN OPEN SYSTEM: Using the Mathcad computer program, the equations for bubble growth in a closed system were first solved with the compliance (C') set equal to zero. As pointed out earlier, this reduces the growth equation (Equation 11) to that for an open system. The results of this solution are shown in Figure 7 for TGP levels of 1.31, 1.27 and 1.165 Atms. Other parameters used in the solution are shown in the figure. It is observed that the growth curve corresponding to a water TGP of 1.27 Atms. agrees closely to the solutions obtained by Epstein and Plesset (1950) for a water TGP of 1.25 Atms. In general, for an open

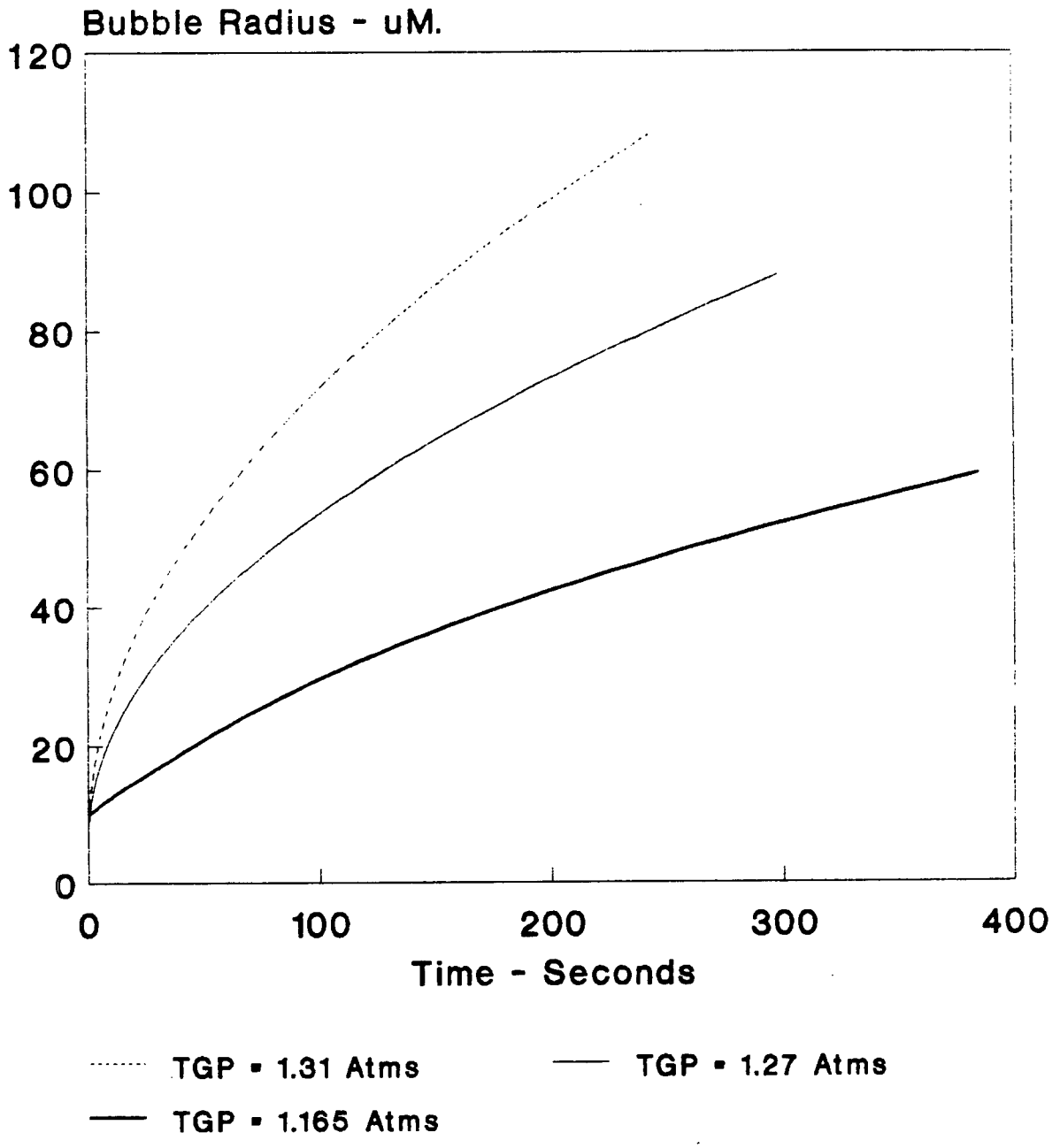
ARTERIAL BUBBLE GROWTH THRESHOLDS TGP VS. WATER pO2 FOR R0 = 12 uM. AND O2 UPTAKE RATIO = .79



Water Depth = 0.0 M.
Water Temperature = 5 - 15 deg. C.
Atmospheric Pressure = 760 mmHg.

3: Variation in Arterial Bubble Growth Thresholds Versus Oxygen Uptake Ratio.

BUBBLE RADIUS VERSUS TIME FOR BUBBLE GROWTH IN AN OPEN SYSTEM



Initial Nuclei Radius = 12 uM.
Temp = 15 deg. C., Depth = 0.0 M.

FIGURE 7: Bubble Radius Versus Time for Bubble Growth in an Open System.

system, a 10 fold increase in bubble radius occurs in a time period of minutes. It should be noted that the solution to the growth rate equations is logarithmic in this case (see Epstein and Plesset, 1950). Therefore, the bubbles continue to grow with the radius becoming infinite.

3.4.4 BUBBLE GROWTH IN A CLOSED SYSTEM: Equation 11 was also solved for a finite compliance and two conditions of P_{OA}. The results are shown in Figures 8 and 9. With the conditions of atmospheric pressure, temperature and critical nuclei shown in the figures, the threshold for bubble growth is 1.12 Atms. The time course for bubble growth at a TGP of 1.20 Atms. is shown in Figure 8, while Figure 9 shows bubble growth for a TGP of 1.316 Atms. In these solutions, the compliance of the system has been chosen somewhat arbitrarily. This is because compliances for the vascular systems of fish are unknown. The value of C' used is $7 \cdot 10^{-5}$ (mmHg./μM^3). Also, total bubble volume is calculated based on an assumption that 1000 bubbles are present in the system. The initial growth of these bubbles appears to follow that of an open system (Figure 7). However, once bubble volume increases system pressure, growth rate declines. It should be noted that, in a closed system, bubble growth does not proceed to an infinite radius. This is because the solution to equation 11 is asymptotic. That is, as bubbles grow, the threshold TGP also increases. This increase continues until system pressure forces the threshold to that corresponding to the actual radius of the growing bubble. Further bubble growth is then suppressed. By comparing Figures 8 and 9, it is seen that the maximum radius which bubbles reach, before growth is suppressed, increases as water TGP (or P_{OA} in this case) increases.

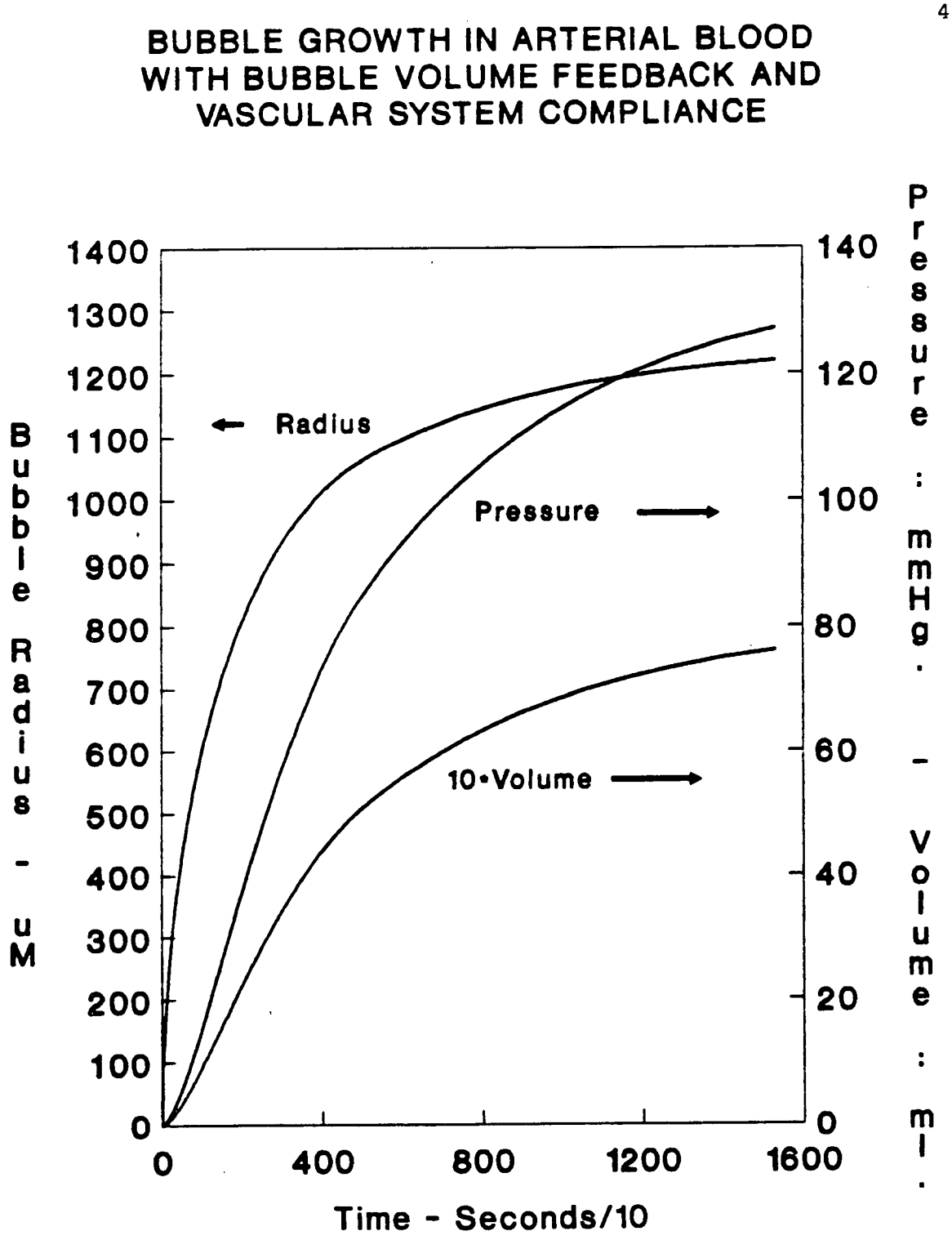


FIGURE 8: Bubble Growth in a Closed System.

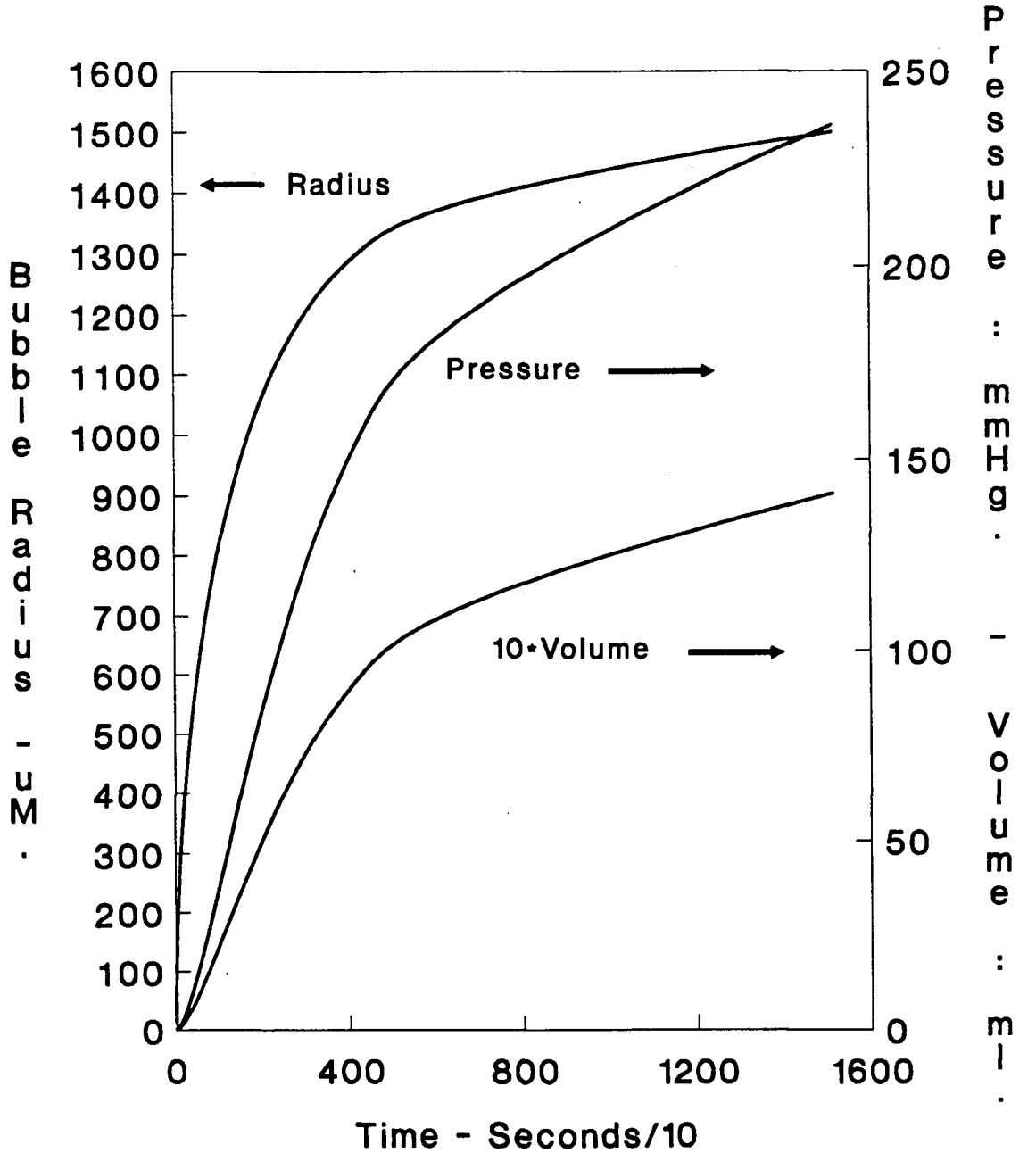


FIGURE 9: Bubble Growth in a Closed System, TGP = 1.316 Atms.

3.5 DISCUSSION

3.5.1 OXYGEN UPTAKE RATIO: Although the data of Figure 5 are quite variable, the values of F in the pO_2 range of 70 to 350 mmHg. have a mean of about 0.79. This range of pO_2 corresponds to that where arterial blood in Rainbow trout is fully saturated with oxygen (Beaumont, 1968 and Cameron, 1971) and hyperoxic conditions where respiratory effort is reduced (Randall and Jones, 1973 and Wood and Jackson, 1980). If, in this range of water pO_2, the gill water flow and blood flow are unaltered, the ratio of arterial pO_2 to water pO_2 should be unvarying. This is due to several factors. First, with constant blood and water flow, the mass transfer coefficients across the gill membrane are constant. Furthermore, as shown by Piper and Scheid (1984), for a gill counter current gas exchange system with constant diffusing capacity:

$$(Pa - Pv)/(Pi - Pv) \rightarrow \text{const.} < 1.0$$

where Pa = partial pressure of oxygen in arterial blood.

Pv = partial pressure of oxygen in venous blood.

Pi = partial pressure of oxygen in inspired water.

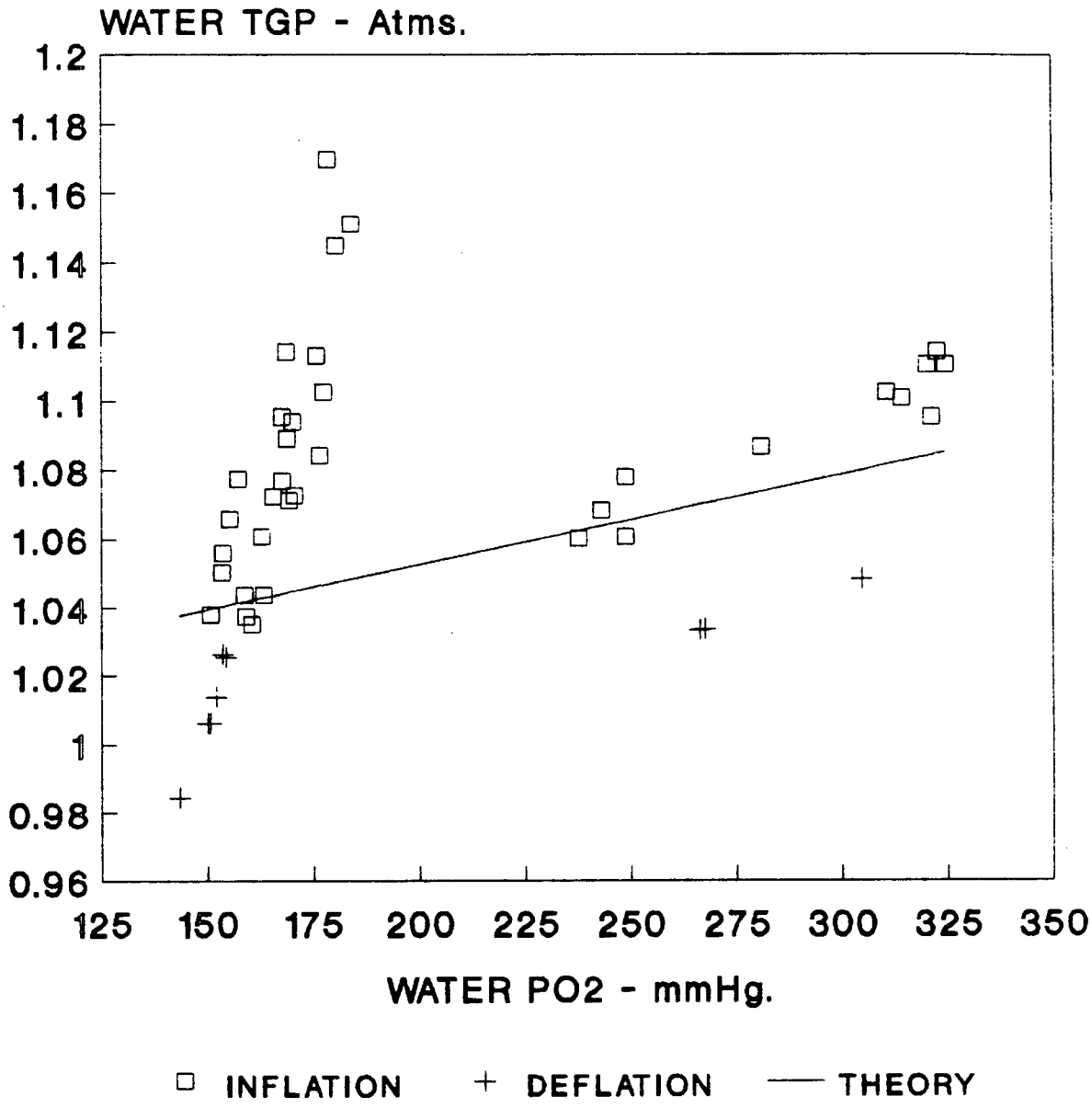
Secondly, most oxygen carried by arterial blood is used in metabolism and most of this oxygen is transported in the bound form. Thus, for fully saturated blood, venous pO_2 levels are low in comparison to either water or arterial levels, regardless of water values. In the limit, as $Pv \rightarrow 0$,

$$(Pa - Pv)/(Pi - Pv) \rightarrow Pa/Pi \rightarrow \text{const.} < 1.0 \qquad \text{Equation 15.}$$

3.5.2 BUBBLE GROWTH THRESHOLD EQUATIONS: With the above definition of F, the bubble growth threshold equations (Equations 4 and 6) are resolved to forms containing an effective nuclei radius as the only unknown dependent variable. In the case of thresholds for swimbladder overinflation, Equation 5 is complete without a nuclei radius. The remaining task was to demonstrate its validity.

In work by Mark Shrimpton, Dave Randall and this author (1988), thresholds for swimbladder overinflation in Rainbow trout were examined experimentally. Fish were exposed to gas supersaturated water while swimbladder pressures were monitored. The results of these experiments are shown in Figure 10. The square symbols in the figure represent those fish exhibiting an increase in swimbladder pressure. The plus symbols are for fish in which declining swimbladder pressures were observed. The region between the two clearly defines the threshold for swimbladder inflation. Also shown in the figure is the theoretical threshold as calculated from Equation 5. The theoretical curve of the figure is for an F of 0.79, a system pressure of 0.0 mmHg., a temperature of 10° C. and sea level atmospheric pressure. Although the experimental data do not densely cover the entire range of water pO_2, it is clear that the theoretical thresholds fall close to but slightly above the experimental thresholds. The reason for this difference is not presently known. However, if the value of F is increased from 0.79 to 0.85, the theoretical threshold corresponds closely to that indicated by the experimental data. It should be noted that this value of F is still within plus one standard deviation of the mean value of F, 0.79, indicated by the data of Figure 5. However, it is not clear why the higher value of F is appropriate for the swimbladder thresholds. Nevertheless, with this adjustment in F, Equation 5 provides an accurate description of thresholds for swimbladder overinflation.

RESPONSE OF SWIMBLADDER TO GAS SUPERSATURATED WATER: COMPARISON OF EXPERIMENTAL DATA WITH THEORY



Experimental Data: Shrimpton, Randall and Fidler (1988)
Theory: Equation 5

FIGURE 10: Swimbladder Inflation Thresholds.

3.5.3 BUBBLE GROWTH RATE: Based on solutions to the bubble growth equations for open and closed systems, the time period for bubble growth is on the order of minutes to a few hours (Figures 7, 8 and 9). Thus, except at very high levels of water TGP, the time period for bubble growth does not appear to be a major component of the total time to mortality. However, this disparity in the time course of the two processes may relate to various assumptions involved in the derivation of the growth rate equations. First, the difference is probably greater than indicated in this analysis. This is because dissolved gases in the cardiovascular system are transported to the bubble by flowing blood. In this case, mass transport is mainly by convection which, for the same concentration difference, is many times more effective than transport by diffusion alone. Thus, initial bubble growth may be more rapid than indicated, which implies an even greater difference between bubble growth times and time to mortality.

As bubbles grow, they should eventually block the arteries in which they are growing. Once arterial blockage occurs, blood flow stops and, as was assumed in the equation derivation, the transport of gases to the bubble is by diffusion. However, at this point, the bubble growth problem becomes one of axial diffusion rather than spherically symmetric diffusion. Also, the effective interfacial diffusion area is reduced by about one half. In addition, there will be a loss of oxygen from the bubble in a downstream direction as tissue metabolism reduces dissolved oxygen concentrations in that direction. These effects will slow the bubble growth process considerably and perhaps account for part of the time difference. Philp, Inwood and Warren (1972) have pointed out that bubble growth in blood may also involve an accumulation of protein components from the blood on the bubble surface. Casillas *et al.* (1975 and 1976) also report that clotting takes place in the blood of Chinook

salmon during decompression. The presence of these organic materials at the bubble surface may slow diffusion and the bubble growth process further.

Thus, there are a number of factors that can explain some of the differences between bubble growth rates and the time required to produce mortality in fish. Further discussion of this subject is delayed until Section 5 where the results of the experimental work are examined. These results add further insight into bubble growth and yield information on the location of certain intravascular bubbles.

Perhaps the most important aspect of the bubble growth model for a closed system is the indication that the volume of growing bubbles can interact with system compliance and increase system pressure. The increase in pressure will then arrest bubble growth. It is possible that increases in system pressure can provide a means for experimentally determining intravascular bubble growth thresholds. For example, if fish are exposed to gradually increasing levels of water TGP, while monitoring blood pressure, a persistent increase in blood pressure may signify the beginning of bubble growth and a TGP threshold. Once the threshold is experimentally defined, the effective radius of vascular system nuclei can be back calculated from Equation 4. This would then allow Equation 4 to be completed. A component of the experimental studies described in Section 5 of this thesis included this technique. An unknown aspect of this response is whether the pressure increase is large enough to be detected at bubble growth thresholds.

In addition to the results shown in Figures 8 and 9, a sensitivity analysis of Equation 11 was performed for TGP levels close to bubble growth thresholds. In particular, for a threshold TGP of 1.12017 Atms. and a water TGP of 1.12039 Atms., bubble growth

is arrested with a system pressure increase of 3.8 mmHg. At a water TGP of 1.123 Atms., bubble growth is arrested by a 15 mmHg. increase in system pressure. Although Equation 11 is approximate and has components such as compliance that are chosen arbitrarily, it nevertheless suggests that, near threshold conditions, significant increases in system pressure may occur as a result of bubble growth. There are other facets to this response, such as the systemic control of blood pressure that will modify these results. However, a discussion of these effects will be postponed until a later section where the results of the experimental work are examined.

3.6 THEORETICAL SUMMARY

In this section, equations that describe various facets of bubble growth in fish exposed to supersaturated water were examined. From this examination it is clear that thresholds exist for the inflation of the swimbladders in Rainbow trout exposed to supersaturated water. Based on experimental data, these thresholds are described adequately by Equation 5 using and oxygen uptake ratio (F) of 0.85.

Theory also predicts the existence of thresholds for intravascular bubble growth, bubble growth in the environmental water and sub-dermal bubble growth in epithelium skin tissue. Intravascular bubble growth involves a coupling of bubble volume with the compliance of the vascular system. This coupling may lead to an increase in system pressure and a suppression of further bubble growth. By combining the system pressure (Ps) in Equation 4 with the radius of nuclei (r_o), the threshold equation for intravascular growth contains one unknown parameter, the effective nucleation site radius, R_o. Similarly, the equation for water/sub-dermal

bubble growth thresholds contains nucleation site radius as the only unknown physiological parameter.

Based on the above analyses, several steps can now be taken to define the effective nuclei radius in the intravascular and water/sub-dermal bubble growth threshold equations. The first is to examine data from the literature and determine if there are thresholds in time to mortality associated with GBT. If so, it should be established that these thresholds can be correlated with bubble growth thresholds as predicted by equations 4 and 6. Thus, there is an experimental requirement to determine the relationship between the predicted bubble growth thresholds, observed bubble growth thresholds, and physiological parameters that relate the two to mortality. It should be re-emphasized that bubble growth thresholds are significant mainly from the standpoint of mortality and stress in fish. These next steps begin with an examination of GBT data from the literature.

4.0 GAS BUBBLE TRAUMA DATABASE

This phase of study involved a review of the literature on dissolved gas supersaturation and GBT in fish. The purpose of the review was to build a database of documented response to supersaturation that could be analyzed for threshold information. A secondary purpose of the review was to extract from the data other relationships that may exist between reported parameters.

By building a database containing many different experimental records, it was anticipated that gaps in data from individual experiments would be filled by data from other experiments. With a more complete description of response, the chances of identifying relationships between parameters and mortality thresholds would be enhanced.

4.1 METHODS AND MATERIALS

The literature review was restricted to five fish species. These included Chinook, Coho, and Sockeye salmon as well as Steelhead and Cutthroat trout. This restriction is based on two factors. The first is the abundance of data on these species compared to the relatively limited data on other species (Weitkamp and Katz, 1980 and Colt, Bouck and Fidler, 1986). The second is the physiological similarity of these animals to each other and to Rainbow trout which were used in the experimental phase of this work.

The review included a re-examination of the data reported by Jensen *et al.* (1985a) as well as additional data not included in their review. In particular, Jensen and co-workers did not include in their database experimental records involving fish exposed

to supersaturation, but without observed mortality. Clearly, water TGP and pO_2 conditions that either produce or fail to produce mortality will be strong indicators of GBT mortality thresholds. Hence, this information, was of particular importance in the database developed in this study.

4.1.1 DATA SOURCES: Table I of Appendix C lists the literature sources from which the GBT database was developed. Complete information on these sources is found in the bibliography of this thesis. The information included in the database is shown in Table II, Appendix C. Each experimental record is given a unique identification consisting of a two digit number corresponding to an Author Number. This is followed by a four digit decimal fraction corresponding to a particular data record of that author. This follows the system used by Jensen *et al.* (1985). In reviewing data from the literature, a number of recording errors were found in the database of Jensen and co-workers. These were corrected and noted. In addition, a number of data records were found to be inappropriate to the purposes of this study. For example, the data of Nebeker *et al.* (1979) are for fish that were exposed to supersaturation while undergoing sudden changes in temperature of up to 17° C. Because of the combined stress condition, these records were excluded from the database developed in this study. Further, the review of Jensen and co-workers includes only recorded data values. That is, there is no interpolation between data points. Many data records from the literature contain information on time to 50 % mortality. On the other hand, other records do not have time to 50% mortality but do have sufficient data at both lower and higher mortalities to allow a reasonable interpolation of the 50% value. That is, at least three and often four adjacent datum points are available to allow second or third order interpolations. The same is true for other levels of mortality. Consequently, this review contains interpolated results

where there are sufficient data. Due to differences in the approach to building this database, the record numbers listed in Appendix C do not always correspond to those of Jensen *et al.* (1985a).

4.1.2 METHODS OF ANALYSIS: The Gas Bubble Trauma database was analyzed using an IBM compatible personal computer running the Borland Reflex database program and the Lotus 123 spreadsheet program. The actual use of the programs depended on whether the analysis required numerical operations or filtering operations. For example, throughout the literature, one encounters two methods for reporting Total Gas Pressure. In some cases, the vapor pressure of water is included in the Total Gas Pressure calculation while in other cases it is not. For consistency, both from the standpoint of reporting and from the standpoint of the physics of the processes involved (Fidler, 1985), all TGP data were corrected to include the vapor pressure of water. In addition, where information permitted, oxygen to nitrogen ratios were converted to the partial pressures of oxygen and nitrogen. The Lotus 123 program was used to perform the calculations needed to obtain these quantities as well as other calculations used in the analysis. The Reflex program was used to develop filters that allow searching for mortality thresholds and relationships between reported parameters in the data. Data filtering is a means of searching the database for records that meet only certain criteria. For example, by restricting the Species Code to a value of 1, only data on Chinook salmon would be selected. Similarly, by setting Species Code equal to 1 and depth to 1.0 or less, only data on Chinook salmon exposed to supersaturation at a depth of one meter or less would be selected.

4.2 RESULTS

From the literature sources listed in Table I, Appendix C, a database of 1013 data records was developed. These records consist of about 500 entries from the database of Jensen *et al.* (1985a). These records were corrected for entry errors and 148 records were eliminated. The eliminations, as mentioned earlier, were data from experiments of combined thermal stress and supersaturation stress (Nebeker *et al.*, 1979). Over 500 new data records were added as a result of this review. The complete tabulation of data is contained in Table III of Appendix C.

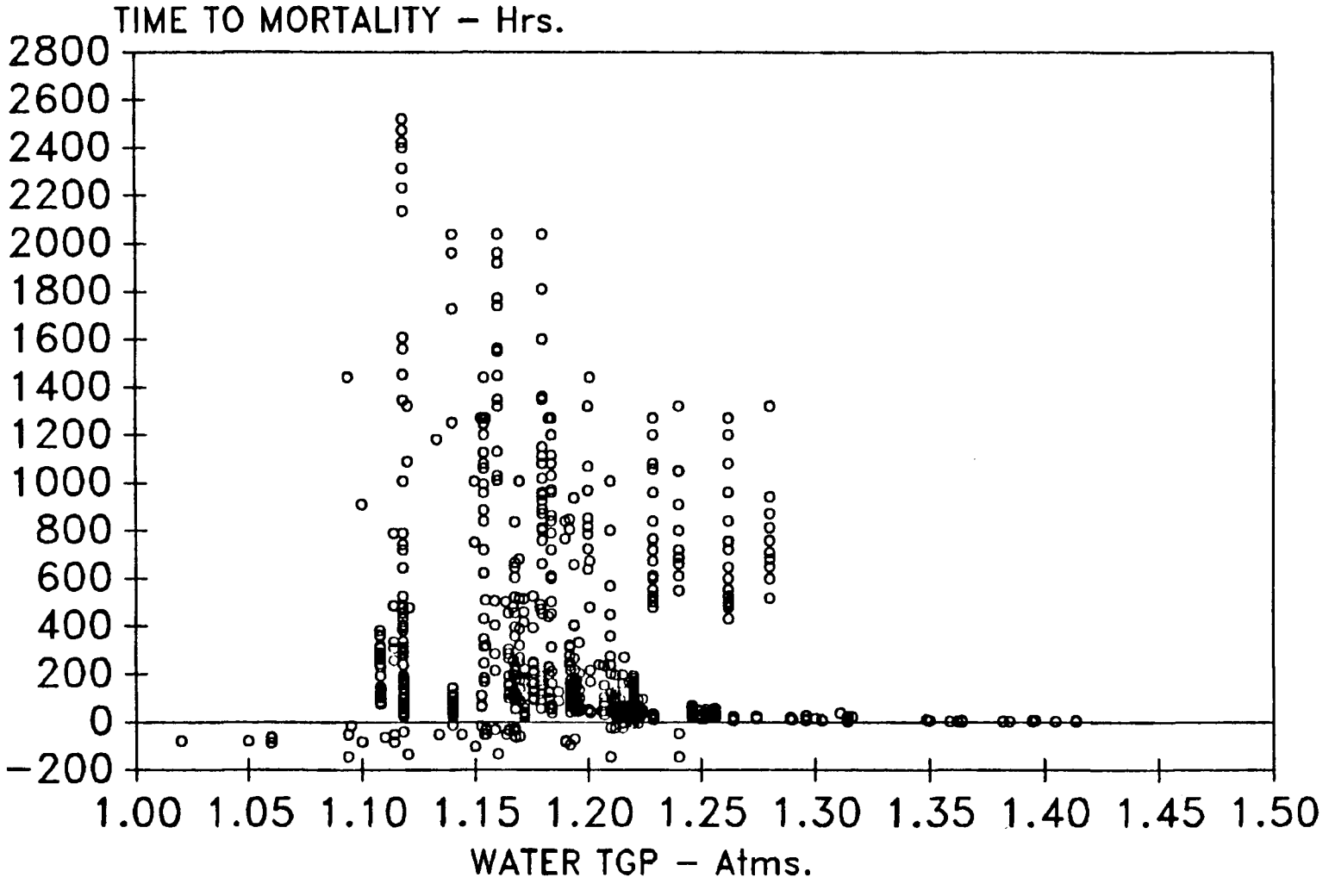
4.2.1 PRELIMINARY FILTERING: To determine if data from the literature exhibit thresholds or other relationships between reported parameters, it was necessary to perform sorting and filtering operations on the database. For example, it is known that salmon and trout eggs are highly resistant to supersaturation and that mortalities occur only at very high levels of TGP (Alderdice and Jensen, 1985 and Rucker, 1975b). To prevent these data from obscuring trends that may be present in data for hatched fish, the first filter application was to restrict the records to those for hatched fish only.

As described in the introduction to this thesis, many experimental records in the literature are based on the premise that dissolved nitrogen is the sole cause of Gas Bubble Trauma. As a result, Total Gas Pressures are not reported in these data. Thus, the second filtering of the database restricted the records to those that report TGP. Figure 11 summarizes the information to this level of filtering where time to mortality is plotted as a function of water TGP. In general, the figure shows there is a lower threshold for mortality near a water TGP of 1.1 Atms. As noted, negative times correspond to experiments where mortalities were not observed. As will be shown,

many of these negative times can be explained in terms of compensation depth available to the fish or high levels of dissolved oxygen in the water. However, other data indicated by negative times are important indicators of threshold levels of TGP. The absolute value of the negative entries, is based on the duration of the experiment divided by -10. To convert this entry back to the duration of the experiment, multiply the negative time to mortality (time of survival) by -10.

The levels of mortality that are included in the database range from a few percent to 100 percent. Because many of the reported data do not contain control experiments, the levels of mortality associated with non-supersaturated conditions are unknown. Furthermore, data on the time to mortality below 20% are quite sparse in the database. In order to establish a level of significance in the response to supersaturation, the database was further filtered to include only records associated with mortality levels between and including 20% and 70%. The 70% level was chosen because data for higher levels of mortality were again sparse and did not yield additional information beyond that within the 20% - 70% levels. A further restriction placed on the data at this point was to include only data for which fish size is known. The reason for this restriction will be discussed in the following sections. Using these filter criteria, the data are as shown in Figure 12. As indicated in the figure, the data have been separated based on fish length. In general, the data show that time to mortality for fish larger than 50 mm. is shorter than for fish smaller than 50 mm. Again, there is strong indication of a threshold for mortality at a TGP of 1.1 Atms.

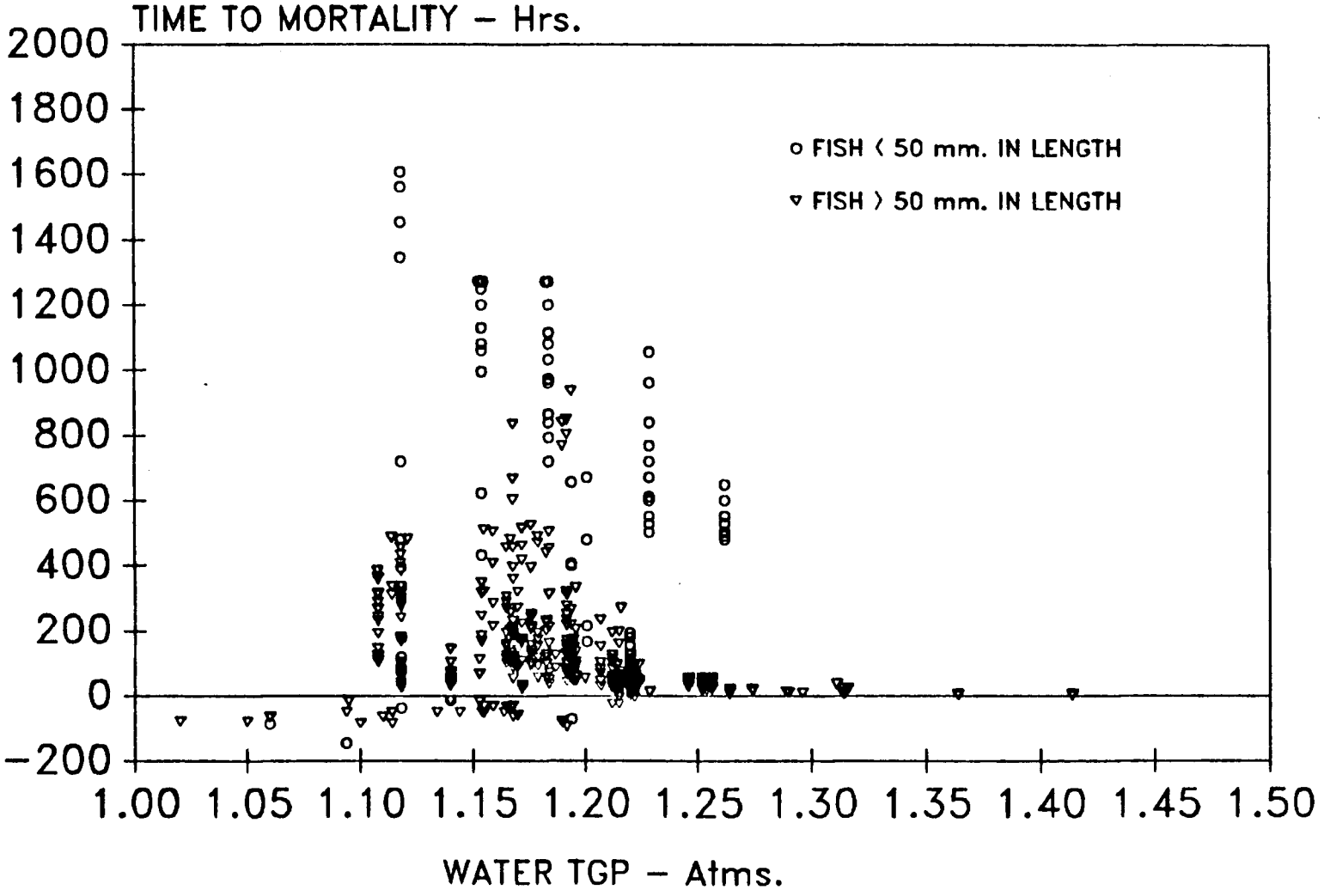
TIME TO MORTALITY VERSUS WATER TGP
FOR ALL FISH IN DATABASE BY L.E. FIDLER



Note: Negative times correspond to experiments with no mortality.

FIGURE 11: Time to Mortality for all Fish in Database by L.E. Fidler

TIME TO MORTALITY VERSUS WATER TGP
MORTALITY RANGE = 20 – 70 %

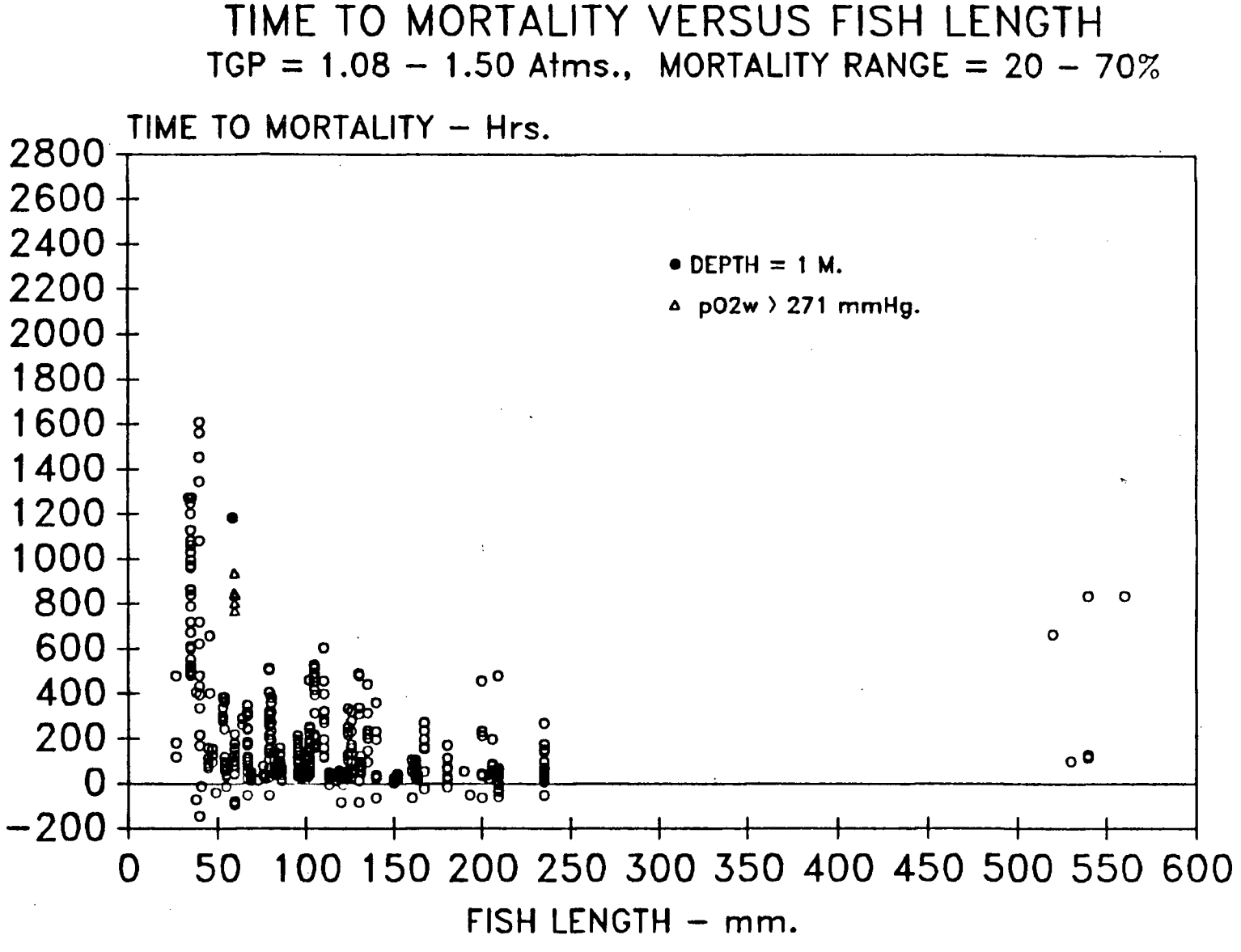


Note: Negative times correspond to experiments with no mortalality.

FIGURE 12: Time to Mortality Versus Water TGP; Mortality Range = 20 - 70%.

The rendering of the database to this point serves as the basis from which all subsequent analyses were performed. In the descriptions that follow, the minimum level of information examined contains Total Gas Pressure and times to mortality between the 20% and 70% levels for hatched fish of known length. Also, the majority of these filtered data correspond to exposure to supersaturation at depths of 0.61 meters or less. In the entire database there are only 10 records for depths greater than 0.61 meters.

4.2.2 UNIQUE DATA SETS: Before considering the database further, it is important to examine six distinct data records. These records will appear repeatedly throughout the analyses that follow. Figure 13, which shows the time to mortality versus fish length, will be used to examine these data. First, the datum point shown by the solid black circle at 1182 hours in Figure 13 (Record No. 879) is unique in that the level of total gas pressure is low (1.13 Atms.), the depth is one meter and the fish are small (i.e. close to 50 mm. in length). It should be noted that this data record yields a time to 50% mortality almost two orders of magnitude greater than data at similar water TGP but slightly shallower depths. Next, five data sets (Records 850, 853, 856, 874, and 875) shown by the five triangular symbols in the figure correspond to a water TGP of 1.19 Atms. and pO_2 levels above 271 mmHg. Again, the times to mortality for these data are considerably above those of data at similar TGP but lower water pO_2 levels. The significance of these data will be explained in the discussion that follows. In the meantime it should be noted that, for fish greater than 50 mm. in length, these data are distinct from the rest in terms of time to mortality and certain water parameters.



Note: Negative times correspond to experiments with no mortality.

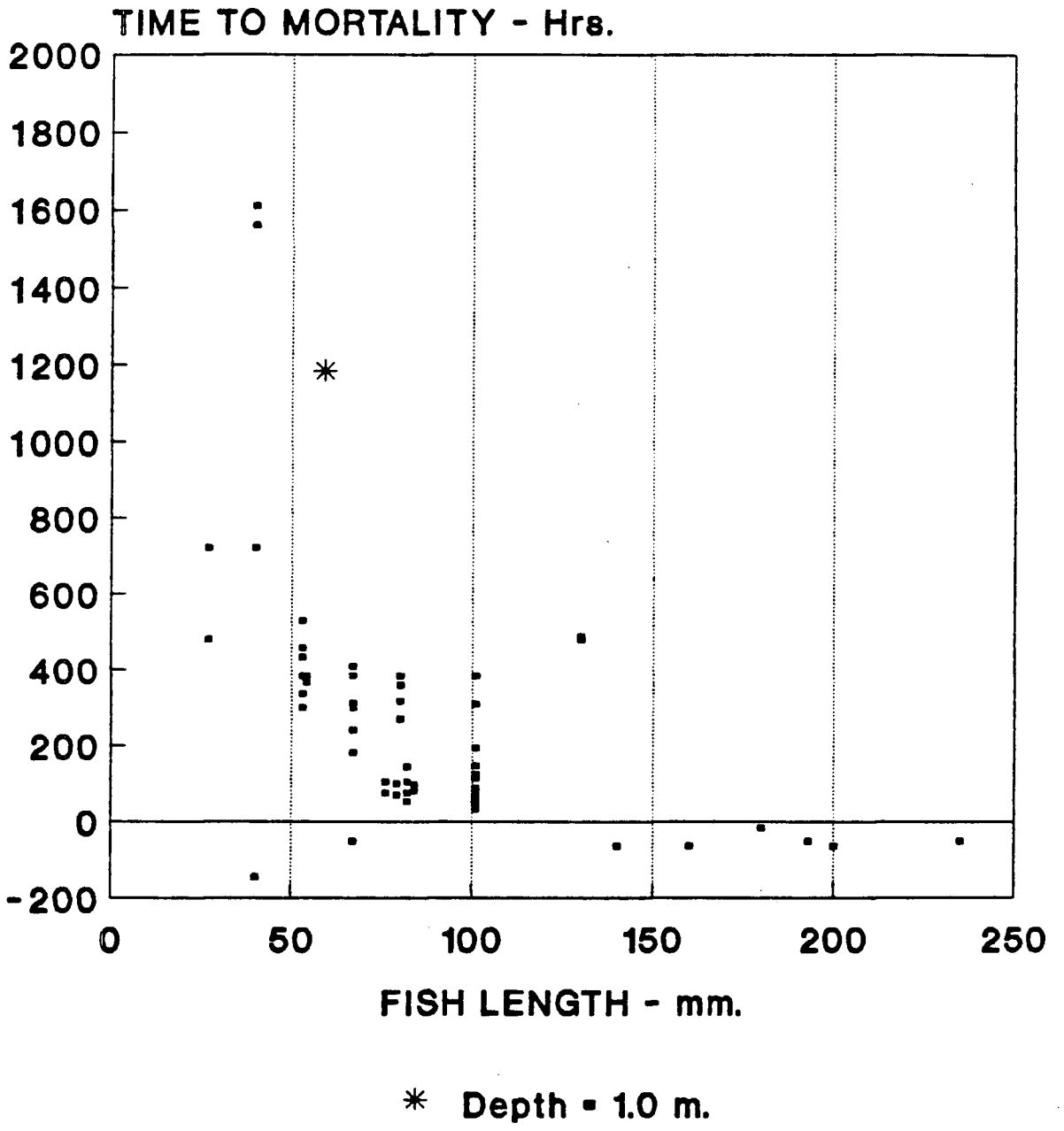
FIGURE 13: Time to Mortality Versus Fish Length; TGP = 1.08 - 1.5 Atms., Mortality Range = 20 -70 %.

4.2.3 EFFECT OF FISH SIZE: It is frequently reported in the literature that small fish are more resistant to supersaturation than large fish (Rucker and Kangas, 1974 and Jensen *et al.*, 1986). To illustrate the effect of fish size on the mortality response, the time to mortality (20 - 70%) is plotted versus fish length in Figure 13. Except for the six data sets mentioned above, it is clear that a significant difference exists between the times to mortality for fish less than 50 mm. in length and for those greater than 50 mm. When this relationship is examined in more detail, it is found that the difference exists independent of water TGP. In Figures 14, 15 and 16 the time to mortality is again plotted versus fish length with the exception that data have been separated based on TGP. Figure 14 gives these results for a TGP range of 1.08 to 1.15 Atms., Figure 15 for a TGP range of 1.15 to 1.20 Atms., and Figure 16 for a TGP range of 1.20 to 1.5 Atms. Although the effect of size is clearly evident in all three ranges, it is most pronounced in the 1.20 to 1.5 Atms. range. These results clearly indicate that 50 mm. is a critical length in time to mortality for these five species.

4.2.4 EFFECT OF FISH SPECIES: The data sets were next filtered according to species. The intent of this separation was to determine if differences in thresholds or other parametric relationships exist based on species.

Fish Less Than 50 mm.: Figure 17 summarizes time to mortality as a function of TGP for fish less than 50 mm. in length. At this level of filtering, the database contains information on Steelhead trout and Chinook and Coho salmon only. From the data of the figure, it is apparent that a mortality threshold exists for all three species at a water TGP of about 1.12 Atms. For Steelhead trout, the range of time to mortality above a TGP of 1.15 Atms. differs from that below 1.15 Atms. That is, between a TGP of 1.1

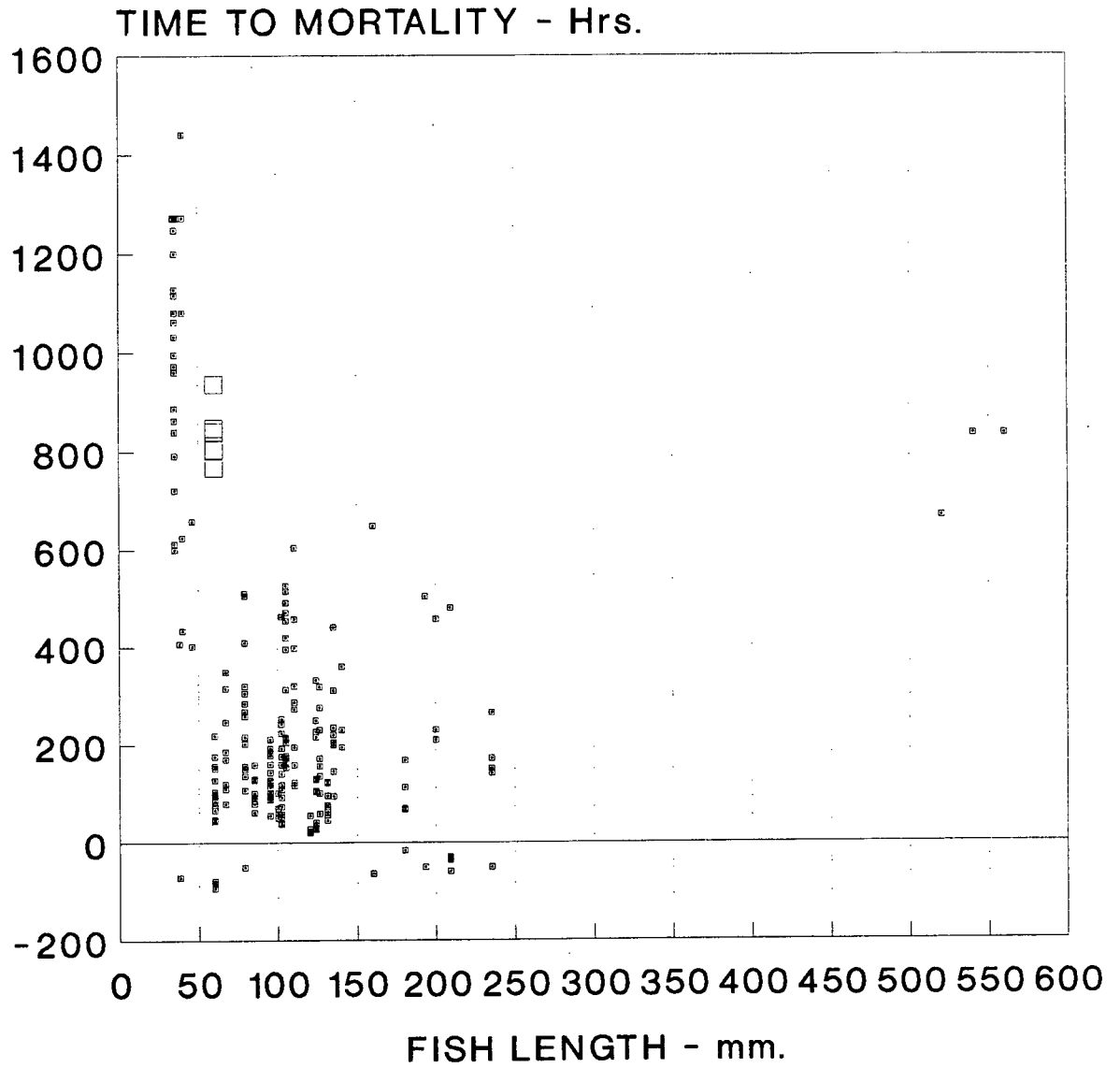
TIME TO MORTALITY VS. FISH LENGTH
ALL FISH: TGP = 1.08 - 1.15 Atms.
MORTALITY RANGE = 20 - 70 %



Note: Negative times correspond to experiments with no mortality

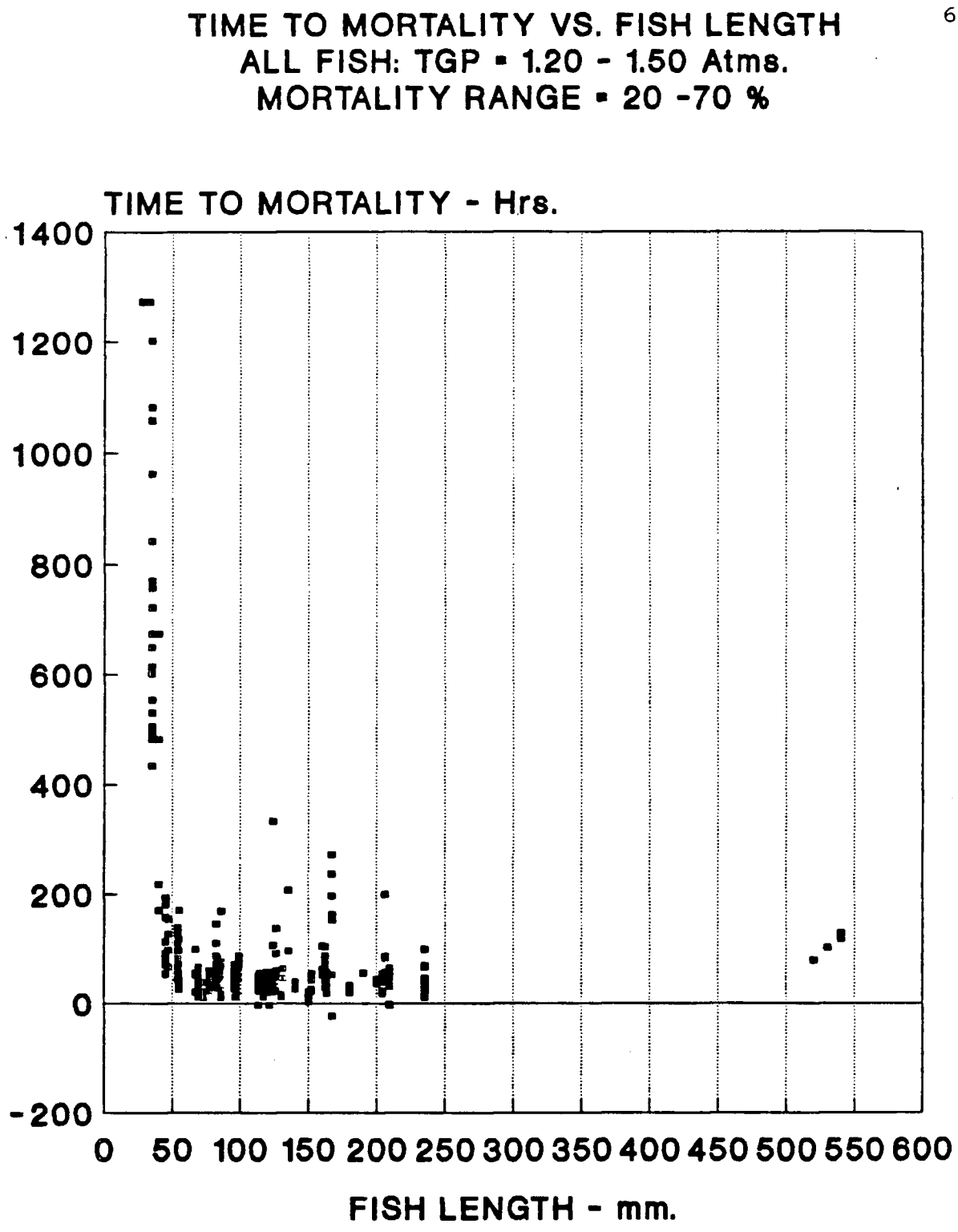
FIGURE 14: Time to Mortality Versus Fish Length; TGP = 1.08 - 1.15 Atms.

TIME TO MORTALITY VS. FISH LENGTH
ALL FISH: TGP = 1.15 - 1.20 Atms.
MORTALITY RANGE = 20 - 70 %



Note: Negative times correspond to experiments with no mortality.

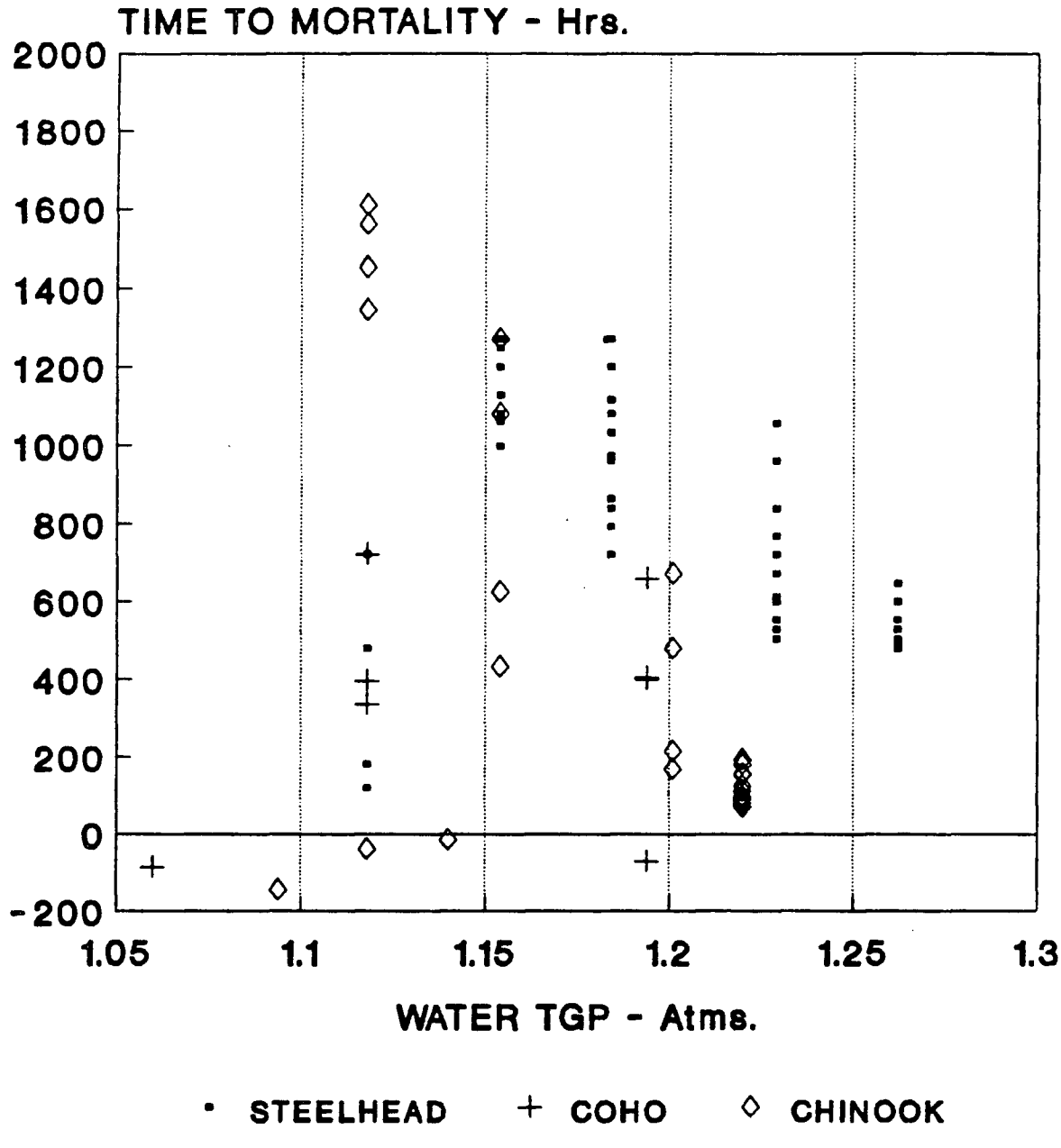
FIGURE 15: Time to Mortality versus Fish Length
TGP = 1.15 - 1.20 Atms.



Note: Negative times correspond to experiments with no mortality

FIGURE 16.: Time to Mortality versus Fish Length; TGP = 1.20 - 1.50 Atms.

TIME TO MORTALITY VERSUS WATER TGP FOR FISH LESS THAN 50 mm. IN LENGTH MORTALITY RANGE 20 - 70 %



Note: Negative times correspond to experiments with no mortality.

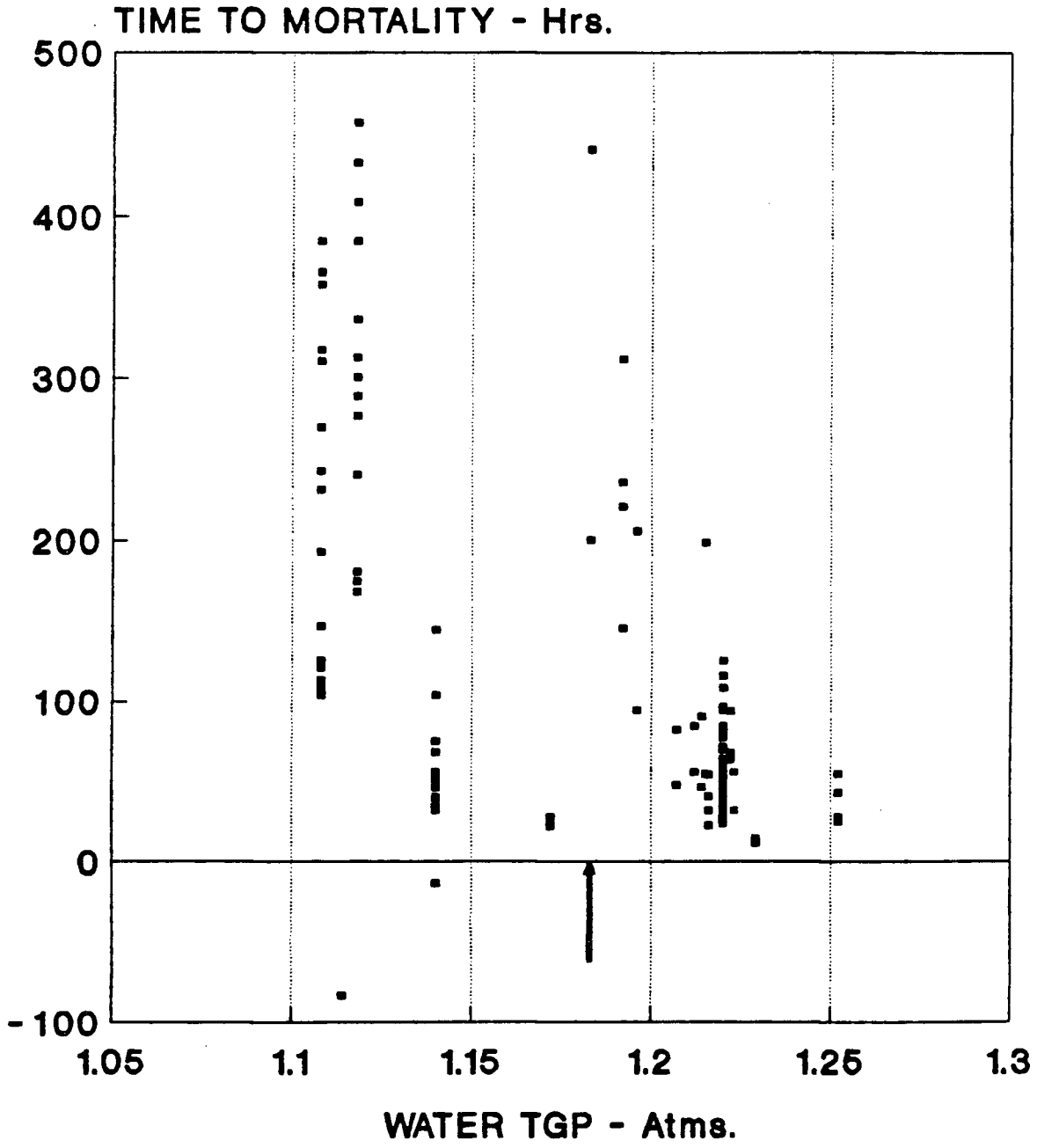
FIGURE 17: Time to Mortality Versus Water TGP for Fish Less Than 50 mm. in Length.

and 1.15 Atms. time to mortality is relatively short compared to that just above 1.15 Atms. As TGP increases beyond 1.15 Atms., time to mortality declines to levels comparable to those below 1.15 Atms. The opposite response is observed in Chinook salmon where mortality levels below 1.15 Atms. are high in comparison to those above 1.15 Atms. For Coho salmon, there is no significant trend in time to mortality above or below 1.15 Atms.

Chinook Salmon Greater Than 50 mm.: Shown in Figure 18 is the response of larger Chinook salmon to supersaturation as indicated by time to mortality versus water TGP. It is clear that a lower threshold for mortality exists at 1.11 Atms. TGP. From this threshold, time to mortality decreases as TGP increases to 1.18 Atms. At 1.18 Atms., time to mortality suddenly increases to levels comparable to those at 1.11 Atms. Above 1.18 Atms., time to mortality again declines with increasing TGP, similar to that between 1.1 and 1.18 Atms. Due to this similarity of response at 1.1 Atms. and 1.18 Atms., there may be another threshold at a TGP of 1.18 Atms for larger Chinook salmon. If so, it is not clear why the mechanism that is responsible for mortality between 1.1 and 1.18 Atms. suddenly becomes less effective at a TGP of 1.18 Atms. In fact, the data suggests a transition to another mechanism for mortality at 1.18 Atms. and above. Another interesting feature of the data is that at both 1.11 Atms. and 1.18 Atms. there is a minimum time to mortality of about 100 hours.

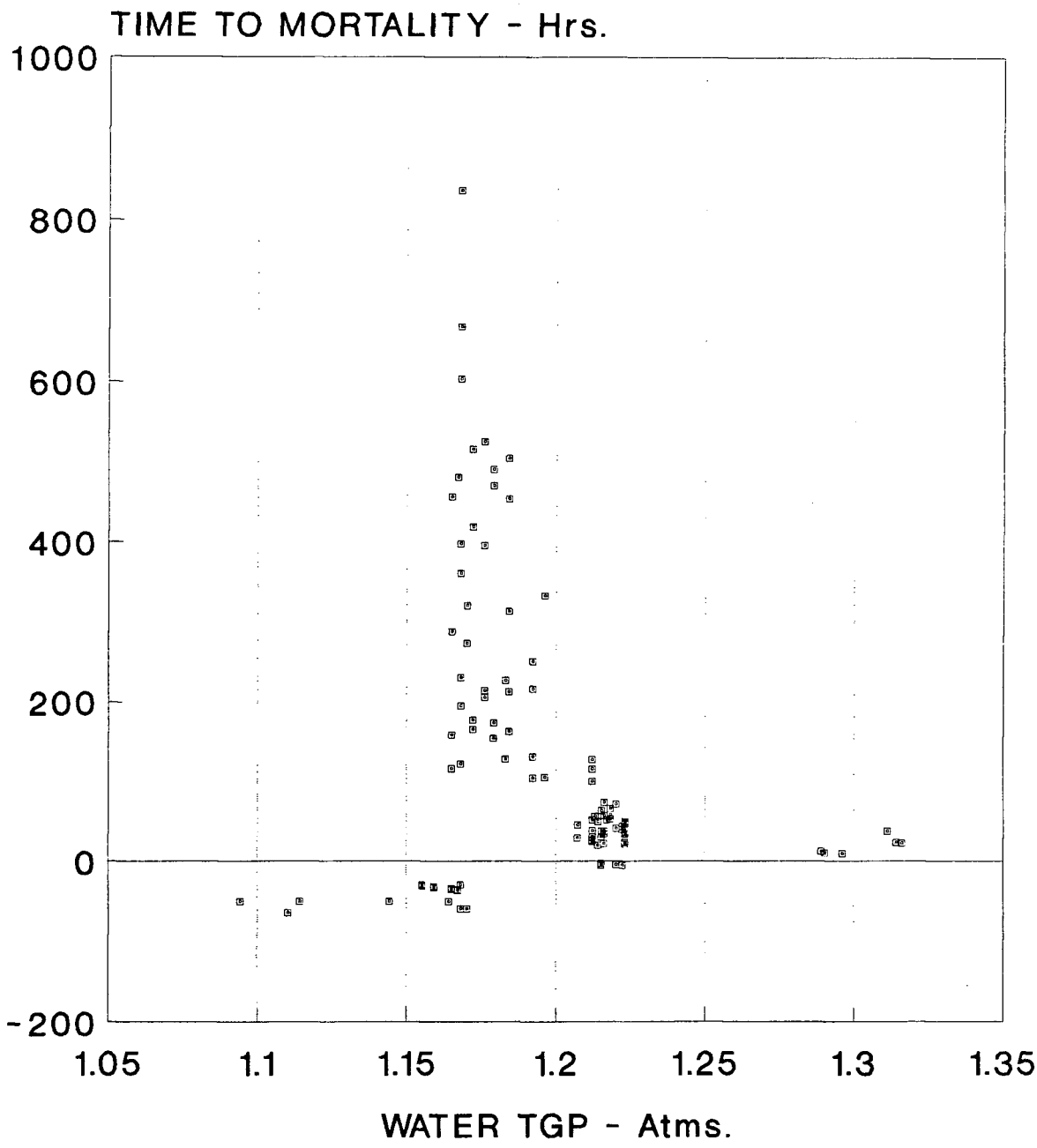
Sockeye Salmon Greater Than 50 mm.: A somewhat different response is observed in Sockeye salmon. This is shown in Figure 19, where again time to mortality is plotted versus water TGP. For Sockeye salmon, there is no evidence of a mortality threshold near the 1.1 Atms. TGP level. However, this may be due to the sparseness of data in the region between 1.1 and 1.17 Atms. On the other hand, there is strong

TIME TO MORTALITY VERSUS WATER TGP
CHINOOK SALMON OVER 50 mm. IN LENGTH
MORTALITY RANGE = 20 - 70 %



Note: Negative times correspond to experiments with no mortality.

FIGURE 18: Time to Mortality Versus Water TGP for Chinook Salmon Over 50 mm. in Length.



Note: Negative times correspond to experiments with no mortality

FIGURE 19: Time to Mortality Versus Water TGP for Sockeye Salmon Over 50 mm in Length.

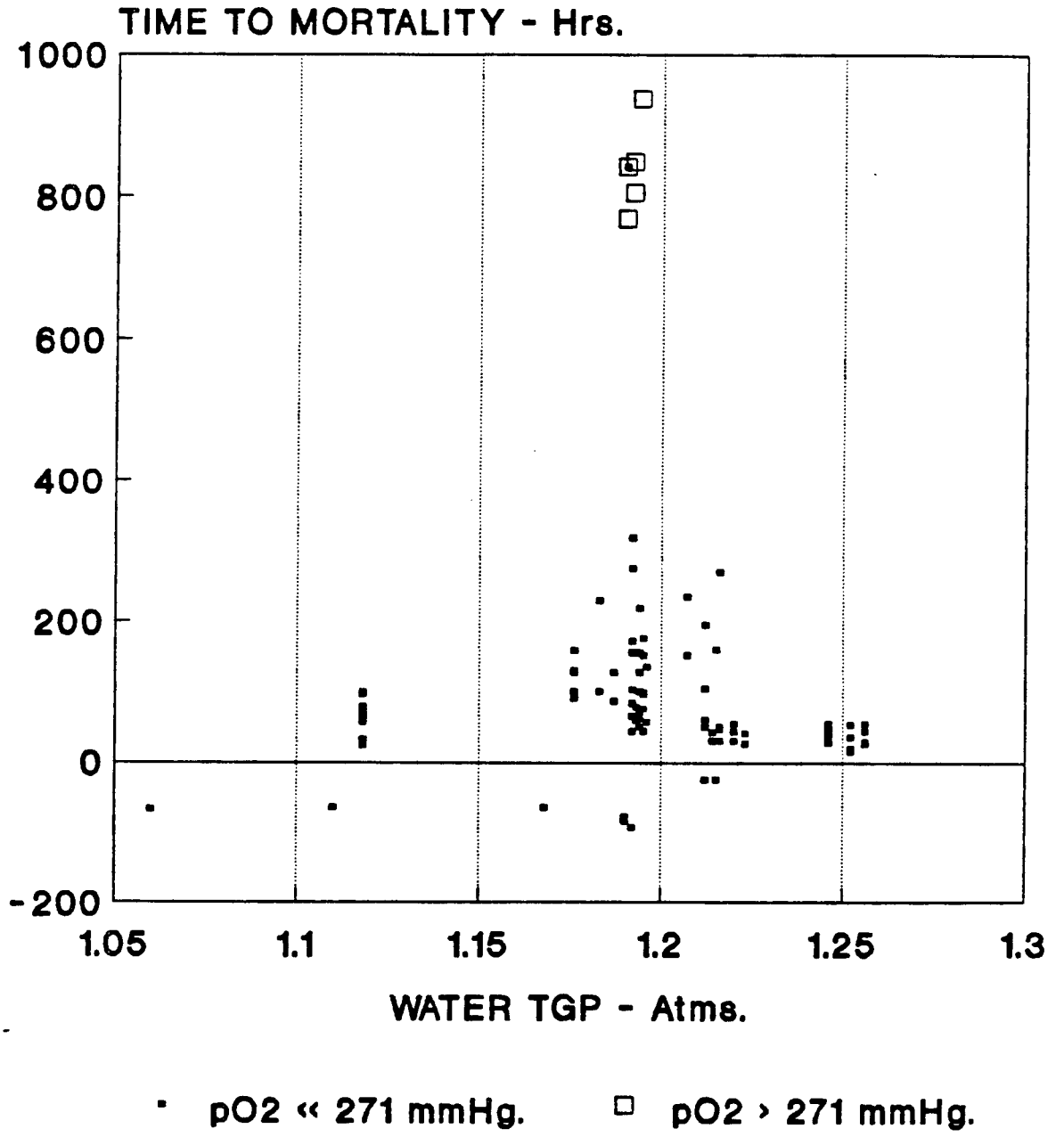
indication of a mortality threshold at a TGP of 1.17 Atms. This is particularly evident from the clustering of both positive and negative data near 1.17 Atms. Also apparent at this threshold is a minimum time to mortality of about 100 hours. This is similar to that observed with Chinook salmon as described above.

Coho Salmon Greater Than 50 mm.: Shown in Figure 20 is the time to mortality versus water TGP for Coho salmon greater than 50 mm. in length. An aspect of these data is the relative scarcity of information in the vicinity of 1.1 to 1.17 Atms. TGP. It is clear there are mortalities at 1.12 and 1.175 Atms. However, there is nothing to suggest that these are in fact thresholds. Also, as described earlier, the five data sets with water pO_2 above 271 mmHg. are significantly higher than all other times of mortality shown in the figure.

Steelhead Trout Greater Than 50 mm.: For Steelhead trout there are sufficient positive and negative data just at and above a water TGP of 1.15 Atms. to suggest this is a threshold for this species (Figure 21). Again a minimum time to mortality is evident at 1.15 Atms. As water TGP approaches 1.1 Atms., from lower values, the combination of negative times to mortality and the sudden increase to positive times at a TGP of 1.1 Atms. suggest this is the lower mortality threshold.

Cutthroat Trout Greater Than 50 mm.: Finally, the data for Cutthroat trout are shown in Figure 22. In this case, there is no information in the database below a water TGP of 1.15 Atms. On the other hand, the data that do exist show a threshold near 1.15 Atms. Once again, there is strong indication of a minimum time to mortality at 1.15 Atms.

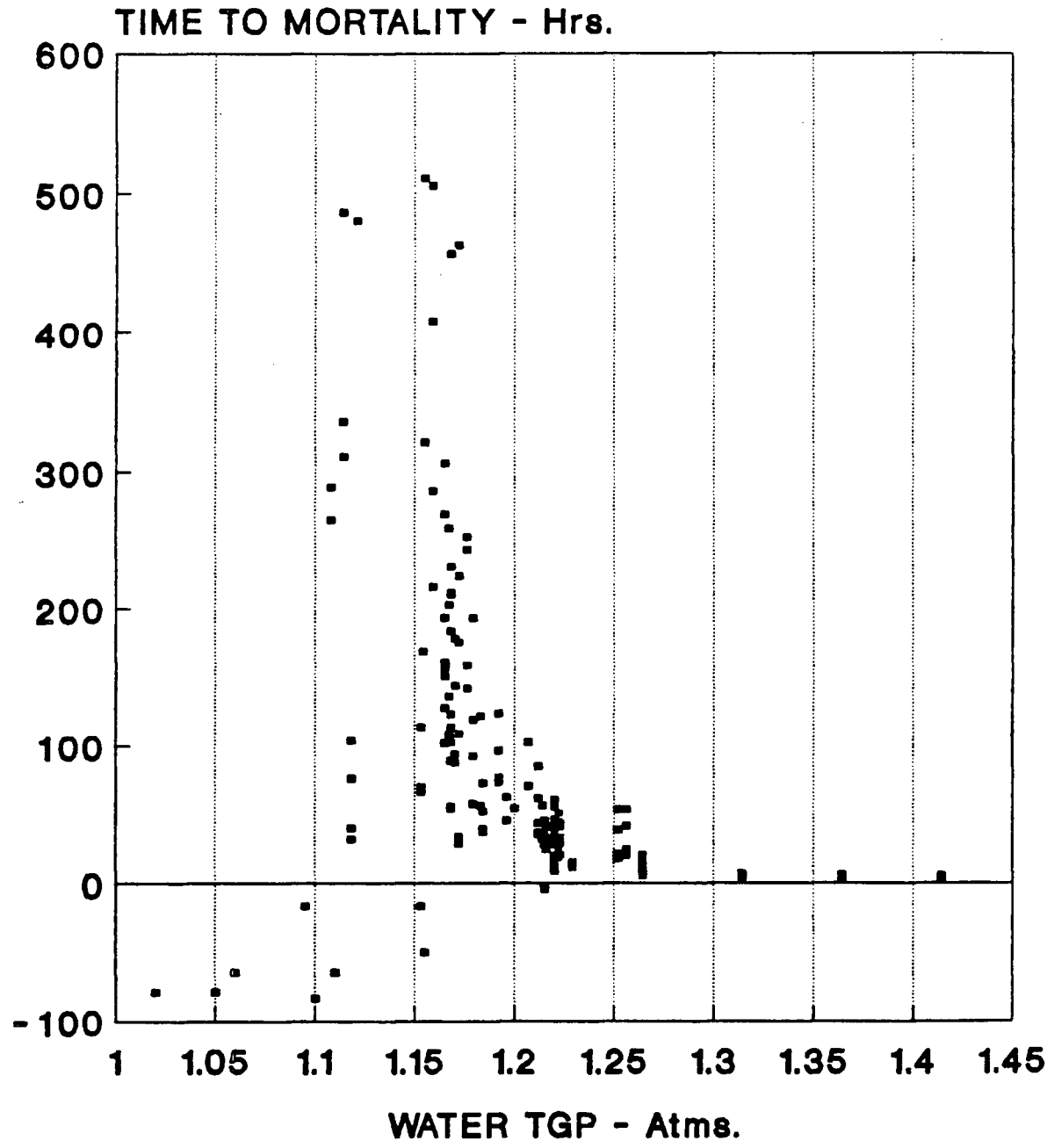
TIME TO MORTALITY VERSUS WATER TGP
COHO SALMON OVER 50 mm. IN LENGTH
MORTALITY RANGE = 20 - 70 %



Note: Negative times correspond to experiments with no mortality.

FIGURE 20: Time to Mortality Versus Water TGP for Coho Salmon Over 50 mm. in Length.

TIME TO MORTALITY VERSUS WATER TGP
STEELHEAD TROUT OVER 50 mm. IN LENGTH
MORTALITY RANGE = 20 - 70 %



Note: Negative times correspond to experiments with no mortality.

FIGURE 21: Time to Mortality Versus Water TGP for Steelhead Trout Over 50 mm. in Length.

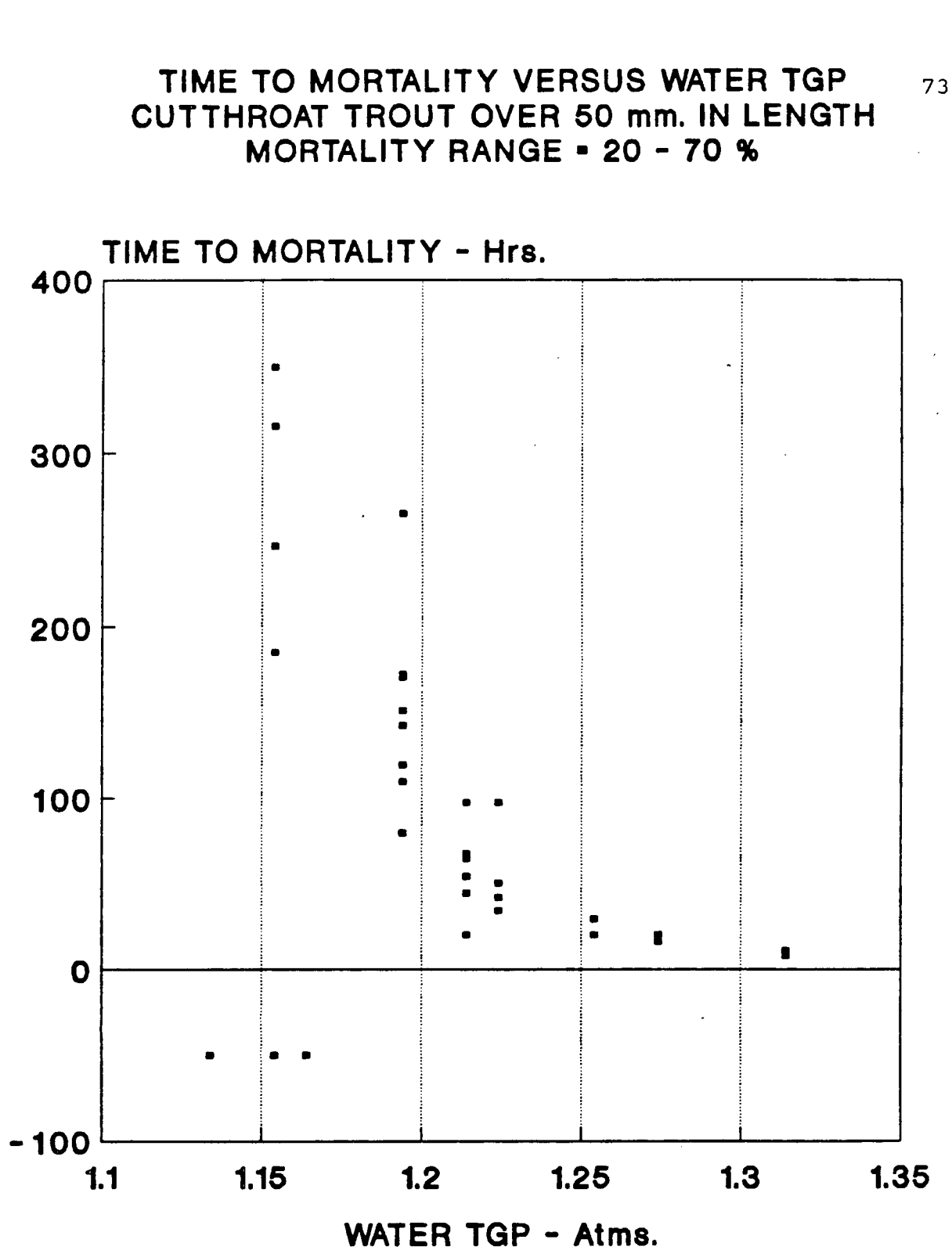
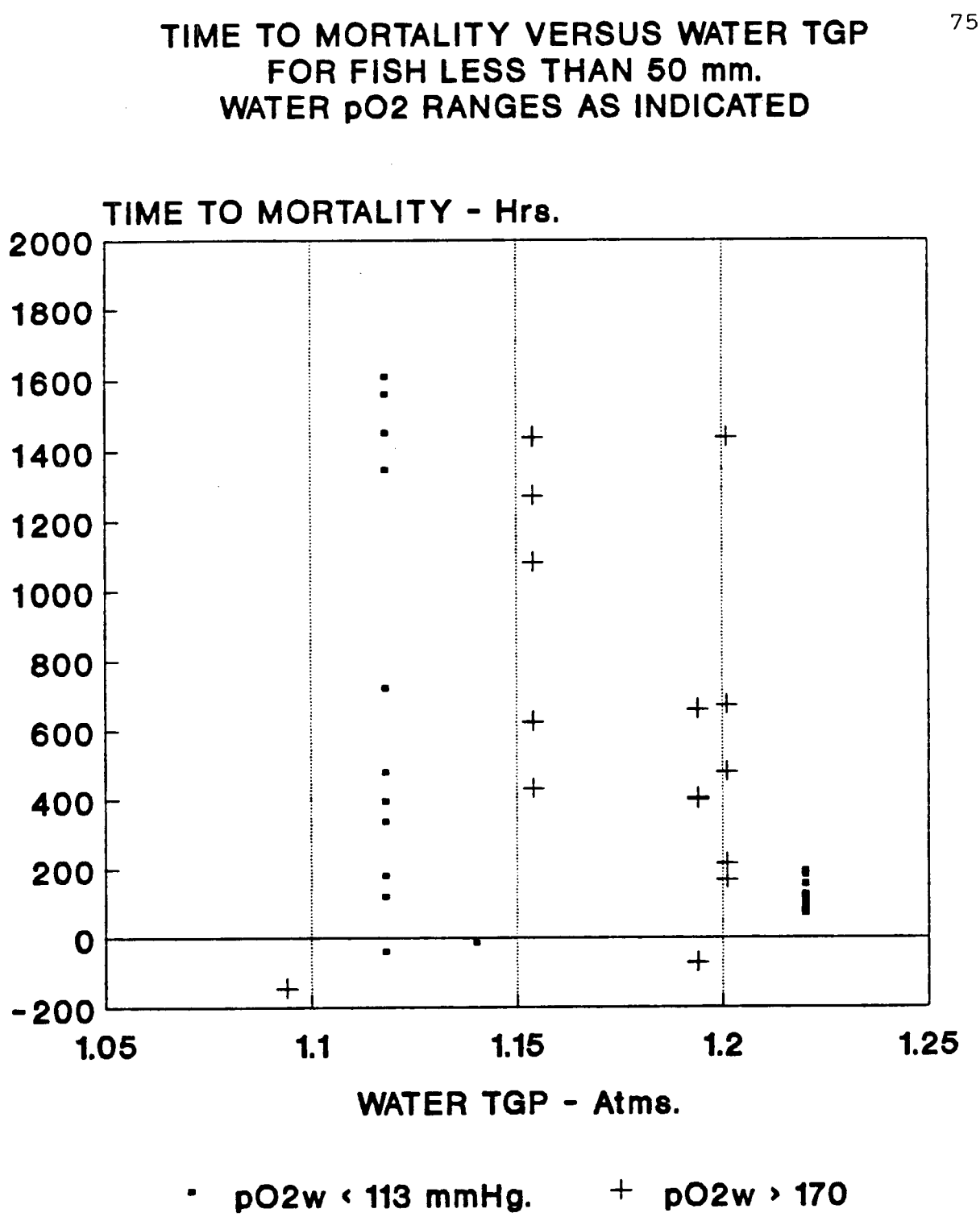


FIGURE 22: Time to Mortality Versus Water TGP for Cutthroat Trout Over 50 mm. in Length.

4.2.5 EFFECTS OF WATER DISSOLVED OXYGEN TENSION: As described in Section 3, the theoretical thresholds for vascular bubble growth show a dependence on the partial pressure of dissolved oxygen in the water. To search for such a dependency, the database was filtered to restrict the records to those containing information on water TGP, pO_2, depth and fish length.

Fish Less Than 50 mm.: An examination of the records show that, for fish less than 50 mm. in length, the water oxygen partial pressure ranges from 111 to 191 mmHg. These data can be subdivided into dominant groupings based on pO_2 greater than 170 mmHg. and less than 113 mmHg. The results of this separation are shown in Figure 23. The data records at this level of filtering contain information on Chinook and Coho salmon and Steelhead trout only. In the figure, it is apparent that no distinct relationship exists between time to mortality and water pO_2 for fish less than 50 mm. in length. There may be a separation of thresholds based on pO_2 at 1.12 and 1.155 Atms. Unfortunately, there are not enough data in this range of TGP to confirm this separation. Also, it is not clear if the separation is for the same threshold at different levels of pO_2 or whether there are two separate thresholds. Yet another complexity associated with these data is the effect of depth on mortality thresholds. This is examined further in section 4.2.6, below.

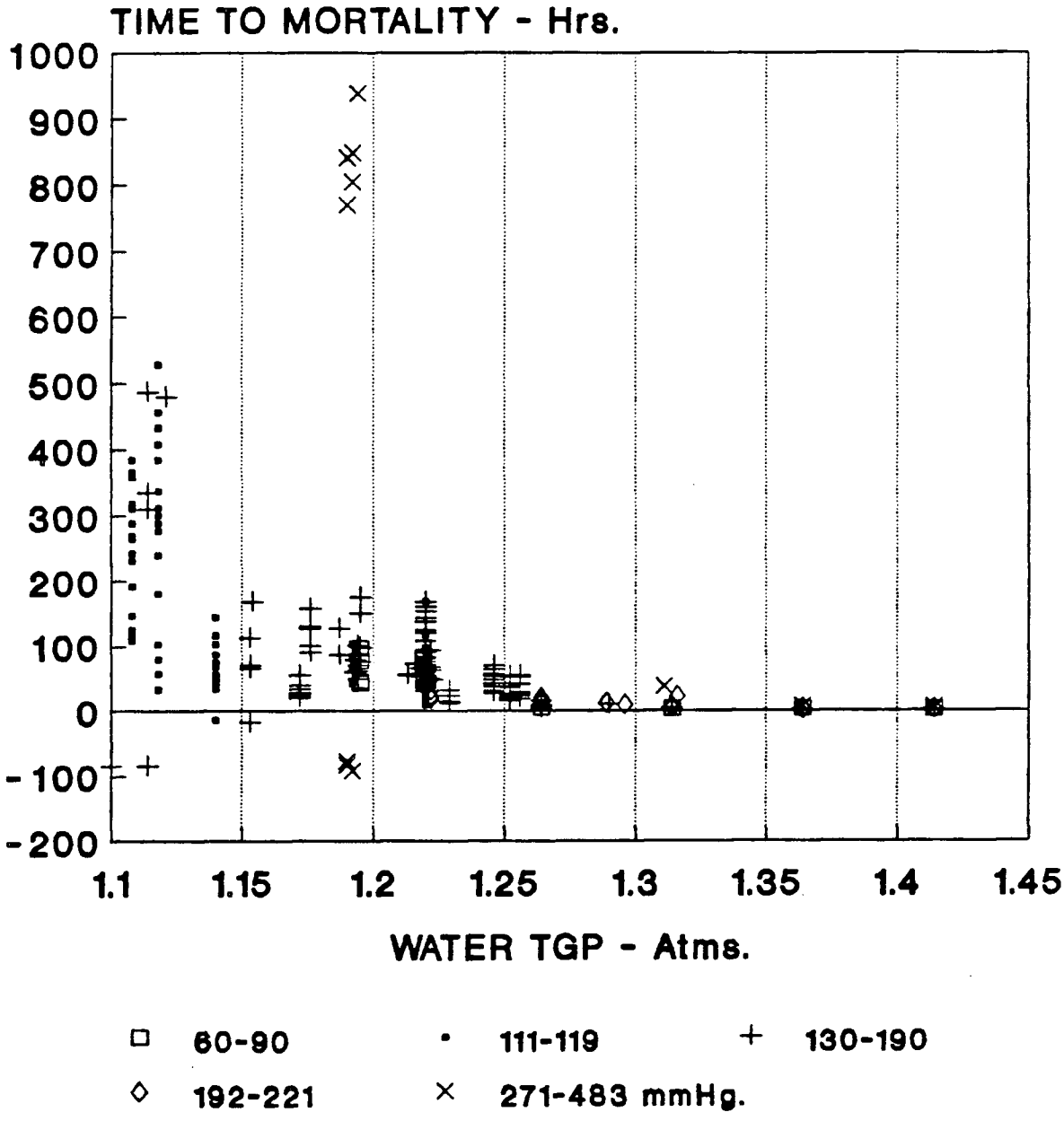
Fish Greater Than 50 mm.: The data records show that water pO_2 ranges from 63 to 458 mmHg. for fish greater than 50 mm. in length. By detailed filtering of these data, the records can be broken into groups based on pO_2 ranges of, 60 to 90, 111 to 119, 130 to 190, 192 to 221 and 271 to 483 mmHg. These data are shown in Figure 24 where, again time to mortality is plotted as a function of water TGP. Except for the



Note: Negative times correspond to experiments with no mortality

FIGURE 23: Time to Mortality Versus Water TGP for Fish Less Than 50 mm.

TIME TO MORTALITY VERSUS WATER TGP FOR FISH GREATER THAN 50 mm. WATER pO2 RANGES AS INDICATED



Note: Negative times correspond to experiments with no mortality.

FIGURE 24: Time to Mortality Versus Water TGP for Fish Greater Than 50 mm.; Water pO_2 Ranges as Indicated.

five records at water pO_2 levels greater than 271 mmHg., there is no clear separation of time to mortality or thresholds based on water pO_2. However, the difficulty in detecting a relationship has to do with the scale at which the data are being examined.

An even finer analysis of the records shows two distinct sets of data that exhibit pO_2 dependency. The first involves the experiments of Nebeker *et al.* (1979a). In four experimental series, water TGP was held constant at 1.264, 1.314, 1.364 and 1.414 Atms. while water pO_2 was varied. The results, this time plotted as time to mortality versus water pO_2, are shown in Figure 25. The legend at the bottom of the figure identifies the data associated with each level of TGP. At each TGP it is evident there is an increase in time to 50% mortality with increasing water pO_2. The most dramatic increase occurs at the lower TGP of 1.264 Atms. Although the data in this range of TGP show time to mortality is dependent on water pO_2, there is nothing to suggest the existence of thresholds. These data and one other set are the only records in the database that show a pO_2 dependency in time to mortality.

Perhaps the most intriguing set of records are those of Rucker *et al.* (1975). These data are for a water TGP of 1.193 Atms. and water pO_2 varying between 80 and 483 mmHg. Figure 26 shows the corresponding times to 25% and 50% mortality plotted against water pO_2. It is apparent in the figure that time to mortality is less than 220 hours for water pO_2 levels between 80 and 249 mmHg. As water pO_2 increases between these two values, the time to 25% and 50% mortality also increases gradually. However, between 249 and 271 mmHg. there is almost a five fold increase in time to 25% mortality. The 50% level of mortality was not reached in an exposure

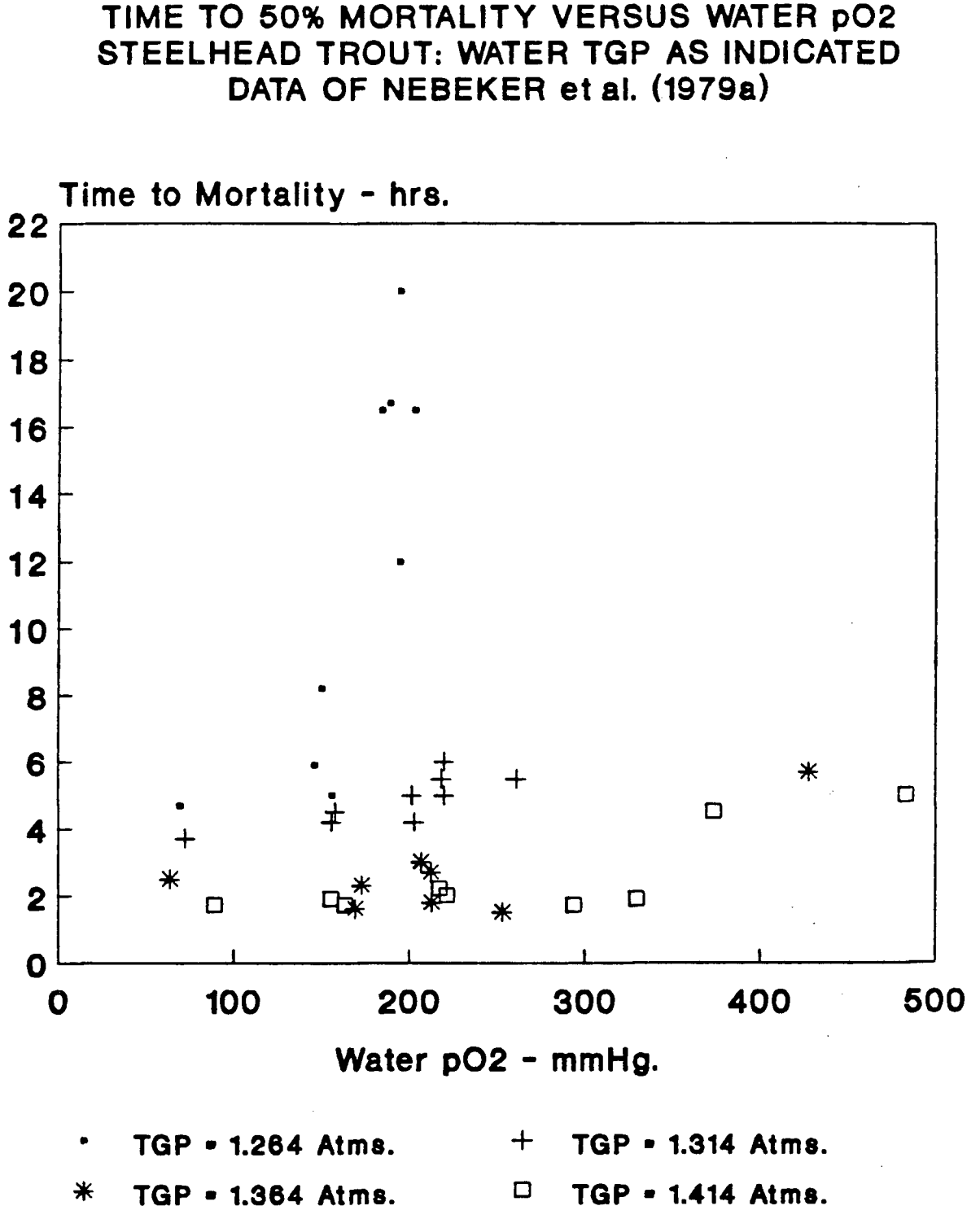
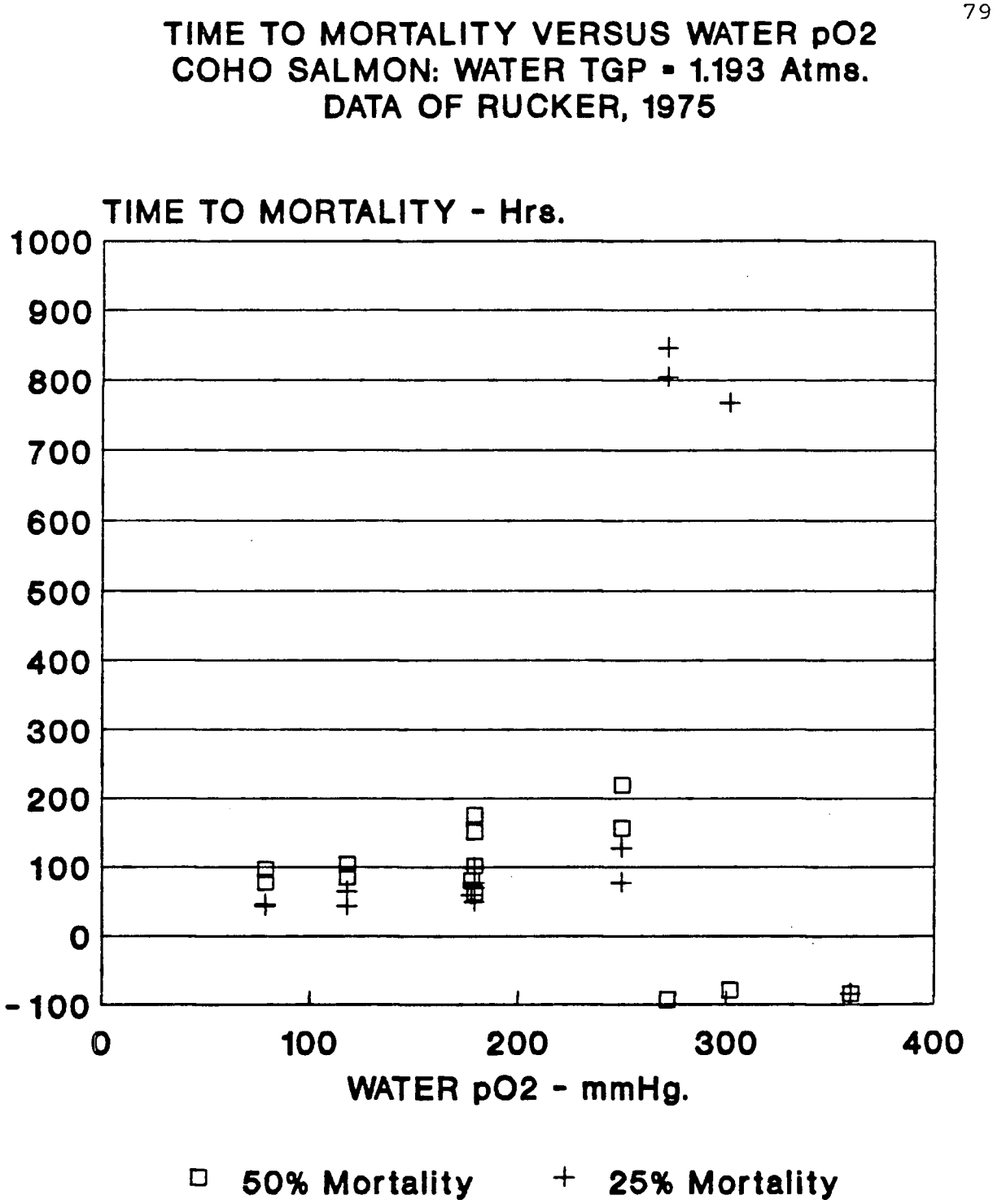


FIGURE 25: Time to 50 % Mortality Versus Water pO_2 for Steelhead Trout: Water TGP as Indicated.



Note: All fish greater than 50 mm.
Negative times correspond to no observed mortality in 1000 hrs.

FIGURE 26: Time to Mortality Versus Water pO_2 for Coho Salmon: Water TGP = 1.193 Atms.

period of 1000 hours once water pO_2 rose above 249 mmHg. (Negative times in the figure). Nor was a 25% mortality reached during the experiment at water pO_2 levels above 300 mmHg. This behavior strongly suggests the existence of a TGP threshold at 1.193 Atms. that is dependent on water pO_2. Further, the threshold is located between a water pO_2 of 249 mmHg. and 271 mmHg. It is this response that made these particular data unique as pointed out early in this analysis.

4.2.6 COMPENSATION DEPTH: As discussed in Section 3, theoretical thresholds for all forms of bubble growth should increase with increasing water depth. If there is sufficient depth, and fish use that depth, the effects of supersaturation can be avoided or reduced. The data of Shrimpton, Randall and Fidler (1988) show that small fish exposed to supersaturated water seek depth to overcome excess buoyancy induced by swimbladder overinflation. Because large fish do not experience the same degree of over buoyancy as small fish (Shrimpton, Randall and Fidler, 1988), they do not seek depth as a means of compensating for supersaturation. In order to examine this effect, the database total gas pressures were corrected for depth. That is, TGP was reduced by the hydrostatic head reported in the data. The correction formula used is:

$$TGP_{corr.} = TGP_{uncorr.} - (73.1 \bullet h / P_{Atms.})$$

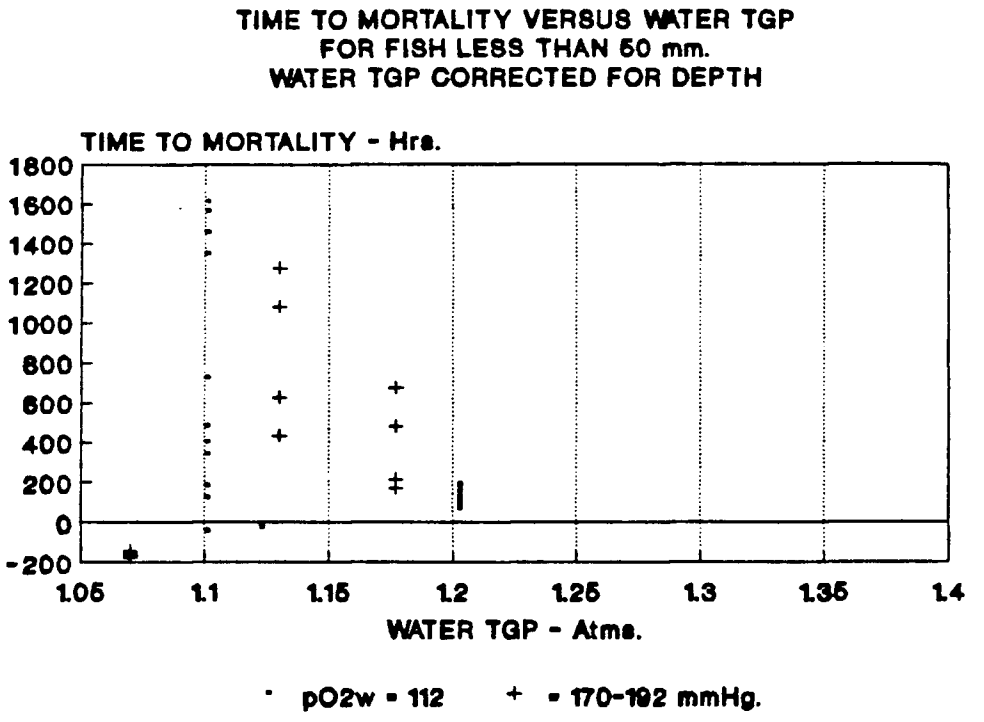
where, TGP is in atmospheres, $P_{Atms.}$ is in mmHg. and h is in meters. The constant, 73.1, combines the density of water and the gravitational constant to yield units of mmHg./m.

The results of this correction are shown in Figure 27a for fish less than 50 mm. The correction produces a horizontal shift of the data to the left on the TGP axis (See

Figure 23 for a comparison of the corrected data with uncorrected data). With the correction of TGP for depth, the apparent lower threshold is shifted from 1.12 Atms. to 1.1 Atms. It will be recalled from previous discussions that this corresponds to the lower threshold of mortality for fish greater than 50 mm. The same correction was applied to records for fish greater than 50 mm. An inspection of Figure 27b again shows a leftward shift of data; however, no other changes appear as a result of the depth correction (See Figure 24 for a comparison of the corrected data with uncorrected data). Based on the work of Shrimpton, Randall and Fidler (1988), a correction of TGP for depth may not be appropriate for fish greater than 50 mm.

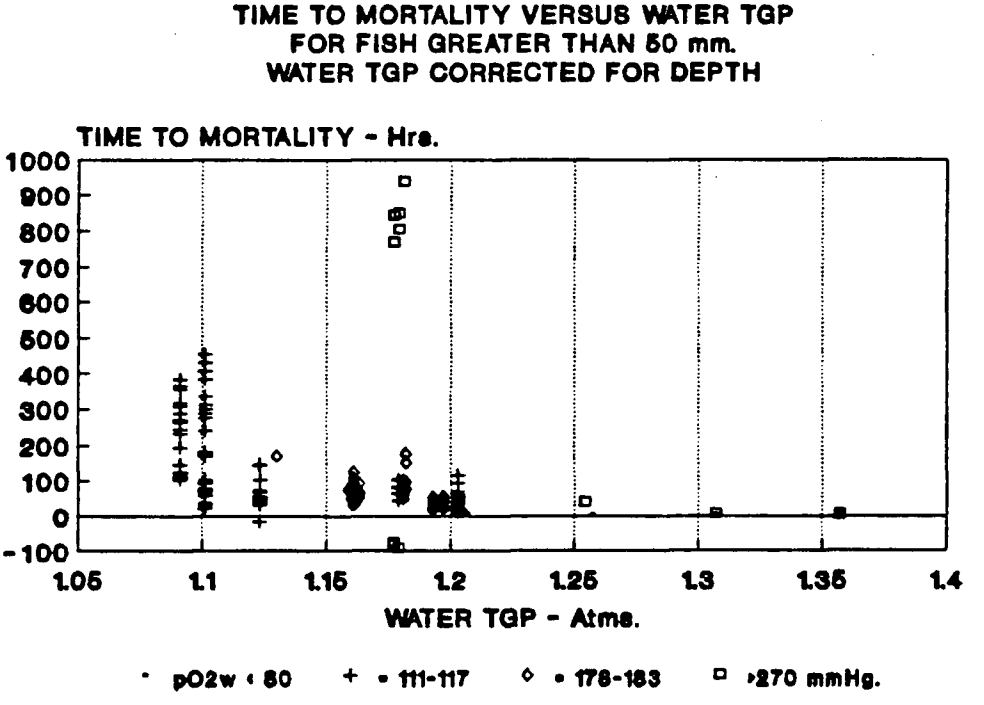
The database was further analyzed for evidence of depth compensation effects. Although there were clear indications of depth compensation by fish, the results were not conclusive because of species variations and unknown levels of water pO_2. Figure 28 shows the best correlation that was obtained and is restricted to fish greater than 50 mm. in length. In the figure, water pO_2 is unknown for all data at a depth of 0.61 meters. With this in mind, the data suggest that depth leads to increased survival time at the same level of TGP.

In the data records, depths range from essentially zero to about four meters. In most cases there is no restriction on the movement of fish within the depth indicated in each record. The one exception is the data of Knittel *et al.* (1980). In these experiments Steelhead trout were exposed to supersaturated water in cages held at specific depths.



Note: Negative times correspond to experiments with no mortality.

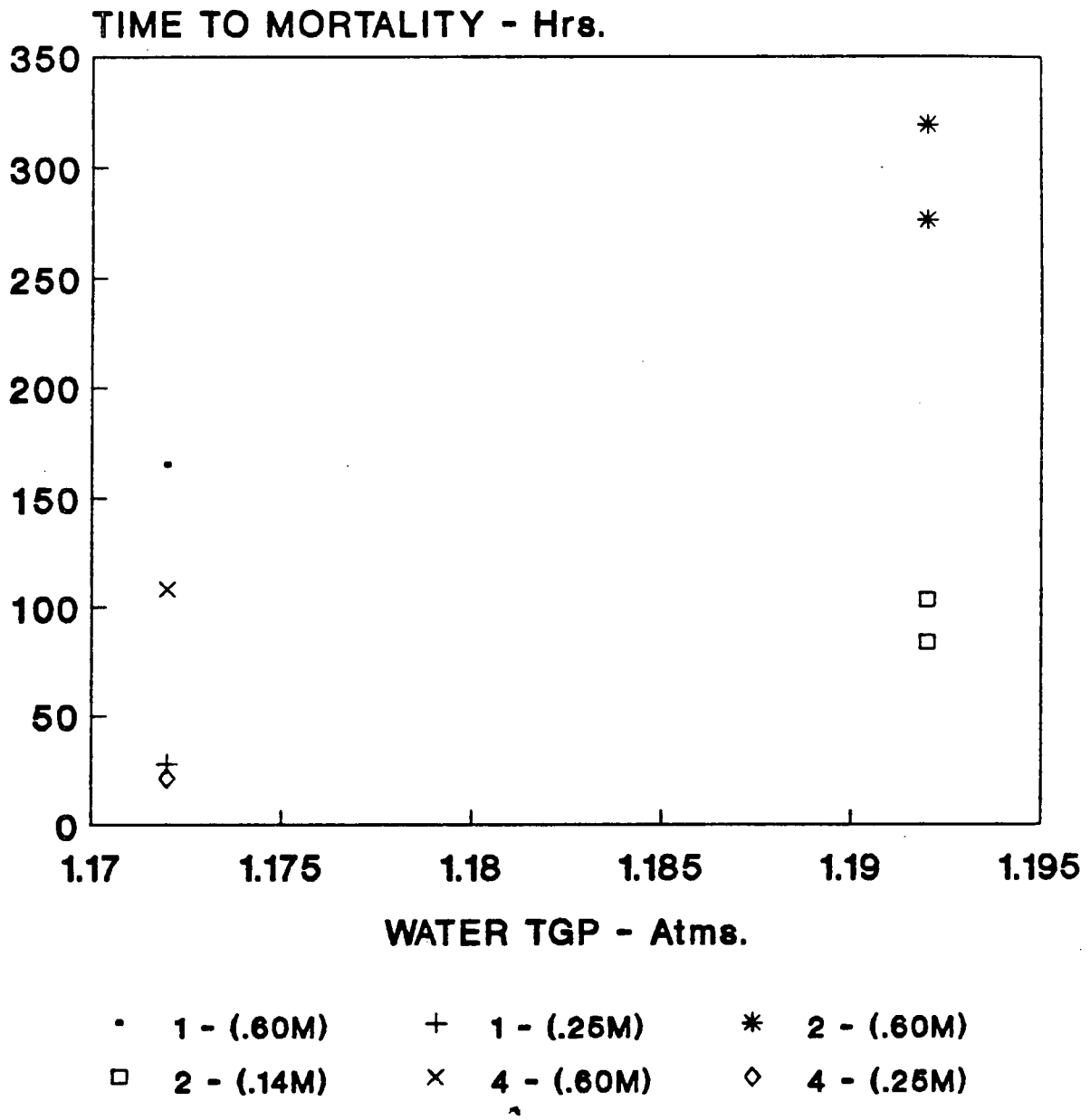
FIGURE 27: Time to Mortality Versus Water TGP for Fish Less Than 50 mm.: Water TGP Corrected for Depth.



Note: Negative times correspond to experiments with no mortality.

FIGURE 27: Time to Mortality Versus Water TGP for Fish Greater Than 50 mm.: Water TGP Corrected for Depth.

TIME TO MORTALITY VERSUS WATER TGP FOR FISH GREATER THAN 50 mm. Chinook ▪ 1, Coho ▪ 2, Steelhead ▪ 4



Depths as indicated in parentheses.
Chinook and Steelhead at 50 % mortality
Coho at 20% mortality

FIGURE 28: Time to Mortality Versus Water TGP for Fish Greater Than 50 mm.; Chinook, Coho and Steelhead Trout.

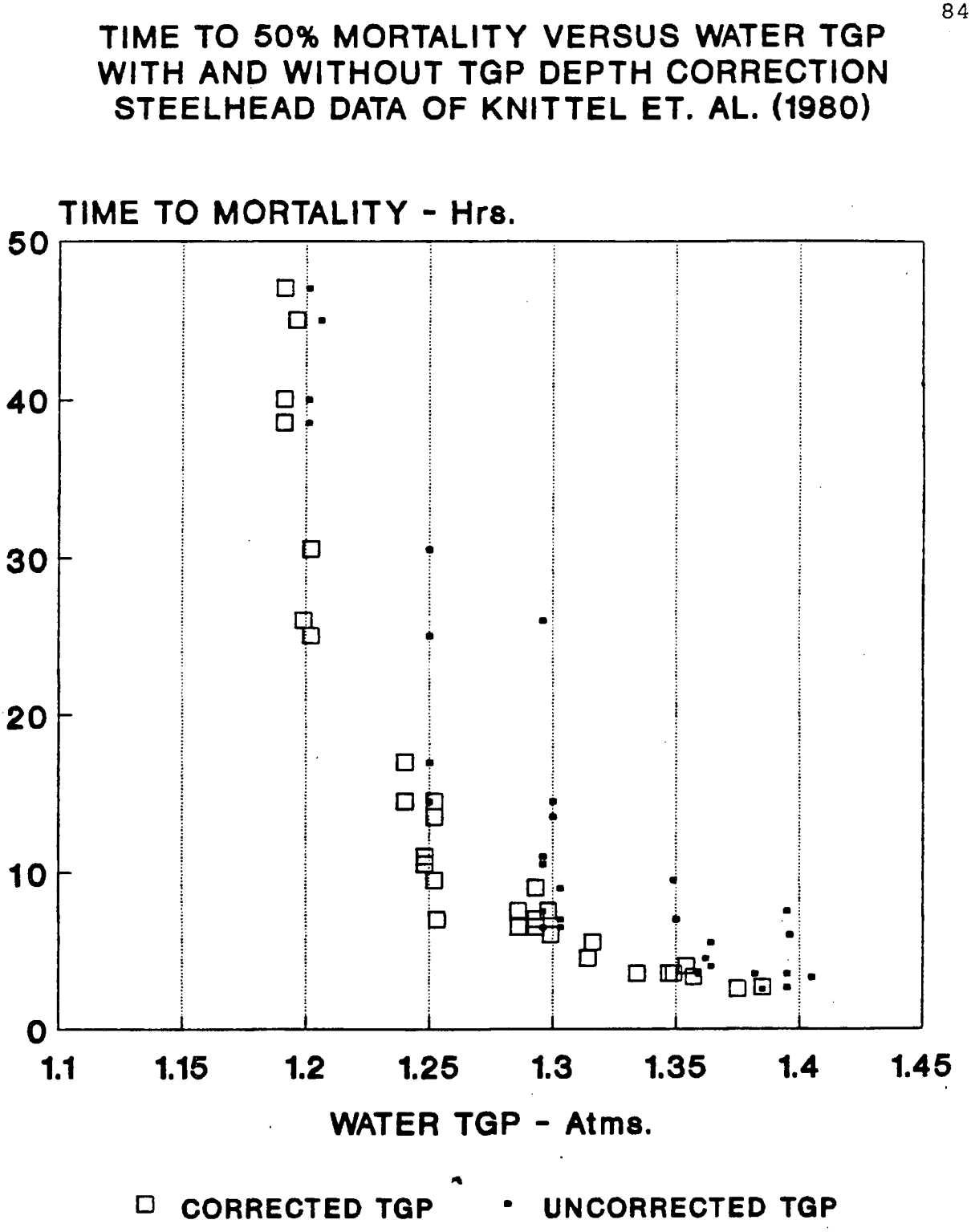


FIGURE 29: Time to 50 % Mortality Versus Water TGP With and Without TGP Depth Correction: Steelhead Trout.

TGP ranged from about 1.19 Atms. to 1.41 Atms. Figure 29 shows the results of these experiments in terms of time to 50% mortality versus water TGP. The solid points correspond to data uncorrected for depth while the open squares are for TGP corrected for depth. It is clear that depth correction significantly reduces the scatter in the data and suggests a hyperbolic or asymptotic form of response. Further, the corrected data show a TGP threshold near 1.19 Atms.. This is well above that indicated in the rest of the Steelhead data (Figure 21). However, water pO_2 is unrecorded in the data of Knittel *et al.* (1980).

4.3 DISCUSSION:

From this analysis, it is clear that the response to supersaturated water by Chinook, Coho and Sockeye salmon along with Steelhead and Cutthroat trout is highly dependent on fish size. The difference in time to mortality between fish greater than 50 mm. and those less than 50 mm. is so strong as to suggest a discontinuous relationship between time to mortality and fish size. This would imply yet another threshold that must be considered when mathematically modeling time to mortality relationships. In an analysis such as that used by Jensen *et al.* (1985), which depends on continuous functions for regression procedures, ignoring such a threshold can severely distort the formulation of the model. Clearly, the solution to this problem is to separate the data based on size and apply the model to the data sets independently. However, this would not solve all problems associated with multiple thresholds. As pointed out in the introduction to this thesis, if there are multiple thresholds dependent on water TGP and pO_2, further separation of the data would be necessary before the models of Jensen *et al.* (1985) could be used successfully. The preceding database analysis strongly suggests the existence of more than a single threshold for mortality in fish exposed to supersaturated water.

It is interesting to compare the predicted response of the models of Jensen *et al.* (1985) with the data upon which the models are based. Figures 30 and 31 show this comparison for their Models 1 and 15 respectively. In Figure 30, where predicted time to 50% mortality is plotted versus TGP, the model describes the data reasonably well only at TGP levels above 1.22 Atms. Between a TGP of 1.15 and 1.22 Atms., the predicted response diverges from the bulk of data in this region. Below a TGP of 1.15 Atms., there is considerable variation between the model and experimental data. In Figure 31 the model predictions are obtained by using the TGP and oxygen to nitrogen ratio of each experimental datum point to predict time to 50% mortality. Again, above a water TGP of 1.25 Atms., the data and model predictions are relatively similar. However, below this level of TGP there are significant differences between the two. Except for the one point at 1182 hours, the model consistently over predicts time to mortality for TGP levels between 1.1 and 1.17 Atms. Between 1.17 and 1.25 Atms., the model tends to under-predict time to mortality.

Although not conclusive for all species examined, the database analysis suggests that two, TGP related, mortality thresholds may be involved in the response of fish to supersaturation. A lower threshold is clearly apparent at a TGP of about 1.1 Atms., while a second threshold may exist in the TGP range of 1.15 to 1.17 Atms. The evidence for the two thresholds is strongest for Chinook salmon greater than 50 mm. in length (Figure 18). The evidence for a higher threshold near a TGP of 1.15 Atms. is particularly clear for Sockeye salmon and Cutthroat trout (Figures 19 and 22 respectively). The data also suggest that the higher threshold varies slightly with fish species.

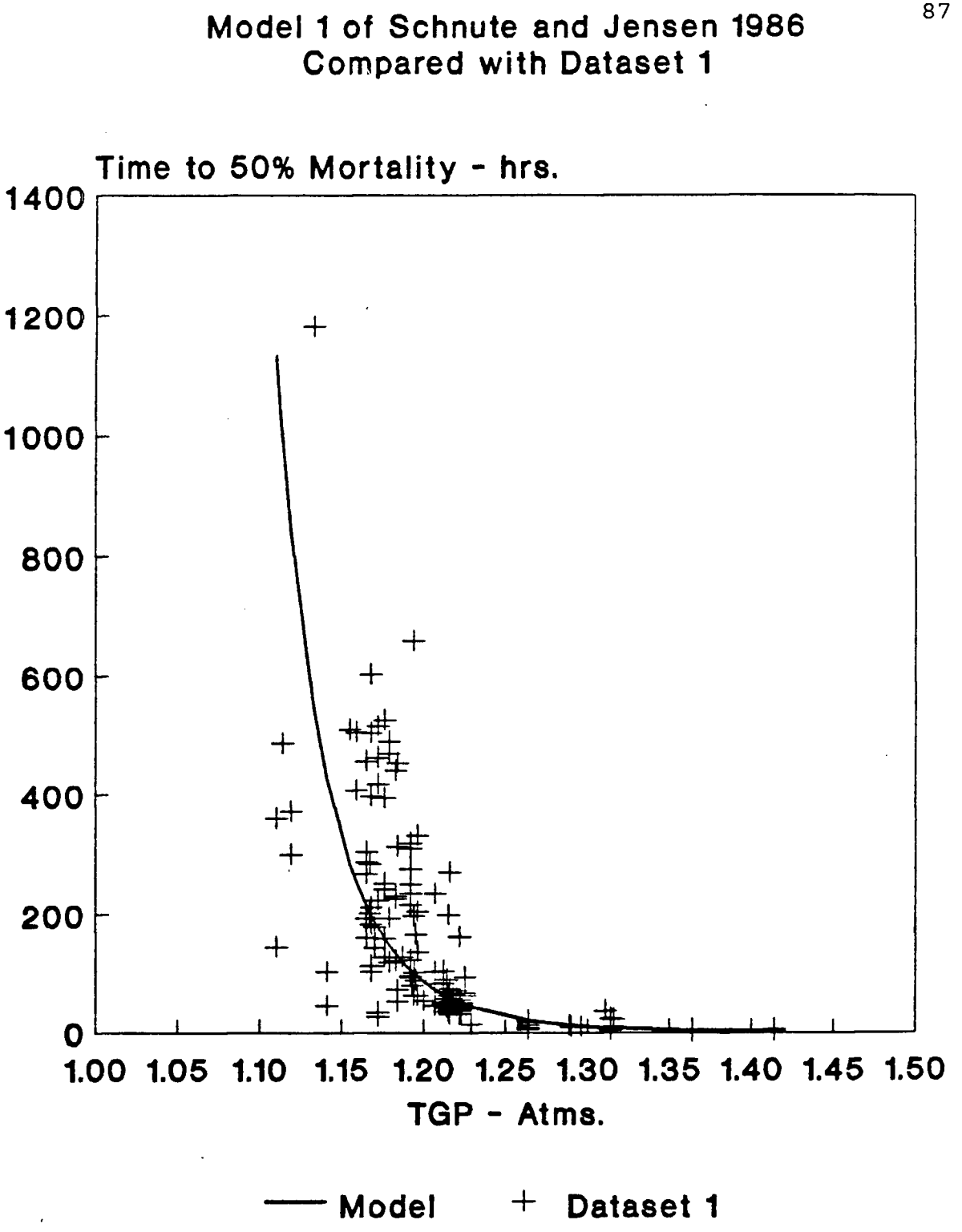


FIGURE 30: Model 1 of Schnute and Jensen 1986

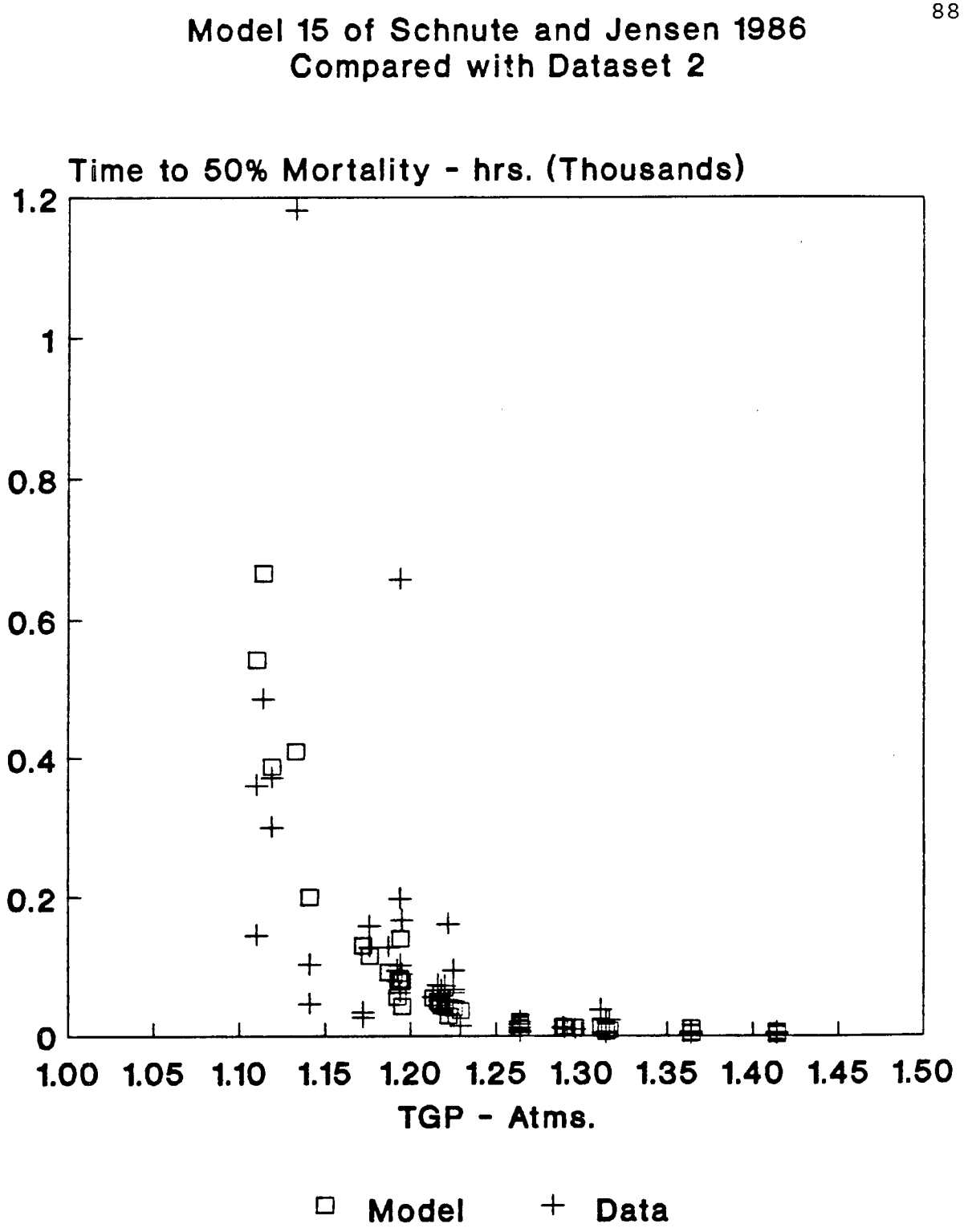


FIGURE 31: Model 15 of Schnute and Jensen 1986

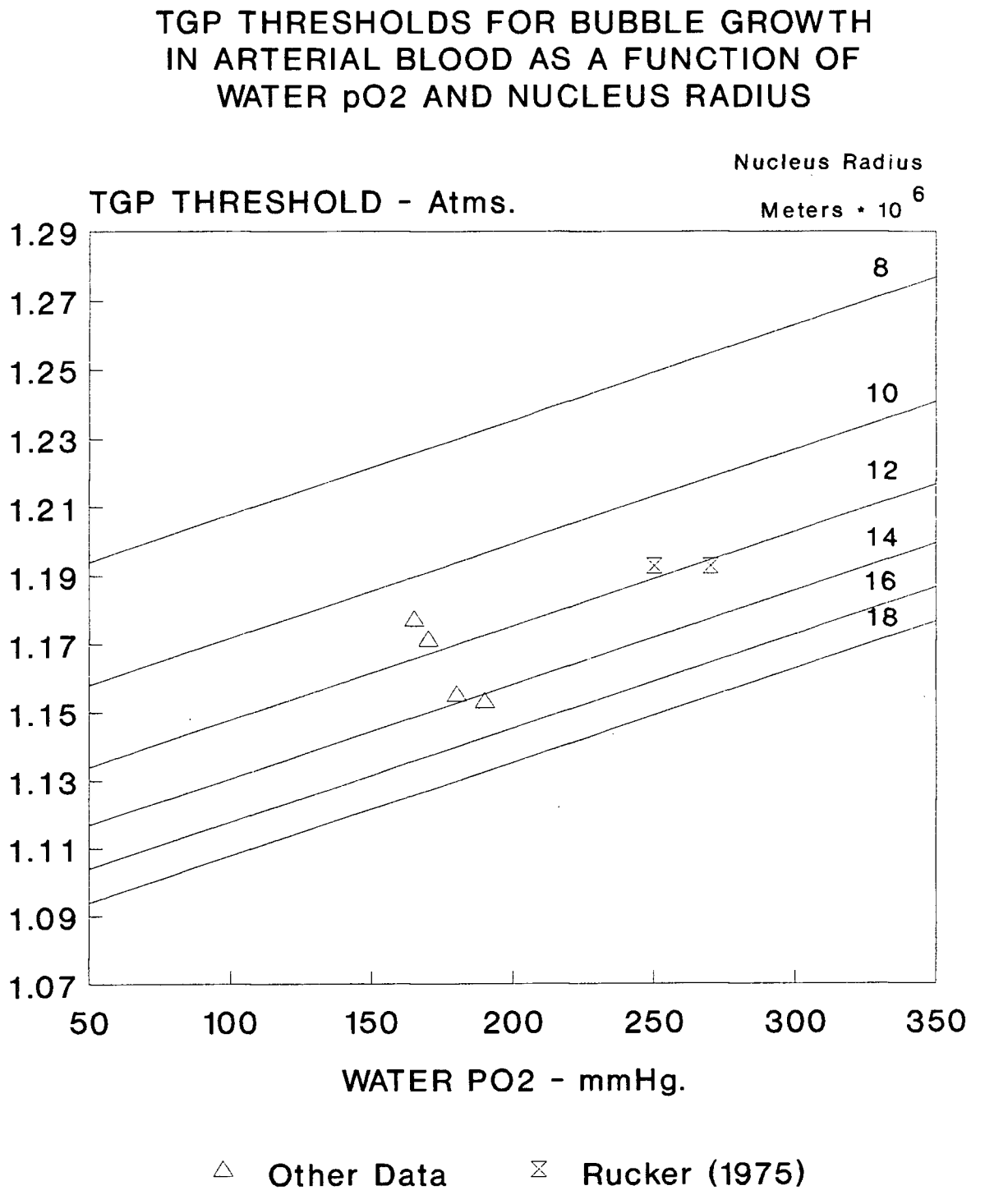
For fish less than 50 mm. in length the only mortality threshold that is apparent, is one at a TGP of 1.12 Atms. However, if depth corrections are applied to this threshold (assuming small fish seek compensation depth), the threshold for zero depth is shifted to 1.1 Atms. (Figure 27a). This places the apparent lower threshold at about the same TGP as for fish greater than 50 mm. with TGP uncorrected for depth.

Although there is evidence for two TGP thresholds for fish greater than 50 mm. in length, the physiological consequences of these thresholds is not clear. Nor is it clear which lethal symptoms are associated with each threshold. As discussed earlier, the data for Chinook salmon greater than 50 mm. in length suggest two separate mortality mechanisms are operating over two different ranges of TGP. Furthermore, at a TGP of 1.17 Atms., there appears to be a transition from one mechanism of mortality to another. This is accompanied by a dramatic increase in time to mortality. It is not clear what these mechanisms are or the reasons for the apparent transition from one to the other. However, the experimental work of this thesis yields further insight into these mechanisms and the nature of the apparent transition. These results are examined in Section 5 of this thesis.

An indication of the cause of mortality at the higher TGP threshold is suggested in the studies of Stroud and Nebeker (1976) on Steelhead trout. In this work, symptoms of GBT were recorded for fish exposed to various levels of water TGP. It was noted that vascular bubbles in the gill arteries did not appear until water TGP rose above 1.15 Atms. Meekin and Turner (1974), made similar observations; however, they reported only dissolved nitrogen concentrations and not TGP. Assuming the apparent threshold at 1.15 Atms. is associated with vascular system bubble growth, the data from Table III, Appendix C, that contain information on water pO_2 can be plotted in

relation to the theoretical bubble growth thresholds (Section 3). This is shown in Figure 32. The data shown as "Other" in the figure correspond to all data in the database between a water TGP of 1.153 and 1.176 Atms. for which water pO_2 is known. In addition, the thresholds indicated in the data of Rucker (1975) are shown. Interestingly, the critical nucleus radius indicated by this cross plotting is on the order of 12 to 14 μM. This radius is about twice the size of erythrocytes for these species and also about twice the size of tissue capillaries. This would imply that the formation of bubbles from nuclei in the vascular system occurs in vessels somewhat larger than capillaries. As was shown in Section 3, where the variation in vascular system pressure was examined, this result would be consistent with the hypothesis that the arterioles just upstream of the capillary tissue beds are the most likely location for bubble formation.

Of the 528 data records containing information on water pO_2, only 77 records (those of Rucker, 1975 and Nebeker *et al.*, 1979a), yield relationships between time to mortality and water pO_2 (Figures 25 and 26). The absence of correlation in the other data is probably due to the wide variation in water TGP in the database combined with a rather sparse range of water pO_2 values. Another complicating factor may be the depth at which fish position themselves in the water column. For example, Dawley *et al.* (1976) found that groups of 40 mm. Chinook salmon fry and 180 mm. Steelhead trout responded to increasing levels of supersaturation by increasing their depth in the water column. However, Steelhead trout held lower positions in the water column than did Chinook salmon for the same TGP. This contrasts somewhat with the data of Shrimpton, Randall and Fidler (1988). These data show that the depth single Rainbow trout fry seek in the water column is proportional to water TGP.



Water Depth • 0.0 M. F • 0.79
Water Temp • 5 - 15 deg. C.
Atmospheric Pressure • 760 mmHg.

FIGURE 32: TGP Thresholds for Bubble Growth in Arterial Blood.

Thus, there may be behavioral characteristics based on species differences and the number of fish that modifies the depth compensation response.

The effect of depth on time to mortality is clearly shown in the data of Knittel *et al.* (1980). By correcting TGP for depth, a much stronger correlation of time to mortality with water TGP is obtained. The hyperbolic or asymptotic form of response indicated in the corrected data is typical of dose response relationships commonly used in toxicology studies (Sprague, 1969 and Warren and Doudoroff, 1971). The form is also consistent with the models used by Jensen *et al.* (1985). However, the data of Knittel *et al.* (1980) correspond to TGP levels at or above the upper TGP threshold indicated in the database. Thus, there data is for a single threshold. Although the correction of TGP for fish restrained to specific depths appears valid, it is seldom that such restrictions can be achieved.

5.0 EXPERIMENTAL STUDIES

5.1 INTRODUCTION:

A theoretical model for vascular system bubble growth was reviewed and expanded in Section 3. The model predicts that the growth of vascular system bubbles is dependent on TGP thresholds. Furthermore, these thresholds were shown to be a function of water pO_2, water depth, barometric pressure and the effective size of nucleation sites in the vascular system. The principal unknown in the model is the effective size of the nucleation sites. If this can be established experimentally, Equation 4 will offer a complete description of thresholds for this type of bubble growth.

It was hypothesized in Section 3 that the growth of bubbles in the vascular system might lead to alterations in vascular system pressure. Because the cardiovascular system is a relatively closed fluid system, pressure should increase as a result of the gas volume added by the growing bubbles. This type of response was modeled mathematically as presented in Equation 11 and shown in Figures 8 and 9. If such a perturbation can be detected experimentally, it would serve as an indicator of bubble growth thresholds. Once thresholds are defined, the effective radius of cardiovascular nuclei can be back calculated from Equation 4.

Also reviewed in Section 3 was the mathematical model for bubble growth in the environmental water or for sub-dermal bubble growth on the external body of fish exposed to supersaturated water. The model predicts that, for nucleation sites comparable in size to those of the vascular system, bubble growth will occur at lower

TGP thresholds than those of the vascular system. However, it is not known if this form of bubble growth can produce mortality in fish. In any case, there is an experimental need to verify the threshold equation for these types of bubble growth.

In the preceding section an analysis of data from the literature implied there are may be two thresholds for mortality in fish exposed to supersaturated water. The higher threshold, in the vicinity of 1.15 to 1.17 Atms. TGP, appears to correlate with other experimental findings regarding the appearance of intravascular bubbles in gill lamella (Stroud and Nebeker, 1976). This and other threshold correlations, plotted in Figure 32, show that if the higher threshold is associated with intravascular bubble growth, the effective size of the nuclei involved is on the order of 12 to 14 μM. in radius.

A lower threshold, in the vicinity of 1.1 Atms., also appears in the literature database. If this threshold is associated with bubble growth in the environmental water or sub-dermal bubbles on the skin of the animal, the effective size of nucleation sites is about 12 μM.

Thus, theory predicts there are multiple thresholds for bubble growth in fish exposed to supersaturated water. Data from the literature suggest the existence of two thresholds for mortality in fish exposed to supersaturated water. However, the two are uncorrelated. That is, there is no clear association of the theoretical bubble growth thresholds and the observed thresholds for mortality. Mortality may be caused by bubble growth. However, other physiological insults resulting from supersaturation may also be responsible. Thus, there is a need to experimentally study the relationship between thresholds for bubble growth and physiological parameters that may establish whether or not bubbles are responsible for death in

fish. The experimental approach to this problem was accomplished in two distinct experimental phases.

5.1.1 PHASE I EXPERIMENTS: The Phase I experiments examined the response of the vascular system to intravascular bubble growth. The purpose was to determine if detectable increases in blood pressure accompany bubble formation and growth. If an increase in pressure is observed, it can be used as an indicator to experimentally search for bubble growth thresholds.

5.1.2 PHASE II EXPERIMENTS: The Phase II experiments involved examining an array of other physiological parameters as indicators of thresholds for bubble growth. In addition to intravascular bubbles, these experiments focused on thresholds for water bubble and sub-dermal bubble growth. In particular, arterial pO_2, pH, hematocrit, and catacholamine levels were surveyed. These were correlated with the severity of GBT symptoms and water TGP and pO_2. Accompanying these experiments was a study of respiratory performance under conditions of dissolved gas supersaturation.

5.2 EXPERIMENTAL MATERIALS AND METHODS

The section that follows contains a description of the materials and methods used in both phases of experimental work. The descriptions are of a general nature and common to many of the experiments. For example, the methods of cannulating fish and the device used to produce supersaturated water are common throughout both phases of experimental work. As each experimental series is examined in detailed, unique procedures associated with that series will be described.

5.2.1 EXPERIMENTAL APPARATUS AND WATER PARAMETERS

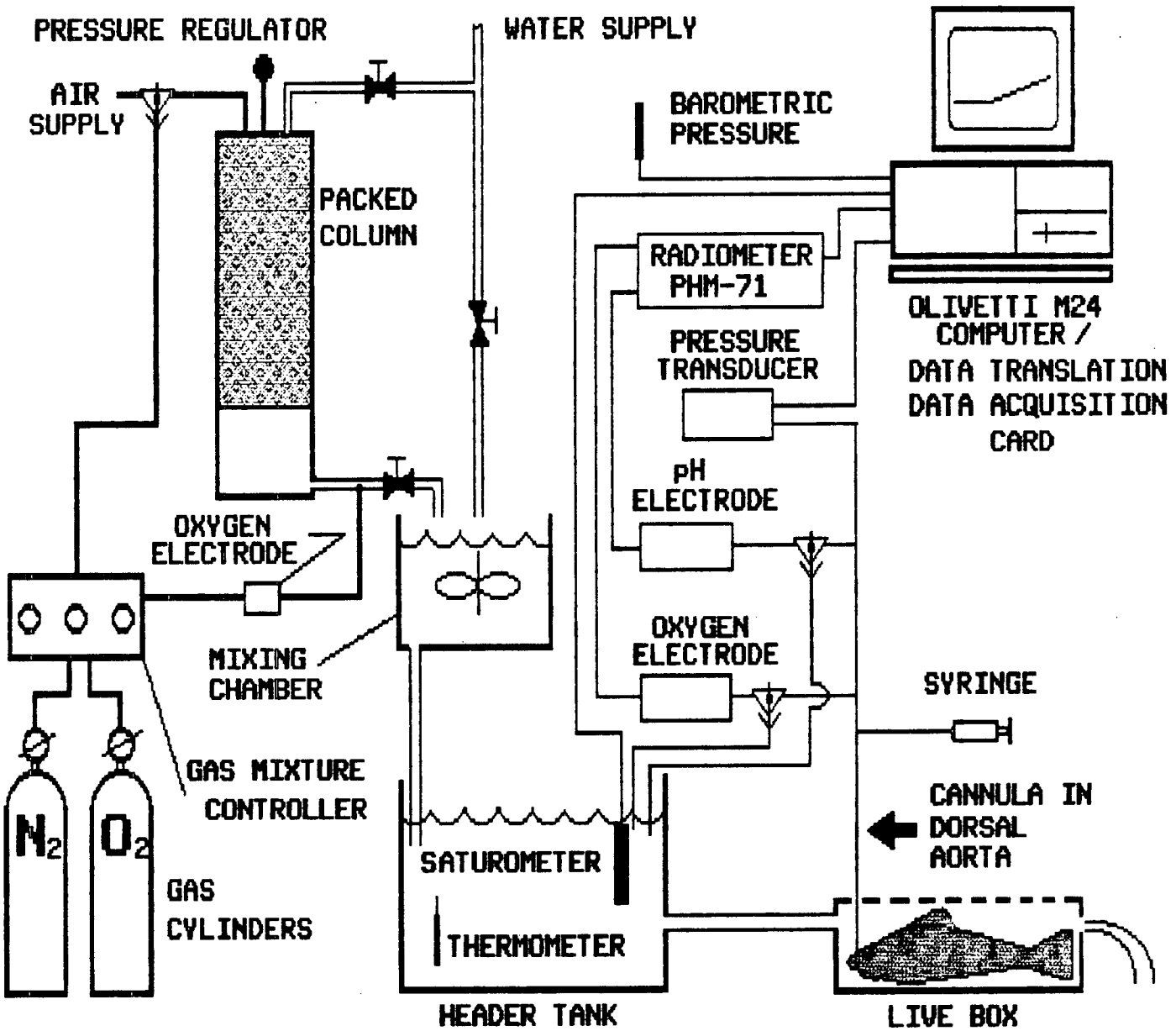
Production of Supersaturaterd Water: A packed column was used to produce supersaturated water for the Phase I and Phase II experiments. This device employs turbulent mixing of gas and water phases under pressure to accomplish the necessary gas exchange (Fidler 1983). The column consisted of a plastic pipe, 2.5 meters long by 200 mm. inside diameter, filled with 1.5 cm. Norton Intalox saddles. The Intalox saddles produce turbulence in water that falls through the column, thereby enhancing the gas exchange process. The column was mounted in a vertical position and sealed at both ends except for water and gas ports. Water supply to the column consisted of two independent flows. The main supply entered at the top of the column and fell over the packing material while a continuous flow of air or pre-selected gas mixtures passed upward through the packing. The second supply, or make-up water, entered at the bottom of the column and mixed with the outflow supply of supersaturated water. A float valve at the foot of the column controlled make-up water flow and assured a uniform discharge from the column. The make-up supply also allowed fine tuning of the system Total Gas Pressure. Pressure regulators upstream in all raw water supply lines insured uniform inlet water flow to the system.

The gas supply to the column was either atmospheric air under pressure or a pressurized mixture of oxygen, nitrogen and water vapor. A three way valve in the gas supply system allowed switching between air and the pre-selected gas mixtures. The mixture ratio of oxygen to nitrogen was controlled by a gas flow mixing system with a pO_2 monitoring control loop. When the gas mixer was used, a Radiometer E5046 thermostated oxygen electrode measured pO_2 in the outflow water from the

column. The monitored electrode signal was used in an electronic feedback system to regulate gas mixture to the column. The setpoint was a pre-selected pO_2 level fixed by a calibrated potentiometer on the controller. A pressure cooker type relief valve at the top of the column controlled pressure within the column. Column pressure could be maintained at a precise level by adjusting the weight of the relief valve. With this system, it was possible to regulate Total Gas Pressure and dissolved oxygen concentration within narrow tolerances for long periods.

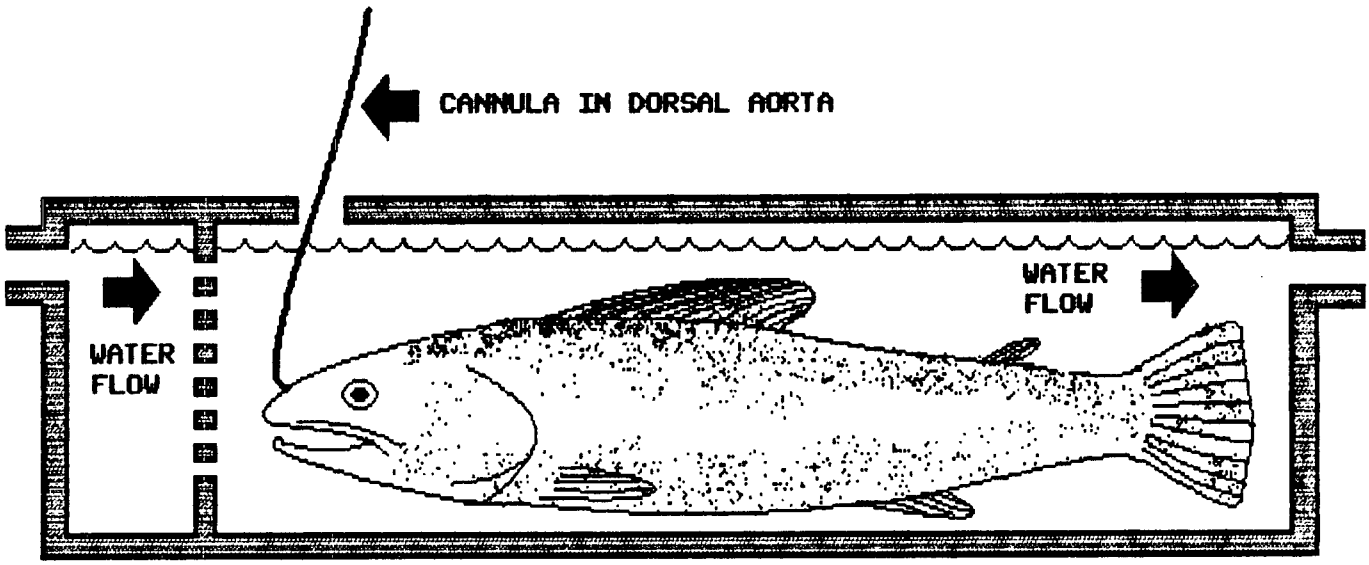
Water from the column flowed into a mixing chamber and then into a head tank. The surface of the water in the head tank was covered with a styrofoam float to reduce dissolved gas loss to the atmosphere. Water for the experiments was drawn from the head tank and introduced into live boxes that contained the experimental animals. Water pH, temperature, total gas pressure and pO_2 were measured in the head tank at a point near the discharge to the live boxes. The general arrangement of the packed column and other experimental apparatus are shown in Figure 33. As shown in the figure, a separate supply of air equilibrated water was available for holding fish in the live boxes before the supersaturation experiments. Figure 34 shows an individual live box with a fish in place. The depth of the box was no more than 10 cm. and the box was open to the atmosphere to prevent hydrostatic head buildup.

Water Dissolved Gas Measurements: Throughout the Phase I and Phase II experiments, water dissolved gas tensions were determined by measuring Total Gas Pressure and pO_2. TGP was measured continuously with a Model 300 Nova Tech Saturometer. Before each experiment the instrument output was set to zero and barometric pressure recorded. All subsequent measurements of TGP were corrected for changes in barometric pressure occurring during the experiment.



EXPERIMENTAL APPARATUS

FIGURE 33: Experimental Apparatus



LIVE BOX

FIGURE 34: Live Box

The sensor portion of the instrument was placed in the head tank supplying water to the live boxes (Figure 33). The sensor was shaken continuously during experiments by a mechanical shaker. This prevented bubble formation on the silastic tubing membrane of the instrument which has been shown to reduce instrument sensitivity (Bouck, 1982).

Dissolved oxygen was monitored continuously by passing a stream of water from the head tank through a Radiometer Model E5046 oxygen electrode. The electrode was housed in a water jacket which maintained the electrode at the experimental water temperature. The electrode electrical output was connected to a Radiometer PHM 71 signal conditioner and meter. The system was calibrated before and at times during each experiment with two standards. The low standard consisted of water that had been completely degassed of oxygen. The high standard was water that had been equilibrated with atmospheric air. The high standard calibration was corrected for the partial pressure of water vapor at the experimental temperature and barometric pressure at the time of calibration.

Water pH and Temperature: Water pH was measured with a Radiometer G279/G2 glass capillary electrode and F497 calomel electrode connected to a Radiometer PHM 71 signal conditioner and meter. Before and following each experimental series, the pH electrode was calibrated using Radiometer precision phosphate buffers S1500 and S1510. Water pH was measured at prescribed intervals during each experimental series. Water temperature was measured with a mercury thermometer.

5.2.2 COMPUTERIZED DATA ACQUISITION SYSTEM: As shown in Figure 33, most experimental measurements were recorded digitally on a personal computer. A Data

Translation DT-2801 analog to digital card, installed internally in the computer, interfaced with the computer main bus. The DT-2801 card converts voltage signals from the various instruments into digital data which were recorded on magnetic media by the Olivetti M24 personal computer. The data collection process and operation of the system was managed by the Laboratory Technologies LabTech Notebook computer program. This software allows complete control of data sampling rates and experimental protocol. Data were stored on floppy diskettes for later analysis with the Lotus 123 spreadsheet computer program.

5.2.3 EXPERIMENTAL ANIMALS AND SURGERY PROCEDURES

Experimental Animals: Rainbow trout (*Salmo gairdneri*), weighing between 220 and 850 grams were obtained from West Creek Trout Farms of Aldergrove British Columbia. The fish were maintained in outdoor tanks at water temperatures ranging from 5 to 15° C., depending on the time of year. The animals were fed once weekly, *ad libitum*, a diet of commercial trout food. When experimental temperatures differed from holding temperatures, fish were acclimated to the experimental temperature by temperature changes of no more than 0.5 °C. per day. The maximum temperature re-acclimation required during any experimental series was 4° C., (i.e. from 5 °C. to 9° C.).

Surgery Procedures: For experiments requiring the measurement of blood parameters, fish were fitted with dorsal aorta cannula. Surgical implantation of the cannula was accomplished with animals anesthetized in a pH buffered (pH = 7.5) solution of tricaine methane sulphonate, MS-222, at a concentration of 1:10,000. Cannulation was performed on a surgery table where the gills of the fish were

continuously irrigated with an oxygenated, half concentration dose (1:20,000) of the anesthetic solution. The temperature of the irrigation solution was the same as the holding temperature for the fish.

Cannulation was performed using the technique of Sovio *et. al.* (1972), in which a polyethylene tube (PE 50) is guided into the dorsal aorta by way of a sharpened steel wire within the cannula. Using a surgical 20 gage needle, a blind puncture is made centrally in the roof of the mouth between the first and second gill arches. The cannula, with the steel wire protruding 3 mm. from the tip, is guided through the puncture and forced through the wall of the aorta. Once in the aorta the wire is slowly withdrawn from the PE tubing while the tubing is fed into the aorta for a distance of 30 to 70 mm depending on the size of the fish. When in place, the cannula is anchored with sutures to the roof of the mouth at two locations. The cannula is then led through a flanged PE 200 sleeve which had been previously forced through the roof of the mouth just ahead of the nares. The PE 200 sleeve is anchored with cotton thread looped around the sleeve and tightly knotted next to the skin where the sleeve exits from the fish. When not in use for blood sampling or pressure measurements, the end of the cannula was plugged with a straight pin to prevent blood loss.

During recovery from surgery, cannula were flushed daily with Cortland saline (Wolf, 1963) containing 10,000 USP units/L of sodium heparine. Following surgery, animals were allowed a recovery period of at least 24 hours before being subjected to an experimental procedure. Before committing an animal to an experimental series, blood pO_2 was measured. If the arterial pO_2 was not above 90 mmHg., the animal was not used. This was a precaution against failure of the animal to fully recover from surgery and as a screen against animals of poor health.

5.2.4 PHYSIOLOGICAL PARAMETERS: Every effort was made to minimize net blood loss by the animals as a result of sampling procedures. For example, when blood was remove for pO_2 analysis it was returned to the animal following the measurement. Except for hematocrit and pH samples, any blood that was permanently removed from the animal was replaced with an equal volume of Cortland saline. The need to minimize blood removal was considered important in order to obtain accurate hematocrit assays and to minimize any effect of sampling on the intravascular bubble growth process.

Blood Pressure: Blood pressure was monitored by connecting the dorsal aorta cannula to a Statham Model 50-B pressure transducer. The transducer was calibrated with a mercury manometer before and following each experiment. The range of calibration was 0 to 100 mmHg. Before connection to the cannula, the transducer pressure chamber and all connecting tubing were filled with Cortland saline containing 10,000 USP units/L of sodium heparine. Three way valves, located on the pressure and vent ports of the transducer, allowed flushing of the system with heparinized saline to remove any bubbles that formed in the tubing or transducer. Care was taken to locate the transducer strain gage diaphragm at about the same level as the lateral line of the fish. This insured that the recorded pressure was the dorsal aorta pressure without hydrostatic components related to elevation differences. The electrical output of the transducer was connected to the computerized data acquisition system. The digitized pressure data were recorded on floppy diskettes for subsequent analysis with the Lotus 123 spreadsheet program.

Partial Pressure of Oxygen in Arterial Blood: Arterial blood pO_2 was measured with a Radiometer E5046 oxygen electrode connected to a Radiometer PHM 71 signal conditioner. The electrode was contained in a housing thermostated to the experimental water temperature. Two techniques were used to draw blood through the electrode. During experiments involving blood pressure measurements, a three way valve in the dorsal aorta cannula allowed blood to bypass the pressure transducer. This blood was drawn through the pO_2 electrode by way of a peristaltic roller pump. Once the pO_2 measurement was complete, the pump was reversed and blood returned to the animal.

For all other experiments, blood was removed from the animal through the cannula using a 1 ml. syringe. The syringe was then connected to the inlet port of the oxygen electrode system and blood forced through the electrode. The outlet port of the electrode was connected to a long loop of PE 90 tubing that had been previously filled with Cortland saline. Once the measurement was complete, the blood was withdrawn from the electrode and PE 90 tubing loop back into the sampling syringe. The blood was then returned to the animal via the cannula.

Arterial Blood pH Measurements: Blood pH was measured with a Radiometer G279/G2 glass capillary pH electrode and K497 calomel electrode thermostated to the experimental water temperature. The electrical output of the electrode was connecter to a Radiometer PHM 71 signal conditioner and meter. Blood from the cannula was drawn into a 1 ml. syringe as described above for measurement of pO_2. Before blood in the PE 90 loop was returned to the sampling syringe, the syringe was disconnected from the pO_2 electrode momentarily and blood drawn directly from the syringe into the pH electrode sampling loop. The syringe was re-connected to the

pO_2 electrode port while the pH was being measured. Blood in the pH loop, about 0.05 ml., was not returned to the animal.

Arterial Blood Hematocrit: Hematocrit was determined by drawing blood samples into microhematocrit tubes. The samples were taken by sampling directly from the dorsal aorta cannula immediately after blood had been drawn into the pH/pO_2 sampling syringe. The net blood loss associated with the hematocrit measurements is estimated to be 0.05 ml. per measurement.

5.2.5 PHASE I EXPERIMENTS: The first experimental series involved a survey of the dorsal aorta pressure during exposure to supersaturation. The acquisition of data involved sampling data at various rates depending on the response of the animal. Two sampling sequences were employed. A fast rate, 30 samples per second, gave clear definition of the system pressure pulses. From this, a mean blood pressure and pulse pressure amplitude could be determined. This sampling rate also allowed precise definition of heart rate. Due to computer storage and memory limitations during analysis, this level of monitoring could be maintained for no more than 3 minutes. For longer periods of monitoring, a sampling rate of one sample every 10 seconds was used. The lower sampling rate allowed data recording for up to 15 hours.

During these experiments, total gas pressure was varied between 1.1 and 1.3 Atms. while oxygen partial pressure ranged from 100 to 225 mmHg. In addition to measuring blood pressure, blood samples were drawn periodically for dissolved oxygen and hematocrit measurements. For this series of experiments, fish were exposed to supersaturation and monitored individually or in pairs.

In most cases heart rate could be calculated directly from the blood pressure recordings taken at 30 samples per second. In other cases, presumably as a result of bubble formation in the vascular system, pulse pressures were erratic and distorted; thereby making heart rate difficult to determine. In these cases, the Fourier transform capability of the Labtech Notebook program was used to transform the time domain pressure data to frequency domain data (See Champenny, 1971 and Rabiner and Gold, 1975 for a discussion of Fourier transforms and power spectral density analysis). This procedure allowed examination of the pressure traces for the frequency content and principal modes.

5.2.6 PHASE II EXPERIMENTS: The Phase II studies involved several experimental series. Each series had individual objectives and correspondingly different experimental techniques for accomplishing these objectives.

Series 1 through 5, Correlation of Physiological Measurements with GBT Symptoms: The objective of experimental series 1 through 5 was to correlate physiological data with bubble growth thresholds and mortality. During exposure to supersaturated water, blood pO_2, hematocrit and pH were monitored in groups of 12 fish (six cannulated and six un-cannulated). The five series consisted of exposure to water TGP levels of 1.10, 1.12, 1.15, 1.17 and 1.19 Atms., and corresponding pO_2 levels of 170, 175, 183, 195 and 201 mmHg. An additional component of these studies involved evaluation of the severity of GBT symptoms at death in each animal. This evaluation included an assessment of severity for:

1.) Extra corporeal bubble formation in the gill lamella. These were bubbles observed in the water between gill lamella and were clearly not internal to the animal.

2.) Intravascular bubble formation in gill lamella. These bubbles were located in the filamental arteries of the gill lamella and formed within the blood medium.

3.) Sub-dermal bubble formation in the buccal cavity. These bubbles, as well as the two forms listed below, were obvious blisters that forced separation of the epithelium tissue layer from the underlying tissue.

4.) Sub-dermal bubble formation on the opercula.

5.) Sub-dermal bubble formation on the fins.

In order to obtain a relative evaluation of symptom severity, an arbitrary scale ranging from 0 to 3 was used. A value of 0 indicates that the particular symptom was absent. A value of 3 indicates the symptom was of the maximum severity observed. As the results of the experiments are examined, photographic examples will be used to illustrate the severity of various symptoms. Visual examinations were made for external symptoms such as sub-dermal blisters on the body, fins and in the mouth.

The examination of extracorporeal gill bubbles and intravascular gill bubbles was done microscopically. The microscopic studies involved excising 4 to 6 samples of gill tissue from each side of the animal, (8 to 10 samples total per animal). The samples were placed on glass slips that had been cooled to the experimental water temperature. The samples were covered with a cooled glass slip and quickly placed under the microscope for examination. The purpose of cooling the glass slips and covers was to prevent bubble formation due to temperature changes. A Wild dissecting microscope equipped with a Leitz photographic system was used to examine and photograph gill and other tissue samples.

Series 6, Catacholamine Assays: The objective of this experimental series was to determine if fish exposed to supersaturated water exhibited symptoms of stress as indicated by blood catacholamine levels. Catacholamine assays were conducted on cannulated fish in which no other blood measurements were made. Only fish that were 500 grams or more were used for these assays. Since repeated samples were needed and each sample was at least 700 μl., it was anticipated that the use of large fish would minimize the effects of blood sampling on bubble formation. For each blood sample taken, an equivalent volume of Cortland saline was returned to the animal to minimize total blood loss. Blood samples were placed in 1.5 ml. vials and cooled in an ice bath. Within five minutes the samples were centrifuged to separate plasma from red blood cells. A minimum of 200 μl. plasma was siphoned into 1 ml. vials. These samples were immediately frozen with liquid nitrogen and stored at a temperature of -80° C. until analysis. Analysis for adrenaline and nor-adrenaline was performed with a High Precision Liquid Chromatograph (Spectra Physics, Model Sp8700) using techniques described by Woodward (1982) and Primmett *et al.* (1986).

Series 7, Respiration Frequency and Ventilation Volume: Various facets of the Phase I and II experiments indicated that acute hypoxia is a factor in the death of fish exposed to supersaturated water. In this situation, other physiological responses such as respiratory performance would serve as confirming indicators of this conclusion. In order to monitor respiration, three un-cannulated fish were fitted with surgical rubber masks sutured around the mouth and snout as described by Cameron and Davis (1970). Masks were installed under surgery using the anesthetic procedures described earlier. The fish were then installed in van Dam respiration monitoring boxes. The boxes, constructed of clear plastic, are divided into two compartments separated by a plastic partition containing a circular hole and flange system. When a fish was placed in the aft section of the box, the flange system allowed the rubber mask to be clamped such that water could flow from the forward compartment to the aft compartment only by way of the gill ventilation system. The arrangement is shown schematically in Figure 35. With the outflow ports in the two sections of the box at the same level, no differential hydrostatic head exists between the sections. By measuring the rate of outflow from the aft section of the box, the rate of respiratory water flow could be determined. A visual measurement of respiratory frequency permitted calculation of the respiratory ventilation volume.

Series A, B, C and 4; Vascular Bubble Growth Threshold Dependency on Water pO_2: The objective of this series was to confirm that vascular system bubble growth thresholds are dependent on water pO_2. In these experiments, water TGP was held constant at 1.15 Atms., while pO_2 was varied. Series A and B corresponded to a water pO_2 of 100 mmHg. Series C was for a water pO_2 of 125 and Series 4 (the same as Series 4 described above) a water pO_2 of 183. Groups of 6 un-cannulated fish were used at each series and the time to mortality for each group was monitored.

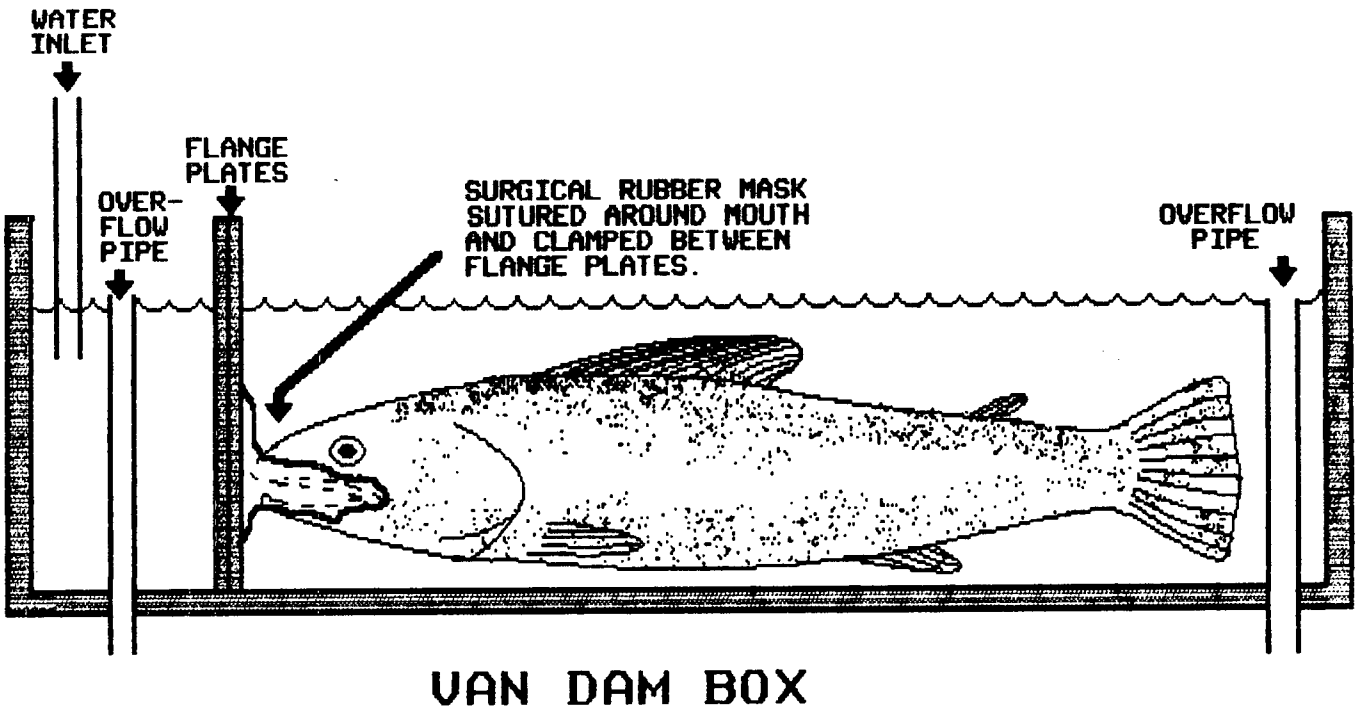


FIGURE 35: Fish in van Dam Respiration Chamber

The intent was to determine if a bubble growth threshold could be detected similar to that shown in the data of Rucker (1975) but at a different water TGP and levels of pO_2. In addition, an examination of gill lamella at death was made to establish if intravascular bubbles were present.

5.3. PHASE I EXPERIMENTAL RESULTS

5.3.1 GENERAL OBSERVATIONS: A total of 27 fish were examined during this series of experiments. Depending on water TGP and the response of the fish, individual experiments lasted as long as 300 hours and as short as 3.5 hours. Although the cannulation and pressure monitoring procedures were easily implemented, obtaining pressure data throughout a complete experiment was often difficult. In general, data could be obtained through the early periods of all experiments. In fact, at water TGP levels below 1.15 Atms., it was frequently possible to obtain pressure recordings for the entire experimental period. However, at TGP levels above 1.20 Atms., cannula become blocked regularly. In many cases it was clear that blood clotting had occurred and was the probable cause of cannula blockage. In most cases, the cannula could not be cleared and pressure measurements were lost. Hence, it was impossible to determine if intravascular bubble growth was modifying blood pressure. The successful recordings, obtained at higher levels of TGP, did show a response indicative of an interaction between bubble growth and system pressure.

Of the successful attempts at monitoring blood pressure, the response to bubble growth was quite variable. However, certain characteristics were often repeated within specific ranges of TGP. For example, at levels of TGP above 1.18 Atms., there was usually an increase in blood pressure, followed by death of the animal. Due to

the unpredictable occurrence and often short duration of this response, it was difficult to get clear definition of blood pressure by way of detailed recordings (i.e. the 30 samples per second sampling rate). Most records of blood pressure were obtained at the lower sampling rate of one sample per 10 seconds and gave only mean pressure levels. Nevertheless, the lower sampling rate provided clear indication of increases in blood pressure, presumably due to bubble growth. This result is in agreement with the predicted response based on the theoretical model for bubble growth in a closed system, Section 3. Also, following death, a residual blood pressure was frequently observed for TGP levels above 1.17 Atms. That is, blood pressure did not drop to zero following death as was the case for TGP levels below 1.17 Atms. The residual blood pressures were low (generally less than 10 mmHg.); however, they were characteristic of the higher levels of TGP.

At TGP levels below 1.15 Atms. only modest increases in blood pressure were observed during the experiments. At the same time, heart rate would usually decrease and only slight increases in hematocrit could be detected. At all levels of TGP above 1.10 Atms., arterial pO_2 declined during each experiment. In addition, microscopic examination of the gills showed considerable numbers of extracorporeal bubbles growing between gill lamella. These bubbles were clearly in the water phase, external to the gill lamella, and of a size that could conceivably block respiratory water flow through the lamella.

At water TGP levels above 1.17 Atms. blood hematocrit rose dramatically during the experiment and reached maximums just before death of the animal. A nearly 85% increase was observed in one animal. As at lower levels of TGP, arterial pO_2 fell during the experiments and reached a minimum just before death of the animal. Also,

as at lower levels of TGP, many extracorporeal bubbles were found between gill lamella. In addition to these bubbles, there was clear evidence of intravascular bubbles in the filamental arteries of the primary lamella. Often, entire primary lamella were blocked with little or no evidence of red blood cells in the arteries of either primary or secondary lamella.

Three fish were tested at a water TGP of 1.1 Atms. and pO_2 levels of 100, 177 and 225 mmHg., for periods ranging from 198 to 250 hours. Although two fish died before the experiment had reached 250 hours of exposure, none of the animals showed any symptoms of GBT. That is, there were no major alterations in blood pressure, arterial pO_2 or hematocrit that could be related to GBT. For this series of experiments, it was concluded that thresholds did not exist for any form of bubble growth below a water TGP of 1.1 Atms.

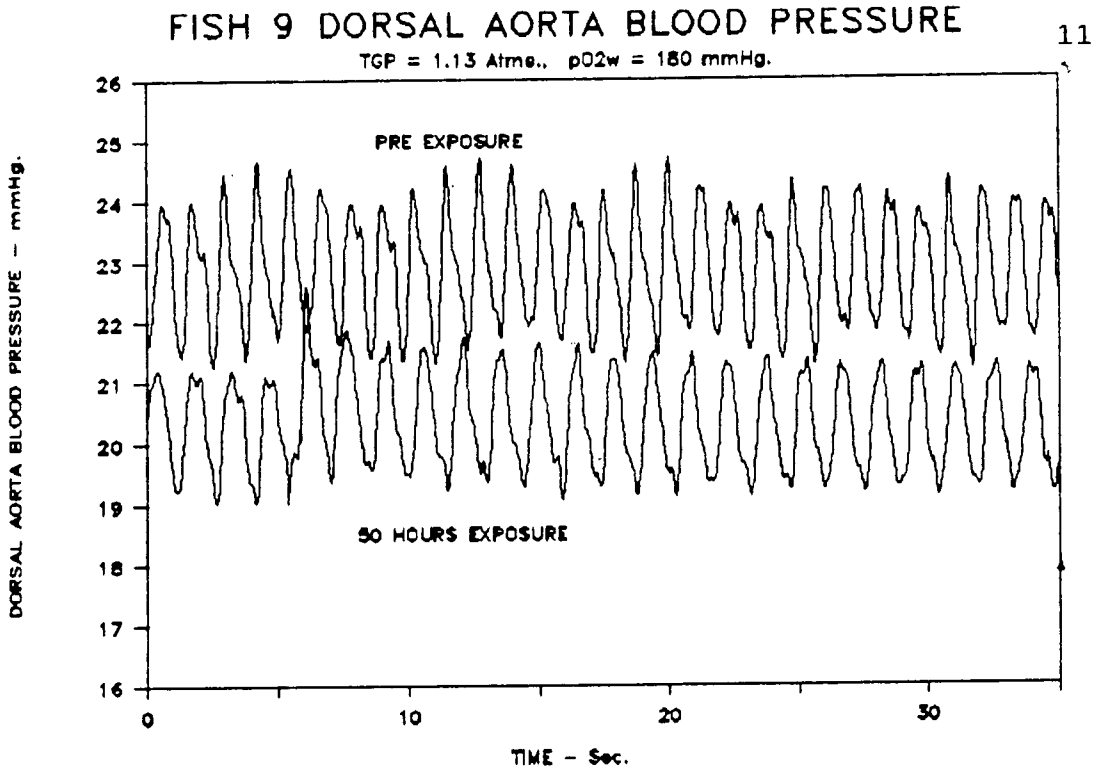
5.3.2 RESPONSE OF INDIVIDUAL FISH: Much of the Phase I experimental effort was exploratory in nature. Also, as mentioned earlier, it was often difficult to get continuous recordings of all measured parameters over the entire duration of an experiment. As a result, many of the data for individual fish were incomplete and cannot be compared directly with data from other fish in the experiments. Thus, tabulated data are not presented. However, to illustrate the variability of the results and at the same time point out important features in the overall response, blood pressure traces of six selected fish will be examined.

Fish No. 9 (TGP = 1.13 Atms., pO_2w = 180 mmHg.): The upper plot of Figure 36 shows the blood pressure recorded before and at 50 hours of exposure for Fish No.

9. Pre-exposure blood pressure was about 23 mmHg. and heart rate is 48 beats per minute, (BPM). After 50 hours of exposure blood pressure dropped to 20 mmHg. and heart rate has decreased slightly to 42 BPM. Later, as shown in the bottom plot, mean blood pressure rose to about 30 mmHg. at 75 hours; however, heart rate had fallen to 36 BPM. The rise in mean blood pressure began just after 50 hours of exposure. Finally, at 125 hours mean blood pressure is 19 mmHg. and heart rate has dropped to 27 BPM. The animal persisted at this level for another 52 hours and then died. During the experiment, blood pO_2 remained near the pre-exposure level of 101 mmHg. until the 125 hour measurement. At that time pO_2 was 75 mmHg. A sample taken a few hours before death showed a pO_2 of 40 mmHg. Hematocrit fraction changed very little during the experiment. An increase was observed from a pre-exposure level of 0.31 to 0.33 near the end of the experiment.

In addition to the vascular system response just described, various external symptoms of GBT were present. At about 125 hours, blisters were well formed on the surface of the opercula and within fin rays. Also, blisters had formed in the lining of the buccal cavity. These blisters became progressively larger with time. Some minor hemorrhaging from external skin lesions was also present. This was observed in all fish that exhibited severe sub-dermal bubble growth, regardless of water TGP. At death there was evidence of extracorporeal bubbles between gill primary and secondary lamella. However, there was no indication of intravascular bubbles or gill damage and blood pressure was zero at death.

This general behavior of blood pressure and other symptoms was observed in four fish; two fish exposed to a TGP level of 1.12 Atms. with pO_2 levels of 178 and 220 mmHg., and two fish at a TGP of 1.14 Atms with pO_2 levels of 150 and 250 mmHg.



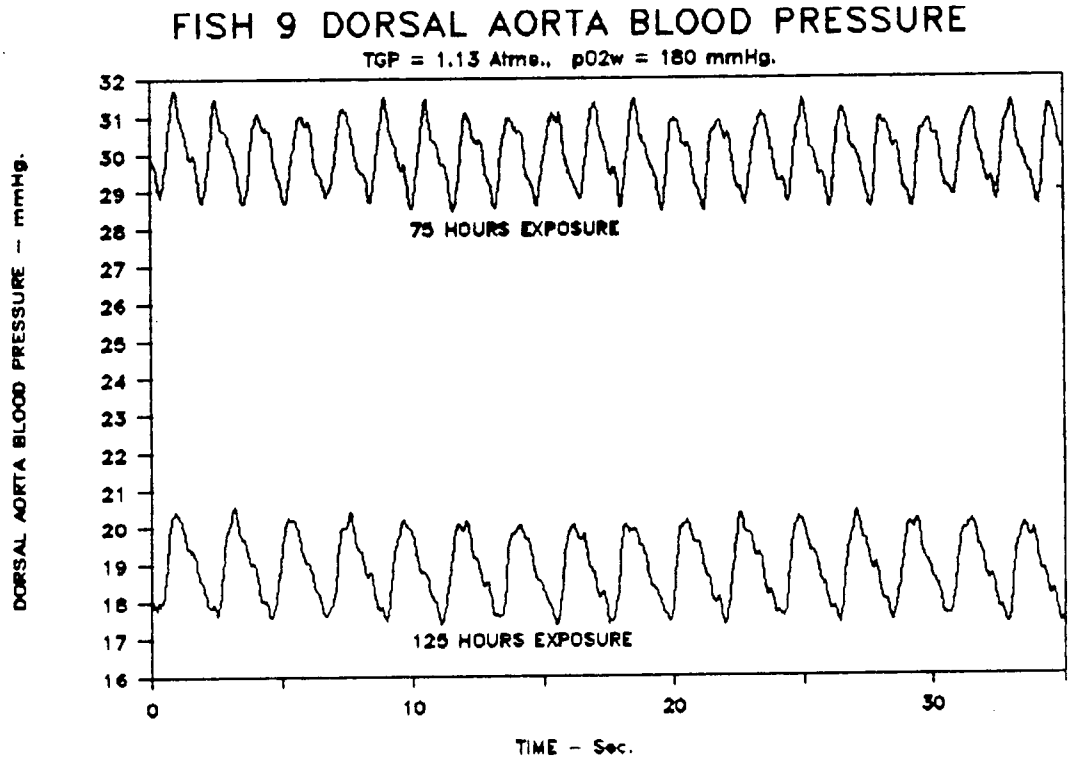
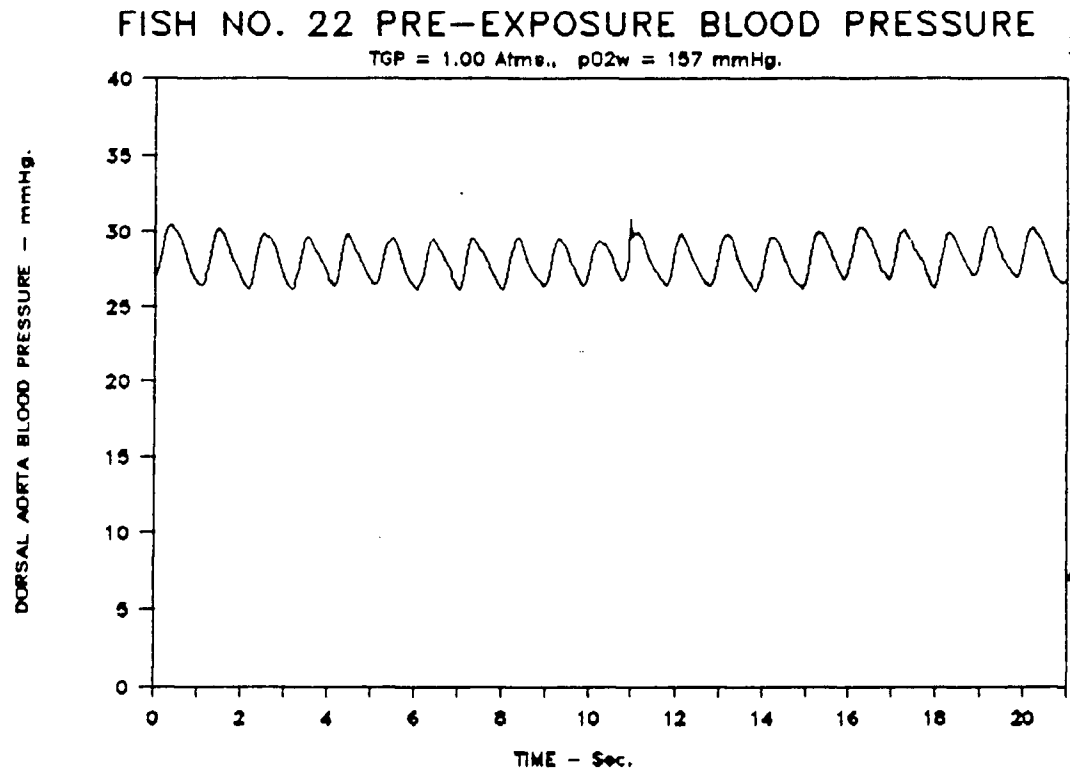


FIGURE 36: Fish 9 Dorsal Aorta Blood Pressure.

Heart rates, hematocrits and arterial pO_2 levels varied between the fish both before and during the experiments. The most noticeable early variation in arterial pO_2 was during first exposure where the pO_2 reflected the variations in water pO_2. In these fish, reductions in heart rate varied both in frequency and the time over which the reduction took place. At the higher levels of pO_2, the fall in heart rate did not take place as early as at the lower levels of pO_2. The other symptoms including extracorporeal bubble growth in the gills and sub-dermal bubble growth on the external surfaces of the fish did not appear to vary significantly with water pO_2.

FISH NO. 22 (TGP = 1.15 Atms., pO_2w = 180 mmHg.): The upper plot of Figure 37 shows blood pressure just before exposure to supersaturated water. Heart rate is uniform at 61 BPM and blood pressure is 28 mmHg. The bottom plot of Figure 37 shows a 2.3 hour record of blood pressure taken after 86 hours of exposure. The monitoring rate is one sample per 10 seconds. It is clear there is little change in mean blood pressure or pulse pressure (the difference between the maximum and minimum pressure). The pulse pressure is about 5 mmHg. Over the next 50 hours mean blood pressure rose to approximately 40 mmHg. and remained there through 200 hours of exposure (upper plot, Figure 38). Beyond 200 hours, blood pressure began to drop accompanied by periodic decreases in heart rate. The lower plot of Figure 38 shows the response at 256 hours, a few hours before death of the fish. Blood pressure was quite variable but on the order of 10 mmHg. Because of the erratic pressure pulse, heart rate could not be determined precisely. A Fourier analysis of this pulse yielded an array of frequencies with 22 and roughly 48 BPM being dominant. Blood pO_2 again declined during the experiment with a drop from 115 mmHg., pre-exposure, to 45 mmHg. at 256 hours. Hematocrit fraction showed no major variation.



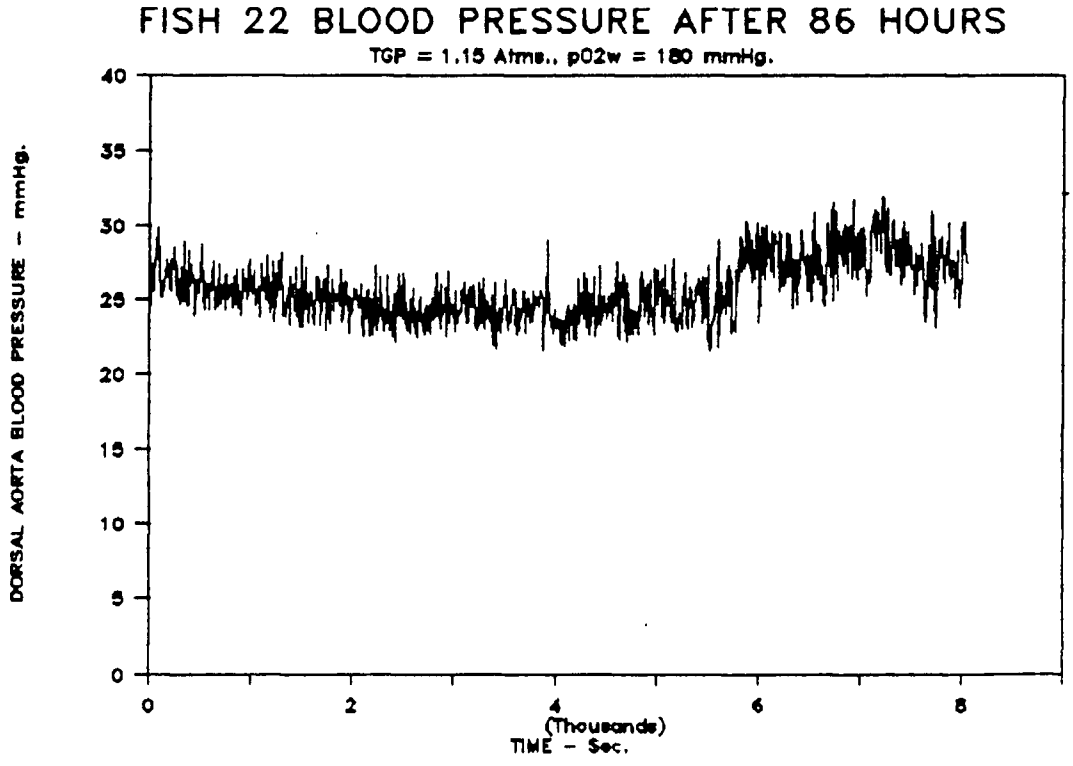
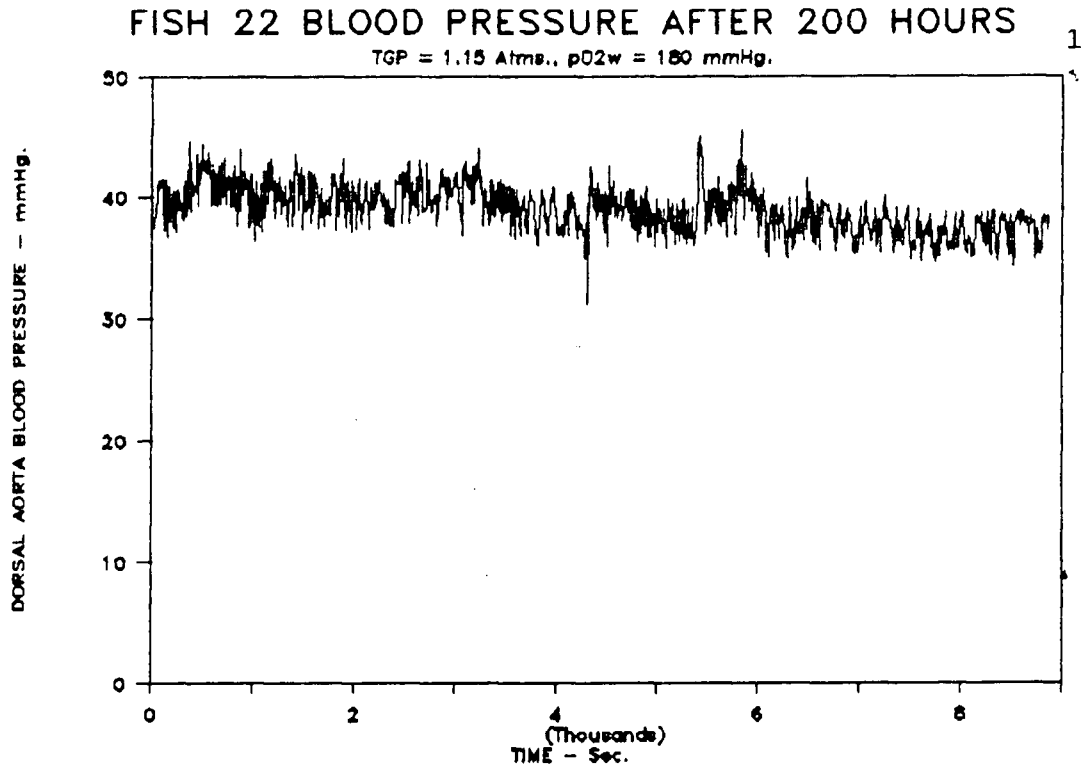


FIGURE 37: Fish 22; Pre-exposure Blood Pressure
Blood Pressure after 86 Hours.



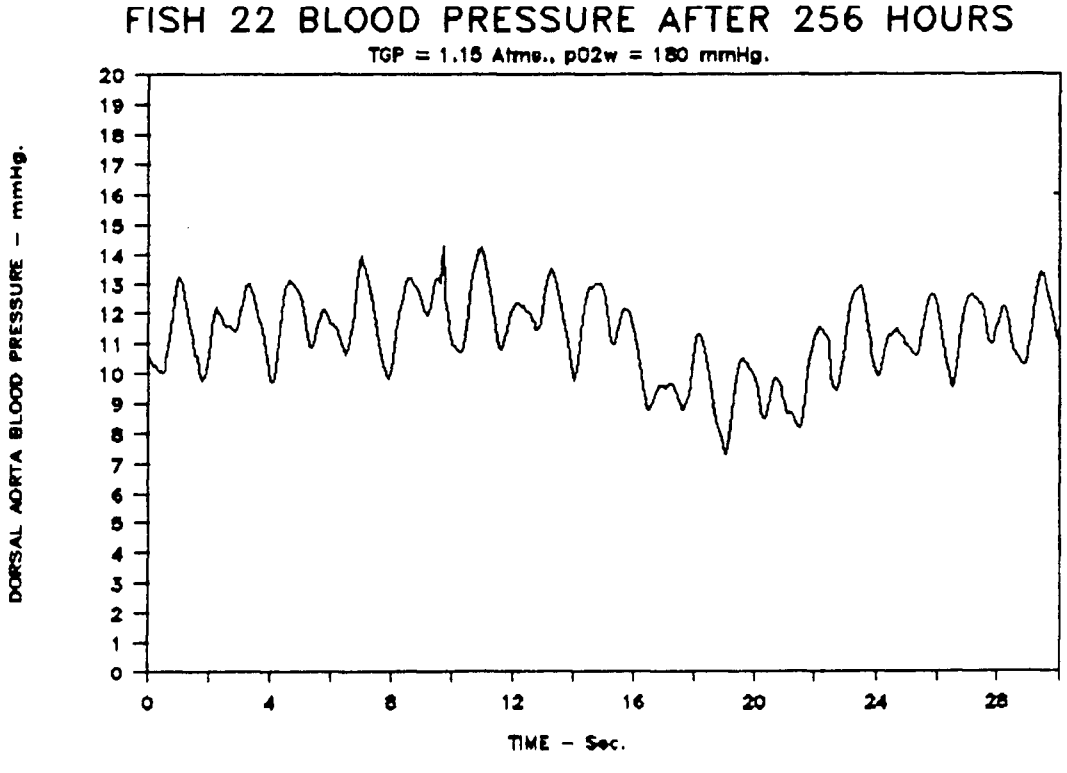


FIGURE 38: Fish 22; Blood Pressure after 200 Hours.
Blood Pressure after 256 Hours.

External symptoms of GBT included blistering of fin rays, opercula flaps and the lining of the mouth. At death, gills again showed evidence of extracorporeal bubbles but no major gill damage. At death, blood pressure returned to zero.

FISH NO. 11 (TGP = 1.18 Atms., pO_2 = 195 mmHg.): Fish 11 showed a blood pressure response that was unique to all the fish tested. Figure 39 shows an array of blood pressure traces taken at periodic intervals. The bottom trace is the pre-exposure blood pressure which has a mean value of approximately 33 mmHg., a pulse pressure of 5 mmHg, and a heart rate of 45 BPM. This response changed very little for 73 hours. At 73 hours of exposure, mean blood pressure rose while pulse pressure declined as shown in the 75 hour trace of Figure 39. Heart rate had decreased to 39 BPM at 75 hours. The rise in mean pressure with decreasing pulse pressure continued through 105 hours. At 105 hours the apparent heart rate was considerably elevated from the pre-exposure heart rate. Fourier analysis of the pressure pulse indicated a dominant frequency in the range of 60 BPM, with higher frequency, lower energy modes also present. The trend in increasing mean blood pressure and declining pulse pressure continued through 115 hours to 117 hours (Figure 40). At 117 hours the mean blood pressure is about 57 mmHg. and pulse pressure is on the order of 2.5 mmHg. Heart rate is extremely difficult to assess from the trace. Fourier analysis of the 115 hour record showed quite variable results depending on the period over which the data were analyzed. Frequencies ranging from 45 BPM to over 90 BPM were present.

The unusual signature of the pressure pulse beyond 75 hours appears to be the result of an interaction between the compliance of growing bubbles and the fluid dynamics of the vascular system.

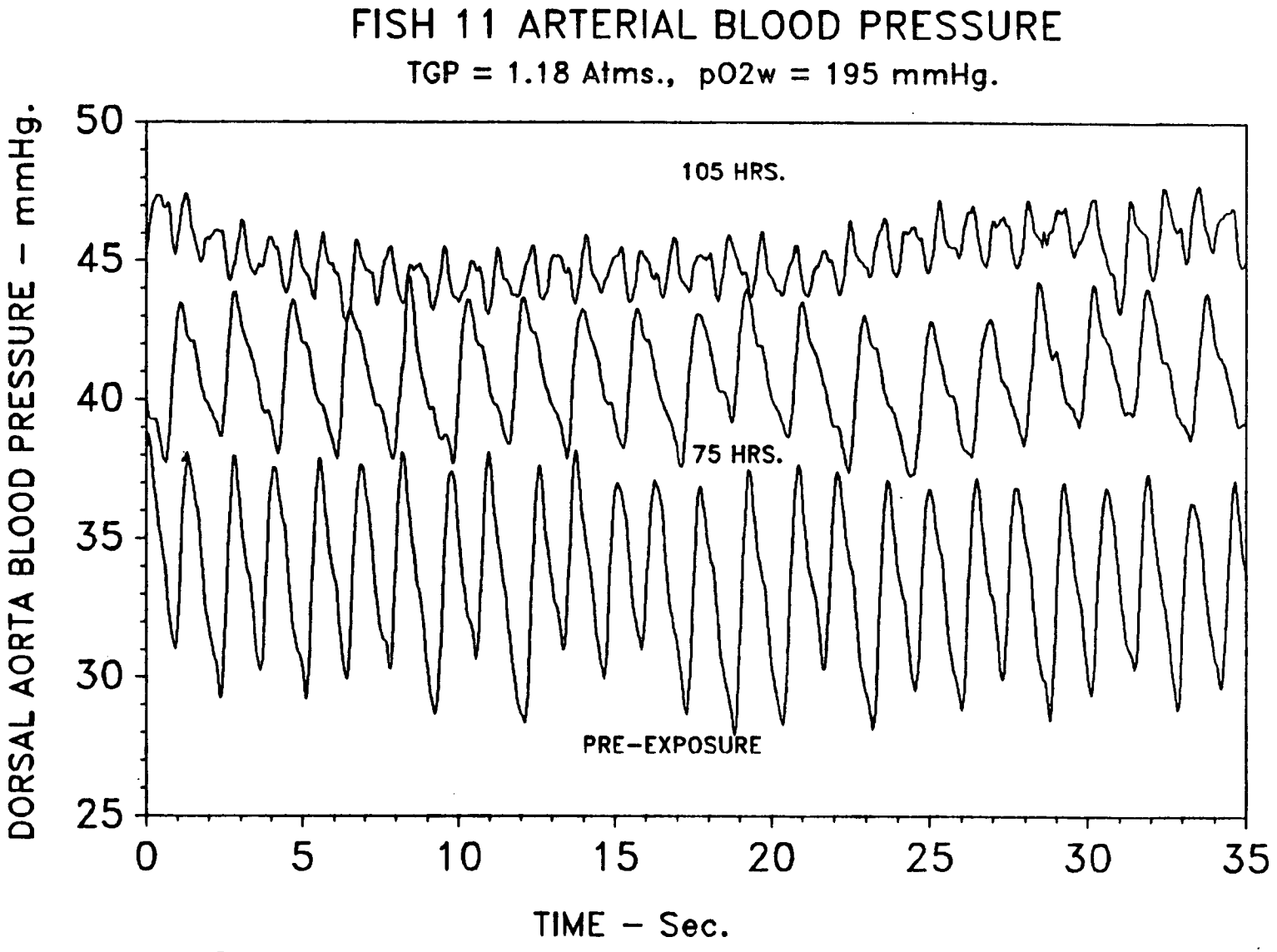


FIGURE 39: Fish 11; Arterial Blood pressure. Pre-exposure, 75 Hours and 105 Hours.

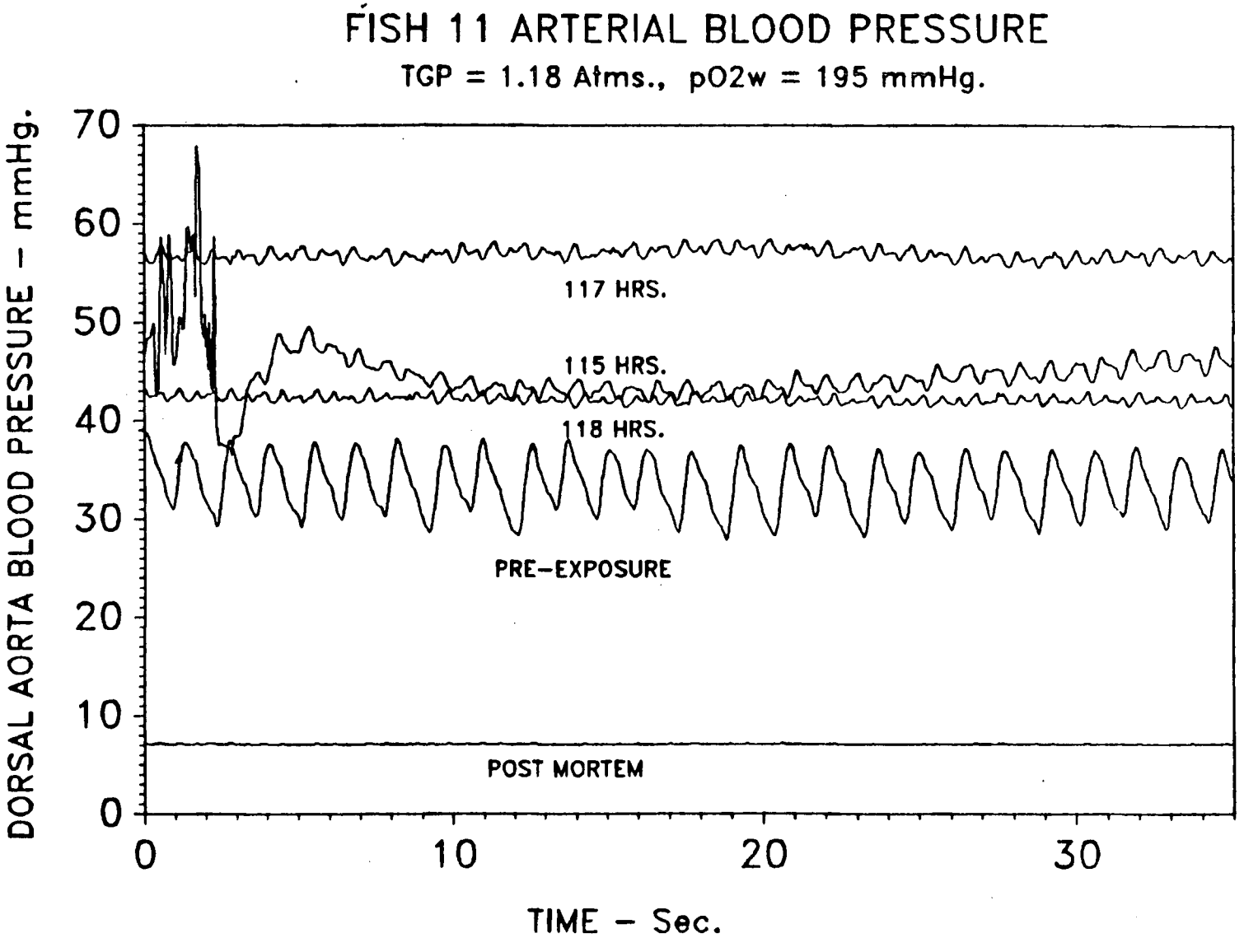
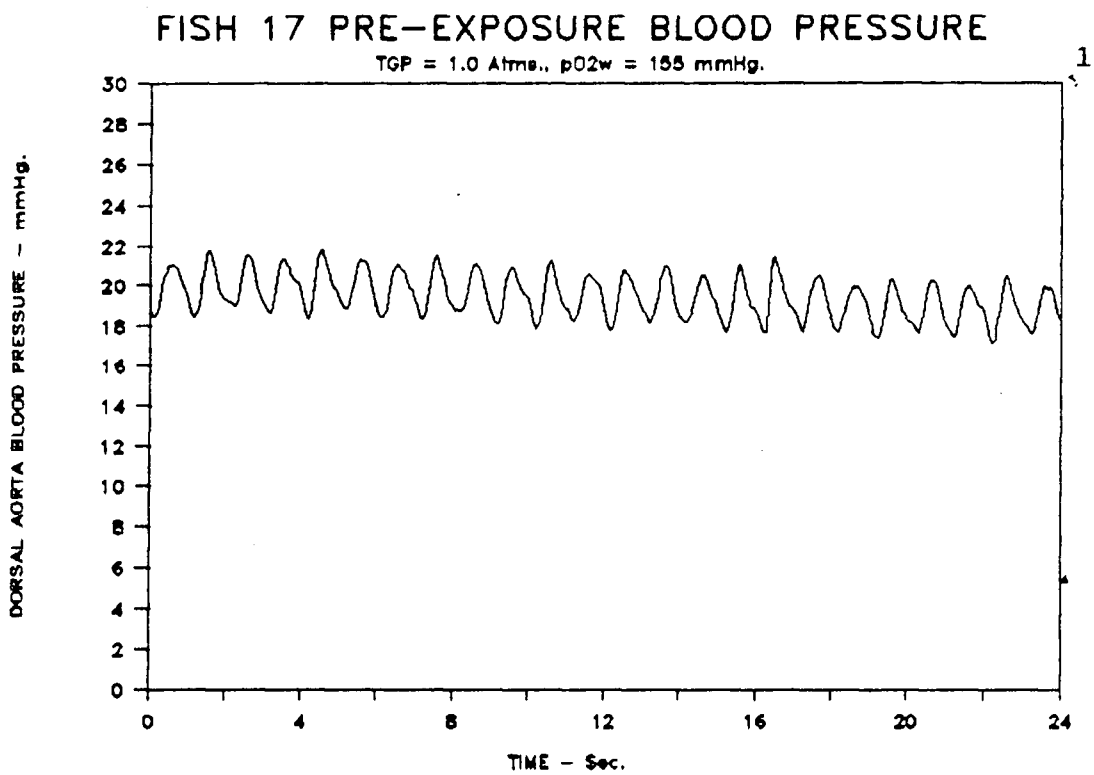


FIGURE 40: Fish 11; Arterial Blood pressure. Pre-exposure, 115 Hours, 117 Hours and 118 Hours.

The general behavior is one of attenuation of lower frequency pressure pulses combined with the development of higher frequency resonant modes.

During this period the fish exhibited periods of violent swimming and thrashing as is evident at the beginning of the 115 hour trace. Beyond 117 hours mean blood pressure dropped rapidly until death just short of 119 hours. In Figure 40 the pre-exposure blood pressure trace is included for comparison. Interestingly, the fish showed a residual blood pressure of 7 mmHg. following death. This residual blood pressure was also observed in other fish at higher levels of TGP. At death, there was evidence of blisters on the opercula and in fin rays as well as some blisters in the mouth of the animal. The extent of blistering at death was not as severe as that seen in fish exposed to lower levels of TGP. Areas of the gill showed evidence of intravascular bubbles and extracorporeal bubbles were present between gill lamella. Arterial pO_2 declined during the experiment from a pre-exposure level of 105 mmHg. to 32 mmHg. at 118 hours. Hematocrit rose from a pre-exposure level of 0.32 to 0.44 at 118 hours.

FISH NO. 17 (TGP = 1.20 Atms., pO_2 = 208 mmHg.): Fish 17 exhibited a pre-exposure mean blood pressure of about 20 mmHg. with a pulse pressure of about 4 mmHg. Heart rate was 60 BPM and fairly uniform (upper trace, Figure 41). Except for a rise in mean pressure to 24 mmHg., there was little change in other characteristics of the pressure trace through 10 hours (lower trace, Figure 41). Some temporary excursions to lower mean blood pressures were seen after 49 hours (upper trace, Figure 42). At 56 hours, mean blood pressure began to rise and over a period of about 58 hours reached a maximum of 55 mmHg. (lower trace, Figure 42).



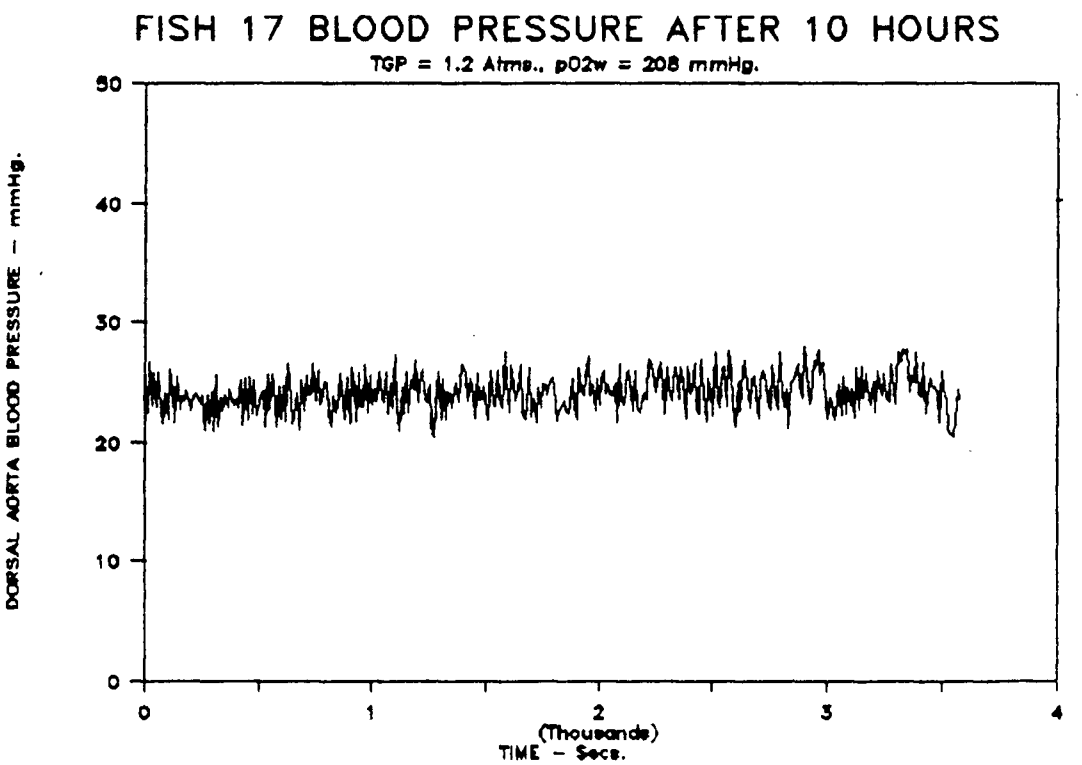
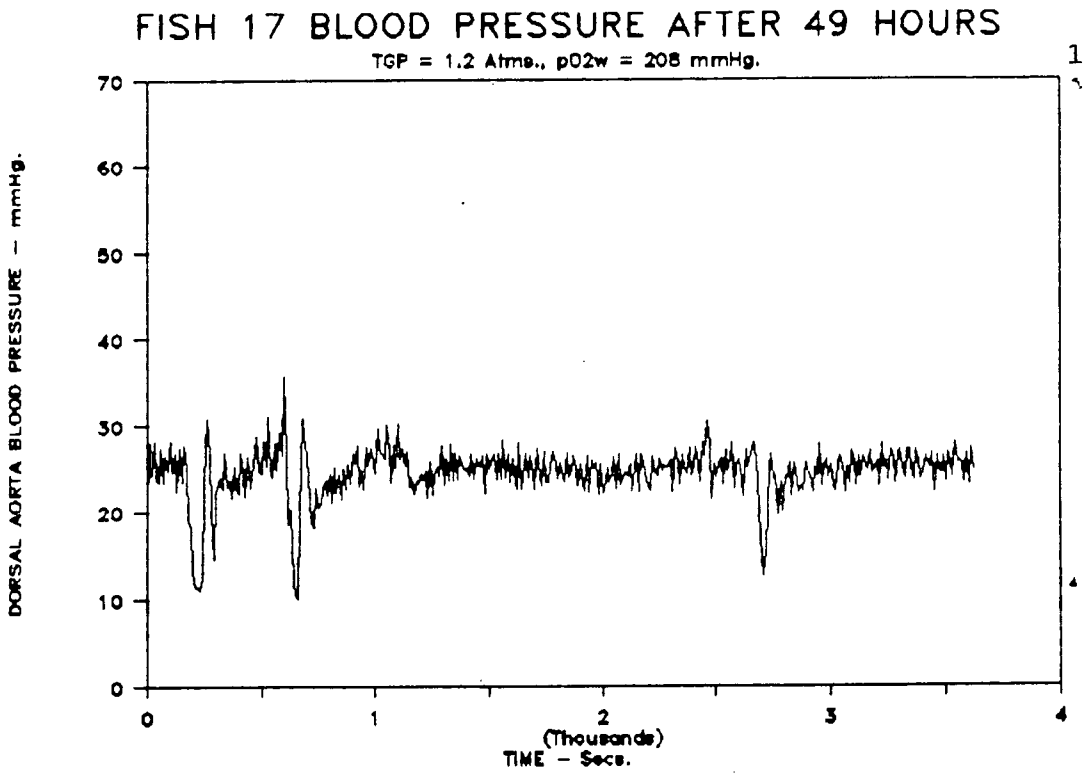


FIGURE 41: Fish 17; Pre-exposure Blood Pressure and at 10 Hours.



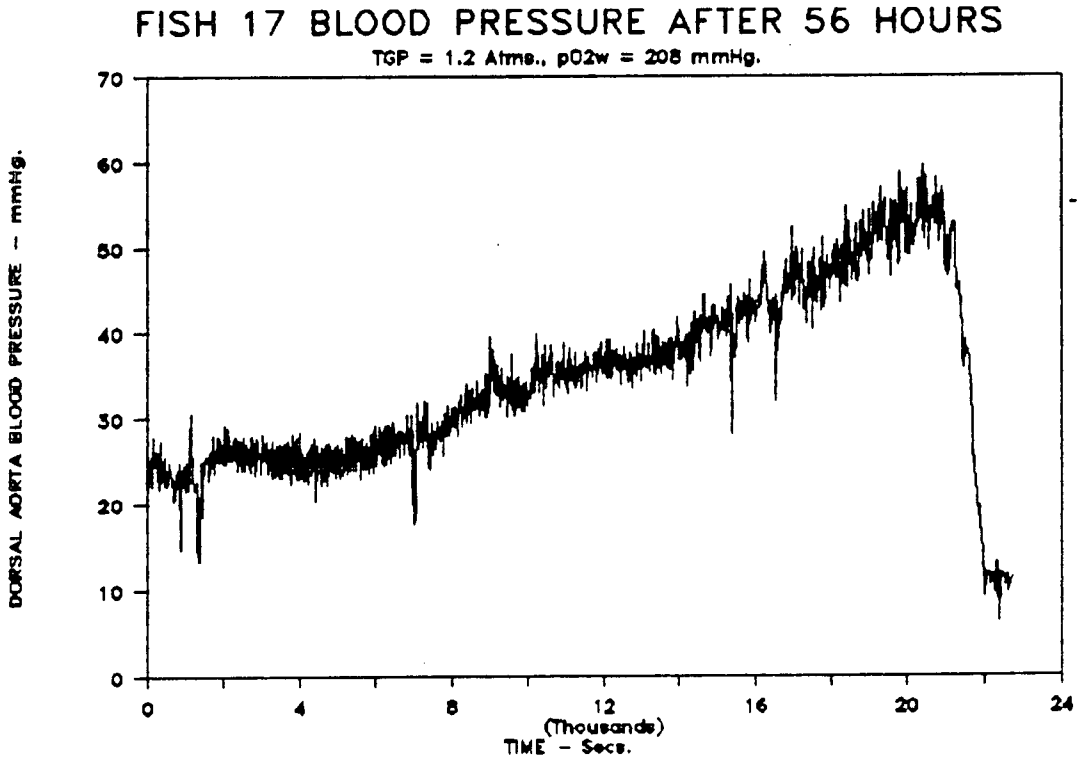


FIGURE 42: Fish 17; Blood Pressure After 49 Hours and 56 Hours.

This was immediately followed by a rapid plunge in blood pressure and death of the fish. A residual blood pressure of about 9 mmHg. existed following death. This fish, like Fish 11, exhibited periods of violent swimming during the rise in blood pressure from 56 hours to death. No external signs of skin blistering were evident in Fish 17. However, there was evidence of intravascular bubbles in the gills and bubbles between gill lamella. Arterial pO_2 dropped from a pre-exposure level of 125 mmHg. to about 60 mmHg. at 56 hours. Hematocrit rose from a pre-exposure level of .22 to .37 at 56 hours.

FISH NO. 6 (TGP = 1.23 Atms., pO_2 = 225 mmHg.): Fish No. 6 exhibited the most violent reaction of all fish tested and experienced death in the shortest time. Shown in the lower left side of Figure 43 is the pre-exposure blood pressure trace. The mean pressure is about 27 mmHg., the pulse pressure around 5 mmHg. and heart rate is uniform at about 60 BPM. Mean blood pressure and heart rate changed little in three hours of exposure. However, at three hours, blood pressure began to rise and, in a fifteen minute period, reached the levels shown in the upper trace of Figure 43. At this time, heart rate was on the order of 40 BPM. The fish became extremely violent as evident in the larger blood pressure fluctuations shown in the figure. Although TGP was immediately reduced to 1.0 Atms., the fish did not recover. Before the animal died three hours later, mean blood pressure declined to about 40 mmHg. The experiment was ended before death of the animal, and it is not known if a residual pressure existed after death. Because of the rapid onset of this reaction, blood pO_2 and hematocrit were not obtained after the pre-exposure levels were measured. There were no signs of external blistering and no effort was made to examine gills for damage or bubble formation.

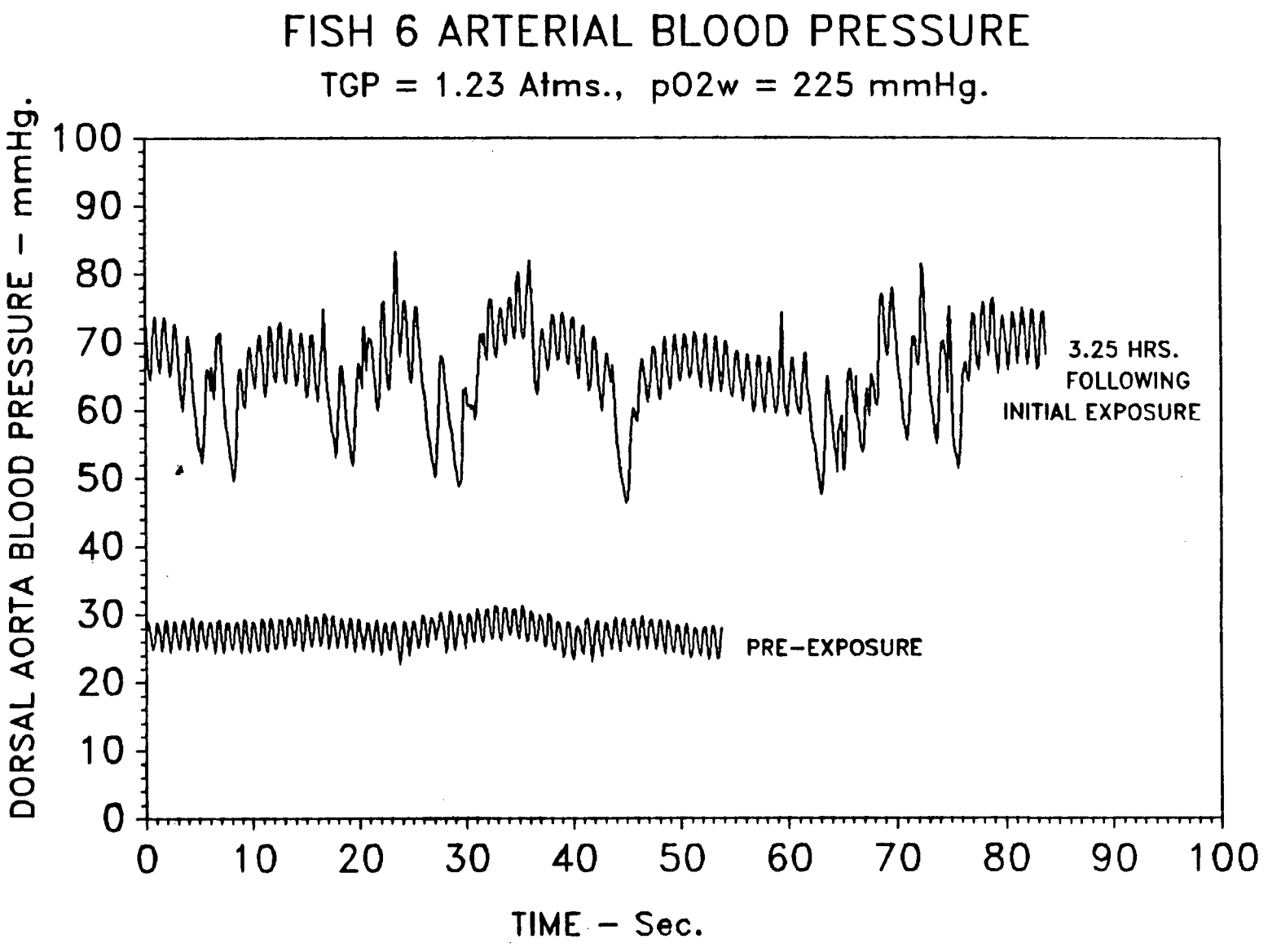
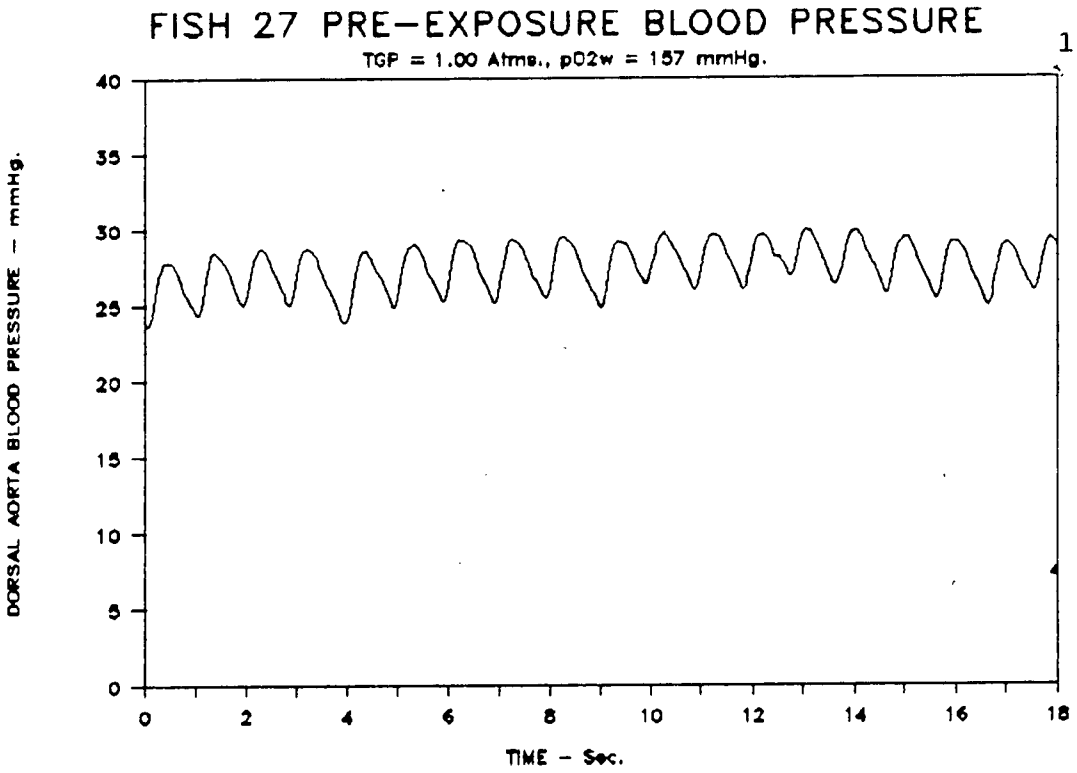


FIGURE 43: Fish 6; Arterial Blood Pressure.

This was the first fish that showed the hoped for response and it was not clear at the time bubbles might be found in gill lamella.

FISH 27 (TGP = 1.25 Atms., pO_2w = 215 mmHg.): The pre-exposure mean blood pressure of Fish No. 27 was about 25 mmHg., pulse pressure was 5 mmHg., and heart rate was 63 BPM (upper plot, Figure 44). Following 7 hours of exposure, mean blood pressure, pulse pressure and heart rate were unchanged. These parameters continued to remain unchanged for another four hours. At that time, mean blood pressure began to rise. Pulse pressure became erratic with large changes in amplitude and heart rate varied considerably (Figure 45). The overall response was similar to Fish No. 6; however, the onset of this response was considerably slower than that of Fish No. 6. Mean blood pressure reached a maximum after about 12.5 hours and then began a slow decline until death of the animal. Following death there was a residual blood pressure of about 3 mmHg. During this experiment, arterial pO_2 declined from a pre-exposure level of 126 mmHg. to about 50 mmHg. at 12 hours. However, the major portion of the decline did not occur until after 7 hours. Hematocrit rose from a pre-exposure level of .21 to a maximum of .39 at 12 hours.

Other fish tested in the TGP range from 1.2 to 1.3 Atms. showed blood pressure behavior similar to those of Fish No's. 6 and 27. However, some fish showed a response similar to Fish No. 17 where there was little elevation of blood pressure during exposure to supersaturation. A repeatable rise in blood pressure could be obtained only at TGP levels above 1.25 Atms. In all cases, there was a decline in arterial pO_2 while hematocrit rose. Gill damage and intravascular bubbles were clearly present; however, there was little evidence of skin blistering. That which did appear was minor and occurred on fish which took the longest time to die.



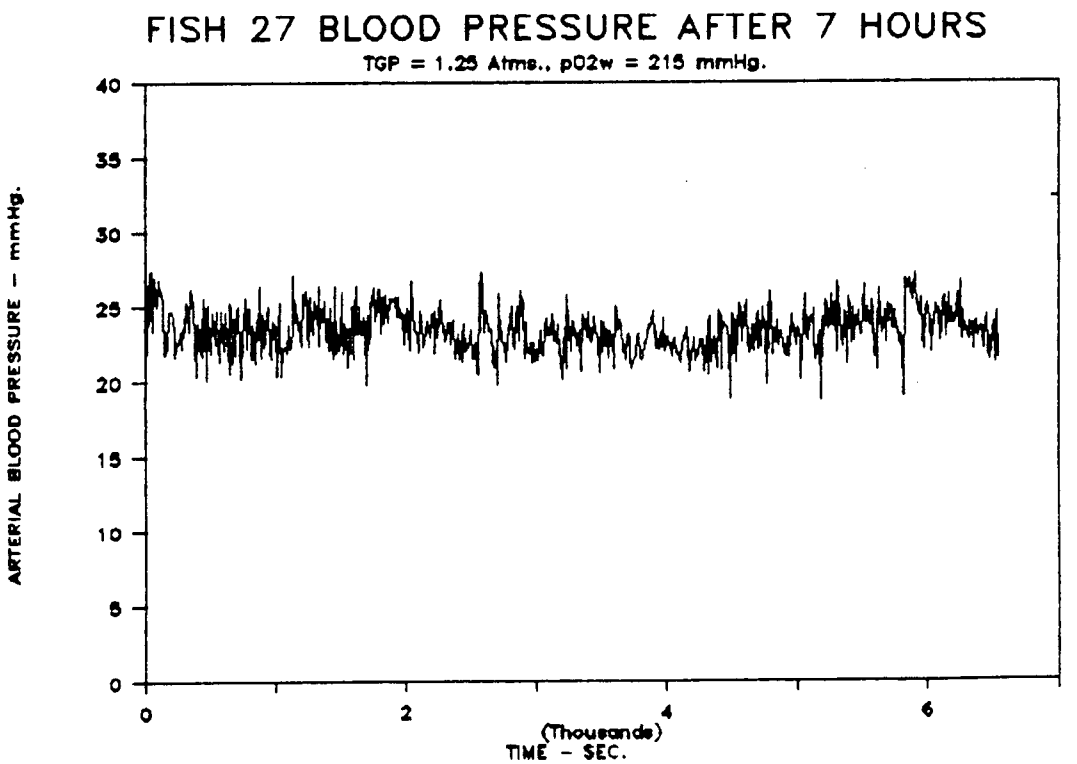


FIGURE 44: Fish 27; Pre-exposure Blood Pressure and After 7 Hours.

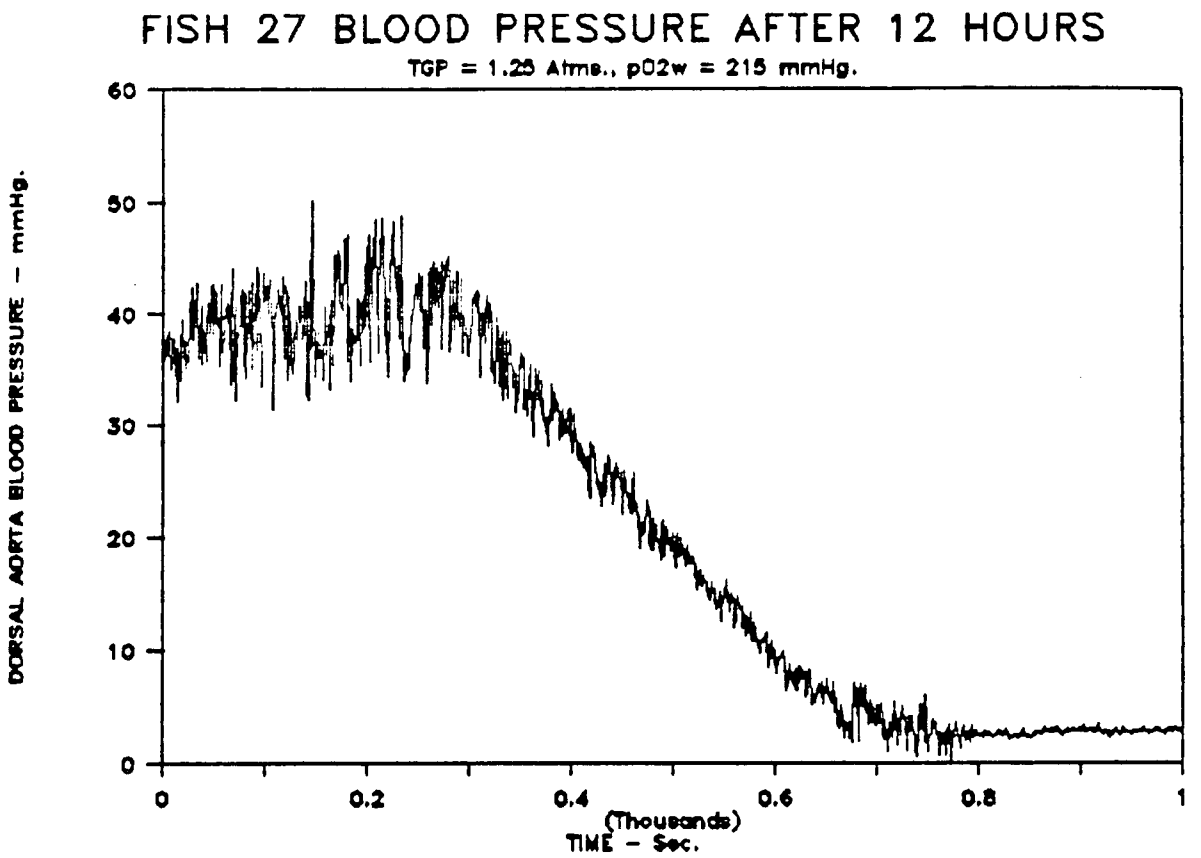


FIGURE 45: Fish 27; Blood Pressure After 12 Hours.

5.4 DISCUSSION OF PHASE I EXPERIMENTS

5.4.1 GENERAL RESPONSE TO SUPERSATURATION: These experiments demonstrate that, above certain levels of TGP, major perturbations in vascular system pressure do occur in fish exposed to supersaturated water. It is presumed that these alterations are the result of bubble growth in the vascular system. Other symptoms such as increased hematocrit, intravascular bubble growth in the gills and residual blood pressure following death support this conclusion.

In general, the cardiovascular response to supersaturation is highly dependent on water TGP. Below a TGP of 1.15 Atms., there is limited response of the vascular system in terms of elevated blood pressure, increased hematocrit, intravascular gill bubbles or residual blood pressure following death. These observations imply that vascular system bubbles are not present below a TGP of 1.15 Atms. Above a TGP of 1.25 Atms., increases in blood pressure and residual blood pressures following death are clearly present. These symptoms, visible evidence of intravascular gill bubbles and elevated hematocrit support the conclusion that bubbles are present in the vascular system. The large increases in hematocrit are interpreted as being the result of bubbles displacing intravascular water. This phenomena will be examined further in the Phase II experimental results.

Between 1.15 and 1.25 Atms. of TGP, response is highly variable; sometimes giving results similar to those at 1.15 Atms. and sometimes similar to those above 1.25 Atms. Because of the large variability in the blood pressure response, it is not a precise indicator of bubble growth thresholds. The one clear result is that, for adult fish, no threshold for bubble growth exists below a TGP of 1.1 Atms.

Other parameters measured during the experiments yield additional information regarding the overall physiological response to supersaturation and GBT. One aspect of these measurements, common to all levels of TGP of 1.12 Atms. and greater, was a decline in arterial pO_2 with time. The implication of this decline is that the transport of oxygen to blood is being blocked by some mechanism, presumably at the gill level. Because many of the symptoms of GBT appear to depend on water TGP levels, they will be examined in more detail based on TGP.

5.4.2 GBT BETWEEN A TGP OF 1.1 AND 1.15 Atms.: In this range of TGP the overall response is that associated with hypoxia. That is, arterial blood pressure, heart rate and pO_2 levels react in a manner typical of fish exposed to hypoxic water. Holeton and Randall (1967a,b) have shown that Rainbow trout exposed to water low in dissolved oxygen exhibit a decline in arterial pO_2. There is an associated rise in blood pressure and a fall in heart rate. There is a large increase in cardiac stroke volume to offset the decline in heart rate and allow maintenance of overall cardiac output. The increased blood pressure is thought to be the result of generalized vasoconstriction while the reduction in heart rate is thought to facilitate the synchronism between respiratory water flow and blood flow through the gills (Randall, 1982a). Except for stroke volume, which was not measured, all other cardiovascular characteristics associated with hypoxia were seen in fish exposed to TGP between 1.12 and 1.15 Atms.

Blockage of Oxygen Transport: As described earlier, several factors indicate that bubble formation in the vascular system does not occur at TGP levels below 1.15 Atms. With the apparent absence of intravascular bubbles, the observed reduction in

arterial pO_2 at lower levels of TGP must be caused by other factors. The most obvious, based on other symptoms, is the blockage of respiratory water flow by blisters in the lining of the mouth, blockage of flow by extracorporeal bubbles that appeared between the gill lamella or both of these effects acting in concert. If either or both of these effects are present, it should be expected that adjustments in ventilation will be made by the fish to offset the reduced transport of oxygen to blood. Holeton and Randall (1967a, b) have shown that, over a wide range of hypoxic conditions, Rainbow trout increased ventilation volume dramatically so that net oxygen uptake does not decrease. If the blockage of respiratory water flow is the cause of declining arterial pO_2, a study of the respiratory function during exposure to supersaturation would help confirm the blockage hypothesis. This question was addressed in the Phase II experiments and will be discussed in Section 5.7.

Bubble Growth Thresholds: Since a response similar to that of hypoxia was observed at a TGP of 1.12 Atms., and no response occurred at 1.10 Atms., there appears to be a GBT threshold between these two levels of TGP. This is also the range of the lower threshold found in the database analysis, Section 4. Based on the earlier discussion of experimental results, this does not appear to be a threshold for bubble growth in the vascular system. However, it was clear the threshold at 1.10 to 1.12 Atms. corresponds to that for sub-dermal bubble growth in the skin and, in particular, the growth of tissue blisters in the buccal cavity. It was not established in these experiments that 1.10 to 1.12 Atms. TGP was also the threshold for the growth of extracorporeal bubbles observed in the water between gill lamella. It was concluded that, in order to determine this threshold, a detailed microscopic examination of the gills is required. This conclusion was also carried forward into the Phase II experimental studies.

Other Observations From the Literature: The external symptoms of GBT observed in these experiments agree with most observations from the literature (Nebeker *et al*, 1976; Rucker, 1975; Dawley and Ebel, 1975; Nebeker and Brett, 1976; Nebeker *et al*., 1980). One contrasting example is the data of Stroud and Nebeker (1976) for Steelhead trout exposed to a TGP of 1.1 Atms. Of the fish sampled at this TGP, the authors report hemorrhages in the gills in 17 out of 29 fish. Yet, microscopic examination of the gill revealed no intravascular bubbles. This is particularly unusual in that, at a TGP of 1.15 Atms., they report only one fish out of 29 had gill hemorrhages and there was still no evidence of intravascular bubbles. At a TGP of 1.20 Atms. they found only one fish out of 19 with gill hemorrhages; however, 18 of these fish showed extensive bubble formation in the gill arteries. The prevalence of hemorrhages at 1.10 Atms. is difficult to explain in terms of intravascular bubbles and implies other mechanisms were responsible for this damage. In the Phase I experiments, there was occasional evidence of gill hemorrhages; however, these were only at the highest TGP levels tested.

In yet other data from the literature there is evidence that, in some environments, the lesions of external tissue caused by sub-dermal bubble growth can act as sites for fungal infections (Weitkamp, 1976). It has been speculated that these infections are a contributing factor in the death of fish (Weitkamp and Katz, 1980). In the Phase I experiments there were no indications of fungal infections in any of the fish, although some skin lesions were present. The presence of fungal infections will no doubt be highly dependent on water quality and general fish health. Therefore, the absence of such infections in these experiments cannot be taken as significant.

Neither in the database analysis of Section 4 nor in other data from the literature is there any evidence that symptoms or mortality are dependent on water pO2 for TGP levels below 1.15 Atms. (Stroud and Nebeker, 1976; Meekin and Turner, 1974; Nebeker et al., 1976; Rucker, 1975; Dawley and Ebel, 1975; Nebeker and Brett, 1976; Nebeker et al., 1980). This is consistent with the results of the Phase I experiments. pO_2 levels varied from 100 to 250 mmHg. in these experiments; however, at any given TGP, there were no detectable differences in symptom severity or time to mortality that could be related to differences in water pO_2.

5.4.3 GBT BETWEEN A TGP OF 1.15 AND 1.25 Atms.: The response of the vascular system in fish exposed to these levels of supersaturation varied considerably; often with contrasting results. Fish No's. 6, 11, 17, and 27 all experienced major increases in arterial blood pressure in this range of TGP. Yet, Fish No. 15 and other fish tested showed no elevated blood pressure in this same TGP range. The only consistent results, above a TGP of 1.18 Atms., were declines in arterial pO_2, elevated hematocrits and the presence of intravascular gill bubbles.

Blockage of Oxygen Transport at the Gills: The decline of arterial pO_2 above a TGP of 1.15 Atms. shows that fish continue to experience reduced oxygen transport to the blood. As suggested above, extracorporeal gill bubbles and blisters in the mouth may be the source of this problem. However, at the higher levels of TGP, blisters of tissue lining the mouth were either not well developed or absent entirely. Presumably, this was because the onset of lethal symptoms was more rapid at higher levels of TGP and buccal cavity blisters did not have time to form before death. Therefore, it is concluded that, at higher levels of TGP, intravascular bubbles, extracorporeal bubbles in the gill lamella or other mechanisms are responsible for the decline in pO_2.

It is not difficult to understand how blockage of respiratory water flow by extracorporeal bubbles would inhibit oxygen transport to the blood. However, the low arterial pO_2 is a more complex problem to explain in terms of blockage of blood flow by intravascular bubbles. First, as pointed out in the theoretical section (Section 3), the gills are not the most likely sites for initial bubble formation. High vascular system pressures in this region would increase the threshold for bubble formation as reflected by the Ps term in Equation 4. The formation of bubbles in the gill vasculature would most likely occur just before death of the animal when blood pressure is falling. Figures 42 and 45 clearly show the drop in blood pressure that precedes death. As shown in the figures, this occurs only during the last few hours or minutes before death; whereas, the decline in arterial pO_2 begins much earlier. The period over which intravascular bubbles existed in the gill vasculature was not examined in the Phase I experiments; however, this problem was addressed in the Phase II studies.

Assuming that bubble formation in the gills does occur early, an explanation for low arterial pO_2 can be developed around reduced blood transit time through the lamella. Because the heart is a form of positive displacement pump, flow continuity requires that blockage of flow in some gill arteries would increase flow velocity in the unblocked arteries. It follows that the time available for oxygen transport is reduced in the unblocked arteries. As a result, blood would not become fully oxygenated. An additional consideration is that under resting conditions, fish do not use all gill lamella for oxygen transport (Booth, 1978; Farrell, 1979 and Farrell, Daxboeck and Randall; 1979). That is, only about 2/3 of the available lamella are perfused with blood (Farrell, Daxboeck and Randall; 1979). The remaining lamella are recruited under conditions of hypoxia or swimming activity. Thus, it appears that fish have the ability

to withstand some blockage of gill blood flow before there is a decline in arterial pO_2. On the other hand, if extracorporeal gill bubbles have already induced hypoxia and full deployment of gill lamella, blockage by intravascular bubbles will have an immediate effect on oxygen transport. It should be remembered that a significant period is involved before a decline in arterial pO_2 is observed. During the Phase I experiments, this time varied from a few hours, at high levels of TGP, to over 100 hours at a TGP level of 1.15 Atms. If bubbles do form early in the gill vasculature, there should be adequate time for their growth to lead to blockage of most of the gill vasculature. In this situation, it is expected that bubbles eventually impact oxygen transport. There remains however, the problem of the high blood pressure in the gills which would suggest other locations for initial bubble formation. The Phase II experiments produced additional insight into intravascular bubble formation in the gills and further discussion of this problem will be postponed until those results are examined.

Other Blockage of the Cardiovascular System: There remains the possibility that bubbles form elsewhere in the cardiovascular system. As shown in the theoretical section, the most likely sites for initial bubble formation are the arterioles just upstream of tissue capillary beds. If this is the case, the explanation of declining arterial pO_2 at high levels of TGP must lie entirely with the extracorporeal gill bubbles. Blockage of tissue and organ capillary beds can have dire consequences in terms of oxygen supply to these areas. However, in the absence of intravascular or extracorporeal bubbles in the gills, it should not effect pO_2 at the dorsal aorta. An extreme case can be envisioned where generalized blockage of all tissue will stop return blood flow to the heart. In this case, oxygen in the dorsal aorta will decline as a result of utilization by red blood cells and vascular system tissue. However, it is

difficult to see how the heart could maintain blood pressure or how the fish could live for the long periods during the gradual decline in arterial pO_2. Thus, in the absence of intravascular gill bubbles, one is left with extracorporeal bubbles blocking respiratory water flow as the most likely explanation of declining arterial pO_2. Again, the Phase II experiments provide further information which help explain the low arterial pO_2.

Systemic Control Functions: The response of the vascular system to intravascular bubble formation appears to involve a conflict of systemic control functions in the animal. As described earlier, hypoxia caused by the blockage of oxygen transport elicits a general peripheral vasoconstriction, reduced heart rate and increased heart stroke volume. On the other hand, the response to rising blood pressure is one of peripheral vasodilation and a reduction of both heart rate and cardiac output (Randall, 1970). In either case, a reduction in heart rate will occur under conditions of hypoxia or elevated blood pressure. This was clearly the case in Fish 11 where heart rate was reduced up until attenuation of the pressure pulse made heart rate ambiguous (Figure 39). In Fish 6 which underwent the most rapid response to bubble formation, heart rate also became progressively slower up until the time of death.

Blood Pressure Response to Bubble Growth: The increase in blood pressure observed in some fish exposed to supersaturation, along with the residual blood pressure at death, is interpreted as being caused by the volume of gas bubbles added to the vascular system. As shown in the theoretical section, the addition of gas to a closed system will cause the pressure to rise. In the cardiovascular systems of fish, as well as in other animals, systemic control of blood pressure will alter this

response somewhat. As pointed out above, the general response to an elevation in blood pressure is a dilation of peripheral circulation and a reduction in both heart rate and cardiac output. In addition, there can be an increase in blood flow to the kidneys which effectively reduces blood volume in other portions of the circulatory system (Eckert, 1983). There may also be reduced secretion of ADH from the pituitary gland which slows filtration rates in the kidney (Eckert, 1983). This also increases water loss which provides still another means of reducing blood volume. The kidney response is observed in many animals; however, it has not been confirmed for fish. Assuming a similar response in fish, it is conceivable that during the initial stages of bubble growth, systemic regulated adjustments to the vascular system could permit bubble growth without major changes in vascular system pressure. It would be anticipated there are limits in the ability of the system to withstand pressure increases due to the addition of bubble gas. The controlling factors would be the limiting balance between the rate of gas addition and the rate of water loss from the system. Certainly, if gas from growing bubbles is being added faster than systemic adjustments can be made, there will be a rise in pressure.

In some environments, there may be other volume adjustments caused by a change in water flux at the gills. In freshwater fish, which are hyper-osmotic relative to their environment, there is a net uptake of water across the gills (Isaia, 1984). This is in the face of a hydrostatic pressure within the animal (i.e. blood pressure) greater than the hydrostatic pressure of the environment. For Rainbow trout, the inward water flux is driven by a colloid osmotic pressure gradient of about 350 mosm. (Holmes and Donaldson, 1969). Because of this high osmotic pressure, there is little chance of a reversal of water flux at the gills of freshwater fish caused by elevated blood pressure. However, an elevated blood pressure will slow this influx somewhat. It will be recalled

that in some experimental fish, there was a doubling or near doubling of blood pressure caused by bubble growth.

For marines fishes, blood plasma is either hypo-osmotic or nearly iso-osmotic relative to sea water. In the first case, there is a net efflux of water across the gills. An elevated blood pressure resulting from intravascular bubbles will cause an increase in this efflux. Fish that are iso-osmotic relative to the environment may begin loosing water across the gills as a result of bubble growth. In either case, increased water loss by the kidneys or across the gills should produce a concentration of red blood cells and other blood components as gas replaces water in the system.

An additional mechanism for water loss is the movement of intravascular water into tissue intercellular spaces. This water flux would be driven by the elevated blood pressure observed at the higher levels of TGP. In this case, opposing colloid osmotic gradients would be considerably less than those involved in the movement of water across the gills. Again, the net effect should be reflected as an increase in hematocrit.

Hematocrit: Hematocrit can also increase as a result of other physiological processes. Under conditions of hypoxia, acid base disturbances have been shown to induce swelling of red blood cells with corresponding increases in hematocrit (Baroin *et al.*, 1984; Nikinmaa, 1982; Nikinmaa and Huestis, 1984; Heming *et al.*, 1987 and Tufts, 1988). It has been suggested that during hypoxia, recruitment of red cells from the spleen and kidney can serve to increase oxygen carrying capacity of the blood (Ostroumova, 1964; Stevens, 1968; Johansen and Hansen, 1967 and Yamamoto, 1980). Such recruitment will also be reflected as an increase in hematocrit. In the Phase I experiments, increases in hematocrit were clearly present

at TGP levels above 1.18 Atms. In fact, hematocrit fractions were found to increase by as much as 85% at the higher levels of TGP. It is unclear as to whether these increases are the result of water displacement, the swelling of red blood cells, red cell recruitment or combinations of all three. However, the answer to this question may lie in observations from the literature.

Weber (1982); Baroin *et al.*, (1984a,b), Nikinmaa and Huestis (1984), Heming *et al.* (1987) and Tufts (1988) have shown that Rainbow trout red blood cells (RBC) swell as part of a defense against intracellular acid-base disturbances. A decline in plasma pH will seriously disrupt the oxygen carrying capacity of Root - Bohr sensitive hemoglobins which are characteristic of Rainbow trout (Riggs, 1979 and Foster and Steen, 1969). This is particularly true for extended exposure to deep hypoxia. That is, a buildup of lactic acid produced by anaerobic metabolism can lead to a metabolic acidosis and reduced blood pH (Holeton and Randall, 1967a,b and Thomas and Hughes, 1982). In pure hypoxia, which is usually accompanied by hyperventilation, there is seldom a problem of CO_2 accumulation in the blood (Holeton and Randall, 1967a,b and Thomas and Hughes, 1982). An accumulation of CO_2 will decrease blood pH (Ferguson & Black, 1941; Cameron, 1971; Cameron and Randall, 1972 and Eddy *et al.*, 1977). This, combined with any metabolic acidosis, will reduce blood oxygen carrying capacity even further. In the case of hypoxia produced by the blockage of respiratory pathways, it can be expected that, as oxygen delivery to the blood is impeded, CO_2 removal from the blood will be restricted. Thus, CO_2 buildup can compound oxygen uptake by reducing blood pH even further. In fish, the problem of blockage can become even more acute since they also use respiratory pathways for removal of metabolites and osmotic regulation. In many ways, the situation is similar to that examined by Holeton and Randall (1967a,b) where Rainbow

trout were subjected to hypoxic water in a closed swimtube respirometer. In this environment, hypoxia was induced through utilization of oxygen by the fish while CO2 and metabolic wastes in the water increased as a result of respiration and other excretory processes. This should have caused a gradual reduction of total CO2 excretion at the gill and compounded the acidosis created by an observed buildup of lactate. Holeton and Randall did observe an increase in blood hematocrit accompanied by red blood cell swelling. However, no evidence could be found for increases in red cell count. Although the authors noted increases in hematocrit caused by exposure to the combined hypoxia/hypercapnia environment, the actual changes are not reported. Also, the time course over which the hypoxia took place was not described. Based on these results it appears that red blood cell swelling will take place in situations involving both metabolic and respiratory acidosis.

In other experiments, involving Rainbow trout exposed to hypoxia, Thomas and Hughes (1982) found hematocrit rose quickly from 0.20 to 0.23 during 24 hours of exposure to a water pO_2 of 60 mmHg. However, in these experiments it was not determine if red blood swelling occurred or if RBC recruitment was present. They did find that blood pH reflected an initial state of respiratory alkalosis which they attributed to hyperventilation. Over a 24 hour period, blood pH gradually returned to normal. In yet other experiments with Rainbow trout exposed to deep hypoxia (water pO_2 of 40 mmHg), Thomas and Hughes (1982) found an initial metabolic acidosis of the blood brought on by lactate buildup. This was followed by a recovery of pH and then a reversal to an alkalotic state. Again a very rapid increase in hematocrit from 0.23 to 0.29 was noted. If it is assumed that the increases in hematocrit observed by Thomas and Hughes are the result of red blood cell swelling, then the maximum change in hematocrit produced by this effect was a 27% increase. Even if a

component of this was due to RBC recruitment, the maximum change would still be 27%. Fish in the Phase I experiments exposed to high levels of TGP showed increases in hematocrit of as much as 85%. Thus, it appears that a significant portion of the observed hematocrit increase is due to other causes such as the displacement of intravascular water in the vascular system by bubble gas.

5.5 PHASE I CONCLUSIONS:

The Phase I experimental studies produced an improved understanding of the physiological response in fish exposed to supersaturated water. At water TGP above 1.1 Atms., the dominant response is one of hypoxia. That is, there is clear evidence that oxygen transport to the blood is being impeded somewhere in the transport pathway. The indications are that between a TGP of 1.1 and 1.15 Atms., the blockage is due to extracorporeal bubbles forming between gill lamella, the formation of blisters in the buccal cavity or a combination of these two. Above a TGP of 1.15, where buccal cavity blisters are less frequent or non existent, intravascular bubbles within the gill lamella are common. These bubbles along with the extracorporeal gill bubbles may be the blockage mechanism at the higher levels of TGP.

These experiments demonstrate that a TGP threshold exists between 1.10 and 1.12 Atms. for the growth of sub-dermal bubbles on the external body surfaces and lining of the mouth. This corresponds to the lower threshold for mortality derived from the database analysis. However, it is not known that buccal cavity blisters are the cause of mortality. This is also the TGP range of thresholds for extracorporeal gill bubble growth. However, this threshold could not be established from the Phase I experimental data. Based on the presence of intravascular bubbles in the gills,

elevated hematocrit and residual blood pressures following death, it appears that a threshold for intravascular bubble growth exists in the TGP range of 1.15 to 1.25 Atms.

Above a TGP of 1.25 Atms., there is clear evidence that blood pressure reflects the presence of growing bubbles in the vascular system. However, between a TGP of 1.15 and 1.25 Atms., the response of blood pressure before death was highly variable. As a result, it was a poor indicator of bubble growth thresholds for the vascular system. The most reliable indicators of intravascular bubble growth were hematocrit and visual evidence of intravascular gill bubbles. Microscopic examination of gill lamella gave the clearest confirmation of the presence of both intravascular and extracorporeal gill bubbles.

From the Phase I studies it was clear that additional experiments were required before intravascular and extracorporeal bubble growth thresholds can be defined. In addition, there remained the task of correlating these thresholds with mortality and the thresholds indicated in the literature database analysis.

5.6 PHASE II EXPERIMENTAL RESULTS:

The Phase II experiments expanded on the Phase I results and provided clearer indications of the various bubble growth thresholds associated with GBT. In addition, the experiments focused on specific physiological responses in fish as a means of defining the relationship between bubble growth and mortality. The results of the Phase II experiments will be presented in the order of the individual experimental series.

5.6.1 SERIES 1 THROUGH 5 EXPERIMENTS: The Series 1 through 5 experiments involved exposing groups of 12 fish (6 cannulated and 6 un-cannulated) to various levels of water TGP and pO_2. External symptoms, internal and external conditions of the gills and blood parameters were surveyed during the experiments. The results are summarized in a series of figures which will be examined next. The individual series will be described in the order of increasing water TGP. Table II lists the abbreviations used in the various bar charts that will be presented. In the results, Fish No's 1 through 6 always correspond to the cannulated fish while Fish No's 7 through 12 are the un-cannulated fish.

TABLE II: ABBREVIATIONS FOR SEVERITY OF SYMPTOMS CHARTS

LAMELLA - Extracorporeal bubbles between gill lamella.

VASCULAR - Intravascular bubbles in gill lamella.

OPERCULA - Sub-dermal bubbles on opercular flaps.

FINS - Sub-dermal bubbles in fin rays.

BUCCAL - Sub-dermal bubbles in the lining of the buccal cavity.

In general, it was found that cannulated fish did not live as long as un-cannulated fish exposed to supersaturated water. Figure 46 summarizes these findings in terms of time to mortality versus water TGP for the two groups. It will be noted that the times to mortality in the figure do not vary greatly in the TGP range of 1.10 through 1.17 Atms. However, at a TGP of 1.19 Atms., time to mortality drops to roughly one half or one third of the time required at lower TGP. Both cannulated and un-cannulated fish showed similar signs of GBT throughout the experiments. However, it was impossible to determine if decreased survival of the cannulated fish was due to the stress of cannulation, increased susceptibility to bubble growth caused by handling and surgery, or the presence of the cannula.

Series 1 (Water TGP = 1.10 Atms., pO_2w = 170 mmHg.): In this series of experiments two fish showed external signs of GBT. Of these, one fish (Fish 4) died early and exhibited a decline in arterial pO_2 which suggested a blockage of oxygen transport as was observed in the Phase I experiments. Furthermore, this fish was the only one to show a significant number of extracorporeal bubbles between gill lamella. Other blood parameters were at normal levels. Although there was a decline in arterial pO_2 at the last measurement, it was not low enough to suggest acute hypoxia. In Figure 47 the variations in arterial pO_2 for the six cannulated fish are shown as a function of time of exposure. Except for Fish 4, only small changes occurred during the experiment. Although three fish died at various times during the experiment, Fish 4 was the only one to show signs of GBT that would be considered lethal. Fish 8 also showed bubble growth between gill lamella. However, there was no evidence of hypoxia and the animal lived without signs of stress for the full 362 hours of the experiment.

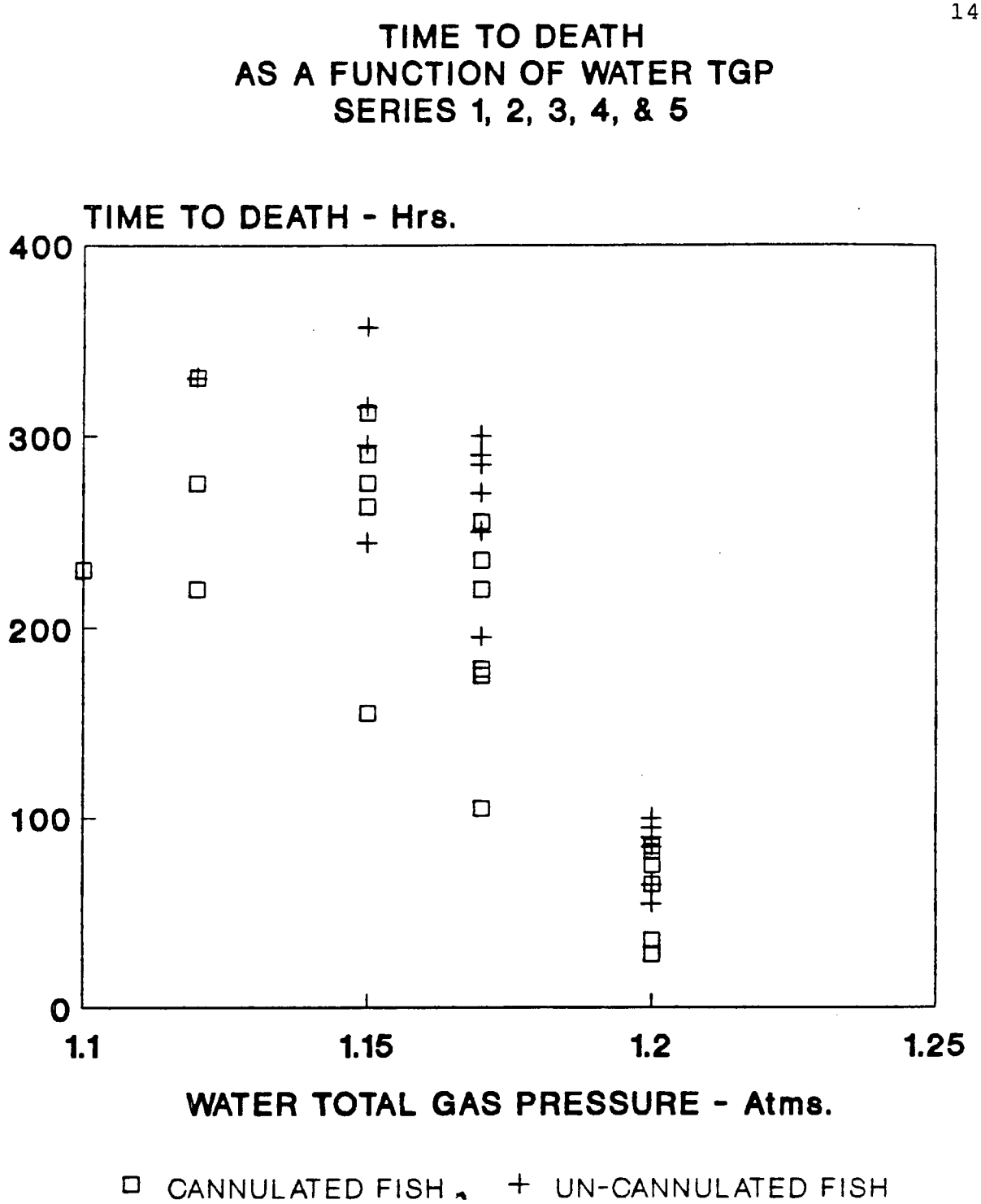


FIGURE 46: Time to Death as a Function of Water TGP.

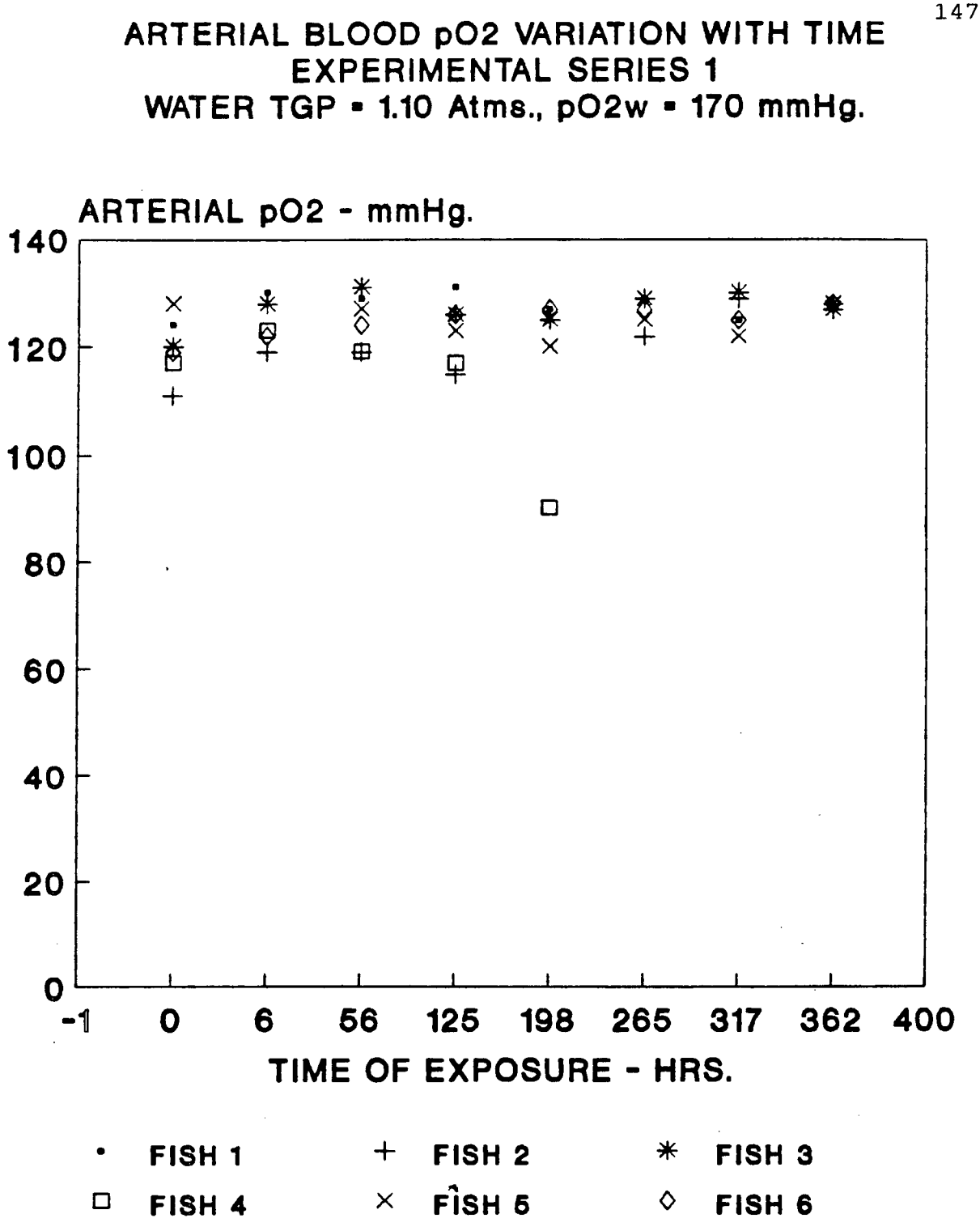
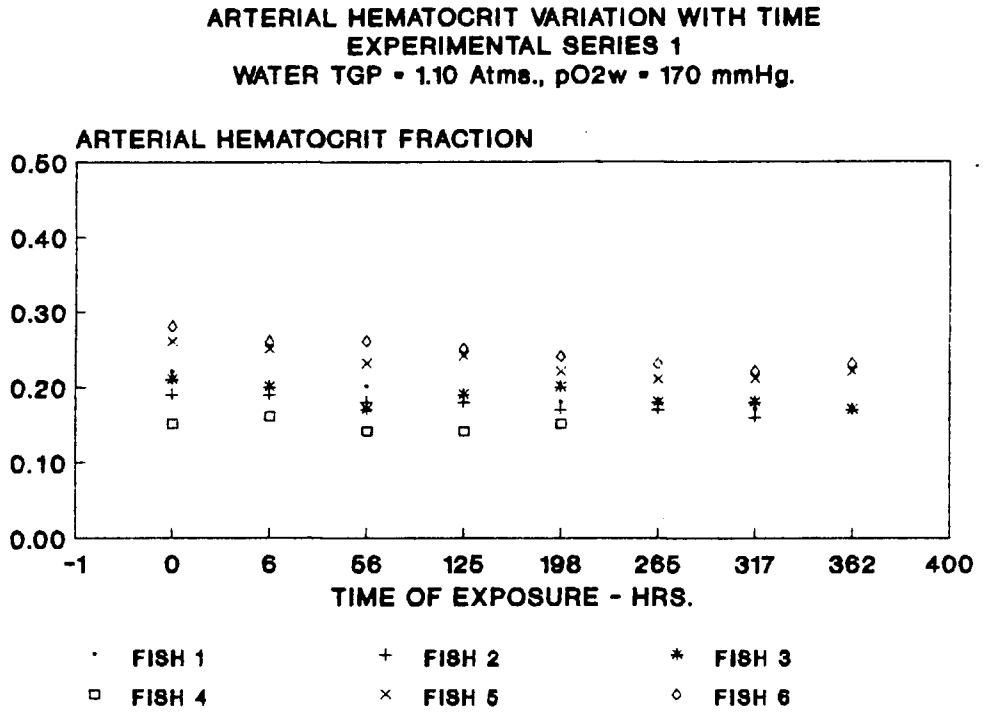


FIGURE 47: Arterial pO_2 Variation With Time, Experimental Series 1.

In Figure 48, the variations in arterial blood pH and hematocrit are shown for the cannulated fish as a function of exposure time. It is clear that little change in either occurred over the 362 hours of exposure. Figure 49 shows the level of severity of various symptoms at death in the 12 animals tested. The scale for these bar charts, and all others to follow, corresponds to the scaling system described in the Experimental Methods Section above. In this experimental series most animals were alive at the end of the experiment and were killed to make the necessary measurements. This procedure was followed in each of the series when fish remained alive through the end of the experiment.

Series 3 (Water TGP = 1.12 Atms., pO_2w = 175 mmHg.): The results of these experiments showed several fish with declines in arterial pO_2 that suggest a blockage of oxygen transport. In Figure 50, arterial pO_2 is shown for the six cannulated fish as a function of exposure time. Except for Fish 1 and Fish 6, major reductions in arterial pO_2 occurred. Furthermore, GBT related mortality occurred in all but three of the cannulated fish and in one of the un-cannulated fish (Figure 46).

In addition to declines in arterial pO_2, there were corresponding declines in arterial blood pH as shown in Figure 51. The decreases in pH correlate with the pO_2 declines of Figure 50. However, arterial hematocrit showed little change in any of the cannulated fish. This observation is consistent with the results of the Phase I experiments at low levels of TGP. In Figure 52, the severity of symptoms at death are shown for the six cannulated fish in the upper plot, and for the six un-cannulated fish in the lower plot. It is clear that in all cases there were external blisters on the opercula and fins, but the degree varied considerably from fish to fish.



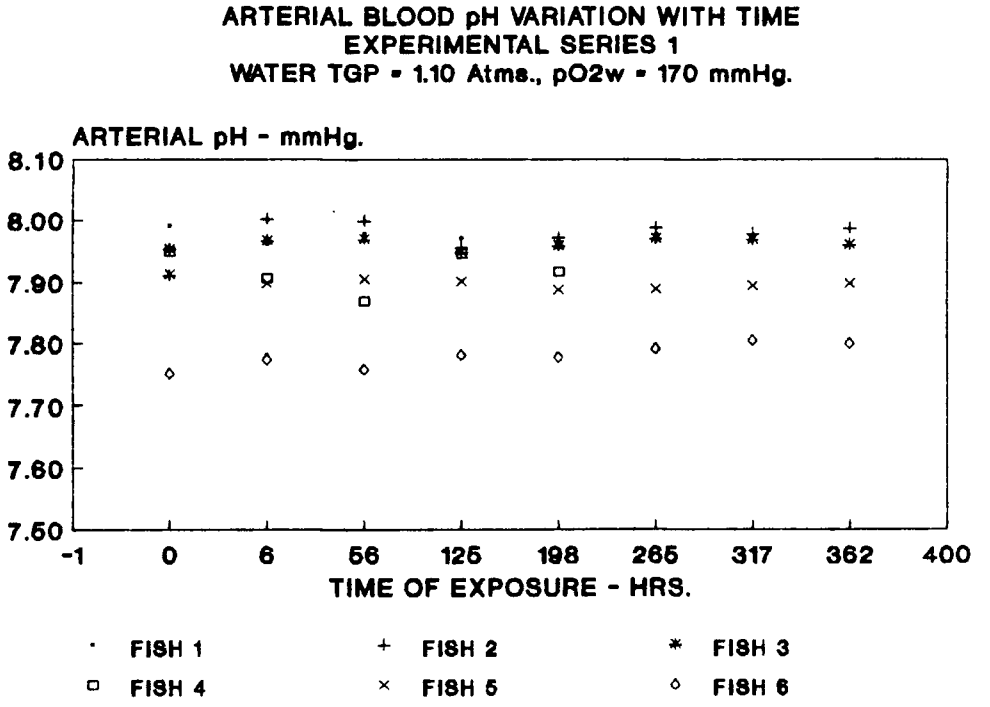


FIGURE 48: Arterial Hematocrit and pH Variation with Time, Series 1.

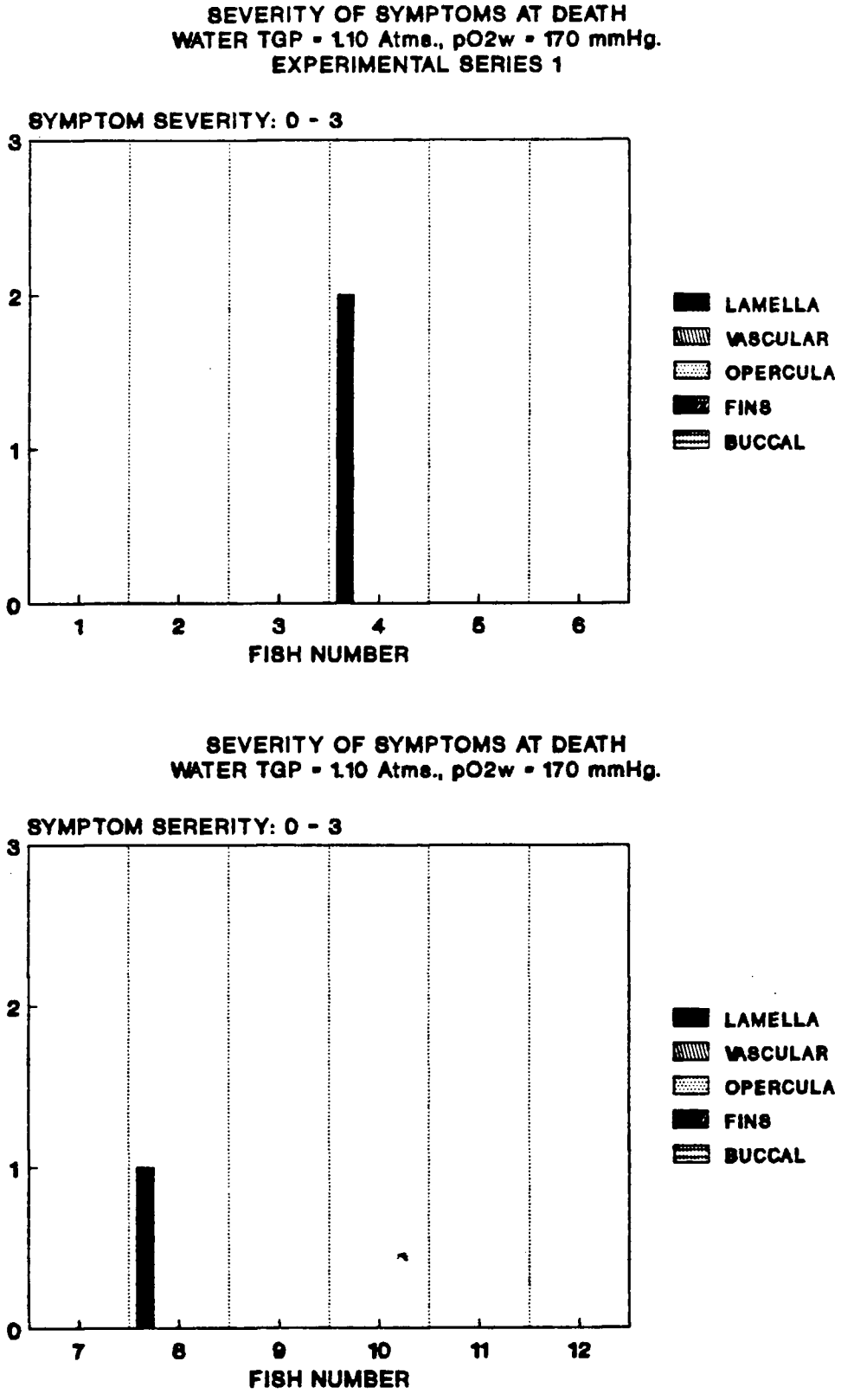


FIGURE 49: Severity of Symptoms at Death, Experimental Series 1

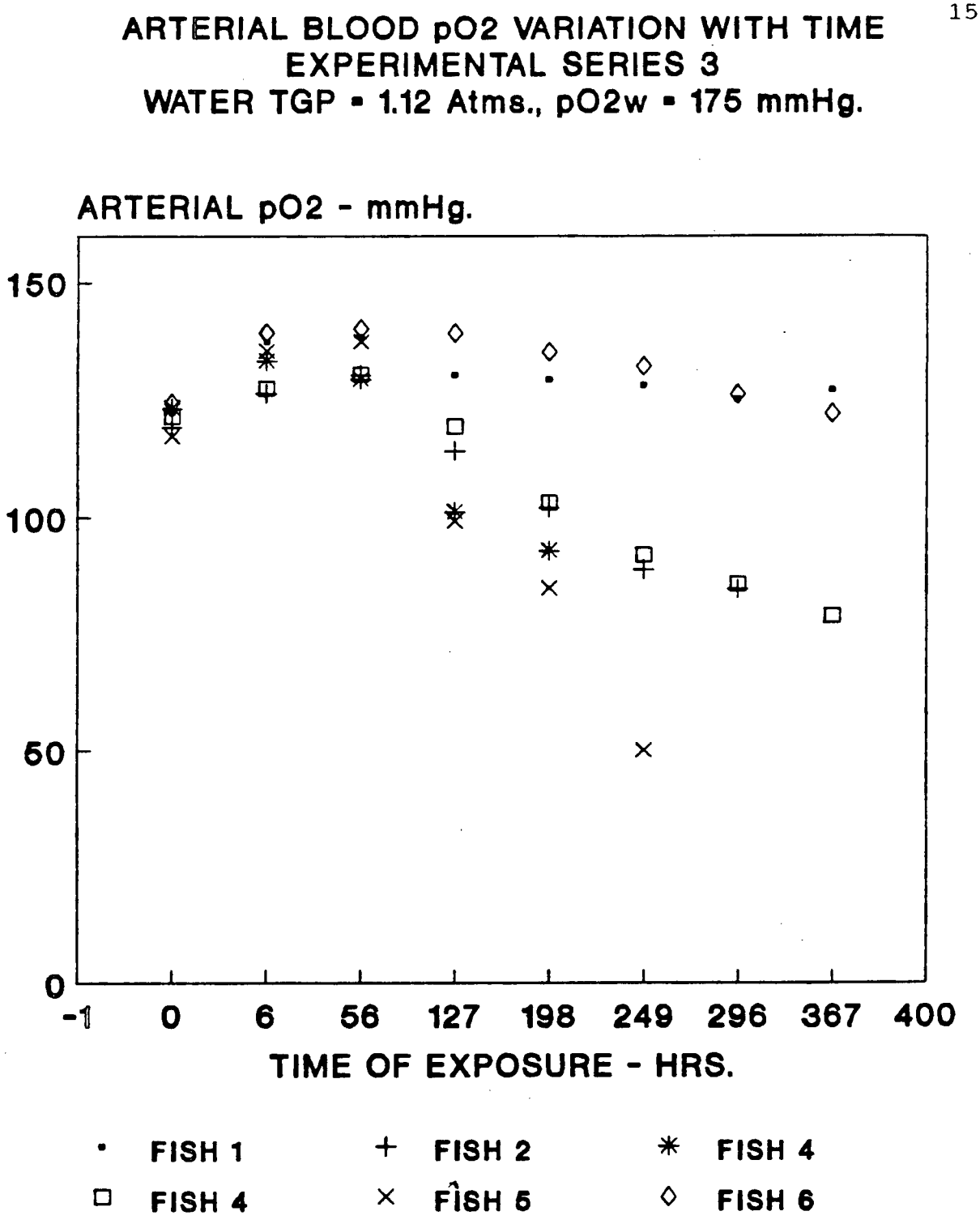
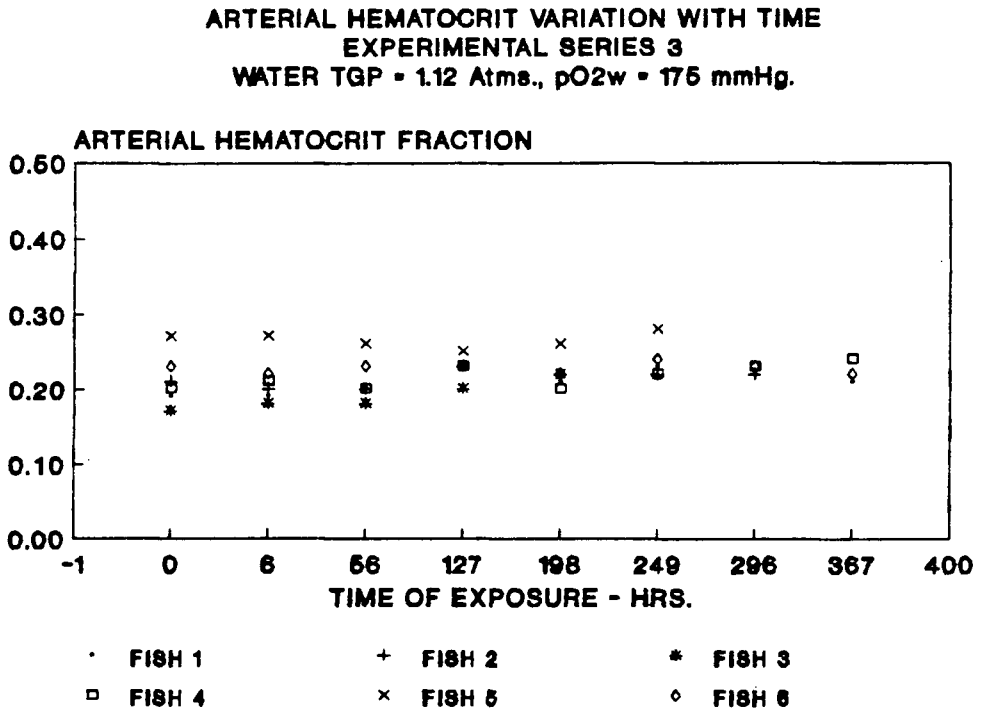


FIGURE 50: Arterial Blood pO_2 Variation with Time, Experimental Series 3.



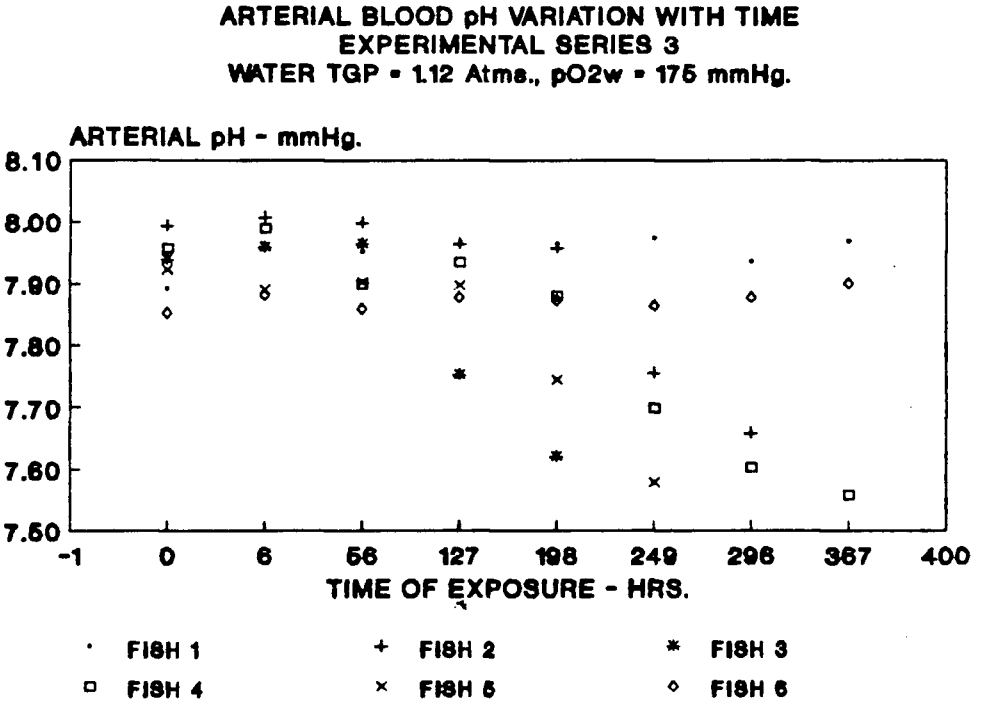


FIGURE 51: Arterial Hematocrit and pH Variation with Time, Series 3.

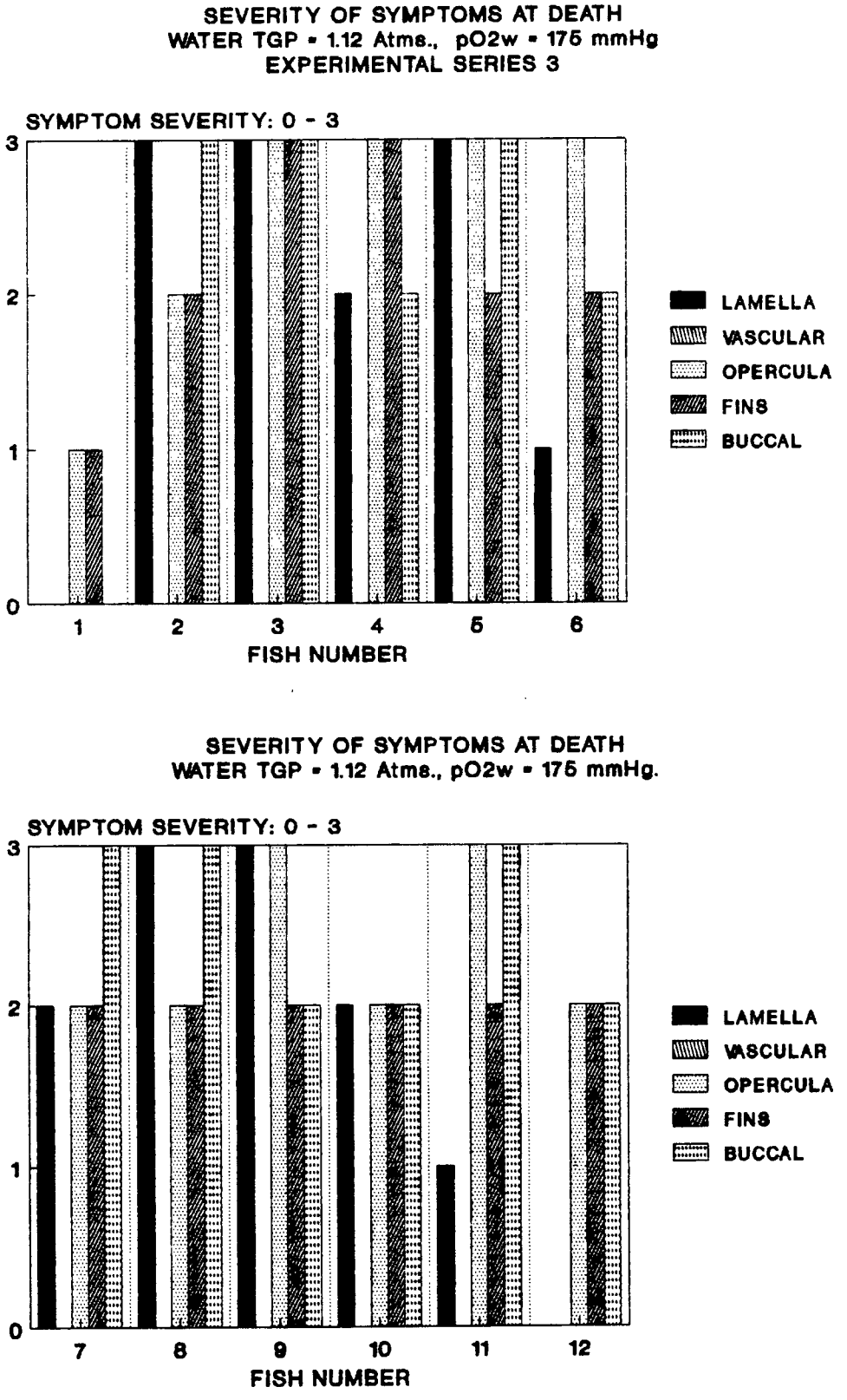


FIGURE 52: Severity of Symptoms at Death, Series 3.

Also, except for Fish No. 1, there were varying degrees of extracorporeal bubble growth between gill lamella accompanied by blisters in the buccal cavity. Figure 53 is a photograph showing an example of the extracorporeal bubbles that were usually present. The bubbles shown in the photograph are typical of those cases assigned a number of one for the level of severity. The bubbles appear to be randomly distributed throughout the primary and secondary lamella. The ones between secondary lamella were quite stable and were difficult to dislodge with a needle. There was no evidence of intravascular bubbles in these gill lamella. The same was true for all fish examined in this series. Figure 54 is a photograph showing large blisters on the upper part of the mouth of one of the experimental animals tested at a TGP of 1.12 Atms. Figure 55, taken of the same animal but at a slightly different angle, shows blisters lining the entrances to the gills. These blisters were of a size and number that would suggest interference with water flow to the gills. In this fish, the severity of buccal cavity blisters was assigned a value of 3. Figures 56 and 57 show examples of blisters (severity level between 2 and 3) that were found on the opercular flaps of fish exposed to the lower levels of TGP. Examples of the blisters occurring between fin rays are shown in Figures 58 and 59. Figure 58 shows a segment of the dorsal fin from one fish while Figure 59 shows the caudal fin of the same animal. In both cases, the level of severity is 2. If one examines the array of symptoms and their severity (Figure 52), it is evident that a correlation exists between the overall severity of symptoms and the time to mortality.

It will be noted that in the first six hours of the experiment, arterial pO_2 is elevated relative to the pre-exposure levels. This reflects the higher levels of dissolved oxygen in supersaturated water. This observation was common to all experiments where experimental water pO_2 was above that of the pre-exposure holding water.

FIGURE 53:

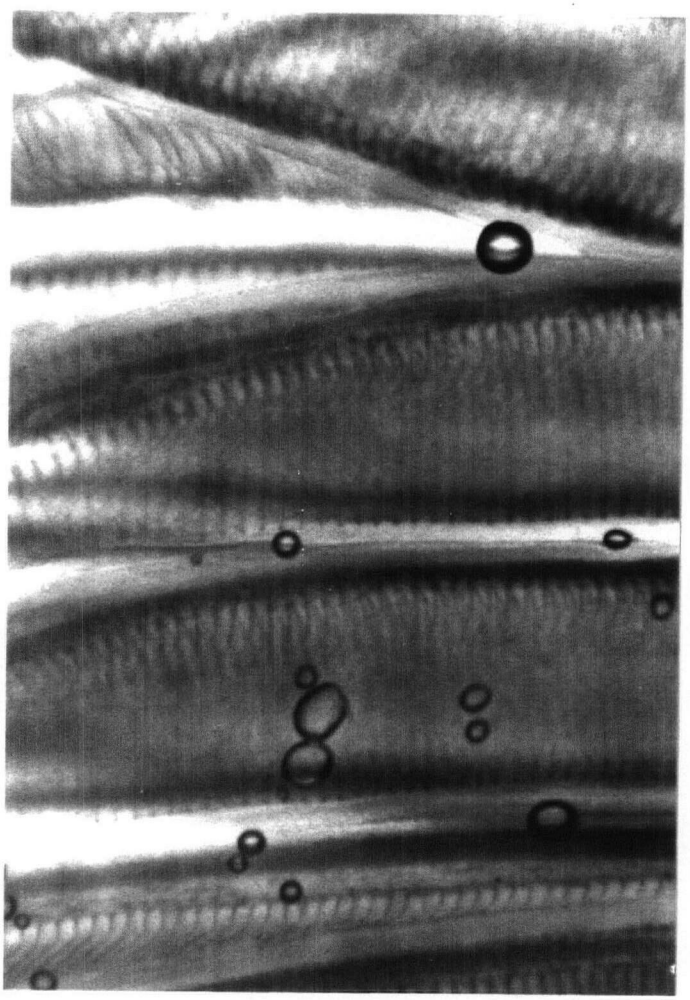


FIGURE 53: Extracorporeal Bubbles in Gills.

FIGURES 54 AND 55:

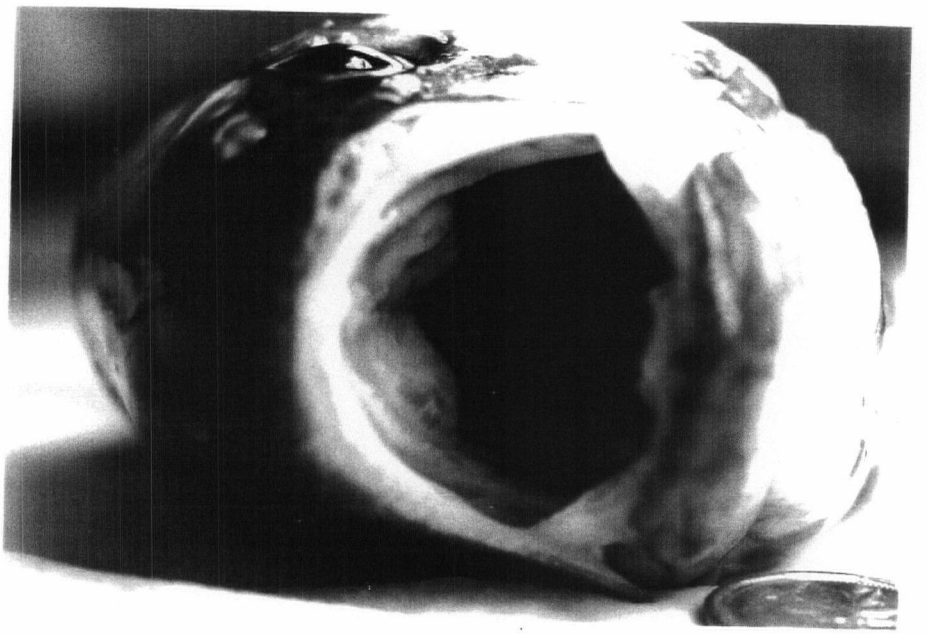


FIGURE 54: Subdermal Bubbles in Mouth.

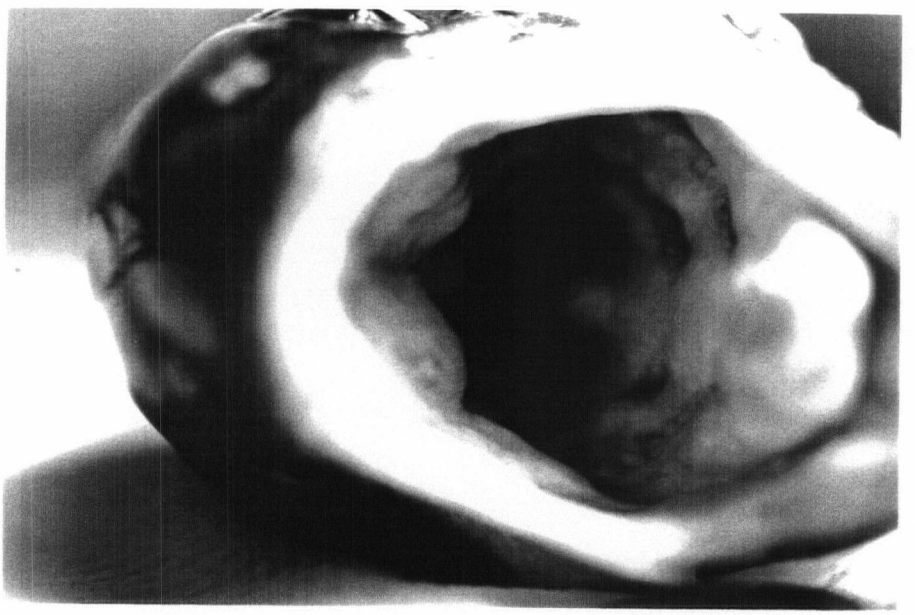


FIGURE 55: Subdermal Bubbles in Mouth.

FIGURES 56 AND 57:

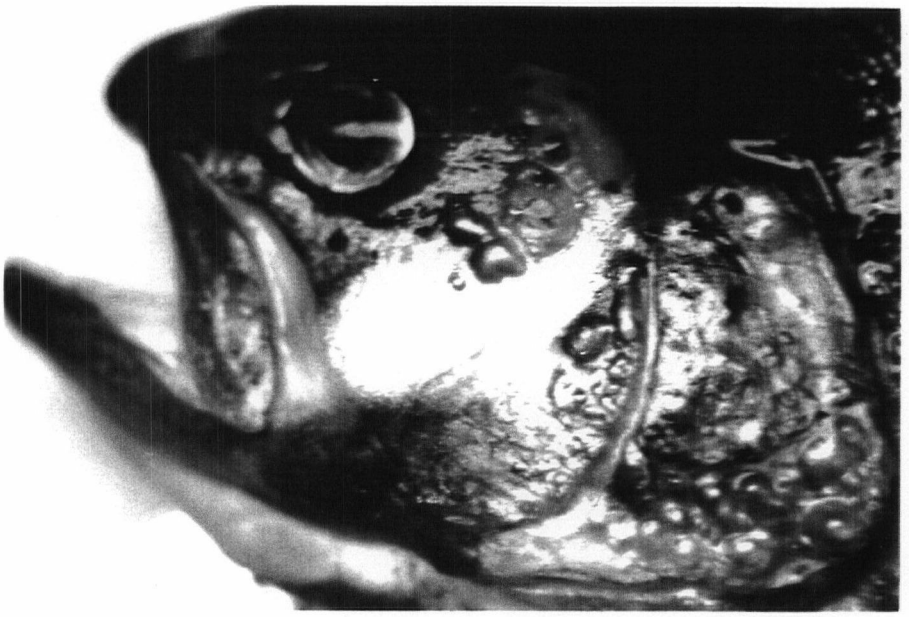


FIGURE 56: Opercular Bubbles.

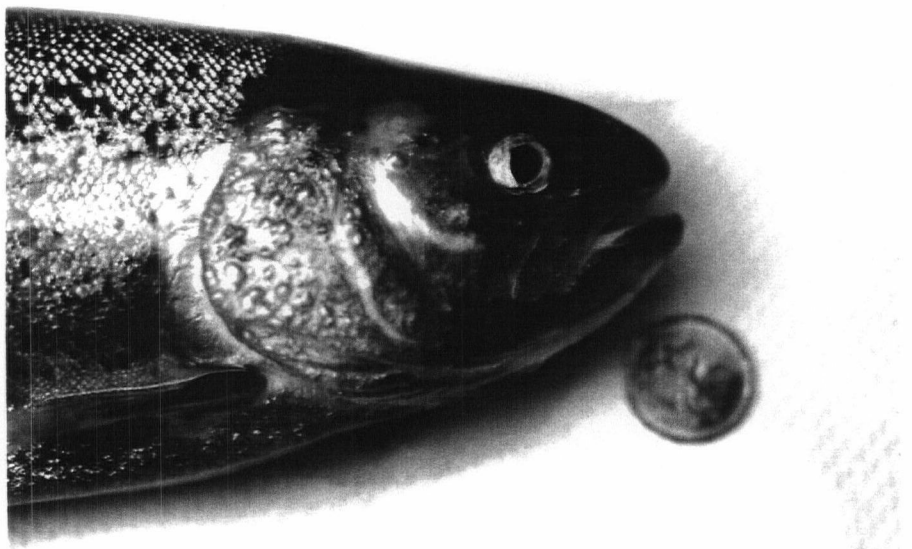


FIGURE 57: Opercular Bubbles.

FIGURES 58 AND 59:

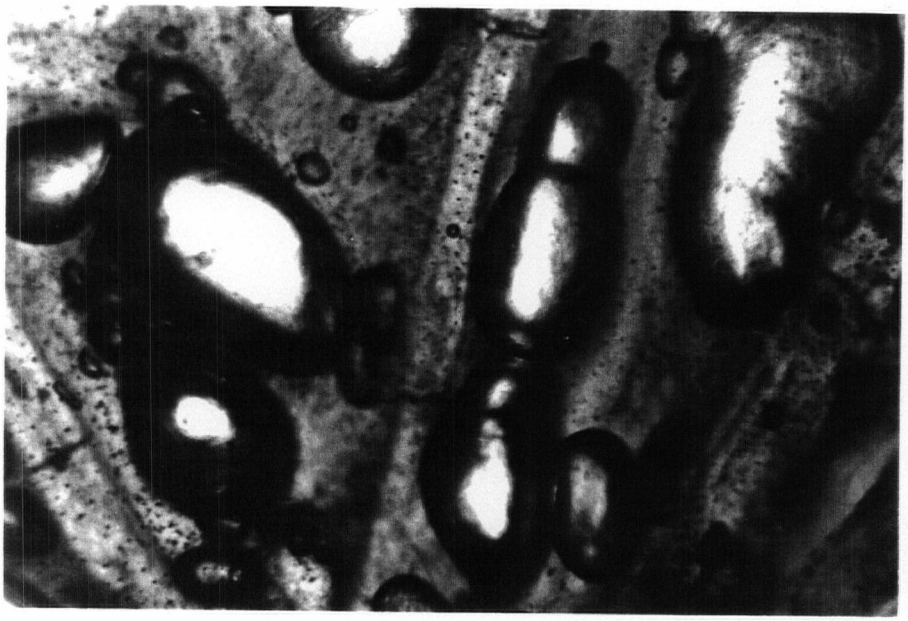


FIGURE 58: Bubbles in Dorsal Fins.

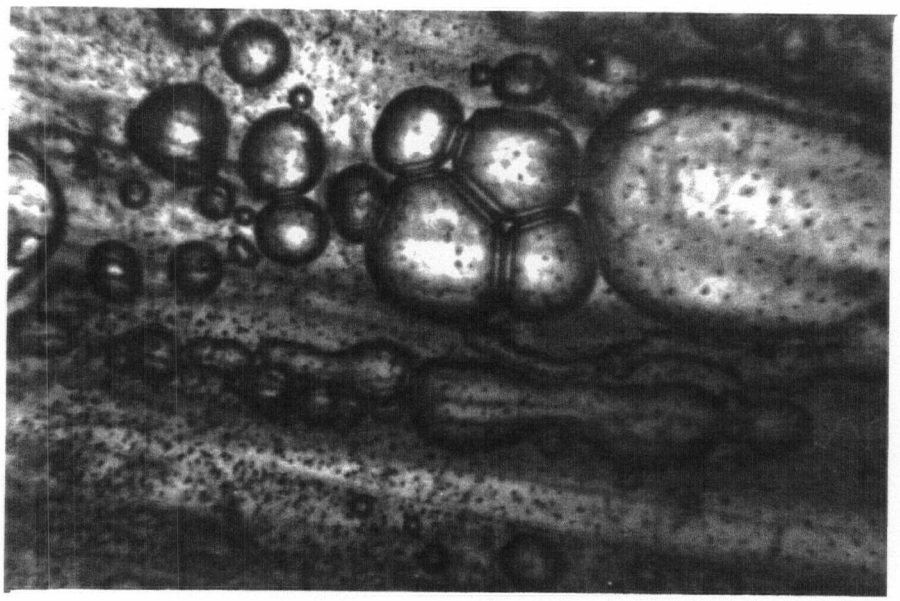


FIGURE 59: Bubbles in Caudal Fins.

Series 4 (Water TGP = 1.15 Atms., pO_2w = 183 mmHg.): The results of this experimental series show that all cannulated fish had varying rates of declining arterial pO_2 (Figure 60). At the time of measurement, none of the levels were considered acute. However, death usually followed the lowest measured value. All but one of the cannulated fish and one of the un-cannulated fish died during the experiment. All fish showed signs of GBT and, as in experimental Series 3, the time to mortality could be correlated with the severity of symptoms. All cannulated fish showed a decline in arterial pH (Figure 61), which corresponded closely to the declining pO_2. Extracorporeal bubbles were present to varying degrees throughout the gills of all fish. Except for Fish No. 3, blood hematocrit showed little variation for the cannulated fish. In Fish No. 3, there was a 67% increase in hematocrit over the course of the experiment. Furthermore, this fish, Fish 4 and Fish 10 were the first to show signs of intravascular bubble growth in the gill arteries (Figure 62). In fish 4 and fish 10, bubble growth was at a minimum level and the times to mortality were not much different from those for fish without vascular bubbles. Figures 63 and 64 are a collection of microscope photographs taken of gill lamella from some of these fish and from fish in series at higher levels of TGP. In all photographs vascular bubbles are clearly present in the filamentary arteries of the primary lamella. Of all the gill lamella examined microscopically, only one fish, exposed to a water TGP of 1.19 Atms.. showed the presence of bubbles in the secondary lamella. These bubbles had clearly grown into the region from the filamentary arteries of the primary lamella. With few exceptions, bubbles appear to start at the distal ends of the filamentary arteries and grow toward the base of the primary lamella.

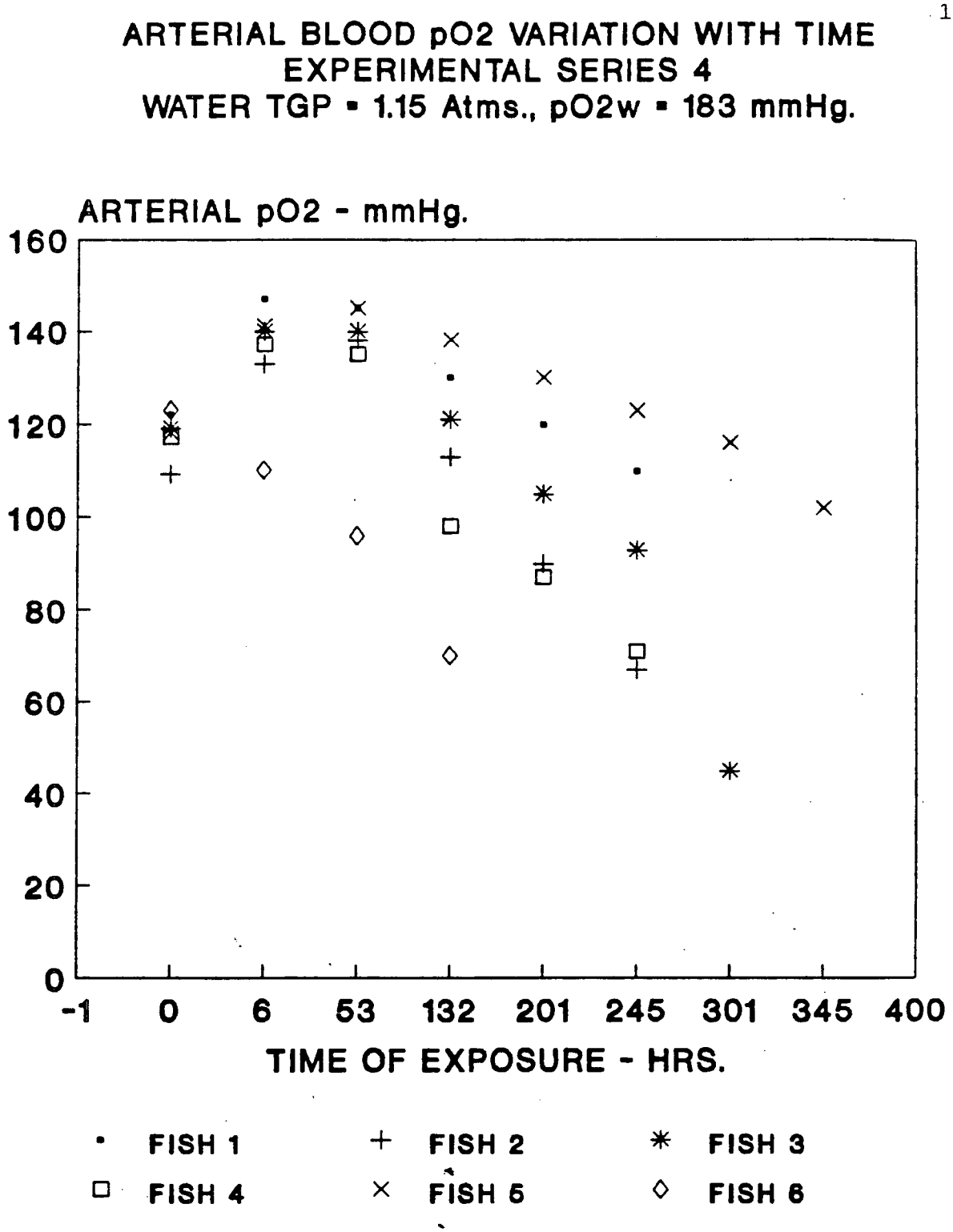
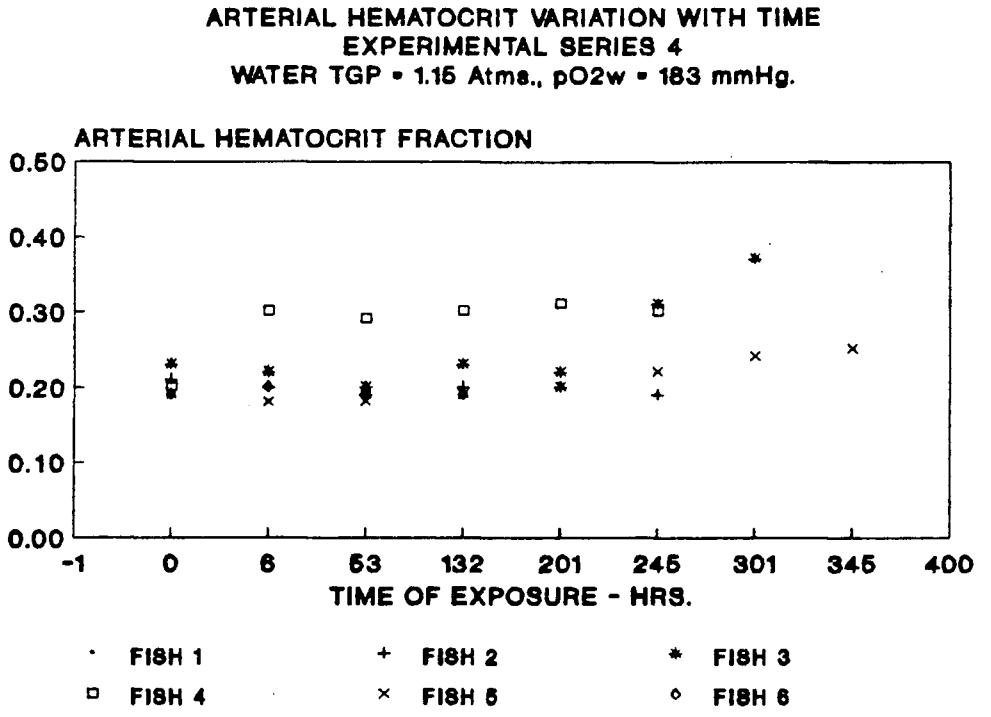


FIGURE 60: Arterial Blood pO_2 Variation with Time, Series 4.



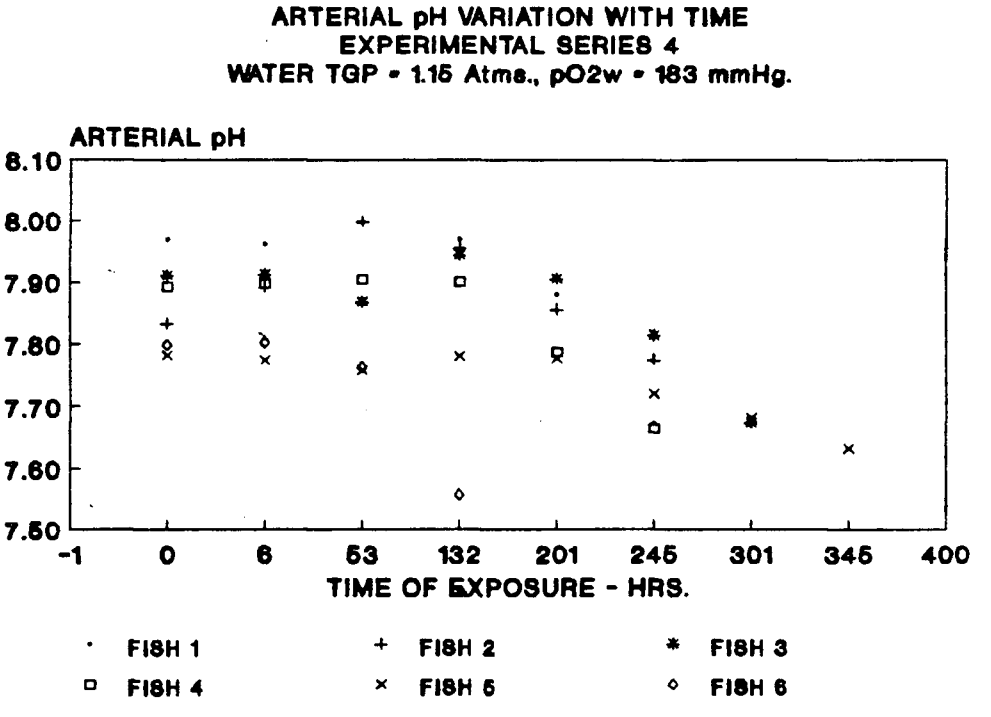


FIGURE 61: Arterial Hematocrit and pH Variation with Time, Series 4.

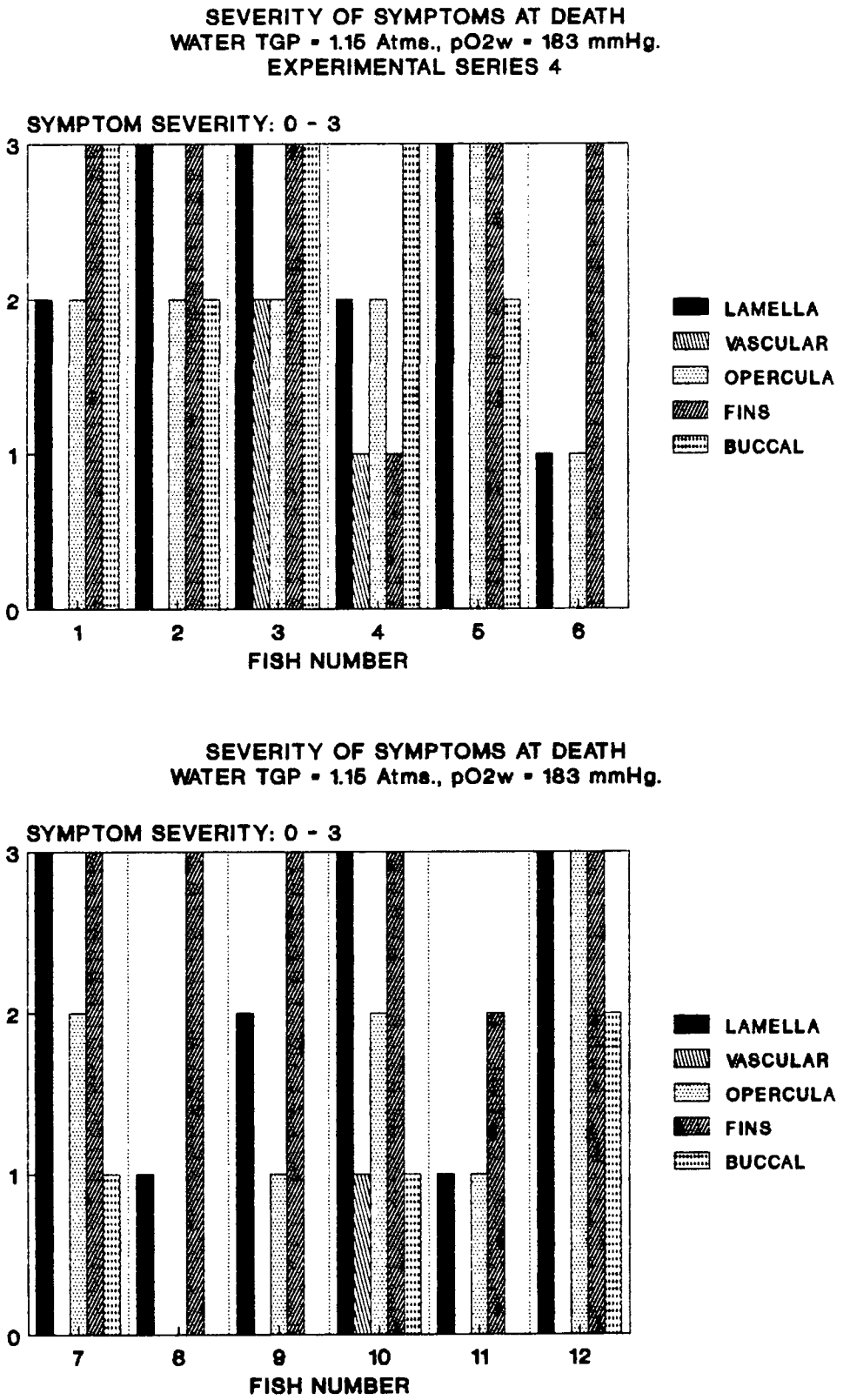


FIGURE 62: Severity of Symptoms at Death, Series 4.

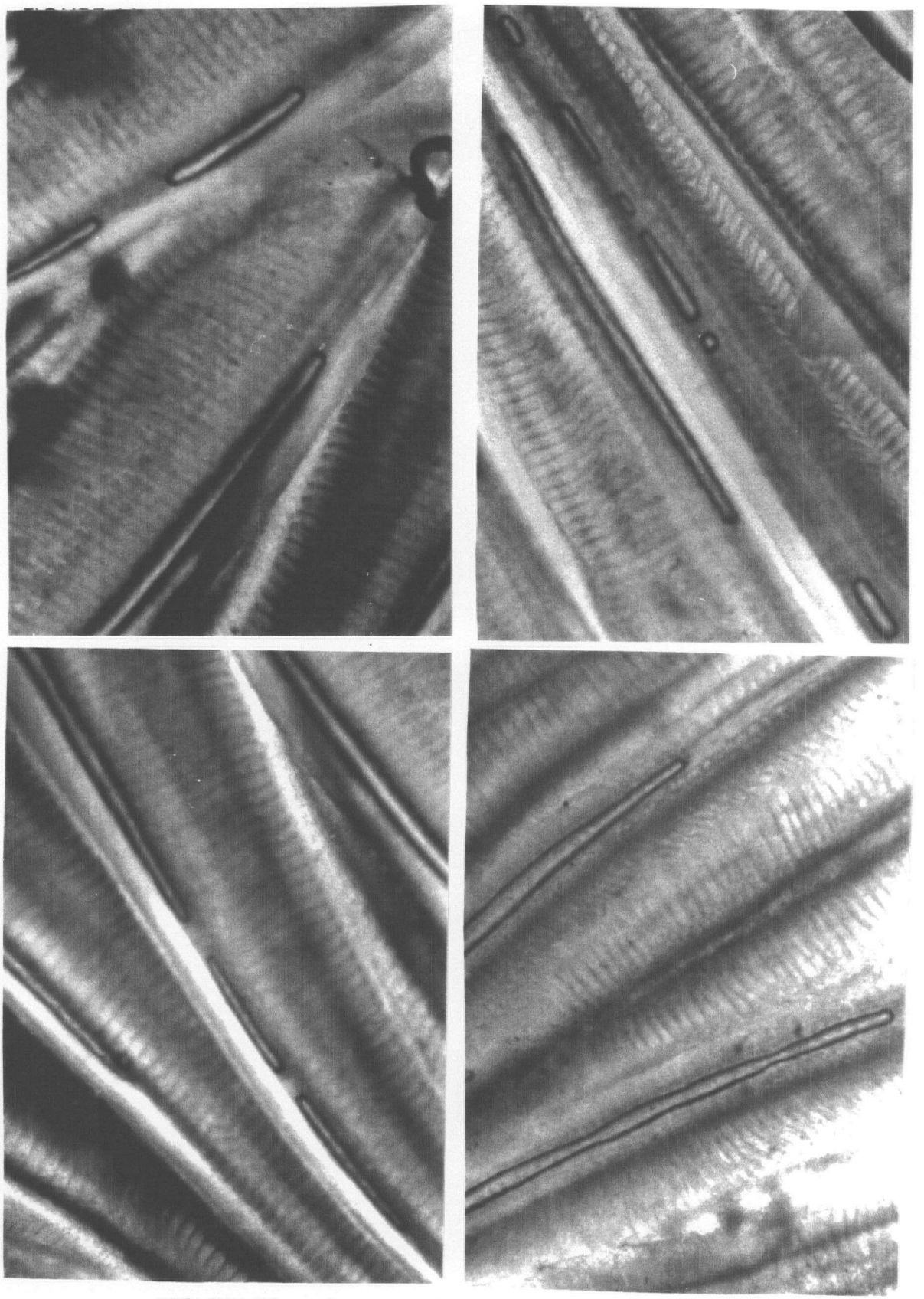


FIGURE 63: a, b, c & d: Vascular Bubbles in Gills.

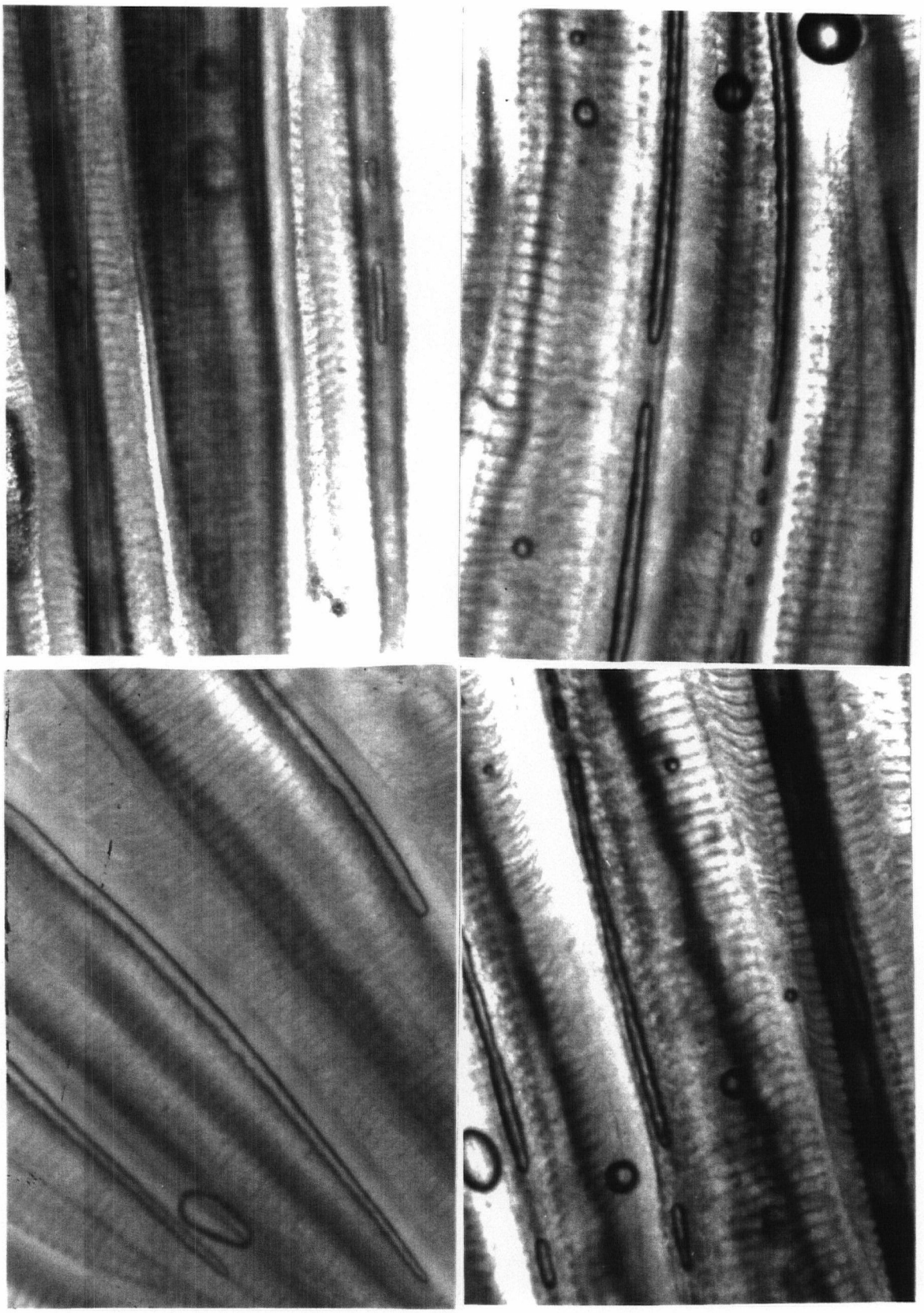


FIGURE 64: a, b, c & d: Vascular Bubbles in Gills.

Series 2 (Water TGP = 1.17 Atms., pO_2w = 195 mmHg.): In the Series 2 experiments every fish showed varying degrees of intravascular bubble growth in gill lamella (Figure 67). Increases in hematocrit were seen in each of the cannulated fish (Figure 66). Arterial blood pH and pO_2 dropped dramatically (Figures 66 and 65, respectively) and all fish died during the experiment (Figure 46). Interestingly, the external signs of GBT, such as blistering of tissue, were not as pronounced as in the experiments at lower levels of TGP. Presumably, this is because fish died from other causes before these symptoms could become well developed. Also, it was clear that the number of extracorporeal bubbles were fewer than at lower levels of TGP. On the other hand, the bubbles that were present appeared to be larger. As with previous experimental Series, a rise in pO_2 during initial stages of exposure was seen in many of the fish. This again reflects the elevated pO_2 levels in the water. As exposure time increased, this initial rise in pO_2 was offset by the apparent blockage of oxygen transport to blood.

Series 5 (Water TGP = 1.19 Atms., pO_2w = 201 mmHg.): At a TGP of 1.19 Atms. the time of survival of all fish declined dramatically (Figure 46). Arterial pO_2 declined rapidly for all cannulated fish (Figure 68) and presumably for the un-cannulated fish as well. Also, there was a high level of intravascular bubble formation in the gills of all fish (Figure 70). As with previous experiments, arterial pH dropped and hematocrit rose, but more rapidly than in any of the other experiments (Figure 69). The trend in decreasing numbers of extracorporeal bubbles in the gills continued in this experimental series. Again, the sizes of the bubbles were larger than those in previous series. Also, very few external blisters were present in these animals, presumably because of the short time of survival.

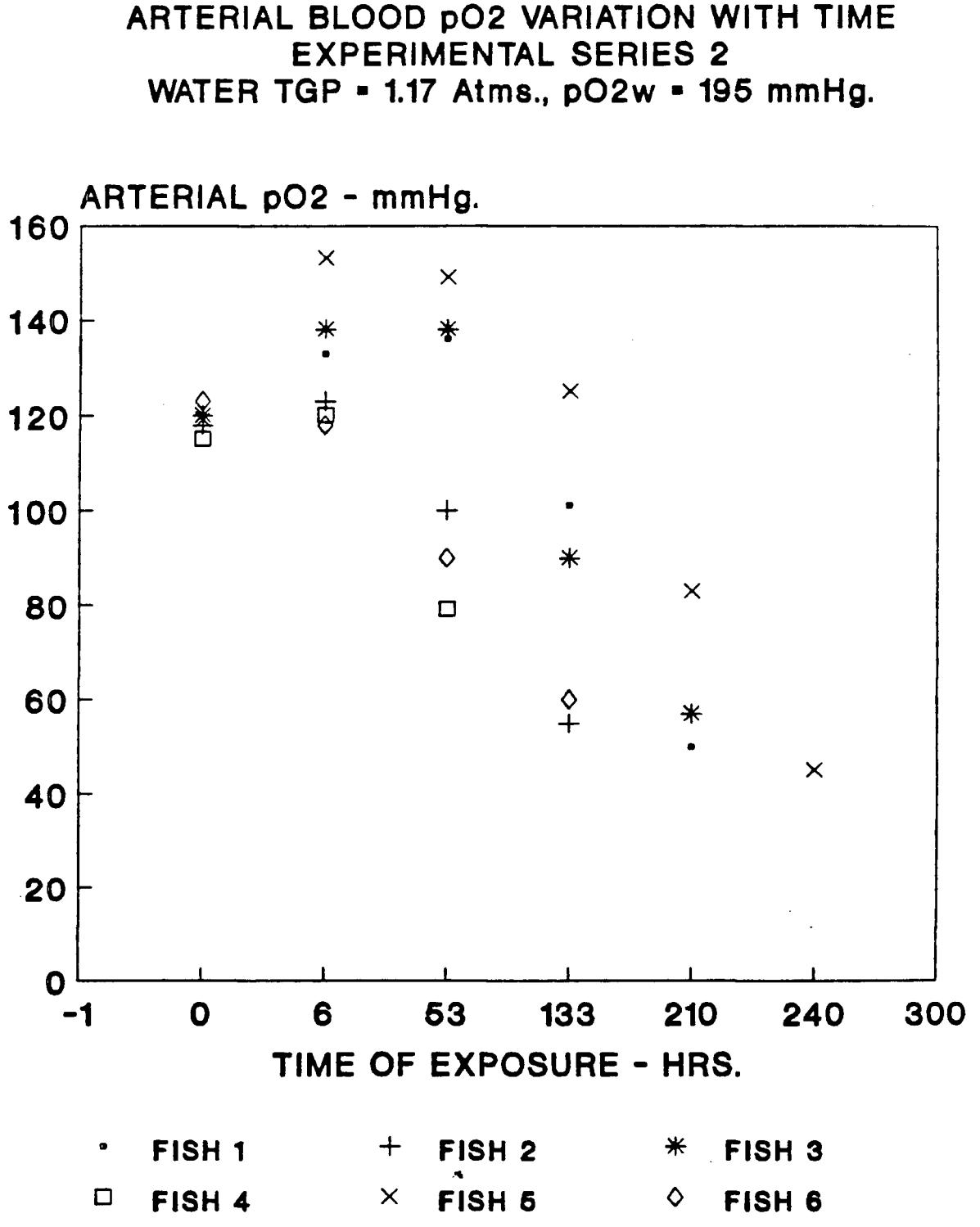
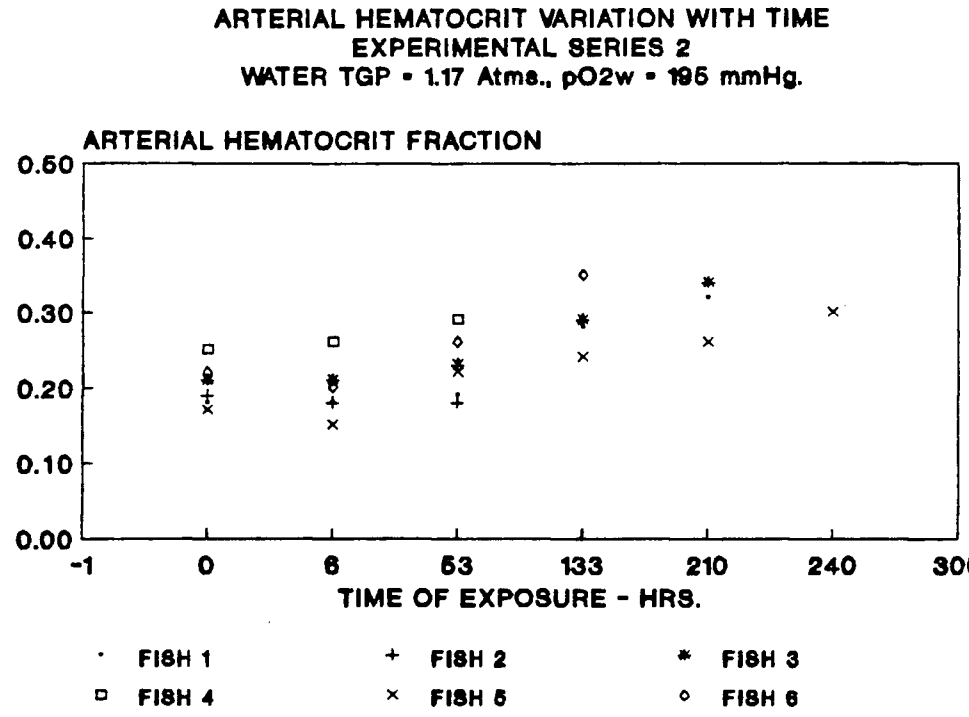


FIGURE 65: Arterial Blood pO_2 Variation with Time, Series 2.



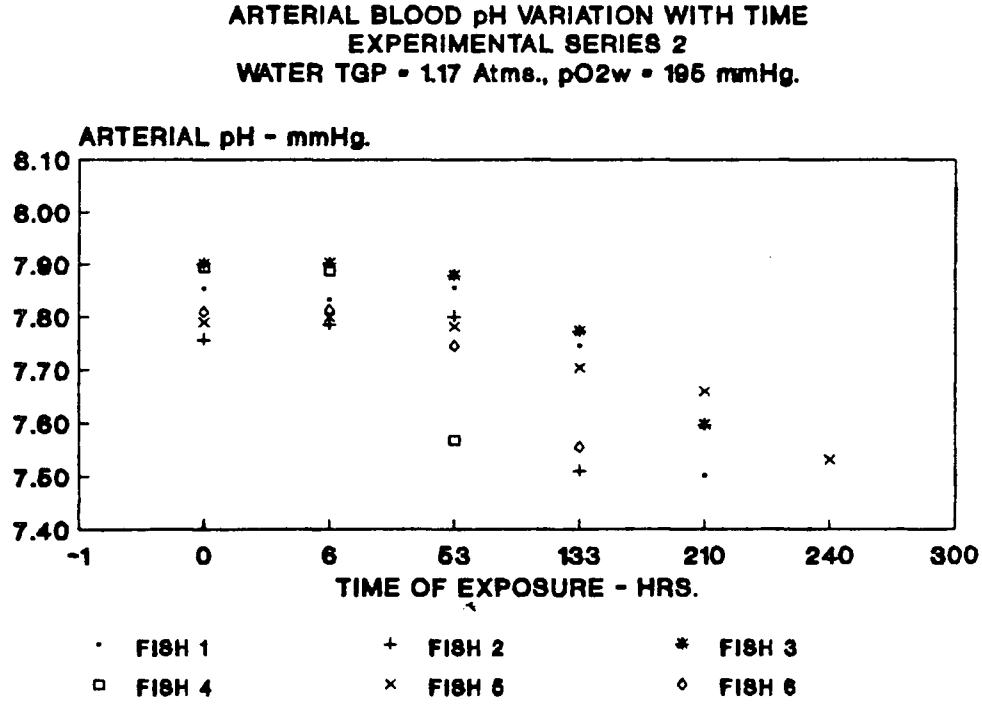


FIGURE 66: Arterial Hematocrit and pH Variation with Time, Series 2.

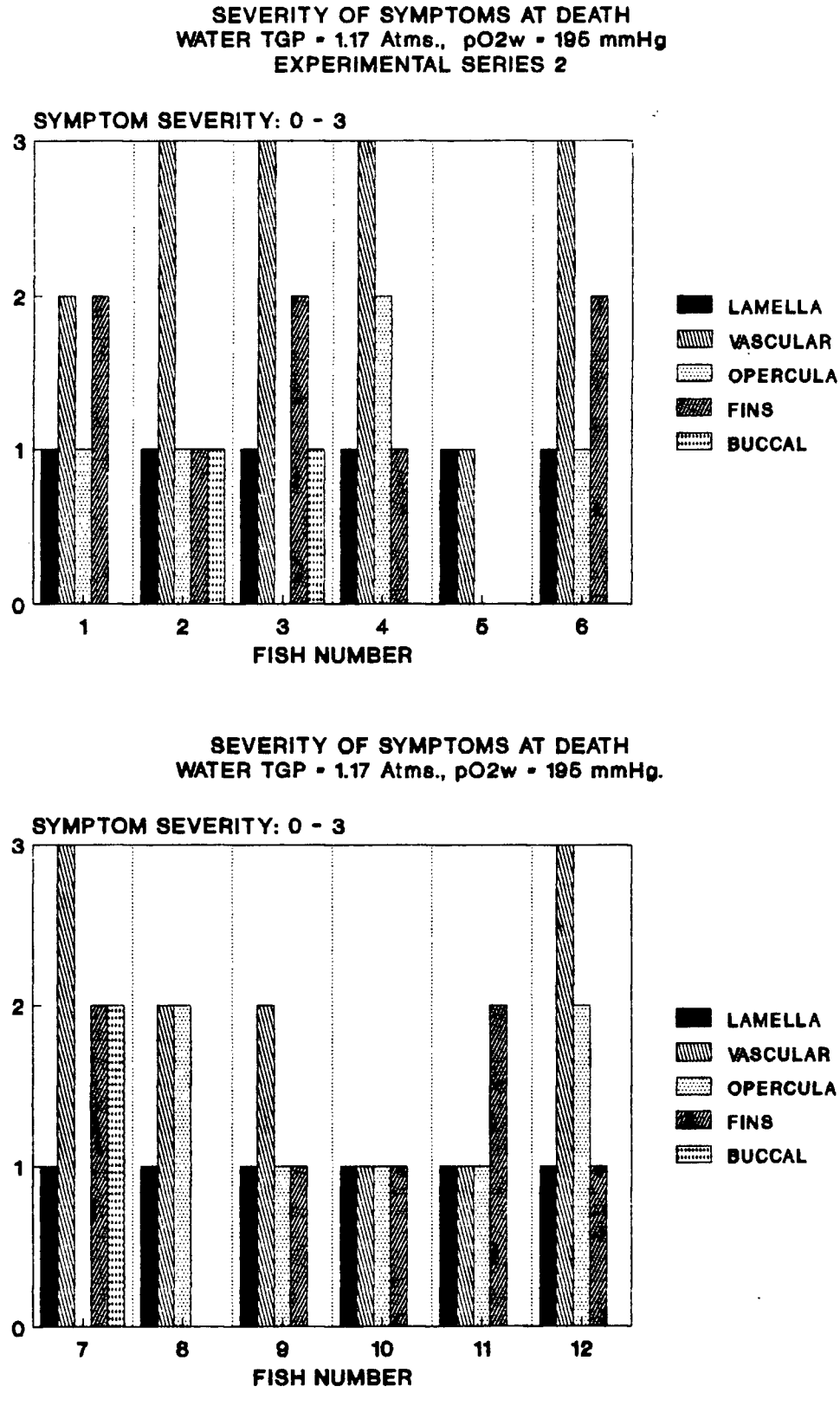


FIGURE 67: Severity of Symptoms at Death, Series 2.

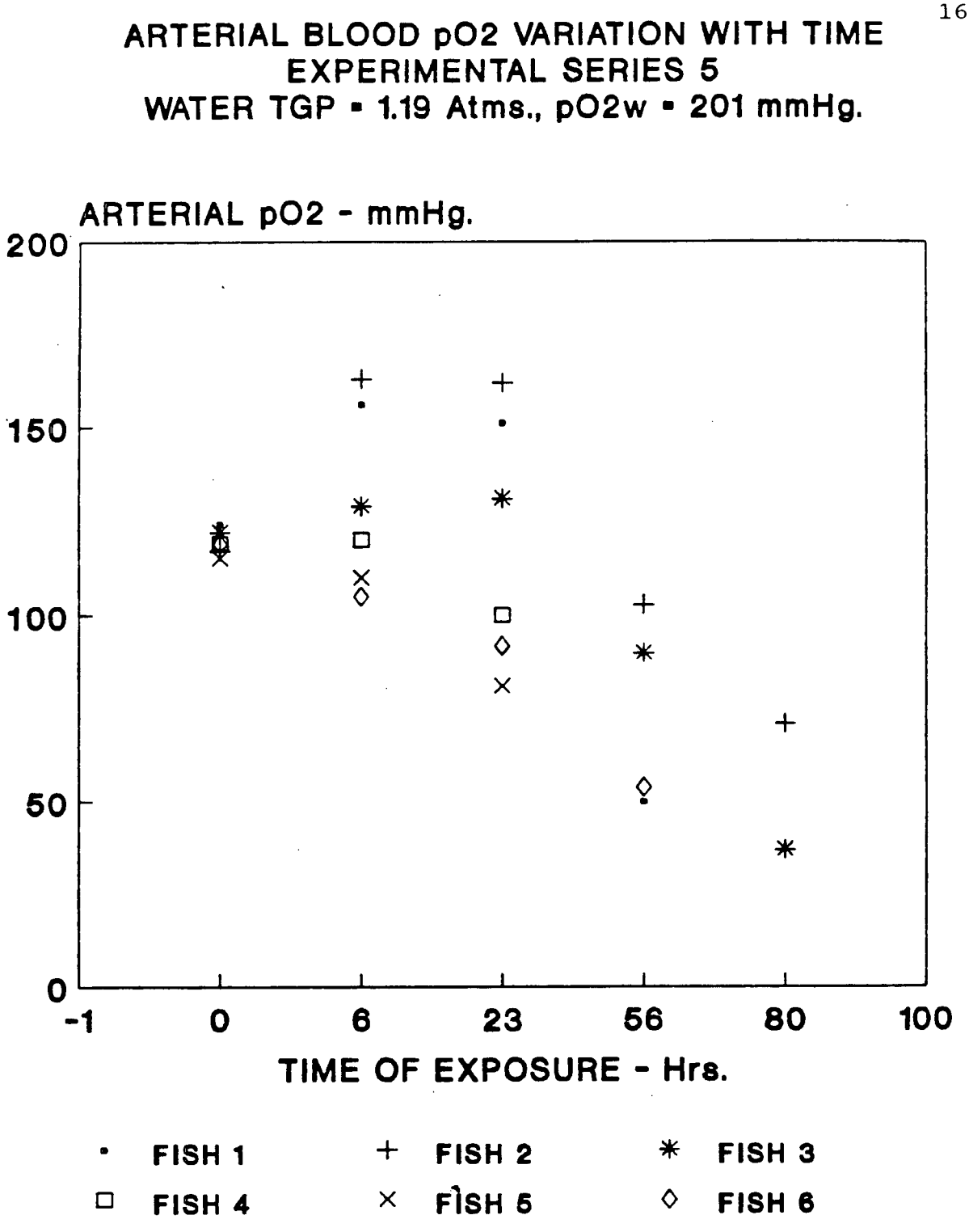
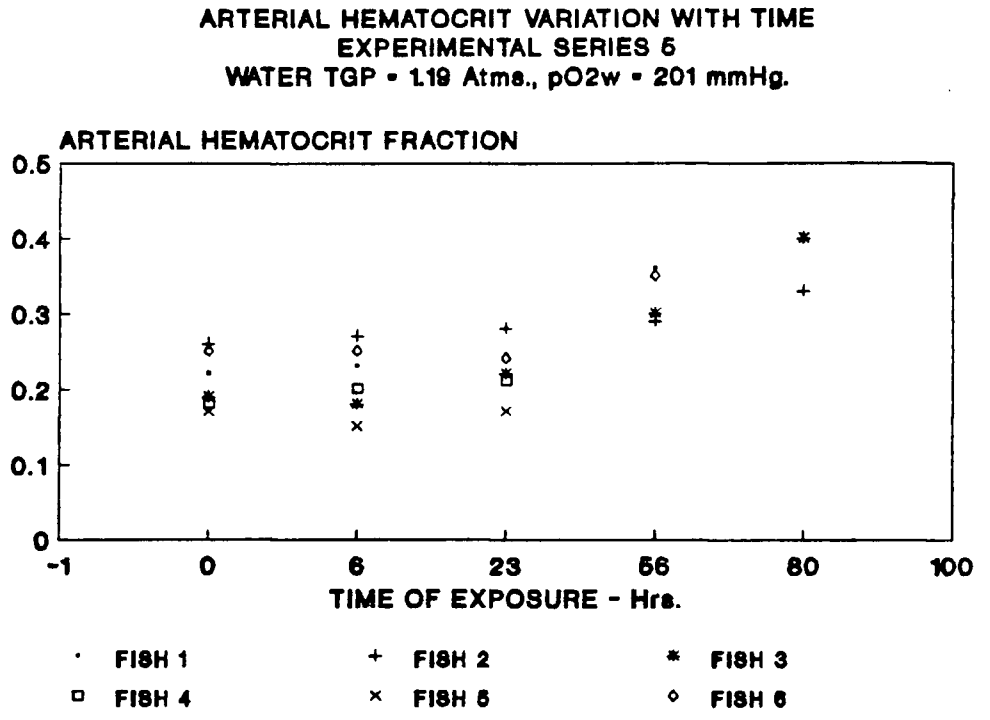


FIGURE 68: Arterial Blood pO_2 Variation with Time, Series 5.



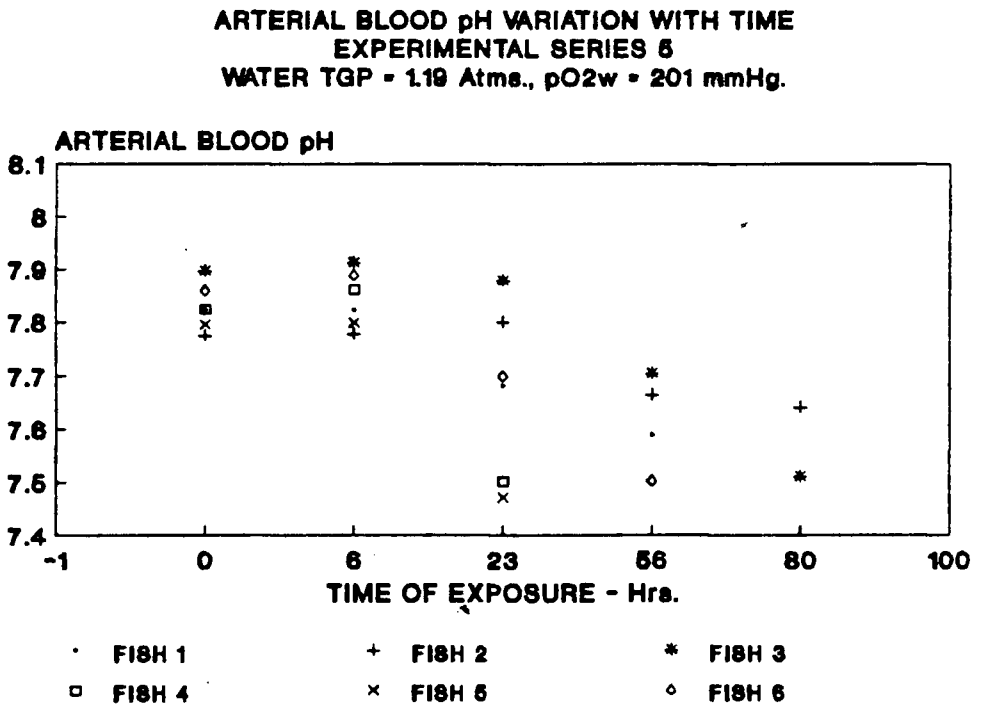


FIGURE 69: Arterial Hematocrit and pH Variation with Time, Series 5.

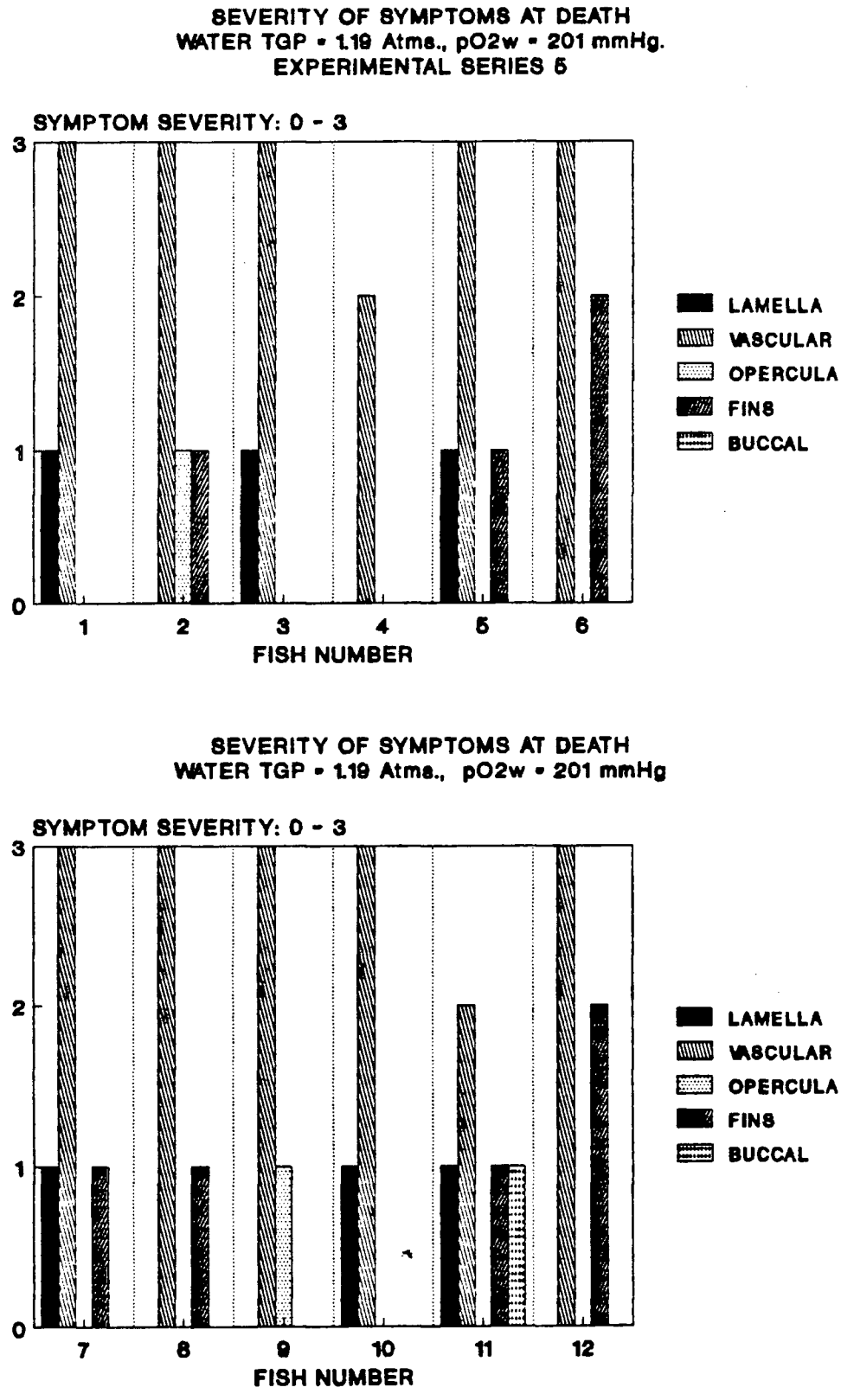


FIGURE 70: Severity of Symptoms at Death, Series 5.

5.6.2 Series 6 (Water TGP = 1.19 Atms., pO_2w = 201 mmHg.): In this series of experiments the levels of adrenaline and noradrenaline were measured in six cannulated fish during exposure to supersaturated water. Figure 71 shows the variation in adrenaline for the six animals as a function of exposure time. In this case, Fish No's. 1, 3, 4 and 5 all showed increasing levels of adrenaline as exposure time increased. Figure 72 shows the variation in noradrenaline with time for the six fish. Only Fish 3 exhibited a consistent increase in noradrenaline with exposure time.

5.6.3 Series 7 (Water TGP = 1.19 Atms., pO_2w = 201 mmHg.): In this experimental series the ventilation performance of three fish was monitored using the van Dam respiratory chambers described in the methods section. Figure 73 shows the variation in both ventilation volume and frequency as a function of the time of exposure. Only one fish lived the full 80 hours of exposure. However, it was clear that all fish showed increases in both ventilation volume and frequency as the exposure period lengthened. In Fish 2, which lived the longest, a decline in both ventilation volume and frequency was measured 20 minutes before the fish died.

5.6.4 Series A, B, C, and Series 4 (Water TGP = 1.15 Atms., pO_2w = 100, 125 and 183 mmHg.): In these experiments, water TGP was held constant while water pO_2 was varied. Times to mortality were monitored along with the condition of gills. Twelve fish, Series A and B, were tested at a pO_2 of 100 mmHg. Experimental Series C involved six fish exposed at a pO_2 of 125 mmHg. The Series 4 data are the same as that for the un-cannulated fish described in Series 4 earlier in this section. The results of these experiments (Figure 74) clearly show there is strong dependence of time to mortality on water pO_2. Furthermore, a significant transition occurs between a pO_2 of 100 and 125 mmhg.

ADRENALINE VARIATION WITH TIME
TGP = 1.19 Atms. pO2 = 201 mmHg.
SERIES 6

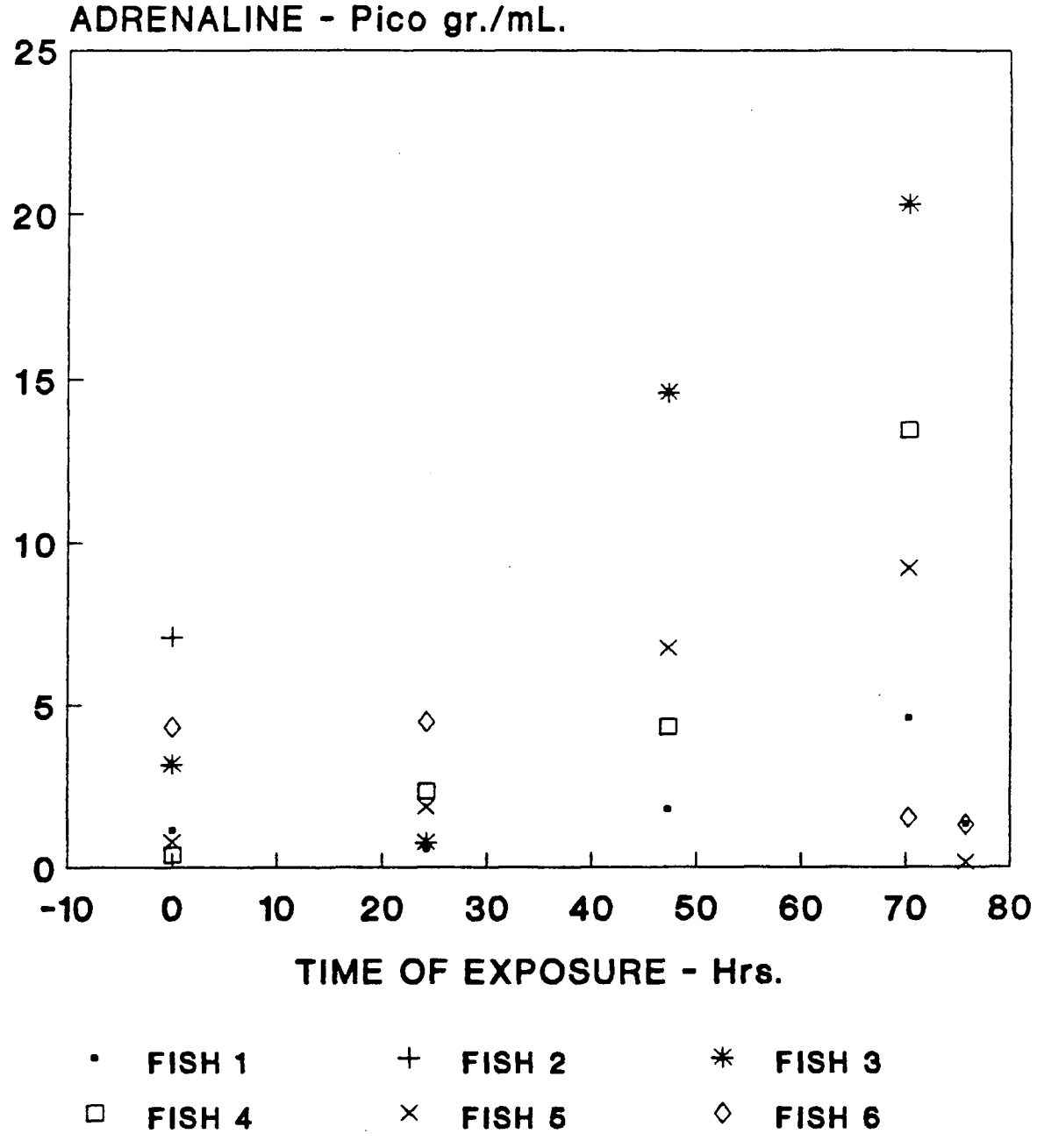


FIGURE 71: Adrenaline Variation with Time, Experimental Series 6.

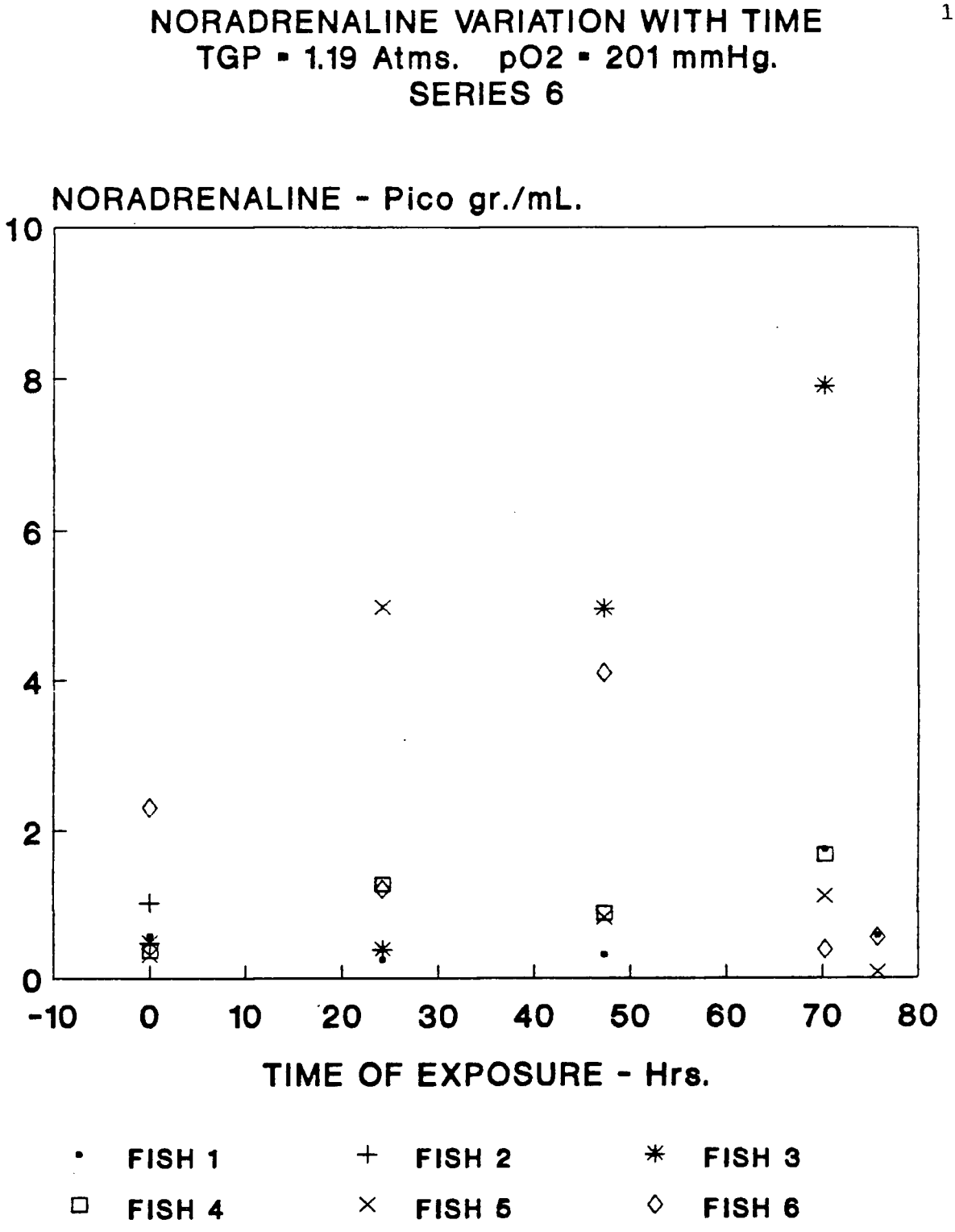
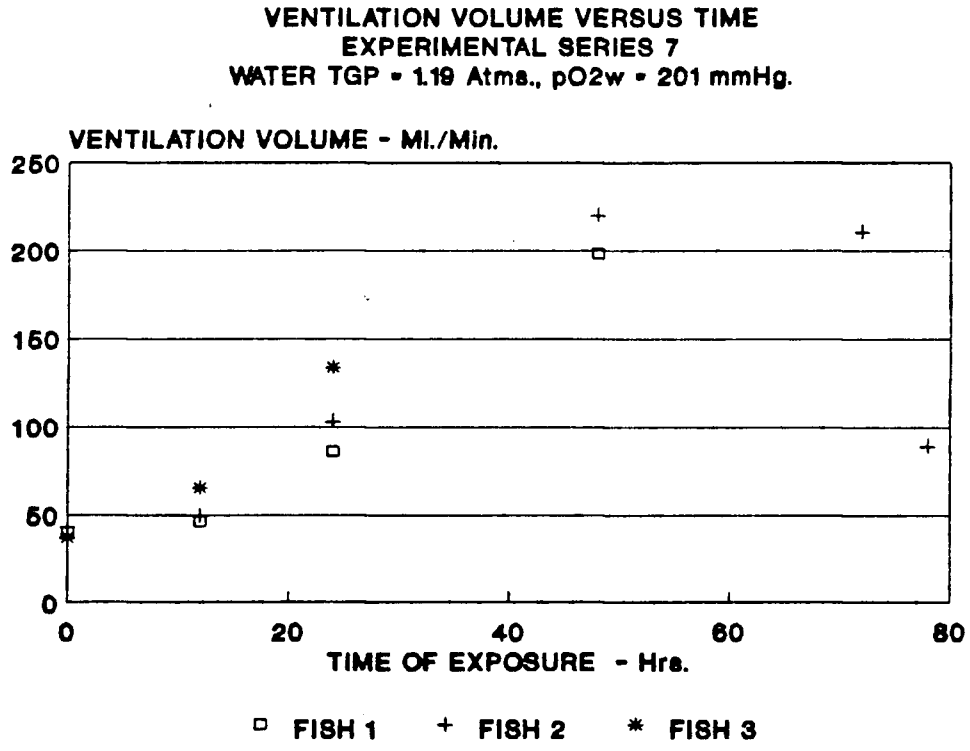
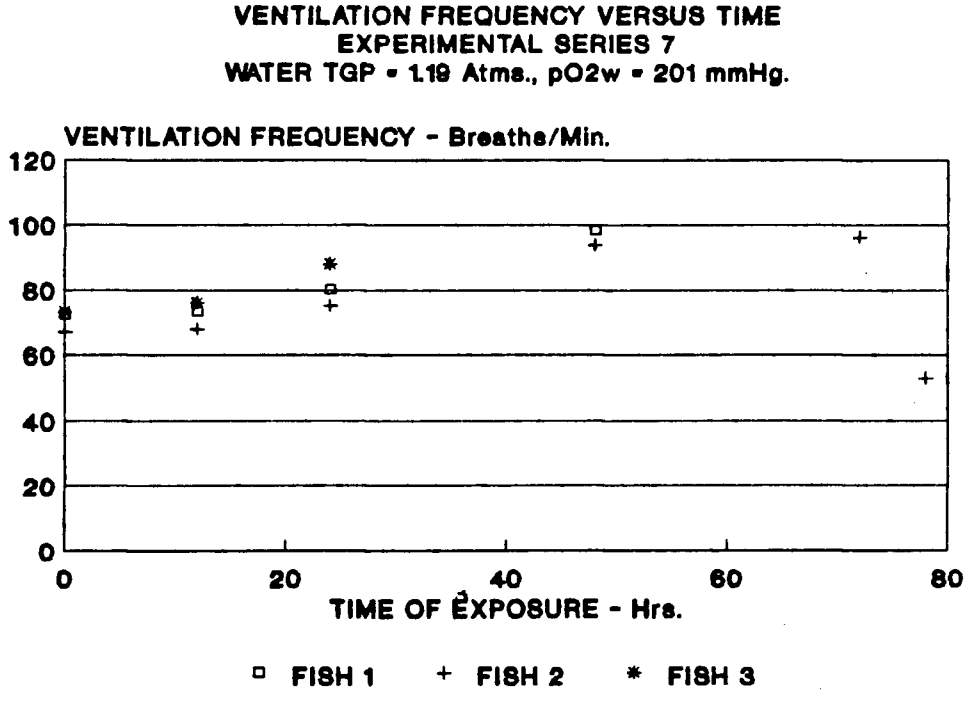


FIGURE 72: Noradrenaline Variation with Time, Experimental Series 6.





NOTE: All fish had vascular system bubbles at death.

FIGURE 73: Ventilation Volume and Frequency Versus Time, Series 7.

As described earlier, this is similar to the results obtained with Coho salmon by Rucker (1975) at a water TGP of 1.19 Atms. In that case, the transition in time to mortality occurred at a water pO_2 of 249 - 275 mmHg. The conclusion drawn was that the strong transition in time to mortality represents a threshold for the cause of mortality. Presumably this was due to bubble growth. In Table III the presence or absence of intravascular gill bubbles at death is shown for the Phase II experimental series A, B, C and 4. These data also confirm that the transition in time to mortality correlates well with the presence of intravascular gill bubbles.

TABLE III: SURVEY OF INTRAVASCULAR GILL BUBBLES

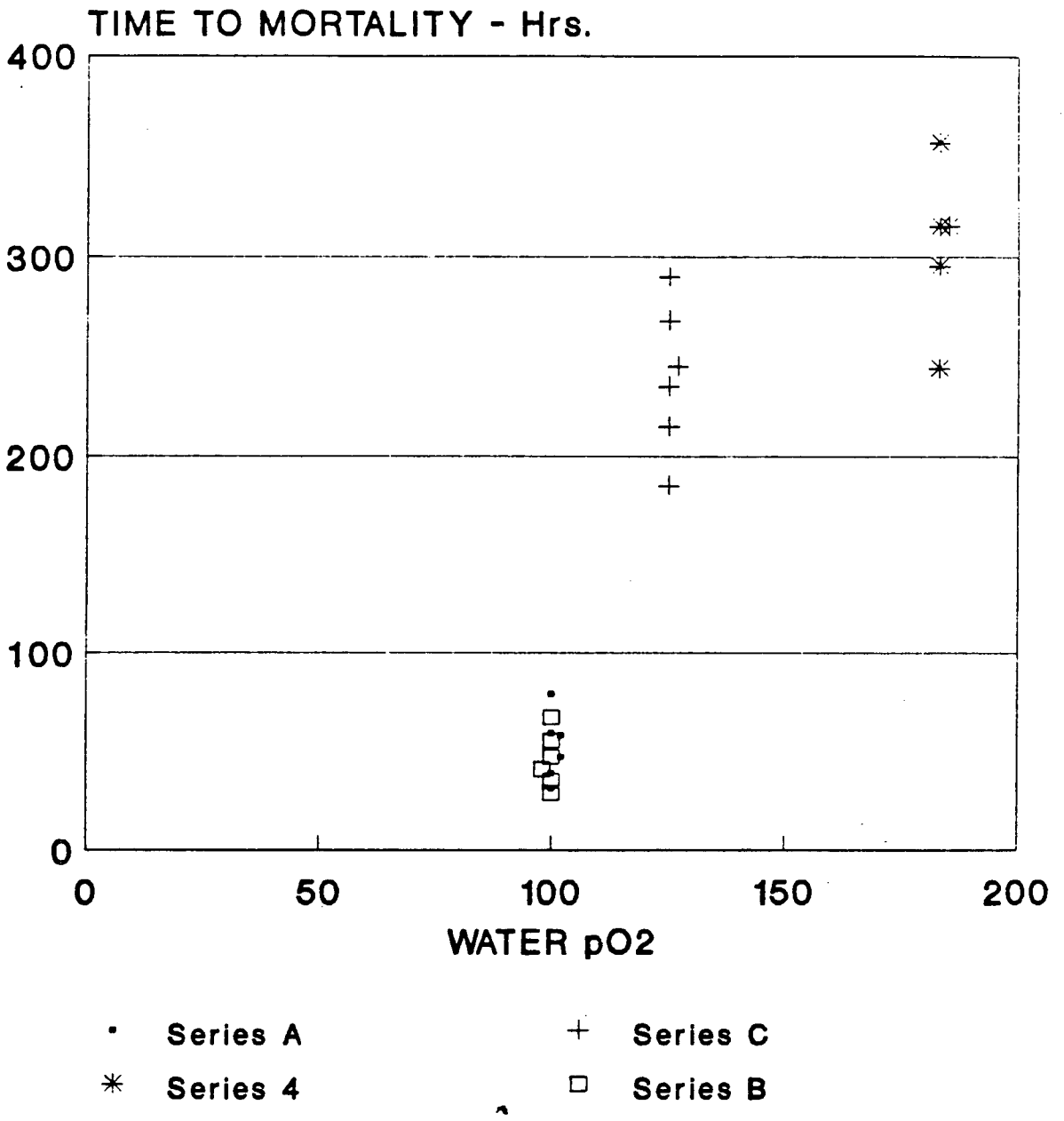
+ indicates bubbles were present.

- indicates bubbles were not present.

pO_2 - in mmHg.

FISH NO.	BUBBLES?	pO_2	FISH NO.	BUBBLES?	pO_2
A1	+	100	C1	-	125
A2	+	100	C2	-	125
A3	+	100	C3	-	125
A4	+	100	C4	+	125
A5	-	100	C5	-	125
A6	+	100	C6	-	125
B1	-	100	4-7	-	183
B2	+	100	4-8	-	183
B3	+	100	4-9	-	183
B4	+	100	4-10	+	183
B5	+	100	4-11	-	183
B6	+	100	4-12	-	183

TIME TO MORTALITY VERSUS WATER pO2
FOR CONSTANT TOTAL GAS PRESSURE
TGP = 1.15 Atms.



Note: One fish in Series 4 did not die in 400 hours.

FIGURE 74: Time to Mortality Versus Water pO_2 for Constant TGP.

5.6.5 OTHER RESULTS: In addition to the results described above, several supplemental studies were performed during the Phase II experiments. For example, tissue samples were periodically taken from muscle, liver and heart for microscopic examination. When intravascular bubbles were found in the gill lamella, intravascular bubbles were frequently found in the other tissue samples as well. Although bubbles were found less frequently in heart and liver tissue, they were again of intravascular origin.

During experiments at a water TGP of 1.19 Atms., three fish, not included in those described above, were prematurely killed when their blood showed an initial rise in hematocrit and before the development of external stress symptoms (i.e. violent swimming). In each case, intravascular bubbles had started to grow in the gill lamella. The degree of bubble development, however, was not as severe as seen in other fish at the same TGP just before death. This observation, combined with the blood pressure response of the Phase I experiments, imply that intravascular bubble growth in the gill lamella begins early and is not the result of declines in blood pressure just before death.

Similarly, two fish exposed to a water TGP of 1.12 Atms. were killed after 24 hours of exposure. This was done to determine if extracorporeal bubbles were present in the gills before declines in pO_2 occurred or the development of severe sub-dermal bubble growth in the mouth. In both fish it was clear that extracorporeal bubbles were present between gill lamella. Again, as with intravascular bubbles, the degree of development was not as severe as that seen much later in the exposure period.

5.7 DISCUSSION OF PHASE II RESULTS

The results of these experiments confirm the observations of the Phase I experimental studies and refine the definition of bubble growth thresholds. In addition, significant information was added to the understanding of bubble growth and the physiological response of fish to this growth.

As with the Phase I experiments, fish that experienced severe forms of sub-dermal bubble growth on the external skin also showed signs of minor hemorrhaging from lesions produced by these bubbles. However, the lesions were not considered to be severe enough, compared to other symptoms, as to contribute significantly to death of any of the animals.

5.7.1 EXTRACORPOREAL AND SUB-DERMAL BUBBLE GROWTH THRESHOLDS: Although fish of the Phase I experiments showed no response to supersaturation at a water TGP of 1.10 Atms., the Phase II experimental animals did show a limited response at 1.10 Atms. This difference between the two experimental Phases was most likely due to the number of fish examined. It was clear in the Phase II studies that the 1.10 to 1.12 Atms. TGP threshold applies not only to sub-dermal bubble growth in external epithelium tissue, but also to the growth of extracorporeal bubbles between gill lamella. However, it was not possible to demonstrate that sub-dermal bubble growth in the mouth, extracorporeal bubble growth in the gill lamella or the two acting in concert was the primary or contributing cause of mortality at the lower TGP levels. However, it was clear that in this range of TGP, arterial pO_2 and pH declined with increasing exposure time; thus. indicating a progressive blockage of the respiratory pathway. The only apparent mechanical means for this blockage were the

sub-dermal bubbles in the mouth and extracorporeal bubbles in the gills. Thus there is at least a correlation of mortality, blockage of respiratory pathways and the two forms of bubble growth in this narrow range of water TGP.

It was not possible to define the lower bubble growth threshold more precisely than the TGP range of 1.10 to 1.12 Atms. This was clearly due to the variability in the response of the animals. The source of this variability is not known. However, because the time to mortality is quite long in this range of TGP, factors such as fish health, handling stress and water quality are probably important considerations. Using this range (1.10 to 1.12 Atms.) for the extracorporeal and sub-dermal bubble growth thresholds, the radius of critical nuclei back calculated from Equation 6 is 12 - 14 μM.

5.7.2 INTRAVASCULAR BUBBLE GROWTH THRESHOLDS: The results of the Phase II experiments clearly show the existence of a threshold for intravascular bubble growth in fish exposed to supersaturated water. This conclusion is based primarily on the observation of bubbles in the gill vasculature and the significant changes in hematocrit. A clear definition of this threshold could not be obtained from the results of the Phase II experiments. Again, the large variability in the response of the animals coupled with unknown variations in stress produced by sub-dermal and extracorporeal bubble growth clouded the response. Furthermore, as shown in the theoretical section, fish exhibit a large variation in the ratio of arterial pO_2 to water pO_2 (the gill uptake ratio in Equation 4). This is no doubt a contributing factor in the variability observed. Nevertheless, the results of the Series 1 through 5 experiments show that the intravascular bubble growth threshold is in the TGP range of 1.15 to 1.19 Atms. for water pO_2 between 185 and 201 mmHg. If only the un-cannulated

fish of these series are considered, the threshold appears to be in a TGP range of 1.16 to 1.19 Atms.

The experimentally derived dependence of intravascular bubble growth thresholds on water pO_2 can be obtained by combining the thresholds indicated in the various series (Series 1 through 5 and Series 7) with the data of Rucker (1975 and Section 5.6.4 above). The combined data are plotted in Figure 75 as a function of water pO_2. Shown in this plot are only data for un-cannulated fish of the Phase II studies. The arrows indicate the range of TGP and pO_2 in which the various thresholds are indicated to lie. The individual data records are identified in the legend on the figure. Also plotted on the graph are the theoretical predictions of Equation 4 for various sizes of nucleation sites. The theoretical curves are for an oxygen uptake ratio of 0.79, a water temperature of 5 to 12 $^{\circ}$ C. and sea level atmospheric pressure. In Figure 75, the experimental thresholds correlate well with theoretical thresholds based on a critical nuclei radius of 12 to 14 μM. This observation is important in terms of the size of arteries in which the bubbles were found. This radius is approximately twice that of Rainbow trout red blood cells (Heming, 1984a; Mott, 1957 and Smith, 1952). As pointed out in the theoretical section, arteries of the secondary lamella have characteristic widths comparable to the diameter of red blood cells. This implies that nuclei and bubbles could not exist in the secondary lamella. As shown in the results section, with one exception, all lamella examined had bubbles only in the filamentary arteries (Figures 63 and 64). In the one gill segment that had a bubble in a secondary lamella, it was clear that its origin was in the much larger filamentary artery supplying the secondary lamella. This was observed in a single fish exposed to the highest level of TGP in the Phase II experiments. Thus, the size of nuclei is consistent with the size of gill vessels in which bubbles were found.

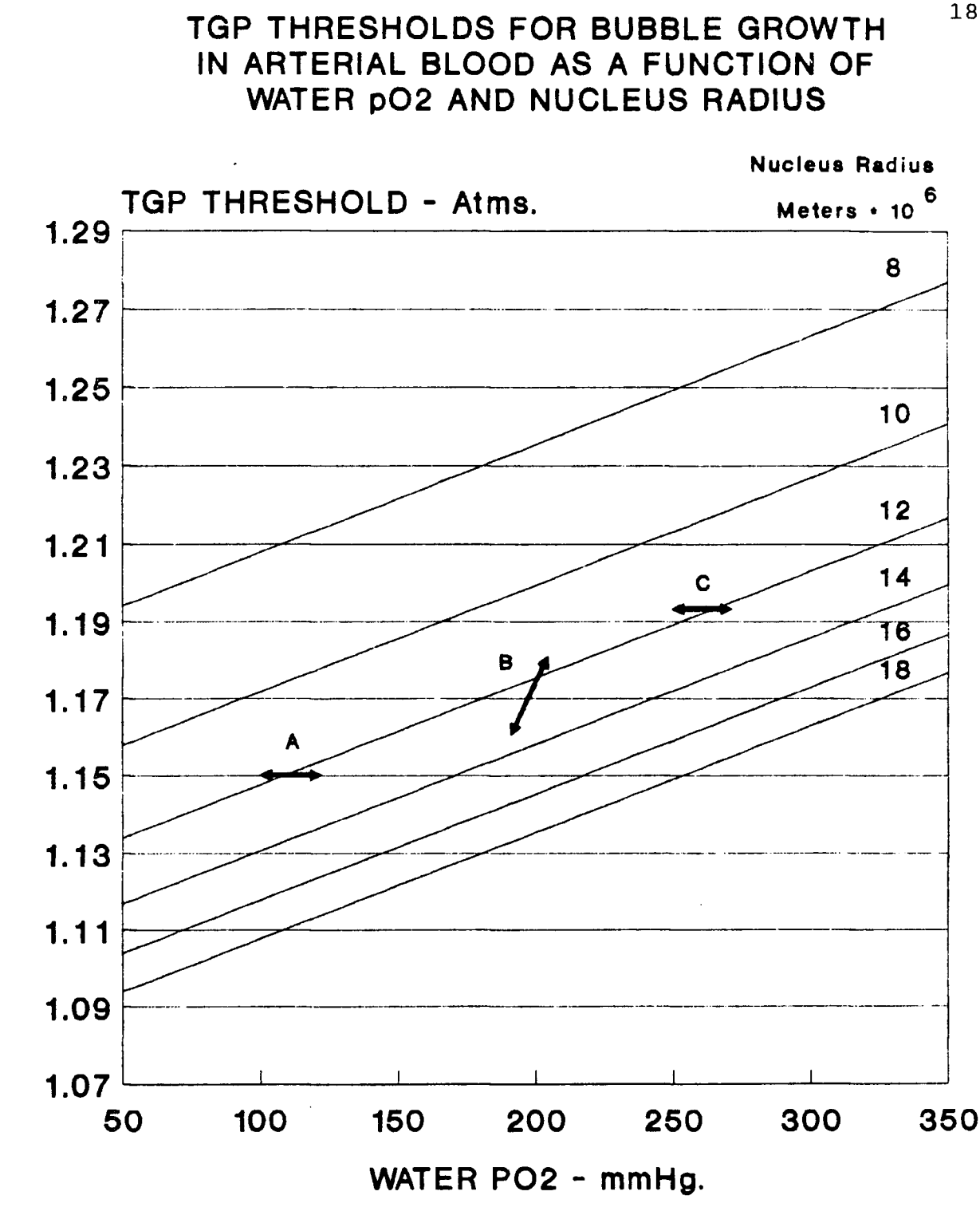


FIGURE 75: TGP Thresholds for Bubble Growth in Arterial Blood.

It should be recognized that bubbles are probably growing in other locations within the animal. As mentioned in the results section above, bubbles were found in muscle, heart and liver tissue samples. Smith (1988) and Nebeker *et al.* (1976b) and Dawley and Ebel (1975) have also shown intravascular bubbles to be present in these organs. As with the gill vascular bubbles, the indicated size of the critical nuclei are larger than the characteristic dimensions of capillary beds. Thus, these nuclei would have to exist in vessels the size of arterioles or larger. Considering the variation of blood pressure and pO_2 in the circulatory system (Section 3, above), the most favorable location still falls to the arterioles just upstream of the capillary beds.

5.7.3 BUBBLE GROWTH AT LOW TGP LEVELS: In the Phase II experiments, there were unique physiological characteristics associated with each form of bubble growth. In many cases these characteristics correlate with time to mortality for each bubble growth threshold.

Sub-dermal Bubble Growth: This form of bubble growth took considerable time to develop at the lower levels of water TGP (1.12 to 1.15 Atms.). However, based on the size of bubbles, it appears that blisters in the buccal cavity grow more quickly than blisters formed on the opercula flaps or other external surfaces. All sub-dermal bubbles were present at TGP levels well below the thresholds for intravascular bubble growth. This implies that the gas transport pathway for this form of bubble growth is directly from the water to skin, and does not involve the circulatory system. If the circulatory system were involved, one would expect sub-dermal bubbles only at the same levels of TGP that initiate intravascular bubble growth. Furthermore, because the bubbles appear just beneath the epithelium cell layer, it is presumed that utilization of oxygen by these cells will have little effect on pO_2 concentrations in the immediate

vicinity of bubble nuclei. In effect, the nuclei involved in these bubbles will see dissolved oxygen tensions close to those of the environmental water. Thus, the value of the uptake ratio (F) will be very close to 1.0. Based on the thresholds indicated for this form of bubble growth, nuclei are again about 12 - 14 μM. in radius, as calculated from Equation 6.

Extracorporeal Bubble Growth Between Gill Lamella: As suggested by the supplemental experiments described in the results section, these bubbles appear to develop early during exposure to supersaturated water. Furthermore, the size of these bubbles appears to be related to water TGP levels with size increasing as TGP increases. This is a reasonable conclusion since, as shown in Section 3, higher TGP levels lead to higher rates of bubble growth. Although extracorporeal bubbles were larger at higher levels of TGP, there were fewer present or they were entirely absent once TGP levels rose above 1.15 Atms. Presumably, this was because the larger bubbles could be easily dislodged by the respiratory water flow. This observation offers an explanation for the two thresholds noted in the database analysis for Chinook salmon over 50 mm. in length. It will be recalled there was a transition from one mechanism of mortality to another at a water TGP of 1.15 Atms. Furthermore, there was a sharp discontinuity in the time to mortality at this TGP. Thus, if extracorporeal bubbles are no longer present above a TGP of 1.15 Atms., sub-dermal bubbles by themselves may not be enough to cause mortality. On the other hand, if a TGP of 1.15 is the threshold for intravascular bubble growth, this mechanism of mortality may become effective above 1.15 Atms. and involve different times to mortality. Thus, a transition from one mechanism of mortality to another may in fact be occurring.

Epithelial Tissue Cells: It is not clear whether extracorporeal bubbles in the gills originate on nuclei associated with the epithelium tissue of the lamella or on nuclei free in the water and carried to those locations by respiration. However, the critical radius back calculated from Equation 6 is again 12 - 14 μM. It is of interest to consider this dimension in relation to the size of tissue cells that make up the mucosal epithelial surfaces of fish gills.

Laurent (1984) describes several types of cells that populate the external surfaces of Rainbow trout gills. It is significant that of these cells, both chloride and squamous cells, have characteristic dimensions on the order of 10 -12 μM. in radius. Chloride cells appear as natural depressions in the epithelial surface. As pointed out by Harvey (1951), nuclei are frequently created by microscopic discontinuities in surfaces. Thus, based on their size and geometry, the chloride cells appear to be likely candidates for bubble nuclei.

When squamous and other epithelial cells die, they are removed from the epithelial surface. The dead cells are eventually replaced by new cells that grow upward from beneath the old cell. However, before the dead cell is completely replaced, there is a temporary discontinuity in the surface about the size of the dead cell. This discontinuity may also become a temporary nucleation site for bubble growth. In Figure 28b of Laurent (1984), both chloride cells and depressions in squamous cells are clearly seen in photographs of the gill external epithelial surface. Although somewhat circumstantial, the cells that make up the external epithelial surface of the gill may, at one time or another, be nucleation sites for extracorporeal bubble growth in the gills.

For intravascular bubble growth, it is not known if nucleation sites are directly associated with the cells lining the circulatory system. The difficulty in establishing such a relationship is that the dimensions of these cells in fish are unknown. The only conclusion to be drawn regarding intravascular bubbles is that the dimensions of the effective nucleation site radius back calculated from the experimental data are of the same order as those of the gill epithelial cells.

It is reasonable to assume that nucleation sites free in the environmental water may be carried into gill lamella by respiratory water flow. For example, water below dam spillways is often filled with bubbles. Many water sources carry a high loading of large silt particles. Thus, nucleation sites of considerable size could be carried into gill lamella and initiate extracorporeal bubble growth at very low levels of TGP. In fact, Bouck (1980) reports observing bubbles in the gills of fish exposed to TGP levels of 1.03 Atms. Unfortunately, he did not specify whether the bubbles were of intravascular or extracorporeal origin.

Time to Mortality: Theory predicts that bubbles grow rapidly in the environmental water (Section 3). The Phase II experiments confirm the presence of these bubbles early in the exposure process. It is difficult, however, to explain the long time to mortality seen in the low ranges of TGP (1.10 to 1.15 Atms.) in terms of extracorporeal bubble growth alone. It appears that the time to mortality is also dependent on the much slower growing sub-dermal bubbles formed in the lining of the mouth. As mentioned in the results section, these bubbles are of a size and number that could easily block the flow of respiratory water. Although this hypothesis cannot be verified with the Phase II results, it is, at present, the most likely explanation for the times to mortality observed in the experiments. This does not imply that

extracorporeal bubbles are uninvolved in mortality. However, it does suggest that extracorporeal bubbles, alone, cannot produce mortality.

5.7.4 BUBBLE GROWTH AT HIGH TGP LEVELS:

Intravascular Bubble Growth: The Phase II experiments demonstrate that, at high levels of TGP, bubbles form in the gill vasculature before the decline in blood pressure that precedes death. Thus, an explanation is needed for this bubble growth in the face of the high vascular system pressures in the gills. As pointed out in the discussion of Phase I results, resting fish perfuse only about 2/3 of the available lamella with blood (Farrell *et al.*, 1979). The remaining lamella are recruited when conditions of hypoxia or exercise demanded additional oxygen. Until that time the lamella are closed off and do not respond until elevated system pressure forces them open (Farrell *et al.*, 1979). Farrell and co-workers observed that the dormant lamella are the secondary lamella lying at the distal ends of the primary lamella. In the Phase II experiments it was noted that intravascular bubble formation also began in the distal ends of the primary lamella. Thus, if these regions are closed off, system pressures may be significantly lower than in the perfused lamella. This would allow bubble growth to begin on nuclei smaller than those required for bubble growth in the higher pressure regions of the gill vasculature (see Figure 1, Section 3). It will be recalled from Section 3 that, once a bubble begins to grow, it effectively becomes a progressively larger nucleation site. It can then continue to grow in the face of lower TGP or increased system pressure. Thus, once the critical size limitation is overcome, bubbles originating in the closed off regions of the lamella can proceed to grow into the perfused, higher pressure regions of the gill vasculature without

collapse. Although much of this argument is based on circumstantial evidence, it offers the most plausible explanation for the existence of these bubbles.

5.7.5 TIME TO MORTALITY: As with mortalities involving combinations of extracorporeal gill and sub-dermal buccal cavity bubble growth, it is difficult to explain the time to mortality based on the time required for intravascular bubbles to grow. As shown in Section 3, times for bubble growth are predicted to be on the order of several hours. On the other hand, except at very high levels of TGP, time to mortality is on the order of a hundred hours or more (Figure 46). There are several possible explanations for the difference between the two. Since the following explanations are not mutually exclusive, the actual cause may involve a combination of these explanations. First, it will be recalled from Section 3 that as bubble volume is added to the vascular system, there will eventually be an increase in system pressure. This was clearly evident in the Phase I experimental results. When system pressure rises, bubble growth will slow or cease completely. The continuation of growth beyond this point will depend on how rapidly water is removed from the cardiovascular system. As shown by the Phase I and Phase II hematocrit measurements, there is a net removal of water from the vascular system at the higher levels of TGP. Thus, bubble growth may be delayed by the process of water removal.

As pointed out earlier, the initially rapid growth of bubbles will occur only until the bubbles block the arteries in which they are located. At that point blood flow will stop and the diffusion of gases to bubbles will be altered. For a bubble in the filamentary arteries of the primary lamella, growth is by direct diffusion of dissolved gases from the water to the bubble. Therefore, these bubbles should continue to grow even after they block the artery. As pointed out earlier, growth may continue even as the

bubbles advance into regions of higher system pressure. In the case of bubbles growing in tissue arteries and arterioles, growth will slow once the artery or arteriole becomes blocked. As pointed out in Section 3, once blood flow stops, the movement of dissolved gas to the bubble will be by diffusion alone through a very long diffusion pathway. Because diffusion gradients continue to decline in this process, rates of bubble growth should slow dramatically.

5.7.6 RESPONSE TO HYPOXIA: As with the Phase I studies, mortality at all levels of TGP above 1.1 Atms. seems to be related to acute hypoxia caused by blockage of respiratory pathways. The mechanisms leading to this blockage appear to be various forms of extracorporeal, sub-dermal and intravascular bubble growth.

Hypoxia between TGP's of 1.10 and 1.15 Atms.: In this range of dissolved gas tension, the decline in pO_2 appears to be the result of reduced delivery of oxygen to the blood caused by blockage of respiratory water flow. The blockage of this flow will also reduce the rate at which fish can remove CO_2 and other metabolic wastes by way of the gills. Therefore, as blood CO_2 concentrations increase, blood pH will decline. It has been shown that, under hypercapnia, Rainbow trout are able to recover from a similar type of respiratory acidosis. That is, with time, they are able to restore blood pH to near normal conditions (Heisler, 1984). This is accomplished through an accumulation of bicarbonate and other ionic adjustments taking place through exchange mechanisms on the gill membrane (Heisler, 1984). In the Phase II experiments, there was no evidence that fish were able to achieve this compensation. This failure to compensate may also be caused by the blockage of respiratory water flow. That is, the blockage impedes the rate of ionic exchanges that normally take place through the gill membrane. In this situation, there would be an internal buildup

of all metabolites including ammonia. Recently, it has been shown that ammonia facilitates the removal of CO_2 (Wright *et al.*, 1987). Ammonia excreted into the water flowing over the gill lamella provides a sink for protons produced by the ionization of carbonic acid. This causes the catalyzed CO_2 - H_2O reaction to favor the production of bicarbonate in the gill water boundary layer. Thus, high CO_2 gradients are maintained between blood and water. A reduction in this gradient would reduce CO_2 excretion by the animal and thereby lower blood pH. These conditions should exist regardless of whether reductions in oxygen uptake and metabolite excretion is the result of reduced water flow over the gills or the blockage of blood flow in portions of the gills.

Response above a TGP of 1.15 Atms.: The Phase II experiments leave little doubt that bubble formation in the gill filamentary arteries occurs above a water TGP of 1.15 to 1.16 Atms. They also confirm that declines in arterial pO_2 and pH continued at the higher levels of TGP with the rate of decline increasing with water TGP. It is also clear that these declines correlate with the appearance of intravascular bubbles. At TGP levels near 1.15 Atms., extracorporeal bubbles are still present in gill lamella and may account for a portion of the pO_2 and pH response. However, as noted in the results, sub-dermal blisters were few or non-existent at the higher levels of TGP. Also, as in the Phase I experiments, extracorporeal bubbles became larger but less numerous as water TGP increased. As discussed earlier, the reason for fewer bubbles may be that the larger bubbles are more easily dislodged by the respiratory water flow. At TGP levels above 1.19 Atms., the number of extracorporeal bubbles were so few that it is difficult to see how they would contribute significantly to the declining arterial pO_2. Thus, the explanation for the declining pO_2 may be the result of reduced blood transit time through the gill lamella. As described in the discussion of Phase I experimental

results, a partial blockage of the gill lamella will increase blood velocities in other lamella. If cardiac output does not fall, blood velocity will increase inversely with the fraction of arteries that are open. That is, velocity will double if half of the arteries are open or quadruple if only one fourth of the arteries are open. Randall (1982) presents information on the equilibration time for the oxygenation of blood in secondary lamella of Rainbow trout. Figure 11 of the reference shows that residence time in secondary lamella must fall below one second before there is a drop in arterial pO_2. Randall calculates that for fully perfused secondary lamella, blood residence time in the lamella is about 3 seconds. Residence time falls to two seconds for a one third blockage of the secondary lamella. The data of Randall (1982) imply a residence time of 0.7 seconds in the secondary lamella is needed to achieve a 60% decline in arterial pO_2. This level of pO_2 decline was common in the Phase II experiments. Assuming an inverse relationship between blockage and residence time, this would correspond to a blockage of at least 75% of the secondary lamella. It was observed that fish in the Series 5 experiments (TGP = 1.19 Atms.), often had three quarters or more of the gill vasculature blocked by bubbles. This would correspond to a level of severity of 3 on the scale used. It will be recalled that bubbles were actually found in the filamentary arteries of the primary lamella. These bubbles would effectively block all secondary lamella at and distal to the bubble. Thus, reduced blood transit time could explain the declines in pO_2 and perhaps the declines in blood pH as well. That is, if oxygen delivery is slowed, it would be reasonable to assume that reduced transit times would also slow the removal of CO_2 and other metabolic wastes.

Respiratory Performance: In addition to the cardiovascular response observed in the various Phase II experiments, hypoxia elicits certain characteristic respiratory adjustments in Rainbow trout. Holeton and Randall (1967a,b) and Thomas and

Hughes (1982) have shown that, under conditions of hypoxia, Rainbow trout will increase respiratory water flow in order to maintain oxygen delivery to the blood. This increase is accomplished by increases in both respiratory frequency and ventilation volume. The largest component of the increase is that in ventilation volume (Holeton and Randall, 1967a,b). This response was clearly observed in the Series 7 experiments where both ventilation volume and frequency increased with time (Figure 65). A comparison was made of the relationship between ventilation volume and frequency of these experiments with data from Davis and Cameron (1971), and Iwama (1986). In the Davis and Cameron experiments, respiration was examined in Rainbow trout exposed to hypoxia. Iwama looked at these parameters as a function of head differences across the respiratory system. The notable difference between the Phase II experimental data and that of Davis and Cameron and Iwama is that fish in the Series 7 experiments had lower ventilation volumes in relation to ventilation frequency. It could be concluded that the lower ventilation volumes of the Series 7 experiments were due to blockage of respiratory water flow by bubbles. However, without a comparison of hematocrit and other physiological parameters among the various fish, this conclusion is somewhat tenuous. On the other hand, the presence of extracorporeal bubbles in the gills will undoubtedly increase the resistance to water flow through the gills. This will eventually require some form of compensatory adjustment by the fish in respiration frequency and/or volume.

Catacholamines: Exercising fish exhibit catacholamine levels at least an order of magnitude greater than those found in fish of the Series 6 experiments (Primmett *et al.*, 1986). Thus, in spite of the trends shown in Figures 71 and 72, the fish do not appear to be highly stressed (as indicated by the catacholamine levels). However, other factors may prevent the measured catacholamine levels from being accurate

indicators of stress. For example, with the blockage of blood flow by bubbles, it is possible that catacholamines released into the blood may not appear at the dorsal aorta. Furthermore, bubbles have been observed in the spinal columns of salmon exposed to supersaturated water (Stroud, Bouck and Nebeker, 1975). These bubbles could interfere with reflex neural pathways and inhibit the release of catacholamines. With this combination of blockage mechanisms it is not surprising that catacholamine levels were not elevated to levels characteristic of stressed fish.

6.0 CONCLUDING DISCUSSION

The two phases of experimental study confirm the existence of thresholds associated with various forms of bubble growth in fish exposed to supersaturated water. These thresholds correlate with thresholds for mortality observed during the experiments as well as with mortality threshold data from the literature. Furthermore, with the definition of a gill oxygen uptake ratio and a critical nucleation site radius, the thresholds can be are predicted using bubble growth threshold equations derived by this author. A mortality threshold occurring at water TGP levels between 1.10 and 1.12 Atms. corresponds to thresholds for the growth of extracorporeal gill bubbles and sub-dermal bubbles in the buccal cavity. A mortality threshold occurring at water TGP levels of 1.15 to 1.18 Atms. correlates with thresholds for intravascular bubble growth occurring in the TGP range of 1.16 to 1.19 Atms.

However, in spite of these correlations, the direct implication of bubbles in the death of fish remains somewhat circumstantial. Still, several experimental observations combined with components of physiological stress measured during the experiments support the hypothesis that bubbles are the principal cause of death. First, all fish exposed to supersaturated water above a TGP of 1.12 Atms. exhibited declines in arterial pO_2 and pH. In most cases, arterial pO_2 measurements taken just before death showed blood pO_2 levels were near or below those corresponding to acute hypoxia for Rainbow trout. This was at times when water pO_2 levels were in equilibrium with atmospheric oxygen or even supersaturated with oxygen. The reductions in blood oxygen tension were clearly due to impaired movement of respiratory gases and not reduced ventilation effort on the part of the fish. Experiments with van Dam respiratory chambers showed that ventilation frequency

and volume were in fact elevated during exposure to supersaturated water. Detailed examination of intravascular as well as extracorporeal bubbles in gill lamella and sub-dermal bubbles in the lining of the mouth indicated that these were the only forms of mechanical blockade that could account for low arterial oxygen. Thus, the only plausible conclusion is that bubbles are involved in the hypoxic response of fish exposed to supersaturated water.

The combination of extracorporeal bubbles in gill lamella and sub-dermal bubbles in the lining of the mouth appear to block respiratory water flow at TGP levels between 1.1 and 1.15 Atms. Above a TGP of 1.15 Atms., intravascular bubbles block blood flow in the filamentary arteries of gill lamella. This should produce an accelerated blood flow in the remaining unblocked arteries. Higher blood velocities reduce transit time in secondary lamella to the point where full oxygenation of the blood does not occur and pO_2 levels drop.

It remains unclear whether hypoxia by itself is the cause of mortality in fish exposed to supersaturated water. As indicated in the discussions of the Phase I and Phase II results, a mechanical blockage of the respiratory pathways more than likely blocks or impedes the removal of CO_2 and ammonia from the animal. In addition, other ionic exchanges at the gill may be disrupted. Therefore, the potential exists for the physiological insult caused by hypoxia to be compounded by other factors. In addition, data from the literature show that neural functions may be disrupted by bubbles forming in the spinal columns of fish. It is conceivable that these bubbles can block the normal responses to the applied stresses and reduce survival capability even further. It should be noted that no GBT related deaths occurred that were not accompanied by decreases in arterial pO2 and pH. Therefore, it is concluded that

hypoxia is a significant contributor to the death of fish exposed to supersaturated water.

The transition from a lower TGP threshold at 1.10 to 1.12 Atms. to an upper threshold between 1.16 and 1.19 Atms. appears to involve a shift in the bubble related mechanisms that lead to mortality. At the lower threshold, sub-dermal bubbles in the lining of the mouth and extracorporeal bubbles in gill lamella appear to be the only forms of blockage to the movement of respiratory gases. As water TGP increases, both the sub-dermal and extracorporeal bubbles become larger while time to mortality decreases. However, at a water TGP above 1.15 Atms., the extracorporeal bubbles become fewer and even disappear as TGP is increased further. This appears to be the result of the larger bubbles being more easily dislodged by respiratory water flow. Furthermore, as water TGP increases above 1.15 Atms., sub-dermal bubbles in the lining of the mouth are not as large at death or are absent entirely. Apparently, this is due to the rapid onset of mortality caused by intravascular bubbles that occur at higher levels of TGP. At water TGP levels somewhere between 1.16 and 1.19 Atms., intravascular bubble formation begins and time to mortality again decreases with increasing TGP. This sequence correlates with data from the literature which suggest that during this transition there is a region of TGP that offers some relief to the fish. That is, for Chinook salmon greater than 50 mm. in length, time to mortality jumps from just a few hours at a TGP of 1.15 Atms. to about 100 hours at slightly higher levels of TGP. However, from the 100 hour level, time to mortality again declines as water TGP increases further. The transition in time to mortality may be due to the reduced number of extracorporeal gill bubbles and smaller sub-dermal bubbles in the mouth.

Using the threshold equations to back calculate an effective nuclei radius, it is found that a radius of 12 to 14 μM. is common to all forms of bubble growth. It was shown that this dimension is also characteristic of epithelium cells lining the mucosal surfaces of the gill. The coincidence of these dimensions is significant and may explain the origin of nucleation sites for bubble growth.

Using 12 μM. as an effective nucleation radius, the theoretical threshold equations can be written in a final form. These are shown below as Equations 16, 17 and 18. The oxygen uptake ratio, F, has been taken as 0.79 for intravascular bubble growth and 0.85 for swimbladder overinflation. These values of F should apply for water pO_2 levels ranging from 70 to 350 mmHg. For higher or lower water pO_2 levels, adjustments will have to be made in the F parameter based on the data of Figure 5 . The equations have been plotted for sea level atmospheric pressure and a water temperature of 5 to 15° C. as shown in Figure 76.

THRESHOLD CRITERIA FOR BUBBLE GROWTH IN THE VASCULAR SYSTEM

$$TGP_{CV} \geq P_{Atm} + 73.1 \cdot h + 0.21 \cdot pO_2 + 83.0$$

Equation 16

THRESHOLD CRITERIA FOR OVERINFLATION OF THE SWIMBLADDER

$$TGP_{SB} \geq P_{Atm} + 73.1 \cdot h + 0.15 \cdot pO_2$$

Equation 17

THRESHOLD CRITERIA FOR BUBBLE GROWTH IN ENVIRONMENTAL WATER

$$TGP_{EW} \geq P_{Atm} + 73.1 \cdot h + 83.0$$

Equation 18

In conclusion, threshold equations 16, 17 and 18 shown plotted in Figure 76 provide useful predictive tools for persons working in the fields of fisheries, aquaculture or environmental impact assessment. For example, hyperoxic environments are now being used to increase carrying capacity of hatchery aquaculture operations. Often this is done by raising dissolved oxygen levels without compensating reductions in dissolved nitrogen levels. Therefore, water can become supersaturated with these dissolved gases. Equations 16, 17 and 18 can be used to determine the limits of oxygenation before symptoms of GBT will appear in fish. In situations where hatchery water is naturally supersaturated, the threshold equations can be used to establish criteria for the design of aeration systems to reduce dissolved gas tensions. Another example of a situation in which the threshold equations would have application involves hydroelectric dams. Often the level of supersaturation below a dam is related to the volume of water that is spilled over the dam (White *et al.*, 1986). In some cases, minor adjustments to the spillway flow can reduce supersaturation below threshold levels and avoid harm to fish populations below the dam. As a final example, unexplained fish kills often occur in the freshwater and marine environments during periods of intense photosynthetic activity. Providing dissolved gas data are available at these times, the threshold equations will show whether or not supersaturation is a factor in these mortalities.

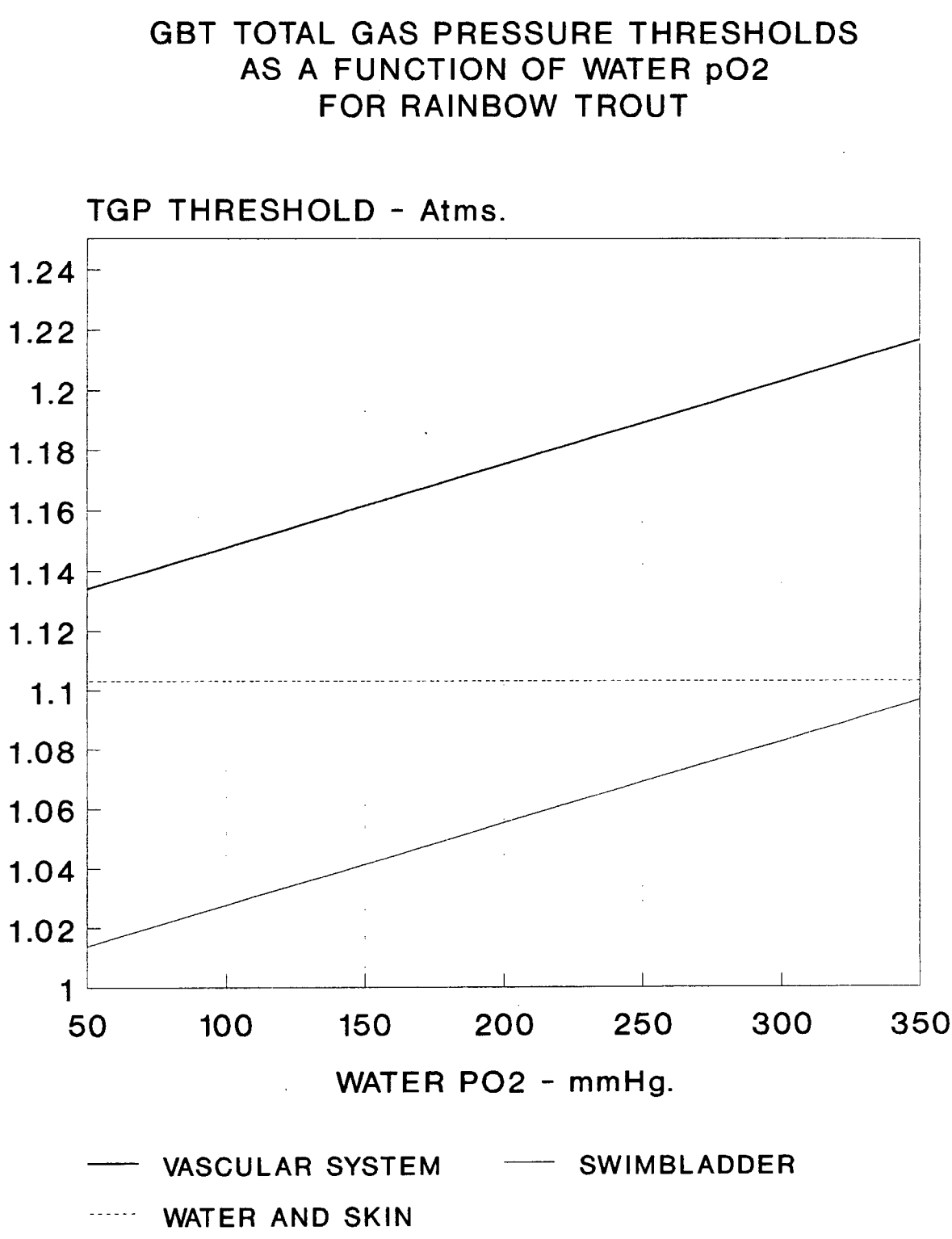


FIGURE 76: Bubble Growth Thresholds as a Function of Water pO_2

REFERENCES

Ackles, K.N. (1973) Proc. Symp. on Blood-bubble Interaction in Decompression Sickness. Canadian DCIEM Conf. 73-CP-960. Canadian Defense Research Board, Downsville, Ont.

Adair, W.D. and Hains, J.J. (1974) Supersaturation values of dissolved gases associated with the occurrence of gas bubble disease in fish in a heated effluent. In. Thermal ecology. Eds. Gibbons, J.W and Sharitz, R.R. U.S.A.E.C. Contribution 030505.

Adamson, A.W. (1967). Physical Chemistry of Surfaces. Academic Press, New York.

Alderdice, D.F. and Jensen (1985) An explanation for the high resistance of incubating salmonid eggs to atmospheric gas supersaturation of water. Aquaculture 49, 85-88.

Alderdice, D.F. and Jensen, J.O.T. (1985) Assessment fo the influence of gas supersaturation on salmonids in the Nechako River in relation to Kemano Completion. Can. Tech. Report Fish. Aquat. Sci. No. 1386.

Alikunhi, K.H.; Ramachadran, V. and Chaudhari, H. (1951) Mortality of carp fry under supersaturation of dissolved oxygen in water. Proc. National Institute of Science, India 17, 261-264.

Altman, P.L. and Dittmer, D.S. (1961). Blood and Other Body Fluids. Federation of American Societies for Experimental Biology.

Altman, P.L. and Dittmer, D.S. (1964). Biological Data Book. Federation of American Societies for Experimental Biology.

Altman, P.L. and Dittmer, D.S. (1971). Respiration and Circulation. Federation of American Societies for Experimental Biology.

Baroin, A.; Garcia-Romeu, F.; Lamarre, T. and Motais, R. (1984) A transient sodium-hydrogen exchange system induced by catacholamines in erythrocytes of rainbow trout, J. Physiol. (London), 356, 21-31.

Becker, C.D. (1973) Columbia river thermal effects study: reactor effluent problems. Journal Of the water pollution control Fed. 45, 850-869.

Beiningen, K.T. and Ebel, W.J. (1970) Effect of the John Day Dam on dissolved nitrogen concentrations and salmon in the Columbia river. Trans. Am. Fish. Soc. 99, 664-671

Berg A. et al. (1984) Supersaturation of dissolved air in the waterways of hydroelectric power plants: causal relationships, detrimental effects and preventative measures. Norwegian Hydrodynamics Laboratories.

Beyer, D.; D'Aoust, B.G. and Smith, L. (1976a) Response of Coho salmon to supersaturation at one atmosphere. in Fickeisen and Schneider (1976) 47-50

Beyer, D.; D'Aoust, B.G. and Smith, L. (1976b) Decompression induced bubble formation in Salmonids. Comparison to gas bubble disease. Undersea Biomedical Res. 3, 321-338

Bird, R.B.; Stewart, W.E. and Lightfoot, E.N. (1960). Transport Phenomena. John Wiley & Sons, New York.

Black, E.C. and Irving, L. (1937) The effect of carbon dioxide upon oxygen capacity of the blood of the carp. Trans. R. Soc. Can. Sect. 5, 29-32.

Blahm, T.H.; McConnell, R.J. and Snyder, G.R. (1973) Effect of gas supersaturated Columbia River water on the survival of juvenile salmonids. Final Report - Part 1. Nat. Mar. Fish. Serv. Environmental Field Station. Prescott, Oregon.

Blahm, T.H.; McConnell, R.J. and Snyder, G.R. (1975) Effect of gas supersaturated Columbia River water on the survival of juvenile Chinook and Coho salmon. NOAA Tech. Rept. NMFS SSRF-688.

Booth, J.H. (1980) The effects of oxygen supply, epinephrine, and acetylcholine on the distribution of blood flow in trout gills. J. exp. Biol. 83, 31-39

Bouck, G.R.; Chapman, G.A.; Schneider, P.W. Jr. and Stevens, D.G. (1970) Observations on gas bubble disease in adult Columbia River sockeye salmon. Pacific Northwest Laboratory, Federal Water Quality Administration, Corvalis, Oregon.

Bouck, G.R. (1980) Etiology of gas bubble disease. Trans. Am. Fish. Soc. 109, 703-707.

Bouck, G.R. (1982) Gasometer: an inexpensive device for continuous monitoring of dissolved gases and supersaturation. Trans. Am. Fish. Soc. 111, 505-516.

Boutilier, R.G.; Heming, T.A. and Iwama, G.K. (1984). Physicochemical parameters for use in fish respiratory physiology. In Fish Physiology, Vol. Xa. Edited by W.S. Hoar and D.J. Randall, Academic Press. 403-430.

Bowser, P.R.; Toal, R.; Robinette, H.R. and Brunson, M.W. (1983) Coelomic distension in channel catfish fingerlings. Prog Fish. Cult. 45, 208-209

Boyer, P.B. (1974) Lower Columbia and lower Snake Rivers, nitrogen gas supersaturation and related data analysis and interpretations. U.S. Army Corps of Engineers, North Pacific Division, Portland Oregon.

Boyle, R. (1670) New pneumatical observations concerning respiration. Philosophical Transactions. 5:2011-2031, 2035-2056.

Cameron, J.N. (1971) Oxygen dissociation characteristics of the blood of rainbow trout, Comp. Biochem. Physiol. 38A, 699-704.

Cameron, J.N. and Davis, J.C. (1970) Gas exchange in rainbow trout (Salmo Gairdneri) with varying blood oxygen capacity. J. Fish. Res. Board. Can. 27, 1069-1085.

Cameron, J.N. and Randall, D.J. (1972) The effect of increased ambient CO_2 tension, CO_2 content and pH in rainbow trout. J. exp. Biol. 57, 673-680.

Casillas, E.; Miller, S.E.; Smith, L.S. and D'Aoust, B.G. (1975). Changes in hemostatic parameters in fish following rapid decompression. Undersea Biomedical Research. 2:267-276.

Casillas, E.; Smith, L.S. and D'Aoust, B.G. (1976a). The response of fish blood cells, particularly thrombocytes, to decompression. Undersea Biomedical Research. 3:273-281.

Casillas, E.; Smith, L.S. and D'Aoust, B.G.(1976b) Effects of stress on salmonid blood clotting mechanisms. in Fickeisen and Schnider (1976) 93-95

Champeney, D.C. (1973). Fourier Transforms and their physical applications. Academic Press, New York.

Colt, J.E. and Cornacchia, J.W. (1984). The effects of dissolved gas supersaturation on larval striped bass, Morone saxatilis (Walbaum). J. Fish dis. 7:15-27.

Colt, J.E. (1983). The computation and reporting of dissolved gas levels. Water Res. 17:841-849.

Colt, J.E. (1984) Computation fo dissolved gas concentrations in water as functions fo temperature, salinity and pressure. Special publication No. 14, Amer. Fish. Soc., Bethesda Md.

Colt, J.E.; Bouck, G and Fidler, L.E. (1986) Review of current literature and research on gas supersaturation and gas bubble trauma. U.S. Dept. of Energy. Bonneville Power Admin. Div. of Fish and Wildlife.

Cornacchia, J.W. and Colt, J.E. (1984) The effects of dissolved gas supersaturation on larval striped bass, *Morone saxatilis*. J. Fish Diseases. 7, 15-27.

Clift, R.; Grace, J.R. and Weber, M.E. (1978). Bubbles, Drops and Particles. Academic Press, New York.

Coutant, C.C. and Genoway, R.B. (1968) Final report on the exploratory study of interaction of increased temperature and nitrogen supersaturation on the mortality of adult salmonids. U.S. Bur. Commercial Fish. Seattle, PAC N.W. Lab. Richland Wash.

Davis, J.C. and Cameron, J.N. (1971) Water flow and gas exchange at the gills of rainbow trout (Salmo gairdneri). J. exp. Biol. 54, 1-18.

Dawley, E.M. and Ebel, W.J. (1975) Effects of various concentrations of dissolved gas on juvenile chinook salmon and steelhead trout. U.S. Fish. Bull. 73, 787-796.

Dawley, E.M.; Schiewe, M. and Monk, B. (1976). Effects of long term exposure to supersaturation of dissolved atmospheric gases on juvenile Chinook salmon and Steelhead trout in deep and shallow tank tests. In: Fickeisen & Schneider (1976): 1-10.

Dawley, E.M. and Ebel, W.J. (1975). Effects of various concentrations of dissolved atmospheric gas on juvenile chinook salmon and steelhead trout. United States National Marine Fisheries service Fishery Bulletin. 73:787-796.

Dell, M.B.; Erho, M.W. (1975) Occurrence of gas bubble disease symptoms on fish in mid Columbia reservoirs. Grant, Douglas, and Chelan County Public Utility Districts. Ephrata and Wenatchee, Washington.

DeMont, J.D. and Miller, R.W. (1972) First reported incidence of gas bubble disease in the heated effluent of a steam electric generating station. Proc. of the Annual Conf. S.E. assoc. of Game and Fosh Comm. 25, 392-399

D'Aoust, B.G. and Smith, L.S. (1974) Bends in fish. Comp. Biochem. and Physiol. 49, 311-321

Ebel, W.J. (1969) Supersaturation of nitrogen in the Columbia River and its effect on salmon and steelhead trout. U.S. Natl. Marine Fish. Serv. Bulletin 68, 1-11

Ebel, W.J.(1971) Dissolved nitrogen concentrations in the Columbia and Snake Rivers in 1970 and their effect on chinook salmon and steelhead trout. NOAA. Tech. Report. NMFS SSRF 646.

Ebel, W.J. (1979) Effects of atmospheric gas supersaturation on survival of fish and evaluation of proposed solutions. 5th. Prog Report, U.S. Army Corps of Engineers.

Ebel, W.J.; Dawley, E.M. and Monk, B.H. (1971) Thermal tolerance of juvenile salmon in relation to nitrogen supersaturation. U.S. fish and Wildlife Service Fisheries Bulletin. 69, 833-843.

Ebel, W.J.; Kroma, R.W. and Raymond, H.L. (1973) Evaluation of fish protective facilities at Little Goose Dam and review of other studies relating to protection of juvenile salmonids in the Columbia and Snake Rivers, 1973. National Marine Fisheries Service. Northwest Fisheries Center, Seattle Washington.

Ebel, W.J.; Raymond, H.L. Monan, G.E.; Farr, W.E. and Tanonaka, G.K. (1975) Effect of atmospheric gas supersaturation caused by dams on salmon and steelhead trout of the Snake and Columbia Rivers. National Marine Fisheries Service. Northwest Fisheries Center, Seattle Washington.

Ebel, W.J.; Beningen, K.T.; Bouck, G.R.; Penrose, W.R. and Weitkamp, D.E. (1979) Gases, total dissolved. In: Thurston, R.V.; Russo, R.C.; Fetterolf, C.M., Edsall, T.A. and Barber, Y.M. editors. A review of the EPA red book: quality criteria for water. American Fisheries Soc.

Ebeling, G. (1954) Sauerstoff-Ubersattigung in fliessender und stehenden Gewassern Vom Wasser. 21, 84-99.

Eckert, R. (1983) Osmoregulation and excretion. In: Animal Physiology, Eckert, R. and Randall, D.J.. W.H. Freeman, San Francisco: 483-543.

Eddy, F.B.; Lomholt, J.P.; Weber, R.E. and Johansen, K. (1977) Blood respiration properties of rainbow trout kept in water of high CO_2 tension. J.exp Biol. 67, 37-47.

Epstein, P.S. and Plesset, M.S. (1950). On the stability of gas bubbles in liquid gas solutions. J. Chem. Phy. 18(11):1505-1509.

Fairbanks, R.B. and Lawton, R.P. (1977) Occurrence of large striped mullet in Cape Cod Bay, Massachusetts. Chesapeake Sci. 18, 309-310.

Fange, R. (1966) Physiology of the swimbladder. Physiol Rev. 46, 299-322.

Farrell, A.P. (1988) Personal Communication.

Farrell, A.P.; Sobin, S.S.; Randall, D.J. and Crosby, S. (1980) Intralamellar blood flow patterns in fish gills. Am. Jour. of Physiol. 239, 428-436.

Farrell, A.P.; Daxboeck, C. and Randall, D.J. (1979) The effect of input pressure and flow on the pattern and resistance to flow in the isolated perfused gill of a teleost fish. Jour. Comp. Physiol. 133, 233-240

Feathers, M.G. and Knable, A.E. (1983) Effects of depressurization upon largemouth bass. North Amer. J. Fish. Man. 3, 86-90

Feigl, E.O. (1974) Physics of the cardiovascular system. In: Ruch, T.C. and Patton, H.D. eds. Physiology and Biophysics. 20th. ed. Vol.2 Philadelphia: Saunders.

Ferguson, J.K.W. and Black, E.C. (1941) The transport of CO_2 in the blood of certain fresh water fishes. Biol. Bull. 80, 139-152.

Fickeisen, D.W. and Schneider, J.M. (1976). Gas Bubble Disease. CONF - 741033. Technical Information Center, Energy Research and Development Administration, Oak Ridge, Tennessee, USA.

Fidler, L.E. (1983) Design and analysis methods for hatchery aerations systems. Report to Dept. Fisheries and Oceans, Canada. Salmon Enhancement Program.

Fidler, L.E. (1985). Biophysical phenomena associated with gas bubble trauma in fish. M.Sc. Thesis, University of British Columbia, Vancouver, B.C., Canada.

Folkow, B. and Neil, E. (1971). Circulation. Oxford University Press.

Foster, R.E. and Steen, J.B. (1969) The rate of the Root shift of ell red cells and haemoglobulin solution. J. Physiol. 204, 259-282.

Harvey, H.H. and Cooper, A.C. (1962) Origin and treatment of a supersaturated river water. Progress Report 9., Intnl. Pacific Salmon Fisheries Commission., Vancouver, Canada.

Harvey, E.N.; Barnes, D.K.; McElroy, W.D. Whiteley, A.H; Pease, D.C. and Cooper, K.W. (1944) Bubble formation in animals. Jour. Cell. and Comp. Physiol. 24,

Harvey, E.N. (1951) Physical factors in bubble formation. In: Decompression sickness. Ed. F.J. Fulton. Saunders, Philadelphia.

Harvey, H.H. (1967) Supersaturation of lake water with a precaution to hatchery usage. Trans. Am. Fish Soc. 96, 194-201.

Harvey, H.H. (1975). Gas disease in fish - a review. In: Chemistry and physics of aqueous solutions. Edited by W.A. Adams. The Electrochemical Society, Princeton, New Jersey. 450-485.

Harvey, H.H (1963) Pressure in the early life stage of salmon. Ph. D. Thesis. The University of British Columbia. Dept. of Zoology.

Hauck, K. (1986) Gas bubble disease due to helicopter transport of pink salmon. Trans. Am. Fish. Soc. 115, 630-635

Heggberget, T.G. (1984) Effect of supersaturated water on fish in river Nidelva, southern Norway. J. Fish. Biol. 24, 65-74

Heisler, N. (1984). Acid - base regulation in fishes. In: Fish Physiology, Vol X. Edited by W.S. Hoar and D.J. Randall. Academic Press. 315-401.

Heming, T.A.: (1984 a). The role of fish erythrocytes in transport and excretion of carbon dioxide. Ph.D. Thesis, University of British Columbia, Vancouver, B.C.

Heming, T.A.: (1984 b). Unpublished Data. Dept. of Zoology, University of British Columbia, Vancouver, B.C.

Heming, T.A.; Randall, D.J. and Mazeaud, M.M. (1987) Effects of adrenaline on ionic equilibria in red blood cells of rainbow trout. Fish Physiol. Biochem. 3(2):83-90.

Hemmingsen, B.B.; Stienberg, N.A. and Hemmingsen, E.A. (1985) Intracellular gas supersaturation tolerances of erythrocytes and research ghosts. Biophys. J., 47, 491-496.

Hemmingsen, B.B. (1986) Promotion of gas bubble formation by ingested nuclei in ciliate, *Tetrahymena pyriformis*. Cell Biophysics. 8, 189-200

Hills, B.A. (1977) Decompression Sickness; John Wiley & Sons. New York.

Hobe, H; Wood, C.M. and Whetly, M.G. (1984) The mechanisms of acid-base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. Resp. Physiol.

Holl, K. (1955) Chemische Untercuchungen an kleinen Fliessgewassern. International Association of theoretical and applied limnology proceedings. 12, 360-372.

Holmes, W.N. and Donaldson, E.M. (1969). The body compartments and the distribution of electrolytes. In: Fish Physiology, Vol. I. Edited by W.S. Hoar and D.J. Randall, Academic Press. 1-79.

Holton, G.F. (1972). Gas exchange in fish with and without hemoglobin. Respir. Physiol. 14:142-150.

Holton, G.F. (1980). Oxygen as an environmental factor of fishes. In: Environmental Physiology of Fishes. Edited by M.A.Ali. Plenum, New York. 7-32.

Holeton G.F. and Randall, D.J. (1967a) The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. exp. Biol. 46, 317-327.

Holeton G.F. and Randall, D.J. (1967b) Changes in blood pressure in the rainbow trout during hypoxia. J. exp. Biol. 46, 297-305.

Hsieh, D.Y. (1965). Some analytical aspects of bubble dynamics. J. Basic Eng. Trans. ASME. Vol. 87 No. 4:991-1005.

Isaia, J. (1984) Water and nonelectrolyte permeation. In: Fish Physiology Vol 10B. eds. Hoar, W.S. and Randall, D.J., Academic Press.

Iwama, G. (1986) Strategies for acid-base regulation in fishes. Ph. D. Thesis. The University of British Columbia. Dept. of Zoology.

Jarnefelt, V.H. (1948) Der Einfluss der Stromschnellen auf den Sauerstoff und Kohlensauregehalt und das pH des Wassers im Flusse Vuoski. International association of theoretical and applied limnology proceedings. 10, 210-215

Jensen, J. (1980). Effect of TGP and total water hardness in Steelhead eggs and alevins. A progress report. Proc. N. W. Fish Culturists Conf. Courtney, B.C., Canada. 15-22.

Jensen, J.O.T.; Halley, A.N. and Schnute, J. (1985) Literature data on salmonid response to gas supersaturation and ancillary factors. Can. Data Report of Fish. and Aquatic Sci. No. 501

Jensen, J.O.T.; Schnute, J. and Alderdice, D.F. (1986). Assessing juvenile salmonid response to gas supersaturation using a general multivariant dose - response model. Can. J. Aqua. Sci. 43:1694-1709.

Johansen. K. and Hansen,D. (1967) Functional anatomy of the hearts of lungfish and amphibians. Am. Zool. 8, 191-210

Johnson, D.W and Katavic, I. (1984) Mortality, growth and swimbladder stress syndrome of sea bass larvae under varied environmental conditions. Aquaculture. 38, 67-78.

Jones, D.R. and Randall, D. J. (1978). The respiratory and circulatory systems during exercise. In: Fish Physiology, Vol. VII. Edited by W.S. Hoar and D.J. Randall, Academic Press. 425-501.

Kiceniuk, J.W. and Jones, D.J. (1977). The oxygen transport system in trout during sustained exercise. J. exp. Biol. 69:247-260.

Kiceniuk, J.W. (1969) Unpublished Data.

Kirsch, R. and Nonnote, G. (1977) Cutaneous respiration in three freshwater teleosts. Resp. Physiol. 29, 339-354

Knittel, M.D.; Chapman, G.A. and Garton, R.R. (1980) Effects of hydrostatic pressure on steelhead survival in air supersaturated water.Trans. Am. Fish. Soc. 109, 755-759.

Kolbeinshavn, A. and Wallace, J.C. (1985) Observations of swimbladder stress syndrome in arctic char induced by inadequate water depth. Aquaculture. 36, 259-261.

Kraul, S. (1983) Results and hypothesis for the propagation of the grey mullet. L. Aquaculture. 30, 273-284.

Laurent, P. (1984) Gill internal morphology. In: Fish Physiology- Vol 10A. Eds. Hoar, W.S. and Randall, D.J., Academic Press.

Lindroth, A. (1957) Abiogenic gas supersaturation of river water. Archiv. fur Hydrobiologie. 53, 589-597.

Makren, A. (1974). Hemoglobins, myoglobins and haptoglobins. In: Clinical Chemistry - Principles and Technics. 2nd. ed. Edited by: R.F. Henry, D.C. Cannon and J.W. Winkelman. Harper and Row. 1128-1135.

Marcello, R.A. Jr. and Fairbanks, R.B. (1976) Gas bubble disease in Atlantic menhaden at a coastal nuclear power plant. In: Fickeisen and Schneider (1976).

Marsh, M.C. (1910) Notes on the dissolved gas content of water in its effects upon fish. Bull. U.S. Bureau of Fisheries. 28, 891-906

Matsue, Y.; Egusa, S.and Saeki (1953) On nitrogen gas contents dissolved in flowing water of artesian wells and springs. Bull. Japanese Soc. Sci. Fish. 19, 439-444.

Mazeaud and Mazeaud (1981) Adrenergic responses to stress in fish. In: Stress in Fish, Ed. A.D, Pickering. Academic Press.

Meekin, T.A. and Turner, B.K. (1974). Tolerance of Salmonid eggs, juveniles and squawfish to supersaturated nitrogen. Wash. Dept. of Fisheries Tech Rept. 12:78-126.

Meisel, S.; Nir, A. and Kerem, D. (1981) Bubble dynamics in perfused tissue undergoing decompression. Resp. Physiol. 43, 89-98.

Miller, R.W. (1974) Incidence and cause of gas bubble disease. In: Gibbons, J.W. and Sharitz, R.R., Ed. Thermal Ecology. Conf. 730505 U.S. EPA, Washington, D.C.

Mott, J.C.: (1957). In: The Physiology of Fishes; Edited by M.E. Brown. Academic Press, New York.

Nakano and Tomlinson (1968) Catacholamine concentrations in rainbow trout, *Salmo gairdneri* in relation to physical disturbances. J. Fish. Res. Bd. Can. 25, 603

Nebeker, A. V.; Bouck, G.R. and Stevens, D.G. (1976) Carbon dioxide and oxygen-nitrogen ratios as factors affecting salmon survival in air supersaturated water. Trans. Am. Fish. Soc. 105, 425-429.

Nebeker, A.V.; Stevens, D.G. and Baker, R.J (1976b) Survival of salmon smolts in sea water after exposure to air supersaturated water. Prog. Fish-Cult. 41, 30-31.

Nebeker. A.V.; Hauck, A.K. and Baker, R.J. (1979) Temperature and oxygen-nitrogen gas ratios affecting fish survival in air supersaturated water. Water Res. 13, 299-303.

Nebeker, A.V.; Stevens, D.G. and Stroud, R.K. (1976) Effects of air supersaturated water on adult sockeye salmon. J. Fish. Res. Board. Ca. 33, 2629-2633.

Nebeker, A.V.; Andros, J.D.; McCrady, J.K. and Stevens, D.G. (1978) Survival of steelhead trout eggs, embryos, and fry in air supersaturated water. J. Fish. Res. Board Can. 35, 261-264.

Nebeker, A.V. and Brett. J.R. (1976) Effects of air supersaturated water on survival of Pacific salmon and steelhead smolts. Trans. Am. Fish. Soc. 105, 338-342.

Newcomb, T.W. (1976). Changes in blood chemistry of juvenile steelhead, *Salmo gairdneri*, following sublethal exposure to nitrogen supersaturation. In: Fickeisen & Schneider (1976): 96-100.

Nikinmaa, M. (1982) The effects of adrenaline on red cell volume and concentration gradient of protons across the red cell membrane in the rainbow trout. Mol. Physiol., 71A, 353-356.

Nikinmaa, M. and Huestis (1884) Adrenergic swelling in nucleated erythrocytes: Cellular mechanisms in a bird, domestic goose and two teleosts, striped bass and rainbow trout. J. exp. Biol., 113, 215-224.

Perry, R.H. and Chilton, C.H. (1973). Chemical Engineers Handbook. 5th. ed. McGraw Hill, New York.

Peterson, H. (1971). Smolt rearing methods, equipment and techniques used successfully in Sweden. Atlantic Salmon Foundation Special Publication Series. 2(1):32-62.

Philp, B.; Inwood, M.J., and Warren, B.A. (1972). Interactions between gas bubbles and components of the blood: Implications in decompression sickness. Aerospace Medicine, Sept. 1972. 946-953.

Piper, J. and Scheid, P. (1984) Model analysis of gas transfer in fish gills. In: Fish Physiology Volume 10A. eds. Hoar, W.S. and Randall, D.J., Academic Press.

Plesset, M.S. and Zwick, S.A. (1954). The growth of vapor bubbles in superheated liquids. J. Appl. Physics. Vol. 25, No. 4.

Plesset, M.S. (1964). In: Cavitation in real fluids. Edited by R. Davis. American Elsevier Co., New York.

Primmett, D.N.R.; Randall, D.J.; Mazeaud, M.M. and Boutilier, R.G. (1986) The role of catacholamines and oxygen transport in rainbow trout (*Salmo gairdneri*). J. exp. Biol. 123, 139-148.

Rabiner, L.R. and Gold, B. (1975). Theory and application of digital signal processing. Prentice Hall, Englewood Cliffs, N.J.

Railo, E.; Nikinmaa, M. and Soivio, A. (1985). Effects of sampling on blood parameters in Rainbow trout, Salmo gairdneri. J. exp. Biol. 126:489-512.

Randall, D.J. (1982). Blood flow through gills. In: Soc. Exp. Biol. Sem. Series 16, Gills. (Ed. D.F. Houlihan; Rankin, C. and Shuttleworth, T.J.). Cambridge University Press. 173-191.

Randall, D.J. (1984). Oxygen and carbon dioxide transfer across fish gills. In: Fish Physiology, Vol X. Edited by W.S. Hoar and D.J. Randall. Academic Press. 263-314.

Randall, D.J. (1970 a). Gas exchange in fish. In: Fish Physiology, Vol IV. Edited by W.S.Hoar and D.J. Randall. Academic Press. 253-292.

Randall, D.J. (1970 b). The circulatory system. In: Fish Physiology, Vol IV. Edited by W.S. Hoar and D.J. Randall. Academic Press. 133-172.

Randall, D.J. and Jones, D.R. (1973). The effect of deafferentation of the pseudobranch on the respiratory response to hypoxia and hyperoxia in the trout. Respiratory Physiology. 17:291-301.

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

Randall, D.J. and Daxboeck, C. (1984) Oxygen and carbon dioxide transfer across fish gills. In: Fish Physiology, Vol. XA. Eds. Hoar, W.S. and Randall, D.J. Academic Press. 263-314.

Reid, R.C,; Prausnitz, J.M. and Sherwood, T.K. (1977). The properties of Gasesand Liquids. 3rd. ed. McGraw Hill Co., New York.

Reintjes, J.W. (1969) Synopsis of biological data on the Atlantic menhaden. Circular 320, U.S. Fish and Wildlife Service.

Renfro, W.C. (1963). Gas bubble mortality of fishes in Galveston Bay, Texas. Trans. Amer. Fish. Soc. 92:320-322.

Riggs, A. (1979) Studies of the hemoglobins of Amazonian fishes. an overview. Comp. Biochem. Physiol. 62A, 257-271.

Rosenthal, H. (1988) personal communication.

Rucker, R.R. (1975a) Gas bubble disease mortalities of coho salmon in water with constant total gas pressure and different oxygen-nitrogen ratios. U.S. fish. Bull. 73, 915-918.

Rucker, R.R. (1975b) Excess nitrogen gas in water not a cause of coagulated yolk disease in chinook salmon. Prog. Fish cult. 37, 101-102.

Rucker, R.R and Tuttle, E.M. (1948) Removal of excess nitrogen in a hatchery water supply. Prog. Fish Cult. 10, 88-90.

Rucker, R.R. and Kangas, P.M. (1974) Effect of nitrogen supersaturated water on coho and chinook salmon. Prog. Fish Cult. 36, 152-159.

Rukavina, J. and Varenika, D. (1956) Air bubble disease of trout of the source of the river Bosna Acta Ichthyologica Bosniae et Hercegovinae 1(7) 5-12.

Schiewe, M.H. (1974). Influence of dissolved atmospheric gas on swimming performance of juvenile chinook salmon. Trans. Amer. Fish. Soc. 103:717-721.

Schmassermann, H. (1951) Untersuchungen uber den Stoffhaushalt Gewasser. Schweizerische Zeitschrift fur Hydrologie. 13, 300-335.

Schnute, J. and McKinnell (1984). A biological meaningful approach to response surface analysis. Can. J. Fish. Aquat. Sci. 41:6.

Shelton, G., Jones, D.R. and Milsom, W.K. (1986) Control of breathing in ectothermic vertebrates. In: Handbook of Physiology - The respiratory system II, American Physiological Society. 857-909.

Shirahata, S. (1966). Experiments on nitrogen gas disease with Rainbow trout fry. Bulletin of the Freshwater Research Laboratory, Tokyo. 15:197-211.

Shrimpton, M.J.; Randall, D.J. and Fidler, L.E. (1988). Factors affecting swimbladder volume in Rainbow trout (*Salmo gairdneri*) held in gas supersaturated water. (In preparation).

Smith, C.E. (1988) Histopathology of gas bubble disease in juvenile Rainbow trout. U.S. Fish and Wildlife Service. Fish Technology Center, Bozeman, Montana. in press.

Smith, F.M. and 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.

Smith, C.G.; Lewis, W.M. and Kaplan, H.M. (1952). Comparative morphologic and physiologic study of fish blood. Prog. Fish Cult. 14:169-172.

Soivio, A.; Westman, K. and Nyholm, K. (1972). Improved methods of dorsal aorta catheterization: Haematological effects followed for three weeks in Rainbow trout, Salmo gairdneri. Finnish Fish Res. 1:11-12.

Sovio, A; Nikinmaa, M.; Nyholm, K. and Westman, K. (1981) The role of gills in the responses of (*Salmo gairdneri*) during moderate hypoxia. Comp. Biochem. Physiol. 70A, 133-139.

Sovio, A.; Westman, K. and Nyholm, K. (1972) Improved methods of dorsal aorta catheterization, Haemotological effects followed fro three weeks in Rainbow trout (*Salmo gairdneri*). Finnish Fish. Res. 1, 11-21.

Sprague, J.B. (1969) Measurement of pollutant toxicity to fish. I. bioassay methods for acute toxicity. Water Res. 3, 793-821.

Spry, D.J. and Wood, C.M. (1985) Ion flux rates, acid-base status, and blood gases in Rainbow trout (*Salmo gairdneri*) exposed to toxic zinc in natural soft water. Can. J. Fish. Aquat. Sci. 42, 1332-1341.

Steen, J.B. (1970) The swim bladder as a hydrostatic organ. In: Fish Physiology Vol. 4., eds. Hoar, W.S. and Randall, D.J., Academic Press.

Steen, J.B. and Sund, T. (1977). Gas deposition by counter-current multiplication in the ell swim-bladder: Experimental verification of a mathematical model. J. Physiol. 267: 697-702.

Stevens, E.D. (1968) Cardiovascular dynamics during swimming in fish, particularly Rainbow trout (*Salmo gairdneri*) Ph. D. thesis, The University of British Columbia. Dept. of Zoology.

Stevens, E.D. and Randall, D.J. (1967). Changes of gas concentrations in blood and water during moderate swimming activity in Rainbow trout. J. exp. Biol. 46:307-315.

Stroud, R.K. and Nebeker, A.V. (1976) A study of the pathogenesis of gas bubble disease in steelhead trout. In: Fickeisen and Schneider (1976)

Stroud, R.K.; Bouck, G.R. and Nebeker, A.V. (1975) Pathology of acute and chronic exposure of salmonid fishes to supersaturated water.In: Adams, W.A., Editor. Chemistry an physics of aqueous gas solutions. The Electrochemical Society. Princeton, N.J., U.S.A.

Supplee, V.G. and Lightner, D.V. (1976) Gas bubble disease due to oxygen supersaturation in raceway reared California brown shrimp. Prog. Fish Cult. 28, 198-199.

Thomas, S. and Hughes, G.M. (1982) A study of the effects of hypoxia on acid-base status of rainbow trout blood using an extracorporeal blood circulation. Resp. Physiol. 49, 371-382.

Tuurala, H. (1983) Structure and blood circulation of the secondary lamella of (*Salmo gairdneri*) gills in relation to oxygen transfer. University of Helsinki, Dept. of Zool/Physiol.

Tufts, B. L. (1988) Ion exchange mechanisms for the control of volume and pH in fish and amphibian erythrocytes. Ph. D. Thesis. The University of British Columbia. Dept. of Zoology.

Van, R.D. and Clark, H.G. (1975) Bubble growth and mechanical properties of tissue in decompression. Undersea Biomedical Research. 2(3), 185-194.

Warren, C. and Doudoroff, P. (1971) Biology and water pollution control. W.B. Saunders Co. Toronto, Ont., 434.

Weber, R.F. (1982) Intraspecific adaptations of hemoglobin function in fish to oxygen availability. In: Exogenous and Endogenous Influences on metabolic and neural Control. eds. Addink, A.D.F. and Sprank, N. 87-102, Pergamon Press.

Weiss, R.F. (1970). The solubility of nitrogen, oxygen and argon in water and sea water. Deep Sea Research. 17:721-735.

White, R.G., Phillips, G. and Liknes, G. (1986). The effects of supersaturation of dissolved gases on the fishery of the Bighorn river downstream of the Yellowtail afterbay dam. Montana Cooperative Fishery Research Unit, Montana State University, Bozeman Montana.

Weitkamp, D.E. and Katz, M. (1980). A review of dissolved gas supersaturation literature. Trans. Am. Fish. Soc. 109:659-702.

Weitkamp, D.E. (1974) Dissolved gas supersaturation in the Columbia River system: salmonid bioassay and depth distribution studies, 1973 and 1974. Parametrix, Inc. Report to Utility Cooperative, Idaho Power Company., Boise, Idaho.

Weitkamp, D.E. (1976) Dissolved gas supersaturation: live cage bioassays of Rock Island Dam, Washington. In Fickeisen and Schneider (1976)

Westman, J.R. and Nigrelli, R.F. (1955) Preliminary studies fo menhaden and their mass mortality in Long Island and New Jersey waters. New York Fish and Game J., 2: 142-153.

White, R.G.; Phillips, G.; Liknes, G. and Sanford, S. (1986) The effects of supersaturation of dissolved gases on the fishery of the Bighorn River downstream of the Yellowtail Dam. Annual report to U.S. Bureau of Reclamation.

Wolf, K. (1963) Physiological salines for freshwater teleosts. Prog Fish Cult. 25, 135-140.

Wood, C.M. and Jackson, E.B. (1980). Blood acid base regulation during environmental hypoxia in the Rainbow trout, Salmo gairdneri. Respir. Physiol. 42:351-372.

Wood, C.M.; Wheatly, M.G. and Hobe, H. (1984) The mechanisms of acid base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. III. Branchial exchanges. Respir. Physiol. 55, 175-192.

Woodbury, L.A. (1941). A sudden mortality of fishes accompanying a supersaturation of oxygen in Lake Waubesa, Wisconsin. Trans. Amer. Fish. Soc. 71:112-117.

Wright, P.A., Heming, T.A. and Randall, D.J. (1986). Downstream pH changes in water flowing over the gills of Rainbow trout. J. exp. Biol. 126:489-512.

Wright, P.A. and Randall, D.J. (1987) Ammonia distribution in fish. Fish Physiol. Biochem. 3 (3), 107-120.

Wyatt, E.J. and Beiningen, K.T. (1969) A nitrogen gas disease catastrophe. Proceedings the 20th. annual northwest fish culture conference. 71-72.

Yamamoto, K.I.; Itzawa, Y. and Kobayashi, H. (1980) Supply of erythrocytes into the circulatory blood from the spleen of exercising fish. Comp. Biochem. Physiol. 65A, 5-11.

Yang, W.J. and Liang, C.Y. (1972) Dynamics of the dissolution of gas bubbles or pockets in tissue. J. Biochem. 5.

Yount, D.E. (1979) Application of a bubble formation model to decompression sickness in rats and humans. Aviation, Space and Environmental Medicine. January.

Yount, D.E. and Yeung, C.M. (1979) Determination of the radii of gas cavitation nuclei by filtering gelatin. J. Acoustic Soc. Am. 65 (6).

Yount, D.E. (1981) A model for microbubble fission surfactant solutions. J. Colloid and Interfacial Science 91 (2)

Zaitsev, Y.P. (1971) Marine Neustonology, Israel Program for Scientific Translations, Jerusalem.

APPENDIX A

DERIVATION OF BUBBLE GROWTH THRESHOLD EQUATIONS

This derivation begins with the development of an equation for the growth of bubbles in the cardiovascular systems of fish exposed to supersaturated water. In the derivation, it will be assumed that bubble growth occurs under isothermal conditions and that inertial effects are negligible (i.e. the growth process is diffusion limited - Hseih, 1965). Furthermore, it will be assumed that the diffusing gases are oxygen, nitrogen and water vapor and that they obey the perfect gas law. In addition, it will be assumed that the principal resistance to gaseous diffusion lies in the water or plasma phase, that the interior of the bubble is of uniform composition and the gas and liquid phases are inviscid. Finally, the partial pressure of water vapor in the bubble will be taken as the saturated vapor pressure of water at the isothermal temperature.

By the perfect gas law the number of moles of oxygen in a bubble (n_{Ob}) within the vascular system is given by:

$$n_{Ob} = \frac{4 \cdot \pi \cdot r^3 \cdot p_{Ob}}{3 \cdot R' \cdot T} \qquad \text{Eq. A1}$$

where r is the radius of the bubble.

Similarly, for nitrogen:

$$n_{Nb} = \frac{4 \cdot \pi \cdot r^3 \cdot p_{Nb}}{3 \cdot R' \cdot T} \qquad \text{Eq. A2}$$

In Equations A1 and A2, p_{Ob} and p_{Nb} are the partial pressures of oxygen and nitrogen in the bubble, R' is the universal gas constant and T is the absolute temperature. The total pressure of gases in the bubble, Pt, is given by:

$$Pt = p_{Ob} + p_{Nb} + pH2O \qquad \text{Eq. A3}$$

where, pH2O is the vapor pressure of water at temperature T.

Laplaces Equation defines the balance of pressure and surface tension forces acting on the bubble and is given by:

$$Pt = Pe + \frac{2 \cdot \sigma}{r} \qquad \text{Eq. A4}$$

where Pe is the pressure in the liquid external to the bubble. For this derivation Pe is defined as:

$$Pe = P_{Atm.} + \rho \cdot g \cdot h + Ps \qquad \text{Eq. A5}$$

where $P_{Atm.}$, ρ, r, h, and Ps are the atmospheric pressure, the density of water, depth of fish in water column and system pressure where bubble growth is occurring. g is the gravitational constant. Equations A3 and A4 can be combined to give:

$$p_{Ob} + p_{Nb} + pH2O = P_{Atm.} + \rho \cdot g \cdot h + Ps + \frac{2 \cdot \sigma}{r} \qquad \text{Eq. A6}$$

Solving for p_{Nb} in Equation A6 and substituting the results in Equation A2 gives:

$$n_{Nb} = \frac{4 \cdot \pi}{3 \cdot R' \cdot T} \cdot (Pe \cdot r^3 + 2 \cdot \sigma \cdot r^2 - p_{Ob} \cdot r^3 - p_{H2O} \cdot r^3) \qquad \text{Eq. A7}$$

Equation A7 can be differentiated with respect to time to give:

$$\frac{dn_{Nb}}{dt} = \frac{4 \cdot \pi}{3 \cdot R' \cdot T} \cdot \left[\left(3 \cdot Pe \cdot r^2 + 4 \cdot \sigma \cdot r - 3 \cdot p_{Ob} \cdot r^2 - 3 \cdot p_{H2O} \cdot r^2 \right) \cdot \frac{dr}{dt} - r^3 \cdot \frac{dp_{Ob}}{dt} \right] \qquad \text{Eq. A8}$$

Equation A1 can be differentiated with respect to time to give:

$$\frac{dn_{Ob}}{dt} = \frac{4 \cdot \pi}{3 \cdot R' \cdot T} \cdot \left[r^3 \cdot \frac{dp_{Ob}}{dt} + 3 \cdot p_{Ob} \cdot \frac{r^2 \cdot dr}{dt} \right] \qquad \text{Eq. A9}$$

Equations A8 and A9 give the time rate of change in the number of moles of oxygen and nitrogen in the bubble. This change must be balanced by the rate of diffusion of these gases into the bubble. The diffusion equations for the movement of gases into the bubble can be written as follows.

$$\frac{dn_{Ob}}{dt} = H_O \cdot k_O \cdot 4 \cdot \pi \cdot r^2 \cdot (p_{Op} - p_{Ob}) \qquad \text{Eq. A10}$$

$$\frac{dn_{Nb}}{dt} = H_N \cdot k_N \cdot 4 \cdot \pi \cdot r^2 \cdot (p_{Np} - p_{Nb}) \qquad \text{Eq. A11}$$

where,

k_O = mass transfer coefficient for oxygen

k_N = mass transfer coefficient for nitrogen

p_{Op} = partial pressure of oxygen in plasma

p_{Np} = partial pressure of nitrogen in plasma

H_O = Henrys constant for oxygen

H_N = Henrys constant for nitrogen

Using Equation A5 and A6, Equation A11 can be written as:

$$\frac{dn_{Nb}}{dt} = H_N \cdot k_N \cdot 4 \cdot \pi \cdot r^2 \cdot \left[p_{Np} - Pe - \frac{2 \cdot \sigma}{r} + p_{Ob} + p_{H2O} \right] \qquad \text{Eq. A12}$$

Equating Equations A9 and A10 and Equations A8 and A12 gives two equations that can be combined into a single equation for the rate of change in bubble radius with time. The resulting equation is:

$$\frac{dr}{dt} = \frac{R' \cdot T \cdot \left[H_O \cdot k_O \cdot \left(p_{Op} - p_{Ob} \right) + H_N \cdot k_N \cdot \left[p_{Np} - \left(Pe + \frac{2 \cdot \sigma}{r} - p_{Ob} - pH2O \right) \right] \right]}{\left(Pe + \frac{4 \cdot \sigma}{3 \cdot r} - pH2O \right)} \qquad \text{Eq. A13}$$

Nitrogen, in the vascular system of a fish, is biologically inert. Once gases in the fish are in a steady state relative to those in the water, p_{Np} should be the same as the partial pressure of nitrogen in the environmental water (p_{N2}). Thus, $p_{Np} = pN2$. On the other hand, oxygen is biologically active and in a state of constant transport from the water to the vascular system. Due to mass transport resistances at the gill membrane and utilization of oxygen by the gill, plasma dissolved oxygen partial pressure will be reduced from that of the water. Defining F as the ratio of the partial pressure of oxygen in the plasma to that of the environmental water, $p_{Op} = F \cdot pO2$.

Finally, the Total Gas Pressure (TGP) in the environmental water is related to the component dissolved gases by:

$$TGP = pO2 + pN2 + pH2O$$

Incorporating these expressions into Equation A13 yields:

$$\frac{dr}{dt} = \frac{R' \cdot T \cdot \left[H_O \cdot k_O \cdot \left(F \cdot pO2 - p_{Ob} \right) + H_N \cdot k_N \cdot \left(Pe + \frac{2 \cdot \sigma}{r} - p_{Ob} + pO2 - TGP \right) \right]}{\left(Pe + \frac{4 \cdot \sigma}{3 \cdot r} - pH2O \right)}$$

Eq. A14

For a bubble that is not growing, dr/dt in the above equation is equal to zero. For bubble growth, dr/dt must be positive. Based on this growth criteria, the following criteria can be developed from Equation A14. For bubble growth:

$$H_O \cdot k_O \cdot (F \cdot p_{O2} - p_{Ob}) + H_N \cdot k_N \cdot \left[Pe + \frac{2 \cdot \sigma}{r} - p_{Ob} + p_{O2} - TGP \right] \geq 0$$

or,

$$TGP \geq Pe + \frac{2 \cdot \sigma}{r_o} - p_{O2} \cdot (K \cdot F - 1) - (1 - K) \cdot p_{Ob} \qquad \text{Eq. A15}$$

where K is defined as: $K = \frac{k_O \cdot H_O}{k_N \cdot H_N}$

and r_o is the initial radius of the bubble nucleus from which growth begins. Expanding Pe, Equation A15 becomes:

$$TGP \geq P_{Atm} + Ps + \rho \cdot g \cdot h + \frac{2 \cdot \sigma}{r_o} - p_{O2} \cdot (K \cdot F - 1) - (1 - K) \cdot p_{Ob}$$

Eq. A16

Thus, Equation A16 is the general threshold equation for bubble growth in the vascular systems of fish exposed to supersaturated water.

Because the swimbladders of physostome fish are highly vascularized with arterial blood, this organ can also grow in volume as a result of the transfer of supersaturated gases from the blood into the bladder. Assuming that the swimbladder of a fish acts like a very large bubble, Equation A16 can be used to define its threshold for overinflation. In this case, the nucleus radius, r_o, is very large. As a result, the surface tension term, $2 \cdot \sigma / r_o$, is very small and can be neglected. Thus, the criteria for overinflation of the swimbladder becomes:

$$TGP \geq P_{Atm.} + Ps + \rho \cdot g \cdot h - pO2 \cdot (K \cdot F - 1) - (1 - K) \cdot p_{Ob}$$

Eq. A17

Finally, for a bubble in the environmental water, the F term in Equation A16 is 1.0 and Ps = 0. Thus, for a bubble in the environmental water, the threshold criteria becomes:

$$TGP \geq P_{Atm.} + \rho \cdot g \cdot h + \frac{2 \cdot \sigma}{r_o} + (K - 1) \cdot (p_{Ob} - pO2) \qquad \text{Eq. A18}$$

APPENDIX B

TABLE I: SOURCES FOR WATER AND DORSAL AORTA pO_2 DATA

AUTHOR CODE	AUTHOR (S)
1	Cameron, J.C. and Davis, J.C. (1970)
2	Eddy, F.B. (1977)
3	Kiceniuk, J.W. and Jones, D.J. (1977)
4	Stevens, E.D. and Randall, D.J. (1967)
5	Kiceniuk, J.W. (1969)
6	Stevens, E.D. (1967)
7	Holton, G.F. and Randall, D.J. (1967)
8	Thomas, S. and Hughes, G.M. (1982)
9	Sovio, A.; Nikinmaa, M.; Nyholm,K. and Westman, K. (1981)
10	Hobe, H.; Wood, C.M. and Whetly, M.G. (1984)
11	Wood, C.M. and Jackson, E.B. (1980)
12	Tuurala, H. (1983)
13	Smith, F.M. and Jones, D.J. (1982)
14	Spry, D.J. and Wood, C.M. (1985)
15	Fidler, L.E. (1988)

Complete references can be found in the bibliography of this thesis.

TABLE II: LIST OF ABBREVIATIONS FOR TABLE III

AUTHORS: Author code as shown in Table I

WATER PO2: pO_2 of environmental water - mmHg.

ARTERIAL PO2: pO_2 of arterial blood at dorsal aorta - mmHg.

TEMP. C.: Water temperature - deg. C.

NO. SAMP.: Number of samples in measurement.

RECORD NO.: Record number assigned to data set.

Std.Err.W.: Standard error in water pO_2 measurement.

Std.Err.A.: Standard error in blood pO_2 measurement.

F RATIO: Ratio of arterial pO_2 to water pO_2.

TABLE III: WATER AND DORSAL AORTA pO2 DATA
FROM THE LITERATURE FOR RAINBOW TROUT

AUTHORS	WATER PO2	ARTERIAL PO2	TEMP. C.	NO. SAMP.	RECORD NO.	Std. Err.W.	Std. Err.A.	F RATIO
1	155.000	133.200			1.001			0.859
2	155.000	117.000			2.001			0.755
3	153.000	137.000	10.00	8	3.001			0.895
4	135.000	85.000			4.001			0.630
5	152.900	137.000	10.00	8	5.001	1.960	4.230	0.896
6	134.000	85.000		13	6.001			0.634
7	30.000	17.600	15.00	27	7.001		2.100	0.587
7	40.000	23.200	15.00	27	7.002		2.100	0.580
7	50.000	26.750	15.00	27	7.003		4.250	0.535
7	60.000	33.050	15.00	27	7.004		3.500	0.551
7	70.000	45.370	15.00	27	7.005		4.570	0.648
7	80.000	60.840	15.00	27	7.006		5.980	0.761
7	90.000	67.600	15.00	27	7.007		5.000	0.751
7	100.000	81.240	15.00	27	7.008		3.870	0.812
7	110.000	87.430	15.00	27	7.009		4.010	0.795
7	120.000	91.450	15.00	27	7.01		5.640	0.762
7	130.000	105.710	15.00	27	7.011		3.020	0.813
7	140.000	111.200	15.00	27	7.012		4.290	0.794
7	150.000	121.340			7.013		8.090	0.809
8	155.000	108.000	15.00	6	8.001	3.000	11.000	0.697
8	60.000	23.000	15.00	6	8.002	3.000	6.000	0.383
8	155.000	115.000	15.00	8	8.003	4.000	10.000	0.742
9	155.000	125.730	9.00	9	9.001		6.190	0.811
9	73.580	68.310	9.00	9	9.002		6.540	0.928
9	73.580	63.240	9.00	9	9.003		3.630	0.859
9	73.580	61.890	9.00	9	9.004		9.450	0.841
9	155.000	117.150	9.00	9	9.005		4.000	0.756
9	155.000	120.600	9.00	9	9.006		4.360	0.778
9	155.000	116.300	9.00	9	9.007		5.300	0.750
9	155.000	120.630	9.00	9	9.008		2.500	0.778
9	155.000	115.550	9.00	9	9.009		2.900	0.745
10	150.400	105.600	13.00	12	10.001			0.702
10	496.000	376.000	13.00	12	10.002			0.758
10	544.000	356.800	13.00	12	10.003			0.656
10	528.000	329.600	13.00	12	10.004			0.624
10	560.000	360.000	13.00	10	10.005			0.643
11	162.100	111.000	15.00	7	11.001	14.730	9.820	0.685
11	407.660	314.300	15.00	7	11.002	12.300	29.460	0.771
11	419.450	315.300	15.00	7	11.003	13.750	18.600	0.752
11	402.750	335.000	15.00	5	11.004	9.820	23.600	0.832
11	429.270	336.900	15.00	5	11.005	12.280	19.640	0.785
11	471.500	324.160	15.00	5	11.006	14.730	14.730	0.688
11	399.800	349.700	15.00	4	11.007	12.230	22.100	0.875
12	153.040	113.280	10.00		12.001			0.740

TABLE III: WATER AND DORSAL AORTA pO2 DATA
FROM THE LITERATURE FOR RAINBOW TROUT

AUTHORS	WATER PO2	ARTERIAL PO2	TEMP. C.	NO. SAMP.	RECORD NO.	Std. Err.W.	Std. Err.A.	F RATIO
12	146.130	120.400	18.00		12.002			0.824
12	51.390	44.410	18.00		12.003			0.864
13	150.040	109.530	8.80		13.001	4.500	3.000	0.730
13	93.020	70.520	8.80		13.002	4.500	6.000	0.758
14	155.000	110.000	15.00		14.001		3.000	0.710
14	155.000	115.000	15.00		14.002		3.000	0.742
15	155.000	124.000	9.50	1	15.007			0.800
15	155.000	111.000	9.50	1	15.008			0.716
15	155.000	120.000	9.50	1	15.009			0.774
15	155.000	117.000	9.50	1	15.01			0.755
15	155.000	128.000	9.50	1	15.011			0.826
15	155.000	119.000	9.50	1	15.012			0.768
15	155.000	124.000	9.50	1	15.013			0.800
15	155.000	117.000	9.50	1	15.014			0.755
15	155.000	122.000	9.50	1	15.015			0.787
15	155.000	119.000	9.50	1	15.016			0.768
15	155.000	115.000	9.50	1	15.017			0.742
15	155.000	119.000	9.50	1	15.018			0.768
15	155.000	121.000	9.50	1	15.019			0.781
15	155.000	122.000	9.50	1	15.02			0.787
15	155.000	119.000	9.50	1	15.021			0.768
15	155.000	123.000	9.50	1	15.022			0.794
15	155.000	121.000	9.50	1	15.023			0.781
15	155.000	117.000	9.50	1	15.024			0.755
15	155.000	124.000	9.50	1	15.025			0.800
15	155.000	122.000	9.50	1	15.026			0.787
15	155.000	109.000	9.50	1	15.027			0.703
15	155.000	119.000	9.50	1	15.028			0.768
15	155.000	117.000	9.50	1	15.029			0.755
15	155.000	118.000	9.50	1	15.03			0.761
15	155.000	123.000	9.50	1	15.031			0.794
15	155.000	120.000	9.50	1	15.032			0.774
15	155.000	123.000	9.50	1	15.033			0.794
15	155.000	118.000	9.50	1	15.034			0.761
15	155.000	120.000	9.50	1	15.035			0.774
15	155.000	125.000	9.50	1	15.036			0.806
15	155.000	118.000	9.50	1				0.761
15	162.000	130.000	9.50	1	15.037			0.802
15	162.000	119.000	9.50	1	15.038			0.735
15	162.000	128.000	9.50	1	15.039			0.790
15	162.000	123.000	9.50	1	15.04			0.759
15	162.000	128.000	9.50	1	15.041			0.790
15	162.000	122.000	9.50	1	15.042			0.753
15	183.000	147.000	9.50	1	15.043			0.803
15	183.000	133.000	9.50	1	15.044			0.727
15	183.000	140.000	9.50	1	15.045			0.765
15	183.000	137.000	9.50	1	15.046			0.749
15	183.000	141.000	9.50	1	15.047			0.770

TABLE III: WATER AND DORSAL AORTA pO2 DATA
FROM THE LITERATURE FOR RAINBOW TROUT

AUTHORS	WATER PO2	ARTERIAL PO2	TEMP. C.	NO. SAMP.	RECORD NO.	Std. Err.W.	Std. Err.A.	F RATIO
15	175.000	137.000	9.50	1				0.783
15	175.000	126.000	9.50	1				0.720
15	175.000	133.000	9.50	1				0.760
15	175.000	127.000	9.50	1				0.726
15	175.000	135.000	9.50	1				0.771
15	175.000	139.000	9.50	1				0.794

APPENDIX C: TABLE I: SOURCE OF DATA FOR GBT DATABASE

Author Code	Author(s)
1	Rucker (1975a)
2	Nebeker *et al.* (1978)
3	Jensen (1980)
4	Ebel (1971)
5	Ebel (1969)
6	Rucker and Kangas (1974)
7	Ebel *et al.* (1971)
8	Dawley *et al.* (1976)
9	Weitkamp (1976)
10	Wyatt and Beiningen (1969)
11	Blahm *et al.* (1974)
12	Meekin and Turner (1974)
13	Dawley and Ebel (1975)
14	Nebeker *et al.* (1979a)
15	Rucker (1975a)
16	Blahm *et al.* (1975)
17	Knittel *et al.* (1980)
18	Stroud and Nebeker (1976)
19	Nebeker *et al.* (1976a)
20	Coutant and Genoway (1968)
21	Nebeker *et al.* (1976b)
22	Nebeker and Brett (1976)
23	Nebeker *et al.* (1979)
24	Nebeker *et al.* (1980)

TABLE II: LIST OF ABBREVIATIONS USED IN GBT DATABASE

Record: Record number in database

Author: Author data set identity number

Species: Species Code; 1 = Chinook, 2 = Coho, 3 = Sockeye,

4 = Steelhead, 5 = Cutthroat.

Stage: Stage Code; 0 = Eggs, 1 = Alevins, 2 = Fry, 3 = Adult

Length: Fish length in mm.

Weight: Fish weight in gr.

Temp.C.: Water temperature in deg. C.

Patm. : Atmospheric pressure in mmHg.

Depth: Water depth in M.

%Mort.: Percent mortality.

Time: Time to mortality in hrs.

TGP%: Total Gas Pressure in percent of Patm.

O2: Partial pressure of dissolved oxygen in mmHg.

N2: Partail pressure of dissolved nitrogen in mmHg.

pH2O: Vapor pressure of water at Temp.C.

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
1	1.001	1	1	0	0	0	7.2	758	0	11	1032	99	149.63	592.88	7.617
2	1.001	1	1	0	0	0	7.2	758	0	19	1560	99	149.63	592.88	7.617
3	1.002	1	1	0	0	0	7.2	758	0	1.4	1560	109.7	154.35	668.96	7.617
4	1.002	1	1	0	0	0	7.2	758	0	1.8	1032	109.7	154.35	668.96	7.617
5	1.003	1	1	0	0	0	7.2	758	0	0.6	1032	118.6	162.23	729.24	7.617
6	1.003	1	1	0	0	0	7.2	758	0	0.4	1032	118.6	162.23	729.24	7.617
7	1.003	1	1	0	0	0	7.2	758	0	0.1	1560	118.6	162.23	729.24	7.617
8	1.003	1	1	0	0	0	7.2	758	0	0.1	1560	118.6	162.23	729.24	7.617
9	2.001	2	4	1	35	0.35	10	754	0.08	60	600	126.2	0	0	9.208
10	2.001	2	4	1	35	0.35	10	754	0.08	90	1080	126.2	0	0	9.208
11	2.001	2	4	1	35	0.35	10	754	0.08	60	600	126.2	0	0	9.208
12	2.001	2	4	1	35	0.35	10	754	0.08	50	552	126.2	0	0	9.208
13	2.001	2	4	1	35	0.35	10	754	0.08	84	840	126.2	0	0	9.208
14	2.001	2	4	1	35	0.35	10	754	0.08	70	648	126.2	0	0	9.208
15	2.001	2	4	1	35	0.35	10	754	0.08	90	1080	126.2	0	0	9.208
16	2.001	2	4	1	35	0.35	10	754	0.08	10	432	126.2	0	0	9.208
17	2.001	2	4	1	35	0.35	10	754	0.08	78	720	126.2	0	0	9.208
18	2.001	2	4	1	35	0.35	10	754	0.08	25	492	126.2	0	0	9.208
19	2.001	2	4	1	35	0.35	10	754	0.08	20	480	126.2	0	0	9.208
20	2.001	2	4	1	35	0.35	10	754	0.08	18	480	126.2	0	0	9.208
21	2.001	2	4	1	35	0.35	10	754	0.08	30	504	126.2	0	0	9.208
22	2.001	2	4	1	35	0.35	10	754	0.08	88	960	126.2	0	0	9.208
23	2.001	2	4	1	35	0.35	10	754	0.08	93	1200	126.2	0	0	9.208
24	2.001	2	4	1	35	0.35	10	754	0.08	40	528	126.2	0	0	9.208
25	2.001	2	4	1	35	0.35	10	754	0.08	80	756	126.2	0	0	9.208
26	2.002	2	4	1	35	0.35	10	754	0.08	10	480	122.9	0	0	9.208
27	2.002	2	4	1	35	0.35	10	754	0.08	50	672	122.9	0	0	9.208
28	2.002	2	4	1	35	0.35	10	754	0.08	20	504	122.9	0	0	9.208
29	2.002	2	4	1	35	0.35	10	754	0.08	10	480	122.9	0	0	9.208
30	2.002	2	4	1	35	0.35	10	754	0.08	68	840	122.9	0	0	9.208
31	2.002	2	4	1	35	0.35	10	754	0.08	55	720	122.9	0	0	9.208
32	2.002	2	4	1	35	0.35	10	754	0.08	40	612	122.9	0	0	9.208
33	2.002	2	4	1	35	0.35	10	754	0.08	60	768	122.9	0	0	9.208
34	2.002	2	4	1	35	0.35	10	754	0.08	38	600	122.9	0	0	9.208
35	2.002	2	4	1	35	0.35	10	754	0.08	70	1056	122.9	0	0	9.208
36	2.002	2	4	1	35	0.35	10	754	0.08	68	960	122.9	0	0	9.208
37	2.002	2	4	1	35	0.35	10	754	0.08	30	552	122.9	0	0	9.208
38	2.002	2	4	1	35	0.35	10	754	0.08	74	1200	122.9	0	0	9.208
39	2.002	2	4	1	35	0.35	10	754	0.08	25	528	122.9	0	0	9.208
40	2.002	2	4	1	35	0.35	10	754	0.08	71	1080	122.9	0	0	9.208
41	2.003	2	4	1	35	0.35	10	754	0.08	63	1200	118.4	0	0	9.208
42	2.003	2	4	1	35	0.35	10	754	0.08	30	864	118.4	0	0	9.208
43	2.003	2	4	1	35	0.35	10	754	0.08	30	840	118.4	0	0	9.208
44	2.003	2	4	1	35	0.35	10	754	0.08	7.5	600	118.4	0	0	9.208
45	2.003	2	4	1	35	0.35	10	754	0.08	20	720	118.4	0	0	9.208
46	2.003	2	4	1	35	0.35	10	754	0.08	40	972	118.4	0	0	9.208
47	2.003	2	4	1	35	0.35	10	754	0.08	20	720	118.4	0	0	9.208
48	2.003	2	4	1	35	0.35	10	754	0.08	25	792	118.4	0	0	9.208
49	2.003	2	4	1	35	0.35	10	754	0.08	10	612	118.4	0	0	9.208
50	2.003	2	4	1	35	0.35	10	754	0.08	50	1032	118.4	0	0	9.208
51	2.003	2	4	1	35	0.35	10	754	0.08	38	960	118.4	0	0	9.208
52	2.003	2	4	1	35	0.35	10	754	0.08	58	1080	118.4	0	0	9.208

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
53	2.003	2	4	1	35	0.35	10	754	0.08	60	1116	118.4	0	0	9.208
54	2.004	2	4	1	35	0.35	10	754	0.08	4	720	115.4	0	0	9.208
55	2.004	2	4	1	35	0.35	10	754	0.08	17	960	115.4	0	0	9.208
56	2.004	2	4	1	35	0.35	10	754	0.08	30	1128	115.4	0	0	9.208
57	2.004	2	4	1	35	0.35	10	754	0.08	25	1062	115.4	0	0	9.208
58	2.004	2	4	1	35	0.35	10	754	0.08	35	1200	115.4	0	0	9.208
59	2.004	2	4	1	35	0.35	10	754	0.08	40	1248	115.4	0	0	9.208
60	2.004	2	4	1	35	0.35	10	754	0.08	27	1080	115.4	0	0	9.208
61	2.004	2	4	1	35	0.35	10	754	0.08	20	996	115.4	0	0	9.208
62	2.004	2	4	1	35	0.35	10	754	0.08	8	840	115.4	0	0	9.208
63	2.004	2	4	1	35	0.35	10	754	0.08	10	888	115.4	0	0	9.208
64	2.005	2	4	1	33	0.3	10	754	0.08	76	1272	122.9	0	0	9.208
65	2.008	2	4	1	36.3	0.4	10	754	0.08	90	1272	126.2	0	0	9.208
66	2.01	2	4	1	27.8	0.2	10	754	0.08	76	1272	122.9	0	0	9.208
67	2.011	2	4	1	34.3	0.4	10	754	0.08	32	1272	115.5	0	0	9.208
68	2.012	2	4	1	35.4	0.4	10	754	0.08	65	1272	118.3	0	0	9.208
69	2.013	2	4	1	36.4	0.5	10	754	0.08	67	1272	118.4	0	0	9.208
70	2.015	2	4	1	35.5	0.4	10	754	0.08	45	1272	115.3	0	0	9.208
71	3.001	3	4	1	29.7	0.2	12	766	0.03	11.8	909.6	110	174.59	659.58	10.517
72	4.001	4	1	2	0	0	12.2	749	0.75	100	168	0	0	740.87	10.657
73	4.001	4	1	2	0	0	12.2	749	0.75	92	48	0	0	740.87	10.657
74	4.001	4	1	2	0	0	12.2	749	0.75	60	24	0	0	740.87	10.657
75	4.003	4	1	2	0	0	13.1	749	4.5	68	168	0	0	754.79	11.305
76	4.004	4	1	2	0	0	14.8	749	0.75	74	24	0	0	753.45	12.624
77	4.004	4	1	2	0	0	14.8	749	0.75	82	48	0	0	753.45	12.624
78	4.004	4	1	2	0	0	14.8	749	0.75	86	48	0	0	753.45	12.624
79	4.004	4	1	2	0	0	14.8	749	0.75	100	168	0	0	753.45	12.624
80	4.004	4	1	2	0	0	14.8	749	0.75	58	24	0	0	753.45	12.624
81	4.004	4	1	2	0	0	14.8	749	0.75	100	168	0	0	753.45	12.624
82	4.005	4	1	2	0	0	14.8	749	1.75	86	168	0	0	753.45	12.624
83	4.007	4	1	2	0	0	14.8	749	4.5	50	168	0	0	753.45	12.624
84	4.008	4	1	2	0	0	18.3	749	0.75	100	24	0	0	756.02	15.772
85	4.009	4	1	2	0	0	18.3	749	1	100	24	0	0	756.02	15.772
86	4.009	4	1	2	0	0	18.3	749	1	56	168	0	0	756.02	15.772
87	4.01	4	1	2	0	0	18.3	749	4	34	24	0	0	756.02	15.772
88	4.011	4	1	2	0	0	18.3	749	4.5	38	168	0	0	756.02	15.772
89	4.012	4	1	2	0	0	12.2	749	4	6	168	0	0	740.87	10.657
90	4.013	4	1	2	0	0	12.2	749	4.5	45	168	0	0	740.87	10.657
91	4.014	4	1	2	0	0	13.1	749	0.75	100	168	0	0	754.79	11.305
92	4.014	4	1	2	0	0	13.1	749	0.75	98	48	0	0	754.79	11.305
93	4.014	4	1	2	0	0	13.1	749	0.75	98	24	0	0	754.79	11.305
94	4.015	4	1	2	0	0	13.1	749	1	98	48	0	0	754.79	11.305
95	4.015	4	1	2	0	0	13.1	749	1	100	168	0	0	754.79	11.305
96	4.015	4	1	2	0	0	13.1	749	1	64	24	0	0	754.79	11.305
97	4.016	4	1	2	0	0	13.1	749	2	40	168	0	0	754.79	11.305
98	4.017	4	1	2	0	0	16.3	749	0.75	50	19.7	128.9	0	0	13.898
99	5.001	5	1	2	0	0	15.9	749	1.5	8	2280	0	0	685.68	13.548
100	5.002	5	1	2	0	0	15.9	749	6	11	2280	0	0	685.68	13.548
101	5.003	5	1	2	0	0	15.9	749	3	2	2280	0	0	685.68	13.548
102	5.004	5	2	2	0	0	0	749	1.5	10	240	0	0	705.8	4.583
103	5.005	5	2	2	0	0	0	749	3	3	240	0	0	705.8	4.583
104	5.007	5	2	2	0	0	0	749	6	11	240	0	0	705.8	4.583

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
105	5.008	5	2	2	0	0	0	749	3	70	240	0	0	823.43	4.583
106	5.009	5	2	2	0	0	0	749	1.5	100	240	0	0	823.43	4.583
107	5.01	5	2	2	0	0	0	749	3.5	3	240	0	0	823.43	4.583
108	5.011	5	2	2	0	0	0	749	6	18	240	0	0	823.43	4.583
109	6.001	6	1	1	0	0	10	760	0	6	1320	112	176.5	664.38	9.208
110	6.001	6	1	1	0	0	10	760	0	20	-132	112	176.5	664.38	9.208
111	6.001	6	1	1	0	0	10	760	0	25	-132	112	176.5	664.38	9.208
112	6.001	6	1	1	0	0	10	760	0	5	1090	112	176.5	664.38	9.208
113	6.002	6	1	1	0	0	10	760	0	5	1010	116	182.81	688.11	9.208
114	6.002	6	1	1	0	0	10	760	0	10	1320	116	182.81	688.11	9.208
115	6.002	6	1	1	0	0	10	760	0	20	-132	116	182.81	688.11	9.208
116	6.002	6	1	1	0	0	10	760	0	25	-132	116	182.81	688.11	9.208
117	6.003	6	1	1	0	0	10	760	0	25	817.5	120	189.11	711.84	9.208
118	6.003	6	1	1	0	0	10	760	0	20	735	120	189.11	711.84	9.208
119	6.003	6	1	1	0	0	10	760	0	50	1070	120	189.11	711.84	9.208
120	6.003	6	1	1	0	0	10	760	0	10	725	120	189.11	711.84	9.208
121	6.003	6	1	1	0	0	10	760	0	30	850	120	189.11	711.84	9.208
122	6.003	6	1	1	0	0	10	760	0	55	1320	120	189.11	711.84	9.208
123	6.003	6	1	1	0	0	10	760	0	40	970	120	189.11	711.84	9.208
124	6.003	6	1	1	0	0	10	760	0	5	640	120	189.11	711.84	9.208
125	6.004	6	1	1	0	0	10	760	0	5	550	124	195.41	735.57	9.208
126	6.004	6	1	1	0	0	10	760	0	60	1050	124	195.41	735.57	9.208
127	6.004	6	1	1	0	0	10	760	0	30	720	124	195.41	735.57	9.208
128	6.004	6	1	1	0	0	10	760	0	40	800	124	195.41	735.57	9.208
129	6.004	6	1	1	0	0	10	760	0	20	660	124	195.41	735.57	9.208
130	6.004	6	1	1	0	0	10	760	0	10	610	124	195.41	735.57	9.208
131	6.004	6	1	1	0	0	10	760	0	50	910	124	195.41	735.57	9.208
132	6.004	6	1	1	0	0	10	760	0	25	690	124	195.41	735.57	9.208
133	6.004	6	1	1	0	0	10	760	0	70	1320	124	195.41	735.57	9.208
134	6.005	6	1	1	0	0	10	760	0	70	940	128	201.72	759.3	9.208
135	6.005	6	1	1	0	0	10	760	0	10	600	128	201.72	759.3	9.208
136	6.005	6	1	1	0	0	10	760	0	50	815	128	201.72	759.3	9.208
137	6.005	6	1	1	0	0	10	760	0	20	650	128	201.72	759.3	9.208
138	6.005	6	1	1	0	0	10	760	0	40	760	128	201.72	759.3	9.208
139	6.005	6	1	1	0	0	10	760	0	25	680	128	201.72	759.3	9.208
140	6.005	6	1	1	0	0	10	760	0	5	520	128	201.72	759.3	9.208
141	6.005	6	1	1	0	0	10	760	0	78	1320	128	201.72	759.3	9.208
142	6.005	6	1	1	0	0	10	760	0	30	710	128	201.72	759.3	9.208
143	6.005	6	1	1	0	0	10	760	0	60	870	128	201.72	759.3	9.208
144	6.006	6	1	1	0	0	8	760	0	25	-204	111	157.84	677.3	8.045
145	6.006	6	1	1	0	0	8	760	0	10	-204	111	157.84	677.3	8.045
146	6.006	6	1	1	0	0	8	760	0	20	-204	111	157.84	677.3	8.045
147	6.007	6	1	1	0	0	8	760	0	10	-204	111	157.84	689.18	8.045
148	6.007	6	1	1	0	0	8	760	0	20	-204	112	157.84	689.18	8.045
149	6.007	6	1	1	0	0	8	760	0	25	-204	112	157.84	689.18	8.045
150	6.008	6	1	1	0	0	8	760	0	25	-204	114	159.41	701.06	8.045
151	6.008	6	1	1	0	0	8	760	0	20	1960	114	159.41	701.06	8.045
152	6.008	6	1	1	0	0	8	760	0	22	2040	114	159.41	701.06	8.045
153	6.008	6	1	1	0	0	8	760	0	10	1730	114	159.41	701.06	8.045
154	6.009	6	1	1	0	0	8	760	0	30	1550	116	160.99	712.94	8.045
155	6.009	6	1	1	0	0	8	760	0	25	1450	116	160.99	712.94	8.045
156	6.009	6	1	1	0	0	8	760	0	10	1130	116	160.99	712.94	8.045

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH20 mmHg
157	6.009	6	1	1	0	0	8	760	0	55	2040	116	160.99	712.94	8.045
158	6.009	6	1	1	0	0	8	760	0	40	1770	116	160.99	712.94	8.045
159	6.009	6	1	1	0	0	8	760	0	20	1350	116	160.99	712.94	8.045
160	6.009	6	1	1	0	0	8	760	0	50	1960	116	160.99	712.94	8.045
161	6.01	6	1	1	0	0	8	760	0	20	890	118	162.57	724.83	8.045
162	6.01	6	1	1	0	0	8	760	0	50	1080	118	162.57	724.83	8.045
163	6.01	6	1	1	0	0	8	760	0	80	1350	118	162.57	724.83	8.045
164	6.01	6	1	1	0	0	8	760	0	10	800	118	162.57	724.83	8.045
165	6.01	6	1	1	0	0	8	760	0	60	1110	118	162.57	724.83	8.045
166	6.01	6	1	1	0	0	8	760	0	25	925	118	162.57	724.83	8.045
167	6.01	6	1	1	0	0	8	760	0	30	960	118	162.57	724.83	8.045
168	6.01	6	1	1	0	0	8	760	0	40	1020	118	162.57	724.83	8.045
169	6.01	6	1	1	0	0	8	760	0	70	1150	118	162.57	724.83	8.045
170	6.01	6	1	1	0	0	8	760	0	90	2040	118	162.57	724.83	8.045
171	6.011	6	2	1	0	0	8	760	0	10	-204	111	157.84	677.3	8.045
172	6.011	6	2	1	0	0	8	760	0	25	-204	111	157.84	677.3	8.045
173	6.011	6	2	1	0	0	8	760	0	20	-204	111	157.84	677.3	8.045
174	6.012	6	2	1	0	0	8	760	0	10	-204	112	157.84	689.18	8.045
175	6.012	6	2	1	0	0	8	760	0	25	-204	112	157.84	689.18	8.045
176	6.012	6	2	1	0	0	8	760	0	20	-204	112	157.84	689.18	8.045
177	6.013	6	2	1	0	0	8	760	0	25	-204	114	159.41	701.06	8.045
178	6.013	6	2	1	0	0	8	760	0	20	2040	114	159.41	701.06	8.045
179	6.013	6	2	1	0	0	8	760	0	10	1250	114	159.41	701.06	8.045
180	6.014	6	2	1	0	0	8	760	0	20	1560	116	160.99	712.94	8.045
181	6.014	6	2	1	0	0	8	760	0	10	1030	116	160.99	712.94	8.045
182	6.014	6	2	1	0	0	8	760	0	30	1920	116	160.99	712.94	8.045
183	6.014	6	2	1	0	0	8	760	0	35	2040	116	160.99	712.94	8.045
184	6.014	6	2	1	0	0	8	760	0	25	1740	116	160.99	712.94	8.045
185	6.015	6	2	1	0	0	8	760	0	40	950	118	162.57	724.83	8.045
186	6.015	6	2	1	0	0	8	760	0	50	1150	118	162.57	724.83	8.045
187	6.015	6	2	1	0	0	8	760	0	30	870	118	162.57	724.83	8.045
188	6.015	6	2	1	0	0	8	760	0	60	1360	118	162.57	724.83	8.045
189	6.015	6	2	1	0	0	8	760	0	10	660	118	162.57	724.83	8.045
190	6.015	6	2	1	0	0	8	760	0	70	1600	118	162.57	724.83	8.045
191	6.015	6	2	1	0	0	8	760	0	87	2040	118	162.57	724.83	8.045
192	6.015	6	2	1	0	0	8	760	0	20	760	118	162.57	724.83	8.045
193	6.015	6	2	1	0	0	8	760	0	25	815	118	162.57	724.83	8.045
194	6.015	6	2	1	0	0	8	760	0	80	1810	118	162.57	724.83	8.045
195	6.016	6	2	1	0	0	8	760	0	10	750	115	157.84	707	8.045
196	6.016	6	2	1	0	0	8	760	0	14	1008	115	157.84	707	8.045
197	6.016	6	2	1	0	0	8	760	0	20	-100.8	115	157.84	707	8.045
198	6.016	6	2	1	0	0	8	760	0	25	-100.8	115	157.84	707	8.045
199	6.017	6	2	1	0	0	8	760	0	40	680	117	160.99	724.83	8.045
200	6.017	6	2	1	0	0	8	760	0	30	515	117	160.99	724.83	8.045
201	6.017	6	2	1	0	0	8	760	0	10	250	117	160.99	724.83	8.045
202	6.017	6	2	1	0	0	8	760	0	25	452.5	118	160.99	724.83	8.045
203	6.017	6	2	1	0	0	8	760	0	50	1008	117	160.99	724.83	8.045
204	6.017	6	2	1	0	0	8	760	0	20	390	117	160.99	724.83	8.045
205	6.018	6	2	1	0	0	8	760	0	10	120	121	162.57	748.59	8.045
206	6.018	6	2	1	0	0	8	760	0	30	275	121	162.57	748.59	8.045
207	6.018	6	2	1	0	0	8	760	0	70	800	121	162.57	748.59	8.045
208	6.018	6	2	1	0	0	8	760	0	20	200	121	162.57	748.59	8.045

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH20 mmHg
209	6.018	6	2	1	0	0	8	760	0	60	570	121	162.57	748.59	8.045
210	6.018	6	2	1	0	0	8	760	0	40	360	121	162.57	748.59	8.045
211	6.018	6	2	1	0	0	8	760	0	50	450	121	162.57	748.59	8.045
212	6.018	6	2	1	0	0	8	760	0	74	1008	121	162.57	748.59	8.045
213	6.018	6	2	1	0	0	8	760	0	25	237.5	1.21	162.57	748.59	8.045
214	7.006	7	1	2	134	23	15	760	0.2	50	11.3	0	0	752.73	12.787
215	7.006	7	1	2	134	23	15	760	0.2	15	8.5	0	0	752.73	12.787
216	7.006	7	1	2	134	23	15	760	0.2	5	8.5	0	0	752.73	12.787
217	7.006	7	1	2	134	23	15	760	0.2	100	15	0	0	752.73	12.787
218	7.007	7	1	2	134	23	20	760	0.2	100	13	0	0	747.94	17.534
219	7.007	7	1	2	134	23	20	760	0.2	5	4.3	0	0	747.94	17.534
220	7.007	7	1	2	134	23	20	760	0.2	15	6	0	0	747.94	17.534
221	7.007	7	1	2	134	23	20	760	0.2	50	7.5	0	0	747.94	17.534
222	7.008	7	1	2	134	23	23	760	0.2	5	2.5	0	0	744.38	21.069
223	7.008	7	1	2	134	23	23	760	0.2	15	5.8	0	0	744.38	21.069
224	7.008	7	1	2	134	23	23	760	0.2	50	7.3	0	0	744.38	21.069
225	7.008	7	1	2	134	23	23	760	0.2	100	11.5	0	0	744.38	21.069
226	7.014	7	2	2	122.7	19.1	23	760	0.2	5	9.3	0	0	744.38	21.069
227	7.014	7	2	2	122.7	19.1	23	760	0.2	100	17.3	0	0	744.38	21.069
228	7.014	7	2	2	122.7	19.1	23	760	0.2	15	8.5	0	0	744.38	21.069
229	7.014	7	2	2	122.7	19.1	23	760	0.2	50	13	0	0	744.38	21.069
230	7.015	7	2	2	122.7	19.1	20	760	0.2	100	15.3	0	0	747.94	17.534
231	7.015	7	2	2	122.7	19.1	20	760	0.2	15	8.3	0	0	747.94	17.534
232	7.015	7	2	2	122.7	19.1	20	760	0.2	50	13.5	0	0	747.94	17.534
233	7.015	7	2	2	122.7	19.1	20	760	0.2	5	6.3	0	0	747.94	17.534
234	7.016	7	2	2	122.7	19.1	15	760	0.2	50	9.3	0	0	752.73	12.787
235	7.016	7	2	2	122.7	19.1	15	760	0.2	50	18.1	0	0	752.73	12.787
236	7.016	7	2	2	122.7	19.1	15	760	0.2	15	7.9	0	0	752.73	12.787
237	7.016	7	2	2	122.7	19.1	15	760	0.2	5	6.9	0	0	752.73	12.787
238	7.016	7	2	2	122.7	19.1	15	760	0.2	5	7	0	0	752.73	12.787
239	7.016	7	2	2	122.7	19.1	15	760	0.2	15	7.2	0	0	752.73	12.787
240	7.017	7	2	2	122.7	19.1	10	760	0.2	5	12.9	0	0	756.33	9.208
241	7.017	7	2	2	122.7	19.1	10	760	0.2	15	14.6	0	0	756.33	9.208
242	7.017	7	2	2	122.7	19.1	10	760	0.2	100	16.5	0	0	756.33	9.208
243	7.017	7	2	2	122.7	19.1	10	760	0.2	5	6.9	0	0	756.33	9.208
244	7.017	7	2	2	122.7	19.1	10	760	0.2	50	10	0	0	756.33	9.208
245	7.017	7	2	2	122.7	19.1	10	760	0.2	15	7.6	0	0	756.33	9.208
246	7.018	7	2	2	122.7	19.1	5	760	0.2	15	10.3	0	0	759.02	6.543
247	7.018	7	2	2	122.7	19.1	5	760	0.2	5	8.8	0	0	759.02	6.543
248	7.018	7	2	2	122.7	19.1	5	760	0.2	50	13.5	0	0	759.02	6.543
249	7.02	7	1	2	129	19	10	760	0.2	5	10	0	0	756.33	9.208
250	7.028	7	4	2	179	54	15	760	0.2	100	11	0	0	752.73	12.787
251	7.028	7	4	2	179	54	15	760	0.2	50	14	0	0	752.73	12.787
252	7.028	7	4	2	179	54	15	760	0.2	50	5.7	0	0	752.73	12.787
253	7.028	7	4	2	179	54	15	760	0.2	100	22	0	0	752.73	12.787
254	7.028	7	4	2	179	54	15	760	0.2	15	3.6	0	0	752.73	12.787
255	7.028	7	4	2	179	54	15	760	0.2	15	10.8	0	0	752.73	12.787
256	7.028	7	4	2	179	54	15	760	0.2	5	2.9	0	0	752.73	12.787
257	7.028	7	4	2	179	54	15	760	0.2	5	9.5	0	0	752.73	12.787
258	7.03	7	4	2	179	54	10	760	0.2	5	3.3	0	0	756.33	9.208
259	7.03	7	4	2	179	54	10	760	0.2	15	4.7	0	0	756.33	9.208
260	7.03	7	4	2	179	54	10	760	0.2	100	11	0	0	756.33	9.208

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH20 mmHg
261	7.03	7	4	2	179	54	10	760	0.2	50	5.1	0	0	756.33	9.208
262	8.001	8	4	2	180	54.8	10	760	0.25	57	168	115.4	182.02	686.33	9.208
263	8.003	8	1	2	40	0.4	10	760	0.25	80	1440	115.4	182.02	686.33	9.208
264	8.004	8	1	2	40	0.4	10	760	0.25	97	1440	120.1	191.79	711.84	9.208
265	8.005	8	1	2	40	0.4	10	760	0.25	18	1440	109.4	170.2	652.52	9.208
266	8.007	8	4	2	180	54.8	10	760	0.25	100	48	122.2	210.78	708.87	9.208
267	8.013	8	1	2	40	0.4	10	760	0.25	20	168	120.1	191.79	711.84	9.208
268	8.014	8	1	2	40	0.4	10	760	0.25	25	216	120.1	191.79	711.84	9.208
269	8.015	8	1	2	40	0.4	10	760	0.25	50	480	120.1	191.79	711.84	9.208
270	8.016	8	1	2	40	0.4	10	760	0.25	70	672	120.1	191.79	711.84	9.208
271	8.017	8	1	2	40	0.4	10	760	0.25	20	432	115.4	182.02	686.33	9.208
272	8.018	8	1	2	40	0.4	10	760	0.25	25	624	115.4	182.02	686.33	9.208
273	8.019	8	1	2	40	0.4	10	760	0.25	50	1080	115.4	182.02	686.33	9.208
274	8.02	8	1	2	40	0.4	10	760	0.25	70	1272	115.4	182.02	686.33	9.208
275	8.021	8	1	2	40	0.4	10	760	0.25	20	-144	109.4	170.2	652.52	9.208
276	8.022	8	1	2	40	0.4	10	760	0.25	25	-144	109.4	170.2	652.52	9.208
277	8.023	8	1	2	40	0.4	10	760	0.25	50	-144	109.4	170.2	652.52	9.208
278	8.024	8	1	2	40	0.4	10	760	0.25	70	-144	109.4	170.2	652.52	9.208
279	8.025	8	4	2	180	54.8	10	760	0.25	20	18	122.2	210.78	708.87	9.208
280	8.026	8	4	2	180	54.8	10	760	0.25	25	21.6	122.2	210.78	708.87	9.208
281	8.027	8	4	2	180	54.8	10	760	0.25	50	28.8	122.2	210.78	708.87	9.208
282	8.028	8	4	2	180	54.8	10	760	0.25	70	31.2	122.2	210.78	708.87	9.208
283	8.029	8	4	2	180	54.8	10	760	0.25	20	66	115.3	189.42	677.44	9.208
284	8.03	8	4	2	180	54.8	10	760	0.25	25	69.6	115.3	189.42	677.44	9.208
285	8.031	8	4	2	180	54.8	10	760	0.25	50	112.8	115.3	189.42	677.44	9.208
286	8.032	8	4	2	180	54.8	10	760	0.25	70	-16.8	115.3	189.42	677.44	9.208
287	8.033	8	4	2	180	54.8	10	760	0.25	20	-16.8	109.5	170.83	652.52	9.208
288	8.034	8	4	2	180	54.8	10	760	0.25	25	-16.8	109.5	170.83	652.52	9.208
289	8.035	8	4	2	180	54.8	10	760	0.25	50	-16.8	109.5	170.83	652.52	9.208
290	8.036	8	4	2	180	54.8	10	760	0.25	70	-16.8	109.5	170.83	652.52	9.208
291	9.001	9	1	2	0	0	0	743	4	20	-144	121	0	0	4.583
292	9.001	9	1	2	0	0	0	743	4	0	-144	121	0	0	4.583
293	9.002	9	1	2	0	0	0	743	3	0	-144	121	0	0	4.583
294	9.002	9	1	2	0	0	0	743	3	20	-24	121	0	0	4.583
295	9.003	9	1	2	0	0	0	743	4	0	-144	124	0	0	4.583
296	9.004	9	1	2	0	0	0	743	3	20	-48	124	0	0	4.583
297	9.005	9	1	2	0	0	0	743	0.1	53	240	121	0	0	4.583
298	9.006	9	1	2	0	0	0	743	0.25	50	48	125	0	0	4.583
299	9.007	9	1	2	0	0	0	743	0.25	50	240	120.5	0	0	4.583
300	9.03	9	1	2	0	0	0	743	4	20	-48	124	0	0	4.583
301	10.001	10	1	2	0	0	0	0	0.6	100	5	0	0	-5.5	4.583
302	11.001	11	1	2	0	0	0	759	2.5	11	1320	0	0	718.26	4.583
303	11.002	11	1	2	0	0	0	759	1	80	1320	0	0	718.26	4.583
304	11.003	11	4	2	0	0	0	759	2.5	6	1320	0	0	718.26	4.583
305	11.004	11	4	2	0	0	0	759	1	80	1320	0	0	718.26	4.583
306	12.001	12	1	1	0	0	11.1	736	0.17	25	2316	111.8	112.78	699.89	9.909
307	12.001	12	1	1	0	0	11.1	736	0.17	40	2424	111.8	112.78	699.89	9.909
308	12.001	12	1	1	0	0	11.1	736	0.17	30	2400	111.8	112.78	699.89	9.909
309	12.001	12	4	0	0	0	11.1	736	0.17	54	792	111.8	112.78	699.89	9.909
310	12.001	12	1	1	0	0	11.1	736	0.17	10	2136	111.8	112.78	699.89	9.909
311	12.001	12	4	0	0	0	11.1	736	0.17	77.3	648	111.8	112.78	699.89	9.909
312	12.001	12	1	1	0	0	11.1	736	0.17	60	2520	111.8	112.78	699.89	9.909

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH20 mmHg
313	12.001	12	1	1	0	0	11.1	736	0.17	50	2472	111.8	112.78	699.89	9.909
314	12.001	12	1	1	0	0	11.1	736	0.17	20	2232	111.8	112.78	699.89	9.909
315	12.001	12	4	0	0	0	11.1	736	0.17	68	744	111.8	112.78	699.89	9.909
316	12.002	12	1	2	54	0	11.1	736	0.17	36	384	110.8	117.35	688.42	9.909
317	12.002	12	1	2	54	0	11.1	736	0.17	30	364.8	110.8	117.35	688.42	9.909
318	12.002	12	1	2	0	0	11.1	736	0.17	10	76.8	110.8	117.35	688.42	9.909
319	12.002	12	1	2	54	0	11.1	736	0.17	20	120	110.8	117.35	688.42	9.909
320	12.002	12	1	2	54	0	11.1	736	0.17	25	242.4	110.8	117.35	688.42	9.909
321	12.003	12	1	2	49	0	11.1	736	0.17	20	-38.4	111.8	112.78	699.89	9.909
322	12.003	12	1	2	49	0	11.1	736	0.17	10	156	111.8	112.78	699.89	9.909
323	12.003	12	1	2	49	0	11.1	736	0.17	25	-38.4	111.8	112.78	699.89	9.909
324	12.004	12	1	2	101	0	11.1	736	0.17	30	112.8	110.8	117.35	688.42	9.909
325	12.004	12	1	2	101	0	11.1	736	0.17	70	309.6	110.8	117.35	688.42	9.909
326	12.004	12	1	2	101	0	11.1	736	0.17	25	108	110.8	117.35	688.42	9.909
327	12.004	12	1	2	101	0	11.1	736	0.17	40	124.8	110.8	117.35	688.42	9.909
328	12.004	12	1	2	101	0	11.1	736	0.17	10	84	110.8	117.35	688.42	9.909
329	12.004	12	1	2	101	0	11.1	736	0.17	76	384	110.8	117.35	688.42	9.909
330	12.004	12	1	2	101	0	11.1	736	0.17	20	103.2	110.8	117.35	688.42	9.909
331	12.004	12	1	2	101	0	11.1	736	0.17	60	192	110.8	117.35	688.42	9.909
332	12.004	12	1	2	101	0	11.1	736	0.17	50	146.4	110.8	117.35	688.42	9.909
333	12.005	12	1	2	80	0	11.1	736	0.17	10	134.4	110.8	117.35	688.42	9.909
334	12.005	12	1	2	80	0	11.1	736	0.17	20	192	110.8	117.35	688.42	9.909
335	12.005	12	1	2	80	0	11.1	736	0.17	40	316.8	110.8	117.35	688.42	9.909
336	12.005	12	1	2	80	0	11.1	736	0.17	25	230.4	110.8	117.35	688.42	9.909
337	12.005	12	1	2	80	0	11.1	736	0.17	30	268.8	110.8	117.35	688.42	9.909
338	12.005	12	1	2	80	0	11.1	736	0.17	57	384	110.8	117.35	688.42	9.909
339	12.005	12	1	2	80	0	11.1	736	0.17	50	357.6	110.8	117.35	688.42	9.909
340	12.006	12	1	2	67	0	11.1	736	0.17	50	312	111.8	112.78	699.89	9.909
341	12.006	12	1	2	67	0	11.1	736	0.17	62	408	111.8	112.78	699.89	9.909
342	12.006	12	1	2	67	0	11.1	736	0.17	25	174	111.8	112.78	699.89	9.909
343	12.006	12	1	2	67	0	11.1	736	0.17	20	168	111.8	112.78	699.89	9.909
344	12.006	12	1	2	67	0	11.1	736	0.17	30	180	111.8	112.78	699.89	9.909
345	12.006	12	1	2	67	0	11.1	736	0.17	40	240	111.8	112.78	699.89	9.909
346	12.006	12	1	2	67	0	11.1	736	0.17	60	384	111.8	112.78	699.89	9.909
347	12.006	12	1	2	67	0	11.1	736	0.17	10	144	111.8	112.78	699.89	9.909
348	12.006	12	1	2	67	0	11.1	736	0.17	50	300	111.8	112.78	699.89	9.909
349	12.007	12	1	2	53	0	11.1	736	0.17	60	432	111.8	112.78	699.89	9.909
350	12.007	12	1	2	53	0	11.1	736	0.17	30	300	111.8	112.78	699.89	9.909
351	12.007	12	1	2	53	0	11.1	736	0.17	25	288	111.8	112.78	699.89	9.909
352	12.007	12	1	2	53	0	11.1	736	0.17	10	192	111.8	112.78	699.89	9.909
353	12.007	12	1	2	53	0	11.1	736	0.17	20	276	111.8	112.78	699.89	9.909
354	12.007	12	1	2	53	0	11.1	736	0.17	50	384	111.8	112.78	699.89	9.909
355	12.007	12	1	2	53	0	11.1	736	0.17	40	336	111.8	112.78	699.89	9.909
356	12.007	12	1	2	53	0	11.1	736	0.17	70	456	111.8	112.78	699.89	9.909
357	12.007	12	1	2	53	0	11.1	736	0.17	75	528	111.8	112.78	699.89	9.909
358	12.008	12	1	2	101	0	11.1	736	0.17	25	32.4	114	111.26	717.11	9.909
359	12.008	12	1	2	101	0	11.1	736	0.17	20	31.2	114	111.26	717.11	9.909
360	12.008	12	1	2	101	0	11.1	736	0.17	10	26.4	114	111.26	717.11	9.909
361	12.008	12	1	2	101	0	11.1	736	0.17	100	144	114	111.26	717.11	9.909
362	12.008	12	1	2	101	0	11.1	736	0.17	40	39.6	114	111.26	717.11	9.909
363	12.008	12	1	2	101	0	11.1	736	0.17	80	86.4	114	111.26	717.11	9.909
364	12.008	12	1	2	101	0	11.1	736	0.17	90	115.2	114	111.26	717.11	9.909

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
365	12.008	12	1	2	101	0	11.1	736	0.17	30	33.6	114	111.26	717.11	9.909
366	12.008	12	1	2	101	0	11.1	736	0.17	50	46.8	114	111.26	717.11	9.909
367	12.008	12	1	2	101	0	11.1	736	0.17	70	67.2	114	111.26	717.11	9.909
368	12.008	12	1	2	101	0	11.1	736	0.17	60	55.2	114	111.26	717.11	9.909
369	12.009	12	1	2	82	0	11.1	736	0.17	40	74.4	114	111.26	717.11	9.909
370	12.009	12	1	2	82	0	11.1	736	0.17	56	144	114	111.26	717.11	9.909
371	12.009	12	1	2	82	0	11.1	736	0.17	25	45	114	111.26	717.11	9.909
372	12.009	12	1	2	82	0	11.1	736	0.17	10	27.6	114	111.26	717.11	9.909
373	12.009	12	1	2	82	0	11.1	736	0.17	50	103.2	114	111.26	717.11	9.909
374	12.009	12	1	2	82	0	11.1	736	0.17	20	38.4	114	111.26	717.11	9.909
375	12.009	12	1	2	82	0	11.1	736	0.17	30	51.6	114	111.26	717.11	9.909
376	12.01	12	1	2	97	0	11.1	736	0.17	100	72	122	112.78	774.47	9.909
377	12.01	12	1	2	97	0	11.1	736	0.17	60	37.2	122	112.78	774.47	9.909
378	12.01	12	1	2	97	0	11.1	736	0.17	70	39.6	122	112.78	774.47	9.909
379	12.01	12	1	2	97	0	11.1	736	0.17	40	28.8	122	112.78	774.47	9.909
380	12.01	12	1	2	97	0	11.1	736	0.17	25	24	122	112.78	774.47	9.909
381	12.01	12	1	2	97	0	11.1	736	0.17	10	9.6	122	112.78	774.47	9.909
382	12.01	12	1	2	97	0	11.1	736	0.17	30	25.2	122	112.78	774.47	9.909
383	12.01	12	1	2	97	0	11.1	736	0.17	80	44.4	122	112.78	774.47	9.909
384	12.01	12	1	2	97	0	11.1	736	0.17	20	22.8	122	112.78	774.47	9.909
385	12.01	12	1	2	97	0	11.1	736	0.17	50	32.4	122	112.78	774.47	9.909
386	12.01	12	1	2	97	0	11.1	736	0.17	90	52.8	122	112.78	774.47	9.909
387	12.011	12	1	2	96	0	11.1	736	0.17	100	96	122	112.78	774.47	9.909
388	12.011	12	1	2	96	0	11.1	736	0.17	60	45.6	122	112.78	774.47	9.909
389	12.011	12	1	2	96	0	11.1	736	0.17	80	60	122	112.78	774.47	9.909
390	12.011	12	1	2	96	0	11.1	736	0.17	30	28.8	122	112.78	774.47	9.909
391	12.011	12	1	2	96	0	11.1	736	0.17	25	26.4	122	112.78	774.47	9.909
392	12.011	12	1	2	96	0	11.1	736	0.17	50	38.4	122	112.78	774.47	9.909
393	12.011	12	1	2	96	0	11.1	736	0.17	70	52.8	122	112.78	774.47	9.909
394	12.011	12	1	2	96	0	11.1	736	0.17	40	32.4	122	112.78	774.47	9.909
395	12.011	12	1	2	96	0	11.1	736	0.17	10	10.8	122	112.78	774.47	9.909
396	12.011	12	1	2	96	0	11.1	736	0.17	20	24	122	112.78	774.47	9.909
397	12.011	12	1	2	96	0	11.1	736	0.17	90	67.2	122	112.78	774.47	9.909
398	12.012	12	1	2	95	0	11.1	736	0.17	25	34.2	122	112.78	774.47	9.909
399	12.012	12	1	2	95	0	11.1	736	0.17	30	37.2	122	112.78	774.47	9.909
400	12.012	12	1	2	95	0	11.1	736	0.17	40	43.2	122	112.78	774.47	9.909
401	12.012	12	1	2	95	0	11.1	736	0.17	70	60	122	112.78	774.47	9.909
402	12.012	12	1	2	95	0	11.1	736	0.17	50	50.4	122	112.78	774.47	9.909
403	12.012	12	1	2	95	0	11.1	736	0.17	10	26.4	122	112.78	774.47	9.909
404	12.012	12	1	2	95	0	11.1	736	0.17	20	31.2	122	112.78	774.47	9.909
405	12.012	12	1	2	95	0	11.1	736	0.17	87	69.6	122	112.78	774.47	9.909
406	12.012	12	1	2	95	0	11.1	736	0.17	80	66	122	112.78	774.47	9.909
407	12.012	12	1	2	95	0	11.1	736	0.17	60	55.2	122	112.78	774.47	9.909
408	12.013	12	1	2	45	0	11.1	736	0.17	64	192	122	112.78	774.47	9.909
409	12.013	12	1	2	45	0	11.1	736	0.17	30	90	122	112.78	774.47	9.909
410	12.013	12	1	2	45	0	11.1	736	0.17	10	52.8	122	112.78	774.47	9.909
411	12.013	12	1	2	45	0	11.1	736	0.17	25	79.8	122	112.78	774.47	9.909
412	12.013	12	1	2	45	0	11.1	736	0.17	50	156	122	112.78	774.47	9.909
413	12.013	12	1	2	45	0	11.1	736	0.17	60	180	122	112.78	774.47	9.909
414	12.013	12	1	2	45	0	11.1	736	0.17	40	110.4	122	112.78	774.47	9.909
415	12.013	12	1	2	45	0	11.1	736	0.17	20	69.6	122	112.78	774.47	9.909
416	12.014	12	2	2	77	0	11.1	736	0.61	25	29.4	124.6	104.41	722.84	9.909

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
417	12.014	12	2	2	77	0	11.1	736	0.61	70	54	124.6	184.41	722.84	9.909
418	12.014	12	2	2	77	0	11.1	736	0.61	20	27.6	124.6	184.41	722.84	9.909
419	12.014	12	2	2	77	0	11.1	736	0.61	40	38.4	124.6	184.41	722.84	9.909
420	12.014	12	2	2	77	0	11.1	736	0.61	80	57.6	124.6	184.41	722.84	9.909
421	12.014	12	2	2	77	0	11.1	736	0.61	60	48	124.6	184.41	722.84	9.909
422	12.014	12	2	2	77	0	11.1	736	0.61	30	31.2	124.6	184.41	722.84	9.909
423	12.014	12	2	2	77	0	11.1	736	0.61	50	43.2	122	192.03	694.16	9.909
424	12.014	12	2	2	77	0	11.1	736	0.61	100	69.6	124.6	184.41	722.84	9.909
425	12.014	12	2	2	77	0	11.1	736	0.61	90	64.8	124.6	184.41	722.84	9.909
426	12.014	12	2	2	77	0	11.1	736	0.61	50	43.2	124.6	184.41	722.84	9.909
427	12.014	12	2	2	77	0	11.1	736	0.61	10	21.6	124.6	184.41	722.84	9.909
428	12.015	12	4	2	69	0	11.1	736	0.61	90	69.6	122	178.32	711.37	9.909
429	12.015	12	4	2	69	0	11.1	736	0.61	10	28.8	122	178.32	711.37	9.909
430	12.015	12	4	2	69	0	11.1	736	0.61	50	50.4	122.2	178.32	711.37	9.909
431	12.015	12	4	2	69	0	11.1	736	0.61	70	60	122	178.32	711.37	9.909
432	12.015	12	4	2	69	0	11.1	736	0.61	20	31.2	122	178.32	711.37	9.909
433	12.015	12	4	2	69	0	11.1	736	0.61	60	55.2	122	178.32	711.37	9.909
434	12.015	12	4	2	69	0	11.1	736	0.61	80	64.8	122	178.32	711.37	9.909
435	12.015	12	4	2	69	0	11.1	736	0.61	30	38.4	122	178.32	711.37	9.909
436	12.015	12	4	2	69	0	11.1	736	0.61	25	34.8	122	178.32	711.37	9.909
437	12.015	12	4	2	69	0	11.1	736	0.61	40	43.2	122	178.32	711.37	9.909
438	12.016	12	1	2	99	0	11.1	736	0.61	80	84	122	178.32	711.37	9.909
439	12.016	12	1	2	99	0	11.1	736	0.61	50	62.4	122.2	178.32	711.37	9.909
440	12.016	12	1	2	99	0	11.1	736	0.61	25	42.6	122	178.32	711.37	9.909
441	12.016	12	1	2	99	0	11.1	736	0.61	30	48	122	178.32	711.37	9.909
442	12.016	12	1	2	99	0	11.1	736	0.61	90	91.2	122	178.32	711.37	9.909
443	12.016	12	1	2	99	0	11.1	736	0.61	100	120	122	178.32	711.37	9.909
444	12.016	12	1	2	99	0	11.1	736	0.61	70	76.8	122	178.32	711.37	9.909
445	12.016	12	1	2	99	0	11.1	736	0.61	20	37.2	122	178.32	711.37	9.909
446	12.016	12	1	2	99	0	11.1	736	0.61	60	69.6	122	178.32	711.37	9.909
447	12.016	12	1	2	99	0	11.1	736	0.61	40	55.2	122	178.32	711.37	9.909
448	12.016	12	1	2	99	0	11.1	736	0.61	50	62.4	122	178.32	711.37	9.909
449	12.016	12	1	2	99	0	11.1	736	0.61	10	21.6	122	178.32	711.37	9.909
450	12.017	12	1	2	82	0	11.1	736	0.61	60	84	122	178.32	711.37	9.909
451	12.017	12	1	2	82	0	11.1	736	0.61	80	144	122	178.32	711.37	9.909
452	12.017	12	1	2	82	0	11.1	736	0.61	10	28.8	122	178.32	711.37	9.909
453	12.017	12	1	2	82	0	11.1	736	0.61	25	41.4	122	178.32	711.37	9.909
454	12.017	12	1	2	82	0	11.1	736	0.61	30	45.6	122	178.32	711.37	9.909
455	12.017	12	1	2	82	0	11.1	736	0.61	90	153.6	122	178.32	711.37	9.909
456	12.017	12	1	2	82	0	11.1	736	0.61	50	69	122	178.32	711.37	9.909
457	12.017	12	1	2	82	0	11.1	736	0.61	20	37.2	122	178.32	711.37	9.909
458	12.017	12	1	2	82	0	11.1	736	0.61	50	67.2	122.2	178.32	711.37	9.909
459	12.017	12	1	2	82	0	11.1	736	0.61	70	108	122	178.32	711.37	9.909
460	12.017	12	1	2	82	0	11.1	736	0.61	40	54	122	178.32	711.37	9.909
461	12.017	12	1	2	82	0	11.1	736	0.61	100	168	122	178.32	711.37	9.909
462	12.018	12	1	2	54	0	11.1	736	0.61	20	56.4	122	178.32	711.37	9.909
463	12.018	12	1	2	54	0	11.1	736	0.61	70	124.8	122	178.32	711.37	9.909
464	12.018	12	1	2	54	0	11.1	736	0.61	30	69.6	122	178.32	711.37	9.909
465	12.018	12	1	2	54	0	11.1	736	0.61	40	80.4	122	178.32	711.37	9.909
466	12.018	12	1	2	54	0	11.1	736	0.61	90	160.8	122	178.32	711.37	9.909
467	12.018	12	1	2	54	0	11.1	736	0.61	60	108	122	178.32	711.37	9.909
468	12.018	12	1	2	54	0	11.1	736	0.61	80	136.8	122	178.32	711.37	9.909

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
469	12.018	12	1	2	54	0	11.1	736	0.61	50	93.6	122.2	178.32	711.37	9.909
470	12.018	12	1	2	54	0	11.1	736	0.61	50	94.2	122	178.32	711.37	9.909
471	12.018	12	1	2	54	0	11.1	736	0.61	25	63	122	178.32	711.37	9.909
472	12.018	12	1	2	54	0	11.1	736	0.61	10	43.2	122	178.32	711.37	9.909
473	12.019	12	1	2	40	0	11.1	736	0.17	32	1608	111.8	112.78	699.89	9.909
474	12.019	12	1	2	40	0	11.1	736	0.17	10	1008	111.8	112.78	699.89	9.909
475	12.019	12	1	2	40	0	11.1	736	0.17	20	1344	111.8	112.78	699.89	9.909
476	12.019	12	1	2	40	0	11.1	736	0.17	25	1452	111.8	112.78	699.89	9.909
477	12.019	12	1	2	40	0	11.1	736	0.17	25	1452	111.8	112.78	699.89	9.909
478	12.019	12	1	2	40	0	11.1	736	0.17	30	1560	111.8	112.78	699.89	9.909
479	12.02	12	1	2	55	0	11.1	736	0.17	10	67.2	114	111.26	717.11	9.909
480	12.02	12	1	2	55	0	11.1	736	0.17	20	-14.4	114	111.26	717.11	9.909
481	12.02	12	1	2	55	0	11.1	736	0.17	25	-14.4	114	111.26	717.11	9.909
482	12.021	12	1	2	41	0	11.1	736	0.17	25	-14.4	114	111.26	717.11	9.909
483	12.021	12	1	2	41	0	11.1	736	0.17	20	-14.4	114	111.26	717.11	9.909
484	12.022	12	1	2	83	0	11.1	736	0.17	10	26.4	122	112.78	774.47	9.909
485	12.022	12	1	2	83	0	11.1	736	0.17	60	62.4	122	112.78	774.47	9.909
486	12.022	12	1	2	83	0	11.1	736	0.17	70	70.8	122	112.78	774.47	9.909
487	12.022	12	1	2	83	0	11.1	736	0.17	50	55.2	122	112.78	774.47	9.909
488	12.022	12	1	2	83	0	11.1	736	0.17	80	81.6	122	112.78	774.47	9.909
489	12.022	12	1	2	83	0	11.1	736	0.17	30	42	122	112.78	774.47	9.909
490	12.022	12	1	2	83	0	11.1	736	0.17	90	93.6	122	112.78	774.47	9.909
491	12.022	12	1	2	83	0	11.1	736	0.17	40	50.4	122	112.78	774.47	9.909
492	12.022	12	1	2	83	0	11.1	736	0.17	20	33.6	122	112.78	774.47	9.909
493	12.022	12	1	2	83	0	11.1	736	0.17	25	37.8	122	112.78	774.47	9.909
494	12.023	12	1	2	55	0	11.1	736	0.17	60	96	122	112.78	774.47	9.909
495	12.023	12	1	2	55	0	11.1	736	0.17	50	69.6	122	112.78	774.47	9.909
496	12.023	12	1	2	55	0	11.1	736	0.17	40	55.2	122	112.78	774.47	9.909
497	12.023	12	1	2	55	0	11.1	736	0.17	10	24	122	112.78	774.47	9.909
498	12.023	12	1	2	55	0	11.1	736	0.17	25	38.4	122	112.78	774.47	9.909
499	12.023	12	1	2	55	0	11.1	736	0.17	76	168	122	112.78	774.47	9.909
500	12.023	12	1	2	55	0	11.1	736	0.17	20	33.6	122	112.78	774.47	9.909
501	12.023	12	1	2	55	0	11.1	736	0.17	30	43.2	122	112.78	774.47	9.909
502	12.023	12	1	2	55	0	11.1	736	0.17	70	115.2	122	112.78	774.47	9.909
503	12.024	12	1	2	47	0	11.1	736	0.17	25	124.8	122	112.78	774.47	9.909
504	12.024	12	1	2	47	0	11.1	736	0.17	10	64.8	122	112.78	774.47	9.909
505	12.024	12	1	2	47	0	11.1	736	0.17	30	153.6	122	112.78	774.47	9.909
506	12.024	12	1	2	47	0	11.1	736	0.17	20	96	122	112.78	774.47	9.909
507	12.025	12	4	2	73	0	11.1	736	0.17	25	-79.2	102	132.59	608.11	9.909
508	12.025	12	4	2	73	0	11.1	736	0.17	20	-79.2	102	132.59	608.11	9.909
509	12.026	12	4	2	77	0	11.1	736	0.17	20	-79.2	105	129.55	631.05	9.909
510	12.026	12	4	2	77	0	11.1	736	0.17	25	-79.2	105	129.55	631.05	9.909
511	12.027	12	4	2	74	0	11.1	736	0.17	25	-64.8	106	129.55	642.53	9.909
512	12.027	12	4	2	74	0	11.1	736	0.17	20	-64.8	106	129.55	642.53	9.909
513	12.028	12	4	2	64	0	11.1	736	0.17	20	264	110.8	117.35	688.42	9.909
514	12.028	12	4	2	64	0	11.1	736	0.17	25	288	110.8	117.35	734.32	9.909
515	12.028	12	4	2	64	0	11.1	736	0.17	10	192	110.8	117.35	688.42	9.909
516	12.029	12	4	2	27	0	11.1	736	0.17	50	480	111.8	112.78	699.89	9.909
517	12.029	12	4	2	27	0	11.1	736	0.17	25	180	111.8	112.78	699.89	9.909
518	12.029	12	4	2	27	0	11.1	736	0.17	20	120	111.8	112.78	699.89	9.909
519	12.029	12	4	2	27	0	11.1	736	0.17	70	720	111.8	112.78	699.89	9.909
520	12.03	12	4	2	76	0	11.1	736	0.17	25	39.6	111.8	112.78	699.89	9.909

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
521	12.03	12	4	2	76	0	11.1	736	0.17	70	103.2	111.8	112.78	699.89	9.909
522	12.03	12	4	2	76	0	11.1	736	0.17	50	75.6	111.8	112.78	699.89	9.909
523	12.03	12	4	2	76	0	11.1	736	0.17	20	31.2	111.8	112.78	699.89	9.909
524	12.031	12	4	2	69	0	11.1	736	0.17	20	10.8	122	112.78	774.47	9.909
525	12.031	12	4	2	69	0	11.1	736	0.17	50	28.8	122	112.78	774.47	9.909
526	12.031	12	4	2	69	0	11.1	736	0.17	70	45.6	122	112.78	774.47	9.909
527	12.031	12	4	2	69	0	11.1	736	0.17	25	13.8	122	112.78	774.47	9.909
528	12.032	12	4	2	73	0	11.1	736	0.17	20	8.4	122	112.78	774.47	9.909
529	12.032	12	4	2	73	0	11.1	736	0.17	50	19.2	122	112.78	774.47	9.909
530	12.032	12	4	2	73	0	11.1	736	0.17	25	10.2	122	112.78	774.47	9.909
531	12.032	12	4	2	73	0	11.1	736	0.17	70	36	122	112.78	774.47	9.909
532	12.033	12	2	2	38	0	11.1	736	0.17	20	-86.4	106	129.55	642.53	9.909
533	12.033	12	2	2	38	0	11.1	736	0.17	25	-86.4	106	129.55	642.53	9.909
534	12.034	12	2	2	84	0	11.1	736	0.17	25	-67.2	106	129.55	642.53	9.909
535	12.034	12	2	2	84	0	11.1	736	0.17	20	-67.2	106	129.55	642.53	9.909
536	12.035	12	2	2	40	0	11.1	736	0.17	25	396	111.8	112.78	699.89	9.909
537	12.035	12	2	2	40	0	11.1	736	0.17	50	720	111.8	112.78	699.89	9.909
538	12.035	12	2	2	40	0	11.1	736	0.17	20	336	111.8	112.78	699.89	9.909
539	12.036	12	2	2	84	0	11.1	736	0.17	20	57.6	111.8	112.78	699.89	9.909
540	12.036	12	2	2	84	0	11.1	736	0.17	70	96	111.8	112.78	699.89	9.909
541	12.036	12	2	2	84	0	11.1	736	0.17	25	61.2	111.8	112.78	699.89	9.909
542	12.036	12	2	2	84	0	11.1	736	0.17	50	79.2	111.8	112.78	699.89	9.909
543	12.037	12	2	2	79	0	11.1	736	0.17	20	24	111.8	112.78	699.89	9.909
544	12.037	12	2	2	79	0	11.1	736	0.17	50	69.6	111.8	112.78	699.89	9.909
545	12.037	12	2	2	79	0	11.1	736	0.17	25	31.8	111.8	112.78	699.89	9.909
546	12.037	12	2	2	79	0	11.1	736	0.17	70	98.4	111.8	112.78	699.89	9.909
547	12.038	12	2	2	163	0	11.1	736	0.61	25	29.4	125.6	179.84	734.32	9.909
548	12.038	12	2	2	163	0	11.1	736	0.61	70	55.2	125.6	179.84	734.32	9.909
549	12.038	12	2	2	163	0	11.1	736	0.61	20	26.4	125.6	179.84	734.32	9.909
550	12.038	12	2	2	163	0	11.1	736	0.61	50	43.2	125.6	179.84	734.32	9.909
551	12.039	12	4	2	152	0	11.1	736	0.61	20	19.2	125.6	179.84	734.32	9.909
552	12.039	12	4	2	152	0	11.1	736	0.61	70	52.8	125.6	179.84	734.32	9.909
553	12.039	12	4	2	152	0	11.1	736	0.61	25	24	125.6	179.84	734.32	9.909
554	12.039	12	4	2	152	0	11.1	736	0.61	50	40.8	125.6	179.84	734.32	9.909
555	12.04	12	2	2	163	0	11.1	736	0.61	20	16	125.2	182.89	728.58	9.909
556	12.04	12	2	2	163	0	11.1	736	0.61	25	19.8	125.2	182.89	728.58	9.909
557	12.04	12	2	2	163	0	11.1	736	0.61	70	52.8	125.2	182.89	728.58	9.909
558	12.04	12	2	2	163	0	11.1	736	0.61	50	36	125.2	182.89	728.58	9.909
559	12.041	12	4	2	204	0	11.1	736	0.61	70	52.8	125.2	182.89	728.58	9.909
560	12.041	12	4	2	204	0	11.1	736	0.61	20	16.8	125.2	182.89	728.58	9.909
561	12.041	12	4	2	204	0	11.1	736	0.61	50	38.4	125.2	182.89	728.58	9.909
562	12.041	12	4	2	204	0	11.1	736	0.61	25	20.4	125.2	182.89	728.58	9.909
563	12.042	12	1	2	114	0	11.1	736	0.61	50	42	125.2	182.89	728.58	9.909
564	12.042	12	1	2	114	0	11.1	736	0.61	20	24	125.2	182.89	728.58	9.909
565	12.042	12	1	2	114	0	11.1	736	0.61	25	27	125.2	182.89	728.58	9.909
566	12.042	12	1	2	114	0	11.1	736	0.61	70	54	125.2	182.89	728.58	9.909
567	13.001	13	1	2	120	16.2	15	760	0.25	10	19.3	117.2	167.03	708.45	12.787
568	13.001	13	1	2	120	16.2	15	760	0.25	20	21.05	117.2	167.03	708.45	12.787
569	13.001	13	1	2	120	16.2	15	760	0.25	25	22	117.2	167.03	708.45	12.787
570	13.001	13	1	2	120	16.2	15	760	0.25	100	55	117.2	167.03	708.45	12.787
571	13.001	13	1	2	120	16.2	15	760	0.25	50	26.9	117.2	167.03	708.45	12.787
572	13.002	13	4	2	124	20.6	15	760	0.25	25	28.6	117.2	167.03	708.45	12.787

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
573	13.002	13	4	2	124	20.6	15	760	0.25	20	27.7	117.2	167.03	708.45	12.787
574	13.002	13	4	2	124	20.6	15	760	0.25	50	33.3	117.2	167.03	708.45	12.787
575	13.002	13	4	2	124	20.6	15	760	0.25	10	26	117.2	167.03	708.45	12.787
576	13.002	13	4	2	124	20.6	15	760	0.25	100	40	117.2	167.03	708.45	12.787
577	13.003	13	4	2	130	19.8	15	760	0.25	10	258	111.4	153.7	678.93	12.787
578	13.003	13	4	2	130	19.8	15	760	0.25	50	486	111.4	153.7	678.93	12.787
579	13.003	13	4	2	130	19.8	15	760	0.25	25	335	111.4	153.7	678.93	12.787
580	13.003	13	4	2	130	19.8	15	760	0.25	100	-84	111.4	153.7	678.93	12.787
581	13.003	13	4	2	130	19.8	15	760	0.25	20	310	111.4	153.7	678.93	12.787
582	13.004	13	1	2	120	13.6	15	760	0.25	25	-84	111.4	153.7	678.93	12.787
583	13.004	13	1	2	120	13.6	15	760	0.25	7	792	111.4	153.7	678.93	12.787
584	13.004	13	1	2	120	13.6	15	760	0.25	20	-84	111.4	153.7	678.93	12.787
585	13.004	13	1	2	120	13.6	15	760	0.25	10	-84	111.4	153.7	678.93	12.787
586	13.005	13	1	2	117	16.8	15	760	0.25	25	11.5	122.9	180.37	737.97	12.787
587	13.005	13	1	2	117	16.8	15	760	0.25	50	13.6	122.9	180.37	737.97	12.787
588	13.005	13	1	2	117	16.8	15	760	0.25	100	32.1	122.9	180.37	737.97	12.787
589	13.005	13	1	2	117	16.8	15	760	0.25	20	11.1	122.9	180.37	737.97	12.787
590	13.005	13	1	2	117	16.8	15	760	0.25	10	10.6	122.9	180.37	737.97	12.787
591	13.007	13	4	2	130	20	15	760	0.25	25	11.7	122.9	180.37	737.97	12.787
592	13.007	13	4	2	130	20	15	760	0.25	50	14.2	122.9	180.37	737.97	12.787
593	13.007	13	4	2	130	20	15	760	0.25	100	23	122.9	180.37	737.97	12.787
594	13.007	13	4	2	130	20	15	760	0.25	10	10.3	122.9	180.37	737.97	12.787
595	13.007	13	4	2	130	20	15	760	0.25	20	11.2	122.9	180.37	737.97	12.787
596	13.008	13	4	2	130	20	15	760	0.25	50	480	112.1	154.96	684.83	12.787
597	13.008	13	4	2	130	20	15	760	0.25	25	-84	110	138.33	684.83	12.787
598	14.001	14	4	2	79	5.8	10	756.5	0.6	20	320	115.5	0	0	9.208
599	14.001	14	4	2	79	5.8	10	756.5	0.6	50	-51	115.5	0	0	9.208
600	14.001	14	4	2	79	5.8	10	756.5	0.6	50	510	115.5	0	0	9.208
601	14.002	14	4	2	79	5.8	12	756.5	0.6	50	408	115.9	0	0	10.517
602	14.002	14	4	2	79	5.8	12	756.5	0.6	50	505	115.9	0	0	10.517
603	14.002	14	4	2	79	5.8	12	756.5	0.6	20	235	115.9	0	0	10.517
604	14.002	14	4	2	79	5.8	12	756.5	0.6	20	215	115.9	0	0	10.517
605	14.003	14	4	2	79	5.8	15	756.5	0.6	20	150	116.5	0	0	12.787
606	14.003	14	4	2	79	5.8	15	756.5	0.6	20	156	116.5	0	0	12.787
607	14.003	14	4	2	79	5.8	15	756.5	0.6	50	305	116.5	0	0	12.787
608	14.003	14	4	2	79	5.8	15	756.5	0.6	50	268	116.5	0	0	12.787
609	14.004	14	4	2	79	5.8	18	756.5	0.6	20	135	116.7	0	0	15.477
610	14.004	14	4	2	79	5.8	18	756.5	0.6	50	202	116.7	0	0	15.477
611	14.004	14	4	2	79	5.8	18	756.5	0.6	20	107	116.7	0	0	15.477
612	14.004	14	4	2	79	5.8	18	756.5	0.6	50	258	116.7	0	0	15.477
613	14.005	14	4	2	102	11.7	9	756.2	0.6	50	462	117.2	0	0	8.609
614	14.005	14	4	2	102	11.7	9	756.2	0.6	20	175	117.2	0	0	8.609
615	14.005	14	4	2	102	11.7	9	756.2	0.6	20	108	117.2	0	0	8.609
616	14.005	14	4	2	102	11.7	9	756.2	0.6	50	223	117.2	0	0	8.609
617	14.006	14	3	2	105	13.9	9	756.2	0.6	50	515	117.2	0	0	8.609
618	14.006	14	3	2	105	13.9	9	756.2	0.6	20	177	117.2	0	0	8.609
619	14.006	14	3	2	105	13.9	9	756.2	0.6	20	165	117.2	0	0	8.609
620	14.006	14	3	2	105	13.9	9	756.2	0.6	50	418	117.2	0	0	8.609
621	14.007	14	4	2	102	11.7	12	756.2	0.6	20	158	117.6	0	0	10.517
622	14.007	14	4	2	102	11.7	12	756.2	0.6	20	141	117.6	0	0	10.517
623	14.007	14	4	2	102	11.7	12	756.2	0.6	50	252	117.6	0	0	10.517
624	14.007	14	4	2	102	11.7	12	756.2	0.6	50	242	117.6	0	0	10.517

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
625	14.008	14	3	2	105	13.9	12	756.2	0.6	20	214	117.6	0	0	10.517
626	14.008	14	3	2	105	13.9	12	756.2	0.6	50	395	117.6	0	0	10.517
627	14.008	14	3	2	105	13.9	12	756.2	0.6	50	525	117.6	0	0	10.517
628	14.008	14	3	2	105	13.9	12	756.2	0.6	20	205	117.6	0	0	10.517
629	14.009	14	4	2	102	11.7	15	756.2	0.6	50	118	117.9	0	0	12.787
630	14.009	14	4	2	102	11.7	15	756.2	0.6	50	193	117.9	0	0	12.787
631	14.009	14	4	2	102	11.7	15	756.2	0.6	20	92	117.9	0	0	12.787
632	14.009	14	4	2	102	11.7	15	756.2	0.6	20	57	117.9	0	0	12.787
633	14.01	14	3	2	105	13.9	15	756.2	0.6	50	470	117.9	0	0	12.787
634	14.01	14	3	2	105	13.9	15	756.2	0.6	20	173	117.9	0	0	12.787
635	14.01	14	3	2	105	13.9	15	756.2	0.6	50	490	117.9	0	0	12.787
636	14.01	14	3	2	105	13.9	15	756.2	0.6	20	154	117.9	0	0	12.787
637	14.011	14	4	2	102	11.7	18	756.2	0.6	50	52	118.4	0	0	15.477
638	14.011	14	4	2	102	11.7	18	756.2	0.6	20	39	118.4	0	0	15.477
639	14.011	14	4	2	102	11.7	18	756.2	0.6	20	37	118.4	0	0	15.477
640	14.011	14	4	2	102	11.7	18	756.2	0.6	50	72	118.4	0	0	15.477
641	14.012	14	3	2	105	13.9	18	756.2	0.6	50	453	118.4	0	0	15.477
642	14.012	14	3	2	105	13.9	18	756.2	0.6	20	162	118.4	0	0	15.477
643	14.012	14	3	2	105	13.9	18	756.2	0.6	50	313	118.4	0	0	15.477
644	14.012	14	3	2	105	13.9	18	756.2	0.6	20	212	118.4	0	0	15.477
645	14.013	14	4	2	95	10.4	9	760.2	0.6	50	193	116.5	0	0	8.609
646	14.013	14	4	2	95	10.4	9	760.2	0.6	50	160	116.5	0	0	8.609
647	14.013	14	4	2	95	10.4	9	760.2	0.6	20	127	116.5	0	0	8.609
648	14.013	14	4	2	95	10.4	9	760.2	0.6	20	101	116.5	0	0	8.609
649	14.014	14	3	2	110	18	9	760.2	0.6	50	287	116.5	0	0	8.609
650	14.014	14	3	2	110	18	9	760.2	0.6	20	158	116.5	0	0	8.609
651	14.014	14	3	2	110	18	9	760.2	0.6	20	116	116.5	0	0	8.609
652	14.014	14	3	2	110	18	9	760.2	0.6	50	456	116.5	0	0	8.609
653	14.015	14	4	2	95	10.4	12	760.2	0.6	20	122	116.8	0	0	10.517
654	14.015	14	4	2	95	10.4	12	760.2	0.6	20	88	116.8	0	0	10.517
655	14.015	14	4	2	95	10.4	12	760.2	0.6	50	183	116.8	0	0	10.517
656	14.015	14	4	2	95	10.4	12	760.2	0.6	50	211	116.8	0	0	10.517
657	14.016	14	3	2	110	18	12	760.2	0.6	20	122	116.8	0	0	10.517
658	14.016	14	3	2	110	18	12	760.2	0.6	20	195	116.8	0	0	10.517
659	14.016	14	3	2	110	18	12	760.2	0.6	50	397	116.8	0	0	10.517
660	14.016	14	3	2	110	18	12	760.2	0.6	50	603	116.8	0	0	10.517
661	14.017	14	4	2	95	10.4	15	760.2	0.6	50	143	117	0	0	12.787
662	14.017	14	4	2	95	10.4	15	760.2	0.6	50	178	117	0	0	12.787
663	14.018	14	4	2	95	10.4	15	760.2	0.6	20	93	117	0	0	12.787
664	14.018	14	4	2	95	10.4	15	760.2	0.6	20	87	117	0	0	12.787
665	14.019	14	4	2	95	10.4	18	760.2	0.6	20	54	116.8	0	0	15.477
666	14.019	14	4	2	95	10.4	18	760.2	0.6	50	113	116.8	0	0	15.477
667	14.019	14	4	2	95	10.4	18	760.2	0.6	50	102	116.8	0	0	15.477
668	14.019	14	4	2	95	10.4	18	760.2	0.6	20	55	116.8	0	0	15.477
669	14.02	14	4	2	113	16.6	9	760.6	0.6	20	35	121.5	0	0	8.609
670	14.02	14	4	2	113	16.6	9	760.6	0.6	50	-4.5	121.5	0	0	8.609
671	14.02	14	4	2	113	16.6	9	760.6	0.6	50	45	121.5	0	0	8.609
672	14.02	14	4	2	113	16.6	9	760.6	0.6	20	29	121.5	0	0	8.609
673	14.021	14	4	2	113	16.6	12	760.6	0.6	20	27	122.2	0	0	10.517
674	14.021	14	4	2	113	16.6	12	760.6	0.6	20	28	122.2	0	0	10.517
675	14.021	14	4	2	113	16.6	12	760.6	0.6	50	40	122.2	0	0	10.517
676	14.021	14	4	2	113	16.6	12	760.6	0.6	50	44	122.2	0	0	10.517

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
677	14.022	14	4	2	113	16.6	15	760.6	0.6	20	28	122.3	0	0	12.787
678	14.022	14	4	2	113	16.6	15	760.6	0.6	50	40	122.3	0	0	12.787
679	14.022	14	4	2	113	16.6	15	760.6	0.6	50	43	122.3	0	0	12.787
680	14.022	14	4	2	113	16.6	15	760.6	0.6	20	30	122.3	0	0	12.787
681	14.023	14	3	2	113	17.1	15	760.6	0.6	50	49	122.3	0	0	12.787
682	14.023	14	3	2	113	17.1	15	760.6	0.6	20	34	122.3	0	0	12.787
683	14.023	14	3	2	113	17.1	15	760.6	0.6	50	36	122.3	0	0	12.787
684	14.023	14	3	2	113	17.1	15	760.6	0.6	20	21	122.3	0	0	12.787
685	14.024	14	4	2	162	40.5	8	756.7	0.6	50	102	120.7	0	0	8.045
686	14.024	14	4	2	162	40.5	8	756.7	0.6	20	70	120.7	0	0	8.045
687	14.025	14	3	2	209	111.5	8	756.7	0.6	50	45	120.7	0	0	8.045
688	14.025	14	3	2	209	111.5	8	756.7	0.6	20	29	120.7	0	0	8.045
689	14.026	14	1	2	206	95.8	8	756.7	0.6	50	82	120.7	0	0	8.045
690	14.027	14	2	2	167	51.6	8	756.7	0.6	20	152	120.7	0	0	8.045
691	14.027	14	2	2	167	51.6	8	756.7	0.6	50	235	120.7	0	0	8.045
692	14.028	14	4	2	124	19.8	18	763.3	0.6	50	32	121.6	0	0	15.477
693	14.028	14	4	2	124	19.8	18	763.3	0.6	20	24	121.6	0	0	15.477
694	14.029	14	3	2	121	19.2	18	763.3	0.6	50	37	121.6	0	0	15.477
695	14.029	14	3	2	121	19.2	18	763.3	0.6	20	22	121.6	0	0	15.477
696	14.03	14	1	2	126	23.8	18	763.3	0.6	50	31	121.6	0	0	15.477
697	14.03	14	1	2	126	23.8	18	763.3	0.6	20	22	121.6	0	0	15.477
698	14.031	14	2	2	119	20.5	18	763.3	0.6	50	46	121.6	0	0	15.477
699	14.031	14	2	2	119	20.5	18	763.3	0.6	20	30	121.6	0	0	15.477
700	14.032	14	4	2	162	40.5	12	756.7	0.6	50	84	121.2	0	0	10.517
701	14.032	14	4	2	162	40.5	12	756.7	0.6	20	61	121.2	0	0	10.517
702	14.033	14	3	2	209	111.5	12	756.7	0.6	50	51	121.2	0	0	10.517
703	14.033	14	3	2	209	111.5	12	756.7	0.6	20	30	121.2	0	0	10.517
704	14.034	14	1	2	206	95.8	12	756.7	0.6	50	84	121.2	0	0	10.517
705	14.034	14	1	2	206	95.8	12	756.7	0.6	20	55	121.2	0	0	10.517
706	14.035	14	2	2	167	51.6	12	756.7	0.6	20	195	121.2	0	0	10.517
707	14.035	14	2	2	167	51.6	12	756.7	0.6	50	-25	121.2	0	0	10.517
708	14.036	14	4	2	162	40.5	16	756.7	0.6	50	35	121.5	0	0	13.634
709	14.036	14	4	2	162	40.5	16	756.7	0.6	20	27	121.5	0	0	13.634
710	14.037	14	3	2	209	111.5	16	756.7	0.6	50	63	121.5	0	0	13.634
711	14.037	14	3	2	209	111.5	16	756.7	0.6	20	37	121.5	0	0	13.634
712	14.038	14	1	2	206	95.8	16	756.7	0.6	20	54	121.5	0	0	13.634
713	14.038	14	1	2	206	95.8	16	756.7	0.6	50	198	121.5	0	0	13.634
714	14.039	14	2	2	167	51.6	16	756.7	0.6	20	160	121.5	0	0	13.634
715	14.039	14	2	2	167	51.6	16	756.7	0.6	50	-25	121.5	0	0	13.634
716	14.04	14	4	2	162	40.5	20	756.7	0.6	50	40	121.6	0	0	17.534
717	14.04	14	4	2	162	40.5	20	756.7	0.6	20	28	121.6	0	0	17.534
718	14.041	14	3	2	209	111.5	20	756.7	0.6	20	34	121.6	0	0	17.534
719	14.041	14	3	2	209	111.5	20	756.7	0.6	50	57	121.6	0	0	17.534
720	14.042	14	1	2	206	95.8	20	756.7	0.6	20	40	121.6	0	0	17.534
721	14.042	14	1	2	206	95.8	20	756.7	0.6	50	53	121.6	0	0	17.534
722	14.043	14	2	2	167	51.6	20	756.7	0.6	50	270	121.6	0	0	17.534
723	14.043	14	2	2	167	51.6	20	756.7	0.6	20	51	121.6	0	0	17.534
724	14.044	14	4	2	124	19.8	10	756.3	0.6	50	56	121.4	0	0	9.208
725	14.044	14	4	2	124	19.8	10	756.3	0.6	20	31	121.4	0	0	9.208
726	14.045	14	3	2	121	19.2	10	756.3	0.6	20	20	121.4	0	0	9.208
727	14.045	14	3	2	121	19.2	10	756.3	0.6	50	49	121.4	0	0	9.208
728	14.046	14	1	2	126	23.8	10	756.3	0.6	20	46	121.4	0	0	9.208

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
729	14.046	14	1	2	126	23.8	10	756.3	0.6	50	90	121.4	0	0	9.208
730	14.047	14	2	2	119	20.5	10	756.3	0.6	50	43	121.4	0	0	9.208
731	14.047	14	2	2	119	20.5	10	756.3	0.6	20	30	121.4	0	0	9.208
732	14.048	14	4	2	124	19.8	12	756.3	0.6	20	20	122.3	0	0	10.517
733	14.048	14	4	2	124	19.8	12	756.3	0.6	50	33	122.3	0	0	10.517
734	14.049	14	3	2	121	19.2	12	756.3	0.6	20	23	122.3	0	0	10.517
735	14.049	14	3	2	121	19.2	12	756.3	0.6	50	46	122.3	0	0	10.517
736	14.05	14	1	2	126	23.8	12	756.3	0.6	20	31	122.3	0	0	10.517
737	14.05	14	1	2	126	23.8	12	756.3	0.6	50	55	122.3	0	0	10.517
738	14.051	14	2	2	119	20.5	12	756.3	0.6	20	26	122.3	0	0	10.517
739	14.052	14	4	2	124	19.8	15	756.3	0.6	20	27	122	0	0	12.787
740	14.052	14	4	2	124	19.8	15	756.3	0.6	50	42	122	0	0	12.787
741	14.053	14	1	2	126	23.8	15	756.3	0.6	20	29	122	0	0	12.787
742	14.053	14	1	2	126	23.8	15	756.3	0.6	50	50	122	0	0	12.787
743	14.054	14	2	2	119	20.5	15	756.3	0.6	50	55	122	0	0	12.787
744	14.054	14	2	2	119	20.5	15	756.3	0.6	20	31	122	0	0	12.787
745	14.055	14	3	2	121	19.2	15	756.3	0.6	20	41	122	0	0	12.787
746	14.055	14	3	2	121	19.2	15	756.3	0.6	50	-4.5	122	0	0	12.787
747	14.056	14	4	2	131	26.3	9	755.4	0.6	50	121	118.3	0	0	8.609
748	14.056	14	4	2	131	26.3	9	755.4	0.6	20	56	118.3	0	0	8.609
749	14.057	14	3	2	124	22.2	9	755.4	0.6	50	226	118.3	0	0	8.609
750	14.057	14	3	2	124	22.2	9	755.4	0.6	20	128	118.3	0	0	8.609
751	14.058	14	1	2	135	32.3	9	755.4	0.6	20	200	118.3	0	0	8.609
752	14.058	14	1	2	135	32.3	9	755.4	0.6	50	440	118.3	0	0	8.609
753	14.059	14	2	2	126	24.1	9	755.4	0.6	20	100	118.3	0	0	8.609
754	14.059	14	2	2	126	24.1	9	755.4	0.6	50	230	118.3	0	0	8.609
755	14.06	14	4	2	131	26.3	12	755.4	0.6	20	73	119.2	0	0	10.517
756	14.06	14	4	2	131	26.3	12	755.4	0.6	50	123	119.2	0	0	10.517
757	14.061	14	3	2	124	22.2	12	755.4	0.6	20	131	119.2	0	0	10.517
758	14.061	14	3	2	124	22.2	12	755.4	0.6	50	250	119.2	0	0	10.517
759	14.062	14	1	2	135	32.3	12	755.4	0.6	20	220	119.2	0	0	10.517
760	14.062	14	1	2	135	32.3	12	755.4	0.6	50	311	119.2	0	0	10.517
761	14.063	14	2	2	126	24.1	12	755.4	0.6	20	156	119.2	0	0	10.517
762	14.063	14	2	2	126	24.1	12	755.4	0.6	50	276	119.2	0	0	10.517
763	14.064	14	4	2	131	26.3	15	755.4	0.6	50	96	119.2	0	0	12.787
764	14.064	14	4	2	131	26.3	15	755.4	0.6	20	76	119.2	0	0	12.787
765	14.065	14	3	2	124	22.2	15	755.4	0.6	20	104	119.2	0	0	12.787
766	14.065	14	3	2	124	22.2	15	755.4	0.6	50	216	119.2	0	0	12.787
767	14.066	14	1	2	135	32.3	15	755.4	0.6	50	235	119.2	0	0	12.787
768	14.066	14	1	2	135	32.3	15	755.4	0.6	20	145	119.2	0	0	12.787
769	14.067	14	2	2	126	24.1	15	755.4	0.6	20	172	119.2	0	0	12.787
770	14.067	14	2	2	126	24.1	15	755.4	0.6	50	319	119.2	0	0	12.787
771	14.068	14	4	2	131	26.3	18	755.4	0.6	20	45	119.6	0	0	15.477
772	14.068	14	4	2	131	26.3	18	755.4	0.6	50	62	119.6	0	0	15.477
773	14.069	14	3	2	124	22.2	18	755.4	0.6	20	105	119.6	0	0	15.477
774	14.069	14	3	2	124	22.2	18	755.4	0.6	50	332	119.6	0	0	15.477
775	14.07	14	1	2	135	32.3	18	755.4	0.6	50	205	119.6	0	0	15.477
776	14.07	14	1	2	135	32.3	18	755.4	0.6	20	94	119.6	0	0	15.477
777	14.071	14	2	2	126	24.1	18	755.4	0.6	20	58	119.6	0	0	15.477
778	14.071	14	2	2	126	24.1	18	755.4	0.6	50	136	119.6	0	0	15.477
779	14.072	14	4	2	150	32	12	758.4	0.6	50	5	131.4	201.56	781.76	10.517
780	14.073	14	4	2	150	32	12	758.4	0.6	50	4.5	131.4	157.61	825.49	10.517

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
781	14.074	14	4	2	150	32	12	758.4	0.6	50	6	131.4	219.77	763.45	10.517
782	14.075	14	4	2	150	32	12	758.4	0.6	50	5.5	131.4	218.2	764.63	10.517
783	14.076	14	4	2	150	32	12	758.4	0.6	50	4.2	131.4	202.82	779.99	10.517
784	14.077	14	4	2	150	32	12	758.4	0.6	50	4.2	131.4	155.72	827.26	10.517
785	14.078	14	4	2	150	32	12	758.4	0.6	50	3.7	131.4	72.05	910.58	10.517
786	14.079	14	4	2	150	32	12	758.4	0.6	50	16.5	126.4	203.13	742.76	10.517
787	14.08	14	4	2	150	32	12	758.4	0.6	50	20	126.4	194.34	751.04	10.517
788	14.081	14	4	2	150	32	12	758.4	0.6	50	12	126.4	194.34	751.04	10.517
789	14.082	14	4	2	150	32	12	758.4	0.6	50	16.7	126.4	188.69	756.95	10.517
790	14.083	14	4	2	150	32	12	758.4	0.6	50	16.5	126.4	184.3	761.08	10.517
791	14.084	14	4	2	150	32	12	758.4	0.6	50	5	126.4	156.2	789.45	10.517
792	14.085	14	4	2	150	32	12	758.4	0.6	50	8.2	126.4	150.23	795.35	10.517
793	14.086	14	4	2	150	32	12	758.4	0.6	50	5.9	126.4	145.99	799.49	10.517
794	14.087	14	4	2	150	32	12	758.4	0.6	50	4.7	126.4	69.39	875.72	10.517
795	14.088	14	4	2	150	32	12	758.4	0.6	50	5	141.4	483.34	574.95	10.517
796	14.089	14	4	2	150	32	12	758.4	0.6	50	2	141.4	221.19	836.72	10.517
797	14.09	14	4	2	150	32	12	758.4	0.6	50	2.2	141.4	216.95	840.85	10.517
798	14.091	14	4	2	150	32	12	758.4	0.6	50	1.7	141.4	163.42	894.63	10.517
799	14.092	14	4	2	150	32	12	758.4	0.6	50	1.9	141.4	155.41	902.31	10.517
800	14.093	14	4	2	150	32	12	758.4	0.6	50	1.7	141.4	88.85	968.49	10.517
801	14.094	14	4	2	150	32	12	758.4	0.6	50	5.7	136.4	427.77	593.27	10.517
802	14.095	14	4	2	150	32	12	758.4	0.6	50	1.5	136.4	253.05	767.58	10.517
803	14.096	14	4	2	150	32	12	758.4	0.6	50	1.8	136.4	212.87	807.76	10.517
804	14.097	14	4	2	150	32	12	758.4	0.6	50	2.7	136.4	212.39	808.35	10.517
805	14.098	14	4	2	150	32	12	758.4	0.6	50	3	136.4	206.74	813.67	10.517
806	14.099	14	4	2	150	32	12	758.4	0.6	50	2.3	136.4	172.84	847.35	10.517
807	14.1	14	4	2	150	32	12	758.4	0.6	50	1.6	136.4	169.23	850.9	10.517
808	14.101	14	4	2	150	32	12	758.4	0.6	50	2.5	136.4	63.58	956.67	10.517
809	14.102	14	4	2	150	32	12	758.4	0.6	50	5.5	131.4	261.22	722.08	10.517
810	14.103	14	4	2	150	32	12	758.4	0.6	50	5	131.4	219.77	763.45	10.517
811	14.104	14	4	2	150	32	12	758.4	0.6	50	4.5	141.4	373.46	684.86	10.517
812	14.105	14	4	2	150	32	12	758.4	0.6	50	1.7	141.4	293.71	764.63	10.517
813	14.106	14	4	2	150	32	12	758.4	0.6	50	1.9	141.4	329.66	728.58	10.517
814	14.107	14	3	2	110	18	15	760.2	0.6	20	272	117	0	0	12.787
815	14.107	14	3	2	110	18	15	760.2	0.6	20	320	117	0	0	12.787
816	14.108	14	3	2	209	111.5	10	756.5	0.6	50	-31	115.5	0	0	9.208
817	14.108	14	3	2	209	111.5	10	756.5	0.6	20	-30	115.5	0	0	9.208
818	14.109	14	3	2	209	111.5	12	756.5	0.6	50	-33	115.9	0	0	10.517
819	14.109	14	3	2	209	111.5	12	756.5	0.6	20	-32	115.9	0	0	10.517
820	14.11	14	3	2	209	111.5	15	756.5	0.6	50	-35	116.5	0	0	12.787
821	14.11	14	3	2	209	111.5	15	756.5	0.6	20	-34	116.5	0	0	12.787
822	14.111	14	3	2	209	111.5	18	756.5	0.6	20	480	116.7	0	0	15.477
823	14.111	14	3	2	209	111.5	18	756.5	0.6	20	-36	116.7	0	0	15.477
824	14.111	14	3	2	209	111.5	18	756.5	0.6	50	-37	116.7	0	0	15.477
825	14.112	14	3	2	209	11.5	15	760.2	0.6	50	-60	117	0	0	12.787
826	14.113	14	3	2	209	11.5	18	760.2	0.6	50	-60	116.8	0	0	15.477
827	14.113	14	3	2	209	11.5	18	760.2	0.6	20	-30	116.8	0	0	15.477
828	14.114	14	3	2	209	111.5	9	760.6	0.6	20	30	121.5	0	0	8.609
829	14.114	14	3	2	209	111.5	9	760.6	0.6	20	-3	121.5	0	0	8.609
830	14.114	14	3	2	209	111.5	9	760.6	0.6	50	-5	121.5	0	0	8.609
831	14.115	14	3	2	209	111.5	12	760.6	0.6	50	-5.1	122.2	0	0	10.517
832	14.2	14	1	2	206	95.8	8	756.7	0.6	20	47	120.7	0	0	8.045

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
833	14.201	14	3	2	113	17.1	12	760.6	0.6	20	43	122.2	0	0	10.517
834	14.201	14	3	2	113	17.1	12	760.6	0.6	20	39	122.2	0	0	10.517
835	14.202	14	2	2	119	20.5	12	763.3	0.6	50	41	122.3	0	0	10.517
836	15.001	15	2	2	60	0	13.6	760	0.14	50	76.8	119.5	78.54	815.92	11.679
837	15.001	15	2	2	60	0	13.6	760	0.14	25	45.6	119.5	78.54	815.92	11.679
838	15.001	15	2	2	60	0	13.6	760	0.14	50	96	119.5	78.54	815.92	11.679
839	15.001	15	2	2	60	0	13.6	760	0.14	25	43.2	119.5	78.54	815.92	11.679
840	15.002	15	2	2	60	0	13.6	760	0.14	25	64.8	119.2	117.8	774.53	11.679
841	15.002	15	2	2	60	0	13.6	760	0.14	50	103.2	119.2	117.8	774.53	11.679
842	15.002	15	2	2	60	0	13.6	760	0.14	25	43.2	119.2	117.8	774.53	11.679
843	15.002	15	2	2	60	0	13.6	760	0.14	50	84	119.2	117.8	774.53	11.679
844	15.003	15	2	2	60	0	13.6	760	0.14	25	76.8	119.5	179.06	715.41	11.679
845	15.003	15	2	2	60	0	13.6	760	0.14	50	175.2	119.5	179.06	715.41	11.679
846	15.003	15	2	2	60	0	13.6	760	0.14	50	151.2	119.5	179.06	715.41	11.679
847	15.003	15	2	2	60	0	13.6	760	0.14	25	98.4	119.5	179.06	715.41	11.679
848	15.004	15	2	2	60	0	13.6	760	0.14	50	156	119.4	249.75	644.46	11.679
849	15.004	15	2	2	60	0	13.6	760	0.14	25	76.8	119.4	249.75	644.46	11.679
850	15.004	15	2	2	60	0	13.6	760	0.14	28	936	119.4	271.74	620.81	11.679
851	15.004	15	2	2	60	0	13.6	760	0.14	50	218.4	119.4	249.75	644.46	11.679
852	15.004	15	2	2	60	0	13.6	760	0.14	25	127.2	119.4	249.75	644.46	11.679
853	15.005	15	2	2	60	0	13.6	760	0.14	25	804	119.2	271.74	620.81	11.679
854	15.005	15	2	2	60	0	13.6	760	0.14	50	-93.6	119.2	271.74	620.81	11.679
855	15.006	15	2	2	85	0	13.6	760	0.14	50	79.2	119.3	177.49	715.41	11.679
856	15.006	15	2	2	60	0	13.6	760	0.14	25	847.2	119.2	271.74	620.81	11.679
857	15.006	15	2	2	85	0	13.6	760	0.14	25	60	119.3	177.49	715.41	11.679
858	15.007	15	2	2	85	0	13.6	760	0.14	25	86.4	118.7	172.78	715.41	11.679
859	15.007	15	2	2	85	0	13.6	760	0.14	50	127	118.7	172.78	715.41	11.679
860	15.008	15	2	2	85	0	13.6	760	0.14	25	101	117.6	164.93	715.41	11.679
861	15.008	15	2	2	85	0	13.6	760	0.14	50	127	117.6	164.93	715.41	11.679
862	15.008	15	2	2	85	0	13.6	760	0.14	25	91.2	117.6	164.93	715.41	11.679
863	15.008	15	2	2	85	0	13.6	760	0.14	50	158	117.6	164.93	715.41	11.679
864	15.008	15	2	2	85	0	13.6	760	0.14	25	130	117.6	164.93	715.41	11.679
865	15.008	15	2	2	85	0	13.6	760	0.14	50	158	117.6	164.93	715.41	11.679
866	15.009	15	2	2	38	0	13.6	760	0.14	25	406	119.4	179.06	715.41	11.679
867	15.01	15	2	2	46	0	13.6	760	0.14	50	658	119.4	179.06	715.41	11.679
868	15.01	15	2	2	46	0	13.6	760	0.14	25	401	119.4	179.06	715.41	11.679
869	15.011	15	2	2	100	0	13.6	760	0.14	50	62	119.4	179.06	715.41	11.679
870	15.011	15	2	2	100	0	13.6	760	0.14	50	101	119.4	179.06	715.41	11.679
871	15.011	15	2	2	100	0	13.6	760	0.14	25	50	119.4	179.06	715.41	11.679
872	15.011	15	2	2	100	0	13.6	760	0.14	25	69.6	119.4	179.06	715.41	11.679
873	15.012	15	2	2	60	0	13.6	760	0.14	25	-84	119	359.7	532.12	11.679
874	15.012	15	2	2	60	0	13.6	760	0.14	20	840	119	359.7	532.12	11.679
875	15.013	15	2	2	60	0	13.6	760	0.14	25	768	119	301.58	591.25	11.679
876	15.014	15	2	2	60	0	13.6	760	0.14	50	-84	119	359.7	532.12	11.679
877	15.015	15	2	2	60	0	13.6	760	0.14	50	-79.2	119	301.58	591.25	11.679
878	15.016	15	2	2	38	0	13.6	760	0.14	50	-72	119.4	179.06	715.41	11.679
879	16.001	16	1	2	59	2.3	8.3	759	1	50	1182	113.3	168.78	682.18	8.211
880	17.001	17	4	2	0	0	10	760	0.1	50	38.5	120.1	0	0	9.208
881	17.001	17	4	2	0	0	10	760	0.1	50	40	120.1	0	0	9.208
882	17.001	17	4	2	0	0	10	760	0.1	50	47	120.1	0	0	9.208
883	17.001	17	4	2	0	0	10	760	0.1	50	45	120.6	0	0	9.208
884	17.002	17	4	2	0	0	10	760	0.1	50	6.5	123.6	0	0	9.208

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
937	22.002	22	2	2	160	51	12	754	0.6	7	648	116.8	0	0	10.517
938	22.003	22	4	2	200	74	12	754	0.6	50	456	116.8	0	0	10.517
939	22.004	22	4	2	200	74	12	754	0.6	50	43	121.2	0	0	10.517
940	22.005	22	3	2	140	36	12	754	0.6	50	38	121.2	0	0	10.517
941	22.006	22	3	2	140	36	12	754	0.6	50	360	116.8	0	0	10.517
942	22.007	22	3	2	160	51	12	754	0.6	50	-64.8	111	0	0	10.517
943	22.008	22	3	2	140	36	12	754	0.6	50	-64.8	111	0	0	10.517
944	22.009	22	4	2	200	74	12	754	0.6	50	-64.8	111	0	0	10.517
945	22.01	22	2	2	160	51	12	754	0.6	20	-64.8	111	0	0	10.517
946	22.011	22	2	2	160	51	12	754	0.6	20	-64.8	116.8	0	0	10.517
947	22.012	22	2	2	160	51	12	754	0.6	20	50	121.2	0	0	10.517
948	22.013	22	2	2	160	51	12	754	0.6	25	-64.8	111	0	0	10.517
949	22.014	22	2	2	160	51	12	754	0.6	25	-64.8	116.8	0	0	10.517
950	22.015	22	2	2	160	51	12	754	0.6	25	60	121.2	0	0	10.517
951	22.016	22	3	2	140	36	12	754	0.6	20	-64.8	111	0	0	10.517
952	22.017	22	3	2	140	36	12	754	0.6	20	195	116.8	0	0	10.517
953	22.018	22	3	2	140	36	12	754	0.6	20	25	121.2	0	0	10.517
954	22.019	22	3	2	140	36	12	754	0.6	25	-64.8	111	0	0	10.517
955	22.02	22	3	2	140	36	12	754	0.6	25	230	116.8	0	0	10.517
956	22.021	22	3	2	140	36	12	754	0.6	25	26	121.2	0	0	10.517
957	22.022	22	4	2	200	74	12	754	0.6	20	-64.8	111	0	0	10.517
958	22.023	22	4	2	200	74	12	754	0.6	20	210	116.8	0	0	10.517
959	22.024	22	4	2	200	74	12	754	0.6	20	35	121.2	0	0	10.517
960	22.025	22	4	2	200	74	12	754	0.6	25	-64.8	111	0	0	10.517
961	22.026	22	4	2	200	74	12	754	0.6	25	230	116.8	0	0	10.517
962	22.027	22	4	2	200	74	12	754	0.6	25	36	121.2	0	0	10.517
963	23.001	23	3	2	193	95.5	12.5	754	0.28	80	504	118.4	0	0	10.869
964	23.002	23	3	2	193	95.5	12.5	754	0.28	60	504	118.4	0	0	10.869
965	23.003	23	3	2	193	95.5	12.5	754	0.28	10	504	116.4	0	0	10.869
966	23.004	23	3	2	193	95.5	12.5	754	0.28	50	-50.4	116.4	0	0	10.869
967	23.005	23	3	2	193	95.5	12.5	754	0.28	20	-50.4	116.4	0	0	10.869
968	23.006	23	3	2	193	95.5	12.5	754	0.28	50	-50.4	114.4	0	0	10.869
969	23.007	23	3	2	193	95.5	12.5	754	0.28	20	-50.4	114.4	0	0	10.869
970	23.008	23	3	2	193	95.5	12.5	754	0.28	50	-50.4	111.4	0	0	10.869
971	23.009	23	3	2	193	95.5	12.5	754	0.28	20	-50.4	111.4	0	0	10.869
972	23.01	23	3	2	193	95.5	12.5	754	0.28	50	-50.4	109.4	0	0	10.869
973	23.011	23	3	2	193	95.5	12.5	754	0.28	20	-50.4	109.4	0	0	10.869
974	24.001	24	5	3	235	121.5	12	760	0.6	50	11	131.4	0	0	10.517
975	24.002	24	5	3	235	121.5	12	760	0.6	50	20	127.4	0	0	10.517
976	24.003	24	5	3	235	121.5	12	760	0.6	50	29	125.4	0	0	10.517
977	24.004	24	5	3	235	121.5	12	760	0.6	50	97	121.4	0	0	10.517
978	24.005	24	5	3	235	121.5	12	760	0.6	50	42	122.4	0	0	10.517
979	24.006	24	5	3	235	121.5	12	760	0.6	50	265	119.4	0	0	10.517
980	24.007	24	5	3	235	121.5	12	760	0.6	50	-50.4	115.4	0	0	10.517
981	24.008	24	5	3	235	121.5	12	760	0.6	50	-50.4	113.4	0	0	10.517
982	24.009	24	5	3	235	121.5	12	760	0.6	50	67	121.4	0	0	10.517
983	24.01	24	5	3	235	121.5	12	760	0.6	50	172	119.4	0	0	10.517
984	24.011	24	5	3	235	121.5	12	760	0.6	50	-50.4	116.4	0	0	10.517
985	24.012	24	5	3	235	121.5	12	760	0.6	50	-50.4	113.4	0	0	10.517
986	24.013	24	5	2	67	3.5	12	760	0.6	50	97	122.4	0	0	10.517
987	24.014	24	5	2	67	3.5	12	760	0.6	50	170	119.4	0	0	10.517
988	24.015	24	5	2	67	3.5	12	760	0.6	50	349	115.4	0	0	10.517

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
885	17.002	17	4	2	0	0	10	760	0.1	50	14.5	125	0	0	9.208
886	17.002	17	4	2	0	0	10	760	0.1	50	7	130.3	0	0	9.208
887	17.002	17	4	2	0	0	10	760	0.1	50	6.5	130.3	0	0	9.208
888	17.002	17	4	2	0	0	10	760	0.1	50	7.5	129.6	0	0	9.208
889	17.002	17	4	2	0	0	10	760	0.1	50	3.5	135.9	0	0	9.208
890	17.002	17	4	2	0	0	10	760	0.1	50	17	125	0	0	9.208
891	17.002	17	4	2	0	0	10	760	0.1	50	9	130.3	0	0	9.208
892	17.002	17	4	2	0	0	10	760	0.1	50	4	136.4	0	0	9.208
893	17.003	17	4	2	0	0	10	760	0.1	50	2.5	138.5	0	0	9.208
894	17.003	17	4	2	0	0	10	760	0.1	50	2.6	139.5	0	0	9.208
895	17.004	17	4	2	0	0	10	760	1	50	6	139.6	0	0	9.208
896	17.005	17	4	2	0	0	10	760	0.5	50	11	129.6	0	0	9.208
897	17.006	17	4	2	0	0	10	760	0.5	50	3.5	139.5	0	0	9.208
898	17.007	17	4	2	0	0	10	760	0.5	50	25	125	0	0	9.208
899	17.007	17	4	2	0	0	10	760	0.5	50	10.5	129.6	0	0	9.208
900	17.007	17	4	2	0	0	10	760	0.5	50	13.5	130	0	0	9.208
901	17.007	17	4	2	0	0	10	760	0.5	50	3.5	138.2	0	0	9.208
902	17.007	17	4	2	0	0	10	760	0.5	50	14.5	130	0	0	9.208
903	17.007	17	4	2	0	0	10	760	0.5	50	30.5	125	0	0	9.208
904	17.007	17	4	2	0	0	10	760	0.5	50	4.5	136.2	0	0	9.208
905	17.007	17	4	2	0	0	10	760	0.5	50	3.3	140.5	0	0	9.208
906	17.007	17	4	2	0	0	10	760	0.5	50	5.5	136.4	0	0	9.208
907	17.008	17	4	2	0	0	10	760	1	50	7.5	139.5	0	0	9.208
908	17.009	17	4	2	0	0	10	760	1	50	26	129.6	0	0	9.208
909	17.009	17	4	2	0	0	10	760	1	50	9.5	134.9	0	0	9.208
910	17.009	17	4	2	0	0	10	760	1	50	7	135	0	0	9.208
911	18.001	18	4	2	190	0	10	754	0.6	50	54	120	0	0	9.208
912	19.001	19	3	2	86	7.7	16	754	0.6	50	10	129	186.95	758.7	13.634
913	19.002	19	3	2	86	7.7	16	754	0.6	50	57.8	121.8	170.32	721.26	13.634
914	19.003	19	3	2	86	7.7	16	754	0.6	50	9.2	129.6	196.45	754.02	13.634
915	19.004	19	3	2	86	7.7	16	754	0.6	50	52.8	121.8	178.09	713.65	13.634
916	19.005	19	3	2	86	7.7	16	754	0.6	50	73.2	121.6	181.04	708.97	13.634
917	19.006	19	3	2	86	7.7	16	754	0.6	50	51.3	121.7	178.09	712.48	13.634
918	19.007	19	3	2	86	7.7	16	754	0.6	50	56.3	121.3	186.02	701.37	13.634
919	19.008	19	3	2	86	7.7	15	754	0.6	50	37	131.1	289.69	671.13	12.787
920	19.009	19	3	2	86	7.7	15	754	0.6	50	22.3	131.6	201.48	761.91	12.787
921	19.01	19	3	2	86	7.7	15	754	0.6	50	23.3	131.4	198.37	764.25	12.787
922	19.011	19	3	2	86	7.7	16	754	0.6	50	48.5	122.3	174.98	720.09	13.634
923	19.012	19	3	2	86	7.7	15	754	0.6	7	167	121.8	265.26	626.63	12.787
924	19.013	19	3	2	86	7.7	15	754	0.6	50	71.6	122	182.34	710.37	12.787
925	19.014	19	3	2	86	7.7	15	754	0.6	50	65.8	121.8	178.14	713.3	12.787
926	19.015	19	3	2	86	7.7	16	754	0.6	50	12.1	128.9	196.58	748.17	13.634
927	20.001	20	1	3	0	0	0	756	0.6	50	240	0	0	700.56	4.583
928	21.001	21	3	3	530	1700	12	754	0.6	20	100	121.2	0	0	10.517
929	21.002	21	3	3	540	1800	12	754	0.6	30	115	121.2	0	0	10.517
930	21.003	21	3	3	540	1900	12	754	0.6	40	127	121.2	0	0	10.517
931	21.004	21	3	3	520	1600	12	754	0.6	10	77	121.2	0	0	10.517
932	21.005	21	3	3	520	1700	12	754	0.6	20	667	116.8	0	0	10.517
933	21.006	21	3	3	560	1700	12	754	0.6	40	835	116.8	0	0	10.517
934	21.007	21	3	3	540	1500	12	754	0.6	30	835	116.8	0	0	10.517
935	21.008	21	3	3	590	2300	12	754	0.6	10	523	116.8	0	0	10.517
936	22.001	22	2	2	160	51	12	754	0.6	50	104	121.2	0	0	10.517

Record	Author	Auth. No.	Species	Stage	Length mm	Weight g	Temp. C	Patm mmHg	Depth m	% Mort.	Time hr	TGP %	O2 mmHg	N2 mmHg	pH2O mmHg
989	24.016	24	5	2	67	3.5	12	760	0.6	50	-50.4	113.4	0	0	10.517
990	24.017	24	5	2	67	3.5	12	760	0.6	50	54	121.4	0	0	10.517
991	24.018	24	5	2	67	3.5	12	760	0.6	50	119	119.4	0	0	10.517
992	24.019	24	5	2	67	3.5	12	760	0.6	50	315	115.4	0	0	10.517
993	24.02	24	5	2	67	3.6	12	760	0.6	50	-50.4	113.4	0	0	10.517
994	24.021	24	5	3	235	121.5	12	760	0.6	20	8	131.4	0	0	10.517
995	24.022	24	5	3	235	121.5	12	760	0.6	20	16	127.4	0	0	10.517
996	24.023	24	5	3	235	121.5	12	760	0.6	20	20	125.4	0	0	10.517
997	24.024	24	5	3	235	121.5	12	760	0.6	20	64	121.4	0	0	10.517
998	24.025	24	5	3	235	121.5	12	760	0.6	20	34	122.4	0	0	10.517
999	24.026	24	5	3	235	121.5	12	760	0.6	20	142	119.4	0	0	10.517
1000	24.027	24	5	3	235	121.5	12	760	0.6	20	-50.4	115.4	0	0	10.517
1001	24.028	24	5	3	235	121.5	12	760	0.6	20	-50.4	113.4	0	0	10.517
1002	24.029	24	5	3	235	121.5	12	760	0.6	20	44	121.4	0	0	10.517
1003	24.03	24	5	3	235	121.5	12	760	0.6	20	151	119.4	0	0	10.517
1004	24.031	24	5	3	235	121.5	12	760	0.6	20	-50.4	116.4	0	0	10.517
1005	24.032	24	5	3	235	121.5	12	760	0.6	20	-50.4	113.4	0	0	10.517
1006	24.033	24	5	2	67	3.5	12	760	0.6	20	50	122.4	0	0	10.517
1007	24.034	24	5	2	67	3.5	12	760	0.6	20	109	119.4	0	0	10.517
1008	24.035	24	5	2	67	3.5	12	760	0.6	20	246	115.4	0	0	10.517
1009	24.036	24	5	2	67	3.5	12	760	0.6	20	-50.4	113.4	0	0	10.517
1010	24.037	24	5	2	67	3.5	12	760	0.6	20	20	121.4	0	0	10.517
1011	24.038	24	5	2	67	3.5	12	760	0.6	20	79	119.4	0	0	10.517
1012	24.039	24	5	2	67	3.5	12	760	0.6	20	185	115.4	0	0	10.517
1013	24.04	24	5	2	67	3.5	12	760	0.6	20	-50.4	113.4	0	0	10.517