THE HEMATOLOGY, METABOLIC RATE, AND AEROBIC SWIMMING CAPACITY OF TRIPLOID BROOK TROUT

by

Evelyn J. Stillwell

B.Sc., University of New Brunswick, 1985
B.Ed., Saint Thomas University, 1990

A Thesis Submitted in Partial Fulfilment of
the Requirements for the Degree of

Master of Science

in the Graduate Academic Unit of Biology

Supervisor:	Tillmann J. Benfey, PhD, Biology
Examining Board:	Timothy Dilworth, Biology, Chair James Kieffer, Biology, Internal Examiner James Sexsmith, Kinesiology, External Examiner

THE UNIVERSITY OF NEW BRUNSWICK

August, 1997

Out of clutter, find simplicity.
From discord find harmony.
In the middle of difficulty lies opportunity.

Albert Einstein
Three rules of work

If all the beasts were gone, we would die from great loneliness of spirit,
for whatever happens to the beast, happens to us. All things are connected.

Chief Seathl

Dedication

To my parents, Ingrid and Llewellyn Stillwell, for their love and support.

Abstract

In this thesis I have examined the effects of artificially induced triploidy on the hematology, metabolic rate, and aerobic swimming capacity of brook trout (*Salvelinus fontinalis*). My results indicate that triploids have a lower metabolic (oxygen consumption) rate than diploids but that diploids and triploids are equivalent in their aerobic swimming capacity (as measured by critical swimming velocity). Diploids and triploids are also similar in their hematology except around the time of diploid ovulation, when diploid females have a lower total blood hemoglobin concentration than triploid females. Hematological results suggest that the blood-oxygen carrying capacity of triploids may not be impaired to the degree previously anticipated. The lower oxygen requirements of triploids may act to offset any existing reduction in blood-oxygen carrying capacity such that the aerobic capacity of diploids and triploids is equivalent.

Preface

This thesis is presented in an "articles" format. Chapter 1 serves as a general introduction providing background information and indicating the main objectives. Chapters 2 to 4 describe the experimental work. Chapter 5 is a general discussion and conclusion chapter serving to relate the individual experiments to one another.

Chapter 2 has been published in *Fish Physiology and Biochemistry*, chapter 3 is currently in press in *The Journal of Fish Biology*, and a portion of chapter 4 is currently being revised for resubmission to *Fish Physiology and Biochemistry*. Letters granting me permission to include published material in my thesis appear in appendix 5 (pp. 80-86). Each manuscript is published/submitted under joint authorship with Dr. Benfey who has critically read and edited each manuscript. Otherwise the experimental design, data collection, statistical analyses, figures, diagrams and text were my efforts. Contributions of other individuals are acknowledged within each manuscript and in the general thesis acknowledgement.

Chapters 2 to 4 are written in the format dictated by the respective publishing journal and contain their own introduction, materials and methods, results, discussion and reference sections. I ask that readers forgive the redundancy inherent in presenting a thesis in an articles format.

Acknowledgements

I would like to thank Dr. Tillmann Benfey for acting as my supervisor throughout this thesis. My graduate student experience was as gratifying as it was in large part thanks to his excellent supervision and the many opportunities he provided. I also thank Tillmann for his thoroughness and patience as an editor; many a red pen met its demise at his hands. My thanks are also extended to Dr. Richard Saunders and Dr. Jim Wiggs, the other members of my supervisory committee, whose critical comments, suggestions, and insight have been invaluable.

This research was financially supported by a research grant from the Natural Sciences and Engineering Research Council. I am grateful to the APICS Aquaculture Committee and the Aquaculture Association of Canada for providing me an opportunity to share my work, for recognising my efforts, and for their supportive and open attitude towards students. I would also like to express my thanks to the department of Biology for supporting my hybrid existence as a graduate student and a technician. In particular I thank Dr. Jim Wiggs and Ms. Angelique Gloss for their patience and support during this time.

Although many people have offered assistance along the way I am indebted to four individuals in particular. Thanks to: Mr. Donald Hornibrook whose skilled hands restored the Blazka respirometer to its former glory; Dr. Roman Mureika whose statistical proficiency and explanatory skills gave me faith that my statistical analyses were correct and almost made me understand why; Mr. Roger Smith whose photographic skills have been invaluable to me for various presentations; Mrs. Robyn O'Keefe, my ever-ready anaesthesiologist and pita-twin, who helped me in many ways. Among other things I owe her thanks for showing me the proper way to bleed a fish.

I would also like to thank past and present members of the fish-wing coffee group (including our token entomologist) for brightening my days with their conversation and "treats".

I am very grateful to Paul (P.J.) Jardine for sharing his views on science and for providing critical insight and constant encouragement. I thank him and Ruth for helping me to maintain balance in my life and for putting a smile on my face at the end of each day.

Finally, I would like to acknowledge my experimental brook trout who always behaved as they should even when I didn't realise it. I thank them for teaching me patience and for helping me to gain an appreciation for their species. This research would not have been possible without them.

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Chapter 1

General Introduction

Background

Most vertebrates are diploids and, as such, contain two set of chromosomes in their somatic cells. Within diploid species of fish cases of triploidy occur spontaneously (Benfey 1989). Such cases typically occur when the second polar body with its full complement of chromosomes is retained within, rather than extruded from, the fertilised egg (Benfey 1989). Resultant zygotes have three sets of chromosomes (one set of paternal origin and two of maternal origin) instead of the normal two. Triploidy has been artificially induced in many fish species by applying a thermal, chemical, or hydrostatic pressure treatment to eggs shortly after fertilisation (Benfey 1989). Although each of these methods has a slightly different mode of action, the ultimate effect is that the second polar body is retained by the egg and triploidy results (Figure 1.1).

Fish cells containing three sets of chromosomes (i.e., triploid cells) are able to undergo normal mitotic division (Figure 1.2) thus triploid fish are viable and grow normally. In contrast, meiotic division does not proceed normally in triploid salmonids (i.e., fish of the family Salmonidae). The presence of homologous triads creates mechanical problems in the cross-over and separation events of meiosis I such that most triploid germ cells are not able to complete meiosis (Figure 1.3) and the few that do are generally aneuploid (Gui *et al.* 1992). Triploid salmonids are, therefore, sterile.

Although sterility confers no obvious advantage to the fish itself, fisheries managers regard sterility as a useful means of controlling cases of undesired reproduction. This presents itself in situations where interbreeding between cultured and wild fish reduces the genetic'fitness' of wild fish populations and in situations where uncontrolled reproduction of introduced fish species results in their establishment in non-native areas. The artificial induction of triploidy is a simple and effective means by which mass-sterilisation can be accomplished.

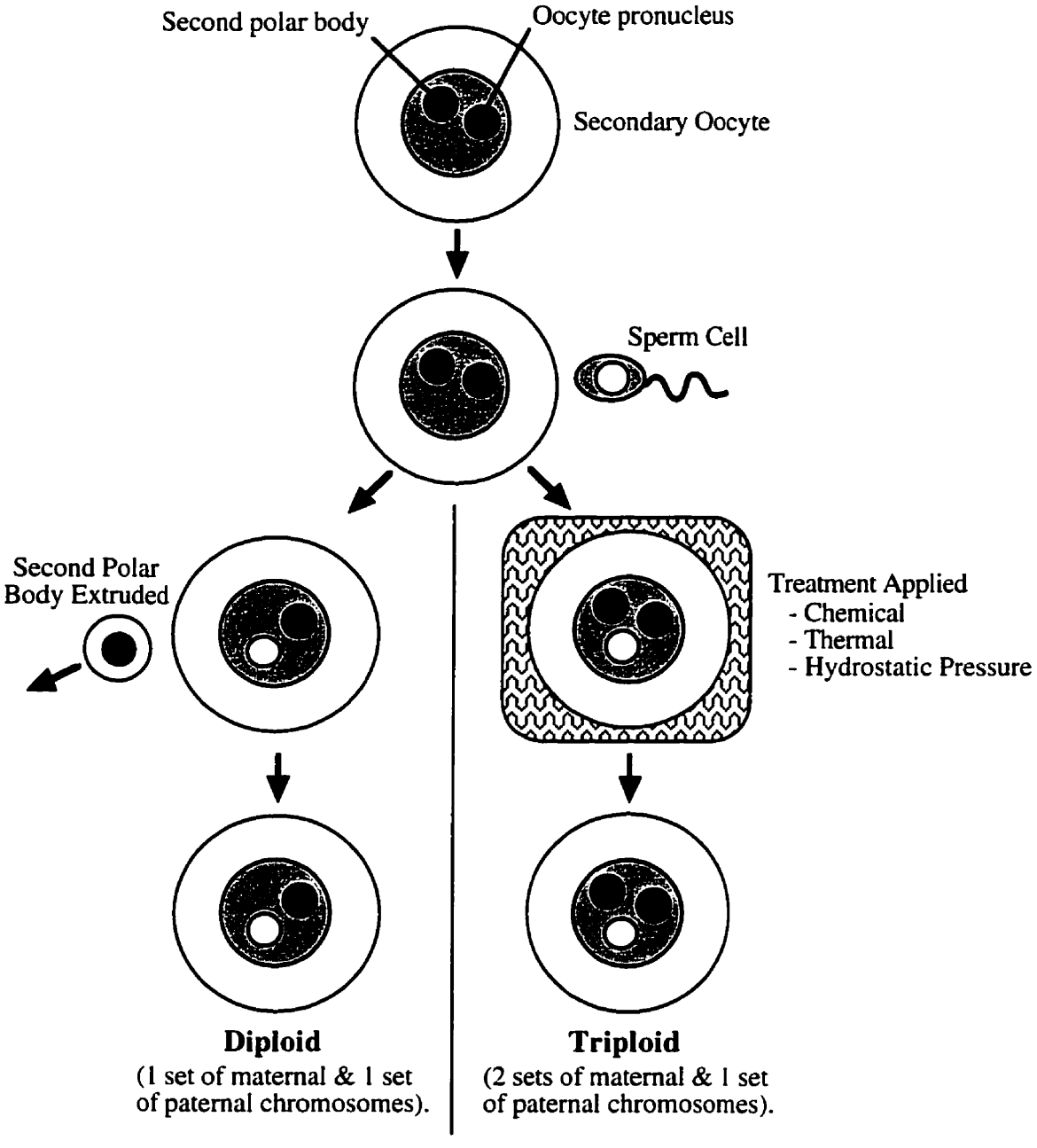
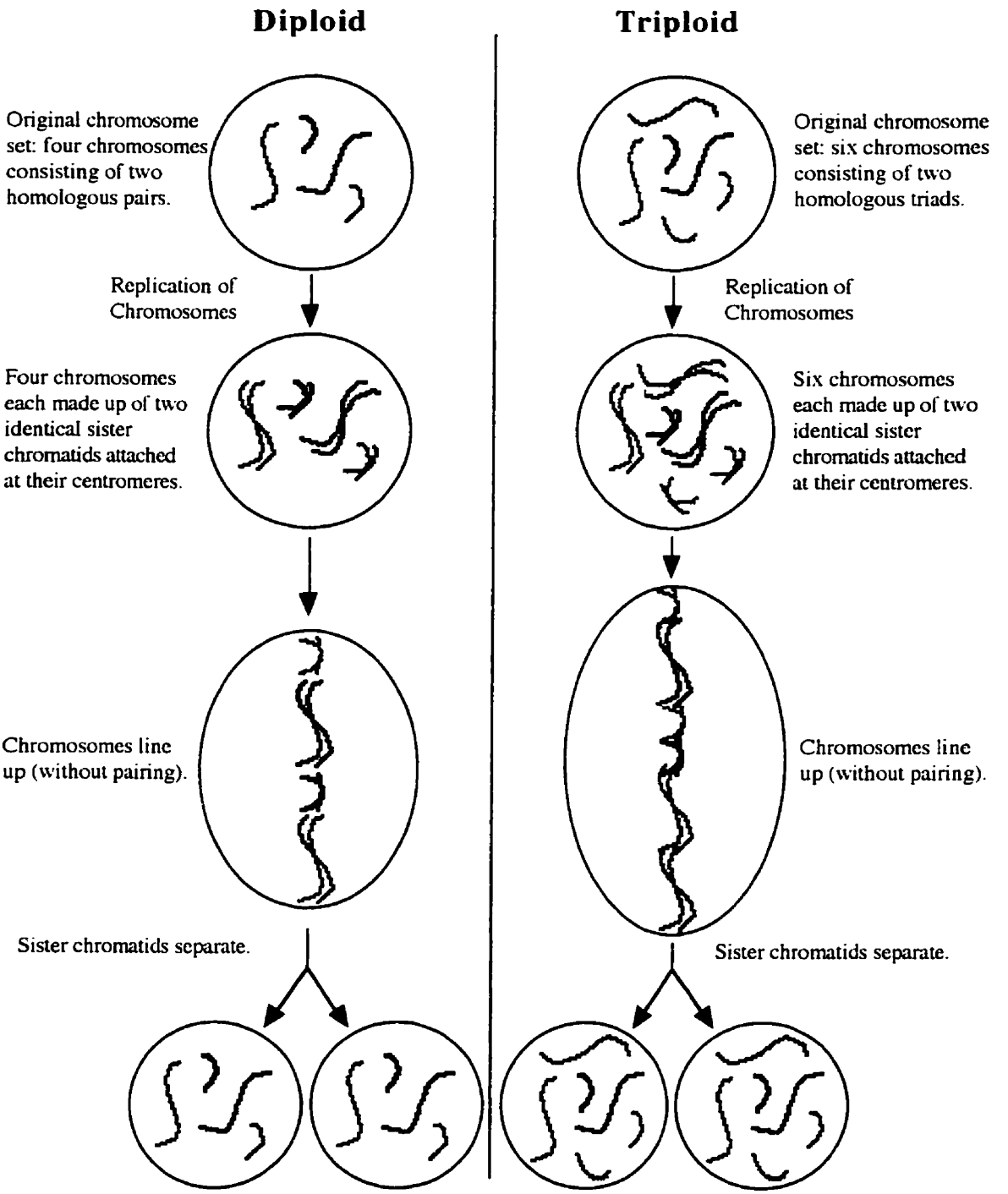


Figure 1.1. The artificial induction of triploidy: the application of a chemical, thermal or hydrostatic pressure treatment shortly after fertilisation causes the second polar body to be retained within the egg and results in the production of a triploid zygote.



Result: Mitosis proceeds normally in triploid cells. In both diploids and triploids each daughter nucleus has chromosomes identical to those in the original nucleus.

Figure 1.2. A comparison of mitotic division in diploid and triploid somatic cells (adapted from Curtis, 1979).

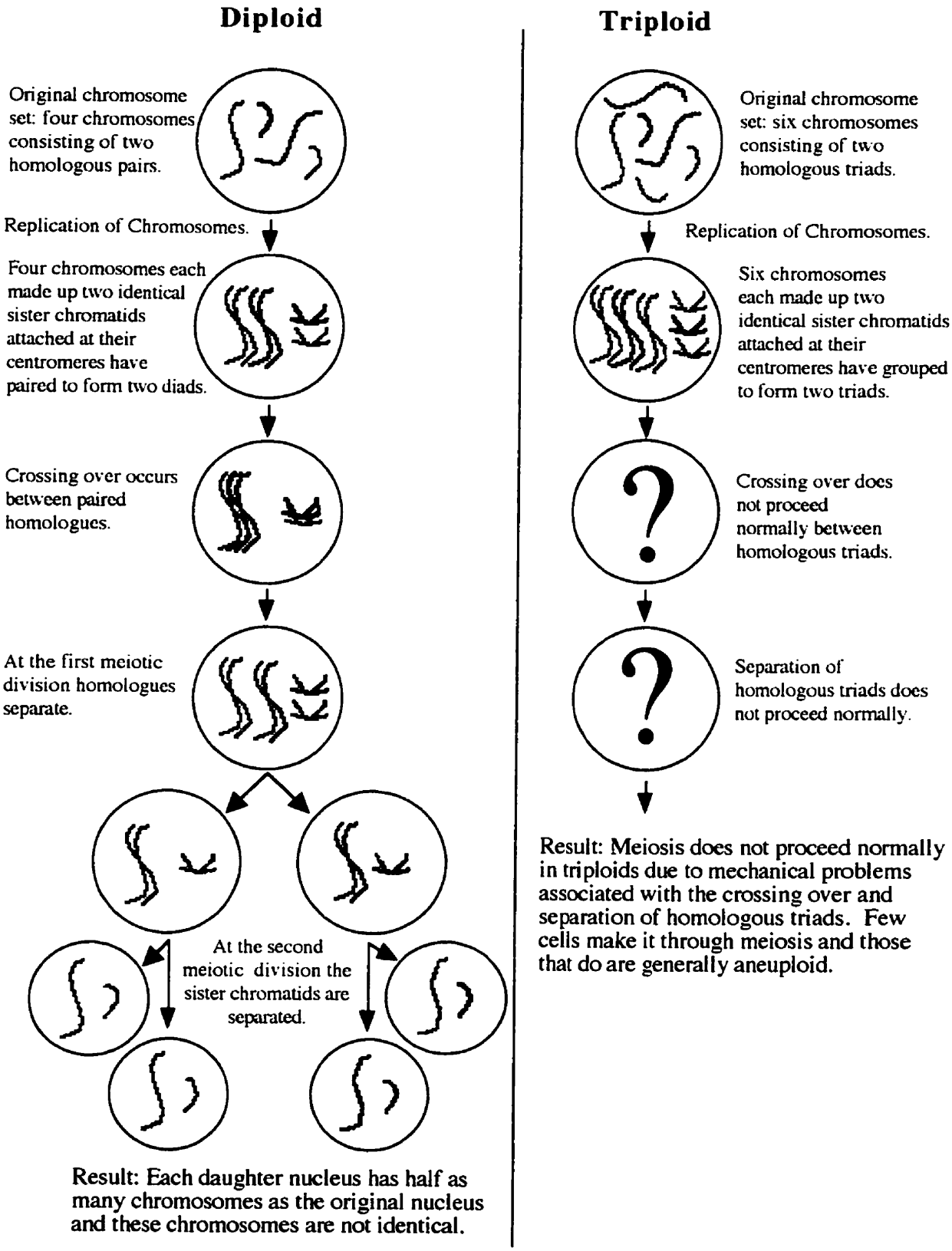


Figure 1.3. A comparison of meiotic division in diploid and triploid germ cells (adapted from Curtis, 1979).

Although both male and female triploids are sterile, the endocrinological effects of triploidy are not the same for both sexes. In female salmonids those steroid hormones directly associated with reproduction are generally produced in the thecal and granulosa cells of the ovarian follicular tissue (Scott 1987); ovarian interstitial cells may also have some degree of steroidogenic activity (Fostier *et al.* 1983). The steroidogenic nature of follicular tissue, however, develops only after germ cells have completed meiosis I. Since triploid oogonia are arrested in meiosis I, follicular development does not occur and triploid ovaries produce no significant amounts of reproductive steroids (Benfey 1997). In male salmonids the interlobular and interstitial cells of the testes are the major steroidogenic sites associated with reproduction (Fostier *et al.* 1983). As the steroidogenic activity of these tissues is not dependent on the germ cells completing meiosis, male triploids have endocrine profiles similar to those of male diploids (Benfey 1997).

The endocrinological differences between male and female triploids manifest themselves in that male triploids develop normal secondary sexual characteristics whereas female triploids remain sexually immature (Benfey 1997). It is this feature that makes female triploids attractive to the aquaculture industry: Diploid males and females as well as triploid males show a deterioration in flesh quality and a decline in growth rate during sexual maturation whereas female triploids, as a result of their suppressed sexual development, show continued growth and maintain high flesh quality (Benfey 1997). Both of these traits are valued in aquaculture.

Aside from producing the endocrinological effects described, triploid induction also results in direct physical changes at the cellular level. The presence of an increased amount of genetic material in triploids results in triploid nuclear size being increased relative to that of diploids. Triploid cell size increases concomitantly resulting in the maintenance of an equivalent cytoplasmic to nuclear volume ratio across ploidy groups (Swarup 1959). Despite this increase in cell size, the body and organ size of triploids is equivalent to that of diploids because triploid cell numbers are reduced. This increase in cell size and decrease in cell number has been noted in various triploid tissues (e.g., epithelium, cartilage, liver,

kidney, retina, brain) and in blood, where both leukocytes and erythrocytes are influenced (Benfey 1997).

An increase in cellular and nuclear size has several potential physiological implications for triploids. An increased diffusion and transport distance in triploids would be expected if cell/nuclear shape is maintained with an increase in size. The surface area to volume ratio of triploid cells and nuclei would also be reduced which in turn could impair the performance of any function limited by surface area (e.g., nutrient and ion exchange, hormone binding, etc.)(Benfey 1997). A decreased surface area to volume ratio might also reduce the energy required by triploids to maintain ionic and osmotic gradients and to produce and repair nuclear and cell membranes (Benfey 1997). One would expect that any changes occurring at the cellular level would translate into effects at the whole animal level.

Several studies have examined the physiological consequences of triploid induction in salmonid fishes. The following provides an overview of the status of physiological research in triploid salmonid metabolism, hematology and swimming performance at the onset of this study. Metabolic rate has been examined as a means of assessing how the described ploidy-related changes at the cellular level influence energetic requirements at the whole animal level. Three studies which measured metabolic rate using indirect calorimetric methods (i.e., by measuring oxygen consumption rate) found no difference in the metabolic rate of diploid and triploid salmonids (Benfey and Sutterlin 1984a, Oliva-Teles and Kaushik 1990, Yamamoto and Iida 1994a).

The induction of triploidy appears to have effects on certain hematological parameters. Several studies have found total blood hemoglobin concentration (TBHC) to be lower in triploid than diploid salmonids (Benfey and Sutterlin 1984b, Graham *et al.* 1985, Small and Randall 1989, Yamamoto and Iida 1994b). Graham *et al.* (1985) also found a 23% reduction in the hemoglobin-oxygen loading ratio of triploid Atlantic salmon (*Salmo salar*) relative to diploid controls; this factor, combined with a lower TBHC, resulted in experimental triploids having blood-oxygen carrying capacity which was only 68% that of

experimental diploids.

If triploid salmonids are equivalent to diploids in their oxygen requirements (i.e., metabolic rate) but inferior in their blood-oxygen carrying capacity, one would presume that triploid aerobic capacity would be impaired. The aerobic capacity of triploid salmonids relative to diploid controls has been assessed by examining indirect biochemical indicators of aerobic swimming capacity (Virtanen *et al.* 1990) and through critical swimming velocity tests (Small and Randall 1989). Based on their findings, Virtanen *et al.* (1990) concluded that the aerobic capacity of triploid rainbow trout (*Oncorhynchus mykiss*) was impaired. This study has been criticised because experimental triploids had a significantly lower hematocrit than the experimental diploids at the onset of the study; it appears that this rather than triploidy *per se* may have lead to the observed reduction in aerobic capacity. Small and Randall (1989), in contrast, found no difference in the critical swimming velocity of diploid and triploid coho salmon (*Oncorhynchus kisutch*) and concluded that the aerobic capacity of diploids and triploids was equivalent.

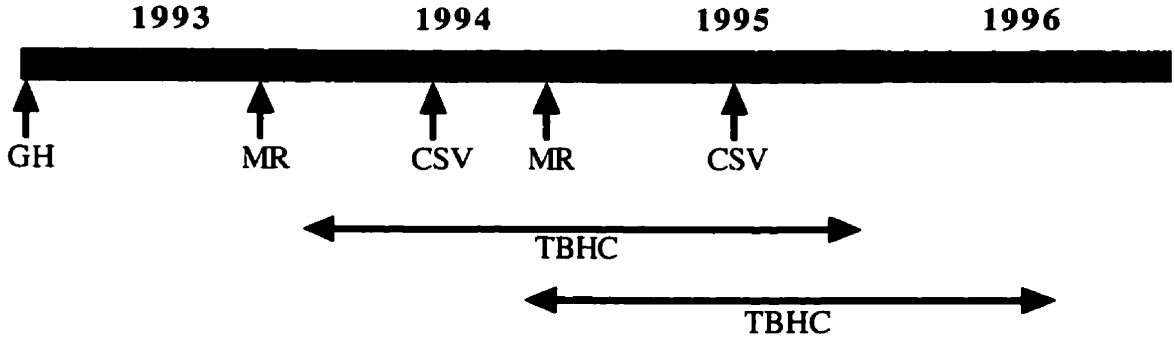
It is unclear how triploids are able to perform as well aerobically as diploids given that they have equivalent oxygen requirements to, but a lower blood-oxygen carrying capacity than, diploids. There appear to be two possible explanations which might account for these findings. One possibility is that triploids have developed mechanisms which enable them to compensate for a lower blood-oxygen carrying capacity. For example, triploids might have a greater swimming efficiency than diploids and may, therefore, require less energy (and less oxygen) to attain the same critical swimming velocities as diploids. Triploids could also have an increased opercular abduction rate which would act to offset the effects of a reduced blood-oxygen carrying capacity. Levels of hemoglobin oxygen-affinity modulating solutes (e.g., nucleoside triphosphates; NTP) may also be altered such that the loading/unloading of oxygen to/from triploid hemoglobin is enhanced. Other compensatory mechanisms are also possible (e.g., increased cardiac output, increased gill irrigation volume, etc.). The second possibility is that conclusions from studies which examined hematological parameters, metabolic rate and/or critical swimming velocity in

diploids and triploids are incorrect. If this is the case it may in part be due to a lack of proper controls between ploidy groups. From the information detailed above it is clear that diploids and triploids are different not only in their ploidy level but also in their endocrinology; males and females also differ endocrinologically within a ploidy group. In most of the aforementioned studies endocrinological differences were not controlled for, therefore, ploidy effects may have been confounded by endocrinological effects. Fish species also varied between studies. If any of the previous physiological findings were species-specific they may have further confused the interpretation of how the various physiological factors interacted with one another.

Objectives

The purpose of this thesis was to explore these possibilities by (1) determining if triploids exhibit compensatory activity in the form of improved swimming efficiency, increased opercular abduction rates and/or altered levels of total blood NTP and (2) examining the hematology, metabolic rate and critical swimming velocity of diploids and triploids under controlled conditions. Endocrinological differences between ploidy and sex groups were controlled for by using sexually immature fish and/or fish of only one sex. A single species of fish (i.e., brook trout, *Salvelinus fontinalis* Mitchill) was used for all experimental work to control for any species-specific effects. Brook trout were specifically chosen as the model salmonid species because the parameters for triploid induction are well understood for this species and because they are relatively easy to rear, hardy in culture and fairly tolerant of experimental manipulation. Brook trout are also an important aquaculture species.

Chronologically, the experimental work proceeded as described in Figure 1.4. The metabolic rate experiment is presented in chapter 2 of this thesis; preliminary results from the critical swimming velocity experiment are also referenced in this chapter. The critical swimming velocity experiment is discussed in detail in chapter 3. The general hematology and TBHC experiments are presented together in chapter 4.



GH: Jan./Feb. 1993.
General hematology was examined (i.e., total blood hemoglobin concentration, NTP levels, cellular and nuclear dimensions, cell number, etc.). Total blood hemoglobin concentration was examined in further detail in the TBHC experiment (see below).

MR: Nov./Dec. 1993.
The metabolic rate experiment was conducted. Possible compensatory mechanisms were examined (i.e., increased gill irrigation rate, improved swimming efficiency). TBHC was measured.
Experiment repeated in Nov./Dec. 1994.

CSV: May/June 1994.
The critical swimming velocity experiment was conducted.
Experiment repeated in June/July 1995.

TBHC: Dec. 1993 - Dec. 1995
Total blood hemoglobin concentration was examined at regular intervals over a two-year period.
Experiment repeated Sept. 1994 - Aug. 1996.

Figure 1.4. The chronology of experimental work.

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Chapter 2

Hemoglobin level, metabolic rate, opercular abduction rate and swimming efficiency in female triploid brook trout (*Salvelinus fontinalis*).

This chapter (excluding Appendix) has been published in the journal *Fish Physiology and Biochemistry*, Volume 15: 377-383 (1996).

Abstract

Metabolic (oxygen consumption) rate, opercular abduction rate and tail beat frequency were determined in two strains of diploid and triploid female brook trout (*Salvelinus fontinalis*) while these fish swam at 0.37 ± 0.02 body lengths/sec in a Blazka respirometer. Total blood hemoglobin level was also measured and opercular condition examined. Total blood hemoglobin levels in diploids and triploids were equal. The opercular abduction rate was the same in diploids and triploids (regardless of whether triploid opercular condition was good or poor) yet triploids had a lower oxygen consumption rate than diploids, indicating that triploids take up less oxygen than diploids per opercular cycle. Tail beat frequency, an indicator of swimming effort, was the same in diploids and triploids, suggesting that triploids require less oxygen than diploids for a similar swimming effort.

Introduction

Diploid and triploid salmonids are virtually indistinguishable at the whole animal level. This observation belies the existence of fundamental differences between diploids and triploids which arise as a consequence of the extra set of chromosomes present in triploid cells. In order to accommodate their extra genetic material, the cell nuclei of triploid fish are larger than those of diploid fish; triploid cells are also enlarged thus conserving the cytoplasmic-nuclear volume ratio (Dunham 1990). The increase in cell size is accompanied by a decrease in cell number; the presence of a smaller number of larger cells has been established in a number of triploid tissues and organs as well as in triploid blood, where both the red and white cells are affected (Benfey 1991). Triploidy also causes gametogenesis to be impaired with the result being that triploid salmonids are sterile (Dunham 1990; Ihssen *et al.* 1990). Furthermore, in female triploids, reproductive endocrinology is altered such that these fish remain sexually immature throughout their lives (Benfey *et al.* 1989).

Differences in the physiology of diploids and triploids have been reported as well. Several studies have found triploid salmonids to have lower total blood hemoglobin levels than diploids (Benfey and Sutterlin 1984a; Graham *et al.* 1985; Small and Randall 1989; Yamamoto and Iida 1994a). In addition, Graham *et al.* (1985) found that triploid Atlantic salmon (*Salmo salar*) had a lower hemoglobin-oxygen loading ratio than diploids. These findings suggest that triploid blood would be inferior to diploid blood in its ability to transport oxygen to metabolically active tissue. Results from several metabolic (oxygen consumption) rate studies suggest that the oxygen requirements of diploid and triploid salmonids are equivalent (Benfey and Sutterlin 1984b; Oliva-Teles and Kaushik 1990; Yamamoto and Iida 1994b). One would expect that equivalent oxygen requirements combined with a presumed inferior blood-oxygen transport capacity would result in triploid salmonids having a lower aerobic capacity (i.e., being disadvantaged aerobically) when compared with diploids.

Three studies have examined the aerobic capacity of triploid salmonids. In one study Virtanen *et al.* (1990) used indirect biochemical indicators and found triploid rainbow trout (*Oncorhynchus mykiss*) to be inferior to diploids in their aerobic swimming capacity. This study has been criticised because the triploids tested had a lower hematocrit than the diploids at the beginning of the experiment, which in itself could account for a reduced aerobic capacity (Stillwell and Benfey 1996). In the other two studies, triploid coho salmon (*O. kisutch*) and brook trout (*Salvelinus fontinalis*) were found to perform as well as their diploid counterparts when tested for critical swimming velocity (Small and Randall 1989; Stillwell and Benfey 1996, respectively).

These findings lead one to question how it is possible for triploid salmonids, with similar oxygen requirements but an apparent inferior blood-oxygen transport capacity, to perform as well as diploids in tests of aerobic swimming capacity. Physiological compensatory mechanisms such as improved respiratory, cardiovascular or circulatory performance in triploids have been proposed to account for such findings (Graham *et al.* 1985; Benfey 1991). A physical compensatory mechanism such as a superior mechanical swimming ability in triploids could also explain these findings.

Our objectives in the present study were as follows:

(A) To examine the total blood hemoglobin level of sexually immature female diploids and triploids since gender and level of sexual maturity may have acted as confounding variables in previous studies in which hemoglobin level was examined.

(B) To compare the sustained metabolic rate (i.e., the metabolic rate at a sustained swimming speed) of diploids and triploids under strictly controlled conditions since factors which may have affected metabolic rate (i.e., gender, fitness level, relative swimming speed and temperature) were not always controlled for in previous studies.

(C) To determine whether triploids abduct their opercula more frequently than diploids as a means to compensate for a lower blood-oxygen transport capacity.

(D) To determine whether triploids require fewer tail beats than diploids to maintain a

similar swimming speed; a superior swimming efficiency/mechanical swimming abiility in triploids may compensate for a lower blood-oxygen transport capacity.
All of the aforementioned were examined in two strains of brook trout, a species in which such work has not been conducted previously .

Materials and Methods

This experiment was conducted once in 1993 with the UNB strain of brook trout and again in 1994 with brook trout obtained from a commercial supplier (Pisciculture des Alleghanys, Inc., St.-Philemon, Quebec). Diploid and triploid cohorts came from the same egg lots (i.e., the same parents); triploidy was induced by subjecting eggs to a hydrostatic pressure treatment. Ploidy level was confirmed by flow cytometric measurement of erythrocytic DNA content.

In both years the experimental diploids and triploids were reared communally from approximately two months post-hatch onwards. Fish were reared in City of Fredericton water which had been dechlorinated, aerated and heated to a constant temperature of 13.6 ± 0.2° C. The fish were fed to satiation once daily at approximately the same time every day (0800 hrs ± 30 min) with a prepared trout pellet (Corey Feed Mills Ltd., Fredericton, N.B.), and were held under artificial light conditions set to match the natural photoperiod.

The 1993 test involved a group of 15 diploids and 15 triploids that were of the same mean size (forklength and weight were respectively 22.4 ± 1.6 cm (standard deviation) and 169.5 ± 45.0 grams for diploids and 21.9 ± 1.3 cm and 149.6 ± 29.7 grams for triploids; $p>0.10$ for both). The 1994 test was conducted with a group of 18 diploids and 10 triploids that were of the same mean size (forklength and weight were respectively 23.0 ± 1.5 cm and 170.0 ± 35.5 grams for diploids and 22.4 ± 1.5 cm and 150.0 ± 37.3 grams for triploids; $p>0.10$ for both). All of the experimental fish were sexually immature (i.e., with no vitellogenic oocytes) nine to ten-month old females.

The experiment was conducted in the same manner in both years. Fish were conditioned for two months prior to being tested in order to establish a comparable baseline fitness level at the beginning of the experiment. Conditioning involved housing fish in a fibreglass circular "doughnut"-style tank in which they swam against a constant water current of 1 body length (forklength) per second. Diploids and triploids were held in the same conditioning tank in order to eliminate tank effects.

A Blazka-type respirometer (as described by Kutty and Saunders 1972) was used to collect metabolic rate data. The speed at which the fish were swimming could be maintained within a narrow range. Temperature was held constant and the level of dissolved oxygen in the water was monitored using an oxygen meter (Oxyguard Handy MkII). The presence of a fish in the inner cylinder of the respirometer causes a localised decrease in the area through which water can pass, in turn causing the water speed to which the fish is exposed to increase. This "blocking effect" was mathematically corrected for when determining the speed at which a fish was swimming (Smit *et al.* 1971).

The experimental protocol was as follows. A fish was removed from the conditioning tank at the same time each day and immediately anaesthetised in a 1% solution of tertiary amyl alcohol. Various body measurements were taken at this time and a small blood sample (approximately 100µL) was collected by caudal vein puncture for later ploidy confirmation and/or hemoglobin analysis. Hemoglobin concentration was determined using a commercial version of the cyanomethemoglobin method (Sigma Chemical Co., Cat No. 525). Once the fish had recovered from the anaesthesia (indicated by the resumption of normal swimming) it was placed into the respirometer for a 15-hour habituation period. During the habituation period the fish was left undisturbed; a dark covering was placed over the respirometer and a light was shone onto the front portion of the respirometer to provide visual orientation. Water temperature and speed were held constant at $13.6 \pm 0.4°$ C and 0.37 ± 0.02 body lengths/sec., respectively. This swimming speed was chosen because it allowed fish to maintain station against the water current while displaying little

spontaneous activity, permitted the visual determination of tail beat frequency, and ensured that metabolism was entirely aerobic (Jobling 1994). By having all fish swim at an equivalent relative, rather than absolute, swimming speed (i.e., equivalent in terms of body lengths/sec rather than cm/sec) activity level was controlled for while the effects of size differences were minimised.

The test period began at the same time each day; by this time the fish had been deprived of food for 24 hours. Oxygen consumption was monitored by measuring the level of dissolved oxygen in the water every 30 minutes. The initial dissolved oxygen level was approximately 9 mg/l and the experiment was terminated when the dissolved oxygen level fell to 6.0 mg/l. When dissolved oxygen levels reached 7.5, 7.0 and 6.5 mg/l, the opercular abduction rate and the tail beat frequency were determined visually using a stopwatch to measure time required per given cycle of each. These data were collected in triplicate at each dissolved oxygen level.

All statistical analyses were performed with SAS (software release 6.06) using the Wilcoxon Rank Sum test.

Results

Although 15 diploids and 15 triploids were used in 1993 (UNB strain), some data points were not obtained. In the case of tail beat frequency and opercular abduction rate, this was due to an awkward location of the fish within the tunnel which made observations difficult without disturbing the fish; in the case of hemoglobin, too small a blood sample was obtained to be assayed. Tail beat frequency, opercular abduction rate and hemoglobin level results for that year , therefore, represent the mean data (± 1 standard deviation) from 13, 14 and 15 diploids and 14, 14 and 13 triploids, respectively. Results presented for the 1994 group (Quebec strain) are means (± 1 standard deviation) for all 18 diploids and 10 triploids.

Triploids from the UNB strain were frequently observed to have incomplete opercula which left the gills partially exposed. These malformations were also observed occasionally in diploids of the UNB strain. Opercular condition in both diploids and triploids from the Quebec strain was very good; opercular malformations were rarely observed. Only those fish from the Quebec strain that had excellent opercular condition were used in the present study.

The hemoglobin level assay was conducted only in 1993 with the UNB strain of fish; total blood hemoglobin levels were not significantly different in diploids and triploids (8.7 ± 0.5 and 8.6 ± 0.6 g/dL respectively, p=0.84). After 1993 a separate experiment was undertaken to examine the hemoglobin levels of diploid and triploid brook trout in more detail. For all other variables results from diploids and triploids of the UNB strain are largely mirrored in diploids and triploids of the Quebec strain. The metabolic rate of triploids was lower than that of diploids in both years (Figure 2.1), however, in 1993 the difference between the UNB diploids and triploids was highly significant (p=0.004) while the difference between Quebec diploids and triploids in 1994 was moderately significant (Schlotzhauer and Littell 1987; p=0.076). In both 1993 and 1994 there was no significant difference in the opercular abduction rate of diploids and triploids (p=0.96 for 1993 and 0.42 for 1994), but as dissolved oxygen levels decreased opercular abduction rate tended to increase (Table 2.1). There were also no significant differences between diploids and triploids in tail beat frequency in both years (p=0.24 for 1993 and 0.57 for 1994), and tail beat frequency did not vary significantly across dissolved oxygen levels (Table 2.1).

Discussion

Similar tail beat frequencies suggest that diploids and triploids are making a similar swimming effort to maintain the same relative swimming speed. At low swim speeds the

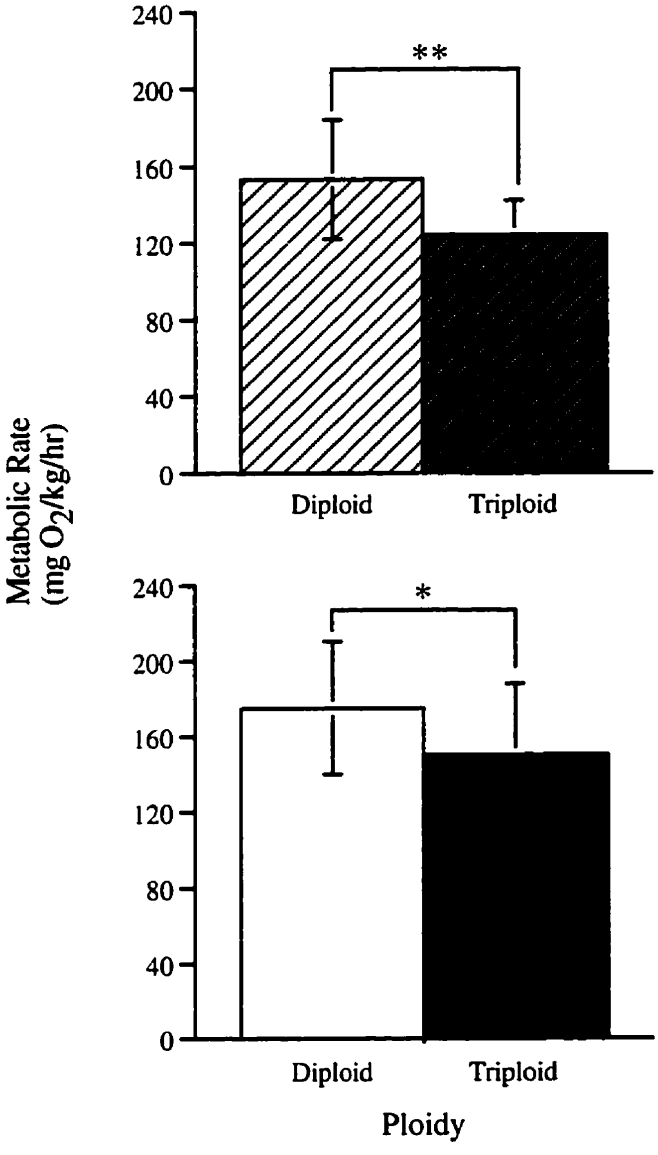

Figure 2.1. Metabolic rate of diploid and triploid brook trout from two strains (upper panel: UNB strain; lower panel: Quebec strain). * ($p<0.10$) and ** ($p<0.01$) indicate the level of significant difference between diploids and triploids.

	DO_2 Level (mgO_2/L)	Opercular Abduction Rate (abductions/min)		Tail Beat Frequency (beats/min)	
		Diploids	Triploids	Diploids	Triploids
UNB Strain	7.5	84.6 ± 6.8	84.7 ± 7.8	113.6 ± 15.7	120.3 ± 16.2
	7.0	87.1 ± 9.5	86.3 ± 8.6	114.9 ± 15.6	118.4 ± 13.6
	6.5	89.4 ± 8.3	88.4 ± 11.0	111.9 ± 13.9	118.7 ± 16.2
Quebec Strain	7.5	85.4 ± 9.6	82.8 ± 7.6	122.5 ± 13.9	123.2 ± 14.3
	7.0	87.1 ± 9.1	83.8 ± 8.7	124.6 ± 13.8	118.5 ± 13.7
	6.5	92.5 ± 12.3	87.9 ± 7.9	119.2 ± 8.8	119.7 ± 11.4

Table 2.1. Opercular abduction rate and tail beat frequency at various dissolved oxygen (DO_2) levels in diploid and triploid brook trout from two strains. Differences between diploids and triploids of each strain were not significant at the 0.10 level.

swimming efficiency/mechanical swimming ability of diploids and triploids, therefore, appears to be equivalent. Our findings do not support the hypothesis that superior triploid swimming efficiency compensates for a lower blood-oxygen transport capacity. It would, however, be valuable to examine the swimming efficiency of diploids and triploids at higher speeds since swimming dynamics may vary with swim speed.

Triploid fish had a lower oxygen consumption rate (i.e., metabolic rate) than diploids (in terms of mg oxygen utilised/ kg of body weight/ hr) to maintain the same relative sustained swimming speed and for the same swimming effort (as measured by tail beat frequency). Considering that triploid organisms have larger cells than diploids, studies relating cell size and metabolic rate may explain the lower metabolic rate of triploids. When erythrocytes from various diploid species were examined, the erythrocytic rate of oxygen uptake was inversely proportional to the erythrocytic volume (Holland and Forster 1966). As well, when the metabolic rate of several species of diploid amphibians with differing cell sizes was examined, those species with larger cells had lower whole animal metabolic rates (Szarski 1976). Szarski (1976, 1983) gave several possible explanations to account for the reduced metabolic rate associated with increased cell size. Large cells have a lower surface area to volume ratio than small cells, therefore a given volume of large cells has less cell membrane than would the same volume of smaller cells. Szarski suggested that because cell membranes, consisting largely of lipids and proteins, are metabolically expensive to make, organisms with large cells may require less energy to make and maintain cell membranes. Szarski also proposed that a reduced surface area to volume ratio could translate into less energy being required to maintain the internal osmotic environment in organisms with large cells than in organisms with smaller cells.

Our metabolic rate results contradict previous findings for salmonids (Benfey and Sutterlin 1984b; Oliva-Teles and Kaushik 1990; Yamamoto and Iida 1994b). This discrepancy may be attributed in part to the experimental protocol: in the present study a number of factors (i.e., fitness level, gender, and temperature) were controlled, swimming speed was held constant with respect to body lengths/sec (rather than cm/sec) and the "blocking effect" was

mathematically corrected for. Neglecting to take these factors into account in previous studies may have led to an inaccurate interpretation of results. Our results indicate a lower metabolic rate in triploids at a fixed swimming speed, however, it would be interesting to determine if the same phenomenon is present at all levels of activity (including a state of no activity). A lower oxygen requirement in triploids across activity levels may compensate for a lower blood-oxygen transport capacity, thus enabling their equivalent aerobic swimming performance.

Our finding of equivalent total blood hemoglobin level in diploids and triploids contradicts findings from certain salmonid studies (Benfey and Sutterlin 1984a; Graham *et al.* 1985; Small and Randall 1989; Yamamoto and Iida 1994a) but supports those of other studies (Biron 1993). Within non-salmonid species total hemoglobin levels are also reported to be the same in diploids and triploids in certain studies (Barker *et al.* 1983; Sezaki *et al.* 1983; Aliah *et al.* 1991; Sezaki *et al.* 1991) but not in others (Parsons 1993). The variability of results suggests that, regardless of ploidy effects, hemoglobin level itself may be extremely variable. Previous studies have indeed shown that hemoglobin levels fluctuate dramatically seasonally and especially during the spawning period (Robertson *et al.* 1961; Sopinska 1983) and that hemoglobin levels also demonstrate a marked gender difference with males tending to have higher hemoglobin levels than females (Robertson *et al.* 1961; Sopinska 1983; Habekov 1991). Since triploid females remain sexually immature, neglecting to control for sexual maturity in experimental diploids may have distorted results in previous studies; neglecting to control for gender in previous studies may have produced further distortions. When gender and/or level of sexual maturity were controlled for as in the Biron (1993) study (in which all-female rainbow trout were tested) and in the present study, hemoglobin levels were the same in both ploidy groups. Concluding that triploid blood is inferior to diploid blood in its oxygen transport capacity on the basis of total blood hemoglobin data seems inappropriate when one considers the effects of the aforementioned factors on results from previous studies and the hemoglobin results obtained in the present study. The hemoglobin-oxygen loading ratio, which can also affect blood-oxygen transport capacity, was not examined in the present study.

Triploid salmonids have been reported to have a higher frequency of jaw and opercular deformities than diploids (Benfey 1996); only opercular abnormalities were observed in the present study. Incomplete opercula were more prevalent in triploids than diploids in the UNB strain (which is likely fairly inbred), however, in the Quebec strain of fish opercular condition was very good in both diploids and triploids. These findings suggest that it is not triploidy *per se* which results in an increase in the frequency of this physical abnormality; rather, it appears that this deformity may be strain-related or related to inbreeding, and inducing triploidy may simply exacerbate an existing problem.

The metabolic rate results indicate that triploids consumed less oxygen than diploids, yet the opercular abduction rate required to obtain this oxygen was the same for both groups. Similar results have been reported by King and Lee (1993). These findings suggest that triploids may have a less efficient respiratory apparatus than diploids. An inefficient respiratory system may be partially attributed to the poorer opercular condition that is frequently observed in triploids; good opercular condition is crucial to the efficient functioning of the respiratory pump. Poor triploid opercular condition does not, however, explain the results obtained from the Quebec strain of fish since all experimental fish from the Quebec strain had excellent opercular condition. It appears that triploids, regardless of opercular condition, may thus have a less efficient respiratory apparatus than diploids. The efficiency of the respiratory apparatus can, however, not be assessed by opercular abduction rate alone; respiratory volume and blood flow through the gills should also be examined (Davis and Randall 1973). Unfortunately this was not possible with the experimental apparatus used in this study.

The possible reduction in the efficiency of the triploid respiratory system (based on opercular abduction rate) may indicate that triploid brook trout are at a physiological disadvantage when compared to their diploid counterparts. This may, however, be directly offset by a lower oxygen requirement in triploids such that aerobic performance is not impaired. Further investigations into the respiratory physiology and aerobic performance

of triploids are therefore warranted.

Acknowledgements

We would like to express our thanks to Mr. Donald Hornibrook and Ms. Robyn O'Keefe for their technical assistance and to Professor Roman Mureika for his assistance with statistical analyses. We are grateful to Dr. R.L. Saunders and Dr. A.J. Wiggs for their input into the experimental design and for their critical reading of this manuscript. Thanks also to the Department of Fisheries and Oceans Biological Station in St. Andrews, N.B., for lending us the Blazka respirometer. This project was supported by funding from an NSERC research grant. The experimental protocol was approved by the U.N.B. Animal Care Committee, meeting Canadian Council of Animal Care guidelines.

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Appendix 2.1

Figure 2.2. A schematic diagram of the Blazka respirometer.

Formulae 2.1, 2.2, 2.3: Formulae used in Chapter 2 (to correct for the 'blocking effect', calculate the cross-sectional area of a fish and calculate metabolic rate).

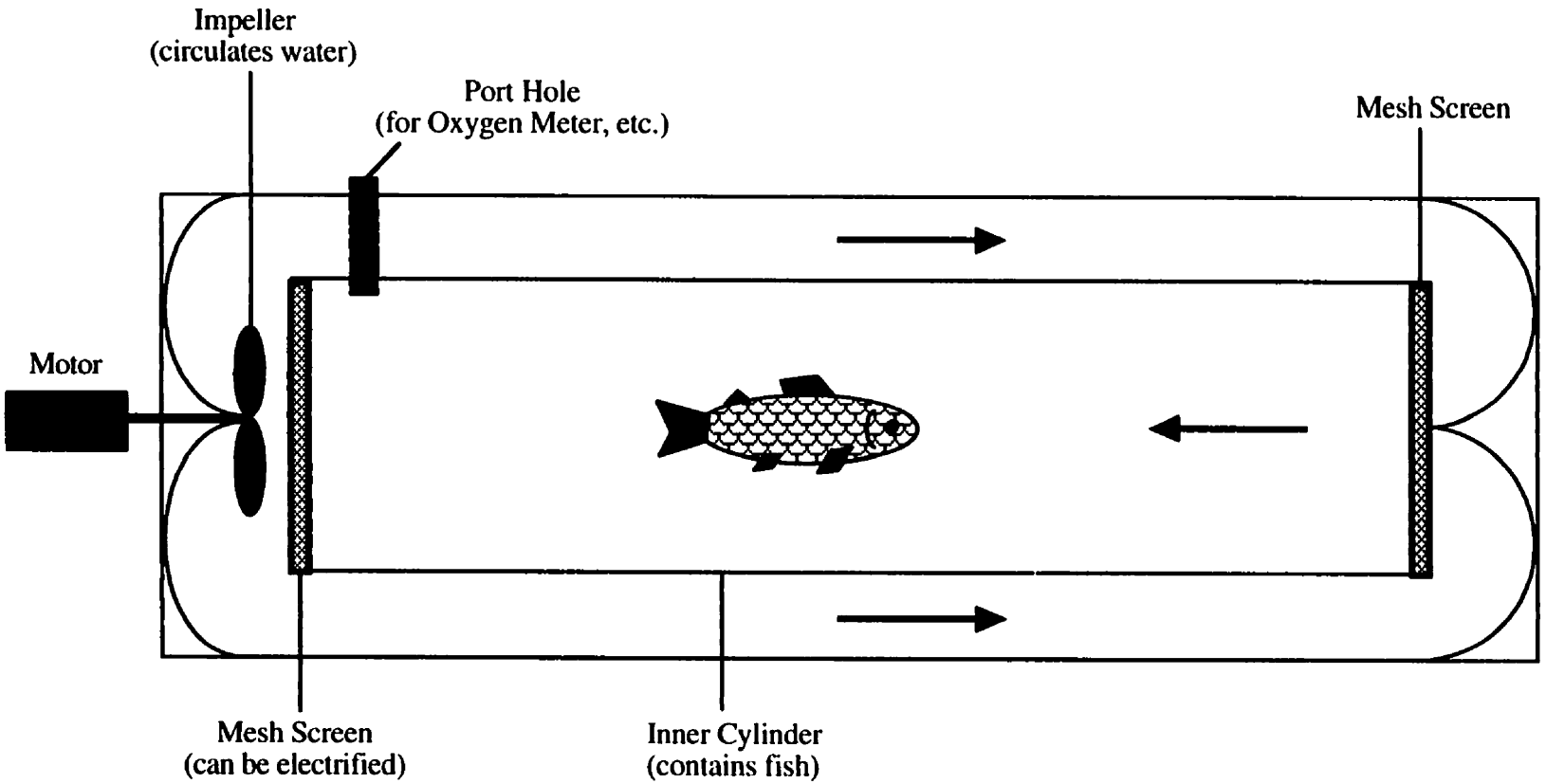

Figure 2.2. A schematic representation of the Blazka-type respirometer used for the metabolic rate and the critical swimming velocity experiments. Arrows indicate the direction of the water current.

Corrected Velocity:

$$V_{CORRECTED} = V_{MEASURED} \bullet \left[1 + \frac{A_{FISH}}{A_{CYLINDER}} \right]$$

Formula 2.1. The formula used to correct for the "blocking effect". $V_{CORRECTED}$ is the corrected water velocity (cm/sec), $V_{MEASURED}$ is the measured water velocity (cm/sec); A_{FISH} is the cross sectional area of the fish (calculated in formula 2.2, below) and $A_{CYLINDER}$ is the cross-sectional area (cm^2) of the inner cylinder of the Blazka respirometer (from Smit *et al.* 1971).

Cross sectional area of fish:

$$A_{FISH} = \pi \bullet 1/2\ D \bullet 1/2\ W$$

Formula 2.2. The formula used to calculate the cross-sectional area (cm^2) of a fish. D is the maximum body depth (cm) and W is the maximum body width (cm).

Metabolic Rate:

$$M.R. = \frac{ROU \bullet BRV}{FW}$$

Formula 2.3. The formula used to calculate the metabolic rate (mg O_2/kg/hr). ROU is the rate of oxygen uptake by the fish (mg O_2/hour), BRV is the volume of the Blazka respirometer (38.7 L) and FW is the weight of the fish (kg).

Chapter 3

The Critical Swimming Velocity of Diploid and Triploid Brook Trout.

This chapter (excluding Appendix) is currently in press in *The Journal of Fish Biology*.

Abstract

No significant difference was found in the critical swimming velocity of diploid and triploid brook trout (*Salvelinus fontinalis*), suggesting that the triploid state is not associated with a reduced aerobic capacity.

Numerous studies have found that cells in the blood, tissues and organs of triploid organisms are fewer in number but larger in size relative to diploids (Benfey, 1997). The rate of gas exchange has been reported to be inversely related to cell size in mammalian and amphibian erythrocytes (Holland and Forster, 1966). Due to their larger size the erythrocytes of triploid fish may, therefore, be less efficient than those of diploids at loading oxygen in the gills and unloading oxygen in metabolically active tissues. An impairment in the efficiency of gas exchange might reduce the aerobic capacity of triploid fish and consequently hinder their performance in culture situations.

Conflicting results were reported in the two studies which examined the aerobic capacity of triploid salmonids. Virtanen *et al.* (1990) examined energy metabolism in diploid and triploid rainbow trout (*Oncorhynchus mykiss*) during prolonged exercise and found that the aerobic capacity of triploids was lower than that of diploids. In contrast, Small and Randall (1989) found the aerobic capacity of diploid and triploid coho salmon (*O. kisutch*) to be equivalent based on critical swimming velocity (C.S.V.). Small and Randall (1989) suggested that their findings might be species-specific.

The objective of the present experiment was to attempt to resolve the conflict that exists regarding the aerobic swimming capacity of triploid salmonids by repeating the Small and Randall (1989) experiment. By using brook trout (*Salvelinus fontinalis*) as our experimental species we hoped to clarify whether or not Small and Randall's findings were species/genera-specific.

The C.S.V. tests were conducted twice: once in 1994 with a mixed-sex group of equivalent-sized three to four month old diploid (n=22) and triploid (n=17) brook trout from the UNB strain (weight: 4.26 ± 2.51 [S.D.] and 4.58 ± 1.69 g respectively, $p>0.05$; fork length: 6.82 ± 1.08 and 7.10 ± 0.91 cm respectively, $p>0.05$), and again in 1995 with a mixed-sex group of equivalent-sized three to four month old diploid (n=12) and triploid

(n=11) brook trout from the Quebec strain (weight: 4.49 ± 0.86 and 4.09 ± 0.50 g respectively, $p>0.05$; fork length: 7.26 ± 0.44 and 7.12 ± 0.33 cm respectively, $p>0.05$). Diploid and triploid cohorts came from the same egg lots (i.e., the same parents); triploidy was induced by hydrostatic pressure treatment.

All fish were conditioned for one month prior to the test period to ensure an equivalent basic fitness level; conditioning involved rearing a mixed-ploidy group of fish in a circular, 42 cm diameter "doughnut"-style tank in which they swam against a water current of 1 body length (BL, equal to fork length in this study) per second. Fish were held in City of Fredericton water which had been dechlorinated and aerated and under artificial light conditions set to match the natural photoperiod. Fish were fed three to four times daily with a prepared trout pellet (Corey Feed Mills Ltd., Fredericton, N.B.).

The C.S.V. tests were conducted in a Blazka-type respirometer (as described by Kutty and Saunders, 1972). The inner sleeve (cylinder) of the respirometer served as a swim chamber in which the fish were contained by means of screens located at either end; an electric current could be applied to the downstream screen. Water was drawn through the inner cylinder and recirculated through the outer cylinder by means of a motor-driven impeller located at the rear of the respirometer. The speed at which the fish swam was regulated by adjusting the motor speed. The respirometer was set up as a flow-through system thus permitting control of water temperature and dissolved oxygen level.

The experimental protocol was as follows: a fish was randomly removed from the conditioning tank and fork length was measured. The fish was then placed in the respirometer for a one-hour habituation/training period during which it swam against a 0.5 $BL \cdot sec^{-1}$ water current (i.e., the downstream screen was electrified and fish resting against this screen received a mild shock sufficient to elicit an avoidance response). The habituation/training period was immediately followed by the test period.

The experimental protocol varied slightly between years. In 1994 the experimental (UNB-

strain) fish were food-deprived for three hours prior to the test period and fish were tested at various times of day. In 1995 the experimental fish were food-deprived for 24 hours prior to testing and all fish were tested at the same time of day (16.00 hrs). UNB-strain fish were not available in 1995 therefore Quebec-strain fish (obtained from Pisciculture des Alleghanys, Inc.) were used.

During the test period the downstream screen was not electrified and water-speed was increased in 0.5 BL•sec^{-1} increments every 30 minutes until the fish was fatigued (defined as the inability to escape from the downstream grid after three consecutive 1-second electrical shocks). Once a fish was fatigued, water speed was rapidly decreased and the fish was removed from the swim tunnel. After a short recovery period the fish was anaesthetised in 1% tertiary amyl alcohol, weight was measured, fork length verified and a small blood sample ($\leq$ 50µL) was collected via caudal vein puncture for ploidy confirmation by flow cytometric measurement of erythrocytic DNA content. The C.S.V. was calculated as per Brett (1964).

All statistical analyses were performed with SAS (software release 6.08) using a two-sample t-test. Data were also analysed using a Wilcoxon Rank Sum test due to the non-normality of some of the data.

The 1994 tests found no significant difference in the C.S.V. of diploids and triploids from the UNB strain (2.31 $\pm$ 0.33 and 2.16 $\pm$ 0.29 BL•sec^{-1} respectively, $p>0.05$ in both statistical tests). There was, however, some concern as to whether testing these fish at various times of day may have affected C.S.V.. Also, prior to the test period the UNB-strain of fish were given a three-hour food-deprivation period and a one-hour adaptation period. Traditionally swim speed tests are conducted following a 24-hour adaptation period and after a fish has been food-deprived for 24 hours (to achieve a post-absorptive state). Glova and McInerney (1977) found that swimming performance was not significantly different in fish given a 1-hour or 24-hour habituation period. It is, however, unclear whether ensuring a post-absorptive state is simply convention or whether it may

actually have an impact on swimming performance. The 3-hour food-deprivation period used in 1994 would not have produced a post-absorptive state.

To address these concerns the experiment was repeated in 1995 with all fish tested at the same time of day (16.00 hrs) and with the customary 24-hour food deprivation period. As previously indicated Quebec-strain fish were used at this time because UNB-strain fish were not available. There was again no significant difference in the C.S.V. of diploids and triploids (1.86 ± 0.21 and 1.73 ± 0.27 BL•sec^{-1} respectively, $p>0.05$ in both statistical tests). The fish tested in 1995, however, had a lower C.S.V. than those tested in 1994. Unfortunately, it is impossible to determine whether the time of testing or length of food-deprivation affected C.S.V. or whether these differences were simply strain-related.

The C.S.V. values in the present study are substantially lower than those obtained by Peterson (1974) who found the C.S.V. of diploid brook trout to be 4-5 BL•sec^{-1}. The test procedures were, however, not identical. Peterson (1974) also does not indicate the source of fish (wild or cultured) nor whether fish were conditioned prior to testing. Test procedure (Farlinger and Beamish, 1977), exercise history (Brett *et al.*, 1958) and source of fish (Vincent, 1960) can have a significant effect on performance in swimming tests. Any single one or a combination of these factors may account for the differences in C.S.V. results reported in the Peterson (1974) study and those presented here.

Our results from both 1994 and 1995 suggest that the aerobic capacity of diploid and triploid brook trout is equivalent. These findings agree with those of Small and Randall (1989) indicating that their results were not in fact species or genera-specific but may apply to salmonids in general. Parsons (1993) found no significant difference in the C.S.V. of diploid and triploid white crappies, *Pomoxis annularis*, suggesting that perhaps all triploid fish show this trend.

Our findings contradict those of Virtanen *et al.* (1990), but their conclusions must be viewed with caution because experimental triploids had a significantly ($p\leq0.01$) lower

hematocrit than diploids at the beginning of the experiment. This is an atypical finding; all other studies that we are aware of report equivalent hematocrits in diploids and triploids (Benfey, 1997). Jones (1971) found that a reduction in hematocrit to 47% of normal resulted in a 34% reduction in maximum swimming speed. It is, therefore, not surprising that triploids in the Virtanen *et al.* (1990) study had a lower aerobic capacity than diploids. Concluding that triploids have a reduced aerobic capacity on the basis of results obtained from these atypical triploids is unwarranted.

The results of the present study suggest that, although triploids have been shown to have larger erythrocytes, they are not impaired in their aerobic swimming capacity as measured by C.S.V.. Triploids may have a physiological mechanism which compensates for a possible impairment in erythrocytic gas exchange such that aerobic capacity is not reduced. We (Stillwell and Benfey, 1996), for example, found that triploid brook trout had a lower metabolic rate (i.e., oxygen requirement) than diploids; a lower oxygen requirement might compensate for a reduction in the efficiency of gas exchange thus enabling an equivalent aerobic performance in both ploidy groups. Alternately, it is possible that erythrocytic size may not affect the efficiency of gas exchange to the level anticipated: Reeves and Park (1993) found that the half-thickness of cells covering a 600-fold volume range was essentially the same. Benfey and Sutterlin (1984) also found that, in Atlantic salmon (*Salmo salar*), triploid erythrocytes had greater length and width than diploid erythrocytes but that erythrocytic height (i.e., thickness) was the same in both ploidy-groups. Since the rate of hemoglobin oxygenation is determined by the half-thickness of a cell (Holland and Forster, 1966; Reeves and Park, 1993), the equivalent thickness of diploid and triploid erythrocytes may translate to an equivalent efficiency of gas exchange and may account for the equivalent aerobic capacity of both ploidies in the present study.

Acknowledgements

We thank Mr. Donald Hornibrook for his technical assistance and Mr. P.J. Jardine, Dr. R.L. Saunders and Dr. A.J. Wiggs for their critical reading of this manuscript. Funding for this project was provided by an NSERC research grant. This project was approved by UNB's Animal Care Committee in accordance with the guidelines of the Canadian Council of Animal Care.

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Appendix 3.1

Formula 3.1: The formula used to calculate the critical swimming velocity.

Critical Swimming Velocity:

$$C.S.V. = V_{MAXIMUM} + \left[\frac{T_{FATIGUE}}{T_{INTERVAL}} \right] \bullet V_{INCREMENT}$$

E.g., A fish that was able to maintain a maximum velocity of 2.0 BL/sec. for 15 minutes would have a C.S.V. of 2.25 L/sec:

$$C.S.V. = 2.0 + \left[\frac{15}{30} \right] \bullet 0.5$$

$$= 2.25 \text{ BL/sec.}$$

Formula 3.1. The formula used to calculate critical swimming velocity (C.S.V.). $V_{MAXIMUM}$ is the maximum velocity attained by the fish (in body lengths [BL]/sec.), $T_{FATIGUE}$ is the length of time (minutes) that the maximum velocity could be maintained, $T_{INTERVAL}$ is the the length of the time interval that a fish swam at each velocity (i.e., 30 minutes) and $V_{INCREMENT}$ is the increment by which water speed was increased in each interval (i.e., 0.5 BL/sec.). From Brett (1964).

Chapter 4

The Hematology of Triploid Brook Trout (*Salvelinus fontinalis*).

A portion of this chapter is currently being revised for resubmission to the journal *Fish Physiology and Biochemistry.*

Abstract

Several hematological parameters were examined in a group of diploid (n=19) and triploid (n=21) female brook trout (*Salvelinus fontinalis*). Triploid erythrocytes and their nuclei were found to be significantly larger than those of diploids (nuclear length: 9.53 ± 0.22 [standard error] and 7.62 ± 0.20 μm; nuclear width: 4.42 ± 0.11 and 3.97 ± 0.10 μm; erythrocyte length 18.37 ± 0.43 and 14.98 ± 0.35 μm; erythrocyte width: 11.29 ± 0.28 and 9.66 ± 0.24 μm, respectively for triploids and diploids, p=0.0001 in each case). This was accompanied by a significant decrease in numbers of triploid erythrocytes relative to diploids (88.42 ± 3.01 and 113.42 ± 2.27 x $10^4/mm^3$ respectively, p=0.0001) such that hematocrit was equivalent in both ploidy groups (39.56 ± 1.14, and 37.55 ± 0.82%, triploids and diploids respectively, p=0.1512). Total blood hemoglobin concentration (TBHC) was significantly higher in triploids than in diploids (9.37 ± 0.29 and 7.85 ± 0.18 g/dL, respectively, p=0.0001) but total blood nucleoside triphosphate levels were equivalent in both ploidy groups (109.34 ± 1.02 and 108.44 ± 0.91 μmol/dL, triploids and diploids respectively, p=0.4035). All parameters except TBHC were consistent with findings reported for triploids of other fish species. The TBHC of diploid and triploid fish was therefore examined in further detail: TBHC was measured every 3-4 months over a two year period in all-female (n=32) and mixed-sex (n=50) groups of diploid and triploid brook trout. TBHC varied significantly over time suggesting that age and/or season affected this parameter. Although the TBHC was generally similar in all sex and ploidy groups, certain differences were observed: around the time of ovulation diploid females had a significantly lower TBHC than their male and their triploid counterparts (e.g., Quebec-strain fish in Nov. 1995: 8.03 ± 0.54, 10.19 ± 0.43 and 9.56 ± 0.23 g/dL, respectively for diploid females, diploid males and triploid females, $p \leq 0.0002$ in each case). Our results suggest that the TBHC differences noted were not related to ploidy or sex differences *per se* but appear instead to reflect endocrinological/physiological differences between groups. The overall similarity in the TBHC of diploid and triploid brook trout suggests that triploid blood should be as effective as diploid blood in meeting biological oxygen requirements and, as such, should not restrict triploid performance.

Introduction

In aquaculture numerous attempts have been made to produce fish which do not show the declines in flesh quality, immunocompetence and growth rate that normally accompany sexual maturation. To date the only economically-feasible and consumer-accepted method of doing this has been to produce female triploids, which remain sexually immature as a result of endocrinological dysfunction (Benfey 1997). Triploid fish are also sterile and, because sterility eliminates concerns over cross-breeding between domestic and wild stocks of fish, appealing to fisheries managers. Despite having these advantages, triploid fish are not cultured extensively because their performance is reported to be inferior to that of diploids under conditions of high biological oxygen demand and/or low oxygen availability (Benfey 1997).

Research on the hematological effects of artificially inducing triploidy (i.e., producing fish with a third set of chromosomes) has generated certain consistent results (Benfey 1997). For example, relative to diploids, triploid erythrocytes and their nuclei are larger in size but erythrocyte numbers are reduced. This balance between cell number and size results in an equivalent hematocrit (packed blood cell volume) in diploids and triploids. Examinations of other hematological parameters have produced conspicuous inconsistencies. The concentration of hemoglobin in triploid blood has been reported to be either equivalent to (Barker *et al.* 1983, Sezaki *et al.* 1983, Aliah *et al.* 1991, Sezaki *et al.* 1991, Biron 1993, Stillwell and Benfey 1996) or lower than that of diploids (Benfey and Sutterlin 1984, Graham *et al.* 1985, Small and Randall 1989, Parsons 1993, Yamamoto and Iida 1994). A lower concentration of hemoglobin in triploid blood has been suggested as a potential factor in the poor performance of triploids when oxygen is limiting.

Total blood hemoglobin concentration (TBHC) is a basic measure of the ability of blood to transport oxygen and, as such, an indicator of an organism's ability to supply oxygen adequately for metabolic processes (Houston 1990). The oxygen affinity of hemoglobin

may, however, be altered in response to affinity-modulating solutes (e.g., H^+, Cl^-, Mg^{2+}, and organic phosphates; Nikinmaa 1992). Of these modulators only nucleoside triphosphate (NTP), which decreases blood-oxygen affinity, has been compared in diploids and triploids: Graham *et al.* (1985) found that, although TBHC was lower in triploids, blood levels of NTP were equivalent in both ploidy groups. These results were, however, based on small sample sizes and pooled blood samples.

The objectives of the present study were two-fold: (1) to determine if the hematology of triploid brook trout (*Salvelinus fontinalis*) is consistent with that of triploids of other salmonid species and (2) to examine the TBHC of diploid and triploid brook trout in further detail in an attempt to understand the inconsistencies reported in the literature for this specific hematological index. Brook trout were selected for this research because this species is used for aquaculture and sport fishing and has proven to be hardy in culture and experimentally robust, and because the parameters for artificial triploid induction in brook trout are clearly understood. Presently little hematological information exists on triploids in this genus of salmonids (the charrs).

Materials and Methods

This study consisted of two experiments. In Experiment I several hematological parameters were examined in a group of forty (19 diploid, 21 triploid) 23-month old female UNB-strain brook trout. In Experiment II the TBHC was monitored at three to four-month intervals in (1) an all-female group of 32 (16 diploid, 16 triploid) UNB-strain fish for 24 months beginning at 9.5 months post-hatch and (2) a mixed-sex group of 50 (25 diploid, 25 triploid) Quebec-strain fish (Pisciculture des Alleghanys, St. Philemon, Quebec) for 23 months starting at 6 months post-hatch. A shortage of UNB-strain fish made it impossible to control for fish-strain in Experiment II. It should be noted that in Experiment I diploid fish were "spawned" (manually stripped of gametes) approximately 7 weeks prior to blood sampling while in Experiment II spawning took place in January and December 1995

(UNB-strain fish) and November 1995 (Quebec-strain fish).

Within each strain and experiment diploid and triploid cohorts were from the same egg lots (i.e., the same parents). Triploidy was induced by subjecting eggs to a 5 minute hydrostatic pressure treatment (9500 psi) approximately 20 minutes after fertilisation. The ploidy of all experimental fish was confirmed through flow cytometric measurement of erythrocyte DNA content (Dr. Everett Chalmers Hospital, Fredericton, N.B.).

In Experiment I diploids and triploids were communally reared in two 75 cm square flow-through tanks for several months prior to blood sampling. In Experiment II diploids and triploids were reared separately until they were large enough to receive passively-integrated transponder (PIT) tags. After this time (roughly 4 months post-hatch) diploids and triploids were reared communally in 1 meter diameter circular flow-through tanks where they swam against a mild water current. All fish were reared in dechlorinated and aerated City of Fredericton water in which the dissolved oxygen level was maintained above 7 mg/L. Water temperature varied from approximately 6°C (winter) to 13°C (summer); the artificial photoperiod was set to follow the natural photoperiod. Fish were fed once daily to satiation with pelleted trout feed (Corey Feed Mills Ltd., Fredericton, N.B.).

Experiment I - Hematological Parameters

Fish were individually netted in random order and restrained in a large sponge which had been lubricated with Stresscoat (Aquarium Pharmaceuticals, Inc., Quebec). Approximately 2.5 mls of blood was collected via caudal vein puncture into a chilled, heparinized 4 ml Vacutainer tube equipped with a sterile 22G1 collection needle; blood sampling required approximately 1 minute per fish. Blood samples were stored on ice and/or refrigerated immediately after collection. Fish were placed into a well-aerated recovery container and returned to their home tank only after all fish in each tank had been sampled. All assays and procedures were completed as expeditiously as possible (NTP, hemoglobin and cortisol assays were started within 1 hour of sampling while hematocrit determination, blood smears and erythrocyte counts were completed within 2, 6 and 18 hours of

sampling, respectively). Results from Korcock *et al.*(1988) indicate that processing blood in the manner described above (i.e., use of heparin as an anticoagulant, storage on ice) should not cause hemolysis nor lead to any significant changes in hematocrit, hemoglobin and NTP levels in the time frame indicated above.

Blood levels of NTP and hemoglobin were determined spectrophotometrically in duplicate for each fish using commercially prepared kits (Sigma Chemical Co., 340-13 and 525-A, respectively). The former, although marketed as an ATP kit, does not distinguish ATP from ITP, GTP or UTP; concentrations of the various triphosphates are thus collectively presented as concentrations of NTP. Technical difficulties prevented NTP from being measured for five experimental fish. Plasma cortisol concentrations were determined by radioimmunoassay (Baxter Clinical Cat. Nos. CA-529).

Hematocrits were measured in triplicate for each fish; blood was collected into microhematocrit tubes and centrifuged at 5°C for 5 minutes at 10,000 x g. Duplicate blood smears were made and, once dry, stained with a commercially prepared Wright-Giemsa stain (Fisher Scientific Ltd). The maximum length and width of 25 erythrocytes and their nuclei were measured per slide, for a total of 50 erythrocytes and their nuclei per fish. Erythrocytes were magnified 1000 times through a Zeiss Universal compound microscope (Carl Zeiss Canada Ltd.). A high resolution video camera (COHU, Inc. Electronics Division, San Diego, CA, USA) mounted on the microscope further magnified the image and transferred it to a video monitor. A final image magnification of 5076 times actual size resulted; image size was calibrated using a stage micrometer. Measurements were made from video images using a computer equipped with a TARGA video adaptor board (Truevision, Inc., Indianapolis, IN, USA) and JAVA Software (Version 1.4; Jandel Scientific, Corte Madera, CA, USA). Nuclear and erythrocytic length-width ratios were calculated to determine basic nuclear and cell shapes.

Erythrocyte counts were made using a Neubauer Improved Bright-line hemocytometer equipped with an optically-plane coverglass. Hendrick's diluting fluid (Houston 1990)

was used to dilute blood 1:200 (v/v) in a standard red blood cell diluting pipette. To ensure consistency in the diluting and counting processes, the dilution process was repeated and two counts were performed per dilution to give quadruplicate erythrocyte counts for each fish sampled.

All data were statistically analysed with SAS software (release 6.11). Data were analysed using the general linear models (GLM) procedure to compare least squares means of the various hematological parameters between ploidy groups. Pearson correlation coefficients were calculated between sampling order and all hematological parameters for both ploidy groups, to determine whether order of removal from a tank affected experimental results.

Experiment II - Total Blood Hemoglobin Concentration

TBHC was determined spectrophotometrically (as described above) in duplicate for each fish. Fish were randomly netted, anesthetized in 1% tertiary amyl alcohol and a small blood sample (~200μL) was collected via caudal vein puncture into a chilled, heparinized, 1cc plastic syringe equipped with a 26G1 needle. After blood-sampling fish were placed into a well-aerated recovery container until all cohorts had been sampled. Although the same fish were repeatedly sampled in this experiment, the sample size varied over time due to technical difficulties and/or mortalities.

Data were statistically analysed with SAS software (release 6.11) using the GLM procedure to conduct repeated measures analysis of variance tests. At each sampling date least-squares means comparisons of TBHC were made. In the all-female (UNB-strain) group comparisons were made between ploidies. In the mixed-sex (Quebec-strain) group comparisons were made between ploidies within each sex and between sexes within each ploidy. Statistical comparisons between groups which varied in both ploidy and sex (e.g., between diploid females and triploid males) were not conducted as the effects of ploidy and sex could not be assessed independently in such cases. The experimentwise error rate was set at α=0.05 for both groups of fish; the comparisonwise error rate was adjusted to α=0.0064 for the all-female group and to α=0.0018 for the mixed-sex group (Sokal and

Rohlf 1981). Pearson correlation coefficients were calculated between sampling order and TBHC for each sex and ploidy group at each sampling date, to determine whether order of removal from a tank affected experimental results.

Results

Experiment I - Hematological Parameters

Nuclear and cellular lengths, widths, and length-width ratios were all significantly greater in triploids than in diploids (Table 4.1). Erythrocyte counts were significantly lower in triploids than in diploids but hematocrit was equivalent in both ploidy groups (Table 4.2). TBHC was significantly higher in triploids than in diploids. There was no significant difference between diploids and triploids in total blood NTP nor plasma cortisol concentration. Erythrocyte count showed a significant positive correlation with sampling order in diploid fish; no other hematological parameter was significantly correlated with sampling order in either ploidy group (Table 4.3). The plasma cortisol concentration generally remained fairly low regardless of sampling order (Figure 4.1).

Erythrocytic Measurement	Diploids	Triploids	*P*-value
Nuclear Length (μm)	7.62 ± 0.20	9.53 ± 0.22	0.0001
Nuclear Width (μm)	3.97 ± 0.10	4.42 ± 0.11	0.0001
Nuclear Length-Width Ratio	1.94 ± 0.01	2.18 ± 0.01	0.0001
Cellular Length (μm)	14.98 ± 0.35	18.37 ± 0.43	0.0001
Cellular Width (μm)	9.66 ± 0.24	11.29 ± 0.28	0.0001
Cellular Length-Width Ratio	1.56 ± 0.01	1.65 ± 0.01	0.0001

Table 4.1: Erythrocytic measurements in an all-female group of diploid (n=19) and triploid (n=21) brook trout (*Salvelinus fontinalis*). Values represent means ± 1 standard error.

Hematological Index	Diploids	Triploids	*P*-value
Erythrocyte Count (x 10^4 / mm^3)	113.42 ± 2.27	88.42 ± 3.01	0.0001
Hematocrit (%)	37.55 ± 0.82	39.56 ± 1.14	0.1512
TBHC (g/dL)	7.85 ± 0.18	9.37 ± 0.29	0.0001
Total Blood NTP (μmol/dL)[a]	108.44 ± 0.91	109.34 ± 1.02	0.4035
Plasma Cortisol Concentration (ng/ml)	19.55 ± 5.06	19.50 ± 3.95	0.9802

a: n=17 for diploids and 18 for triploids.

Table 4.2: Hematological indices in an all-female group of diploid (n=19) and triploid (n=21) brook trout (*Salvelinus fontinalis*). Values represent means ± 1 standard error.

Hematological Parameter	Diploids			Triploids		
	r	r^2	*P*	r	r^2	*P*
Nuclear Length	0.3748	0.1405	0.1138	-0.1187	0.0141	0.6085
Nuclear Width	0.2244	0.0504	0.3556	-0.2729	0.0745	0.2314
Nuclear L-W Ratio	0.2942	0.0866	0.2215	0.0508	0.0026	0.8268
Cellular Length	0.2649	0.0702	0.2730	-0.0456	0.0021	0.8445
Cellular Width	0.2046	0.0419	0.4008	-0.1298	0.0168	0.5750
Cellular L-W Ratio	0.1269	0.0161	0.6046	0.0499	0.0025	0.8298
Erythrocyte Count	0.5981	0.3577	0.0068*	-0.1639	0.0269	0.4777
Hematocrit	0.2792	0.0780	0.2470	-0.3117	0.0972	0.1690
TBHC	0.3706	0.1373	0.1183	-0.1481	0.0219	0.5218
Total Blood NTP[a]	-0.2559	0.0655	0.3214	-0.3859	0.1489	0.1137
Plasma Cortisol Conc.	0.3176	0.1009	0.1852	0.0778	0.0061	0.7376

a: n=17 for diploids and 18 for triploids.

Table 4.3. The correlation between various hematological parameters and sampling order in all-female diploid (n=19) and triploid (n=21) brook trout. r = the Pearson correlation coefficient, *P* = the associated significance level. * represents a significant value ($p \leq 0.05$).

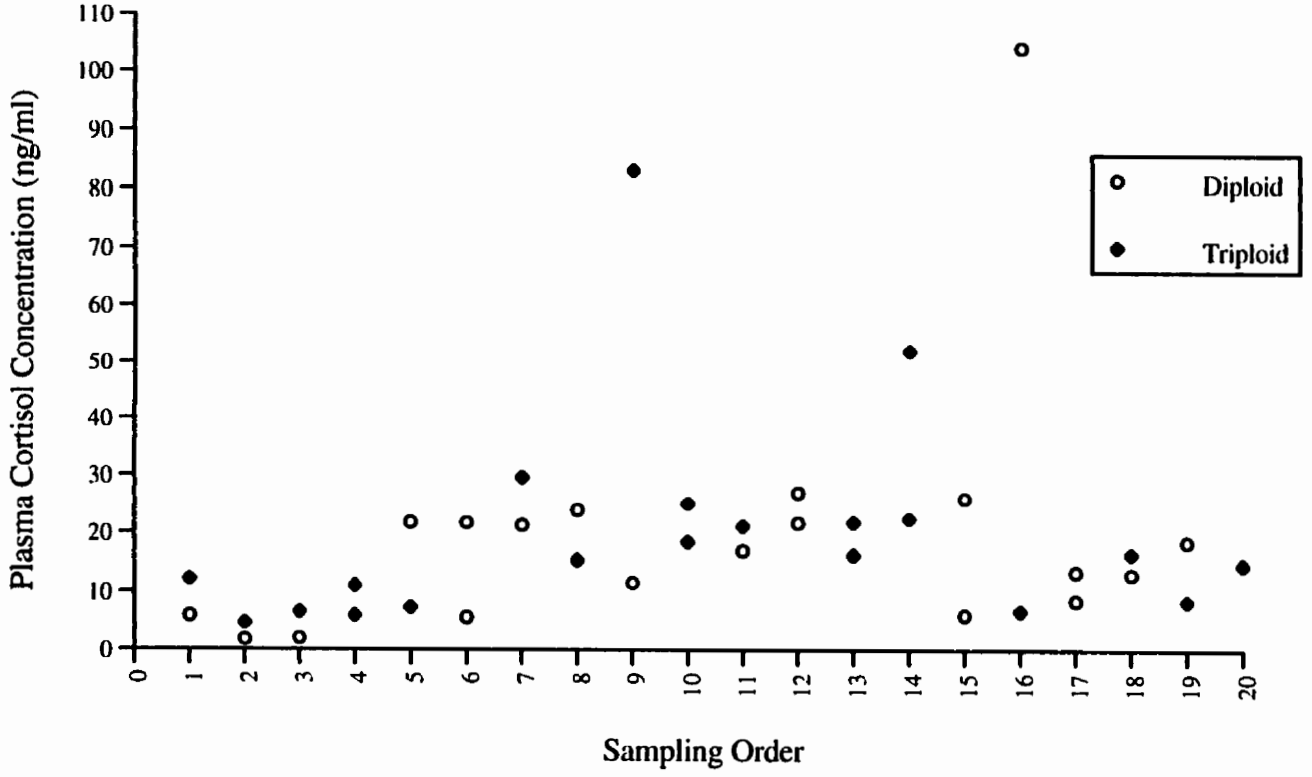

Figure 4.1. The relationship between plasma cortisol concentration and sampling order in an all-female group of diploid (n=19) and triploid (n=21) brook trout.

Experiment II - Total Blood Hemoglobin Concentration

Sampling date was found to have a significant effect on TBHC in both the all-female (UNB-strain) and mixed sex (Quebec-strain) groups of fish (p=0.0001 for both; Figures 4.2 and 4.3). In the all-female group the TBHC profiles of diploids and triploids were almost identical (Figure 4.2). The only ploidy-related difference appeared in April 1995 (~12 weeks after diploid spawning) when diploids had a lower TBHC than triploids (p=0.0066; marginally significant).

In the mixed-sex group of fish no significant difference was noted in the TBHC profiles of diploid and triploid males (Figure 4.3). Differences in the TBHC profiles of diploid and triploid females existed but were present only at or following diploid spawning (November 1995 and April 1996, respectively); in both cases diploid females had a significantly lower TBHC than did triploid females (p=0.0002 and 0.0001, respectively). No sex-related difference in TBHC was observed in triploids, but the TBHC of diploid females was significantly lower than that of diploid males at and following spawning (November 1995 and April 1996; p=0.0001 and 0.0009, respectively).

Only two significant correlations between sampling order and TBHC were found (Tables 4.4 and 4.5) and the direction of the correlation was negative in one case (UNB-strain diploids, Dec. 1993) and positive in the other (Quebec-strain triploids, April 1996).

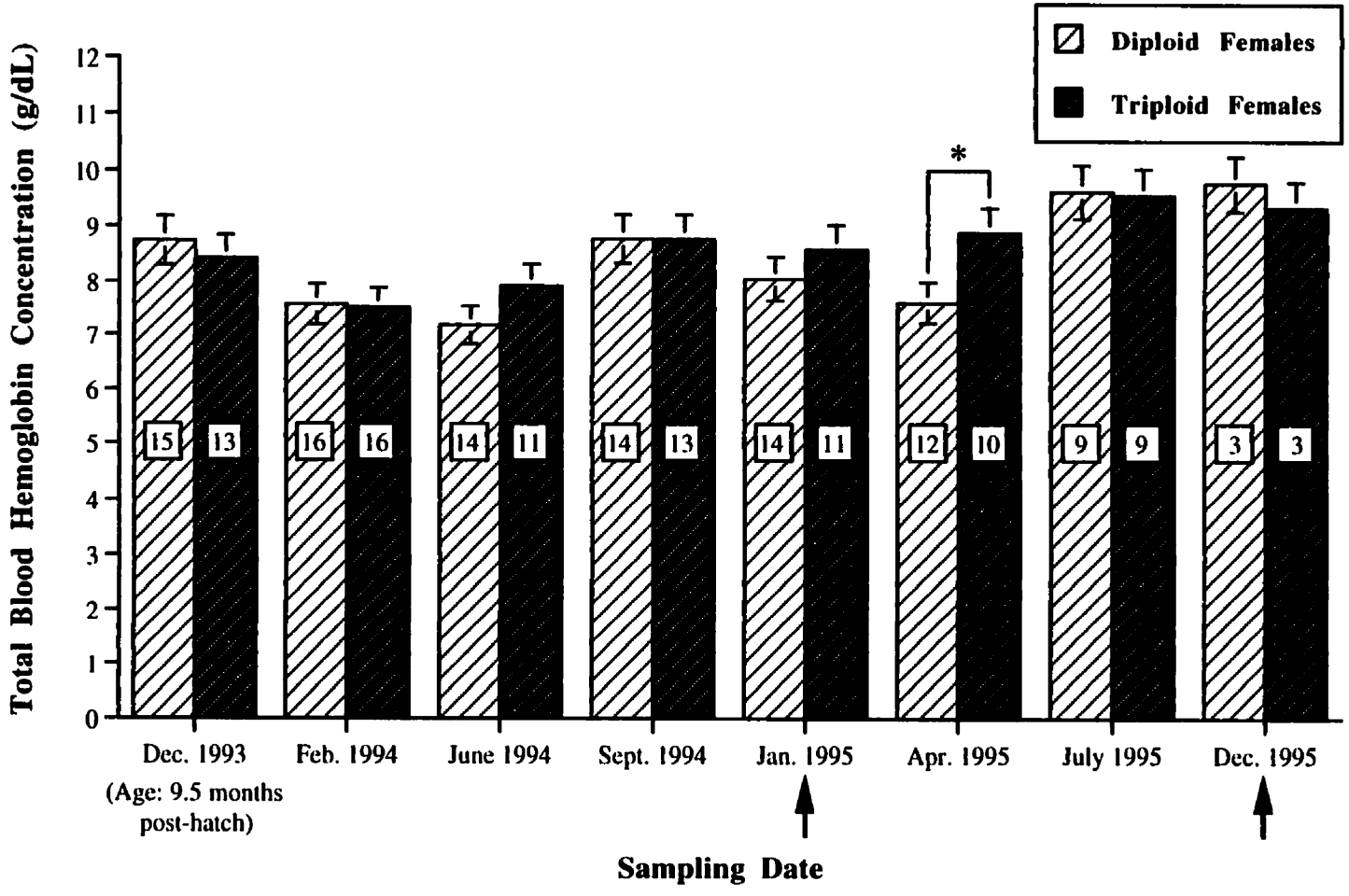

Figure 4.2: Total blood hemoglobin concentrations in an all-female (UNB-strain) group of diploid and triploid brook trout (*Salvelinus fontinalis*); bars represent means (± 1 standard error). Sample sizes are indicated by the numbers on the histogram. * represents a marginally significant difference ($p \leq 0.0131$). Sampling dates marked with arrows indicate dates when diploids were "spawned" (i.e., stripped of eggs).

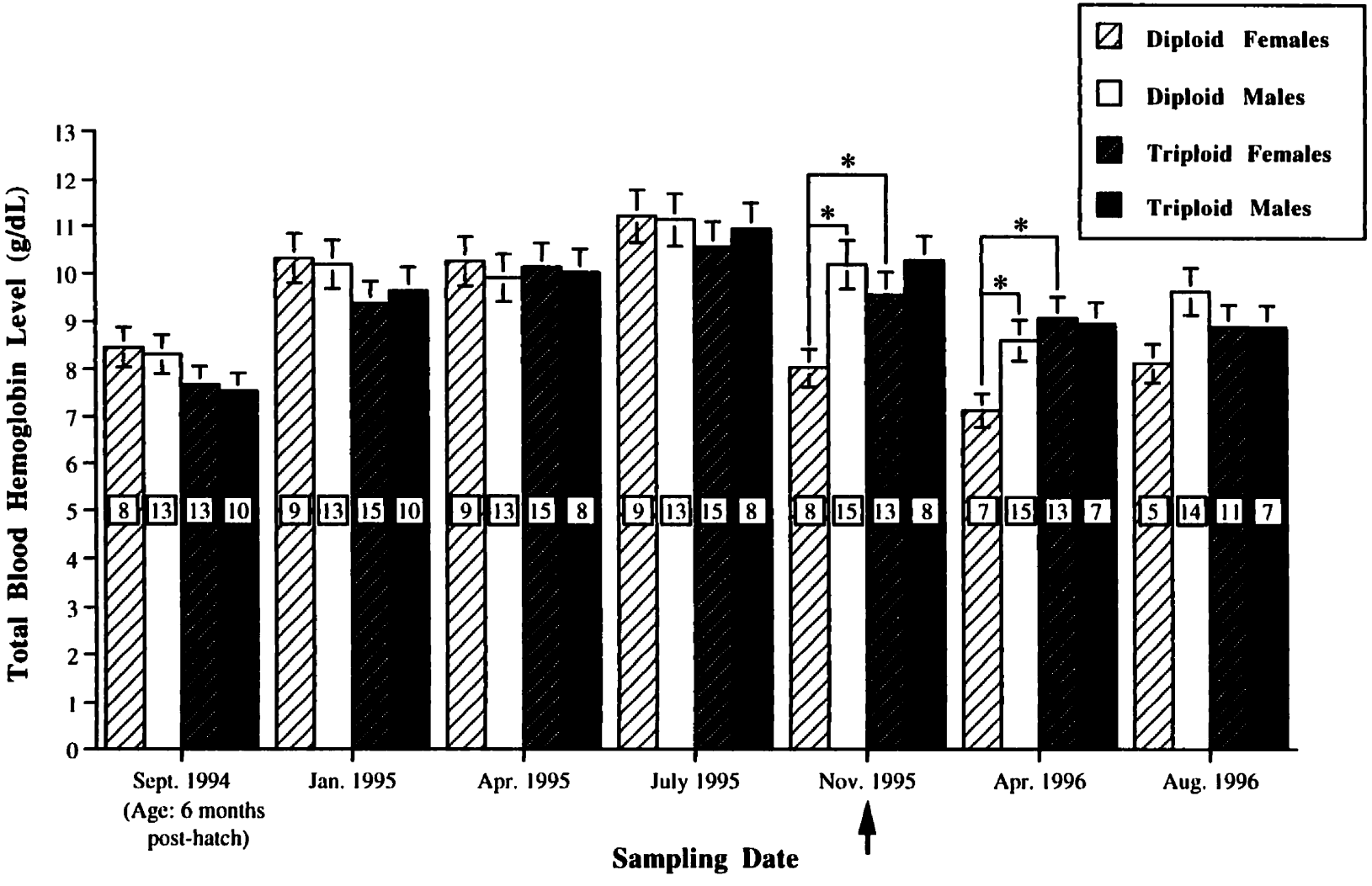

Figure 4.3: Total blood hemoglobin concentrations in a mixed-sex (Quebec-strain) group of diploid and triploid brook trout (*Salvelinus fontinalis*); bars represent means (± 1 standard error). Sample sizes are indicated by the numbers on the histogram. * represents a significant difference ($p \leq 0.0018$). The sampling date marked with an arrow indicates a date when diploids were "spawned" (i.e., stripped of gametes).

Sampling Date	Diploids			Triploids		
	r	r^2	*P*	r	r^2	*P*
Dec 1993	-0.5505	0.3031	0.0335*	0.3928	0.1543	0.1843
Feb 1994	n/a	n/a	n/a	n/a	n/a	n/a
June 1994	0.0667	0.0044	0.8208	0.4227	0.1787	0.2236
Sept 1994	-0.0003	0.0000	0.9993	-0.0228	0.0005	0.9412
Jan 1995	-0.0739	0.0055	0.8017	0.3666	0.1344	0.2674
April 1995	-0.2117	0.0448	0.5090	0.3811	0.1452	0.2773
July 1995	0.2399	0.0576	0.5340	-0.0339	0.0011	0.9311
Dec 1995	-0.5647	0.3189	0.6180	0.4069	0.1656	0.7332

Table 4.4. The correlation between total blood hemoglobin concentration and sampling order in UNB-strain (all-female) diploid and triploid brook trout. Sample sizes are as indicated in Figure 4.2. r = the Pearson correlation coefficient, *P* = the associated significance level. n/a indicates that sampling order data were not available. * represents a significant value ($p \leq 0.05$).

Sampling Date	Sex	Diploids r	Diploids r^2	Diploids *P*	Triploids r	Triploids r^2	Triploids *P*
Sept 1994	F	-0.6801	0.4625	0.0635	0.3014	0.0908	0.3170
	M	-0.3416	0.1167	0.2533	-0.3010	0.0906	0.3980
Jan 1995	F	0.6469	0.4185	0.0597	0.4082	0.1666	0.1309
	M	-0.2662	0.0709	0.3794	-0.2772	0.0768	0.4382
Apr 1995	F	0.1669	0.0279	0.6677	0.5124	0.2626	0.0508
	M	0.3823	0.1462	0.1974	0.6425	0.4128	0.0858
July 1995	F	0.5610	0.3147	0.1161	-0.1624	0.0264	0.5630
	M	-0.4178	0.1746	0.1554	0.2443	0.0597	0.5599
Nov 1995	F	0.1588	0.0252	0.7072	0.4611	0.2126	0.0836
	M	0.2983	0.0890	0.3223	0.1621	0.0263	0.7014
April 1996	F	-0.3192	0.1019	0.4852	0.6432	0.4137	0.0097*
	M	0.0747	0.0056	0.8084	-0.0199	0.0004	0.9662
Aug 1996	F	0.2831	0.0801	0.6445	0.1755	0.0308	0.5485
	M	0.3696	0.1366	0.2633	-0.0175	0.0003	0.9703

Table 4.5. The correlation between total blood hemoglobin concentration and sampling order in Quebec-strain (mixed-sex) diploid and triploid brook trout. Sample sizes are as indicated in Figure 4.3. r = the Pearson correlation coefficient, *P* = the associated significance level. * represents a significant value ($p \leq 0.05$).

Discussion

Experiment I - Hematological Parameters

Consistent with previous literature, our results indicate that triploid erythrocytes and their nuclei are significantly larger than those of diploids. The greater nuclear size is a function of the increased amount of genetic material present in triploid nuclei (Benfey 1997) while a greater triploid cell size results from the nuclear-cytoplasmic ratio being maintained at a comparable level in diploids and triploids (Benfey 1997).

The data for length-width ratio indicate that triploidy also affects cell and nuclear shape; relative to their width, triploid erythrocytes and nuclei are significantly longer than those of diploids. This observation has been reported previously (Benfey 1997) but, since based on blood smear preparations, may not reflect the *in vivo* situation. It is not clear to what extent cell height (i.e., thickness) was affected by triploid induction since this parameter could not be directly measured in our study. If, as literature suggests (Reeves and Park 1993, Benfey 1997), increases in cell length and width in larger cells are not accompanied by a decrease in cell height, triploid cells should have a lower surface area to volume ratio than diploid cells. The performance of any function limited by surface area would, therefore, be expected to be reduced in triploids. Interestingly, studies on polyploid cell populations have found that nuclei with the highest ploidy also have highly branched membranes (Brodsky and Uryvaeva 1978). It would be worthwhile to investigate whether a comparable situation exists in the cell membranes of triploid organisms as a means of increasing cell surface area.

Our results, in accordance with those from previous studies (Benfey 1997), found erythrocyte numbers to be significantly lower in triploids than diploids while hematocrits were equivalent in both ploidies. The decrease in cell number in triploid fish appears be under the control of an inherent mechanism which acts to maintain a standard organ and body size despite an increase in cell size. This mechanism is operational in most animal species but appears to be absent in plants where triploidy causes giantism (Swarup 1959);

aside from this, little is known about the nature of this mechanism. An equivalent hematocrit indicates that this mechanism is functional at the level of the blood.

Significant differences in TBHC related to ploidy have been previously reported, however, the direction of the difference in this case (i.e., a higher TBHC in triploids than diploids) does not agree with any result previously reported in the literature. As indicated above, our experimental diploids, unlike the triploids, had ovulated and been stripped of eggs approximately seven weeks prior to the date of blood sampling. It may be that endocrinological and/or physiological changes associated with final maturation and ovulation in diploids, rather than ploidy differences *per se*, were responsible for the observed difference in TBHC. This was further investigated in Experiment II and will be discussed below.

Blood NTP levels were equivalent in both ploidy groups. These findings agree with those of Graham *et al.* (1985). NTP acts to stabilize the deoxygenated form of hemoglobin thereby reducing hemoglobin's oxygen affinity. Higher blood concentrations of NTP, therefore, improve the ability of hemoglobin to unload oxygen within tissues (Nikinmaa 1992). It is thus interesting that, although the diploids had a lower TBHC than triploids in this study, this was not physiologically compensated for by an alteration in the oxygen affinity of the hemoglobin as measured by levels of total blood NTP. It should be noted, however, that NTP is just one of several solutes which can modulate oxygen affinity. A lower TBHC may have been compensated for through altered levels of other oxygen affinity modulators or by some other means entirely (e.g., increased cardiac output, increased gill irrigation rate, etc.).

Netting fish from a tank acts as a stressor not only to the particular fish being sampled but also to cohorts in the same tank (Strange *et al.* 1977, Biron and Benfey 1994). Fish sampled (i.e., netted) later in a sampling regime may consequently be exposed to more stress than those sampled early on. Environmental stress is known to produce a neuroendocrine response in fish: the sympathetico-chromaffin and hypothalamic-pituitary-

interrenal networks are stimulated causing, respectively, catecholamines and corticosteroids (e.g., cortisol) to be released into the blood (Mazeaud *et al.* 1977). These substances may, in turn, affect hematological variables. Although it appears that diploids and triploids respond similarly to acute stress and to the stress of cohort sampling (Biron and Benfey 1994), there was some concern that the order in which fish were sampled may have affected the hematological parameters examined.

The relatively low plasma cortisol concentrations observed suggest that fish were not excessively stressed by the netting and blood sampling procedures. The maintenance of relatively low cortisol levels across sampling order and the lack of a significant positive correlation between cortisol level and sampling order suggests that stress from cohort sampling was minimal. Further, the general absence of significant correlations between the various other hematological parameters and sampling order suggests that sampling order had little impact on the hematology of either ploidy group. In light of these findings, the presence of a significant positive correlation between sampling order and erythrocyte count in diploid fish is difficult to reconcile, especially considering the lack of a corresponding significant positive correlation with hematocrit. Interestingly, if the highest and lowest erythrocyte count values are removed from the diploid data set, the correlation between diploid erythrocyte count and sampling order becomes non-significant (p=0.0621). Considering this fact it is unlikely that the impact of sampling order negates the conclusion that erythrocyte numbers are greater in diploids than triploids, as has been demonstrated in numerous other species (Benfey 1997).

Experiment II - Total Blood Hemoglobin Concentration

The TBHC of both the all-female (UNB-strain) and mixed-sex (Quebec-strain) groups of fish varied significantly with sampling date. Age/size differences and seasonal environmental variations (i.e., changes in temperature and photoperiod) existed between sampling dates. These factors are known to affect TBHC (Glomski *et al.* 1992) and,

therefore, likely account for the fluctuations observed in TBHC over time.

In general the TBHC profiles of diploids and triploids of both sexes were very similar. The only significant sex or ploidy-related differences in TBHC noted were present at or following diploid spawning, when diploid females had a significantly lower TBHC than diploid males or triploid females. The similarity in the TBHC profile of triploid males and females, as well as in that of diploid and triploid males, suggests that neither sex nor ploidy differences alone affect TBHC. It appears instead that the endocrinological/physiological changes present in diploid females around the time of spawning were responsible for the reduction in TBHC.

The endocrinology of female fish is significantly affected by ploidy: unlike those of diploid females, the ovaries of triploid females produce no significant amounts of reproductive steroids. Diploid females, therefore, mature sexually and spawn while triploid females do not (Benfey 1997). Ploidy-related differences in female endocrinology are most pronounced in the pre-spawning period when levels of reproductive steroids become significantly elevated in diploid females but remain negligible in triploid females (Benfey *et al.* 1989). Male endocrinology is affected to a lesser degree by ploidy: triploid males have reproductive endocrine profiles which are similar to those of diploid males, consequently, males of both ploidies mature sexually and demonstrate spawning behaviour (Benfey 1997). The overall effect is that testosterone is produced in diploid and triploid males and in diploid females, but 17ß-estradiol is produced only in diploid females. Neither testosterone nor 17ß-estradiol are produced to a significant degree in triploid females (Benfey 1997).

In mammals testosterone acts to stimulate erythropoiesis (Mirand *et al.* 1965) whereas estrogens are known to be potent inhibitors of erythropoiesis (Dukes and Goldwasser 1961, Blobel *et al.* 1995); evidence of similar controls over erythrocyte production exists in teleost fish (Pickering 1986). Erythrocytes synthesize hemoglobin as they develop and mature (Fänge 1986). Therefore, factors which influence erythropoiesis also affect TBHC.

TBHC may exhibit a delayed or prolonged response to factors affecting erythropoiesis since fish erythrocytes can survive for up to 150 days in circulation (Hevesy *et al.* 1964). The influence of estrogens on erythropoiesis and consequently on hemoglobin synthesis may explain why the TBHC decreases in diploid females at or following spawning while no such decrease is evident in triploid females or in males of either ploidy.

Increased levels of estrogens also stimulate the production of vitellogenin in female fish (Sumpter 1984). This yolk-protein has been shown to have an iron-binding capacity in several fish species (Hara 1976, Hara et al. 1983, Tsioros et al. 1996). It is hypothesized that the large amount of iron required for maturation of eggs is provided maternally via vitellogenin (Hara 1976, Tsioros et al. 1996). Maternal iron reserves may, therefore, be diminished during vitellogenesis and the amount of iron available for hemoglobin sythesis in the female may consequently be reduced at this time. Vitellogenesis is also an energetically demanding process. Metabolic energy is typically diverted to gonadal development and egg production to such a degree that somatic growth and tissue quality are dramatically reduced at this time (Aksnes *et al.* 1986). In diploid females both the iron-binding capacity of vitellogenin and the metabolic demands of vitellogenesis may thus create physiological conditions which favour the suppression of erythropoiesis/hemoglobin synthesis. Triploid females would not be expected to show this pattern since they do not produce any significant amounts of vitellogenin (Benfey 1997).

Differences in the TBHC profiles of diploid and triploid females from the mixed-sex (Quebec-strain) group occurred at and following diploid spawning (Figure 4.3) while in females from the all-female (UNB-strain) group TBHC differences between ploidies were less pronounced and occurred only after the spawning event (Figure 4.2). This disparity may simply represent strain differences or may be related to the respective presence and absence of cohabitating males. Male pheromonal and tactile stimuli have been shown to stimulate ovarian development and cause an elevation in the level of 17ß-estradiol in females of several fish species (Van Weerd and Richter 1991).

As in experiment I, the general lack of significant correlation between TBHC and sampling order observed in this experiment suggests that sampling order had little impact on the TBHC findings presented.

In conclusion, our study finds the hematology of triploid brook trout to be generally consistent with the hematology of triploids of other salmonid species. Our study also offers some insight into the inconsistencies reported in TBHC between ploidy groups. Previously reported differences in the TBHC of diploid and triploid fish appear to reflect differences in the endocrinological profiles of these groups rather than ploidy differences *per se*. Differences in the TBHC of males and females also appear to be related to endocrinological and/or physiological differences between sexes rather than to sex differences *per se*. These findings emphasize the importance of controlling for sex, level of sexual maturity, and factors related to spawning when comparing TBHC in diploid and triploid (or male and female) fish. These factors appear not to have been controlled for in many of the previous studies in which the TBHC of diploids and triploids was compared (Sezaki *et al.* 1983, Benfey and Sutterlin 1984, Graham *et al.* 1985, Small and Randall 1989, Sezaki *et al.* 1991, Parsons 1993, Yamamoto and Iida 1994). It is interesting to note that in studies where sex and/or level of sexual maturity were controlled, TBHC is reported to be equivalent in diploids and triploids (Barker *et al.* 1983, Aliah *et al.* 1991, Biron 1993, Stillwell and Benfey 1996). Although not the definitive measure of oxygen carrying capacity, the overall similarity in the TBHC of diploids and triploids in this study suggests that triploid performance should not be restricted by blood-oxygen transport capacity.

Acknowledgements

We are grateful to Michel Biron, Mark Deeley, Lisa McCabe, Robyn O'Keefe and Kevin Richards for their technical assistance. We thank Dr. R. Mureika for statistical advice and Dr. A.J. Wiggs and Dr. R. Saunders for their critical reading of this manuscript. This research was supported by a Research Grant from the Natural Sciences and Engineering

Research Council. The experimental protocol was approved by the U.N.B. Animal Care Committee meeting Canadian Council of Animal Care guidelines.

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Appendix 4.1

Figure 4.4: Diploid and triploid brook trout erythrocytes.

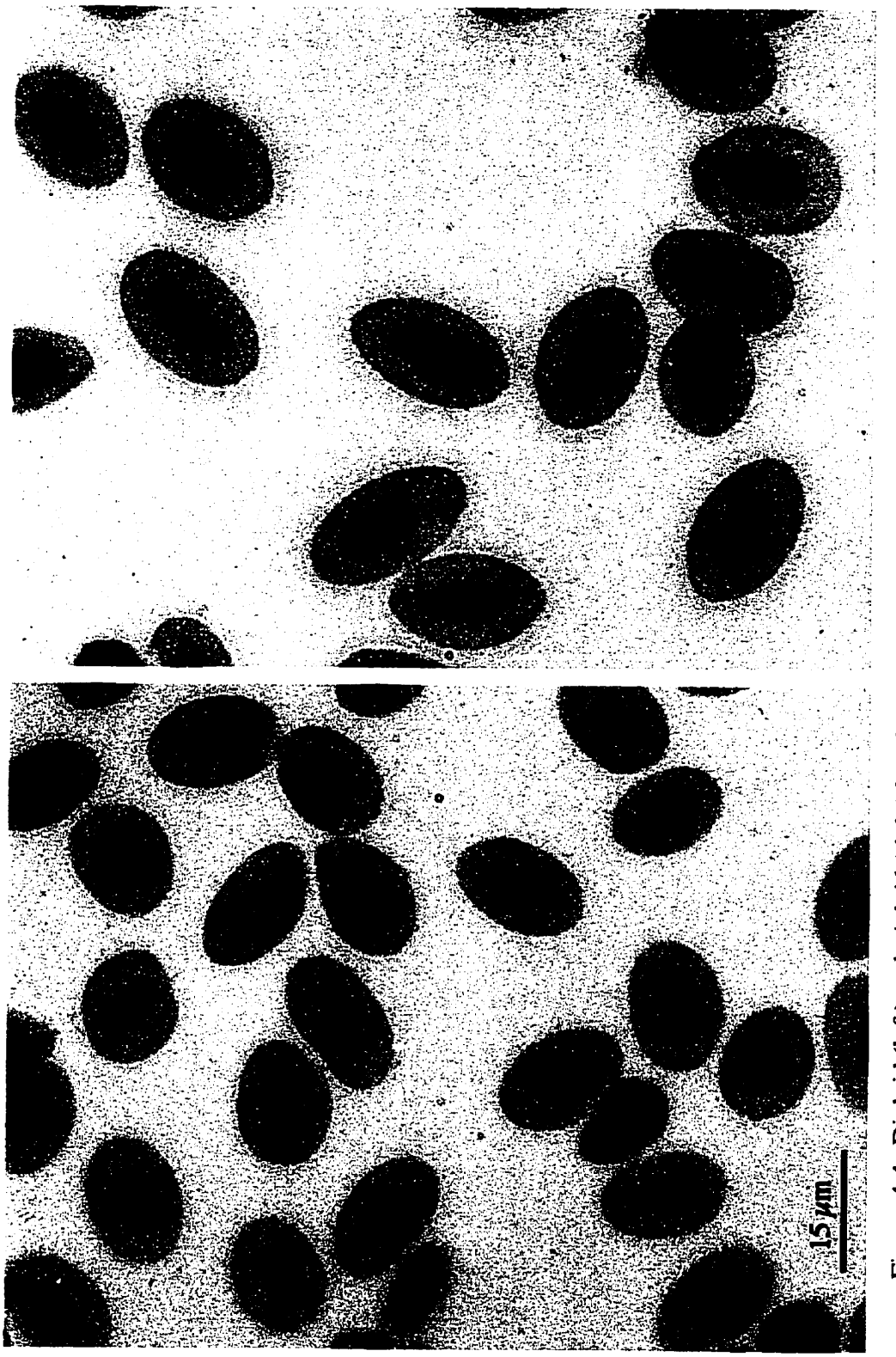

Figure 4.4. Diploid (left) and triploid (right) brook trout erythrocytes.

Chapter 5

General Discussion and Conclusions

At the onset of this thesis, research indicated that diploid and triploid salmonids had equivalent oxygen requirements but that triploids had a lower blood-oxygen carrying capacity than diploids. In spite of these factors triploid salmonids performed as well as diploids in tests of aerobic capacity (i.e., critical swimming velocity tests). There appeared to be two possible explanations to account for these findings: either triploids had developed mechanisms to compensate for their lower blood-oxygen carrying capacity or conclusions from studies which compared the hematology, metabolic rate and critical swimming velocity of diploids and triploids were incorrect. I set out to investigate these options experimentally in diploid and triploid brook trout (*Salvelinus fontinalis*).

I conducted hematological, metabolic rate and critical swimming velocity experiments to assess if conclusions from previous studies were correct. An examination of hematological parameters (chapter 4, experiment 1) revealed that triploid brook trout hematology was generally consistent with that of triploids of other salmonid species. This indicated that brook trout are a suitable model in which to investigate the physiological effects of triploid induction in salmonids. The one hematological inconsistency found was in TBHC. In contrast to previous studies where TBHC was significantly lower in triploids than diploids (Benfey and Sutterlin 1984a, Graham *et al.* 1985, Small and Randall 1989, Yamamoto and Iida 1994a), I found TBHC to be significantly higher in triploids than in diploids. TBHC was also examined in the metabolic rate experiment (chapter 2) and, in this case, TBHC was equivalent in both ploidy groups; this also did not correspond with previous results. Although in both experiments fish were of one sex (i.e., females) and all triploids were sexually immature, in the hematological experiment diploids were sexually mature and had recently ovulated whereas in the metabolic rate experiment diploids were sexually immature. It appeared that factors related to ovulation or sexual maturity were somehow affecting TBHC; this was examined in the TBHC experiment (chapter 4, experiment 2).

In the TBHC experiment the TBHC profiles of diploids and triploids were generally similar, however, the TBHC of diploid females decreased during/after ovulation while

TBHC of all other groups remained high. These results suggest that ploidy itself has little effect on TBHC. Instead it appears that endocrinological/physiological changes related to ovulation are responsible for the noted differences in TBHC. These findings underline the importance of controlling for level of sexual maturity and factors related to ovulation when comparing TBHC between ploidy groups. These findings also suggest that the blood-oxygen carrying capacity of triploids (which is derived from the TBHC and the hemoglobin-oxygen loading ratio) may not be reduced to the degree proposed by Graham *et al.* (1985). The results from the Graham *et al.* (1985) study are based on small sample sizes and pooled blood samples and have not been verified in other salmonid species. These considerations combined with the current findings suggest that results from the Graham *et al.* (1985) study should be verified before being accepted as fact.

My investigation of critical swimming velocity (C.S.V.; chapter 3) and metabolic rate (chapter 2) indicates that triploid brook trout have an equal C.S.V. and a lower sustained metabolic rate relative to their diploid counterparts. The former supports the critical swimming velocity results of Small and Randall (1989) and indicates that aerobic capacity is not affected by ploidy level. The latter contradicts results from previous studies (Benfey and Sutterlin 1984b, Oliva-Teles and Kaushik 1990, Yamamoto and Iida 1994b) in which diploids and triploids were found to have an equivalent metabolic (oxygen consumption) rate. This discrepancy might be related to the fact that numerous factors which might have affected results, or the interpretation thereof, had not been controlled for in previous studies but were regulated in my experiment (e.g., sex, level of sexual maturity, fitness level, temperature, etc.). The lower oxygen requirements of triploids may serve to offset any reduction in blood-oxygen transport capacity and enable triploids to be equivalent to diploids in their aerobic swimming performance.

In order to determine if triploids have mechanisms to compensate for their potentially lower blood-oxygen carrying capacity, I examined total blood NTP levels (chapter 4, experiment 1), opercular abduction rate (chapter 2), and swimming efficiency (chapter 3) in diploid and triploid brook trout. In all cases, there was no difference in these parameters between

ploidy-groups. These findings suggest that a compensatory mechanism is not present in triploids. This lack of a compensatory mechanism might be expected if, as discussed above, the blood-oxygen carrying capacity of triploids was not reduced to the degree proposed by Graham *et al.* (1985). I did not, however, conduct an exhaustive survey and other unidentified compensatory mechanisms may be active.

In conclusion, results from this thesis indicate that, relative to diploid brook trout, triploids have a lower oxygen requirement but an equivalent C.S.V.. The lower oxygen requirements of triploids might have offset any possible reduction in triploid blood-oxygen carrying capacity and enabled an equivalent aerobic capacity. My TBHC results, however, suggest that triploid blood-oxygen carrying capacity may, in fact, not be impaired to the degree previously anticipated.

I believe that the single most valuable contribution of this thesis is the recognition of the importance of controlling endocrinological/physiological factors when comparing diploid and triploid organisms. Any conclusions related to ploidy that are garnered from research in which endocrinological/physiological factors are not considered will, at best, be equivocal.

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Yamamoto, A. and Iida, T. 1994b. Oxygen consumption and hypoxic tolerance of triploid rainbow trout. Fish Pathol. 29: 245-251.

Appendix 5

Letters of Permission to include Chapters 2 and 3:

Chapter 2:
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Chapter 3:
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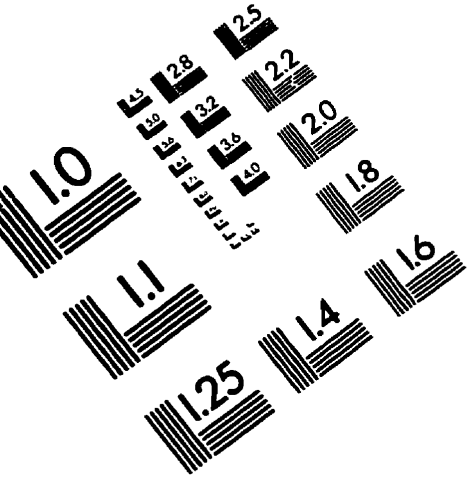
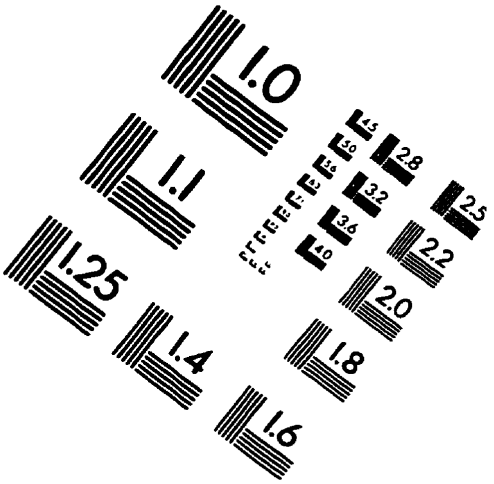
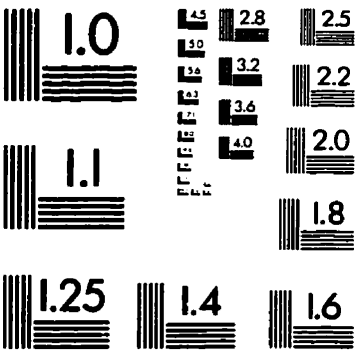
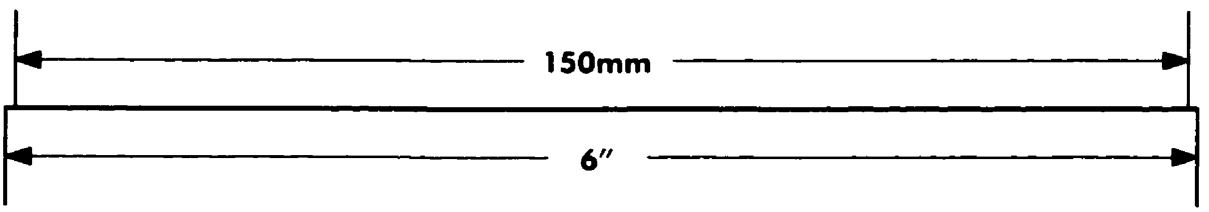
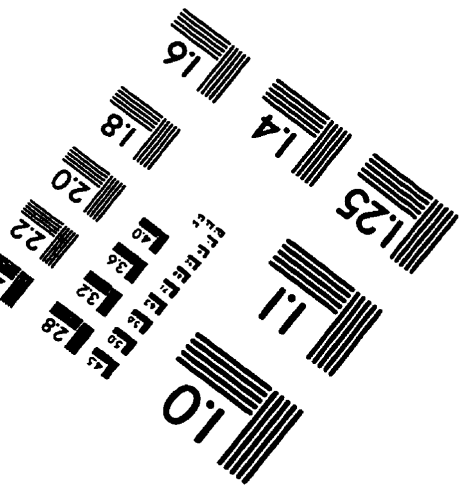
IMAGE EVALUATION TEST TARGET (QA-3)

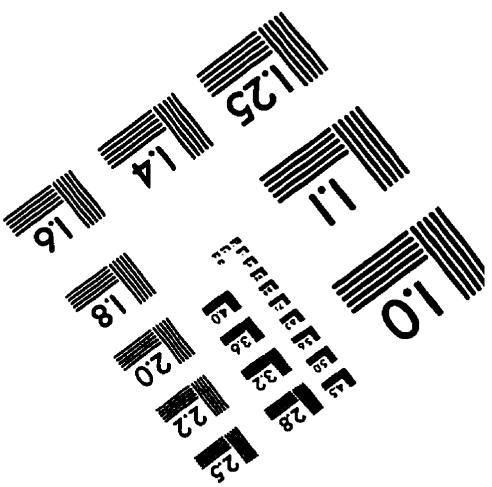












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