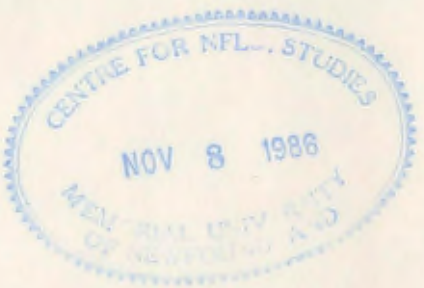
THE PRODUCTION OF TRIPLOID LANDLOCKED ATLANTIC SALMON (SALMO SALAR L.) AND THEIR POTENTIAL FOR AQUACULTURE

TILLMAN J. BENFEY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THE PRODUCTION OF TRIPLOID LANDLOCKED ATLANTIC SALMON (*SALMO SALAR* L.) AND THEIR POTENTIAL FOR AQUACULTURE

BY

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ABSTRACT

Heat shocks of 5 minutes at 32°C or hydrostatic pressure shocks of 3 or 6 minutes at 7.0×10^4 kPa (10,150 p.s.i.), when completed within 20 minutes of fertilization at 10°C, were found to induce 100% triploidy with 70-90% survival (relative to controls) in eggs of landlocked Atlantic salmon (*Salmo salar* L.). The identical heat shock yielded substantially lower numbers of triploids when applied 25 to 45 minutes after fertilization. Pressure shocks of longer duration (9 to 15 minutes at 7.0×10^4 kPa) or higher magnitude (6 minutes at 7.9×10^4 to 10.5×10^4 kPa) resulted in 100% mortality prior to hatching. The duration of the effective period within which heat shocks could be used to induce triploidy was found to be temperature dependent, being longer at 6.5°C than at 10°C. Attempts to induce polyploidy with cytochalasin B were unsuccessful.

Karyotyping was not a useful method for the routine identification of triploids because it was time-consuming and inconsistent. The use of a microspectrophotometer to measure the DNA content of Feulgen-stained erythrocytes, although time-consuming, clearly distinguished triploid from diploid fish. Blood cell sizing by means of a Coulter Counter Channelyzer was a highly effective alternative to screen for triploids, being both fast and accurate. This

technique was routinely used to identify triploid individuals. The use of various erythrocyte dimensions measured from blood smears was a valid method for identifying triploids, but was also time-consuming. No mosaic polyploids were found, and the existence of such fish is questioned.

Triploid fish had a greater mean erythrocyte volume (MCV) but lower erythrocyte count than diploids; the haematocrit was thus the same in diploids and triploids. Although the total blood haemoglobin content and the mean corpuscular haemoglobin concentration (MCHC) were lower in triploids than in diploids, the mean corpuscular haemoglobin content (MCH) was higher than that of diploids. The increase in triploid MCV was mainly due to an increase in cell length; there was only a minor increase in cell width and no increase in cell height. The nucleus of triploid erythrocytes occupied a greater percentage of the corpuscular volume than did the diploid nucleus. Mean cytoplasmic haemoglobin concentration was found to be the same for diploids and triploids when this was taken into account.

The rates of oxygen consumption by diploid and triploid fish were the same, as was the oxygen tension at asphyxiation. Growth rates (measured as change in weight with time) prior to spawning were the same for diploids and

triploids, but diploids were consistently shorter in fork length, thus having a higher condition factor. The gonadosomatic index (GSI) of diploid females was 13 times greater than that of triploid females, but the GSI of diploid males was only 1.9 times greater than that of triploid males. Triploid ovaries had the appearance of undifferentiated gonads, but triploid testes were well-developed. Gonad histology revealed that diploid ovaries were packed with hundreds of previtellogenic oocytes (stages 1-4), whereas triploid ovaries contained mostly undifferentiated oogonia. Every triploid ovary examined, however, contained at least one oocyte having the typical diploid appearance, indicating that triploid females were not sterile. Triploid testes contained all the elements present in the diploid testes, but development appeared to be delayed in comparison to that of the diploids.

It is concluded that triploid females may be of benefit to salmonid aquaculture, but that further studies of growth and maturation are required.

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PREFACE

Even if it were merely to complete the general picture, information on a number of problems should be gathered: (1) the occurrence and frequency of polyploidy in different groups of animals; (2) the origin of these exceptional individuals, in other words, the mechanisms that are responsible for their production; (3) the effectiveness of methods for the experimental induction of polyploidy; (4) the general effects of polyploidy on cell size, body size, and viability, and on general physiology and biochemistry of the organism; (5) the occurrence of qualitative effects, which are added to the more obvious quantitative consequences.

(Fankhauser, 1945a, page 21)

The day may come when chromosome-doubling will be commonplace. If that day comes, it will be because the process has real advantages over normal reproduction; but those advantages are yet to be proven.

(Blish, 1961, page 1)

1. INTRODUCTION

Rationale for Using Triploid Fish in Aquaculture

When salmonids mature under culture conditions, energy allocated to somatic growth is diverted to gonadal growth, total growth is reduced, flesh quality deteriorates, and post-spawning mortality is often high or total (Gardner, 1976; Edwards, 1978; Sutterlin and Merrill, 1978; Naevdal et al., 1981; Naevdal, 1983). As all these aspects of maturation are detrimental in commercial aquaculture, various techniques have been considered to delay or prevent maturation (Chevassus et al., 1979a, 1979b; Stanley, 1979, 1981; Donaldson and Hunter, 1982; Yamazaki, 1983). One of these techniques, first suggested for use in salmonid culture by Purdom and Lincoln (1973), is the use of triploid fish. The induction of triploidy is just one of several methods of chromosome manipulation presently being considered under the general topic of genetic engineering in fish (Purdom, 1983).

Triploids are expected to be sterile, since the meiotic development of their gametes is disrupted. Normal meiosis, as observed in a diploid individual, is characterized by the pairing of homologous chromosomes at the equatorial plate. Chromosome pairs are then separated by a bipolar spindle apparatus during the first meiotic division. In the absence

of a tripolar spindle, triploids would presumably form either non-viable aneuploid gametes, or no gametes at all (Purdom and Lincoln, 1973; Purdom, 1976; Stanley, 1979, 1981). Although tripolar spindles have been observed in naturally occurring triploid individuals of the silver crucian carp, Carassius auratus gibelio, and apparently serve to maintain triploidy in this population (Cherfas, 1966, 1969), they have never been described in any other species of naturally occurring or induced triploid fish (Uzzell, 1970; Cimino, 1972; Schultz, 1980; Chourrout, 1982b).

Origin of Triploids

Spontaneous Triploidy.

Theoretically, there are numerous routes by which triploidy can arise in an individual (Beatty, 1957). In all cases where triploid individuals have been observed in bisexual, diploid species of fish, retention of the second polar body has been cited as the presumed cause of triploidy. Extrusion of the second polar body is a process which normally occurs in fish shortly after fertilization of the egg (Figure 1). Prior to fertilization, development of the egg is arrested at metaphase of the second meiotic division. Activation by a fertilizing spermatozoon allows meiosis to go to completion, with extrusion of the second polar body and resulting formation of the haploid egg

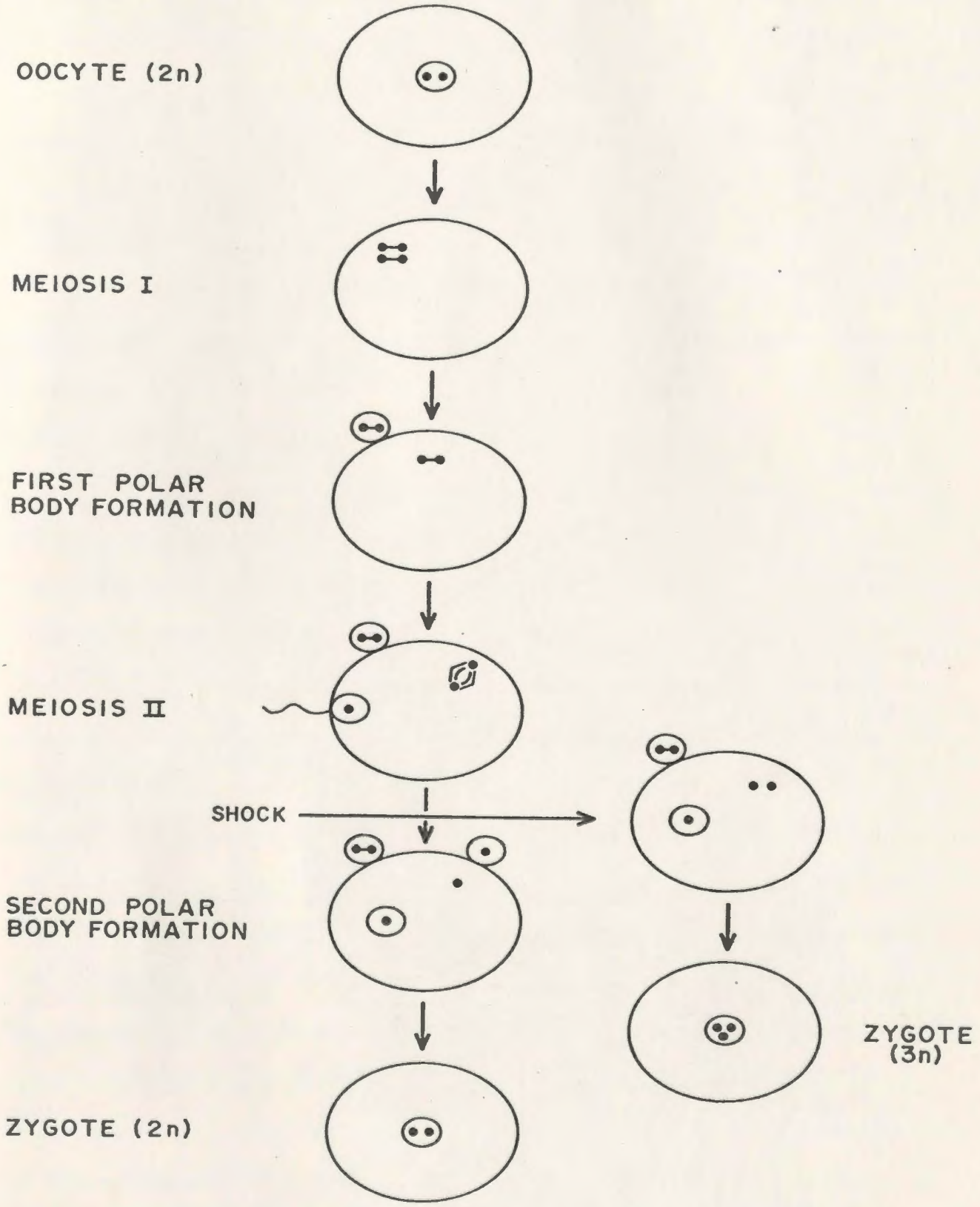

Figure 1: Chromosome manipulation leading to triploidy.

pronucleus. Fusion of the egg and spermatozoon pronuclei results in the formation of the diploid zygote. This process is apparently the same for all teleosts (Makino and Ozima, 1943; Ginzburg, 1968; Ojima and Makino, 1978; Masui and Clarke, 1979; Wallace and Selman, 1981).

Retention of the second polar body during meiotic development provides the egg with two haploid chromosome complements of maternal origin (Figure 1). Upon fertilization, the resulting zygote will be triploid, and either male (XXY) or female (XXX) (Thorgaard and Gall, 1979). If the activating spermatozoon is genetically inert (i.e., lacking functional DNA), a diploid zygote results which contains chromosomes of solely maternal origin. This process is a form of gynogenesis, and has been reviewed for fish by several authors (Golovinskaya, 1969; Stanley and Sneed, 1974; Stanley, 1979, 1981; Chourrout, 1982b). For species in which the female is homogametic, such as rainbow trout, Salmo gairdneri (Thorgaard, 1977; Okada et al., 1979) and coho salmon, Oncorhynchus kisutch (Hunter et al., 1982), gynogenetic diploids should invariably be female (XX). This has been confirmed for both these species (Chourrout and Quillet, 1982; Refstie et al., 1982).

Spontaneous retention of the second polar body occasionally results when eggs develop parthenogenetically. This has been observed in salmonids (Melander and Montén,

1950; Purdom, 1969; Tsoi, 1972; Chourrout, 1980), sturgeons (Romashov et al., 1963), the loach, Misgurnus fossilis (Romashov and Belaieva, 1965), cyprinids (Cherfas, 1975; Stanley et al., 1975; Stanley 1976a, 1976b, 1976c; Nagy et al., 1978), and the zebra fish, Brachydanio rerio (Streisinger et al., 1981). In parthenogenesis, haploid eggs are induced to develop in the absence of paternal chromosomes. This generally leads to mortality prior to or just after hatching in the salmonids, with larvae characterized by the "haploid syndrome" of microcephaly, twisted tail, and a short, thickened body (Arai et al., 1979; Onozato, 1982; Refstie et al., 1982; Onozato and Yamaha, 1983). A small percentage of parthenogenetic larvae (usually less than 1%) may continue to develop, and appear identical to their maternal parent. These individuals are gynogenetic diploids which have arisen through the spontaneous retention of the second polar body, and thus possess only maternal chromosomes (Thompson et al., 1981).

The occurrence of both triploids and gynogenetic diploids is common in certain species of unisexual fish which have evolved complex modes of reproduction (Schultz, 1980). However, the vast majority of fish species are bisexual diploids. Of such fish, only two spontaneous triploids have been found in the wild: one individual each of the California roach, Hesperoleucus symmetricus (Gold and Avise, 1976), and the Japanese common loach, Misgurnus

anguillicaudatus (Ojima and Takai, 1979). Triploid salmonids have never been encountered in the wild, but no specific attempt has been made to locate such fish. Both triploids and mosaic polyploids are regularly found in small numbers among hatchery reared salmonids (Svärdson, 1945; Chernenko, 1968; Cuellar and Uyeno, 1972; Grammeltvedt, 1974; Allen and Stanley, 1978; Thorgaard and Gall, 1979; Thorgaard et al., 1982; Utter et al., 1983).

Interspecific hybridization frequently results in the production of triploid or gynogenetic diploid individuals through retention of the second polar body (Chevassus, 1983). This has been reported in salmonids (Svärdson, 1945; Buss and Wright, 1956; Uyeno, 1972; Capanna et al., 1974; Onozato, 1981a), flatfish (Purdom and Lincoln, 1974), and cyprinids (Makeyeva, 1975; Vasil'ev et al., 1975; Makeeva, 1976; Stanley et al., 1976; Cherfas and Ilyasova, 1980), but the low incidence of such fish among these hybrids indicates that they may have arisen through spontaneous retention of the second polar body, rather than due to hybridization itself. The hybridization of female grass carp, Ctenopharyngodon idella, with male bighead carp, Aristichthys nobilis, results in a high incidence (up to 100%) of triploid offspring (Márián and Krasznai, 1978; Beck et al., 1980; Sutton et al., 1981; Beck and Biggers, 1982; Allen and Stanley, 1983). This is the only fish hybrid for which such a phenomenon has been described, and

its mechanism has not yet been determined.

Induced Triploidy.

The classic experiments by Gerhard Fankhauser of Princeton University (reviewed by Fankhauser, 1945a) demonstrated that extrusion of the second polar body could be blocked in salamanders by subjecting the eggs to thermal shocks shortly after fertilization. Makino and Ozima (1943) were the first to use this technique in fish, using cold shocks to successfully prevent extrusion of the second polar body in eggs of the carp, Cyprinus carpio. Subsequent research has demonstrated that cold shocks can be effectively used to block extrusion of the second polar body in three-spined stickleback, Gasterosteus aculeatus (Swarup, 1956, 1959a), loach, Misgurnus fossilis (Romashov and Belaieva, 1965), flatfish (Purdom, 1969, 1972; Purdom and Lincoln, 1973, 1974; Purdom, 1976; Purdom et al., 1976; Hoornbeek and Burke, 1981; Lincoln, 1981a, 1981b), cyprinids (Stanley and Sneed, 1974; Cherfas, 1975; Nagy et al., 1978; Ojima and Makino, 1978; Gervai et al., 1980a, 1980b; Meriwether, 1980; Tsoi, 1981), blue tilapia, Tilapia aurea (Valenti, 1975), and channel catfish, Ictalurus punctatus (Wolters et al., 1981b).

The use of heat shocks to produce triploid or gynogenetic diploid fish has received less attention. Heat shocks have been used to prevent extrusion of the second

polar body in fertilized eggs of the three-spined stickleback (Swarup, 1956, 1959a), loach (Romashov and Belaieva, 1965), sturgeon (Vasetskii, 1967), blue tilapia (Valenti, 1975), grass carp (Stanley, 1979) and zebra fish (Streisinger et al., 1981).

The first attempts to induce retention of the second polar body in salmonids were carried out by Svärdson (1945). He subjected whitefish, Coregonus lavaretus, and Atlantic salmon, Salmo salar, eggs to cold shocks shortly after fertilization with the milt of either whitefish or brown trout, Salmo trutta, respectively. In this way, a few triploid whitefish and Atlantic salmon x brown trout hybrids were produced, as well as some apparently gynogenetic diploid Atlantic salmon in which the brown trout chromosomes had not been incorporated into the genome. Subsequent research has shown that cold shocks are relatively ineffective in inducing triploidy or gynogenetic diploidy in salmonids (Vassileva-Dryanovska and Belcheva, 1965; Purdom and Lincoln, 1973; Lincoln et al., 1974; Chourrout, 1980; Lemoine and Smith, 1980; Refstie et al., 1982). Heat shocks, on the other hand, readily prevent extrusion of the second polar body in salmonids (Chourrout, 1980; Thorgaard et al., 1981; Chourrout, 1982c; Chourrout and Quillet, 1982; Lincoln and Scott, 1983; Thorgaard et al., 1983; Utter et al., 1983).

Hydrostatic pressure has been demonstrated to prevent extrusion of the second polar body in fertilized eggs of amphibians (Dasgupta, 1962; Ferrier and Jaylet, 1978; Jaylet and Ferrier, 1978; Müller et al., 1978; Tompkins, 1978; Gillespie and Armstrong, 1979). Stanley et al. (1975) were unable to produce gynogenetic diploid cyprinids using pressures of 0.3 to 1.2 X 10^4 kPa, but Onozato (1981b) successfully prevented extrusion of the second polar body in rainbow trout at 6.4 to 6.9 X 10^4 kPa, as did Streisinger et al. (1981) in the zebra fish at 5.5 X 10^4 kPa. Triploid rainbow trout have apparently been produced through hydrostatic pressure treatment (Chourrout, 1982b; Yamazaki, 1983), but the details of these experiments have not yet been reported.

The cytological mechanism whereby extrusion of the second polar body is prevented has received little attention. Cold shocks have been shown to slow or stop the anaphase separation of chromosomes long enough that the restitution nucleus incorporates what would have been both the egg pronucleus and the second polar body (Barber and Callan, 1943; Makino and Ozima, 1943). Heat shocks release the spindle apparatus from the egg cortex, and normal anaphase separation of chromosomes then occurs below the surface of the egg. Two pronuclei are formed, and these subsequently fuse with the spermatozoon pronucleus, giving rise to a triploid zygote (Fankhauser and Godwin, 1948;

Sládeček and Lanzová, 1959). Hydrostatic pressure causes solation of the egg cortex and spindle, after which the chromosomes either remain in their original position, or sink into the interior of the egg. In either case, separation of the chromosomes is blocked long enough that they are all incorporated by the restitution nucleus (Dasgupta, 1962).

Induced Tetraploidy and the Breeding of Triploids.

Purdom (1972) suggested that triploid fish could be produced by crossing the diploid gametes of a tetraploid fish with the haploid gametes of a diploid fish. Subsequent attempts to produce tetraploid flatfish, using cold shocks to block the first mitotic cleavage of the fertilized eggs, were unsuccessful (Purdom, 1972; Purdom and Lincoln, 1973). There was evidence of spindle disruption when cold shocks were applied to rainbow trout eggs at the time of first cleavage, but no tetraploids were observed (Purdom and Lincoln, 1973; Lincoln et al., 1974). Heat shocks, on the other hand, have been used to produce tetraploid sturgeon, Acipenser guldenstadti (Vasetskii, 1967), and rainbow trout (Thorgaard et al., 1981; Chourrout, 1982a). Tetraploid sturgeon were only grown to the larval stage, and their morphology was not described (Vasetskii, 1967). Tetraploid rainbow trout larvae, identified by karotype, were deformed and none were grown to maturity (Thorgaard et al., 1981; Chourrout, 1982a, Thorgaard et al., 1982).

Cytochalasin B, which inhibits cytokinesis but not mitosis (Defendi and Stoker, 1973), has been used in attempts to block first cleavage and thereby produce tetraploids (Refstie et al., 1977; Allen and Stanley, 1979; Refstie, 1981). Although the original experiments apparently yielded only mosaic polyploids (Refstie et al., 1977; Allen and Stanley, 1979), Refstie (1981) reported the production of tetraploid rainbow trout using cytochalasin B. The reproductive viability of these fish has not as yet been reported, and their classification as tetraploids has been questioned (Purdom, 1983).

The mitotic-inhibitor colchicine, used extensively in plant research, has generally been considered too toxic for inducing polyploidy in animals (Eigsti and Dunstan, 1955). Nevertheless, colchicine treatment has yielded mosaic polyploids in the medaka, Oryzias melastigma (Sriramulu, 1962), yellow perch, Perca fluviatilis, and rainbow trout (Lieder, 1964), numerous centrarchid species (Roberts, 1965), and the brook trout, Salvelinus fontinalis (Smith and Lemoine, 1979). The successful production of tetraploid fish using colchicine has not been reported.

To date, no one has succeeded in producing triploid fish by the crossing of tetraploids with diploids. In amphibians, viable tetraploids can be produced using thermal shocks (Sanada, 1951; Fischberg, 1958; Kawamura and

Nishioka, 1960; Jaylet, 1972; Gaillard and Jaylet, 1975) or hydrostatic pressure (Reinschmidt et al., 1979), and triploid individuals have successfully been produced by the crossing of tetraploids with diploids (Humphrey and Fankhauser, 1949; Fankhauser, 1952; Fankhauser and Humphrey, 1954; Beetschen, 1962; Kawamura and Nishioka, 1963; Beetschen, 1967; Beçak et al., 1968; Danzmann and Bogart, 1982, 1983).

Identification of Triploids

Most studies on genetic manipulation in fish have used direct chromosome counts to determine ploidy level. Karyotypes can be obtained through squash preparations (Simon, 1964; McPhail and Jones, 1966; Denton and Howell, 1969; Kligerman and Bloom, 1977) or lymphocyte culture (Barker, 1972; Grammeltvedt, 1974, 1975; Wolters et al., 1981a; Blaxhall 1983a, 1983b, 1983c; Hartley and Horne, 1983). The difficulties associated with karyotyping fish, and the relative merits of these two techniques, have recently been reviewed (Blaxhall, 1975; Gold, 1979). It is difficult to obtain consistently good squash preparations, and the individual generally has to be sacrificed after colchicine treatment. Lymphocyte culture requires a minimum of 1 ml of blood to provide sufficient numbers of lymphocytes, and is therefore not practical for juvenile fish.

Chromosome number can be determined indirectly by the spectrophotometric measurement of the DNA content of individual cells. This can be done rapidly and accurately on a large number of cells using a flow cytometer (Thorgaard et al., 1982; Allen, 1983; Allen and Stanley, 1983; Utter et al., 1983), or on a smaller number of cells using a microspectrophotometer (Rasch et al., 1965; 1970; Cimino, 1974; Nagy et al., 1978; Gervai et al., 1980a, 1980b; Lincoln, 1981a, 1981b).

The number of nucleoli per cell is determined genetically, and is related to ploidy level (Fankhauser and Humphrey, 1943; Branch and Berns, 1976). This fact has been used to distinguish between haploid and diploid sturgeons (Romashov et al., 1963) and cyprinids (Cherfas, 1975; Cherfas and Ilyasova, 1980; Tsoi, 1981), but has never been used to identify triploid fish. Triploid amphibians, on the other hand, are regularly identified by the number of nucleoli per cell (Kawahara, 1978; Müller et al., 1978; Gillespie and Armstrong, 1979; Cassidy and Blackler, 1980; Tashiro et al., 1983).

Electrophoresis has been used to distinguish between diploid and triploid forms of the grass carp x bighead carp hybrid (Magee and Philipp, 1982; Beck et al., 1983) and between diploids and triploids of certain unisexual fish species (Abramoff et al., 1968; Balsano et al., 1972;

Vrijenhoek, 1975; Liu et al., 1978, 1980; Monaco et al., 1982; Sezaki et al., 1983). This technique has also been used to identify diploid cyprinids of normal, gynogenetic and androgenetic origin (Stanley et al., 1976; Cherfas and Truveller, 1978), but never to identify triploids of non-hybrid origin. Such fish could theoretically be identified based on electrophoretic staining intensity, as has been demonstrated in diploid and triploid forms of the soft-shell clam, Mya arenaria (Allen et al., 1982).

It has been demonstrated in fish of known ploidy that the cellular and nuclear dimensions of triploid erythrocytes are greater than those of diploids (Swarup, 1959b; Cherfas, 1966, 1969; Cimino, 1973; Sezaki et al., 1977; Liu et al., 1978, 1980; Wolters et al., 1982a; Beck and Biggers, 1983; Sezaki et al., 1983). On this basis, erythrocyte measurements have been used to detect or verify triploidy in flatfish (Purdom, 1972; Purdom and Lincoln, 1973; Lincoln, 1981a, 1981b), blue tilapia (Valenti, 1975), carp (Meriwether, 1980), and salmonids (Allen and Stanley, 1978, 1979; Thorgaard and Gall, 1979; Lemoine and Smith, 1980; Refstie, 1981; Refstie et al., 1982; Lincoln and Scott, 1983). However, due to the large variation associated with the small number of cells which can feasibly be measured from a blood smear, several authors have questioned the validity of using erythrocyte measurements to identify triploid salmonids (Thorgaard and Gall, 1979; Lemoine and

Smith, 1980; Thorgaard et al., 1982; Wolters et al., 1982a; Utter et al., 1983).

The nuclear volume of cartilage cells is greater in triploid than in diploid three-spined stickleback (Swarup, 1959b), and this criterion has been used to identify haploid and gynogenetic diploid salmonids through the measurement of nuclear size in histological sections (Purdom, 1969; Arai et al., 1979). Similarly, the nuclear size of epidermal cells, obtained after severing the tail, has been used to identify sturgeons of various ploidy levels (Romashov et al., 1963; Vasetskii, 1967). Finally, triploid and gynogenetic diploid flatfish hybrids have been identified based on larval pigmentation (Purdom, 1972; Purdom and Lincoln, 1973, 1974; Purdom, 1976; Lincoln, 1981a, 1981b) and unspecified meristics (Purdom, 1972).

Effect of Triploidy on Cell Size

Since the classic study by Boveri (1905), numerous authors have demonstrated that the nuclear and cellular volume of animal cells is directly related to both their chromosome number (Fankhauser, 1941; Bŏŏk, 1945; Fankhauser, 1945a, 1945b; Briggs, 1947; Beatty and Fischberg, 1951; Fankhauser, 1952; Swarup, 1959b; Bachmann and Cowden, 1965; Epstein, 1967) and their DNA content (Commoner, 1964; McKinnell and Bachmann, 1965;

Bachmann and Cowden, 1967; Sullivan and Garcia, 1970; Pedersen, 1971; Fontana, 1976; Bachmann and Nishioka, 1978; Kuramoto, 1981). However, triploid individuals are generally no larger than diploids of the same age, due to a reduction in cell numbers (Fankhauser, 1941; Böök, 1945; Fankhauser, 1945a, 1945b; Briggs, 1947; Beatty and Fischberg, 1951; Fankhauser, 1952; Swarup, 1959b).

A characteristic increase in erythrocyte cellular and nuclear size associated with polyploidy has been documented in amphibians (Fankhauser, 1938; Fankhauser and Humphrey, 1942; Böök, 1945; Davison, 1957, 1959; Uzzell, 1963, 1964; Wilbur, 1976; Günther, 1977) and fish (Swarup, 1959b; Cherfas, 1966, 1969; Cimino, 1973; Sezaki et al., 1977; Liu et al., 1978; Sezaki and Kobayasi, 1978; Liu et al., 1980; Wolters et al., 1982a; Beck and Biggers, 1983; Sezaki et al., 1983). The increase in triploid erythrocyte size is mainly due to an increase in the length of both the cell and its nucleus, with a relatively smaller increase in their widths (Davison, 1957, 1959; Cherfas, 1966, 1969; Sezaki et al., 1977; Sezaki and Kobayasi, 1978; Wolters et al., 1982a, Beck and Biggers, 1983). Furthermore, there is apparently no increase in erythrocyte thickness associated with triploidy (Davison, 1957, 1959; Uzzell, 1964). Thus, although the erythrocyte nuclei of triploids are larger in all three dimensions, the cells themselves are only larger in two dimensions.

The only species of fish for which a detailed study has been conducted on cell size and number in the various organs is the three-spined stickleback (Swarup, 1959b). In addition to having larger erythrocytes, triploid stickleback have larger cartilage cells, cartilage cell nuclei, and brain cell nuclei than diploids. Furthermore, cell numbers are reduced in proportion to cell size in the hind brain, cartilagenous occipital arch, and pronephric duct. In amphibians, triploidy causes an increase in nuclear and cellular size, with a concomitant decrease in cell number, in the epidermis, cartilage, pronephric tubule, lens and retina, cerebral and spinal ganglia, sensory cells of the lateral line, and the lachrymal, lingual and abdominal glands (Fankhauser, 1938, 1941; Book, 1945; Fankhauser, 1945a, 1945b; Briggs, 1947; Fankhauser, 1952; Costello and Holmquist, 1957). The only exception to this general situation may be the notochord, but the evidence is not clear (Fankhauser, 1941; Swarup, 1959b).

Haematology of Triploids

Information on the haematology of triploid animals is restricted to triploid salamanders, Pleurodeles waltlii, produced by cold shock or by the crossing of tetraploid females with diploid males (Deparis and Beetschen, 1965; Deparis et al., 1966, 1975); spontaneously arising triploid chickens, Gallus domesticus (Abdel-Hameed, 1972); and

natural populations of the triploid goldfish, Carassius auratus langsdorfi (Sezaki et al., 1983).

In all three, triploids were found to have a greater mean erythrocyte volume (MCV) but a lower erythrocyte count than diploids. Haematocrit (i.e., packed cell volume) was the same for diploids and triploids, as was the haemoglobin content of whole blood. The mean corpuscular haemoglobin content (MCH) of triploid erythrocytes was higher than that of diploids, but mean corpuscular haemoglobin concentration (MCHC) was the same for diploids and triploids.

Abdel-Hameed (1972) suggested that the decrease in erythrocyte count observed in triploid chickens was due to a homeostatic mechanism ("canalization") acting to maintain the same haemoglobin concentration in the blood of triploids as is found in diploids. Such a homeostatic mechanism would be necessary only if triploid erythrocytes exhibit an increase in enzyme activity proportional to their increased gene content. This is the case in fruit flies, Drosophila melanogaster (Lucchesi and Rawls, 1973), and goldfish (Sezaki et al., 1983), but apparently not in salamanders (Audit et al., 1976). Complete homeostasic regulation of haemoglobin concentration is maintained in triploid chickens (Abdel-Hameed, 1972), salamanders (Deparis et al., 1975), and goldfish (Sezaki et al., 1983), but not in salamanders of higher ploidy (Deparis et al., 1975).

The rate of oxygen uptake by isolated erythrocytes is a function of cell thickness, haemoglobin concentration, velocity constant of the reaction, and diffusion coefficient of oxygen within the cytoplasm and across the cell membrane (Holland and Forster, 1966). Since diploid and triploid erythrocytes have the same thickness (Davison, 1957, 1959; Uzzell, 1964) and haemoglobin concentration (Deparis et al., 1966; Abdel-Hameed, 1972; Deparis et al., 1975; Sezaki et al., 1983), the rate of oxygen uptake by diploid and triploid erythrocytes should theoretically be the same. On the basis of haematology alone, one would therefore expect no difference in oxygen utilization between diploid and triploid fish, but other physiological differences could influence gas transport and exchange. Davison (1957, 1959) found that capillary diameter was the same in diploid and triploid newts, Triturus viridescens, suggesting that triploid erythrocytes undergo greater deformation when passing through them, thus increasing vascular resistance.

Oxygen Utilization by Triploids

The research by Swarup (1959c) on three-spined stickleback represents the only experimental work which has been conducted on oxygen consumption in triploid animals. Based on the decreased surface area to volume ratio of triploid erythrocytes, he suggested that triploids should have a lower rate of oxygen consumption. Unfortunately,

this hypothesis was invalid. Diploid and triploid fish have the same erythrocyte thickness and haematocrit, indicating that the total surface area of erythrocytes available for oxygen uptake must be the same. In subsequent experiments, Swarup found that there was no difference in oxygen consumption between diploids and triploids when calculated per unit body weight, but that triploids consumed less oxygen than diploids per unit surface area of the fish.

Under conditions of intensive culture in cages, fish are often inadvertently subjected to low oxygen levels (Stevenson, 1980; Randall et al., 1982; Soderberg, 1982). In addition to the direct threat of asphyxiation, such fish may be subjected to prolonged periods of stress leading to decreased growth and increased susceptibility to disease. It is therefore important to determine whether triploids will adapt to hypoxic conditions as well as diploids. The respiratory and circulatory responses of fish to hypoxia have recently been reviewed (Shelton, 1970; Hughes, 1973; Randall, 1982; Randall et al., 1982), but no information is available on how well triploid animals survive under hypoxic conditions.

Growth of Triploids

In juvenile fish of the same age, diploid and triploid individuals are generally the same size (Swarup, 1959b;

Cuellar and Uyeno, 1972; Purdom, 1972, 1976; Gold and Avise, 1976; Gervai et al., 1980b). The only reported exception is the blue tilapia, where polyploid individuals of uncertain ploidy were significantly larger than diploids at 14 weeks (Valenti, 1975). In the channel catfish, triploids had a better food conversion efficiency, and consequently were heavier than diploids at 8 months, by which time diploids had begun to mature (Wolters et al., 1982b).

Prior to maturation, the growth rate of diploid rainbow trout is generally greater than than that of triploids or tetraploids (Thorgaard and Gall, 1979; Refstie, 1981; Thorgaard et al., 1982). Only a single sterile triploid female was available to which the growth of maturing diploids could be compared (Thorgaard and Gall, 1979). This female continued to grow during the spawning period, whereas the diploid females ceased to grow with the onset of maturation. Two months prior to spawning, two diploid controls weighed 16% more than this triploid, but by one week after spawning the triploid female was 21% heavier than these diploids.

A similar pattern has been observed in flatfish, where diploids cease to grow in both length and weight several months prior to spawning, whereas triploids continue to grow during this period (Purdom, 1976; Lincoln, 1981c). Diploid

plaice, Pleuronectes platessa, x flounder, Platichthys flesus hybrids had a higher condition factor than triploids prior to spawning, but this dropped rapidly with spawning, and all fish had the same condition factor by the end of the spawning period. Percentage food conversion was the same for diploids and triploids over the entire pre-spawning and spawning period, indicating that sterile triploids were putting food energy into somatic growth, whereas diploids had diverted it to gonadal growth. This was supported by the observation that triploids had a significantly heavier fillet weight than diploids after the spawning period had ended. However, the diploids grew rapidly after spawning, and within two months they were equal to triploids in both length and weight (Lincoln, 1981c).

Maturation of Triploids

Research on the maturation of triploid amphibians has revealed that females are generally sterile, but males usually develop testes, and may even produce spermatozoa (Fankhauser, 1940, 1941; Kawamura, 1951a, 1951b; Smith, 1958). An apparent exception is the axolotl, Ambystoma mexicanum, in which triploid males are usually sterile, but triploid females frequently produce eggs (Humphrey and Fankhauser, 1949; Fankhauser and Humphrey, 1950, 1954). When the milt of a triploid is used to fertilize the eggs of a diploid, the offspring generally have aneuploid chromosome

numbers intermediate between the diploid and triploid chromosome number, and are characterized by reduced viability, abnormal development, and reduced growth (Humphrey and Fankhauser, 1949; Kawamura, 1951a, 1951b; Fankhauser and Humphrey, 1954). Similar results are usually obtained when the eggs of a triploid are fertilized with the milt of a diploid, but tetraploid offspring are occasionally produced due to the production of unreduced, triploid eggs by the triploid female (Humphrey and Fankhauser, 1949; Fankhauser and Humphrey, 1950).

This same sexual dimorphism with respect to maturation is apparent in triploid fish. Diploid and triploid testes were identical in histological appearance in juvenile plaice x flounder hybrids and rainbow trout, but whereas the ovaries of diploids were already packed with developing oocytes, those of the triploids were devoid of them (Purdom, 1972; Purdom and Lincoln, 1973; Lincoln and Scott, 1983; Yamazaki, 1983). In 20-month-old carp and 8-month-old channel catfish the difference in gonadal development between sexes was not as apparent: both triploid males and females showed signs of reduced gonad size relative to diploids (Gervai et al., 1980b; Wolters et al., 1982b).

In a study of 2-year-old rainbow trout, Thorgaard and Gall (1979) found that both diploid and triploid males had developed the secondary sexual characteristics associated

with steroidogenesis and sexual maturation, as had the diploid females. The single triploid female which was available did not show any secondary sexual characteristics. Examination of the gonads at the time when diploids were spawning revealed that triploid males had partially developed testes, but the females had undeveloped gonads.

In plaice and plaice x flounder hybrids, 3- and 4-year-old triploid females had a greatly reduced gonadosomatic index (GSI) in comparison with diploids, and none had ovulated by 5 years of age (Purdom, 1976; Lincoln, 1981b). However, in 3-year-old triploid males, the testes were found to be the same size as those of diploid males (Lincoln, 1981a). Abnormal spermatogenesis was observed in the testes of the triploid hybrids, yet normal spermatogenesis was apparently occurring in the testes of the triploid plaice. When eggs from normal diploids were fertilized with the milt of these triploid males, they developed to the gastrula stage, at which point mortality increased rapidly. Most embryos died prior to hatching, and none survived longer than 48 hours after hatching (Lincoln, 1981a). Although the chromosome numbers of these embryos were not determined, they were probably aneuploid, as observed when similar crosses were carried out in amphibians (Humphrey and Fankhauser, 1949; Kawamura, 1951a, 1951b; Fankhauser and Humphrey, 1954). Occasional oocytes were encountered in the triploid ovaries of both flatfish

(Lincoln, 1981b) and channel catfish (Wolters et al., 1982b), but it is not known whether they would have developed into eggs.

It is apparent that triploid males probably offer no advantage in aquaculture as regards avoiding the deleterious side-effects of maturation, but triploid females could be useful in this respect. All-female populations of fish can be produced by hormonal treatment (Chevassus et al., 1979a; Stanley, 1979, 1981; Donaldson and Hunter, 1982; Yamazaki, 1983). This technique has been combined with chromosome manipulation to produce all-female triploid rainbow trout (Lincoln and Scott, 1983). Such an approach will likely prove to be the most advantageous for aquacultural purposes.

Research Objectives

The objectives of this research were to develop efficient techniques for the production and early screening of triploid Atlantic salmon, and to evaluate the suitability of such fish for aquaculture. This thesis has been divided into separate chapters, each describing a specific set of experiments designed to meet the general objectives. Details of fish husbandry are not included, since this is beyond the scope of the thesis. The Marine Sciences Research Laboratory possesses facilities for the hatchery rearing of salmonids, but the reader should bear in mind

that rearing Atlantic salmon generally entails substantial losses of fish. In each experiment it was therefore necessary to use as small a number of fish as possible, so as to maintain sufficient numbers for subsequent experimentation.

2. INDUCTION OF TRIPLOIDY

Objectives

The Atlantic salmon is of great economic importance in aquaculture (Edwards, 1978; Sutterlin and Merrill, 1978; Sutterlin et al., 1981). Unfortunately, it has proven to be a difficult species in which to induce triploidy. Svärdson (1945) obtained two triploid individuals from 30 eggs subjected to cold shocks shortly after fertilization. Subsequent attempts by Lincoln et al. (1974) to prevent extrusion of the second polar body by means of cold shocks were unsuccessful. Cytochalasin B treatment was used to produce presumed mosaic polyploids, but no triploids were reported (Refstie et al., 1977; Allen and Stanley, 1979). Recently, Purdom (1983) reported the use of heat shocks to induce triploidy in Atlantic salmon, but the details of this research have not been published. The purpose of this experiment was to develop effective techniques for the production of triploid Atlantic salmon.

Three techniques for inducing triploidy were evaluated: heat shock, cytochalasin B treatment, and hydrostatic pressure shock. Heat shocks applied shortly after fertilization readily induce triploidy in rainbow trout (Chourrout, 1980; Thorgaard et al., 1981; Chourrout and Quillet, 1982; Lincoln and Scott, 1983) and Pacific salmon

(Utter et al., 1983), and have also been used to induce tetraploidy in rainbow trout (Thorgaard et al., 1981; Chourrout, 1982a). The use of cytochalasin B to induce polyploidy has been very successful in bivalves (Stanley et al., 1981; Allen et al., 1982), but has not proven effective in inducing triploidy or tetraploidy in salmonids (Refstie et al., 1977; Allen and Stanley, 1979; Refstie, 1981). Hydrostatic pressure has apparently been used to produce triploid rainbow trout (Chourrout, 1982b; Yamazaki, 1983), but the details of these experiments have not been reported. The application of hydrostatic pressure shocks shortly after fertilization readily induces triploidy in amphibians (Ferrier and Jaylet, 1978; Müller et al., 1978; Tompkins, 1978; Gillespie and Armstrong, 1979).

Four variables must be considered when developing a successful protocol for inducing triploidy: the preshock duration and temperature, and the duration and magnitude of the shock itself. The shock must be applied at the correct stage of meiotic development in order to effectively block extrusion of the second polar body, hence the importance of preshock duration and temperature. In rainbow trout eggs incubated at 10^{o}C, heat shocks applied within the first 45 minutes of fertilization are effective in blocking extrusion of the second polar body (Chourrout, 1980; Thorgaard et al., 1981; Chourrout, 1982c; Chourrout and Quillet, 1982; Lincoln and Scott, 1983). The exact time of polar body

extrusion in Atlantic salmon eggs has not been determined, but the initial rate of development of Atlantic salmon embryos is similar to that of rainbow trout (Battle, 1944; Knight, 1963; Vernier, 1969), and the protocol is therefore likely to be similar.

Materials and Methods

Heat Shock.

Separate heat shock experiments were conducted in the fall of 1981 and 1982. The eggs of 40 females and milt of about 10 males were used in the first experiment (HS1), and the gametes of 5 females and 4 males were used in the second experiment (HS2). The preshock incubation temperature was 6.5°C (±0.5°C) for HS1 and 10.0°C (±0.5°C) for HS2. The former represented ambient temperature of hatchery water at that date, whereas the latter was used as a basis of comparison with published methods for inducing triploidy in rainbow trout.

Heat shocks were conducted according to the scheme outlined in the first three columns of Tables 1 and 2. The preshock duration encompassed the period between fertilization of the eggs and administration of the shock. Eggs were thoroughly mixed by hand to ensure random distribution prior to the removal of subsamples for shocking. Shocks were administered by suspending the eggs

in a nylon mesh basket submerged in a water bath at the appropriate temperature. Control "shocks" were administered at 6.5°C in HS1 and 10.0°C in HS2 (each ± 0.5°C). An additional heat shock was conducted at 430 minutes after fertilization in HS1, in an attempt to induce tetraploidy by blocking the first mitotic cleavage of the embryos.

Cytochalasin B.

Cytochalasin B (Sigma Chemical Co.) was diluted to 10 mg/l in 2.5% dimethyl sulphoxide (DMSO, BDH Chemicals Ltd.), as suggested by Allen and Stanley (1979). Controls consisted of either 2.5% DMSO, or hatchery water alone. The eggs of 20 females and milt of 10 males were used. Both the pre-treatment and treatment temperatures were held at 7.0°C (±0.5°C). Treatment consisted of placing the eggs in a whirlpak plastic bag containing the appropriate solution. Half the eggs were treated from 15 minutes to 4 hours after fertilization, to induce triploidy through retention of the second polar body, and the other half from 4 to 15 hours after fertilization, to induce tetraploidy by inhibiting the first mitotic cleavage.

Hydrostatic Pressure Shock.

The design and use of the pressure chamber and hydraulic press were identical to that described by Dasgupta (1962), except that the chamber containing the eggs was filled with hatchery water at 10.0°C (±0.5°C). Due to the

asynchronous spawning of broodstock, experiments were carried out as eggs became available. Pressure shocks were always applied 15 minutes after fertilization and incubation at 10.0°C (±0.5°C), with the magnitude and duration of the shock being the two variables tested. In the first experiment (1 female x 5 males), 6 minute shocks were carried out at 3.5, 4.4, 5.3, 6.1 or 7.0×10^4 kPa (1kPa = 0.145 p.s.i.). In the second experiment (4 females x 10 males), shocks of 3, 6, 9, 12 or 15 minutes were applied at 7.0×10^4 kPa. In the third and fourth experiments (each 1 female x 4 males), the eggs were subjected to 6 minutes at 7.9, 8.8, 9.7 or 10.5×10^4 kPa.

Incubation and Rearing Conditions of Eggs.

All experimental groups were kept in separate baskets suspended in the same incubation system. Mortality was recorded daily, and all dead eggs or fish were removed. Due to space restrictions, all replicates from the HS1 and cytochalasin B experiments had to be combined after hatching before being screened to determine their ploidy. In the HS2 and hydrostatic pressure experiments, each replicate was kept separate and all surviving fish were sacrificed at the yolk-sac stage to determine their ploidy. The HS1 and cytochalasin B treated fish were reared for two years, using standard hatchery techniques (e.g., Leitritz and Lewis, 1980; Stevenson, 1980). These fish were used in subsequent experiments on the identification, haematology, oxygen

utilization, growth and gonadal development of triploid Atlantic salmon (Chapters 3 to 7).

Results

The use of heat shocks to induce triploidy was most effective when the shock was applied within 30 minutes of fertilization at a preshock temperature of 6.5°C (HS1; Table 1 and Figure 2), or within 15 minutes of fertilization at a preshock temperature of 10.0°C (HS2; Table 2 and Figure 3). Heat shocks applied at later times after fertilization yielded decreasing numbers of triploids. The most effective heat shock was found to be 5 minutes at 32.0°C, which yielded all-triploid groups in both HS1 and HS2. The survival to hatching of treated fish was generally lower than the survival to 24 hours, even when calculated relative to controls (i.e., control survival as 100%). The mortality within 24 hours of treatment was indicative of subsequent survival to hatch, as indicated by the parallel nature of the survival curves (Figures 2 and 3).

Cytochalasin B treatment yielded no tetraploids and only a few triploids (Table 3). No mosaic polyploids were observed. The survival of treated fish was generally lower than that of controls.

Hydrostatic pressure shocks of 3 or 6 minutes at

Table 1: Survival and ploidy of heat shocked fish (HS1).

preshock duration (min)	shock duration (min)	shock temp. (°C, ±0.5)	starting number of eggs	survival to 24 hr (%)	survival to hatch (%)	number sampled	% diploid	% triploid
15	1	36	527	34.7± 0.9	10.4± 2.3	12	25.0	75.0
15	5	32	501	29.7± 1.7	1.9± 1.0	3	33.3	66.7
15	10	28	492	51.9± 6.8	15.1± 3.6	21	76.2	23.8
30	1	36	463	44.1± 3.2	21.4± 1.8	18	88.9	11.1
30	5	32	432	36.2± 3.2	7.8± 4.2	4	0.0	100.0
30	10	28	450	59.9± 1.4	19.1± 6.7	23	87.0	13.0
45	1	36	409	34.5± 0.7	18.3± 2.3	22	100.0	0.0
45	5	32	389	38.5± 2.4	12.6± 2.7	19	57.9	42.1
45	10	28	407	60.8± 3.4	24.2± 4.0	25	92.0	8.0
60	1	36	402	47.0± 4.1	26.9± 2.5	31	96.8	3.2
60	5	32	395	14.2± 2.7	0.0	0	-	-
60	10	28	400	59.0± 0.8	17.3± 1.0	24	100.0	0.0
	control		387	95.1± 0.1	41.1±41.5	0	-	-
	control		403	97.3± 2.1	45.7±23.7	0	-	-
430	1	36	397	72.9± 7.8	19.9± 2.7	15	100.0	0.0
430	5	32	418	5.7± 1.2	0.0	0	-	-
430	10	28	388	51.2±15.6	14.0± 4.3	16	100.0	0.0
	control		390	89.7± 8.6	39.3±12.6	0	-	-
	control		414	96.4± 1.8	40.9± 4.3	0	-	-

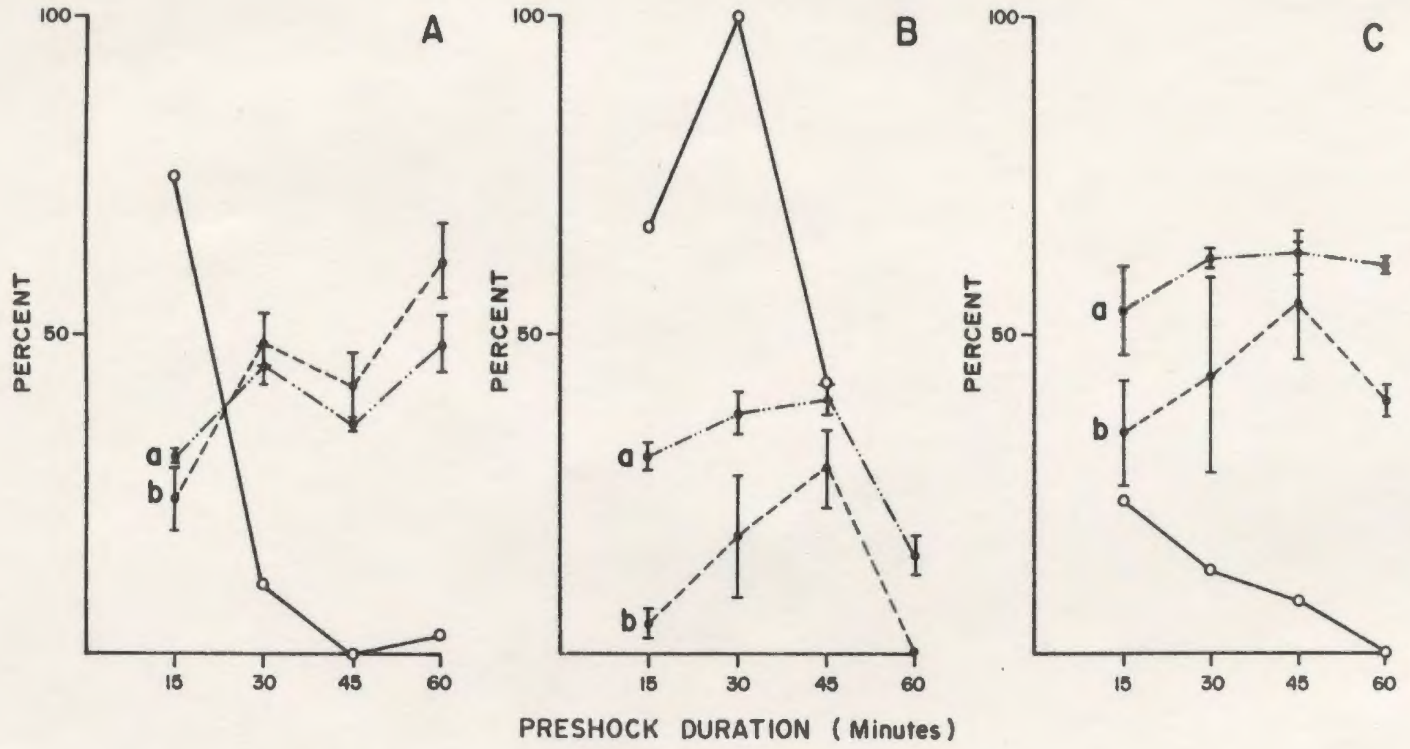

Figure 2: Relative survival and incidence of triploidy in heat shocked fish (HS1) (Heat shocks: A = 1 min at 36°C, B = 5 min at 32°C, C = 10 min at 28°C; a = survival to 24 hr, b = survival to hatching, open circle = triploidy; bars represent standard deviations, 3 replicates in each case).

Table 2: Survival and ploidy of heat shocked fish (HS2).

preshock duration (min)	shock duration (min)	shock temp. (°C, ±0.5)	starting number of eggs	survival to 24 hr (%)	survival to hatch (%)	number sampled	% diploid	% triploid
5	5	32	124	70.2	12.1	9	0.0	100.0
5	5	32	99	71.7	7.1	5	0.0	100.0
	control		105	100.0	36.2	30	100.0	0.0
15	5	32	100	92.0	40.0	30	0.0	100.0
15	5	32	77	96.1	27.3	13	0.0	100.0
	control		81	100.0	43.2	28	96.4	3.6
25	5	32	82	98.8	23.2	9	66.7	33.3
25	5	32	91	100.0	33.0	16	81.3	18.8
	control		62	100.0	33.9	19	89.5	10.5
35	5	32	105	99.0	30.5	25	76.0	24.0
35	5	32	70	100.0	27.1	12	91.7	8.3
	control		65	100.0	23.0	14	92.9	7.1
45	5	32	86	100.0	14.0	9	100.0	0.0
45	5	32	84	100.0	26.2	16	87.5	12.5
	control		68	100.0	25.0	8	100.0	0.0

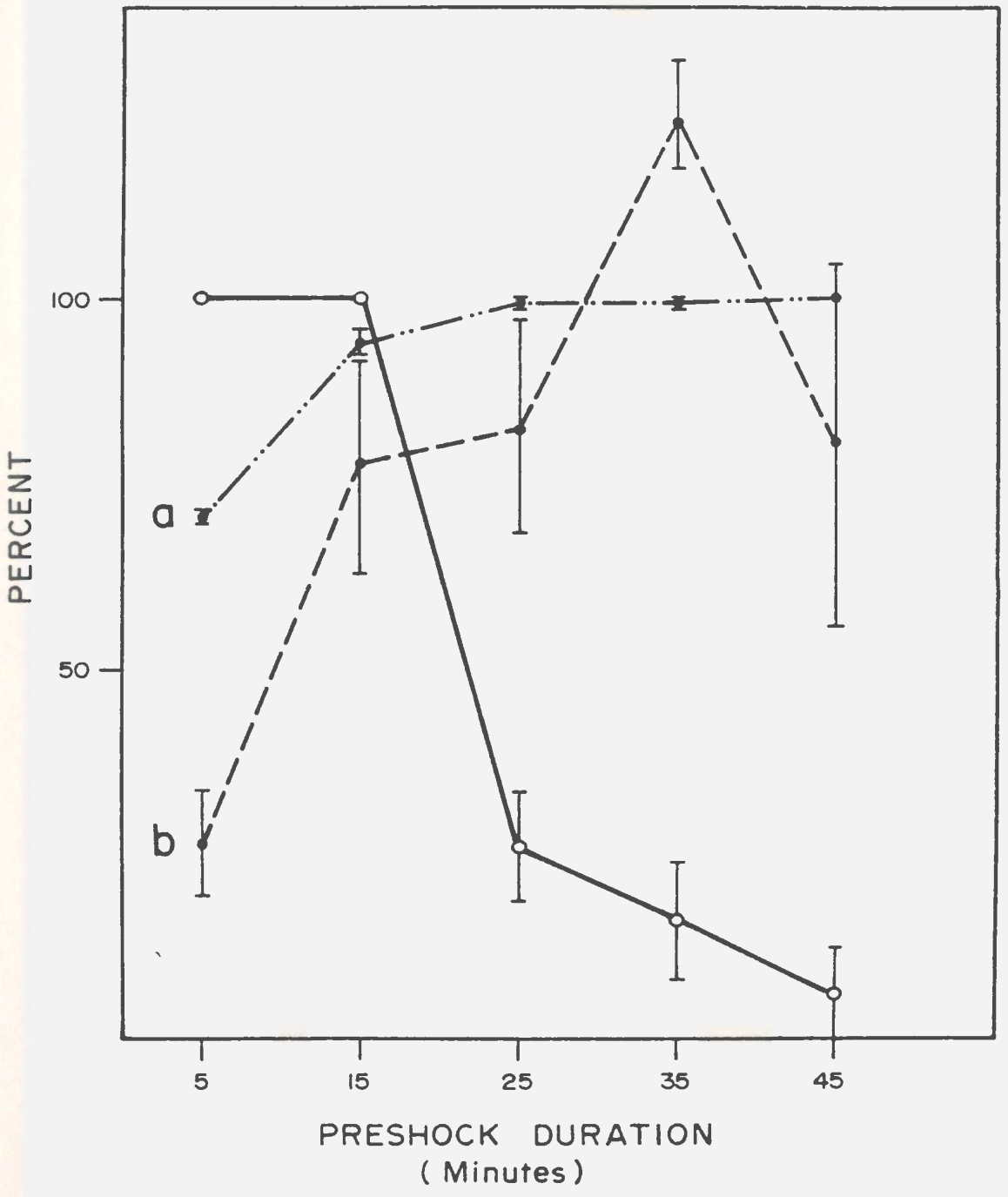

Figure 3: Relative survival and incidence of triploidy in heat shocked fish (HS2) (a = survival to 24 hr, b = survival to hatching, open circle = triploidy; bars represent ranges, 2 replicates in each case).

Table 3: Survival and ploidy of cytochalasin B treated fish.

preshock duration (hr)	shock duration (hr)	starting number of eggs	survival to 24 hr (%)	survival to hatch (%)	number sampled	% diploid	% triploid
0.25	3.75	589	54.5± 7.7	20.6±18.8	44	95.5	4.5
DMSO	control	576	93.1± 2.4	22.5±29.8	0	-	-
water	control	569	94.0± 1.8	17.0±20.1	0	-	-
4.0	11.0	460	71.1± 2.8	23.5± 4.1	51	98.0	2.0
DMSO	control	456	86.6± 6.1	39.2±10.5	0	-	-
water	control	480	92.5± 2.7	37.8± 9.8	0	-	-

Table 4: Survival and ploidy of hydrostatic pressure shocked fish.

shock duration (min)	shock pressure ($X10^4$ kPa)	starting number of eggs	survival to 24 hr (%)	survival to hatch (%)	number sampled	% diploid	% triploid
6	3.5	75	93.3	4.0	0	-	-
6	4.4	68	97.1	0.0	0	-	-
6	5.3	48	93.8	0.0	0	-	-
6	6.1	49	98.0	0.0	0	-	-
6	7.0	40	92.5	0.0	0	-	-
control		15	93.3	0.0	0	-	-
3	7.0	101	93.1	27.7	21	0.0	100.0
6	7.0	92	100.0	30.4	18	0.0	100.0
9	7.0	74	97.3	1.4	0	-	-
12	7.0	90	97.8	0.0	0	-	-
15	7.0	96	95.8	0.0	0	-	-
control		67	100.0	34.3	3	100.0	0.0
6	7.9	92	94.6	0.0	0	-	-
6	8.8	90	94.4	0.0	0	-	-
6	9.7	114	64.0	0.0	0	-	-
6	10.5	94	29.8	0.0	0	-	-
control		100	98.0	33.0	12	91.7	8.3
6	9.7	127	100.0	0.0	0	-	-
6	9.7	138	100.0	0.0	0	-	-
control		149	100.0	34.2	12	100.0	0.0

7.0×10^4 kPa, applied at 15 minutes after fertilization and incubation at 10°C, yielded all-triploid groups, with 81 and 89% relative survival to hatch, respectively (Table 4). Pressure shocks of longer duration (9 to 15 minutes at 7.0×10^4 kPa), or higher magnitude (6 minutes at 7.9 to 10.5×10^4 kPa) resulted in 100% mortality prior to hatching. In the experiment conducted at lower pressures, all the treatment and control eggs died before hatching, which indicates that the eggs were of inferior quality. It is therefore not possible to report on the effectiveness of lower pressures to induce triploidy. Survival to 24 hours of pressure-treated eggs was generally good, and gave no indication of subsequent survival to hatch.

The average survival to hatching of control eggs was 34.1% (±8.2% SD), which is typical for this stock of Atlantic salmon when maintained in captivity (A.M. Sutterlin, pers. comm.). A low incidence of triploidy was observed in these controls (5 out of 126 fish, representing 4.0% of all those sampled).

Discussion

All-triploid groups of Atlantic salmon were produced by means of either heat shocks (5 minutes at 32.0°C) or hydrostatic pressure shocks (3 or 6 minutes at 7.0×10^4 kPa) applied within 15 minutes of fertilization at

a preshock temperature of 10.0°C. This is the first time that the induction of triploidy with 100% success has been reported in this species. Furthermore, at the time that these experiments were conceived, planned and initiated, there were no published reports dealing with the use of hydrostatic pressure to produce triploid fish. Other authors have since reported the induction of triploidy in rainbow trout by means of hydrostatic pressure shock, but specific details of the technique and its effectiveness are lacking (Chourrout, 1982b; Yamazaki, 1983).

The only other salmonid species in which the production of all-triploid groups has been reported is the rainbow trout. In this species, heat shocks of 20 minutes at 26°C, applied 25 minutes after fertilization and incubation at 10°C, yielded all-triploid groups with survival to hatching slightly better than that of controls (Chourrout and Quillet, 1982). Heat shocks of 10 or 15 minutes at 27°C or 28°C, applied at various times within the first 45 minutes after fertilization and incubation at 10°C, also yielded all-triploid groups (Lincoln and Scott, 1983). In the latter case, mortality of treated and control eggs was not recorded, but it was reported that the survival to hatch of treated eggs was lower than that of controls.

The data presented in the present study indicate that all-triploid groups of Atlantic salmon can be produced with

80 to 90% relative survival to hatch, by means of either heat or hydrostatic pressure shocks. With further refinement of these techniques, it may be possible to produce all-triploid groups with survival rates identical to that of controls.

The optimum time to apply heat shocks to prevent extrusion of the second polar body in Atlantic salmon was found to be temperature dependent, and restricted to the time intervals of 15 or 30 minutes after fertilization at preshock temperatures of 10.0°C or 6.5°C, respectively. In rainbow trout, heat shocks applied as late as 45 minutes after fertilization at a preshock temperature of 10.0°C are effective in blocking extrusion of the second polar body (Lincoln and Scott, 1983). This indicates that polar body formation proceeds more rapidly in Atlantic salmon than it does in rainbow trout, making the duration of the preshock interval for such experiments more critical in Atlantic salmon. By using lower temperatures during the preshock interval, it may be possible to prolong the effective period of shock.

The induction of tetraploidy by either heat shock or cytochalasin B treatment was unsuccessful. It is possible that tetraploids were produced, but died before the alevins were examined. Tetraploid rainbow trout have been produced by heat shocks, but were generally deformed and did not

survive long after hatching (Thorgaard et al., 1981; Chourrout, 1982a; Thorgaard et al., 1982). No mosaic polyploid individuals were found among the cytochalasin B treated fish, although similar treatments have apparently yielded mosaic polyploid and tetraploid salmonids (Refstie et al., 1977; Allen and Stanley, 1979; Refstie, 1981). Three triploid individuals were found, representing 3.2% of the fish which received cytochalasin B treatment. The production of triploids cannot be attributed to this treatment, since 4.0% of the controls were also found to be triploids. Triploids may have been inadvertently transferred to the control groups during routine removal of dead eggs and alevins. Alternatively, these triploids may have arisen spontaneously, a phenomenon known to occur in salmonid hatcheries (Cuellar and Uyeno, 1972; Grammeltvedt, 1974; Allen and Stanley, 1978; Thorgaard and Gall, 1979; Thorgaard et al., 1982; Utter et al., 1983).

The data presented here indicate that hydrostatic pressure treatment was the most effective technique for inducing triploidy in Atlantic salmon. The same conclusion has been reached by researchers working on the induction of triploidy in amphibians; they found that hydrostatic pressure treatment was less harmful to the eggs than heat shock (Müller et al., 1978; Gillespie and Armstrong, 1979). However, heat shocking may be a more practical technique, as it requires no specialized equipment and can accommodate

larger batches of eggs. The large-scale production of triploid Atlantic salmon by means of heat shocks would appear feasible at the commercial level.

3. IDENTIFICATION OF TRIPLOIDS

Objectives

Since the induction of triploidy in fish is rarely completely successful, techniques are required for the rapid screening of triploid individuals. Karyotyping is impractical for this purpose, as it is time-consuming, inconsistent, and often requires sacrifice of the fish (Blaxhall, 1975; Gold, 1979). The quantification of cellular DNA content by either microspectrophotometry (e.g., Rasch et al., 1965, 1970; Cimino, 1974) or flow cytometry (Thorgaard et al., 1982; Allen, 1983; Allen and Stanley, 1983; Utter et al., 1983), is a viable alternative, but these techniques require equipment which may not be readily available.

The dimensions of the erythrocyte cell and nucleus are related to ploidy in teleost fish, and several authors have used measurements of erythrocyte nuclear volume, obtained from blood smears, as the sole criterion to identify polyploids (Valenti, 1975; Allen and Stanley, 1978, 1979; Meriwether, 1980; Refstie, 1981; Refstie et al., 1982; Lincoln and Scott, 1983). This technique requires no special equipment or expertise, making it ideal for field work, or for use by commercial fish farmers. However, the validity of its use to identify polyploid salmonids

correctly has been questioned (Thorgaard and Gall, 1979; Lemoine and Smith, 1980; Thorgaard et al., 1982; Wolters et al., 1982a; Utter et al., 1983). Of particular concern is the accuracy of this method for correctly identifying mosaic polyploids and tetraploids.

As an alternative, erythrocyte volume can be measured by means of an electronic particle-sizing instrument, such as a Coulter Counter. Such instruments are regularly used for this purpose in clinical haematology (Wintrobe et al., 1974), but have never been used to identify triploid animals. In the Coulter Counter, cells are drawn singly through a 100 μm aperture, the opening of which is spanned by two platinum electrodes (Figure 4). The cells are poorer electrical conductors than the saline solution in which they are diluted, and therefore produce changes in impedance proportional to their size as they pass through the aperture (Wintrobe et al., 1974). The resulting electrical impulses are counted by the Coulter Counter and accumulated into set interval sizes by the Coulter Channelyzer. The cell size distribution can then be plotted on a chart recorder.

The purpose of the research described in this chapter was to develop an effective technique by which the Coulter Counter and Channelyzer could be used to identify triploid fish. Karyotyping and microspectrophotometry were to be used to confirm triploidy. In addition, the accuracy of

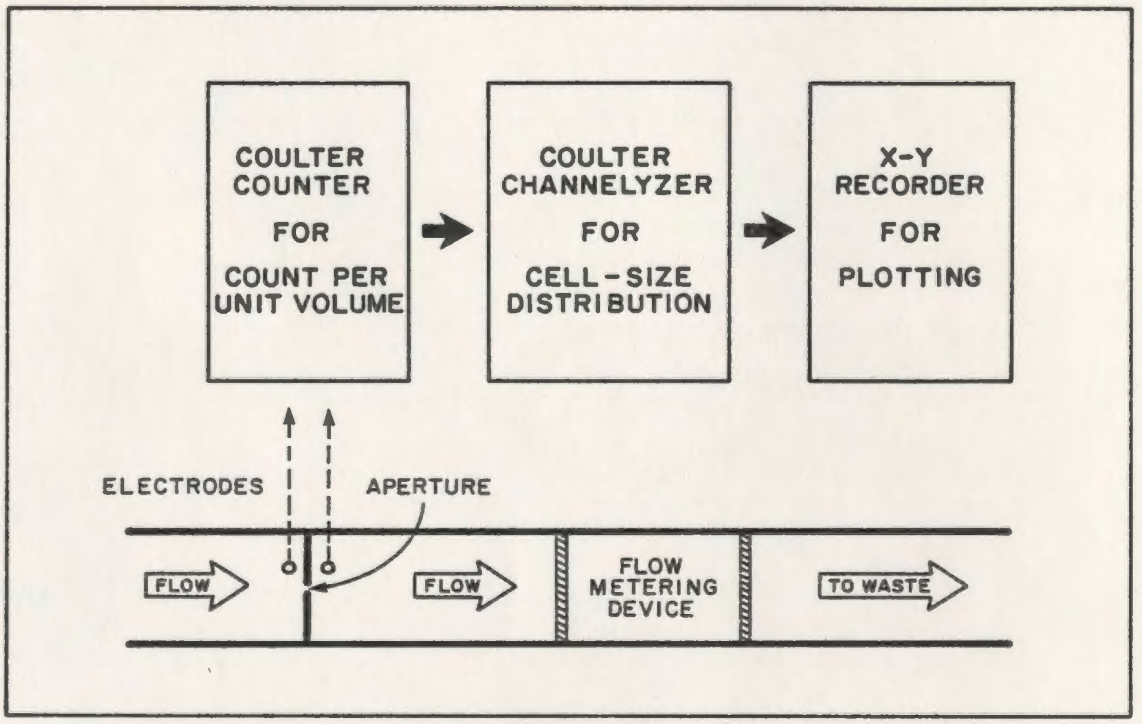

Figure 4: Schematic diagram of the Coulter Counter and Channelyzer (after Wintrobe et al., 1974).

correctly identifying triploid fish by means of erythrocyte dimensions obtained from blood smears was to be evaluated.

Materials and Methods

Karyotyping.

The technique used for karyotyping fish followed that described by Kligerman and Bloom (1977). Live fish were held for 18 hours at ambient temperature in an aerated jar containing 1.25 mM colchicine solution made up in hatchery water. After pithing the fish, its gills were dissected out, placed into a hypotonic solution of 0.4% KCl for 1 hour, blotted on tissue paper, and placed in Carnoy's fixative (3 parts absolute ethanol : 1 part glacial acetic acid) for 15 minutes. A cell suspension was prepared by adding about 7 drops of 50% acetic acid to the gills and gently mincing them for 1 minute with a scalpel. This suspension was drawn up in a microhaematocrit capillary tube, and 3 or 4 drops were delivered from a height of about 20 cms onto a microscope slide heated to 47°C. After air drying, the slides were stained for 30 minutes in 4% Giemsa stain (Humason, 1972) made up in 0.01M phosphate buffer, rinsed briefly with distilled water, and allowed to air dry. The preparations were mounted in Permount beneath a cover slip, and chromosome spreads were located under the microscope, using oil immersion.

Microspectrophotometry.

Preparation of the slides for microspectrophotometry was based on the techniques used by Rasch et al. (1970) and Cimino (1974). A blood smear was prepared from each experimental fish on one half of a microscope slide, and a reference smear (common to all slides) from a single adult fish was placed on the other half of each slide. After air-drying, the cells were fixed for 20 minutes in Carnoy's fixative, hydrolyzed for 45 minutes in 5N HCl at 23°C, and stained for 2 hours in the dark in de Tomasi's Schiff's Reagent (Pearse, 1968). On each slide, the relative DNA contents of 25 nuclei per experimental fish and 25 nuclei per reference fish were determined using a Zeiss microspectrophotometer (Carl Zeiss, Oberkochen, West Germany). Transmittance was measured by the two-wavelength procedure. This technique, and the theory behind it, has been the subject of several reviews (e.g., Rasch and Rasch, 1970; Berlyn and Cecich, 1976; Van Oostveldt and Boeken, 1976). Absolute DNA content was calculated using a diploid DNA content of 7.52 pg/cell for Atlantic salmon, based on the data of Schmidtke et al. (1976a, 1976b).

Coulter Counter Analysis

The reproducibility of channelyzed sizing in Coulter Counter analysis necessitates that particle size, specifically erythrocyte volume in this study, remains constant from the time that the sample is taken until it is

analyzed. The application of Coulter Counter sizing therefore required development of a heparinized saline solution isosmotic with the blood of Atlantic salmon. The original choice was the saline used by Holmes and Stott for the blood of cutthroat trout, Salmo clarkii, having a freezing point depression of 0.58°C (Lockwood, 1961). However, it was found that Atlantic salmon erythrocytes swelled when diluted in this saline. A DigiMatic Model 3 Osmometer (Advanced Instruments Inc., Needham Heights, Massachusetts) was therefore used to measure the freezing point depression of the blood plasma of 5 adult Atlantic salmon, and of triplicate samples of Holmes and Stott saline to which had been added 0, 1, 2, 3, 4 or 5% sucrose (w/v). These data were then used to determine the optimum concentration of sucrose required to make the saline isosmotic with the blood of Atlantic salmon. Sodium heparin (Sigma Chemical Co., St. Louis, Missouri) was added to this saline at a concentration of 100 U.S.P. standard units/ml, to act as anticoagulant. The saline was filtered through a glass microfibre filter (Whatman GF/F) to remove contaminants and undissolved solutes.

Blood samples were withdrawn from the caudal vein of unanaesthetized fish using a heparinized 10 µl syringe (Hamilton Co., Reno, Nevada) fitted with a 30 gauge needle, and diluted to 1:20,000 in the modified Holmes and Stott saline. Erythrocyte volume was determined from the cells in

this sample, using a Coulter Counter Model Z_B and Coulter Channelyzer II (Coulter Electronics Inc., Hialeah, Florida). The channelyzer was used to accumulate individual erythrocyte volumes into 5.2 μm^3 intervals. Sizing was ended when the peak interval contained 10,000 cells, or when the sample had been used up. In either case, the individual volumes of 150,000 to 200,000 erythrocytes were determined for each fish. The channelyzer integrated the area of the erythrocyte volume-frequency curve, thereby giving median erythrocyte volume for that fish. This entire procedure was completed in under 5 minutes per fish.

Erythrocyte Dimensions

Blood smears were obtained from experimental fish, stained for 15 minutes with Wright's stain (Humason, 1972), rinsed with distilled water, and air dried. The outlines of 50 erythrocytes and their nuclei were traced from each blood smear using a Zeiss microscope and camera lucida. Observations of erythrocyte wet mounts made in this study and SEM photographs of rainbow trout erythrocytes presented by Yamamoto and Iuchi (1975) indicated that salmonid erythrocytes are the shape of elliptical discs. Erythrocyte surface area was therefore calculated as πab, where a and b are the major and minor semi-axes of the cell, respectively. It has been suggested that erythrocyte nuclei are the shape of oblate spheroids (Valenti, 1975; Allen and Stanley, 1978, 1979; Wolters et al., 1982a; Beck and Biggers,

1983), and nuclear volume was therefore calculated as $4/3(\pi ab^2)$, where a and b are the major and minor semi-axes of the nucleus, respectively. Discriminant function analysis was used (Statistical Analysis System; SAS Institute Inc., 1982) to determine whether each of the 6 parameters (i.e., cell major axis, minor axis and surface area, and nucleus major axis, minor axis and volume) could be used to distinguish between diploid and triploid fish.

Results

The results obtained for karyotyping were very inconsistent, with the identical treatment yielding numerous resolvable chromosome spreads in one individual and none in the next. Since it was not possible, despite some considerable degree of effort, to obtain reproducible results in this study by means of karyotyping, this technique was not used to identify triploid fish. An example of one of the better chromosome spreads obtained is presented in Figure 5.

The use of the osmometer revealed that the freezing point depression of Holmes and Stott saline, as described by Lockwood (1961), was 0.51°C. The mean freezing point depression of the 5 samples of Atlantic salmon blood was found to be 0.58°C (±0.02°C, SD), identical to that of cutthroat trout. The addition of 1.34% sucrose (w/v) to the

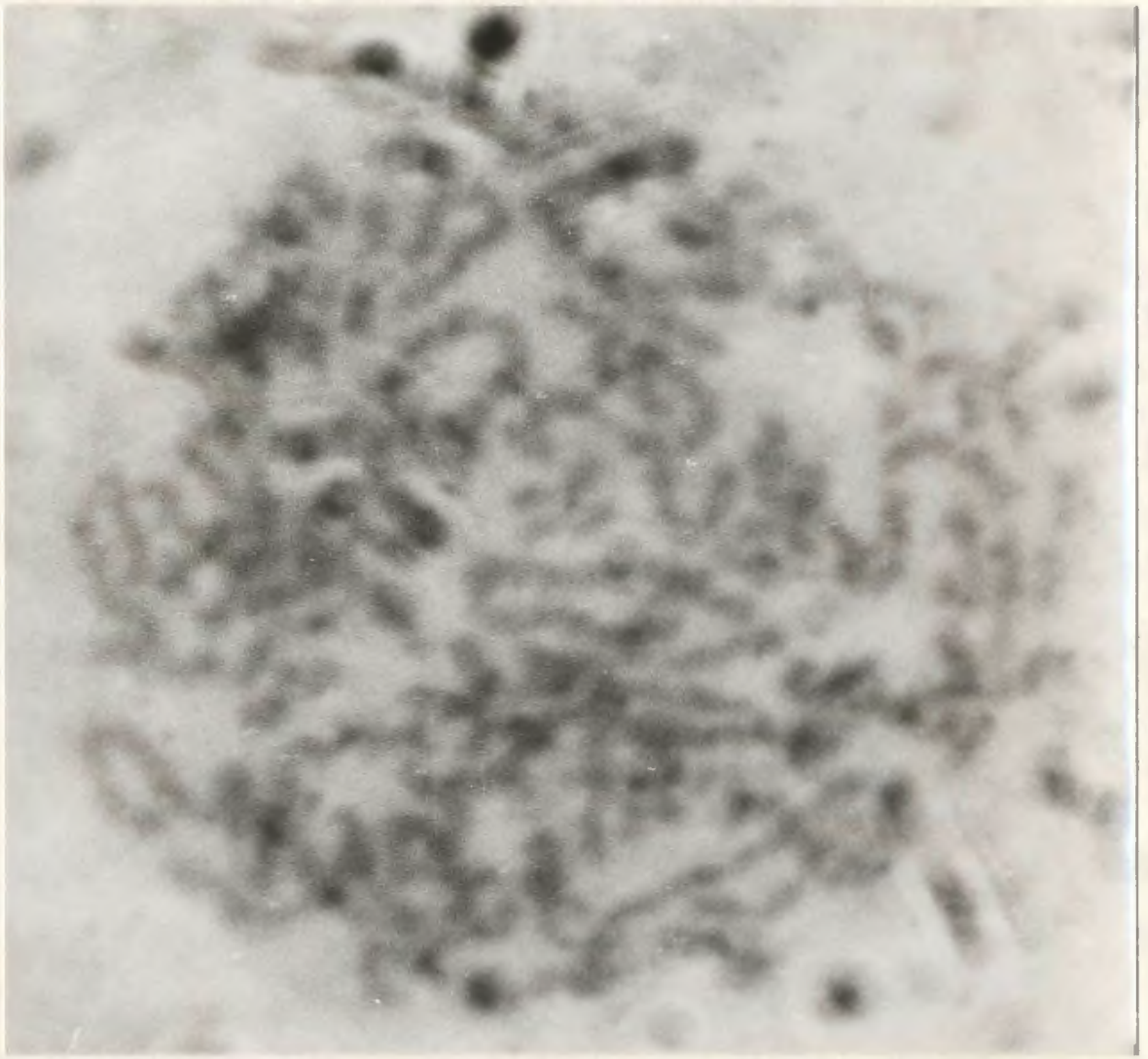

Figure 5: Diploid karyotype of an Atlantic salmon alevin.

Holmes and Stott saline increased the osmolality sufficiently to make it isosmotic with the blood of Atlantic salmon.

The results of the various erythrocyte measurements made have been summarized in Table 5. Although the two groups could be clearly distinguished on the basis of DNA content, the ratio of diploid to triploid DNA content was 1:2.2, rather than the expected 1:1.5 ratio. Analysis by the Coulter Counter revealed that the erythrocyte size distributions of diploid and triploid fish were very uniform (Figure 6), and that the median erythrocyte volume of the triploids was 1.38 times that of the diploids.

Of the 6 variables measured from the blood smears, all but cell minor axis could be used by discriminant analysis to classify correctly all 20 fish as diploid or triploid. The use of cell minor axis resulted in the misclassification of one diploid fish as a triploid. The increase in size associated with triploidy was found to be highly significant ($P<0.001$) for each of these 6 erythrocyte dimensions, but it is apparent that the cell and nucleus minor axes are the poorest predictors of ploidy.

Table 5: DNA content and dimensions of diploid and triploid erythrocytes (mean ± 95% confidence limits; n=10 in all cases; % correct classification based on discriminant function analysis).

	diploid	triploid	F*	% correct classification
DNA content (pg/cell)	6.56 ± 0.84	14.63 ± 3.23	22.5	
median erythrocyte volume (μm^3)	190.8 ± 5.7	263.1 ± 5.9	297	
cell major axis (μm)	14.73 ± 0.31	18.28 ± 0.27	283	100
cell minor axis (μm)	8.84 ± 0.15	9.68 ± 0.09	90.8	95
cell surface area (μm^2)	102.4 ± 3.3	139.0 ± 2.6	286	100
nucleus major axis (μm)	6.65 ± 0.13	8.40 ± 0.14	329	100
nucleus minor axis (μm)	3.60 ± 0.05	4.01 ± 0.05	124	100
nucleus volume (μm^3)	45.8 ± 1.8	71.5 ± 2.9	224	100

* $P < 0.001$ in all cases.

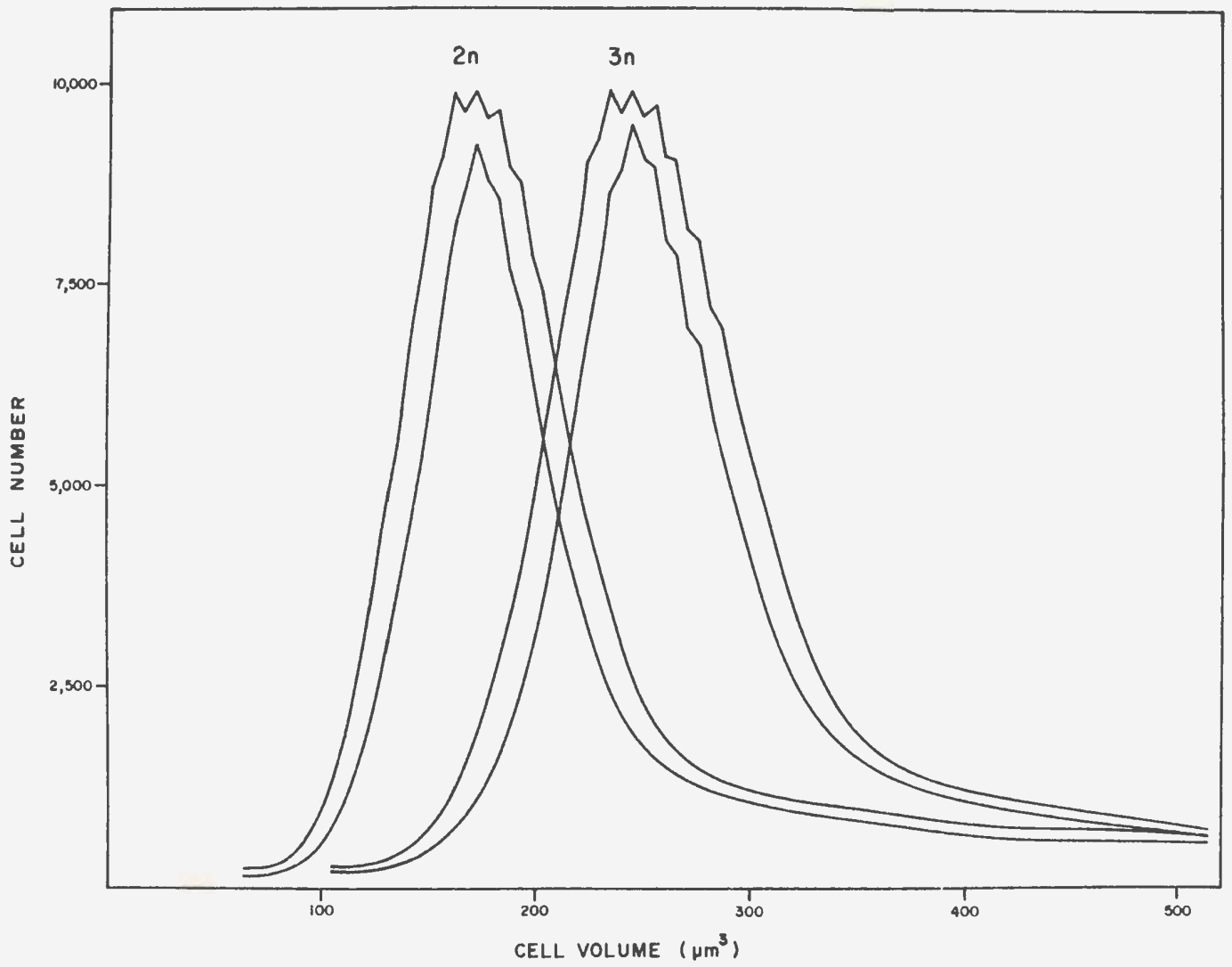

Figure 6: Size distribution of diploid and triploid erythrocytes, obtained through Coulter Counter analysis (plotted as 95% confidence limits about the mean).

Discussion

In this study microspectrophotometry was used to measure the DNA content of Feulgen-stained erythrocytes of diploid and triploid Atlantic salmon. This is the first time that this technique has been used to identify triploid salmonids, but it is regularly used to identify triploid poeciliids (e.g., Rasch et al., 1965, 1970; Cimino, 1974), and has also been used to screen experimentally produced triploid and gynogenetic diploid cyprinids (Nagy et al., 1978; Gervai et al., 1980a, 1980b) and triploid flatfish (Lincoln, 1981a, 1981b). The value for triploid DNA content obtained by this method was more than double the diploid value, whereas only a 50% increase was expected. Nevertheless, it is reasonable to assume that these fish were triploid, based on the general non-viability of both aneuploids (Gervai et al., 1980a; Lincoln, 1981a) and tetraploids (Thorgaard et al., 1981; Chourrout, 1982a; Thorgaard et al., 1982). The cytoplasm of Feulgen-stained erythrocytes was noticeably darker in triploids than in diploids, and this may have caused a decrease in transmittance through the triploid nuclei greater than that due solely to the increase in DNA content. Previous studies employing microspectrophotometry to screen experimentally-produced triploids (i.e., Gervai et al., 1980b; Lincoln, 1981a, 1981b) have failed to report the ratio of diploid to triploid DNA content, and the data from

the present study can therefore not be compared. Further substantiation of the triploid status of these fish was obtained upon examination of their gonads (Chapter 7).

The use of a Coulter Counter and Channelyzer to measure erythrocyte volume proved to be a rapid and accurate method for the identification of triploids, and this technique was routinely used to screen experimental fish. Regardless of their age, there was no overlap in median erythrocyte volume between diploid and triploid fish (Figure 7). The separation of the two groups became better as the fish became older, perhaps a reflection of the facility with which an uncontaminated blood sample can be withdrawn from a fish as it becomes larger. Coulter Counter analysis is comparable to flow cytometry for both speed and the amount of data that can be collected for an individual fish.

The effect of ploidy on each of the six erythrocyte dimensions measured from the blood smears was found to be highly significant, but the cell and nucleus minor axes were the poorest predictors of ploidy. This can be related to the fact that the increase in erythrocyte cell and nucleus volume associated with triploidy is mainly the result of an increase in length (Cherfas, 1966, 1969; Sezaki et al., 1977; Wolters et al., 1982a; Beck and Biggers, 1983). Previous studies have demonstrated that the identification of triploid fish can be based solely on the measure of the

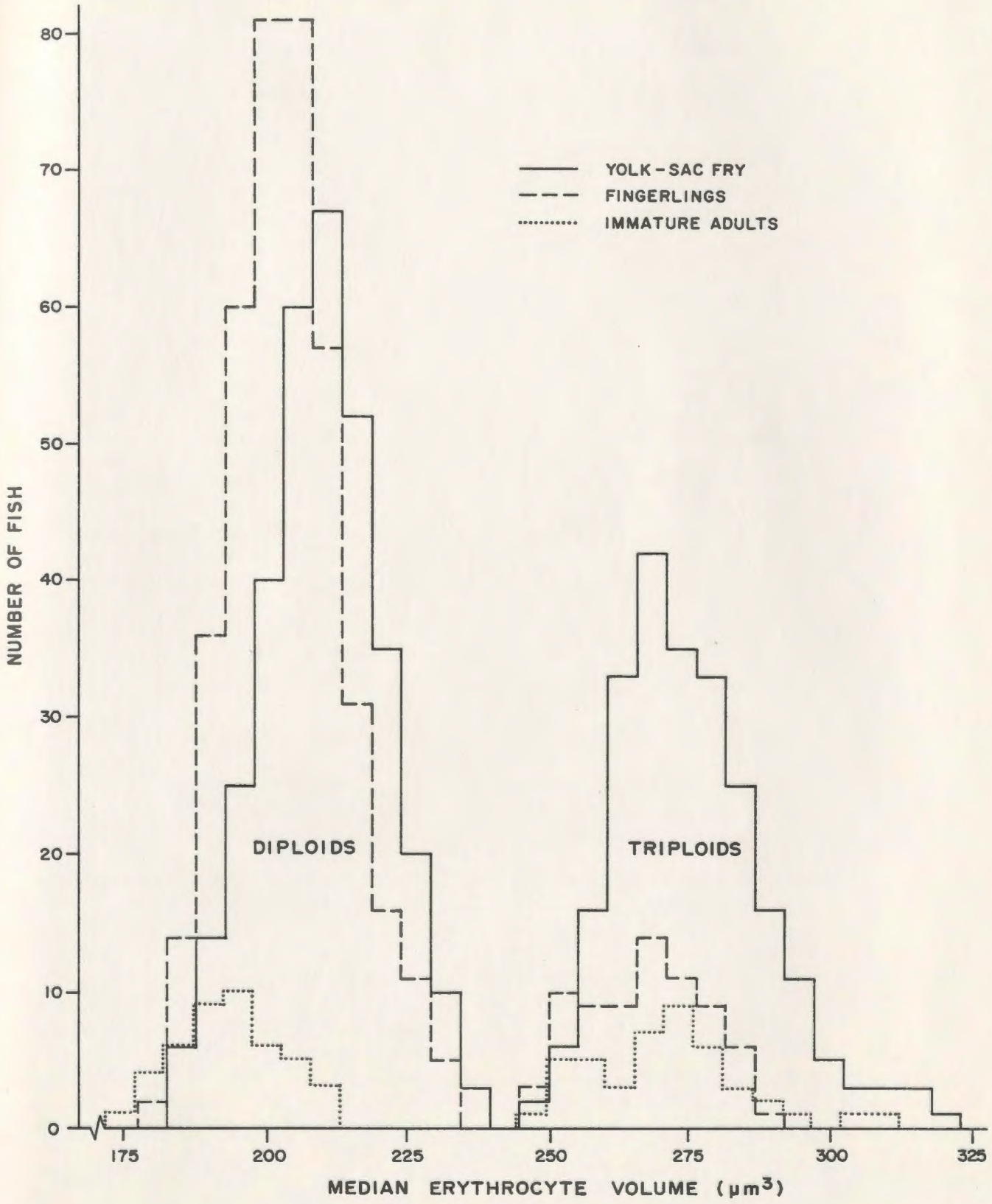

Figure 7: Median erythrocyte volume of diploid and triploid fish at various stages of development.

major axis of either the cell or its nucleus (Cimino, 1973; Sezaki et al., 1977; Liu et al., 1978; Wolters et al., 1982a; Beck and Biggers, 1983), and the data presented here for Atlantic salmon support this. The calculation of cell surface area or nuclear volume, which necessitates the measurement of a second axis, does not increase the accuracy of identifying triploids.

In the course of screening several hundred fish by sizing erythrocytes with the Coulter Counter, no conclusive evidence of mosaic polyploidy was obtained. Mosaic polyploid salmonids have been described as arising spontaneously (Allen and Stanley, 1978) or by chemical treatment with cytochalasin B (Refstie et al., 1977; Allen and Stanley, 1979) or colchicine (Lemoine and Smith, 1979). The identification of such fish as mosaic polyploids was based on a small number of chromosome counts or nuclear volume measurements. In the present study, bimodal erythrocyte size distributions were occasionally encountered in yolk-sac fry, but there is no reason to suppose that such fish were mosaic polyploids. Examination of the blood of one such fish revealed binucleated erythrocytes which were apparently undergoing amitotic division in the peripheral blood. No such bimodal erythrocyte size distributions were encountered in fish which survived beyond yolk absorption. The use of flow cytometry to screen triploid salmonids has also failed to support the existence of mosaic polyploids

(Thorgaard et al., 1982; Allen, 1983; Utter et al., 1983). In fact, when the Atlantic salmon originally described as mosaic polyploids by Allen and Stanley (1979) were reevaluated several years later by means of flow cytometry, no such mosaic polyploids were found (Allen, 1983). This leads one to question the accuracy of using a small number of measurements (frequently less than 10), either by karyotyping or from blood smears, to identify mosaic polyploid salmonids.

4. HAEMATOLOGY OF TRIPLOIDS

Objectives

In the previous chapter it was demonstrated that the erythrocytes of triploid Atlantic salmon are larger than those of diploids. Although there is abundant evidence that this is a characteristic feature of triploid animals in general, little information is available regarding the influence this may have on the oxygen utilization capabilities of such animals. The purpose of the research described in this chapter was to examine in greater detail the effects of triploidy on erythrocyte dimensions, and to determine what influence this has on the haematology of triploid Atlantic salmon. Such information can be used to make predictions of possible changes in oxygen utilization arising from induced triploidy.

Materials and Methods

Ten diploid and ten triploid fingerlings (12 months post-hatch) were used in this experiment. A single blood sample was withdrawn from the caudal vein of each unanaesthetized fish using a 10 µl syringe fitted with a 30 gauge needle, and subdivided as follows. Haemoglobin content was determined spectrophotometrically, using 2 µl of blood, by the Drabkin technique (Anon., 1980) modified to

account for the small volume of blood. Median erythrocyte volume (MCV) and erythrocyte count were determined on the Coulter Counter and Channelyzer, using 1 µl of blood. Haematocrit was measured using 2 to 3 µl of blood drawn up in a heparinized Strumia capillary tube (Sherwood Medical Industries, St. Louis, Missouri) and centifuged for 5 minutes. The remaining blood was used to make a blood smear which was stained for 15 minutes with Wright's stain (Humason, 1972), rinsed with distilled water, and air dried.

Mean corpuscular haemoglobin content (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulae

$$MCH = \frac{\text{haemoglobin (g/100ml) X 10}}{\text{erythrocyte count (million/mm}^3\text{)}} \text{ , and}$$

$$MCHC = \frac{\text{MCH (pg) X 100}}{\text{MCV } (\mu m^3)} .$$

This formula for MCHC was considered to be more accurate than the commonly used method of dividing total blood haemoglobin by haematocrit (e.g., Deparis et al., 1975), since it is based on a direct measurement of erythrocyte volume.

Erythrocyte dimensions were measured using the Zeiss microscope and camera lucida, and cell surface area and nuclear volume were calculated using the formulae presented in Chapter 3. Cell height was calculated by dividing MCV by the cell surface area. The cytoplasmic volume of

erythrocytes was calculated by subtracting nucleus volume from MCV. This value for cytoplasmic volume was substituted into the denominator of the formula for MCHC given above, thereby giving a measure of the mean cytoplasmic haemoglobin concentration.

Results

The haematological characteristics of diploid and triploid fish are summarized in Table 6 and Figure 8. Compared to the diploid condition, there was a 42% increase in MCV and 37% decrease in erythrocyte count associated with triploidy; as a result, haematocrit was the same for diploids and triploids. The total blood haemoglobin content of triploids was significantly lower than that of the diploids, but MCH was significantly higher. Triploids had a significantly lower MCHC than diploids.

It is apparent (Table 7 and Figures 9 and 10) that the increase in triploid MCV was mainly due to an increase in cell length, although there was also an increase in cell width. Cell height was the same for diploid and triploid erythrocytes. The increase in nuclear volume of triploid erythrocytes was also found to be mainly due to an increase in length. The nucleus was found to occupy 27.8% ($\pm$0.8%, SD) of the triploid cell volume, but only 23.0% ($\pm$1.8%, SD) of the diploid cell volume. When MCHC was recalculated to

Table 6: Haematological parameters of diploid and triploid fish (mean ± 95% confidence limits, n=12 in all cases).

	diploid	triploid	significance
MCV (μm^3)	195.9 ± 4.7	277.3 ± 7.6	$P < 0.001$
erythrocyte count (million/mm^3)	1.08 ± 0.05	0.68 ± 0.05	$P < 0.001$
haematocrit (%)	30.9 ± 2.1	29.7 ± 2.6	$0.50 > P > 0.25$
haemoglobin content (g/100 ml)	7.52 ± 0.38	5.87 ± 0.53	$P < 0.001$
MCH (pg)	69.8 ± 3.5	85.6 ± 4.8	$P < 0.001$
corpuscular MCHC (pg/μm^3)x100	35.6 ± 1.3	30.9 ± 1.7	$P < 0.001$
cytoplasmic MCHC (pg/μm^3)x100	46.0 ± 1.2	42.6 ± 2.6	$0.05 > P > 0.025$

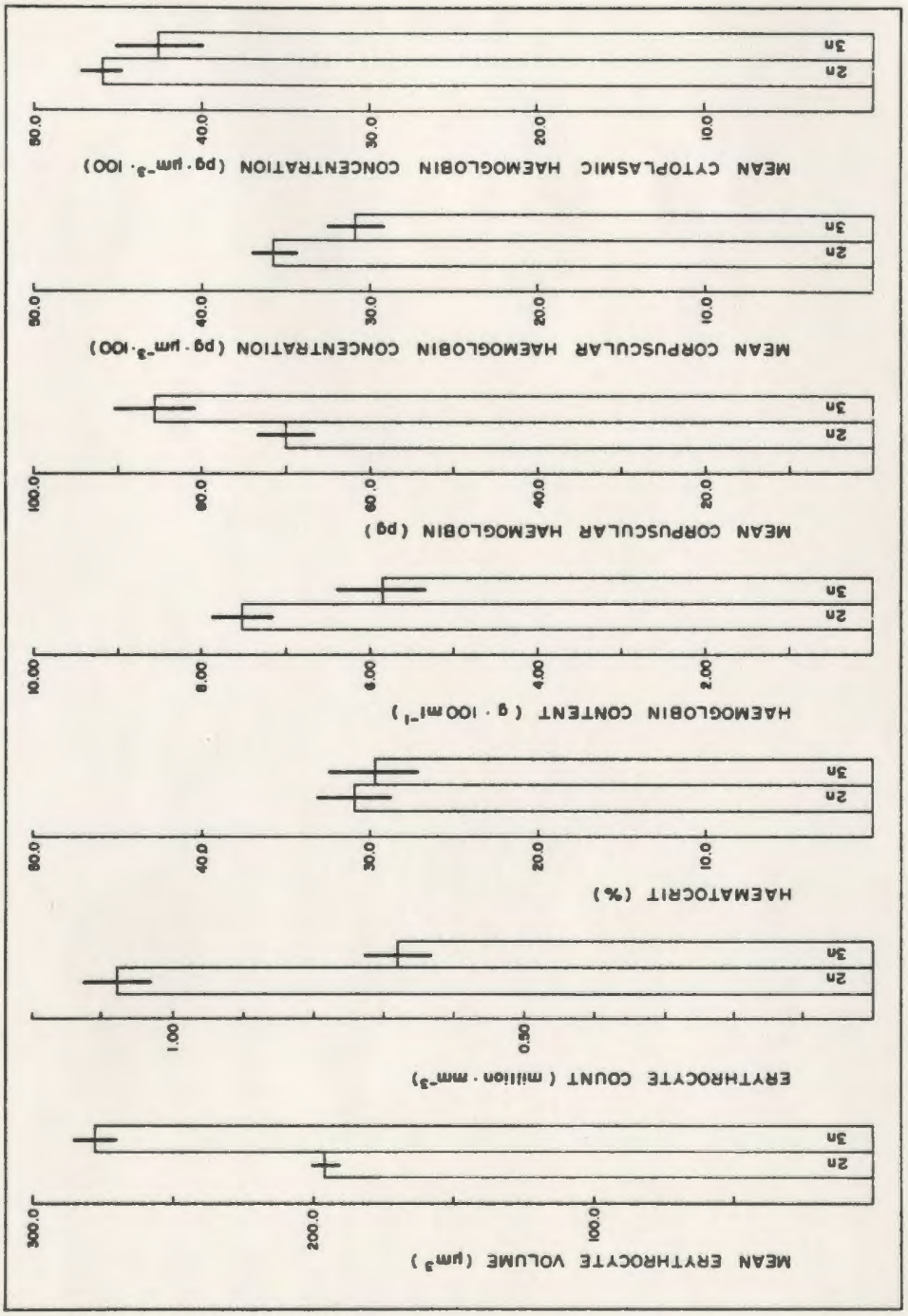

Figure 8: Haematological parameters of diploid and triploid fish (mean ± 95% confidence limits, n=12 in all cases).

Table 7: Dimensions of diploid and triploid erythrocytes (mean ± 95% confidence limits, n=10 in all cases).

	diploid	triploid	significance
cell surface area (μm^2)	100.8 ± 4.5	141.3 ± 5.0	$P < 0.001$
cell major axis (μm)	14.70 ± 0.34	18.40 ± 0.54	$P < 0.001$
cell minor axis (μm)	8.70 ± 0.22	9.76 ± 0.22	$P < 0.001$
cell height (μm)	1.98 ± 0.11	1.99 ± 0.07	$P > 0.75$
nucleus volume (μm^3)	45.4 ± 2.8	77.9 ± 3.1	$P < 0.001$
nucleus major axis (μm)	6.66 ± 0.10	8.72 ± 0.20	$P < 0.001$
nucleus minor axis (μm)	3.60 ± 0.10	4.10 ± 0.08	$P < 0.001$

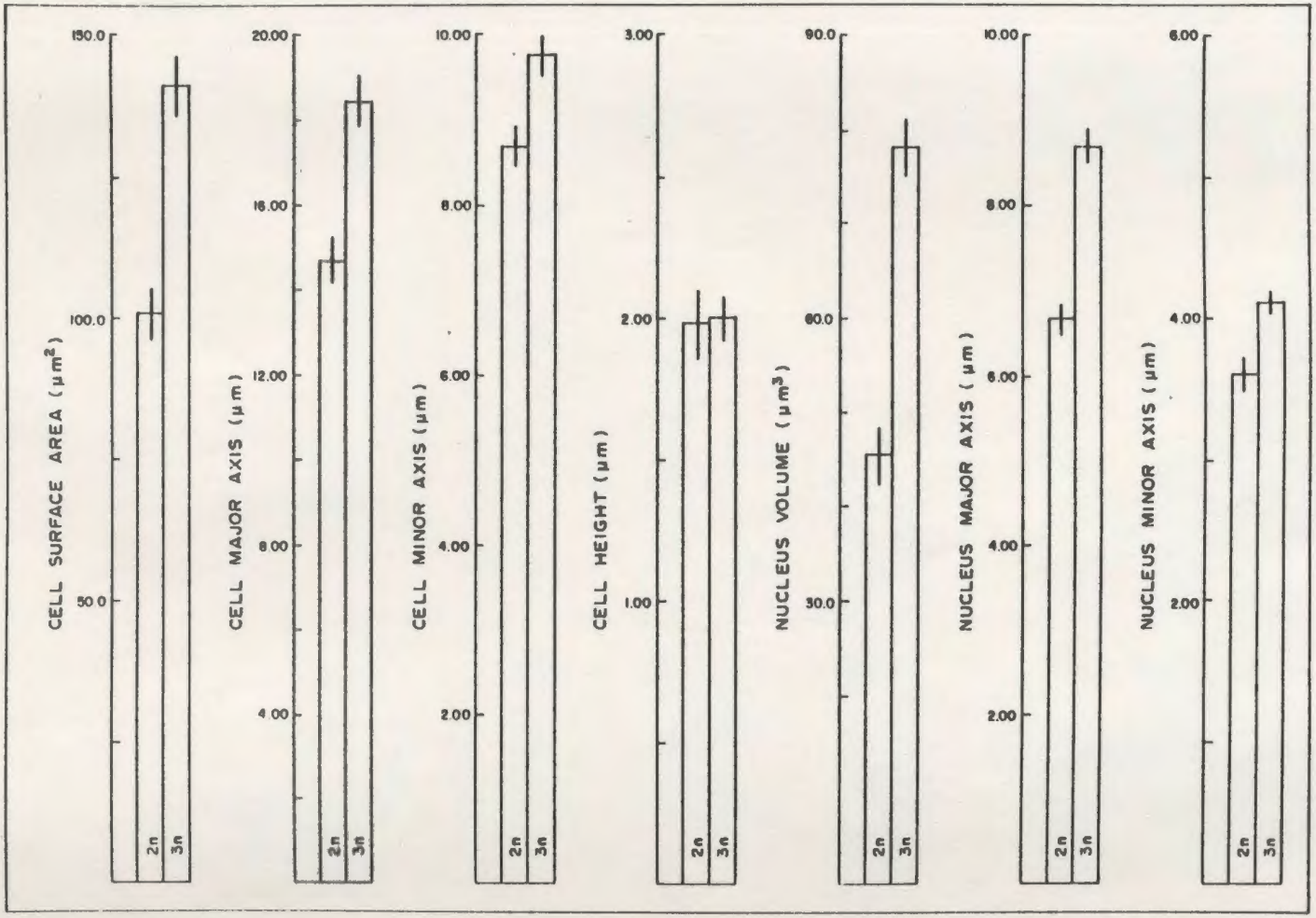

Figure 9: Dimensions of diploid and triploid erythrocytes (mean ± 95% confidence limits, n=10 in all cases).

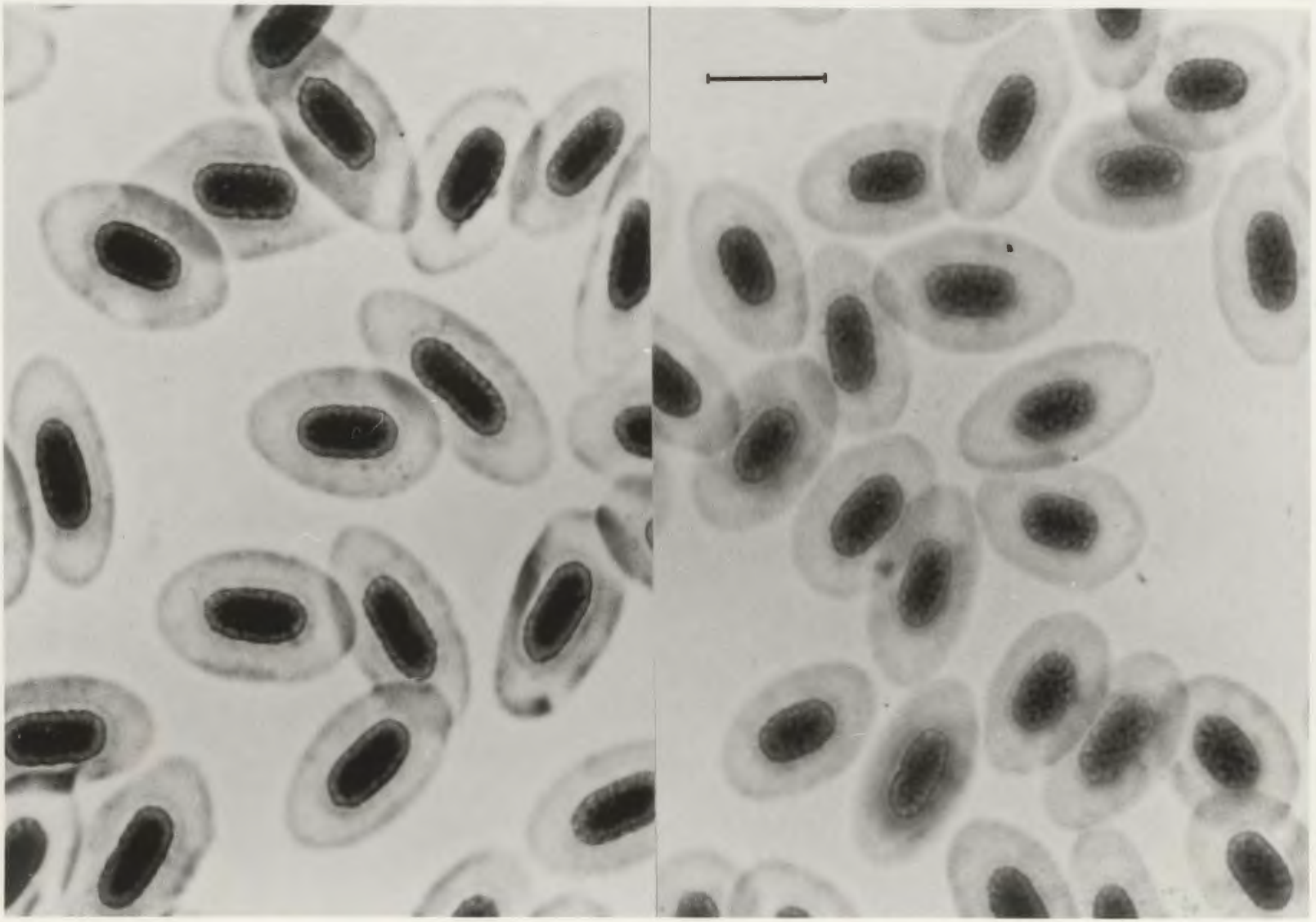

Figure 10: Diploid and triploid erythrocytes, stained with Wright's stain (diploids on the left; bar = 10 μm).

take this into account, the mean cytoplasmic haemoglobin concentration of triploids was only 7% lower than that of diploids, and not significantly different at the 95% confidence level.

Discussion

Incorporation of the triploid genome generally causes an increase in individual cell size, but triploid animals as a whole are no larger than diploids due to a reduction in cell numbers (Fankhauser, 1941, 1945b; Swarup, 1959b). Such a regulatory mechanism has previously been demonstrated for erythrocyte numbers in triploid salamanders (Deparis and Beetschen, 1965; Deparis et al., 1966, 1975), chickens (Abdel-Hameed, 1972) and goldfish (Sezaki et al., 1983), and now in triploid Atlantic salmon.

As expected, the MCH of triploid Atlantic salmon was higher than that of diploids. However, in contrast to the results of other researchers (Deparis et al., 1966; Abdel-Hameed, 1972; Deparis et al., 1975; Sezaki et al., 1983), both the total blood haemoglobin content and the MCHC were lower in triploid than in diploid Atlantic salmon. Thus, as in tetraploid and pentaploid salamanders (Deparis et al., 1975), complete homeostasis of haemoglobin concentration has not been achieved by triploid Atlantic salmon.

As in cyprinids (Cherfas, 1966, 1969; Sezaki et al., 1977; Beck and Biggers, 1983) and channel catfish (Wolters et al., 1982a), the major effect of triploidy on MCV was an increase in cell length. There was no increase in cell height, a feature shared with triploid amphibians (Davison, 1957, 1959; Uzzell, 1964), but not previously described in fish. The increase in erythrocyte nuclear volume of triploids was also mainly due to an increase in length of the nucleus. As pointed out by Wolters et al. (1982a), the shape of the erythrocyte nucleus is thus not the same for diploids and triploids, and this is true for the erythrocyte cell as well. In addition, the nucleus of triploid erythrocytes occupies a greater percentage of cell volume than it does in diploid erythrocytes. As a result, the mean cytoplasmic haemoglobin concentration is not significantly different between diploids and triploids.

Assuming that both the velocity constant for the uptake of oxygen by haemoglobin and the diffusion coefficient of oxygen within the cytoplasm are the same for diploids and triploids, the rate of oxygen uptake by erythrocytes should be a function solely of cell thickness and haemoglobin concentration (Holland and Forster, 1966). Since the diploid and triploid erythrocytes examined in this study were found to have the same thickness and similar haemoglobin concentrations, it follows that oxygen uptake by these two types of erythrocytes should be comparable.

5. OXYGEN UTILIZATION BY TRIPLOIDS

Objectives

In the previous chapter it was suggested that at the cellular level oxygen uptake by diploid and triploid erythrocytes should be the same. However, the haemoglobin content of whole blood was significantly lower in triploid fish, indicating that oxygen carrying capacity of the blood might be reduced relative to that of the diploids. Such a reduction in oxygen carrying capacity could have an influence on the ability of triploid fish to survive under hypoxic conditions. The purpose of the research described in this chapter was to examine oxygen utilization by triploid fish over a range of oxygen tensions as an index of fitness for intensive hatchery and cage culture.

Materials and Methods

Oxygen consumption rates were determined at 20 months post-hatch for 8 diploid and 8 triploid fish. Individual fish were placed into a 3 litre respiration flask equipped with a magnetic stirrer and held at 15^{o}C for the duration of the experiment. The respiration flask remained open to a surrounding water bath for a minimum of 12 hours, permitting the fish to acclimate in oxygen-saturated water after handling. The flask was then sealed and oxygen tension was

measured continuously using a Radiometer PHM71 analyzer and micro-electrode (Radiometer, Copenhagen). The experiment was terminated at the point of asphyxiation, defined as when the fish ceased all mouth and opercular movement, and oxygen consumption had dropped to zero.

Oxygen consumption rates were calculated over PO_2 (partial pressure of oxygen) intervals of 10 mm Hg. The PO_2 at saturation was recalculated daily using the formulae provided by Davis (1975) to account for changes in atmospheric pressure reported by the St. John's Weather Office. After asphyxiation, the wet weight and fork length of the fish were recorded and the gonads removed. The gonads were weighed and placed in Bouin's fixative (Humason, 1972) for gonad histology (Chapter 7). The fish were dried at 70-80 °C for a minimum of 36 hours before measurement of dry weight. Oxygen consumption rates were calculated as mg oxygen consumed per gram dry weight of fish per hour.

Results

The data for oxygen utilization by diploid and triploid fish are summarized in Table 8 and Figure 11. Single factor analysis of variance revealed that there was no significant difference between sexes for either oxygen consumption or PO_2 at asphyxiation, and sexes were therefore combined in subsequent analysis. Neither the oxygen consumption rate

Table 8: Oxygen utilization by diploid and triploid fish (mean ± 95% confidence limits, n=8 in all cases).

P_{O_2} (mm Hg)	Oxygen Consumption Rate (mg/g/hr)		
	diploid	triploid	significance
150	1.41 ± 0.34	1.17 ± 0.30	0.50 > P > 0.25
140	1.21 ± 0.26	1.22 ± 0.20	P > 0.75
130	1.19 ± 0.21	1.24 ± 0.17	0.75 > P > 0.50
120	1.11 ± 0.22	1.23 ± 0.21	0.50 > P > 0.25
110	1.16 ± 0.30	1.17 ± 0.20	P > 0.75
100	1.12 ± 0.22	1.12 ± 0.16	P > 0.75
90	0.99 ± 0.20	1.02 ± 0.15	P > 0.75
80	0.90 ± 0.13	0.85 ± 0.13	0.75 > P > 0.50
70	0.82 ± 0.09	0.77 ± 0.12	0.75 > P > 0.50
60	0.77 ± 0.10	0.70 ± 0.10	0.50 > P > 0.25
50	0.61 ± 0.05	0.63 ± 0.09	P > 0.75
40	0.52 ± 0.07	0.52 ± 0.08	P > 0.75
30	0.39 ± 0.09	0.43 ± 0.06	0.50 > P > 0.25
P_{O_2} at death (mm Hg)	23.1 ± 2.1	23.4 ± 2.7	P > 0.75

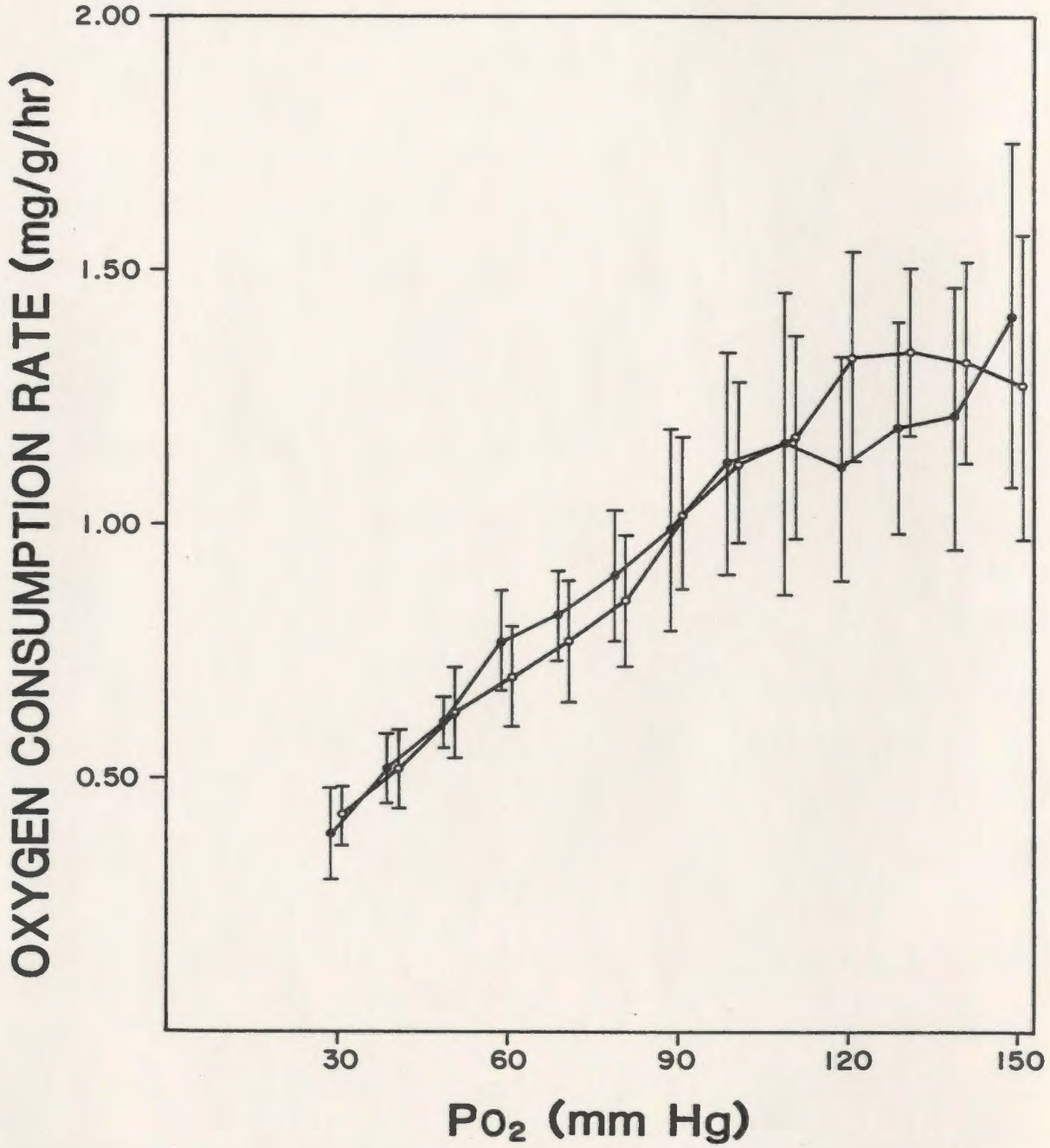

Figure 11: Oxygen consumption rates of diploid and triploid fish (mean ± 95% confidence limits, n=8 in all cases; open circles = triploids, closed circles = diploids).

nor the PO_2 at asphyxiation were significantly different between diploids and triploids. Although not distinct, respiratory independence was apparently maintained by both diploids and triploids until the oxygen tension reached 100 mm Hg. Respiratory dependence became quite apparent below 100 mm Hg.

Discussion

In spite of the lower haemoglobin content of their blood, triploid fish were no different from diploids in their ability to utilize oxygen. This was confirmed under both normoxic and hypoxic conditions. The activity of the fish was not controlled, since this allowed the fish to choose their own response to the depletion of oxygen. Although not generally the method used for measuring oxygen consumption by fish, it has the advantage of serving as a model for the response of fish to oxygen depletion in a hatchery environment. The results indicate that under conditions of reduced oxygen, triploids fare just as well as diploids.

Swarup's study of oxygen consumption in triploid three-spined stickleback (Swarup, 1959c) is the only other experimental work conducted on oxygen utilization by triploid animals. Swarup assumed that triploid individuals had a lesser total surface area of erythrocytes than

diploids, based on the increase in triploid erythrocyte size. Clearly, this is not the case in Atlantic salmon where both the thickness of the erythrocytes and the haematocrit are the same for diploid and triploid individuals. The contention made by Swarup that oxygen consumption should be lower in triploid than in diploid stickleback can therefore not be applied to Atlantic salmon.

Swarup found that the oxygen consumption rates of diploid and triploid stickleback were the same when calculated per unit body wet weight, but that triploids consumed less oxygen than diploids per unit body surface area. Oxygen consumption rates in fish are generally calculated as a function of body weight rather than surface area (Shelton, 1970; Hughes, 1973; Hoar and Hickman, 1975), suggesting that of the two methods used by Swarup, calculation per unit body weight was the more valid. Thus, the data indicate that oxygen consumption rates are the same for diploids and triploids in both three-spined stickleback and Atlantic salmon.

6. GROWTH OF TRIPLOIDS

Objectives

The only experimental data available on growth rates in triploid fish are those reported by Purdom (1976) and Lincoln (1981c) for female plaice x flounder hybrids. Prior to the onset of maturation, growth rates were identical for diploids and triploids in these two studies. However, diploid females ceased to grow as their ovaries began to mature, and somatic growth was not resumed until after spawning was completed. The period of no growth in the diploid females lasted about 6 months. Triploid females, which were sterile, continued to grow over this 6 month period.

In addition to measuring growth rates, Lincoln (1981c) measured the condition factor of the fish in his study. In immature fish, the condition factor of diploids and triploids was the same, but in the diploid females condition factor increased as spawning approached. In triploid females, on the other hand, condition factor remained at a constant low level. Thus, for about a 9 month period, the diploids had a higher condition factor than the triploids. By the end of the spawning period, the condition factor of the diploids had returned to its original lower level.

Data on the growth rates of triploid fish are of obvious importance if such fish are to be considered for use in aquaculture. Several authors have suggested that prior to maturation, diploid salmonids exhibit better growth rates than triploids (Thorgaard and Gall, 1979; Refstie, 1981; Thorgaard et al., 1982), but no experimental evidence is available to support this. The purpose of the research described in this chapter was to examine the growth rates of diploid and triploid Atlantic salmon, in order to assess their growth potential under culture conditions.

Materials and Methods

At 9 months after hatching, all 39 surviving triploids were placed in a 100 X 100 X 42 cm self-cleaning fiberglas tank receiving a constant flow of water at ambient temperatures. On the same day, an equal number of known diploids of approximately the same size (judged by eye) were placed in an identical tank beside that of the triploids. Artificial lighting was used to simulate approximate natural daylengths. Both groups were fed excess rations simultaneously, 2 to 3 times a day in the winter and 3 to 4 times a day in the summer.

The weight (W) of each fish was measured at 13 intervals over a 9 month period. In the final 6 intervals, length (L) was also recorded and condition factor (CF) was

calculated using the formula

$$CF = \frac{W \times 100}{L^3} .$$

The growth experiment was terminated after 9 months, at which time some of the fish were used for experiments on oxygen consumption (Chapter 5). Additional information on weight and condition factor was obtained from these fish after asphyxiation.

Single factor analysis of variance was used to determine the level of significance of any differences encountered in weight, length or condition factor between diploids and triploids.

Results

No growth was shown by any of the fish over the first 5 intervals (Table 9 and Figure 12), coinciding with the lowest water temperatures during the winter. The triploid fish had a higher initial growth rate in the spring, and were significantly heavier than the diploids by May 29. However, the growth rate of the diploids soon increased and remained similar to that of the triploid fish for the duration of the experiment.

Diploids were consistently shorter than triploids (Table 10 and Figure 13), and thus had a higher condition

Table 9: Growth in weight of diploid and triploid fish (mean ± 95% confidence limits, n in brackets).

	weight		
date	diploid	triploid	significance
10 DEC 82	1.20 ± 0.11 (39)	1.26 ± 0.13 (39)	n.s.
11 JAN 83	1.29 ± 0.12 (37)	1.39 ± 0.16 (39)	n.s.
11 FEB 83	1.31 ± 0.13 (36)	1.43 ± 0.16 (38)	n.s.
13 MAR 83	1.28 ± 0.14 (34)	1.40 ± 0.16 (38)	n.s.
10 APR 83	1.32 ± 0.15 (33)	1.45 ± 0.17 (37)	n.s.
06 MAY 83	1.69 ± 0.21 (29)	2.01 ± 0.23 (33)	n.s.
29 MAY 83	2.26 ± 0.30 (29)	2.89 ± 0.36 (31)	$0.025 > P > 0.010$
16 JUN 83	3.68 ± 0.49 (29)	4.16 ± 0.52 (31)	n.s.
05 JUL 83	6.47 ± 0.87 (28)	6.78 ± 0.90 (32)	n.s.
18 JUL 83	10.31 ± 1.36 (28)	10.29 ± 1.41 (31)	n.s.
07 AUG 83	13.61 ± 1.91 (25)	14.08 ± 2.01 (30)	n.s.
30 AUG 83	17.44 ± 2.79 (25)	18.46 ± 2.81 (30)	n.s.
19 SEP 83	20.29 ± 3.67 (23)	20.67 ± 3.55 (28)	n.s.

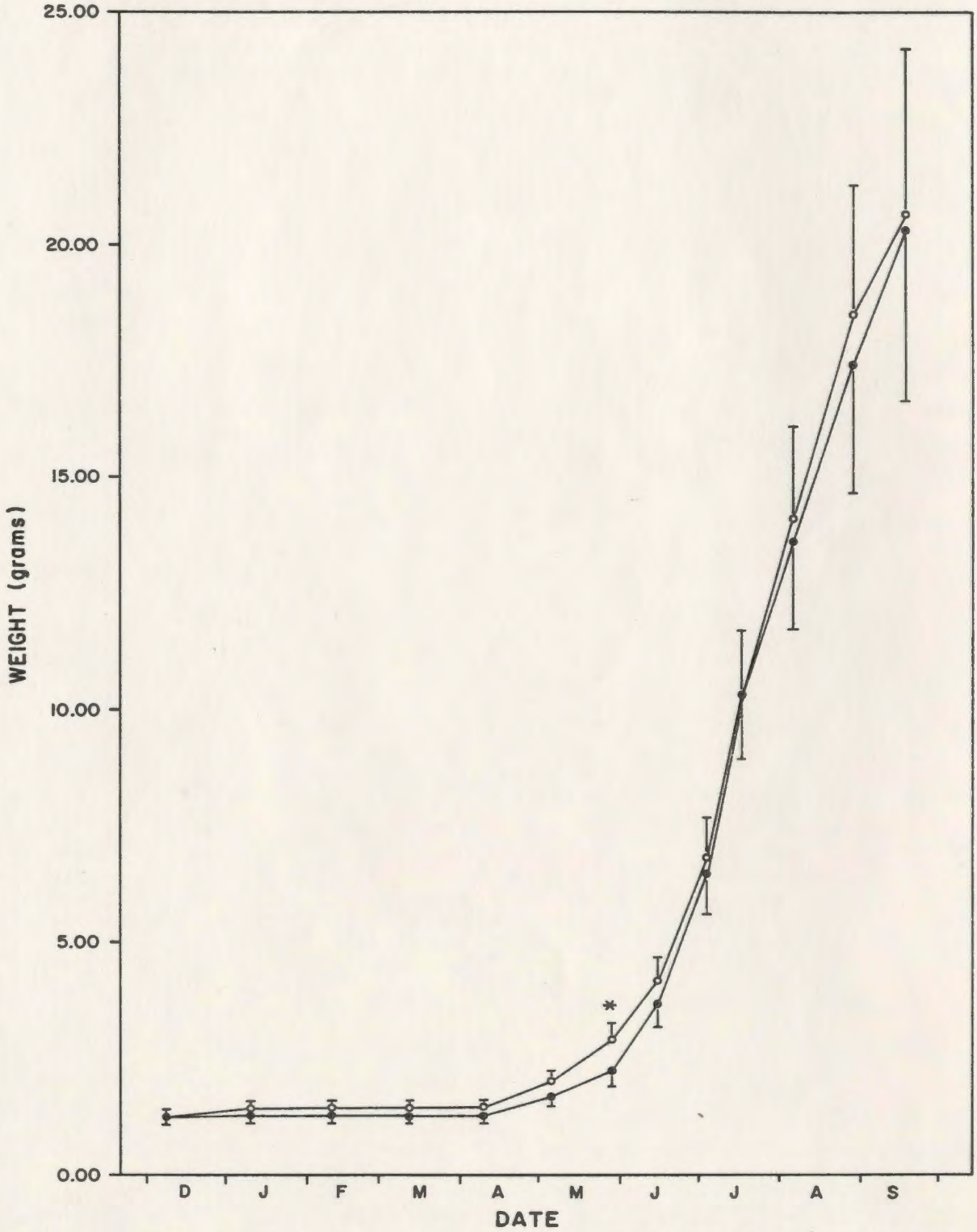

Figure 12: Growth in weight of diploid and triploid fish (mean ± 95% confidence limits, n in Table 9; open circles = triploids, closed circles = diploids; *: $P < 0.025$).

Table 10: Length of diploid and triploid fish (mean ± 95% confidence limits, n in brackets).

	length (cm)		
date	diploid	triploid	significance
16 JUN 83	6.8 ± 0.3 (29)	7.3 ± 0.4 (31)	0.05 > P > 0.025
05 JUL 83	8.0 ± 0.4 (28)	8.3 ± 0.5 (32)	n.s.
18 JUL 83	9.0 ± 0.4 (28)	9.3 ± 0.5 (31)	n.s.
07 AUG 83	10.0 ± 0.5 (25)	10.4 ± 0.6 (30)	n.s.
30 AUG 83	10.9 ± 0.6 (25)	11.2 ± 0.6 (30)	n.s.
19 SEP 83	11.5 ± 0.7 (23)	11.8 ± 0.8 (28)	n.s.

Table 11: Condition factor of diploid and triploid fish (mean ± 95% confidence limits, n in brackets).

	condition factor		
date	diploid	triploid	significance
16 JUN 83	1.15 ± 0.05 (29)	1.03 ± 0.04 (31)	P < 0.001
05 JUL 83	1.21 ± 0.05 (28)	1.13 ± 0.05 (32)	0.050 > P > 0.025
18 JUL 83	1.36 ± 0.07 (28)	1.21 ± 0.03 (31)	P < 0.001
07 AUG 83	1.29 ± 0.06 (25)	1.17 ± 0.04 (30)	P < 0.001
30 AUG 83	1.28 ± 0.06 (25)	1.21 ± 0.04 (30)	n.s.
19 SEP 83	1.25 ± 0.07 (23)	1.14 ± 0.04 (28)	0.005 > P > 0.001

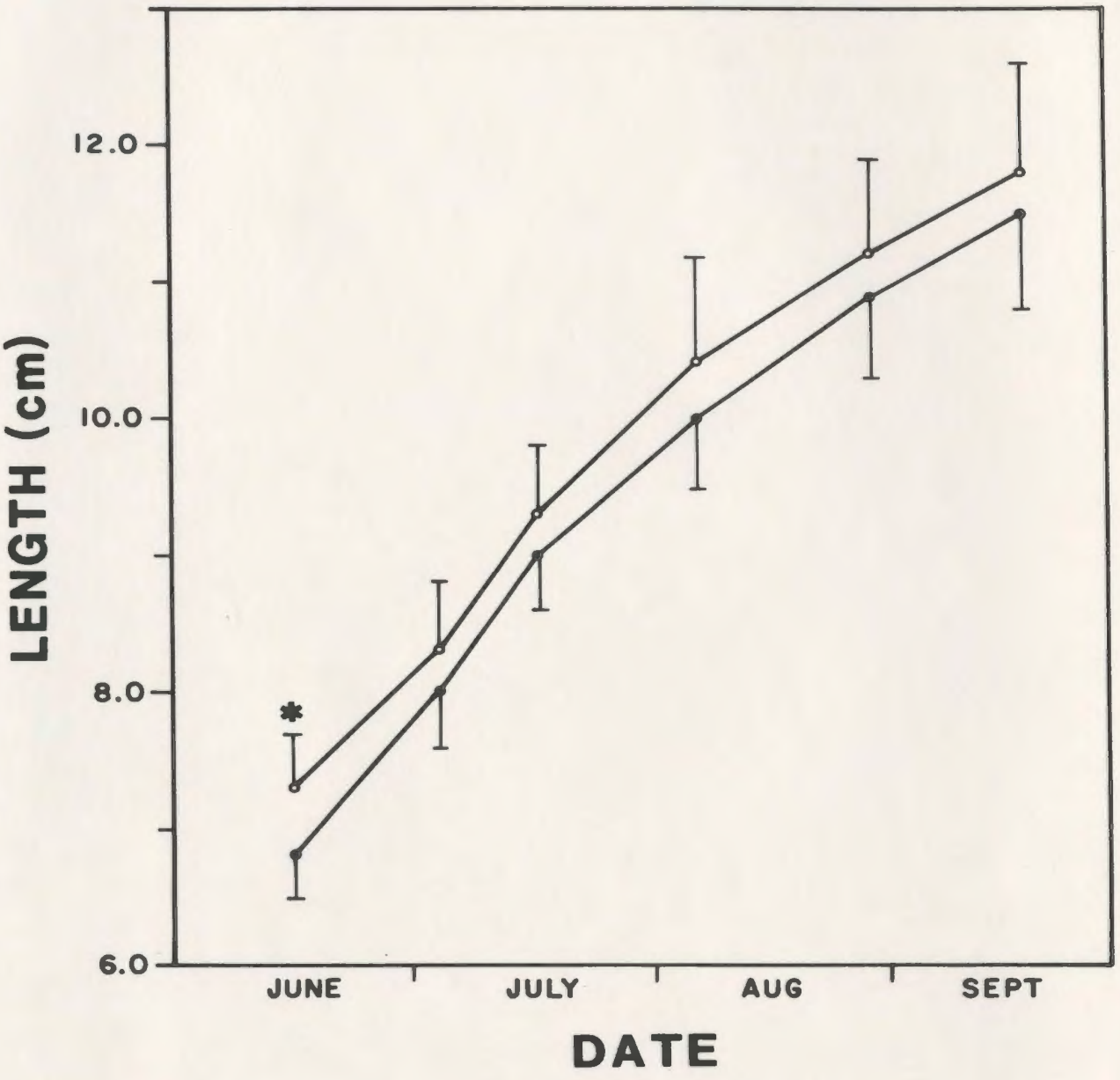

Figure 13: Length of diploid and triploid fish (mean ± 95% confidence limits, n in Table 10; open circles = triploids, closed circles = diploids; *: $P < 0.05$).

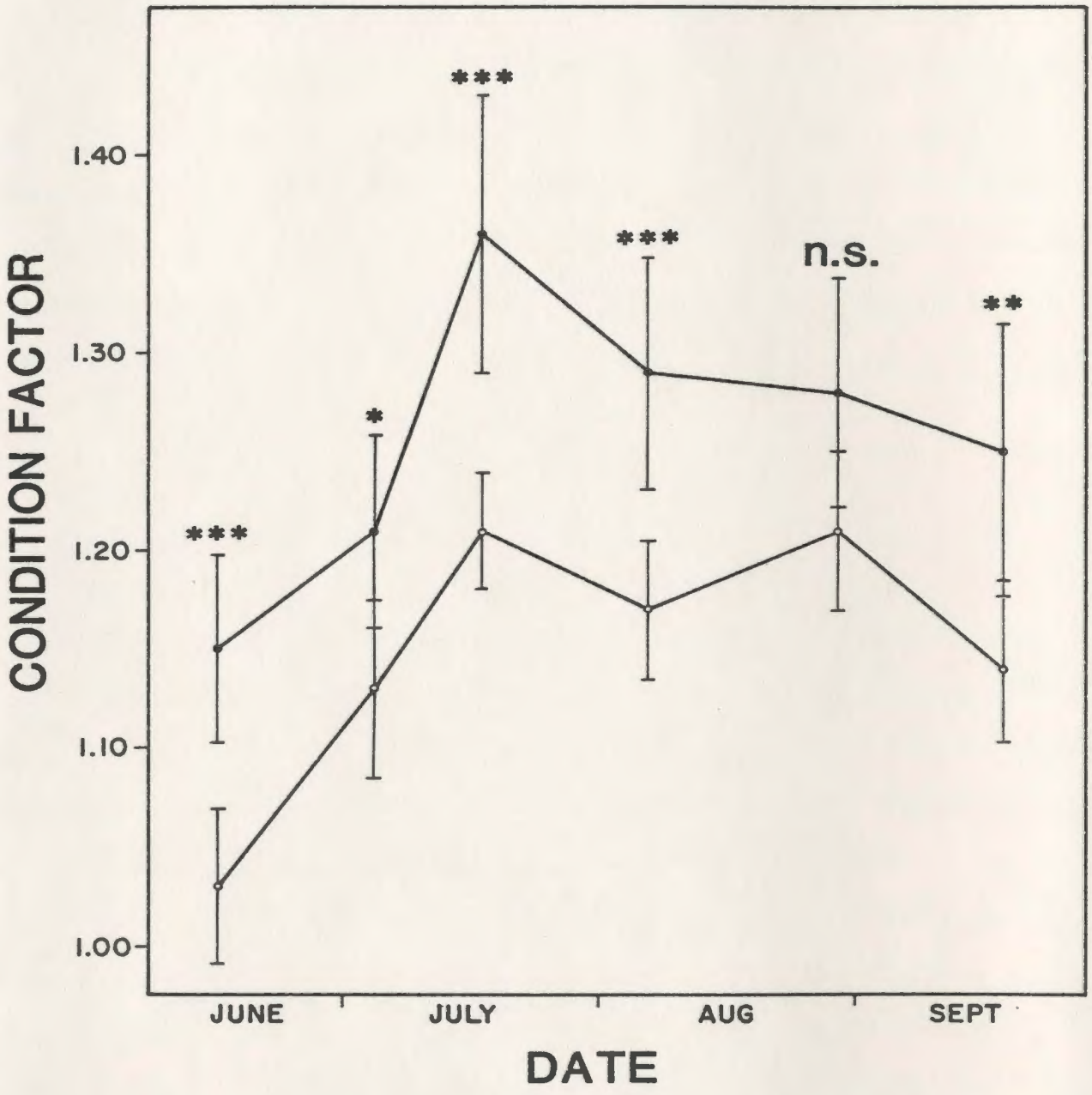

Figure 14: Condition factor of diploid and triploid fish (mean ± 95% confidence limits, n in Table 11; open circles = triploids, closed circles = diploids; ***: $P < 0.001$, **: $P < 0.005$, *: $P < 0.05$, n.s.: not significant).

factor over the entire sampling period (Table 11 and Figure 14). Condition factor for both groups reached a maximum in mid-July, corresponding to the period of greatest growth, and then began to fall as the growth rates diminished towards the end of the summer. Examination of the fish used in the oxygen consumption experiments revealed that for both sexes the diploids had a higher condition factor than the triploids (diploid females: 1.21 ± 0.27 SD; triploid females: 1.07 ± 0.11; diploid males: 1.19 ± 0.08; triploid males: 1.10 ± 0.11), but these differences were not statistically significant.

Discussion

Growth rates are virtually identical for immature diploid and triploid Atlantic salmon. Similar results have been reported for plaice x flounder hybrids (Purdom, 1976; Lincoln, 1981c). It is not suprising that growth rates were the same for diploids and triploids prior to maturation, since no energy is diverted to gonadal development in either group. However, if triploids are sterile, they can be expected to show continued growth over the period when maturing diploids exhibit reduced or no growth. It was not possible to carry on the growth experiment with Atlantic salmon long enough to confirm this, but this was certainly the case in female triploid plaice x flounder hybrids (Purdom, 1976; Lincoln, 1981c). These flatfish hybrids

spawn in the spring, and are hence subject to growth depression at a time when non-maturing fish are expected to begin rapid growth. Because Atlantic salmon spawn in the fall, maturing fish are likely to show reduced growth in late summer and fall, at a time when immature or sterile fish are also growing at a slower rate. It is therefore unlikely that the effect of triploidy on growth in Atlantic salmon will be as apparent as it is in flatfish. Nevertheless, it may give the triploids enough of an advantage going into the winter to result in a better survival rate over this critical period, and more rapid growth in the subsequent spring.

The influence of triploidy on condition factor in Atlantic salmon closely resembles the situation in flatfish (Lincoln 1981c). In both species, diploids exhibited an elevated condition factor prior to spawning. The data obtained by Lincoln for flatfish are easier to interpret than those described here, since he used only female fish. The effect of ploidy on gonadal development was far greater in female flatfish than in males, (Lincoln, 1981a, 1981b), and it was therefore expected that differences in condition factor would be more apparent in the females. The data obtained for gonad development (Chapter 7) revealed that this was the case for Atlantic salmon as well, but it was not possible to distinguish the sex of the fish used in the growth study described here. Data collected on a smaller

number of fish of known sex used in the oxygen consumption experiments indicated that the condition factor was apparently lower for triploids than for diploids in both sexes, but this was not statistically significant in either case.

7. GONADAL DEVELOPMENT IN TRIPLOIDS

Objectives

Since the primary goal of inducing triploidy has been to produce sterile fish for aquaculture, it is of obvious importance to examine gonadal development in such fish to determine whether or not they are truly sterile. Triploid male rainbow trout develop testes and show the same secondary sexual characteristics observed in maturing diploid trout. Triploid females, on the other hand, apparently do not develop ovaries, retaining only the string-like gonads characteristic of sexually undifferentiated fish (Thorgaard and Gall, 1979). Gonad histology has revealed that the ovaries of triploid rainbow trout are devoid of oocytes at a time when diploid ovaries are packed with oocytes in advanced stages of development (Lincoln and Scott, 1983; Yamazaki, 1983). No other information is available on maturation in triploid salmonids.

Studies of triploid flatfish (Purdom, 1972; Lincoln, 1981a, 1981b), carp (Gervai et al., 1980b) and channel catfish (Wolters et al., 1982b) have revealed that although ovarian development is greatly retarded compared to that of the diploids, small numbers of oocytes are generally produced by triploid ovaries in all these species. Gonadal

development in triploid males is also retarded relative to that of the diploids, but not nearly to the extent of that reported for the females. Triploid male plaice have produced spermatozoa with the ability to activate eggs, but none of the resulting embryos survive long after hatching (Lincoln, 1981a).

The purpose of the research described in this chapter was to examine gonadal development in diploid and triploid Atlantic salmon, to determine whether triploidy induces sterility in this species.

Materials and Methods

Gonadal development was examined in the 16 fish sacrificed for the oxygen consumption experiments (Chapter 5). An additional 4 fish were sacrificed at the same time, to provide data on 5 individuals of each sex for both diploid and triploid fish. Total wet weight of the fish was determined prior to the removal of the gonads. The gonads were then excised and weighed for the calculation of gonadosomatic index (GSI) by the formula

$$GSI = \frac{(\text{gonad weight}) \times 100\%}{(\text{total weight} - \text{gonad weight})} .$$

The gonads were then fixed for a minimum of 24 hours in Bouin's fixative (Humason, 1972) prior to preparation for histology. Standard techniques of dehydrating, embedding,

sectioning and staining were used (Humason, 1972). Sections were cut at 7μm and stained in Gill's haematoxylin and eosin. Identification of cell types in the gonads was based on the terminology used for rainbow trout (van den Hurk et al., 1978; van den Hurk and Peute, 1979: van den Hurk and Slof, 1981).

Results

Gonadal development was reduced in triploids of both sexes compared to that of diploids (Figure 15). The GSI of diploid females was 13 times greater than that of triploid females (0.31% ± 0.04% SD versus 0.024% ± 0.007%), but in the males the GSI of diploids was only 1.9 times greater than that of triploids (5.8% ± 2.0% versus 3.0% ± 0.4%). This difference in GSI was statistically significant for both sexes. Triploid ovaries had the appearance of undifferentiated gonads, typical of those seen in juvenile salmonids. Triploid testes, although well-developed, did not have the smooth surface typical of diploid testes, and appeared to be more heavily vascularized.

Gonad histology of the females (Figure 16) and males (Figure 17) revealed morphological differences between diploids and triploids of both sexes. Diploid ovaries were packed with hundreds of previtellogenic oocytes (stages 1 to 4), whereas triploid ovaries were composed mostly of

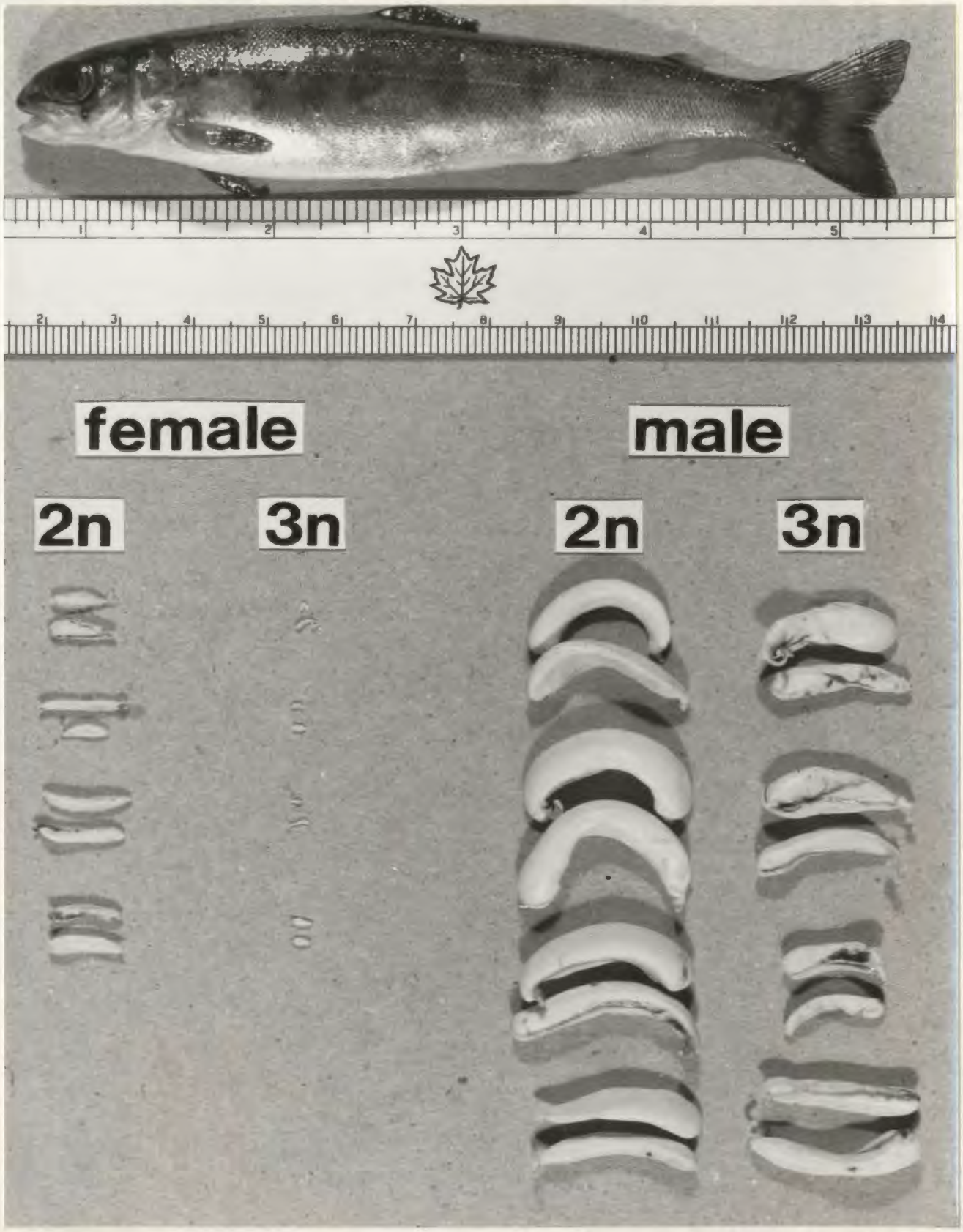

Figure 15: Gonad size in diploid and triploid fish.

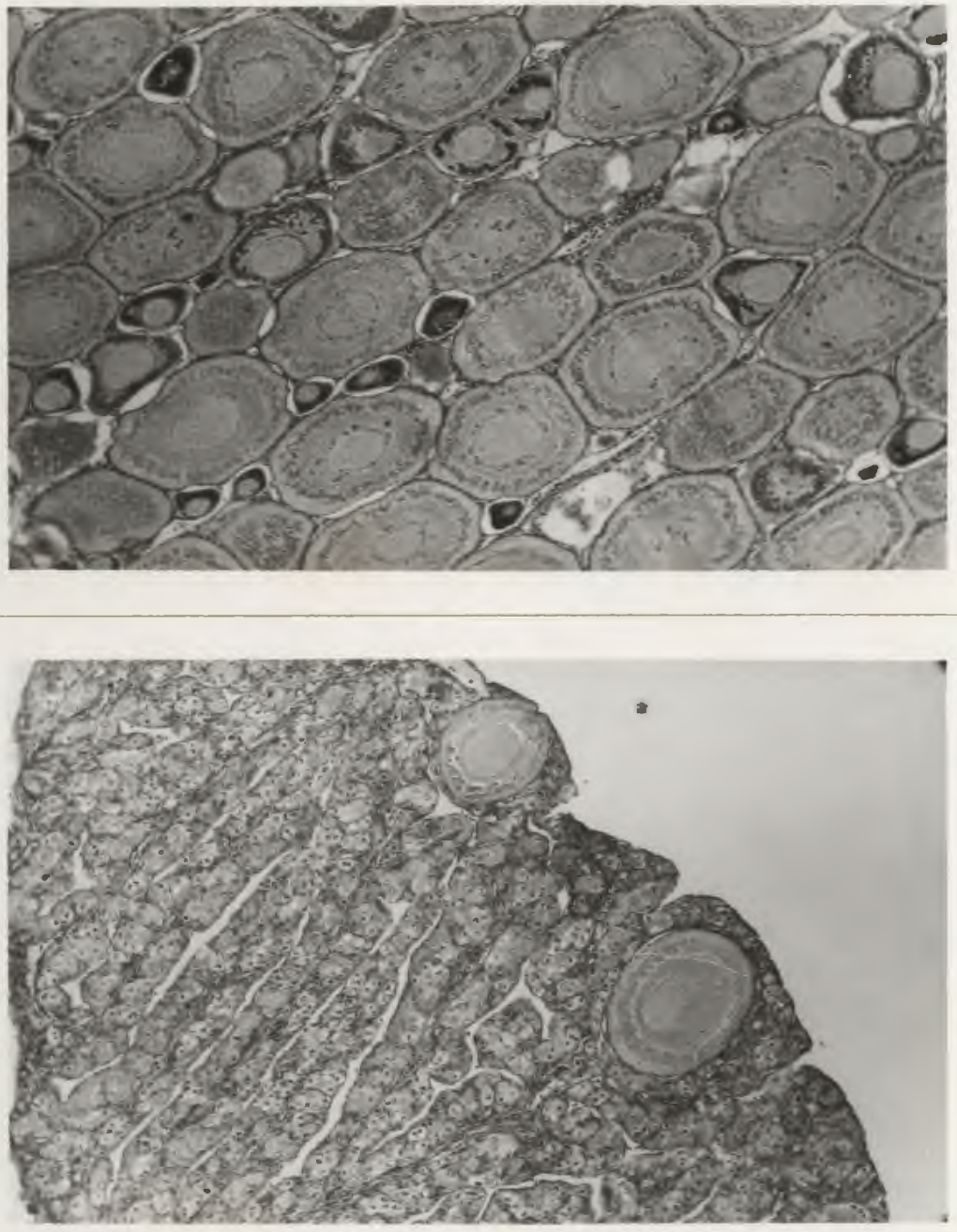

Figure 16: Transverse sections of diploid and triploid ovaries (diploid on top; X 40).

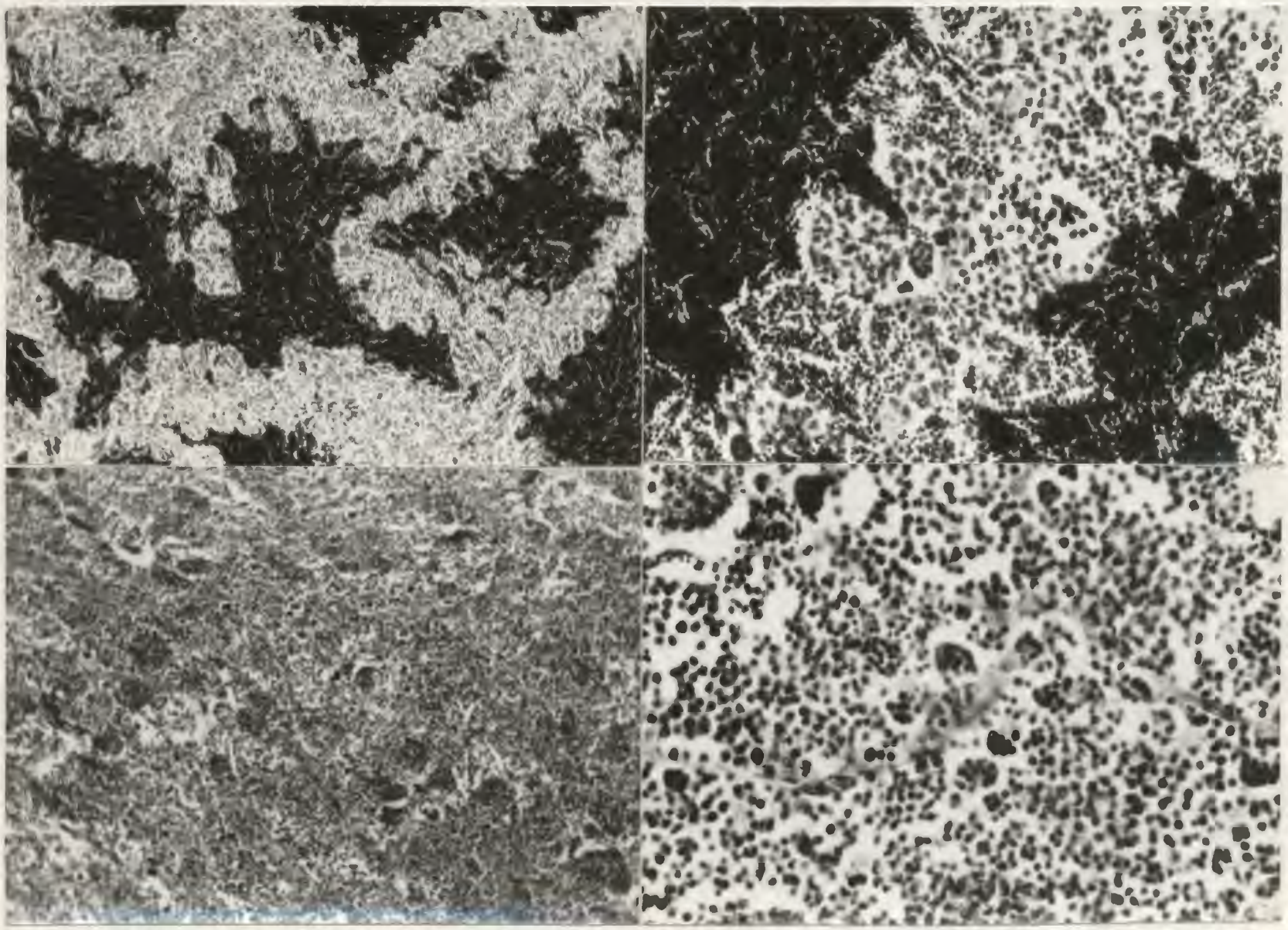

Figure 17: Transverse sections of diploid and triploid testes, showing lightly-stained spermatogonia and spermatocytes and darkly-stained spermatids (diploid on top; left side X 40, right side X 160).

oogonia. However, each triploid female possessed a few (from 1 to 12) oocytes developed as far as stages 2 and 3. These oocytes were indistinguishable from the oocytes of diploid ovaries. Diploid testes contained large bundles of spermatids, indicating that these fish were approaching spermiation. Most of the cells in the triploid testes were at the spermatogonium and spermatocyte stages, and these testes had the appearance of delayed development compared to the diploids.

Discussion

Gonadal development in triploid Atlantic salmon was greatly reduced in females and slightly reduced in the males. Such sexual dimorphism with respect to gonadal development has previously been described in triploid fish (Purdom, 1972; Thorgaard and Gall, 1979; Gervai et al., 1980b; Lincoln, 1981a, 1981b; Wolters et al., 1982b) and amphibians (Fankhauser, 1940, 1941; Kawamura, 1951a, 1951b; Smith, 1958). In external appearance, triploid ovaries resembled the undifferentiated gonads of juvenile salmonids, indicating that they were probably sterile, as is the case in triploid rainbow trout (Thorgaard and Gall, 1979; Lincoln and Scott, 1983; Yamazaki, 1983). However, gonad histology revealed that a small number of oocytes were produced by triploid ovaries, and these fish were thus not truly sterile. Histological examination of the triploid

testes revealed that although these fish were not as advanced as the diploid males, they may eventually have spermiated. These results are identical to those obtained for triploid flatfish (Lincoln, 1981a, 1981b).

Oogenesis in salmonids, as in teleosts in general, begins with the proliferation of oogonia by mitoses. Oogonia then enter meiosis I to become primary oocytes. At this point meiosis is blocked at metaphase and does not resume until after the oocytes have matured and been ovulated. At ovulation meiosis I goes to completion with extrusion of the first polar body, and the eggs enter meiosis II. The second meiotic division is again blocked at metaphase and does not resume until fertilization of the eggs (Tokarz, 1978; Wallace and Selman, 1981). The lack of oocytes in triploid Atlantic salmon suggests that the blockage to oogenesis occurs at the time of oogonial transformation into primary oocytes, probably through obstruction of the meiotic mechanism. However, occasional oocytes are formed in triploid Atlantic salmon, as in most other teleosts in which triploidy has been induced (Purdom, 1972; Gervai et al., 1980b; Lincoln, 1981b; Wolters et al., 1982b). The only apparent exception is the rainbow trout (Lincoln and Scott, 1983; Yamazaki, 1983).

The oocytes encountered in diploid females were all previtellogenic, and these fish would have spawned in

another year (Sutterlin, pers. comm.). Numerous stage 4 oocytes were present, indicating that steroidogenesis associated with maturation had likely begun (van den Hurk and Peute, 1979). The few oocytes observed in triploid females had reached stage 3, but no stage 4 oocytes were encountered. It is possible that with further maturation of the oocytes, steroidogenesis would also have begun in the triploid females.

The meiotic stages of spermatogenesis are similar to those of oogenesis, but are apparently not as susceptible to blockage through the induction of triploidy. In amphibians, the spermatozoa produced by triploid males are generally aneuploid (Humphrey and Fankhauser, 1949; Kawamura, 1951a, 1951b; Fankhauser and Humphrey, 1954), as is probably the case in triploid flatfish (Lincoln, 1981a). It appears that triploid spermatogonia readily undergo meiotic division, but that chromosome partitioning is disrupted, resulting in the formation of aneuploid spermatocytes. These spermatocytes are able to develop into spermatozoa and are thus capable of activating eggs. However, in so doing, they transmit an incomplete chromosome complement, thus forming non-viable embryos.

Triploidy can thus be used to reduce gonadal development in Atlantic salmon, but triploids are not sterile. This may be of advantage to aquaculture, since an

increased amount of energy will be retained for somatic growth. However, triploids may develop some of the other disadvantageous traits of maturation. This is apparently the case in triploid rainbow trout, in which males develop all the characteristics of sexually mature diploids, even though gonad growth is reduced (Thorgaard and Gall, 1979). It is not yet clear what influence the formation of a small number of oocytes may have on maturation in triploid females.

8. GENERAL DISCUSSION AND CONCLUSIONS

In a review of his research on chromosome manipulation in amphibians, Fankhauser (1945a) suggested five major areas of study important to an understanding of the general effects of ploidy in animals (see Preface). Fankhauser's research dealt with the effects of changes in chromosome number on developmental biology, whereas the general aim of induced polyploidy in fish has been to influence reproductive processes for commercial purposes. Specifically, my work has dealt with the evaluation of induced triploidy as a means of producing sterile Atlantic salmon for aquacultural purposes. In so doing, I have considered four of Fankhauser's principles:

1. Triploidy was induced using physical shocks to block extrusion of the second polar body. In fish, this process occurs shortly after fertilization, at a time when eggs are easily obtained and treated. Retention of the second polar body, with its haploid chromosome complement, yields a triploid zygote upon fusion of the egg and spermatozoon pronuclei.

2. Heat and hydrostatic pressure shocks were found to be highly effective means for inducing triploidy. It is apparent that the Atlantic salmon is readily amenable to chromosome manipulation, thus making it a useful tool for

genetic engineering.

3. Triploids were found to have larger erythrocytes than diploids, and on this basis triploids were routinely identified using a Coulter Counter and Channelyzer to measure erythrocyte volume. Examination of the haematology of the triploid fish revealed a homeostatic mechanism acting to regulate haemoglobin content at the cellular level, but the haemoglobin content of triploid blood was lower than that of the diploids. Diploid and triploid fish were identical in external appearance.

4. Both oxygen consumption and growth were the same in hatchery reared diploid and triploid fish. Triploidy inhibited gonadal development, especially in the females, but in neither sex were the triploids sterile. This fact has been well documented in triploid amphibians, but has only recently been confirmed in triploid fish.

My work has shown that the production of triploid Atlantic salmon is feasible at the commercial level, and that triploidy has no apparent detrimental effect on the fitness of such fish under culture conditions. Retardation of gonadal development is apparent in triploids of both sexes, but is far more striking in the females. Nevertheless, the fact that neither male nor female triploid Atlantic salmon were sterile raises doubts concerning their

usefulness for aquaculture. It is clear that triploidy confers no advantage to the males in this respect, but with the substantial reduction in ovarian development, triploid females may be of benefit. In commercial salmonid farming it is the males that generally mature at a smaller size and earlier age, and the most likely application of triploidy to fish culture may therefore be in conjunction with treatments producing all-female populations, as suggested by Lincoln and Scott (1983).

If further research demonstrates that triploid Atlantic salmon do not meet the expectations of commercial fish farming, their use in sea ranching might be considered. Sterile salmon are expected to remain at sea since they have no stimulus to return to freshwater for spawning (Donaldson and Hunter, 1982), and will thus provide the commercial fishery with large salmon of excellent quality. Although triploid females are not sterile, it may be that the reduction in ovarian development is sufficient to suppress migratory behaviour, and that such fish will remain at sea. If this is the case, triploid females could be a valuable addition to the commercial salmon fishery.

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