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The effects of temperature and time of first feeding on egg and fry development in Atlantic salmon, Salmo salar L.

by

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A thesis submitted to the University of Stirling for the degree of Doctor of Philosophy.

Institute of Aquaculture
University of Stirling

October, 1994

UNIVERSITY OF STIRLING

ABSTRACT

Doctor of Philosophy

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The first part of this study investigated the effects of varying temperature regimes within the range of 8-22°C on the development and survival of Atlantic salmon (Salmo salar L.) eggs and alevins. The temperature tolerance of eggs was lower than that of alevins: egg mortality increased above 11°C and no eggs survived to eyeing or to hatching at 16 and 14°C, respectively; alevin mortality increased above 16°C and no alevins survived at 22°C. Optimal survivals of eggs and alevins occurred at 8-11°C and 10-14°C, respectively. Subsequent survival at later stages of development was largely determined by survival at earlier stages. Developmental abnormalities among eggs (pin-eyed eggs) and alevins (abnormal hatching and yolk-sac oedema) appeared to be temperature-dependent.

Development time in days from fertilisation to eyeing, hatching and maximum alevin wet weight (MAWW) varied inversely with temperature. The sum of degree-days from fertilisation to eyeing and to MAWW was similar at all temperatures, but declined with increasing temperature from eyeing to hatching. The hatching period was similar for all temperatures except 8°C where it was significantly longer. Although alevin size at hatching was not temperature-dependent within the range of 8-12°C, alevin size at MAWW decreased progressively with increasing temperature (10-20°C) during the alevin stage.

Fry size at first feeding did not affect their subsequent growth rate or survival. Advanced fry which were fed earliest grew at similar rates to those produced at lower temperatures and attained the greatest weight. Biomass gain was more dependent upon survival than upon mean fish weight.

The second part of this study investigated the effects of timing of first feeding on fry growth and survival. Alevins fed prior to final yolk resorption were larger and had lower mortalities than those fed after MAWW. Although the "window" of first feeding opportunity lasted several weeks, delaying feeding beyond MAWW reduced absolute growth. A 5-week delay led to mortalities approaching 60%. However, first feeding can be delayed beyond MAWW for 1-2 weeks at 10°C without adversely affecting subsequent survival or growth rate.

PREFACE

This thesis comprised 8 years of part-time research work under the supervision of Professor N.R. Bromage. Following 2 years of registration at the University of Aston in Birmingham, the registration was transferred to the University of Stirling for the remainder of it's term.

The study was conducted at Sparsholt College, Hampshire where the author is employed as a lecturer in Fishery Studies in the Department of Fish, Game and Wildlife Management. The experimental work was conducted at the College's salmonid hatchery, which has proven to be a valuable resource for applied research work, particularly because of it's protected groundwater supply which provides a controlled environment for experimental studies. The external supervisor at Sparsholt College was the Principal, Dr L. Norman.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the supervision given him by Professor N.R. Bromage and Dr L. Norman, together with the considerable support given by his family throughout.

This study was supported by Sparsholt College, Hampshire.

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GLOSSARY OF TERMS AND ABBREVIATIONS

Alevin: yolk-sac fry; life stage of salmonid species from hatching to the start of exogenous feeding; fish are dependent on yolk reserves.

Alkalinity: measure of the capacity of a mineral solution to neutralise hydrogen ions.

Caudal fin: tail fin.

Chorion: shell of egg.

Degree-day: one degree-day equals 1°C above 0°C for 24 hours.

Embryo: developing fish prior to hatching.

Eyed egg: when retinal pigmentation is first visible without magnification, the eggs are designated as "eyed".

Fork length: straight line distance between the tip of snout and point of division of the caudal fin.

Fry: stage beginning with the independence from yolk-sac as a primary source of nutrition and commencement of exogenous feeding. This stage terminates when parr marks are clearly visible.

Gametes: sex cells (sperm and ova).

Globulin: one of a group of proteins insoluble in water, but soluble in dilute solutions of neutral salts.

Grilse: adult salmon reaching sexual maturity after one sea winter.

Hatching: alevins which have completely escaped from the chorion are considered to have hatched, even though some died immediately after emerging.

Incubation time/period: time taken from initial fertilisation of egg to, and including, the median hatching day in the hatching period; period of embryonic development.

Iodophor: iodine-based solution used for disinfection of eggs.

Larva: immature form, which must undergo a change of appearance or metamorphosis before reaching the adult state.

Lethal: mortal; causing total mortality.

Lipid: any of a group of organic compounds consisting of the fats and other substances of similar properties which are insoluble in water.

Malachite green: fungicide commonly used to control Saprolegnia parasitica.

MAWW: maximum alevin wet weight.

Milt: sperm-bearing fluid of fishes.

Mortality: death.

Oedema: a condition characterised by an excess of watery fluid collecting in the cavities or tissues of the body.

Osmoregulation: process by which stable osmotic pressures are maintained in the body.

Parr: juvenile salmon with distinctive vertical marks on flanks.

S½: half-year-old salmon smolt.

S1: one-year-old salmon smolt.

S2: two-year-old salmon smolt.

Shocking (of eggs): physical agitation of eyed eggs, which ruptures the yolk membranes and turns unfertilised eggs white.

Smolt: fully silvered juvenile salmon during its first seaward migration, and is physiologically capable of surviving transition from freshwater to seawater.

Standard length: straight line distance between the tip of snout and the posterior end of the last caudal vertebra.

Stocking density: biomass per unit volume.

Subacute: having effects less severe than those observed at lethal temperatures.

Sublethal: not killing.

Swim-up: post-alevin stage when fry rise to the surface to inflate the swim-bladder and commence feeding; more pronounced in trout than salmon.

Tolerance: ability to survive indefinitely at a given temperature.

Total length: straight-line distance between the tip of snout and the terminal end of the caudal fin.

Upper temperature limit: highest temperature permitting indefinite survival.

Water hardening: process by which an egg absorbs water into the perivitelline space.

LIST OF SCIENTIFIC AND COMMON NAMES OF SALMONID SPECIES

Scientific name	Common name
Coregonus albula	vendace
Coregonus artedii	lake herring
Hucho hucho	huchen
Oncorhynchus gorbuscha	pink salmon
Oncorhynchus keta	chum salmon
Oncorhynchus kisutch	coho salmon
Oncorhynchus masou	masou salmon
Oncorhynchus mykiss (formerly *Salmo gairdneri*)	rainbow trout
Oncorhynchus nerka	sockeye salmon
Oncorhynchus tshawytscha	chinook salmon
Salmo salar	Atlantic salmon
Salmo trutta	brown trout, sea trout
Salvelinus alpinus	Arctic char
Salvelinus clarki	cutthroat trout
Salvelinus fontinalis	brook char, brook trout
Salvelinus namaycush	lake trout
Thymallus thymallus	grayling

Chapter 1 General Introduction

This study investigated the effects of two important factors on egg and fry development in Atlantic salmon: firstly, the effects of varying temperature regimes on the development and survival of eggs and alevins; and secondly, the influence of time of first feeding on fry survival and growth. Temperature is the main environmental factor affecting the development rates of salmon eggs and alevins, while the transition to first feeding is a critical stage in determining the ultimate growth and survival of fry. The determination of optimal conditions for the early development of salmon will contribute to significant economic improvements in salmon rearing practices. The value of the information produced in this study lies primarily in its application to promoting more efficient rearing techniques in the production of rapidly growing fry, which will subsequently become smolts at an early age.

In salmon hatcheries, the prime objective is to produce large parr which will attain smolt status after one year. This is because one-year-old smolts (S1) are preferred to smolts from older age classes (S2, S3...) primarily because S1 smolts occupy the freshwater facilities for only one year and hence are cheaper to produce. Because production time is reduced, manpower can be used to better advantage. Feed costs are also less for one-year-old smolts. Additionally, S1 smolts produce proportionally fewer grilse than S2 or S3 smolts (Ritter, 1975); in ranching programmes they yield as many adult returns as do S2 smolts of the same size (Peterson, 1973). There are also indications that S1 smolts have a greater resistance to furunculosis (Aeromonas

salmonicida) than S2 smolts (Piggins, 1986).

The decision whether or not salmon smoltify in the spring is taken some 8-9 months before the event when bimodality is established - this is the segregation into two distinct sub-populations (upper and lower modes) which grow at different rates (Thorpe et al., 1980; Stefansson et al., 1990). Bimodal segregation becomes evident in late summer or early autumn of their first year (Thorpe et al., 1980). The upper mode fish continue to grow throughout the year while the lower mode fish show reduced feeding activity (Higgins and Talbot, 1985; Metcalfe et al., 1986; Metcalfe and Thorpe, 1992a), leading to reduced growth rate. Higher growth rate among the upper mode fish is associated with smoltification in the next spring (Higgins, 1985), while smoltification among the lower mode fish will occur at least one year later. It is therefore important to identify the optimal hatchery conditions which will promote early growth so that a greater proportion of salmon parr enter the upper mode group when bimodality becomes established. Both temperature and time of first feeding are two factors which can be manipulated in salmon hatcheries to promote early feeding and growth, thereby increasing the proportion of fish entering the upper mode and ultimately the number of S1 smolts produced. The employment of warmed water is of particular benefit to salmon hatcheries located in sub-arctic regions, where low ambient temperatures severely restrict the potential for S1 production.

Although salmon are normally reared for at least one year in freshwater before being tranferred to seawater facilities in the spring, there has been an upsurge of interest in the

use of half-year-old smolts ($S\frac{1}{2}$) for on-growing. This would lead to considerable economic benefits by reducing further the protracted production cycle which is normally two and a half years for grilse and three years for 2-sea-winter salmon. Clearly, the ability to produce $S\frac{1}{2}$ smolts is very much dependent upon the attainment of rapid growth in the hatchery. In an increasingly competitive salmon farming industry, it is important that the full potential for growth improvement and survival is achieved to provide maximum fish production at minimum cost.

In addition to providing valuable temperature data for the planning of salmon production strategies in hatcheries, the information produced in this thesis is also essential for studies on the effects of thermal pollution on natural populations of salmon. In this respect, thermal criteria need to be determined which will safeguard any sensitive stages of development in the life cycle of the Atlantic salmon. This study will provide detailed information on the temperature tolerances of eggs and alevins.

Chapter 2 Effects of varying temperature regimes on the development and survival of Atlantic salmon (*Salmo salar* L.) eggs and alevins.

2.1 INTRODUCTION AND OBJECTIVES

Temperature is the primary environmental factor affecting the various life processes of poikilothermic animals. All fish species have a specific optimal temperature range for development, growth and survival (reviewed by Blaxter, 1969).

Salmonid species are well represented in temperate and sub-arctic regions of the world and because of their adaptations for survival in cold environments, are well suited for aquaculture in such areas. However, very low freshwater temperatures retard development and growth and are an economic constraint to culture: generally, low temperatures prolong and high temperatures decrease development times. Growth and survival of salmonid fry in hatcheries requires considerable attention from an economic point of view, to provide maximum production at minimum cost. The use of elevated water temperatures in hatcheries offers promise of further increasing production, while at the same time reducing production costs.

Although considerable applied research and development work has been undertaken to increase fish production by using warmed water to accelerate the development and growth of salmonids during their freshwater rearing phase (Markus, 1962; Donaldson and Brannon, 1976; Ingebrigtsen and Torrissen, 1980; Kjolseth, 1980; Møller and Bjerk, 1980; Reid, 1980; Sumari and Westman, 1980; Brannon et al., 1982; Isaksson, 1985; Hiroi et al., 1988; Kazakov et al., 1988), relatively few detailed studies of temperature effects during early development have been conducted, and consequently the

temperature limits for the different stages of development in many species of salmonid have not been clearly defined.

The extent to which salmonid eggs and alevins can adapt to varying temperature conditions is largely unknown. In general, salmonid species show rather narrow ranges of temperature tolerance, suggesting that such adaptive ability is restricted. In hatcheries using surface water supplies at ambient temperatures, salmon eggs are normally incubated at temperatures below 8°C. Hatcheries using groundwater supplies have shown that newly-fertilised Atlantic salmon eggs can tolerate a constant 10°C throughout the incubation period but the upper temperature limit has not been precisely identified. Thus, further studies are required to investigate the effects of temperatures exceeding 10°C on egg and alevin development and survival. This study will attempt to establish the upper temperature limits for Atlantic salmon eggs and alevins. It will also examine any effects that egg and alevin development at high temperatures might have on the subsequent survival and growth rates of fry.

Under hatchery conditions, it may be possible to control and maintain water temperatures within a narrow range for optimal egg and alevin development. This is of considerable importance because discrete stages of development may have different temperature limits and optimal ranges. It is therefore important to investigate the effects of high temperatures at known critical periods of development: during egg incubation, blastulation and gastrulation are particularly sensitive stages (Johnson and Brice, 1953; Post et al., 1974; Jensen and Alderdice, 1983, 1989; Rosenberg,

1985; Johnson et al., 1989). Following hatching, alevin growth and development during the period of yolk dependence is an important determinant of later survival because any environmental factor that affects yolk conversion efficiency could affect alevin size and condition, and consequently their ability to begin exogenous feeding. If the rearing temperature is increased much above the optimal range in an attempt to increase the rate of development, a decrease in survival may result.

In addition to providing data for the planning of fish production strategies in hatcheries, such information is also needed for studies on the effects of thermal pollution in rivers and streams, which is having an increasingly negative impact on the reproduction of many species of salmonid. Thermal pollution is caused by the periodic or continuous discharge of warm cooling water from industrial installations. Additionally, natural sources of heated water may expose salmonids to high temperatures during critical stages of development. A study by Kaya (1977) reported that geothermally heated waters increased downstream temperatures by about 10.5°C and detrimentally affected the reproductive biology of both brown and rainbow trout populations. In this field, little data on lethal temperatures are available for the Atlantic salmon.

The effects of temperature on the early development of salmonids have been established during years of artificial propagation in hatcheries and related experimental studies. Reviews of the various literature pertaining to the effects of temperature on development rates among salmonids have been

made by Hayes (1949), Hayes et al. (1953), Alderdice and Velsen (1978), and more recently by Crisp (1988). One of the earliest studies was conducted by Ainsworth (1859) and determined the development times from fertilisation to hatching for brook char eggs within the temperature range of 3-12°C. Ainsworth (1859) published some fragmentary data showing that high incubation temperatures increased the rate of development in brook char eggs. These findings were confirmed by later studies (Green, 1870; Wallich, 1901; Embody, 1934; Seymour, 1956) which investigated the effect of temperature on the development rates of several salmonid species.

Various early attempts were made to formulate a precise index of development in relation to temperature. Ainsworth (1859) and Green (1870) were the first to offer a rule showing the relationship between temperature and the incubation time for fish eggs. Green (1870) stated that the incubation time required for brook char eggs to hatch was relatively constant for any given temperature. Later, Wallich (1901), having studied the development times of eggs from various salmonid hatcheries, published an account of the relationship between temperature and development time for chinook salmon and formulated the concept of thermal units, defining a thermal unit (or degree-day) as an increase of 1°F above 32°F (the freezing point of freshwater) for a period of one day. Wallich (1901) reported that, within the temperature range of 5-10°C, there was a linear relationship between temperature and development time. Degree-days are now generally determined from the product of development time in

days to reach any given stage of development and the mean temperature in degrees Celsius above 0°C during that time. Although the relationship between temperature and development time appears to be non-linear (Battle, 1944; Garside, 1966; Alderdice and Velsen, 1978; Crisp, 1981; Jungwirth and Winkler, 1984; Humpesch, 1985a; Tang et al., 1987) towards the upper and lower temperature limits for each species, the degree-day concept is still widely used by hatchery managers because it is the simplest and most practical means of accounting for changing temperatures during development.

Various empirical models (Garside, 1966; Peterson et al., 1977; Alderdice and Velsen, 1978; Jungwirth and Winkler, 1984; Tang et al., 1987; Crisp, 1988; Kaeriyama, 1989) have been formulated to describe more precisely the dependence of development times of salmonid eggs and alevins on water temperatures. The present study will examine the relationship between temperature and development times for Atlantic salmon eggs and alevins at temperatures equal to, and greater than, 8°C.

Incubation temperatures not only influence development rates of salmonid eggs and alevins (Embody, 1934; Crisp, 1981, 1988; Jungwirth and Winkler, 1984) but also affect the size of alevins at hatching (Gray, 1928b; Garside, 1966; Timoshina, 1972; Hamor and Garside, 1977; Peterson et al., 1977; Heming, 1982; Murray and Beacham, 1986) and MAWW (Murray, 1980; Heming, 1982). During development, incubation temperatures influence the size of salmonid embryos and alevins as a result of temperature-dependent changes in the efficiency of yolk conversion to body tissues (Gray, 1928a;

Marr, 1966; Hamor and Garside, 1977). Temperature regimes during development may also have a significant impact on subsequent hatching and survival of salmonid alevins (Combs and Burrows, 1957; Bishai, 1960; Markus, 1962; Brannon, 1965; Bams, 1969; Fowler, 1972 ; Peterson et al., 1977; Gunnes, 1979; Murray and Beacham, 1986). Although the association between temperature and development times for eggs and alevins is widely documented for many species of salmonid, few studies have attempted to assess the functional relationship between temperature and the duration of development and survival at temperatures near the upper limit. The most detailed study was conducted by Peterson et al. (1977) on the effects of systematic temperature variation during the development of Atlantic salmon eggs and alevins. However, the only available experimental data on egg and alevin development in Atlantic salmon has been obtained from experiments conducted at temperatures up to 12°C (Markus, 1962; Hamor and Garside, 1976; Foda and Henderson, 1977; Peterson et al., 1977; Gunnes, 1979). Furthermore, few studies have tested a series of closely-spaced temperatures. Hence, predictions of development times at higher temperatures are based on extrapolations from reported data and not from observations. Furthermore, many studies have focused on predicting and monitoring development rates during egg incubation, and have not examined the alevin stage. Most studies have been directed toward establishing optimal hatchery temperatures rather than the determination of the upper temperature limit for incubation. The upper temperature limits have not been clearly identified for many species of

salmonid, including the Atlantic salmon, nor have relationships been established between those temperatures and development times.

Studies by Markus (1962), Peterson et al. (1977) and Gunnes (1979) have shown that egg mortality increased significantly at a constant temperature of 12°C. However, no published work has been found which has investigated temperatures greater than 12°C on egg survival. Among other salmonid species, the upper temperature limits for eggs are more clearly defined. At 12°C, severe mortalities have been reported for brown trout (Jungwirth and Winkler, 1984), brook char (Hokanson et al., 1973), and Arctic char (Swift, 1965; Jungwirth and Winkler, 1984; Humpesch, 1985a). In the genus Oncorhynchus, the upper temperature limits are higher, ranging from about 14-17°C, depending on species: for chinook salmon (Garling and Masterson, 1985), coho salmon (Tang et al., 1987), chum salmon (Hiroi et al., 1988) and rainbow trout (Timoshina, 1972; Kwain, 1975a, b; Humpesch, 1985a), the upper temperature limits are about 14-15°C, whilst for sockeye salmon, the upper limit is closer to 17°C (Combs, 1965).

Few workers have investigated the influence of temperature variation from fertilisation through embryonic development to MAWW: the majority of studies have largely focused on survival during the incubation period from initial fertilisation to hatching. However, the few that have investigated the effects of high temperatures on the survival of salmonid alevins, have demonstrated that alevins have greater thermal tolerance than eggs. Bishai (1960) reported that the upper temperature limits for Atlantic salmon and

brown trout alevins were between 20-23°C, depending on the degree of thermal acclimation. Though brook char alevins have a similar level of thermal tolerance (McCormick et al., 1972), Arctic char alevins suffer high mortalities at 12°C (Wallace and Aasjord, 1984b). For species within the genus Oncorhynchus, the upper temperature limits have not been established. Although high levels of alevin mortality have been reported for rainbow trout (Timoshina, 1972), chinook salmon (Heming, 1982; Garling and Masterson, 1985), coho salmon (Tang et al., 1987) and chum salmon (Beacham and Murray, 1985) at temperatures of 12-15°C, these losses appear to be caused by the indirect effects of adverse temperatures during egg incubation, rather than the direct effects of high temperatures during the alevin stage. Little attention has been given to the effects of accelerated development on the subsequent survival and growth of fry both during and after the critical transition from endogenous to exogenous nutrition.

In hatcheries located in the northern parts of the Atlantic salmon's natural range, juvenile salmon are reared under low temperature conditions for the greater part of the year and these temperatures arrest or retard their development and growth. In northern parts of Norway, conditions are not favourable for conventional smolt production, with average air temperatures being minus 4°C during February and only 14°C during July. With Arctic river water temperatures close to freezing point, hatching generally occurs during the period from April to June. Arctic rivers such as the river Alta, which lies to the north of Tromsø, have a relatively

constant water temperature of only 0.1°C during the winter months (Wallace and Heggberget, 1988). Where hatcheries have a water supply colder than about 4.5°C, and where 60 or more days are required for absorption of the yolk-sac following hatching, Markus (1962) has reported that fry become sensitive and predisposed to certain diseases resulting in high losses.

There are a number of advantages to be gained from reducing the hatchery rearing period using controlled temperature regimes. Isaksson (1985) reported that in Iceland the elevation of egg incubation and alevin rearing temperatures to 10°C advanced the time of first feeding by 4 months. Clearly, advancing development at this time will extend the growing period for fry and parr, thereby producing a higher proportion of one-year-old smolts. Furthermore, by maximising the growing period, larger one-year-old smolts can be produced. Because of very cold seawater temperatures in sub-arctic regions and consequent low growth rates, it is necessary to use a larger smolt in ocean pens in order to obtain a 3.5 kg Atlantic salmon after 18-19 months at sea (Sutterlin, 1980). The use of warm water in hatcheries will also prevent problems caused by freezing water supplies in winter.

Consideration must also be given to the subsequent survival at sea of salmon smolts reared in a temperature-controlled environment. Donaldson and Brannon (1976) stated that the adult return rates from coho salmon smolts produced at high temperatures were very good, with the majority of returning adults being 2-year-old instead of 3-year-old, which is the

normal age for non-advanced returning stock. Ocean survival of larger Atlantic salmon smolts released from eastern Canadian hatcheries (Sutterlin, 1980) has been shown to be greater than smaller smolts due to an improved osmoregulatory function among larger individuals (Hoar, 1976). Clearly, temperature can be manipulated in hatcheries to produce larger S1 smolts which will show improved seawater survival. This will be of considerable economic benefit to marine farming and ocean ranching operations.

The subject of rearing "zero-age" or S½ smolts (autumn smolts) is being reviewed with increased interest by fisheries managers and Atlantic salmon farmers. The ability to transfer Atlantic salmon to seawater in the autumn would be of considerable benefit to the industry, as it would reduce the rearing time and eliminate the problem of overlapping year-classes in the hatchery. Furthermore, it would also allow fish to be transferred outside the normal "window" of smoltification in the spring; this would provide a more consistent supply of market-size salmon thereby improving the cash flow for salmon producers. Isaksson (1985) considered the prospects of producing zero-age smolts of Atlantic salmon using geothermal resources while Brannon et al. (1982) reported that zero-age coho salmon smolts could be produced by using an incubation temperature of 11°C; first feeding commenced in February and by May the parr were already 11 g.

More recent studies (Bergheim et al., 1990; Bjerknes et al., 1992; Solbakken et al., 1994; Thrush et al., 1994) have shown that, providing parr have reached the critical size

threshold for smoltification to proceed (Johnston and Saunders, 1981; Nicieza et al., 1991), temperature and photoperiod can be manipulated to regulate the timing of smoltification in Atlantic salmon.

It is apparent that considerable benefits can be gained by using warmed water in Atlantic salmon hatcheries. However, the extent to which egg and alevin development can be accelerated without causing significant mortalities has not been fully established. Consequently, one of the main objectives of this study is to define the upper temperature limits for Atlantic salmon eggs and alevins.

Measures taken to counteract the low temperature constraint to aquaculture include the use of heating systems and thermal effluents to raise ambient water temperatures. Consequently, heating systems are employed primarily to enhance the production of one-year-old smolts, particularly in Polar regions of Norway, where smolts would naturally be S4s or even S5s. Similarly, in salmon hatcheries in the north eastern region of the United States and Canada, where ambient surface water temperatures are as low as 0.6°C in streams and only slightly higher in lakes (about 3°C), it is necessary to incorporate water heating systems (Sutterlin, 1980). Where heating systems are installed in hatcheries the temperature is generally maintained within the range of 6-10°C.

Heating systems employed by salmon farms generally include the use of conventional systems such as oil-fired low pressure boilers (Markus, 1962) to raise the temperature of natural waters. The heat is then recovered from the waste water using a heat exchanger and the efficiency of heat

recovery may be improved by the incorporation of a heat pump. An alternative although less common approach to raising temperatures in salmon hatcheries involves the employment of water recirculation technology. Using a recirculation system, Mϕller and Bjerk (1980) reported that Atlantic salmon eggs were incubated at 7°C rising to 11°C at hatching. The alevins were then maintained at 11°C throughout the yolk-sac and the early feeding period. Using this temperature regime, mortality from fertilisation to hatching was 48% with a further 2% during the alevin stage. The high egg mortality was caused by a lack of stock husbandry knowledge and experience by hatchery operators, and was not attributed to any temperature effects.

Another solution to the problems imposed by low freshwater temperatures includes the use of heat exchange systems using seawater (Kjolseth, 1980), which is naturally warmer than freshwater during the winter months.

A further possibility includes the utilisation of waste heat from hydroelectric and nuclear power stations (i.e. energy dissipated as heat into cooling water and discarded) to accelerate the production of Atlantic salmon smolts for ocean ranching or marine farming. The utilisation of waste heat in salmonid culture has been employed in several countries, including Norway (Ingebrigtsen and Torrissen, 1980), Canada (Reid, 1980; Sutterlin, 1980), the United States (Donaldson and Brannon, 1976) and Russia (Kazakov et al., 1988). Generally, the heated effluent water is first passed through a heat exchange system and then degassed before being supplied to salmon stocks. Degassing of water

which is supersaturated with dissolved nitrogen is required to prevent gas bubble trauma (Rucker and Hodgeboom, 1953; Nebeker et al., 1978) among salmonid alevins and fry. Where heating systems are used in hatcheries, further water quality problems can arise from the presence of heavy metals which are highly toxic to salmonids, even at very low concentrations (Wildish et al., 1971; Spear and Pierce, 1979; Sutterlin, 1980). Copper leaching from heating coils is a potential problem, particularly in acid waters. Attention must therefore be given to the use of heating systems which are manufactured from non-toxic materials (e.g. stainless steel).

In Icelandic hatcheries, the production of one-year-old Atlantic salmon smolts, primarily for use in salmon ranching programmes, is dependent upon the use of geothermal water from hot springs (Isaksson, 1985). Because the temperature of geothermal water is extremely high (50-100°C) and often contains toxic levels of sulphur, heat exchange systems are used to heat pure sources of cool groundwater.

In view of the increasing cost of energy, it is important to determine whether the cost of manipulating water temperature is justifiable. From an assessment of thermal effluent aquaculture facilities in Canada, Reid (1980) concluded that economic viability is possible, but that capital and running costs must be kept to a minimum, particularly for small-scale hatchery operations. Jungwirth and Winkler (1984) reported that with low investment costs for heating, high commercial gains can be achieved, while Peterson (1973) stated that the economic costs incurred

during intensive rearing of salmon smolts were lower than those required for production under naturally low temperature conditions.

Clearly, the additional costs incurred by the use of heating systems to reduce development times and increase growth rates, must be offset by the production of a significantly higher proportion of salmon smolts in year one. Using heating systems, temperatures can be manipulated to ensure that fry are ready to feed at optimal times relative to the hatchery's water-heating budget when minimal quantities of water must be heated, or when ambient temperatures are adequate for first feeding. For accelerated smolt production to be cost-effective, fry must be ready to feed as soon as possible, but not so early that excessive water heating costs will be incurred to stimulate first feeding.

Thus, because temperature can be regulated in salmonid hatcheries by installing heating systems or using warm water from industrial effluents or geothermal sources, it is important to establish the optimal temperature regimes for the planning of cost-effective fish production strategies. The results of this study will provide hatchery managers with important data required for formulating optimal temperature regimes for maximising the production of Atlantic salmon smolts.

This study was undertaken to determine the extent by which the early stages of development in Atlantic salmon can be accelerated by heating water, without causing significant mortality. The investigation studies the consequences of

systematic temperature variation on the development of Atlantic salmon eggs and alevins. An important objective was to establish the upper temperature limits for Atlantic salmon eggs and alevins. Development times (in days and degree-days) and mortalities were recorded from fertilisation to eyeing, from eyeing to hatching, and from hatching to MAWW - the three important operational stages which occur in hatcheries. The temperature was held constant during each of these three stages of development. It was also important to examine any interactions that might occur between the effects of temperature during one stage of development and another, as well as examining the effects of varying temperature regimes on the duration of the hatching period and on alevin size at hatching and at MAWW.

At MAWW it is vital to confirm that the resulting "accelerated" alevin has all the survival and growth potential of its unprovoked sibling. The transition from a diet of yolk material to exogenous feeding is much more important than hatching in determining the ultimate survival of a salmonid embryo (Balon, 1984). Having obtained early fry, further investigation into the comparative sensitivity between these advanced fry and those of a normal hatch was conducted. At MAWW, fry were transferred to rearing tanks for first feeding; MAWW has been closely associated with the optimal time to begin exogenous feeding (Marr, 1965; Heming, 1979; Heming et al., 1982), although hatchery managers tend to feed alevins near the lower limit of the range of predicted times to MAWW, probably because the decision to initiate feeding is based on the appearance of the most

advanced alevins in a batch of fish. Following MAWW, fry survival and growth rates were monitored during several weeks of feeding to ensure that fry were capable of changing to exogenous sources of food.

The effect of temperature on the net biomass gain (product of the number of fish extant and their mean individual weight) was also examined for alevins at MAWW and for fry at the end of the study.

In summary, the specific objectives of this study were to determine:

(a) The upper temperature limits for eggs and alevins.

(b) The effects of varying temperature regimes on:

  (i) The development times from fertilisation to eyeing, hatching and MAWW.

  (ii) The duration of the hatching period.

  (iii) Alevin size at hatching and MAWW.

(c) Whether the temperature regimes during egg and alevin development affect the subsequent survival and growth rates of fry.

(d) The optimal temperature regimes for maximum biomass gain.

Four series of experiments were designed to compare and contrast the development and survival of Atlantic salmon eggs and alevins at varying temperature regimes within the range of 8-22°C. No such investigations have so far been systematically undertaken for Atlantic salmon.

2.2 MATERIALS AND METHODS

2.2.1 SOURCE OF STOCK AND METHODOLOGY OF GAMETE COLLECTION

Atlantic salmon (Salmo salar L.) gametes were obtained from adult broodstock which were caught by electro-fishing in the Rivers Test and Itchen, Hampshire, England, UK and by electro-fishing or trapping (in-scale fixed trap) in the River Thames, Berkshire, England, UK. Broodstock were generally held in captivity for a short period of time until ready for stripping. Broodstock details are shown in Table 2.2.1. To reduce any paternal effects on the performance of fish, milt from one male was used to fertilise the eggs of a single hen. Eggs were fertilised by the dry fertilisation method (Leitritz and Lewis, 1976) and, following washing and water hardening, were transported to the hatchery. Eggs reached the hatchery within 3 hours of stripping, where they were disinfected in a 50 ppm solution of iodine (Buffodine; Evans Vanodine International Ltd., Preston, Lancashire, England, UK) for 10 minutes prior to being transferred to the experimental system. For each experiment eggs were derived from a single hen. A random sample of 50 eggs was withdrawn from each batch of eggs for measurement of their outside diameter using a micrometer. Table 2.2.1 shows the derivation of eggs, date of stripping, and egg size for each of the batches used in the various experiments.

Table 2.2.1 Details of stock used in experimental Series 1-4: river of broodstock origin, weight and age of female broodstock, date eggs stripped and set (date experiment commenced), mean egg size (outside diameter) and broodstock holding system prior to stripping.

Expt. Series	River of origin	Wt. female broodstock (kg)	Age (yrs)	Date ova set	Egg dia. (mm)	Holding system
1	Test	3.6	1.2	30.12.86	5.8	Round tank
2	Test	3.3	2.1	22.12.87	5.7	None
3	Test	5.5	1.2	11.01.89	6.0	Raceway
4A	Thames	2.9	2.1	12.11.89	5.6	FW cage
4B	Thames	2.4	1.1	15.12.89	5.5	FW cage
4C	Itchen	2.3	1.1	09.01.90	5.6	None

2.2.2 EXPERIMENTAL APPARATUS AND DESIGN

2.2.2.1 Egg incubation and alevin development

The experimental apparatus comprised 5 independent constant-temperature units as shown in Fig. 2.2.1. Each unit consisted of an insulated polythene water-bath of 54 litres capacity (base = 40 x 45 cm, depth = 30 cm) in which was suspended a rectangular permeable tray (base = 40 x 30 cm, height = 6 cm), divided into 40 identical cells (5 x 6 x 6 cm) each holding up to 100 fry. The water depth in each bath was set at 27 cm by means of an external stand-pipe, giving a standardised water volume of 48.6 litres in each bath. The trays and cells were prefabricated from sheets of 2 mm perforated polyvinyl chloride (PVC). The water depth in each cell was 4 cm. Each tray was fitted with a PVC lid to prevent fry from escaping. Of the 5 constant-temperature units employed in this investigation, one unit incorporated a cooling system which maintained sub-ambient temperatures, a second was maintained at the ambient groundwater temperature (approximately 10°C), and the remaining 3 units incorporated heating systems which maintained water temperatures above that of ambient.

The cooling system employed in unit 1 comprised a refrigeration compressor with a stainless steel coil (Techne Dip Cooler RU200, Techne (Cambridge Ltd., Cambridge, England, UK). The temperature in the water-bath was accurately controlled by an electrical thermometer and relay (Tetcol, The Electrical Thermometer Co. Ltd., Thetford, Norfolk,

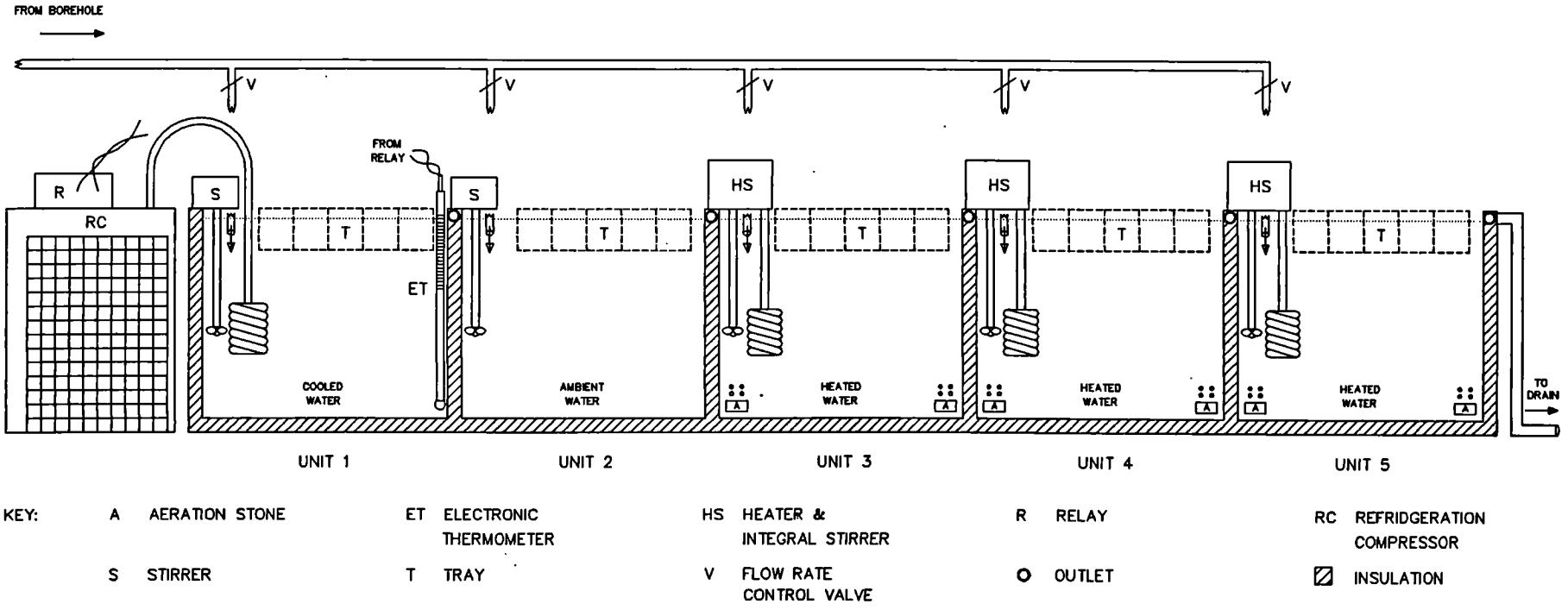

Fig. 2.2.1 Diagram of the experimental apparatus used in the temperature tolerance studies on Atlantic salmon (Salmo salar L.) eggs and alevins. For Series 4, units 1 and 2 were maintained at the ambient water temperature.

England, UK) connected to the refrigeration compressor. In order to maintain a constant temperature throughout the water-bath, a stirrer (Grant S2, Grant Instruments (Cambridge) Ltd., Cambridge, England, UK) was positioned next to the cooling coil.

Unit 2 was maintained at the ambient groundwater temperature of 10°C. A stirrer (Grant S2) was incorporated to generate an efficient circulation throughout the water-bath and tray.

Elevated water temperatures were maintained in each of units 3-5 with a thermostatically-controlled electric heater and integral stirrer (Grant Type SU6; Grant Instruments (Cambridge) Ltd., Cambridge, England, UK). The copper heating coils were plated with stainless steel. The practice of heating water can cause supersaturation with dissolved gases, primarily nitrogen and oxygen, due to lower gas solubility at higher temperatures. If aeration-stripping techniques are not employed to remove excess gases, a potential hazard is created for developing alevins (Rucker and Hodgeboom, 1953; Nebeker et al., 1978). To prevent gas bubble trauma among alevins and fry in the 3 heated units (units 3-5), the water was continuously aerated. Aeration to each of the 3 units was supplied by 4 aquarium air stones connected to a twin piston, low pressure aeration pump having a maximum pressure of 35kPa and output of 1 L/min of free air.

Each unit was provided with a continuous single pass water flow which was controlled by an individual 19 mm gate valve. The rate of flow through each water-bath was measured and adjusted daily to maintain a target value of 1 L/min/1000

eggs or alevins. Flow rates were measured with a stopwatch and graduated cylinder and the velocity of flow through the trays was carefully adjusted to prevent rolling of eggs. The passage of a few drops of dye (zinc-free malachite green) added to the water supply of each unit demonstrated that water circulated efficiently throughout all cells in a tray, prior to running to waste. No water was recirculated.

A black light-proof cover was placed over the whole apparatus; eggs and alevins were kept in total darkness except for brief periods of examination each day.

2.2.2.2 Fry rearing

The experimental facility for fry rearing studies (Figs. 2.2.2 and 2.2.3) comprised three banks of twelve identical tanks with internal diameters of 15, 22 and 30 cm, and respective depths of 12.0, 14.0 and 16.5 cm. The tanks were made of unplasticised polyvinyl chloride (uPVC), smooth on the inside, with self-coloured industrial grey base and sides. An external vertical stand-pipe drain system similar to that described by Parker and Jackson (1980) was incorporated to allow accurate standardisation of water depths (7.0, 8.0 and 9.0 cm, respectively), together with a rapid draw-down for routine flushing.

Water entered each tank tangentially through a single 5 mm orifice with water and detritus voided from each tank by a central, vertically-screened drain. A low-velocity current, maintained by a water flow rate of 1 L/min to each tank, facilitated feed distribution and provided an efficient self-

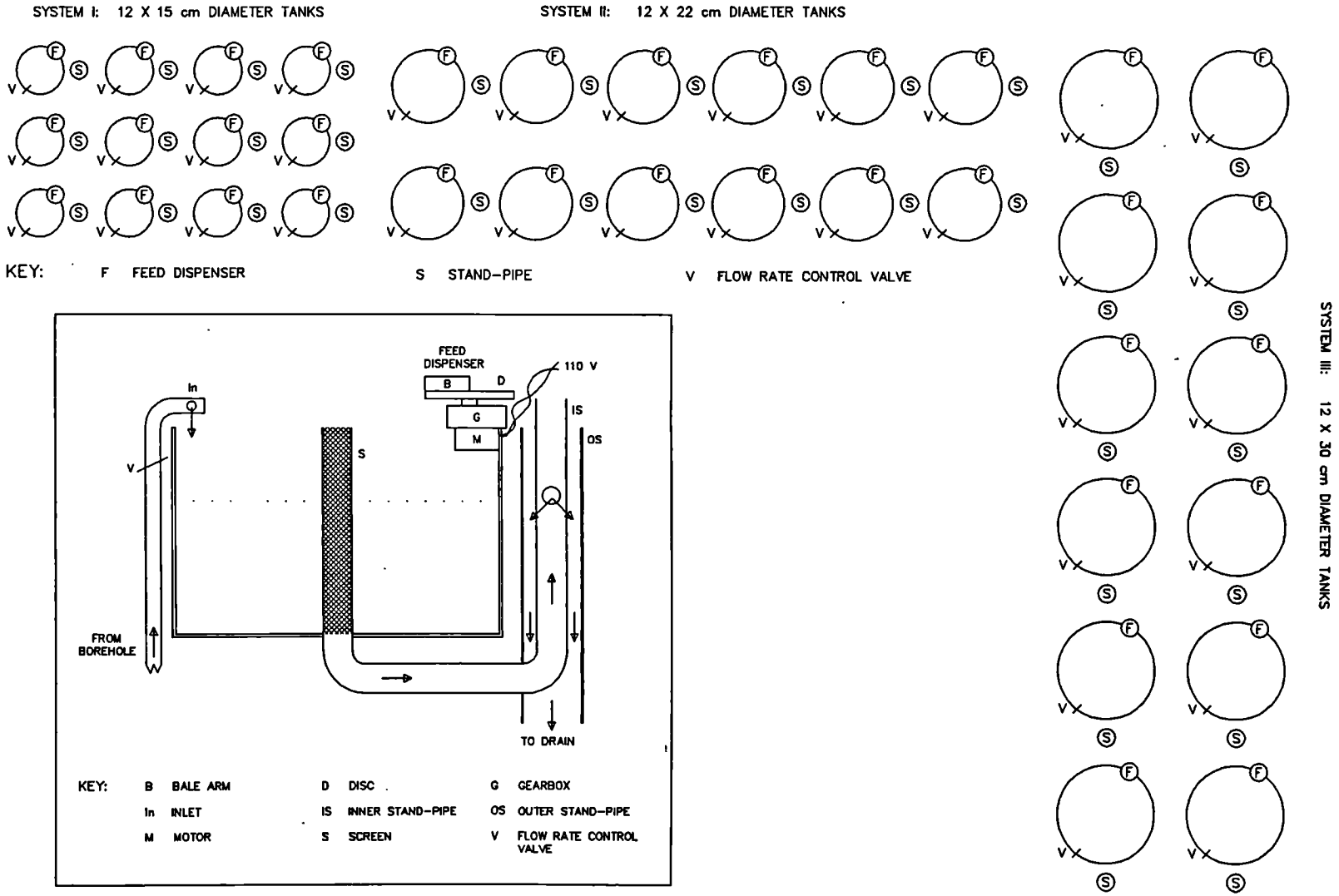

Fig. 2.2.2 Experimental systems for fry rearing studies. Insert shows standardised fry rearing tank with vertical stand-pipe drain and automatic feed dispenser.

Fig. 2.2.3 Photograph of the experimental system for fry rearing studies. A representative section of system II (22 cm diameter tanks) is shown.

cleaning action.

Since the purpose of the experimental design was to maximise feeding opportunity and allow accurate control of feeding input per day, an automatic feed dispenser was designed, which would release small quantities of feed particles continuously during the feeding period. The feed dispenser (Figs. 2.2.2 and 2.2.3) consisted of a 65 mm diameter smooth uPVC disc, mounted above a geared 110V AC electric motor (RS Components Ltd., Corby, Northants, England, UK). The disc was connected to the motor/gearbox (RS Components Ltd.) assembly by a central output shaft. The gearing was adjusted to provide an output shaft speed of 1 revolution every 12 hours. As the disc rotated, a uPVC bale arm pushed feed particles off the edge of the disc into the tank. All feed dispensers operated synchronously via a 110V AC industrial transformer connected to the mains electricity supply.

2.2.2.3 Water quality monitoring

Borehole water drawn from a limestone aquifer was supplied to the experimental apparatus. Monthly analysis of the water supply (Southern Water Authority PLC, Winchester, Hampshire, England, UK) showed that the water quality was constant (Table 2.2.2).

The dissolved oxygen concentration in the inlet water from the borehole supply was 100% of air saturation. During the course of the study, the dissolved oxygen concentrations were determined daily from the outflows of each constant-

Table 2.2.2 Water quality analysis of borehole supply (mg/L unless stated).

Dissolved Oxygen	11.4	Total Zinc	0.0068
Temperature (°C)	10.6	Total Iron	0.0200
pH	7.69	Total Cadmium	0.0016
Alkalinity	244	Total Chromium	0.0008
Calcium	87.10	Total Copper	0.0083
Total Ammoniacal N	0.010	Total Lead	0.0009
Ortho-phosphate	0.010	Total Nickel	0.0012
Nitrate	9.970	Total Manganese	0.0100
Nitrite	0.010	Magnesium	1.82
Chloride	16.3	Sodium	7.62
Conductivity (S)	571	Potassium	0.010

temperature bath with an oxygen meter (pHOX series 67; pHOX Systems Ltd., Shefford, Bedfordshire, England, UK). The dissolved oxygen concentrations were always close to 100% air saturation (range of 95-105%).

Water temperatures in the units were measured to an accuracy of 0.1°C with a calibrated electronic thermister probe (Jenway 2053 and 111P probe; Jenway Ltd., Felsted, Essex, England, UK) and checked with a tested 58 cm immersion mercury thermometer. Measurements were taken and recorded twice daily and adjustments to the temperature control systems, which never exceeded 0.5°C, made daily as necessary. Temperatures were found to be identical in all cells within the same tray. Nominal test temperatures and actual temperatures for the various experiments are shown in Figs. 2.2.4-2.2.7. The range of departure from the means, and the deviation of such departures, were not considered sufficient to influence the results attributed to the long-term exposures to the reported mean temperatures.

2.2.2.4 Experimental design

This study comprised 4 separate but related series of experiments (Series 1-4), which were conducted in consecutive years. Several of the same temperature regimes were used in different series: all experiments included egg incubation, alevin, and fry development at a constant 10°C, a temperature which is known from experience to be acceptable for normal development and survival. In Series 1, lots of newly-fertilised eggs were subjected to constant temperatures of

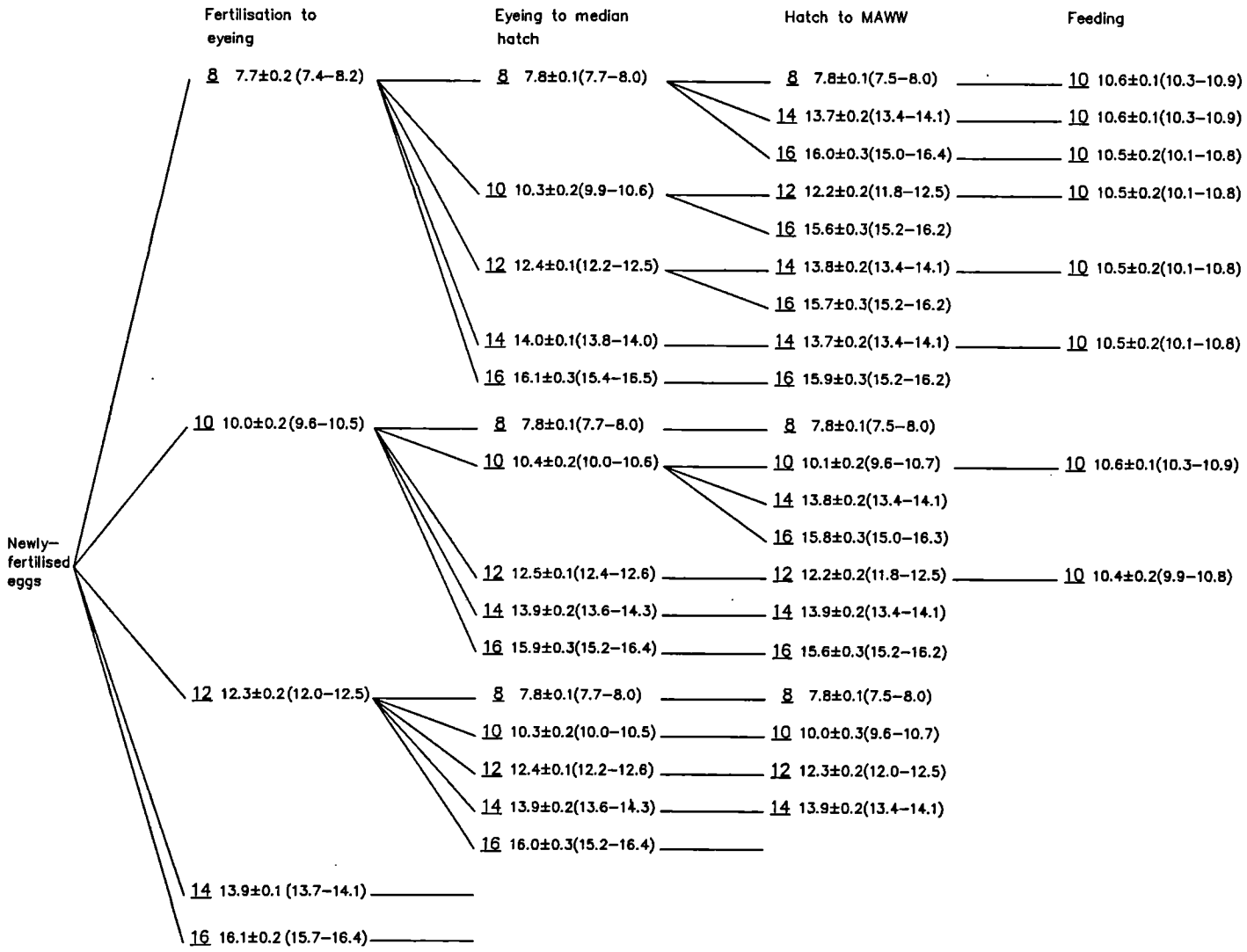

Fig. 2.2.4 Experimental protocol for Series 1 showing target temperatures (underlined), mean experimental temperatures ±SD (°C) and temperature ranges (in parentheses) for each period of development.

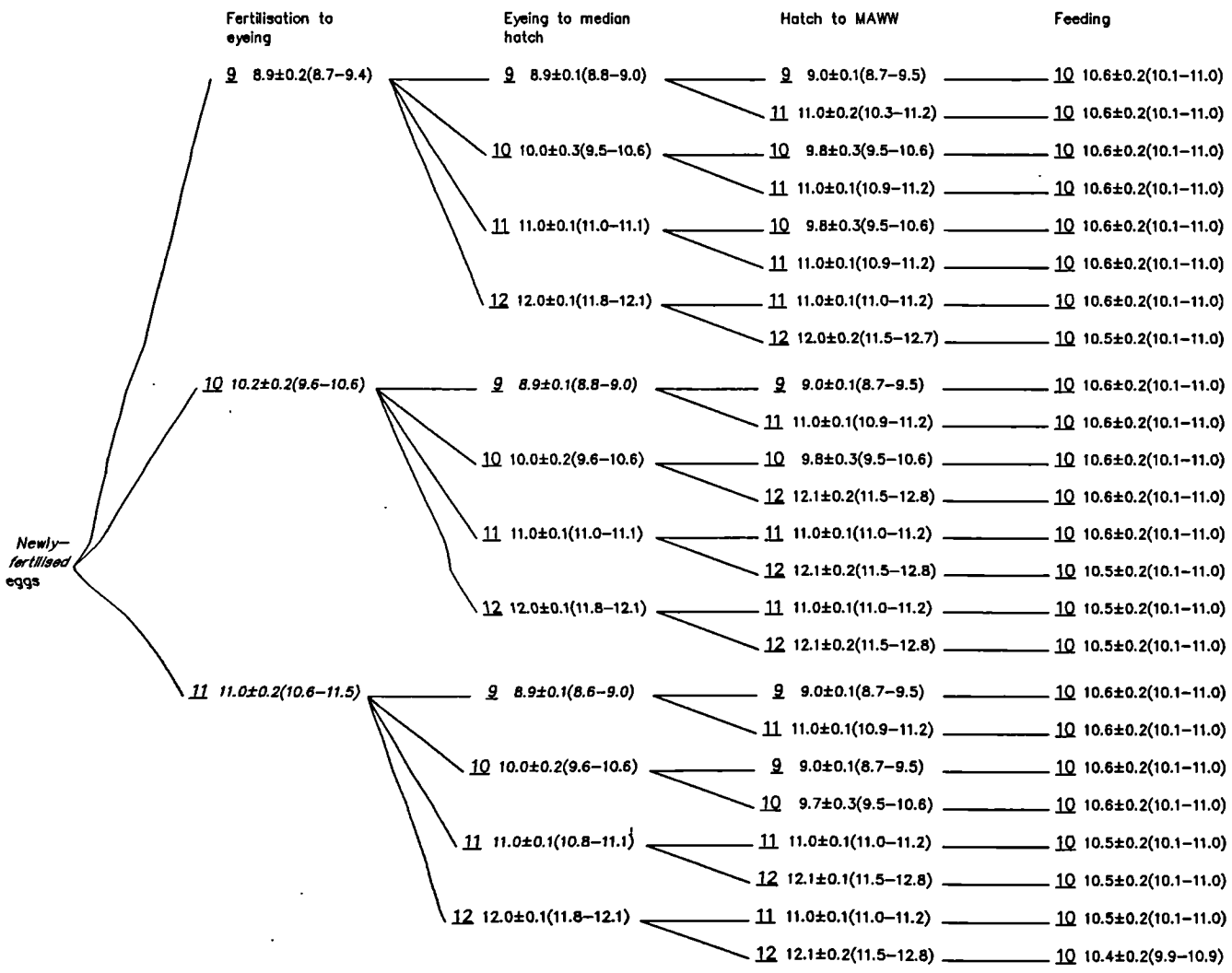

Fig. 2.2.5 Experimental protocol for Series 2 showing target temperatures (underlined), mean experimental temperatures ±SD (°C) and temperature ranges (in parentheses) for each period of development.

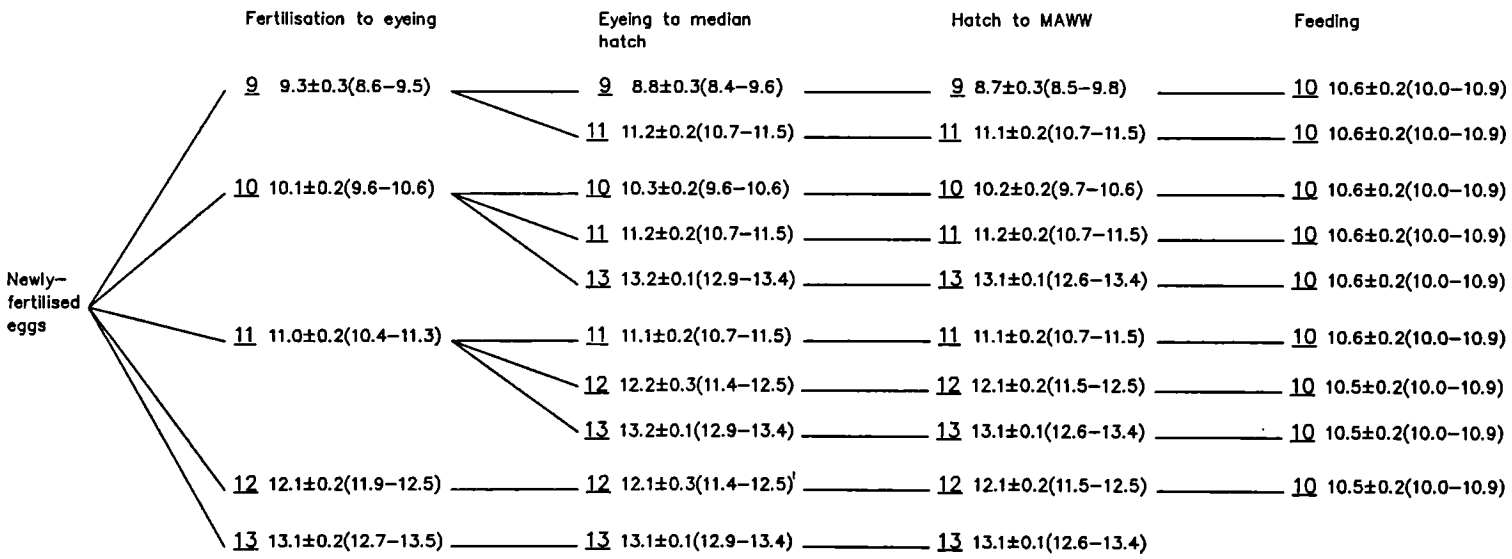

Fig. 2.2.6 Experimental protocol for Series 3 showing target temperatures (underlined), mean experimental temperatures ±SD (°C) and temperature ranges (in parentheses) for each period of development.

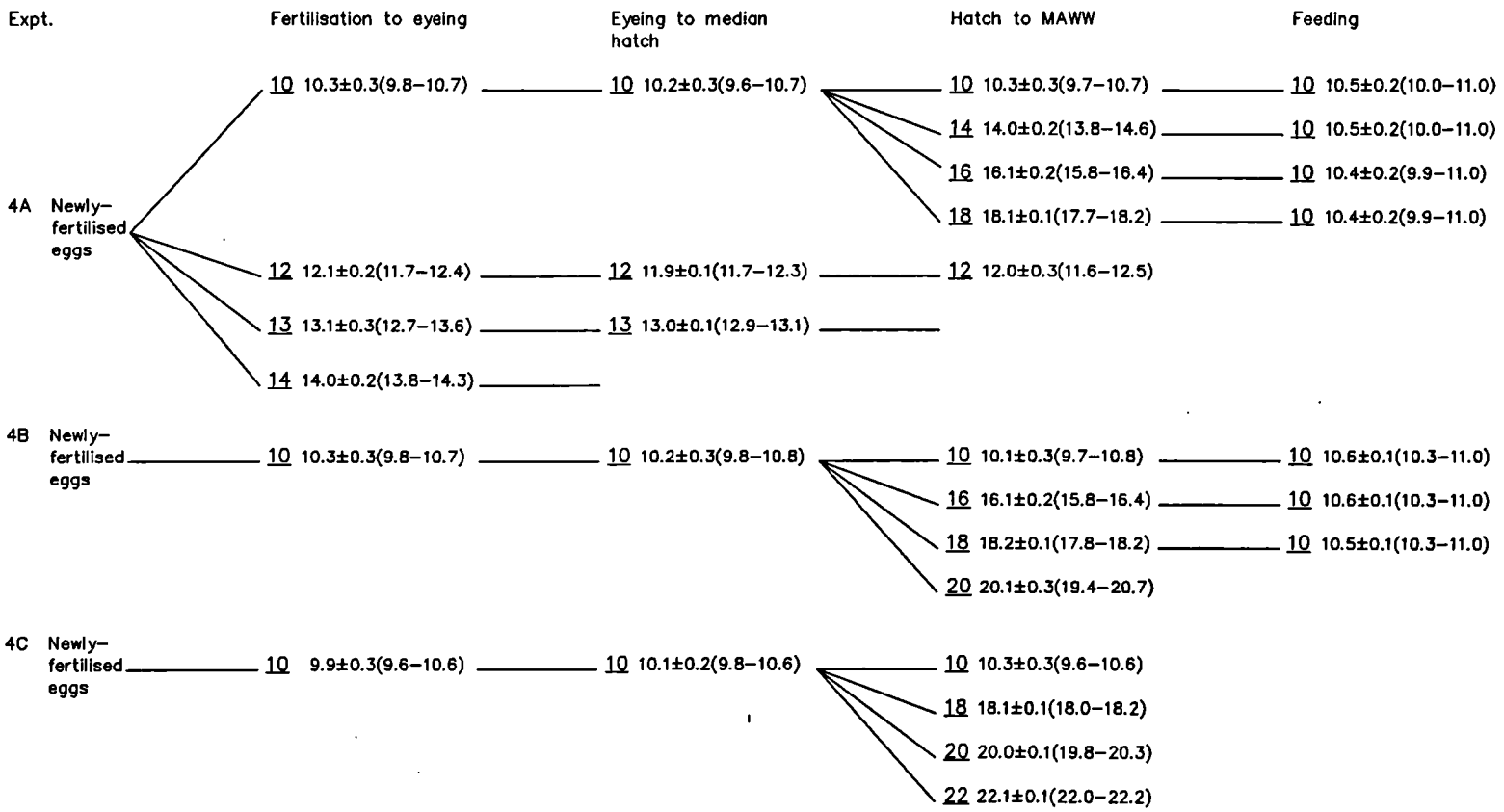

Fig. 2.2.7 Experimental protocols for Series 4 showing target temperatures (underlined), mean experimental temperatures ±SD (°C) and temperature ranges (in parentheses) for each period of development.

12, 14 or 16°C in order to establish a base for future trials.

The mean temperatures for all experiments were always within 0.6°C of the various target temperatures (8, 9, 10, 11, 12, 13, 14, 16, 18, 20, 22°C), and the standard deviations were 0.3°C or less. The data were analysed in relation to the observed temperatures recorded during experimentation (Figs. 2.2.4-2.2.7), but for ease of presentation the target temperatures are referred to in the text. In addition, the temperature regime of any given group from fertilisation to eyeing will be termed FE; that from eyeing to hatching as EH; and that from hatching to the attainment of maximum alevin wet weight (MAWW) as HM. During the subsequent feeding period, all groups were reared at the ambient temperature of a constant 10°C. Throughout each of the periods of development, each group was held at a constant temperature. In each unit measured volumes of newly-fertilised eggs, taken at random from a single batch, were placed in separate cells within each of the incubation trays. A randomised block design was used to assign aliquots of eggs to the appropriate temperature regime.

Each lot (one of 3 replicates) comprised approximately 70 eggs (mean of 71, range of 66-76). The number of eggs was determined following the method described by Brofeldt (1935) in Table 2.2.3. The eggs, lying in a monolayer, were constantly perfused with flowing water.

The remainder of the eggs from each of the batches used in this study were incubated at the ambient water temperature of 10°C in expanded aluminium mesh egg incubation baskets (46 x

Table 2.2.3 Volumetric estimation of the number of water-hardened eggs in relation to egg size as determined by the number of eggs which, when laid side by side, occupy a length of 25 cm (adapted from Brofeldt, 1935).

Vol. of eggs (mL)	Number of eggs per 25 cm									
	40	41	42	43	44	45	46	47	48	49
10	48	52	56	60	64	68	73	78	83	88
20	96	104	112	120	128	136	146	156	166	176
30	144	156	168	180	192	204	219	234	249	264
40	192	208	224	240	256	272	293	312	332	352
50	240	260	280	300	320	340	365	390	415	440

46 x 7.5 cm), placed in a glass-fibre hatching trough (213 x 46 x 15 cm) through which the water flow was maintained at 1 L/min/1000 eggs. Survival was closely monitored to determine whether the experimental systems or procedures had any effect on mortality rates.

When 100% of the eggs had eyed (after shocking) at a particular temperature, lots of eggs were counted and transferred to further test temperatures as shown in Figs. 2.2.4-2.2.7.

With the onset of hatching, the number of embryos which hatched each day in each lot was recorded and the median hatching time was determined. The number of days (or degree-days) taken to reach the median hatching day was considered as the incubation time. When 50% of the eggs within a lot were hatched, the newly-hatched alevins were transferred en masse to further test temperatures, with further alevins, which emerged subsequently, added on a twice daily basis.

After hatching, the alevins were provided with a washed and disinfected igneous gravel substrate, with a particle size of 1-2 cm in diameter. The near spherical shape and uniform size of the gravel provided an even distribution of shelter for the alevins. Considerable work has been conducted on the use of substrate incubators in Pacific salmon species, genus Oncorhynchus (Brannon, 1965; Jochimsen and Bedell, 1968; Emadi, 1973; Witzel and MacCrimmon, 1981; Fuss and Johnson, 1982, 1988; Bams, 1983), char, genus Salvelinus (Peterson and Metcalfe, 1977; Alanärä, 1993), and Atlantic salmon (Marr, 1965; Leon, 1975; Leon and Bonney, 1979; Eriksson and Wetslund, 1983; Hansen and Mϕller, 1985; Pepper and

Stansbury, 1985; Brännäs, 1989; Sveier and Raae, 1992). These studies have shown that the provision of a rearing substrate during the alevin stage results in considerable benefits of improved growth and increased yolk absorption rate, prevention of yolk-sac constrictions, and enhanced growth and survival during subsequent feeding. A yolk-sac constriction is a narrow pass in the yolk-sac, which is generally caused by hyperactivity of the alevin reared in the absence of a substrate. The yolk-sac mass behind the constriction is only partially utilised by the alevin. Dumas (1966) gives detailed drawings of alevins with yolk-sac constrictions. In order to prevent any problems arising from the lack of a rearing substrate, alevins were reared in a gravel substrate until their transfer, as fry, to the first-feeding tanks.

When the temperature for a particular lot was to be changed, the eggs or alevins were carefully syphoned into a beaker and acclimated as necessary (described below), before being hand counted into the new temperature.

Within each series of experiments, each group experienced a unique combination of temperatures from fertilisation to MAWW (Figs. 2.2.4-2.2.7). At MAWW, duplicate lots of fry were transferred to rearing tanks at the ambient temperature of 10°C.

2.2.2.5 Thermal acclimation

Whenever lots of eggs, alevins, or fry were transferred from one temperature to another, the acclimation rate used

was 1°C per hour.

Several workers, in similar experiments, have used slower acclimation rates of: 0.5°C per hour for Atlantic salmon eggs (Brännäs, 1988); 1°C per day for Arctic char alevins (Wallace and Aasjord, 1984b) and for Atlantic salmon at first feeding (Peterson and Martin-Robichaud, 1989); and 0.5°C every 3 days for pink salmon eggs and alevins (Murray and Beacham, 1986). However, Hokanson et al. (1973) and McCormick et al. (1972) used an acclimation rate for brook trout eggs and alevins which approximated to 3°C per hour while Tang et al. (1987), examining the influence of water temperature extremes on coho salmon egg and alevin mortality, found that abrupt changes in temperature, ranging from an increase of 8.4°C to a decrease of 6.2°C, resulted in little or no increase in mortality. Furthermore, studies on induced polyploidy by heat shocking (Benfey and Sutterlin, 1984; Johnstone, 1985) have shown that newly-fertilised Atlantic salmon eggs can survive sudden temperature changes in excess of 16°C.

It was concluded that an acclimation rate of 1°C per hour was acceptable for the present investigations, and did not represent a factor affecting the subsequent mortality rates of eggs, alevins, or fry.

2.2.3 MORTALITIES

At all stages of development, mortalities from all lots were recorded daily and removed to prevent the colonisation and spread of fungus (particularly Saprolegnia parasitica) to viable stock.

Mortality of eggs was judged by the presence of a partially or completely opaque yolk mass, or by a pronounced whitening of the cells of the embryo.

With the aid of a torch fitted with a red lens, mortalities were removed using a hollow glass tube and rubber suction bulb. The use of prophylactic treatments, such as zinc-free malachite green, to control the spread of fungus, was unnecessary. The removal of dead eggs during the sensitive period of embryonic development from fertilisation to eyeing (Post et al., 1974; Jensen and Alderdice, 1983; Johnson et al., 1989), was exercised with extreme care, to prevent physical disturbance of adjacent viable eggs. Eggs were always held as a monolayer, to allow their easy recognition and removal.

All dead eggs were placed in a clearing solution which comprised equal proportions of methanol, acetic acid and water, and were inspected using a binocular microscope at X20 magnification to determine those which were unfertilised. This method can be used approximately 5 degree-days after fertilisation, when fertilised eggs can be seen to have undergone division to the 4-cell stage of development. At later stages (approximately 70 degree-days after fertilisation), the developing embryo can be seen clearly as

a thick white line (the neural keel) when placed in clearing solution. In practice, the majority of unfertilised eggs were identified and removed from the trays, following physical shocking of eyed eggs. This is a standard hatchery procedure that facilitates separation of fertilised from unfertilised eggs, on the basis that unfertilised eggs turn white. Whiteness is caused by the precipitation of globulin following rupture of the delicate yolk membrane.

Alevin mortality was recognised by a lack of response to stimulation and cessation of a heart beat. Alevins and fry showing developmental abnormalities or physical deformities were registered as mortalities.

Fry mortality was generally caused by bacterial gill disease (myxobacterial invasion of the gill filaments). Because losses were low, no medication was administered to any of the experimental groups.

The various mortality data were used to determine percentage mortality rates at each test temperature during each period of development: fertilisation to eyeing; eyeing to hatching; hatching to MAWW; and fry rearing. Unfertilised eggs were excluded from the mortality calculations.

2.2.4 DEVELOPMENT AND GROWTH

2.2.4.1 Rate of development

The progression of the investigation was measured both in days and degree-days (sum of mean daily temperatures). The rate of development was determined by recording the number of days and degree-days elapsed between fertilisation and three successive stages of development: eyeing, hatching, and MAWW. These stages were chosen because they are considered by hatchery operators to be key stages in the development of salmonid eggs and alevins; they are also relatively straightforward to assess. It is recognised that hatching may occur at slightly different stages of differentiation and growth (Garside, 1959) and hatching can be externally manipulated to occur at different times, provided that the embryo is sufficiently developed to survive outside the egg: a temperature change of 0.7°C can provoke salmonid eggs to hatch prematurely (Gray, 1928b; Heggberget and Wallace, 1984). Nevertheless, accurate prediction of hatching time is of practical value to salmon hatchery managers.

There is some variation in the objectivity and accuracy with which "eyeing" - the first readily detectable appearance of the eye pigment within the egg - can be defined. Because this is a rather vague criterion, individual assessments are likely to show slight variation and this may account for some of the variation in development times shown between experimental series for eggs incubated at identical temperatures.

Eyeing, hatching and MAWW as stages for making comparisons remain, however, useful criteria, and have immediate meaning when deciding on the optimal conditions for hatchery practice.

2.2.4.2 Determination of median hatching time

With the onset of hatching, the number of viable alevins which hatched each day in each treatment was recorded, and the median hatching times determined. The median was chosen because it is less sensitive than the mean to the occurrence of extreme values, and it is commonly used in development studies. The median hatching time was the time at which 50% of the viable eggs in a population had hatched. Because the observations were taken at discrete times, the population median was derived by linear interpolation between the distributions of hatching frequency, provided by the interval counts of hatched alevins, for each temperature. These data were also used to determine the influence of temperature on the duration of the hatching period from the commencement to completion of hatching (0-100% hatching).

2.2.4.3 Weight and length determinations

In all cases fresh specimens were used immediately for weight and length determinations. Preservation in a solution of formalin was never used to store material for subsequent analysis, because this has been shown to have a measurable effect on fish weight and length, compared with that of live

fish (Heming and Preston, 1981; Hay, 1982; Heming et al., 1982; Pepper and Stansbury, 1985). Fish decrease in length and increase in weight during preservation in formalin, the magnitude of these changes being a function of the duration of preservation and the preservative concentration.

In this study weight has been used as the principal unit of measurement because linear measurements are subject to variations in proportionality, depending on the individual's condition factor (Brown, 1957).

2.2.4.4 Hatched weight

Within one day of median hatching, a random sample of 10 alevins was withdrawn from each treatment for subsequent determination of their wet weights. Whole alevins were weighed to the nearest 1.0 mg on an analytical balance (Sartorius Model 2842, Sartorius Ltd., Epsom, Surrey, England, UK). The repeated measurement of a test weight (5 g) to the nearest 0.1 mg demonstrated that the analytical balance was accurate to within 1.0 mg (mean±SE = 5.0002 g±0.0003; CV (%) = 0.012).

2.2.4.5 Determination of MAWW

To determine when MAWW was reached, random samples of 10 alevins were removed and weighed from each treatment temperature commencing 700 degree-days after fertilisation, and at regular intervals of 30 degree-days thereafter, until the attainment of MAWW. This sampling protocol ensured that

samples were taken more frequently at higher temperatures to compensate for differential rates of development.

2.2.4.6 Alevin and fry length

In Series 4, measurements were taken for length (total, standard, and fork) and weight of newly-hatched alevins and fry at MAWW.

Samples of 10 fish from each treatment were anaesthetised and measured to the nearest 0.1 mm under a binocular microscope fitted with an eyepiece graticule. Alevin and fry lengths were measured as the straight line distance between the tip of the snout and: the posterior end of the last caudal vertebra (standard length); the point of division of the caudal fin (fork length); and the terminal end of the caudal fin (total length).

Determination of fork length appears to be the most satisfactory and widely used measurement of salmonid length (Peterson et al., 1977; Heming, 1982; Heming et al., 1982; Rombough, 1985; Beacham and Murray, 1988).

The fork length of newly-hatched alevins was measured from the tip of the snout to the midpoint of the outer margin of the tail. This measurement corresponds to the fork length in older alevins, where the tail is more noticeably forked.

Heming (1982) found that changes in alevin length in chinook salmon closely parallel changes in alevin weight. Hurley and Brannon (1969) reported a similar relationship in sockeye salmon. Thus, in situations where the measurement of body weight is impractical, fork length is the next best

measure of size.

2.2.4.7 Growth of fry

Fry from each temperature regime were subsequently reared at the ambient temperature of the normal hatchery supply (approximately 10°C) until 1200 degree-days after fertilisation was reached. At MAWW, duplicate lots of 50 fry were taken randomly from each treatment and, following thermal acclimation, were transferred to separate tanks for first feeding. The allocation of treatment lots to feeding tanks was done using a randomised block design. 24 hours after transfer to tanks for first feeding, all fry received the same proprietary diet (Table 2.2.4). All groups received a ration size of 5% of body wet weight per day, which was fed continuously using an automatic feed dispenser, for 12 hours each day (0700-1900h). Following first feeding, growth was monitored by taking individual test weights (wet and dry weights) of 10 fry from each group. Total stock weight in each tank was recalculated each time the fish were weighed, and the feed ration size was adjusted accordingly.

Once feeding had commenced, it was found that the percentage water content of the fry did not change significantly, (mean±SD of 81.0%±1.0). Hence, the wet weight is a simple multiple of the dry weight. It was therefore unnecessary to consider both measures of weight; the wet weight values have been selected for treatment because this did not involve killing the fish. Using the same procedure, the wet weights of fry from each temperature regime were also

Table 2.2.4 Proximate composition and particle mean size and range of proprietary diet (BP Nutrition (UK) Ltd., Northwich, Cheshire, England, UK).

Moisture	8.0	%
Crude protein	54.0	%
Crude oil	15.0	%
Crude ash	10.0	%
Fibre	1.0	%
Carbohydrate	12.0	%
Particle mean size	0.4	mm
Particle size range	0.3-0.6	mm

measured at 110 days and 1200 degree-days after fertilisation to determine whether any differences in growth rates from the various temperature regimes were caused by temporal effects, or by other factors such as differential yolk conversion efficiences, during egg and alevin development. At the termination of each experiment fry were fasted for a period of 24 hours prior to final measurements.

In cases where an intermediate growth observation (determination of mean wet weight) was made between the time of first feeding, and before the end of the experiment, a sample of 10 fry was used to establish growth of the population (mean wet weight of the group) to this point. Fish were anaesthetised in a 350 ppm solution of 2-phenoxyethanol prior to measurement. No mortalities were attributed to the use of anaesthetics.

2.2.4.8 Sampling protocol for determining wet and dry tissue weights

(a) Wet weight

In view of the difficulty in determining with reasonable objectivity the appropriate endpoint for blotting or removal of surface water from wet tissue before measuring weight, it was necessary to standardise the blotting procedure. For a period of 30 s at room temperature (18-20°C), tissues (eggs, alevins, fry) were superficially dried between 2 sheets of filter paper (Whatman 540, hardened ashless, 18.5 cm diameter circles). Following blotting, each fish within a sample was

placed on an individual glass slide of known weight, prior to being weighed to the nearest 1.0 mg. In order to estimate the possible error associated with this technique, the blotting procedure was carried out three times on each individual within a sub-sample of 10 fish. The coefficient of variation (%) for the repeated measurements of eggs, alevins and fry within the sub-samples were calculated as 0.7, 1.0, and 0.9, respectively.

(b) Dry weight

Dry weights of alevins and fry were determined following desiccation at 60°C for 48 hours. Alevins or fry within a sample were given a lethal dose of 2-phenoxyethanol (1000 ppm). Following rinsing in distilled water, fish were individually blotted dry and weighed to the nearest 1.0 mg. Prior to weighing, samples were held in a silica gel desiccator to prevent hydration of the tissue. Wallace and Aasjord (1984b) showed that hydration of dried tissue could lead to a weight increment of between 2-4% within 10 minutes of removal from the oven.

Having determined both wet and dry weights for an individual fish within a sample, the percentage water content of tissue could then be calculated as follows:

$$\text{Water content (\%)} = (\text{dry tissue weight}/\text{wet tissue weight}) \times 100$$

2.2.5 DATA ANALYSIS AND PRESENTATION

The effects of temperature on development, survival and subsequent growth were examined for each experiment (Series 1-4). Because each experiment was conducted as an independent entity using different batches of eggs, no statistical comparisons were made between experiments.

Standard treatment of the data included analysis of variance (ANOVAR) and Student-Newman-Keuls (SNK) tests. Differences present at the 5% level of probability ($P \leq 0.05$), were judged to be significant in all tests. Where no significant differences in the measured parameters were found between replicate groups, data from replicates were pooled.

2.2.5.1 Mortality

One of the main endpoints used in this investigation to quantify the responses to temperature was mortality: this was the criterion used for establishing the upper temperature limit for eggs and alevins. A significant rise in mortality above the expected level, as determined by ANOVAR, indicated that normal development had not taken place. Because temperature was the only variable in each experiment, any increase in mortality was attributed to this factor.

For the analysis of variation in the mortality rates of eggs, alevins and fry, rates were calculated for each group as percentages, and then an arc sine percentage transformation used to normalise the data distribution (Steel and Torrie, 1980). Transformed mortality data were then

tested by ANOVAR and SNK, where appropriate.

Percentage mortality data are reported in the text as arithmetic means (prior to transformation), with their standard deviations.

2.2.5.2 Hatching

Owing to the fact that the median time to 50% hatch is normally distributed, even in adverse circumstances, the use of a logarithmic transformation was not considered necessary for statistical evaluation.

2.2.5.3 Growth rate

Instantaneous rates of growth (% wet weight increment per day) were calculated for each group during the feeding period as follows:

$$Gw = \frac{\log_e Wt - \log_e Wo}{t} \times 100\%$$

(Bagenal and Tesch, 1978)

where Gw = specific growth rate in % body weight per day; Wo = weight at time 0; Wt = weight at time t; t = time in days.

For each group, daily growth rate was expressed as a percentage of the initial weight at MAWW.

2.2.5.4 Biomass

Net biomass was calculated for alevins at MAWW, and for fry at 110 days and 1200 degree-days after fertilisation. For each of the three stages of development, net biomass was derived from the product of the number of fish extant and their mean individual wet weight.

The various data from groups held under different temperature regimes were first tested for significance using a one-way analysis of variance. When "F" was significant at $P<0.05$, the sources of specific differences between individual group means were then identified using the SNK test. ANOVAR and SNK tests were used to analyse differences between groups held under different temperature regimes in the following areas of investigation:

(a) Individual and cumulative mortalities during egg, alevin and fry stages of development.

(b) Hatching periods in days and degree-days.

(c) Alevin weights at hatching; alevin lengths and weights at MAWW.

(d) Fry weights at 110 days and 1200 degree-days after fertilisation.

(e) Specific growth rates of fry from first feeding (MAWW) to 1200 degree-days after fertilisation.

(f) Biomass of alevins at MAWW and fry at 110 days and 1200 degree-days after fertilisation.

In order to present meaningful interpretation of the data, a suitable temperature regime for normal rates of mortality, growth and net biomass gain was designated, within which the

departure from those rates expected was not greater than that shown by the SNK test when $P<0.05$. The temperature regime designated as a control was a constant 10°C (10-10-10). Included in the various tables of results are means of replicates ±1SD. Asterisks indicate means shown by ANOVAR and SNK tests to be significantly different (* $P<0.05$, ** $P<0.01$, *** $P<0.001$) from the mean of the control group.

The various data were evaluated using a computerised statistical programme (Tadpole III, Biosoft Ltd., Cambridge, England, UK).

Histograms and line graphs were plotted using Harvard Graphics (Software Publishing Europe, London, England, UK).

Diagrams were prepared using Autocad (Autodesk Ltd., Guildford, Surrey, England, UK).

2.3 RESULTS

2.3.1 MORTALITY

The results of the various experiments on the temperature tolerances of salmon eggs and alevins are shown in Tables 2.3.1-2.3.6 and Figs. 2.3.1-2.3.6. Egg, alevin and fry mortalities are shown for four periods of development at different water temperatures. Tables 2.3.1-2.3.6 give percentage mortality data and cumulative values based on the number of eggs within a group at the beginning of each experiment. The histograms in Figs. 2.3.1-2.3.6 show mean cumulative mortalities.

The first figure in each triplicate of temperatures (e.g. 8-8-8) is the temperature from fertilisation to eyeing (FE), the second, that from eyeing to hatching (EH) and the third, that from hatching to the attainment of MAWW (HM). Following MAWW, fry were reared under ambient temperature conditions of 10°C. Temperature was held constant throughout each of the four periods of development.

All values shown are the means of 3 replicates. There was generally a high degree of consistency between treatment replicates. Egg mortality data do not include unfertilised eggs. All dead eggs were inspected to determine those which were unfertilised, using the method described in section 2.2.3.

2.3.1.1 Egg and alevin mortality

(a) Mortality of eggs from fertilisation to eyeing

Series 1 (Table 2.3.1, Fig. 2.3.1)

Mortality was lowest for newly-fertilised eggs incubated at the lowest temperature tested (8°C), and there was a progressive increase in mortality with increasing temperature. Percentage mortalities were 2.0, 6.5 and 29.1 at egg incubation temperatures of 8, 10 and 12°C, respectively. Total mortalities of eggs occurred at temperatures of 14 and 16°C. Mortalities were very significantly lower ($P<0.001$) at 8 and 10°C than at 12°C. There were also significant differences ($P<0.01$) between levels of mortality at 8 and 10°C. Thus, temperatures below 12°C produced the lowest mortality rates of eggs. No eggs survived to eyeing at the two highest temperatures tested (14 and 16°C), although at each of these temperatures some early development did take place. Because total mortality occurred in groups of eggs incubated at 14°C, this temperature was established as the upper temperature for further experiments.

Series 2 (Table 2.3.2, Fig. 2.3.2)

In agreement with Series 1, there was a progressive increase in the mortality of eggs with increasing temperature. Mean egg mortalities were 2.6% at 9°C, 4.7% at 10°C and 11.0% at 11°C. Mortality was significantly higher

Table 2.3.1 Series 1 - mean mortality and mean cumulative mortality (in parentheses) ±SD (%) of salmon eggs, alevins and fry during four periods of development under different temperature regimes. The percentages shown are based on the numbers of viable eggs at the beginning of the experiment. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001. Data were arc sine transformed prior to testing by ANOVAR and SNK.

Temp. regime (°C)	Mean mortality±SD (%)			
	Eggs		Alevins	Fry
	Fert-eyeing	Eyeing-hatch	Hatch-MAWW	MAWW-1200°d
8-8-8	2.0±0.1**	11.0±0.1 (13.0±0.1)	0.0 (13.0±0.1)	0.0 (13.0±0.1)
8-8-14	2.0±0.1**	11.0±0.1 (13.0±0.1)	0.0 (13.0±0.1)	0.0 (13.0±0.1)
8-8-16	2.0±0.1**	11.0±0.1 (13.0±0.1)	1.8±1.7 (14.8±2.5)	0.4±0.3 (15.2±0.6)
8-10-12	2.0±0.1**	3.8±1.8 (5.8±1.8**)	0.0 (5.8±0.9**)	2.0±1.9 (7.8±2.1**)
8-10-16	2.0±0.1**	3.8±1.8 (5.8±1.8**)	9.8±2.1 (15.6±2.9)	NT
8-12-14	2.0±0.1**	3.0±1.0 (5.0±1.4**)	5.7±2.1 (10.7±3.0)	0.5±0.1 (11.2±0.8)
8-12-16	2.0±0.1**	3.0±1.0 (5.0±1.4**)	20.6±3.3*** (25.6±4.7)	NT
8-14-14	2.0±0.1**	42.3±2.1*** (44.3±2.1***)	17.7±6.5** (62.0±6.5***)	0.2±0.1 (62.2±0.1***)
8-16-16	2.0±0.1**	80.9±3.2*** (82.9±2.3***)	10.5±0.7 (93.4±0.7***)	LV
10-8-8	6.5±0.1	6.0±0.7 (12.5±0.7)	5.6±0.8 (18.1±1.1)	NT
10-10-10	6.5±0.1	6.6±1.8 (13.1±1.8)	3.4±0.6 (16.5±0.8)	0.0 (16.5±0.7)
10-10-14	6.5±0.1	6.6±1.8 (13.1±1.8)	3.9±1.3 (17.0±1.8)	NT
10-10-16	6.5±0.1	6.6±1.8 (13.1±1.8)	7.2±0.3 (20.3±0.3)	NT

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Table 2.3.1 (continued)

Temp. regime (°C)	Mean mortality±SD (%)			
	Eggs		Alevins	Fry
	Fert-eyeing	Eyeing-hatch	Hatch-MAWW	MAWW-1200°d
10-12-12	6.5±0.1	19.4±4.3 (25.9±4.3*)	37.0±6.2*** (62.9±6.2***)	2.6±2.5 (65.5±3.7***)
10-14-14	6.5±0.1	32.9±4.5*** (39.4±4.4***)	51.0±3.0*** (90.4±2.1***)	LV
10-16-16	6.5±0.1	85.6±3.5*** (92.1±3.5***)	7.9±0.1 (100.0 ***)	LV
12-8-8	29.1±0.1***	49.6±3.6*** (78.7±3.6***)	10.7±2.7 (89.4±2.7***)	LV
12-10-10	29.1±0.1***	36.3±4.1*** (65.4±4.1***)	17.3±3.5** (82.7±3.5***)	LV
12-12-12	29.1±0.1***	50.5±3.5*** (79.6±3.5***)	15.4±1.6** (95.0±1.6***)	LV
12-14-14	29.1±0.1***	63.0±3.0*** (92.1±3.0***)	7.9±0.1 (100.0 ***)	LV
12-16	29.1±0.1***	70.9±0.1*** (100.0 ***)	NV	NV
14	100.0 ***	NV	NV	NV
16	100.0 ***	NV	NV	NV

NT: Not tested (experimental systems unavailable).
LV: Low viability (mortality >70%). Insufficient number of fish available for fry rearing phase of study.
NV: Not viable (total mortality).

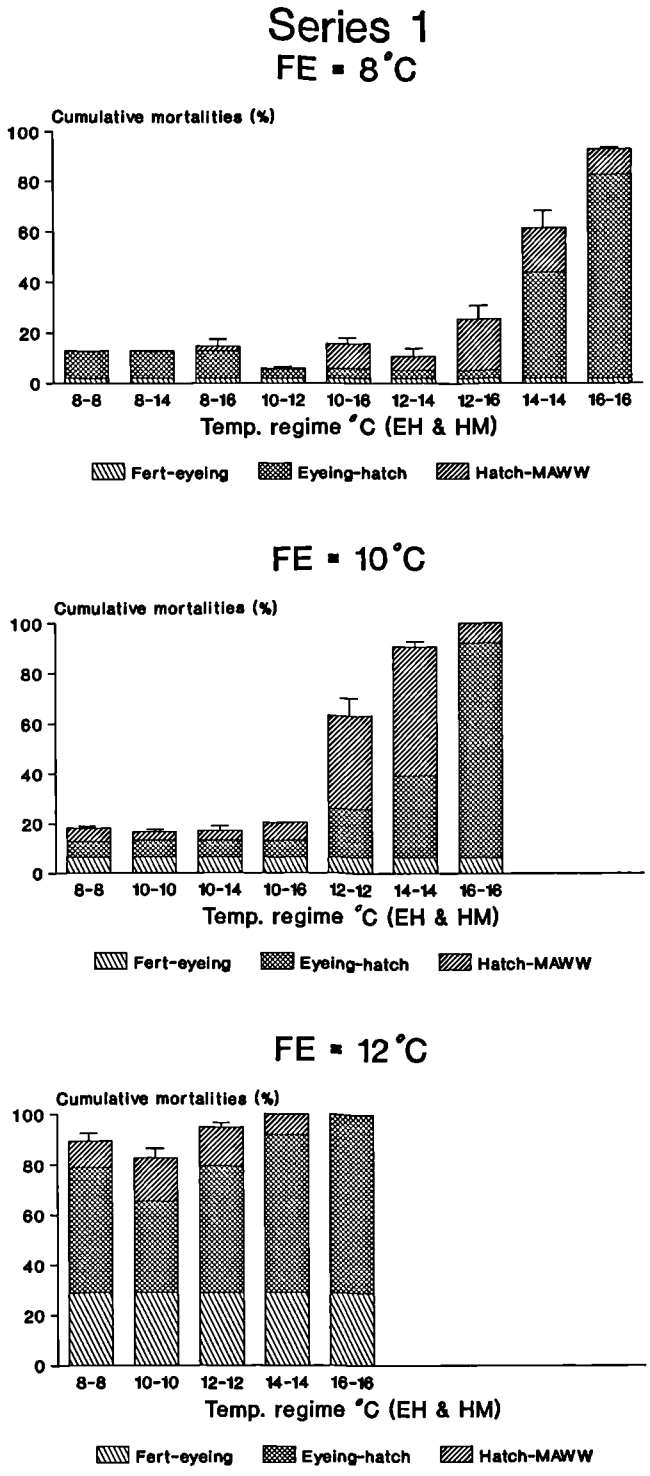

Fig. 2.3.1 Cumulative mortalities ±SD (%) in Series 1 at eyeing, total hatching and MAWW, expressed as a percentage of eggs at the start of the experiment, plotted against different temperature regimes. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Percentage values are the means of 3 replicate treatments.

Table 2.3.2 Series 2 - mean mortality and mean cumulative mortality (in parentheses) ±SD (%) of salmon eggs, alevins and fry during four periods of development under different temperature regimes. The percentages shown are based on the numbers of viable eggs at the beginning of the experiment. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001. Data were arc sine transformed prior to testing by ANOVAR and SNK.

Temp. regime (°C)	Mean mortality±SD (%)			
	Eggs		Alevins	Fry
	Fert-eyeing	Eyeing-hatch	Hatch-MAWW	MAWW-1200°d
9-9-9	2.6±0.1	1.0±0.2 (3.6±0.3)	1.4±1.3 (5.0±2.0)	0.0 (5.0±2.0)
9-9-11	2.6±0.1	1.0±0.2 (3.6±0.3)	0.5±0.4 (4.1±0.7)	0.6±0.2 (4.7±0.3)
9-10-10	2.6±0.1	1.1±0.2 (3.7±0.3)	0.0 (3.7±0.4)	0.6±0.2 (4.3±0.3)
9-10-11	2.6±0.1	1.1±0.2 (3.7±0.3)	0.5±0.4 (4.2±0.7)	0.1±0.1 (4.3±0.2)
9-11-10	2.6±0.1	0.9±0.4 (3.5±0.4)	0.0 (3.5±0.2)	1.1±0.1 (4.6±0.2)
9-11-11	2.6±0.1	0.9±0.4 (3.5±0.4)	1.0±0.8 (4.5±0.4)	3.0±0.5 (7.5±0.7)
9-12-11	2.6±0.1	1.0±0.1 (3.6±0.1)	0.0 (3.6±0.8)	7.8±0.8** (11.4±1.1)
9-12-12	2.6±0.1	1.0±0.1 (3.6±0.1)	0.0 (3.6±0.3)	0.0 (3.6±0.3)
10-9-9	4.7±0.1	2.4±1.0 (7.1±1.0)	0.5±0.4 (7.6±0.4)	0.0 (7.6±0.4)
10-9-11	4.7±0.1	2.4±1.0 (7.1±1.0)	1.1±0.1 (8.2±0.2)	0.1±0.1 (8.3±0.2)
10-10-10	4.7±0.1	1.3±0.3 (6.0±0.4)	1.3±0.4 (7.3±0.6)	0.1±0.1 (7.4±0.2)
10-10-12	4.7±0.1	1.3±0.3 (6.0±0.4)	0.0 (6.0±0.5)	4.7±0.7** (10.7±1.0)
10-11-11	4.7±0.1	2.1±0.7 (6.8±0.7)	0.4±0.3 (7.2±0.6)	2.3±0.3 (9.5±0.4)

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Table 2.3.2 (continued)

Temp. regime (°C)	Mean mortality±SD (%)			
	Eggs		Alevins	Fry
	Fert-eyeing	Eyeing-hatch	Hatch-MAWW	MAWW-1200°d
10-11-12	4.7±0.1	2.1±0.7 (6.8±0.7)	0.4±0.3 (7.2±0.6)	1.9±0.4 (9.1±0.6)
10-12-11	4.7±0.1	2.6±0.9 (7.3±0.9)	0.4±0.3 (7.7±0.6)	0.4±0.1 (8.1±0.1)
10-12-12	4.7±0.1	2.6±0.9 (7.3±0.9)	1.5±0.7 (8.8±1.0)	2.8±2.0 (11.6±2.8)
11-9-9	11.0±2.0**	3.6±0.2 (14.6±0.3**)	0.8±0.7 (15.4±1.1**)	0.4±0.3 (15.8±0.6**)
11-9-11	11.0±2.0**	3.6±0.2 (14.6±0.3**)	0.7±0.6 (15.3±1.0**)	2.6±0.6 (17.9±0.8**)
11-10-9	11.0±2.0**	5.6±0.1 (16.6±0.1**)	0.0 (16.6±0.2**)	0.5±0.4 (17.1±0.7**)
11-10-10	11.0±2.0**	5.6±0.1 (16.6±0.1**)	1.2±1.1 (17.8±1.7**)	0.4±0.3 (18.2±0.6**)
11-11-11	11.0±2.0**	3.1±0.9 (14.1±0.9**)	0.0 (14.1±0.7**)	2.8±0.8 (16.9±1.1**)
11-11-12	11.0±2.0**	3.1±0.9 (14.1±0.9**)	1.1±0.4 (15.2±0.6**)	2.9±1.3 (18.1±1.8**)
11-12-11	11.0±2.0**	2.0±1.6 (13.0±1.6**)	1.0±0.3 (14.0±0.4**)	1.5±0.8 (15.5±1.1**)
11-12-12	11.0±2.0**	2.0±1.6 (13.0±1.6**)	1.0±0.3 (14.0±0.4**)	3.4±0.4 (17.4±0.5**)

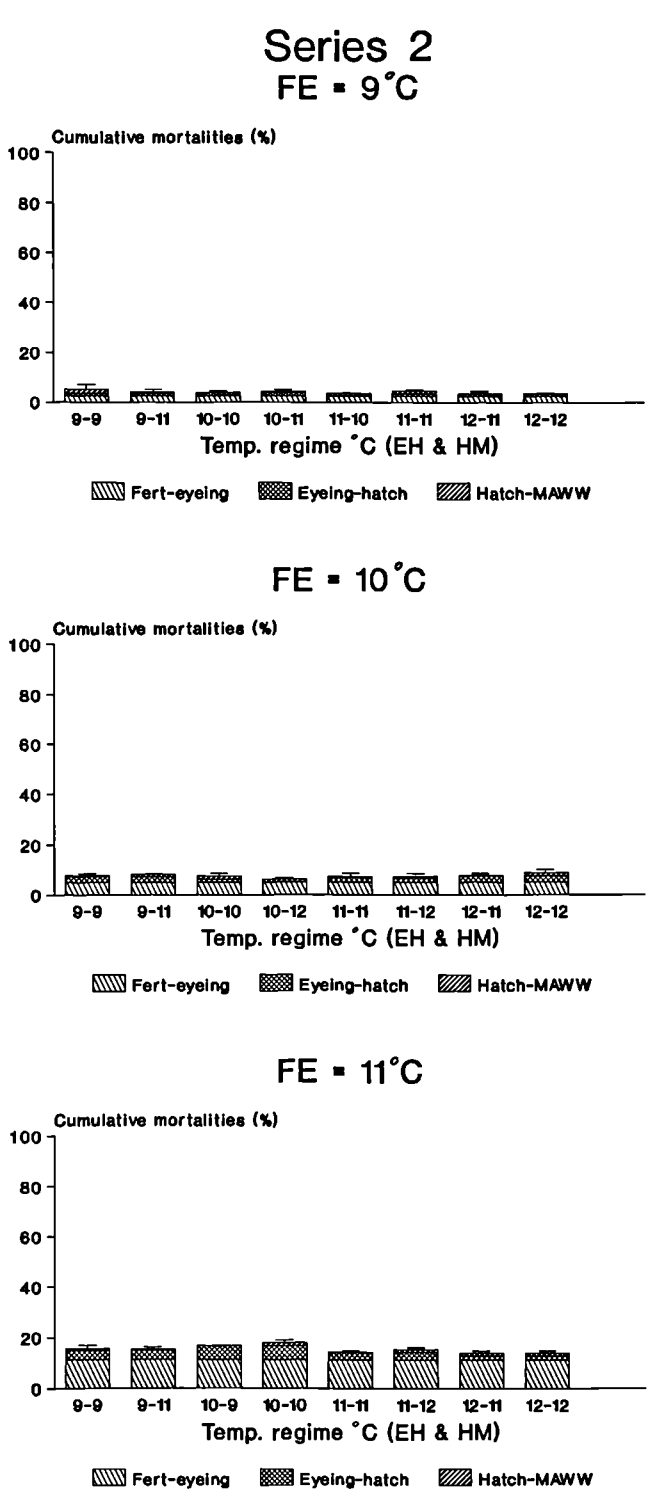


Fig. 2.3.2 Cumulative mortalities ±SD (%) in Series 2 at eyeing, total hatching and MAWW, expressed as a percentage of eggs at the start of the experiment, plotted against different temperature regimes. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Percentage values are the means of 3 replicate treatments.

($P<0.01$) at 11°C than at the two lower temperatures tested. Differences in percentage mortality data between temperatures of 9 and 10°C were not significant.

Series 3 (Table 2.3.3, Fig. 2.3.3)

Mortality was again shown to increase with increasing temperature within the range of 9-13°C. Mortalities were less than 5% in groups of eggs incubated at 12°C and lower. However, a highly significant increase ($P<0.001$) in the level of mortality occurred at 13°C (21.8%). Differences in mortality for the 9 and 12°C incubates were also significant ($P<0.01$).

It appears that 13°C is near to the incipient lethal temperature for incubating salmon eggs.

Series 4A (Table 2.3.4, Fig. 2.3.4)

Within Series 4, the only comparative study of mortality in relation to different egg incubation temperatures (constant 10, 12, 13 and 14°C) was undertaken in Series 4A. There was a very marked increase in the level of mortality with increasing temperature. Mortalities to eyeing ranged from 3.0% at 10°C to 100% at 14°C. There were highly significant differences ($P<0.001$) in mortality between each of the four temperatures (10, 12, 13, 14°C) tested.

Table 2.3.3 Series 3 - mean mortality and mean cumulative mortality (in parentheses) ±SD (%) of salmon eggs, alevins and fry during four periods of development under different temperature regimes. The percentages shown are based on the numbers of viable eggs at the beginning of the experiment. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001. Data were arc sine transformed prior to testing by ANOVAR and SNK.

Temp. regime (°C)	Mean mortality±SD (%)			
	Eggs		Alevins	Fry
	Fert-eyeing	Eyeing-hatch	Hatch-MAWW	MAWW-1200°d
9-9-9	0.4±0.3	3.9±1.2 (4.3±1.2)	2.7±0.8 (7.0±0.8)	1.6±1.0 (8.6±1.4)
9-11-11	0.4±0.3	5.7±1.0 (6.1±1.0)	1.8±0.8 (7.9±1.1)	2.5±0.1 (10.4±0.3)
10-10-10	1.5±0.1	5.1±1.8 (6.6±1.8)	2.3±0.3 (8.9±0.4)	5.5±3.6 (14.4±3.6)
10-11-11	1.5±0.1	7.1±2.2 (8.6±2.2)	2.9±0.4 (11.5±0.6)	2.7±0.9 (14.2±2.6)
10-13-13	1.5±0.1	8.3±2.7 (9.8±2.7)	6.6±2.7 (16.4±2.7*)	11.1±3.7 (27.5±3.7*)
11-11-11	1.6±0.3	6.9±1.5 (8.5±0.8)	6.0±1.5 (14.5±1.5)	0.9±0.3 (15.4±0.4)
11-12-12	1.6±0.3	7.8±1.5 (9.4±1.5)	5.7±1.9 (15.1±2.7)	3.0±0.6 (18.1±0.8)
11-13-13	1.6±0.3	9.1±2.7 (10.7±2.7)	19.2±2.7** (29.9±3.8**)	1.8±0.4 (31.7±0.6**)
12-12-12	4.5±1.0	20.4±3.9*** (24.9±3.9***)	16.7±2.5** (41.6±5.3***)	6.4±1.7 (48.0±2.4**)
13-13-13	21.8±7.2***	66.3±4.2*** (88.1±5.8***)	8.3±3.4 (96.4±3.4***)	LV

LV: Low viability (mortality >70%). Insufficient number of fish available for fry rearing phase of study.

Series 3

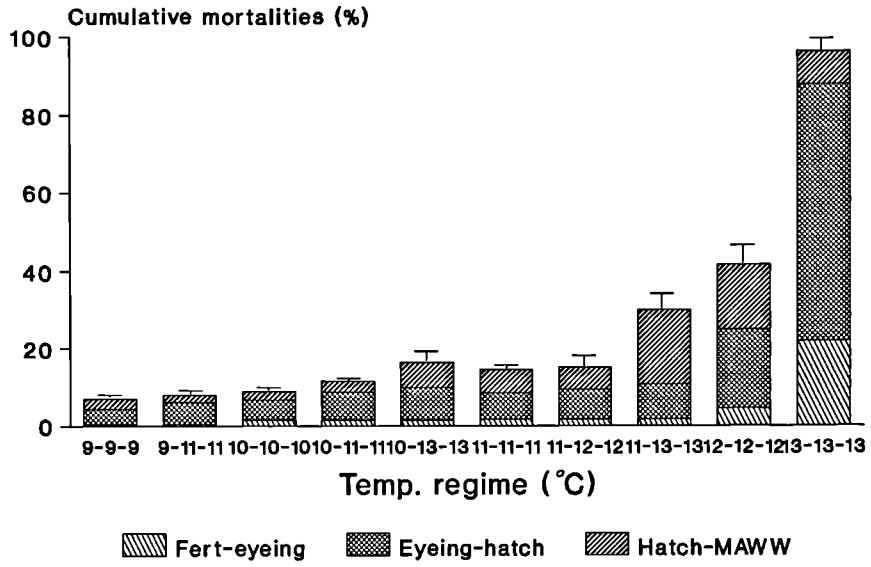


Fig. 2.3.3 Cumulative mortalities ±SD (%) in Series 3 at eyeing, total hatching and MAWW, expressed as a percentage of eggs at the start of the experiment, plotted against different temperature regimes. Percentage values are the means of 3 replicate treatments.

Table 2.3.4 Series 4A - mean mortality and mean cumulative mortality (in parentheses) ±SD (%) of salmon eggs, alevins and fry during four periods of development under different temperature regimes. The percentages shown are based on the numbers of viable eggs at the beginning of the experiment. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001. Data were arc sine transformed prior to testing by ANOVAR and SNK.

Temp. regime (°C)	Mean mortality±SD (%)			
	Eggs		Alevins	Fry
	Fert-eyeing	Eyeing-hatch	Hatch-MAWW	MAWW-1200°d
10-10-10	3.0±0.6	5.6±1.5 (8.6±1.5)	0.9±0.1 (9.5±0.1)	3.6±0.2 (13.1±0.3)
10-10-14	3.0±0.6	5.6±1.5 (8.6±1.5)	1.7±0.6 (10.3±0.6)	2.8±2.7 (13.1±2.9)
10-10-16	3.0±0.6	5.6±1.5 (8.6±1.5)	2.3±1.3 (10.9±1.8)	2.3±0.6 (13.2±0.5)
10-10-18	3.0±0.6	5.6±1.5 (8.6±1.5)	3.3±2.1 (11.9±2.1)	2.6±1.9 (14.5±1.9)
12-12-12	18.6±1.9***	61.1±7.1*** (79.7±7.1***)	3.1±2.3 (82.8±2.3***)	LV
13-13	79.0±4.6***	21.0 *** (100.0 ***)	NV	NV
14	100.0 ***	NV	NV	NV

LV: Low viability (mortality >70%). Insufficient number of fish available for fry rearing phase of study.
NV: Not viable (total mortality).

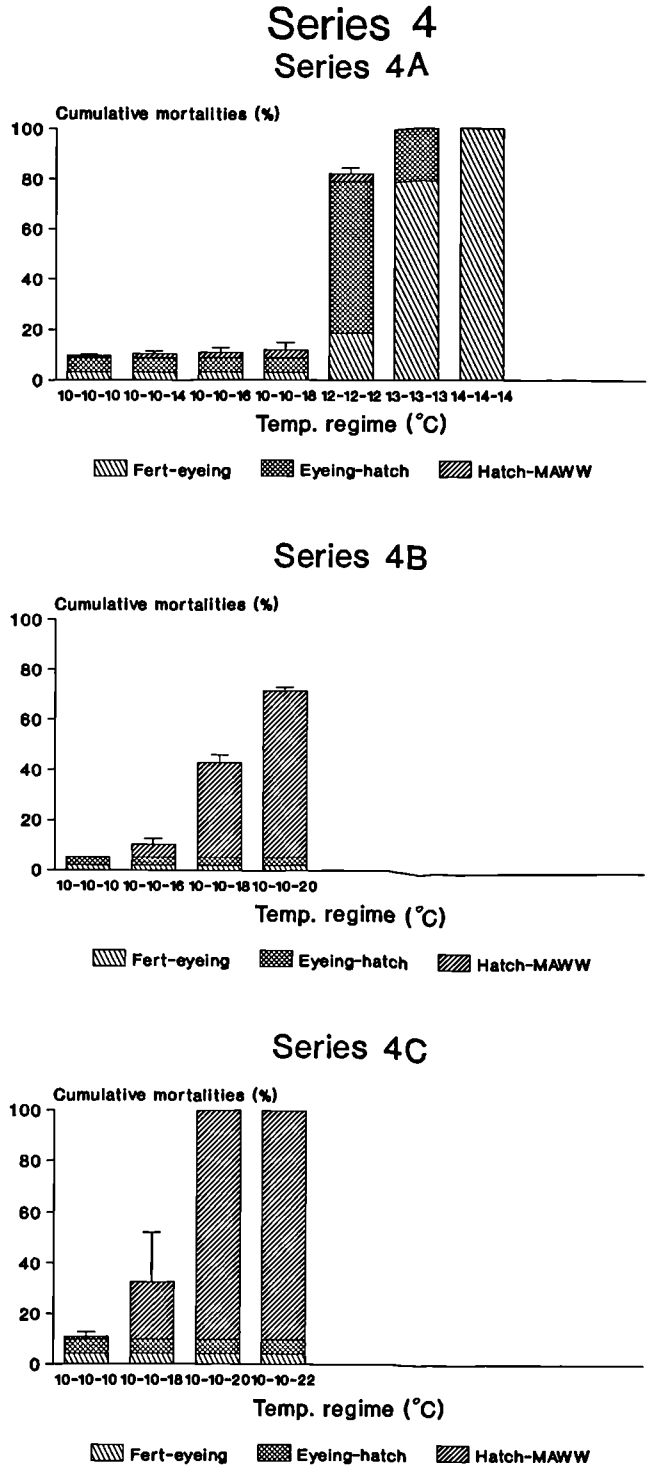


Fig. 2.3.4 Cumulative mortalities ±SD (%) in Series 4 at eyeing, total hatching and MAWW, expressed as a percentage of eggs at the start of the experiment, plotted against different temperature regimes. Percentage values are the means of 3 replicate treatments.

(b) Mortality of eggs from eyeing to hatching

Series 1 (Table 2.3.1, Fig. 2.3.1)

For the particular temperature regimes examined in Series 1, survival was significantly higher at 10°C (8-10) and 12°C (8-12), where respective hatching rates were 94.2 and 95.0%. Although survival to hatching was lower (87.0%) at 8°C (8-8), the differences between the transformed data were not shown to be significant. For temperatures of 14°C (8-14) and 16°C (8-16), mortalities were progressively more severe, and differences were shown to be highly significant ($P<0.001$).

The extent of egg mortality from eyeing to hatching was largely determined by the temperature from fertilisation to eyeing. The mortality data showed losses under ambient conditions (10°C) of between 5.8-65.4% to hatching, depending on the initial incubation temperature (8, 10 or 12°C) from fertilisation to eyeing. When the temperature from fertilisation to eyeing was 12°C, the highest mortality was subsequently recorded from eyeing to hatching. Eggs incubated at 8 or 10°C until eyeing, and then exposed to 16°C, experienced over 80% mortality to hatching. Over the range of temperatures (EH) examined (8-16°C), total viable hatch was less than 35% when FE=12°C, and no hatching occurred at 16°C. This effect was highly significant ($P<0.001$) at 12°C, where 95.0% of the 8-12 eggs hatched normally, compared to 74.1 and 20.4% normal hatch for the 10-12 and 12-12 groups, respectively. Therefore, high egg mortalities in groups where EH is 12°C or less were attributable specifically to a high

FE. The same influence of temperature (from fertilisation to eyeing) on subsequent levels of mortality was again clearly demonstrated where EH was 14 and 16°C. When FE was 8°C, hatching success was 55.7% at 14°C and only 17.1% at 16°C. When FE was increased to 12°C, hatching success was further reduced to 7.9 and 0%, respectively.

It appears that eggs are more sensitive to higher temperatures during the second period of development (EH), when incubated at higher temperatures from fertilisation to eyeing. The upper EH temperature for normal hatch was raised to 12°C when FE was 8°C. Thus, the upper temperature limit during the eyed stage can be increased by lowering the temperature from fertilisation to eyeing. However, irrespective of the temperature from fertilisation, raising the temperature above 12°C following eyeing resulted in a sharp increase in the level of mortality. At 16°C few eggs hatched successfully when FE was 8 or 10°C, and none hatched when FE was 12°C.

A greater percentage of eggs hatched normally when exposed to temperature extremes at the eyed stage, than at the previous stage of development. This response was significant at temperatures of 12, 14, and 16°C ($P<0.01$).

Of the three groups of eggs which were maintained at constant temperatures (8, 10 and 12°C) from fertilisation until hatching, the levels of mortality were very similar at 8°C (13.0%) and 10°C (13.1%), but very significantly greater ($P<0.001$) at 12°C (79.6%).

During the hatching period (first to last hatch), excepting the 8°C incubates, a greater number of alevins survived in

the central portion of the hatching distribution compared to the extremes of the range ($P<0.01$). Mortalities at this time were highest in the tests involving temperatures greater than 10°C. Mortality occurred primarily during the hatching period, following the appearance of a small rupture in the chorion over the region of the head, or yolk-sac, of the embryo. The majority of mortalities occurred in embryos that partly hatched with the head, or yolk-sac, emerging from the chorion first. Tail-first is the usual process. At the lowest temperature regime tested (8-8), most mortalities occurred in full-term embryos that died unhatched.

Series 2 (Table 2.3.2, Fig. 2.3.2)

The second series of experiments duplicated the middle range of temperatures tested in Series 1.

ANOVAR showed no significant differences in egg mortality within the range of temperatures tested (9-12°C) during the eyed stage of development. However, mean cumulative mortalities of eggs from fertilisation to hatching were very significantly higher ($P<0.001$) under all temperature regimes when FE=11°C. This was due to a higher level of mortality (11.0%) at 11°C during the first period of incubation. When egg mortalities for all groups with FE=9°C, but with EH varying from 9-12°C, were pooled and tested against two other similarly pooled groups for which FE=10 and 11°C, mean mortalities for the pooled groups were 3.6, 6.8 and 14.6%, respectively. There were highly significant differences between each of the pooled groups ($P<0.001$). Clearly, the

production of viable alevins was highest when the temperature from fertilisation to eyeing was 9°C.

When eggs were held at constant temperatures of 9, 10 and 11°C, percentage mortalities from fertilisation to hatching were 3.6, 6.0 and 14.1, respectively - these differences were very significant ($P<0.01$).

Series 3 (Table 2.3.3, Fig. 2.3.3)

The level of egg mortality from eyeing to hatching was again shown to be influenced by the temperature from fertilisation to eyeing. A gradual increase in cumulative mortalities to hatching within the temperature range of 9-11°C was followed by very significant increases ($P<0.001$) at 12°C (24.9%) and 13°C (88.1%).

Among the 5 groups of eggs incubated at constant temperatures from fertilisation to hatching, very significant increases ($P<0.001$) in mortality were again shown at 12 and 13°C. At these temperatures, high mortalities occurred just prior to and during hatching.

Series 4A (Table 2.3.4, Fig. 2.3.4)

Mortality was excessive at 12°C (12-12) and total mortality occurred at 13°C (13-13). Differences between the levels of mortality during egg incubation at the ambient temperature of 10°C (8.6%), and at 12°C (79.7%) and 13°C (100%), were highly significant ($P<0.001$).

(c) Alevin mortality

Series 1 (Table 2.3.1, Fig. 2.3.1)

The lowest mortalities occurred in groups of alevins derived from eggs incubated at 8 or 10°C. Significantly higher mortalities ($P<0.01$) occurred in groups where egg incubation occurred at temperatures of 12°C and greater. Pearson's product moment correlation coefficient (r) showed a high statistical association ($P<0.01$) between the levels of egg mortality and alevin mortality within groups ($r = 0.835$).

At temperatures of 12-16°C, alevin survival was improved when egg incubation temperatures were equal to, or less than, 10°C: for groups held at temperature regimes of 8-8-16 and 10-10-16, alevin mortalities were 1.8 and 7.2%, respectively; while temperature regimes of 8-12-16 and 10-12-12 gave rise to mortalities of 20.6 and 37.0%, respectively.

Alevins were generally less sensitive to high temperatures within the range of 12-16°C than eggs. Excluding data from groups in which the egg incubation temperature was higher than 10°C, all temperatures tested during the alevin stage within Series 1 (8-16°C) gave rise to high alevin survival. Mean alevin mortalities for these groups ranged from 0% for temperature regimes of 8-8-8, 8-8-14, 8-10-12, to 9.8% for the group held at 8-10-16. The upper temperature limit for alevins was not established in this series.

Series 2 (Table 2.3.2, Fig. 2.3.2)

Within the narrow temperature range tested in this series (9-12°C), alevin mortalities were very low - less than 2% (range of 0-1.5%). Thus, temperatures within the range of 9-12°C during egg incubation appeared to have no influence on the subsequent survival of alevins. However, mean cumulative mortalities of eggs and alevins from fertilisation to MAWW were significantly higher ($P<0.01$) when FE=11°C due to the higher level of egg mortality (11%) occurring at that temperature.

Series 3 (Tables 2.3.3, Fig. 2.3.3)

In agreement with Series 1, an association was shown between the temperature regime during egg incubation and subsequent alevin mortality. For those groups held at a common temperature regime from eyeing to MAWW (11-11, 12-12 and 13-13), the level of alevin mortality increased significantly ($P<0.01$) with increasing temperature from fertilisation to eyeing.

For the 5 groups held at constant temperatures (9, 10, 11, 12 and 13°C) throughout development, cumulative mortalities to MAWW (Fig. 2.3.3) increased gradually from 7.0% at 9°C to 14.5% at 11°C, but showed a very significant increase ($P<0.001$) to levels of 41.6 and 96.4% at 12 and 13°C, respectively.

Temperatures of 12 and 13°C only caused high alevin mortalities when the egg incubation temperatures were of

similar magnitude.

Series 4A (Table 2.3.4, Fig. 2.3.4)

The alevins held in unheated water (ambient conditions of 10°C) suffered lower losses than those in warmed water.

Following egg incubation at a constant 10°C, subsequent levels of alevin mortality were similar within the temperature range tested (10-18°C).

Highest cumulative mortality occurred in the group held at a constant 12°C (12-12-12) from fertilisation to MAWW. This difference was shown to be highly significant at $P<0.001$.

Series 4B (Table 2.3.5, Fig. 2.3.4)

Alevins held at 10°C suffered no mortality and at 16°C mortality was relatively low (5.1%). However, at higher temperatures alevin mortalities increased sharply: mortalities were very significantly greater ($P<0.001$) in the groups held at 18°C (37.8%) and 20°C (66.3%).

Series 4C (Table 2.3.6, Fig. 2.3.4)

The results of this experiment confirmed those of the two previous experiments conducted in Series 4. Highest survivals were again attained by the alevins held at the ambient temperature of 10°C. Mortality was 32.7% at 18°C, and total mortality occurred at 20 and 22°C. Alevin mortality was very significantly higher at 18°C than 10°C ($P<0.001$).

Table 2.3.5 Series 4B - mean mortality and mean cumulative mortality (in parentheses) ±SD (%) of salmon eggs, alevins and fry during four periods of development under different temperature regimes. The percentages shown are based on the numbers of viable eggs at the beginning of the experiment. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001. Data were arc sine transformed prior to testing by ANOVAR and SNK.

Temp. regime (°C)	Mean mortality±SD (%)			
	Eggs		Alevins	Fry
	Fert-eyeing	Eyeing-hatch	Hatch-MAWW	MAWW-1200°d
10-10-10	2.0±0.1	3.1±0.1 (5.1±0.1)	0.0 (5.1±0.1)	2.5±1.2 (7.6±1.7)
10-10-16	2.0±0.1	3.1±0.1 (5.1±0.1)	5.1±2.9 (10.2±2.9)	9.2±0.2** (19.4±0.1**)
10-10-18	2.0±0.1	3.1±0.1 (5.1±0.1)	37.8±2.1*** (42.9±2.1***)	28.9±1.1*** (71.8±1.6***)
10-10-20	2.0±0.1	3.1±0.1 (5.1±0.1)	66.3±1.0*** (71.4±1.0***)	LV

LV: Low viability (mortality >70%). Insufficient number of fish available for fry rearing phase of study.

Table 2.3.6 Series 4C - mean mortality and mean cumulative mortality (in parentheses) ±SD (%) of salmon eggs, alevins and fry during four periods of development under different temperature regimes. The percentages shown are based on the numbers of viable eggs at the beginning of the experiment. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001. Data were arc sine transformed prior to testing by ANOVAR and SNK.

Temp. regime (°C)	Mean mortality±SD (%)			
	Eggs		Alevins	Fry
	Fert-eyeing	Eyeing-hatch	Hatch-MAWW	MAWW-1200°d
10-10-10	4.1±0.1	5.6±0.1 (9.7±0.1)	1.0±1.0 (10.7±2.0)	NT
10-10-18	4.1±0.1	5.6±0.1 (9.7±0.1)	23.0±1.0*** (32.7±16.5***)	NT
10-10-20	4.1±0.1	5.6±0.1 (9.7±0.1)	90.3±0.1*** (100.0 ***)	NV
10-10-22	4.1±0.1	5.6±0.1 (9.7±0.1)	90.3±0.1*** (100.0 ***)	NV

NT: Not tested (because of high alevin mortalities within Series 4C, no further testing was conducted).
NV: Not viable (total mortality).

Within this series of experiments, alevin mortality was predominantly caused by severe oedema of the yolk-sac ("blue sac disease").

2.3.1.2 Fry mortality

The objective of this part of the investigation was to examine the effect of the various temperature regimes during egg and alevin development on subsequent fry survival from first feeding (MAWW) to 1200 degree-days after fertilisation. The temperature tolerance of fry was not tested in this investigation. All fry rearing studies were conducted at the ambient temperature (10°C).

Series 1 (Table 2.3.1)

Relatively few groups of fry were tested in Series 1 because the survival of fish in many groups was low (less than 30% surviving) or nil. A further 5 groups of fry (8-10-16, 8-12-16, 10-8-8, 10-10-14 and 10-10-16) were not tested because the fry rearing systems were being utilised by a concurrent experiment. Mortalities were very low in those groups tested, ranging from 0-2.6% (mean of 0.7%). There appeared to be no association between the temperature regimes experienced by groups from fertilisation to MAWW and their subsequent fry mortality.

Cumulative mortalities were very significantly higher ($P<0.001$) in those groups (8-14-14 and 10-12-12) which had suffered proportionally higher mortalities during earlier

periods of development.

Series 2 (Table 2.3.2)

Groups of fry from all 24 temperature regimes were tested in this series. Fry mortalities were generally low ranging from 0-7.8% (mean of 1.7%). Mortalities in groups held at 9-12-11 (7.8%) and 10-10-12 (4.7%) were shown to be significantly greater ($P<0.01$) than the other groups tested. However, these losses were mainly due to other factors; bacterial gill disease was diagnosed in fry within both groups. Because disease-related mortality was low, no medication was administered to any of these groups. There were no apparent trends among the data.

Cumulative mortalities were again significantly higher ($P<0.01$) in all groups held at 11°C from fertilisation to eyeing.

Series 3 (Table 2.3.3)

Excepting the group maintained at a constant 13°C (13-13-13), where cumulative egg and alevin mortality was excessive (96.4%), all groups of fry were tested in Series 3. Mortalities ranged from 0.9 (11-11-11) to 11.1% (10-13-13), with a mean mortality of 3.9%. The main losses were again attributed to bacterial gill disease. There appeared to be no association between the mortality within groups of fry and their temperature regime from fertilisation to MAWW.

Cumulative mortalities were significantly higher ($P<0.01$)

in groups 11-13-13 and 12-12-12, where losses were high from eyeing to MAWW.

Series 4A (Table 2.3.4)

Levels of mortality in the 4 groups of fry tested were very similar (range of 2.3-3.6%; mean of 2.8%). Clearly, the influence of temperature within the range of 10-18°C during alevin development had no apparent effect on subsequent fry mortality.

Series 4B (Table 2.3.5)

The pattern of fry mortality was similar to that between hatching and MAWW. The temperature experienced by alevins had a marked effect on the subsequent level of fry mortality. Differences in mortality between the 3 groups tested were very significant ($P<0.01$). It was noted that many of the alevins in the groups held at 18°C and particularly those held at 20°C, retained an abnormally high proportion of yolk material at the attainment of MAWW. This condition accounted for the majority of losses which occurred during both the alevin and fry stages of development.

Series 4C (Table 2.3.6)

Because alevin mortalities were excessive at the higher temperatures tested (18, 20 and 22°C), fry rearing studies were not conducted in this experiment.

2.3.1.3 Comparison of mortality data from Series 1-4 (Tables 2.3.1-2.3.6, Figs. 2.3.1-2.3.6).

No statistical comparisons were performed between the various series because the experiments were conducted in different years using different genetic stocks. Furthermore, variations in experimental conditions may have occurred between years.

The results of the four series of experiments show that egg incubation temperature had a marked effect on egg and alevin survival. When comparing experimental series, common temperature regimes generally had a consistent effect on egg and alevin survival. The main difference in levels of egg mortality within the the four series occurred between those groups incubated at temperatures less than 12°C, and those incubated at 12°C and above. Within all series of experiments, low mortalities occurred from fertilisation to hatching at the ambient temperature of 10°C: mean egg mortality for all series was 8.2%, ranging from 5.1% in Series 4B to 13.1% in Series 1. At 10°C, alevin mortalities were again consistently low within all series of experiments, with an overall mean of 1.5% and a range of 0% (Series 4B) to 3.4% (Series 1).

However, tolerance to high temperatures did vary slightly between corresponding groups from different experimental series. The greatest variation in levels of egg mortality between the four series occurred at the temperature extremes. Series 3 showed less mortality as a group when compared with the other three series. It appears that the stock used in

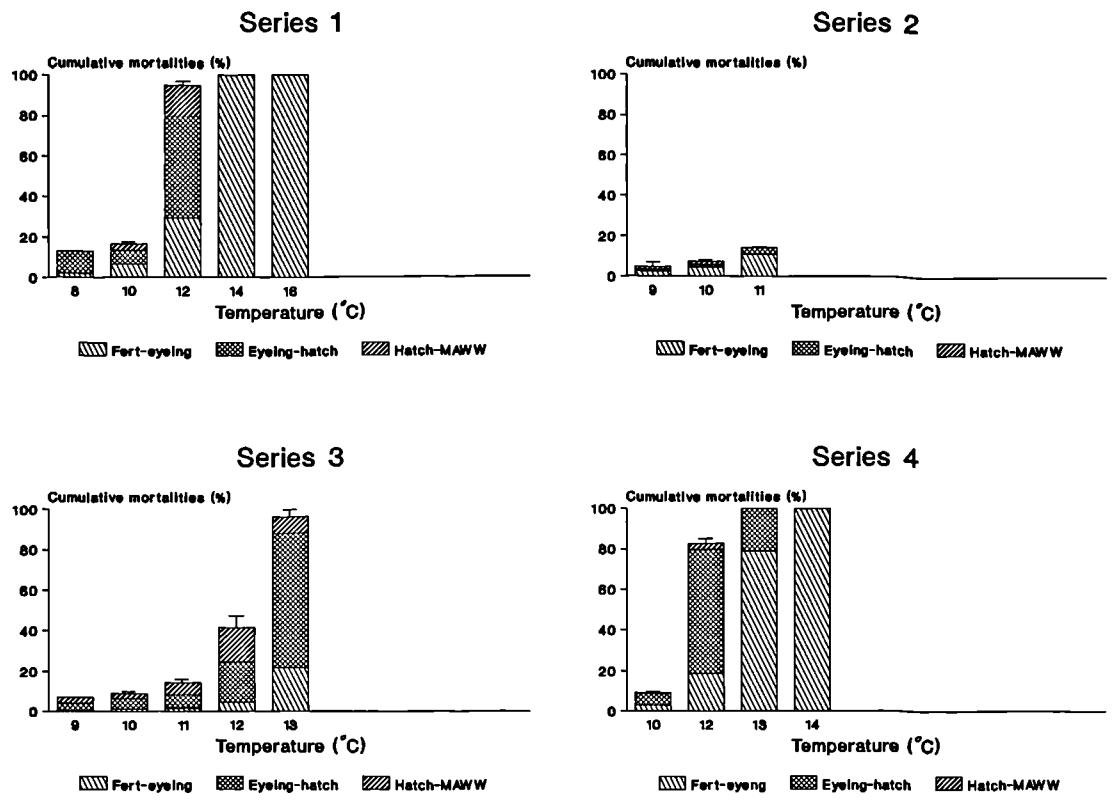


Fig. 2.3.5 Cumulative mortalities ±SD (%) at eyeing, total hatching and MAWW, expressed as a percentage of eggs at the start of the experiment plotted against different constant temperature regimes tested in Series 1-4. Percentage values are the means of 3 replicate treatments.

Series 3 was less sensitive to temperature extremes than the stocks used in the other series. At 12°C, mortality from fertilisation to eyeing was markedly higher ($P<0.001$) than at the lower temperatures tested in Series 1 and 4A (29.1 and 18.6%, respectively), but was only slightly higher, although still significantly greater ($P<0.05$), than the lowest temperature tested in Series 3 (4.5%).

At a constant 12°C, Series 1 eggs had a 20.4% hatching success and a 5.0% alevin survival to MAWW, while Series 3 eggs had a 75.1% hatching success and 58.4% alevin survival to MAWW. At the same temperature, hatching success and alevin survival in Series 4A was 20.3 and 17.2%, respectively. Within all series, at 12°C a higher proportion of the mortality occurred during the process of hatching. Clearly, temperatures below 12°C are recommended for the incubation of eggs. At 13°C, cumulative mortality to MAWW was virtually complete (96.4%) in Series 3, and was total in Series 4A. At 14°C, egg mortality was 100% in both Series 1 and 4A. All eggs died before eyeing.

In each of the 3 experiments conducted in Series 4 (A-C), optimal survival (>90%) to MAWW occurred at 10°C. Low alevin mortality (3.3%) occurred at 18°C in Series 4A. In contrast, high alevin mortality occurred in the group maintained at 18°C in Series 4B (37.8%) and 4C (23.0%). Mortality was excessive at 20°C in Series 4B and reached 100% at the same temperature in Series 4C.

Within all four series, there were few significant differences in mortality between groups of fry held at the ambient temperature (10°C). Excepting Series 4B, where fry

mortality was 28.9% for the group held at 10-10-18, mortalities were low in all groups within all series, ranging from 0-11.1% (Tables 2.3.1-2.3.5).

2.3.1.4 Mortality of eggs and alevins in hatching troughs

Among the 6 lots of surplus eggs (one from each experiment) placed in the glass-fibre hatching troughs under ambient temperature conditions (10°C) at the beginning of the various experiments, percentage mortalities from fertilisation to MAWW were 17.6 (Series 1), 8.3 (Series 2), 8.2 (Series 3), 10.2 (Series 4A), 7.2 (Series 4B), and 12.1 (Series 4C), demonstrating that the experimental systems and procedures were not detrimental to the survival of eggs or alevins. Indeed, mortalities in the hatching troughs generally exceeded those in the experimental lots. To some extent, this can be explained by the inclusion of unfertilised eggs in the mortality figures for the hatching troughs.

2.3.1.5 Patterns of mortality

Mortalities occurred more randomly during the course of development in eggs incubated at 8-11°C than those incubated at higher temperatures, where definite patterns of mortality were apparent (Fig. 2.3.6). The majority of mortalities occurred at two separate phases of development: the first phase of high mortality was detected between 50-150 degree-days after fertilisation, depending on temperature, and the second phase coincided with hatching. Mortality increased

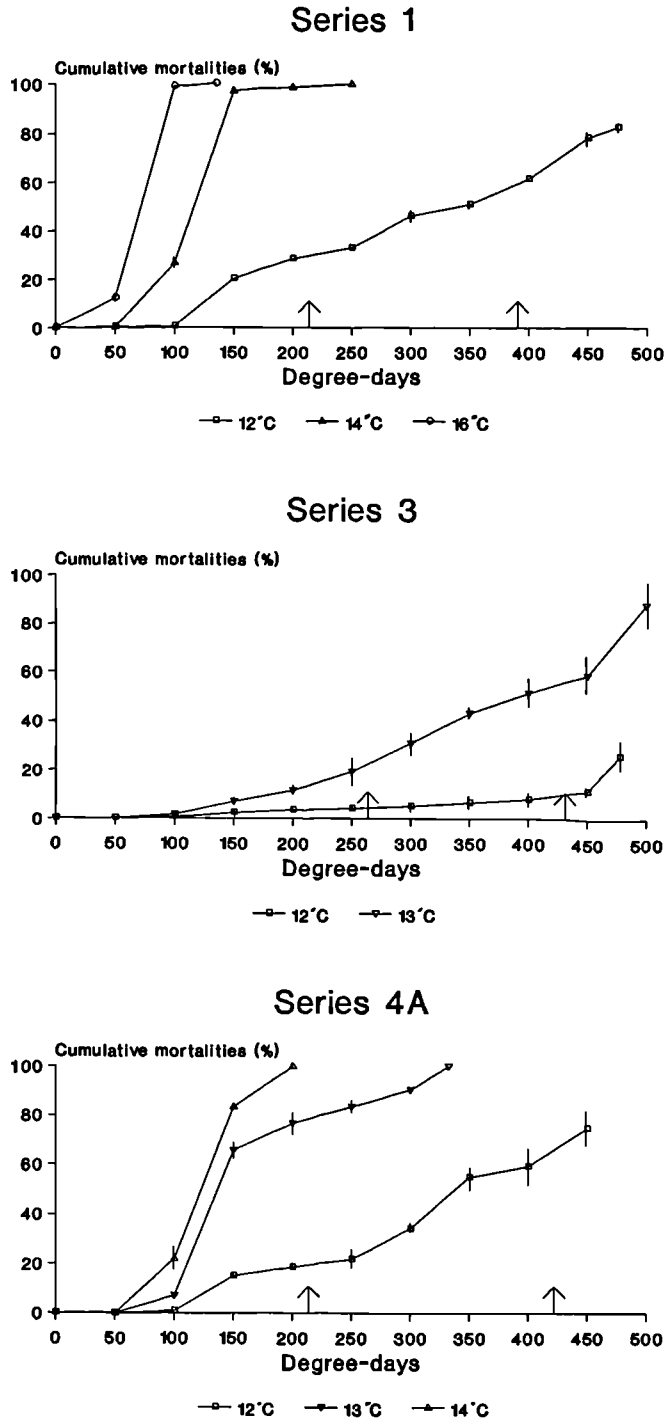


Fig. 2.3.6 Cumulative mortalities ±SD (%) of eggs and alevins at subacute and lethal constant temperatures of 12°C (Series 1, 3 & 4A), 13°C (Series 3 & 4A) 14°C (Series 1 & 4A) and 16°C (Series 1) plotted against development time in degree-days after fertilisation. Percentage values are the means of 3 replicate treatments. Arrows indicate the approximate times to eyeing and 50% hatching.

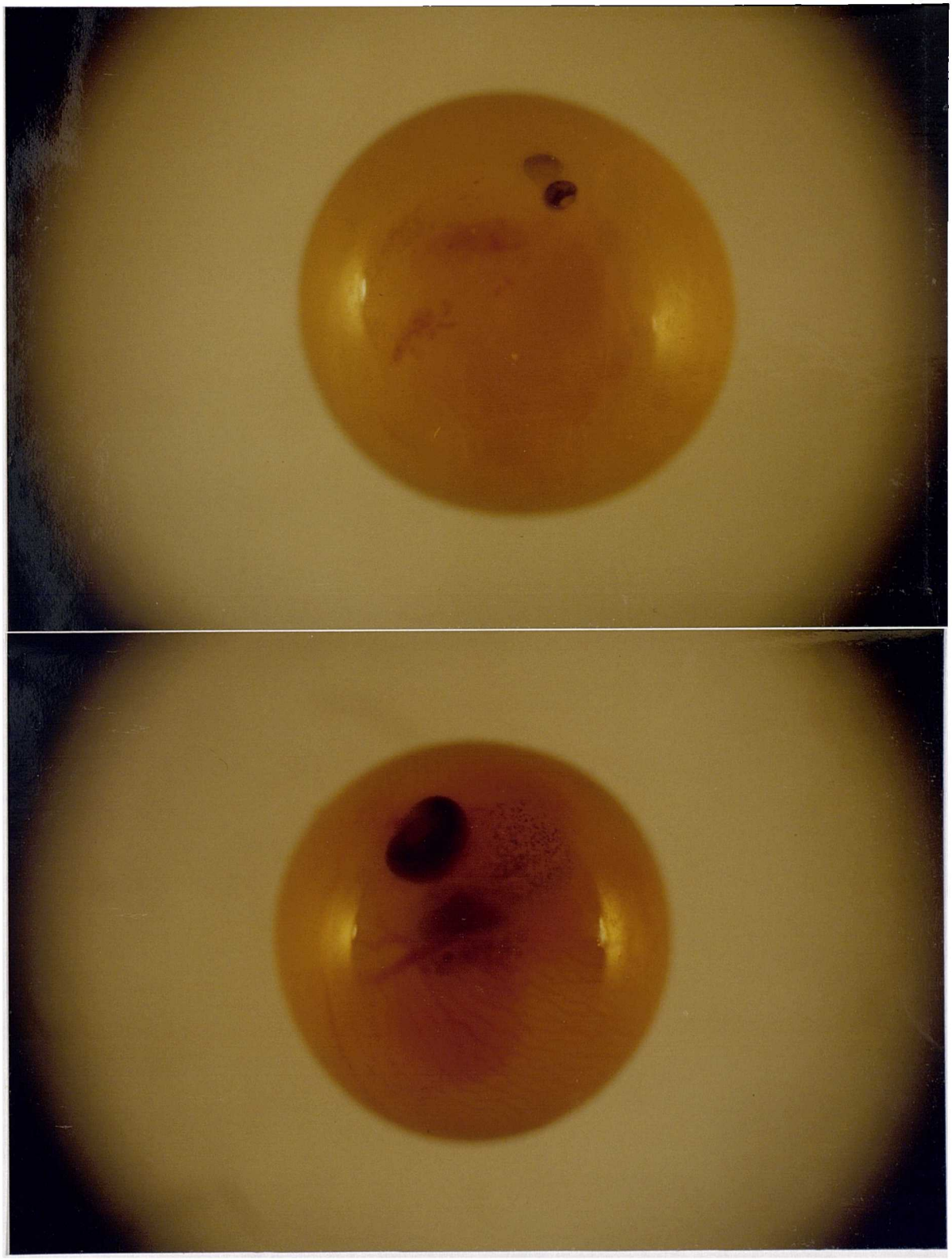
shortly before hatching began and remained high during the hatching period. With increasing temperature, a progressively larger proportion of eggs died at the earlier phase of development. At 14 and 16°C all eggs died at this time.

2.3.1.6 Abnormal development of eggs and alevins

A number of morphological abnormalities of eggs and alevins were observed, though not precisely diagnosed, in the experiments described in this study. Although under all temperature regimes various structural defects were observed in some of the eggs and alevins, abnormalities were generally more prevalent at higher temperatures. These defects were generally similar at different temperatures and mainly included incomplete eye pigmentation, body deformations and pericardial oedema. Similar developmental abnormalities have also been observed at normal temperatures under experimental conditions (Marten, 1992) and in salmonid hatcheries.

The most frequently observed malformation of developing embryos was a partial failure of the development of retinal pigment deposition in the eyes (Fig. 2.3.7). The eyes were very lightly pigmented and only occasionally did hatching occur; surviving alevins were small, lightly pigmented, and had poorly developed vascular systems. This condition was more prevalent when the temperature from fertilisation to eyeing was equal to or greater than 10°C. At the lowest temperatures tested in Series 1-3 (8-9°C) a number of eggs appeared to develop to full-term embryos, but did not hatch and were classified as non-viable eggs. This abnormality was

Fig. 2.3.7 Photographs of eyed eggs close to hatching (400 degree-days after fertilisation) showing: top, abnormal egg with incomplete retinal pigmentation and; bottom, normal egg with complete retinal pigmentation.



never observed at higher temperatures.

Among all groups within all experimental series, some newly-hatched alevins showed abnormal development. At any given temperature, the first eggs that hatched produced alevins which were generally small and seldom viable. These alevins were designated as premature and were more prevalent at temperatures greater than 11°C. At all temperatures a proportion of the eggs hatched head or yolk-sac first (Fig. 2.3.8). This abnormality was more apparent following egg incubation at temperatures of 12°C and above.

A greater number of abnormalities were observed among alevins from groups which had been incubated near the upper temperature limit: thus, eggs incubated at 12°C and above yielded higher frequencies of abnormal alevins than those incubated at lower temperatures. At all temperatures tested during this investigation, a frequent form of abnormality among newly-hatched alevins was a prominent curvature of the spinal column (Fig. 2.3.9). Unless spinal curvature was slight, alevins retained this contorted posture throughout their subsequent development.

At the highest egg incubation temperatures in Series 1 (14 and 16°C), abnormalities characterised by a virtual absence of the lower jaw or, by its retarded development, were apparent in some newly-hatched alevins. Many malformed alevins continued to develop up to and through MAWW, but were unable to feed because the lower jaw was non-functional. The occurrence of this abnormality was only observed in Series 1 and accounted to some extent for the higher level of mortality experienced in this series compared to the other

Fig. 2.3.8 Photographs of hatching eggs (430 degree-days after fertilisation) showing: top, abnormal head-first hatch and; bottom, normal tail-first hatch.

Fig. 2.3.9 Photograph of alevins (460-540 degree-days after fertilisation) showing abnormal curvature of the spine.

series. It is unclear why this particular type of deformity was confined to Series 1.

In groups where newly-hatched alevins were deformed, such alevins were included in counts of non-viable eggs. Clearly, these developmental abnormalities occurred during the egg incubation period but were only apparent at hatching.

During the alevin stage, severe oedema of the yolk-sac (Fig. 2.3.10) was particularly common when temperatures were high (16°C and greater) following hatching. Oedema of the pericardial cavity was caused by fluid distension of the vitelline membrane. In most instances where this condition occurred, the head was bent downward and the lower jaw was permanently agape. The majority of alevins afflicted with this condition subsequently died prior to final yolk-sac absorption.

No subsequent abnormalities were observed in any of the groups of fry that had been superficially normal at the time of first feeding.

Fig. 2.3.10 Photograph of alevin (590 degree-days after fertilisation) showing severe oedema of the yolk sac.

2.3.1.7 Summary of Results: Mortality (2.3.1)

Egg and alevin mortality

The results of the four series of experiments showed that maximum survival to hatching within the range of temperatures tested (8-16°C) occurred between 8-11°C. The maximum temperature which gave a high rate of egg survival at a constant temperature throughout the incubation period was 11°C. Mortalities were less than 17% at 8-11°C, but above this range mortalities were considerably greater, increasing to 24.9-79.7, 88.1-100.0, and 100%, at egg incubation temperatures of 12, 13 and 14°C, respectively.

The capacity to tolerate high temperatures was dependent upon the stage of development of salmon. Few mortalities at any stage of development occurred at 11°C or less. Near maximum percentage survival to hatching (87% or greater) was achieved during egg incubation at 8-10°C; above 11°C, effects were a function of temperature, duration of exposure, and stage of development, and at temperatures above 12°C, egg survival was severely reduced. Temperature was shown to have a more appreciable effect on survival during egg incubation than after hatching: eggs undergoing early development were the least tolerant and alevins were the most tolerant, surviving exposure in varying degrees to 18°C. When increased mortality occurred at high temperatures from fertilisation to eyeing, the adverse effects of these high temperatures continued to be reflected in the mortality data throughout the eyed egg and alevin stages of development. Oedema of the

yolk-sac caused much of the mortality during the alevin stage.

From the foregoing series of investigations it is apparent that the upper incipient lethal temperatures for salmon eggs and alevins were 14 and 20°C, respectively. The long-term upper lethal temperatures for eggs and alevins were 12-14°C and 18-20°C, respectively. Eggs should not be continuously exposed to temperatures greater than 11°C.

Fry mortality

In general, there was little or no association between the thermal history of groups of fish from fertilisation to MAWW and their subsequent fry mortality.

2.3.2 DEVELOPMENT AND GROWTH

2.3.2.1 Development times

The times required to reach various stages of development at different combinations of temperature are given in Tables 2.3.7-2.3.12, and the relationship between temperature and mean development times is shown in Figs. 2.3.11-2.3.14. Development times are expressed as the mean number of days and sums of degree-days required by eggs and alevins to reach eyeing, 50% hatching, and MAWW.

It is apparent that the duration of development in days has a direct relationship with temperature. Development rate to any given stage of development was directly dependent on temperature, being accelerated at high temperatures: in Series 1, development time from fertilisation to eyeing decreased from 29.4 days at 8°C to 17.4 days at 12°C. The time from eyeing to hatching was similarly affected by temperature over the range tested. The total time from fertilisation to 50% hatching varied between 59.2 and 31.6 days at constant temperatures of 8 and 12°C, respectively. The period from mean hatching to MAWW was also dependent on temperature, with higher temperatures accelerating development. The time required to absorb the yolk declined with increasing temperature from 40.5 days at 10°C, to 23.5 days at 18°C (Series 4A, Table 2.3.10). The time in days to the attainment of successive stages of development was inversely related to temperature: at constant temperatures the time required to attain specific stages of development

Table 2.3.7 Series 1 - mean development times in days and degree-days from fertilisation to eyeing, to 50% hatching, and to MAWW at various temperatures.

Temp. regime (°C)	Development times					
	Fert-eyeing		Fert-hatch		Fert-MAWW	
	Days	°Days	Days	°Days	Days	°Days
8-8-8	29.4	227.0	59.2	459.9	110.0	856.1
8-8-14	29.4	227.0	59.2	459.9	83.2	788.7
8-8-16	29.4	227.0	59.2	459.9	82.8	837.0
8-10-12	29.4	227.0	48.9	428.1	75.9	757.8
8-10-16	29.4	227.0	48.9	428.1	76.0	850.9
8-12-14	29.4	227.0	43.0	395.9	72.9	809.1
8-12-16	29.4	227.0	43.0	395.9	73.1	868.1
8-14-14	29.4	227.0	41.0	388.9	72.0	813.6
8-16-16	29.4	227.0	39.3	386.0	NV	NV
10-8-8	23.4	233.9	52.7	462.9	97.0	809.0
10-10-10	23.4	233.9	41.1	418.0	82.9	840.2
10-10-14	23.4	233.9	41.1	418.0	67.9	787.8
10-10-16	23.4	233.9	41.1	418.0	68.1	844.6
10-12-12	23.4	233.9	36.3	395.1	68.0	781.8
10-14-14	23.4	233.9	34.4	386.8	63.9	796.9
10-16-16	23.4	233.9	34.6	411.8	NV	NV
12-8-8	17.4	213.5	49.0	460.0	97.1	835.7
12-10-10	17.4	213.5	36.4	409.2	82.9	874.2
12-12-12	17.4	213.5	31.6	390.0	68.0	838.1
12-14-14	17.4	213.5	30.7	398.4	NV	NV
12-16	17.4	213.5	NV	NV	NV	NV
14	NV	NV	NV	NV	NV	NV
16	NV	NV	NV	NV	NV	NV

NV: Not viable (total mortality).

Table 2.3.8 Series 2 - mean development times in days and degree-days from fertilisation to eyeing, to 50% hatching, and to MAWW at various temperatures.

Temp. regime (°C)	Development times					
	Fert-eyeing		Fert-hatch		Fert-MAWW	
	Days	°Days	Days	°Days	Days	°Days
9-9-9	24.5	217.9	50.0	444.9	90.8	813.2
9-9-11	24.5	217.9	50.0	444.9	83.2	807.1
9-10-10	24.5	217.9	45.1	423.7	83.2	796.2
9-10-11	24.5	217.9	45.1	423.7	80.1	808.1
9-11-10	24.5	217.9	41.3	403.0	79.9	781.8
9-11-11	24.5	217.9	41.3	403.0	77.0	797.2
9-12-11	24.5	217.9	39.4	395.6	74.8	785.0
9-12-12	24.5	217.9	39.4	395.6	72.1	789.3
10-9-9	21.5	218.8	46.0	436.2	91.4	844.8
10-9-11	21.5	218.8	46.0	436.2	80.2	811.8
10-10-10	21.5	218.8	41.6	420.8	80.1	796.3
10-10-12	21.5	218.8	41.6	420.8	73.2	803.2
10-11-11	21.5	218.8	37.7	397.5	74.0	797.9
10-11-12	21.5	218.8	37.7	397.5	71.2	802.8
10-12-11	21.5	218.8	36.2	394.1	71.8	785.7
10-12-12	21.5	218.8	36.2	394.1	70.1	804.3
11-9-9	19.5	213.8	44.0	430.8	86.2	809.1
11-9-11	19.5	213.8	44.0	430.8	77.0	795.5
11-10-9	19.5	213.8	39.8	416.7	82.8	804.3
11-10-10	19.5	213.8	39.8	416.7	76.8	775.6
11-11-11	19.5	213.8	36.3	398.9	72.1	792.6
11-11-12	19.5	213.8	36.3	398.9	70.0	805.2
11-12-11	19.5	213.8	34.1	388.3	71.2	795.5
11-12-12	19.5	213.8	34.1	388.3	68.1	799.0

Table 2.3.9 Series 3 - mean development times in days and degree-days from fertilisation to eyeing, to 50% hatching, and to MAWW at various temperatures.

Temp. regime (°C)	Development times					
	Fert-eyeing		Fert-hatch		Fert-MAWW	
	Days	°Days	Days	°Days	Days	°Days
9-9-9	27.5	255.6	50.8	459.9	99.8	886.2
9-11-11	27.5	255.6	42.1	419.0	84.1	883.7
10-10-10	24.5	247.5	43.0	438.7	89.0	908.3
10-11-11	24.5	247.5	40.0	420.6	83.1	903.3
10-13-13	24.5	247.5	36.5	405.9	75.9	921.7
11-11-11	23.5	258.2	37.9	418.7	81.6	909.1
11-12-12	23.5	258.2	36.1	411.2	78.0	917.6
11-13-13	23.5	258.2	34.1	397.9	74.9	932.5
12-12-12	21.5	259.5	34.2	412.9	75.1	906.1
13-13-13	20.5	268.3	33.8	443.0	68.0	890.5

Table 2.3.10 Series 4A - mean development times in days and degree-days from fertilisation to eyeing, to 50% hatching, and to MAWW at various temperatures.

Temp. regime (°C)	Development times					
	Fert-eyeing		Fert-hatch		Fert-MAWW	
	Days	°Days	Days	°Days	Days	°Days
10-10-10	23.4	241.0	43.0	440.0	83.5	857.2
10-10-14	23.4	241.0	43.0	440.0	72.5	852.2
10-10-16	23.4	241.0	43.0	440.0	69.5	866.3
10-10-18	23.4	241.0	43.0	440.0	66.5	865.3
12-12-12	17.5	210.9	35.0	420.2	ND	ND
13-13-13	16.5	216.4	NV	NV	NV	NV
14	NV	NV	NV	NV	NV	NV

ND: Not determined (severe oedema of yolk-sac prevented meaningful determination of MAWW).
NV: Not viable (total mortality).

Table 2.3.11 Series 4B - mean development times in days and degree-days from fertilisation to eyeing, to 50% hatching, and to MAWW at various temperatures.

Temp. regime (°C)	Development times					
	Fert-eyeing		Fert-hatch		Fert-MAWW	
	Days	°Days	Days	°Days	Days	°Days
10-10-10	23.2	239.1	42.5	435.9	81.5	829.8
10-10-16	23.2	239.1	42.5	435.9	70.5	886.7
10-10-18	23.2	239.1	42.5	435.9	66.5	872.7
10-10-20	23.2	239.1	42.5	435.9	ND	ND

ND: Not determined (severe oedema of yolk-sac prevented meaningful determination of MAWW).

Table 2.3.12 Series 4C - mean development times in days and degree-days from fertilisation to eyeing, to 50% hatching, and to MAWW at various temperatures.

Temp. regime (°C)	Development times					
	Fert-eyeing		Fert-hatch		Fert-MAWW	
	Days	°Days	Days	°Days	Days	°Days
10-10-10	24.4	241.6	43.4	434.7	82.4	836.4
10-10-18	24.4	241.6	43.4	434.7	71.4	941.5
10-10-20	24.4	241.6	43.4	434.7	NV	NV
10-10-22	24.4	241.6	43.4	434.7	NV	NV

NV: Not viable (total mortality).

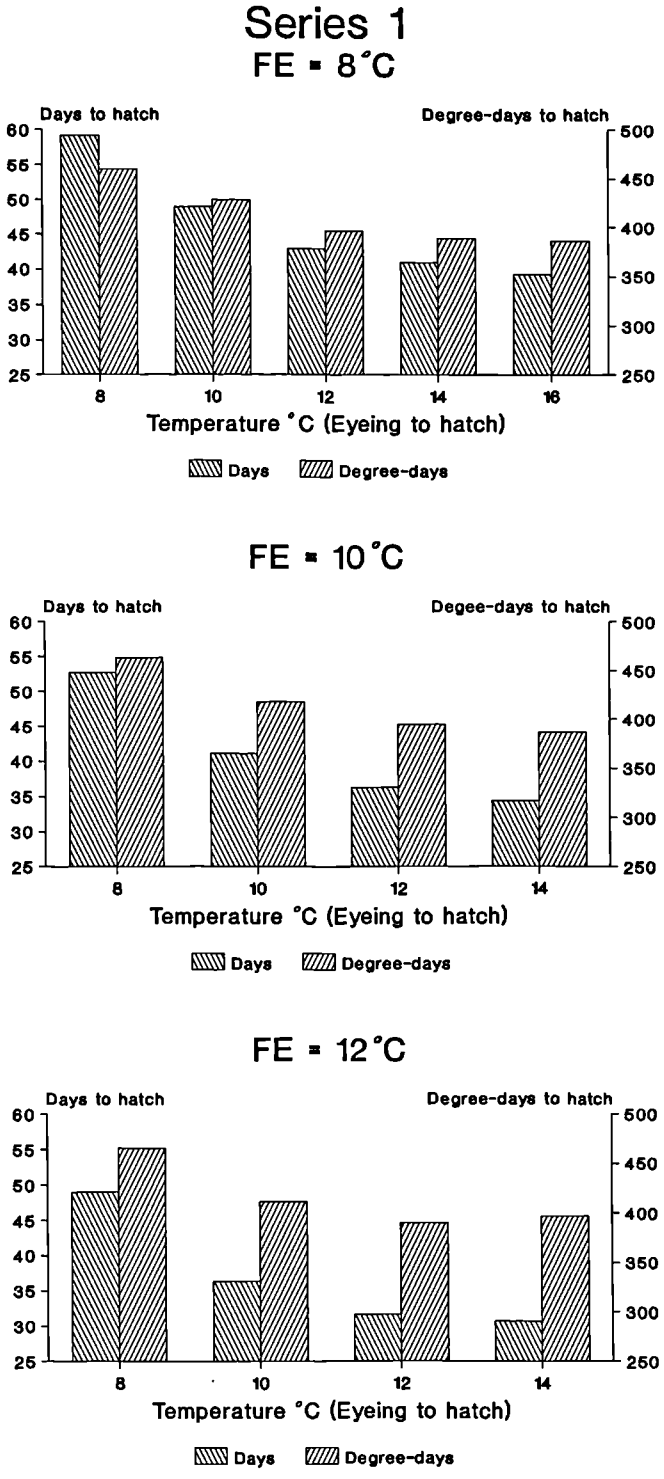


Fig. 2.3.11 Series 1 - relationship between temperature and development times in days and degree-days from fertilisation to 50% hatching. The temperature from fertilisation to eyeing is shown as FE.

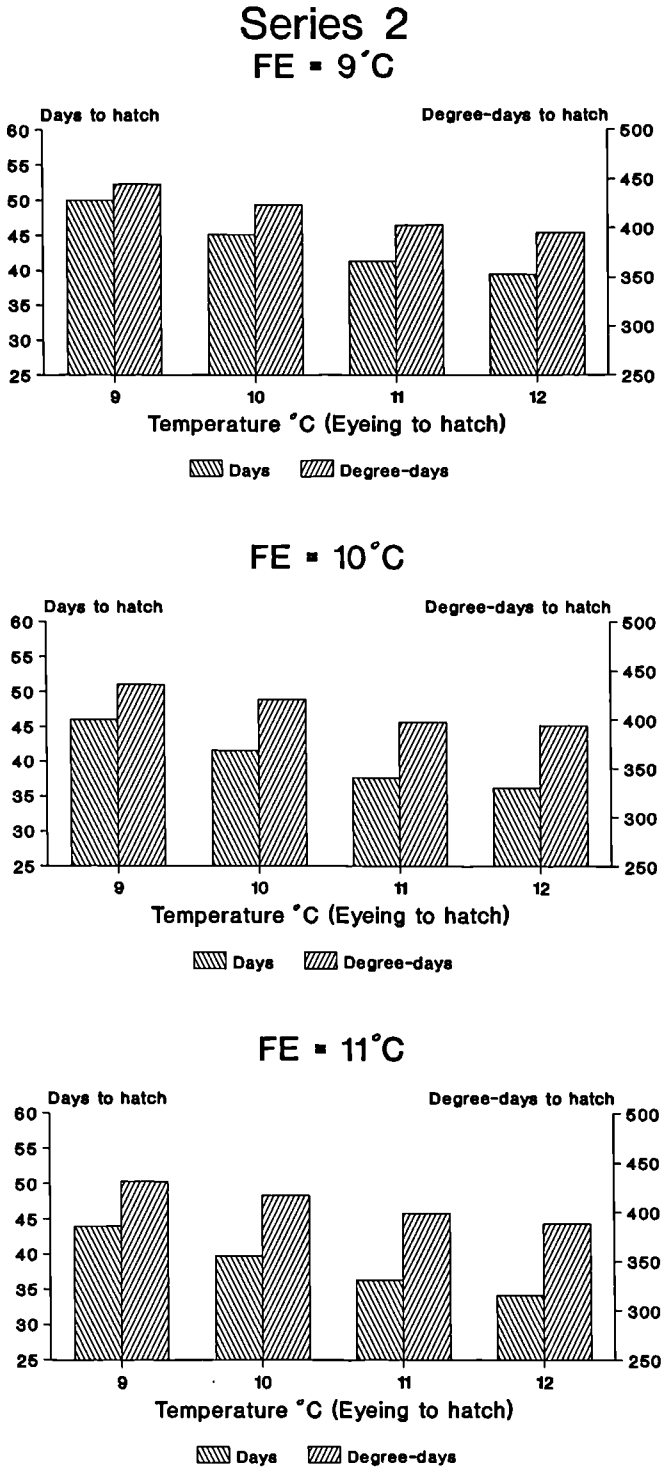


Fig. 2.3.12 Series 2 - relationship between temperature and development times in days and degree-days from fertilisation to 50% hatching. The temperature from fertilisation to eyeing is shown as FE.

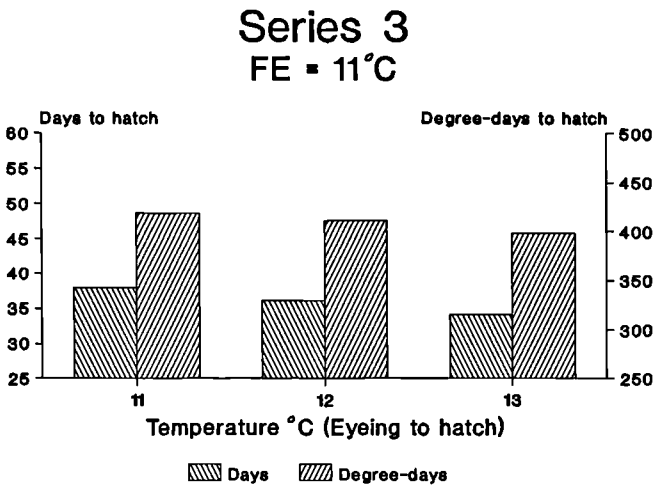


Fig. 2.3.13 Series 3 - relationship between temperature and development times in days and degree-days from fertilisation to 50% hatching. The temperature from fertilisation to eyeing is shown as FE.

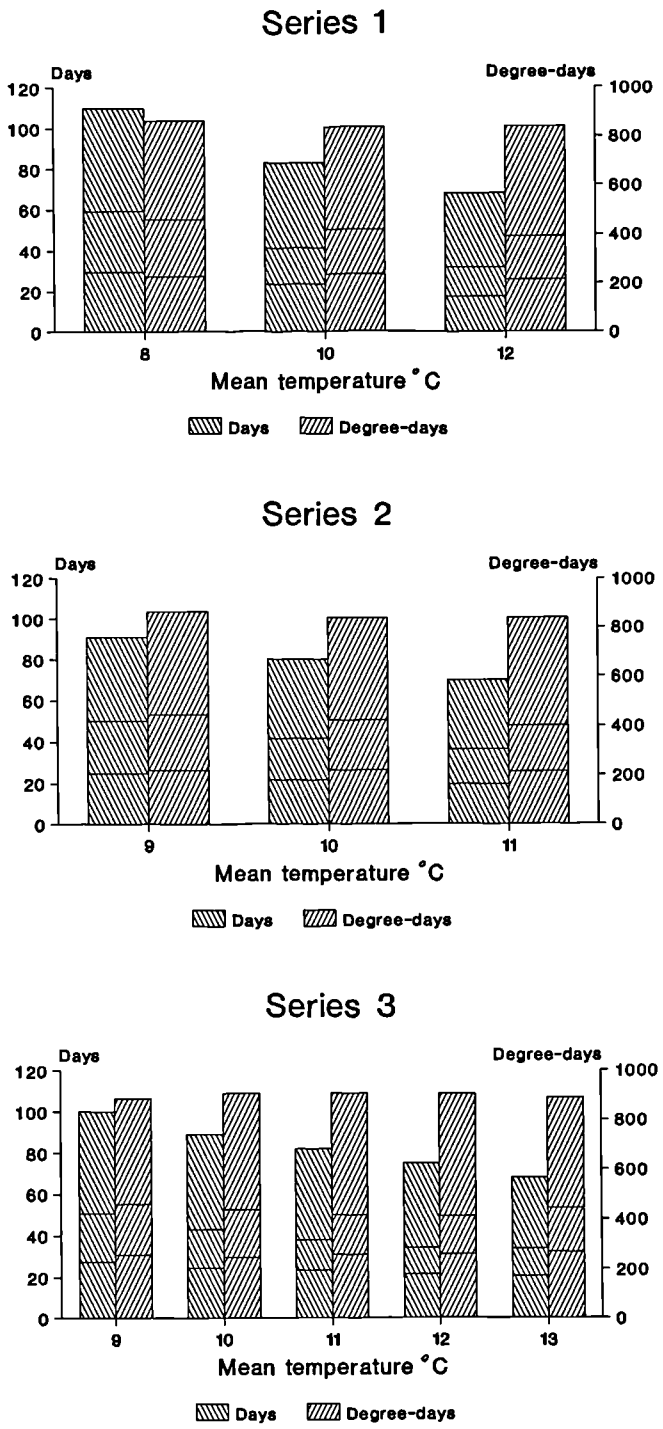


Fig. 2.3.14 Relationship between temperature and development times in days and degree-days from fertilisation to eyeing, hatching and MAWW in Series 1-3. Temperatures were constant from fertilisation to MAWW.

decreased, on average, by about 10% for every 1°C increase in temperature.

An evaluation of the empirical data showed that the relationship between temperature and the rate of development is best represented curvilinearly for the relatively narrow temperature range tested in this study (Tables 2.3.7-2.3.12, Figs. 2.3.11-2.3.13). Thus, the acceleration of the rate of development through the range of temperatures is not uniform.

The relationships between temperature and development times in days at constant temperatures (8-13°C) during the three periods of development can be expressed proportionally as follows:

$$FE/FH \times 100 = 55.2 \pm 4.1\%$$

and

$$FM/FH \times 100 = 199.5 \pm 10.9\%$$

where FE, FH, and FM represent the periods from fertilisation to eyeing, 50% hatch, and MAWW, respectively.

The relationship between temperature and development time in degree-days (Tables 2.3.7-2.3.12, Figs. 2.3.11-2.3.14) was relatively constant from fertilisation to eyeing, but showed a decline from eyeing to hatching, indicating that the acceleration of the rate of development through the range of temperatures is not uniform, but decreases gradually at higher temperatures during this latter stage of development. Development times in degree-days from hatching to MAWW were

relatively constant throughout most of the temperature range tested, but increased with increasing temperature towards the upper end of the temperature range in Series 1, 4B and 4C. Delayed attainment of MAWW at these temperatures was attributed to a yolk abnormality, which caused an accumulation of fluid in the yolk-sac. Similarly, at constant temperatures from fertilisation to MAWW (Fig. 2.3.14), the cumulative number of degree-days was relatively constant over the range of temperatures examined in this study (8-13°C). Any differences between the development times in degree-days were small and without consistent trend.

When comparing temperature regimes with the same mean egg incubation temperatures (e.g. in Series 1: 8-12, 10-10 and 12-8; Series 2: 9-11, 10-10 and 11-9; Series 3: 11-13 and 12-12), eggs incubated at constant temperatures hatched slightly earlier than those incubated under declining temperatures, but later than those incubated under increasing temperature regimes (Tables 2.3.7-2.3.9). Differences in development times in degree-days from fertilisation to hatching between increasing and declining temperature regimes were significant ($P<0.05$).

The development times at any given temperature varied slightly among the 4 series of experiments. The eggs and alevins from the stock used in Series 3 developed more slowly than those used in the other series: MAWW was attained after 89.0 days (908.3 degree-days) at 10°C, compared to a mean of 82.1 days (832.0 degree-days) for the other 3 series of experiments. At a constant 10°C (10-10-10), the development times in degree-days for the 4 series of experiments

correspond within 14% of each other for FE, 10% for EH, and 26% for HM. The wider divergence in development times during the alevin stage is due largely to the prolonged period of development during this stage in Series 3.

2.3.2.2 Hatching period

The relationship between temperature and the length of the hatching period (first to last hatch) and the progression of hatching is shown in Tables 2.3.13-2.3.16 and Figs. 2.3.15-2.3.16.

In Series 1 (Table 2.3.13) the hatching period was very significantly longer ($P<0.001$) at the lowest temperature tested (8°C): 90% of the eggs hatched during a period of 9.4 days (73.5 degree-days), while the same proportion of eggs hatched within 3.0-4.0 days (31.0-50.5 degree-days) within the temperature range of 10-16°C. Thus, the hatching period showed little variation in length within the temperature range of 10-16°C, and any differences were not significant.

Hatching periods for the other three series of experiments (Tables 2.3.14-2.3.16, Figs. 2.3.15-2.3.16) were very similar to those found at temperatures greater than 8°C in Series 1. There was no apparent relationship between temperature and hatching period within the range of 9-16°C: any increase in temperature above 9°C did not reduce the duration of the hatching period.

At 8°C (Series 1) the progression of hatching was highly asynchronous. The hatching frequency distribution was positively skewed, there being a tendency for it to rise

Table 2.3.13 Series 1 - mean hatching times ±SD (%) in days and degree-days from first hatch to 10, 50, 90 and 100% hatch at different temperatures. Asterisks indicate a mean significantly different from other means: * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. (°C)		Eggs hatched±SD (%)			
		10	50	90	100
8	Days	0.9±0.5	2.9±1.7	9.4±4.0	20.0±3.1 ***
	°Days	6.8±3.8	22.1±13.4	73.5±30.8	156.2±23.8***
10	Days	0.9±0.5	1.8±0.6	3.0±0.9	4.3±1.4
	°Days	9.4±4.7	18.8±6.1	31.0±9.2	44.4±14.2
12	Days	1.1±0.4	2.0±0.6	4.0±2.4	5.8±2.7
	°Days	13.9±5.2	24.8±7.1	50.3±29.6	72.5±33.5
14	Days	0.6±0.4	1.9±1.0	3.0±1.7	4.5±1.3
	°Days	8.0±5.4	26.5±13.8	41.5±23.2	62.4±17.6
16	Days	0.6±0.3	1.9±1.2	3.2±1.7	4.2±1.6
	°Days	9.1±5.7	30.6±18.2	50.5±26.5	66.7±24.7

Table 2.3.14 Series 2 - mean hatching times ±SD (%) in days and degree-days from first hatch to 10, 50, 90 and 100% hatch at different temperatures. ANOVAR showed that any differences between the mean hatching times of groups were not significant.

Temp. (°C)		Eggs hatched±SD (%)			
		10	50	90	100
9	Days	1.8±0.8	3.1±0.8	4.2±0.9	5.5±1.4
	°Days	16.2±7.5	28.0±7.4	37.6±7.8	49.1±12.2
10	Days	2.2±0.7	3.3±0.9	4.4±1.5	7.3±1.6
	°Days	22.4±6.7	33.0±8.9	44.0±15.0	72.8±16.0
11	Days	1.7±0.4	2.5±0.4	3.7±0.5	5.5±0.9
	°Days	18.4±4.8	27.3±5.0	40.3±5.6	60.5±10.4
12	Days	1.5±0.8	2.1±0.8	3.0±0.9	4.4±1.4
	°Days	18.1±9.9	24.6±9.5	35.7±10.5	52.4±16.9

Table 2.3.15 Series 3 - mean hatching times ±SD (%) in days and degree-days from first hatch to 10, 50, 90 and 100% hatch at different temperatures. ANOVAR showed that any differences between the mean hatching times of groups were not significant.

Temp. (°C)		Eggs hatched±SD (%)			
		10	50	90	100
9	Days	2.6±0.6	3.8±0.6	4.4±0.6	6.5±0.6
	°Days	22.2±5.2	32.3±5.2	37.6±5.2	57.8±5.6
10	Days	1.4±0.4	2.5±0.4	3.6±1.1	7.5±0.7
	°Days	15.0±4.5	25.8±4.0	36.9±10.5	73.5±6.6
11	Days	1.2±0.4	2.2±0.4	3.2±0.7	7.7±2.5
	°Days	13.9±4.2	25.3±4.1	35.8±7.0	86.6±27.8
12	Days	1.9±0.6	3.1±0.7	5.0±1.4	8.1±1.7
	°Days	23.5±6.6	38.3±8.0	61.8±17.2	97.7±19.3
13	Days	1.1±0.3	2.5±1.4	3.6±1.6	6.7±1.2
	°Days	13.8±3.3	33.1±18.6	47.5±21.4	87.7±16.0

Table 2.3.16 Series 4A - mean hatching times ±SD (%) in days and degree-days from first hatch to 10, 50, 90 and 100% hatch at different temperatures.

Temp. (°C)		Eggs hatched±SD (%)			
		10	50	90	100
10	Days	1.5±0.8	2.7±1.0	3.8±1.3	6.3±2.1
	°Days	15.8±8.1	25.9±9.6	37.4±12.9	60.7±19.9
12	Days	1.1±0.3	2.2±0.5	3.6±0.8	5.3±0.6
	°Days	14.6±3.9	28.0±6.2	43.4±8.9	63.5±6.9

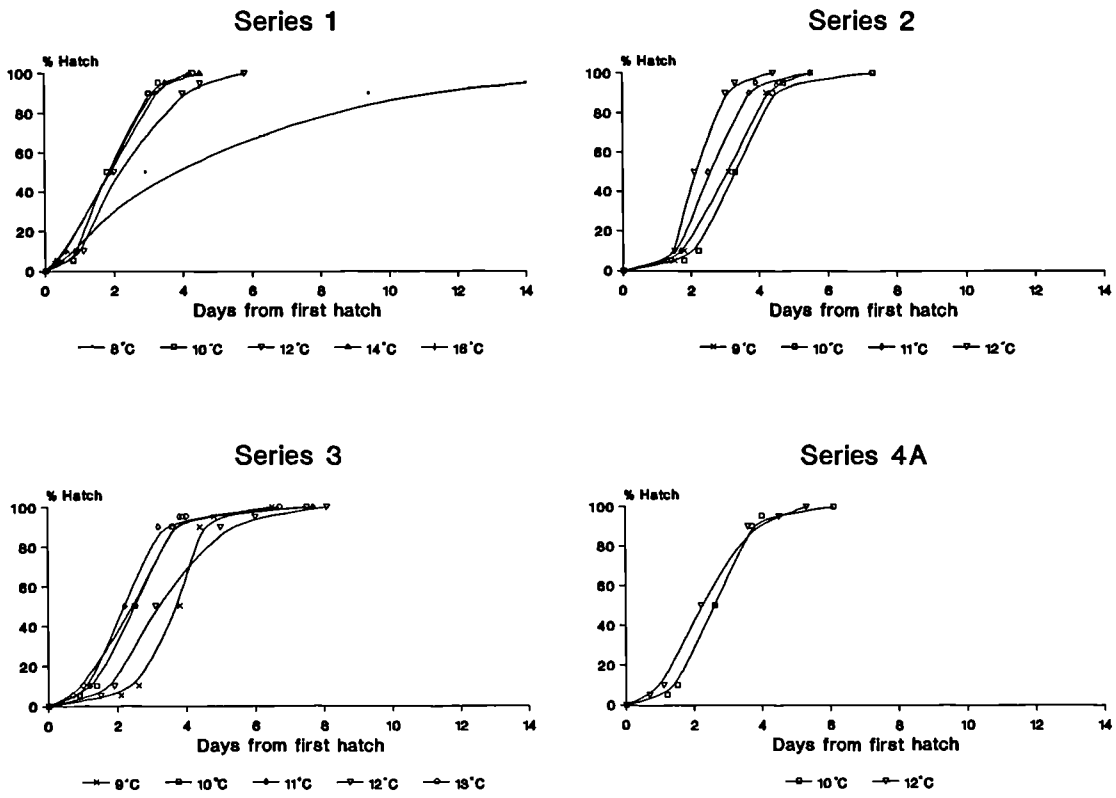


Fig. 2.3.15 Progression of hatching in days from first hatch at different temperatures in Series 1-4.

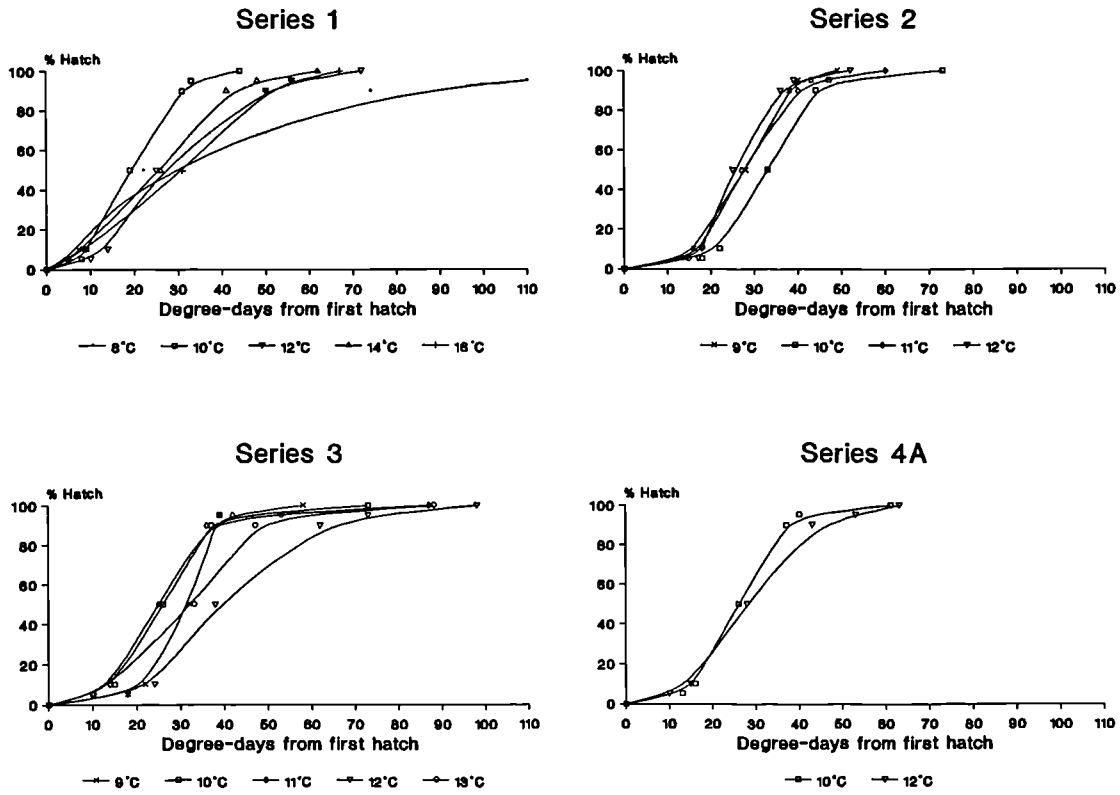


Fig. 2.3.16 Progression of hatching in degree-days from first hatch at different temperatures in Series 1-4.

abruptly, reach a maximum, and attenuate more slowly. For hatching temperatures greater than 8°C, the data provided reasonably normal hatching frequency distributions. In general, 80% of the eggs in most groups within all series hatched within 1.5 days (20 degree-days) of the mean hatching time. However, at the lowest temperature (8°C), 80% of the eggs hatched within 4.2 days (33.3 degree-days) of the mean hatching time.

2.3.2.3 Alevin size at hatching and MAWW

(a) Alevin weight at hatching - Series 1 and 2

Alevin wet weights at hatching for different combinations of temperature during egg incubation are presented in Table 2.3.17 and Fig. 2.3.17 for Series 1 (temperature range of 8-16°C), and in Table 2.3.18 and Fig. 2.3.18 for Series 2 (temperature range of 9-12°C).

There was little difference in mean alevin weights at hatching between groups of eggs incubated under different temperature regimes. Mean alevin weights ranged from 0.111-0.123 g in Series 1, and from 0.106-0.108 g in Series 2. No significant differences in alevin weights at hatching were detected among the groups within Series 1 or 2, and no particular trends were apparent.

(b) Alevin weight at MAWW

Mean wet weights at MAWW for the different temperature

Table 2.3.17 Series 1 - mean individual wet weight of newly-hatched alevins ±SD (g) following incubation of eggs under different temperature regimes. ANOVAR showed that any differences between the mean weights of alevins in groups were not significant.

Temp. regime (°C)	Mean wet wt. ±SD (g)	Temp. regime (°C)	Mean wet wt. ±SD (g)
8-8	0.119±0.001	10-10	0.115±0.003
8-10	0.111±0.003	10-12	0.114±0.002
8-12	0.114±0.004	10-14	0.118±0.002
8-14	0.112±0.002	12-8	0.120±0.002
8-16	0.117±0.004	12-10	0.117±0.003
10-8	0.117±0.004	12-12	0.123±0.004

Table 2.3.18 Series 2 - mean individual wet weight of newly-hatched alevins ±SD (g) following incubation of eggs under different temperature regimes. ANOVAR showed that any differences between the mean weights of alevins in groups were not significant.

Temp. regime (°C)	Mean wet wt. ±SD (g)	Temp. regime (°C)	Mean wet wt. ±SD (g)
9-9	0.107±0.001	10-11	0.107±0.002
9-10	0.106±0.002	10-12	0.107±0.001
9-11	0.106±0.001	11-9	0.106±0.001
9-12	0.106±0.001	11-10	0.107±0.002
10-9	0.108±0.001	11-11	0.106±0.001
10-10	0.107±0.001	11-12	0.107±0.001

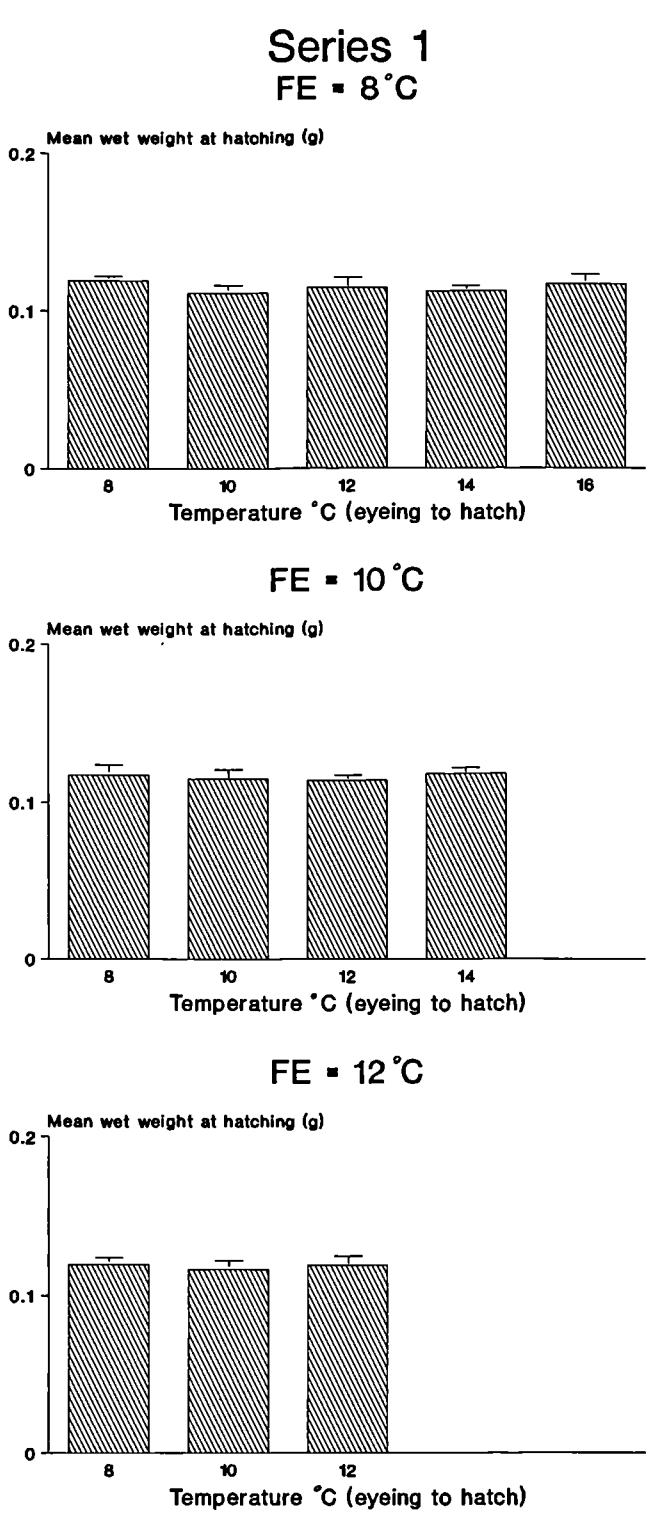


Fig. 2.3.17 Mean individual wet weight of newly-hatched alevins ±SD (g) under different temperature regimes in Series 1. The temperature from fertilisation to eyeing is shown as FE.

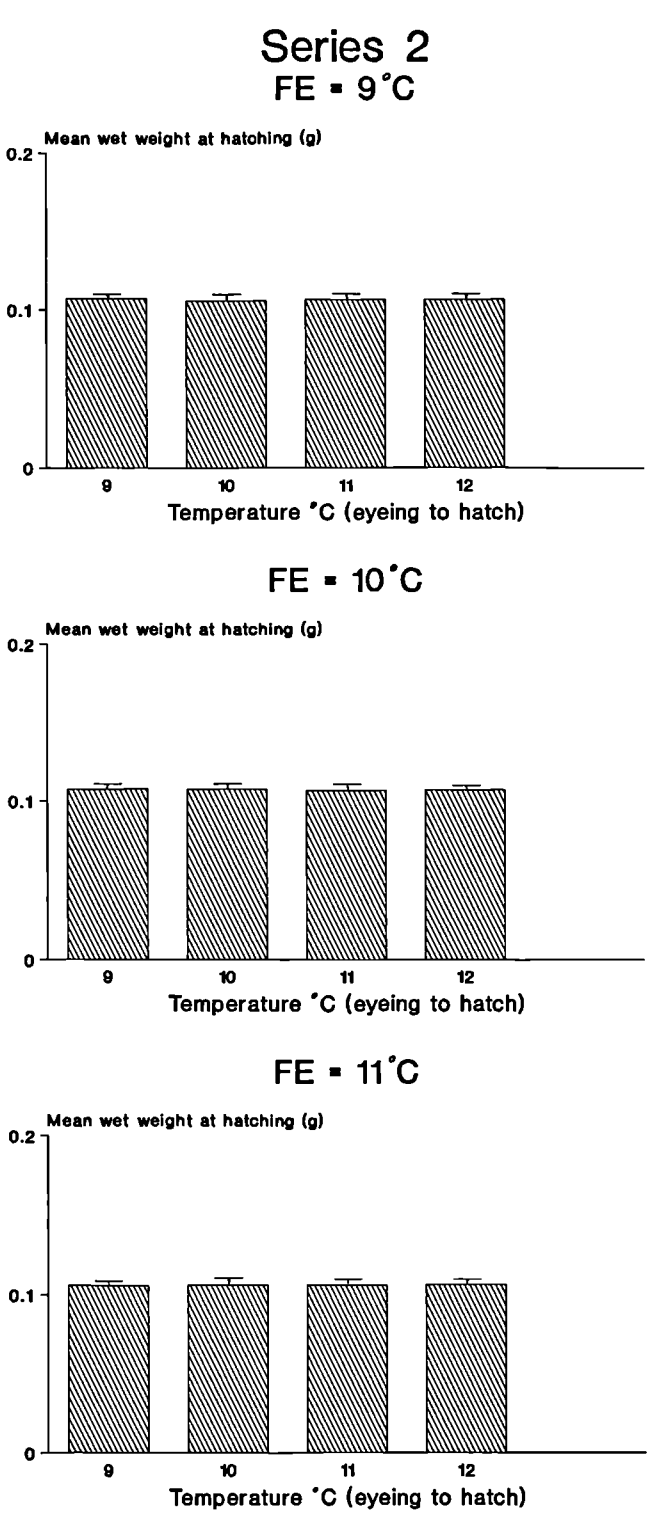


Fig. 2.3.18 Mean individual wet weight of newly-hatched alevins ±SD (g) under different temperature regimes in Series 2. The temperature from fertilisation to eyeing is shown as FE.

regimes within the four series are shown in Tables 2.3.19-2.3.24, and are presented graphically in Figs. 2.3.19-2.3.22. The mean percentage water content of alevins at MAWW is also included for Series 2 (Table 2.3.20) and Series 4 fish (Tables 2.3.22-2.3.24).

In contrast to dry matter, alevin wet weight increases steadily from hatching to MAWW at all temperatures. Weight increase is primarily due to changes in the water content of the alevins, as relatively dense yolk (50% water by weight) is converted into less dense tissues (84% water). This results in an increase in the water content of alevins from about 63% at hatching to 81% at MAWW.

Series 1 (Table 2.3.19, Fig. 2.3.19)

Mean individual weights at MAWW ranged from 0.136-0.172 g and were largely dependent upon the temperature during the alevin stage. MAWW at any given temperature regime during egg incubation showed a general decline with increasing temperature during the alevin stage: for a constant 10°C (10-10), MAWW decreased significantly ($P<0.01$) with increasing temperature during the alevin stage from 0.158 g at 10°C (10-10-10), to 0.143 g at 14°C (10-10-14), and 0.136 g at 16°C (10-10-16).

MAWW was greatest in Series 1 when the temperature regimes incorporated a temperature combination of 8-8, even when the subsequent temperature during the alevin stage was high (e.g. 8-8-14 and 8-8-16). MAWW in these groups was significantly greater ($P<0.05$) than the other groups incubated at 8°C from

Table 2.3.19 Series 1 - mean individual wet weights ±SD (g) of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean individual wet weight±SD (g)		
	MAWW	110 days	1200°days
8-8-8	0.167±0.004*	0.167±0.004***	0.205±0.006
8-8-14	0.168±0.003*	0.205±0.007	0.233±0.007
8-8-16	0.159±0.001	0.209±0.006	0.209±0.006
8-10-12	0.151±0.001	0.209±0.006	0.227±0.006
8-10-16	0.144±0.001**	NT	NT
8-12-14	0.150±0.004	0.217±0.004	0.214±0.004
8-12-16	0.141±0.004**	NT	NT
8-14-14	0.146±0.007	0.201±0.015	0.198±0.015
8-16-16	0.140±0.001**	LV	LV
10-8-8	0.167±0.001*	NT	NT
10-10-10	0.158±0.003	0.204±0.007	0.228±0.007
10-10-14	0.143±0.002**	NT	NT
10-10-16	0.136±0.003***	NT	NT
10-12-12	0.153±0.004	0.241±0.007**	0.226±0.007
10-14-14	0.141±0.005**	LV	LV
10-16-16	NV	NV	NV
12-8-8	0.172±0.001*	LV	LV
12-10-10	0.157±0.004	LV	LV
12-12-12	0.147±0.006*	LV	LV
12-14-14	NV	NV	NV
12-16	NV	NV	NV

continued overleaf

Table 2.3.19 (continued)

Temp. regime (°C)	Mean individual wet weight±SD (g)		
	MAWW	110 days	1200°days
14	NV	NV	NV
16	NV	NV	NV

NT: Not tested (experimental systems unavailable).
LV: Low viability (mortality>70%). Insufficient number of fish available for fry rearing phase of study.
NV: Not viable (total mortality).

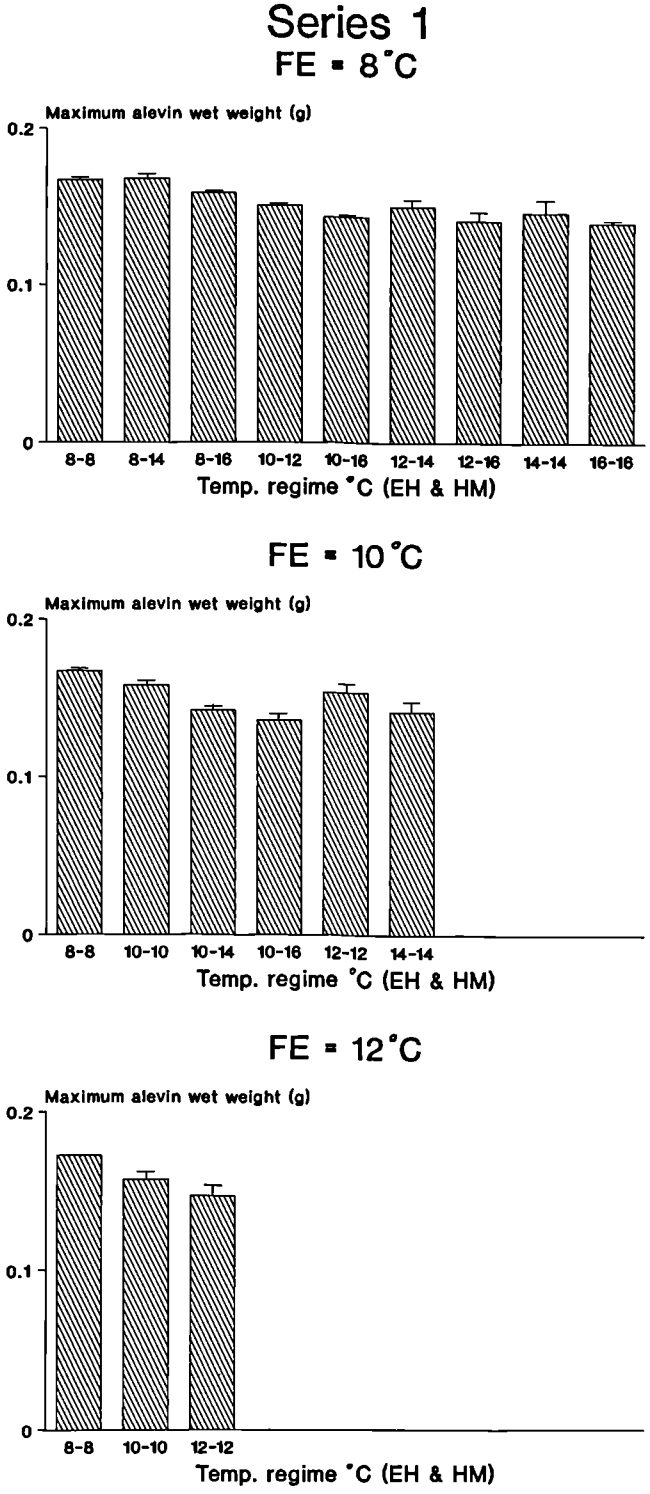


Fig. 2.3.19 Mean individual wet weight of alevins ±SD (g) at MAWW under different temperature regimes in Series 1. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Values are the means of 3 replicate treatments.

fertilisation to eyeing. It appears that low incubation temperatures may, to some extent, compensate for decreased MAWW induced by high temperatures during the alevin stage. When egg incubation temperatures were higher than 8°C, subsequent MAWW appeared to be more dependent on the temperature during the alevin stage.

In Series 1, size differences at MAWW among the three groups maintained at constant temperatures throughout their development (0.167, 0.158, and 0.147 g, at 8, 10, and 12°C, respectively) were also shown to be significant ($P<0.05$).

Series 2 (Table 2.3.20, Fig. 2.3.20)

Mean wet weights at MAWW under the different temperature regimes were more uniform (range of 0.139-0.161 g) than those observed in Series 1. This was due to the comparatively narrow temperature range (9-12°C) used in this series. MAWW was greatest (0.161 g) at temperature regimes of a constant 10°C (10-10-10) and 11-12-12, with those groups held at 10-10-12 (0.139 g), 10-12-12 (0.143 g), and 11-9-9 (0.142 g), being significantly lighter ($P<0.01$). However, decreased water content of alevins within these groups (Table 2.3.20) may account for some of the differences. Mean weights at MAWW of alevins held at the 3 constant temperature regimes tested in Series 2 (0.149 g at 9°C, 0.161 g at 10°C, and 0.158 g at 11°C) were not significantly different. Further analysis of the data revealed few significant size differences between the various groups of alevins, and no particular trends were apparent.

Table 2.3.20 Series 2 - water content ±SD (%) and mean individual wet weights ±SD (g) of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Water content at MAWW±SD(%)	Mean individual wet weight±SD (g)		
		MAWW	110 days	1200°days
9-9-9	79.9±0.1	0.149±0.002	0.189±0.004***	0.293±0.007***
9-9-11	80.3±0.1	0.155±0.001	0.325±0.002	0.429±0.004
9-10-10	79.6±0.6	0.158±0.003	0.325±0.002	0.437±0.004
9-10-11	79.9±0.6	0.156±0.002	0.347±0.005	0.419±0.006
9-11-10	79.5±0.2	0.154±0.004	0.348±0.004	0.451±0.007
9-11-11	79.8±0.5	0.150±0.006	0.344±0.004	0.391±0.004
9-12-11	79.2±0.4	0.148±0.004	0.342±0.006	0.375±0.007
9-12-12	80.8±0.1	0.157±0.001	0.322±0.001	0.328±0.001**
10-9-9	81.1±0.2	0.152±0.001	0.194±0.001***	0.282±0.002***
10-9-11	80.6±0.2	0.158±0.002	0.347±0.004	0.412±0.006
10-10-10	80.0±0.1	0.161±0.003	0.346±0.005	0.426±0.006
10-10-12	78.7±0.5	0.139±0.001***	0.351±0.001	0.360±0.001
10-11-11	79.5±0.1	0.148±0.003	0.358±0.002	0.376±0.001
10-11-12	79.9±0.2	0.156±0.003	0.379±0.034	0.373±0.033
10-12-11	83.2±0.1	0.154±0.003	0.413±0.007***	0.424±0.008
10-12-12	79.4±0.1	0.143±0.003**	0.327±0.013	0.312±0.012**
11-9-9	79.0±0.3	0.142±0.002**	0.230±0.008***	0.299±0.009***
11-9-11	80.6±0.2	0.156±0.004	0.343±0.003	0.389±0.004
11-10-9	78.9±0.6	0.146±0.002*	0.328±0.003	0.447±0.004
11-10-10	79.1±0.1	0.156±0.001	0.324±0.004	0.376±0.004
11-11-11	83.5±0.1	0.158±0.003	0.375±0.003	0.381±0.003

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Table 2.3.20 (continued)

Temp. regime (°C)	Water content at MAWW±SD(%)	Mean individual wet weight±SD (g)		
		MAWW	110 days	1200°days
11-11-12	79.6±0.5	0.146±0.004*	0.315±0.001	0.301±0.001***
11-12-11	79.5±0.1	0.151±0.001	0.366±0.011	0.361±0.011
11-12-12	81.2±0.5	0.161±0.001	0.404±0.006**	0.374±0.006

Table 2.3.21 Series 3 - mean individual wet weights ±SD (g) of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean individual wet weight±SD (g)		
	MAWW	110 days	1200°days
9-9-9	0.160±0.002	0.194±0.007**	0.281±0.012
9-11-11	0.161±0.003	0.254±0.026	0.270±0.027
10-10-10	0.153±0.001	0.225±0.006	0.256±0.007
10-11-11	0.161±0.002	0.252±0.001	0.257±0.001
10-13-13	0.154±0.001	0.286±0.008**	0.248±0.007
11-11-11	0.159±0.002	0.247±0.010	0.244±0.010
11-12-12	0.156±0.001	0.277±0.016**	0.251±0.015
11-13-13	0.149±0.003	0.255±0.006	0.218±0.005
12-12-12	0.155±0.006	0.261±0.007	0.233±0.006
13-13-13	0.144±0.001**	LV	LV

LV: Low viability (mortality>70%). Insufficient number of fish available for fry rearing phase of study.

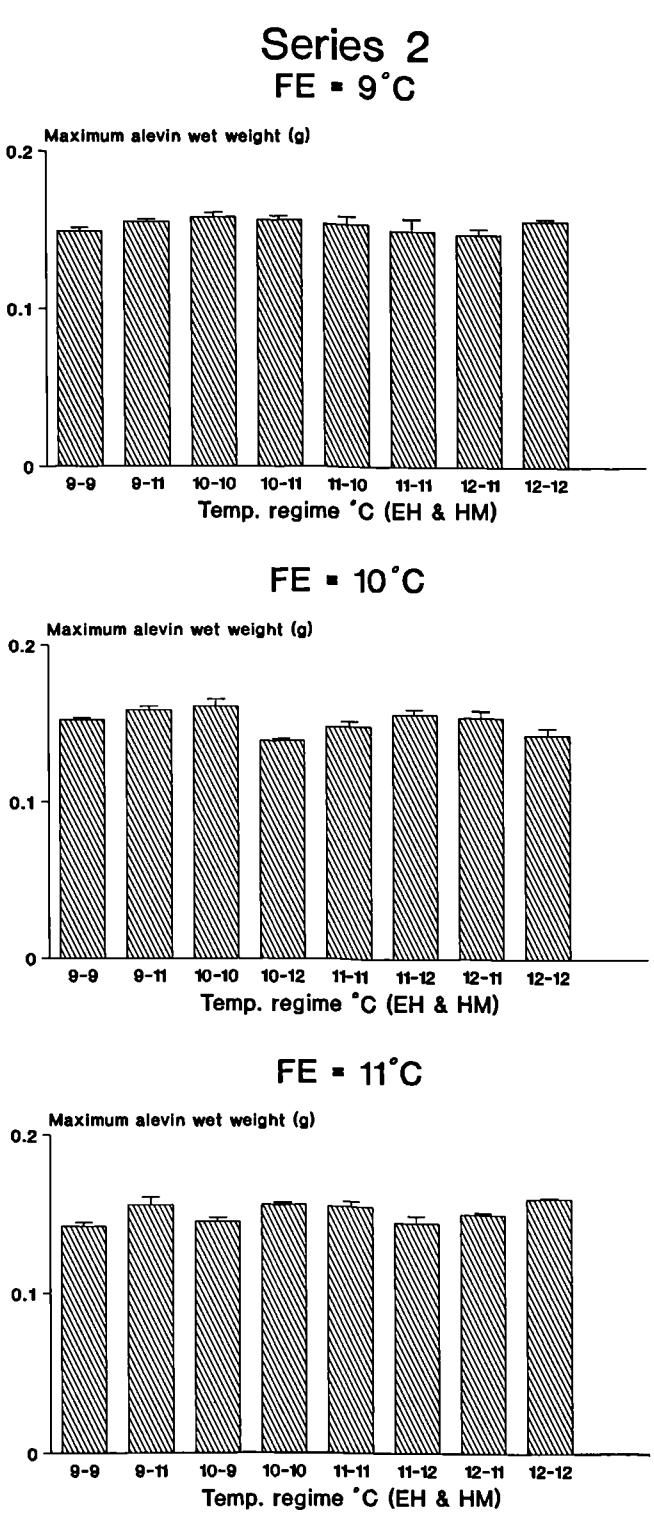

Fig. 2.3.20 Mean individual wet weight of alevins ±SD (g) at MAWW under different temperature regimes in Series 2. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Values are the means of 3 replicate treatments.

Series 3 (Table 2.3.21, Fig. 2.3.21)

In Series 3, the range of values of mean weights at MAWW (0.144-0.161 g) was also relatively narrow. The weights of alevins held at the constant temperature regimes of 9, 10, 11 and 12°C were similar, but at 13°C alevins were significantly smaller ($P<0.01$) than those groups held at lower temperatures.

Series 4 (Tables 2.3.22-2.3.24, Fig. 2.3.22)

In the 3 experiments conducted in Series 4, temperature during the alevin stage had a very significant effect on the actual MAWW achieved. MAWW was consistently greatest at 10°C and declined significantly ($P<0.01$) at the higher temperatures tested (16 and 18°C). At MAWW, the weights of alevins held at 18°C during the alevin stage were 93% (Series 4A), 74% (Series 4B) and 73% (Series 4C), of the weights at 10°C. Whilst the lower alevin wet weights were predominantly caused by a reduced dry matter content, in some cases lower weights were also partly due to a reduced water content (Tables 2.3.23-2.3.24). The crests of the curves of alevin wet weight against time are broad, and it is occasionally difficult to obtain reliable estimates of MAWW because of the variability in alevin weight and the limited number of samplings.

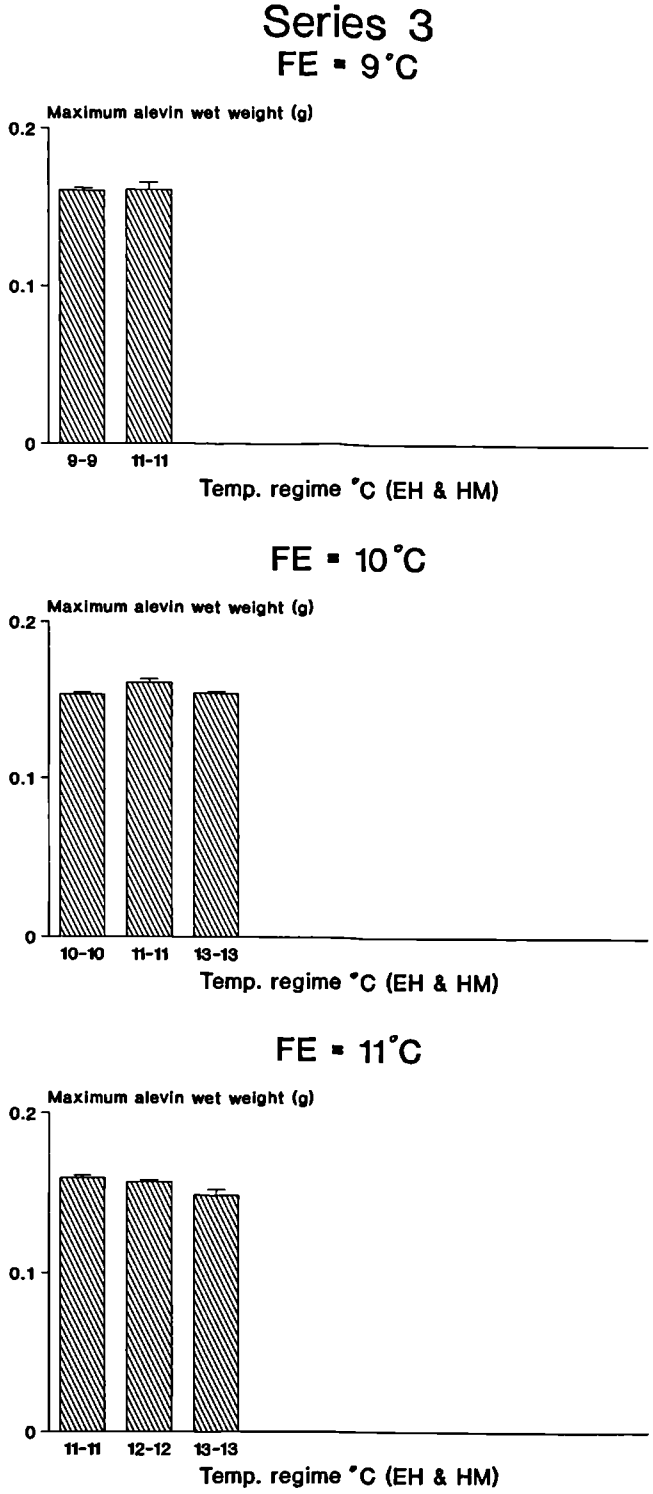

Fig. 2.3.21 Mean individual wet weight of alevins ±SD (g) at MAWW under different temperature regimes in Series 3. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Values are the means of 3 replicate treatments.

Table 2.3.22 Series 4A - water content ±SD (%) and mean individual wet weights ±SD (g) of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Water content at MAWW±SD(%)	Mean individual wet weight±SD (g)		
		MAWW	110 days	1200°days
10-10-10	79.8±0.1	0.149±0.001	0.300±0.001	0.346±0.001
10-10-14	77.6±0.4	0.143±0.002	0.429±0.019***	0.374±0.017
10-10-16	79.2±0.9	0.138±0.003**	0.457±0.034***	0.362±0.027
10-10-18	78.4±0.3	0.139±0.003**	0.466±0.016***	0.351±0.012
12-12-12	79.7±0.7	0.147±0.002	LV	LV
13-13	NV	NV	NV	NV
14	NV	NV	NV	NV

LV: Low viability (mortality>70%). Insufficient number of fish available for fry rearing phase of study.
NV: Not viable (total mortality).

Table 2.3.23 Series 4B - water content ±SD (%) and mean individual wet weights ±SD (g) of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Water content at MAWW±SD(%)	Mean individual wet weight±SD (g)		
		MAWW	110 days	1200°days
10-10-10	80.5±0.4	0.146±0.005	0.257±0.010	0.295±0.011
10-10-16	80.3±0.3	0.120±0.006***	0.266±0.005	0.223±0.004***
10-10-18	76.8±0.9	0.108±0.002***	0.141±0.003**	0.131±0.003***
10-10-20	ND	ND	LV	LV

ND: Not determined (severe oedema of yolk-sac prevented meaningful determination of MAWW).
LV: Low viability (mortality>70%). Insufficient number of fish available for fry rearing phase of study.

Table 2.3.24 Series 4C - water content ±SD (%) and mean individual wet weight of alevins ±SD (g) at MAWW under different temperature regimes. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Water content at MAWW±SD (%)	Mean individual weight±SD (g)
10-10-10	81.0±0.3	0.140±0.005
10-10-18	79.3±0.3	0.103±0.005***
10-10-20	NV	NV
10-10-22	NV	NV

NV: Not viable (total mortality).

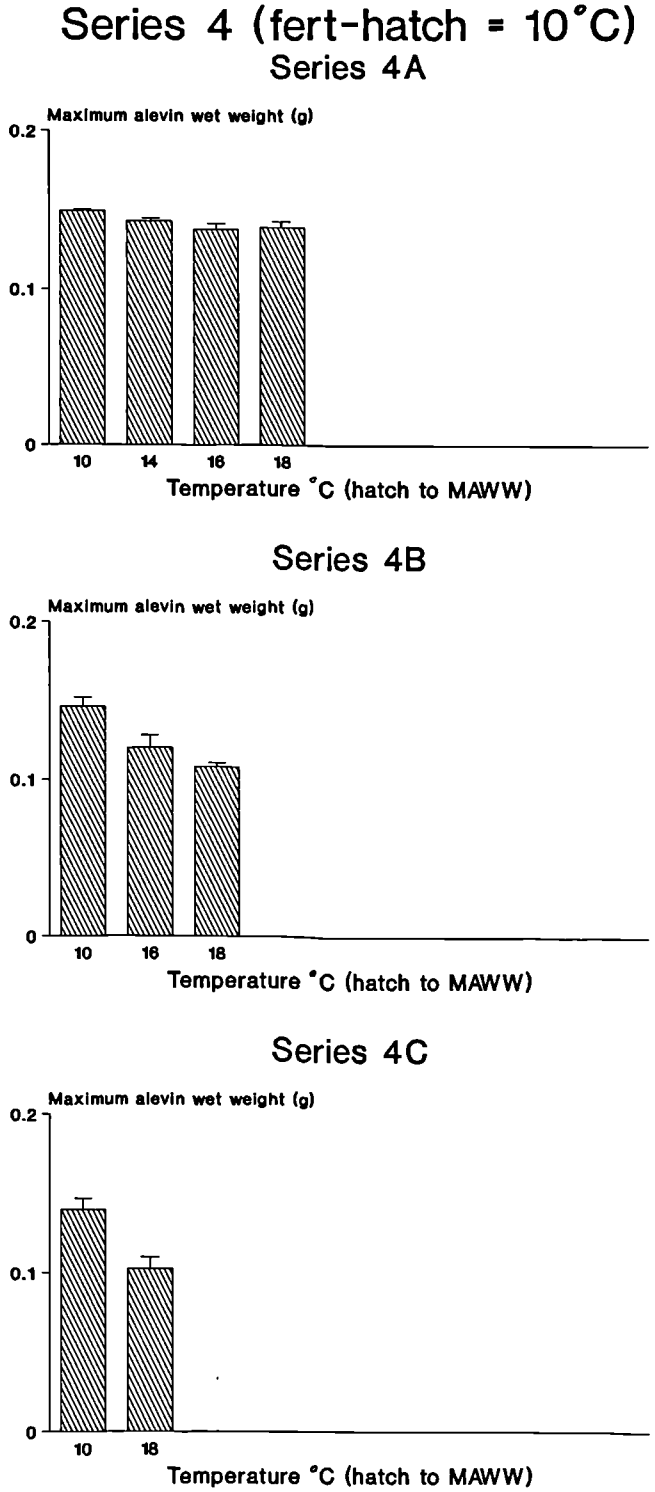

Fig. 2.3.22 Mean individual wet weight of alevins ±SD (g) at MAWW under different temperature regimes in Series 4. The temperature from fertilisation to hatching was 10°C. Values are the means of 3 replicate treatments.

(c) Length at MAWW - Series 4

(Tables 2.3.25-2.3.27, Fig. 2.3.23)

Tables 2.3.25-2.3.27 and Fig. 2.3.23 show mean alevin lengths (standard, fork and total) at MAWW for different temperatures during the alevin stage (10-18°C) in Series 4.

Temperature had a significant effect ($P<0.01$) on alevin length at MAWW, with the longest alevins consistently produced at 10°C, those at 14 and 16°C intermediate in length, and those at 18°C being the shortest. The influence of temperature on alevin length was less pronounced in experiment 4A than in either 4B or 4C. In experiment 4A, alevin lengths, as measured by standard, fork or total length, decreased by a mean of 0.26 mm for every 1°C increase in temperature during the alevin stage. This compares with a decrease in length of 0.47 and 0.50 mm in experiments 4B and 4C, respectively.

Determinations of alevin length were not made when experimental temperatures exceeded 18°C (Series 4B, Table 2.3.26; Series 4C, Table 2.3.27) because alevin survival was either very low or nil. Furthermore, the majority of surviving alevins (Series 4B) suffered severe oedema of the yolk-sac which prevented an accurate determination of MAWW.

Thus, the effect of temperature during the alevin stage on alevin length and weight was similar. Temperature had a dominant influence on alevin size at temperatures above 10°C, with alevin size decreasing significantly at higher temperatures. Alevins were both shortest and lightest at 18°C.

Table 2.3.25 Series 4A - mean standard, fork and total lengths of alevins ±SD (mm) at MAWW under different temperatures from hatching to MAWW. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean individual length±SD (mm)		
	Standard	Fork	Total
10-10-10	24.8±0.3	27.8±0.2	28.6±0.3
10-10-14	24.3±0.2	27.4±0.2	28.2±0.2
10-10-16	23.8±0.2**	26.6±0.3**	27.5±0.3**
10-10-18	23.3±0.1**	26.0±0.1**	27.2±0.1**

Table 2.3.26 Series 4B - mean standard, fork and total lengths of alevins ±SD (mm) at MAWW under different temperatures from hatching to MAWW. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean individual length±SD (mm)		
	Standard	Fork	Total
10-10-10	23.8±0.2	26.8±0.3	27.4±0.3
10-10-16	22.8±0.3	25.7±0.4	26.7±0.3
10-10-18	21.3±0.7***	23.8±0.6***	24.4±0.7***
10-10-20	ND	ND	ND

ND: Not determined (severe oedema of yolk-sac prevented meaningful determination of MAWW).

Table 2.3.27 Series 4C - mean standard, fork and total lengths of alevins ±SD (mm) at MAWW under different temperatures from hatching to MAWW. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean individual length±SD (mm)		
	Standard	Fork	Total
10-10-10	23.8±0.3	26.6±0.3	27.8±0.4
10-10-18	21.1±0.5***	23.7±0.4***	24.4±0.5***
10-10-20	NV	NV	NV
10-10-22	NV	NV	NV

NV: Not viable (total mortality).

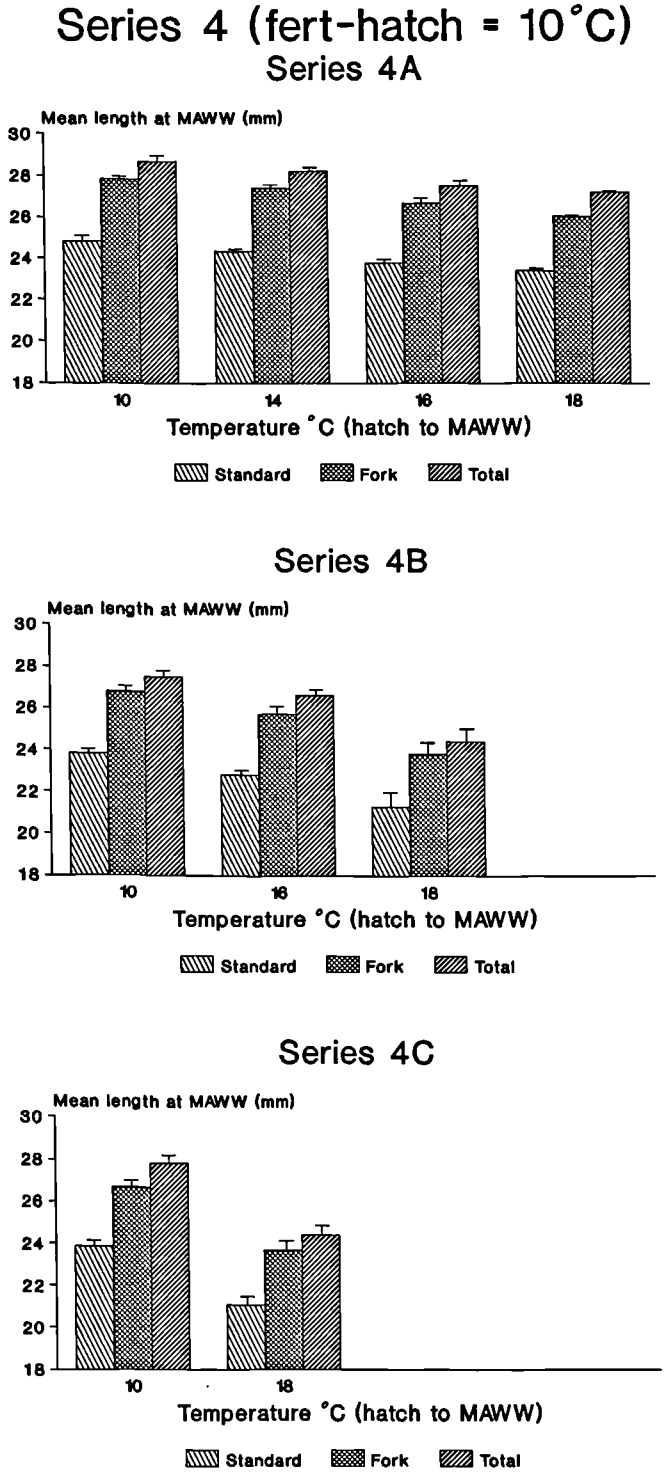

Fig. 2.3.23 Mean standard, fork and total lengths of alevins ±SD (mm) at MAWW under different temperature regimes in Series 4. The temperature from fertilisation to hatching was 10 °C. Values are the means of 3 replicate treatments.

2.3.2.4 Fry growth

Tables 2.3.19-2.3.23 and Figs. 2.3.24-2.3.27 show the effects of previous temperature regimes on the subsequent growth of fry during a period of exogenous feeding from first feeding (MAWW) to 110 days and 1200 degree-days after fertilisation. All groups of fry were held at a common temperature of 10°C (ambient) during the feeding period.

Although temperature had a significant effect on alevin size at first feeding, it appeared to have no effect on the timing of exogenous feeding. First observed feeding occurred soon after the attainment of MAWW, when alevins still had residual yolk material in the body cavity. At this time, growth was derived both from endogenous and exogenous sources of nutrition. Because yolk has a lower water content (about 56%, Peterson & Metcalfe, 1977) than tissue (about 82.5%), the determination of growth based on the measurement of wet weight will have given rise to some experimental error. However, the proportion of fry wet weight that comprised yolk was assumed to be small, and consequently the error slight.

The mean weights attained by fry at 110 days and 1200 degree-days after fertilisation were calculated for all groups which were reared beyond the alevin stage.

(a) Mean wet weight of fry at 110 days after fertilisation

Series 1 (Table 2.3.19, Fig. 2.3.24)

At 110 days after fertilisation, fry derived from the group maintained at 10-12-12 weighed significantly more ($P<0.01$), and those from group 8-8-8 very significantly less ($P<0.001$), than the other groups tested. The mean weights of the 6 intermediate groups of fry were very similar, ranging from 0.201-0.217 g.

Series 2 (Table 2.3.20, Fig. 2.3.25)

At 110 days after fertilisation, the highest mean weights of fry were attained by the groups held at 10-12-11 (0.413 g) and 11-12-12 (0.404 g). These 2 groups of fry were significantly heavier than the other groups tested ($P<0.01$). The lowest mean weights of fry in this series occurred in the 3 groups held at a constant 9°C from eyeing until MAWW. Here, mean weights of fry were very significantly lower than the other groups tested ($P<0.001$). There was a progressive increase in the mean weights of fry among these groups with increasing temperature from fertilisation to eyeing.

Series 3 (Table 2.3.21, Fig. 2.3.26)

Similar results were obtained in Series 3, where significantly lower ($P<0.01$) mean weights of fry at 110 days

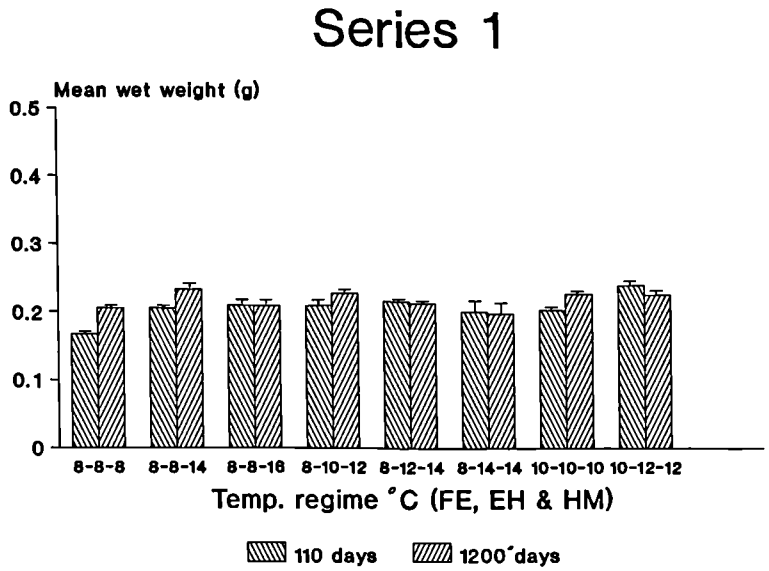

Fig. 2.3.24 Series 1 - mean individual wet weights of fry ±SD (g) at 110 days and 1200 degree-days after fertilisation in relation to different temperature regimes from fertilisation to MAWW. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Following the alevin stage (MAWW), fry were reared at 10°C.

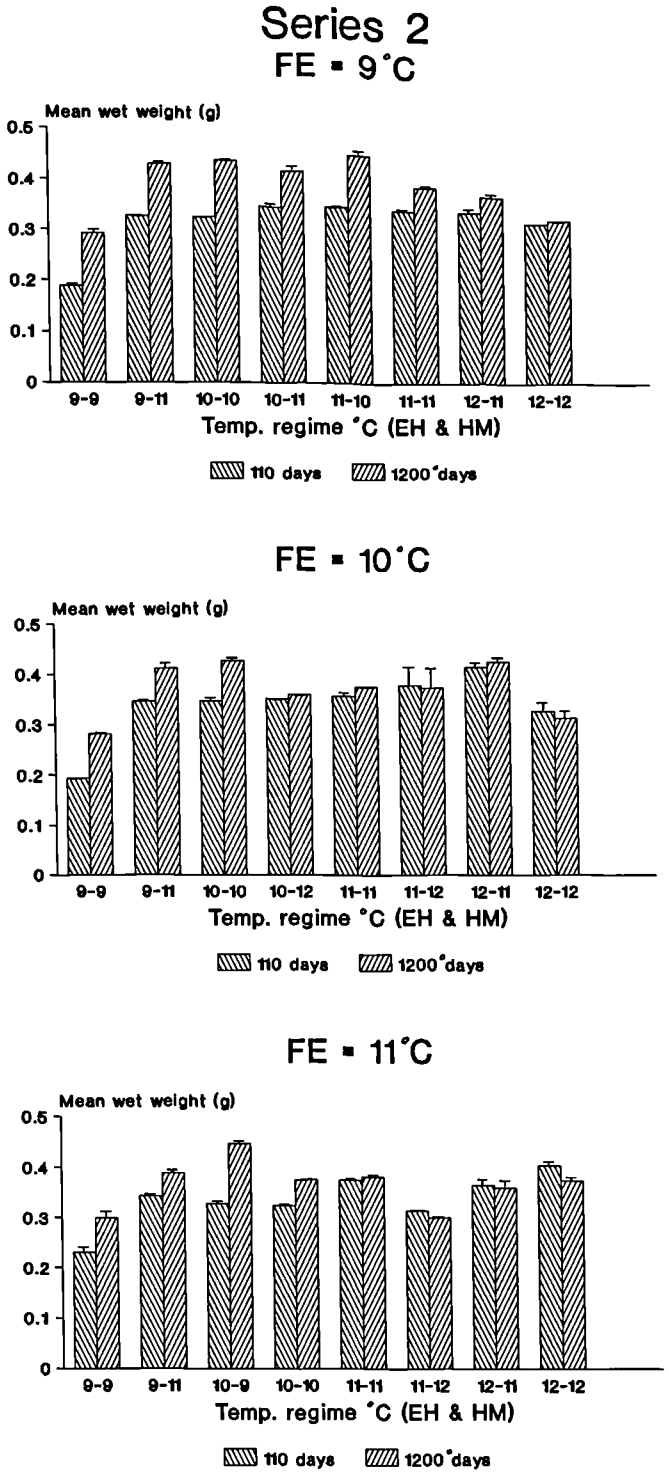

Fig. 2.3.25 Series 2 - mean individual wet weights of fry ±SD (g) at 110 days and 1200 degree-days after fertilisation in relation to different temperature regimes from fertilisation to MAWW. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Following the alevin stage (MAWW), fry were reared at 10°C.

Series 3

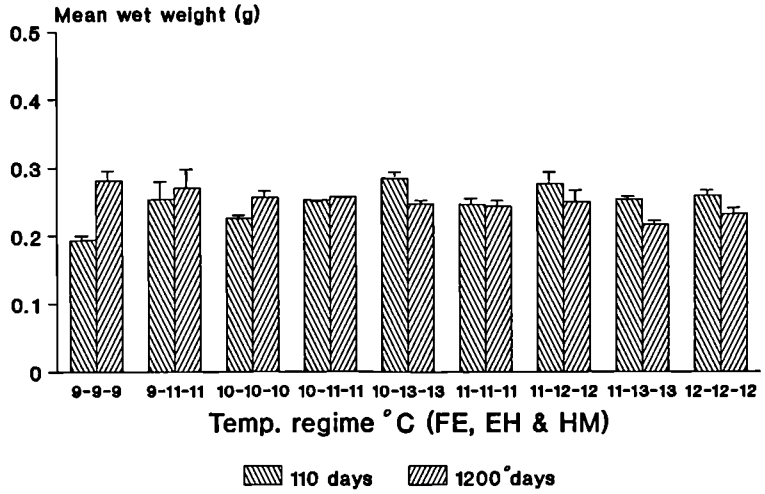

Fig. 2.3.26 Series 3 - mean individual wet weights of fry ±SD (g) at 110 days and 1200 degree-days after fertilisation in relation to different temperature regimes from fertilisation to MAWW. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Following the alevin stage (MAWW), fry were reared at 10 °C.

after fertilisation once again occurred in the group held at the lowest temperature regime (9-9-9). The highest mean weights of fry occurred in those groups held at 10-13-13 (0.286 g) and 11-12-12 (0.277 g).

Series 4A (Table 2.3.22, Fig. 2.3.27)

Similarly, in Series 4A the mean weight of fry at 110 days after fertilisation was very significantly lower ($P<0.001$) in the group held at the lowest temperature regime of a constant 10°C (10-10-10).

Series 4B (Table 2.3.23, Fig. 2.3.27)

In Series 4B however, a temperature of 18°C during the alevin stage gave rise to a very significant decrease ($P<0.001$) in the subsequent mean weight of fry at 110 days after fertilisation. This temperature has already been shown to be close to the upper limit for alevin development.

The stock tested in Series 4A (Table 2.3.22) appeared to be more tolerant of temperature extremes than that of Series 4B.

(b) Mean wet weight of fry at 1200 degree-days after fertilisation

Series 1 (Table 2.3.19, Fig. 2.3.24)

In Series 1, the mean weights of groups of fry at 1200 degree-days after fertilisation were relatively uniform and

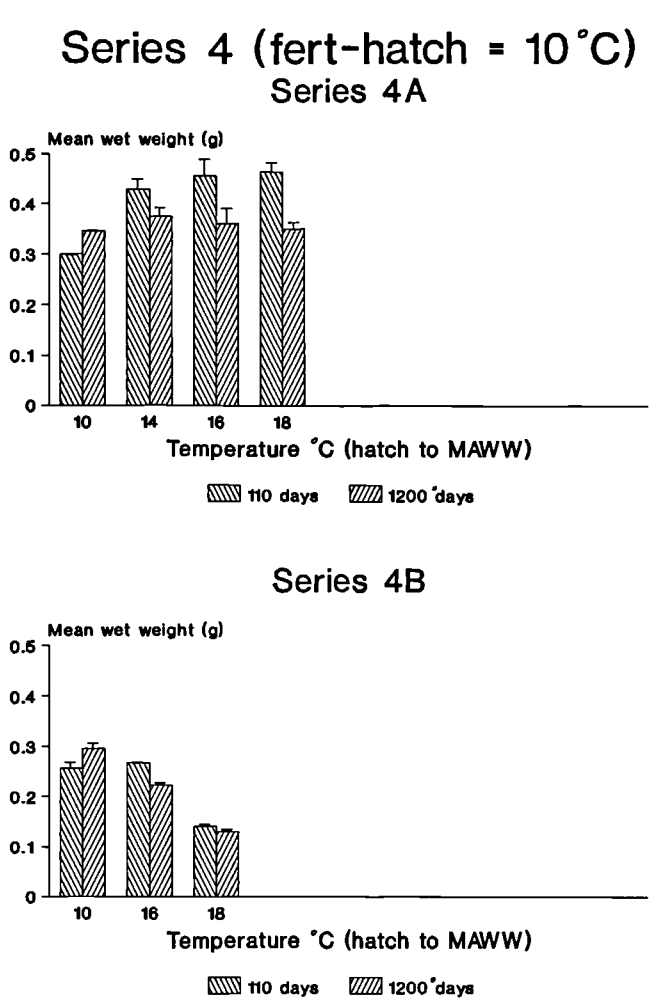

Fig. 2.3.27 Series 4 - mean individual wet weights of fry ±SD (g) at 110 days and 1200 degree-days after fertilisation in relation to different temperatures from hatching to MAWW. The temperature from fertilisation to hatching was 10°C. Following the alevin stage (MAWW), fry were reared at 10°C.

no differences were shown to be significant.

Series 2 (Table 2.3.20. Fig. 2.3.25)

In Series 2, very significant size differences ($P<0.001$) were shown between the 3 groups held at the lower temperature regimes of 9-9-9 (0.293 g), 10-9-9 (0.282 g) and 11-9-9 (0.299 g), and the other groups tested (mean weight of 0.388 g) within the series. At first feeding, it was observed that the feeding responses of these 3 groups of fry were less pronounced than those of the early feeding groups.

Although significantly lower mean weights of fry at 1200 degree-days after fertilisation ($P<0.01$) were also demonstrated for those groups held at 9-12-12 (0.328 g), 10-12-12 (0.312 g), and 11-11-12 (0.301 g), no particular trends were apparent.

Series 3 (Table 2.3.21, Fig. 2.3.26)

Irrespective of the different thermal histories to which groups had been exposed, the mean weights of fry from most groups at 1200 degree-days after fertilisation were relatively uniform.

In contrast to Series 2, the group maintained at the lowest temperature regime of 9-9-9 in Series 3, gave rise to the largest fry at 1200 degree-days after fertilisation.

Series 4A-B (Tables 2.3.22-2.3.23, Fig. 2.3.27)

No differences in the mean weights of groups of fry at 1200 degree-days after fertilisation were found to be significant within Series 4A (Table 2.3.22). However, in Series 4B (Table 2.3.23) mean weights showed a very significant decline ($P<0.001$) with increasing temperature during the alevin stage. A temperature of 18°C during the alevin stage was shown to have a very marked effect on the subsequent growth of fry. Thus, it appears that individual stocks differ in their tolerances to temperature extremes.

Table 2.3.23 showed that in Series 4B, large alevins at MAWW maintained their weight differential during the feeding period. Pearson's product moment correlation coefficient (r) for weight at MAWW and final weight (1200 degree-days after fertilisation) was 0.93. However, any association between MAWW and final weight was less apparent in the other series, where r was 0.38, 0.41, 0.70, and -0.40, for Series 1, 2, 3, and 4A, respectively.

(c) Specific growth rates of fry
(Tables 2.3.28-2.3.32)

The specific growth rates of fry within a series were generally similar for groups previously held under different temperature regimes from fertilisation to MAWW. However, some differences in growth rate did occur in Series 2 (Table 2.3.29), where very significantly lower ($P<0.001$) growth rates were shown in those groups maintained at 9°C from

Table 2.3.28 Series 1 - specific growth rates (Gw) of fry ±SD (%/day) from first feeding (MAWW) to 1200 degree-days after fertilisation, following development from fertilisation to MAWW under different temperature regimes. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Gw±SD (%/day)	Temp. regime (°C)	Gw±SD (%/day)
8-8-8	0.645±0.06*	8-12-14	0.954±0.08
8-8-14	0.896±0.13	8-14-14	0.754±0.10
8-8-16	0.789±0.08	10-10-10	1.067±0.04
8-10-12	0.974±0.09	10-12-12	0.967±0.14

Table 2.3.29 Series 2 - specific growth rates (Gw) of fry ±SD (%/day) from first feeding (MAWW) to 1200 degree-days after fertilisation, following development from fertilisation to MAWW under different temperature regimes. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Gw±SD (%/day)	Temp. regime (°C)	Gw±SD (%/day)
9-9-9	1.838±0.06***	10-11-11	2.464±0.04
9-9-11	2.749±0.02	10-11-12	2.285±0.27
9-10-10	2.667±0.03	10-12-11	2.596±0.11
9-10-11	2.662±0.08	10-12-12	2.069±0.21***
9-11-10	2.719±0.11	11-9-9	2.003±0.06***
9-11-11	2.524±0.15	11-9-11	2.403±0.06
9-12-11	2.387±0.13	11-10-9	3.011±0.01
9-12-12	1.877±0.02***	11-10-10	2.215±0.02
10-9-9	1.817±0.01***	11-11-11	2.268±0.05
10-9-11	2.612±0.08	11-11-12	1.928±0.08***
10-10-10	2.559±0.08	11-12-11	2.265±0.09
10-10-12	2.519±0.01	11-12-12	2.178±0.02

Table 2.3.30 Series 3 - specific growth rates (Gw) of fry ±SD (%/day) from first feeding (MAWW) to 1200 degree-days after fertilisation, following development from fertilisation to MAWW under different temperature regimes. ANOVAR showed that any differences between the growth rates of fry in groups were not significant.

Temp. regime (°C)	Gw±SD (%/day)	Temp. regime (°C)	Gw±SD (%/day)
9-9-9	1.889±0.21	11-11-11	1.562±0.18
9-11-11	1.735±0.42	11-12-12	1.766±0.28
10-10-10	1.858±0.12	11-13-13	1.514±0.21
10-11-11	1.653±0.05	12-12-12	1.464±0.01
10-13-13	1.810±0.07		

Table 2.3.31 Series 4A - specific growth rates (Gw) of fry ±SD (%/day) from first feeding (MAWW) to 1200 degree-days after fertilisation, following development from fertilisation to MAWW under different temperature regimes. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Gw±SD (%/day)	Temp. regime (°C)	Gw±SD (%/day)
10-10-10	2.615±0.07	10-10-16	2.855±0.09
10-10-14	2.903±0.01*	10-10-18	2.683±0.03

Table 2.3.32 Series 4B - specific growth rates (Gw) of fry ±SD (%/day) from first feeding (MAWW) to 1200 degree-days after fertilisation, following development from fertilisation to MAWW under different temperature regimes. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Gw±SD (%/day)	Temp. regime (°C)	Gw±SD (%/day)
10-10-10	1.977±0.27	10-10-18	0.629±0.17***
10-10-16	1.945±0.26		

eyeing to MAWW, together with the groups held at 9-12-12, 10-12-12 and 11-11-12. In Series 4B (Table 2.3.32), very significant differences ($P<0.001$) were also shown between the group held at 10-10-18 and the other 2 groups.

A comparison of fry growth rates for different series of experiments showed that, following development of groups at the common temperature regime of a constant 10°C (10-10-10), a large variation occurred: growth rates ranged from 1.067 in Series 1 to 2.615 in Series 4A (overall mean of 2.015).

2.3.2.5 Biomass gain

Tables 2.3.33-2.3.38 and Figs. 2.3.28-2.3.35 show the mean biomass (g), calculated as the product of the number of fish extant and the mean individual fish wet weight, at MAWW, at 110 days, and at 1200 degree-days after fertilisation.

(a) Biomass at MAWW

Series 1 (Table 2.3.33, Fig. 2.3.28)

At MAWW, the biomasses of all groups maintained at 12°C from fertilisation to hatching, together with those groups maintained at a constant 14 and 16°C from eyeing to MAWW, and the group held at 10-12-12 were very significantly lower ($P<0.001$) than the control group (10-10-10). The biomasses of groups 8-12-16 and 10-10-16 were also significantly lower than the remaining groups ($P<0.05$). The lowest biomasses occurred in groups where weight loss through mortality was

Table 2.3.33 Series 1 - mean biomass ±SD (g), as wet weight, of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean biomass±SD (g)		
	MAWW	110 days	1200°days
8-8-8	14.53±0.13	14.53±0.13	17.81±0.49
8-8-14	14.62±0.20	17.80±0.49	20.24±0.62
8-8-16	13.55±0.40	17.71±0.60	17.71±0.60
8-10-12	14.22±0.13	19.31±0.06	20.98±0.11
8-10-16	12.13±0.37	NT	NT
8-12-14	13.39±0.82	19.27±0.10	18.99±0.10
8-12-16	10.49±0.34*	NT	NT
8-14-14	5.57±0.95***	7.60±1.04***	7.47±0.77***
8-16-16	0.92±0.09***	LV	LV
10-8-8	13.70±0.19	NT	NT
10-10-10	13.19±0.10	17.03±0.47	19.06±0.59
10-10-14	11.83±0.44	NT	NT
10-10-16	10.84±0.18*	NT	NT
10-12-12	5.69±0.86***	8.30±1.13***	7.81±1.07***
10-14-14	1.36±0.26***	LV	LV
10-16-16	0 ***	NV	NV
12-8-8	1.83±0.47***	LV	LV
12-10-10	2.72±0.51***	LV	LV
12-12-12	0.73±0.26***	LV	LV
12-14-14	0 ***	NV	NV

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Table 2.3.33 (continued)

Temp. regime (°C)	Mean biomass±SD (g)			
	MAWW		110 days	1200°days
12-16	0	***	NV	NV
14	0	***	NV	NV
16	0	***	NV	NV

NT: Not tested (experimental systems unavailable).
LV: Low viability (mortality>70%). Insufficient number of fish available for fry rearing phase of study.
NV: Not viable (total mortality).

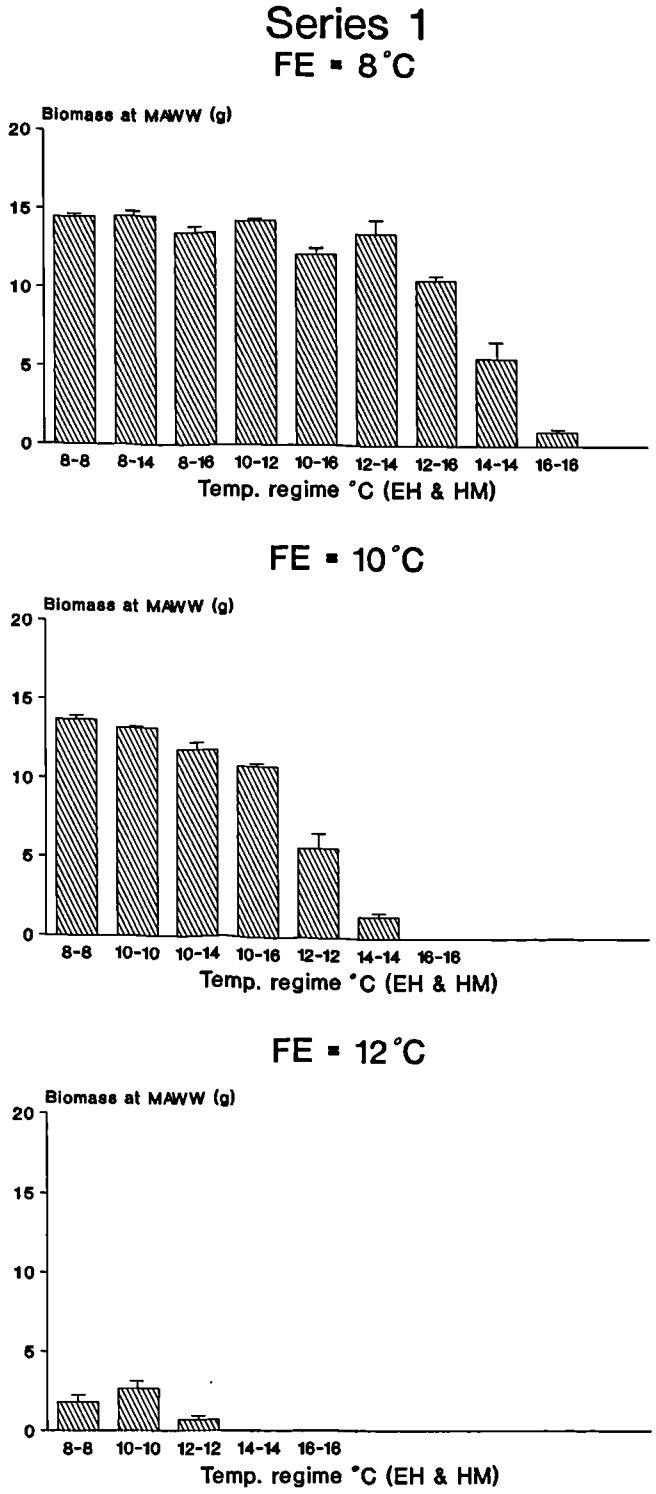

Fig. 2.3.28 Mean biomass, as wet weight, of alevins ±SD (g) at MAWW in relation to different temperature regimes tested in Series 1. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM.

high: this was apparent at temperatures equal to and greater than 12°C from fertilisation to eyeing, and temperatures exceeding 12°C from eyeing to hatching. For common temperature regimes from fertilisation to hatching (e.g. 8-8, 8-10, 10-10...) there was a progressive decrease in biomass with increasing temperature during the alevin stage.

Series 2 (Table 2.3.34, Fig. 2.3.29)

At MAWW, the biomasses of all groups within Series 2 were relatively uniform ranging from 12.03 g (11-9-9) to 15.22 g (9-10-10), with an overall mean biomass for the series of 13.87 g. The biomasses of groups held at 11°C from fertilisation to eyeing were generally lower than the other groups, and when the biomasses at MAWW for all groups with a common temperature from fertilisation to eyeing (i.e. 9, 10 and 11°C) were pooled, the means for the 3 pooled groups showed a decline with increasing temperature. The mean biomass for pooled groups was slightly lower at 10°C (13.99 g) than at 9°C (14.74 g), but was significantly lower ($P<0.01$) at 11°C (12.88 g).

Series 3 (Table 2.3.35, Fig. 2.3.30)

At MAWW, the biomasses were significantly lower ($P<0.01$) than the control in the group held at 11-13-13 together with those groups where the temperature from fertilisation to eyeing exceeded 11°C. A comparison of the 5 constant temperature regimes (9, 10, 11, 12 and 13°C) showed a

Table 2.3.34 Series 2 - mean biomass ±SD (g), as wet weight, of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean biomass±SD (g)		
	MAWW	110 days	1200°days
9-9-9	14.19±0.12	17.85±0.60***	27.60±1.00***
9-9-11	14.87±0.13	31.02±0.30	40.90±0.46
9-10-10	15.22±0.22	31.07±0.29	41.80±0.46
9-10-11	14.98±0.08	33.26±0.47	40.10±0.60
9-11-10	14.84±0.40	33.57±0.35	43.50±0.62
9-11-11	14.34±0.91	31.85±0.08	36.20±0.05
9-12-11	14.30±0.49	30.28±0.88	33.20±1.05
9-12-12	15.16±0.07	31.04±0.14	31.60±0.14
10-9-9	14.05±0.03	17.93±0.13***	26.02±0.20***
10-9-11	14.54±0.18	31.82±0.44	37.80±0.64
10-10-10	14.90±0.36	32.09±0.51	39.50±0.65
10-10-12	13.08±0.04*	31.32±0.28	32.11±0.23
10-11-11	13.75±0.33	32.36±0.04	34.10±0.04
10-11-12	14.44±0.21	34.41±2.99	33.90±2.90
10-12-11	14.18±0.20	37.95±0.71	38.90±0.78
10-12-12	13.00±0.43*	28.89±0.70	27.60±0.62***
11-9-9	12.03±0.03**	19.39±0.70***	25.20±1.12***
11-9-11	13.20±0.56	28.16±0.06	32.00±0.04
11-10-9	12.14±0.22**	27.20±0.02	37.10±0.04
11-10-10	12.92±0.18	26.53±0.53	30.70±0.56*

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Table 2.3.34 (continued)

Temp. regime (°C)	Mean biomass±SD (g)		
	MAWW	110 days	1200°days
11-11-11	13.55±0.35	31.15±0.18	31.60±0.20
11-11-12	12.35±0.52**	25.83±0.64*	24.60±0.61***
11-12-11	12.97±0.18	30.91±1.73	30.50±1.73*
11-12-12	13.87±0.04	33.40±0.73	30.90±0.65*

Table 2.3.35 Series 3 - mean biomass ±SD (g), as wet weight, of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean biomass±SD (g)		
	MAWW	110 days	1200°days
9-9-9	14.91±0.26	17.77±0.74	25.65±1.05
9-11-11	14.83±0.44	22.78±2.28	24.23±2.42
10-10-10	13.97±0.13	19.28±1.69	21.91±1.90
10-11-11	14.22±0.28	21.63±0.28	22.07±0.28
10-13-13	12.90±0.71	20.74±2.12	17.95±1.84
11-11-11	13.61±0.26	20.91±1.27	20.64±1.34
11-12-12	13.24±0.54	22.73±1.55	20.55±1.98
11-13-13	10.42±0.82**	17.41±0.64	14.92±0.57**
12-12-12	9.05±1.03**	13.57±0.21**	12.14±0.21**
13-13-13	0.52±0.49***	LV	LV

LV: Low viability (mortality>70%).

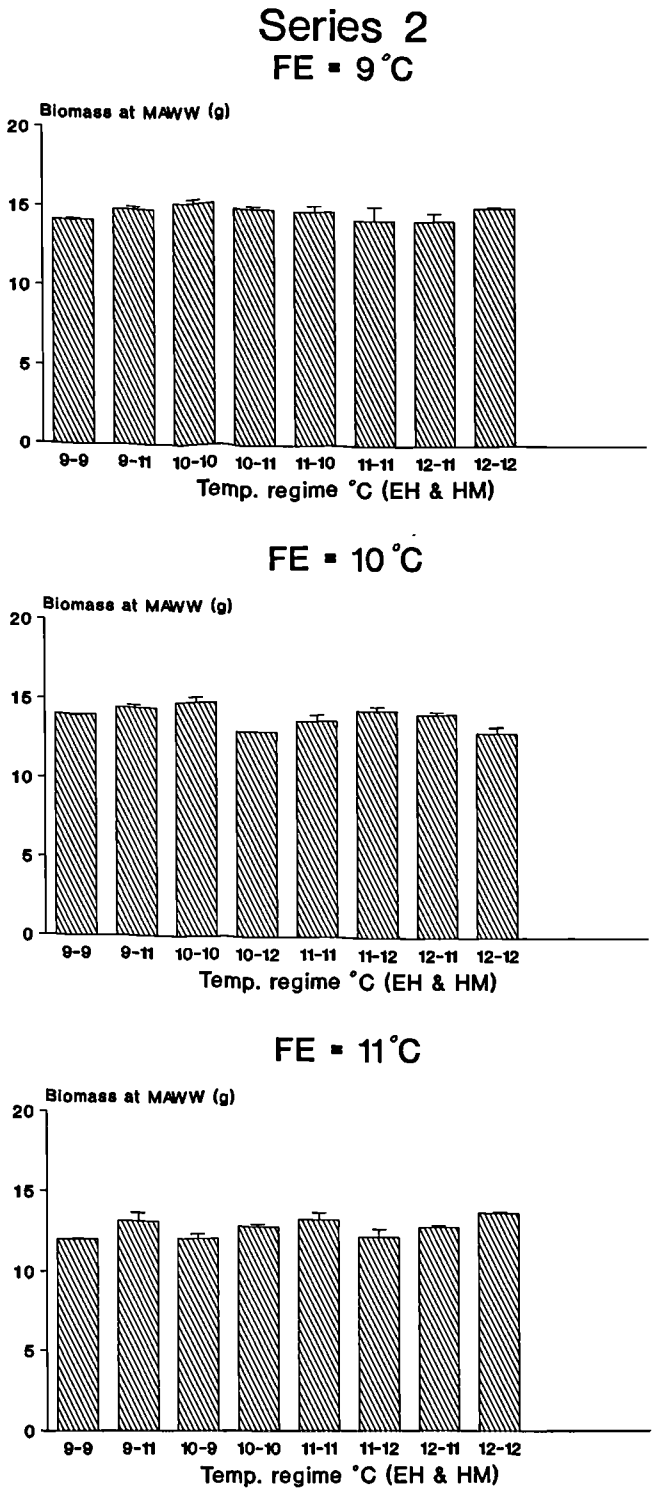

Fig. 2.3.29 Mean biomass, as wet weight, of alevins ±SD (g) at MAWW in relation to different temperature regimes tested in Series 2. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM.

Series 3

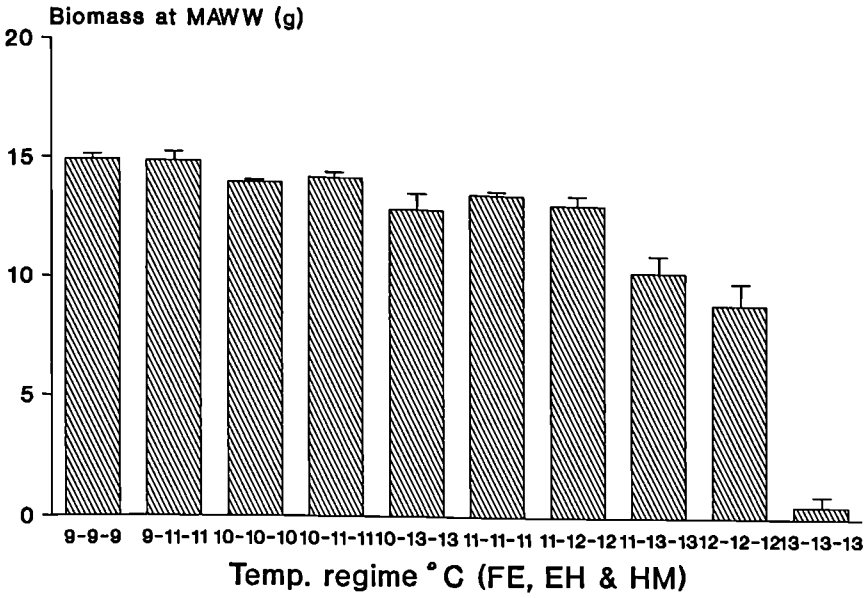

Fig. 2.3.30 Mean biomass, as wet weight, of alevins ±SD (g) at MAWW in relation to different temperature regimes tested in Series 3. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM.

decrease in biomass with increasing temperature. The decrease in biomass was slight between 9-11°C, but showed a marked decline ($P<0.01$) at the 2 higher temperatures.

Series 4A-C (Tables 2.3.36-2.3.38, Fig. 2.3.31)

As with previous series, Series 4A showed that biomass at MAWW (Table 2.3.36) was very significantly reduced ($P<0.001$) at a temperature of 12°C or greater from fertilisation to eyeing.

Following a temperature regime of a constant 10°C from fertilisation to hatching, biomass at MAWW showed a progressive decrease with increasing temperature during the alevin stage. Although not significant in Series 4A, this effect was very marked in Series 4B (Table 2.3.37, Fig. 2.3.31) and 4C (Table 2.3.38, Fig. 2.3.31), where $P<0.001$. In Series 4C, alevin survival was very low at 18°C and was nil at the higher temperatures tested. Consequently, the mean biomass at MAWW of the group held at 10°C during the alevin stage was nearly twice that of the group held at 18°C, and the biomasses were nil for the other 2 groups.

(b) Biomass at 110 days after fertilisation

Series 1 (Table 2.3.33, Fig. 2.3.32)

At 110 days after fertilisation, the highest biomasses were attained by the groups held at 8-10-12 and 8-12-14, and the lowest biomasses occurred in groups 8-14-14 and 10-12-12,

Table 2.3.36 Series 4A - mean biomass ±SD (g), as wet weight, of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean biomass±SD (g)		
	MAWW	110 days	1200°days
10-10-10	13.51±0.28	26.10±0.04	30.10±0.02
10-10-14	12.83±0.19	37.29±2.59***	32.49±2.27
10-10-16	12.29±0.36	39.63±3.16***	31.38±2.50
10-10-18	12.26±0.32	39.80±0.61***	30.00±0.46
12-12-12	2.53±0.37***	LV	LV
13-13	0 ***	NV	NV
14	0 ***	NV	NV

LV: Low viability (mortality>70%). Insufficient number of fish available for fry rearing study.
NV: Not viable (total mortality).

Table 2.3.37 Series 4B - mean biomass ±SD (g), as wet weight, of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Following the alevin stage (MAWW), fry rearing was conducted at 10°C. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean biomass±SD (g)		
	MAWW	110 days	1200°days
10-10-10	13.88±0.40	23.77±0.81	27.26±0.94
10-10-16	10.80±0.22**	21.48±0.35**	17.99±0.31**
10-10-18	6.19±0.11***	3.97±0.24***	3.68±0.23***
10-10-20	ND	LV	LV

ND: Not determined (severe oedema of yolk-sac prevented meaningful determination of MAWW).
LV: Low viability (mortality>70%). Insufficient number of fish available for fry rearing phase of study.

Table 2.3.38 Series 4C - mean biomass ±SD (g), as wet weight, of alevins at MAWW under different temperature regimes and fry at 110 days and 1200 degree-days after fertilisation. Asterisks indicate means significantly different from mean of control group (10-10-10): * P<0.05, ** P<0.01, *** P<0.001 (ANOVAR and SNK tests).

Temp. regime (°C)	Mean biomass±SD (g)		
	MAWW	110 days	1200°days
10-10-10	12.47±0.32	NT	NT
10-10-18	6.91±1.82***	NT	NT
10-10-20	0	NV	NV
10-10-22	0	NV	NV

NT: Not tested (further testing inappropriate because total mortality occurred in groups held at 20 & 22°C).
NV: Not viable (total mortality).

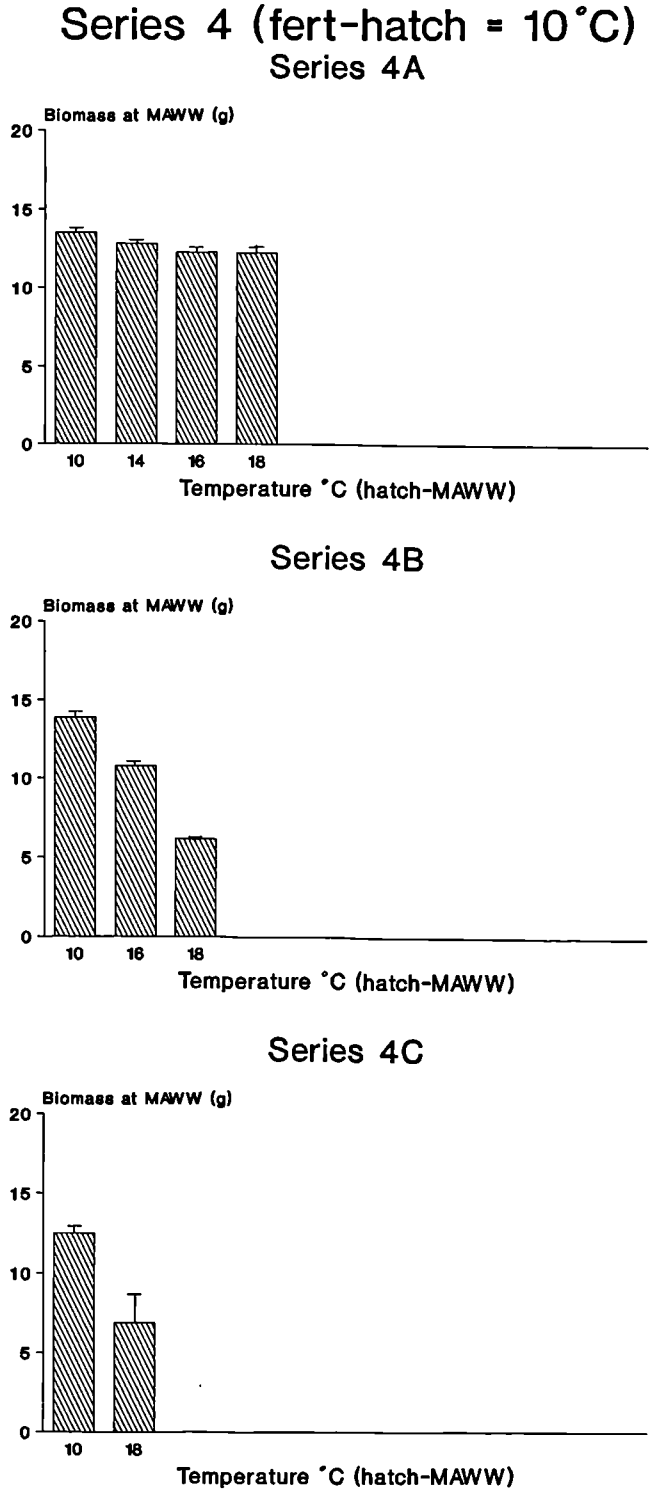

Fig. 2.3.31 Mean biomass, as wet weight, of alevins ±SD (g) at MAWW in relation to different temperature regimes tested in Series 4. The temperature from fertilisation to hatching was 10°C.

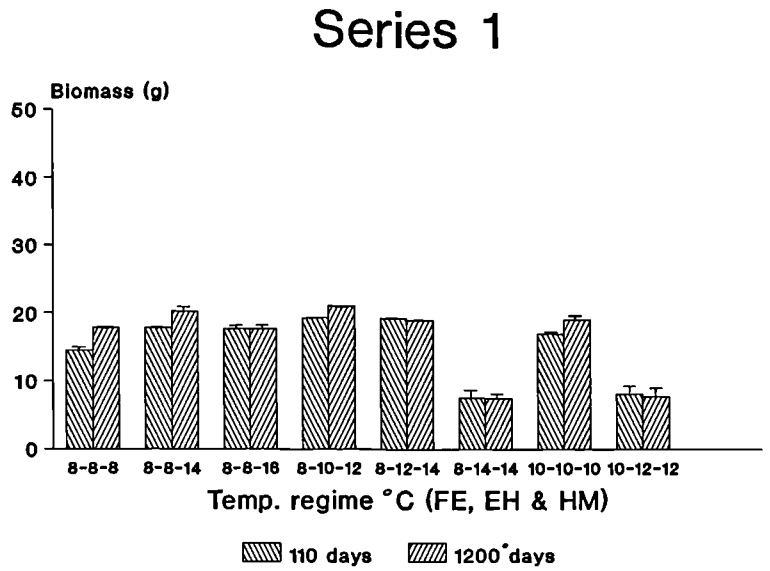

Fig. 2.3.32 Series 1 - mean biomass, as wet weight, of fry ±SD (g) at 110 days and 1200 degree-days after fertilisation in relation to different temperature regimes from fertilisation to MAWW. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Following the alevin stage (MAWW), fry were reared at 10°C.

where survivals from eyeing to MAWW were low. There were very significant differences ($P<0.001$) between the 2 groups having the lowest biomasses and the remaining groups. For the group maintained at a constant 8°C (8-8-8), biomass was intermediate in magnitude, and was significantly different ($P<0.01$) from the highest and lowest groups. Survival was high in this group due to the fact that the fry were still utilising their yolk reserves, so that mortality associated with transfer to exogenous feeding was postponed. However, the prolonged duration of development at 8°C meant that fry weight was low because the fry had only just attained MAWW.

Series 2 (Table 2.3.34, Fig. 2.3.33)

At 110 days after fertilisation, the biomasses of the 3 groups held at 9°C following eyeing (i.e. 9-9-9, 10-9-9 and 11-9-9) were very significantly lower ($P<0.001$) than the remaining groups, because of retarded growth due to their poor feeding responses during first feeding. Excepting these groups, there was little variation in the biomasses attained by different groups held at common temperatures from fertilisation to eyeing. Though the mean biomass for pooled groups was higher at the intermediate temperature of 10°C (30.85 g) than either 9°C (29.99 g) or 11°C (27.81 g), these differences were not significant.

Series 3 (Table 2.3.35, Fig. 2.3.34)

At 110 days after fertilisation, biomass was highest for

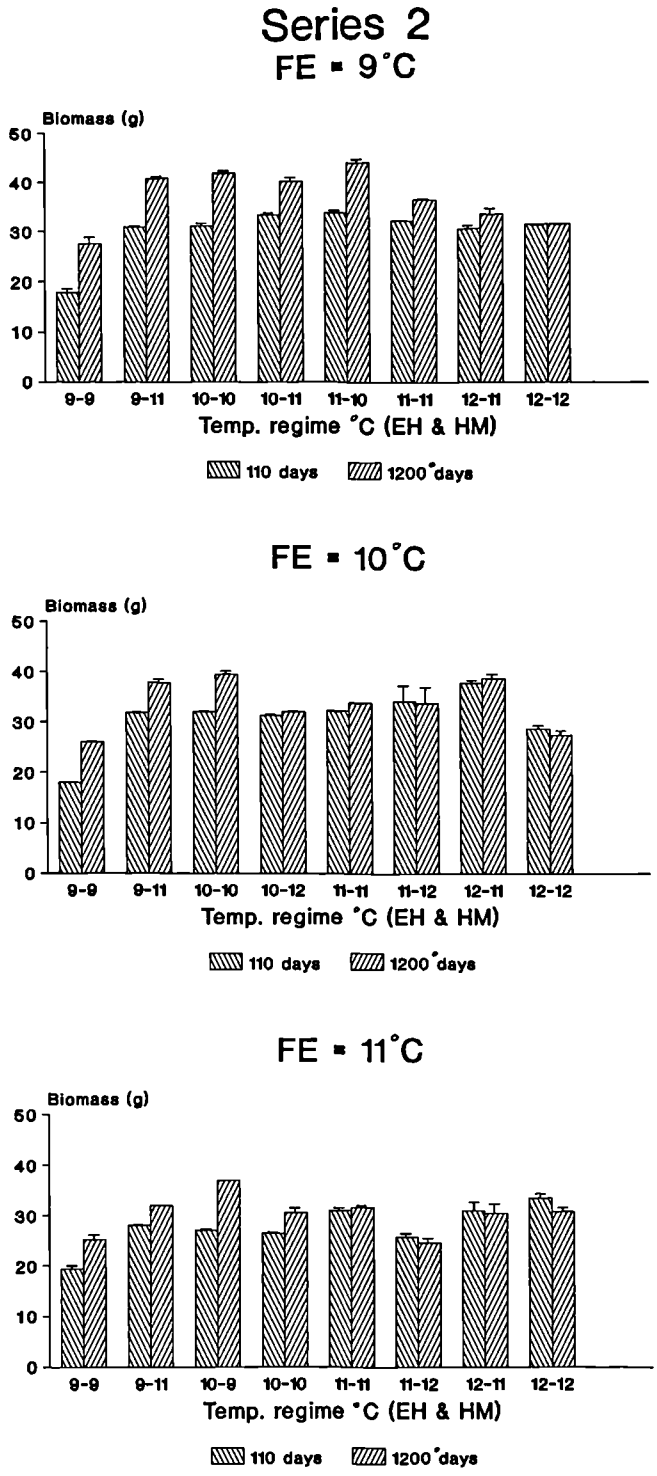

Fig. 2.3.33 Series 2 - mean biomass, as wet weight, of fry ±SD (g) at 110 days and 1200 degree-days after fertilisation in relation to different temperature regimes from fertilisation to MAWW. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Following the alevin stage (MAWW), fry were reared at 10°C.

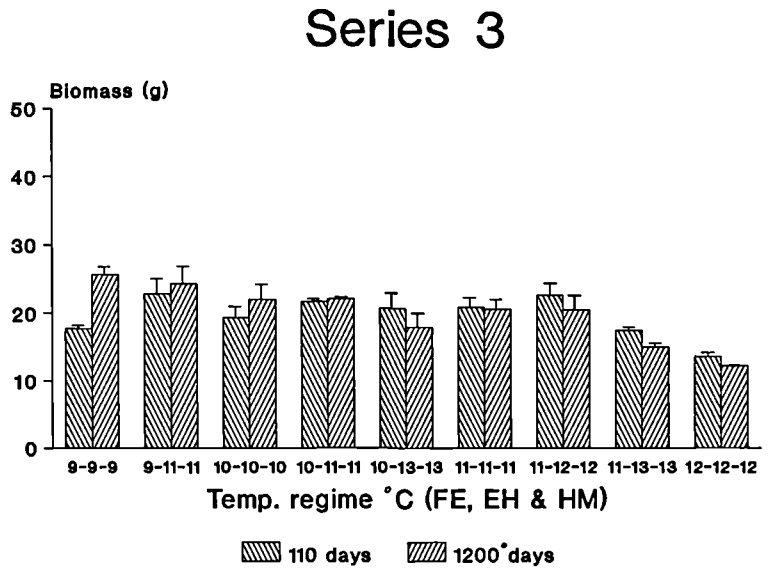

Fig. 2.3.34 Series 3 - mean biomass, as wet weight, of fry ±SD (g) at 110 days and 1200 degree-days after fertilisation in relation to different temperature regimes from fertilisation to MAWW. The temperature from fertilisation to eyeing is shown as FE; that from eyeing to hatching as EH; that from hatching to MAWW as HM. Following the alevin stage (MAWW), fry were reared at 10 °C.

the groups maintained at 9-11-11 and 11-12-12, and was significantly lower ($P<0.01$) for the group held at a constant 12°C. Although the biomasses of the groups held at 9-9-9 and 11-13-13 were lower than those of the intermediate groups, the differences were not significant.

Series 4A-C (Tables 2.3.36-2.3.38, Fig. 2.3.35)

In Series 4A, biomass at 110 days after fertilisation (Table 2.3.36, Fig. 2.3.35) increased with increasing temperature during the alevin stage, and raising the temperature from 10 to 14°C during this stage gave rise to a very significant increase ($P<0.001$) in biomass. At higher temperatures though, further increases in biomass were slight and were not shown to be significant.

However, the results of Series 4B (Table 2.3.37, Fig. 2.3.35) and 4C (Table 2.3.38) do not support those of Series 4A. In Series 4B, the biomass decreased very significantly ($P<0.001$) with increasing temperature during the alevin stage. Because of the very low survival of fry at the higher temperatures tested within Series 4C, no determinations were made for biomass at 110 days after fertilisation.

(c) Biomass at 1200 degree-days after fertilisation

Series 1 (Table 2.3.33, Fig. 2.3.32)

At 1200 degree-days after fertilisation, the biomasses of all groups except 8-14-14 and 10-12-12 were similar, ranging

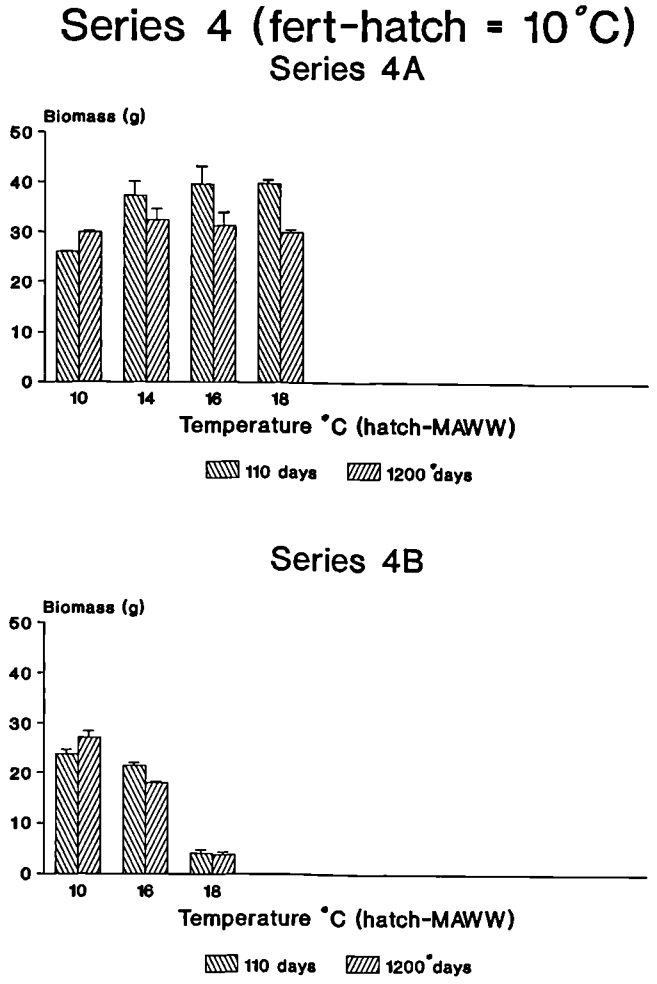

Fig. 2.3.35 Series 4 - mean biomass, as wet weight, of fry ±SD (g) at 110 days and 1200 degree-days after fertilisation in relation to different temperature regimes from fertilisation to MAWW. The temperature from fertilisation was 10°C. Following the alevin stage (MAWW), fry were reared at 10°C.

from 17.71 g (8-8-16) to 20.98 g (8-10-12), with a mean of 19.13 g. The biomasses of groups 8-14-14 (7.47 g) and 10-12-12 (7.81 g) were very significantly lower ($P<0.001$) than the other groups, because of the high levels of mortality experienced in these groups.

Series 2 (Table 2.3.34, Fig. 2.3.33)

At 1200 degree-days after fertilisation, the results were similar to those for biomass at 110 days after fertilisation. However, in addition to the 3 groups held at a constant 9°C following eyeing, the biomasses were also very significantly lower in those groups maintained at 10-12-12 and 11-11-12 ($P<0.001$); and 11-10-10, 11-12-11 and 11-12-12 ($P<0.05$). For pooled groups, mean biomass decreased progressively from 36.86g at 9°C, to 33.74 g at 10°C, and 30.33 g at 11°C. ANOVAR demonstrated a significant difference ($P<0.01$) in biomass between the 9 and 11°C groups. Thus, a temperature of 11°C from fertilisation to eyeing appeared to exceed the optimal temperature for net biomass gain.

Series 3 (Table 2.3.35, Fig. 2.3.34)

At 1200 degree-days after fertilisation, the highest biomass was attained by the group held at a constant 9°C, and biomass showed a general decline with increasing constant temperatures (10, 11 and 12°C). The biomasses of groups held at 11-13-13 and a constant 12°C were significantly lower ($P<0.01$) than the other groups tested.

Series 4A-C (Tables 2.3.36-2.3.38, Fig. 2.3.35)

At 1200 degree-days after fertilisation, the biomasses were very similar (range of 30.00-32.49 g; mean of 30.99 g) for the 4 groups tested in Series 4A (Table 2.3.36, Fig. 2.3.35).

However, the results of Series 4B and 4C do not support those of Series 4A. In Series 4B (Table 2.3.37, Fig. 2.3.35), the biomass decreased very significantly ($P<0.001$) with increasing temperature during the alevin stage, and because of the very low survival of fry at the higher temperatures tested within Series 4C, no determinations were made for biomass at 1200 degree-days after fertilisation (Table 2.3.38).

2.3.2.6 Summary of Results: Development and growth (2.3.2)

- Development times

The time in days to the attainment of successive stages of development was inversely related to temperature.

Development times in degree-days under different temperature regimes during the periods from fertilisation to eyeing, and hatching to MAWW, were relatively uniform, but showed a decline during the intermediate period of development (eyeing to hatching) as temperature increased.

- Hatching period

Excepting the 8°C incubates, the duration of the hatching period was not influenced by temperatures within the range of 9-16°C.

- Alevin size

Alevin weight at hatching and at MAWW

Alevin wet weight at hatching was not dependent upon incubation temperature within the range tested (8-16°C) in this study.

In Series 1 and 4, mean weights at MAWW were largely dependent upon the temperature during the alevin stage. High temperatures during the alevin stage, particularly within the range of 16-18°C, generally gave rise to significantly lower

mean weights of alevins at MAWW. Because of the narrower temperature ranges tested in Series 2 and 3 (9-13°C), no clear trends were apparent.

Alevin length at MAWW

In Series 4A-C, a temperature of 18°C during the alevin stage resulted in a marked decrease in alevin length at MAWW.

- Fry growth

At 110 days after fertilisation, those groups of fry attaining the highest mean individual weights generally included the more advanced early-feeding groups, which had been held at high temperatures during egg and alevin development. Clearly, groups held at high temperatures during the periods from fertilisation to MAWW were ready to begin feeding earlier, and were therefore fed for a longer period than those groups held at lower temperatures. In Series 2, several groups of fry achieved mean weights at 110 days after fertilisation which were more than twice that of the group held at a constant 9°C from fertilisation to MAWW.

At 1200 degree-days after fertilisation, the mean weights of fry in most groups within a series were relatively uniform, and generally independent of the temperature regime during egg and alevin development.

Specific growth rates of fry within a series were generally

quite similar for groups held under different temperature regimes during egg and alevin development. However, the mean specific growth rate of fry in Series 1 was considerably lower than the means from the other 3 series.

- Biomass gain

The net biomass gain for groups of alevins at MAWW, and fry at 110 days and 1200 degree-days after fertilisation, in relation to different temperature regimes from fertilisation to MAWW was more dependent upon the levels of egg and alevin mortality, than upon the mean weights attained by fish within a group.

2.4 DISCUSSION

2.4.1 MORTALITY

Among the four series of experiments, the response to temperature, as determined by survival, was quite consistent. The least consistent effects of temperature on survival occurred during the alevin stage.

2.4.1.1 Egg mortality

Atlantic salmon eggs appear to be unable to tolerate temperatures much above their normal range. Although eggs hatched at constant temperatures within the range of 8-13°C, mortalities were generally high at 12°C, and very high at 13°C. At temperatures below 12°C normal development occurred, without an increase in egg mortality. A comparison of published results with those of the present study showing mortalities of Atlantic salmon eggs incubated at high temperatures is presented in Table 2.4.1. It is apparent that a high percentage of Atlantic salmon eggs hatch successfully at constant temperatures equal to, or less than, 11°C. Peterson et al. (1977) showed that the mortality of Atlantic salmon eggs up to hatching at 10-12°C was significantly higher than at 4, 6, and 8°C. Similarly, Gunnes (1979) reported increased egg mortality at 12°C, and recommended that 10°C was the highest acceptable temperature for egg incubation. An earlier study by Markus (1962), showed that the mortality of eggs incubated at 10.1°C was "low" (not

Table 2.4.1 A comparison of published data with the combined results from this study (means of all series), showing percentage mortalities of Atlantic salmon eggs from fertilisation to hatching at constant temperatures within the range of 8-16°C. Temperatures are rounded to the nearest 1°C and mortalities to the nearest 1%.

Source	Temperature (°C)							
	8	9	10	11	12	13	14	16
Markus, 1962	-	-	"low"	-	<50	-	-	-
Hamor and Garside, 1976	-	-	8	-	-	-	-	-
Foda and Henderson, 1977	-	-	20	-	-	-	-	-
Peterson et al., 1977	6	-	7	-	14	-	-	-
Gunnes, 1979	14	-	14	-	66	-	-	-
This study	13	4	8	11	61	94	100	100

stated), whilst at 12.3°C eggs suffered mortalities approaching 50% and many that hatched were not viable. Foda and Henderson (1977) tested three incubation temperatures (4.0, 7.2 and 10.0°C) and found that the percentage mortality increased progressively with increasing temperature from 9.2 at 4.0°C, to 19.6 at 10.0°C. No published work has been found which has tested the effects of constant incubation temperatures greater than 12°C on the survival of Atlantic salmon eggs from fertilisation to hatching.

Other studies have established that salmonid eggs exhibit species-specific and relatively narrow optimal temperature ranges. For brown trout eggs, temperatures in excess of 12°C cause substantial mortalities (Jungwirth and Winkler, 1984), and temperatures of 15°C and above are rapidly lethal (Humpesch, 1985a).

In the genus Salvelinus, the upper temperature limit for brook char eggs is similar to that for brown trout. Studies by Hokanson et al. (1973) and Humpesch (1985a) reported severe mortalities of brook char eggs at 12°C (40-46% mortality) and 14°C (60% mortality), respectively, with total mortality occurring in both investigations at 15°C. The tolerance of Arctic char eggs to high temperatures has been shown to be less than that of other salmonids: studies by Swift (1965), Jungwirth and Winkler (1984), and Humpesch (1985a), have reported total mortality at incubation temperatures of 12-13°C. For lake trout eggs, Dwyer (1987) reported a high level of mortality at 9.8°C (37.7%). However, it should be noted that within the same investigation, mortality was of a similar magnitude (33.9%) at an incubation

temperature of 6.4°C.

In the genus Oncorhynchus, Combs and Burrows (1957) reported that the upper temperature limit for the normal development of chinook salmon eggs was between 14.2°C (6.1% mortality) and 15.6°C (12.4% mortality). For the same species, Heming (1982) reported 10.7% mortality among eggs incubated at 12°C, and Johnson and Brice (1953) concluded that eggs could be incubated safely at a mean temperature below 12.3°C, but sustained excessive mortalities at temperatures above 15.7°C. Garling and Masterson (1985) reported similar results, with a 76.9% mortality among chinook salmon eggs incubated at 15.0°C.

Combs (1965) showed that the upper temperature limit for sockeye salmon eggs was approximately 16.9°C (83.0% mortality). For coho salmon eggs, Tang et al. (1987) demonstrated that the upper temperature limit for eggs was 14.4°C (85-99% mortality). The upper temperature limits are less well defined for pink and chum salmon eggs. Very low levels of mortality have been reported for pink salmon eggs incubated at high temperatures: at 12°C, Beacham and Murray (1988) reported a 4% mortality from fertilisation to hatching; and at an initial incubation temperature of 15°C, decreasing thereafter by 0.5°C every 3 days until hatching, Murray and Beacham (1986) reported a mortality of only 1.7%. For chum salmon eggs incubated at 12°C, mortality was 12-23%, depending on egg size (Beacham and Murray, 1985). Hiroi et al. (1988) concluded that the upper temperature limit for chum salmon eggs lay within the range of 10-14°C.

Numerous studies have investigated the effect of

temperature on the survival of rainbow trout eggs: Garside (1966) reported an overall temperature range of 2.5-17.5°C for rainbow trout eggs; Timoshina (1972) found that the upper temperature limit for rainbow trout eggs was close to 13°C (40.7-47.3% mortality); Orska (1962) stated that 14.5°C was near the maximum temperature for rainbow trout eggs, and Ishida (1985) reported poor survival of eggs and premature hatching at 15°C. Kamler and Kato (1983) showed that within the range of 9-14°C, the mortality of rainbow trout eggs was only slightly lower at intermediate temperatures of 10-12°C (60-62%), than at 9°C (72%) and 14°C (70%). Similarly, Lebedeva and Meshkov (1969) reported that the upper temperature limit for rainbow trout eggs was 14°C. In studies conducted by Kwain (1975a, b), an incubation temperature of 10°C resulted in mortalities of rainbow trout eggs below 15%, whilst at 15°C mortalities approached 60%. However, at very similar incubation temperatures, Embody (1934), Rombough (1986), and Kashiwagi et al. (1987) reported considerably lower levels of mortality in rainbow trout eggs (<10% at 15.5°C, 15% at 15.1°C, and 20% at 16°C, respectively). Total mortality of rainbow trout eggs has been shown to occur at 18°C (Kashiwagi et al., 1987), and 19°C (Humpesch, 1985a). In the latter study, 75% mortality occurred at 18°C.

The tolerance of huchen eggs to high incubation temperatures appears to be very similar to that of brook char: Humpesch (1985a) reported 80% mortality at 15°C, with total mortality occurring at 16°C.

Thus, the eggs of species within the genus Oncorhynchus show the greatest tolerance to high temperatures ($\leq$14°C);

brown trout, brook char, and huchen eggs are less tolerant than those of Oncorhynchus species (≤13°C), though more tolerant than Atlantic salmon eggs; Arctic char eggs are the least tolerant of high temperatures (<12°C).

2.4.1.2 Alevin mortality

Providing that Atlantic salmon eggs were incubated at temperatures below 12°C, the subsequent mortalities of alevins were low for all temperatures below 16°C during the alevin stage. Above 16°C, alevin mortalities were significantly greater in most, but not all, experiments. The upper temperature limit for newly-hatched alevins was between 18-20°C. A comparison of published results with those from the present study showing mortalities of Atlantic salmon during the alevin stage at high temperatures is presented in Table 2.4.2. The results of the investigation conducted by Bishai (1960) are similar to the present findings: it was concluded that the temperature limit for alevins occurred at 20-22°C. Bishai (1960) demonstrated that newly-hatched Atlantic salmon alevins suffered no mortality during 16 days at 20°C, and that the upper temperature limit for alevins held at 5-6°C, and gradually heated to the target temperatures (22-26°C) in 6 hours, was 22°C. Bishai (1960) also found little acclimation influence on subsequent 7-day TL50 (highest temperature at which 50% of the population can survive for 7 days) determinations. An increase in acclimation from 5-6°C (7-day TL50 = 22.0-22.5°C) to 10 and 20°C increased the 7-day TL50 values to only 23°C, for both

Table 2.4.2 A comparison of published data and the results from this study (means of all series), showing percentage mortalities of Atlantic salmon during the alevin stage (hatching to final yolk absorption) at constant temperatures within the range of 8-22°C. The numerators show the egg incubation temperatures and the denominators show the temperatures during the alevin stage. Temperatures are rounded to the near 1°C and mortalities to the nearest 1%.

Source	Temperature (°C)																						
	8							9		10								11		12			
	8	10	12	14	16	20	22	9	11	8	10	12	14	16	18	20	22	11	12	8	10	12
Bishai, 1960	-	-	-	-	-	0	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Foda and Henderson, 1977	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-
Peterson *et al*, 1977	0	0	3	-	-	-	-	-	-	0	0	0	-	-	-	-	-	-	-	4	0	0
[a]	2	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	32
Gunnes, 1979	5	11	7	-	-	-	-	-	-	16	26	42	-	-	-	-	-	-	-	29	46	61
Brännäs, 1988	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	15
[b]	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	10
This study	0	-	-	0	2	-	-	2	1	-	2	0	3	5	23	85	100	4	1	-	-	38

[a] Results from a second experiment

[b] Results from a sibling group

the 10 and 20°C acclimations. No other studies have been found which investigated the effects of temperatures greater than 12°C during alevin development in Atlantic salmon. The alevin mortalities reported by Peterson et al. (1977), Gunnes (1979), and Brännäs (1988) appear to be associated with the detrimental effects of high temperatures, particularly 12°C, during earlier stages of development, rather than during the alevin stage.

Among other salmonid species, Gray (1928b) reported that brown trout mortality was "high" (not stated) during the later stages of alevin development at 17°C, while for the same species, Bishai (1960) reported no alevin mortality at 20°C, but total mortality at 22°C.

In the genus Salvelinus, Wallace and Aasjord (1984b) reported that 12°C was excessive (48.6% mortality) for Arctic char alevins. McCormick et al. (1972) reported a 7-day TL50 and a 1-day TL50 for newly-hatched brook char alevins at temperatures of 20 and 22-24°C, respectively.

Within the genus Oncorhynchus, few studies have attempted to identify the upper temperature limits for alevins. Although several investigators have reported high levels of alevin mortality within the range of 12-15°C, these can generally be attributed to the detrimental effects of high temperatures during egg incubation. Thus, Heming (1982) reported that 12°C was excessive for the rearing of chinook salmon because of greater alevin mortality (15.4%) at that temperature. For the same species, Garling and Masterson (1985) reported 26.3% alevin mortality at 15°C. In both of these studies, temperatures were held constant throughout egg

and alevin development, and alevin mortalities were associated with adverse temperatures during egg incubation.

For coho salmon held at a constant 14°C throughout egg and alevin development, Tang et al. (1987) reported alevin mortalities of 20 and 35% for one salmon stock, and 100% for a second stock. For pink salmon, Beacham and Murray (1988) reported 2% alevin mortality at a constant 12°C during egg and alevin development, while 0.7% alevin mortality was reported by Godin (1980) at 15°C. In the latter study, eggs were incubated at 8°C. Beacham and Murray (1985) reported that chum salmon alevin survival increased with decreasing temperature, with 100% survival observed at 4°C. At a constant 12°C throughout development, alevin mortalities ranged from 24-31% depending on egg size.

For rainbow trout held at constant temperatures during egg and alevin development, Rombough (1986) reported an alevin mortality below 5% at 15°C, while at 13°C, Timoshina (1972) reported alevin mortalities of 63.7-65.4%. However, Timoshina (1972) also reported high alevin mortality (48.0%) at 10°C. For the same species, Danzmann and Ferguson (1988) reported a significantly higher alevin mortality at a constant 12°C than at 8°C.

Thus, salmonid alevins exceed eggs in their tolerance to high temperatures. The upper temperature limit for the alevins of Atlantic salmon, brown trout and brook char is about 20°C. The few studies conducted for species within the genus Oncorhynchus indicate that the upper temperature limits for alevins exceed 15°C.

2.4.1.3 Fry mortality

Throughout this study, no clear correlation was shown between the thermal history of eggs and alevins, and subsequent fry mortality. The only latent effects of previous temperature regimes on fry survival were shown in Series 4B, where alevins held at 16 and 18°C contracted a yolk-sac deformity. However, Gunnes (1979) stated that the substantial differences in mortality that occurred in different groups of Atlantic salmon fry during the first 6 weeks of feeding must have been associated with the temperature differences (8, 10, 12°C) experienced during egg and alevin development.

Though not investigated here, it appears that juvenile salmonids have greater temperature tolerance than do alevins: the upper limit appears to lie within the range of 21-26°C, depending on the species and the acclimation temperature. Siemien and Carline (1991) reported mortalities during the first 3 weeks of feeding of 5.8, 12.5, 20.9, and 16.9% for Atlantic salmon fry reared at constant temperatures of 10, 14, 18 and 22°C, respectively. Bidgood and Berst (1969) found that the upper lethal temperature for 4-9 cm rainbow trout was between 25-26°C, following thermal acclimation at 15°C. Brett (1952) determined the temperature limits for five Oncorhynchus species (chinook, chum, coho, pink and sockeye salmon) within the size range of 0.3-1.6g: the upper temperature limit for each species acclimated at 5°C was in the range of 21.2-22.9°C. For acclimation temperatures ranging from 5-24°C, there were significant differences between species in their resistance to high temperatures. No

species could tolerate temperatures exceeding 25.1°C, when exposed for one week following full acclimation. McCormick et al. (1972) reported that the upper temperature limit for "swim-up" brook char fry (7-day TL50 of 24°C) was greater than that for newly-hatched alevins (7-day TL50 of 20°C). Because newly-hatched brook char alevins failed to demonstrate a relationship between acclimation temperature and temperature tolerance, McCormick et al. (1972) postulated that the physiological mechanism responsible for acclimation is not developed at this early stage of development. Earlier studies by Bishai (1960) and Mantelman (1960) support this hypothesis: Bishai (1960) reported that the upper temperature limit was not increased when Atlantic salmon and brown trout alevins were first acclimated at 10 and 20°C before testing at 22-26°C; and Mantelman (1960) reported that rainbow trout alevins failed to respond in a temperature-selective manner to a temperature gradient as do fry following "swim-up".

Thus, the findings of the present investigation, together with those from other studies, show that the temperature tolerance of salmonids increases as development proceeds; there is a marked increase in temperature tolerance after hatching followed by a further, though relatively small, increase in the level of tolerance following "swim-up".

2.4.1.4 Patterns of mortality

At subacute and lethal egg incubation temperatures, a peak period of mortality occurred during early development. High mortalities were first observed between 50-150 degree-days

after fertilisation, and remained high during blastulation and gastrulation. Table 2.4.3 presents a broad outline of the embryogenesis of Atlantic salmon, showing the general scheme of early ontogenesis in relation to development times in degree-days. Mortalities decreased immediately before or during organogenesis, and remained low until hatching at all incubation temperatures except the highest (12°C and above). Hayes (1949) demonstrated that a period of maximum sensitivity in Atlantic salmon occurred during the early embryonic stages, prior to closure of the blastopore. Battle (1944) also stated that increased mortality during salmonid embryonic development occurs during epiboly. Resistance develops when the vitelline membrane enclosing the yolk is replaced by a heavy layer of ectoderm, endoderm and mesoderm.

The results of studies on other salmonid species are similar to those reported here. Hiroi et al. (1988) examined the influence of high temperatures (10, 14, 16, 18 and 20°C) on the mortality of newly-fertilised chum salmon eggs. Following fertilisation at 10°C, eggs were exposed during "water hardening" to high temperatures for a period of 2 hours. At 14°C and greater, mortalities were excessive, and occurred during early development (0-200 degree-days); at 10°C egg mortality was low. Studies by Combs (1965) on sockeye and chinook salmon, and by Tang et al. (1987) on coho salmon, also found that the effects of lethal temperatures during egg incubation occurred before closure of the blastopore. Combs (1965) found that, following the 128-cell stage of development, both sockeye and chinook salmon demonstrated a greater range of temperature tolerances.

Table 2.4.3 Stages in the embryogenesis of Atlantic salmon from fertilisation to hatching. Development times are given in degree-days.

Development times (°days)	Developmental stage
0	
	Bipolar differentiation
	Cleavage and definition of germ layers
	Development and growth of blastodisc
50	Appearance of embryonic shield and germ ring
	Axis formation
	Neural keel; somite formation begins
	Optic anlagen and brain vesicles defined
100	Closure of germ ring over yolk plug
	Heart and brain differentiation
150	Somitogenesis complete
	Heart beating; formation of hind-gut and branchial primordia
	Blood circulation through aortic arches
200	Retinal pigmentation
	Pectoral fins, gills and nares evident
250	Mouth open; yolk sac vascularisation
300	Body melanised
350	Development of operculae
	Bile in intestine
400	Pectoral fin movement
	Jaw and hyobranchial movement; formation of gill lamellae and pelvic fins
450	Hatching glands active on head and anterior yolk sac

(Adapted from Klinkhardt et al., 1987)

Similarly, in brook char, Hokanson et al. (1973) and Marten (1992) reported that the stage of development at which the embryo is exposed to extreme temperatures was a significant factor in determining temperature tolerance, and demonstrated that the tolerance increased as development progressed. Hokanson et al. (1973) found that the temperature limit for brook char eyed eggs was 1°C higher than that of earlier phases of development. Furthermore, in agreement with the results of this investigation, both studies demonstrated that the temperature during the first period of incubation (fertilisation to eyeing), significantly affected the number of brook char eggs that survived the second period of incubation (eyeing to hatching).

At high temperatures, mortalities again increased shortly before hatching began, and remained high throughout the hatching period. An early study by Hayes (1930) reported the existence of this second period of sensitivity prior to hatching, when the embryo first becomes opaque. Later, Hayes (1949) reported that mortality was substantially greater at this time under high temperature conditions.

Sensitivity to high temperatures is not only influenced by the stage of development, but also by the exposure time. The various effects of temperature described by these experiments apply only to continuous exposures to constant temperatures within the three periods of development examined. Within these periods of development, there appear to be intervals when tolerance to short-term exposure to high temperatures is increased. Numerous studies have shown that salmonid eggs can tolerate short-term temperature extremes. Chinook salmon eggs

can withstand temperature shocks approaching 26°C for 2 hours, although the subsequent frequency of abnormalities is relatively high (Brett, 1956; Bishai, 1960; Neitzel and Becker, 1985).

Studies on induced triploidy by heat shocking (Benfey and Sutterlin, 1984; Johnstone, 1985) have also demonstrated that newly-fertilised Atlantic salmon eggs can survive a short-term exposure (up to 20 minutes) to temperatures approaching 32°C.

Thus, salmonid eggs show a high level of temperature tolerance during early cleavage. Studying the temperature tolerance of chinook salmon eggs and alevins acclimated to 10°C, Neitzel and Becker (1985) showed that abrupt increases in temperature to above 22°C for periods of 1-8 hours reduced the survival of cleavage eggs (56 degree-days after fertilisation); embryos (281 degree-days after fertilisation) survived an 8-hour exposure to 25°C and a 2-hour exposure to 26.5°C; alevins (566 and 837 degree-days after fertilisation) tolerated a 4-hour exposure to 23.5°C and 1-hour exposure to 25.0°C. However, Bishai (1960) reported that the tolerance of salmonid alevins appeared not to change with age. Smith (1957) stated that both Atlantic salmon and rainbow trout were tolerant of a short-term increase in temperature during hatching. For brook char alevins, McCormick et al. (1972) reported that temperatures of 18°C and above were detrimental, except for short-term exposure; newly-hatched alevins can tolerate 20°C or slightly above, but at such temperatures longer term exposure may give rise to substantial mortalities. McCormick et al. (1972) also showed

that "swim-up" brook char fry were more tolerant of a short-term exposure to 25°C than alevins. Bidgood (1980a, b) showed that the sensitivity of rainbow trout to short-term fluctuations in temperature decreases at the fry stage, soon after the yolk-sac has been fully absorbed.

Thus, both the timing of exposure and the precise stage of development at which eggs and alevins are subjected to high temperatures are important determinants of the resulting level of mortality. However, further precision in the identification of specific intervals of increased or reduced sensitivity to high temperatures would be of little practical value to hatchery managers, except during very early development (within 1 hour of fertilisation) when the practice of heat shocking is sometimes employed to produce triploid salmonid stocks.

2.4.1.5 Optimal temperatures for eggs and alevins

The optimal temperature ranges giving the highest levels of survival for Atlantic salmon eggs and alevins, within the temperatures tested in the four series of experiments (8-22°C), were 8-11°C and 8-16°C, respectively. Thus, following hatching, it is possible to increase the temperature by several degrees Celsius to advance development, without increasing alevin mortality. Gunnes (1979) found that 8-10°C was optimal for both egg and alevin stages of Atlantic salmon development. However, Gunnes' investigation only examined three constant temperatures (8, 10 and 12°C). A more wide-ranging investigation conducted by Peterson et al. (1977),

showed that the optimal temperatures for Atlantic salmon eggs and alevins were 4+8°C and 6-12°C, respectively, (temperature range tested was 2-12°C). Vernidub (1963), in a study directed towards establishing the optimal temperatures for the early development of Atlantic salmon, concluded that each particular stage of development has a specific optimal temperature range: these were 2-4°C during the early stages of development, 5-6°C towards the time of hatching, 8-10°C following hatching, and 10-12°C during the transition to exogenous feeding. Brännäs (1988) studied the survival of Atlantic salmon alevins at three constant temperatures (6, 10 and 12°C) and found that 10°C was optimal.

Among other salmonid species, Beacham and Murray (1988) reported marked differences in the levels of mortality of pink salmon eggs at different temperatures (4, 8, 12°C), with the highest survival occurring at 8 and 12°C, and the lowest at 4°C. Kwain (1975a), studying the effects of various combinations of light and temperature on rainbow trout eggs, reported that the lowest mortality rates occurred at 7 and 10°C (and light intensities of 0.2 and 20 lux). A second investigation by Kwain (1975b) on the effect of temperature (5, 10, 15°C) and acidity on the survival of rainbow trout eggs from fertilisation to hatching, again reported that mortality (in the experimental control at pH 6.9) was lowest at the intermediate temperature of 10°C (14%). Timoshina (1972) found that, although the survival of rainbow trout at constant temperatures of 5 and 7°C was high, better results were obtained by gradually raising the temperature: following egg incubation at 5.0-5.3°C to eyeing, the temperature was

raised to 11.5°C towards hatching, and then gradually raised to 13°C during the alevin stage. Timoshina (1972) concluded that the optimal temperatures for rainbow trout eggs and alevins change during the course of development: temperatures of 10-13°C were unsuitable during early development, but optimal towards the time of hatching and during the alevin stage. Lebedeva and Meshkov (1969) established that the optimal temperature for the development of rainbow trout eggs was between 3-9°C. For the same species, Kawajiri (1928) found that 9°C was the optimal temperature for eggs.

The temperature requirements of species within the genus Salvelinus appear to be lower than those of other salmonids. Ostergaard (1987) found that the survival of lake trout eggs, alevins and fry was higher between 3.8-7.8°C (mean of 54%) compared with 8.4-10.6°C (mean of 18%). Jungwirth and Winkler (1984) reported that the optimal temperature range for Arctic char eggs was 5-8°C, while for the same species, Gruber and Weiser (1983) stated that the optimal range for eggs and alevins was 4-8°C. Similarly, Swift (1965) found that 4-8°C was optimal for Arctic char eggs and alevins, and Wallace and Aasjord (1984b), that 3-8°C was optimal for alevins. The brook char appears to be a less stenothermic species: McCormick et al. (1972) reported that a suitable temperature range for the survival and growth of brook char alevins to fry was 9.8-15.4°C, and stated that the optimal range was 12.4-15.4°C.

Humpesch (1985a) investigated the temperature requirements of five salmonid species and stated that although hatching success was found to vary considerably within the temperature

ranges tested, the optimal ranges for greatest survival from fertilisation to hatching were: 3-5°C for Arctic char; 3-8°C for brook char; 4-8°C for brown trout; 7-8°C for huchen; and 8-12°C for rainbow trout.

Thus, the optimal temperature ranges for the survival of Atlantic salmon eggs and alevins are 4-11°C and 6-16°C, respectively. Excepting the Arctic char, the relatively few published data suggest that the temperature optima for the eggs and alevins of other salmonid species are similar to those for Atlantic salmon.

2.4.1.6 Abnormalities of eggs and alevins

The results of the various experiments showed that eggs incubated at temperatures of 12°C and greater were generally not viable; eggs which did hatch, and particularly those hatching prematurely, showed a high incidence of developmental abnormalities which had not necessarily been manifest prior to hatching. Particularly noteworthy was the occurrence of spinal malformation which was only apparent at hatching. Common forms of abnormality included slight or, in extreme cases, pronounced curvature of the trunk region of the spinal column.

Many studies have reported that morphological abnormalities can be induced by temperature extremes during early development. Hayes et al. (1953) reported that when Atlantic salmon eggs were incubated from Pelluet's (Pelluet, 1944) stage 6 (about 150-200 degree-days after fertilisation) at 11.5°C, the surviving alevins showed a marked distortion of

the spine. Similar abnormalities occurred when more advanced eggs were exposed to 15.5°C. The effect was less pronounced when eggs were incubated from beyond Pelluet's stage 6 at 11.5°C, and the spinal flexure disappeared as subsequent development proceeded. However, because alevins hatching from eggs incubated from Pelluet's stage 11 (about 350-400 degree-days after fertilisation) and beyond at 14.1°C showed no evidence of body curvature, Hayes et al. (1953) concluded that the critical period of susceptibility to temperature-induced spinal deformation was passed. Similarly, Marten (1992) inferred that developmental abnormalities were induced in brook char embryos by high temperatures during the period of incubation from fertilisation to eyeing.

Hiroi et al. (1988) reported that the main abnormality occurring among chum salmon eggs held for 2 hours at 14 and 16°C, following fertilisation at 10°C, was twinning ("Siamese twins": a conjoined twin condition), while those eggs held for the same period of time at 18 and 20°C frequently resulted in spinal malformation. Following the heat treatments, the four groups of eggs were incubated at a constant 10°C. Kuramoto et al. (1988) reported that twinning was apparent among chum salmon incubated at 15°C during the formation of the embryonic shield (embryonic body formation), 69 degree-days after fertilisation. Exposure of the eggs immediately after fertilisation to high temperature was considered to have induced the condition. Smith (1957) stated that twinning and axial duplication became apparent at gastrulation. This form of abnormality was not observed in the present investigation.

The "pin-eyed" condition (incomplete retinal pigmentation), commonly observed in the present study has not been previously reported in Atlantic salmon. However, Timoshina (1972) described a similar condition occurring in newly-hatched rainbow trout alevins hatched from eggs incubated at 10 and 13°C. Alevins were small, relatively inactive, and had a poorly developed vascular system; the head and body were lightly pigmented, the paired fins were poorly developed, and haemorrhages were frequently observed on the head and body. Spinal deformities were also apparent, and considerable mortalities were prevalent during the alevin stage. In comparison, Timoshina (1972) found that alevins from eggs incubated at 5 and 7°C were larger and more viable: alevins were active, had a well developed vascular system, a well pigmented body and head, and clear fin definition. Haemorrhages on the body were absent, and subsequent alevin survival was high.

The type of jaw deformity observed in newly-hatched alevins in Series 1, has not been reported in other salmonid studies. In newly-hatched alevins the lower jaw is normally shorter than the upper jaw and, as development proceeds, it extends forward beyond the upper jaw. However, at 14 and 16°C (Series 1 only) the lower jaw was virtually absent or rudimentary in form.

Among alevins held at 20°C in experimental Series 4B, mortality was generally caused by oedema of the yolk-sac which is indicative of a yolk resorption deficiency. Following high temperatures during the alevin stage, similar abnormalities of the yolk-sac have been observed during first

feeding in brown trout (Gray, 1926) and rainbow trout (Smith, 1947).

A commonly observed abnormality afflicting Atlantic and Pacific species of salmon is the development of yolk-sac constrictions in alevins. Gunnes (1979) stated that while the highest level of yolk-sac constrictions occurred in Atlantic salmon alevins held at 12°C, they were also present under the other temperature regimes tested (8 and 10°C). Dumas (1966) also reported the occurrence of yolk-sac constrictions in Atlantic salmon alevins at temperatures of 8.4 and 11.8°C, and Gunnes (1979) reported that their presence in Atlantic salmon alevins did not seem to influence the subsequent growth rate of the resulting fry. This type of yolk-sac abnormality was not observed in this investigation because alevins were provided with a suitable substrate during the alevin stage (see section 2.2.2.4).

Thus, temperature-induced abnormalities most commonly occurred during egg and alevin development at temperatures exceeding 10°C and 16°C, respectively. In the present study these included spinal malformation, incomplete eye pigmentation and oedema of the yolk-sac.

2.4.1.7 Causes of mortality

The onset of mortalities among eggs incubated at 12, 14 and 16°C was approximately 50-100 degree-days after fertilisation, which coincides with epiboly. The exact cause(s) of mortality due to adverse temperatures outside of the optimal range has not been fully established. A number of

investigators have attributed mortalities at different stages of development to various factors. It has been postulated that, excepting genetic deformities, abnormalities frequently result from differential rates of specific developmental processes. Hayes (1949) suggested that the cause of mortality in Atlantic salmon due to adverse temperatures may be explained by a lack of coordination between the processes of embryonic growth and differentiation in organogenesis, or by significant dislocations in the order of morphological differentiation (Hayes and Pelluet, 1945; Hayes et al., 1953). This was explained by the differential action of high temperatures on the rates of sensitive metabolic processes leading to a degree of unbalance which cannot be tolerated by the embryo. The temperature limit at which no further acceleration of development occurs in response to increasing temperature appears to vary from one developmental process to another: at incubation temperatures of 11°C and above, Hayes et al. (1953) found that the accelerating effect of high temperatures no longer operated for certain morphological events such as eyeing, yolk-sac vascularisation and circulation. Later studies have confirmed that high temperatures cause accelerated differentiation of tissues and organs, but retard the growth of these structures relative to the action of low temperatures (Garside, 1959, 1966).

Thus, ontogenesis of salmonid fish species can occur normally only within strictly defined temperature limits, beyond which it ceases; at temperature extremes the order of differentiation may change, or growth may not relate to differentiation in the normal manner. There is a limit to the

extent to which the processes of development can be uncoupled, without causing abnormality or mortality. When incubation temperatures are outside the optimal range, development appears to be inhibited at early stages, generally near the time of gastrulation. This results in either mortality of the embryo, or the development of abnormalities. Further deviation from the optimal range causes a marked increase in mortality.

It has also been suggested that mortality is caused by the detrimental effects of high temperatures on enzyme activities. Marten (1992) stated that high temperatures may affect temperature-dependent enzyme processes involved in organogenesis; Torrissen and Torrissen (1984) reported a significant effect of temperature on the enzyme activities (digestive proteases) of Atlantic salmon parr. However, because the temperature limits for eggs and alevins are lower than those for fry or older fish, it is unlikely that this is the main factor.

Mortality could also be due to the coagulation of proteins or some effect on lipids (Hayes et al., 1953). Johnson and Brice (1953) stated that "coagulated yolk disease" was the cause of high mortality of chinook salmon fry from eggs incubated at high temperatures. It is more likely that mortality is caused by the disruption of cellular membranes because during early stages of development embryos are unable to adjust the lipid composition of their cell membranes (Terner et al., 1968) in response to temperatures outside their normal range, leading to a break down in ionic regulation. In support of this theory, Hayes et al. (1973)

showed that throughout normal embryonic development in rainbow trout, lipid composition was stable.

At high incubation temperatures, precocious hatching was a common cause of mortality. It has been suggested that alevins hatch precociously as a result of premature partial digestion of the chorion by proteolytic enzymes (Gray, 1928a). Hayes (1942, 1949) suggested that a precocious extension of the softening process described by Hein (1907) leads to premature rupture of the chorion, which generally results in mortality of the embryo. It may be that premature rupturing of the chorion is also associated with intensified movements of the embryo at high temperatures (Hayes, 1942; Knight, 1963; Timoshina, 1972).

The hatching of alevins head-first or yolk-sac first was prevalent at temperatures of 12°C and above, whilst at lower temperatures of 8-9°C, the chorion tended to rupture simultaneously throughout its entire length allowing rapid and easy release from it. For those alevins hatching head-first, the hatching process was prolonged because the alevins were unable to free themselves from the chorion and mortality generally followed. Few head-first and no yolk-first alevins survived. Hayes et al. (1953) reported that normal hatching can also be prevented at low temperature extremes, with many alevins again tending to emerge from the egg either head-first or yolk-sac first.

Among alevins held at temperatures of 16°C and above, particularly in Series 4, mortalities were generally attributed to severe oedema of the yolk-sac. Markus (1962) reported a similar condition, referred to as "blue sac"

(together with white spots in the yolk-sac), in Atlantic salmon alevins held at a constant 12.3°C; Peterson et al. (1977) observed that this condition also occurred in Atlantic salmon alevins held at low temperatures. The exact cause of oedema in alevins is unknown: Peterson et al. (1977) suggested that it might be associated with osmotic failure; Laale (1981) stated that a fluid imbalance resulting from circulatory irregularities is common and may lead to oedema of the pericardial and peritoneal cavities.

For the stenothermic Arctic char, Wallace and Aasjord (1984b) suggested that extreme temperatures caused high mortality by reducing the yolk conversion efficiency to such an extent that the yolk reserve, or possibly some vital component, is exhausted before the alevin reaches the fry stage. There were no indications of alevin mortalities induced by yolk exhaustion in the present study. This may be due to the greater quantity of yolk reserves in Atlantic salmon alevins compared with Arctic char, which will delay the onset of starvation during the transition from endogenous to exogenous feeding.

It has been reported that temperature has a more pronounced effect on metabolic rates of embryos and alevins than those of juveniles or adults: values for Q_{10} approximating 2.0 have been reported in adult salmonids (Fry, 1971) compared with values of 2.2-5.8 (Gruber and Weiser, 1983; Rombough 1988; Oliva-Teles and Kaushik, 1990) for embryos and alevins. The more pronounced effect of temperature during early development reflects narrower ranges of temperature tolerance. Fry (1957) demonstrated that active metabolism can

decline near the upper limits of temperature tolerance. The greater sensitivity of alevins to high temperatures compared with older fish may be related to differences in the characteristics of their respective haemoglobins (Iuchi, 1973).

Many studies have indicated that temperature-related egg and alevin mortality may be influenced by other water quality parameters, such as dissolved oxygen concentration and acidity. A number of investigators have reported various sublethal effects of low oxygen levels in salmonid eggs and alevins; these include retarded development (Garside, 1959, 1966) and growth (Silver et al., 1963) and the premature emergence of alevins from redds (Bailey et al., 1980). Low water flows can also give rise to similar effects.

Salmonid eggs can tolerate very low oxygen levels prior to gastrulation (Rombough, 1988), but during the transition to gastrulation (approximately 50 degree-days after fertilisation) there is an increased oxygen demand, and consequently an increased sensitivity to the effects of high temperatures.

Studies on Atlantic salmon by Hayes (1949) and Hayes et al. (1951) have shown that at high temperatures, mortalities occurring immediately before hatching may be due to asphyxiation, because the rate of diffusion of oxygen through the chorion was insufficient to meet the respiratory requirements of the developing embryo. Hayes et al. (1951) found that at high temperatures, a limiting oxygen tension was established as development proceeded: above 10°C there was a decrease in the diffusion rate through the chorion; the

diffusion coefficient was constant within the range of 4-10°C, but decreased significantly at 12 and 14°C. With the loss of the chorion at hatching, the limiting oxygen tension was lowered again. Bishai (1960) found that Atlantic salmon could survive very low levels of oxygen following hatching, but this ability decreased as development proceeded.

Similarly, in rainbow trout Rombough (1986, 1988) found that critical oxygen levels increased progressively during egg development from low levels at fertilisation (less than 1 mg/L) to a maximum just prior to hatching. Critical levels rapidly decreased by 2-3 mg/L at hatching, and then declined gradually to reach stable levels midway through the alevin stage. At all stages of development critical levels increased with increasing temperature as a direct result of increased metabolic demand. Rombough (1986) estimated the maximum critical values for rainbow trout eggs at constant temperatures of 9.1, 12.0 and 15.1°C, as 8.7, 9.5 and 10.2 mg/L, respectively; and for alevins at the same temperatures, as 3.1, 3.7, and 4.5 mg/L, respectively. A later study by Rombough (1988) estimated that critical dissolved oxygen levels were in excess of 100% air saturation during the final stages of egg development (just before hatching) at 15°C.

Thus, two periods of potential oxygen limitation have been shown to exist for Atlantic salmon and rainbow trout: just before hatching when oxygen consumption is low but the critical level is high; and during final yolk absorption when the metabolic rate is maximal.

At high temperatures many eggs which died during the period from eyeing to hatching did so during, or immediately after,

hatching. A similar study by Swift (1965) also reported high losses of Arctic char eggs during the hatching period. Garside (1966) suggested that mortalities of rainbow trout and brook char eggs may have been caused by premature hatching, induced by limiting oxygen levels within the chorion of the egg. An early study by Gray (1928b) showed that the effect of temperature on the hatching mechanism in brown trout eggs was relatively greater than that of other developmental processes, resulting in precocious hatching at high temperatures.

In the present study, mortalities occurring at temperatures exceeding 12°C may have been caused by asphyxiation, or by the induction of precocious hatching, even though the dissolved oxygen concentration was continually maintained at a level close to 100% air saturation. Thus, it is important that the ambient dissolved oxygen levels should not fall below 100% air saturation during early development at high temperatures.

A further factor affecting hatching success is the movements of the embryo which are thought to assist respiration. The intensity of movement of the embryo would be expected to increase at high temperatures. However, Smith (1957) reported that at high incubation temperatures there is a cessation of normal movements of contraction and rotation of the embryo within the chorion.

Some workers have suggested that the physical movement of eggs during periods of high sensitivity may have been a significant cause of mortality in some experimental studies. In the present study, salmon were handled at three different

stages of development: as newly-fertilised eggs (up to 4 hours after fertilisation); as eyed eggs; and as newly-hatched alevins. Johnson and Brice (1953), studying the effects of transportation on the mortality of chinook salmon eggs, found that newly-fertilised eggs could be transported safely for up to 6.5 hours. There are two particularly sensitive periods during later development when high mortalities may occur: in the development of Atlantic salmon eggs, Hayes and Armstrong (1942) found that the first period corresponded with closure of the blastopore and the second with hatching, and Swift (1965) observed similar periods of sensitivity for Arctic char eggs. Steuert (1906) found that brown trout eggs, taking 50 days to hatch, were most susceptible to physical shock 10-17 days after fertilisation. Post et al. (1974) demonstrated that rainbow trout eggs were only sensitive to physical shock prior to eyeing. Johnson et al. (1989), studying the relationship between development time from initial fertilisation and sensitivity to handling stress in coho salmon and rainbow trout, reported that coho salmon eggs were most sensitive to handling between 85-145 degree-days (peak sensitivity at 98-115 degree-days) after fertilisation, whilst rainbow trout eggs were most sensitive during the period from 91-107 degree-days (peak at 98 degree-days) after fertilisation. The interval of increased sensitivity coincides with the period of blastodisc overgrowth (Velsen, 1980). Hein (1907) demonstrated that brook char were very sensitive to mechanical shocking from 111-194 degree-days after fertilisation. Similar findings were reported by Smirnov (1975) for chum, pink and coho

salmon, and Jensen and Alderdice (1983) working on coho salmon.

A later study by Jensen and Alderdice (1989) found that the physical shock sensitivity of eggs from 6 Oncorhynchus species (sockeye, chinook, chum, coho, pink salmon and rainbow trout) increased as embryonic development proceeded. Maximum sensitivity occurred during epiboly (70-100 degree-days at 10°C after fertilisation). Following yolk plug closure, the level of sensitivity declined rapidly. Rosenberg (1985) studied the effect of physical shock on the survival of coho salmon eggs from eyeing to near hatching and confirmed that eggs are indeed resistant to physical stress throughout this stage of development. Ballard (1973) stated that it was important to prevent the rolling over of eggs (rainbow trout and brook char), at least until epiboly has completed the formation of the ectodermal-mesodermal yolk-sac, otherwise the rolling movement ruptures the delicate yolk membrane.

From the foregoing review of the literature pertaining to the effects of physical movement on egg mortality in salmonids, it can be concluded that the experimental protocols used in this study did not subject eggs to physical movement during sensitive periods of development. Although discrete periods of sensitivity to high temperatures do appear to coincide with those periods of high physical sensitivity, no losses were attributed to the physical movement of eggs in this study.

In the present study, there were clear differences in the overall levels of mortality among the four series of

experiments. These differences may be explained by a number of factors, including any variations in the experimental conditions, and the use of different stocks (Rivers Test, Itchen and Thames). Kanis et al. (1976) showed that there were significant differences in egg, alevin and fry mortalities between different strains of Atlantic salmon. Similarly, Gunnes (1979) reported that mortalities of Atlantic salmon eggs incubated at 12°C were substantially higher than those reported in a similar study by Peterson et al. (1977) at the same incubation temperature (Table 2.4 1), and suggested that the Norwegian salmon strain tested was adapted to lower incubation temperatures. Tang et al. (1987) demonstrated differences in the upper temperature limits for eggs and alevins between two stocks of coho salmon, which may reflect genetic differences imposed by a difference of 6°C in their environmental temperatures. Beacham and Murray (1985) also demonstrated substantial variation in chum salmon egg and alevin survival rates among families: survival rates among families within a size class could vary from 45-100% at a given temperature, and variation was accentuated as temperatures became more extreme (4 and 12°C).

Further studies by Beacham and Murray (1986) and Murray (1988) demonstrated significant differences in egg and alevin survival in relation to temperature between families within stocks of pink salmon. Survival rates also varied significantly among stocks within broodlines, indicating that stocks are not all equally adapted to the same environment. Hence, although there is some evidence for adaptive radiation, salmonid species appear to be relatively

conservative in their adaptations to temperature.

Differences in egg and alevin survival may also be explained, in part, by different broodstock maturation and spawning temperatures. It has been shown that the quality of oocytes is improved when Atlantic salmon broodstock are held at optimal temperatures (Klinkhardt et al., 1987). For the same species, Taranger and Hansen (1993) showed that high temperatures (13-14°C) during the spawning season inhibited ovulation and had a detrimental effect on the survival of eggs. Smith et al. (1983) found that fertilisation rates of cutthroat trout eggs could be increased significantly by holding the female broodstock at temperatures within the range of 2-10°C; at warmer groundwater temperatures (10±2.0°C) eggs showed a distinct variation in size, many eggs were opaque and embedded in ovarian tissue, and atretic eggs were apparent. Consequently, broodstock were difficult to strip, and repeated stripping was necessary.

Kaya (1977) reported that geothermally heated waters (geysers and hot springs) discharging into the Firehole river (Yellowstone National Park), increased downstream temperatures by about 10.5°C, and detrimentally affected the reproductive biology of brown trout and rainbow trout populations: the annual temperature range varied from approximately 12°C in winter to 25°C in summer, and pre-spawning atresia of eggs was common among brown trout adults. Of the two salmonid species present, only rainbow trout eggs were tolerant of an incubation temperature of 13.3°C.

For the production of good quality rainbow trout eggs, Leitritz and Lewis (1976) stated that broodstock should not

be held at temperatures greater than 13.3°C, and preferably not above 12.2°C, for a period of 2-6 months before spawning. Hokanson et al. (1973) determined the temperature requirements for reproduction in brook char: although males produced motile sperm at 19°C, and ovulation and spawning occurred at 16°C, it was recommended that 12.2°C should not be exceeded during the breeding season, and that a mean temperature below 8.9°C was required for optimal spawning behaviour, gamete viability, and embryo survival.

Some studies have also suggested that the variation in the survival rates of eggs and alevins at a given temperature, may depend on other aspects of egg quality. Various triploidy studies (Benfey and Sutterlin, 1984; Johnstone, 1985) have demonstrated that factors such as egg size and the time of stripping, in relation to the timing of ovulation, appear to affect subsequent survival. Springate et al. (1985) found no correlation between egg size and subsequent survival in rainbow trout; similarly, Galkina (1969) found no relationship between the size of rainbow trout eggs from broodstock of the same age and survival. However, when very small eggs (35 mg and less) from first spawning broodstock were examined, survival was lower. Beacham and Murray (1985) showed that differences in survival rates of chum salmon at a given temperature, increased as female broodstock size increased: egg survival rates from small broodstock were 88% at 12°C and 97% at 8°C, a differential of 9%; while those from larger broodstock were 77 and 96%, respectively, a differential of 19%. However, eggs from large broodstock were more sensitive to temperature extremes than eggs from small

broodstock. Higher mortality rates among large eggs may be associated with the increased duration of epiboly: eggs having relatively greater quantities of yolk take longer to complete epiboly (Smirnov, 1975).

Studies by Bagenal (1969), Gall (1974), Pitman (1979), Escaffre and Bergot (1984) and Wallace and Aasjord (1984a), have reported that salmonid alevins from large eggs have numerous advantages over those hatching from small eggs, including improved survival and longer survival when starved following yolk exhaustion.

In the present study, the least sensitivity to high temperatures was shown by the salmon stock having the largest egg size (Series 3). It may be that the high level of temperature tolerance shown by pink salmon eggs (Murray and Beacham, 1986) is associated with their relatively large egg size. However, the relatively few data provided by the present study are insufficient to evaluate any effects that egg size may have had on temperature tolerance.

Garling and Masterson (1985) reported considerable variation in the temperature tolerance of chinook salmon eggs from individual female broodstock, and speculated that this may be due to the presence of unspecified trace constituents in the yolk.

Lebedeva and Meshkov (1969) stated that the thermal resistance of rainbow trout eggs was associated with the intensity of pigmentation; the greater the degree of carotenoid pigmentation, the higher was their resistance. Galkina (1969) also stated that the thermal resistance of pale-coloured rainbow trout eggs was lower than that of

brightly coloured eggs. However, in both studies egg colouration was assessed subjectively, and no attempt was made to determine egg carotenoid levels (Craik, 1985).

A number of investigators have stated that the more intensive development of fungus (Saprolegnia parasitica) at high water temperatures was responsible for mortalities. Peterson et al. (1977) stated that fungal infections were probably responsible for mortalities of Atlantic salmon eggs incubated at 10-12°C. Foda and Henderson (1977) also stated that a secondary fungal infection could have contributed to the mortalities recorded during their experiments with Atlantic salmon eggs and alevins at temperatures of 4-15°C. Beacham and Murray (1985) reported that chum salmon alevin mortality occurred a few days after hatching at 12°C, and was associated with a fungal infection of the gills. Timoshina (1972) stated that when temperature rises rapidly at the beginning of development, the mortality of rainbow trout eggs may be caused by the rapid proliferation of Saprolegnia.

Fungal infections were not encountered in the present investigation, and mortalities were always attributed to other factors. The only disease condition observed during this investigation was bacterial gill disease, which infected small numbers of salmon fry in experimental Series 2-3. Bacterial gill disease is an external myxobacterial infection of the gills (Wakabayashi, 1980) which is generally associated with the presence of an environmental irritant, such as high levels of suspended solids or ammonia. It is a very common problem in salmonid hatcheries during first feeding when fine food particles can irritate the gills,

causing congestion with mucus and tissue hyperplasia of the respiratory epithelium. Because disease-related fry mortalities were always low, no medication was administered to any of the stocks.

In summary of the effects of temperature on egg and alevin mortality, it is apparent that salmon alevins exceed eggs in their temperature tolerance by 4-6°C, depending on their thermal history. No eggs survived to eyeing or hatching at temperatures of 16 and 14°C, respectively, whilst the upper temperature limit for newly-hatched alevins was between 20-22°C.

The subsequent survival of later stages of development was largely determined by survival at earlier stages; thus, the most favourable temperatures from eyeing to hatching and hatching to MAWW, varied according to the temperatures experienced during earlier incubation.

Optimal egg and alevin survival occurred within the temperature ranges of 8-11°C and 8-16°C, respectively.

Under all temperature regimes, mortalities were generally greatest during a short phase of development prior to eyeing, and during hatching.

Several forms of abnormality were observed, including "pin-eyed" eggs at temperatures above 10°C, whilst at temperatures greater than 11°C incomplete hatching was a common abnormality. Spinal deformities were observed under most temperature regimes. High temperatures (particularly 20°C) during the alevin stage frequently caused severe oedema of the yolk-sac resulting in high mortalities.

No correlation was demonstrated between the thermal history

of eggs and alevins and subsequent fry mortality. This study did not investigate the temperature tolerance of fry.

2.4.2 DEVELOPMENT AND GROWTH

2.4.2.1 Development times

In all series of experiments the development rate, measured by the number of days required to reach a given stage of development, was accelerated by increasing temperature. There was an inverse relationship between temperature and development time: the period from fertilisation to median hatch ranged from a mean of 59.2 days at 8°C to 33.6 days at 12°C; following hatching, alevins reached MAWW after a mean of 40.8 days at 10°C and 25.2 days at 18°C.

A comparison of published results with those from the present study showing the development times of Atlantic salmon eggs and alevins at different temperatures is presented in Table 2.4.4. It is apparent that the development times reported here are in general agreement with the results from other studies, and confirm that at high temperatures a markedly shorter time is required to reach a given stage of development. Comparing constant temperature regimes used by Peterson et al., 1977 (7.9, 10.0, and 11.7°C) and Gunnes, 1979 (8.0, 10.0, 12.0°C) with similar regimes tested in the present investigation, it is apparent that the observed development times from fertilisation to hatching are similar. A comparison between corresponding values for development times from hatching to MAWW shows more variation, and this may reflect the more subjective criteria used to define MAWW. The practical determination of the attainment of MAWW is less precise than that of hatching. The method used here to

Table 2.4.4 A comparison of published data with the combined results from this study (mean of all series), showing development times in days for Atlantic salmon from fertilisation to hatching (upper figures) and hatching to final yolk absorption (lower figures) at constant temperatures within the range of 8-18°C. Temperatures are rounded to the nearest 1°C and development times to the nearest day.

Source		Temperature (°C)								
		8	9	10	11	12	13	14	16	18
Hamor and Garside, 1976	[a]	-	-	43	-	-	-			
		-	-	-	-	-	-	-	-	-
Foda and Henderson, 1977	[c]	-	-	58	-	-	-			
		-	-	35	-	-	-	-	-	-
Peterson et al., 1977	[a]	62	-	47	-	34	-			
		51	-	41	-	36	-	-	-	-
Gunnes, 1979	[c]	57	-	49	-	42	-			
		36	-	30	-	24	-	-	-	-
Klinkhardt et al., 1987	[a]	86	-	-	-	-	-			
		-	-	-	-	-	-	-	-	-
Brännäs, 1988	[c]	-	-	-	-	-	-			
		-	-	32	-	23	-	-	-	-
Kane, 1988	[b]	63	-	48	-	37	-			
		48	-	-	-	-	-	-	-	-
Jenson et al., 1989	[c]	-	-	-	-	-	-			
		-	30	24	22	-	-	-	-	-
This study	[a]	59	50	42	37	34	34			
		48	44	40	37	34	34	29	27	25

[a] Development times relate to 50% hatch.
[b] Development times relate to 90% hatch.
[c] Development times relate to 100% hatch.

determine MAWW was described in section 2.2.4.5.

Because temperature has a fundamental role on metabolic control, it has a very marked effect on the overall development rate: high temperatures produced a shortened development time, whilst at lower temperatures the embryos develop more slowly. Even a relatively small increase in temperature can greatly decrease the development time: an increase of 2°C will decrease the development time from fertilisation to MAWW by more than 2 weeks. Changes of this magnitude will enhance first year growth, thereby increasing the proportion of one-year-old salmon smolts produced.

The effect of temperature on the rate of development of Atlantic salmon is similar to that of other salmonid species (Crisp, 1981, 1988): development times decrease exponentially with increasing temperature. Kuramoto et al. (1988) studied histologically the early development of chum salmon embryos at 3 incubation temperatures (5, 8, 15°C), and reported that development rates were influenced to a greater extent by low, rather than high temperatures. There are slightly different responses to temperature among different salmonid species as well as between specific strains: brown trout eggs hatch 108 days after fertilisation at 3°C and 37 days at 10°C (Wood, 1931); at the same temperatures, rainbow trout eggs hatch in 85 and 34 days, respectively (Lebedeva and Meshkov, 1969).

Development times in degree-days to hatching under varying temperature regimes with similar mean temperatures were longer under decreasing temperature regimes, and shorter under increasing temperature regimes. Development times in degree-days under constant temperature regimes were of

intermediate duration. Peterson et al. (1977) demonstrated the same relationship between varying and constant temperature regimes and development times for Atlantic salmon eggs.

Among other salmonid species, Alderdice and Velsen (1978) concluded from their comparison of ambient and constant incubation temperatures for chinook salmon eggs that development times were more prolonged at constant temperatures than under varying temperatures, having the same mean values. However, Murray and Beacham (1986) found that for pink salmon eggs incubated under constant and increasing temperature regimes, development time to hatching was extended when compared with a decreasing temperature regime. These differences may be due to species differences, or possibly to the greater temperature range (2-15°C) investigated by Murray and Beacham (1986).

The present study showed that the development time in degree-days from hatching to MAWW depended solely on the temperature during that period of development, and was not affected by any temperature regime that the egg had experienced. Hence, alevin development proceeded at a constant rate for a given temperature.

Some variation in development times at the same temperatures was shown among the four series of experiments conducted in this study; in particular, development rates were slower in Series 3 compared with the other three series. These differences may be due to a number of factors. Any differences in the experimental conditions between the four series may have accounted for some variation in development

times. The use of different spawning stocks (Rivers Test, Itchen and Thames) may also explain some of this variation. Peterson et al. (1977) reported consistent differences in development times between two Atlantic salmon stocks at any given temperature. However, they noted that the experiments were conducted in different years, and therefore may have been exposed to slightly different experimental methodology. Excepting any significant experimental differences, Peterson et al. (1977) suggested that the differences in development times may have been due to differences in the temperature-dependence of development, or possibly different temperature coefficients of the hatching enzyme.

Differences in egg development rates may also be explained, in part, by different broodstock maturation and spawning temperatures. This may lead to differences in the developmental stages of newly-stripped batches of eggs. Embody (1934) reported substantial differences in the hatching period among stocks from different female broodstock within a given strain (a difference of 4.3% from the mean values). Embody (1934) also stated that there was considerable variation in the hatching period even within individuals from the same stock, and suggested that this was the result of differential development of eggs in the ovaries. Although all eggs within a single batch are exactly the same age, the embryos develop at slightly different rates, these differences appearing as early as the first cleavage stages. Knight (1963) showed that there was considerable variation in the rate of development of rainbow trout eggs during the first few days of incubation: at 12.3°C

some eggs were still at the 32-cell stage 24 hours after fertilisation, while the majority of the embryos were blastoderms. However, Garside (1959) has shown that lake trout embryos held at a given temperature appear to hatch at the same anatomical stage of development.

Egg size is another factor which can influence development times. Studying the effect of egg size on development rates in rainbow trout, Escaffre and Bergot (1984) showed that although development time to hatching was not influenced by egg size, MAWW was attained earlier for small eggs. Similarly, Rombough (1985) reported that the development time required to reach MAWW in chinook salmon was considerably shorter for small eggs (200 mg) compared to large eggs (500 mg). Similar observations have been reported for rainbow trout (Smith, 1958), brown trout (Bagenal, 1969) and Arctic char (Wallace and Aasjord, 1984a). In the present study, development times to hatching were similar at the same temperatures in all experimental series, but MAWW was attained later for the largest eggs (Series 3). However, Kazakov (1981) found no differences in the rates of development between large and small Atlantic salmon eggs: the embryos reached identical stages of development simultaneously. Similarly, Beacham et al. (1985), studying the effect of egg size on the early development of chum and coho salmon, found no significant differences between egg size and hatching time or the duration of yolk absorption.

Genetic factors may also account for some of the variation in development times shown in Table 2.4.4. Given the wide geographic range of the species, substantial adaptive

radiation in respect of temperatures for spawning and egg incubation would be expected. Brännäs (1988) stated that the development rates of Atlantic salmon show evidence of an evolutionary adaptation to different environmental conditions; comparing differences between development rates of Baltic and Canadian Atlantic salmon stocks, it was suggested that Baltic stocks have adapted physiological responses to sharper seasonal changes in their environment. A comparison between development rates in northern and southern Atlantic salmon populations indicates that there may be a variety of locally adapted populations within the species (Gunnes, 1979; Peterson et al., 1977; Brännäs, 1988). Thus, the mean times from fertilisation to final yolk absorption (from Table 2.4.4) were 113, 88 and 70 days at 8, 10 and 12°C, respectively, for Atlantic salmon from southern Canada (Peterson et al., 1977), whereas the Norwegian strain studied by Gunnes (1979) only required 93, 79 and 66 days at the same respective temperatures. Comparing development times in Atlantic salmon from hatching to "swim-up", Brännäs (1988) also found that development was more rapid in the Norwegian stock used by Gunnes (1979), and Heggberget and Wallace (1984) postulated that some Norwegian strains of Atlantic salmon may be "cold-adapted" in respect of their egg incubation times. Similarly, Nævdal et al. (1978) suggested that different strains of Atlantic salmon may have different optimal temperatures for growth. In this respect, Torrissen and Torrissen (1984) demonstrated variations in the digestive proteases of Atlantic salmon from different strains.

However, in a later study by Wallace and Heggberget (1988)

which compared development rates from fertilisation to hatching in different Norwegian stocks of Atlantic salmon, no evidence of adaptation to temperature was apparent at any of the different localities. A similar result was obtained from a study by Beacham and Murray (1985) on egg development in chum salmon.

Variations in development times reported here and in the literature may also be due to other factors: one complicating factor is that hatching can occur at different developmental stages. It may be accelerated or retarded by a number of factors. Hatching is not a distinct stage of development because its precise timing is temperature-dependent (Hayes et al., 1953). Gray (1928b) noted that the processes of embryonic development and hatching may have different temperature relationships; hatching is a physiological event and not an anatomical stage of development.

Because the reported development times in degree-days to hatching have been shown to vary, hatching may not always be a good indicator of the embryo's developmental stage (Gray, 1928b; Hayes and Armstrong, 1942; Hayes et al., 1953; Smith, 1957). Various environmental factors such as oxygen levels and physical shock may also advance or delay the precise time of hatching. Such effects would cause variation in the hatching time further to that caused by incubation temperature alone and would also, as a consequence, influence the development time from hatching to MAWW. Clearly, the use of temperature models to predict the development times of salmonids can only be a general guide for anticipating development times. Published data on development times to

hatching should therefore be compared with caution.

2.4.2.2 Development times in relation to temperature units

Throughout this investigation, development times in degree-days under different temperature regimes during the periods from fertilisation to eyeing and hatching to MAWW were relatively uniform. However, development times declined during the period from eyeing to hatching as temperature increased. Although Wallich (1901) originally concluded that the sum of the temperature units from fertilisation to hatching was constant at all temperatures within the normal range for eggs, he also reported on the inconsistency with which temperature units predict hatching times at temperatures outwith this range.

Further studies, which have been directed at assessing the limitations of the use of the temperature unit for salmonid development, have also contradicted the assumptions of linearity in the degree-days concept (Battle, 1944; Garside, 1966; Alderdice and Velsen, 1978; Crisp, 1981; Jungwirth and Winkler, 1984; Humpesch, 1985b; Tang et al., 1987). Plots of published data showing development times in degree-days from fertilisation to hatching against temperature for Atlantic salmon are presented in Fig. 2.4.1, and for four other salmonid species in Fig. 2.4.2. Development times from the present study (combined data from all four series of experiments) are also shown in Fig. 2.4.1. Battle (1944) observed the same morphological stage of development in 2 groups of Atlantic salmon embryos that had developed for 136

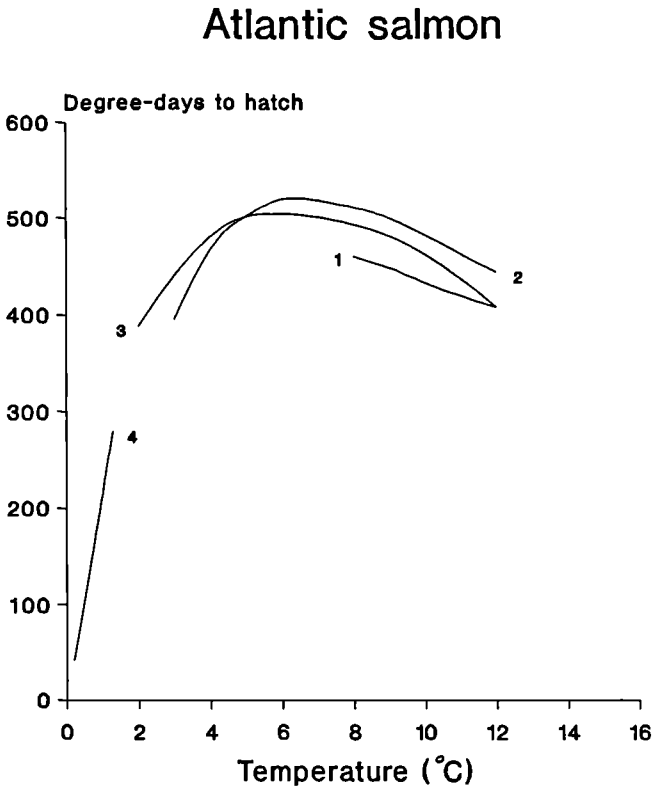

Fig. 2.4.1 The relationship between temperature and degree-days from fertilisation to hatching for Atlantic salmon. 1, Present study; 2, Kane (1988); 3, Peterson et al. (1977); 4, combined data from Heggberget & Wallace (1984) and Wallace & Heggberget (1988).

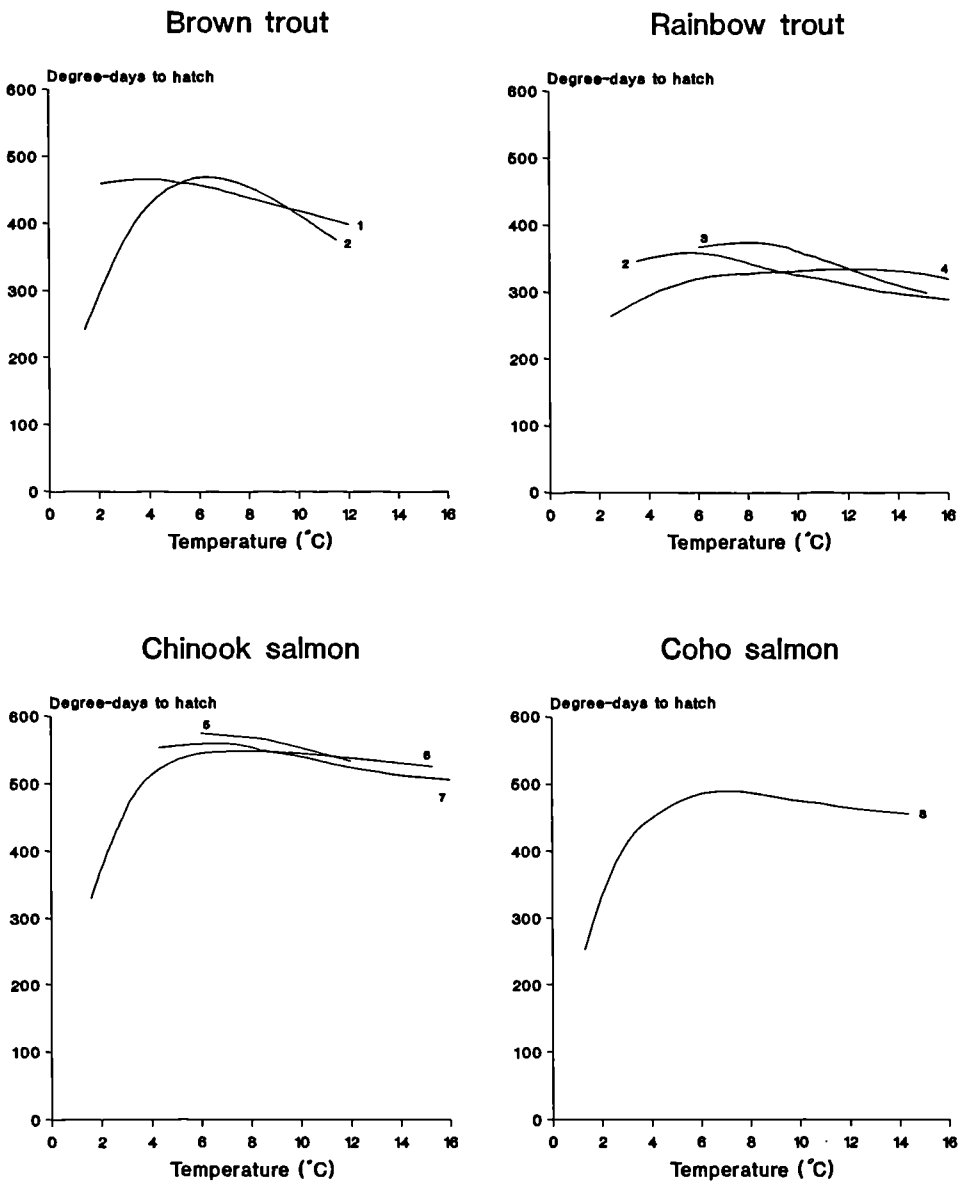

Fig. 2.4.2 The relationship between temperature and degree-days from fertilisation to hatching for four salmonid species. 1, Jungwirth & Winkler (1984); 2, Embody (1934); 3, Rombough (1986); 4, Garside (1966); 5, Heming (1982); 6, Burrows (from Alderdice & Velsen, 1978); 7, Seymour (1956); 8, Tang et al. (1987).

and 170 degree-days from fertilisation, and stated that the use of degree-days to determine comparable stages in embryos was unsatisfactory. Garside (1966) stated that development rate was not a linear function of temperature throughout the entire temperature range, and concluded that the use of degree-days was therefore inappropriate as a criterion for comparing equal proportions of development. As an alternative, Garside (1966) suggested that morphological criteria could be used. However, the problem of uncoupling the developmental processes at different temperatures (as discussed in section 2.4.1.7) may invalidate the use of structural development as a means of direct comparison.

Alderdice and Velsen (1978) developed three non-linear equations based on data extracted from a review of the literature on the development of chinook salmon eggs. Crisp (1981), using data for five salmonid species (brook char, brown trout, rainbow trout, Atlantic salmon and chinook salmon), also concluded that the relationship between temperature and development was non-linear. Tang et al. (1987) stated that the relationship between temperature and development time in coho salmon was also non-linear, particularly for extreme temperatures. Peterson et al. (1977) found that the cumulative number of degree-days from fertilisation to hatching for Atlantic salmon eggs could vary from less than 400 to over 500 for different temperature regimes within the range of 2-12°C. Similarly, Hayes (1949) observed that the developmental velocity curve (i.e. degree-days concept) deviated at high and low temperature extremes, and stated that this was caused by developmental interference

which gave rise to abnormal development. Thus, it is clear that the development rates of salmonid eggs are not a linear function of temperature throughout the entire temperature range for each species.

When a wide temperature range is considered, a curvilinear relationship between temperature and development time to hatching has been shown for a number of salmonid species (Crisp, 1981; Jungwirth and Winkler, 1984; Humpesch, 1985b). Simple linearity between temperature and development time only occurs over a narrow temperature range. Furthermore, there are often inflections even of the curvilinear relationship at extreme temperatures.

Numerous empirical models have been used to describe the relationship between temperature and development time for most salmonid species (Garside, 1966; Peterson et al., 1977; Alderdice and Velsen, 1978; Jungwirth and Winkler, 1984; Tang et al., 1987; Crisp, 1988; Kaeriyama, 1989). These regression equations have been formulated for predicting development times to different stages of development including eyeing, hatching and MAWW at constant temperatures, as well as different combinations of temperatures. Though the form of curvilinear model fitted for each species varies between studies, predictions of development times are similar over most of the temperature range. Garside (1966) demonstrated graphically that the curve showing the relationship between temperature and development rate was an elongate sigmoid. Kaeriyama (1989) used the following formula to describe a similar relationship for chum salmon eggs:

$(x+1.0)y = 576$

where, x = water temperature (°C) and y = days from fertilisation to hatching.

Kamler and Kato (1983) stated that the influence of temperature on development time in rainbow trout could be simply expressed using the development rate, which is the reciprocal of the number of days.

In many studies a power function with temperature correction, often termed the Belehrádek function (Jungwirth and Winkler, 1984), produces a good fit for much of the temperature range. However, Jungwirth and Winkler (1984) studied the temperature dependence of egg development in 4 salmonid species, and concluded that the use of degree-days was applicable only in mathematical equations (as in the type of relationship described by Belehrádek, 1930), where the exponent of the power function is equal to 1 (characteristic of late autumn-spawning salmonids). The main difference occurred at the low end of the temperature range, where the relationship between temperature and development time was non-linear. Crisp (1988) suggested that discrepancies in the data presented by many workers were caused by experimental error associated with the difficulties of precise temperature control at temperatures below 3°C. Crisp (1988) calculated that the relationships between development times from fertilisation to eyeing (FE), fertilisation to hatching (FH), and fertilisation to MAWW (FM) at constant temperatures were approximately linear, and that over most of the normal temperature range broad relationships for Atlantic salmon

were as follows:

FE = 0.5 x FH and FM = 1.7 x FH

Although the data from the present study correspond closely with those data generated by Crisp (1988) for the prediction of egg development times in Atlantic salmon, the predicted development times for alevins were generally underestimated by 5-15%.

Since change in development rate is not a linear function of temperature throughout the entire range, the use of the term degree-day should be confined to the intermediate temperatures where the time-temperature relationship is relatively linear (Figs. 2.4.1 and 2.4.2). Nevertheless, the degree-day concept is still of practical use for predicting development times. Although this simple model is known to be inaccurate, particularly at very low temperatures, it is still widely used in hatcheries because of its ease of use.

Early workers (Johansen and Krogh, 1914; Hayes, 1949) have tended to consider only the central, linear segment of the relationship between temperature and development time, which they have extrapolated linearly to the intercept on the x-axis to determine empirically the temperature of developmental or biological zero. However, later studies conducted at very low incubation temperatures indicate that the true biological zero would be lower than those obtained by linear extrapolations: Garside (1959) stated that the developmental zero for lake trout was -1.5°C, and Kamler and Kato (1983) computed developmental zero for rainbow trout as

-1.6 to -1.7°C. Hence, thermal sums should be based on the biological zero of the species. Heggberget and Wallace (1984) reported considerable discrepancies between observed and predicted hatching times for Atlantic salmon at very low incubation temperatures: eggs incubated at a mean temperature of 0.65°C hatched 232 days after fertilisation (150.8 degree-days). Because the water temperature was rising at the time of hatching, it was suggested that this acted as a stimulus which triggered the hatching mechanism. In a later study, Wallace and Heggberget (1988) observed that hatching times from fertilisation were 260 days (44 degree-days) and 211 days (211 degree-days) at mean temperatures of 0.17 and 1.0°C, respectively. Embody (1934) observed that the incubation period required for brown trout eggs was 148 days at 1.7°C (251.6 degree-days); the normal incubation period is 41 days at 10°C (410 degree-days). Hence, the prediction of development times from assessments made at very low temperatures greatly underestimates the number of days required from fertilisation to hatching.

Hamor and Garside (1976) stated that when temperature-controlled development times were transformed to rates of development, there were typically 3 phases: a sigmoidal relation in which the long central segment is virtually linear, while the tails reflect slow progress at low and high temperatures (Garside, 1966).

In the present study, development was only observed over the upper segment of the temperature range; hence, development times were shown to decline with increasing temperature (Fig. 2.4.1).

The time required to reach any given stage of development can also differ under different environmental conditions. Several studies have examined fry emergence times from deep-substrate incubators and simulated gravel redds in relation to temperature (Godin, 1980; Heming, 1982; Brännäs, 1987, 1988). Brännäs (1987) found that water temperature not only influenced the period of time Atlantic salmon remained in the gravel but also the duration of emergence: development times from hatching to emergence decreased with increasing temperature, with 50% of fry emerging after 35 days (248-280 degree-days), 24 days (276 degree-days), and 16 days (232 degree-days) at temperatures of 7.1-8.0, 11.5 and 14.5°C, respectively. The period of emergence also decreased with increasing temperature: following the onset of emergence, 50% of fry had emerged from the gravel within 5, 7, and 10 days at 14.5, 11.5, and 7.0°C, respectively. Yolk reserves retained by fry during the main period of emergence were less than 10%. The development times reported by Brännäs (1987) for Atlantic salmon from hatching to emergence were similar to those observed from hatching to MAWW in this study. Heming (1982) demonstrated that chinook salmon emerged from simulated redds at a stage close to MAWW. A second study by Brännäs (1988) again demonstrated that high temperatures during the alevin stage led to earlier emergence of Atlantic salmon fry from gravel; early emerging fry retained greater yolk reserves than emerging fry held at low temperatures. Similarly, Heming (1982) studied emergence times for chinook salmon fry within the temperature range of 6-12°C, and found that it occurred earlier in their development at high

temperatures. In contrast, Godin (1980) found that the period of emergence for pink salmon fry was more prolonged at high temperatures. This may reflect species differences.

A further environmental factor which has been shown to influence development times in salmonids is oxygen. Because the solubility of oxygen in water is directly related to temperature, it is important to consider the effects of low oxygen conditions (arising when water temperatures are high) on egg and alevin development. Various studies have demonstrated that the control of development rates by temperature can be modified by the level of dissolved oxygen; low oxygen levels cause a reduction in the rate of development (Alderdice et al., 1958; Garside, 1959, 1966; Brooke and Colby, 1980), while high levels can accelerate the development rate. However, if development is already at an advanced stage, a decrease in the level of dissolved oxygen can induce earlier hatching (Alderdice et al., 1958). Hamor and Garside (1976) have shown that the retarding effect of low dissolved oxygen was more pronounced in Atlantic salmon at 10°C than 5°C. The development rate was also shown to be more influenced by low oxygen levels at later stages of development. This effect has also been demonstrated for three other salmonid species (Garside, 1959, 1966).

Garside (1966) also found that development in hypoxic conditions delayed development once the earlier stages of development had occurred. Silver et al. (1963) stated that both chinook salmon and rainbow trout embryos can compensate for a reduction in ambient oxygen levels by a concomitant reduction in the rate of developmental metabolism, which

gives rise to retarded but otherwise normal development. Similarly, Alderdice et al. (1958) subjected chum salmon eggs to low oxygen levels continuously for 7 days (approximately 12% of the incubation time), and found that the metabolic rate of embryos decreased with decreasing oxygen levels; this caused a decrease in the rate of development.

Further sublethal effects of hypoxia have also been shown to occur, including reduced vitelline circulation (Garside, 1959), reduced yolk conversion efficiency (Hamor and Garside, 1977), reduced size at hatching (Silver et al., 1963) and teratogenesis (Alderdice et al., 1958).

Smith (1957) stated that salmonid eggs can resist relatively prolonged periods of low oxygen because dissolved oxygen is stored in the perivitelline fluid. However, when a rapid temperature rise occurs, the oxygen supply in the perivitelline space can become depleted because the chorion then acts like a diffusion barrier; a shortage of oxygen can then lead to mortality or to various sublethal effects. Devilliers and Rosenberg (1953) found that pre-gastrular rainbow trout eggs were not affected by anaerobic conditions for 48 hours, but after 96 hours development was retarded, and after 7 days mortality occurred. Following gastrulation, there was a marked increase in the oxygen demand of the developing embryo. Similarly, Hamor and Garside (1977) found that the development of Atlantic salmon was retarded progressively and quantitatively at low dissolved oxygen levels; the oxygen supply did not satisfy the increasing demand as differentiation proceeded so that oxygen became limiting; this resulted in a decrease in the specific growth

rate of the developing embryo. Thus, the efficiency of yolk utilisation is reduced at low levels of dissolved oxygen. The effect of oxygen levels on the efficiency of yolk conversion is discussed more fully in section 2.4.2.4 (b).

Thus, at high egg incubation temperatures development rates may be slowed by the effect of temperature on oxygen solubility, particularly when the oxygen concentration is not fully saturated.

2.4.2.3 Hatching period

Hatching was not a simultaneous event; within the temperature range of 9-16°C, the mean hatching period from first to last hatch was 4-8 days (40-100 degree-days). However, at 8°C the hatching period was more prolonged (20 days, 156 degree-days). Excepting the 8°C incubates, the hatching period was not dependent upon temperature. However, a number of studies have shown that the duration of the hatching period in salmonids is temperature-dependent, the degree of protraction increasing progressively with decreasing temperature. At hatching temperatures of 2, 5, 7, 10 and 13°C, Timoshina (1972) reported that the hatching periods for rainbow trout eggs were 25 days (50 degree-days), 14 days (70 degree-days), 12 days (84 degree-days), 7 days (70 degree-days) and 5 days (65 degree-days), respectively. For the same species, Embody (1934) reported that the hatching periods were 3-4 days (60 degree-days) at 17.6°C, and 14 days (80 degree-days) at 5.7°C. Following the incubation of brown trout eggs at 10°C until just before

hatching, Gray (1928b) reported hatching periods of 28 days (140 degree-days), 10 days (90 degree-days), and 1 day (15 degree-days) at hatching temperatures of 5, 9 and 15°C, respectively. Similarly, Gruber and Wieser (1983) reported that the hatching periods for Arctic char were 23 days (92 degree-days) and 13 days (104 degree-days) at 4 and 8°C, respectively.

Although the hatching periods found in the present study were very similar to those reported for other salmonid species at the same temperatures, this study did not show a progressive decrease in the hatching period with increasing temperature: the hatching period was extended only at the lowest temperature tested (8°C). It may be that the influence of temperature on the duration of the hatching period is significant only at temperatures below about 9°C. The more synchronous hatching of salmon eggs, resulting from the use of high hatching temperatures, would be of benefit to the management of salmon hatcheries.

In summary of the effects of temperature on development, it is apparent that temperature has a profound influence on egg and alevin development times. There was an inverse relationship between temperature and the development time in days. The total number of degree-days required from fertilisation to eyeing and to MAWW were similar at all temperatures, but declined during the period from eyeing to hatching as temperature increased.

Although various studies have shown that the relationship between temperature and the development times for salmonid eggs is curvilinear due to the differential effects of

temperature on the development rate at either end of the temperature range for each species, the degree-days concept is still widely used by hatchery operators because of its practical value.

The variation in development times to hatching and to MAWW at specific temperatures shown here and in other studies, may be due to experimental differences associated with the use of different broodstocks; differences in genetic strain, age and size of broodstock, maturation and spawning temperatures, and broodstock condition, can all influence development times.

Although development times are primarily affected by temperature, other environmental parameters, including oxygen and the presence of a rearing substrate, can also influence the development times of eggs and alevins.

The hatching period was of similar duration (4-8 days) at all temperatures tested in this study with the exception of 8°C, where the hatching period was significantly longer (20 days).

2.4.2.4 Alevin size at hatching and MAWW

Egg incubation temperatures within the range tested (mean of 8-12°C) in this study had no effect on the mean weight of newly-hatched alevins. The mean wet weights of alevins hatched from eggs incubated at different temperatures were generally within 5% of the mean hatching weight for all groups. These results do not support the findings of many similar studies (Gray, 1928b; Garside, 1966; Bailey and Evans, 1971; Timoshina, 1972; Hamor and Garside, 1977;

Peterson et al., 1977; Heming, 1982; Murray and Beacham, 1986), which have shown that the size of newly-hatched alevins is temperature-dependent. This may be because the range of different temperature combinations tested here (8-16°C) was too narrow to detect any significant size differences.

Gruber and Wieser (1983) reported that the size of newly-hatched Arctic char alevins did not vary with temperature: as with the present study, the temperature range tested was probably too limited (4 and 8°C) to detect a difference. Most studies have demonstrated that maximum lengths and weights of newly-hatched alevins have occurred at the lower end of the temperature range for each species. Generally, these studies have shown that significant size differences occurred only at egg incubation temperatures below 8°C. In the present study, 8°C was the lowest temperature tested. The size of newly-hatched alevins has been shown by other studies to be inversely related to temperature; the lower the egg incubation temperature, the greater the length of newly-hatched alevins. Peterson et al. (1977) found that the temperature which yielded the largest newly-hatched Atlantic salmon alevins was 6°C. A high temperature of 12°C has been shown to result in newly-hatched salmonid alevins of comparatively low body weight (Timoshina, 1972; Peterson et al., 1977; Gunnes, 1979; Murray, 1980; Heming, 1982).

Peterson et al. (1977) showed that alevin size at hatching was positively correlated with development time measured in degree-days: the greater the number of accumulated degree-days to hatching, the greater the alevin length at hatching.

Peterson et al. (1977) also reported that different temperature combinations during the periods from fertilisation to eyeing, and eyeing to hatching, will yield different sizes of newly-hatched Atlantic salmon alevins; the temperature from fertilisation to eyeing had a significant, but lesser effect on alevin size at hatching than the temperature from eyeing to hatching.

Similar results with other salmonid species including vendace (Luczynski and Kirklewska, 1984), pink salmon (Murray and Beacham, 1986), coho salmon (Tang et al., 1987), and brook char (Marten, 1992) have also demonstrated that high temperatures from eyeing to hatching produced significantly smaller newly-hatched alevins. Murray and Beacham (1986) found that decreasing temperature regimes produced larger pink salmon alevins and fry than increasing temperature regimes. Murray (1980) reported similar results for coho salmon and rainbow trout alevins and fry.

Larger size is not always reflected in total body length: Beacham and Murray (1985) found that, although chum salmon alevins were shorter at hatching following egg incubation at 12°C, they had the highest body weight; as egg incubation temperatures increased (from 4 to 12°C), newly-hatched alevins had converted more of their yolk reserves into body tissue. Beacham and Murray (1985) also found that although temperature had a significant effect on alevin body weight at hatching, there were no significant differences in weight at the time of first feeding. Alevins produced at 4°C had lower body weights at hatching than those produced at 12°C, but grew more rapidly after hatching than the 12°C alevins;

consequently, at the time of first feeding, the mean weights were similar for the three groups of fry derived from eggs incubated at 4, 8 and 12°C.

Peterson et al. (1977) stated that the larger size of newly-hatched alevins, resulting from a low temperature from eyeing to hatching, also led to larger fry at first feeding. Murray and Beacham (1986) also demonstrated that temperature had a significant effect on the size of pink salmon alevins at MAWW: alevins from decreasing temperature regimes (e.g. 14-5-5) were larger than those from increasing temperature regimes (e.g. 5-14-14). They also found that the size gains resulting from egg incubation at low temperatures were maintained even when temperatures were increased during the alevin stage. Similarly, Gray (1928b) demonstrated that brown trout alevins were smaller following egg incubation at high temperatures, and that the size advantage gained from incubating eggs at low temperatures was maintained throughout the alevin stage. Although fry produced at high temperatures are smaller, feeding begins earlier than fry produced at lower temperatures. For the Atlantic salmon, Pavlov (1985) showed that any size gain at first feeding resulting from incubating eggs at low temperatures is eliminated by the extended period of feeding.

Thus, although no correlation was shown here between temperature and alevin size at hatching, studies which have been conducted at lower temperatures have shown that alevin size at hatching is inversely related to temperature. The main effect of temperature during egg incubation has been shown to occur from eyeing to hatching.

(a) Precocious hatching

Various studies have interpreted the shorter lengths of newly-hatched alevins produced at high temperatures as being indicative of precocious hatching. Studies by Peterson et al. (1977) and Pavlov (1985) suggested that Atlantic salmon eggs incubated at high temperatures hatched at a less advanced stage of development. Similarly, Heming (1982) stated that the size of newly-hatched chinook salmon alevins was smaller when the eggs were incubated at 12°C because alevins hatched at a slightly earlier stage of development than alevins produced at lower temperatures. Because most of the development of the hatching gland occurs just prior to hatching (Hayes, 1942; Klinkhardt et al., 1987), the temperature from eyeing to hatching will have more effect on the length of newly-hatched alevins than will the temperature from fertilisation to eyeing. Gray (1928b) showed that larger brown trout alevins were produced when eggs were incubated at low temperatures because the hatching process was delayed.

Various studies have demonstrated that the level of dissolved oxygen is also of prime importance during the hatching process: low oxygen levels have been shown to stimulate hatching in salmonids. The influence of oxygen on the hatching process was described earlier in section 2.4.2.2.

Thus, various studies have indicated that precocious hatching induced by adverse environmental conditions (high temperature and low oxygen) may contribute to the smaller size of newly-hatched alevins.

(b) Effect of temperature on yolk conversion efficiency

The decrease in development times with increasing temperature is caused by the accelerating effect of high temperature on metabolic rate and depletion of the energy reserves. Although development rates were temperature-dependent, this study showed no significant effects of egg incubation temperature on the weight of newly-hatched alevins. However, this study did show that alevin size at MAWW was dependent upon temperature during yolk-sac absorption within the range tested (10-20°C); mean lengths and weights of alevins at MAWW were progressively and significantly reduced at temperatures above 10°C. At 20°C, surviving alevins averaged little more than half the weight of those held at 10°C. The direct relationship shown here between alevin size at MAWW and temperature within the range of 10-20°C (Series 4) suggests that yolk conversion efficiency decreases progressively with increasing temperature.

Numerous metabolic studies conducted on salmonid species have confirmed that water temperature is the main environmental factor influencing the efficiency with which yolk is transformed into body tissues. At low temperatures a more efficient conversion of endogenous yolk reserves occurs, resulting in alevins which are larger at hatching and MAWW. Yolk reserves are utilised to provide structural materials for ontogenetic development as well as energy for maintenance, activity and growth. Yolk conversion efficiency

is generally measured in terms of the proportion of yolk energy transformed into body tissues; during development, the yolk reserves decrease with time as a function of its conversion into an energy source for metabolic processes and growth of tissues.

Hayes and Pelluet (1945) investigated the effect of temperature within the range of 0.2-16°C on the yolk conversion efficiency of Atlantic salmon alevins and found that during the early alevin stage, efficiency was low (42%) at all temperatures below 5°C, but showed a linear increase with increasing temperature to 60% at 16°C. Similarly, Marr (1966) reported that Atlantic salmon alevins grow less efficiently at low temperatures: Marr (1966) measured the yolk conversion efficiencies of Atlantic salmon between 7.6-14.3°C, and found that the optimal efficiency occurred at 10°C (70%); at the upper and lower ends of the temperature range tested, the efficiencies were only 64 and 65%, respectively. Gunnes (1979) found that Atlantic salmon alevins hatching at 12°C were smaller than alevins from groups incubated at lower temperatures (8 and 10°C), and concluded that this was due to decreased yolk conversion efficiency at 12°C. Similarly, Peterson and Metcalfe (1977) found that newly-hatched Atlantic salmon alevins were larger when the eggs had been incubated at low temperatures, and stated that this implied less efficient use of yolk material at high temperatures; following hatching, however, the yolk conversion efficiencies were similar at 4, 8 and 12°C. The same study found that the specific gravity of newly-hatched alevins was nearly constant at all three temperatures,

although alevins produced from eggs incubated at 4°C were considerably longer than those produced at 12°C. However, during yolk-sac absorption, alevins held at 12°C had a lower specific gravity (more hydrated), implying that yolk reserves were depleted.

In a later study, Peterson and Metcalfe (1979) reported that newly-hatched Atlantic salmon alevins showed low levels of locomotor activity at low temperatures (less than 6°C), and that activity increases with increasing temperature. Because locomotor activity increased as development proceeds, alevins have an increasing demand for energy towards the end of yolk-sac absorption. Thus, locomotor activity of Atlantic salmon alevins competes with growth for energy stored in the yolk. Consequently, where rearing conditions lead to excessive activity, alevins may be smaller at MAWW (Marr, 1963, 1965). The use of a rearing substrate for alevins in the present study will have reduced the energy expenditure associated with locomotor activity, particularly at high temperatures.

Hamor and Garside (1977) showed that yolk conversion efficiency decreased in Atlantic salmon eggs with increasing temperature, but increased with increasing temperature during the alevin stage: the yolk conversion efficiency (by wet weight calculation) during egg incubation was greater at 5°C (102%) than at 10°C (91%), whilst during the alevin stage it was greater at 10°C (139%) than at 5°C (133%). After hatching, yolk conversion efficiency increased sharply at both temperatures, leading to more rapid growth of the alevins. Greater efficiency during the alevin stage of

development appears to be due to the removal of the limiting zona radiata, and the continued development of respiratory structures and vascularisation of the yolk-sac (Hagenmaier, 1974); this allows improved uptake of oxygen to meet the higher metabolic demand of the increasingly active and rapidly growing alevin. Low temperatures were necessary for efficient yolk conversion during the egg stage, but following hatching higher temperatures enhanced the yolk conversion efficiency. Although Hamor and Garside (1977) reported reduced rates of oxygen consumption (as a measure of metabolic rate) by Atlantic salmon eggs at 5°C compared to 10°C, the total oxygen consumption for egg incubation was very similar at each temperature. Thus, the slower rate of oxygen consumption at 5°C was offset by the additional development time required during egg incubation. Although total oxygen consumption was similar at 5 and 10°C, the size of newly-hatched alevins was significantly greater following egg incubation at the lower temperature. As a result, relatively more oxygen was utilised for maintenance (rather than for conversion into structural materials) at 10°C compared to 5°C.

For the production of Atlantic salmon alevins of optimal length and weight at hatching, Klinkhardt et al. (1987) recommended that eggs should be incubated about 5°C from fertilisation until just before eyeing, followed by an increase to 10-13°C until hatching. Klinkhardt et al. (1987) showed that motor activity in Atlantic salmon embryos is closely related to the temperature. General activity of embryos increased significantly at high temperatures; the

heart rate, which is an expression of the metabolic rate, was particularly sensitive to temperature change, together with increased movements of the eyes and the pectoral fins. The movement of the pectoral fins, required to maintain a constant circulation of the perivitelline fluid within the chorion, started later but quickly reached a higher pulse rate at 8°C compared to 3°C; the intensity of pectoral fin movements was greatest at 8-10°C, and decreased markedly above and below this range. Movements of the embryos exhibit no such relationship with temperature, because of the restrictions to movement imposed by the lack of space within the chorion. Klinkhardt et al. (1987) also reported that the total oxygen consumption during egg incubation was approximately 40% higher in Atlantic salmon at 8°C compared to 3°C.

Vernidub (1963) showed that discrete stages of embryonic development in Atlantic salmon had different temperature requirements, and that the optimal temperature tended to increase as development progressed. The optimal temperature recommended by Vernidub (1963) for all stages of development was below 10°C, while Marr (1966) found that yolk conversion efficiency of Atlantic salmon embryo was in fact greatest at 10°C. Gunnes (1979) stated that the optimal temperature during the alevin stage was dependent upon the temperature during egg incubation. When eggs were incubated at 10°C it was recommended that following hatching, the temperature should be reduced to 8°C. The present study showed that 10°C was an optimal temperature throughout development from fertilisation to MAWW.

Marr (1966) also measured yolk conversion efficiencies of brown trout alevins maintained at temperatures 4-12°C, and found that the greatest efficiency occurred at 10°C. The yolk conversion efficiencies of alevins maintained at 4 and 10°C were 57 and 81%, respectively. Thus, the yolk conversion efficiencies of Atlantic salmon and brown trout alevins appear to be maximal at 10°C. Gray (1928b) incubated brown trout eggs at 5-16°C and found that although the growth of brown trout embryos was more rapid at high temperatures, the final size of alevins at final yolk absorption decreased with increasing temperature. Within the range of 5-16°C, the corresponding size range of fry at MAWW was 135-95 mm. Gray (1928b) stated that this was due to a greater requirement for yolk reserves for maintenance, rather than for the development of tissues at high temperatures, and concluded that the temperature range for optimal yolk conversion efficiency of brown trout lay within the range of 5-12°C. Outside this range, there is a differential effect of temperature on rates of metabolism and growth: at both high and low temperature extremes, a large amount of yolk is utilised in the process of respiration, leaving less for tissue growth, with the result that alevins are small at hatching. Gray (1928b) also stated that MAWW in brown trout would occur at an earlier stage of development at high temperatures. Wood (1931) determined average yolk conversion efficiencies of brown trout alevins from hatching to final yolk absorption at 3°C (54.5%), 7°C (65.0%) and 12°C (64.5%). Having combined these results with those given by Gray (1928b), which reported a value for yolk conversion

efficiency of 63% at 10°C (although for a slightly different stage of development), Wood (1931) concluded that alevins held at 3°C used a greater quantity of yolk for maintenance than those at higher temperatures, and that efficiencies were nearly constant within the range of 7-12°C. However, Marr (1966) recalculated Wood's data and concluded that the yolk conversion efficiency was not, in fact, constant between 7-12°C, but was highest at 10°C with a value of 81%.

Conversely, Kamler and Kato (1983) found that the yolk conversion efficiencies of rainbow trout embryos and alevins increased progressively with increasing temperature within the range of 9-14°C. Most other studies have shown an optimal temperature range of maximum efficiencies, above and below which efficiencies are reduced. Evidently, the highest temperature (14°C) tested by Kamler and Kato (1983) did not exceed the optimal range for yolk conversion efficiency.

Oliva-Teles and Kaushik (1990) examined the effect of temperature on the yolk conversion efficiency of rainbow trout, and reported that even short-term changes in temperature had a considerable influence on the respiratory metabolism during early development. This effect was more pronounced among eggs than alevins: the mean Q_{10} values for eggs and alevins were 5.8 and 2.2, respectively.

Among other species in the genus Oncorhynchus, Beacham and Murray (1985) reported that following egg incubation at 3 different temperatures (4, 8 and 12°C), newly-hatched chum salmon alevins having the heaviest body weight were those incubated at 12°C, and the lightest alevins were those incubated at 4°C.

Heming (1982) and Heming et al. (1982) examined the yolk conversion efficiency of chinook salmon within the temperature range of 6-12°C, and demonstrated that at final yolk absorption, the longest and heaviest alevins were produced at the lowest temperature. At 12°C, the alevins hatched with slightly more yolk than those hatched at 6-10°C, suggesting that hatching was precocious at the higher temperature. Beacham and Murray (1988) also found that the yolk weight of newly-hatched pink salmon alevins was influenced by temperature, with alevins hatching at 12°C having a greater quantity of yolk than those hatched at 4 and 8°C.

In agreement with Gray (1928b), Heming (1982) also found that MAWW occurred earlier in development at high temperatures. Heming (1982) stated that the yolk conversion efficiency varied markedly during development. Instantaneous rates of yolk conversion efficiency reached a peak just prior to hatching, and then declined to 0% at maximum alevin dry weight. A reduction in efficiency as development proceeds may be due to the relatively higher metabolic demands of heavier alevins together with an increased level of activity. High temperatures reduced the pre-hatching maximum, and following hatching, 12°C markedly reduced efficiency.

For the Arctic char, Gruber and Weiser (1983) showed that although alevin weight at hatching was only slightly higher following egg incubation temperature at 4°C compared to 8°C, the yolk conversion efficiency during the alevin stage was markedly higher at 4°C (35%) than 8°C (12%). Gruber and Weiser (1983) reported that the effect of temperature on the

metabolic rate of Arctic char alevins was greater than that for other salmonids, and suggested that this was caused by greater levels of locomotor activity. Wallace and Aasjord (1984b) found that the highest yolk conversion efficiency of alevins occurred at 6°C, and was reduced by approximately 15% between 8-12°C. A similar reduction in yolk conversion efficiency between 8-12°C was reported by Heming (1982) in chinook salmon. Wallace and Aasjord (1984b) also found that the body weight of Arctic char alevins decreased towards the end of yolk absorption at 8 and 12°C, and suggested that the energy demand for metabolism at these temperatures could not be met by the remaining yolk reserves. The alevins then metabolised energy from resorption of their body tissues, while still utilising endogenous yolk reserves; at 3 and 6°C, alevins continued to gain weight until yolk depletion. Heming (1982) also found that chinook salmon exhibited a metabolic energy deficit prior to final yolk absorption at temperatures of 6-12°C.

The preceding review of the extensive literature pertaining to the effects of temperature on yolk conversion efficiencies of salmonids during early development shows that there are discrete differences between different species. Most studies have demonstrated that high temperatures produced smaller alevins (in terms of weight and length) at hatching and MAWW than those produced at low temperatures. The size of alevins and fry is dependent upon the yolk conversion efficiency of yolk to body tissues, which in turn is dependent upon the temperature during development (Gray, 1928a, b; Marr, 1966; Hamor and Garside, 1976, 1977; Peterson et al., 1977; Heming,

1982; Beacham and Murray, 1985). The size of newly-hatched alevins is also a function of the development time in degree-days to hatching (Peterson et al., 1977; Heming, 1982). To some extent, alevins were smaller at hatching following egg incubation at high temperatures because hatching occurred at an earlier developmental stage than at lower temperatures.

A reduction in yolk conversion efficiency at high temperatures appears to be caused by increased maintenance costs (Gray, 1928b; Hamor and Garside, 1977; Heming, 1982). As a result, size based on dry matter content of the alevins is inversely related to temperature. At high temperatures, the yolk reserves cannot meet the increased metabolic demands for the maintenance of growth, and existing body tissue is mobilised to meet the deficit. The yolk conversion efficiency is generally between 40-70%, depending on temperature. Thus, there appear to be two compensating factors which affect the temperature-dependent changes in yolk conversion efficiences: the effect of temperature on the development time, and its effect on the metabolic rate.

In some salmonid studies, yolk conversion efficiency has been shown to increase with increasing temperature; in others it decreased with increasing temperatures, and in others maximum efficiency occurred at intermediate temperatures. However these differences are generally due to differences in the methods used to measure efficiency, together with differences in the temperature ranges and periods of development studied. Marr (1966) stated that the use of average yolk conversion efficiency can be misleading because it is not constant throughout development: it has been shown

to decrease with time as development proceeds at all temperatures. During the early stages of embryonic development, relatively less yolk is used for maintenance than for growth, giving a high yolk conversion efficiency. The rate of yolk conversion has been found to be relatively slow just prior to hatching, and also in later development when the yolk reserves are almost exhausted. When comparing the effects of temperature on yolk conversion efficiency, it is often difficult to make direct comparisons between the results of studies which have considered slightly different periods of development. Because instantaneous rates of yolk conversion efficiency have been found to vary substantially during development (Heming, 1982), estimates of efficiency will depend on the precise period of development examined. While many workers including Heming (1982), Gray (1928a, b), Hayes (1949) and Smith (1957) have considered the entire period of development from fertilisation to final yolk absorption, the period investigated by Marr (1966) was from 15-80% tissue-to-alevin-dry-weight ratio.

The varying amount of yolk material remaining at MAWW (Heming, 1982) can also affect estimates of yolk conversion efficiency. Differences in the patterns of yolk conversion among the salmonids reported in the literature may also reflect interspecific differences in their biology and/or variations in experimental design and methodology.

From the foregoing review of the literature and the results of this study, there appears to be a relatively narrow intermediate range of temperatures for each salmonid species within which the yolk conversion efficiency is optimised. At

temperatures above and below this range, metabolic demands are proportionally greater than those utilised for tissue growth, giving rise to reduced alevin size.

Alevin size at hatching and MAWW will not be determined solely by the influence of water temperature. It can also be influenced by the level of dissolved oxygen (Garside, 1959, 1966; Silver et al., 1963; Hamor and Garside, 1977; Brooke and Colby, 1980; Gruber and Weiser, 1983). Given the effect of oxygen availability on hatching, as discussed previously (section 2.4.2.2), it is likely that any factor which affects metabolic rate will also influence hatching by causing alterations in the rate of oxygen consumption; consequently, the minimum level of dissolved oxygen required for continued egg incubation may change.

Hamor and Garside (1977) showed that the weight of newly-hatched Atlantic salmon alevins was affected by various combinations of temperature and dissolved oxygen: development rate retarded by low temperatures produced larger alevins, while development rate retarded by low levels of dissolved oxygen produced smaller alevins. The mean dry weight of newly-hatched alevins derived from eggs incubated in fully saturated water maintained at a constant 5°C was 22.1 mg, while those from eggs incubated at a constant 10°C had a mean weight of 14.5 mg. When the dissolved oxygen level was reduced to 30% saturation, the mean dry weights of newly-hatched alevins were 11.9 mg at 5°C and 10.0 mg at 10°C.

Gruber and Wieser (1983) reported that low dissolved oxygen has the same limiting effect on the growth of Arctic char embryos: embryos developing at low levels hatched at a

smaller size than those developing at high oxygen levels.

Because oxygen levels were always maintained close to 100% saturation in the present study, yolk conversion efficiency would not have been influenced by low oxygen conditions.

Yolk conversion efficiency can also be affected by other environmental factors, including water velocity (Silver et al., 1963), light intensity and substrate contour (Marr, 1963, 1965; Peterson and Metcalfe, 1977). Efficiency can be increased by holding eggs and alevins in darkness and by providing a suitable rearing substrate for alevins - these environmental requirements were met in the present study (see section 2.2.2.4). The influence of water velocity on alevin size would be expected to be small when dissolved oxygen levels are within the normal range for development. However, under low oxygen conditions, water velocities can have a significant effect on alevin size. Silver et al. (1963) found substantial differences in the mean lengths of newly-hatched chinook salmon alevins following exposures to various combinations of dissolved oxygen and water velocity: mean length increased with increasing levels of dissolved oxygen and water velocity and it was inferred that low water velocity limits oxygen uptake by the developing embryo. Hamor and Garside (1976) have also shown that the water velocity influences the availability of oxygen for developing Atlantic salmon embryos.

Thus, fish size and development rate from fertilisation to MAWW are functions of the amount of yolk present, the rate of yolk absorption, and the yolk conversion efficiency. Although the amount of yolk present is determined ultimately by egg

size, both the rates of absorption and yolk conversion efficiency depend primarily upon water temperature, and to a lesser extent upon other environmental factors. Though the rate of yolk absorption is directly related to temperature, the effect of temperature on yolk conversion efficiency is more variable. In the present study, yolk conversion efficiency during the alevin stage was optimal at 10°C, but declined progressively at higher temperatures. At 20°C, surviving alevins at MAWW were about half the weight of those held at 10°C. Because alevins were provided with a rearing substrate, the lower yolk conversion efficiency would be caused by greater energy demands for metabolic processes rather than by increased levels of alevin activity at high temperatures.

Clearly, the effect of temperature on yolk absorption has important implications in the selection of optimal temperatures for egg incubation and alevin development in Atlantic salmon hatcheries. Presumably, larger fish have an initial advantage when exogenous feeding begins. It may be advantageous to maintain Atlantic salmon eggs and alevins at low temperatures to increase the yolk conversion efficiency, thereby producing large first-feeding fry. However, this would prolong the egg and alevin stages of development which will reduce the duration of the subsequent feeding period.

2.4.2.5 Growth of fry

First feeding began earlier in groups of Atlantic salmon fry derived from eggs and alevins held at high temperatures.

This led to an extended feeding period among advanced groups of fry which gave rise to a significant weight gain by the end of the study. In Series 1, a further 4 weeks of feeding was gained by groups held at 10°C from fertilisation to MAWW compared with those held at 8°C. Because alevins are more tolerant than eggs to high temperatures, the temperature can be increased to 16°C after hatching; in Series 4, MAWW was reached about 2 weeks earlier at 16°C compared with 10°C. Given a specific growth rate of 1.84% per day for fry (mean of all series), a 2-week extension to the feeding period would result in a weight gain of 30%, whilst a 4-week extension would lead to a weight gain of almost 70%. Although advanced fry were smaller than those produced at lower temperatures, this size difference was quickly lost after feeding commenced and the size of fry at first feeding (MAWW) was not a determinant of their subsequent growth rate.

Although some differences in growth rates between the various groups within a series were significant, no particular trends were apparent: advanced fry to which feed had been presented earliest generally grew at very similar rates to those produced at lower temperatures, and had attained the highest mean weights at 110 days after fertilisation. The mean weights of fry at 1200 degree-days after fertilisation were generally similar, regardless of the temperature regimes experienced by the various groups during egg and alevin development. Gunnes (1979) also found that the temperature regime (combinations of 8, 10 and 12°C) during egg and alevin development had little bearing on the total number of degree-days required for Atlantic salmon fry to

reach a given weight.

Although the temperature regime prior to first feeding had no apparent effect on the growth rate of fry during early feeding, a study by Peterson and Martin-Robichaud (1989) showed that the growth rate of Atlantic salmon fry can be influenced by lower temperatures than those tested in the present study. It was found that the growth rate of first feeding fry derived from eggs and alevins held at 10°C was greater than those from eggs and alevins held at 4°C.

In fish growth studies, investigators have used various methods to measure the growth of fish. Fish size has been measured in terms of length as defined by standard, fork, and total length, and in terms of total weight as defined by wet, and dry weight. Clearly, there is a requirement for uniformity in the measurement of growth to allow straightforward comparisons between growth data from different studies. The use of standard, fork and total length gives no information on the condition of the fish. On the other hand, the use of wet weight introduces the error of varying water content (Peterson and Metcalfe, 1977); variations in the total water content occur as dense yolk is converted into relatively more hydrated body tissue.

Although dry weight is the most satisfactory measure of growth, the use of wet weight of fish, as a parameter for determining the timing of first feeding (MAWW), does not require dissection of yolk from alevin, which is difficult to perform during the final stages of yolk resorption. The growth of fry at first feeding is influenced initially by the quantity of yolk material retained in the body cavity; this

may be quite variable when MAWW is reached (Heming, 1982). Thus, growth is derived during first feeding from both endogenous and exogenous sources of nutrition. Although there are limitations associated with the use of total wet weight as a means of monitoring growth, it has been shown that once the endogenous yolk supply has been fully utilised and the fry rely on an exogenous source of nutrition, there was a good correlation between wet and dry weights.

Throughout this study, the use of wet weight for the determination of growth was considered reliable. However, in order to allow direct comparisons with data from other studies, alevin length at MAWW was also determined in some of the later experiments (Series 4).

In the present study, fry rearing was conducted at a constant 10°C, which is lower than the optimal temperature for the growth of Atlantic salmon. The temperature range over which feeding occurs in juvenile Atlantic salmon is 6-20°C (Elliott, 1981): Refstie (1979) recommended that the minimum temperature required for first feeding in both Atlantic salmon and rainbow trout was 7-8°C; similarly, Peterson and Martin-Robichaud (1989) reported that the feeding response of Atlantic salmon fry during first feeding was inhibited at 4°C, though feeding was observed at 8°C. In natural conditions juvenile Atlantic salmon reduce or stop feeding at temperatures below 7°C (Jensen et al., 1989). Liao and Mayo (1972) stated that the optimal temperature range for Atlantic salmon during the first-feeding period was 10-18°C, whilst Peterson and Martin-Robichaud (1989) reported an optimal range of 16-20°C. The mean specific growth rate of Atlantic

salmon fry in the present study was 1.84% per day at a constant 10°C. At the same temperature, Siemien and Carline (1991) showed that the mean specific growth rate of Atlantic salmon fry was 0.5% per day during the first 3 weeks of feeding. Within a more optimal temperature range (10-16°C), Stefansson et al. (1990) showed that the specific growth rate of Atlantic salmon fry was 2.4% per day during the first 3 months of feeding.

These growth data compare poorly with those published by Austreng et al. (1987), which reported specific growth rates for Atlantic salmon fry weighing 0.15-0.80 g of 2.6% at 10°C and 3.1% per day at 12°C. However, these growth data were derived from domesticated salmon stocks which had been specifically selected for increased growth. Furthermore, the data were taken from groups of fish performing best throughout 12 different feeding trials. Similarly, for a genetically fast growing strain of Atlantic salmon selected over a period of 20 years, Isaksson (1985) reported that the specific growth rate for fry weighing 0.15-1.2 g was 3.4% per day at 12.5°C. At a similar temperature, Sveier and Raae (1992) reported specific growth rates of 4.3-5.0% per day for Atlantic salmon fry. Peterson and Martin-Robichaud (1989) reported a maximum specific growth rate of 1.6% per day for Atlantic salmon fry at an optimal temperature of 18.6°C, but predicted that under ideal conditions, maximal growth rates in excess of 3.5% per day can be achieved by newly-fed Atlantic salmon fry. Refstie and Kittelsen (1976) stated that the mean weight of Atlantic salmon fry during 6 weeks of rearing at temperatures of 8.5-13.5°C increased from 0.13 g

to 0.58 g in one strain and from 0.21 g to 0.89 g in another, representing mean specific growth rates of 3.5 and 1.4% per day, respectively. The latter figure is similar to the growth rate of Atlantic salmon fry reported by Thorpe et al. (1982) and is comparable with the growth data in the present study.

It is apparent that large differences in the specific growth rate of Atlantic salmon fry have been reported in the literature. Though temperature differences may account for some of this variation, many studies have reported very different specific growth rates at similar temperatures. Genetic differences between strains of Atlantic salmon have commonly been cited to explain differences in growth rates (Refstie and Kittelsen, 1976; Nævdal et al., 1978; Ryman and Ståhl, 1981; Thorpe and Mitchell, 1981; Isaksson, 1985; Torrissen and Torrissen, 1984).

Among other salmonid species, Wallace and Aasjord (1984b) reported that 8°C was optimal for first feeding in Arctic char. Heming et al. (1982) studied the effect of temperature (6, 8, 10, 12°C) on first feeding in chinook salmon and reported that temperature and the timing of food presentation interacted to create a zone of optimal feeding. The initial utilisation of food for growth did not occur until some time after feeding began; nor did it coincide with any specific stage of development, but rather it occurred earlier, and at a less advanced stage of development, at high temperatures. Because food utilisation occurred earlier in development at high temperatures it may be that temperature affects the production and/or the action of digestive enzymes. Indeed, Torrissen and Torrissen (1984) reported that protease enzymes

in the digestive tract of Atlantic salmon fry were significantly affected by temperature, and showed that peak enzyme activity occurred within the temperature range of 9-12.6°C, depending on differences between strains.

Several studies have investigated the influence of temperature on the growth of Atlantic salmon parr. Wankowski and Thorpe (1979) reported optimal growth of 4-20 cm Atlantic salmon at 17°C, while Dwyer and Piper (1987) and Farmer et al. (1983) reported optimal growth of 1 g parr at 16°C. Similar temperature optima have been established for the growth of rainbow trout (Hokanson et al., 1977; Papst et al., 1982) and brown trout (Elliott, 1975).

Clearly, the full growth potential of Atlantic salmon fry in the present study was not achieved because fry rearing was conducted at 10°C. Had the water temperature during feeding been nearer to the optimal temperature (16-20°C) for maximal growth in first feeding Atlantic salmon fry, the weight increments between the early and late feeding groups of fry would have been greater.

2.4.2.6 Biomass gain

In general, the biomass (product of the number of fish extant and mean fish weight) of groups of alevins at MAWW and fry at 110 days and 1200 degree-days after fertilisation in relation to different temperature regimes from fertilisation to MAWW, was largely dependent upon the levels of egg and alevin mortality rather than the mean weights attained by fish within a group. Thus, although the mean weights of

advanced groups of fry at 110 days after fertilisation were often greater than later feeding groups, the levels of mortality among the more advanced groups of fry produced at high temperatures were generally more severe than among those groups produced at lower temperatures. The highest mortalities, and consequently the lowest biomasses, occurred in groups where the temperature exceeded 11°C from fertilisation to eyeing and exceeded 12°C from eyeing to hatching; following hatching, biomasses were lowest when the temperature exceeded 16°C.

A study by Gunnes (1979) also showed that although the mean weight of Atlantic salmon fry produced at 12°C during egg and alevin development was greater (by virtue of an extended feeding period) than those produced at 8 and 10°C, mortality was severe at 12°C. Consequently, the net biomass gains of the 8 and 10°C groups were considerably greater (about 9-fold) than the 12°C group. At the same temperatures, the results of a study by Peterson et al. (1977) also indicated that high mortality at 12°C would lead to a very low biomass at MAWW. Because Peterson et al. (1977) used alevin length (rather than weight) as a measure of growth, it was not possible to calculate actual figures for biomass from their data.

The results of various other salmonid studies (McCormick et al., 1972; Timoshina, 1972; Heming, 1982; Kamler and Kato, 1983; Jungwirth and Winkler, 1984; Garling and Masterson, 1985; Humpesch, 1985a; Kwain, 1985a; Kashiwagi et al., 1987; Tang et al., 1987) have also shown that the potential weight gain associated with the early attainment of MAWW, following

egg and alevin development at high temperatures, is generally compromised by increased levels of mortality.

Thus, there are two compensating factors which determine the net biomass gain: on the one hand the effect of high temperatures shortening the time to reach MAWW thereby extending the feeding period for fry, and on the other the increasing levels of mortality at high temperatures. Maximum biomass gain will occur at temperatures near the upper end of the optimal temperature range for each species.

In the Atlantic salmon farming industry, there is currently a growing demand for autumn smolts ($S\frac{1}{2}s$) - these can be produced only when first feeding commences at an early time, thereby increasing the potential for growth by extending the growing season. This objective can be realised by using the highest recommended water temperature for egg and alevin development. The results from this study showed that biomass gain was maximal when egg and alevin development occurred at 10-11°C and 14-16°C, respectively.

2.5 CONCLUSIONS

Following the incubation of Atlantic salmon eggs at varying temperature regimes within the range of 8-16°C, it was established that mortality increased above 11°C and no eggs survived to hatch above 13°C. The upper temperature limit for eggs was sharply defined: at 14°C no eggs survived to eyeing, and at 13°C very few eggs hatched. Under all temperature regimes, mortalities were generally greatest during a short phase of development prior to eyeing, and during hatching. The subsequent survival of later stages of development was largely determined by survival during earlier stages; thus, the most favourable temperatures from eyeing to hatching and from hatching to MAWW were dependent upon the temperature experienced during the previous stages of development. Mortalities at later stages of development were generally high when the eggs were incubated at 12°C from the outset. Within the temperature range tested, optimal survival occurred at 8-11°C: for any temperature combination within this range, survival from fertilisation to hatching would be expected to exceed 85%.

The temperature tolerance of eggs was more limited than alevins; the upper lethal temperatures for eggs and alevins were 14 and 22°C, respectively. Alevin mortalities were low at 16°C, indicating that alevins exceed eggs in their temperature tolerance by 4-6°C, depending on their previous temperature history. Above 16°C alevin survival was significantly reduced compared with lower temperatures in most, but not all, experiments.

Although the present study did not investigate the upper limit of temperature tolerance for fry, other studies indicate that it lies between 21-26°C, depending on the acclimation temperature and the level of dissolved oxygen. Further work is required to establish the upper lethal temperature for Atlantic salmon fry.

Several forms of abnormality were observed in this study. Although some abnormalities (e.g. spinal deformities) were commonly observed under most temperature regimes, the incidence of "pin-eyed" eggs was greater above 9°C. In order to confirm whether this condition is induced by incubating eggs at high temperatures, it would be necessary to conduct a further study to examine the incidence of this abnormality over a wider temperature range (e.g. 4-13°C). Above 11°C, there was generally a higher incidence of abnormalities (particularly incomplete hatching) observed during hatching leading to increased alevin mortality. Various studies have indicated that mortality during hatching is frequently caused by precocious hatching induced by hypoxic conditions within the egg at high temperatures. It would be of interest to establish whether egg incubation at supersaturated dissolved oxygen levels would reduce the prevalence of precocious hatching at high temperatures.

High temperatures during the alevin stage frequently gave rise to severe oedema of the yolk-sac, resulting in slower development and increased mortality; in particular, alevins held above 16°C had a high incidence of yolk-sac abnormalities and demonstrated reduced feeding success. Although not observed in the present study, the development

of yolk-sac constrictions is a very common abnormality among Atlantic salmon alevins. Because it is a chronic condition resulting from high alevin activity, particularly at high temperatures, it is essential to provide alevins with a substrate (alevin support system) until MAWW is reached.

In all instances the rate of development, as measured by the number of days required to reach any given stage of development, was accelerated by increasing temperature. Development times from fertilisation to median hatch ranged from 59 days at 8°C to 34 days at 12°C; following hatching, alevins reached MAWW after 41 days at 10°C and 25 days at 18°C.

The total number of degree-days required from fertilisation to eyeing and to MAWW was similar at all temperatures, but declined during the period from eyeing to hatching as temperature increased.

The duration of the hatching period (time between hatching of the first and last eggs) were similar at all temperatures investigated except 8°C, where it was significantly longer: the hatching period was 20 days (156 degree-days) at 8°C and 4-8 days (44-98 degree-days) at 9-16°C. The more synchronous hatching of eggs, associated with the use of at high hatching temperatures, would be of benefit to the management of Atlantic salmon hatcheries.

Within the temperature range tested (8-16°C) in this study, the weight of newly-hatched alevins was independent of temperature. Because other salmonid studies have shown an inverse relationship between the size of newly-hatched alevins at lower incubation temperatures, it is concluded

that the temperatures tested here were probably too high to detect a significant effect. However, alevin size at MAWW was shown to be dependent upon temperature during yolk-sac absorption within the range tested (10-20°C); mean lengths and weights of alevins at MAWW decreased progressively and significantly with increasing temperatures. At 20°C, surviving alevins averaged little more than half the weight of those held at 10°C. Thus, within the range of 10-18°C, compromises are made between rapid development and maximum alevin size at first feeding. Above 18°C, survival is severely reduced.

Although alevins expend more energy for metabolism at the expense of growth at high temperatures, this is offset by the significantly earlier attainment of MAWW. Early fry, although generally smaller at first feeding than those produced at lower temperatures, quickly overcame this size difference soon after feeding commenced. Advanced fry to which feed had been presented earliest grew at very similar rates to those produced at lower temperatures and had attained the greatest weight at 110 days after fertilisation. The extended feeding period resulting from egg and alevin development at high temperatures gave rise to a significant growth advantage. The mean weights of fry at 1200 degree-days after fertilisation were generally similar, irrespective of the temperature regimes experienced by the various groups during egg and alevin development.

The specific growth rates of fry were not influenced by temperature regimes during the egg and alevin stages. The size of fry at first feeding (MAWW) was not a determinant of

their subsequent growth rate or survival.

The net biomass gains (product of the number of fish extant and mean fish weight) for groups of alevins at MAWW and fry at 110 days and 1200 degree-days after fertilisation were more dependent upon survival rates than upon mean fish weights.

The results of this study, together with those from similar studies (Peterson et al., 1977; Gunnes, 1979; Brännäs, 1988; Kane, 1988), showed some variation in the development times and mortality rates for Atlantic salmon eggs and alevins at the same temperatures. Although a number of environmental and genetic factors have been cited to explain these differences, there is clearly scope for conducting further studies in this area.

Further studies are also required to investigate the effects of low temperatures on the early development of Atlantic salmon. There is commercial interest in the use of low temperature egg incubation as a means of spreading the availability of Atlantic salmon eggs for overseas markets: currently, Chile's rapidly expanding Atlantic salmon industry has a high requirement for eyed eggs during the summer months. Although studies by Peterson et al. (1977), Heggberget and Wallace (1984) and Wallace and Heggberget (1988) have reported development times and survival rates for Atlantic salmon eggs and alevins at low temperatures, the subsequent growth and survival of the resulting fry has not been tested. Clearly, the suitability of low temperature regimes for Atlantic salmon eggs and alevins can be determined only once this information is known.

This study clearly demonstrates that temperature regimes can be manipulated to enhance survival, control development times, and increase biomass, thereby improving the efficiency of Atlantic salmon production. The main benefit of using high temperatures during egg and alevin development is to shorten the time from fertilisation to first feeding. Early feeding will allow a hatchery operator to realise the full potential for growth in order to maximise the production of S1 and particularly S½ salmon smolts. It is therefore important to use the highest recommended temperature whenever possible. However, caution must be exercised by hatchery operators when egg and alevin development is conducted at high temperatures. Any failure of the heating system could prove damaging, depending on the duration, severity of the temperature change and stage of development. Peterson et al. (1977) warned that a sudden decrease in the egg incubation temperature could lead to oedema in alevins. Equally, a rapid rise in temperature could prove lethal to eggs and alevins.

Consideration must also be given to other criteria such as the costs of installation and running of heating systems in hatcheries in relation to the potential increase in production. In this respect, it is important that salmon fry are not produced too early, when the ambient water temperature is still very low; otherwise excessive water heating costs may be incurred by the increased water flow rates required by fry. Because the water requirement for salmon fry production in hatcheries is low, it is likely that with relatively low investment costs for heating and careful fish production planning, high commercial gains can be

achieved. Clearly, a detailed financial appraisal would be required to assess the feasibilty of this type of operation, involving factors beyond the scope of this investigation.

Chapter 3 Influence of the timing of first feeding on the survival and growth of hatchery-reared Atlantic salmon, *Salmo salar* L.

3.1 INTRODUCTION AND OBJECTIVES

Although in recent years there have been very significant improvements in the survival and quality of reared Atlantic salmon smolts, there is still scope for improvement, particularly during first feeding. The transition from endogenous to exogenous nutrition is a critical stage in determining the ultimate growth and survival of salmonid fry (Balon, 1984; Poston, 1988).

During the early development of the Atlantic salmon farming industry, very significant mortality problems were experienced during first feeding. Refstie (1979) considered that a first feeding mortality of 20% was an "acceptable" loss at that time. Bergstrom (1973) reported that Atlantic salmon fry commonly suffered mortalities of 20-50% during their first 5 weeks of feeding. Similarly, Rumsey and Ketola (1975), Gunnes (1979), Lemm and Hendrix (1981) and Lemm (1983) reported mortalities often in excess of 30%. Somewhat later, Hansen and Mϕller (1985) stated that the Atlantic salmon farming industry still experienced significant mortality problems during first feeding. More recent studies have demonstrated significant improvements in the survival of fry during first feeding. During the first 3 weeks of feeding, Siemien and Carline (1991) reported mean mortalities of 6 and 12% for Atlantic salmon fry reared 10 and 14°C, respectively. Similarly, Sveier and Raae (1992) reported that mortality in Atlantic salmon alevins and fry during the period from hatching to approximately one month after first feeding (674 degree-days after hatch) was less than 10%.

There is no published information on current first feeding success in the Atlantic salmon farming industry although it is generally thought to be approximately 80-90%.

There is extensive literature on the critical transition to exogenous feeding dating back over a century to the time when salmonid culture first began (Green, 1878; Porter, 1878; Thomas, 1879; Henshall, 1904; Atkins, 1905, 1906). Whilst numerous investigations have studied Pacific salmon species (genus Oncorhynchus), relatively few data exist for the Atlantic salmon.

In salmonids, various criteria have been used to identify the optimal timing of first feeding. Early studies by Henshall (1904), Atkins (1905, 1906) and White (1915) proposed that first feeding should commence following disappearance of the visible yolk-sac. However, a study by Brown and Buck (1939) concluded that the size of the yolk-sac bore no correlation with first feeding time in rainbow trout, brook char or grayling fry; the quantity of yolk remaining varied greatly during the early feeding period both within and between the 3 species studied. The transition to exogenous feeding in salmonids is generally associated with the inflation of the swimbladder, hence the term "swim-up" is commonly used to describe this stage. Leach (1923) suggested that brook char begin to feed when the yolk-sac diminishes sufficiently for the fry to swim-up. Brown and Buck (1939) found that feeding could occur at or just prior to swim-up in brown trout, rainbow trout, cutthroat trout, brook char and grayling. For the Arctic char, Wallace and Aasjord (1984b) reported that fry began feeding actively 40-50 degree-days

(at 10°C) after swim-up although the yolk-sac was not exhausted until a further 2 weeks (140 degree-days at 10°C) had elapsed. Because swim-up is a behavioural response, its recognition is subjective (Hodson and Blunt, 1981). Although the onset of swim-up behaviour provides a good indication of the optimal time to begin feeding in many salmonid species, particularly the rainbow trout where an easily-recognised swim-up stage occurs, it is of limited value in Atlantic salmon where there is often little or no evidence of swim-up behaviour (Nortvedt, 1986).

That exogenous feeding in salmonids can occur some time before complete yolk absorption was demonstrated by Henshall (1904). Feeding liver to grayling alevins, Henshall concluded that stronger fry were produced when feeding began prior to complete yolk absorption. Holm (1986) reported high levels of feeding activity among Atlantic salmon fry long before yolk-sac exhaustion (12-24% of the total fry weight comprised yolk). Leach (1923) reported exogenous feeding during yolk absorption in hatchery-reared brook char. Marr (1966) defined experimentally that Atlantic salmon alevins began feeding when the tissue-to-alevin-dry-weight ratio was about 0.85. Kane (1988) stated that Atlantic salmon fry were ready to begin feeding when the yolk comprised 5% of the total alevin wet weight. At a constant 10°C, Kane calculated that this corresponded to 827 degree-days after fertilisation. Similarly, Thorpe et al. (1984) reported that Atlantic salmon first fed when 3% of the alevin weight comprised yolk. Wallace and Aasjord (1984a, b) reported that Arctic char alevins will feed while still absorbing the last 14% of their

yolk supply; alevins receiving food earliest, grew more rapidly than those fed at yolk exhaustion. No correlation was found between the timing of feeding and early fry mortality. However, a more recent study by Alanärä (1993) showed that the optimal time to commence feeding Arctic char (in respect of growth and survival) was when 55% of the yolk remained. Palmer et al. (1951) found that feeding during yolk absorption in sockeye salmon and chinook salmon enhanced their subsequent growth rates. Studying the influence of the timing of feeding in rainbow trout, Escaffre and Bergot (1985) found that early feeding (from the oesophagus opening) gave rise to the largest fry. Clearly, salmonid alevins are capable of feeding prior to complete yolk absorption; however the precise stage of yolk absorption at which alevins are first capable of feeding is unclear. For the Atlantic salmon, the proportion of yolk to total alevin weight at first feeding has been shown to vary from 3% (Thorpe et al., 1984) to 24% (Holm, 1986).

Studies which have investigated the timing of first feeding in relation to the attainment of maximum alevin wet weight (MAWW) have provided more consistent results. These studies have indicated that the optimal time to start feeding salmonid fry is at, or close to, MAWW. For the Atlantic salmon (Marr, 1965) and chinook salmon (Heming, 1979; Rombough, 1985), feeding at MAWW has resulted in optimal survival and growth of fry. Heming (1979) concluded that the optimal time to start feeding chinook salmon fry was just prior to MAWW (40 degree-days before MAWW) because the normal levelling off to zero growth (in wet weight) at MAWW did not

occur; instead, growth was maintained throughout the transition to exogenous feeding. Similarly, Bams (1983) stated that emergence and initiation of feeding at MAWW did not maximise growth in chum salmon fry and demonstrated that steady growth occurred only when feeding commenced 44 degree-days before MAWW. Early emergence proved advantageous because the extended feeding period gave rise to larger chum salmon fry after 8 weeks of feeding. Thus, many studies have concluded that the optimal time to commence feeding salmonid fry is at, or just before, the attainment of MAWW. Consequently, MAWW will be used in this study as an objective measure of alevin development in relation to the timing of first feeding.

Numerous studies have investigated the effects of delayed first feeding on subsequent survival and growth in salmonids. Delaying feeding until yolk reserves are nearly depleted has been found to promote a more efficient transition to exogenous feeding in rainbow trout (Twongo and MacCrimmon, 1976; MacCrimmon and Twongo, 1980) and sockeye salmon (Harvey, 1966). However, Heming (1982) stated that chinook salmon alevins encountered a metabolic energy deficit when 10 mg (as dry weight) of the yolk material was still present, leading to weight loss resulting from the resorption of body tissues. A period of weight loss prior to complete yolk absorption has also been shown in brown trout (Gray, 1926) and Atlantic salmon (Marr, 1966). However, Hurley and Brannon (1969) found that sockeye salmon continued to grow until yolk reserves were exhausted. Delaying the food supply much beyond yolk exhaustion caused increased mortalities in chinook

salmon (Palmer et al., 1951; Heming et al., 1982), rainbow trout (Twongo and MacCrimmon, 1976), sockeye salmon (Hurley and Brannon, 1969) and Atlantic salmon (Atkins, 1905; Marr, 1965). Delays in initial food presentation following complete yolk-sac absorption have also resulted in decreased final weight gain (Palmer et al., 1951; Marr, 1965; Hurley and Brannon, 1969; Twongo and MacCrimmon, 1976; Leon and Bonney, 1979; Wallace and Aasjord, 1984b). The results of these studies have consistently shown that delaying first feeding until the yolk-sac has been completely absorbed has increased the mortalities and depressed the growth rate of salmonid fry.

However, studies by Atkins (1906), Twongo and MacCrimmon (1976) and Bams (1983) showed that once food was introduced, the growth rate of starved fry was superior to that of groups of fry fed earlier. Similarly, Bilton and Robins (1973) reported that starved sockeye salmon fry attained similar weights to the control group within 8 weeks. Thus, starved fry, once provided with food, appear to retain the capacity to develop and grow at rates greater than those of fry receiving food at the optimal time. Because of the scarcity of available data, further work is required to confirm that Atlantic salmon fry demonstrate recovery growth following a period of food deprivation. The duration of the period of food deprivation from which fry can recover also requires further investigation.

In salmon hatcheries, the time for first feeding of fry is generally determined by the hatchery manager's personal judgement and practical experience. The timing of first

feeding is generally based on rather subjective criteria, such as observed behavioural changes or visual estimation of the remaining yolk. However, a common principle is that fry must be given an unlimited opportunity to feed at an early stage in yolk-sac absorption when first capable of exogenous feeding. Peterson and Shreedharan (1992) have shown significant differences in the timing of first feeding of Atlantic salmon among various hatcheries; the mean water content of fry varied from 74-83%, and the proportion of yolk remaining varied from 0-33%. Peterson and Shreedharan (1992) concluded that a water content of 80% is a suitable criterion for use in determining the optimal time to commence feeding Atlantic salmon fry. Hodson and Blunt (1981) stated that the water content of rainbow trout fry at swim-up was also 80-81%. It is apparent that the degree of variation in the timing of first feeding among Atlantic salmon hatcheries is high; feeding is generally commenced at some rather arbitrary time before complete yolk-sac absorption.

Hatchery operators frequently feed stock at the earliest opportunity in order to reduce the prevalence of "pinhead" mortalities. In hatcheries, emaciated fry are commonly referred to as "pinheads" because the head appears abnormally large in relation to the rest of the body. This condition results from failure of salmonid fry to feed following final absorption of the yolk-sac and can result in a high level of mortality. Once this point is reached, starved fry become progressively emaciated, even when food is available, and finally die. Blaxter and Hempel (1963) termed this point the "point-of-no-return", which was the stage after final yolk

absorption at which herring (Clupea harengus L.) larvae became too weak to capture and utilise suitable prey when it became available.

Because mortalities among hatchery-reared salmonid fry are generally attributed to inadequate or inefficient feeding responses at the onset of exogenous feeding, the alevins' environment is continuously saturated with fine food particles, often throughout an artificially extended photoperiod, so that the fish are encouraged to feed before the critical point-of-no-return is reached. However, starvation due to exhaustion of the endogenous reserves and the physical inability to feed may not be the main cause of mortality, because a number of studies (Atkins, 1906; Palmer et al., 1951; Bilton and Robins, 1973; Twongo and MacCrimmon, 1976) have shown that salmonid fry can survive a prolonged period of food deprivation. A proportion of fry die even though abundant food is present. Significant mortality may result from the irritation of the gill epithelium by a high suspended solid loading associated with excessive feeding; this gives rise to gill congestion and tissue hyperplasia, predisposing the fry to bacterial gill disease, fungus and various ecto-parasites (Roberts and Shepherd, 1986). The resulting mortality can commonly exceed 20% of the population. Clearly, growth will also be impaired at this time. Furthermore, Aasjord and Wallace (1980) found that approximately 7% of the mortality among Arctic char fry fed a dry feed was associated with gastric obstruction, caused by large particles (indigestible roughage) lodging in the anterior alimentary canal; this led to starvation and tissue

damage. Wankowski (1979) stated that Atlantic salmon fry showed less particle size selection than older fish. Although Atlantic salmon are less prone to this problem due to their larger size, Aasjord and Wallace (1980) state that fry require particles within the size range of 0.4-0.6 mm; elongate roughage particles passing through sieves during the manufacture of fry feeds are often 0.8-2.3 mm in length.

Although some fry mortality at first feeding will be associated with physical injury and congestion of the pharynx (which may lead to disease problems), further factors may be important. Indeed, recent studies by Metcalfe and Thorpe (1992b) and Metcalfe (1994) suggest that social interactions between fry may cause suppressed feeding activity by subordinate fry. Clearly, the factors which predispose salmonid fry to the pinhead condition are not completely understood and require further investigation.

Early feeding can be an advantage against the onset of starvation, but only if the alevins are functionally capable of digesting the food. A study of the ontogeny of the alimentary tract of coregonid alevins by Loewe and Eckmann (1988) found that the alevins were capable of ingesting external food long before yolk-sac exhaustion; at this time, the buccal cavity and oesophagus were nearly completely developed. It has been suggested that early contact with food can enhance first feeding behaviour in sockeye salmon (Hurley and Brannon, 1969) and Arctic char (Wallace and Aasjord, 1984b; Wallace et al., 1988; Alanärä, 1993). These studies recommend that a period of trial and error learning is required by alevins before feeding can be successfully

initiated. However, Coughlin (1991) reported that Atlantic salmon fry fed effectively within 2-3 days of first receiving food. Similarly, Twongo and MacCrimmon (1976) and Heming et al. (1982) found that an early association with food was not required for the development of feeding behaviour in rainbow trout or chinook salmon. Although premature feeding appears to be necessary for the ontogenetic development of first feeding among some salmonid species, it may not be of direct benefit to Atlantic salmon fry.

In wild fish populations, it is uncertain when salmonids normally begin to feed exogenously. Information in the literature is conflicting and a large variation is indicated. Several workers have stated that feeding commences before emergence from the gravel (Hayes, 1949; Disler, 1953; Dill, 1967; Rogers, 1968; Smirnov, 1975), and this may have significant survival value (Salo and Baycliff, 1958; Thomas and Shelton, 1968). Dill (1967) concluded that the growth of wild sockeye salmon fry could be enhanced by pre-emergent feeding. Other workers have reported that salmonid fry only commence feeding following voluntary emergence when MAWW is reached (Harvey, 1966; Bams, 1969, 1970; Heming, 1982), at which time yolk reserves are near to exhaustion (Brannon, 1967; McCart, 1967; Mead and Woodall, 1968; Balon, 1975; Bardonnet et al., 1993). Because the emergence of siblings from redds is not a simultaneous event - approximately 2 weeks at 10°C (Brännäs, 1988) - this indicates that the anatomical and behavioural development of first feeding is asynchronous. Consequently, in hatcheries an early transfer to first-feeding tanks will stress fry that are not yet ready

to feed, whilst a late transfer may reduce growth among more advanced fry. For this reason, various studies have evaluated the use of simulated redds (Mason, 1976; Brännäs, 1989) and deep substrate incubators (Bams, 1969; Pepper and Stansbury, 1985; Sveier and Raae, 1992; Alanärä, 1993) to achieve spontaneous first feeding in salmonid fry; this relies on the self-initiating emergence of the individual fry at the optimal time of feeding. Compared with conventional hatchery methods, Brännäs (1989) found that the growth and survival of Atlantic salmon was generally higher when held in simulated redds. However, in the same study brown trout fry had difficulties emerging from the substrate, which led to low growth and high mortality.

Although deep-substrate incubators can provide a number of potential benefits in hatcheries, their use on a commercial scale would present various operational difficulties. Because the routine inspection of stock would not be possible, the number of fish surviving would be largely unknown. In order to control the spread of fungal infection, it would be necessary to administer routine prophyllactic treatments. Furthermore, a study by Pepper and Stansbury (1985) reported water circulation deficiencies associated with the clogging of the interstices within deep-substrate incubators. This could lead to pockets of low dissolved oxygen resulting in high alevin mortality. Salmonid fry may also be reluctant or unable to leave the substrate at the appropriate time. Studies by Brännäs (1989) and Sveier and Raae (1992) have demonstrated that the provision of substrates for Atlantic salmon alevins has a substantial influence on the timing of

first feeding; emergence only occurred when the yolk sacs were almost completely absorbed. Similarly, Bams (1983) reported that chum salmon fry emerged from deep-substrate incubators later than those reared without substrates or in shallow substrates. In order to overcome these difficulties in hatcheries, it is recommended that shallow substrates are used. These provide the main benefits of deep-substrate incubators, but also allow close inspection of the stock by hatchery operators. For these reasons, alevins were provided with a shallow substrate in the present study.

Although a number of studies have attempted to determine the optimal time to initiate feeding in Atlantic salmon (Marr, 1965; Coughlin, 1991; Sveier and Raae, 1992), excepting early studies by Atkins (1905, 1906), there is limited published information available on the consequencies of delayed feeding on fry growth and survival. The present study was conducted to investigate the relationship between the timing of first feeding and the subsequent growth response and survival of Atlantic salmon fry. The specific objectives of this study were to determine:

(a) The optimal timing of first feeding as measured by the survival and growth of fry following feeding.

(b) To what extent, if any, a delay in food introduction contributes to non-feeding and consequent point-of-no-return.

(c) Whether a period of association with food is required for the development of normal feeding behaviour.

(d) To what extent, if any, the specific growth rate of fry deprived of food is greater than that of early-fed

fry, enabling late-fed fry to reduce or eliminate any size advantage gained by those fed early.

The results of this study will contribute to an understanding of the mortality associated with the onset of feeding as well as the growth of fry in relation to the timing of feeding. Fundamental studies of this nature can be expected to contribute to significant economic improvements in salmon rearing practices.

3.2 MATERIALS AND METHODS

3.2.1 SOURCE AND REARING HISTORY OF STOCK

On 27.12.86 Atlantic salmon gametes were stripped manually from wild broodstock caught by electro-fishing in the River Test, Hampshire, England, UK. The eggs originated from a single hen weighing 2.8 kg. To reduce any paternal effects on the performance of fish, the eggs were fertilised using the milt from one male fish. Eggs were fertilised by the standard dry fertilisation method (Leitritz and Lewis, 1976) and, following washing and water hardening, were transported to the hatchery. Two hours after stripping, eggs were received at the hatchery where they were disinfected and measured using the procedure described in section 2.2.1. The mean egg diameter was 5.6 mm. The eggs were divided randomly into three batches of approximately 1500 eggs and each batch was incubated in an expanded aluminium mesh basket (46 x 46 x 7.5 cm) placed in a glass-fibre hatching trough (213 x 46 x 13 cm). Borehole water of constant quality (Table 2.2.2) was supplied at an initial flow rate of 0.4 L/min/1000 eggs, increased to 1 L/min/1000 eggs during the hatching period.

Following hatching (43 days at 10°C to 50% hatch), alevins were provided with a gravel substrate in which they remained until required for experimental work. The various benefits to salmonid alevins of providing a rearing substrate were described in section 2.2.2.4. Egg and alevin mortalities were removed and recorded daily, using the procedure described in section 2.2.3. Before the initiation of feeding, mean egg and

alevin mortalities (±SD) in the hatching trough were 8.1%±1.6 and 1.5%±0.4, respectively.

The water temperature and the dissolved oxygen concentration were determined twice daily using the procedure outlined in section 2.2.2.3. During the course of the study, the mean temperature ±SD was 10.0°C±0.2 (range of 9.6-10.5°C), and the dissolved oxygen concentration was always in excess of 93% air saturation. Cumulative degree-days (sum of mean daily temperatures) were calculated from fertilisation and 50% hatching.

3.2.2 EXPERIMENTAL DESIGN

Fry rearing was conducted using the experimental procedure described in section 2.2.2.2. The fry were reared in systems II (12 x 22 cm diameter tanks) and III (12 x 30 cm diameter tanks) of the experimental facility shown in Figs. 2.2.2 and 2.2.3. The respective water depths in each system were 8.0 and 9.0 cm, and the water flow rate to each tank was maintained at 1 L/min throughout the study.

At the beginning of the study, each of the twelve 22 cm diameter tanks was stocked with 200 sibling fry. In order to accommodate the increasing biomass, after 12 weeks each lot was transferred as a group to a 30 cm diameter tank, selected at random, where they were reared for a further 7 weeks.

3.2.2.1 Feeding regime

Salmon fry were fed on a proprietary diet (Table 2.2.4) at

a feeding rate of 5% of body weight per day using an automatic feed dispenser (Figs. 2.2.2 and 2.2.4) which fed each tank for 12 hours each day (0700-1900h) under a natural photoperiod from March to July. Total stock weight in each tank was re-calculated each time the fish were weighed and the food ration size adjusted accordingly. The procedure used to calculate the stock weight in each tank was described in section 2.2.4.7.

3.2.2.2 Timing of first feeding

Food was first presented to triplicate lots of 200 salmon fry at four different stages (Groups I-IV) of development. The experimental area was divided into three blocks and each block contained a single replication of the experiment. The timing of initial food presentation for each of the four groups (I-IV) was as shown in Table 3.2.1.

To determine the percentage wet weight ratio of yolk to total alevin weight, alevins within a sample (n=10) were given a lethal dose of anaesthetic (1000 ppm 2-phenoxyethanol) before being rinsed in distilled water and individually blotted dry (as described in section 2.2.4.8). Alevins were placed on glass slides of known weight before being weighed to the nearest 0.1 mg on an analytical balance (Sartorius, model number AC120S). The measurement of yolk weight required the dissection of the yolk-sac from each alevin, a process which was conducted on a glass slide of known weight. With the aid of a binocular microscope, the yolk material was carefully dissected from each alevin and

Table 3.2.1 Timing of initial food presentation for Groups I-IV.

Gp	°Days after:		Mean wt.±SD(mg)		%Water content ±SD	%Yolk±SD	
	Fert.	50%hatch	Wet	Dry		Wet	Dry
I	805	375	152.9±7.6	34.4±2.3	77.5±1.1	13±4	37±9
II	978	548	153.8±10.9	27.8±2.5	81.9±0.8	ND	ND
III	1083	653	139.2±6.4	24.1±1.5	82.7±0.5	ND	ND
IV	1230	800	120.5±7.7	19.0±2.3	84.2±1.3	ND	ND

ND: Not determined

the fish reweighed. During yolk removal, the alevin was held by the tail with forceps, whilst a small incision was made behind the liver, close to where the yolk-sac is attached to the alevin posteriorly. Using a dissecting needle, the entire yolk-sac was then stripped off as a unit by gently teasing it towards the anterior of the alevin. Following dissection, each slide was weighed twice: firstly, with alevin and yolk; and secondly with yolk only.

Dry weights were recorded following oven drying (60°C, 48h) and desiccation of the material for 1 hour at 20°C. Desiccation is required to prevent rehydration of the dried sample, which can result in weight gains of 2-4% (Wallace and Aasjord, 1984b). Wet and dry weights of the alevins and yolk-excised alevins were then calculated by subtracting the original weights of the slides. The percentage water content was calculated from the mean wet and dry weights of alevins.

Routine sub-sampling every 2 days (from 350 degree-days after hatching) indicated that a maximum alevin wet weight (MAWW) of 162.35 mg±8.80 (mean±1 SD) and a mean dry weight of 32.21 mg±1.83 was reached 435 degree-days after hatching, when the percentage yolk-to-total-alevin-dry-weight ratio was 11.72%±8.79. A determination of the ratio of yolk to total alevin weight was not made for Groups II-IV because accurate removal of yolk material is difficult during final resorption when the abdominal wall has thickened (Hodson and Blunt, 1986). Alevin and yolk weights can only be measured accurately when the shape of the yolk-sac is still well defined and the yolk easily removed. Groups II-IV were fed after MAWW at which time the yolk was visibly exhausted.

However, yolk may still remain within the body cavity at this time. In practice the actual point of yolk exhaustion occurs sometime after MAWW is reached.

Group IV alevins were fed prior to any mortalities resulting from starvation. Further monitoring of unfed stock continued for 947 degree-days after hatching, when mortalities resulting from severe emaciation had reached 20% of the population. At this time, wet and dry mean weights were 108.16 mg±11.23 and 14.34 mg±2.12, respectively.

3.2.2.3 Growth monitoring and survival

At weekly intervals during the first 7 weeks of the study, and fortnightly thereafter, a sample of fry representing 10% of the population was randomly removed from each tank for estimates of wet weight. Fish were rapidly anaesthetised in a 350 ppm solution of 2-phenoxyethanol, surface-dried on absorbent paper, and individually weighed to within 0.01 g using a Sartorius balance (model number L2200P). At the end of the study, fry were starved for 24 hours prior to final weighing of every individual in each tank. Dry weights of fry were recorded during the first 7 weeks of the study and at its conclusion after 19 weeks. Mortalities were removed and recorded daily. When a fish died in a group, one fish was removed at random from every other lot to maintain the same number of individuals in each tank.

3.2.3 DATA ANALYSIS AND PRESENTATION

Differences between replicate tank fish weights were tested by analysis of variance (ANOVAR) and, where differences were insignificant at the 0.05 level of probability, experimental data were pooled for subsequent comparisons. The various pooled data were tested separately by ANOVAR and where F was significant, differences between pairs of groups then tested by calculating the least significant differences (LSD). During the first 4 weeks of the study, the test weight data from unfed groups were combined prior to testing by ANOVAR against that from fed groups.

At the termination of the experimental period, all fish were individually weighed and a weight-frequency distribution was constructed for each set of data. All distributions were tested for their degree of kurtosis, and skewness was measured by Pearson's 2nd coefficient of skewness (Snedecor and Cochran, 1967):

3 x (mean-median/standard deviation)

Pearson's product moment correlation coefficient (r) was used to measure the degree of linear relationship between wet and dry weights of fry.

Arithmetical line graphs were plotted for mean live fish weights and growth rates (Gw) against time:

$$Gw = \frac{(\log_e Wt - \log_e Wo)}{t} \times 100\%$$

(Bagenal and Tesch, 1978)

where Gw = specific growth rate in % body weight per day; Wo = weight at time 0; Wt = weight at time t; t = time in days. The coefficient of variation (CV) expressed as a percentage, was plotted against time:

CV (%) = (standard deviation/mean) x 100

Percentage mortality data were transformed by an arc sine percentage transformation.

Data analyses were executed using two computerised statistical programmes: Tadpole III (Biosoft Ltd., Cambridge, England, UK) was used for ANOVAR whilst the standard deviation, coefficient of variation, and tests for skewness and kurtosis were evaluated using a Unistat statistical programme (Unisoft Ltd., London, England, UK). Histograms and line graphs were plotted using Harvard Graphics (Software Publishing Europe, London, England, UK).

3.3 RESULTS

3.3.1 GROWTH OF FRY

Growth profiles for salmon fry and changes in the specific growth rate with time in relation to the timing of first feeding are shown in Figs. 3.3.1 and 3.3.2, respectively. ANOVAR and LSDs of growth and specific growth data are presented in Tables 3.3.1 and 3.3.2, respectively. The initial weight gain common to all groups was due to an increase in water content which was 63.0%±1.0 at hatching, rising to 81.0%±0.5 at MAWW. It is clear that the highest mean weight was attained by fry fed prior to complete yolk resorption (Group I). Delaying feeding beyond this time resulted in progressively lower final mean weights (Table 3.3.3). Because no statistically significant differences were found between replicates within each treatment group, the growth data for replicates were pooled before comparisons between treatments were made. From week 2, ANOVAR (Table 3.3.1) showed that there were highly significant differences in growth between the groups ($P<0.001$). Excepting a single sampling on week 17 (Groups II and III, where $P=0.50$), the LSD method showed marked differences between the growth data from each group. Once a size difference was established between groups it was maintained until the end of the experimental period.

From the onset of feeding, Group I showed a slight decrease in specific growth rate before rising gradually to a peak of 2.29% after 7 weeks. Following MAWW, unfed Groups II, III and

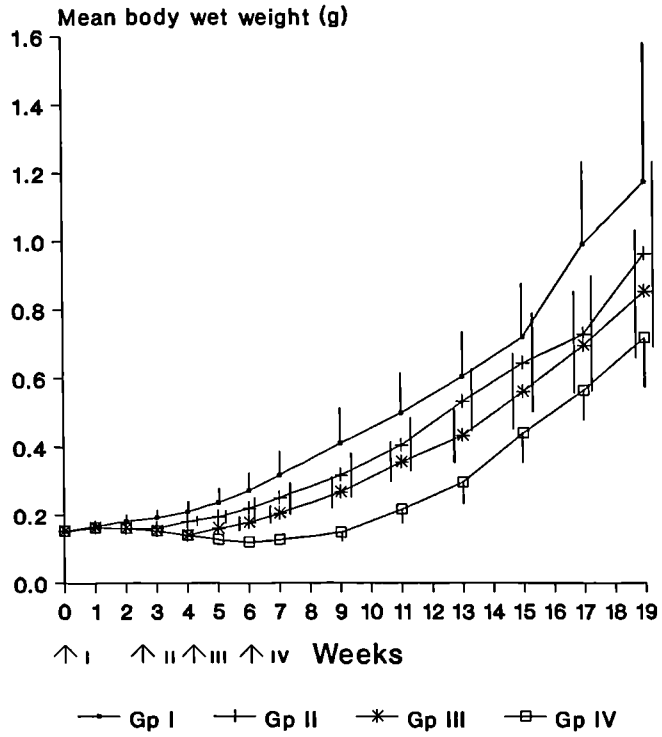

Fig. 3.3.1 Effect of the timing of first feeding on the growth (mean wet weight ±SD) of Atlantic salmon fry. Feeding commenced 375 (Gp I), 548 (Gp II), 653 (Gp III) and 800 (Gp IV) degree-days after 50% hatch. Arrows denote the timing of first feeding (from Koss and Bromage, 1990).

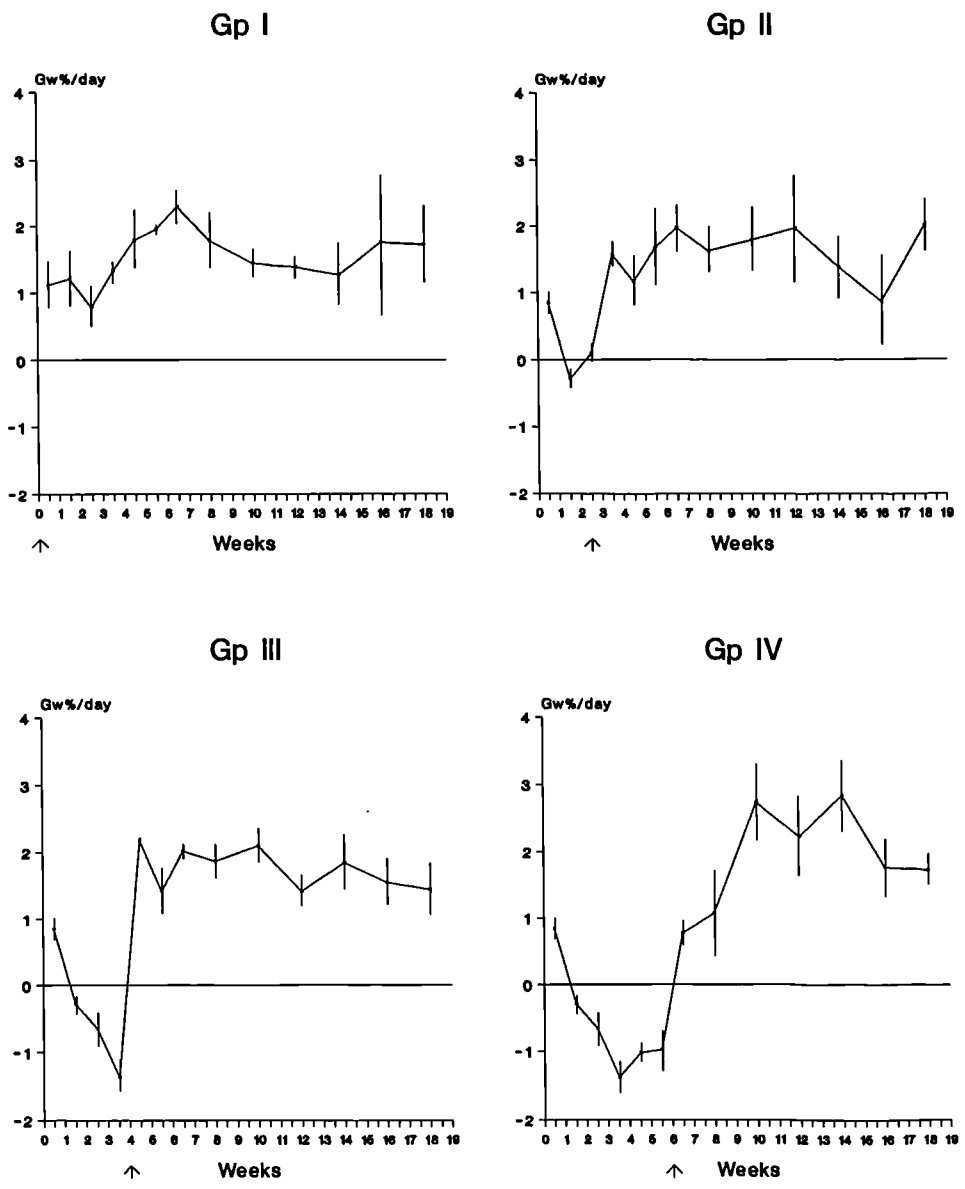

Fig. 3.3.2 Effect of the timing of first feeding on the specific growth rate (%/day ±SD) of Atlantic salmon fry. Feeding commenced 375 (Gp I), 548 (GpII), 653 (Gp III) and 800 (Gp IV) degree-days after 50% hatch. Arrows denote the timing of first feeding.

Table 3.3.1 ANOVAR and least significant differences (LSD) for Atlantic salmon growth data over the 19 weeks of the experiment in relation to the timing of first feeding.

Week	ANOVAR		
	F	Sign.	LSD[a]
1	1.92	NS	NS
2	269.00	P<0.001	-
3	68.35	P<0.001	-
4	284.85	P<0.001	-
5	57.75	P<0.001	-
6	139.94	P<0.001	-
7	195.88	P<0.001	-
9	485.32	P<0.001	-
11	131.82	P<0.001	-
13	103.83	P<0.001	-
15	41.07	P<0.01	-
17	37.97	P<0.01	II&III(P=0.50)
19	57.68	P<0.001	-

[a] Significant unless stated

Table 3.3.2 ANOVAR and least significant differences (LSD) for Atlantic salmon specific growth data over the 19 weeks of the experiment in relation to the timing of first feeding.

Week	ANOVAR		
	F	Sign.	LSD[b]
1	1.35	NS	-
2	33.40	P<0.001	I&II,I&III,I&IV(P<0.001)
3	23.80	P<0.001	I&II(P<0.05); II&III,II&IV(P<0.01); I&III,I&IV(P<0.001);
4	198.25	P<0.001	I&III,I&IV,II&III,II&IV (P<0.001)
5	67.33	P<0.001	I&II(P<0.05); II&III(P<0.01); I&IV,II&IV,III&IV(P<0.001)
6	38.29	P<0.001	I&IV,II&IV,III&IV(P<0.001)
7	25.95	P<0.001	I&IV,II&IV,III&IV(P<0.001)
8	1.98	NS	-
10	5.43	P<0.05	II&IV(P<0.05); I&IV(P<0.01)
12	1.95	NS	-
14	7.20	P<0.05	III&IV(P<0.01); I&IV,II&IV(P<0.001)
16	1.30	NS	-
18	0.89	NS	-

[b] Not significant unless stated

Table 3.3.3 Mean wet weight, standard deviation and number of the four groups (I-IV) of Atlantic salmon fry at the end of the experiment (week 19), and coefficient of variation and analyses of distribution.

Group	I	II	III	IV
Mean (g)	1.17	0.96	0.85	0.72
SD	0.40	0.30	0.22	0.17
n	265	262	258	266
CV (%)	34.36	31.62	26.02	23.91
Skewness	0.47	0.52	0.43	0.34
Kurtosis	4.74	3.89	3.08	3.44

IV lost weight whilst energy for maintenance was derived from the resorption of body tissues. Clearly, the differences in mean specific growth rates between unfed and fed groups were very significant (Table 3.3.2). The specific growth rates in Groups II and III increased sharply as soon as feeding commenced and thereafter remained relatively stable. Following first feeding, Group IV demonstrated 2 weeks of steady growth before rising to a rate of 2.74% during week 10. Once all groups were feeding (weeks 6-19), the specific growth rates of Groups I-III were similar. However, during weeks 10 and 14, the specific growth rate of Group IV was significantly greater than the other groups (Fig. 3.3.2 and Table 3.3.2). The mean values for the specific growth rate from the time of first feeding until the end of the study increased progressively as feeding was delayed: 1.53, 1.60, 1.73 and 1.96% for Groups I-IV, respectively. Excepting the difference in specific growth rate between Groups I and II, these differences were very significant ($P<0.01$). During the final 4 weeks of the study, the growth rates of Groups I and IV were identical. At the end of the study, the mean wet weight of fry in Group I was 18, 27, and 38% higher than that of fry in Groups II, III, and IV, respectively.

The relocation of stock to the system of larger tanks on week 12 had no apparent effect on the growth or survival of fry.

From weight-frequency distributions for final growth data presented in Fig. 3.3.3, it is apparent that later feeding gives rise to a uniformity of size. This is confirmed by a progressive decrease in levels of variation when feeding was

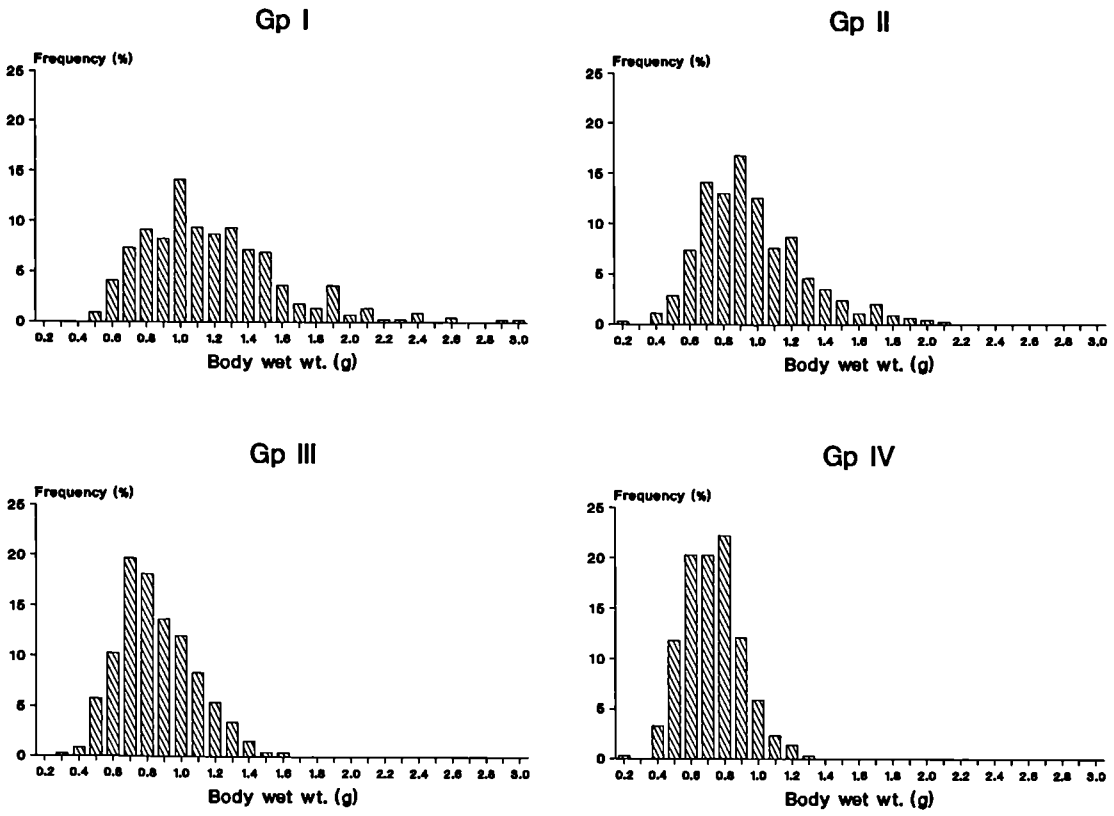

Fig. 3.3.3 Percentage weight-frequency distributions for the four groups of Atlantic salmon fry at the end of the experiment (19 weeks) (from Koss and Bromage, 1990).

delayed. The coefficient of variation (Fig. 3.3.4) increased steadily in all groups once feeding was initiated.

The shape of the weight-frequency distributions seemed little affected by the timing of first feeding. All distributions were leptokurtic and showed slight positive skewing (Table 3.3.3), but none of these were significantly different.

Pearson's product moment correlation coefficient (r) showed a very high statistical association ($P<0.001$) between wet and dry weights for fed fry ($r=0.987\pm0.013$), but for alevins the degree of correlation was lower ($r=0.800\pm0.085$; $P<0.01$).

3.3.2 SURVIVAL OF FRY

Weekly and cumulative weekly percentage fry mortality data are shown in Fig. 3.3.5 and ANOVAR and LSDs of arc sine percentage transformed cumulative mortality data are presented in Table 3.3.4. Because no statistically significant differences were found between replicates within each treatment group, the mortality data for replicates were pooled before comparisons between treatments were made. During the first 6 weeks of the study, mortalities were low in all groups but then showed a marked increase in Groups III and IV, peaking during weeks 10 and 12, respectively. After 10 weeks, the cumulative percentage mortality was significantly higher ($P<0.01$) in Group IV than in each of the other groups. Mortalities increased progressively as the duration of food deprivation was extended. Fry losses were attributed to debilitation caused by severe starvation.

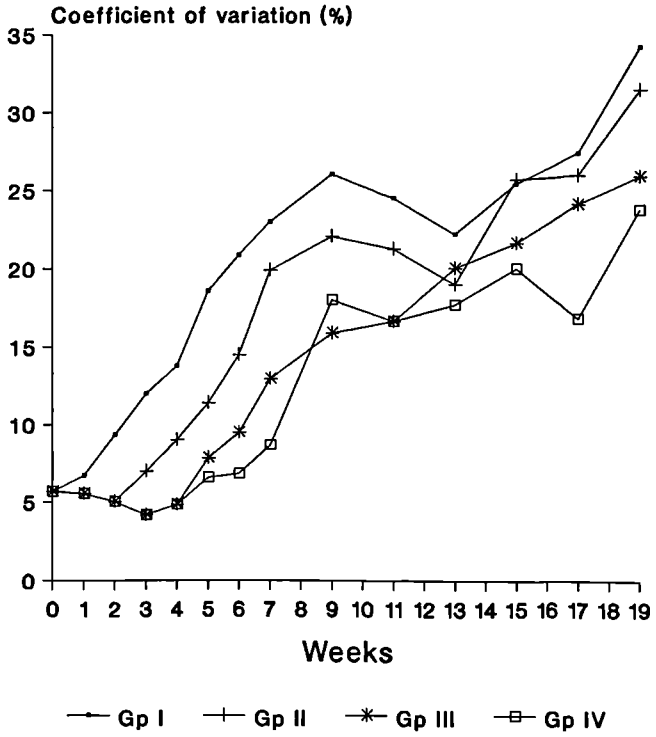

Fig. 3.3.4 Changes in the coefficient of variation for weight with time for the four groups of Atlantic salmon (from Koss and Bromage, 1990).

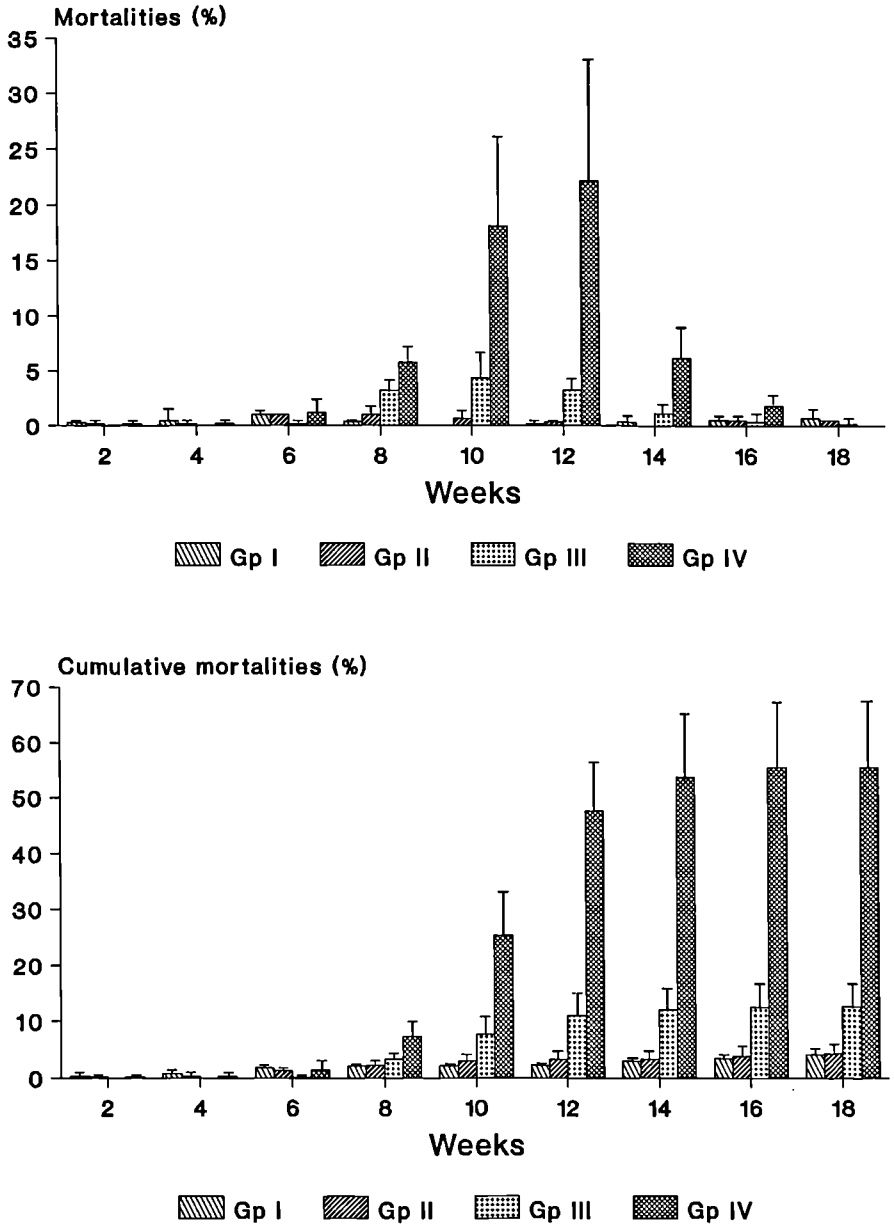

Fig. 3.3.5 Top, 2-weekly and bottom, cumulative mortalities ±SD (%) of the four groups of Atlantic salmon fry (from Koss and Bromage, 1990).

Table 3.3.4 ANOVAR and least significant differences (LSD) of arc sine transformed cumulative mortality data for Atlantic salmon fry over the 19 weeks of the experiment in relation to the timing of first feeding (Groups I-IV).

Week	ANOVAR		
	F	Sign.	LSD[b]
2	0.73	NS	-
4	4.45	NS	-
6	4.29	NS	-
8	9.51	P<0.05	III&IV(P<0.05); I&IV,II&IV(P<0.01)
10	22.79	P<0.01	I&III(P<0.05);III&IV(P<0.01); I&IV,II&IV(P<0.001)
12	55.47	P<0.001	II&III(P<0.05);I&III(P<0.01); I&IV,II&IV,III&IV(P<0.001)
14	52.20	P<0.001	I&III,II&III(P<0.05); I&IV,II&IV,III&IV(P<0.001)
16	50.25	P<0.001	I&III,II&III(P<0.05); I&IV,II&IV,III&IV(P<0.001)
19	48.02	P<0.001	I&III,II&III(P<0.05); I&IV,II&IV,III&IV(P<0.001)

[b] Not significant unless stated

Whilst cumulative mortalities in Groups I and II were very similar throughout the course of the study, significant differences ($P<0.05$) were found between Group III and Groups I and II during weeks 12-18. After 18 weeks, fry losses among all groups had returned to their pre-feeding levels.

3.4 DISCUSSION

3.4.1 GROWTH OF FRY

The present investigation clearly demonstrated that the highest mean weight was achieved when Atlantic salmon fry were fed prior to final yolk resorption. The greater size achieved by fry in Group I at the end of the experiment was primarily attributed to the longer feeding period. Fry in Group I were fed 375 degree-days after 50% hatch (805 degree-days after fertilisation). Similarly, Poston (1988) reported that first feeding at 800 degree-days after fertilisation optimised the growth of Atlantic salmon fry. This is close to the time at which Atlantic salmon are ready to begin feeding; at a constant 10°C, Kane (1988) stated that feeding commenced 360 degree-days after 90% hatch (827 degree-days after fertilisation). A recent study by Sveier and Raae (1992) also showed that growth was higher for Atlantic salmon fed early (259 degree-days after hatch) than for those fed one week later (344 degree-days after hatch). This agrees with the findings from studies conducted with other salmonid species including sockeye salmon (Palmer et al., 1951; Hurley and Brannon, 1969), chum salmon (Bams, 1983), chinook salmon (Palmer et al., 1951; Heming et al., 1982), rainbow trout (Twongo and MacCrimmon, 1976; Escaffre and Bergot, 1985) and Arctic char (Wallace and Aasjord, 1984a, b; Loewe and Eckmann, 1988; Alanärä, 1993).

The initial growth advantage gained by the earliest-fed group (Group I) was maintained throughout the 19 week

experimental period ($P<0.001$). However, because the specific growth rates of the later-fed groups (III-IV) were higher than that of Group I, part of this size difference was lost in the course of time. In a similar study, Twongo and MacCrimmon (1976) found that rainbow trout fry fed 2-4 weeks after swim-up showed the most rapid rate of growth, while Bilton and Robins (1973) reported that sockeye salmon fry deprived of food for 1-3 weeks caught up with the control group within 8 weeks.

Similarly, studies which have examined the growth of fry hatching from eggs of different sizes have shown that although large eggs gave rise to large first-feeding fry, the size difference was not maintained. For the Atlantic salmon, Lindroth (1972) and Thorpe et al. (1984) showed that the size difference was lost through the first year of growth, whilst in rainbow trout, Springate and Bromage (1985) demonstrated that the correlation between egg size and fry weight was lost within 4 weeks of first feeding.

Experiments with fingerling rainbow trout (Weatherley and Gill, 1981) and 9-month-old rainbow trout (Dobson and Holmes, 1984) have also demonstrated that recovery or compensatory growth occurs following periods of restricted rations or starvation. However, Hurley and Brannon (1969) found similar growth rates for sockeye salmon fry fed before and after (approximately 150-750 degree-days after hatch) yolk absorption, and Heming et al. (1982) reported that growth rates of chinook salmon fry were unaffected by the precise timing of first feeding. Differences in the final size of fry were attributed to the initial size difference together with

the extended feeding period of those fed early, rather than to differential growth rates of fry. Similarly, Escaffre and Bergot (1985) found that the end weight of 2-month-old rainbow trout fry was related to their weight at first feeding and the number of feeding days thereafter; fry fed early maintained their growth advantage over those fed late. During the first 8 months of feeding, Brown (1946) found little difference in the specific growth rates of large and small brown trout fry. However, since alevins had different initial weights, larger fry grew larger than smaller fry.

Thus, in studies where fish were subjected to periods of food deprivation, compensatory growth has occurred following feeding. However, in most of these studies the period of compensatory growth was of insufficient magnitude or duration for the starved fish to catch up with the controls. In those studies where the initial size difference of fry was related to egg size, the growth advantage gained by fry derived from large eggs was not maintained during their subsequent growth.

The specific growth rates of Atlantic salmon fry observed in this experiment (mean of 1.71%/day) were similar to those reported in the four series of experiments reported in Chapter 2 (mean of 1.84%/day), but are generally lower than those of other studies (reviewed earlier in section 2.4.2.8).

The observed increased uniformity of size associated with later first feeding is in agreement with the findings of Twongo and MacCrimmon (1976). Fish fed early showed a variable response to food, while among deprived groups of fry an immediate, vigorous feeding response was observed. These results do not support the hypothesis which postulates that

early contact with food provides a learning experience of importance for the development of first feeding behaviour in salmonids (Hurley and Brannon, 1969; Wallace and Aasjord, 1984b; Wallace et al., 1988; Alanärä, 1993). In a study investigating the development of feeding behaviour in Atlantic salmon, Coughlin (1991) also concluded that fry quickly developed an effective feeding response to commercial salmon feed. Although a period of early contact with food has been shown to enhance first feeding success among some salmonid species, particularly the Arctic char, it is not required by Atlantic salmon fry.

The use of body wet weight as a measure of tissue growth proved reliable once fry had commenced feeding. However, the water content of tissues is more variable during the alevin stage when yolk is being converted into hydrated tissue (Gray, 1926; Peterson and Metcalfe, 1977). Hodson and Blunt (1986) found that the conversion of yolk protein to body tissues in rainbow trout involved an increase in percentage water content by more than 50%, leading to a linear increase in the wet weight of alevins. The water content of Atlantic salmon alevins is maximal (82%) at terminal yolk absorption (Peterson and Martin-Robichaud, 1989). Phillips and Dumas (1959) reported a slightly lower water content of 81% in brown trout alevins at final yolk absorption.

Various studies have indicated that the optimal time to first feed Atlantic salmon is when the water content of the fry is approximately 80% (Marr, 1966; Peterson and Martin-Robichaud, 1989; Sveier and Raae, 1992). However, Storebakken and Austreng (1987) found a lower water content of 78.3% for

Atlantic salmon fry at the time of first feeding, indicating that a higher proportion of yolk material was present. It may be that feeding was commenced prematurely in their study. Once feeding is established, the water content remains constant (81%) unless fish are starved. A period of starvation will increase the water content; Escaffre and Bergot (1984) reported that the water content of starved rainbow trout alevins was 91.4%. In the present study, the water content of fry starved for 7 weeks beyond MAWW was 86.7%. Heming (1982) suggested that fork length is a more accurate measure of growth during the alevin stage. Once the endogenous food supply has been exhausted and the fry are feeding on an external food source, the use of total wet weight becomes a more reliable method of monitoring the growth of fry.

3.4.2 SURVIVAL OF FRY

This study showed that provided feeding was commenced within about one week of MAWW (Groups I and II), mortalities were less than 5% during the period of first feeding. Similarly, Poston (1988) reported that optimal survival of Atlantic salmon fry occurred when feeding commenced 800 degree-days after fertilisation (here, Group I was fed 805 degree-days after fertilisation). Although mortalities were higher in fry fed after a period of food deprivation, clear differences only occurred when fry were starved for 3 weeks beyond MAWW (Groups III and IV). Twongo and MacCrimmon (1976) also reported a higher mortality in rainbow trout fry

deprived of food for several weeks after yolk exhaustion, although the differences were not significant, while Heming et al. (1982) did find a marked increase in the mortality of chinook salmon fry when first feeding was delayed until after MAWW. Conversely, Hurley and Brannon (1969) found that sockeye salmon fed prior to yolk exhaustion suffered the highest mortality, with progressively lower losses among alevins fed later. Other studies on brook char and Atlantic salmon (Atkins, 1906), sockeye salmon (Bilton and Robins, 1973), and Arctic char (Wallace and Aasjord, 1984b), have shown little correlation between the timing of first feeding and mortality. Thus, a short-term delay beyond MAWW does not reduce fry survival; only after several weeks of starvation beyond MAWW will mortalities become significantly higher than those fed at the optimal time.

The "point-of-no-return" (Blaxter and Hempel, 1963) beyond which survival could not occur even when food was provided, was reached by over 50% of the population deprived of food for 6 weeks (Group IV). In support of these findings, Atkins (1906), Palmer et al. (1951), Bilton and Robins (1973) and Twongo and MacCrimmon (1976) have demonstrated that fry are tolerant of relatively long periods of food deprivation well beyond MAWW. The larvae of striped bass, Morone saxatilis Walbaum (Doroshev, 1970; Davies, 1973; Rogers and Westin, 1981) and the Californian grunion, Leuresthes tenius Ayres (May, 1971), deprived of a source of exogenous food show a similar resilience. It appears that the oil globule component of the yolk material functions as an energy reserve that can be conserved when food is not available (Dabrowski, 1976;

Eldridge et al., 1981; Loewe and Eckmann, 1988). Heming (1982) noted that the oil globule in chinook salmon alevins was not fully utilised until the remaining yolk was virtually exhausted. The larvae of turbot, Scophthalamus maeoticus (Spectorova et al., 1974), and the snakehead, Channa striatus Bloch (Arul, 1991), show a similar preferential absorption of yolk material. For hatchery-reared Atlantic salmon, the retention of an energy reserve by fry will prolong the period or "window" of opportunity for the development of exogenous feeding. In the wild, this ability to survive initial food deprivation would give Atlantic salmon fry an important survival advantage when the natural food supply is fluctuating or intermittent.

There appears to be little evidence in support of the commonly held supposition that delayed first feeding induces non-feeding with consequent "pinhead" losses: fry have an inherent capacity to withstand several weeks of starvation during which time they retain the ability to feed. However, fry deprived of food much beyond MAWW will suffer a growth penalty.

The interval between yolk absorption and irreversible starvation will be influenced by many factors including temperature, egg size, species and the provision of rearing substrates for alevins. The effect of temperature and egg size on the timing of first feeding has been investigated in chinook salmon (Heming, 1982; Heming et al., 1982; Rombough, 1985). Fry from large eggs reach their maximum size and reabsorb their yolk reserves later than fry from small eggs. The greater yolk reserves associated with larger eggs delay

the irreversible starvation compared with fry from small eggs. Wallace and Aasjord (1984a) stated that the higher growth rate of Arctic char fry from large eggs was explained by their earlier first feeding, and that the low growth rate among small fry was the result of delayed first feeding. Because pinhead mortality was not apparent among fry from large eggs, this condition may be caused by an inadequate yolk supply or possibly a less efficient yolk conversion among small eggs. Although a number of studies have investigated the influence of egg size on fry survival in rainbow trout (Gall, 1974; Pitman, 1979; Springate and Bromage, 1985), chinook salmon (Fowler, 1972) and Arctic char (Wallace and Aasjord, 1984a), comparable studies for Atlantic salmon are scarce. A study by Glebe et al. (1979) showed no correlation between egg size and fry survival for Atlantic salmon; however, the results of this study may have been influenced by genetic effects because eggs were derived from a number of different strains. Clearly, further work is required to determine whether egg size has a significant effect on first feeding success in Atlantic salmon.

A study by MacCrimmon and Twongo (1980) indicated that social interactions (agonistic behaviour) between rainbow trout were responsible for the failure of some fry to initiate feeding. Studies by Brown (1946, 1957) have indicated that variations in size among brown trout fry arise at first feeding when some fry begin feeding earlier than others; these fry acquire an initial advantage in size and become dominant within the population. The existence of social hierarchies can be demonstrated by evidence of an

increasing coefficient of variation with time and the development of a skewed distribution (Purdom, 1974; Jobling and Wandsvik, 1983). In the present study, there were no differences in the coefficient of variation between the various groups and none of the distributions were significantly skewed. Studies by Gunnes (1976) and Jobling and Wandsvik (1983) have indicated that dominance hierarchies start to appear only at a much later stage of development. Gunnes (1976) demonstrated that marked skewing did not develop until more than 6 months after hatching, when the mean weight of fry was approximately 2.5 g. This is further confirmed by studies conducted by Thorpe et al. (1980) and Kristinsson (1984) on the development of bimodality in Atlantic salmon. However, recent studies by Metcalfe and Thorpe (1992b) and Metcalfe (1994) have indicated that differences in dominance status among Atlantic salmon are established within the first few weeks of feeding, when fish with intrinsically higher metabolic rates (irrespective of their initial size) become dominant. Given the apparent effect that dominant fry have in suppressing the feeding behaviour of subordinates, it appears that the failure of some fry to begin feeding can be attributed to the establishment of dominance hierarchies at the time of first feeding. Whether or not salmon fry-rearing methods in hatcheries can be modified to reduce agonistic behaviour, a cause of suppressed feeding behaviour by subordinate fry, is an area of study which requires further investigation.

Experimental initiation of feeding much before MAWW is reached has not been shown to produce any growth or survival

advantage in chinook salmon (Heming et al., 1982) or sockeye salmon fry (Hurley and Brannon, 1969); Harvey (1966) did report an initial weight gain in sockeye salmon, but this growth advantage was only maintained for a short time. Palmer et al. (1951) also found that feeding before complete yolk absorption had little effect on the growth rates of sockeye salmon or chinook salmon. For optimal growth and survival in Atlantic salmon (Marr, 1965) and chinook salmon (Heming, 1979; Rombough, 1985) it is recommended that alevins are fed at MAWW. Marr (1966) defined experimentally that MAWW occurs in Atlantic salmon alevins when the tissue-to-alevin-dry-weight ratio is about 0.85.

The attainment of MAWW is associated with the disappearance of the oesophageal mucus plug which occludes the alimentary tract anterior to the pneumatic duct (Battle, 1942; Prakash, 1961; Twongo and MacCrimmon, 1977; Bardonnet et al., 1993) and with the time at which taste buds mature (Twongo and MacCrimmon, 1976, 1977). MAWW also coincides with the development of certain behavioural characteristics indicative of feeding activity (MacCrimmon and Twongo, 1980). At this time, salmonid alevins switch from a strong to a weak negative phototaxis (Stuart, 1953; Woodhead, 1957; Ali, 1975; Mason, 1976; Dill, 1977). Heming (1982) demonstrated that chinook salmon emerged from simulated redds at a stage close to MAWW. Although various studies have determined the optimal timing of first feeding in salmonid fry in relation to a number of events including fry emergence from gravel redds and substrates, swim-up, and yolk-sac depletion, it would appear that the timing of these events broadly coincide with

the attainment of MAWW. Although MAWW is a good indicator for establishing the optimal timing of first feeding, it does require several measurements during development to determine a functional relationship.

Although premature feeding commonly occurs in hatcheries, it appears to offer little, if any, benefit to alevins. Indeed, the practice of feeding alevins much before yolk exhaustion is highly questionable in view of several studies which have associated premature feeding with increased mortalities (Atkins, 1905; Hurley and Brannon, 1969; Hansen and Mϕller, 1985) and reduced growth (Hansen and Torrissen, 1985). Twongo and MacCrimmon (1977) implied that premature feeding can result in tissue hyperplasia of the oropharyngeal mucosa and gill epithelium. Ochiai et al. (1977) found that premature feeding of ayu (Plecoglossus altivelis) resulted in the entry of food particles into the pneumatic duct during initial filling of the swim bladder, which apparently gave rise to bacterial infection of the swim bladder and alimentary canal. If the digestive system of salmonids reaches full maturity only at yolk exhaustion (Prakash, 1961), it is conceivable that feeding before this time will lead to further disease problems in the alimentary tract.

Premature feeding at this time will also lead to the accumulation of uneaten food in tanks, causing a deterioration in water quality by way of increased suspended solids, ammonia, and biochemical oxygen demand. This will further predispose fry to the development of bacterial gill disease - a condition which commonly occurs in salmonid hatcheries and is associated with high levels of suspended

solids which physically abrade the delicate oropharyngeal mucosa (Twongo and MacCrimmon, 1977) and gill epithelium. The consequent congestion of the pharynx and gills resulting from a combination of tissue hyperplasia, the accumulation of mucus and bacterial disease leads to respiratory stress.

These studies show that the first feeding of Atlantic salmon much before the attainment of MAWW offers little or no benefit to alevins, but instead predisposes them to various gill problems which can lead to reduced growth and mortality. Therefore, if feeding can be delayed without any significant effects on the survival or growth of fry, the incidence of these problems will be reduced.

3.5 CONCLUSIONS

The mean weight of fry was highest when feeding commenced prior to final yolk-sac absorption and close to the attainment of MAWW. The results presented in this study do not support the concept that delayed food presentation induces non-feeding or stunting. A short-term delay beyond MAWW did not affect mortality and feeding commenced as soon as food was available. Fry subjected to several weeks of fasting still retained the ability to feed. However, after 5 weeks of food deprivation, less than 50% of fry retained the capacity to recover from starvation, even though the fry can survive for more than 4 weeks thereafter. Undoubtedly, a more prolonged period of fasting would have led to severe debilitation and a general inability to feed.

Although the specific growth rates of deprived groups demonstrated a period of enhanced, compensatory growth, this did not negate the initial growth advantage of early-fed groups of fry.

The majority of "pinhead" mortalities commonly associated with the failure of fry to initiate feeding may be caused more by a combination of respiratory tissue hyperplasia, the accumulation of mucus and consequent bacterial disease than from an inability to commence feeding induced by a brief period of food deprivation. The results of recent studies also indicate that non-feeding may be caused by agonistic behaviour between fry at first feeding, which may suppress the normal feeding activity of subordinate fry.

The optimal timing of first feeding will be when a high

proportion of the stock is capable of feeding, and prior to any significant suppression of growth or point-of-no-return when irreversible starvation will have begun. First feeding Atlantic salmon fry at this time will maximise the growth and survival of fry, thereby reducing the cost of smolt production by increasing the proportions of potential S½s and S1s in the stock.

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APPENDIX

Publication

A copy of the attached scientific paper entitled "Influence of the timing of initial feeding on the survival and growth of hatchery-reared Atlantic salmon (Salmo salar L.)" was published in the international journal of "Aquaculture" in 1990. It is based on the experimental work reported in Chapter 3 of this thesis.

Aquaculture, 89 (1990) 149–163
Elsevier Science Publishers B.V., Amsterdam

Influence of the timing of initial feeding on the survival and growth of hatchery-reared Atlantic salmon (*Salmo salar* L.)

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(Accepted 20 December 1989)

ABSTRACT

Koss, D.R. and Bromage, N.R., 1990. Influence of the timing of initial feeding on the survival and growth of hatchery-reared Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 89: 149–163.

This study investigated the effects of timing of initial feeding on growth and survival of Atlantic salmon (*Salmo salar* L.) fry. Food was first presented to triplicate groups of 200 salmon fry at four different stages in development (375, 548, 653, 800 degree-days post-hatching). Alevins fed prior to final yolk resorption were significantly larger and had lower mortalities than those fed after the stage at which the maximum alevin wet weight (MAWW) was achieved (circa 435 degree-days post-hatching). Although the "window" of initial feeding opportunity lasted several weeks, delaying feeding much beyond MAWW reduced absolute growth. A 5-week delay led to mortalities which approached 60%. However, initial feeding can be delayed 1–2 weeks at 10°C without adversely affecting subsequent survival or specific growth rate.

INTRODUCTION

The production of low-cost, high-quality Atlantic salmon (*Salmo salar* L.) smolts for stock enhancement programmes, ocean ranching and especially sea water ongrowing systems is currently of great importance.

In commercial hatcheries the prime objective is to produce large parr which will attain smolt status after 1 year. This is because 1-year-old smolts (S1) are preferred to smolts from older age classes (S2, S3...) primarily because S1s only occupy the freshwater facilities for 1 year and hence are cheaper to produce. Additionally, S1 smolts produce proportionally fewer grilse than S2s or S3s (Ritter, 1975); in ranching programmes they yield as many adult returns as do S2 smolts of the same size (Peterson, 1973). There are also indications that S1 smolts have a greater resistance to furunculosis (*Aeromonas*

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0044-8486/90/$03.50

salmonicida) than S2s (Piggins, 1986). Size is a key factor determining the age at which salmon smoltify. Parr must reach a critical threshold size before smoltification can proceed (Elson, 1957; Parry, 1960; Johnston and Eales, 1970; Saunders and Henderson, 1970; Knutson and Grav, 1975; Wedemeyer et al., 1980; Mahnken et al., 1982). Parr failing to reach this minimum size will remain as parr until the following spring. Thorpe et al. (1980, 1982) postulated that parr must reach a size-related developmental stage before entering a faster growing phase in the autumn and subsequently smoltify in the spring. Hence the decision whether or not to smoltify in the spring is taken 8–9 months before the event.

Although in recent years there have been very significant improvements in the survival and quality of reared Atlantic salmon smolts there is still considerable scope for improvement particularly during the critical first feeding period during which time the salmon farming industry still experiences significant mortality problems (Hansen and Moller, 1985). Refstie (1979) considers that an initial feeding mortality of 20% is an "acceptable" loss at this time. Bergstrom (1973) reported that Atlantic salmon fry commonly suffer mortalities of 20–50% during their first 5 weeks of feeding. Similarly, Rumsey and Ketola (1975) and Lemm (1983) reported survivals which were often less than 70%. There is no published information of first feeding success in the salmon farming industry although it is generally thought to be approximately 80–90%.

There is extensive literature on the critical transition to exogenous feeding dating back over a century to the time when salmonid culture first began (Green, 1878; Porter, 1878; Thomas, 1879; Henshall, 1904; Atkins, 1905, 1906). Whilst numerous investigations have studied Pacific salmon species (genus *Oncorhynchus*), relatively few data exist for the Atlantic salmon.

The objective of the present study was to investigate the relationship between the timing of initial feeding and the growth response and survival of Atlantic salmon fry. Fundamental studies of this nature can be expected to contribute to significant economic improvements in salmon-rearing practices.

MATERIALS AND METHODS

Source and rearing history of stock

Atlantic salmon (*Salmo salar* L.) ova derived from wild broodstock caught by electro-fishing were fertilised by the standard dry fertilisation method and following washing and water hardening were incubated in expanded aluminium mesh baskets placed in a California fibre-glass trough. Borehole water of constant quality (Table 1) was supplied at an initial flow rate of 0.4 l/min/1000 ova, increased to 1 l/min/1000 ova during the hatching period. Follow-

TABLE 1

Water quality analyses of borehole supply (mg/l unless otherwise stated)

Dissolved oxygen	11.4	Total Zn	0.0068
Temperature (°C)	10.6	Total Fe	0.020
pH	7.69	Total Cd	0.0016
Alkalinity	244	Total Cr	0.0008
Calcium	87.10	Total Cu	0.0083
Ammoniacal N	0.010	Total Pb	0.0009
O-phosphate	0.010	Total Ni	0.0012
Nitrate	9.970	Total Mn	0.010
Nitrite	0.010	Magnesium	1.82
Chloride	16.3	Sodium	7.62
Conductivity (μs/cm)	571	Potassium	0.010

ing hatching (43 days at 10°C to 50% hatch) alevins were provided with a gravel substrate in which they remained until required for experimental work.

Experimental design

The experimental facility comprised two banks of twelve identical rearing tanks with internal diameters of 22.5 cm and 30.5 cm and respective depths of 14.0 cm and 16.5 cm. The tanks were made of uPVC, smooth on the inside with self-coloured industrial grey base and sides. An external vertical standpipe drain system similar to that described by Parker and Jackson (1980) was incorporated to allow accurate standardisation of water depths (8.0 cm and 9.0 cm, respectively) together with a rapid draw-down for routine flushing.

Water entered each tank tangentially through a single 5-mm orifice. Water and detritus were voided from each tank by a central vertically screened drain. A low-velocity current maintained by a water flow rate of 1 l/min to each tank facilitated food distribution and provided an efficient self-cleaning action.

At the beginning of the study each of the twelve 22.5 cm tanks was stocked with 200 sibling fry. After 12 weeks each lot was transferred as a group to a 30-cm tank system, selected at random, where they were reared for a further 7 weeks.

Automatic feeding system

Salmon fry were fed on a standard commercially available diet (BP Nutrition) at a feeding rate recommended by the manufacturer using an automatic feeder which fed each tank for 12 h each day (07.00–19.00 h) under a natural photoperiod from March to July. Total stock weight in each tank was recalculated each time the fish were weighed and the food ration size adjusted accordingly.

TABLE 2

Timing of initial food presentation for groups I–IV

Group	Degree-days from 50% hatch	Mean wt. ± s.d. (mg)		% Water content	% Yolk ± s.d.	
		wet	dry		wet	dry
I	375	152.9 ± 7.6	34.4 ± 2.3	77.5 ± 1.1	13 ± 4	37 ± 9
II	548	153.8 ± 10.9	27.8 ± 2.5	81.9 ± 0.8	ND	ND
III	653	139.2 ± 6.4	24.1 ± 1.5	82.7 ± 0.5	ND	ND
IV	800	120.5 ± 7.7	19.0 ± 2.3	84.2 ± 1.3	ND	ND

Timing of initial feeding

Food was first presented to triplicate lots of 200 salmon fry at four different stages (groups I–IV) in development. The experimental area was divided into three blocks and each block contained a single replication of the experiment. the timing of initial food presentation for each of the four groups (I–IV) was as shown in Table 2. To determine the percentage wet weight ratio of yolk to total alevin weight, alevins within a sample were given a lethal dose of anaesthetic before being individually blotted dry and weighed to the nearest 0.1 mg on an analytical balance. With the aid of a binocular microscope the yolk material was carefully dissected from each alevin and the fish reweighed. Dry weights were recorded following oven drying (60°C, 48 h) and desiccation of the material.

Routine subsampling indicated that a maximum alevin wet weight (MAWW) of 162.35 mg ± 8.80 (mean ± 1 s.d.) and a mean dry weight of 32.21 mg ± 1.83 was reached 435 degree-days post-hatching when the percentage dry weight ratio of yolk to total alevin was 11.72% ± 8.79. A determination of the ratio of yolk to total alevin weight was not made for groups II–IV because accurate removal of yolk material is difficult during final resorption when the abdominal wall has thickened. Groups II–IV were fed after MAWW at which time the yolk was visibly exhausted. In practice the actual point of yolk exhaustion occurs sometime after MAWW is reached.

Group IV alevins were fed prior to any mortalities resulting from starvation. Further monitoring of unfed stock continued for 947 degree-days post-hatching when mortalities resulting from severe emaciation had reached 20% of the population. At this time wet and dry mean weights were 108.16 mg ± 11.23 and 14.34 mg ± 2.12, respectively.

Growth monitoring and survival

At weekly intervals during the initial 7 weeks of the study and fortnightly thereafter a sample of fry representing 10% of the population was randomly removed from each tank for estimates of wet weight. Fish were rapidly anaesthetised in a 350 ppm solution of 2-phenoxyethanol, surface-dried on ab-

sorbent paper, and individually weighed to within 0.01 g. At the end of the study, fry were starved for 24 h prior to final weighing of every individual in each tank. Dry weights of fry were recorded during the first 8 weeks of the study and at its conclusion after 19 weeks. Mortalities were removed and recorded daily. When a fish died in a group, one fish was removed at random from every other lot to maintain the same number of individuals in each tank.

Data analysis

Differences between replicate tank fish weights were tested by analysis of variance (ANOVAR) and where differences were insignificant at the 0.05 level of probability experimental data were pooled for subsequent comparisons. The various pooled data were tested separately by ANOVAR and where F was significant, differences between pairs of groups then tested by calculating the least significant differences (LSD). During the first 4 weeks of the study the test weight data from unfed groups were combined prior to testing by ANOVAR against that from fed groups.

At the termination of the experimental period all fish were individually weighed and a weight–frequency distribution was constructed for each set of data. All distributions were tested for their degree of kurtosis, and skewness was measured by Pearson's 2nd coefficient of skewness (Snedecor and Cochran, 1967): $=3\times$ (mean − median/standard deviation).

Pearson's product moment correlation coefficient (r) was used to measure the degree of linear relationship between wet and dry weights of fry.

Arithmetical line graphs were plotted for mean live fish weights and growth rates (G_w) against time:

$$G_w = \frac{\log_e W_t - \log_e W_o}{t} \times 100\%$$

G_w = specific growth rate in % body weight per day; W_o = weight at time 0; W_t = weight at time t; t = time in days. The coefficient of variation (CV) expressed as a percentage, was plotted against time:
CV (%) = (standard deviation/mean) × 100.

Percentage mortality data were transformed by an arcsin percentage transformation.

Data analyses were performed using two computer statistical packages. ANOVAR was run on a Tadpole III Statistics Package (Biosoft Ltd., 1987) while the standard deviation, coefficient of variation and tests for skewness and kurtosis were performed on a Unistat Statistics Package (Unisoft Ltd., 1984).

RESULTS

Growth

Growth profiles for salmon fry and changes in the specific growth rate with time in relation to the timing of initial feeding are shown in Fig. 1. The initial

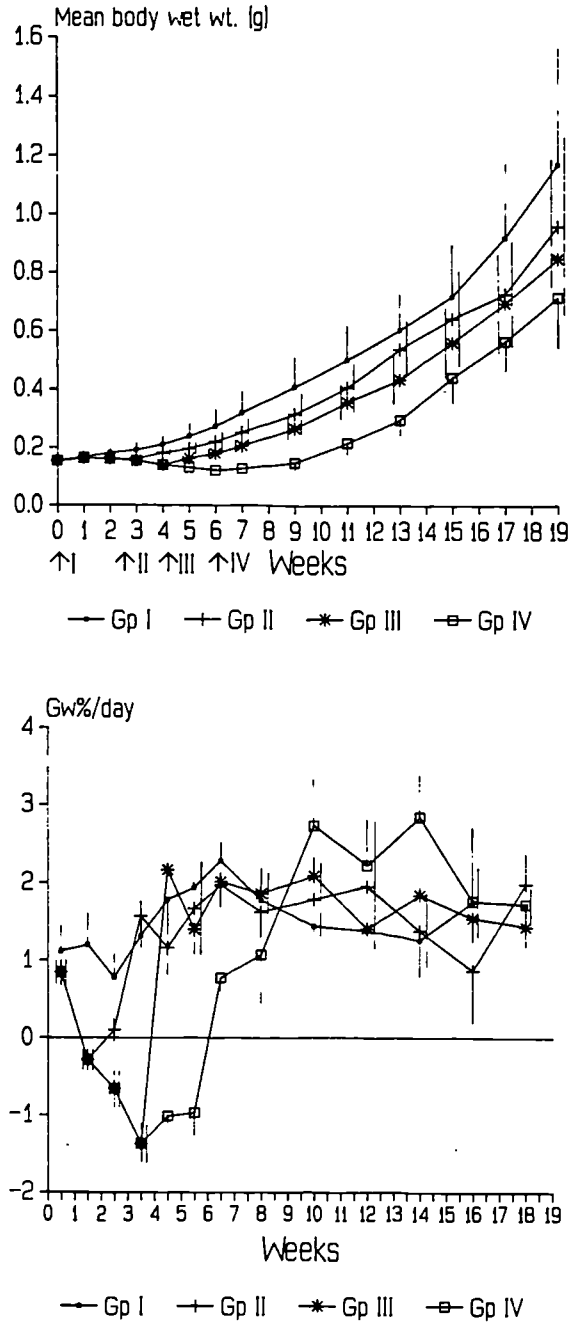

Fig. 1. Top, effect of the time of initial feeding (groups I–IV) on the growth (mean wet weight ± s.d.) of Atlantic salmon fry; bottom, changes in specific growth rate ± s.d. with time. Arrows denote the timing of initial feeding.

weight gain common to all groups was due to an increase in water content which was 63.0% ± 1.0 at hatching, rising to 81.0% ± 0.5 at MAWW. It is clear that the highest mean weight was attained by fry fed prior to complete yolk resorption (group I). Delaying feeding beyond this time resulted in progressively lower final mean weights (Table 3). From week 2, ANOVAR showed

TABLE 3

Mean wet weight, standard deviation and number of the four groups (I–IV) of Atlantic salmon fry at the end of the experiment (week 19)

Group	I	II	III	IV
Mean (g)	1.17	0.96	0.85	0.72
s.d.	0.40	0.30	0.22	0.17
n	265	262	258	266

that there were highly significant differences in growth between the groups ($P<0.001$). Excepting a single sampling on week 17 (groups II and III, where $P=0.50$), the LSD method showed marked differences between the growth data from each group. Once a size difference was established between groups it was maintained until the end of the experimental period.

From the onset of feeding, group I showed a slight decrease in specific growth rate before rising gradually to a peak of 2.29% after 7 weeks. Following MAWW, unfed groups II, III and IV lost weight whilst energy for maintenance was derived from the resorption of body tissues. The specific growth rates in groups II and III increased sharply as soon as feeding was commenced and thereafter remained relatively stable.

Following initial feeding group IV demonstrated 2 weeks of steady growth before rising to a rate of 2.74% during week 10. Although the mean values for the specific growth rate from the time of initial feeding until the end of the study increased progressively as feeding was delayed (1.53, 1.60, 1.73 and 1.96% for groups I–IV respectively), the growth rates of groups I and IV were identical during the final 4 weeks of the study.

The relocation of stock to the system of larger tanks on week 12 had no apparent effect on the growth or survival of fry.

From weight–frequency distributions for final growth data presented in Fig. 2 it is apparent that later feeding gives rise to a uniformity of size. This is confirmed by a progressive decrease in levels of variance as feeding was delayed. The coefficient of variation (Fig. 3) increased steadily in all groups once feeding was initiated.

All distributions were leptokurtic and showed slight positive skewing but none of these were significantly different.

Pearson's product moment correlation coefficient (r) showed a very high statistical association ($P<0.001$) between wet and dry weights for fed fry ($r=0.987\pm0.013$) but for alevins the degree of correlation was lower ($r=0.800\pm0.085; P<0.01$).

Survival

Weekly and cumulative weekly percentage fry mortality data are shown in Fig. 4 and ANOVAR and LSDs of transformed cumulative mortality data are

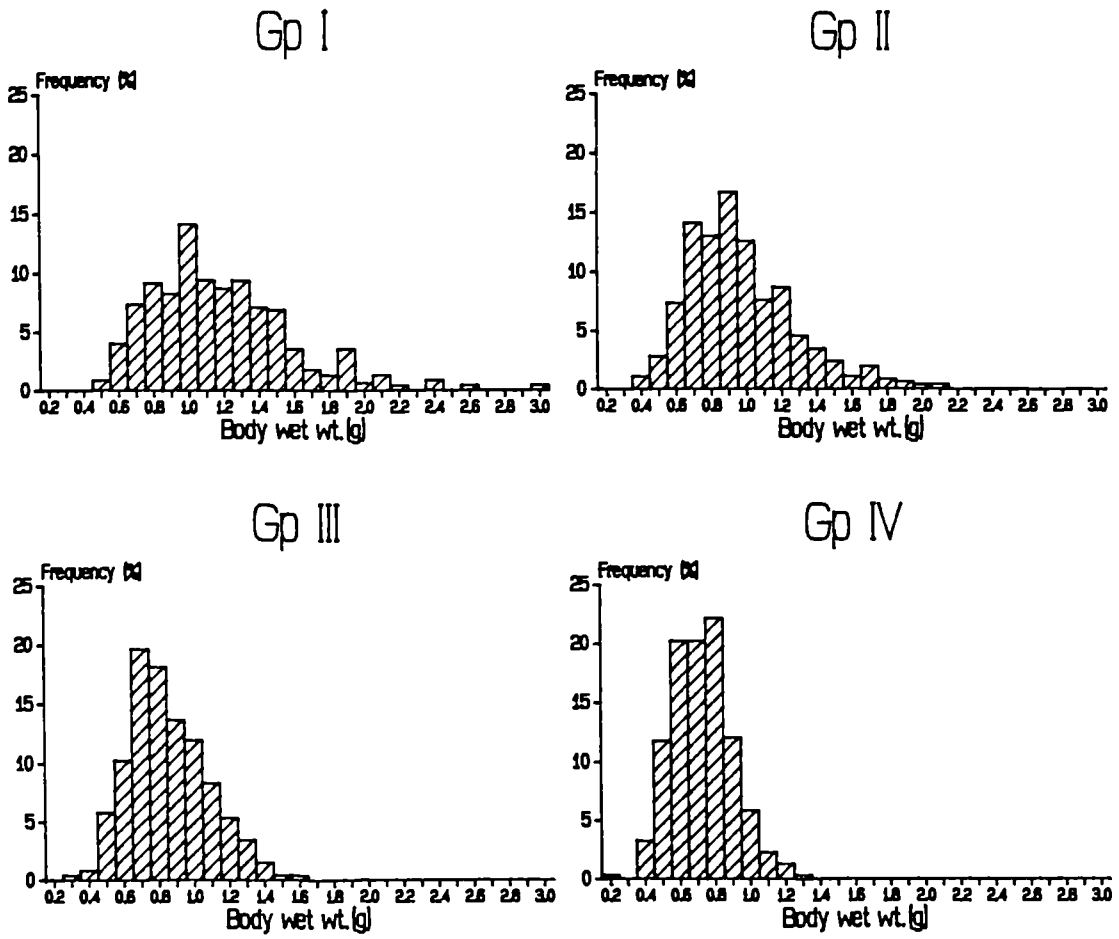

Fig. 2. Percentage weight–frequency distributions for four groups of Atlantic salmon fry at the end of the experiment (19 weeks).

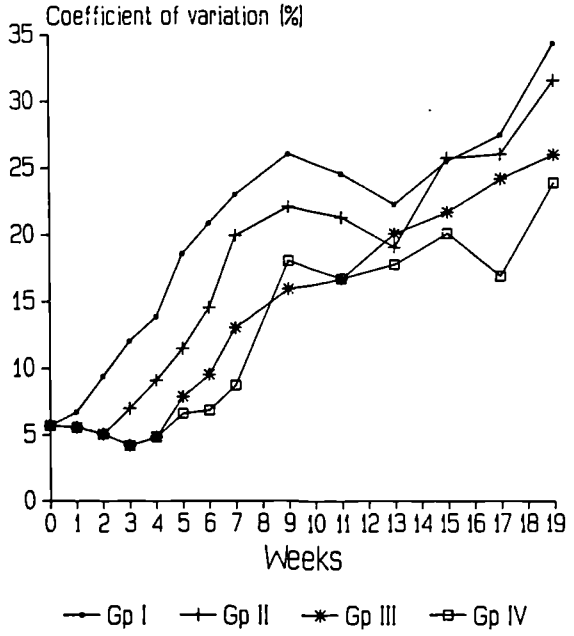

Fig. 3. Changes in the coefficient of variation for weight with time for the four groups of Atlantic salmon.

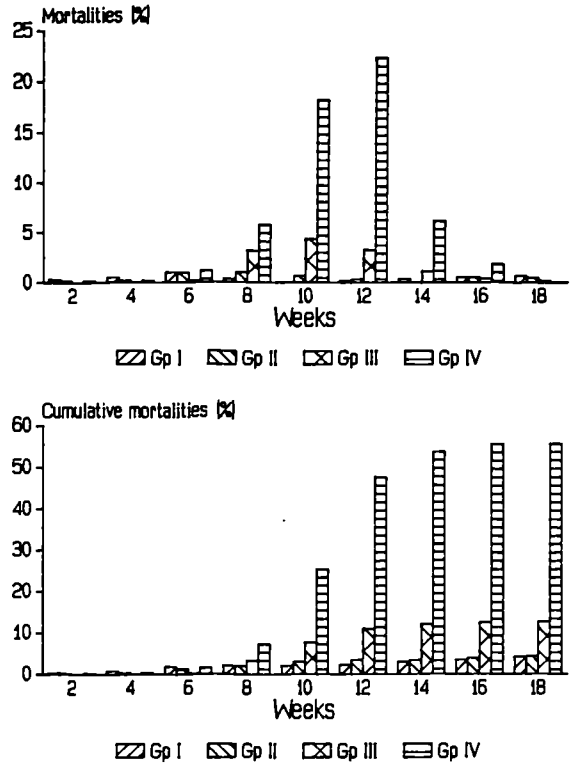

Fig. 4. Top, 2-weekly and bottom, cumulative mortalities (%) of the groups of Atlantic salmon fry.

TABLE 4

ANOVAR and least significant differences (LSD) of arcsin-transformed cumulative mortality data for Atlantic salmon fry over the 19 weeks of the experiment in relation to the timing of initial feeding (groups I–IV)

Week	ANOVAR		LSD[a]
	F	Sign	
2	0.73	n.s.	–
4	4.45	n.s.	–
6	4.29	n.s.	–
8	9.51	$P<0.05$	III & IV($P<0.05$); I & IV, II & IV($P<0.01$)
10	22.79	$P<0.01$	I & III($P<0.05$); III & IV($P<0.01$); I & IV, II & IV($P<0.001$)
12	55.47	$P<0.001$	II & III($P<0.5$); I & III($P<0.01$); I & IV, II & IV, III & IV($P<0.001$)
14	52.20	$P<0.001$	I & III, II & III($P<0.05$); I & IV, II & IV, III & IV($P<0.001$)
16	50.25	$P<0.001$	I & III, II & III($P<0.05$); I & IV, II & IV, III & IV($P<0.001$)
19	48.02	$P<0.001$	I & III, II & III($P<0.05$); I &IV, II & IV, III & IV($P<0.001$)

[a]Not significant unless stated.

presented in Table 4. During the first 6 weeks of the study, mortalities among all groups were low in number but then showed a marked increase in groups III and IV, peaking during weeks 10 and 12, respectively. After 10 weeks, the cumulative percentage mortality was significantly higher ($P<0.01$) in group IV than in any of the other groups. Mortalities increased progressively as the duration of food deprivation was extended. Fry losses were attributed to debilitation caused by severe starvation. Whilst cumulative mortalities among groups I and II were very similar throughout the course of the study, significant differences ($P<0.05$) were found between group III and groups I and II during weeks 12–18. After 18 weeks, fry losses among all groups had returned to their pre-feeding levels.

DISCUSSION

The present investigation clearly demonstrated that the highest mean weight was achieved when Atlantic salmon fry were fed prior to final yolk resorption. This is in agreement with studies conducted with sockeye salmon, *Oncorhynchus nerka* (Hurley and Brannon, 1969), chinook salmon, *Oncorhynchus tshawytscha* (Heming et al., 1982), sockeye and chinook salmon (Palmer et al., 1951) and with rainbow trout, *Oncorhynchus mykiss* (Twonge and MacCrimmon, 1976).

The initial weight advantage gained by the earliest-fed group was maintained throughout the 19-week experimental period ($P<0.001$). However, because the specific growth rate of the later-fed groups (III–IV) was higher than that of group I, part of this size advantage was lost in the course of time. In a similar study Twongo and MacCrimmon (1976) found that rainbow trout alevins fed 2–4 weeks after swim-up showed the most rapid rate of growth while Bilton and Robins (1973) reported that sockeye salmon alevins deprived of food for 1–3 weeks caught up with the control group by the end of 8 weeks. However, Hurley and Brannon (1969) found similar growth rates for sockeye salmon fry fed before and after (approximately 150–750 degree-days post-hatching) yolk absorption and Heming et al. (1982) reported that growth rates of chinook fry were unaffected by the precise timing of initial food presentation.

The observed increased uniformity of size associated with later initial feeding is in agreement with the findings of Twongo and MacCrimmon (1976). Fish fed early showed a variable response to food while among deprived groups of fry an immediate, vigorous feeding response was observed. These results do not support the hypothesis of Hurley and Brannon (1969) that early contact with food provides a learning experience of importance for the development of initial feeding behavior.

The use of body wet weight as a measure of tissue growth proved reliable once fry had commenced feeding. However, the water content of tissues is

more variable during the alevin stage when yolk is being converted into hydrated tissue. Heming (1982) suggested that fork length is a more accurate measure of growth during this period.

Although mortalities were higher in fry fed after a period of food deprivation, clear differences occurred only when fry were starved for 3 weeks beyond MAWW (groups III and IV). Twongo and MacCrimmon (1976) also reported a higher mortility in rainbow trout fry deprived of food for several weeks after yolk exhaustion although the differences were not significant, while Heming et al. (1982) did find a marked increase in the mortality of chinook salmon fry when initial feeding was delayed until after MAWW. Conversely, Hurley and Brannon (1969) found that sockeye salmon fed prior to yolk exhaustion suffered the highest mortality with progressively lower losses among alevins fed later. Other studies on the brook char (*Salvelinus fontinalis* L.) and Atlantic salmon (Atkins, 1906), sockeye salmon (Bilton and Robins, 1973), and Arctic char, *Salvelinus alpinus* L. (Wallace and Aasjord, 1984) have shown little correlation between the timing of the initiation of feeding and mortality.

The "point of no return" (Blaxter and Hempel, 1963) beyond which survival could not occur even when food was provided was reached by over 50% of the population deprived of food for 6 weeks (group IV). In support of these findings Atkins(1906), Palmer et al. (1951), Bilton and Robins (1973) and Twongo and MacCrimmon (1976) have demonstrated that alevins are tolerant of relatively long periods of food deprivation well beyond MAWW. Striped bass (*Morone saxatilis*) larvae deprived of a source of exogenous food show a similar resilience (Doreshev, 1970; Davies, 1973; Rogers and Westin, 1981). In explanation, Eldridge et al. (1981) postulated that the oil globule component of the yolk material functions as an energy reserve that can be conserved when food is not available. Heming (1982) noted that the oil globule in chinook salmon alevins was not fully utilised until the remaining yolk was virtually exhausted. Turbot (*Scophthalmus maeoticus*) larvae show a similar preferential absorption of yolk material (Spectorova et al., 1974).

There appears to be little evidence in support of the commonly held supposition that delayed initial feeding induces non-feeding with consequent "pinhead" losses. Alevins have an inherent capacity to withstand several weeks of starvation during which time they retain the ability to feed. However, fry deprived of food much beyond MAWW will suffer a growth penalty.

Experimental initiation of feeding much before MAWW is reached has not been shown to produce any growth or survival advantage in chinook (Heming et al., 1982) or sockeye fry (Hurley and Brannon, 1969) although Harvey (1966) did report an initial weight gain in sockeye salmon but this growth advantage was only maintained for a short time. Palmer et al. (1951) also found that feeding before complete yolk absorption has little effect on the growth rate of sockeye and chinook salmon.

For optimum growth and survival in Atlantic salmon (Marr, 1965) and chinook salmon (Heming, 1979; Rombough, 1985) it is recommended that alevins are fed at MAWW. Marr (1966) defined experimentally that MAWW occurs in Atlantic salmon alevins when the embryo dry weight to total alevin weight ratio is about 0.85. The attainment of MAWW is associated with the disappearance of the oesophageal plug which occludes the alimentary tract anterior to the pneumatic duct (Battle, 1942; Prakash, 1961; Twongo and MacCrimmon, 1977) and with the time at which taste buds mature (Twongo and MacCrimmon, 1976, 1977). MAWW also coincides with the development of certain behavioural characteristics indicative of feeding activity (MacCrimmon and Twongo, 1980). At this time alevins switch from a strong to a weak negative phototaxis (Mason, 1976; Dill, 1977). Heming (1982) demonstrated that chinook salmon emerged from simulated redds at a stage close to MAWW. Although precocious feeding commonly occurs in hatcheries, when feed is presented prematurely it appears to offer little if any benefit to alevins. Indeed, the practice of feeding alevins much before yolk exhaustion is highly questionable in view of several studies which have associated premature feeding with increased mortalities (Atkins, 1905; Hurley and Brannon, 1969; Hansen and Moller, 1985) and reduced growth (Hansen and Torrissen, 1985). Twongo and MacCrimmon (1977) implied that premature feeding can result in tissue hyperplasia of the oropharyngeal mucosa and gill epithelium. Ochiai et al. (1977) found that premature feeding of ayu (*Plecoglossus altivelis*) resulted in the entry of food particles into the pneumatic duct during intial filling of the swim bladder which apparently gave rise to bacterial infection of the swim bladder and viscera. If the digestive system of salmonids only reaches full maturity at yolk exhaustion (Prakash, 1961) it is conceivable that feeding before this time will lead to further disease problems in the alimentary tract.

The majority of "pinhead" mortalities commonly associated with the failure of fry to initiate feeding, may be caused more by a combination of respiratory tissue hyperplasia, the accumulation of mucus and bacterial disease than by an inability to commence feeding induced by a brief period of food deprivation.

ACKNOWLEDGEMENTS

This study was supported by Sparsholt College, Hampshire, and the Hampshire County Council.

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