Environmental and parental influences on the body size of N.E. Atlantic herring, *Clupea harengus*, larvae.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by:

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CONTENTS

ABSTRACT

Morley, S. A. (1998). Environmental and parental influences on the size of herring larvae. Ph.D. thesis submitted to the University of Liverpool for the degree of Doctor of Philosophy

Investigations were carried out into the effects of mean egg dry weight and incubation temperature on the size of larvae from four N.E. Atlantic herring stocks (Buchan, Manx, Clyde and Celtic Sea).

Hatching characterisitics (length, weight and yolk volume) of Buchan, Manx and Clyde herring were investigated. The time of hatching was inversely related to incubation temperature, although there was some variation between experiments in the date of peak hatching. The total length of larvae increased through the hatching period. In all experiments mean egg dry weight per female was strongly related to the average length, weight and yolk volume of larvae at hatching. The same regression model could be applied to all stocks. There were, however, stock-specific responses of hatching characteristics to incubation temperature although a reduction in length at hatching at higher temperatures was the most consistent response. Development at low temperature resulted in a modification of the length-weight relationship; larvae of the same weight were longer at lower temperatures. Both the increase in length of larvae during the hatching period and the variation in the timing of peak hatching have implications for the comparison of larvae hatching at different temperatures.

The otoliths of Manx herring larvae [from "large" (> 0.33mg mean dry weight) and "small" (<0.25mg mean dry weight) eggs] were marked with either alizarin complexone or calcein so that larvae from pairs of large and small egg batches could be reared under identical conditions (at both 10 and 13.5°C) and relative growth monitored. Within each rearing tank large eggs generally produced larger larvae at hatch (length and weight) with higher growth rates (both weight and length specific). There were significant differences both between eggs from different females and between rearing tanks that confounded the comparisons between rearing temperatures. Fultons Condition factor is not thought to be a good measure of nutritional condition of herring larvae smaller than 15mm total length but may be used as a relative measure of body reserves (RCF) and give an indication of ability to withstand periods of poor feeding. This is indicated by a period of high mortality of larvae hatched from small eggs at 10°C, which corresponded with the time period when these larvae had the lowest body reserves.

Video recording of the foraging behaviour of laboratory reared herring larvae was used to investigate differences between the feeding strategies of groups of larvae of the same size but different ages, i.e. fast and slow growers. Slow growing larvae searched larger areas, thus expending more energy, than fast growing larvae, but there was no difference in food acquisition. The difference in behaviour tended to increase through development. A simple energetics calculation suggested that approximately 50% of the difference in growth rate could be explained by the extra swimming costs of slower growing larvae.

The size of Celtic Sea and Manx herring eggs were experimentally reduced in order to investigate if the volume of yolk in each egg determines the size of hatching larvae. Length at hatch was determined by the volume of yolk in each egg but body weight was not. The development and chemical composition of embryos and larvae needs to be investigated in a further series of experiments.

All results are discussed in terms of the influence of larval size on survival.

CHAPTER 1:

GENERAL INTRODUCTION

One of the keys to successful fisheries management is the ability to quantify the biomass of a commercial stock and to predict the proportion of that stock which can be sustainably exploited (Fritz *et al.*, 1990). Much research has been concentrated on understanding the dynamics of fish populations and although the supporting evidence is still fragmentary (e.g. Campana, 1996) several hypotheses have been constructed that recognise the importance of larval size for survival during early life history stages. These theories tend to discuss survival, as does this thesis, in terms of the relative size of larvae within one species. To compare between species requires the consideration of other ecological factors that may cause differential survival of larvae.

Most of these theories, and much of the literature, use length as their measure of larval size. Although both dry weight and diameter are commonly used to measure egg size, the herring literature largely quotes egg dry weight. Unless otherwise stated these definitions will be used throughout this thesis.

Variable mortality

Large interannual fluctuations in year class strength, which were recognised as early as 1914 by Hjort (Solemdal, 1997), are still a major obstacle to fisheries management. Variable mortality during the early life history stages of fish has long been hypothesised as an important determinant of these interannual variations (Hjort, 1914) although research continues as the link is still not proven (Leggett and Deblois, 1994, Bailey and Houde, 1989). A number of different causes of mortality have been

identified but much of the focus has been placed on starvation and predation (see Heath, 1992 for a review).

Starvation

Starvation acts during the whole first year but particularly at the critical period of first feeding when larvae swap from endogenous to exogenous energy supplies (Hjort, 1914). Small larvae, which have lower body reserves, are thought to be more susceptible to starvation, particularly under conditions when food is limiting (Folkvord *et al.*, 1997, Heinrich, 1988, Miller, *et al.*, 1988, Rosenburg and Haugen, 1982). Starving larvae are also more susceptible to predation (Checkley, 1984) which is thought by many to be the principal source of mortality of larvae (Bailey and Houde, 1989).

Predation

Although it is the main cause of mortality throughout the life of a fish, predation pressure is also usually greater during early life history stages. As fish increase in size they grow to be larger than the gape of a number of potential predators, and as predator size is negatively correlated with predator numbers, so predation risk is generally assumed to reduce as larvae grow (Bailey and Houde, 1989). However, larvae that are larger at hatch will become active sooner and will have a higher food encounter rate (Miller *et al.*, 1988). Although this may initially increase their encounter rate with any particular predator (Paradis *et al.*, 1996), they

will also have a better chance of escape (Batty *et al.*, 1993, Batty and Blaxter, 1992, Miller *et al.*, 1988).

Environmental influence

Interannual fluctuations in year class strength may be correlated to interannual variations in the environment. The longevity of winter ice cover (Michaud *et al.*, 1996), wind indicies (Lappalainen and Lehtonen, 1995, Pihl, 1990), current patterns (Fargo, 1994), day length (Bohling *et al.*, 1991), but most frequently temperature (Lappalainen and Lehtonen, 1995, Francis, 1993, Bohling *et al.*, 1991, Ellertsen *et al.*, 1989, Thompson and Hilden, 1989, Saetersdal and Loeng, 1987) have all been correlated with year class strength. Interannual temperature fluctuations can be quite marked, for example in the North Channel the mean temperature in March is known to vary from 4.9-9.8°C between years (Jones and Jeffs, 1991). Temperature also varies on much longer time scales and global ocean temperatures have been fluctuating since the oceans were formed (Johnston and Ball, 1997). With the ever increasing human population there is now the potential for anthropogenic perturbations of climate, which may be the cause of the current phase of global warming (Johnston and Ball, 1997).

The temperature that eggs and larvae experience will alter the rates of many biochemical and physiological processes (Blaxter, 1992). As the acute Q_{10} for many of these biological processes is typically 2-3 (Taylor *et al.*, 1997), temperature can be seen to have profound effects on larval energetics and growth (Houde, 1989). For a wide range of ectotherms, the "developmental temperature-size rule" applies. This

theory states that body size is smaller in organisms reared at higher temperatures (Atkinson and Sibly, 1997). The physiological basis of this theory is still under investigation but one current hypothesis argues that size of organisms at higher temperatures is limited by the higher oxygen demands of tissues at these higher temperatures. At higher temperatures metabolic rates are faster but the rate of diffusion of oxygen changes little. Body size is therefore limited by the need to maintain a high surface area-volume ratio to ensure enough oxygen can diffuse to each cell (Atkinson and Sibly, 1997).

Environmental temperature also affects the behaviour of larvae. For example, behavioural flexability of herring larval feeding strikes is reduced in larvae reared at low temperatures (Morley and Batty, 1996). The influences of temperature on both behaviour and growth rate of larvae and their predators may well alter the survival probabilities of larvae by altering the balance of predator-prey interactions.

Maternal effects

Egg size is just one possible "maternal effect" that could determine the characteristics of larvae at hatching (Høie, 1997). Fecundity usually increases with the size and age of the female as does egg size. However, females of any size face the trade off between investing more energy into fewer offspring or partitioning their energy amongst a larger number of offspring. Larger larvae can only be produced at the expense of fecundity (Smith and Fretwell, 1974). The environmental conditions that females experience can affect the size of eggs that they produce, and therefore the fecundity. For example, well fed Pacific herring produced larger eggs than

starved females (Hay and Brett, 1988). However, of particular interest to this study is the correlation of fecundity and egg weight with mean seawater temperature (Miller *et al.*, 1995, Winters, *et al.*, 1993, Tanasichuk and Ware, 1987). Tanasichuk and Ware (1987) found that, in any given year, warmer temperatures 60-90 days before spawning were positively correlated with fecundity and negatively correlated with egg size. Miller *et al.* (1995) showed that in Atlantic cod, *Gadus morhua*, which spawn over an extended period and therefore spawn into water which varies in temperature from 2-14°C, egg size is also negatively correlated with temperature. There is also a latitudinal correlation between egg size and temperature, with larger eggs being produced at higher latitudes (Chambers, 1997). The size of eggs strongly influences the size of larvae at hatch (see chapter 3 for full discussion of this issue).

Growth variation

There is substantial variation between the growth rates of individual larvae, even from within the same batch of eggs (see chapter 3 for full discussion of this issue). This variation in growth may tend to compensate for the initial size differences and reduce the variation between individuals, compensatory growth (Atchley, 1984), alternatively it may tend to increase the variation in size between individuals, depensatory growth. The magnitude of length variation also has important survival implications. Simulation models have shown that selection for the fastest growing individuals is stronger from populations with a greater degree of length variation between individuals and when predation pressure is higher (Rice *et al.*, 1993). However, as will discussed throughout this thesis, the heterogeneity of

aquatic ecosystems will ensure that fast growth will not always have a selective advantage (Meekan and Fortier, 1996).

The subsequent growth rates of larvae will also have important implications for survival. Because larger larvae are thought to be less susceptible to predation, faster growth rates should result in larvae growing out of vulnerable stages more rapidly and lead to better survival (Meekan and Fortier, 1996, Hovenkamp, 1992, Titus and Mosegaard, 1991, Post and Prankevicius, 1987, Rice *et al.*, 1987, Crecco and Savoy, 1985, Rosenburg and Haugen, 1982). For example, assuming a constant mortality on larvae of $0.1d^{-1}$, a two fold variation in stage duration of larval Atlantic menhaden, *Brevoortia tyrannus*, could result in a 70 fold variation in the numbers of larvae surviving to metamorphosis (Maillet and Checkley, 1991). Age at metamorphosis is generally more variable than length at metamorphosis (Chambers and Leggett, 1987) and, although temperature can affect the relative timing of ontogeny (Johnston, 1993), within a species body size is usually a good indicator of developmental stage (Fuiman and Higgs, 1997).

Herring

Herring are a widespread, pelagic, schooling clupeid which have been the subject of a fishery by man since the early days of civilisation (Blaxter, 1990). Herring are found in both the north Atlantic and the North Pacific although there are some indications that the populations in the two oceans are separate species (Blaxter, 1990). There are a number of N.E. Atlantic herring, *Clupea harengus* L., stocks that spawn around the coast of the British Isles and Ireland; the stocks used in this study

are shown in fig. 1.1. Each stock spawns in a specific geographic location at a specific time of year (Parrish and Saville, 1965), although the fishery may be based on shoals of mixed race (Blaxter, 1990). Herring are iteroparous and produce only one batch of eggs a year (Chambers and Leggett, 1996). The eggs are demersal and adhere to gravel substrates on the spawning grounds (Blaxter, 1990). This is important for the experimental manipulation of herring eggs as they also adhere to glass plates (Hill and Johnston, 1997). Herring are still a commercially important species, although the majority of the catch is sold on to Eastern European factory ships, the "Klondykers" (Blaxter, 1990). Herring have been successfully reared for experimental investigations for many years (Blaxter, 1956) and therefore present a good model organism for experiments into growth and survival of fish larvae.

Aims

The specific aims of this study are to focus on the effect of egg size and temperature on the size of larvae both at hatch and then during early growth. Survival of larvae after different treatments is either measured directly or discussed in terms of predator-prey interactions. Chapter 3 investigates the effects of egg size and temperature on the hatching characteristics of herring larvae. The effect of temperature and egg size on the survival of embryos, time to hatch and the hatching characteristics (total length, yolk volume and total weight) of larvae is quantified. Although many studies have reported either the effect of egg size or temperature on the size of hatching larvae few have reported on the interaction between the two. In particular the study focuses on the differences between stocks spawning at different

Figure 1.1. Herring stocks used during this study. 1) Clyde herring, which spawn in March, 2) Buchan herring spawn in August, 3) Manx herring spawn in September, 4) Celtic Sea herring spawn in January. See Parrish and Saville (1965) for details of other stocks.

geographic locations and at different times of the year. Chapter 4 investigates if initial size advantage is maintained during early growth. Using published protocols, trials were conducted to develop a procedure for marking otoliths of live herring with either alizarin complexone or calcein solutions. The otoliths of embryos were marked to allow batches of large and small eggs to be reared in the same tanks. Eggs of two sizes (> 0.33mg and < 0.25mg mean dry weight) were incubated at two temperatures (10 and 13.5°C) to create initial size differences between larvae. The maintenance of initial size differences was investigated, along with the relative survival of larvae from different treatments. A relative condition factor was calculated so that the body reserves of larvae could be compared between larvae of different ages and from different treatments. In Chapter 5 video techniques were developed that allowed in-situ filming of larvae within their rearing tanks. The study focussed on the behaviour of foraging larvae to investigate possible behavioural differences between larvae with different growth rates. Video analysis programmes were provided by Mike Burrows (Dunstaffnage Marine Laboratory). In Chapter 6 the mechanism underlying the relationship between egg size and larval size at hatch was tested. Yolk reduction techniques were developed in collaboration with Peter Tytler (Stirling University). Yolk was removed from recently fertilised embryos and the effect on the size of larvae at hatch was recorded.

CHAPTER 2:

GENERAL MATERIALS AND METHODS

This section includes protocols that are common to all experiments:

Herring Rearing

Ripe adult herring were caught on their spawning grounds, fig. 1.1. Gonads were dissected out of the adults and placed into sterile 100ml polystyrene containers which were transported back to the laboratory on ice, so that eggs were maintained at a temperature of between 1 and 4°C. Ten unfertilised eggs from each female were placed into a pre-weighed vial and then dried to constant weight *in vacuo* over anhydrous silica gel at room temperature. The mean dry weight of these ten eggs was measured using a Perkin-Elmer AD-2Z autobalance. The drying and weighing procedure was repeated until dry weights were constant (two measurements agreed to ±15μg). Egg batches were then selected as appropriate for each experiment (see methods chapters 2-4).

Once eggs had been selected, sterile glass plates or slides were placed flat on the bottom of containers under a depth of 4-5cm of seawater. Eggs, which become adhesive on contact with water, were dispersed and adhered in a single layer onto the glass. Eggs were fertilized by placing glass plates and slides into a sperm bath into which the gonads of 5 or 6 males had been dissociated to achieve a sperm density of approximately $5x10^8.ml^{-1}$. Using sperm from a mixture of males made the fertilisation conditions closer to those found in nature, although this approach precludes analysis of paternal influences on offspring. The paternal influence on hatching characteristics has, however, been shown to be small, e.g. in capelin,

Mallotus villosus Mueller, it was found that there was no male effect on size at hatching (Chambers *et al.*, 1989).

Eggs were incubated in recirculation systems, which had a constant bleed through of fresh seawater to remove waste products. Eggs were incubated at temperatures chosen to include the variation about the historical mean temperature (e.g. Jones and Jeffs, 1991) at the spawning ground for each stock at the time of spawning (see each chapter). Temperature was controlled by heating or cooling of water in a sump tank as appropriate. Temperature control from day to day was accurate to ± 0.2°C but because of periodic larger scale fluctuations the long term accuracy was ± 0.5°C. A 12:12h light:dark cycle, with a light intensity of approximately 100 lux, was maintained for all experiments. From 2 days before total yolk sac exhaustion larvae were fed daily, initially on rotifers enriched with algae and then on *Artemia salina* 1st instar nauplii. Larvae were fed once a day at 14.00h. The quantity of food was adjusted daily according to the number of larvae in each tank so that roughly the same number of food particles were being fed to each fish.

Otolith marking

For experiments in chapters 4 and 5 batches of larvae were reared in the same tanks and distinguished by marking of their otoliths. At approximately 100 degree days post fertilisation the otoliths of the embryos from each female were marked with either alizarin complexone (Umino *et al.*, 1996) or calcein (Leffler and Shaw, 1992). The glass plates were removed from the rearing tanks and immersed for 6 hours in either a $50mgl^{-1}$ solution of alizarin complexone or a $150mgl^{-1}$ solution of calcein.

Both of these markers were incorporated into the calcium matrix of the otolith and embryos could be clearly distinguished when viewed using a Zeiss Axioscope epiflourescence microscope (filter set 09, excitation 450-490nm and transmission >520nm). Alizarin complexone appears as an orange band and calcein as a green band. In smaller larvae, typically <18mm total length, otolith fluorescence was viewed through the body wall of fresh specimens, otherwise squash preparations of rehydrated larvae were used.

Age conversion

To allow comparisons of the age of embryos reared at different temperatures ages were converted into a common unit, physiological age (A). The units of physiological age are "day degrees" which are calculated using a biological zero of -1°C (Blaxter, 1956):

$$A = (t_1 - t_0).(T + 1) \qquad (2.1)$$

where T is the temperature (°C). t_0 is either taken as the day of fertilisation (Chapter 3 and 5) or the day of hatch (Chapter 4).

Statistical analysis

The same probability threshold for significance (p=0.05) was used for all statistical analyses except where indicated in the text. In all cases significant

relationships are indicated by the inclusion of a * symbol. Common abbreviations used throughout the thesis are listed in Table 2.1.

Table 2.1. Commonly used abbreviations

Symbol	Description
r	Pearsons product moment co-efficient
r^2	Co-efficient of determination
N	sample size
C.M.H.	Cochran-Mantel Haenszel test
F	the F-statisitic from ANCOVA and ANOVA
p	the probability

CHAPTER 3:

THE EFFECTS OF EGG SIZE AND INCUBATION TEMPERATURE ON THE HATCHING CHARACTERISTICS AND EARLY GROWTH OF LARVAL ATLANTIC HERRING, *CLUPEA HARENGUS* L.

INTRODUCTION

Both egg size and incubation temperature are major determinants of the length, weight and yolk sac volume of hatching fish larvae. Variation in sea temperature and egg size will cause variation in these characteristics of hatching larvae, which may affect foraging ability and susceptibility to starvation or predation (e.g. Pepin and Miller, 1993); mortality dependant on size at hatch may be a factor contributing to variation in year class strength.

Egg size is one possible "maternal effect" that could determine the characteristics of larvae at hatching (Høie, 1997). Further variation in life history traits can occur at a number of different levels (Chambers, 1997): between years (Hay and Brett, 1988, Tanasichuk and Ware, 1987, Hislop *et al.*, 1978), between species (e.g. Shirota, 1970), between stocks (e.g. Hempel and Blaxter, 1967), between individuals within a stock (e.g. Blaxter and Hempel, 1963) or between batches from an individual female (e.g. Kjesbu *et al.*, 1996). However, eggs produced within a single batch tend to be more uniform in size than between batches (e.g. Kjesbu *et al.*, 1996, Blaxter and Hempel, 1963). The egg size appears to be directly related to both larval size and yolk reserves (e.g. Baynes and Howell, 1996, Blaxter and Hempel, 1963).

Within the natural range of temperatures experienced by a species, the most obvious physiological effect of increased temperature is more rapid development of embryos (e.g. Hempel and Blaxter, 1967). Incubation temperature can also have a direct effect on the survival of embryos. Both higher and lower incubation

temperatures can lead to better survival but the effect varies between species (e.g. Buckley *et al.*, 1990, Beacham and Murray, 1985, Laurence and Rogers, 1976). Incubation temperature can also affect the size and yolk volumes of hatching fish larvae, both longer or shorter larvae can hatch at lower incubation temperatures, depending on the species (e.g. Canino, 1994, Beacham and Murray, 1985, Johns *et al.*, 1981, Johns and Howell, 1980). Across species, egg size is inversely related to time to hatch (Pauly and Pullin, 1988) but this effect is slight or absent within a species (Hutchings, 1991, Miranda *et al.*, 1990, Beacham and Murray, 1985, de Ciechomski, 1966, Blaxter and Hempel, 1963).

Studies that have considered developmental rates of fish embryos have focussed either on temperature or egg size effects on the developing embryo and have concentrated on species-specific responses. This study aims to investigate the interactions between egg size and temperature on the timing of hatching, and the characteristics of the larvae at hatching (length, weight and yolk volume). Further this study considers the effects of egg size and temperature on development in three different stocks of N.E. Atlantic herring larvae (*Clupea harengus* L.).

N.E. Atlantic herring are classified into stocks that spawn in different seasons and at different geographical locations (Parrish and Saville, 1965). Although studies on adult herring show that the majority of genetic variation is within rather than between stocks (Smith *et al.*, 1990) it is known that there are significant allelic (Jorstad *et al.*, 1991), morphological, ecological and behavioural differences (Parrish and Saville, 1965) between adults from different stocks. Another aim of the present study is to investigate whether there are differences in the interaction of egg size and

temperature on hatching larvae which may be indicators of stock-specific adaptations to the local environment (Beacham and Murray, 1987).

MATERIALS AND METHODS

Rearing

Egg batches from individual females, representative of the range of available egg sizes, were selected for each experiment. Eggs were incubated at a range of temperatures chosen to include the variation about the historical mean temperature (e.g. Jones and Jeffs, 1991) at the spawning ground for each stock at the time of spawning (Table 3.1).

Survival

At each temperature 3-5 replicate microscope slides of Buchan 1994 embryos, for each of 32 females, were fertilised. Photographs were taken at intervals from fertilisation until hatching and in each photograph the number eggs surviving as a proportion of the number of fertilised eggs was counted.

Hatching characteristics

Before hatching, eggs were transferred from slide racks to 1 litre floating cylindrical containers with a 63μm mesh floor to allow water exchange. 80% of the water in each 1 litre beaker was replaced twice daily. Newly hatched larvae were

Table 3.1. Details of incubation studies of Atlantic herring embryos. N: number of females.

Experiment	Stock	Year	N for each incubation temperature, °C						Range of mean egg dry weights per stock, mg
			°C	N	°C	N	°C	N	
1	Buchan	1994	8	32	12	32	15	32	0.12-0.19
2	Manx	1994	10	28	13.5	14	17	13	0.28-0.4
3	Clyde	1994	5	16	8	16	12	16	0.28-0.39
4	Clyde	1995	5	18	8	15	12	18	0.3-0.44

removed daily, counted and their total length and maximum yolk-sac width and yolk-sac length were measured on a subsample of up to 10 individuals. Yolk volume (Y_v) was calculated as:

$$Y_V = \frac{4}{3}\pi * \left(\frac{Y_L}{2}\right) * \left(\frac{Y_W}{2}\right)^2 \qquad (3.1)$$

where Y_L = yolk length (mm) and Y_W = yolk width (mm).

The mean dry weight of larvae was then measured as for eggs. The mean and standard deviation of hatch length and yolk volume were calculated for each day for each female.

Eggs of the Clyde stock (1995) were incubated at each of the three temperatures and followed for the whole hatching period. The mean and standard deviation of each characteristic (all females combined) were calculated for each day of the hatching period. For the other experiments - Buchan stock (1994), Manx stock (1994) and Clyde stock (1994), daily measurements were continued until the day when the maximum number of larvae hatched. Mean values of hatching characteristics on this day ("peak hatch") were used for further analysis except for Clyde (1995) larvae (see Results section). From the data of the number of larvae hatching on each day, the date of 50% cumulative larval hatch was also calculated.

Early growth

Batches of Clyde 1995 larvae (Mean dry egg weights: 0.50mg and 0.44mg at 12, 8 and 5°C; 0.33mg at 5°C) were reared for approximately 70 days post hatch

(0.33mg eggs did not survive at 12 or 8°C). The lengths and dry weights of between 10 and 20 larvae were measured at approximately fortnightly intervals.

Statistical analysis

Analyses were conducted to test if (1) fertilisation at a common temperature and then transfer to separate incubation temperature had any effect on the initial fertilisation rates and (2) if there was any significant effect of incubation temperature on hatching success. Cochran-Mantel Haenszel (CMH) tests, controlling for mean egg dry weight, were carried out using the SAS procedure FREQ (SAS Institute Inc., 1988).

The significant factors affecting the three hatching characteristics, total length, dry weight and yolk volume (natural log. transformed data were used to ensure normality) were tested for with an analysis of covariance, using the SAS procedure GLM (SAS Institute Inc., 1988). A model was first fitted to each experiment separately, treating temperature as a continuous variable. To investigate general relationships between the four experiments a further model was fitted to all the data for each hatching characteristic. Incubation temperature was treated as a discrete variable and for each stock temperatures were classified as high, medium and low regardless of magnitude. This allowed comparisons of the response of each herring stock to its naturally experienced mean temperature and to a similar range in temperature about that mean.

Three CMH tests were conducted and so the probability threshold was reduced ($p=0.02$) using a Bonferroni approach (Sokal and Rohlf, 1995).

RESULTS

Survival

There were differences in the fertilisation rates of eggs placed into each of the three temperatures, with higher fertilisation rates at the lower temperature (Table 3.2). Variation in fertilisation rates between females (range of fertilisation rates: 15°C, 14-89%; 12°C, 18-89%; 8°C, 14-87%) was far greater than the variation in fertilisation rates between replicate slides for a single female (15°C, 1-12%; 12°C, 2-28%; 8°C, 1-26%).

The percentage survival of Buchan embryos was only weakly, but significantly, correlated with egg dry weight at 91 degree days for embryos incubated at 12°C (R^2=0.12, F=*4.24, n=32) and at 45 degree days for embryos incubated at 8°C (R^2=0.14, F=*4.97, n=32). Incubation temperature had a significant effect on the hatching success of larvae with a higher percentage survival to hatching at lower temperatures. The majority of mortality occurred after 90 degree days, during the later stages of development (Table 3.3).

Table 3.2. Fertilisation rates and survival to hatch of Buchan 1994 embryos. Mean: the mean percentage survival; S.D.: the standard deviation; N: the number of femlaes (for fertilisation rates the sample size includes 3-5 replicate slides for each female); C.M.H.: the Cochran-Mantel-Haenszel statistic.

Temperature /°C	Mean	S.D.	N	Temperatures contrasts, °C	C.M.H.
Fertilisation rate					
15	60.15	18.50	113	15v12	11.16*
12	61.42	16.99	95	15v8	67.92*
8	64.14	17.28	104	12v8	18.21*
Survival					
15	2.73	3.45	24	15v12	415.33*
12	6.71	7.4	24	15v8	4681.40*
8	25.12	12.04	23	12v8	2212.96*

Table 3.3. Percentage survival of Buchan herring embryos from fertilisation to hatch. Age in days from fertilisation was converted to degree days. The number of eggs fertilised was taken as the initial value for 100% survival at day 0. Temp: incubation temperatures of 15, 12 and 8°C; S.D. standard deviation; Mean: average of 3-5 replicate slides of eggs from each of N females.

Temp/°C	Age (Degree days)	0	18	32	45	65	80	91	99	130	144	171
15	Mean	100		94.4			73.3				2.7	
	S.D.	-		8.0			16.7				3.4	
	N	31		28			31				24	
12	Mean	100				85.0		60.6		6.7		
	S.D.	-				12.9		22.0		7.4		
	N	32				32		32		24		
8	Mean	100	94.0		87.4				59.0			25.12
	S.D.	-	7.0		11.9				22.8			12.04
	N	32	12		32				11			23

Hatching characteristics

Time to 50% hatch (t_h) was inversely related to incubation temperature, for all stocks:

$$t_h = 43.93e^{-0.11(T)} \qquad (3.2)$$

where T = the temperature (°C). The least squares regression equation described over 92% of the variation in the time to hatch (R^2=0.92, n=164, F=*1949).

The timing of peak hatch, however, varied between 126 and 174 degree days after fertilisation (Table 3.4). Peak hatch for larvae reared at 5°C was generally later than for larvae reared at other temperatures. This is with the exception of Clyde (1995) larvae which hatched earlier (in degree days) at 5°C and later at 8°C. The importance of variations in peak hatching time were apparent when hatching characteristics were followed for the whole hatching period (fig. 3.1 and 3.2). For clarity the data are also presented with age in days on the x axis (fig. 3.1). The faster development of larvae at higher temperatures is clearly shown by the shorter time to hatch at higher temepratures. At all three temperatures larvae that hatch earlier were shorter (fig. 3.2). Length increased quite rapidly through the middle of the hatching period before either becoming constant, at 5°C and 8°C, or, at 12°C, reducing towards the end of the hatching period. On hatching larvae incubated at 5°C (Equation 3.3) were consistently longer than those incubated at 8°C (Equation 3.4).

$L = -0.0006A^2 + 0.22A - 10.08$, R^2=0.82, n=9, F=*13.32 (3.3)

$L = -0.0005A^2 + 0.19A - 7.52$, R^2=0.82, n=9, F=*13.59 (3.4)

Where L = total length (mm) and A = physiological age (degree days)

Table 3.4. Time of peak hatch (degree days) at each incubation temperature and for each stock.

Temperature	Buchan 1994	Manx 1994	Clyde 1994	Clyde 1995
High	144	126	156	143
Medium	130	145	135	162
Low	171	154	174	144

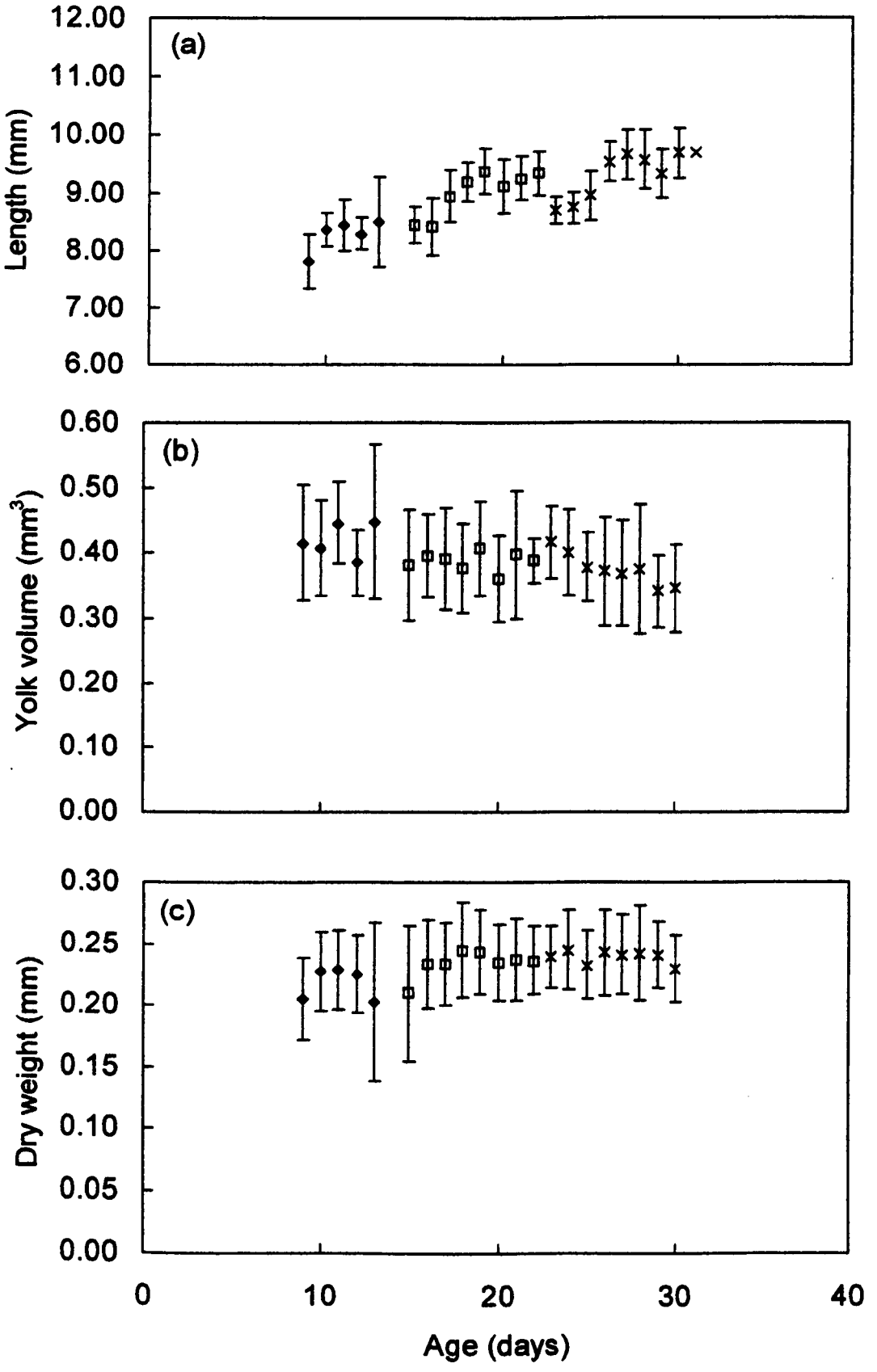

Figure 3.1. (a) length, (b) yolk volume, and (c) weight of hatching Clyde 1995 larvae through the duration of the hatching period. Incubated at (x) 5°C, (□) 8°C and (◆) 12°C. Mean ±1 s.d. are shown.

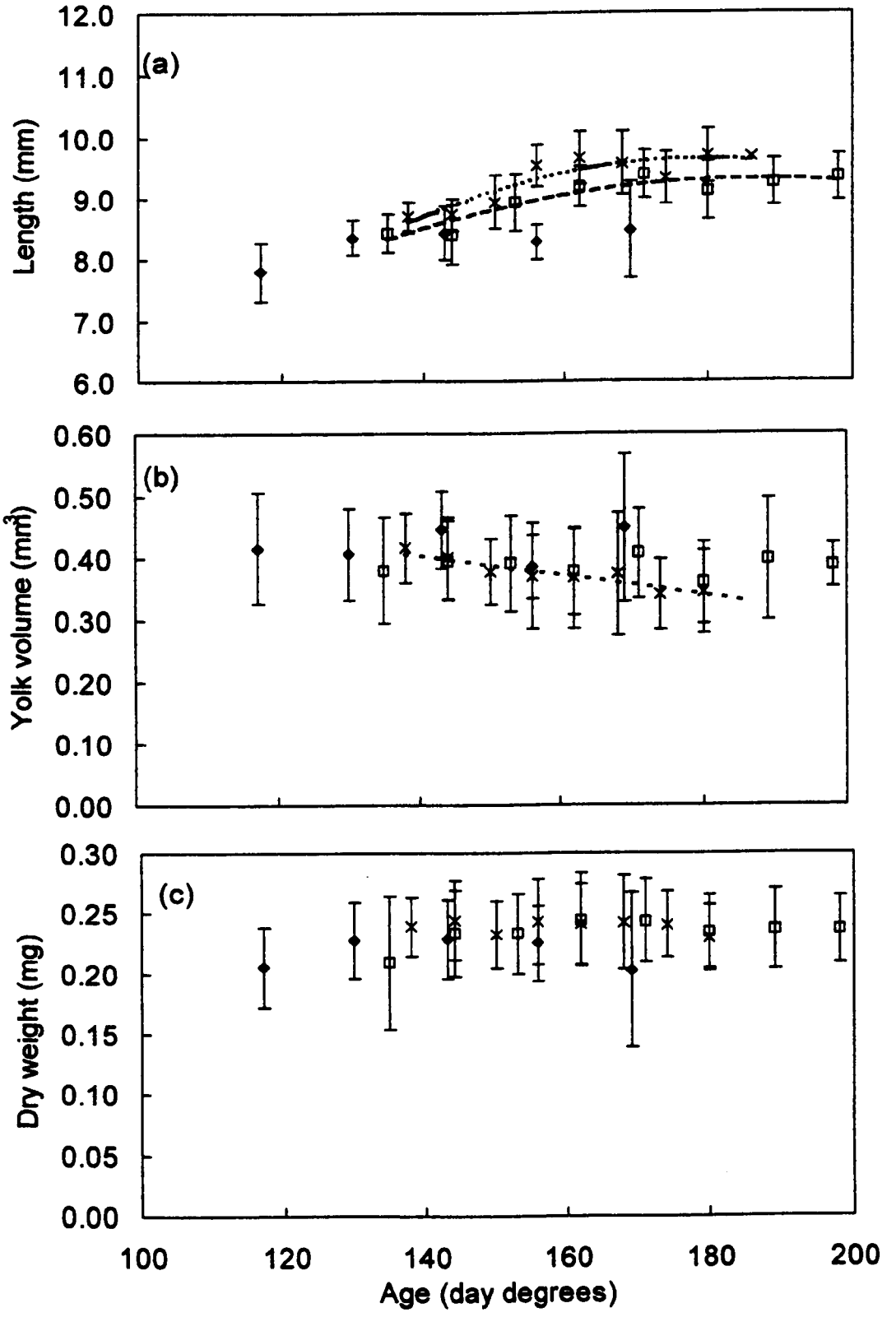

Figure 3.2. (a) length, (b) yolk volume, and (c) weight of hatching Clyde 1995 larvae through the duration of the hatching period. Incubated at (x, ...) 5°C, (□, ----) 8°C and (◆) 12°C. Mean ±1 s.d. are shown.

Although there was some overlap of lengths for larvae hatching between 130 and 140 degree days, 12°C larvae were generally the shortest on hatch. However, the early peak hatch of 5°C Clyde (1995) larvae resulted in a shorter hatch length being recorded than for 8°C. Hence peak hatch date for Clyde (1995) larvae was not representative of the interaction between incubation temperature and egg dry weight for this experiment. For comparison with peak hatch data from the other experiments the average hatching characteristics of Clyde (1995) larvae after the initial rapid increase in hatch length were used.

There was little change in either total weight or yolk volume during the hatching period, except for larvae incubated at 5°C for which there was a negative correlation between the length of incubation and yolk volume (fig 3.2b). Hatch length was positively correlated with egg dry weight and the length of larvae at peak hatch also depended upon the incubation temperature in all cases except Manx 1994 (fig. 3.3, Table 3.5). For a given egg dry weight, hatch length was negatively correlated with incubation temperature. For all experiments females with heavier eggs also had heavier larvae (fig. 3.4 and Table 3.5). For a given egg size the hatch weight of larvae from Buchan and Clyde (1995) experiments was negatively correlated with incubation temperature (fig. 3.4).

Yolk-sac volume at hatch was positively correlated with egg dry weight in all experiments (fig. 3.5 and Table 3.5). Yolk volume was negatively correlated with incubation temperature for Buchan larvae but positively correlated with temperature for Clyde (1995) larvae.

For three of the four experiments (except Manx) the length-weight relationship varied with incubation temperature (fig. 3.6). Larvae hatching at the high

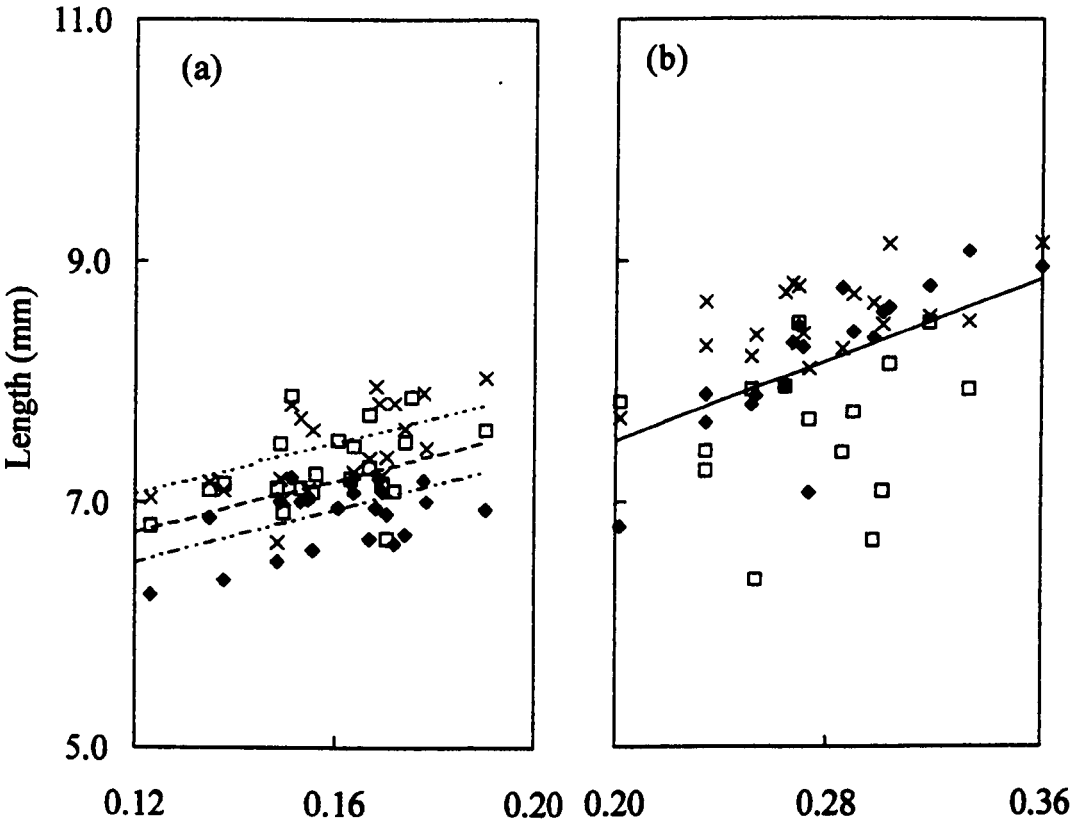

Figure 3.3. Analysis of covariance model fits to the relationship between the length of larvae at peak hatch (except Clyde 1995 larvae, see results section) and egg size. (a) Buchan, (b) Manx, (c) Clyde 1994 and (d) Clyde 1995, incubated at (x, ...) low temperature, (□, ----) medium temperature and (◆, -..-) high temperature, (—) all temperatures combined.

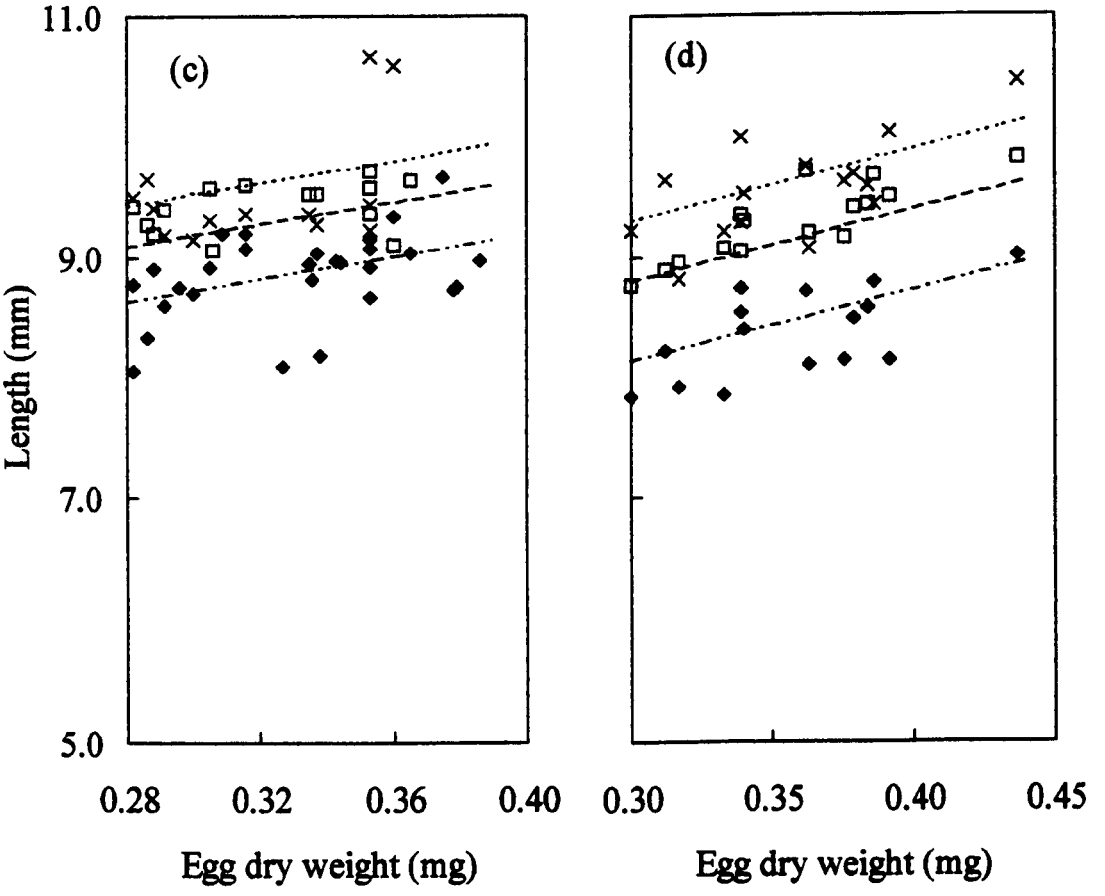

Figure 3.3 (cont.)

Table 3.5. Effects of egg size and temperature on length, weight and yolk volume on hatch, each stock treated separately as determined by analysis of covariance. Temp: incubation temperature; Egg size: mean egg dry weight; both Temp amd Egg size are continuous variables. No interaction terms were significant. F: the F-Statistic from SAS GLM procedure; r^2: the coefficient of determination. The regression fits generated by regression analysis are shown in figs. 3.2, 3.3, and 3.4. * indicates significant value.

Stock	Variable name	Length		Weight		Yolk volume	
		F	r^2	F	r^2	F	r^2
Buchan	Temp.	39.11*		8.14*		9.11*	
1994	Egg size	20.19*		41.01*		61.26*	
	Model	29.65*	0.49	24.33*	0.55	33.56*	0.44
Manx	Temp.	3.10		0.54		0.74	
1994	Egg size	15.20*		50.70*		318.68*	
	Model	9.15*	0.28	25.62*	0.87	159.71*	0.52
Clyde	Temp.	49.84*		5.42		1.13	
1994	Egg size	8.96*		21.41*		50.26*	
	Model	26.65*	0.51	11.97*	0.52	27.68*	0.32
Clyde	Temp.	121.24*		4.08*		20.34*	
1995	Egg size	28.92*		77.47*		371.77*	
	Model	74.92*	0.77	40.77*	0.90	196.06*	0.64

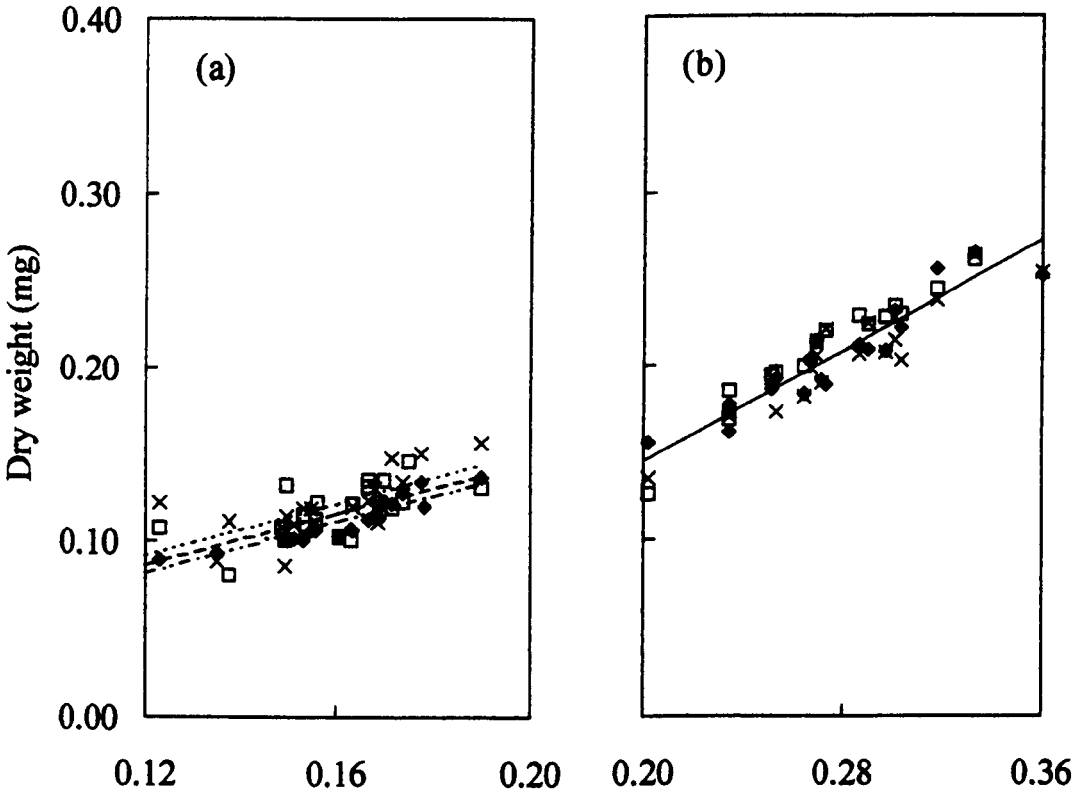

Figure 3.4. Analysis of covariance model fits to the relationship between the total body weight of larvae at peak hatch (except Clyde 1995 larvae, see results section) and egg size. (a) Buchan 1994, (b) Manx 1994, (c) Clyde 1994 and (d) Clyde 1995, incubated at (x, ...) low temperature, (□, ----) medium temperature and (◆, -..-) high temperature, (—) all temperatures combined.

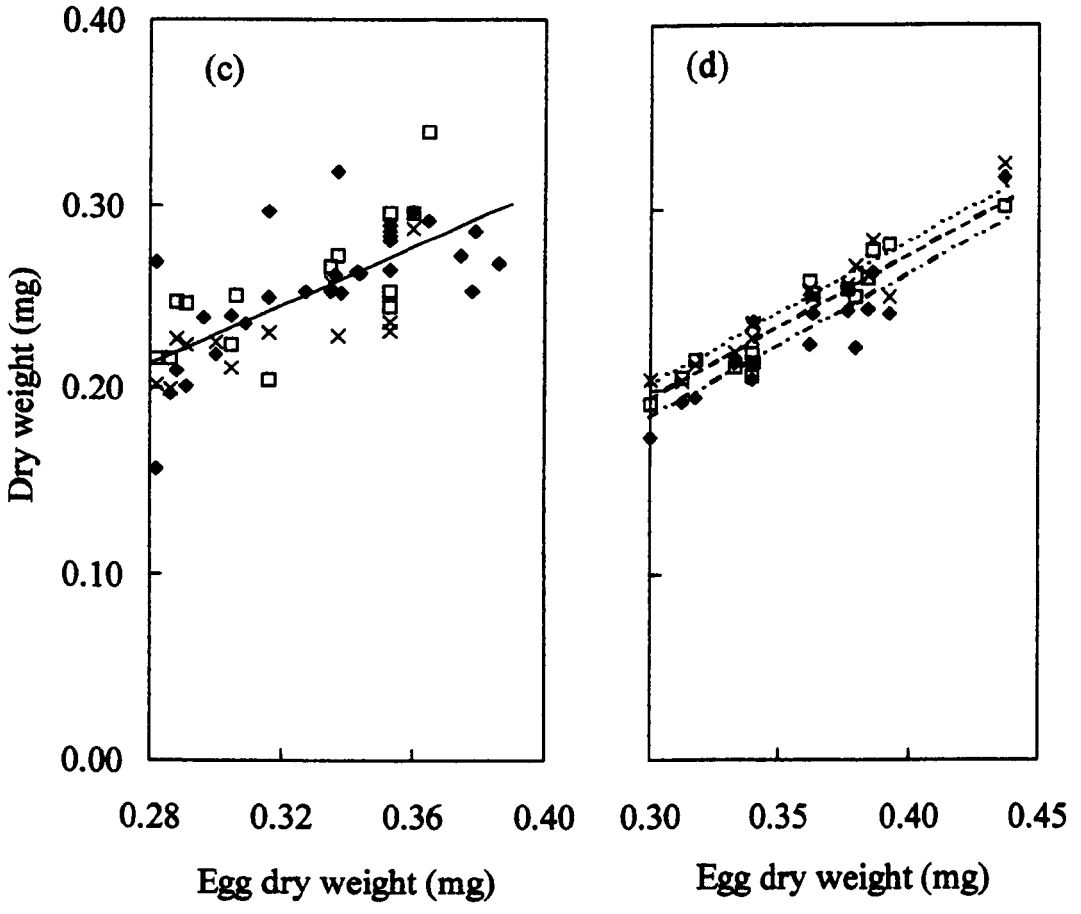

Figure 3.4 (cont.)

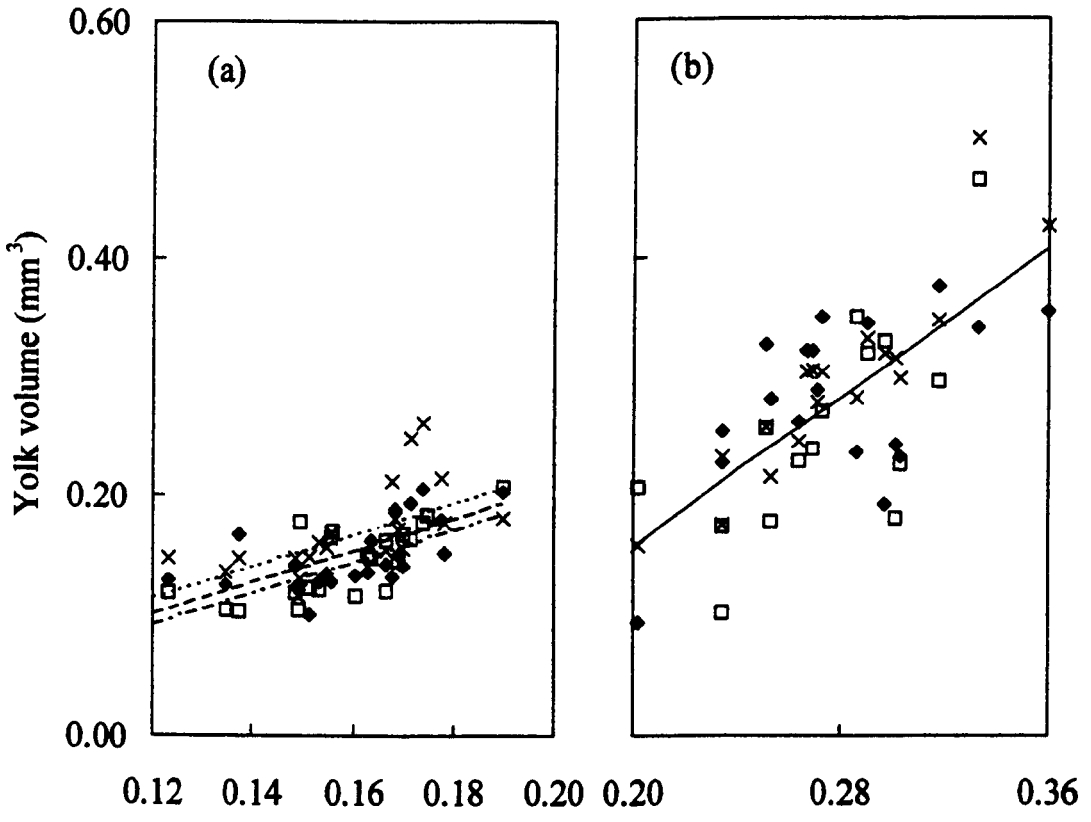

Figure 3.5. Analysis of covariance model fits to the relationship between the yolk volume of larvae at peak hatch (except Clyde 1995 larvae, see results section) and egg size. (a) Buchan 1994, (b) Manx 1994, (c) Clyde 1994 and (d) Clyde 1995, incubated at (x, ...) low temperature, (□, ----) medium temperature and (◆, -..-) high temperature, (—) all temperatures combined.

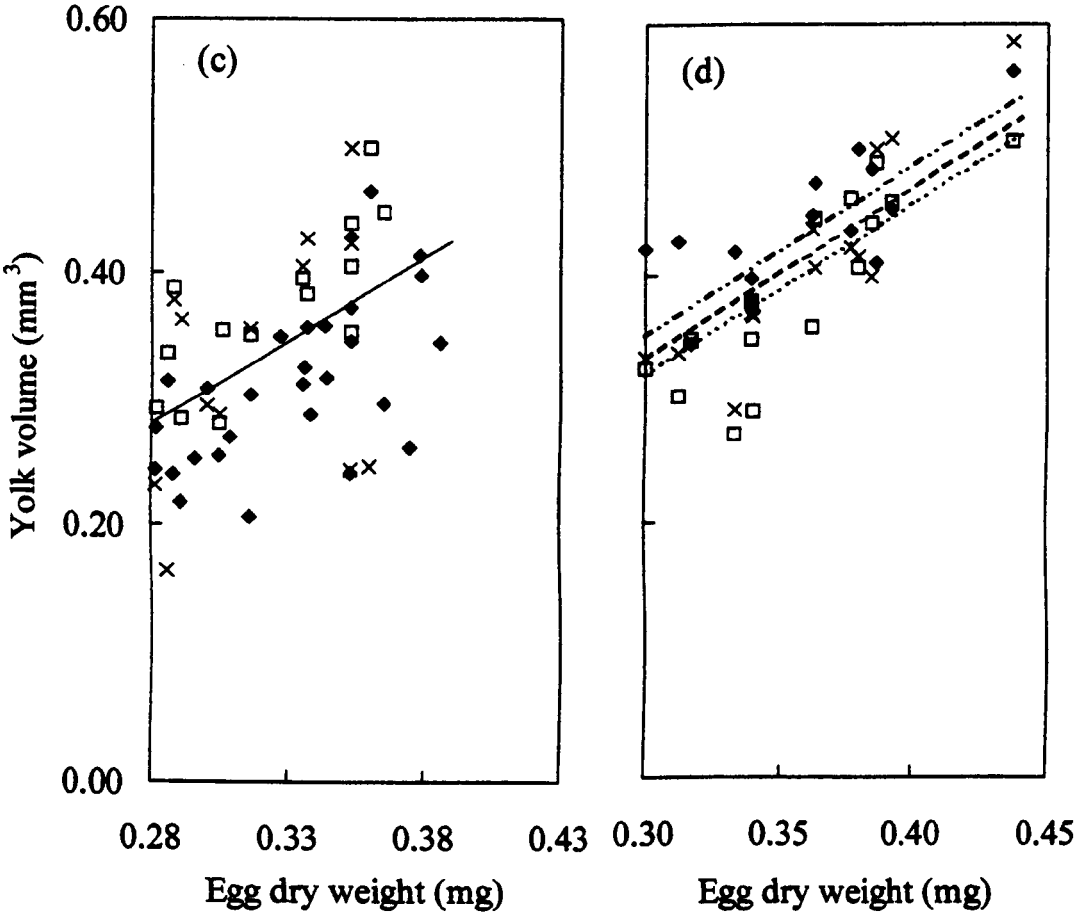

Figure 3.5 (cont.)

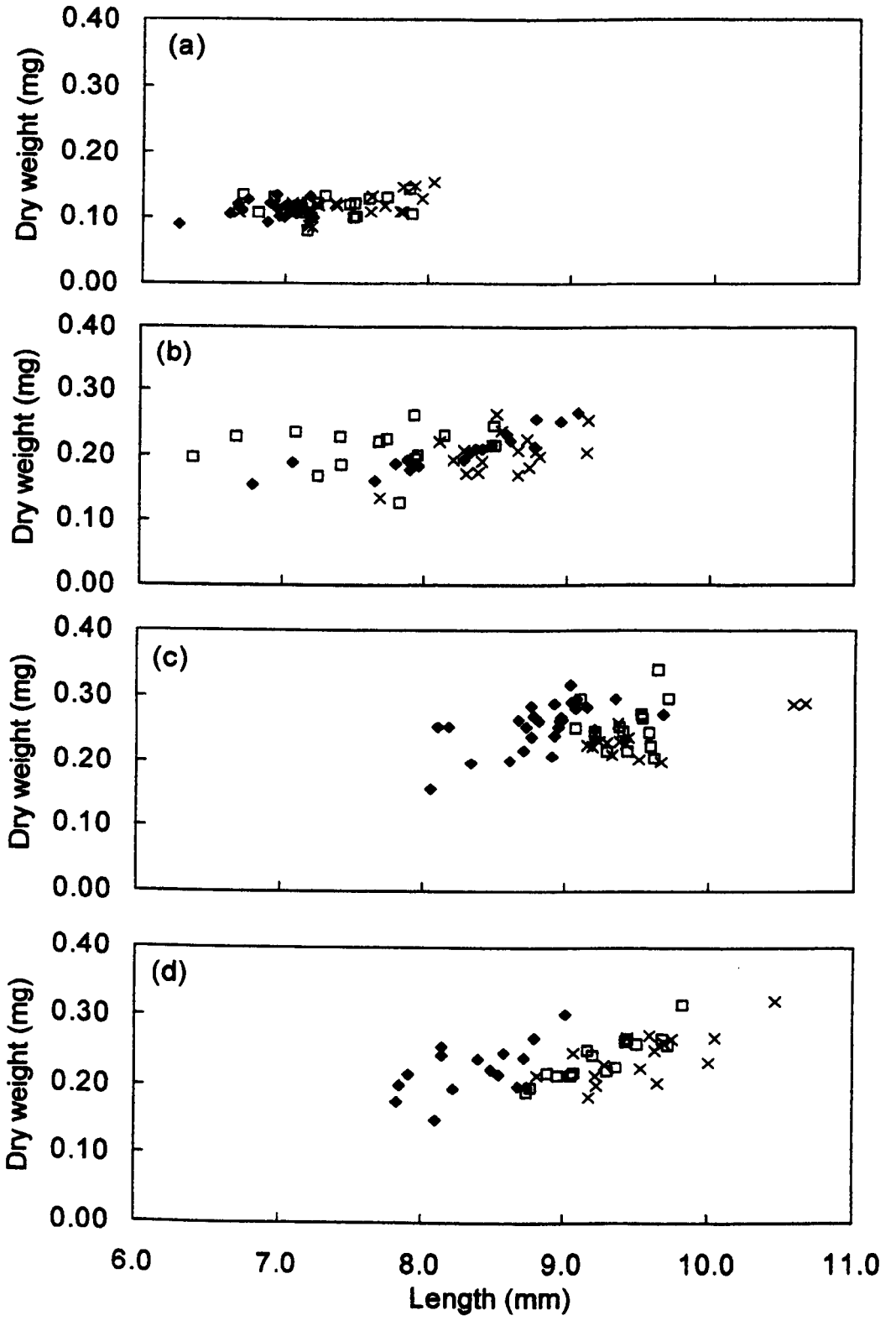

Figure 3.6. Length weight relationships for (a) Buchan 1994, (b) Manx 1994, (c) Clyde 1994 and (d) Clyde 1995 larvae, incubated at at (x) 5°C, (□) 8°C and (◆) 12°C.

temperature were morphometrically different, i.e. shorter for a given mass than larvae reared at the low temperature.

The models fitted to all four experiments combined showed that one model can describe the relationship between dry egg weight and each of the three hatching characteristics (Table 3.6). Only hatch length showed consistent combined effects of both egg size and incubation temperature. Hatch length was negatively correlated to incubation temperature at each of the three temperatures:

$$\text{high temperature} \quad L = 12.06Me^{0.30} \tag{3.5}$$

$$\text{medium temperature} \quad L = 12.42Me^{0.30} \tag{3.6}$$

$$\text{low temperature} \quad L = 12.94Me^{0.30} \tag{3.7}$$

Where L = total length (mm) and M_e = egg dry weight (mg). The fitted line for hatch length had an exponent of 0.30 (standard error ±0.01). The fitted relationship between egg dry weight and total dry weight of larvae at hatch had an exponent of 1.00:

$$W = 0.72Me^{1.00} \tag{3.8}$$

where W = total larval weight (mg). An increase in egg dry weight of 1 mg resulted in an increase of 0.72 mg in total larval weight. The fitted line for yolk has an exponent of 1.16:

$$Y_V = 1.26Me^{1.16} \tag{3.9}$$

Table 3.6. Effects of temperature and egg size on length , weight and yolk volume on hatch, all stocks combined as determined by analysis of covariance. Temp: incubation temperature; Egg size: mean egg dry egg weight; r^2: the coefficient of determination. Temp and stock are both class variables. The natural logarithms of Egg size, total length, weight and yolk volume are continuous variables. The interaction terms for egg size*Temp and eggsize*stock were non-significant.

Variable name	Ln (length)		Ln (weight)		Ln(yolk volume)	
	F	r^2	F	r^2	F	r^2
Temp.	5.78*		1.12		1.60	
Stock	0.11		1.24		0.58	
Egg size	50.38*		130.68*		417.63*	
Model	97.34*	0.84	95.37*	0.84	331.53*	0.95

Where Y_V = yolk volume (mm^3). Larvae from larger eggs will therefore hatch with an increasing proportion of their mass in the form of yolk sac.

Clyde (1995) larvae reared at the highest temperature remained shorter for a given mass than those reared at the lowest temperature during the early growth phase (fig. 3.7).

DISCUSSION

There was a wide range of fertilisation and survival rates between eggs from different Buchan females, but this was not linked to initial egg size. Eggs incubated at the highest temperature had slightly lower fertilisation rates but there was much greater variation in survival to hatch. The highest survival occurred at the lowest incubation temperature, possibly due to the lower oxygen consumption rates of embryos at lower temperatures as development proceeds more slowly (Forrester and Alderdice, 1966).

As expected, incubation temperature is the major factor influencing the rate of development. Egg size and stock explained less than 8% of the variation in the timing of hatch. Larvae incubated at 14.5°C hatched between 6 and 10 days post fertilisation whilst larvae incubated at 5°C hatched between 23 and 25 days post fertilisation (Blaxter and Hempel (1963) recorded a value 7.5 days for incubation at 14.5°C and 24 days at 5°C).

The timing of the day of peak hatch was more variable than the date of 50% cumulative hatching. In particular, Clyde larvae incubated at 5°C hatched earlier than

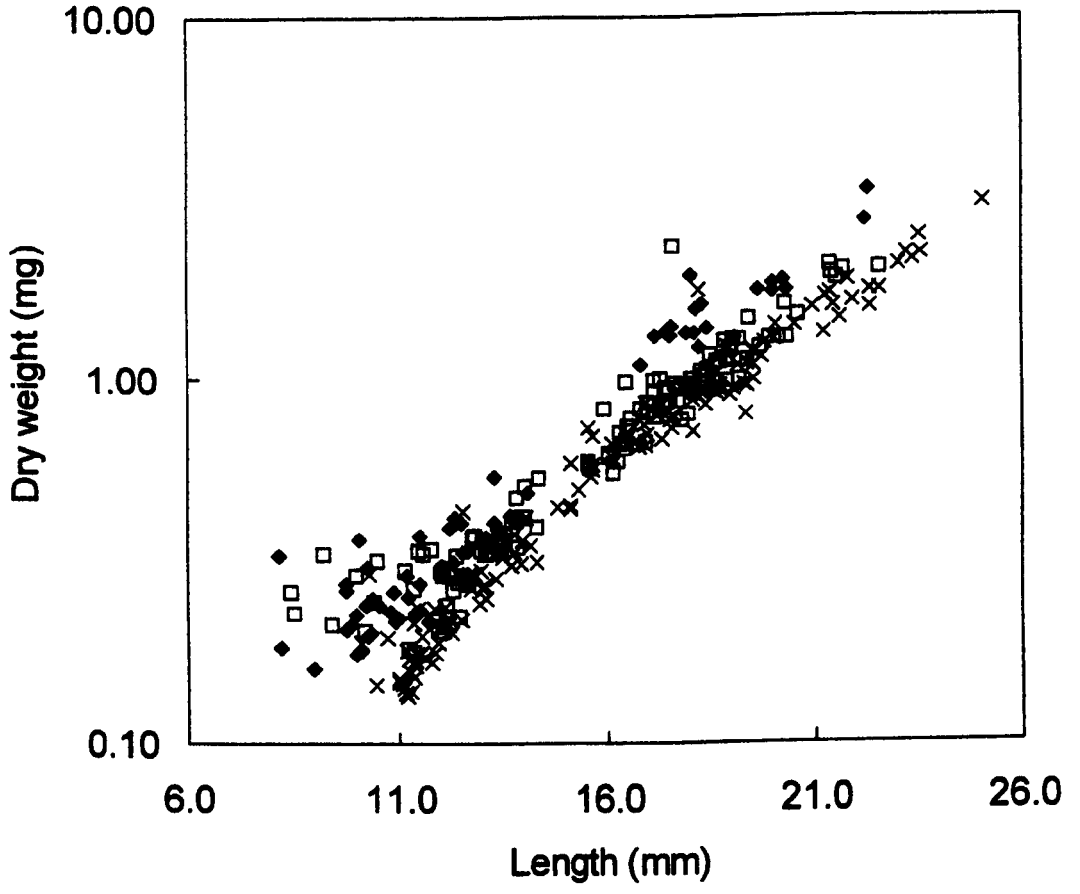

Figure 3.7. Length weight relationships for Clyde 1995 larvae incubated at (x) 5°C, (□) 8°C and (◆) 12°C, during the first two months of growth.

predicted in 1995. Blaxter (1956) also found that herring reared in 1954, and incubated at 8.5 and 5.5°C, hatched earlier than expected. There are many factors known to cause embryos either to hatch early (e.g. reduced oxygen, Keckieis *et al.*, 1996; sewage sludge Costello, 1989) or to delay hatch (e.g. reduced oxygen, Barry *et al.*, 1995). Development and hatching are not always linked because hatching is not strictly a developmental stage. It is however still an important life history event, because it denotes the transition from an encased embryo to a free swimming larva. Temperature alters the relative timing of organ development in herring larvae (Johnston, 1993) and the timing of other early life history events has also been shown to vary, even under controlled conditions (Chambers and Leggett, 1987, 1989; Chambers *et al.*, 1988).

If comparisons between stocks had been made using data from the day of peak hatch, without detailed investigation of the whole of the hatch period, then the effect of temperature on the hatch length of Clyde larvae would have appeared to differ between 1994 and 1995 larvae. However, although larvae which hatched during peak hatch were younger, and thus smaller than usual, the maximum size of larvae over the whole hatch period conformed to the pattern seen for other years and stocks. The few studies of larval characteristics through the hatch period generally report an increase in larval size throughout this period (Chambers *et al.*, 1989, Ryland *et al.*, 1975, Forrester and Alderdice, 1966, Blaxter, 1956). Alderdice and Velson (1971) reported a similar increase but with a slight reduction in the length of the last larvae to hatch. The general increase in length through the hatch period is also accompanied by a reduction in yolk volume at hatch (Chambers *et al.*, 1989) and larvae are therefore hatching at a more advanced stage as development progresses.

However, Bengtson *et al.* (1987) observed that late hatching *Menidia menidia* (L.) larvae were shorter than those hatching earlier.

Longer, heavier herring larvae, with larger yolk reserves, hatched from larger eggs. For many other fish species larger eggs produce larvae that are longer (e.g. Baynes and Howell, 1996, Vieira and Johnston, 1992, Buckley *et al.*, 1991), and heavier (e.g. Araujo-Lima, 1994, Kazakov, 1981), often with more yolk (e.g. Ojanguren *et al.*, 1996, McEvoy and McEvoy, 1991, Miller *et al.*, 1988).

It is clear that the quantity of yolk invested in each egg has important implications for larvae after hatching. It is generally accepted that larger, faster growing larvae will suffer lower mortality (Grimes and Isley, 1996) and as the length advantage of larvae from larger eggs generally persists until yolk-sac absorption (Ojanguren *et al.*, 1996, AraujoLima, 1994, Thorpe *et al.*, 1984), egg size will therefore affect both initial feeding success and early survival.

Although there were differences in the interaction of temperature and egg size on the hatching characteristics of larvae from different stocks, the overall model (equations 6-11) suggests that the factors governing the relationship between egg size and the hatching characteristics of larvae are common to all three herring stocks in this study. This confirms earlier suggestions for N. E. Atlantic herring stocks (Blaxter and Hempel, 1963).

Hatch length has an allometric relationship with egg size (a steeper slope for smaller eggs)(Equations 3.5-3.7). The implication is that differences in egg size have a greater effect on the Buchan stock than they do on the Clyde stock. This may conteract the lower variation in length and weight at hatch of larvae from small batches of eggs (Blaxter and Hempel, 1963).

The relationship between egg size and hatch length was expected to have a slope of 0.33 (the cube root of the relationship between egg size and total weight, which had a slope of 1.00) but it was actually lower than predicted (0.30 ± 0.02; estimate ± 2 S.E.). However, the increased yolk available to larger eggs is not all transferred directly into body tissue but a greater proportion of the weight of these heavier larvae remains in the form of yolk (fig. 3.8). Yolk volume was converted to mass using a relationship between yolk volume and dry weight of newly fertilized eggs (R.S. Batty, unpublished data):

$$Y_W = 0.2583 Y_V \qquad (3.10)$$

where Y_W = yolk weight and Y_v = yolk volume. The mass of the chorion was subtracted from mean egg dry weight before the conversion to a volume (calculated from: Blaxter and Hempel, 1966).

Increased body reserves, either as body tissue or yolk supply, might be expected to increase the resistance to starvation of larvae in the absence of food (Hutchings, 1991, Miller *et al.*, 1988, Rana, 1985, Bagenal, 1969, Blaxter and Hempel, 1963). However, the size of yolk reserves is not necessarily related to the point of no return (Chambers *et al.*, 1989, Araujo-Lima, 1994 and de Ciechomski, 1966). Because larger eggs also give rise to larvae with more body tissue and these larger larvae are thought to require more energy for maintenance; yolk reserves of larvae from both small and large eggs may be exhausted after similar periods of time.

The combined effects of incubation temperature and egg size are more easily detected in some herring stocks than others. However, the effect of temperature on hatch length was more predictable than its effect on either body weight or yolk volume at hatch and a single model, treating incubation temperature as a discrete

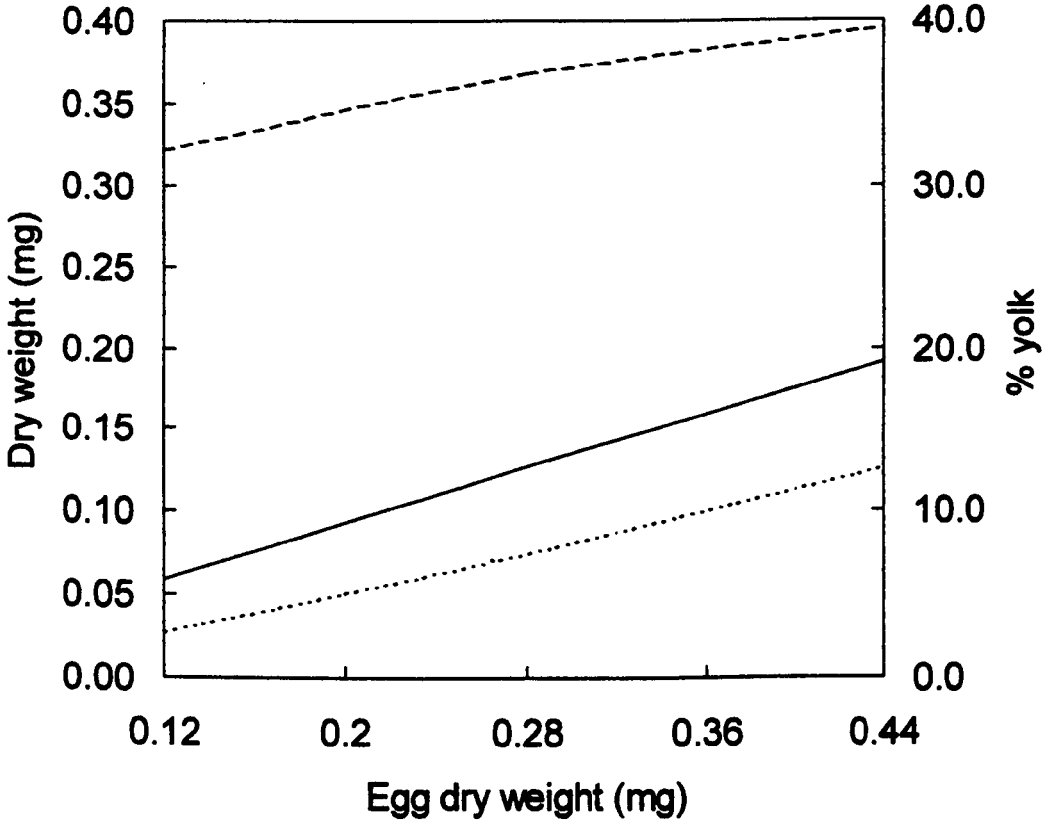

Figure 3.8. The relationship between body weight (.....), total weight (—) and yolk weight as a percentage of total weight (----), with egg size. Calculated from results of SAS procedure GLM. Yolk volume was converted to yolk weight (see discussion).

variable regardless of magnitude, was adequate to describe the majority of the variation in the relationship between hatch length and egg size for all stocks. A similar change in incubation temperature caused a similar difference in the hatch length of all larvae, independent of the stock.

Previous studies on herring larvae have generally shown an inverse relationship between incubation temperature and hatch length (Vieira and Johnston, 1992, Blaxter and Hempel, 1961, Blaxter, 1956, Meyer, 1878). Hatch length and incubation temperature have been found to vary inversely in some fish species (Beacham and Murray, 1985, Ryland *et al.*, 1975, Alderdice and Velsen, 1971) but directly (Canino, 1994, Laurance and Howell, 1981, Marangos *et al.*, 1986) or not at all (Johns and Howell, 1980, Laurence and Rogers, 1976) in others. Eggs incubated at intermediate temperatures have also been found to result in longer larvae (Johns *et al.*, 1981, Laurence and Rogers, 1976).

The effects of incubation temperature on hatch weight have been found to be inconsistent across species. Hatch weight may increase (Beacham and Murray, 1985), decrease (Baynes and Howell, 1996 and Gray, 1928) or not respond (Johns *et al.*, 1981) to incubation temperature. Both negative (Buchan and Clyde 1995) and zero correlations (Manx and Clyde 1994) were found for different experiments in this study.

In this study herring larvae showed negative (Buchan), positive (Clyde 1995) and lack (Manx and Clyde 1994) of correlations between incubation temperature and yolk volume. For different fish species yolk volume is also reported to be both negatively (Baynes and Howell, 1996, May, 1974, Alderdice and Velsen, 1971) and

positively (Alderdice and Forrester, 1971, Blaxter and Hempel, 1961) correlated with incubation temperature.

The pattern, at lower temperatures, of longer larvae hatching with less yolk and at a more advanced developmental stage, is thought to be due to delayed hatching at these temperatures (Canino, 1994, Chambers *et al.*, 1989, Bengtson *et al.*, 1987, Pavlov, 1984). This could explain the effect of temperature on the hatch length of Atlantic herring but does not explain its effect on weight or yolk volume at hatching. Instead larvae of a given mass, if incubated at the lower temperatures, are generally longer at hatching compared to those incubated at the higher temperatures. For Clyde (1995) larvae this length-weight relationship was maintained during the early growth phase.

The effect of incubation temperature on both hatch length and the time to hatch may have important implications for cohort survival. At higher incubation temperatures peak hatch contains a higher proportion of the total number of hatchlings. As they will all be smaller larvae, we assume that they each have a reduced chance of survival due to their small size. Lower incubation temperatures extend the duration of the hatching period and so peak hatch will contain a lower proportion of the total cohort, therefore we speculate that the environment (preditor-prey field, wind strength e.t.c.) on the day of peak hatch will have less of an impact on cohort survival at lower temperatures.

This study has shown that phenotypes of hatching larvae show both similarities and significant differences between stocks of Atlantic herring. Few previous studies have used this approach. Blaxter's (1960) study compared the tolerance of lethal temperatures between Spring and Autumn herring larvae and

found no significant differences in their survival. A more extensive study of chum salmon (*Onchorhynchus keta* Walbaum) stocks (Beacham and Murray, 1987) found inter-stock differences in the responses of embryos and larvae to incubation temperature. Stocks that naturally spawn in colder waters (those spawning later in the season) had better embryonic survival at lower experimental incubation temperatures. Temperature and egg size caused stock-specific responses of larval size at hatch, although the relationship was broadly similar for all stocks (Beacham and Murray, 1987). This pattern of similarities and differences between stocks agrees with our findings for herring larvae and might help to explaining the discrepancies among findings in the literature.

CHAPTER 4:

LARGER LARVAE, FROM LARGER EGGS, GROW FASTER

INTRODUCTION

Both starvation and predation of fish larvae are thought to be size dependant (Grimes and Isley, 1996, Paradis *et al.,* 1996). Although as larvae increase in size their vulnerability to a specific predator often follows a dome shaped curve (Van der Veer, *et al.*, 1997), larger larvae have a reduced number of potential predators and so in general mortality due to predation reduces as larvae grow (Houde, 1997, Bailey and Houde, 1989). High variation in growth rates between individuals is also thought to reduce predation on a cohort of larvae (Rice *et al.*, 1993). Smaller and slower growing individuals are more susceptible to mortality due to starvation (Rosenberg and Haugen, 1982), particularly during the switch from endogenous to exogenous feeding (Cushing, 1975). Larval size should therefore be strongly correlated with the survival probabilities of larvae, particularly during the first few weeks after hatching.

Egg size and incubation temperature are important determinants of the size of hatching fish larvae (Chambers, 1997). On average longer herring larvae, often with a heavier body and more yolk, hatch from larger eggs. However, within this average relationship there is considerable variation both between and within batches of larvae (e.g. Chambers and Leggett, 1996). Body size is inversely related to temperature in many ectotherms (Atkinson and Sibly, 1997). This "developmental temperature-size rule" also generally applies to fish larvae (Blaxter, 1992).

If initial size differences are maintained until yolk sac absorption they may affect survival (Ojanguren *et al.*, 1996, Araujo-Lima, 1994, Thorpe *et al.*, 1984). However although size differences persist in some studies (Chambers and Miller, 1995, Rosenburg and Haugan, 1982) in other studies the sizes of larvae converge

later in development (Thorpe *et al.*, 1984, Springate and Bromage, 1985, Reagan and Conley, 1977).

In the present study the otoliths of Atlantic herring (*Clupea harengus* L.) embryos were marked so that batches of eggs could be reared in the same tanks and therefore under identical conditions. Pairs of "large" and "small" egg batches (in terms of mean egg weight) were reared at both 10 and 13.5°C to create size differences between larvae at hatch. Lengths and weights of each set of progeny were monitored through early development to investigate if initial differences persisted. The relative survival of progeny was investigated to look for evidence of selective mortality.

MATERIALS AND METHODS

Ripe adult herring were caught from their spawning ground, Douglas Bank off the Isle of Man in September 1995. Eggs from a total of six females, three with "large" eggs (mean ≥ 0.33mg) and three with "small" eggs (mean ≤ 0.25mg), were selected. Egg plates containing either eggs from one large mean egg weight and one small mean egg weight female were placed together into each of six 100l tanks and incubated at either 10 or 13.5°C (Table 4.1). The two batches were distinguished by marking of their otoliths during the embryonic stage (see Chapter 2).

Larvae were sampled periodically, every 10-20 degree days during the yolk sac and early feeding stages and then approximately every 100-150 degree days thereafter. Larvae were anaesthetised with benzocaine and live total lengths measured. Larvae were then dried and weighed (method as for eggs except that each larva was weighed individually).

Table 4.1. The mean size of eggs for each female pair, marked with either alizarin complexone or calcein, and then reared at either 10 or 13.5°C.

Female Pair	Marker	Mean egg dry weight (mg)	
		10°C	13.5°C
1	Alizarin	0.23	0.33
	Calcein	0.33	0.23
2	Alizarin	0.25	0.34
	Calcein	0.34	0.25
3	Alizarin	0.25	0.33
	Calcein	0.33	0.25

Fultons condition factor (K) was calculated as:

$$K = 100.W.L^{-3} \qquad (4.1)$$

where W is larval dry weight (mg), L is the total length of larvae (mm) (Bagenal and Tesch, 1978). A second order, least squares, polynomial was fitted to the relationship of condition factor with age, for all larvae in this experiment. A relative condition factor (RCF) was then calculated as:

$$RCF = K.K_p^{-1} \qquad (4.2)$$

where K_p is the predicted condition factor calculated from the regression equation.

The proportion of survivors from either small or large eggs, were pooled for each of three time periods and for each rearing temperature. Three time periods: "early" (<400 degree days) "mid." (400-800 degree days) and "late" (>800 degree days) were chosen because they are approximately even time intervals and because the early period included the yolk sac stage and first feeding.

In a control experiment, the otoliths of two further batches of embryos were marked to investigate the relative effect of the two marking compounds. In one tank eggs of mean size 0.27mg were marked with calcein and eggs of mean size 0.32mg marked with alizarin complexone. The reverse marking scheme was used for eggs reared in another tank. Relative survival and length of larvae marked with both compounds was monitored from marking until 144 day degrees.

Statistical treatment

The effects of the otolith marking compounds on hatching length or survival of control larvae were tested with student t-tests or log likelihood tests respectively (Zar, 1984).

Total length, standard deviation of length, weight and relative condition factor of larvae from the different treatments were compared using the SAS procedure GLM (SAS Institute, 1988), with an analysis of covariance model. The probability acceptance levels for ANCOVA were reduced to $p=0.001$ to allow for repeated sampling of larvae from the same rearing tanks (maximum of 36 repeated measures). The residuals from each test were subjected to a Shapiro-Wilk test for normality. The residuals of total length were non-normally distributed and transformations failed to normalise the data. The analysis was, therefore, carried out using the ranks of total length. Regression analysis of the variables affecting total length, which included only the significant factors from the analysis using ranked data, was then conducted. The residuals from analysis of covariance of both $\log_{10}$ transformed weight and condition factor, and the standard deviation of length were normally distributed. In all models, age, in degree days, was the covariate, with the continuous variable mean egg size and the discrete variables rearing temperature and female pair as factors. Although eggs are referred to throughout as either large or small, a greater coefficient of determination was obtained by treating mean egg size as a continuous variable rather than as a discrete variable. Female pair was a variable created to incorporate maternal effects other than mean egg size but this variable also includes any tank effects, caused by differences in rearing conditions between tanks.

A separate analysis was carried out for condition factors before and after 450 degree days. This was due to the difference in slope before and after this age.

The additional null hypothesis, that there was no difference in the number of surviving larvae from small and large eggs between each time period was tested using a χ^2 test. Tests were performed separately for each rearing temperature.

RESULTS

The two marking compounds, alizarin complexone and calcein, had no significant effect on the survival of larvae during the first week after hatch (Table 4.2). The marking procedure also had no significant effect on the total length of larvae at hatch (Table 4.3).

Survival of larvae from female pair 3 at 13.5°C was low and so larvae from this tank were only sampled for 25 days (fig. 4.1f).

Despite the considerable overlap in the size of individual larvae hatching from different sized eggs, there were differences between the population means. Mean length was significantly affected by all three variables: mean egg size, rearing temperature and female pair (fig.4.1 and 4.2, Tables 4.4 and 4.5). When age was calculated in days, female pair 1 had a higher length specific growth rate at 10°C but there was little difference in the growth rates of female pairs 2 and 3 reared at either 10 or 13.5°C (fig. 4.1). However, when age was converted to degree days, which accounts for the expected faster growth at higher temperatures, longer larvae hatched both from larger eggs and at the lower temperature. These longer larvae had a higher length specific growth rate (fig. 4.2).

Table 4.2. The relative survival of control larvae marked with either alizarin complexone or calcein from hatch until 144 degree days through development. G: the log likelihood statistic (no test was significant).

Age (degree days)	Mean egg dry weight (mg)	No. larvae		G
		Alizarin	Calcein	
0	0.32	18	19	0.37
	0.27	1	2	
31	0.32	6	5	0.45
	0.27	6	4	
144	0.32	8	8	0.00
	0.27	2	2	

Table 4.3. The effect of the otolith markers alizarin complexone and calcein on the total length of larvae at 31 degree days post hatch. T: t-test statistic, s.d.: standard deviation; N: sample size

Mean egg dry weight (mg)	Marker	Mean total length /mm	s.d.	N	T
0.32	Alizarin	9.01	0.84	6	0.45
	Calcein	9.22	0.58	5	
0.27	Alizarin	8.77	0.61	6	1.03
	Calcein	9.34	1.17	4	

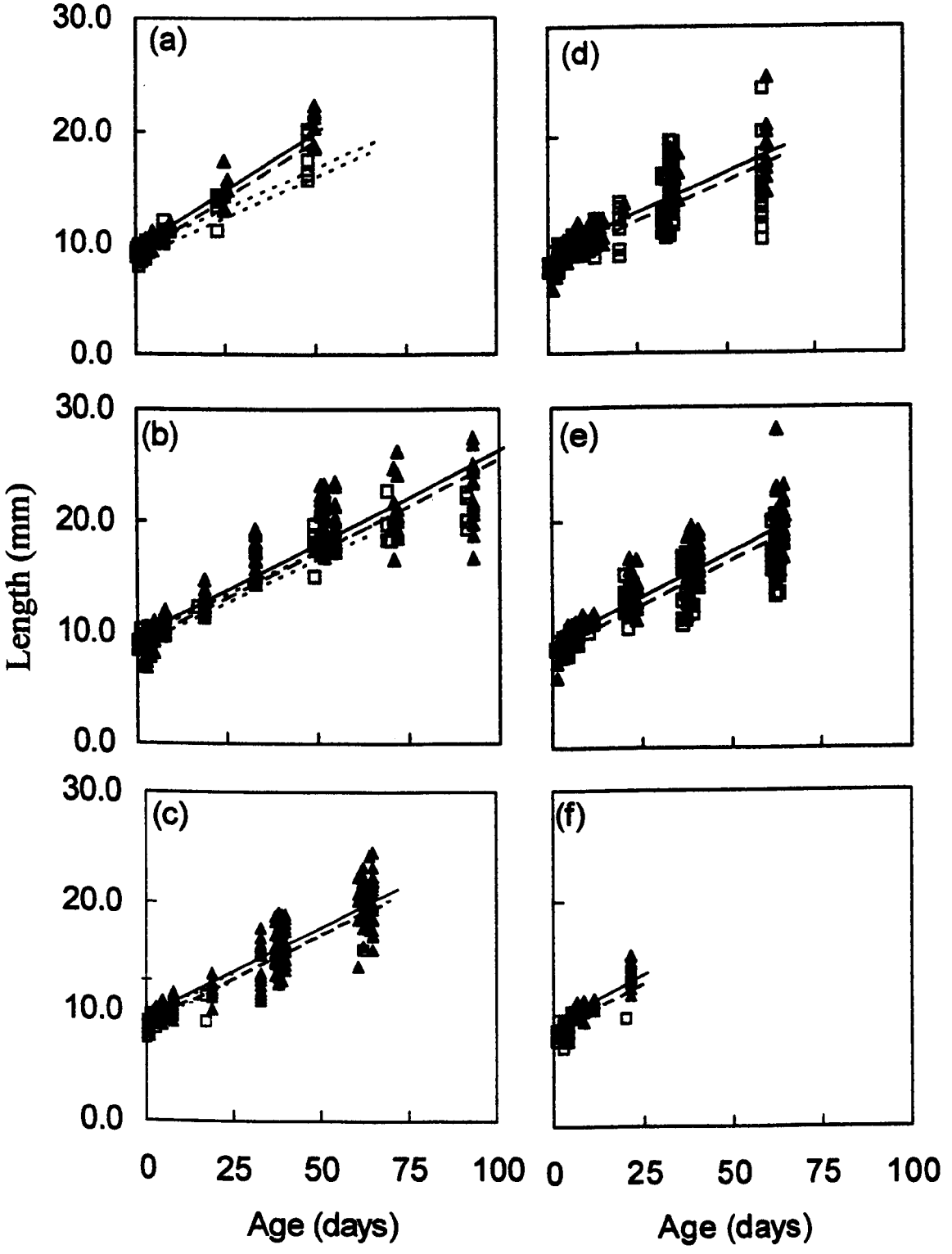

Figure 4.1. The growth, in total length, of larvae hatched from large (▲, —) and small eggs (□, -----). Fitted regression lines include the factors that were significant in an analysis of covariance model (Table 4.4) but were recalculated with age in days. The fitted lines for larvae reared at 13.5°C are marked on the graphs for larvae reared at 10°C (.....). Female pair 1 reared at (a) 10°C (d) 13.5°C, female pair 2 reared at (b) 10°C (e) 13.5°C and female pair 3 reared at (c) 10°C (f) 13.5°C. The data for large eggs are offset by +20 degree days.

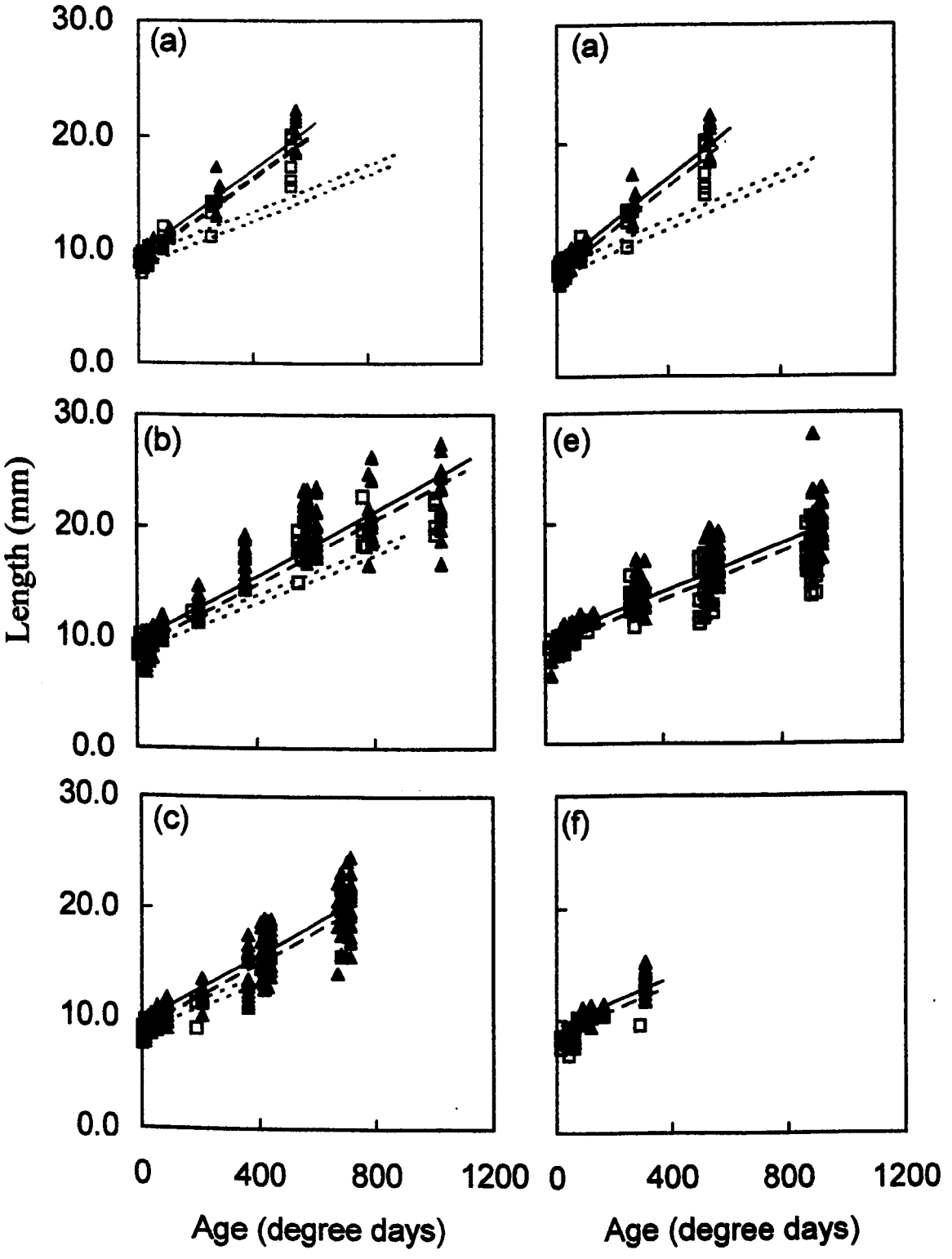

Figure 4.2. The growth, in total length, of larvae hatched from large (▲, ——) and small eggs (□, -----). Lines are fitted lines from an analysis of covariance model. The fitted lines for larvae reared at 13.5°C are marked on the graphs for larvae reared at 10°C (.....). Female pair 1 reared at (a) 10°C (d) 13.5°C, female pair 2 reared at (b) 10°C (e) 13.5°C and female pair 3 reared at (c) 10°C (f) 13.5°C. The data for large eggs are offset by +20 degree days.

Table 4.4. Results of Analysis of Covariance (F) on the variables affecting rank of total length (only significant factors are shown). Age was the covariate. Size: mean egg dry weight; Temp: rearing temperature; Pair: female pair; DF: degrees of freedom; s.s.: sum of squares; r^2: coefficient of determination

Source	DF	s.s.	F	r^2
Model	21	139800386	*290.22	0.83
Error	1242	28489632	-	-
Between egg size				
Size	1	1847857	*80.56	-
Temp	1	282291	*12.31	-
Size*Temp	1	370658	*16.16	-
Within egg size				
Age	1	375626	*16.38	-
Age*Temp*Pair	5	1795519	*39.14	-
				-

Table 4.5. Regression fits (intercept and slope) for total length against age including the significant factors from an analysis of covariance model (Table 4.4). Age in degree days.

Female pair	Mean egg dry weight (mg)	10°C		13.5°C	
		Intercept	Slope	Intercept	Slope
1	0.325	9.81	0.019	9.55	0.010
	0.232	8.73	0.019	8.55	0.010
2	0.335	9.93	0.015	9.65	0.011
	0.247	8.90	0.015	8.71	0.011
3	0.334	9.91	0.015	9.65	0.011
	0.247	8.90	0.015	8.71	0.011

The mean variation of length increased as larvae grew (Table 4.6) but was not significantly affected by any other factor. The relationship was described by the equation, s.d. = 0.0023A + 0.49, where s.d. is the standard deviation of length and A is the age in degree days.

The increase in larval weight with time was the same regardless of whether age was calculated in days or degree days (Table 4.7 and 4.8, fig. 4.3, fig. 4.4). Incubation temperature and female pair both affected the weight of larvae at hatch but egg size did not. Larvae from female pairs 1 and 3 were heavier at hatch when incubated at 10°C rather than 13.5°C and these heavier larvae increased in weight at the same rate (female pair 1) or faster (female pair 3). Although, larvae of female pair 2 were heavier at hatch when incubated at 13.5°C, they grew more slowly than those reared at 10°C. However, the calculated regression for female pair 2 at 10°C was a poor fit to the weight at hatch data (fig. 4.3b); the intercept was lower than any of the data points and the slope was correspondingly much steeper.

The geometric regression for the length-weight relationship for all larvae was, $W = 0.000098L^{3.3}$. Larval condition factor decreased from hatching to 450 degree days, a total length of 15mm, before increasing again (fig. 4.5). The relationship was described by the equation, $K = 0.000000039A^2 - 0.000034A + 0.024$, although age only described 31% of the variation in condition factor ($R^2 = 0.31$, n=394, F=157.88).

34% of the variation in relative condition factor was explained by the analysis of covariance model. Egg size was the only significant factor affecting RCF and this was only significant during the early period, before 450 degree days (Table 4.9). Larvae from small eggs were in a lower relative condition before 450 degree days.

Table 4.6. Results of Analysis of Covariance (F) on the variables affecting the standard deviation of length (only significant factors are shown). Age (in degree days) was the covariate. DF: degrees of freedom; s.s.: sum of squares; r^2: coefficient of determination.

Source	DF	s.s.	F	r^2
Model	14	57.83	*29.20	0.80
Error	100	14.15	-	-
Within egg size				
Age	1	2.59	*18.29	-

Table 4.7. Results of Analysis of Covariance (F) on the variables affecting $\log_{10}$ dry weight (only significant factors are shown). Age (in degree days) was the covariate; Temp: rearing temperature; Pair: female pair; DF: degrees of freedom; s.s.: sum of squares; r^2: coefficient of determination.

Source	DF	s.s.	F	r^2
Model	14	69.35	*133.57	0.73
Error	680	25.22	-	-
Within egg size				
Temp*Pair	2	0.94	*12.61	
Age*Pair	2	1.08	*14.57	

Table 4.8. Regression fits (intercept and slope) for dry weight against age including the significant factors from an analysis of covariance model (table 4.7). Age in degree days

Female pair	10°C		13.5°C	
	Intercept	Slope	Intercept	Slope
1	0.22	0.0015	0.13	0.0015
2	0.13	0.0064	0.19	0.0014
3	0.31	0.0017	0.17	0.0011

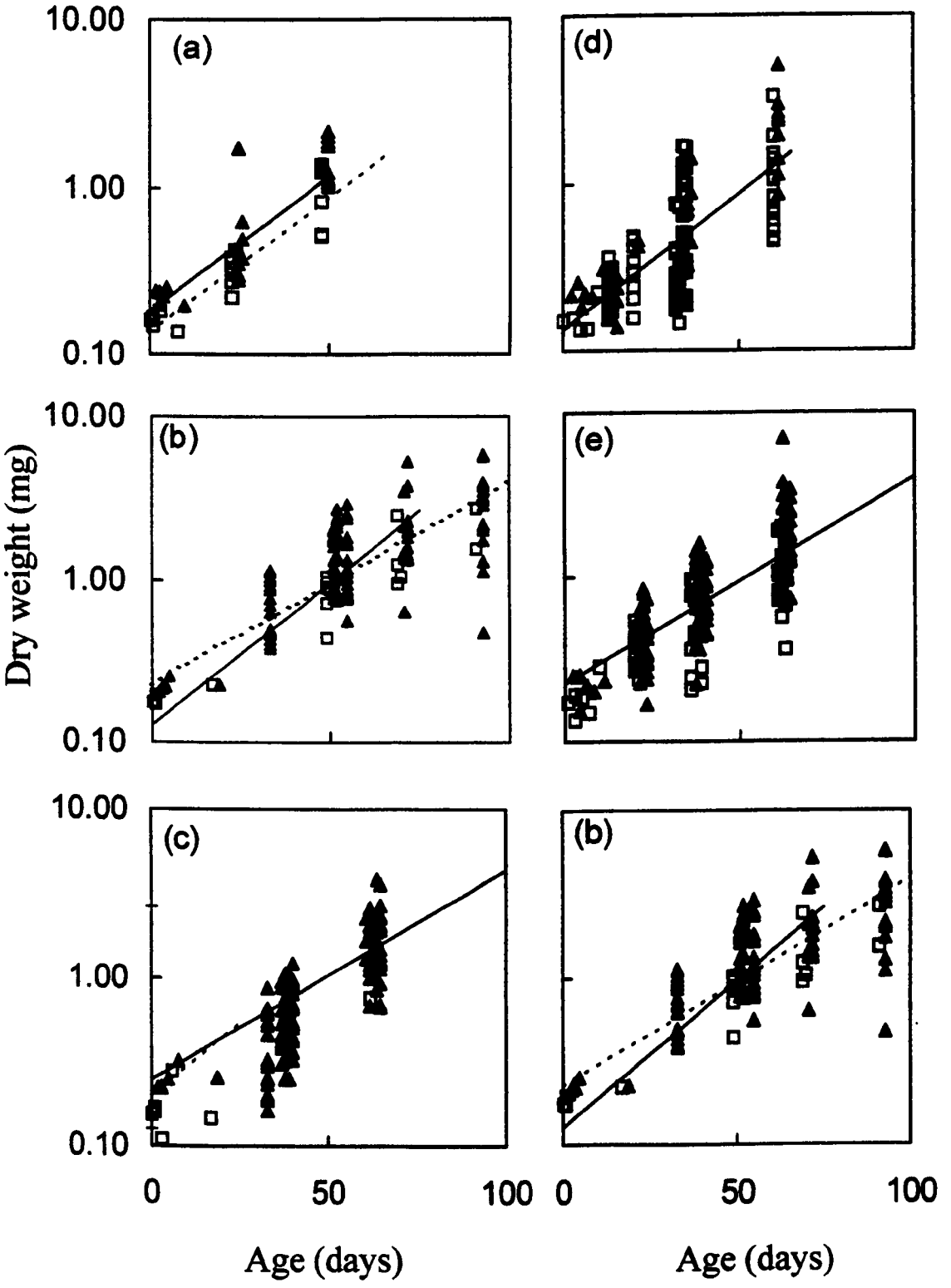

Figure 4.3. The growth, in dry weight, of larvae hatched from large (▲, —) and small eggs (□, -----). Fitted regression lines include the factors that were significant in an analysis of covariance model (Table 4.7) but were recalculated with age in days. Lines are fitted lines from an analysis of covariance model. The fitted lines for larvae reared at 13.5°C are marked on the graphs for larvae reared at 10°C (.....). Female pair 1 reared at (a) 10°C (d) 13.5°C, female pair 2 reared at (b) 10°C (e) 13.5°C and female pair 3 reared at (c) 10°C (f) 13.5°C. The data for large eggs are offset by +20 degree days.

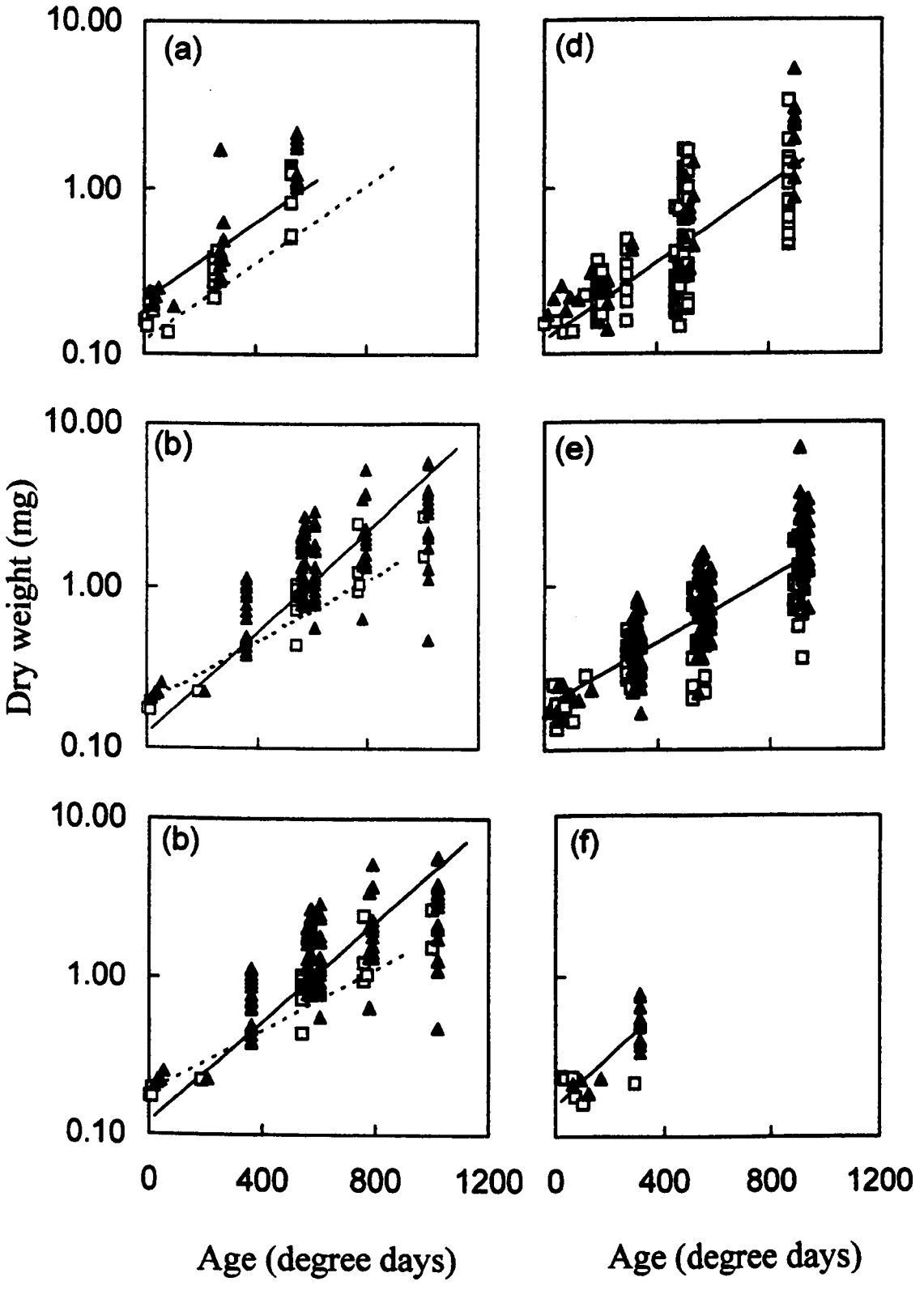

Figure 4.4. The growth, in dry weight, of larvae hatched from large (▲, —) and small eggs (□, -----). Lines are fitted lines from an analysis of covariance model. The fitted lines for larvae reared at 13.5°C are marked on the graphs for larvae reared at 10°C (.....). Female pair 1 reared at (a) 10°C (d) 13.5°C, female pair 2 reared at (b) 10°C (e) 13.5°C and female pair 3 reared at (c) 10°C (f) 13.5°C. The data for large eggs are offset by +20 degree days.

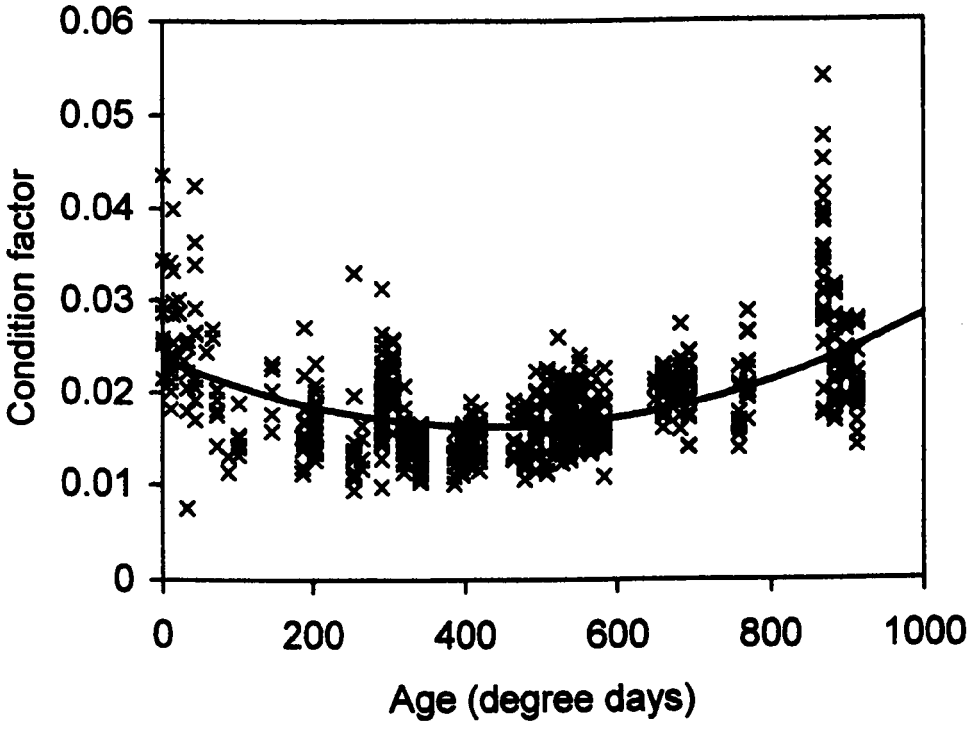

Figure 4.5. Condition factor of all larvae combined. The least squared polynomial regression fit is shown.

Table 4.9. Results of Analysis of Covariance (F) on the variables affecting $\log_{10}$ relative condition factor (only significant factors are shown). Age was the covariate (degree days). Size: mean egg dry weight; s.s.: sum of squares; r^2: coefficient of determination.

Source	< 450 degree days				> 450 degree days			
	DF	s.s	F	r^2	DF	s.s.	F	r^2
Model	21	1.12	*6.90	0.34	16	1.14	*11.95	0.34
Error	287	2.21	-	-	369	2.20	-	-
Between egg size								
Size	1	0.12	*15.11	-	-	-	-	-

The relationship was described by the equation, $\log_{10}(RCF) = 0.17Me - 0.050$. The relative condition factor of all larvae reared at 13.5°C and those reared at 10°C after 450 degree days remained constant, at approximately 1.0. This means that the condition factor of these larvae followed the pattern predicted for all larvae.

There was no significant difference in the relative survival of larvae from small or large eggs reared at 13.5°C (Table 4.10). However, larvae from small eggs suffered significantly higher mortality at 10°C. The majority of this mortality occurred during the early or mid period.

DISCUSSION

Both alizarin complexone and calcein were not detrimental to survival, 100% efficient and long lasting otolith markers for herring larvae. This otolith marking technique allowed the comparison of two groups of larvae from different sized eggs under identical conditions which is very rare in growth studies of larval fishes. The importance of rearing batches under identical conditions was highlighted by the female pair variable, which was a significant factor in most relationships.

There was a large degree of variation between individual herring larvae and therefore much overlap between the lengths and weights of progeny from large and small eggs. However, despite this variation between the progeny of individual females the mean weights and lengths showed consistent differences, particularly within paired comparisons. Larvae from large eggs were initially longer and grew faster. Although there was no significant effect of egg size on weight at hatch, the longer and heavier larvae hatching at the lower temperature tended to grow faster. Mean values for the hatching characteristics of larval herring (length, weight and

Table 4.10. The relative survival of larvae from small and large eggs reared at 10 and 13.5°C during each of the three time periods: Early: < 400 degree days; Mid.: between 400 and 800 degree days; Late: >800 degree days; χ^2 is the chi-squared statistic.

Time period	10°C		13.5°C	
	Small eggs (No. of larvae)	Large eggs (No. of larvae)	Small eggs (No. of larvae)	Large eggs (No. of larvae)
Early	132	202	143	181
Mid.	0	106	53	65
Late	22	155	87	100
χ^2	*87.91		0.27	

yolk volume) are known to be positively correlated with dry egg weight (Blaxter and Hempel, 1963).

There were significant differences between female pairs that affected the comparisons between rearing temperatures. Despite careful attempts to control conditions between rearing tanks there were still significant differences which could have been due to differences between rearing tanks or between the responses of progeny from different batches. The mean response of body size at hatch to incubation temperature shows considerable variation between egg batches (see Chapter 3).

In the present study larval condition factor decreased from hatch to a minimum at approximately 450 degree days (when larvae were between 14 and 17 mm total length) before increasing again. A similar drop in condition factor was found for sea caught (Blaxter, 1971, Vilela and Zijlstra, 1971, Hempel and Blaxter, 1963) and laboratory reared (Blaxter, 1971) herring larvae with the minimum condition factor occurring at a similar size (standard length of fixed larvae; 10-13 mm). The initial drop in condition will in part be due to the utilisation of yolk by larvae. However, condition does not start to increase until well after yolk sac exhaustion. Blaxter (1971) attributed this low condition factor to a change in the length-weight relationship of sea caught herring larvae which increase more rapidly in length, relative to weight, during the early growth phase. In the present study length also showed a greater variation between treatments than weight.

The length-weight relationship is also size dependant and shows that larvae pass through different growth phases. The power coefficient of the dry weight to length relationship, 3.3 was slightly lower than the reported range for laboratory reared herring, 3.8-4.73 (Checkley, 1984). However, if the length-weight relationship

for larvae in the present study is recalculated, excluding larvae smaller than 15mm, a value of 4.2 is obtained. Marshall *et al.* (1937) also found reared herring larvae smaller than 15mm total length to have a different length-weight relationship to those larger than 15mm.

A condition factor of 0.013 has been quoted as indicative of well fed herring larvae (Checkley, 1984). However, the dependency of condition factor on developmental stage means that it is not a good absolute measure of the nutritional condition of herring larvae. Calculation of a relative condition factor, which takes into account these changes in the length-weight relationship through development, does allow comparisons between groups of larvae. De Silva (1973) also found discontinuities in the relationship between condition factor and length of reared herring larvae.

Condition factor does however give a measure of larval body reserves, and may indicate stages when larvae are more susceptible to starvation. The combination of small hatch size of larvae reared at 10°C and the lower relative condition factor of larvae from small eggs before 450 degree days, may account for their relatively high mortality; especially as the lowest condition factor of these larvae coincided with the period of high mortality. Fish larvae are thought to suffer from size-selective mortality even in the absence of predators (Paradis, *et al.*, 1996). Smaller individual larval herring suffered selective mortality in conditions of low food availability (Folkvord *et al.*, 1997). Size selective mortality has also been ascribed to starvation in *Heterandria formosa* (Henrich, 1988) and in larval turbot (Rosenberg and Haugen, 1982).

In simulation models Rice *et al.* (1993) found that larger, faster growing individuals and populations with greater size variation between individuals are more

likely to survive predation. Longer herring larvae with faster growth hatched from large eggs in this study. As starving larvae are also more susceptible to predation (Checkley, 1984), herring larvae from small eggs, which encounter low environmental temperatures may be especially vulnerable to predation when feeding conditions are poor.

However, although in a theoretical ecosystem larger larvae are expected to have an increased chance of survival, aquatic ecosystems are heterogeneous (e.g. Paradis *et al.*, 1996). The conditions that larvae encounter could vary considerably, and may differ to the extent that selection is strongest for a larval phenotype other than large size. Larval body size may be reduced at higher temperatures due to physical constraints of processes such as diffusion (Atkinson and Sibly, 1997).

The experimental design of this study only allowed analysis to be performed at the group level and individual larvae could not be followed. Chambers *et al.* (1989) found differences within their analyses of hatching Capelin, *Mallotus villosus*, depending upon whether data was analysed at the group or the individual level. As natural selection works at the level of the individual they state that extrapolating from analysis of groups to individuals may be misleading.

However, it is possible to backcalculate the size ranks of individual larvae from daily otolith increments (Folkvord *et al.*, 1997, Chambers and Miller, 1995, Rosenburg and Haugan, 1982). In experiments where feeding conditions have remained constant this technique has shown that the size ranks of individual larvae are maintained for up to 5 weeks, with initially longer larvae remaining longer. This tends to agree with the findings of Chambers and Leggett (1996) who managed to recreate most of the variation in length of young of year fish simply by applying an exponential growth function to the size variation of larvae after early mortality.

Although they stress that this is an oversimplification it indicates how important even small initial size differences may be and how these differences can be propagated through development to affect survival.

CHAPTER 5:

A BEHAVIOURAL BASIS FOR GROWTH RATE VARIATION IN HERRING *CLUPEA HARENGUS* LARVAE.

INTRODUCTION

Growth rate depends on a number of interacting physiological processes which include the rate of food consumption, the ability to utilise the dietary components of that food and the efficiency of metabolism which results in nutrients and energy becoming available for body growth (Atchley, 1984, McCarthy, 1980). Between individuals of the same fish species, growth and developmental stage correlate well with body size (Fuiman and Higgs, 1997). The change in length through time can therefore be used as a measure of growth.

Size at age of fish larvae is highly variable both in culture (Chambers and Leggett, 1987, Chambers *et al.*, 1988, Purdon *et al.*, 1972) and in the wild (Munk, 1993, Maillet and Checkley, 1991). This variation between individuals usually increases through the larval phase (Sakagawa and Kimura, 1976, Beyer and Lawrence, 1980, Hunter and Kimbrell, 1980). In many freshwater fish species differential growth rates of individuals can be explained by behavioural interactions between these individuals. For example, aggressive interactions between salmonid larvae lead to the development of a dominance hierarchy of individuals. Dominant individuals obtain a higher proportion of the available food ration and therefore grow faster (e.g. Heland, 1991). However, despite the lack of behavioural interactions between individual Atlantic herring larvae, *Clupea harengus* L., there is still variation between the growth rates of individuals from the same batch of eggs (Pangiotaki and Geffen, 1992).

Herring larvae feed on planktonic prey much smaller than themselves and searching for prey is therefore the most important component of the predation cycle

(O'Brien *et al.*, 1990). Efficiency of foraging will, therefore, have a large effect on the total energy budget. In another planktivorous fish, adult bleak, *Alburnus alburnus*, foraging speeds were found to be close to the theoretical speed that maximises growth (Ware, 1975). This study aims to investigate if differences in foraging strategy, volume of water searched and food intake, correlate with growth rate. By comparing these parameters for larvae of the same length, but of different ages, fast and slow growing larvae herring larvae can be compared.

MATERIALS AND METHODS

Larval rearing

Ripe adult herring were caught from two spawning areas, Douglas Bank off the Isle of Man (September 1994 and 1995), and Ballantrae Bank in the Firth of Clyde (March 1995). Both batches of Manx 1995 eggs were reared in the same tank and distinguished by marking of their otoliths (See Chapter 2 for detailed methodology). In total seven batches of eggs were reared (Table 5.1).

Filming protocol

Experimental filming commenced after the period of high mortality at first feeding 1 hour prior to filming an array of infra-red light emitting diodes was lowered into the selected rearing tank. Infra-red light was chosen as it is outside the visual range of herring larvae and would therefore not affect larval behaviour. Using an array of lights that could be moved between tanks meant that larvae did not have

Table 5.1 Origin, mean egg size and rearing temperature of the seven batches of *Clupea harengus*.

Stock	Year	Batch	Mean dry egg weight (mg)	Rearing temperatures (°C)
Manx	1994	1	0.36	10
		2	0.29	10
Clyde	1995	3	0.50	8,5
		4	0.44	12,8,5
		5	0.33	12,5
Manx	1995	6	0.34	13.5
		7	0.25	13.5

to be moved from their rearing tanks but could be filmed in-situ with the minimum of disturbance. The resulting bright field image of larvae was recorded onto conventional video recording at 25 frames a second via a Vista video camera with a 90 mm telephoto macro lens suspended above the tank, which gave an approximately $4mm^2$ field of view.

Filming of larval feeding behaviour was conducted over a 4 month period at intervals chosen so that consecutive samples contained larvae with overlapping length distributions. In this way larvae of the same size but different ages, i.e. fast and slow growing larvae, could be compared. Larvae were filmed before the introduction of the daily feed at 11.00h in the morning and then again at 14.00h just after the introduction of the daily feed. The same batch were filmed for two (or three) consecutive days and these four (or six) periods combined constituted one sampling period. Each filming period continued until either five fish that had just been filmed had been captured (twenty larvae were captured for Manx 1995 experiments) or 1 hour had elapsed.

Video analysis

In addition to the five sequences of larvae that had been captured, up to a further 15 sequences were analysed during each filming period (only sequences of captured larvae were used for Manx 1995 experiments in which progeny of large and small eggs were identified by otolith marking). Videos were analysed sequentially from the start of each filming period and only sequences of larvae that remained within focus for the duration of that sequence, and were therefore swimming in the horizontal plain, were selected for analysis. The maximum length of each larva,

during each swimming sequence, was measured from the video. A linear regression line was calculated to predict total length from this maximum length. A separate regression was calculated for both Manx 1994 and Clyde 1995 larvae.

The x,y screen co-ordinates of the head position of larvae were digitised from every second frame of each video sequence (at 80 ms intervals). Data were smoothed by taking a weighted average of the current point plus two points before and after (Harper and Blake, 1989). Mean swimming velocities (V) for active periods were calculated as:

$$V = \sqrt{\left(\frac{dx^2}{dt} + \frac{dy^2}{dt}\right)} \qquad (5.1)$$

the distance moved by larvae in the x and y direction (mm), t is time (s). The distance moved in both x and y relative to the water was calculated as, e.g. for x:

$$\frac{dx}{dt} = \frac{dx'}{dt} - \frac{dx^d}{dt} \qquad (5.2)$$

where x' is the actual distance moved by each larva and x^d is the current velocity during each filming sequence which was calculated from the movement of each larva during non-swimming periods. A threshold, a constant value for each sequence, typically $<2 mm.s^{-1}$ was used to detect non-swimming periods.

Herring larvae may be either cruise (MacKenzie and Kiørboe, 1995) or saltatory searchers (H. Browman, Marine Institute Austevoll, *Pers. Com.*). For the purposes of this study herring larvae were assumed to be cruise searchers so that the volume of water searched per second (S) was directly proportional to the distance swum.

$$S = v.A.P \qquad (5.3)$$

where v is the mean swimming speed during active periods, A is the proportion of time spent active and P a measure of larval visual field was calculated as:

$$P = 2/3\pi(1.55L - 8.06)^2 \quad (5.4)$$

where L is total larval length (Blaxter and Staines, 1971, Rosenthal and Hempel, 1970).

The length of food in the gut of each larva could be seen clearly on the video sequences and was measured at the same time as the maximum length. An index of food volume (F) was calculated as:

$$F = L_f.g \quad (5.5)$$

where L_f is the length of food in the gut (mm) and g is the gut cross sectional area.

To calculate gut cross sectional area ventral and lateral views of the mid gut of herring larvae were recorded at 63x magnification via a video camera mounted in an Olympus SZ-STS stereo zoom microscope. Thirty two larvae of total length range 11-18mm and with different volumes of food in the gut were filmed. From these videos gut height (h) and width (w) were measured at four points spread at even intervals along the midgut and cross sectional area, which was assumed to be oval, was calculated.

Data analysis

Larvae which had no food in the gut and those that remained inactive for the whole filming sequence were removed from the analysis as they were assumed to be non-foraging larvae and may have been moribund.

Pearsons product moment correlation coefficients were calculated to identify which of the variables: mean egg dry weight, rearing temperature, age, stock, food volume, volume searched and fed/unfed (before and after the introduction of food), were correlated with total length.

The effects of age and length on both search volume and food index data were tested using analysis of covariance (SAS institute, 1988). A Shapiro-Wilks analysis showed that the residuals from an analysis of covariance model fit to $\log_{10}$ search volume were normally distributed. The residuals for the model fit to $\log_{10}$ food index were non-normally distributed and so the model was run using the rank of food index. A second order polynomial best described the relationships. The significant factors from the analysis using ranks were then included in a model using $\log_{10}$ food index. The probability acceptance levels for ANCOVA were adjusted to account for the number of repeated measurements on larvae from the same rearing tanks. The maximum number of repeated measures was 36 and so p=0.001 was calculated as the significance level.

RESULTS

There was considerable variation in growth rates between individual herring larvae (fig. 5.1). Larvae took between 200 and 1100 degree days to grow to 17mm in length. The relationship between maximum length and calculated total length for Manx 1994 larvae was described by the linear function $L = 0.63L_m + 7.48$ (R^2=0.46, n=157, F=*132.79) and for Clyde larvae by the linear function $L = 0.93L_m + 2.63$ (R^2=0.57, n=300, F=*399.75), where L_m is the maximum length.

Gut cross sectional area increased with total length of each larva, the relationship was described by a least squares second order polynomial fit, $g = 0.00093L^2 - 0.010L - 0.027$. This calculation was based on the least squares linear regression relationships fitted to predict gut width from gut height,

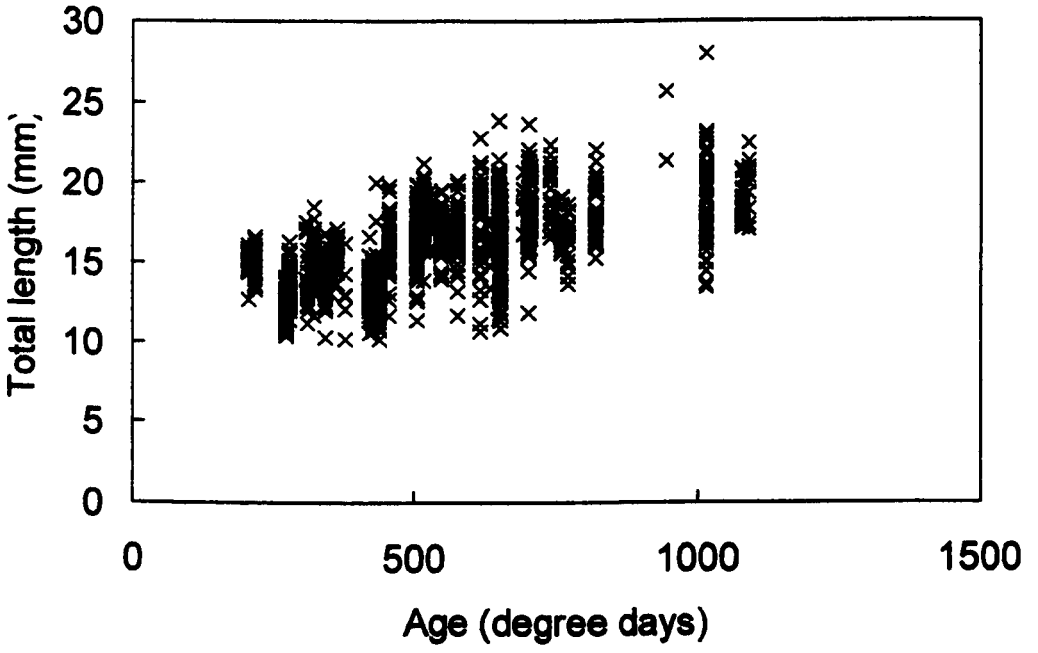

Figure 5.1. Calculated total length against age for herring larvae showing the variation between individuals.

$w = 0.59h + 0.025$, R^2=0.62, F=*170.37; and mean gut height (h_m) from total fish length, $h_m = 0.022lL - 0.14$, R^2=0.56, F=*37.96.

The index of food consumption, age and volume searched were the main correlates with fish length (Table 5.2). Spawning stock, mean egg dry weight, rearing temperature and feeding were only weakly correlated with total length and so they were not included in further analysis.

Both longer and slower growing larvae swam faster. Search volume (S) increased with both increasing age (A) and total length (L), the least squared linear fit was described by the equation, $\log_{10}S = 0.00040A + 0.11L + 1.53$ (Table 5.3, fig. 5.2). However, Food index was only related to total length, $\log_{10}F = -0.0050L^2 + 0.27L - 3.78$.

DISCUSSION

Despite the vast technological improvement and other differences in methodology between the present study and that of Rosenthal and Hempel (1970), the search volumes of herring larvae were in close agreement. Our predicted value of $6.3L.h^{-1}$ for a 13 mm larva, of average growth rate, is within the range of search volumes, 3-8 $L.h^{-1}$ for 12-14mm herring larvae, presented by Rosenthal and Hempel (1970). Although Rosenthal and Hempel (1970) found an almost three fold variaton in the volume searched by larvae they did not attempt to explain this variation in terms of its effect on growth rate.

The lack of influence of rearing temperature on search strategy agrees with the findings of investigations into the effect of temperature on feeding "S-strikes" (Morley and Batty, 1996). The speed of feeding "S-strikes" remained relatively

Table 5.2. Factors correlating with total length. r: Pearsons product moment correlation coefficients.

Correlate	r
Food index	0.64
Age	0.65
Search volume	0.66
Stock	0.18
Mean egg dry weight	0.04
Rearing temperature	0.10
Fed/unfed	0.01

Table 5.3. Analysis of covariance of the effect of age and length on both the $\log_{10}$ search volume and the rank of food intake. D.F.: degrees of freedom; s.s.: sums of squares; F: the analysis of covariance F statistic; r^2: the coefficient of determination.

$\log_{10}$ S					Rank food index			
Source	D.F.	s.s.	F	r^2	D.F.	s.s.	F	r^2
Model	3	160.09	*964.69	0.69	4	51802373	*289.39	0.52
Error	1328	73.46			1054	47168236		
A	1	4.94	*4.94		-	-	-	
L	1	155.10	*2803.86		1	2302367	*51.45	
L^2	-	-	-		1	694224	*15.51	

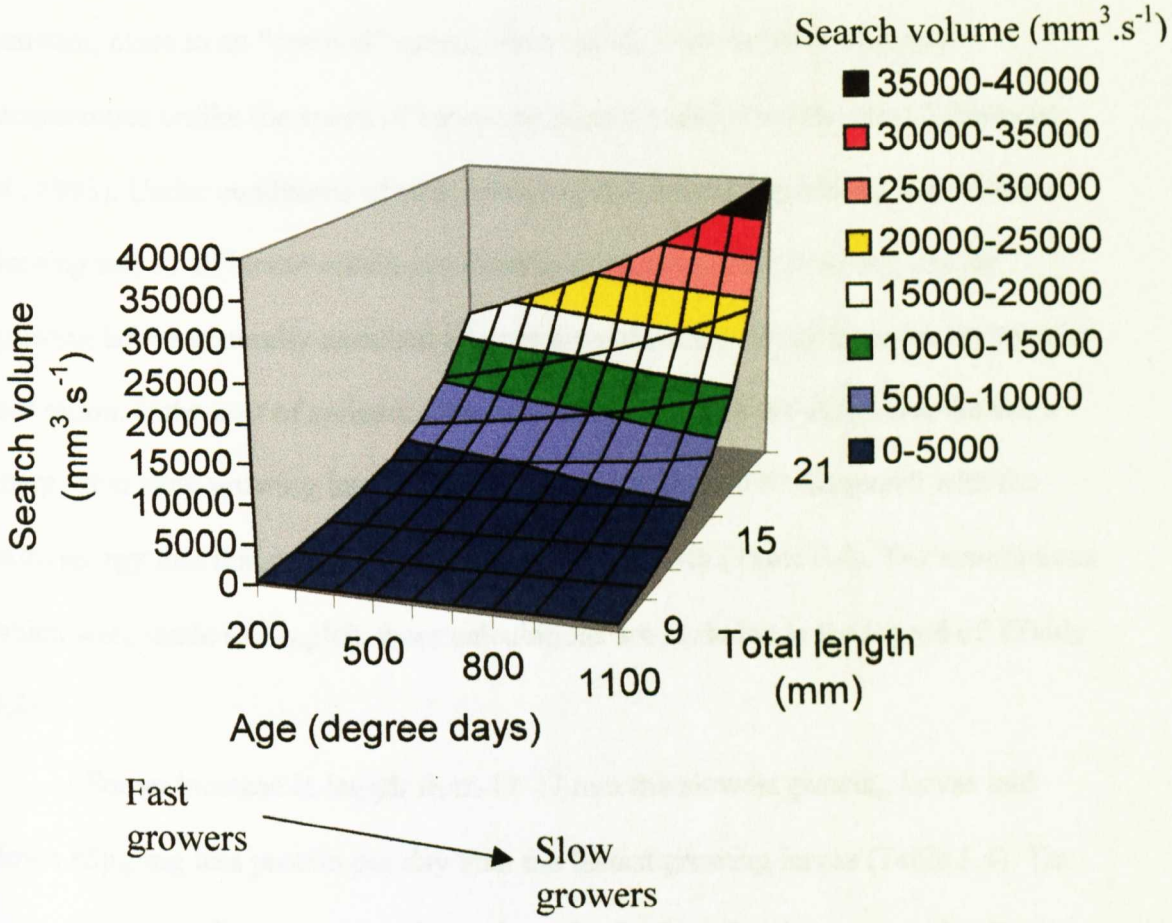

Figure 5.2. The variation in search volume of herring larvae with both length and age. The fitted surface from an analysis of covariance model is shown. As indicated on the figure, faster growing larvae are those that reach a given size at a younger age (to the left of the x-axis).

constant, close to an "optimal" speed, when larvae were tested at different temperatures unlike the speed of escape responses which was maximised (Batty *et al.*, 1993). Under conditions of tank culture in the current experiment food was not limiting and so all larvae obtained a close to maximal ration, however, slower growing larvae generally searched a larger area, thus expending more energy to gain that ration. If the cost of swimming and the cost of growth are estimated, the extra energy that slow growing larvae invest into swimming can be compared with the extra energy that fast growing larvae invest into growth (Table 5.4). The assumptions which were made to simplify these calculations are included in the legend of (Table 5.4).

For an increase in length from 12-17 mm the slowest growing larvae laid down 85μg/mg less protein per day than the fastest growing larvae (Table 5.4). The cost of the extra distance covered searching for food by the slowest growing larvae was equivalent to approximately 50% of the reduction in growth. The less efficient searching of slow growing larvae may therefore account for a large proportion of the variation in growth rate between fast and slow growers.

When the 95% confidence intervals of the mean search volume for each length class are plotted, exponential regression lines, fitted by least squares, describe both the upper (R^2= 0.99, n=12, F=*912.76) and lower (R^2=1.00, n=12, F=*3298.68) limits (fig. 5.3). The difference in behaviour between fast and slow growing larvae increases through early development. This implies that the lengths of fast and slow growing larvae will also be diverging. As the majority of the batches of larvae used in this experiment were reared in separate tanks the growth of individual batches could not be compared.

Table 5.4. Estimation of the energy (in terms of protein) invested into both growth and searching by the fastest and slowest growing larvae. The length-weight (L-W) relationship is taken from de Silva (1973). The cost of swimming was calculated from Yin, Batty, Franklin and Johnston (unpublished data). The protein equivalent was 1.38g O_2 per g protein (West *et al.*, 1966). The cost of growth was assumed to be 42% (Kamler, 1992) and growth was assumed to be in terms of protein only. An average developmental temperature of 10°C was used to calculate t for fast and slow growing larvae. Early growth in length was assumed to be linear and a least squares regression fit of length at age was extrapolated to estimate the length of larvae at time t=0 ($L = 0.0077A + 11.86$, r^2=0.42, n=1369,F=*972.21).

Parameter	Fast growth	Slow growth	Difference in cost	units
Growth				
L at t=0	12	12		mm
L at t=t	17	17		mm
W at t=0	0.22	0.22		mg
W at t=t	0.95	0.95		mg
t	18.18	100		days
Cost (protein)	114.12	20.75	93.37	$\mu g.mg^{-1}.day^{-1}$

Table 5.4 (cont.)

Swimming				
$O_2.L^{-1}$	0.004	0.004		$\mu g.O_2.mg^{-1}$
$L.s^{-1}$	0.30	0.69		$L.s^{-1}$
$L.day^{-1}$	12960	29808		$L.day^{-1}$
Cost (protein)	37.57	86.40	48.83	$\mu g.mg^{-1}.day^{-1}$

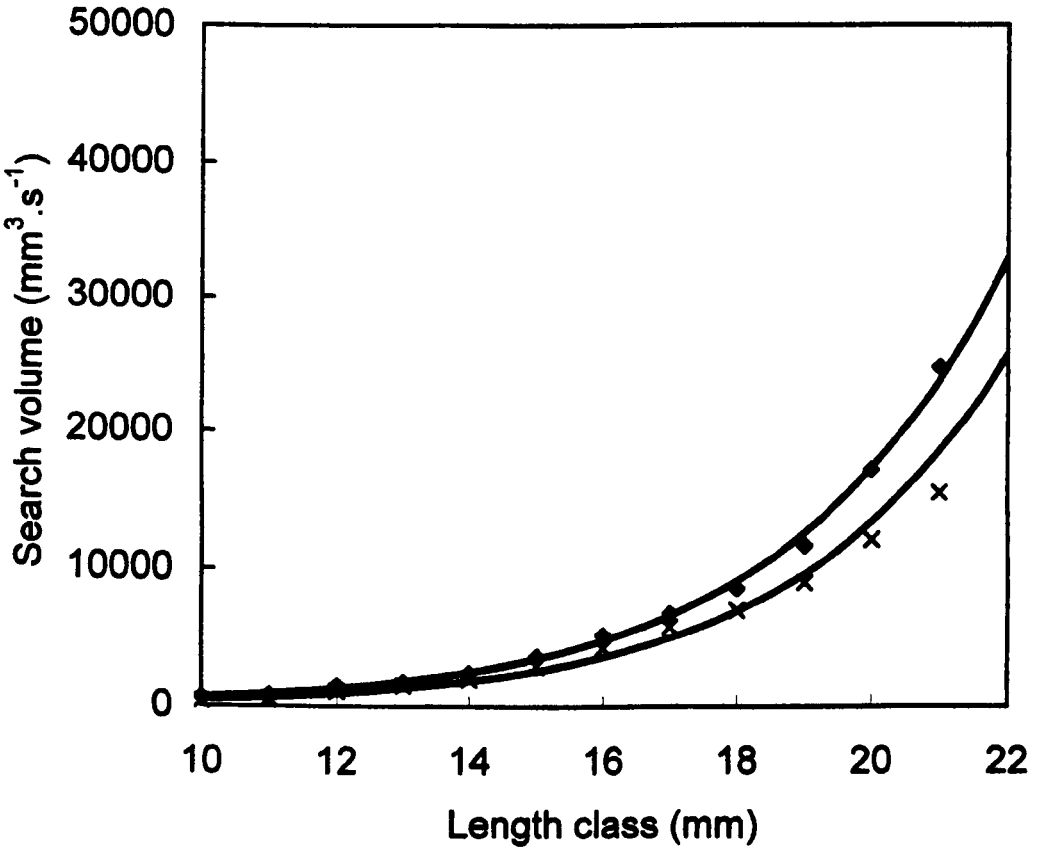

Figure 5.3. The upper and lower 95% confidence intervals of mean search volume for each 1 mm size category. Fitted exponential curves are fitted by least squares.

Although tank culture provides a very specific set of conditions for larval growth, we have demonstrated that under these conditions, differences in search behaviour can account for a large proportion of the observed variation in growth. Although the heterogeneity of aquatic ecosystems will result in larvae experiencing a wide range of potential environmental conditions (e.g. Paradis *et al.*, 1996), variations in foraging behaviour may favour certain groups of larvae at certain times during development.

Larval searching behaviour alters in response to their environment. For example when they encounter patches of prey sardine larvae swim more slowly and turn through larger angles thus increasing their chances of remaining within patches of food (Hunter and Thomas, 1974). The ability to implement changes in search strategy may also vary between groups.

In this study individual larvae could not be identified and it was necessary to group larvae into sub-populations of larvae with different growth rates at a given time. However, studies have shown that larvae with initially faster growth rates tend to maintain this faster growth (Folkvord *et al.*, 1997, Chambers and Miller, 1995, Rosenburg and Haugan 1982). Chambers and Leggett (1996) were able to recreate the majority of variation in size of 1 year old juveniles simply by applying exponential growth to the size variability recorded for larvae after first feeding mortality. This indicates how the structure of a population can be determined by a potential bottleneck, such as first feeding. This study has highlighted a mechanism whereby behavioural differences, and not just initial size, could act to maintain or enhance variation in growth rate between larvae.

CHAPTER 6:

THE EFFECT OF MANIPULATING EGG SIZE ON THE HATCHING CHARACTERISTICS OF HERRING *CLUPEA HARENGUS* L.

INTRODUCTION

It is well documented that longer, heavier fish larvae with more yolk hatch from larger eggs (Blaxter and Hempel, 1963). The size of hatching larvae will be determined by the chemical composition of the eggs, the genotype of the parents and the environment experienced during development. The volume of yolk invested into each egg, one of the key maternal factors, is proportional to the nutrition available for growth of the embryo. The volume of yolk is therefore likely to determine larval size. However, this hypothesis is as yet untested in fish (Chambers, 1997). In a study of *Sceloporus* lizards Sinervo (1989) removed known volumes of yolk from eggs and compared the hatchlings with those of unmanipulated eggs. Using this technique it was possible to determine the proportion of hatch length determined directly by the volume of yolk and that determined by other factors. Figure 6.1 (modified from Sinervo, 1989) explains the theory behind this technique by showing the two extreme outcomes of experimental manipulation of size. When size is manipulated and the response of the trait follows the mean response of the population, outcome A results, that is the trait being studied is directly proportional to size. When size is manipulated but there is no effect on the response of the trait, outcome B results, i.e. the trait is unrelated to size and is determined by some other factor. In this study we reduced the size of herring eggs by removing yolk (2 quantities removed; "little" and "lot") soon after fertilisation. The effect of yolk reduction on the length, body weight and yolk weight of larvae at hatch was investigated.

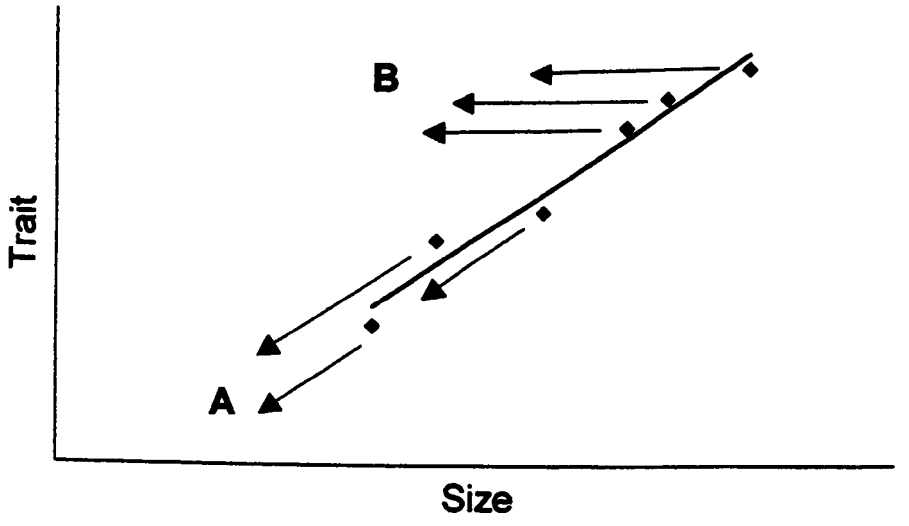

Figure 6.1. Two theoretical responses (A and B) of a trait to the manipulation of size (modified from Sinervo, 1989). See text for full explanation.

MATERIALS AND METHODS

Ripe adult herring were caught from two spawning stocks, Manx herring (September 1996) and Celtic Sea herring (January 1997 and 1998). After fertilisation embryos were washed with 11ppt sterile seawater (11ppt) which is iso-osmotic to the cells of herring larvae (Peter Tytler, Stirling University, *Pers. Coms.*). 11ppt was used to reduce both the risk of infection and osmotic stress on embryos after piercing of the chorion. Embryos were maintained in 1 litre plastic containers, half filled with 11ppt, and maintained at 13°C. Full water changes were effected on alternate days.

Experimental manipulations of yolk volume were conducted within 4 hours of fertilisation (before the 2 cell-stage; Hill and Johnston, 1997). Yolk was removed using capillary tubes, the tips of which had been drawn into fine needles using a PHL-1 (World Precision Instruments). Capillaries were half filled with 11 ppt and the remainder of the capillary tube was filled with degassed silicone oil. The meniscus formed at the oil and water interface was always positioned in the straight portion of the capillary tube. The tip of each needle was broken off to a diameter of between 30 and 50μm; the finest that would withdraw yolk without blocking. The needle was then attached to a microprocessor-controlled nanolitre injector (World Precision Instruments) and the piston of the injector was extended into the silicon oil, expelling some of the seawater from the needle. If after attachment the needle contained any air bubbles a new needle was selected and the procedure repeated. The needle was inserted through the chorion of each egg using a micro-manipulator which allowed fine scale movements in all three dimensions. Small quantities of yolk could then be drawn into the needle by controlled withdrawal of the piston. As the plunger was withdrawn the distance the meniscus moved (0.31 and 0.61mm for the two

treatments) was measured using a microscope, with an eyepiece graticule (mag. x 25), which was mounted at right angles to the glass needle.

The mass of distilled water in sections of seven capillary tubes (M_w) were calculated as:

$$M_w = M_f - M_c \qquad (6.1)$$

where M_f is the mass of the capillary tube full of distilled water and M_c is the mass of the empty capillary tube. By multiplying the mass of distilled water by its density, 1.0 gcm^{-3}, the internal volume (V_i) of the capillary tube was calculated.

The mass of yolk (M_y) in an egg of known dry weight (M_e), i.e. total mass of the egg minus the weight of the chorion, was calculated as $M_y = 0.88M_e - 0.012$ (Blaxter and Hempel, 1966). Yolk volume (Y_v) was then converted to dry mass (Batty, unpublished data) using the least squares regression, $M_y = 0.26Y_v$ (r^2=0.58, F=*30.92, n=23). Assuming 100% efficiency of yolk removal the two treatments removed 0.038 and 0.076mg of yolk from eggs. The efficiency of yolk removal was not calculated but a granular fluid could clearly be seen entering the syringe. After each manipulation the needle was emptied of yolk and the meniscus reset to the start position on the eyepiece graticule. The expelled liquid could clearly be seen to be lipid based.

As well as the two yolk reductions, a sham treatment was conducted during which eggs were pierced with the needle but no yolk was removed. Before each of the three treatments a diagram was drawn of each replicate slide so that the position of each treated egg was known. All non-treated and damaged eggs were removed within two days of the start of each experiment. Control slides were unmanipulated.

On the day of hatching larvae were removed from the incubation pots, anaesthetised with benzocaine and live total length and yolk volume were measured.

Yolk volume was measured as in Equation 3.1. The dry weights of larvae were measured as for eggs except that larvae were weighed individually (except for 0.44mg eggs).

RESULTS

The lengths of capillary tubes (L_c) explained over 99% of the variation in internal volume (V_i):

$$V_i = 0.48L_c \ (r^2 = 1.00, F=*1501, n = 7) \qquad (6.2)$$

From the least squares regression the two treatments were calculated to have removed 0.15 and 0.29mm^3 of fluid.

There was also no difference in the time to hatch of manipulated and unmanipulated eggs. Survival of treated eggs was generally poor, ranging from 0-50% (Table 6.1). There were also large differences in survival between replicates. However, there were no significant differences between the lengths of larvae hatching from replicate slides (Table 6.2) and so data for each replicate were combined.

There was only one significant difference between treated and untreated eggs; larvae hatching after a lot of yolk had been removed from 0.35mg eggs were shorter than both control and sham treated larvae (Table 6.3). However there was a general decrease in the length and body weight of larvae at hatch in the sequence control → sham → little → lot. There was no apparent pattern for the mean weight of the yolk sac at hatch.

Table 6.1. The number of treated eggs and the number surviving to hatch for each experimental treatment. Egg Size: mean egg dry weight (mg); Exp.: experimental treatment

Egg Size	Exp.	n		Egg Size	Exp.	n	
		Treated	Hatch			Treated	Hatch
0.35	sham	40	0	0.44	sham	35	10
		26	12				
					little	39	0
	little	21	0			35	6
	lot	20	10		lot	35	0
		20	9				
0.37	sham	36	0	0.49	sham	39	0
		21	4			20	0
	little	12	4		little	14	4
		14	4			26	0
	lot	25	5		lot	32	4
		24	0			11	0

Table 6.2. T-tests comparing the mean lengths of larvae hatching from replicate slides for each treatment. n: the number of larvae hatching; T: the t-statistic.

Mean egg dry weight (mg)	Treatment	Replicate	Mean length (mm)	n	T
0.35	lot	1	8.15	9	
		2	8.16	10	0.04
0.37	little	1	8.75	4	
		2	9.18	4	2.07
0.49	control	1	9.07	12	
		2	9.17	11	0.54

Mean egg dry weight (mg)	Source	Treatment	Mean length (mm)	SE of length	Mean body weight (mg)	SE of body weight	Mean yolk sac weight (mg)	SE of yolk sac weight	N
0.35	Manx 1996	control	8.81a	0.15	0.114	0.0032	0.090	0.019	22
		sham	8.71ab	0.16	0.099	0.0075	0.096	0.022	12
		lot	8.16b	0.20	0.109	0.0058	0.089	0.026	19
0.37	Manx 1996	control	8.92	0.31	0.116	0.0037	0.096	0.0044	12
		sham	8.46	0.86	0.114	0.0037	0.094	0.0182	4
		little	8.96	0.12	0.083	0.0108	0.074	0.0093	8
		lot	8.25	0.31	0.104	0.0131	0.094	0.0091	5
0.44	Celtic Sea 1997	control	9.07	0.37	0.181	-	0.116	0.012	18
		sham	8.76	0.21	0.164	-	0.132	0.012	10
		little	8.57	0.41	0.180	-	0.116	0.017	6
0.49	Celtic Sea 1998	control	9.12	0.084	0.178	0.0054	0.172	0.0061	23
		little	8.98	0.068	0.157	0.0056	0.180	0.0145	4
		lot	8.91	0.23	0.168	0.0298	0.154	0.0131	4

Table 6.3. The mean and standard errors of length, yolk weight and body weight at hatch. Source: is the stock and year the eggs were collected; n: the number of larvae hatching; SE: the standard error; a and b denote significant differences as tested with a Kruskal Wallace analysis followed by a Dunns test (Zar, 1984; Q=3.115)

The two treatments removed a greater proportion of the yolk from small eggs than large eggs. Therefore, if the quantity of yolk determines each hatching character there should be a greater change in the characters of small eggs. This is only true for hatch length (Table 6.3).

DISCUSSION

We have developed a technique for manipulating egg size that embryos can survive through to hatch. However, mortality of embryos was still high and varied between replicate slides. As was found in chapter 3 there was also a high degree of variability in hatching characteristics between individuals. The variation may have been increased by errors in the quantity of yolk removed from each egg.

If the efficiency of yolk removal is assumed to be 100%, then the mean egg dry weight of each treated egg can be recalculated after yolk removal. This highlights the response of total length but not weight to yolk manipulation (fig. 6.2). Length responds as predicted in outcome A (fig. 6.1), i.e. it is directly determined by the volume of yolk in an egg, whilst yolk weight and body weight respond more closely to the situation predicted in outcome B, and are not directly determined by egg yolk. Although yolk removal was probably not 100% efficient, length was clearly affected more by the manipulation process than either body weight or yolk weight, which was contrary to the predicted result. Previous studies by Sinervo (1990; *Sceloporus* lizards) and Sinervo and McEdward (1988; sea urchins) found that hatchling mass was directly proportional to the volume of yolk in each egg.

In our study, as a greater proportion of yolk was removed from eggs the range of mean hatch lengths also increased. The sham treatment also generally caused a

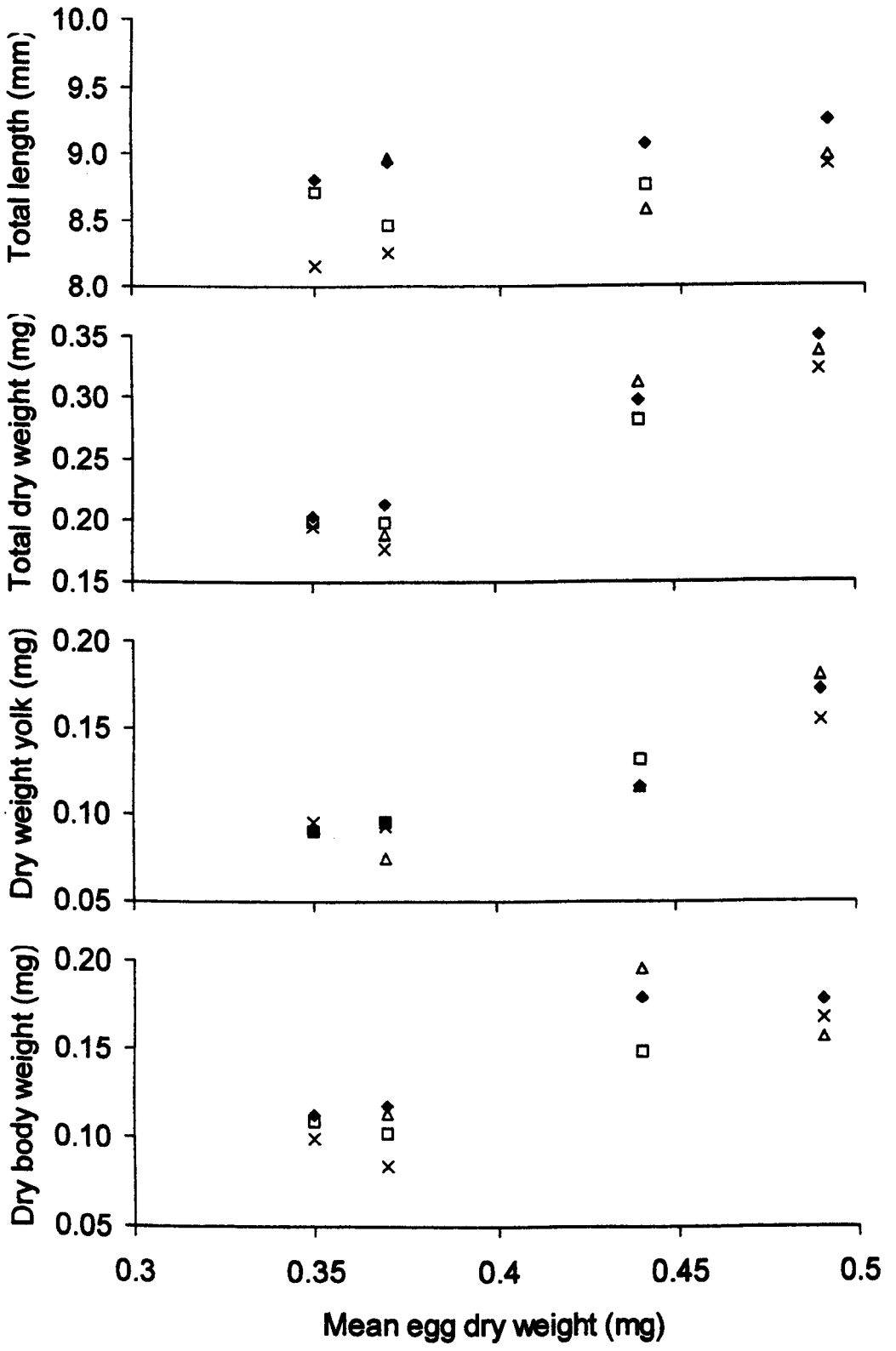

Figure 6.2. The mean hatching characteristics, total length, total dry weight, yolk dry weight and body dry weight against the mean size of unmanipulated eggs. ♦ control, □ sham, Δ little, x lot.

reduction in the length of larvae at hatch, which is thought to be due to the action of piercing the chorion, which often displaced a small quantity of yolk.

It is possible that the development of embryos was affected by the experimental procedure. Inserting a needle into eggs at the stage when the cytoplasm was separating into the animal and vegetal poles may have interrupted the process of development. The more yolk that the treatment removed, the longer the treatment took, and so egg development may have suffered greater disruption. This may, for example, have resulted in fewer cells developing. Histological analysis of larvae at hatch would enable the number and sizes of cells to be examined. The chemical composition of the embryo, particularly of the yolk, was not investigated in this study and so it is possible that some of the weight lost due to yolk removal was replaced with salt from the surrounding water. This is particularly relevant to the measurement of yolk mass at hatch, which was just calculated from the external volume of the yolk sac. The rate of yolk utilisation and the survival time of larvae on endogenous food supplies will also give an indication of the chemical composition of the yolk sac. Assessing the chemical composition of the fluid removed from eggs could also test the efficiency of the yolk removal process.

The results of the investigations in this study are promising and highlight the need for further work in this area. If a reliable technique could be developed for yolk removal (and perhaps for yolk addition to eggs), then genetically identical larvae could be produced that are morphologically different at hatch. The offspring could then be used for investigations into the 'bigger is better' hypothesis.

GENERAL DISCUSSION

The investigations in this thesis have highlighted the influence of both mean egg size and rearing temperature on size at hatching and during early growth. Although the number of larvae surviving to recruitment will be a function of mortality over all life history stages, the investigations in this thesis have concentrated on the early life stages when mortality is at its highest (Bailey and Houde, 1989). Small fluctuations in mortality during early life stages may have a large effect on the numbers of recruits.

The size of larvae at hatching is clearly affected by both the initial egg size and incubation temperature, although the effect on length is more predictable than weight. It was possible to fit one relationship that described the response of length at hatch to egg size at the mean sea temperature that they experience in the wild. Larvae of all stocks exhibit the same decrease in length at hatch after a similar rise in temperature. The weight of larvae at hatch showed a less consistent response between stocks. More experiments using a wider range of temperatures would aid the investigation into whether each stock is adapted to the mean sea temperature at the time of spawning.

Where temperature had a significant effect on the size of larvae at hatch the response tended to follow the "temperature size development rule" with smaller larvae hatching at higher temperatures (Atkinson and Sibley, 1997). The smaller size of larvae at higher temperatures may in part be due to them hatching at an earlier stage. As discussed in chapter 3 this is likely to have a major impact on the survival of larvae during the yolk sac period. However, at higher temperatures growth will be faster (McCarthy and Houlihan, 1996) and so larvae may rapidly outgrow this initial

size disadvantage due to a higher incubation temperature. Increased water temperature will also affect both predators and prey and so survival of larvae is likely to be the result of a combination of temperature effects (Blaxter, 1992).

Size selective predation and its importance for the survival of fish larvae is a theory central to this thesis which therefore deserves further discussion. The central tenement of this theory is that smaller fish larvae are more susceptible to predation than larger ones (Grimes and Isely, 1996). However, some recent studies have shown that under certain conditions larger larvae may suffer a higher mortality due to predation than smaller larvae (Litvak and Leggett, 1992, Pepin *et al.*, 1992, Høenig *et al.*, 1990, Fuiman, 1989). However, all experimental studies provide a specific set of controlled conditions which may not accurately reflect those in the wild. Of particular importance to the discussion of the 'bigger is better hypothesis' is that predation studies often only use a narrow size range of predator, often of a single species. Under these conditions the relative size of predator and prey are crucial to the outcome of predatory attacks (Paradis *et al.*, 1996, Miller *et al.*, 1988). If the predator to prey size ratio is very high, greater than 15-17, then the preys' ability to escape attacks will be minimal (Miller *et al.*, 1988). In situations when the available prey are very much smaller than the predator it is not surprising that predators select the larger individuals. These prey items are not only more visible, probably more active and therefore more likely to be encountered, but will also provide the greatest energy return (Bertram, 1996). As larvae develop their escape capabilities will increase and except in situations where the ratio of predator to prey is very high, a dome shaped vulnerability curve will result (Van der Veer *et al.*, 1997). Under natural conditions predator numbers will also be negatively correlated with predator size (Bailey and Houde, 1989). The resulting predator-prey field, caused by

overlapping domes of susceptibility will maintain the overall higher predation mortality of small prey items. However, controlled experiments do highlight how short term variability in the predator assemblage (size and number of predators) could dramatically alter larval vulnerability to predation (Rice *et al.*, 1997). As already discussed, this may be of particular importance for hatching larvae.

Our study has also shown that initial differences in size tend to be maintained through early growth. If, as the "bigger is better" hypothesis suggests (Bailey and Houde, 1989), there is such a strong selective advantage for larger body size then females laying small eggs will not persist in the population. As already discussed in terms of the predator assemblage, the heterogeneity of marine environments will ensure that the target of selection will also vary considerably in both time and space (Paradis *et al.*, 1996). For example Meekan and Fortier (1996) found evidence that selection for fast growth in Atlantic cod larvae, *Gadus morhua*, was stronger in some years than in others. This is supported by a review by Bertram (1993; cited in Meekan and Fortier, 1996) which only found direct evidence of selection for faster growth in 3 out of 42 studies reviewed. Meekan and Fortier (1996) argue that in years when selection for fast growth is weak there may be a selective advantage to produce more smaller eggs. Mechanisms such as this will work to maintain the variation in egg size between individuals within any population.

In our studies, in the absence of predators, and with excess food available, there was an indication of selective mortality of larvae with the lowest body reserves. This mortality also occurred during the early life stages, at around the timing of first feeding, and so provides evidence to support the critical period hypothesis (Hjort, 1914). However, a monoculture of *Artemia* was used as the prey for herring larvae in our study. *Artemia* is relatively large and may have increased the difference in

growth rates between larvae from large and small eggs. When cod and herring larvae were fed on a mixed diet of rotifers, *Artemia* and wild plankton, the presence of larger prey items in the diet resulted in a skewed size distribution dominated by larger individuals (Geffen, 1996).

The correlation between feeding behaviour and growth rate differences between larvae suggests an area for further research. Our study only investigated the rate of searching by larvae and related that to the ration of food attained. If herring are saltatory searchers (H. Browman, Marine Institute Austevoll, *Pers. Com.*). then detailed analysis of search strategies, for example switching to area restricted search (O'Brien *et al.*, 1990), may be found to influence growth. It also possible that there could be some physiological process, such as metabolic rate, that determines differences in growth rate between individuals.

The behavioural experiments in this study were conducted under carefully controlled laboratory conditions. The importance of variation in feeding behaviour in determining growth rates in the sea may depend on the magnitude of the variation in behaviour in relation to the magnitude of the variation in prey concentration in the wild.

The mechanisms driving recruitment, particularly the importance of starvation mortality, will also vary among taxa (Lasker, 1987). For example California grunion, *Leuresthes tenuis*, larvae can survive up to 20 days without food and so starvation is unlikely to be a factor in their mortality (May, 1971). The mechanisms determining survival and ultimately recruitment in herring may therefore be different to those determining recruitment in other species (Lasker, 1987).

The yolk reduction experiments suggest an interesting area for future research. Investigations need to be accompanied by a range of chemical and histological analyses to try and explain why the length at hatch, but not the weight, appears to be determined by the volume of yolk in an egg. The technique for yolk removal needs to be refined, in particular the survival of larvae needs to be improved and the efficiency of yolk removal quantified. Access to glass needles with tips of a more consistent diameter should improve the technique.

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THE EFFECTS OF TEMPERATURE ON "S-STRIKE" FEEDING OF LARVAL HERRING, *CLUPEA HARENGUS L.*

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Both Manx autumn-spawning and Clyde spring-spawning stocks of Atlantic herring were reared at a range of temperatures (Manx 10 and 13°C; Clyde 5, 8 and 12°C). The behaviour of larvae, feeding on *Artemia* nauplii at a range of test temperatures (7.0, 10.0 and 13.0°C for Manx and 6.8, 9.3 and 13.0°C for Clyde herring) was recorded using a high speed video system at 200 frames per second. The maximum velocities of feeding 'S-strikes' (measured during frame-by-frame analyses) were positively correlated with body length, capture time and attack distance. In contrast to burst swimming speeds reported elsewhere, strike velocities showed only a weak relationship with test temperature. Both strike behaviour and velocity altered as larvae developed and both were modified by rearing temperature.

Keywords: 'S-strike'; herring; larvae; temperature

INTRODUCTION

During the early life history of fish, predator — prey interactions are generally accepted as the most important factors determining future year-class strength (Heath, 1992). As larvae develop, so do the capabilities of many of their sensory and locomotor systems. Mechanisms of larval locomotion are fundamental to the success of larvae both as predators and as prey.

It has been suggested that where overlapping ranges of species occupy similar ecological niches, small changes in climate can alter the competitive advantage between the species and hence their abundance or distribution within that area. Southward *et al.* (1988) showed that over a 400 year period the relative distributions of herring and sardines around the coasts of Devon and Cornwall were correlated with sea temperature, with sardines extending further eastwards, at the expense of herring when the climate was warmer, and *vice versa*. On a

shorter time scale the temperature at which larvae develop and reach first feeding can vary considerably from year to year. A 42 year series of temperatures taken from the North Channel, an area close to Ballantrae Bank (the spawning site of the Clyde herring used in this study), shows that the mean temperature during March ranged from 4.8–9.8°C (Jones and Jeffs, 1991). Altering the rearing-temperture regime of herring, within the naturally experienced range, is known to affect various biochemical, physiological and behavioural processes (see Blaxter, 1993, for review).

It is well documented that the speed of both burst swimming (Webb 1978, Fuiman 1986, Johnston *et al.*, 1991, Batty and Blaxter 1992) and endurance swimming (Fry 1971, Beamish 1978, Videler and Wardle 1991) are related to ambient temperature. For examle, Batty *et al.* (1993) found that the maximum velocity of the escape responses of herring larvae was dependent on test temperature (over the range 5–17°C) but not on the rearing temperature (over the range 5–15°C). However, despite the lack of an effect of rearing temperature on the escape performance, Vieira and Johnston (1992) found differences in the development of muscle fibres of these same larvae. Total muscle bulk, however, was not affected by rearing temperature.

In response to a predatory stimulus herring larvae employ a 'C-start' escape response. The larva bends into a 'C' shape and then swims rapidly, usually in a direction away from the source of danger (Batty *et al.*, 1993). Clupeid larvae are pelagic and feed on small planktonic prey (Blaxter, 1965) with a well defined attack event, the 'S-strike.' The characteristics of this strike have been documented for larval anchovy by Hunter (1972) and for herring larvae by Rosenthal and Hempel (1970). An attack sequence can be broken down into two stages; the coiling and dart phases. Once a prey item has been observed larvae orientate their heads to facilitate optimum binocular vision of the prey item and coil their bodies into an 'S' shape. This set position is held for 1–3 seconds, during which time larvae are able to follow movements of prey by making small adjustments of their position using sculling movements of the paired fins and the caudal fin (Blaxter, 1965). The dart phase of the anchovy strike (Hunter, 1972) starts with a rapid backwards movement of the tip of the tail resulting in rapid forward movement of the head. The mouth opens during this phase and if the strike is successful, the prey item is engulfed within 16 ms.

This study aimed to investigate the relative importance of the different stages of the 'S-strike,' in particular the degree of coil and potential thrust generated from this. If larvae can judge the distance from which they are attacking, the degree of coil might be expected to depend upon the distance between a larva and its prey. This study also set out to investigate how both rearing and test temperature affects the characteristics of 'S-strikes'. As ambient water tempera-

ture increases, resistance forces acting on larvae reduce, and muscle contraction times shorten. Larvae would be expected to respond in one of two ways. They would either maintain the same strike characteristics and achieve a faster maximum velocity, similar to that seen with escape burst swimming responses of larvae (Batty *et al.*, 1993), or if they are capable of responding to their environment, alter the strike, resulting in the maintenance of a constant velocity.

METHODS

Larval Rearing

Ripe adult herring were caught from two spawning areas, Douglas Bank off the Isle of Man (September 1993), and Ballantrae Bank in the Clyde (March 1994). The gonads were dissected out and eggs fertilized in the laboratory with a mixture of sperm from at least four males (Blaxter, 1968). Eggs were then immediately transferred to the rearing tanks, ensuring that eggs from at least six females were represented at each of the temperature regimes shown in Table I. Spring herring were reared using a gradually increasing temperature regime which followed the seasonal rise in sea temperatures while still maintaining the same temperature differential between rearing treatments. This was necessary as herring will not survive if reared at a constant 5°C. Table I shows the test temperatures for the trials.

TABLE I Rearing and test details

Rearing temperature			*Length (mm)*	
On date of experiment	*History*	*Test Temperature*	*Range*	*Mean*
CLYDE				
12.6 ± 0.3	12.0 ± 0.9	13.0	14.3–18.7	16.7
12.8 ± 0.2	12.0 ± 0.9	9.1	14.3–21.4	18.4
12.7 ± 0.2	12.0 ± 0.9	6.7	15.7–16.7	16.8
9.9 ± 0.4	8.4 ± 0.7	9.2	15.7–21.4	18.6
10.0 ± 0.6	8.4 ± 0.7	13.0	17.1–20.2	18.8
7.3 ± 0.4	5.7 ± 0.8	13.0	14.4–19.1	16.4
7.6 ± 0.3	5.7 ± 0.9	9.2	14.5–20.2	17.4
11.0 ± 0.2	8.8 ± 1.1	9.0	16.4–22.8	19.4
11.0 ± 0.1	8.9 ± 1.1	13.0	19.0–21.8	20.5
11.2 ± 0.1	8.9 ± 1.1	6.9	18.5–20.8	19.5
15.6 ± 0.1	12.8 ± 1.4	13.0	17.2–23.3	20.0
15.7 ± 0.1	12.9 ± 1.5	9.3	22.9–29.1	25.7
15.7 ± 0.1	12.9 ± 1.5	6.8	21.0–26.5	23.7
10.0 ± 0.5	6.8 ± 1.8	9.0	18.3–23.6	21.9
10.0 ± 0.5	6.9 ± 1.8	13.0	19.3–22.4	20.9

TABLE I *(Continued)*

Rearing temperature			*Length (mm)*	
On date of experiment	*History*	*Test Temperature*	*Range*	*Mean*
MANX				
13.4 ± 0.6	13.4 ± 0.6	10.0	10.3–13.8	12.5
13.5 ± 0.5	13.5 ± 0.5	7.0	11.1–15.0	13.4
13.5 ± 0.5	13.5 ± 0.5	13.0	12.8–16.1	14.4
9.6 ± 0.4	9.6 ± 0.4	13.0	11.0–12.7	12.1
9.6 ± 0.4	9.6 ± 0.4	10.0	11.0–12.9	11.9
9.6 ± 0.4	9.6 ± 0.4	7.0	11.2–12.8	11.8

TABLE II Results of SAS General Linear Model procedure to investigate the significant factors affecting the two satges of the 'S-strike,' the coil phase (C/S) ratio and the dart phase (maximum velocity). Only two interaction terms were significant. A significant interaction term means that the model had a significantly different combinations of the two variables. Rearing temperature was a class variable whilst attack distance, C/S ratio, body length, test temperature and maximum velocity were continous variables

Dependent variable	*Independent variable*	*Clyde P>F*	*Manx P>F*
C/S ratio	Attack distance	0.14	
	Rearing Temperature	<0.01	
	Test Temperature	<0.13	
	Body length	<0.01	
	Attack distance × Rearing temperature	<0.01	
	Model	<0.01	0.07
Maximum velocity	Attack distance	<0.01	
	Rearing temperature	0.07	
	C/S Ratio	0.88	
	Body length	0.55	
	Test temperature	0.36	
	C/S ratio × Rearing temperature	<0.01	
	Model	<0.01	0.12

Prey Speed

The swimming speed of *Artemia sp.* was tested at temperatures of 4.5, 10.4, 10.5 and 19.8°C. Sequences of swimming behaviour were recorded using conventional video, recording at 50 frames·s^{-1}. Three swimming sequences were analysed for each of 8–10 nauplii. The maximum velocity and average distance moved in 20 ms was calculated.

Experiments

Filming was conducted in an air conditioned room using an NAC H.S.V-400 High Speed Video (H.S.V.) system. A 450 mm diameter tank, filled with seawater

to a depth of 80 mm, with retroreflective material (Scotchlite, 3M Corp.) on the base was used in conjunction with a half silvered mirror which reflected light from a stroboscope (synchronised with the H.S.V.) down onto the larvae to produce a silhouette image (Batty, 1984). Forty to fifty larvae were captured from the main rearing tanks and then allowed 1 h to recover from the stress of capture before being moved to the experimental room and left to acclimate to the test temperature in a holding tank. Larvae were placed in the experimental tank and then allowed another hour to recover from handling stress before *Artemia sp.* were introduced. Filming then commenced at a recording speed of 200 frames·s^{-1}. Trial experiments using food densities of 5.2, 11.5 and 28.0 ml^{-1} were carried out to determine suitable densities for the trials. An intermediate food density of approximately 10 ml^{-1} was used for all experiments.

Analysis

Upon initiation of feeding, larvae coil their bodies into an 'S' shape. At this stage larvae sometimes aborted the strike, straightened their bodies and resumed swimming. The straightening of a larva was difficult to distinguish from some of the slower strikes and so each 'S-strike' was replayed and only those that resulted in successful prey capture were further analysed. Video recordings were analysed frame by frame and the position of the tip of the snout was recorded from each frame, starting one frame before the initiation of the strike. Velocity vectors were calculated from a regression of the X position of the snout on time and a regression of its Y position on time for the first four points after the initiation of movement (5–20 ms). The resultant velocity vector (direction and magnitude) was calculated from these regression equations. During a typical strike sequence larvae moved an average of 30 pixels. By repeatedly analysing such a sequence we estimated the measurement error (Harper and Blake, 1989), and calculated the coefficient of variation for the location of each pixel, which was 4.1%.

The ratio of the distance between the head and tail of a larva at the set position to its body length was calculated as a measure of body coil and was called the 'coiled-to-straight length ratio' (C/S ratio). The distance between the head of a larva at the set position and the prey item was referred to as the attack distance. The number of 5 ms frames from the initiation of the strike to capture of the *Artemia sp.* was calculated as the time to capture. To highlight the effect of both time to capture and attack distance on maximum velocity, larvae were separated into those striking with maximum velocities less than or greater than 160 mm·s^{-1} for Clyde and 180 mm·s^{-1} for Manx larvae. These velocities were chosen as they gave the greatest significant difference (χ^2 analysis) between slower and faster strikes.

The SAS procedure GLM (SAS Institute Inc., 1988) with an analysis of covariance model was used to test the significant factors affecting the two 'S-strike' stages: 1) the effects of attack distance, body length, test and rearing temperatures on C/S ratio; and 2) the effects of C/S ratio, attack distance, body length, test and rearing temperatures on velocity. Due to the dependence of velocity on body length, length specific values were used in the model.

RESULTS

Effect of Food Density

Manx herring reared at 10°C and tested at 7°C were fed with *Artemia sp.* at three densities; 5.2, 11.5 and 28.0 ml^{-1}. Food density over this range had no effect on the maximum velocity of striking herring (Kruskal Wallis test: $H = 1.76$, $P = 0.42$).

Prey Speed

Maximum velocity of *Artemia sp.* nauplii (length = 420 ± 50 µm) ranged from 4.7 ± 1.4 $mm \cdot s^{-1}$ at a test temperature of 4.5°C to 6.5 ± 1.8 $mm \cdot s^{-1}$ at a test temperature of 19.8°C. These velocities were maxima and were maintained for only 20 ms during which time the *Artemia sp.* covered an average maximum distance of 130 µm.

Time to Capture

The majority of prey were captured within 15 ms by both Manx and Clyde larvae (Figure 1a, b), although prey capture occurred between 5 to 30 ms after the initiation of the feeding strike. Manx larvae had significantly more short capture times than Clyde larvae ($P < 0.01$, $\chi^2 = 31.94$). However, as will be shown later, this is largely due to Manx larvae being shorter than Clyde larvae. The fastest strikes of Clyde larvae (faster than 160 $mm \cdot s^{-1}$) tended to result in the shortest time to capture of prey items ($P < 0.01$, $\chi^2 = 12.32$; Figure 1a). For Manx herring there was no significant difference between the capture times of larvae using velocities faster or slower than 180 $mm \cdot s^{-1}$ ($P > 0.75$, $\chi^2 = 0.07$; Figure 1b).

Attack Distance

Both Clyde and Manx larvae attacked the majority of prey from a distance of between 0.6 and 2.0 mm (Figure 1c, d). There was a significant difference between

the overall distributions of attack distance for Manx and Clyde larvae ($P < 0.01$, $\chi^2 = 20.43$), Manx larvae having a higher proportion of short attack distances. A χ^2 analysis for Clyde larvae showed that larvae using strike speeds faster than 160 mm·s^{-1} attacked prey from significantly further away than those using strike speeds of less than 160 mm·s^{-1} ($P < 0.01$, $c^2 = 20.65$). A χ^2 test for Manx larvae showed a similar pattern, there was a significant difference ($P < 0.01$, $\chi^2 = 10.63$; Figure 1d) between fish using strikes faster and slower than 180 mm·s^{-1}.

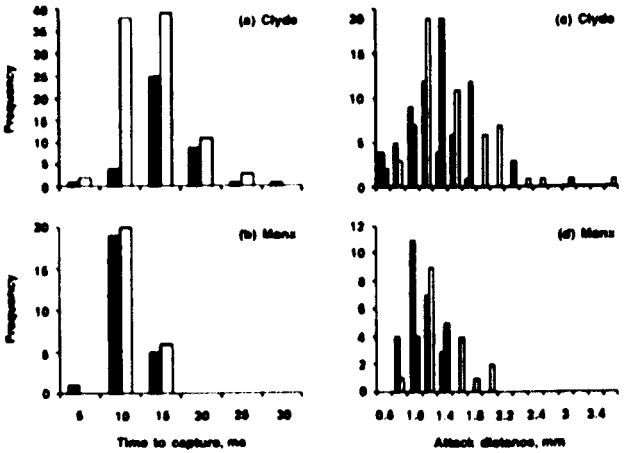

FIGURE 1 Prey capture times (ms) for (a) Clyde larvae and (b) Manx larvae and attack distances (mm) for (c) Clyde larvae and (d) Manx larvae. Strikes for Clyde larvae are split into those with maximum velocities faster (□) and slower than 160 mm·s^{-1} (■). Strikes for Clyde larvae are split into those with maximum velocities faster (□) and slower than 180 mm·s^{-1} (■).

Maximum Velocity

Although there were some differences between treatments (Figure 2a–c), there was no clear trend between maximum velocity and test temperature. The results of a Kruskal Wallis test followed by a non-parametric multiple comparison test (Zar, 1984) on the maximum velocities for the various treatments of Clyde larvae showed that larvae reared at 13°C and tested at 13°C had a higher maximum velocity than fish reared at 13°C and tested at 9°C (Figure 2a). Also, fish tested and reared at 13°C had a higher maximum velocity ($P < 0.01$, $Q = 3.08$; Figure 2a–c) than fish tested at 13°C but reared at 9°C ($P < 0.05$, $Q = 2.87$) or those tested at 13°C but reared at 6°C ($P < 0.05$, $Q = 2.81$).

Within a rearing temperature, test temperature had no effect on the maximum velocity of Manx larvae (high, $P = 0.09$, $H = 4.93$; low, $P = 0.27$, $H = 2.59$). However, there were differences between rearing temperatures. Manx larvae

reared at 13°C had higher maximum velocities than those reared at 10°C when tested at both low ($P < 0.01$, $H = 6.64$) and high ($P = 0.03$, $H = 4.93$) temperatures. Clyde larvae were tested again later in development and three treatments had maximum velocities significantly faster than those recorded in earlier trials: larvae reared at low temperatures and tested at both high temperature ($P < 0.01$, $T = 16.00$; Figure 2c) and medium temperature ($P < 0.01$, $T = 7.08$; Figure 2c)

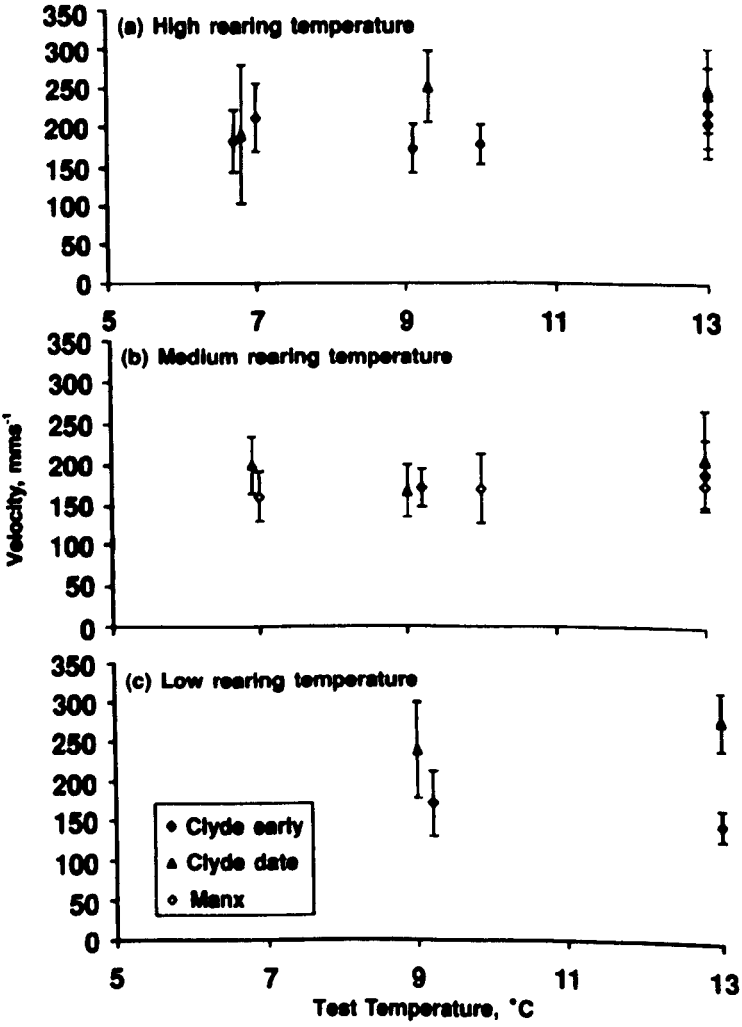

FIGURE 2 Mean maximum strike velocity ($mm \cdot s^{-1}$ ± 1 standard deviation) for larvae reared at (a) high temperature (b) medium temperature (c) low temperature. Clyde early larvae were tested at 500 day-degrees, Clyde late larvae were tested at 850 day-degrees.

and larvae reared at high temperature and tested at medium temperature ($P < 0.01$, $T = 7.35$; Figure 2a).

The maximum velocity of strikes was positively correlated with fish length (Figure 3) and the range of velocities increased with increasing body length. However, when velocities were converted to specific speeds (body lengths per second, $L \cdot s^{-1}$) the majority of maximum velocities, for all lengths of fish, lay within 6–18 $L \cdot s^{-1}$ (Figure 3). There was generally a steeper relationship between velocity and length for Clyde larvae reared at the lowest temperature ($R^2 = 0.44$, $F = 38.18$, $P < 0.01$) than for other treatments of Clyde larvae. Investigating this relationship further it was seen that the C/S ratio, a measure of the tightness of body coil, of fish reared at the lowest temperature was positively correlated with the maximum velocity of strike ($R^2 = 0.46$, $P < 0.01$, $F = 41.84$; Figure 4a). Also there was a positive,

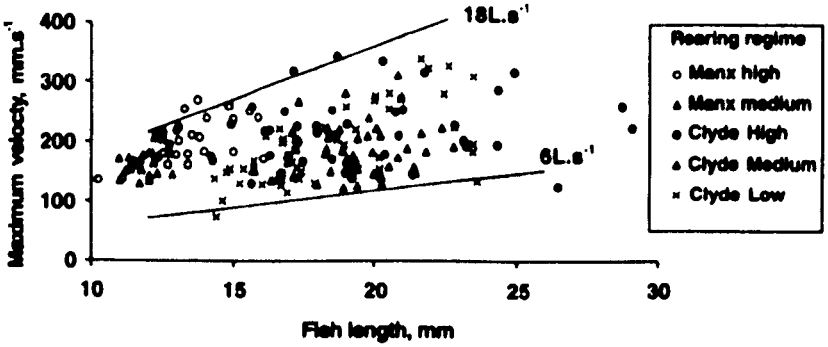

FIGURE 3 The relationship between fish length and maximum velocity ($mm \cdot s^{-1}$) for all larvae. The 6 and 18 body length per second ($L \cdot s^{-1}$) specific speeds are marked on the graph.

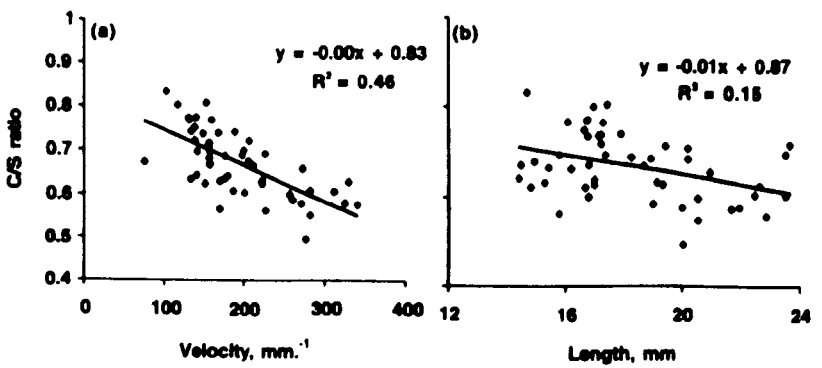

FIGURE 4 Clyde herring reared at low temperature. (a) relationship between C/S ratio and maximum velocity ($mm \cdot s^{-1}$) and (b) relationship between C/S ratio and fish length (mm). Linear regressions equations and coefficients of determination are shown.

although weak ($R^2 = 0.15$), correlation between fish length and C/S ratio for larvae reared at 5°C ($P < 0.01$, $F = 8.69$; Figure 4b).

Factors Affecting Components of the 'S-strike'

The SAS GLM models for Manx larvae were not significant (overall model pit: C/S ratio, $P = 0.07$; maximum velocity, $P = 0.12$). However, the C/S ratio of Clyde larvae (all rearing temperatures combined) was significantly affected by the rearing temperature ($P < 0.01$), body length ($P < 0.01$) and an interaction between attack distance and rearing temperature ($P < 0.01$). When the model was re-run separately for each rearing temperature, the only significant factors were for fish reared at the low temperature (attack distance; $P > 0.01$; body length, $P < 0.01$). Larvae were more tightly coiled when attacking prey from further away and older, longer, larvae were also more tightly coiled for a given attack distance (C/S ratio = 1.05 – 1.91 × Attack distance – 0.01 × Body length).

Velocity of Clyde larvae was significantly (all rearing temperatures combined; overall model fit, $P < 0.01$) affected by attack distance ($P < 0.01$) and an interaction between C/S ratio and rearing temperature ($P < 0.01$). When re-run separately for each rearing temperature the model was not significant for fish reared at high temperature. For larvae reared at both the low ($P < 0.01$) and medium temperatures ($P = 0.02$) maximum velocity increased when attacks were aimed at more distant prey and when the body was more coiled (medium, Maximum velocity = 14.09 + 27.58 × Attack distance – 10.15C/S ratio; low, Maximum velocity = 20.35 + 36.68 × Attack distance – 18.51C/S ratio). Larvae reared at low temperature were using generally higher maximum velocities for a given attack distance and C/S ratio than those reared at medium temperature.

DISCUSSION

The feeding strike of 5–20 mm northern anchovy (*Engraulis mordax*) larvae has been filmed and analysed by Hunter (1972). Using a cine photography technique which recorded at 128 frames·s^{-1} he calculated prey capture times of 7.8 – 15.6 ms. This fits within the range of prey capture times recorded for both Manx and Clyde herring (5–20 ms) when strikes were recorded at 200 frames·s^{-1}. Hunter (1972) noted that although larger anchovy struck at prey from further away, they tended to do so with a higher maximum velocity leading to a relatively constant time to capture. As locomotor systems developed, longer herring larvae were able to strike using higher maximum velocities and overall this gave them

a wider range of strike speeds. There is also some evidence, at least for Clyde larvae reared at the lowest temperature, that longer and therefore older larvae, used a tighter body coil to attack prey from a greater distance. This is further evidenced by the fact that the Manx larvae used in this study, which were generally shorter and therefore at an earlier stage of development, tended to attack prey closer to them. However, unlike the findings reported for anchovy, there was still a tendency for faster strikes to lead to a shorter time to capture.

The maximum velocity of *Artemia sp.* nauplii measured in this study is comparable with the study of Pryor and Epifanio (1993) who recorded velocities of 5.9 ± 0.10 mm·s^{-1} for nauplii of a similar size to those used for our experiments (length: 449 ± 4.9 mm) but tested at a slightly higher temperature of 20°C. These velocities are low when compared to the maximum speed of the 'S-strike' which averaged 120–240 mm·s^{-1}.

Blaxter (1965) published an equation for mouth gape of yolk-sac herring larvae which, when extrapolated, estimates the mouth gape of a 10 mm larva (the smallest larva in this study) to be 370 mm. *Artemia sp.* are longer than the mouth width of this Manx larva, and the smallest Clyde larva (14.3 mm) would still not have a large enough gape (530 µm), as an *Artemia sp.* directly in front of the mouth would still be able to move out of range of the gape within the duration of the strike (*Artemia sp.* can move approximately 130 µm in 20 ms, the maximum duration of most dart phases). So only larger larvae would be able to take *Artemia sp.* moving perpendicular to them and most of the larvae tested in these experiments must be taking *Artemia sp.* head on (*Artemia sp.* width = 260 µm; (Hunter, 1977). Blaxter (1965) found that herring larvae took copepods head on as do anchovy larvae feeding on *Artemia sp.* (Hunter, 1977). If this is the case, larvae would have to adjust the distance over which they are to dart as the prey item moves either towards or away from them. Clyde larvae alter the strike by adjusting the tightness of coil (the C/S ratio) to suit the attack distance.

The fact that the tightness of coil is positively related to velocity, at least in fish reared at low temperatures, suggests that in some way the 'S' shape is used to drive the dart phase. The coil of the body creates a position which allows muscle to shorten further, for a longer period, and from which larvae can use muscular contraction to straighten rapidly and strike. Observations on anaesthetised larvae, whose muscular activity is obviously suppressed, show that they will straighten when bent into a coil (pers. obs.). This suggests that some energy could be stored in the coil and be released to aid the forward motion of the head. However, an energy store is clearly not the only mechanism working as C/S ratio is not related to velocity for all treatments.

As already mentioned larvae reared at low temperatures showed less variability in many of their strike characteristics; principally in the attack distance and

the tightness of body coil. At higher temperatures maximum muscle contraction times are faster but larvae reared at higher temperatures have a learned ability to alter muscle output, as well as the body coil, in order to maintain a constant strike speed. Fish reared at low temperature retain a more rigid repertoire of strike characteristics. They have a limited ability to alter this pattern of behaviour, even when transferred to water of a higher temperature.

It is possible that some of the variation between fish reared at different temperatures is due to differences in muscle structure. Vieira and Johnston (1992) found a positive relationship between the number of muscle fibres and the rearing temperature of herring larvae, those reared at 5°C having fewer larger diameter fibres than larvae reared at 15°C. However, the overall muscle bulk remained fairly constant.

The effect of test temperature is small and there was no consistent trend of test temperature on the overall velocity, unlike the clear relationship seen with escape burst swimming (Batty *et al.*, 1993). Escape burst swimming is a result of fast muscle contraction as the larva bends into a 'C-start' and then swims off rapidly away from the predator. The aim of the escape response is to maximise the speed of burst swimming thus increasing the chances of successful predator evasion, escape velocity therefore increases directly as the potential for muscular activity increases at high temperatures (Batty and Blaxter, 1992). The lack of an overall relationship between test temperature and the maximum speed of 'S-strike' burst swimming suggests that larvae are to a certain degree capable of sensing the ambient temperature and they respond by altering the characteristics of their strike. A number of species of fish, including herring, are known to be able to sense temperature differences of less than one degree (Shelord and Power, 1915; Bull, 1936). The vertical distribution experiments of Batty (1994) with larval herring and Olla and Davis (1990) with Alaska pollack larvae have indicated that larvae are able to sense temperature and respond by altering their swimming behaviour to remain in the upper warmer layer above a thermocline. The ability to alter strike behaviour, and hence compensate for temperature changes, would be important to organisms performing daily vertical migrations, particularly if these migrations cause them to move through a thermocline.

As discussed, the speed of *Artemia sp.* nauplii is low when compared to the strike velocities of herring larvae. It is therefore not surprising that larvae alter the strike characteristics in order to maintain a relatively constant strike velocity which will result in an acceptable capture success without wasting energy. However, the compensation is incomplete and strike characteristics appear to be set, to a certain degree, by the temperature regime larvae have experienced. These experiments suggest that larvae spawned and developing in cooler years may have a reduced behavioural flexibility.

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