Lee, Who Seung (2010) *Effect of growth trajectories on adult performance and lifespan in three-spined sticklebacks.*
PhD thesis.

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EFFECT OF GROWTH TRAJECTORIES ON ADULT PERFORMANCE AND LIFESPAN IN THREE-SPINED STICKLEBACKS

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THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

COLLEGE OF MEDICAL, VETERINARY AND LIFE SCIENCES

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SEPTEMBER 2010

DECLARATION

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ABSTRACT

Changes in environmental conditions in early life can cause changes in the tempo and pattern of growth and development in animals. Natural selection favours processes that enable animals to make decisions that maximise Darwinian fitness. These decisions are influenced by trade-offs between current and future benefits. An episode of poor conditions (i.e. reduced nutrition, low temperature and changes in photoperiod) is generally linked to a slowing of growth. If adequate conditions are restored after this episode, growth rate is accelerated and normal adult size can be reached; in other words, 'compensatory' growth occurs. Compensatory growth has benefits in enabling a return to the typical size-at-age growth trajectory. Although this ability to alter growth rate provides a degree of adaptability, there is now increasing evidence that resource allocation to rapid growth carries various long-term costs. While there is experimental evidence that poor environmental conditions in early life can induce subsequent compensatory growth, little is known about the long-term effects of compensatory growth on locomotor and reproductive performance, and on lifespan.

In this thesis, I investigated how different growth trajectories affected subsequent performance (i.e. locomotory capability, reproduction and lifespan), and how any such effects were influenced by the perceived time until the key life history event of reproduction. Using juvenile three-spined sticklebacks *Gasterosteus aculeatus*, I showed that temperature manipulations early in life in three temperature treatments (low, intermediate and high, independent of food supply) or food restriction (with a constant temperature) affected skeletal growth trajectory not only during the manipulation itself, but also during a subsequent compensatory phase. To investigate the effects of time of year, all experimental groups of temperature and food manipulations were replicated at different seasonal periods (= Winter or Spring); to manipulate apparent time of year while holding initial size and maturity constant, a photoperiod manipulation was also undertaken at both seasonal times (ambient or delayed photoperiod).

While there was compensatory growth (i.e. accelerated growth) in the food manipulation, temperature manipulations induced both positive compensatory growth (i.e. growth acceleration following exposure to low temperature) and also 'negative' compensatory growth (decelerated growth following exposure to high temperature). The outcome of these changes was that fish in all treatment groups reached the same average

size by sexual maturity, despite having different growth patterns. However, early growth trajectories influenced both pre-breeding swimming endurance and its decline over the course of the breeding season, such that swimming ability was negatively correlated with compensatory growth whereas 'negative' compensatory growth reduced swimming ability less (Chapter 2). Reproductive investment (males: sexual ornaments and ability to build nests; females: first clutch size and mean egg size) was negatively affected by compensatory growth; positive effects of 'negative' compensatory growth on reproduction were found (Chapter 3). Interestingly, the effects of growth rate on subsequent swimming and reproductive performance were greater when the perceived, or actual, time until the breeding season was shorter (Chapter 2 and 3). These results implied that increased metabolic rates and cellular damage (e.g. oxidative stress) induced by compensatory growth negatively affected subsequent performance, while decelerated growth reduced the damage levels and so later performance was less affected.

Under food manipulation, there were similar patterns: compensatory growth (i.e. accelerated growth) negatively affected locomotor and reproductive performance and the time until the breeding season altered the effects on performance (Chapter 4). To further examine trade-offs between growth rate and fitness parameters such as future reproductive investment and rates of senescence, I developed four theoretical models of increasing complexity with different growth-damage scenarios, ranging from assuming that the animal maximises growth regardless of any costs, through assuming a relationship between growth rate and mortality risk, to assuming growth leads to damage accumulation and that the animal is able to apportion resources between somatic growth, gonadal growth and investment in repair of damage. The models predicted that growth trajectories strongly influenced future reproductive investment irrespective of body size at the time of breeding, presumably due to the effects of damage accumulation in the run up to the breeding season; the predictions of the most complex model were closest to the experimental data on egg production (Chapter 5).

Lifespan was different among treatment groups and also influenced by early growth trajectories. Compensatory growth negatively affected lifespan whereas 'negative' compensatory growth extended lifespan. Lifespan in female sticklebacks was positively related to egg production. Male sticklebacks lived for a shorter time when they showed less growth between their first and second breeding seasons, and a greater change in the duration of having a red throat between the first and second breeding season (an indicator

of reproductive senescence). The costs of compensation were strongest when the perceived time until breeding was shortest (Chapter 6).

Consequently, this thesis shows that environment conditions in early life have substantial effects on subsequent performances and lifespan. Moreover, results in this thesis strongly support the time-stress hypothesis, that is the time available until the onset of a key life history event, in this case reproduction, influences outcomes.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 EARLY GROWTH AND SENESCENCE

The concept of trade-offs is a central tenet in life history theory, Organisms must allocate limited resources across various competing demands. One important, but surprisingly understudied, trade-off is that between growth and longevity. According to recent studies in several taxa (Rollo 2002; Metcalfe and Monaghan 2003; Ricklefs 2006; Inness and Metcalfe 2008), there is a positive correlation between early growth rate and rate of senescence (both variables being corrected for body size): faster growth in early life is linked to a more rapid increase in mortality with adult age, excluding extrinsic causes of death. This appears to hold within and across species. The aim of this thesis is therefore to carry out experimental investigations of this link between ontogeny and life history. The focus of this study is on environmental effects and how these influence intra-specific variation in the optimal resolution of the growth-lifespan trade-off through changes in performance.

Several potential factors might be responsible for the link between faster growth in early life stages and an accelerated rate of ageing. With respect to environmental effects within species, the basic prediction is that the adult phenotype will be altered by the rate of early growth (independently of final adult size), and that this leads to differences in the rate of senescence. In what ways might the adult phenotype change? It could be that rapid growth itself causes increased metabolic rate in adulthood (Criscuolo et al. 2008) and oxidative stress that then speeds the rate of cellular damage (Jennings et al. 2000; Monaghan and Haussmann 2006) or muscle wastage (Kamel 2003); alternatively, or perhaps in addition, a faster overall rate of early growth might cause a mismatch in the relative growth and development of component tissues/organs, producing a suboptimal adult phenotype (Martell et al. 2006) that would then fail sooner. When environmental circumstances favour accelerating growth, for example to reach a threshold size by some key time in the season, the 'speed' of growing may be more important than the 'quality'. In the European starling *Sturnus vulgaris*, for instance, rapidly grown feathers were found to

be shorter and lighter than those grown more slowly (Dawson et al. 2000); in the pumpkinseed sunfish *Lepomis gibbosus* fast growth was found to be associated with thinner and weaker scales (Arendt et al. 2001), and rapid growth rate clearly induced declined muscular capacity for mature function (proportion of protein) in galliform birds (Shea et al. 2007). Whatever the mechanism, the prediction is that the adult phenotype varies with the rate of early growth (independently of final adult size), and that this variation leads to differences in the rate of senescence.

Growth rate can represent the outcome of interactions between biotic and abiotic factors operating on behavioural and physiological processes (Weatherley and Gill 1987; Jobling 1994). Biotic factors (e.g. predator, age, activity and weight) are the living components, while abiotic factors (e.g. temperature, light, oxygenation, water and pH) are the nonliving components of the environment. In species breeding in seasonal environments, especially, growth rate can vary in relation to a number of environmental factors such as food availability, temperature and/or photoperiod (Weatherley and Gill 1987; Wootton 1998). It has been found that growth is faster towards the end of the breeding season since individuals born late need to attain a certain body size before the onset of winter (Wootton 1976; 1998). Thus eggs laid at different times in the breeding season develop at different rates (Weatherley and Gill 1987; Wootton 1998), and so are predicted to result in different phenotypes. For instance, the larvae of the European pilchard *Sardina pilchardus* that hatch at different times of the breeding season develop different numbers of slow and fast muscle fibres in relation to their body length (Catalán et al. 2004). Hence, poor environmental conditions can lead to slower rates of growth and development and delayed maturation, and thereby decreased fitness since body size has positive effect on survival and reproductive success and also late reproduction increases generation time and can decrease the reproductive lifespan (Roff 1992).

1.2 COMPENSATORY GROWTH IN EARLY LIFE

In early life, changes in environmental conditions can cause changes in the tempo and pattern of growth and development. Reduced nutrition is generally linked to a slowing of growth. If adequate food supplies are restored after food restriction or starvation, growth rate is accelerated and normal adult size can be reached (e.g. Dobson and Holmes 1984; Miglavs and Jobling 1989; Quinton and Blake 1990); in other words, 'compensatory' or 'catch-up' growth occurs. Compensatory growth induced by poor early conditions has been

observed in numerous studies with several species: insects (De Block et al. 2008), fish (Álvarez and Metcalfe 2005), amphibians (Orizaola et al. 2010; Squires et al. 2010), reptiles (Le Galliard et al. 2005; Radder et al. 2007), birds (Blount et al. 2003; Arnold et al. 2007) and mammals (Wilson and Osbourn 1960; Hornick et al. 2000). Recent research (Merry 1995; Arendt 1997; Eriksson et al. 1999; Morgan et al. 2000; Rollo 2002) suggests that such compensatory growth brings a variety of identifiable costs, although it can of course carry several benefits. From an ecological or evolutionary perspective, I would expect that the optimal growth pattern following an episode of poor nutrition depends on the balance of these fitness costs and benefits (i.e. the net fitness return). With respect to benefits, first of all, increases in body size can improve short-term survival chances (Metcalfe and Monaghan 2003). Such benefits might be most marked during the juvenile phases, but could extend throughout life. For instance, the predation risk of animals with smaller bodies may be increased because small animals are easier for predators to catch, kill and consume, and so the duration of the vulnerable period is reduced when growth to a large size is fast (Arendt 1997). Secondly, the increased total energy reserves that can be stored by a larger body can reduce the risk of dying of starvation (Ludsin and DeVries 1997). For example, Kirk (1997) showed that allometric patterns of energy storage and respiration rate in planktonic rotifers lead to the prediction that larger species should have greater starvation resistance than smaller species. Thirdly, fast growth can lead to an increase in expected reproductive success. In many species (especially, where male reproductive success is influenced by success in combat with other males), larger males have greater reproductive success because they are preferred by females or out-compete other males for access to females (Roff 1992). In females, it is fecundity that often increases with body size (Wootton 1998). For example, small male elephant seals *Mirounga leonia* may never reproduce at all, whereas small females may still obtain a place in a male harem (Galimberti et al. 2007). However, sex differences in the benefits of rapid growth have been little studied.

As mentioned above, there are some costs linked to rapid growth. According to several studies, the costs may be manifested in terms of poorer locomotor performance (Billerbeck et al. 2001), impaired future energy deposition (Morgan and Metcalfe 2001), reduced reproductive capacity in females (Holmgren 2003) and greater risk of adult disease (Hales and Ozanne 2003). Moreover, accelerated growth can reduce the maximum lifespan despite rapid gains in body size improving survival chances (Birkhead et al. 1999; Metcalfe and Monaghan 2003; Ozanne and Hales 2004). At the cellular level, faster

growth appears to induce increased oxidative stress (Merry 1995; Rollo 2002) and metabolic rate (Criscuolo et al. 2008). It is also been correlated with reduced investment in protein maintenance (Morgan et al. 2000) and more rapid rates of telomere abrasion, which may in turn be linked to rates of oxidative damage (Jennings et al. 2000; Hall et al. 2003). In other words, rapidly grown structures may induce developmental errors or weaknesses (Arendt 1997; Blanckenhorn 2000). This is evident also in humans. The highest death rates from coronary heart disease occurs in boys who were thin at birth but whose weight caught up so that they had an average or above average body mass from the age of 7 years (Eriksson et al. 1999). Altogether, across a broad range of taxa, there is now good evidence that compensatory growth in early life can negatively affect the performance of the phenotype in adult life.

1.3 ENVIRONMENTAL FACTORS IN EARLY GROWTH

The following are the main environmental factors on which I concentrate in this thesis, since these are known to be important, and amenable to experimental manipulations.

Food availability

Food availability varies considerably over small spatial and temporal scales, and induces variation in growth rates (e.g. James 1991; Madsen and Shine 2000; Lemos-Espinal et al. 2003; Beukers-Stewart and Jones 2004). Several studies of rapid growth in early life have focused on the influence of food deprivation (Ali and Wootton 2000; Maclean and Metcalfe 2001; Morgan and Metcalfe 2001; Zhu et al. 2003; Ozanne and Hales 2004; Alvarez and Metcalfe 2005; Mangel and Munch 2005; Skalski et al. 2005; Bize et al. 2006; Myszkowski et al. 2006; Walling et al. 2007; Auer et al. 2010). According to Lindström (1999) and Metcalfe and Monaghan (2001), poor early nutrition has a negative effect on many adult life-history traits such as body size, survival and secondary sexual trait expression. However, if conditions improve, it has also been demonstrated that organisms are capable of undertaking compensatory strategies to alleviate some of the effects of poor early nutrition (Nicieza and Metcalfe 1997; Birkhead et al. 1999). The fact that growth rates are often kept below the physiological maximum indicates that rapid growth can be costly (Arendt 1997). For example, Bull and Metcalfe (1997) found that juvenile Atlantic salmon *Salmo salar* became hyperphagic after a period of food deprivation during which

they had lost some of their fat reserves. The energy loss rate seemed to affect primarily the duration of the hyperphagic response rather than its magnitude (i.e. intensity of feeding). However, poor early nutrition and subsequent growth compensation does not necessarily result in changes in life-history traits. Walling et al. (2007) showed that growth compensation in adult male green swordtails *Xiphophorus helleri* did not cause reduced sexual attractiveness nor a greater deterioration in secondary sexual characters at older ages than in continuously well-fed males. Nevertheless, a range of traits need to be studied in order to assess the overall impact, and in studies that manipulate growth by altering food intake it can be difficult in the study of compensatory growth to separate the effects of the initial food deficit from those of the subsequent growth acceleration.

Temperature

Environmental temperature has major effects on all animals, but in the case of ectotherms it is especially important due to its effects on growth and metabolism (Guderley 1994). Compensatory growth can thus be induced in ectotherms by a change in environmental temperature, since temperature can directly affect growth rate throughout changing in physiology and resource allocation (Weatherley and Gill 1987; Charnov and Gillooly 2003). For instance, Maclean and Metcalfe (2001) observed accelerated growth rate in juvenile Atlantic salmon subjected to lower temperature (8.4 °C) for three weeks followed by 20 weeks in the same temperature as the controls (16.4 °C). Although they showed compensatory growth in response to changed temperature, they concluded that compensatory growth after a period of irregularly low temperature without food restriction is not controlled by the same mechanism as in food deprivation. While food restriction directly reduces growth due to a reduced energy intake, thermal conditions can limit the rate of growth and development, because colder temperatures limit the rate at which ectotherms can capture and digest food, and temperatures close to upper tolerance limits result in poorer growth due to high metabolic costs (Brett 1979; Gadomski and Caddell 1991). Temperature acclimation leads many species to adjust tissue metabolic capacities (Rome et al. 1984; Sisson and Sidell 1987), but changes in environmental temperature have effects on energy budgets (Guderley 2004), lipid levels and activity patterns (Hurst et al. 2005). For instance, the specific growth rate of juvenile Pacific halibut *Hippoglossus stenolepis* was significantly affected by temperature treatment; growth at 2°C was less than 25% of growth at 10°C (Hurst et al. 2005). This result suggested that the variation in

temperature in their study affected energy levels (low lipid level) and activity (low feeding strike) and that low temperature induced compensatory growth.

Perception of time of year

The perception of time of year is an important factor influencing the timing of life history events (e.g. mating, laying and hatching). It may be important to match pre-programmed schedules to maximise fitness, particularly those of growth and reproduction. So, most animals may try to alter their life-history strategy when expected environmental conditions are changed because undertaking reproduction at the wrong time can reduce fitness. For instance, Visser et al. (1998) found that warmer springs (i.e. a changing abiotic factor) lead to mistimed reproduction in great tits *Parus major*. Photoperiod is also one of the most important abiotic factors affecting growth and survival (Bamabe 1990; Battaglene 1995; Hart et al. 1996; Boeuf and Le Bail 1999). Many diurnal animals are visual predators and therefore require light for feeding (Boeuf and Le Bail 1999). Additionally, several species are responsive to changes in photoperiod with alterations of growth rate, which is generally directly related to day-length (Boeuf and Le Bail 1999). For example, long day length has been found to increase growth of a frog *Rana tigrina* (Saidapur and Hoque 1995), larval rabbitfish *Siganus guttatus* (Duray and Kohno 1988), sea bass *Dicentrarchus labrax* (Barahonafernandes 1979; Ronazani-Cerqueira and Chatain 1991) and collared lemmings *Dicrostonyx groenlandicus* (Hasler et al. 1976). Conversely, growth and survival can be reduced under an extended day-length (Barahonafernandes 1979; Ronazani-Cerqueira and Chatain 1991). Metcalfe and Monaghan (2001) suggested that photoperiod variation can induce compensatory growth as a consequence of a perception of timing of year, since the time of the season may be a crucial factor in determining growth opportunity. Metcalfe et al. (Metcalfe et al. 2002) hypothesized that the degree and rate of compensatory growth would be affected by the amount of time available to restore body size after a period of disturbed growth (so- called 'time-stress'). However, the effects of time-stress on compensatory growth and subsequent fitness (particularly reproduction and lifespan) are currently equivocal (De Block et al. 2008).

1.4 PHENOTYPIC EFFECT: LOCOMOTORY PERFORMANCE

An important route whereby changes in growth rate can influence the performance of the adult phenotype is through changes in metabolic processes, which have profound implications for energy budgets and behaviour. In many species, acclimation to low temperatures increases tissue aerobic capacities (for reviews see Guderley 1990; Johnston 1993; Guderley and St Pierre 1996). Variation in food availability modifies the energetic status of tissues and leads to shifts in tissue metabolic capacities in migratory birds (Schmidt-Nielsen 1990; Jenni-Eiermann et al. 2002) and fish (Sullivan and Somero 1980; Guderley 1994). It is well documented that metabolic rates decrease in starved animals. For instance, starvation in great knots *Calidris tenuirostris* during long-distance migratory flight induces declined basal metabolic rate (Battley et al. 2001). Wieser et al. (1992) found that starved cyprinids (3 species) were saving energy by reducing locomotor activity and by reducing the cost of maintenance functions. They suggested that metabolic rate does not immediately return to the level of the continuously fed fish when fish are transferred from starvation or restricted feeding to satiation feeding. Acclimation to changed photoperiod modifies the rates of respiration in sunfish *Lepomis gibbosus* and also shifts brain, gill, and muscle tissues (Roberts 1964). Moreover, altered photoperiods modify swimming speed of juvenile largemouth bass *Micropterus salmoides* (Kolok 1991). These changes in muscle metabolic capacities are likely to modify locomotor capacities (i.e. flight in birds, running in mammals and swimming in fish). Low maintenance costs, together with high food and energy uptake, would lead to a larger amount of energy being available for growth. Thus mass increase would be faster during the initial phase of recovery. It is also possible that catabolic process slow down while anabolic processes are accelerated and this causes the rapid growth rates during compensatory phase (Jobling 1994). More detailed experimental studies investigating these effects are needed, and I examine the effects of compensatory growth on locomotor performance in this thesis.

1.5 REPRODUCTIVE INVESTMENT

On reaching sexual maturity, secondary sexual characteristics are considered to be important, state dependent signals. In a natural environment overloaded with information, it is necessary for sexually active males to communicate their availability to potential mates (Tinbergen 1951; Andersson 1994). Accordingly, sexual selection favours signals that most effectively stimulate the recipient: that is, those that are noticed more quickly,

give an indication of male quality, or are simply able to advertise the male's presence over a greater distance (Sargent et al. 1998). It is well documented that brighter nuptial colouration in males is more successful at attracting females (e.g. in zebra finches *Taeniopygia guttata* (Mcgraw et al. 2003), great frigatebirds *Fregata minor* (Juola et al. 2008), three-spined sticklebacks *Gasterosteus aculeatus* (Bakker and Mundwiler 1994), lizards *Psammodromus algirus* (Martin and Forsman 1999). These findings support the concept of sexually dimorphic signal acting as an indicator of male quality. It is well known that the biochemical basis of male stickleback sexual coloration is carotenoid pigments (Matsuno and Katsuyama 1976; Wedekind et al. 1998). Carotenoids, which are important antioxidants, can be acquired only through the diet and prey items that contain high concentrations may not be those ideally selected from an energetic perspective, possibly limiting their availability in natural environments (Olson and Owens 1998; Pike et al. 2007). Before the breeding season (i.e. the first winter or early spring) begins, therefore, food availability or environmental conditions strongly influence the brightness of a male's nuptial colouration. Where an animal has undergone compensatory growth, such sexual signals, if they are honest, might be altered as a consequence. Alternatively, individuals may invest heavily in maintaining the signal quality at the expense of other attributes such as lifespan. For example, zebra finches that underwent compensatory growth maintained their carotenoid-based sexual signal strength in adulthood, despite a reduction in plasma levels and likely knock-on negative effects on lifespan (Blount et al. 2003). However, this potential phenotypic effect of compensatory growth has received little attention to date. I therefore investigated the effect of compensatory growth on sexual signals in sticklebacks in my experimental programme.

1.6 THE LIFE-HISTORY OF THREE-SPINED STICKLEBACKS

Three-spined sticklebacks are small teleost fish, abundant in marine and coastal freshwater habitats. Sticklebacks have distinct and well-studied reproductive behaviours (Peichel and Boughman 2003). Sticklebacks become reproductively active from late April until July: initially both the male and female develop a blue eye colouration and males also show red nuptial colouration on their throat. At the onset of the breeding season the males begin to build a nest and the females develop eggs. Generally males in nest-building fishes construct the nest alone and then solicit mating from multiple females. Nest construction by male sticklebacks is well documented (Wootton 1976; Rowland 1994). Using

filamentous algae and other vegetation, males cover and mix them with a glue, produced in the kidney (Jakobsson et al. 1999), on a sandy substratum and make a tunnel through which the female can pass during spawning (Wootton 1976). It is known that the nest structures can act as extended ornamental traits that reflect male physiology (Barber et al. 2001) and can be one of the key sexual cues used by females (Ostlund-Nilsson and Holmlund 2003). After completing the nest, male sticklebacks court gravid females and attempt to lead them back to the nest to spawn, and then fertilize the eggs.

Three-spined sticklebacks are an ideal study species for these research topics. First of all, they are a short-lived species so making it possible to study the entire lifecycle. This is particularly the case with riverine populations in western Scotland where they are generally annual, with the majority of fish dying after a single breeding season (Chellappa et al. 1989). Secondly, sticklebacks are found over a wide range of latitudes (e.g. freshwater populations are found from southern Spain to Iceland, see Wootton 1976), and so are exposed to a range of breeding temperatures; even fish from a single population can experience very different temperatures early in development due to the species breeding in shallow water and producing repeated clutches over an extended period (May-July), a time when water temperatures are rising rapidly. Finally, sticklebacks have nuptial colouration and show mate selection. The life-history strategies are clearly sensitive and flexible under changing conditions.

1.7 AIMS OF THESIS

This thesis combines field and laboratory studies, and a correlative and experimental approach to relate environmentally-induced variation in early growth and development rate to (a) subsequent performance of the adult phenotype, (b) the pattern of decline in this performance in late life, and (c) lifespan. I manipulated four factors to affect growth, reproduction and lifespan: temperature during growth (low, intermediate or high), photoperiod (ambient or delayed), food (restricted or normal) and conducted my experimental at different times in the season (winter or spring). Using these experiments, I aimed to investigate how early growth trajectories induced by environmental conditions affect adult performance (i.e. locomotor and reproduction) and lifespan, and also how the perception of (or actual) time of year influences the degree of, and effects of, compensatory growth. A comprehensive series of related treatments has allowed me to investigate for the first time the effect of growth trajectories on diverse aspects of

reproductive investment in both sexes, and over multiple breeding seasons. I also developed a range of life-history models to understand the trade-off faced by ectotherms between early growth and damage in relation to both temperature and food supply, taking into account the level of activity required to obtain a given amount of food and the resulting pattern of energy allocation.

The objectives of this thesis were to address the following questions:

1) DO EFFECTS OF TEMPERATURE ON EARLY GROWTH TRAJECTORY INFLUENCE SUBSEQUENT CHANGES IN LOCOMOTOR PERFORMANCE, INDEPENDENT OF ANY EFFECT OF NUTRITION? DOES TIME OF SEASON INFLUENCE THE TRADE OFF BETWEEN GROWTH AND LOCOMOTOR PERFORMANCE? (Chapter 2)

2) DO DIFFERING GROWTH TRAJECTORIES AND LEVELS OF 'AVAILABLE TIME' INFLUENCE REPRODUCTIVE INVESTMENT? (Chapter 3)

3) DOES COMPENSATORY GROWTH INDUCED BY EARLY POOR NUTRITION AFFECT SWIMMING ENDURANCE (A SHORT-TERM CONSEQUENCE) AND REPRODUCTIVE INVESTMENT (A LONG-TERM CONSEQUENCE), AND IS THE EXTENT OF THE NEGATIVE EFFECTS OF COMPENSATORY GROWTH RELATED TO THE LEVEL OF TIME STRESS THE ANIMAL PERCEIVES ITSELF TO BE UNDER? (Chapter 4)

4) IS EARLY GROWTH RATE LIKELY TO CAUSE LONG-TERM EFFECTS THROUGH THE ACCUMULATION OF PHYSIOLOGICAL DAMAGE, AND DOES THIS TRADE-OFF BETWEEN GROWTH TEMPO AND DAMAGE LEVEL INFLUENCE OPTIMAL LIFE-HISTORY STRATEGIES? (Chapter 5)

5) DO EARLY GROWTH TRAJECTORIES INDEPENDENT OF NUTRITIONAL EFFECTS INFLUENCE LIFESPAN? DOES PERCEPTION OF TIME AVAILABLE UNTIL THE BREEDING SEASON INFLUENCE THE TRADE-OFF BETWEEN EARLY GROWTH AND REPRODUCTIVE INVESTMENT, AND ARE THERE CONSEQUENCES FOR LONGEVITY? (Chapter 6)

I conclude the thesis with a general discussion in which the results of these experimental studies are related to previous findings and potential future directions are identified (Chapter 7).

CHAPTER 2

THE TRADE-OFF BETWEEN GROWTH RATE AND LOCOMOTOR PERFORMANCE VARIES WITH PERCEIVED TIME UNTIL BREEDING

2.1 ABSTRACT

Environmental circumstances can cause changes in early growth patterns that subsequently affect the adult phenotype. Here I investigated how different growth trajectories affected subsequent locomotor performance, and how any such effects were influenced by the perceived time until the key life history event of reproduction. Using juvenile three-spined sticklebacks *Gasterosteus aculeatus*, I show that a brief period of manipulated temperature in early life (independent of food supply) caused effects on skeletal growth trajectory not only during the manipulation itself, but also during a subsequent compensatory phase. The outcome of these changes was that fish in all treatment groups reached the same average size by sexual maturity, despite having different growth patterns. However, their growth trajectory had impacts on both pre-breeding swimming endurance and its decline over the course of the breeding season, such that swimming ability was negatively correlated with skeletal growth rate during the compensation period. I also show for the first time that 'negative compensation' (i.e. a decelerating growth trajectory) led to an improved swimming performance compared to steadily-growing controls. Replicate experiments and photoperiod manipulations moreover revealed that the effects of growth rate on subsequent swimming performance were greater when the perceived time until the breeding season was shorter. These results show that the costs of accelerated or decelerated growth can last well beyond the time over which growth rates differ, and are affected by the time available until an approaching life history event such as reproduction, possibly because of the time available to repair damage.

2.2 INTRODUCTION

Environmental circumstances in early life can cause changes in the tempo and pattern of growth and development (Weatherley and Gill 1987). Whilst an episode of poor conditions causes a slowing of growth, if adequate supplies are subsequently restored, normal adult size can still be reached by growth acceleration (Dobson and Holmes 1984; Miglavs and Jobling 1989; Quinton and Blake 1990; Gotthard 2008). A large body size may often be beneficial (e.g. through reduced predation rate or increased fecundity (Arendt 1997), or increased prey choice (Ludsin and DeVries 1997). However, recent studies in several taxa have shown that the accelerated growth rate required to achieve a large size after a period of poor growth can carry costs: for instance, faster growth in early life is linked to a more rapid increase in mortality with adult age (Rollo 2002; Metcalfe and Monaghan 2003; Ricklefs 2006). Therefore animals may face continual trade-offs between the benefits and costs of growth compensation.

While many studies of the effect of growth rate on later performance have focussed on the level or quality of nutrition (Miglavs and Jobling 1989; Álvarez and Metcalfe 2005; Inness and Metcalfe 2008), this is not the only cause of variation in growth rate. For ectotherms, environmental temperature has major effects on growth and metabolism, independent of food supply (Guderley 1994). Thermal conditions can limit their rate of growth and development, since colder temperatures limit the rate at which they can capture and digest food while temperatures close to upper tolerance limits result in poorer growth due to high metabolic costs (Brett 1979; Gadomski and Caddell 1991). Temperature acclimation leads many species to adjust tissue metabolic capacities (Rome et al. 1984; Sisson and Sidell 1987), but changes in environmental temperature have effects on energy budgets (Guderley 2004), lipid levels and activity patterns (i.e. changes in foraging rate) (Hurst et al. 2005). As a result, a period of atypically cold temperatures can cause animals to drop below their normal growth trajectory, even if food has been freely available throughout (Nicieza and Metcalfe 1997), since they cannot swim as fast to capture moving food items and intervals between meals are longer due to the reduced speed of digestion (Wootton 1998).

Growth opportunity may also be influenced by seasonal factors (Boeuf and Le Bail 1999). Photoperiod is one of the most important abiotic factors affecting growth and survival (Battaglene 1995; Boeuf and Le Bail 1999). Many diurnal animals are visual predators and therefore require light for feeding, so that the duration of daylight may

constrain food intake by limiting the period of daily foraging (Blaxter 1980). The photoperiod also indicates the time of year, and hence potentially the time available until the end of the growing season or some other event when body size or reserves are strongly linked to fitness (e.g. hibernation, reproduction). Animals may therefore be expected to increase their growth rate if there is a reduction in the perceived time available before such key life history events (Nylin and Gotthard 1998; Metcalfe and Monaghan 2001). However, responses of animals to photoperiod cues of growth opportunity are poorly understood: while an experimental shift in the photoperiod (simulating a shorter time until the end of the growing season) has been found to cause accelerated growth in some insect larvae (Nylin and Gotthard 1998), other studies (e.g. De Block et al. 2008) found that the rate of compensatory growth in response to an earlier period of either food shortage or cool temperatures was not stronger under time stress.

Changes in growth rate may also result in physiological changes in the animal. For instance, the accelerated growth of fish due to changing temperatures influences muscle cellularity and development (Galloway et al. 1999; Johnston 2003). In many species, adjustment to low temperatures also increases tissue aerobic capacities (Guderley 1990; Johnston 1993; Guderley and St Pierre 1996). Such effects on metabolism and musculature are likely to lead to changes in locomotor performance, and indeed rapid growth has been associated with reduced sustained and burst swimming performance in fish (Kolok 1991; Johnston 1993; Billerbeck et al. 2001; Arnott et al. 2006). However, the studies to date have tended to make a single measurement of locomotor performance (usually during a period of rapid growth), and so the longer term consequences of changes in growth trajectory are little known.

The aim of this present study was to investigate how different growth trajectories affected locomotor performance (in both the short and long term), and how any such effects were dependent on the time available until a point when body size has known fitness consequences (i.e. the breeding season). For this study, I chose three-spined sticklebacks *Gasterosteus aculeatus* since they are known to exhibit compensatory growth (Wootton 1998; Álvarez and Metcalfe 2005; Inness and Metcalfe 2008), possibly as a result of their reproductive success being size-dependent (Kraak and Bakker 1998; Kraak et al. 1999), and their swimming performance has previously been found to be compromised by compensatory growth (Álvarez and Metcalfe 2005; 2007). However, in these earlier studies the compensatory growth was induced by changing food levels, and so the effect might have been due to the earlier period of undernutrition rather than the

compensation *per se*. Therefore, in the present study food was always available *ad lib.* (so fish were always in good nutritional condition) and the different growth patterns (i.e. acceleration, deceleration, and steady) were induced by manipulations of environmental temperature. I was thus able to test how effects of temperature on early growth trajectory influenced subsequent changes in locomotor performance, independent of any effect of nutrition. I examined the effect of seasonal influences on the trade off between growth and locomotor performance by both manipulating photoperiod and by conducting the experiment at two different times of the season – winter and spring. This enabled me to examine the effects of perceived time available until the breeding season on both growth response and its effect on swimming performance.

2.3 METHODS

Fish and rearing conditions

The breeding season of sticklebacks in the source population begins in May. Therefore, in order to see whether the compensatory growth response differs (i.e. is more marked, with stronger effects on swimming performance) when the time available for growth prior to the onset of the breeding season is short, the experiment was run twice, with the main manipulation of growth rates through temperature occurring either a long (= Winter experiment) or short (= Spring experiment) time before the start of the breeding season. For the Winter experiment, three-spined sticklebacks were captured with a dip net and minnow traps in the River Endrick, Scotland, UK (56°04′N, 4°23′W) on 1 November 2007. Fish for the corresponding Spring experiment were captured from the same location on 29 January 2008. On both occasions, all fish were initially transferred to acclimatization aquaria (80 L and density 2 fish·L^{-1}) for 3 weeks and fed *ad libitum* with frozen chironomid larvae (i.e. 10% of body mass). The temperature was initially maintained at 9.7 ± 0.1°C prior to the start of experiments, while the photoperiod was initially ambient.

Winter Experiment

On 21 November 2007, fish for the Winter experiment were anaesthetized and measured for standard length (±0.01 mm) and wet mass (±0.001 g). Fish were then sorted into groups of five (of differing size, to aid identification; regular measurements throughout the

experiment confirmed that size ranks never changed within a tank), with each group of five fish in a separate tank (335 × 170 × 185 mm). Each tank was provided with aeration, a filter and artificial plants. 25% of the total volume of water (1.75 L) was changed every week. I also added 62.5 mL of seawater per tank to prevent the risk of whitespot infection *Ichthyophthirius multifiliis*. Four replicate tanks of five fish were assigned randomly to each of three manipulations, defined in relation to temperature: high (14°C), low (6°C) and intermediate (10°C). These temperature manipulations were applied for a four week period (Period 1), following which all fish were transferred to 10°C (Period 2) (Table 2.1). On 16 May, by which time males had started to develop their breeding coloration (reddish throats) and females to become gravid, the temperature was changed to 14°C to allow the fish to breed (Period 3). Food rations (chironomid larvae) were provided *ad libitum* once per day throughout the experiment.

TABLE 2.1 Description of treatments. Note that following the four week manipulation period (Period 1) and all fish were kept at 10°C (Period 2) until the start of the breeding season (Period 3). During the breeding season, they were kept at 14°C. Fish were fed *ad libitum* throughout the experiment. The entire experiment was run twice, with different fish, starting in November (= Winter experiment) and February (= Spring experiment).

Group	Temperature manipulation			Photoperiod manipulation
	Period 1	Period 2	Period 3	
HTA	High (14°C)			Ambient
HTD	High (14°C)			Delayed (35days)
ITA	Intermediate (10°C)	10°C	14°C	Ambient
ITD	Intermediate (10°C)			Delayed (35days)
LTA	Low (10°C)			Ambient
LTD	Low (10°C)			Delayed (35days)

In order to examine the extent to which an alteration in the perception of time of year (and hence time until breeding) influences the growth response, the above three groups were replicated under two different photoperiod regimes: the fish were either given the current natural photoperiod regime for this latitude (= ambient photoperiod treatment) or were transferred to a day length which was 2 h longer at the start of the manipulation, corresponding to a point 35 days earlier in the autumn (= delayed photoperiod treatment). The photoperiod for all fish was achieved using fluorescent lights controlled by electronic

timers, with blackout plastic sheeting around the tanks being used to achieve independent lighting regimes. The photoperiod in the ambient and delayed treatment groups then changed at the ambient and delayed (-35 days) seasonal rates of progression respectively, so that the photoperiod cue received by the fish in the delayed treatment would suggest that they were continually at a stage 35 days earlier in the season (i.e. initially late autumn instead of early winter), and thus had a longer growth period ahead prior to the breeding season.

Thus, overall within this Winter experiment there were six manipulation groups (3 temperature × 2 photoperiod treatments, each with 4 replicate tanks), which enabled me to examine the effect of temperature-induced compensatory growth on swimming performance, and whether the magnitude of the response was influenced by perceived time until the breeding season. Since the intermediate temperature manipulation groups experienced no temperature change, being held at 10°C until the breeding season, I predicted that they would experience steady growth; the low temperature manipulation groups had a four week period at 6°C followed by 10°C, so were expected to show slowed growth followed by (compensatory) growth acceleration; and the high temperature manipulation groups were expected to show the opposite growth pattern (faster growth for 4 weeks followed by a deceleration). If the response of the fish was influenced by nearness to the onset of the breeding season, then I would expect fish in the delayed photoperiod manipulation groups to show weaker compensatory responses than their corresponding group exposed to an ambient photoperiod.

The fish were re-measured for length and mass every 2 weeks during the temperature manipulations and every 3 weeks thereafter; all fish were starved for 24 h prior to measuring to prevent inflation of measured mass due to stomach contents. The length reached at the end of the temperature manipulation (i.e. Period 1) is referred to as the "manipulated fish length". Tanks were inspected daily in order to monitor mortality rates throughout the experiment.

On 16 May, the fish were sexed on the basis of their coloration, and males that had developed the typical sexual ornamentation (blue eye coloration and reddish throats (Wootton 1976) were moved to individual tanks, which were of the same size and arrangement as their group tank but with the addition of a Petri dish containing fine sand (i.e. a nesting dish) and nesting material (50 × 5 cm lengths of thread). Once most males had built nests, each was shown a gravid female enclosed in a Plexiglas container for 5 min

twice daily for 4 weeks to prompt full expression of nuptial coloration (Pike et al. 2007). Females were kept in their original group tanks and were stripped of clutches of eggs whenever they became fully gravid. Data on the effect of the experimental manipulations on reproductive performance will be presented in Chapter 3.

Spring Experiment

In order to examine whether the outcome was influenced by the stage the fish had reached when the experiment began, I repeated the above period using fish caught in January. In the Spring experiment the same process of measuring and assigning wild-caught fish to groups of 5 per tank was carried out on 21 February 2008, with 4 tanks being randomly assigned to the same 6 manipulations as before. All details of the experimental set-up were exactly as in the Winter Experiment, except that this time the fish in the delayed photoperiod treatment were transferred to a day length which was initially 2 h shorter at the start of the manipulation, corresponding to a point 35 days earlier in the spring than the current date. The ambient photoperiod treatment fish experienced a photoperiod that tracked the natural seasonal progression, while the delayed photoperiod group experienced the same rate of change of the seasons except that it always appeared to be 35 days earlier in the year than was actually the case. Period 2 in the Spring experiment commenced on 20 March and Period 3 on 3 July, with males again being separated into individual tanks when they had developed signs of breeding coloration.

Analysis of growth rate

In order to compare the effect of growth rates between the two experiments, I calculated each fish's relative growth rate, which controlled for seasonal and ontogenetic differences in growth rate between the experiments. I first determined the typical growth pattern for unperturbed fish during the compensatory period in each experiment, by using the data for the Intermediate temperature group to regress gain in length over interval t on initial length L_i, both axes being on a logarithmic scale. The resulting regression equation for each experiment was then used to predict the expected growth during the compensatory period for all fish:

$$\ln[G_E] = m[\ln(L_i)] + c$$

where G_E is the expected gain in length over the compensatory period (Period 2) if no compensation occurred, $\ln(L_i)$ is the logarithm of initial length, and *m* and *c* are the regression parameters determined from the data for Intermediate temperature fish. The relative growth rate was calculated as:

$$relative\ growth\ rate = [\ln(G_O) - \ln(G_E)] / \ln(G_E)$$

where G_O, the observed gain in length over the compensatory period, is given by (L_c - L_i). Mean values for relative growth rate were then calculated for each sex within each treatment group.

Swimming performance

I quantified swimming performance as the length of time a fish could swim against a constant strong current of water; this measure of swimming stamina has been used in a range of previous studies (Ojanguren and Braña 2000; 2003; Royle et al. 2006) including of sticklebacks (Álvarez and Metcalfe 2005), and the full details of the experimental setup are given in Álvarez and Metcalfe (2005) and Royle et al. (2006). Swimming performance in both experiments was measured twice: 1) when fish in the different manipulation groups had finished the phase of compensatory growth and had converged on the same mean size prior to breeding, and 2) 18 weeks later (after the breeding season). The swimming trials were conducted inside a temperature-controlled room that maintained the temperature the same as in the holding tanks. One fish at a time was placed into a cylindrical swimming chamber (50 cm long, 20 cm diameter). The fish was initially subjected for 5 min to a moderate water velocity (17.0 cm s^{-1}) to allow it time to adapt to the apparatus. The water velocity was then increased to 34.9 cm s^{-1} (slightly greater than the maximum that could be sustained by sticklebacks, based on pilot trials) and the time taken until fatigue was recorded. A fish was deemed to be exhausted when it was forced back against the fine mesh grid at the downstream end of the compartment for more than 5 s (Ryan 1988) and was no longer able to continue swimming, despite my tapping the side of the chamber (Ojanguren and Braña 2000). I immediately turned off the pump and the fish was allowed 5 min recuperation time before being measured (length and body mass) and returned to its original tank. As a measure of recovery rate, I recorded the opercular ventilation rate (beats min^{-1}) during the 5 min recuperation time, and also recorded the time elapsed until the fish first began to move again. All fish quickly recovered and were swimming normally again

within 2-5 min. Swimming endurance was defined as the amount of time that a fish swam at the highest flow rate. All experiments were performed under license from the UK Home Office (PIL 60/11377).

Statistical analysis

I used multivariate analysis of variance (MANOVA) to test for differences in body length and mass: a) at the beginning of each experiment, b) at the end of the temperature manipulation (Period 1), and c) t days later (see section on growth rates above), when fish in the different manipulation groups had apparently finished the phase of compensatory growth. The effect of manipulations on swimming endurance and recovery time was analyzed in both experiments using a general linear mixed model (GLMM) with treatment (Low, Intermediate or High temperature, denoted LT, IT and HT respectively), photoperiod (Ambient or Delayed) and sex (male or female) as fixed effects, tank as random factor to control for tank effects, and body length at the time of the first swimming test and breeding season growth (i.e. increase in length between the first and second swimming tests) as covariates, plus all interactions among variables. Temporal changes in swimming endurance and recovery time were calculated as the difference in values measured before and after breeding. To test the effect of relative growth rate on the change in swimming endurance over the breeding period, I used a general linear model (GLM) based on the mean value for each sex within each treatment group as data points, with change in swimming endurance as the dependent variable, sex and experiment (winter or spring) as factors and relative growth rate as a covariate. In all analyses non-significant variables were sequentially dropped from the analyses so that the final models only included significant terms. All means are presented with standard errors and all of the analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, Illinois).

2.4 RESULTS

Compensatory growth response after temperature treatment

At the beginning of both experiments there were no differences in length or mass among temperature manipulation groups (low temperature (LT), intermediate temperature (IT) or high temperature (HT)) (MANOVA, Winter: Wilk's λ=0.954, $F_{4,232}$=1.34, P=0.26, Spring:

Wilk's $\lambda=0.994$, $F_{4,232}=0.17$, $P=0.96$), or between photoperiod treatments in each temperature group (ambient photoperiod *vs.* delayed photoperiod (MANOVA, Winter: Wilk's $\lambda=0.990$, $F_{2,232}=0.55$, $P=0.58$, Spring: Wilk's $\lambda=0.997$, $F_{2,232}=0.19$, $P=0.83$). However, by the end of the temperature manipulation period (day 28, end of Period 1), there were significant differences in length and mass among temperature manipulation groups in both experiments (Winter: Wilk's $\lambda=0.867$, $F_{4,224}=4.02$, $P=0.004$, Spring: Wilk's $\lambda=0.812$, $F_{4,218}=5.82$, $P<0.001$, Fig. 2.1), whereas there were no effects of photoperiod on length or mass (Winter: Wilk's $\lambda=0.999$, $F_{2,224}=0.04$, $P=0.96$, Spring: Wilk's $\lambda=0.977$, $F_{2,218}=1.23$, $P=0.30$). In the Winter experiment, LT fish were 6.6% smaller in standard length (ANOVA, $F_{1,76}=4.67$, $P=0.034$) and 28.3% lighter in mass ($F_{1,76}=8.12$, $P=0.006$) than HT fish at the end of Period 1. Similarly, in the Spring experiment, at the end of Period 1 LT fish were 5.3% smaller in length ($F_{1,71}=3.68$, $P=0.059$) and 30.1% lighter in mass ($F_{1,71}=10.11$, $P=0.002$) than HT fish. In both experiments, IT fish were intermediate in size and mass between HT and LT fish.

During the 4 week period of temperature manipulation, the mortality in the Winter experiment was 0%, 10% and 10% for the LT, IT and HT temperature manipulation respectively; there was no difference in mortality among treatments ($\chi^2=2.069$, d.f.=2, $P=0.36$). In contrast, there was a treatment effect on mortality in the Spring experiment ($\chi^2=14.87$, d.f.=2, $P=0.001$). This was due to 35% mortality in the HT group as compared with zero mortality in the other two groups. This higher mortality (7 fish) in the HT group was almost entirely due to all 5 fish in one tank dying suddenly on 8 March 2008, for unknown reasons (all other mortality in the experiments was spread across tanks and days in no clear pattern). Note that the data for fish that subsequently died during the course of the study were excluded from all analyses, to ensure that none of the statistics on growth rate etc would be biased by any differential mortality rates.

The size differences did not persist once the fish were transferred to the same conditions (10°C) at the end of Period 1: compensatory growth occurred (in terms of both accelerated growth in LT groups and decelerated growth in HT groups, relative to IT fish) such that the growth trajectories of the different temperature treatment groups converged (Fig. 2.1). In the Winter experiment, the significant differences in size among temperature groups had disappeared 15 weeks after the end of the manipulation period (comparison of sizes at 15 weeks: Wilk's $\lambda=0.946$, $F_{4,182}=1.29$, $P=0.28$); in the Spring experiment, the compensation was quicker and the corresponding time for size differences to disappear was 12 weeks (Wilk's $\lambda=0.985$, $F_{4,188}=0.37$, $P=0.83$). While growth rate during the

compensatory growth (Period 2) was slower for delayed compared to ambient photoperiod treatment fish in the Winter experiment (GLM, effect of photoperiod: $F_{1,95}=7.77$, $P=0.006$), there was no effect of photoperiod on growth rate in the Spring experiment ($F_{1,98}=0.63$, $P=0.431$).
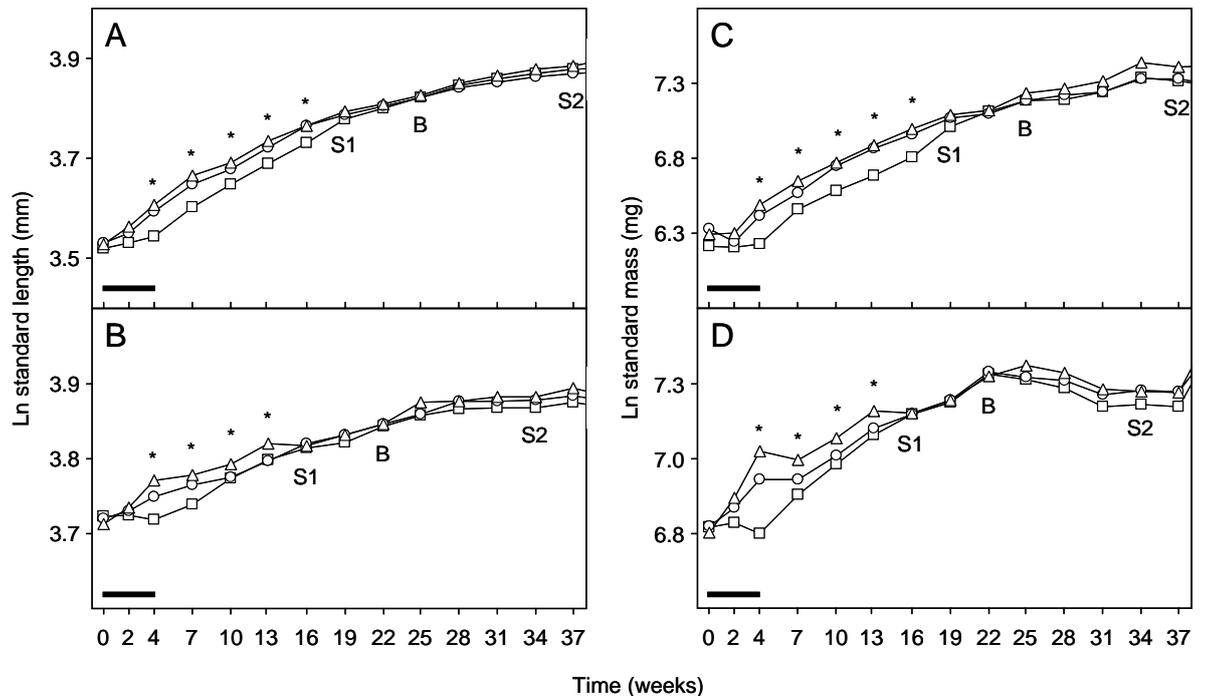
FIG. 2.1 Growth trajectories (logarithm of standard length in mm and of wet mass in mg) of three-spined sticklebacks *Gasterosteus aculeatus* in the Winter (A and C) and Spring (B and D) experiments. Note that the two experiments started on different days, so that Day 1 is 21 November in (A and C) and 21 February in (B and D). The thick horizontal line indicates the period of temperature manipulation (28 days, △ - 14°C, ○ - 10°C, □ - 6°C). After this period, the temperature in all three groups was kept at 10°C until the start of the breeding season ('B'), at which point the temperature was raised to 14°C and male sticklebacks were isolated from female sticklebacks (see Methods for more details). 'S1' and 'S2' indicate the timing of the swimming trials (i.e. at the end of the period of compensatory growth and 18 weeks later, after the breeding season). Asterisks indicate significant differences among treatment groups ($P<0.05$).

Swimming endurance

Endurance was first measured when the fish from the different manipulation groups had approximately converged in mean size (i.e. growth compensation was complete). For the Winter experiment, endurance was measured an average of 114.5 (range 112-117) days after the end of the temperature manipulation, while for the Spring experiment the

measurements were on average 93.5 (range 91-96) days after the manipulation had finished. At this first measurement of swimming performance there was no difference between temperature treatments in endurance in the Winter experiment (GLMM, $F_{2,91}$=0.32, P=0.724), but there was a significant difference in the Spring experiment, with HT fish having the greatest swimming endurance (Fig. 2.2 and Table 2.2). In both experiments, photoperiod influenced endurance (the longest endurance being shown by delayed treatment fish) and there were positive effects of body length at the time of the swimming test on endurance (i.e. larger fish had greater endurance; Table 2.2). Sex did not influence pre-breeding swimming endurance directly in either experiment (Table 2.2). In the Spring experiment, there were significant interactions between temperature and photoperiod, and between photoperiod and body length at the time of the first swimming test: the effects of both temperature and body length were greater under the delayed photoperiod (Fig. 2.2 and Table 2.2).

TABLE 2.2 Results of general linear mixed model analyses examining initial swimming endurance in relation to temperature treatment, photoperiod treatment, sex and body length at the time of the test in the Winter and Spring experiments. Non-significant variables were sequentially dropped from analyses apart from main effects occurring in significant interactions. Tank was included as a random factor.

Experiment	Final model	F	d.f.	P
Winter	Photoperiod	4.60	1, 91	0.035
	Body length at first test	102.99	1, 91	<0.001
Spring	Temperature	5.61	1, 90	0.005
	Photoperiod	5.27	1, 90	0.024
	Body length at first test	206.29	1, 90	<0.001
	Temperature × photoperiod	7.77	1, 90	0.001
	Photoperiod × body length at first test	5.31	1, 90	0.024
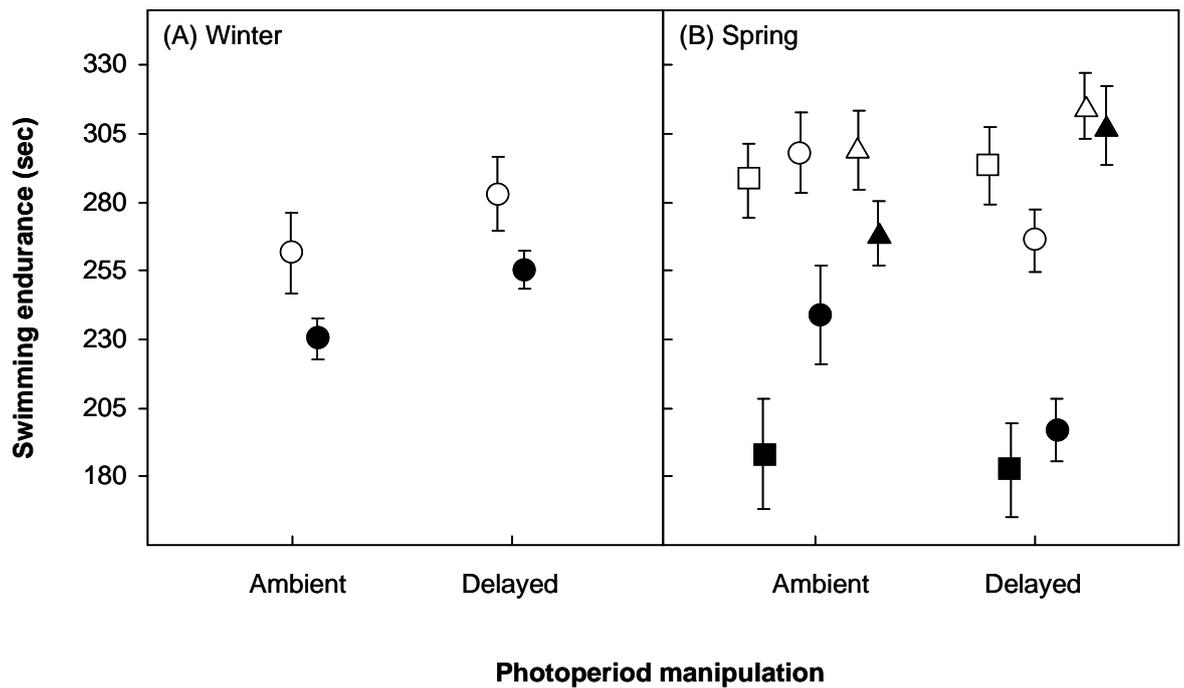
Fig. 2.2 Mean ± SE swimming endurance (sec) of three-spined sticklebacks after an earlier 4-week period of temperature manipulation (low - square, intermediate - circle and high - triangle in panel (B)) (open symbols) and after the breeding season (filled symbols) in relation to photoperiod treatment (ambient or delayed). In separate experiments the temperatures were manipulated in either (A) the Winter or (B) the Spring; this first measurement of swimming endurance was obtained once the growth trajectories had converged after the end of the manipulation (see Fig. 2.1). Data are expressed as least square means (using fish length at time of testing as the covariate) to control for differences in body size among tested fish: data are combined for the temperature treatment groups in the Winter experiment since results did not differ – see text and Table 2.2 for analyses.

When tested again at the end of the breeding season, the average swimming endurance of all categories of fish had declined. The within-individual change in endurance over the course of the breeding season was analyzed using GLMM models, with the same terms as before plus breeding season growth as a covariate. The change in endurance did not differ between temperature treatment groups in the Winter experiment ($F_{2,85}$=3.01, P=0.055), but there was a significant temperature treatment effect in the Spring experiment: LT fish showed the biggest decline in endurance while HT fish declined least (Fig. 2.3 and Table 2.3). In the Winter experiment, breeding season growth and the interaction between body length at the time of the first test and breeding season growth both influenced the change in swimming endurance (Table 2.3): the smallest

decrease in endurance was shown by those fish that grew most during the breeding season, especially if they were amongst the largest at the start of the season. However, there were no significant effects of photoperiod, sex or other interactions on the change in swimming endurance (Table 2.3). In the Spring experiment, the change in swimming endurance over the breeding season was significantly influenced by body length at the time of the first swimming test: larger fish at the time of the first test showed less of a decrease in endurance (Table 2.3). There was also a significant interaction between photoperiod, sex and body length at the time of the first swimming test (Table 2.3). The patterns were therefore complex, but overall the decrease in endurance was greatest in females from the ambient photoperiod group that were smallest at the time of the first swimming test.

TABLE 2.3 Change in swimming endurance over the breeding season in relation to temperature treatment, photoperiod treatment, sex, body length at the time of the first swimming test and breeding season growth (i.e. length at the second swimming test − length at the first swimming test) in the Winter and Spring experiments. Non-significant variables were sequentially dropped from analyses apart from main effects occurring in significant interactions. Tank was included as a random factor.

Experiment	Final model	F	d.f.	P
Winter	Body length at the first test	1.59	1, 81.40	0.211
	Breeding season growth	5.60	1, 83.79	0.020
	Body length at first test × Breeding season growth	5.45	1, 83.95	0.022
Spring	Temperature	32.62	2, 14.75	<0.001
	Photoperiod	2.49	1, 58.48	0.120
	Sex	1.32	1, 66.36	0.255
	Body length at first test	3.53	1, 61.57	0.065
	Photoperiod × sex × body length at first test	4.33	3, 65.96	0.008
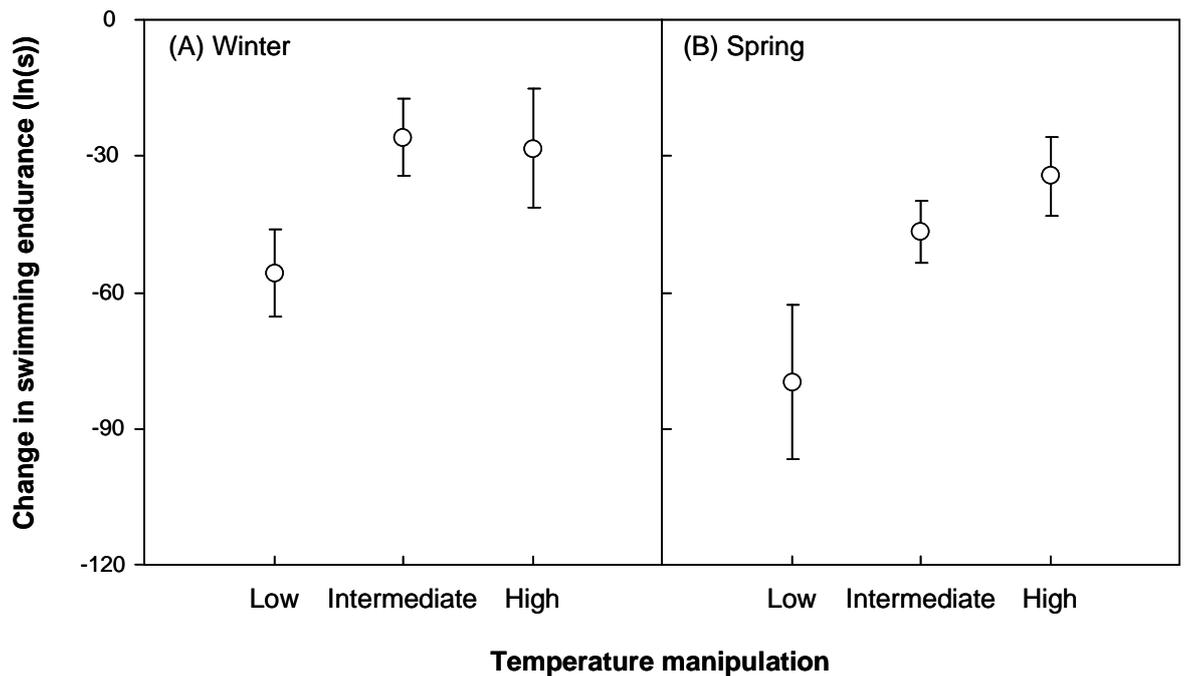
FIG. 2.3 Change (Mean ± SE) in swimming endurance (sec) of three-spined sticklebacks over the breeding season in relation to temperature treatment (low, intermediate and high); in two separate experiments temperatures were manipulated for four weeks in either (A) the Winter or (B) the Spring prior to the breeding season. Data are expressed as in Fig. 2.2; negative values indicate that swimming endurance was poorer at the end of the breeding season. See text for analyses.

To summarise the trends across both experiments, I analysed the effect of relative growth rate (see Methods) on the change in swimming endurance, using the mean value for each sex within each treatment group as data points (so $N = 2$ sexes $\times$ 3 temperatures $\times$ 2 photoperiods $\times$ 2 experiments = 24). The sexes were separated since there was a significant interaction involving sex and body size (see preceding paragraph) and females tended to grow more than males over the breeding season. The data were analysed by GLM, with change in swimming endurance as the dependent variable, sex and experiment (winter or spring) as factors and relative growth rate as a covariate. The estimated decrease in swimming endurance tended to be greater in males (-0.26 ± 0.04) than in females (-0.16 ± 0.04), but this effect of sex was marginal ($F_{1,24} = 4.30$, $P=0.051$), so it was dropped from the model. The change in swimming endurance was negatively affected by relative growth rate (i.e. the faster the relative grow rate of a treatment group, the bigger the reduction in swimming performance over the breeding season; $F_{1,24} = 14.85$, $P<0.001$). However, for a given rate of growth, the adverse effect on swimming was stronger in the Spring

experiment (Fig. 2.4; $F_{1,24} = 18.73$, $P<0.001$). The interaction between season of experiment and relative growth rate was not significant ($F_{1,24} = 0.56$, $P = 0.462$).
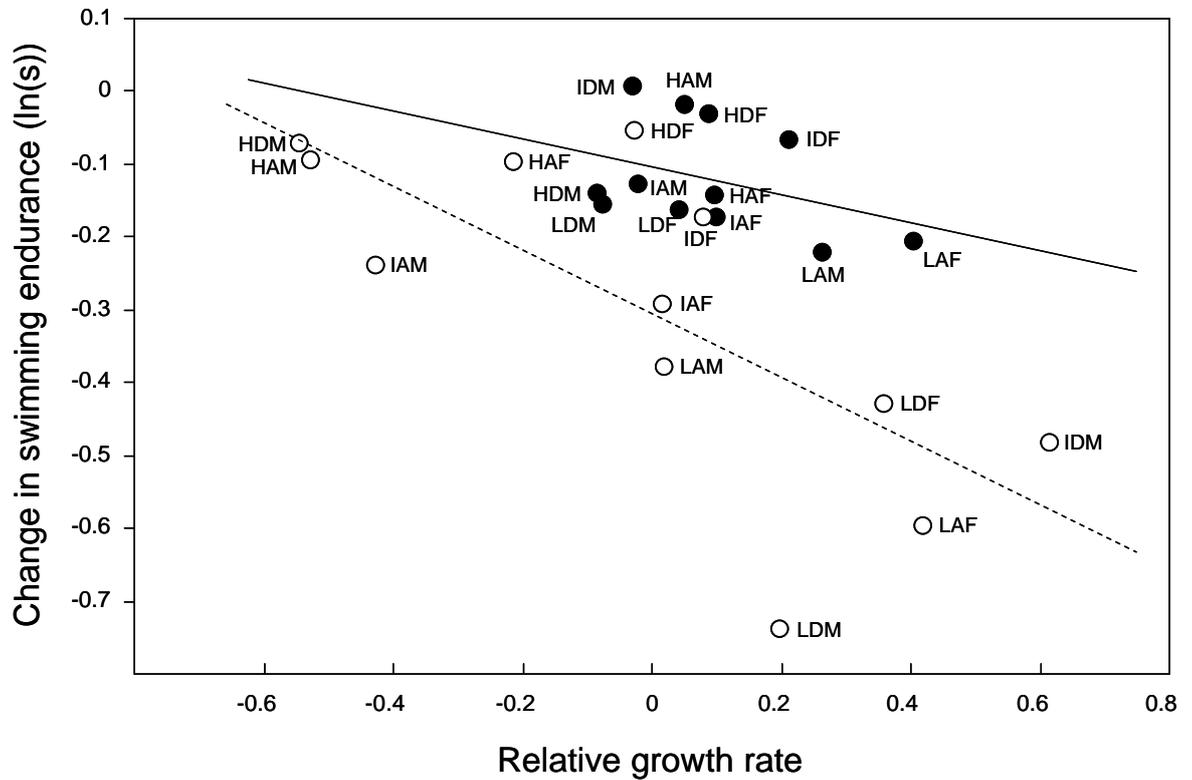
FIG. 2.4 Change in swimming endurance (s, ln transformed) of three-spined sticklebacks over the breeding season in relation to relative growth rate during the compensation period (see Methods). Growth rate is expressed relative to that of the mean for Intermediate fish (see text for details). Mean values are plotted for each sex within each treatment group in both the Winter (filled circles, solid regression line) and Spring (open circles; dashed line) experiment. Each group name is indicated by initials: L-low temperature, I-intermediate temperature, H-high temperature; A-ambient photoperiod, D-delayed photoperiod; M-male and F-female, e.g. 'HDF' indicates data for females in the high temperature and delayed photoperiod group. Note that swimming performance tended to decline least in delayed photoperiod groups and those previously exposed to higher temperatures.

Recovery time

The final models analysing recovery time (after removal of non-significant terms) showed no effect of temperature treatment in the Winter experiment (GLMM $F_{2,90}=0.34$, $P=0.715$) but a significant effect in the Spring experiment, with LT fish taking longer to recover (Fig.

2.5 and Table 2.4). In both experiments, there was a significant effect of photoperiod on recovery times, with fish in the delayed photoperiod treatment recovering fastest (Table 2.4). While there was no effect of body length at the time of the first swimming test on recovery time in the Winter experiment, this term was significant in the Spring experiment, with larger fish recovering faster (Table 2.4). However there was a significant interaction between photoperiod and body length at the time of testing in both experiments, with the slowest recovery being in shorter fish from the ambient photoperiod group (Table 2.4). In neither experiment did other interaction terms or sex have significant effects on recovery time (Table 2.4).

TABLE 2.4 Recovery time after the first swimming test in relation to temperature treatment, photoperiod treatment, sex and body length at the time of the test in the Winter and Spring experiments. Non-significant variables were dropped from the final model apart from main effects occurring in significant interactions. Tank was included as a random factor.

Experiment	Final model	F	d.f.	P
Winter	Photoperiod	9.92	1, 90	0.002
	Body length at first test	2.01	1, 90	0.160
	Photoperiod × body length at first test	9.90	1, 90	0.002
Spring	Temperature	14.61	2, 92	<0.001
	Photoperiod	9.05	1, 92	0.003
	Body length at first test	185.26	1, 92	<0.001
	Photoperiod × body length at first test	9.37	1, 92	0.003
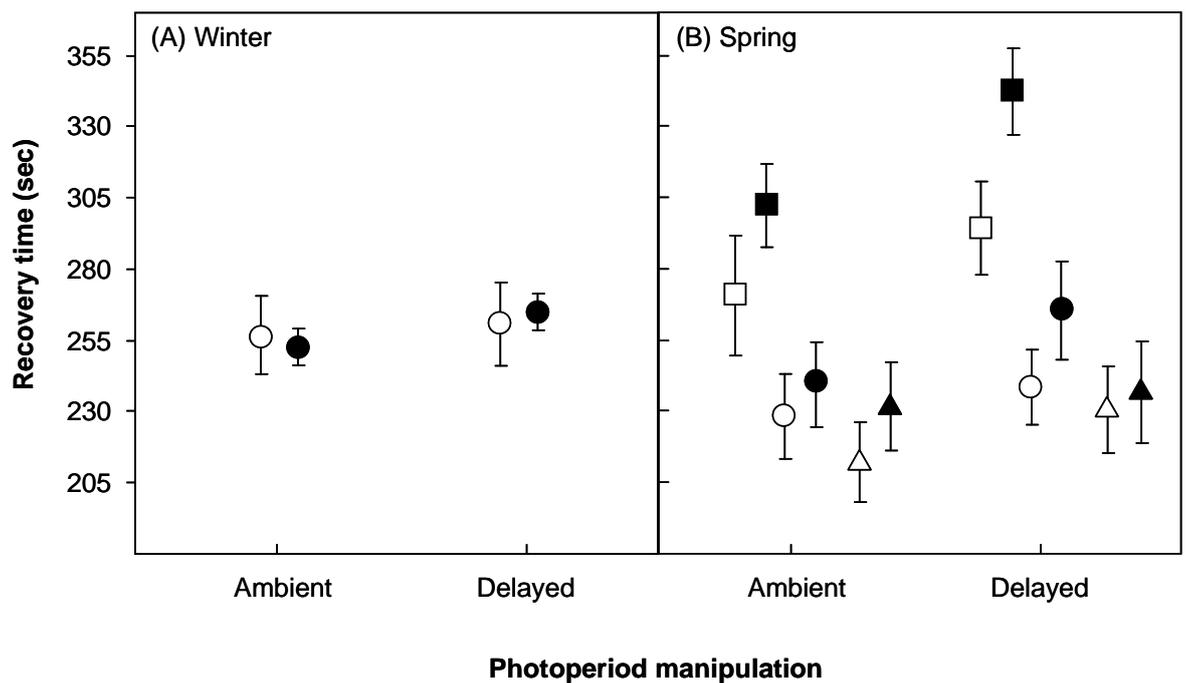
FIG. 2.5 Mean ± SE recovery time (sec) of three-spined sticklebacks after the first swimming endurance trial (open symbols) and after the second trial (closed symbols) in relation to photoperiod treatment (ambient and delayed), measured after growth compensation in relation to temperature manipulation (low - square, intermediate - circle and high – triangle in panel (B)). (A) Winter experiment; (B) Spring experiment. Data are expressed as in Fig. 2.2 – see text and Table 2.4 for analyses.

Recovery times tended to be longer when the fish were re-tested for swimming endurance at the end of the breeding season (Fig. 2.6). Using GLMM, with the same terms as before plus breeding season growth as a covariate, I analyzed the change in recovery time over the course of the breeding season. While the change in recovery time did not differ between temperature groups in the Winter experiment ($F_{2,80}$=0.41, P=0.665), it was significant in the Spring experiment, with HT fish recovering fastest (Table 2.5). The photoperiod treatment was significant in both experiments, with fish in the delayed photoperiod recovering fastest (Table 2.5). In the Winter experiment the change in recovery time was not affected by body length at the time of the first swimming test, whereas in the Spring experiment larger fish showed less of a reduction in recovery time (Table 2.5). An interaction between photoperiod and body length at the time of the initial test influenced the change in recovery time (Table 2.5), with larger fish in the delayed photoperiod group showing least increase in recovery time. There were significant effects of interactions between temperature and sex in the Winter experiment, and between

temperature and body length at initial testing in the Spring experiment (Table 2.5): HT males and bigger HT fish showed least increase in recovery time respectively. In neither experiment was the effect of sex significant.

TABLE 2.5 Change over the breeding season in time to recover from a swimming endurance trial, in relation to temperature treatment, photoperiod treatment, sex, body length at the time of the first swimming test and breeding season growth (i.e. change in length between the first and second swimming tests) in the Winter and Spring experiments. Non-significant variables were dropped from analyses apart from main effects occurring in significant interactions. Tank was included as a random factor.

Experiment	Final model	*F*	d.f.	*P*
Winter	Temperature	0.41	2, 80	0.665
	Photoperiod	9.06	1, 80	0.003
	Sex	<0.001	1, 80	0.976
	Body length at first test	1.18	1, 80	0.280
	Temperature × sex	4.18	1, 80	0.019
	Photoperiod × body length at first test	8.96	1, 80	0.004
Spring	Temperature	6.69	2, 58.70	0.002
	Photoperiod	8.86	1, 58.52	0.004
	Body length at first test	17.84	1, 58.52	<0.001
	Temperature × body length at first test	6.95	2, 58.60	0.002
	Photoperiod × body length at first test	8.97	1, 58.40	0.004
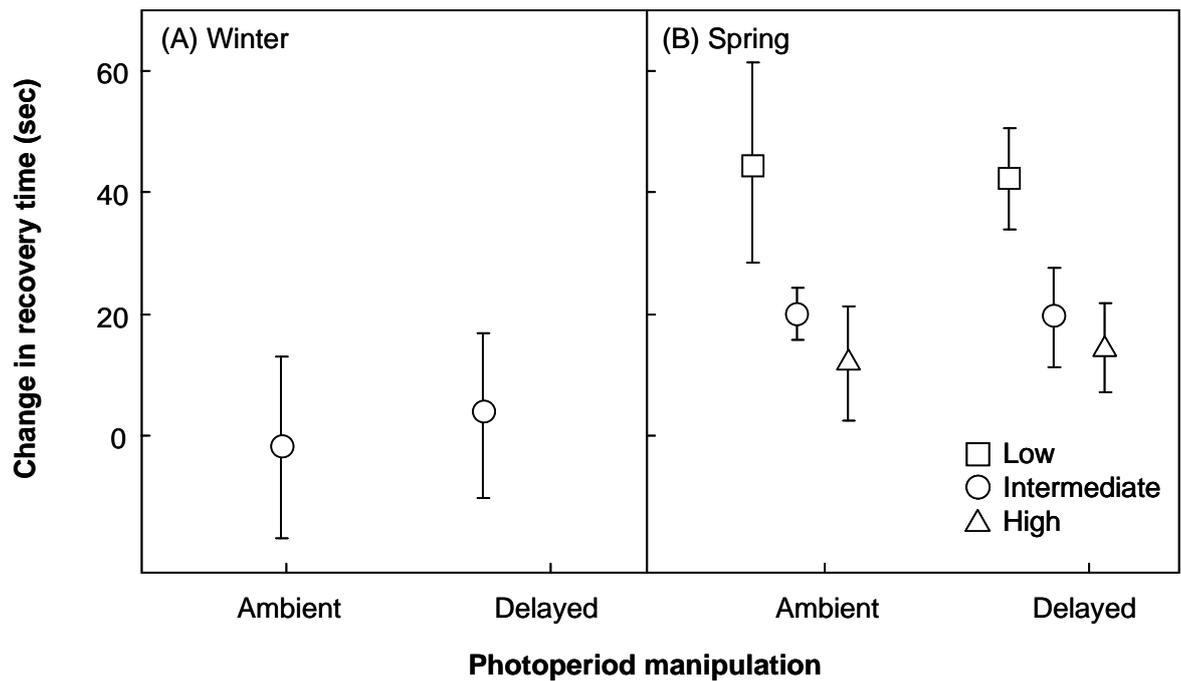
FIG. 2.6 Change over the breeding season in the time (sec) taken by three-spined sticklebacks to recover from a swimming endurance trial, shown in relation to temperature treatment (low, intermediate and high). (A) Winter experiment; (B) Spring experiment. Data are expressed as in Fig. 2.2 and see text and Table 2.5 for analyses; positive values indicate that fish were slower to recover after the breeding season.

2.5 DISCUSSION

Environmental temperatures in early life are known to exert strong effects on the life history of sticklebacks (Wootton 1998). This study found that a brief (4 week) period of manipulated temperature caused effects on growth trajectory not only during the manipulation itself (as would be expected for an ectotherm), but also on subsequent growth trajectories. The growth rates affected body length as well as body mass and so were not simply a change in levels of energy storage. Those fish experiencing the low temperature treatment would have grown slowly because of a reduction in the ability of the fish to process food and synthesis new tissues (Bone and Moore 2007). When the temperature increased again they showed growth acceleration, while fish on the high temperature treatment showed a subsequent growth deceleration when returned to the intermediate temperature. These changes in growth were not simply a physiological response to the new temperature, since growth trajectories of the three treatment groups converged rather than ran parallel to each other, even though all were under the same environmental conditions at

the time. The accelerated growth of the LT fish was presumably due to increased food consumption, which may have been at a level higher than would normally be expected for that temperature (i.e. hyperphagia, as has recently been shown in juvenile brown flounder *Paralichthys olivaceus* (Huang et al. 2008)). Conversely, the HT fish may have exhibited a slightly suppressed food intake until their growth trajectory had converged with that of the IT fish as control fish. There was thus compensatory growth, in both directions, in fish that had earlier experienced a phase of fast or slow growth, even though none of the fish had experienced any food shortage at any time.

It has previously been shown that compensatory growth prompted by changes in nutrition may subsequently affect a range of fitness traits, including locomotor performance (Álvarez and Metcalfe 2005; 2007). The results of the present study support previous work showing that accelerated growth could result in costs to swimming performance in later life, especially if the growth acceleration occurred close to the breeding season (Álvarez and Metcalfe 2005); in the present experiment, stronger effects of the same manipulation were found in the Spring than the Winter experiment (see Fig. 2.4), despite the fact that growth rates were similar. However, I also show for the first time that 'negative compensation' (i.e. a decelerating growth trajectory) led to an improved swimming performance compared to steadily-growing controls. This beneficial effect of a decelerated growth trajectory on both swimming (shown here) and reproductive investment (Chapter 3) may explain why such fish showed a reduced growth rate despite other advantages of larger size during the upcoming breeding season. Swimming capacity in fish is affected by body size and muscle energy reserves (Guderley 2004), but given that there was no difference in body size or nutrition between treatment groups at the time of testing, the treatment effects on swimming performance may instead be related to muscle structure. It is well known that accelerated growth negatively affects muscle cellularity and development (Galloway et al. 1999; Johnston et al. 2002). The development of new muscle fibres is constrained during ontogeny: for instance, the number of fast muscle fibres reaches an asymptote before the fish is half its size at sexual maturity, so that subsequent increases in the size of the muscles can only be achieved by expansion of existing muscle fibres (Johnston 2006). Differences in the timing of muscle fibre recruitment during development have been shown to lead to different compositions of white and red muscle fibres (Johnston 2006): for instance, fast-growing fish have higher percentages of small-diameter white muscle fibres and greater numbers of similar-diameter red muscle fibres than slow-growing fish (Valente et al. 1999). Such growth-induced differences in muscle

structure have been shown in herring *Clupea harengus* to translate into differences in swimming performance that persist even when fish are subsequently growing at the same rate (Johnston et al. 2001). Effects of embryonic conditions on muscle development and subsequent motor performance are not restricted to fish: effects of early growth rate on tail muscle fibre numbers and swimming performance have been found in tadpoles of both toads (Arendt and Hoang 2005) and frogs (Watkins and Vraspir 2006), and it has been suggested that such a trade-off between early growth rate and locomotor performance is common to all vertebrates (Arendt 2003).

The acceleration of growth induced by the LT regime might also have increased the level of damage incurred during development of myotomal muscle, for instance through higher levels of oxidative stress. Recently Pike et al. (2007) showed that growing sticklebacks that had a reduced access to dietary antioxidants were less able to invest in defence against oxidative stress, which can cause damage to a wide range of biomolecules. The modification of anabolic processes that allow an acceleration of tissue growth may involve a diversion of resources towards the synthesis of new protein and away from repair of existing tissues (Morgan et al. 2000). Fish on the LT regime might therefore accumulate more damage, leading to impaired muscle function, whereas those experiencing a decelerated growth trajectory (HT fish) might have been able to invest proportionally more resources into repair (even than the IT fish) and so would have a lower level of damage. Such effects were not restricted only to the time fish were able to swim against a strong current, since the recovery time was longer in accelerated than decelerated growth groups, even though they had spent less time swimming.

The effects of growth trajectory on locomotor performance were evident at the time of the first swimming trial at the end of the compensation period, but they were amplified later in life, after the breeding season. The breeding season for three-spined sticklebacks lasts from late April until July or August: during this time females produce a sequence of clutches of c.100 eggs which they lay in nests that are built by males, who then provide all the care (e.g. nest aeration by fanning, defence against predators and cannibals) for the eggs and young fry (Wootton 1976). The breeding period is thus costly for both sexes (Pike et al. 2007). Similar reproductive costs have been shown to include a temporary impairment of locomotor abilities during the breeding season across a range of organisms (e.g. whelks (Brokordt et al. 2003)), passerine birds (Lee et al. 1996; Veasey et al. 2000; Kullberg et al. 2002). In the present study all groups showed on average a poorer swimming endurance (coupled with a slower recovery) after the breeding season, but this

was accentuated in the groups that had earlier exhibited the fastest growth rate. Therefore the cost of accelerated growth lasted well beyond the time over which growth rates differed between treatment groups.

The prevailing photoperiod can influence the time available per day for feeding activity, and so can affect growth rate. However, it also indicates the time of year and hence time available before key life history events. Metcalfe et al. (2002) hypothesized that animals should be sensitive to the amount of time available when altering their growth trajectory to compensate for a period of perturbed growth, showing a stronger compensation (and hence potentially greater long term costs of compensation) when the time until an approaching life history event such as reproduction was shorter. These results provide strong support for the hypothesis. Firstly, the effect of the temperature treatment was much stronger in the Spring experiment, where the time available from the end of the manipulation until the breeding season was shorter. Secondly, while the photoperiod manipulation with the Winter and Spring experiments had little effect on growth rates during the compensation period, it did affect initial swimming endurance, recovery time, and the change in recovery time over the breeding season. In each case the fish that perceived a greater time from the temperature manipulation until the breeding season (i.e. the delayed photoperiod group) showed the better performance. Given that there was no effect of the photoperiod manipulation on growth rates, these effects on swimming performance may have been due to differential investment in somatic repair: the delayed photoperiod groups would have had a longer time in which to repair any damage in the run up to the breeding season, and may have had a different balance of investment between somatic repair and gonad growth, hence a slower accumulation of cellular damage (Jennings et al. 2000). However, this remains speculation at this stage without further detailed study.

CHAPTER 3

EARLY CONDITION AND REPRODUCTIVE INVESTMENT: COMPENSATORY GROWTH TRAJECTORIES AFFECT SUBSEQUENT BREEDING ORNAMENTATION AND PERFORMANCE

3.1 ABSTRACT

Early environmental conditions can influence the tempo and pattern of growth and development. Whilst poor conditions generally cause slower growth, a normal adult size can still be reached if growth accelerates once conditions improve. However, it is known that such accelerated growth may have deleterious effects later in life. Here, I investigate for the first time how manipulations of growth trajectories affect subsequent breeding performance. During juvenile life, I subjected three-spined sticklebacks to short periods of manipulated temperature (high, intermediate or low), after which all fish had the same temperature regime. In order to test how the perceived time until the onset of the spawning season affected their responses, half of the fish in each treatment were kept on a delayed photoperiod (two months behind ambient). I found that all manipulated fish showed full growth compensation. Those compensating for low temperatures earlier in life (i.e. who showed an accelerating growth trajectory) had reduced reproductive investment (males: sexual ornaments and ability to build nests; females: first clutch size and mean egg size). Moreover, the costs of compensation were strongest when the perceived time until breeding was shortest. In contrast, those fish exposed to high temperatures early in life showed 'negative compensation' (i.e. a decelerating growth trajectory) and an improved breeding performance compared to those experiencing intermediate temperatures. These results clearly demonstrate that relatively fast growth impairs breeding potential, and is therefore likely to carry a fitness cost through this route in addition to effects mediated through reduced life expectancy.

3.2 INTRODUCTION

It is clear that environmental conditions can change an animal's investment in growth and reproduction (Weatherley and Gill 1987). The influence of food supply is obvious, but other environmental factors such as ambient temperature and predation risk can also modify the costs and benefits of alternative patterns of resource allocation. Changing environmental conditions, even if only experienced briefly, can thus influence the pattern of phenotypic expression much later in life (e.g. sexual ornamentation and breeding investment) and can thus influence the evolution of different traits (Candolin and Heuschele 2008; Monaghan 2008). It has recently been well documented that phases of rapid growth early in life (as a result of fluctuations in food supply) can have many long-term (and often detrimental) consequences, in particular affecting locomotor ability and lifespan (Álvarez and Metcalfe 2005; Ricklefs 2006; Criscuolo et al. 2008; Inness and Metcalfe 2008). Changes in growth trajectory can occur naturally when animals experience a brief period of unfavourable growth conditions but then exhibit compensatory growth when conditions improve (Weatherley and Gill 1987; Metcalfe and Monaghan 2001); the reasons for the deleterious effects of the accelerated growth are often unknown but might include increased tissue or molecular damage arising through elevated levels of oxidative stress (Monaghan et al. 2009; Metcalfe and Alonso-Alvarez 2010).

However, the trade off between the potential benefits of rapid growth and the longer term costs is poorly understood (especially since most studies have measured only a limited number of traits). Moreover, since the manipulation of growth has invariably been achieved by alteration of food availability, it is often not clear if the long-term effects are a consequence of a restriction in the diet rather than an alteration in growth *per se*. Ectotherms provide a system in which this problem can be avoided, since their maximal growth rate is directly related to temperature (Guderley 1994); it is thus possible to experimentally manipulate growth rates through alterations of ambient temperature even when food is unlimited (and hence the nutritional condition of the animals is broadly unaffected). While the slowed metabolism induced in ectotherms by lower ambient temperatures can have advantages such as a slowed locomotor senescence and longer lifespan (Valenzano et al. 2006; Brugnano et al. 2009), there are often predation or reproductive costs associated with a smaller body size (Wootton 1976; Rowland 1994). Therefore ectotherms that have experienced a period of reduced growth as a result of atypically cold temperatures may exhibit compensatory growth to return to the typical size-at-age growth trajectory when temperatures return to their seasonal norm (Nicieza and

Metcalfe 1997). However, the long-term consequences of such temperature-induced alterations in growth trajectory have received little attention, although I have shown effects on locomotor performance (Chapter 2).

While the effects of growth trajectory on traits related to health and longevity are reasonably well documented, there is a surprising lack of information on how it influences reproductive performance Obviously, the costs and benefits of reproductive traits depend on the prevailing environmental conditions (Emlen and Oring 1977). Natural selection may favour growth patterns that have long-term costs (for instance in terms of lifespan) if these are more than offset by reproductive benefits (e.g. if larger individuals have greater mate choice or breeding opportunities or rate of offspring production). However, the growth trajectory may itself affect reproductive success, independent from an effect of size by the time of reproduction. For instance, compensatory growth induced by manipulation of food availability has been linked with a reduction in the incidence of sexual maturation (Morgan and Metcalfe 2001) and reduced rate of offspring production (Auer et al. 2010), although in both cases it is possible that the effects were due to the early period of food restriction rather than any acceleration of growth. There is therefore a need for more detailed studies on the effects of juvenile growth on reproduction, including examination of patterns of investment in both sexes. This investment includes secondary sexual colouration as well as offspring production; the relative scheduling of reproductive effort between successive breeding bouts may also be affected, since growth rate may influence the rate of senescence (Valenzano et al. 2006; Inness and Metcalfe 2008) which could then affect changes in reproductive effort with age (Auer et al. 2010).

A further factor that could affect the fitness consequences of variation in growth rate is the time of year. Metcalfe et al. (2002) hypothesized that the amount of time available to restore body size after a period of disturbed growth would influence the degree and rate of compensatory growth: growth acceleration was predicted to be more pronounced when less time was available until a key life history event such as metamorphosis or reproduction (so-called 'time stress'). However, it can also be argued that a given degree of growth acceleration would have more severe consequences when the time stress, since there would be less time to repair any molecular or tissue damage that had occurred as a result of accelerated growth (Metcalfe and Alonso-Alvarez 2010). There is evidence of such effects in three-spined sticklebacks *Gasterosteus aculeatus*: compensatory growth had greater deleterious effects on swimming endurance in fish that underwent the growth acceleration closer to the breeding season (Álvarez and Metcalfe

2005), and my photoperiod manipulation experiments show that this effect is more pronounced when the fish's own perceived time available until breeding is reduced (Chapter 2). This potentially greater cost (as well as benefit) of compensation when the animal is under increased time stress may explain why the rate of compensatory growth in Odonate larvae in response to an earlier period of either food shortage or cool temperatures was not stronger under conditions of time stress (De Block et al. 2008).

The objective of this present study was to investigate by means of experimental manipulations the effect of differing growth trajectories and levels of 'available time' on reproductive investment. By using a seasonally-breeding ectotherm (the three-spined stickleback) I was able to alter growth trajectories and perceptions of time until the breeding season by means of temperature and photoperiod manipulations respectively; the effect of time available from the growth perturbation until breeding was also investigated directly by replicating the experiment in different seasons. This comprehensive series of related treatments has allowed me to investigate for the first time the effect of growth trajectories on diverse aspects of reproductive investment in both sexes, and over multiple breeding seasons. Moreover, by manipulating growth by means of ambient temperature rather than food, and by including decelerating as well as linear and accelerating growth trajectories under differing degrees of time stress, I am able for the first time to evaluate effects of growth trajectory independent of effects of nutrition or final body size. The results clearly demonstrate the strong effects of growth trajectory on reproductive investment.

3.3 METHODS

The fish from the previously described experiments (Chapter 2) were examined during the breeding season. The photoperiod and temperature manipulations were the same as described in Chapter 2, while Period 3 was the first breeding season, which started when the males began to show sexual colouration (=red throat colouration) or female signs of being gravid (16 May 2008 for the Winter experiment and 3 July 2008 for the Spring experiment). Period 4 (non-breeding) began when fish had ceased breeding and Period 5 (second breeding season) began on 6 May 2009 for normal photoperiod fish and 1 June 2009 for those on the delayed photoperiod.

The fish were re-measured for length and mass every 2 weeks during the temperature manipulations and every 3 weeks thereafter; all fish were starved for 24 h prior to measuring to prevent variation in the weight of stomach contents. Tanks were inspected daily in order to monitor mortality rates throughout the experiment; virtually all fish (92% of a total sample size of 240) had died before the third breeding season so this manuscript reports only the results of the first two seasons and the effects of the experimental treatments on lifespan will be covered in Chapter 6.

TABLE **3.1** Description of temperature and photoperiod treatments. Note that following the four week manipulation period (Period 1), all fish were kept at 10°C (Period 2) until the start of the first breeding season (Period 3). Fish were kept at 14°C during both the first and second breeding seasons (Periods 3 and 5 respectively), and at 10°C during the intervening non-breeding season (Period 4). Normal food rations (fed *ad libitum*) were provided throughout.

| Group | Photoperiod manipulation | Temperature manipulation | | | | |
		Period 1	Period 2	Period 3	Period 4	Period 5
HA	Ambient	High (14°C)	10°C	14°C	10°C	14°C
IA		Intermediate (10°C)				
LA		Low (6°C)				
HD	Delayed (35 days)	High (14°C)				
ID		Intermediate (10°C)				
LD		Low (6°C)				

Reproductive investment

The colour of the eye sclera in both male and female sticklebacks changes from silver to blue or blue-green at the onset of the breeding season, while males in most populations also develop a pronounced red throat (Barber et al. 2001). At the start of the first and second breeding seasons (Periods 3 and 5) I moved males that had started to develop blue eye colouration and signs of a red throat to their own individual tank, which was of the same size and arrangement as their group tank, with the addition of a Petri dish containing fine sand (i.e. a nesting dish) and nesting material (50 × 5cm thread). I checked the status of nest building for the first week after placing males in individual tanks and recorded the day when the nest was completed. The rate of nest building was scored from 7 (completed on day 1) to 1 (completed on day 7); males scored 0 if the nest was still not completed on

day 7. Once most males had built nests, each was shown a gravid female enclosed in a Plexiglas container for 5 min twice daily for 4 weeks to prompt full expression of nuptial colouration (Pike et al. 2007). Females were kept in their original group tanks and were stripped of clutches of eggs whenever they became fully gravid. The same procedures were followed in the second breeding season.

At the end of the temperature manipulation (beginning of Period 2; 25 December 2007 for the Winter experiment and 25 March 2008 for the Spring experiment), I began a weekly scoring of eye colouration until at the end of the second breeding season (Period 5) using a 5 point scale from 0 (no blue colouration) to 4 (strong bright blue colouration) (Boughman 2007). All fish were scored by myself in standard lighting conditions against a standard background.

From the onset of the breeding season (Periods 3 and 5) I also took weekly photographs of the red throats of the males. Previous work (Inness and Metcalfe 2008) has shown that the intensity of red throat colouration in this population remains high for about 4 months and then declines sharply, therefore photographs were taken for 16 weeks in each season. The red throat colouration in male was measured using a standardized photography protocol described by Frischknecht (1993). Male sticklebacks were placed within a small tank ($170 \times 70 \times 105$ mm) containing 50 mL water. I covered a white board on the top of the tank as a standard background and photographed the red throat colour area in the ventral area of fish from below the tank using a Panasonic DMC-FX12 Digital camera (3072×2304 pixels, shutter speed 1/2000s, f2.8) with greyscale standards (black, grey, and white). Illumination was provided using two full spectrum daylight bulbs angled at 45° to the tank. In all photographic sessions, the relative position of both lamps and camera was kept constant and the same person photographed all fish on the same day. The time taken from capturing a male in his original tank to taking the photograph took less than 60 s and so would not have influenced the measurement of colour patterns (Laurin and Scott 2009).

Using the measure function in Image J 1.41 software (National Institutes of Health, USA), I selected the red throat colour area based on colour similarities, as described by Barber et al. (2000). The score for redness, greenness, and blueness of the selected area on the fish was obtained using the RGB Measure plug-in. To reduce the effect of variation in light or tone between pictures, all values were then standardized by dividing them by the value of the colour levels obtained for the standard in each picture as described by Inness and Metcalfe (2008). The intensity of red throat colour (R) was calculated using the

equation, $R = red_{STD}/(red_{STD}+green_{STD}+blue_{STD})$ where red_{STD}, $green_{STD}$ and $blue_{STD}$ are the standardized values for the red, green and blue channels (Inness and Metcalfe 2008). High values of R indicate a high proportion of the total image brightness is made up of the red channel (Barber et al. 2000).

I observed females daily and removed any that appeared gravid (i.e. had a grossly distended abdomen) from their tanks. These were stripped of eggs under light anaesthetic (benzocaine) following the protocol of Ali and Wootton (Ali and Wootton 1999). All eggs were collected in a Petri dish and the mass of the clutch recorded. Wootton (1973) has shown that fecundity is related to body size, therefore I recorded the standard length and pre- and post-stripping weights of the female, and then the female was returned to her tank. The total number of eggs (= clutch size) was counted, and the mean diameter of a sample of eggs was measured to 0.1 mm using a dissecting microscope and graticule. The mass of an individual egg was also calculated as the clutch mass divided by the clutch size.

Calculation of compensatory growth rate

In both experiments, compensatory growth rate (% per day) after the temperature manipulation was calculated as: *compensatory growth rate* $= 100 \cdot [ln(L_c \cdot L_i^{-1})] \cdot t^{-1}$ where L_i was the initial length at the end of Period 1 and L_c was the standard length when fish in the different manipulation groups had finished the phase of compensatory growth and had appeared to converge on the same mean size prior to breeding (based on inspection of growth trajectories). t was the interval in days between L_c and L_i, being 105 days in the Winter experiment and 84 days in the Spring experiment.

Statistical analysis

I used multivariate analysis of variance (MANOVA) to test for differences in body length and mass at the beginning of each experiment, before fish had been placed in their treatment tanks, and at the end of the compensatory period. In order to analyze effects of temperature and photoperiod manipulations on compensatory growth rate in both experiments, I used general linear mixed models (GLMM) with temperature (high H, intermediate I or low L) and photoperiod (ambient A or delayed D) as fixed effects and tank (i.e. replicate number, to control for lack of independence among the 5 fish from the

same tank) as a random factor, plus all interactions between variables. The effect of temperature and photoperiod manipulations on breeding ornamentation (blue eye colouration in both sexes, red throat colouration and rate of nest building in males) and breeding investment (individual egg mass and size of 1st clutch in females) were similarly analyzed using GLMM, this time also including age (first or second breeding season) and season (winter or spring) as fixed effects and fish length (manipulated length at the end of Period 1 for analysis of breeding ornamentation and length at time of spawning for analysis of egg investment in females) and compensatory growth rate (over Period 2) as covariates, plus all interactions. Fish identity was also included as a random factor in analyses with repeated measures from the same fish.

The investment in sexual ornamentation in each breeding season was quantified as the duration (in weeks) that fish sustained the intensity of (a) their blue eyes above a threshold, taken to be an eye colour of 3 in males and 2 in females, and (b) their red throats (males only) above a threshold that was the mean red throat colouration of males in the first breeding season.

In all analyses non-significant variables were sequentially dropped so that the final models only included significant terms (or terms that were components of significant interactions). All means are presented with standard errors and all analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, Illinois).

3.4 RESULTS

Compensatory growth rate

At the beginning of both experiments there were no differences in mean standard length or mass in the sticklebacks allocated to the temperature treatment groups (MANOVA, Winter: Wilk's λ=0.954, $F_{4,232}$=1.34, P=0.26, Spring: Wilk's λ=0.994, $F_{4,232}$=0.17, P=0.96) or the photoperiod treatments (Winter: Wilk's λ=0.990, $F_{2,232}$=0.55, P=0.58, Spring: Wilk's λ=0.997, $F_{2,232}$=0.19, P=0.83). The analysis of the Winter experiment showed that, while fish length at the end of the manipulation period (4 weeks, period 1) did not differ between photoperiod treatments (General Linear Mixed Model, $F_{1,19.44}$=0.65, P=0.431) or sexes ($F_{1,79.62}$=0.01, P=0.917), as expected there were significant differences in length among temperature manipulation groups: fish kept at the colder temperatures were significantly shorter than the fish in the other groups ($F_{2,92.83}$=4.27, P=0.017; see also

Chapter 2). There was a positive effect of initial length at the start of the Winter experiment on the length at the end of the manipulation period (=manipulated length) ($F_{1,91.86}$=3261.46, P<0.001), with this effect being greater in the HT treatment (interaction between temperature treatment and initial length: $F_{2,91.89}$=3.14, P=0.048). As with the analysis of length, mass by the end of the manipulation period of the Winter experiment (=manipulated mass) differed significantly between temperature treatments (HT > LT; $F_{2,19.93}$=15.34, P<0.001) but was unaffected by photoperiod ($F_{1,18.38}$=0.17, P=0.684) or sex ($F_{1,87.21}$=1.09, P=0.300). Likewise, manipulated mass was positively related to body mass at the start of the Winter experiment ($F_{1,78.62}$=1032.17, P<0.001).

In the Spring experiment, length at the end of the manipulation period was significantly different among both temperature treatment ($F_{2,15.76}$=70.21, P<0.001) and photoperiod treatment groups ($F_{1,15.93}$=6.47, P=0.022). While there was no effect of sex on manipulation length ($F_{1,90.61}$=2.60, P=0.112), there was a significant temperature by sex interaction ($F_{2,90.44}$=3.87, P=0.024), arising primarily from HT females being larger than other categories. While the manipulated mass was not influenced by photoperiod treatment ($F_{1,90}$<0.01, P=0.954) and did not differ between males and females ($F_{1,90}$=1.67, P=0.200), there was a significant effect of the temperature manipulation ($F_{2,90}$=85.22, P<0.001). Manipulated mass in this experiment was also positively related to initial mass ($F_{1,90}$=1840.84, P=0.022) and there were significant interactions between temperature and photoperiod ($F_{2,90}$=5.94, P=0.004) and between temperature and sex ($F_{2,90}$=3.76, P=0.027).

During Period 2, compensatory growth in length occurred, with accelerated growth in LT fish, decelerated growth in HT and steady growth in IT fish. This led to a convergence in growth trajectories, such that the significant length differences among groups had disappeared after 15 weeks at a common temperature in the Winter experiment and after 12 weeks in the Spring experiment (Winter: Wilk's λ=0.946, $F_{4,182}$=1.29, P=0.277, Spring: Wilk's λ=0.985, $F_{4,188}$=0.37, P=0.83; see Chapter 2 for more details). In both experiments, growth rate during Period 2 (=compensatory growth rate) was thus influenced by the earlier temperature treatment (Table 3.2 and Fig. 3.1), being faster in LT fish than in HT fish. While there was no effect of photoperiod on compensatory growth rate in the Spring experiment (GLMM, $F_{1,94}$=0.93, P=0.339), it had an effect in the Winter experiment (Table 3.2 and Fig. 3.2), with fish under the ambient photoperiod growing faster than those under the delayed photoperiod. Moreover, there was a significant interaction between temperature and photoperiod in the Winter experiment, with the temperature treatment differences in growth rate being much greater under the ambient

than the delayed photoperiod; this interaction was not significant in the Spring experiment (Table 3.2 and Fig. 3.2).

Table 3.2 Analyses of growth rate during the compensation period (Period 2) in relation to temperature and photoperiod treatments. Separate analyses were conducted for the Winter and Spring experiments. The full GLMMs included temperature and photoperiod treatments as fixed effects and tank as a random effect, plus their interaction, but non-significant variables were dropped from the final model.

Experiment	Final model	F	d.f.	P
Winter	Temperature	11.87	2, 17.43	0.001
	Photoperiod	6.89	1, 17.66	0.017
	Temperature × photoperiod	12.12	2, 17.43	0.001
Spring	Temperature	14.98	2, 95	<0.001

Blue eye colouration of males and females

The period over which fish maintained a blue eye colour above the threshold level was longer in the first breeding season (= 2008) than in the second (= 2009), and longer in the Winter experiment than in the Spring experiment (Table 3.3, Figs. 3.2 and 3.3). Analysing the sexes separately, there was a significant effect of both temperature and photoperiod treatments on the duration of blue eye colour in both males and females, with HT temperature and D photoperiod fish maintaining their colouration for longer (Table 3.3, Figs. 3.2 and 3.3). There was no interaction between temperature and photoperiod (General Linear Mixed Model, male: $F_{2,55.54}=1.80$, $P=0.175$; female: $F_{2,90.91}=0.79$, $P=0.455$). In females, the duration of blue colouration was significantly shorter in those fish that had grown most rapidly during the period of compensation (Period 2), but there was no such effect in males (Table 3.3). In both sexes there were significant interactions between age and photoperiod (Table 3.3), with the photoperiod having a greater effect when the fish were older. In males, the season in which the experiment took place had the strongest effect in their first year (when Winter fish had a longer period of colouration that those undergoing compensatory growth in the Spring; Table 3.3 and Fig. 3.2). In females, there were significant interactions between compensatory growth rate and age (Table 3.3),

females with high compensatory growth rates having the shortest period of breeding colouration.
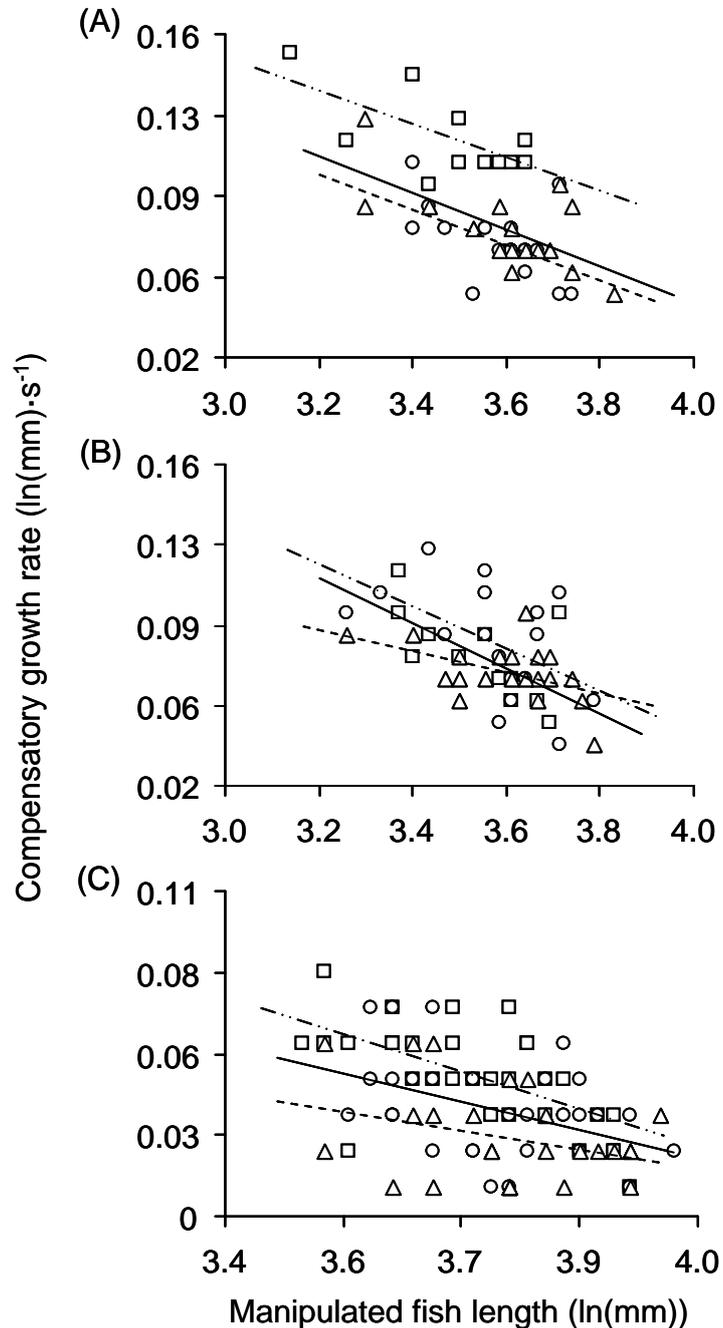
Fig. 3.1 Growth rates (in length) of three-spined sticklebacks (*Gasterosteus aculeatus*) during the compensation period in Period 2 in relation to length at the end of the temperature manipulation (high (14°C) – triangle and dash line, intermediate (10°C) – circle and solid line and low (6°C) – square and double dash line) in (A and B) the Winter experiment and (C) the Spring experiment. In the Winter experiment, data are shown separately for the (A) ambient and (B) delayed photoperiod treatment, but in the Spring experiment these are combined since in that experiment there was no effect of photoperiod treatment on growth. See Table 3.2 for statistical analysis.

TABLE 3.3 General Linear Mixed Model analyses of the duration of blue eye colouration of male and female sticklebacks. The full GLMMs included age (first or second breeding season), season (Winter or Spring experiment), temperature and photoperiod treatment as fixed effects and manipulated fish length (at the end of the temperature manipulation, ln transformed), compensatory growth rate as covariates and tank as a random effect, plus interactions. Non-significant variables were dropped from the final model.

Sex	Final model	F	d.f.	P
Male	Age	90.59	1, 38.71	<0.001
	Season	58.02	1, 61.74	<0.001
	Temperature	7.90	2, 55.83	0.001
	Photoperiod	2.36	1, 64.29	0.130
	Age × season	22.16	1, 38.20	<0.001
	Age × photoperiod	6.47	1, 36.82	0.015
Female	Age	25.55	1, 92.39	<0.001
	Season	5.39	1, 91.90	0.023
	Temperature	17.98	2, 94.73	<0.001
	Photoperiod	13.99	1, 94.70	<0.001
	Compensatory growth rate	2.52	1, 95.94	0.116
	Age × photoperiod	8.18	1, 91.75	0.005
	Age × compensatory growth rate	55.97	1, 97.25	<0.001
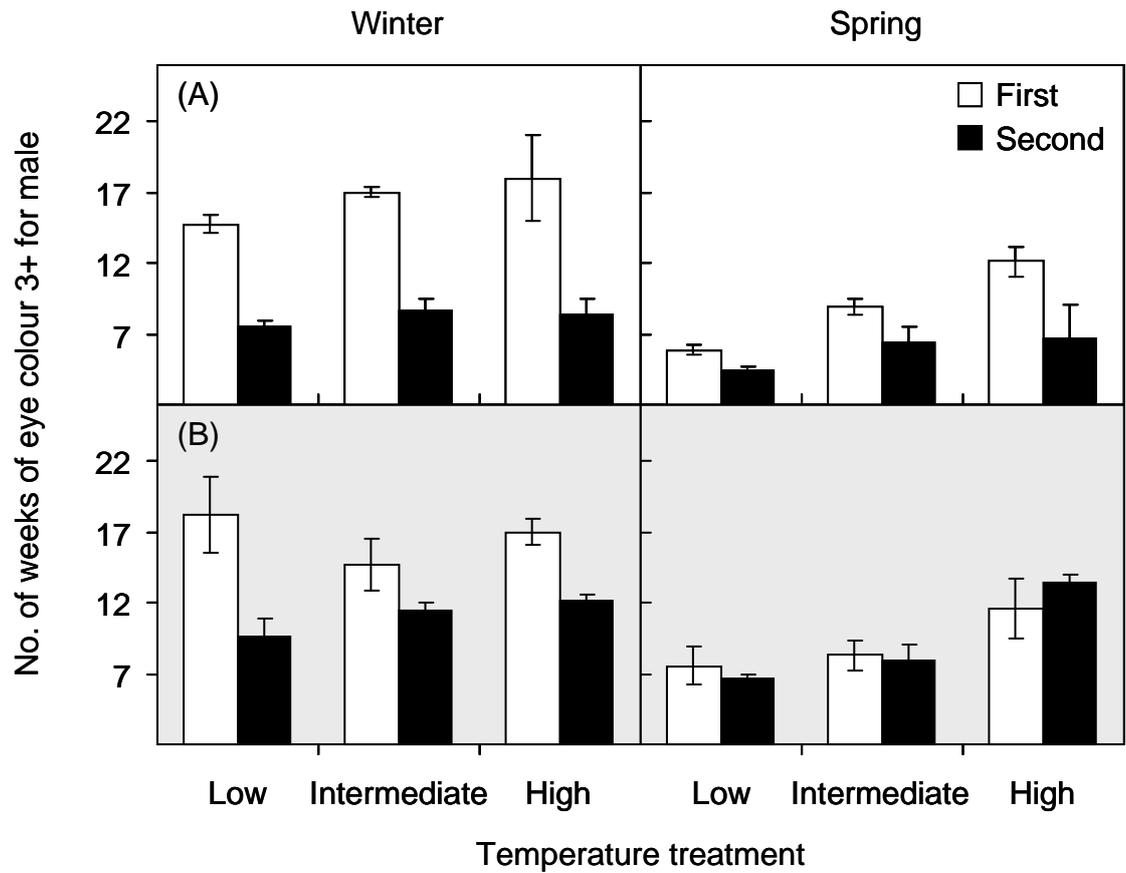
FIG. 3.2 No. of weeks that male three-spined sticklebacks maintained a strong blue eye colour (score 3 or 4) in their first and second breeding seasons, in relation to temperature manipulation (low, intermediate and high) and photoperiod regime ((A) ambient and (B) delayed) in both the Winter (left panels) and Spring (right panels) experiments. Data plotted as means ± SE. See Table 3.3 for statistical analysis.
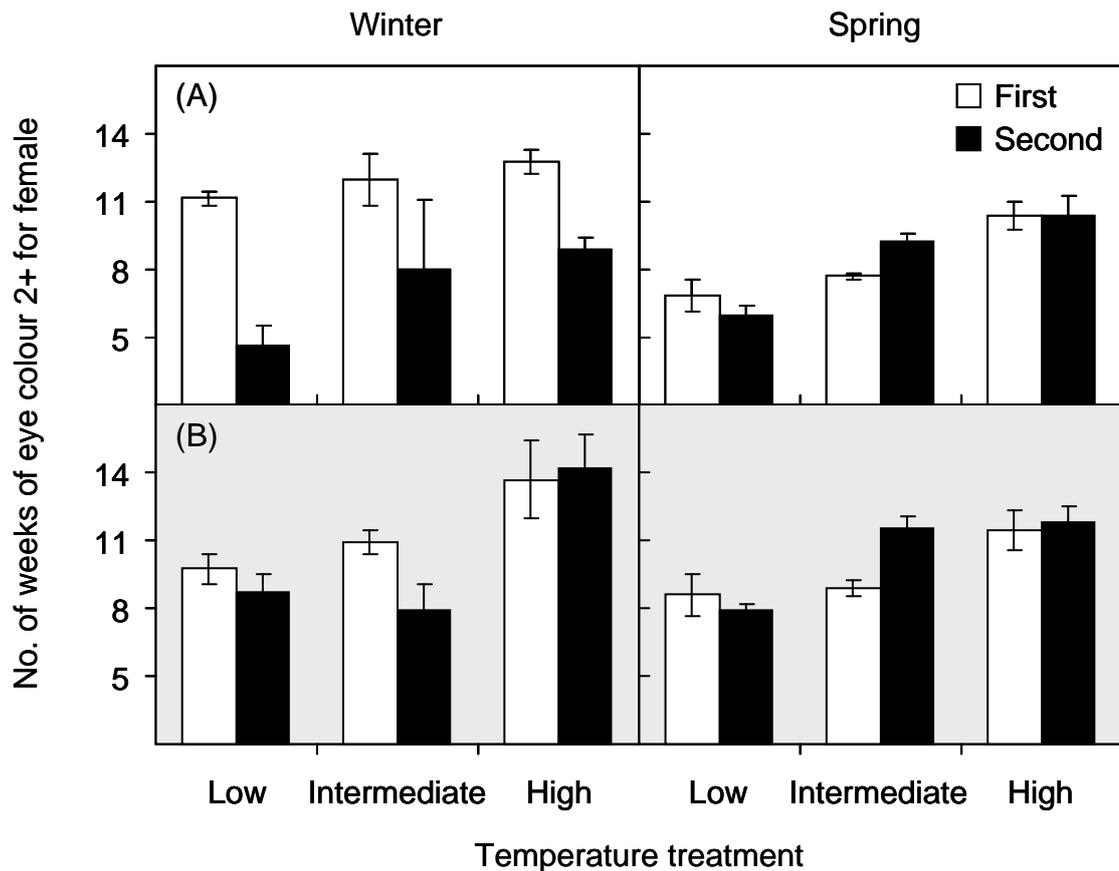
FIG. 3.3 No. of weeks that female three-spined sticklebacks maintained a strong blue eye colour (score over 2) in their first and second breeding seasons, in relation to temperature manipulation (low, intermediate and high) and photoperiod regime ((A) ambient and (B) delayed) in both the Winter (left panels) and Spring (right panels) experiments. Data plotted as means ± SE. See Table 3.3 for statistical analysis.

Red throat colouration of males

Overall, the males showed similar temporal patterns in their red throat intensity as they did in their eye colouration. Males were able to maintain their red throats for longer in their first breeding season than in the second (if they survived that long), and for longer if the period of growth manipulation and subsequent compensation was in the Winter rather than the Spring (Table 3.4 and Fig. 3.4). While the photoperiod manipulation had no direct effect, there was a significant effect of temperature treatment, with HT males maintaining their red throats for longer than LT fish (Table 3.4). There was also an interaction between age and photoperiod (Table 3.4): males under the delayed photoperiod had shorter periods of redness than those under ambient conditions in their first year, but the opposite in their second year. However, there were no effects of fish length at the end of the manipulation

period ($F_{1,61.87}$=0.38, P=0.539), compensatory growth rate ($F_{1,60.18}$=0.01, P=0.918) or other interactions on the duration of red colouration (Table 3.4).

TABLE 3.4 General Linear Mixed Model analyses of the duration of red throat colouration of male sticklebacks in relation to age, season, temperature and photoperiod treatment, manipulated fish length and compensatory growth rate after the 4 weeks of temperature manipulation, plus tank as a random effect. Details of variables as in Table 3.3; non-significant variables were dropped from the final model.

Final model	F	d.f.	P
Age	12.69	1, 60.80	0.001
Season	4.27	1, 63.51	0.043
Temperature	62.44	2, 65.12	<0.001
Photoperiod	0.06	1, 67.68	0.805
Age × photoperiod	22.79	1, 60.96	<0.001

Rate of nest building by males

On average, males completed nests within 2.4±0.2 days of receiving nest material in their first breeding season (Winter: 2.4±0.2 days and Spring: 2.3±0.2 days) and 3.0±0.3 days in their second breeding season (Winter: 2.9±0.2 days and Spring: 3.1±0.3 days). However, there were significant effects of age and season on the time to build nests (Table 3.5): males were faster in their first breeding season than the second, and faster in the Spring than the Winter (Fig. 3.5). The time to completion was also influenced by temperature treatment and the interaction between age and temperature (Table 3.5): HT males built nests faster than LT males, and the effects were stronger in the second breeding season (Fig. 3.5). There was also a significant effect of manipulated fish length, and significant interactions between manipulated fish length and both season and temperature (Table 3.5): fish that were larger at the end of the temperature manipulation built nests faster, particularly in their first breeding season or if they were in the HT treatment group. However, growth rate had no independent effect once the effects of treatment and manipulated fish length were taken into account ($F_{1,75.60}$=0.002, P=0.968).

TABLE 3.5 General Linear Mixed Model analyses of time taken by male sticklebacks to build nests in relation to age, season, temperature and photoperiod treatment, manipulated fish length and compensatory growth rate, plus tank as a random effect. Details of variables as in Table 3.3; non-significant variables were dropped from the final model.

Final model	F	d.f.	P
Age	21.69	1, 55.34	<0.001
Season	9.93	1, 72.96	0.002
Temperature	8.56	2, 71.13	<0.001
Photoperiod	0.08	1, 82.55	0.773
Manipulated fish length	19.62	1, 70.75	<0.001
Age × temperature	10.44	2, 56.75	<0.001
Season × manipulated fish length	10.41	1, 72.70	0.002
Temperature × manipulated fish length	8.38	2, 71.62	0.001
Age × photoperiod	7.06	1, 58.73	0.010
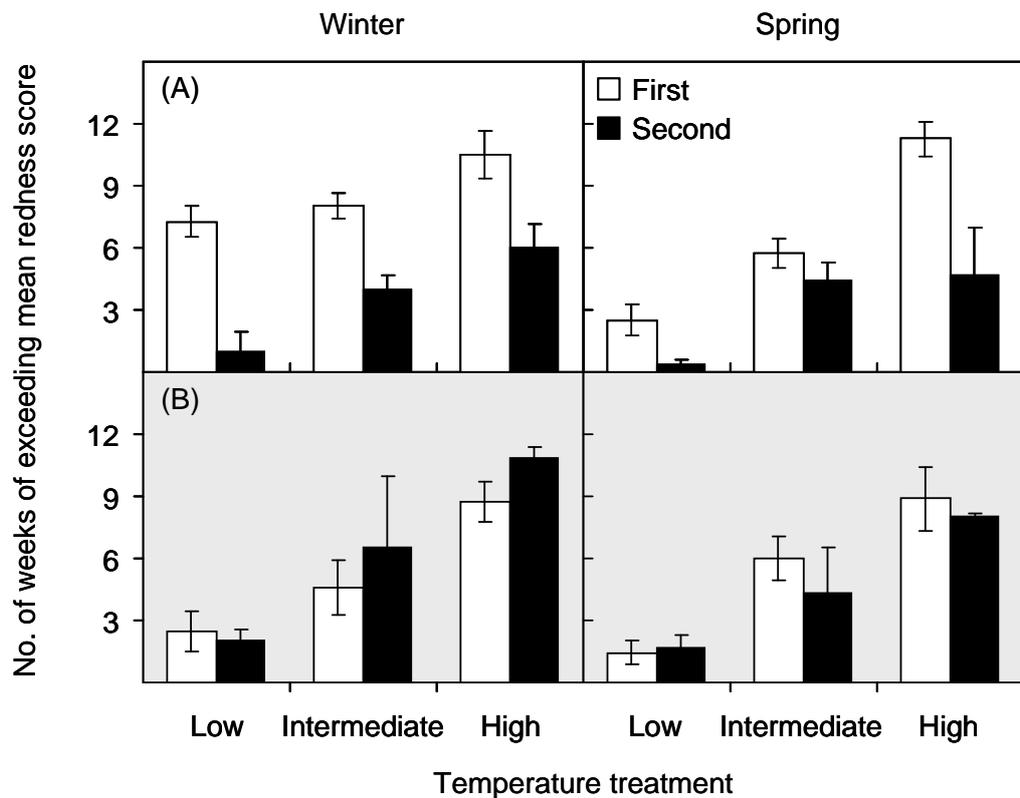
FIG. 3.4 No. of weeks that male three-spined sticklebacks maintained a strong red throat colour (exceeding mean score) in their first and second breeding seasons, in relation to temperature manipulation (low, intermediate and high) and photoperiod regime ((A) ambient and (B) delayed) in both the Winter (left panels) and Spring (right panels) experiments. Data plotted as means ± SE. See Table 3.4 for statistical analysis.
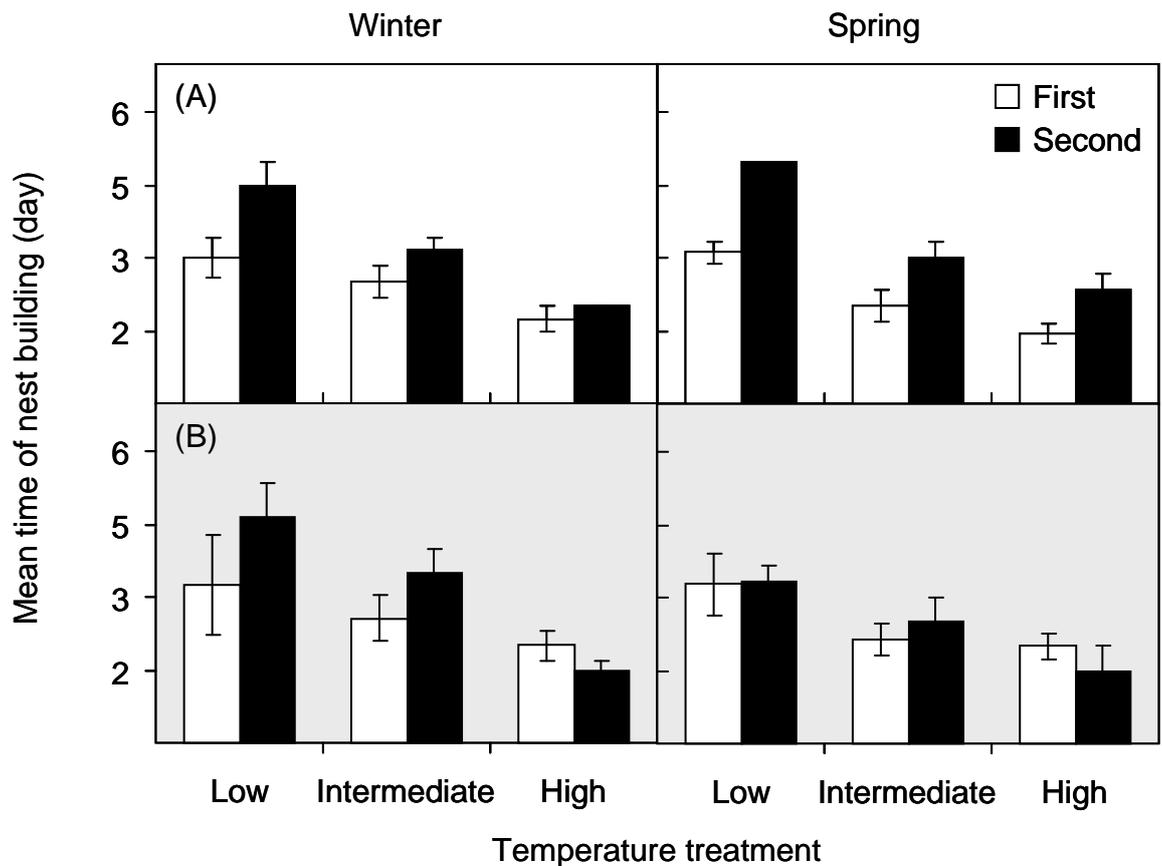
FIG. 3.5 Time taken by male three-spined sticklebacks to build a nest (days, mean ± SE) in relation to temperature (low, intermediate and high) and photoperiod manipulations (A: ambient, B: delayed) in both the Winter (left panels) and Spring experiments (right panels). See Table 3.5 for statistical analysis.

Egg investment by females

A total of 36 and 37 females, in the Winter and Spring experiments respectively, spawned in their first breeding season, but only 17 from the Winter experiment and 20 from the Spring experiment spawned in the second season (38 in the Winter experiment and 42 in the Spring experiment were still alive at that time). Therefore, I only analysed egg investment for the first breeding season, and measurements of individual egg mass and clutch size are only based on the first clutch since the number of clutches varied between females (mean number of clutches per female = 1.24±0.09). The mean mass of an egg was heavier in first clutches from females from the Winter (3.88±0.02mg) than the Spring experiment (3.12±0.02mg; Table 6). There were also effects of temperature treatment (with HT females producing heavier eggs) and photoperiod treatment (eggs in the delayed

photoperiod group being heavier), plus significant interactions between season and temperature (with the lightest eggs being in LT females from the Spring experiment) and between season and photoperiod (eggs in the ambient photoperiod from the Spring experiment being lighter, Table 3.6). There was no significant interaction between temperature and photoperiod ($F_{2,61}$=0.458, P=0.635). The female's body length at the time of spawning had a significant positive effect on the mean mass of her eggs in the Spring but not the Winter experiment (Table 3.6 and Fig. 3.6). Overall, the eggs of LT females were lighter and those of HT females were heaviest (Fig. 3.6).

TABLE 3.6 General Linear Mixed Model analysis of the size of a female's 1st clutch and mean mass of each egg from that clutch, in relation to season, temperature and photoperiod treatment, length at the time of spawning (ln transformed) and compensatory growth rate. Details of the variables as in Table 3.3; tank and fish identity were included as random effects, plus all interactions. Non-significant variables were dropped from the final model.

	Final model	F	d.f.	P
Mass of each egg	Season	103.09	1, 63	<0.001
	Temperature	6.51	2, 63	0.003
	Photoperiod	4.98	1, 63	0.029
	Fish length at spawning	99.55	1, 63	<0.001
	Season × temperature	7.27	2, 63	0.001
	Season × photoperiod	4.46	1, 63	0.039
	Season × fish length at spawning	95.88	1, 63	<0.001
Size of 1st clutch	Season	14.95	1, 66	<0.001
	Temperature	10.05	2, 66	<0.001
	Photoperiod	13.08	1, 66	0.001
	Temperature × photoperiod	3.43	2, 66	0.038

The number of eggs in the first clutch was different between the Winter (71.1±3.6) and Spring experiments (62.9±3.5; Table 3.6). There was also a significant effect of temperature treatment on the number of eggs (Table 3.6), with HT fish spawning more eggs than LT fish (Fig. 3.7). Photoperiod also affected the clutch size (Table 3.6), as

females under the delayed photoperiod produced more eggs (Fig. 3.7). Perhaps surprisingly, there was no effect of female length at the time of spawning on the size of the first clutch ($F_{1,651}$=1.668, P=0.201). The interaction between temperature and photoperiod treatments affected the clutch size, with HT fish under the delayed photoperiod producing the largest first clutches (Table 3.6).
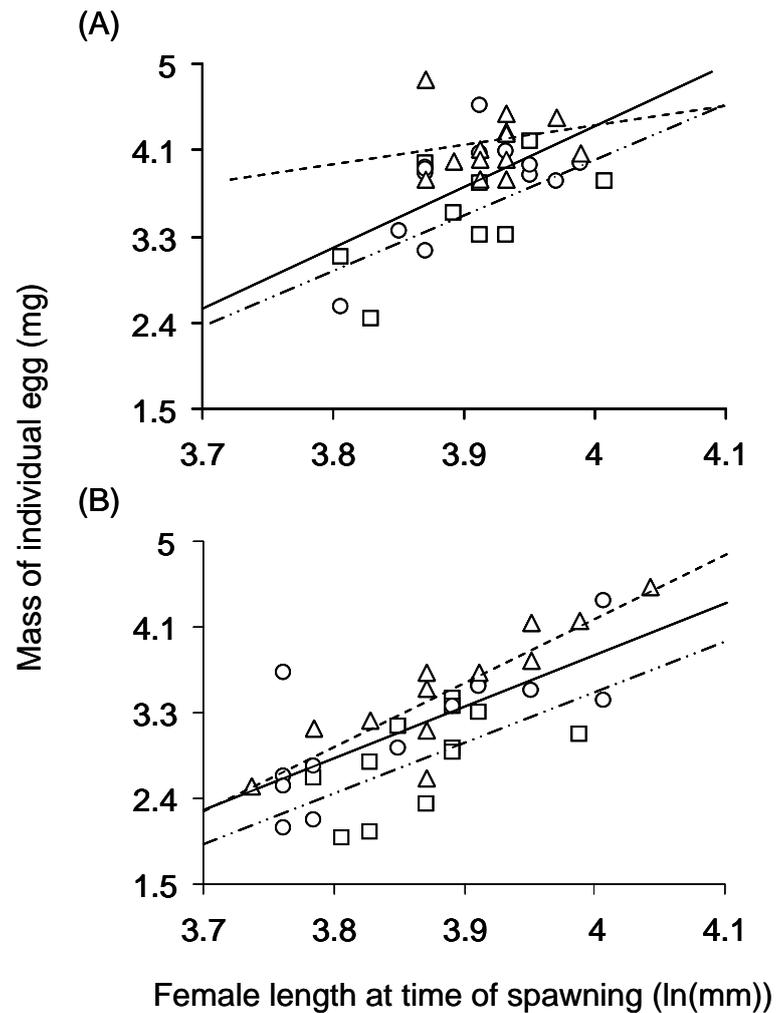
FIG. 3.6 Mean mass of individual eggs from the first clutch (mg, mean ± SE) of female three-spined sticklebacks in relation to their length at time of spawning (mm, ln transformed). Values are plotted for each temperature treatment group (high (14°C) – triangle and dash line, intermediate (10°C) – circle and solid line and low (6°C) – square and double dash line), in the (A) Winter and (B) Spring experiments. See Table 3.6 for statistical analysis.

To examine changes in reproductive investment between seasons, I analysed the effects of the treatments on the proportion of a female's total egg production that she

spawned in the first season (= first season egg proportion). This analysis was restricted to females that were still alive at the time of the second season. There were no effects of temperature or photoperiod treatment on the first season proportion, but an effect of female length at the time of the first breeding season (Table 3.7): large females invested more in that first breeding season than in the second. While there was no effect of the time of the temperature manipulation (Winter or Spring), there was a significant interaction between season and temperature treatment (Table 3.7): in the Winter experiment, the first season egg proportion was higher in LT females than in HT females, but the opposite pattern was evident in the Spring (Fig. 3.8). The first season proportion was affected by compensatory growth rate (Table 3.7), females with high compensatory growth rates producing a greater proportion of their eggs in the first breeding season.

TABLE **3.7** General Linear Mixed Model analysis of the factors influencing the proportion of a female's total egg production (arcsine square root transformed) that she spawned in the first breeding season. Season, temperature and photoperiod treatment were considered as factors, length at time of spawning (ln transformed) and compensatory growth rate as covariates, and tank as a random factor. Non-significant variables were dropped from the final model.

Final model	F	d.f.	P
Season	0.02	1, 69.12	0.878
Temperature	0.20	2, 34.02	0.818
Fish length at spawning	11.65	1, 73.05	0.001
Compensatory growth rate	4.00	1, 77.50	0.023
Season × temperature	4.30	2, 30.17	0.049
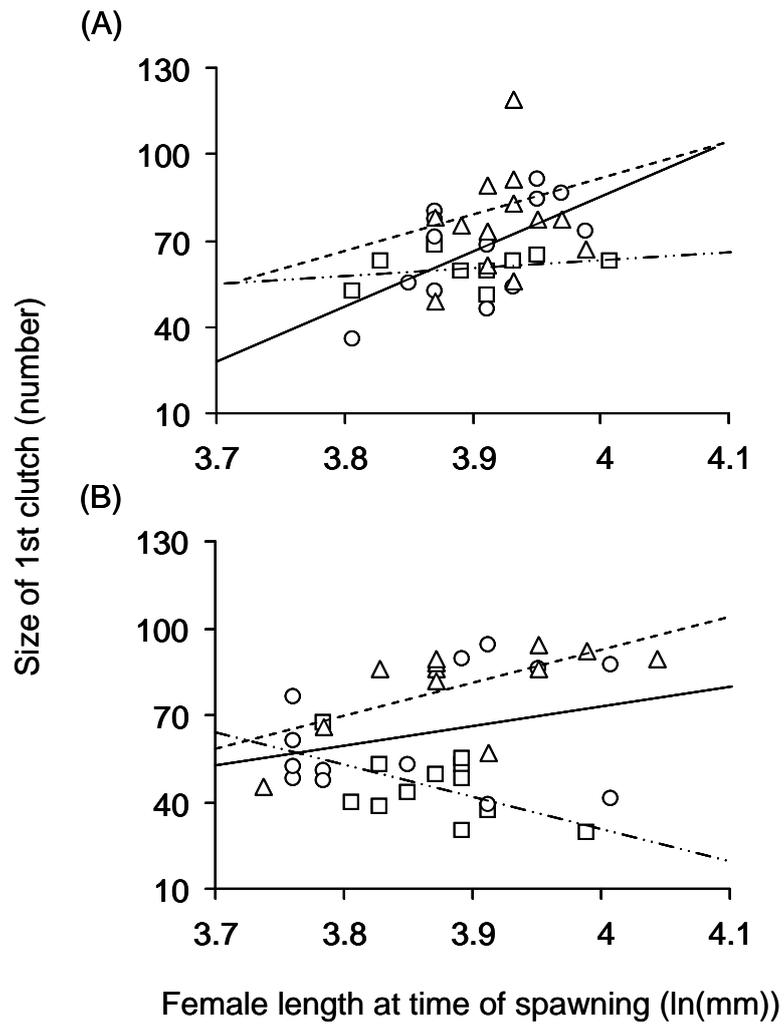
FIG. 3.7 The size of a female's first clutch in relation to her length at the time of spawning (mm, ln transformed). Values are plotted for each temperature treatment group (high (14°C) – triangle and dash line, intermediate (10°C) – circle and solid line and low (6°C) – square and double dash line), in the (A) Winter and (B) Spring experiments. See Table 3.6 for statistical analysis.
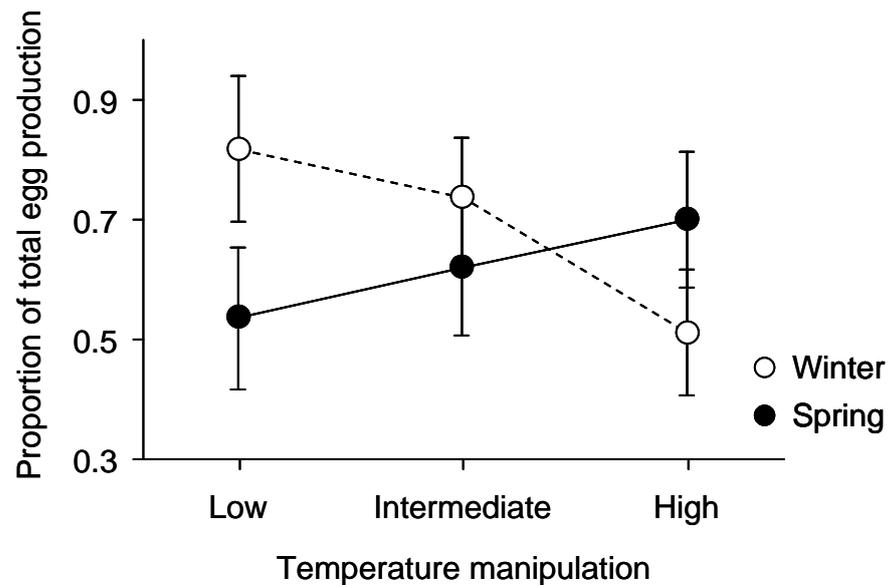
FIG. 3.8 The proportion of a female's total egg production (over two years) that she laid during the first breeding season, in relation to temperature treatment (low, intermediate and high) in both the Winter and Spring experiments. Data plotted as means ± SE; see Table 3.7 for statistical analysis.

3.5 DISCUSSION

While the temperature manipulations during period 1 (4 weeks) gave rise to a slowing of growth in the low temperature fish, and an increase in the higher temperature fish, relative to the intermediate temperature group, there was no effect of photoperiod treatment. Accordingly, by the end of the manipulation period, the fish differed in size. This episode of reduced or accelerated growth was then followed by a compensatory growth trajectory, resulting in growth acceleration and deceleration depending on whether the fish had been exposed to low or high temperatures during period 1. The time until the onset of breeding had an effect only in the Winter experiment, moderating the rate of the compensatory adjustment.

Although the negative effects of accelerated compensatory growth on reproduction have previously been documented in fish after periods of food deprivation (Morgan and Metcalfe 2001; Auer et al. 2010), this study is the first to report such effects without any food restriction. I found that growth rate in early life influenced long-term reproductive investment in both sexes and over multiple breeding seasons. Moreover the different amount of time available until breeding modified the effects of early growth trajectory on reproductive investment.

Temperature, which directly affects growth rate in ectotherms, can exert its effects through changes in physiology and resource allocation (Weatherley and Gill 1987; Gillooly et al. 2001; Morgan and Metcalfe 2001; Charnov and Gillooly 2003). For instance, accelerated growth due to increases in temperature can have negative effects on muscle cellularity and development (Galloway et al. 1999; Johnston et al. 2002), which may explain the consequent reduction in locomotor performance (Chapter 2). Alternatively, resource allocation patterns may be indirectly influenced by growth tempo. Hyperphagia in juvenile brown flounder *Paralichthys olivaceus* was induced when temperature were increased (Huang et al. 2008) which may lead to increased levels of cellular damage. High levels of damage as a result of rapid growth (e.g. oxidative stress level; De Block and Stoks 2008) may have negative consequences for future reproductive investment. Pike et al. (2007) showed that a lowered dietary intake of antioxidants results in a reduced investment in breeding ornamentation due to the high level of oxidative stress. Conversely, I have shown that the decline in locomotory performance over the breeding season is less in fish whose growth has decelerated (see Chapter 2). This suggests that damage is reduced when growth is slowed. Hence changes in metabolism associated with fluctuations in early growth rate may affect the accumulation of physiological damage (= stress) and hence the performance of somatic and reproductive structures (e.g. accelerated growth rate negatively affects the development of somatic structure, Ricklefs et al. 1994; Arendt 2003). Conversely, decelerated growth rate may induce a reduction in metabolic costs and accumulated damage and so give rise to a positive effect on reproductive investment due to the reduced requirement for investment in repair.

Reproductive timing is sensitive to environmental conditions (e.g. temperature, photoperiod and season). In particular the decision to begin breeding in ectotherms is directly affected by temperature and photoperiod (Wootton 1976; Weatherley and Gill 1987). Natural selection may favour life-history strategies that result in body condition and size being aligned to the timing of breeding in order to maximise fitness. These findings support the time-stress hypothesis that the perceived amount of time available until breeding season influences the rate of compensatory growth (Metcalfe et al. 2002). Effects of compensatory growth on reproductive investment (= duration of nuptial colouration, egg mass and number) were affected by photoperiod and time of the season: effects were less if the time apparently available was greater (being maximal in the delayed photoperiod treatment in the Winter experiment, resulting in reduced time-stress), but were increased if the time was apparently short (i.e. under normal photoperiod in the Spring experiment,

resulting in increased time-stress). I found similar effects of time-stress on locomotor performance – see Chapter 2.

It is well known that fitness components (e.g. reproductive success and lifespan) tend to decline with age once animals reach their middle adulthood, possibly as a consequence of damage accumulation, and also that the rate of decline with age may be affected by environmental conditions and the accumulated damage level, but that this may vary between individuals. For instance, in the Alpine Swift *Apus melba*, males that survived to the next breeding season tended to be more resistant to oxidative stress, and females with higher resistance to oxidative stress laid larger clutches (Bize et al. 2006). I found that the reproductive investment in the second breeding season tended on average to be less than in the first breeding season; interestingly the magnitude of the difference between the two was affected by both compensatory growth rate and the time period over which growth adjustment occurred (i.e. time stress): in the Winter experiment, when the time stress was less pronounced, accelerated growth was associated with a greater concentrated investment in the first breeding season, whereas in the Spring experiment (= greater time stress) females in the low temperature group showed a greater tendency to spread their reproductive investment over two breeding seasons whereas female from the high temperature group (who might be expected to have lower levels of damage) invested more in the first breeding season. Therefore I suggest that the interaction between growth rate and the degree of time stress prior to the breeding season should affect the rate of reproductive senescence. The effects on lifespan will be presented in Chapter 6.

In conclusion, early environmental conditions affected growth rate in early life and this was associated with long-term effects on reproductive investment in both sexes and over multiple breeding seasons. Moreover, the time available until the onset of the breeding season (= time stress) influenced the degree of change in growth trajectories and hence the magnitude of the effects on reproductive investment. Further study is needed to determine how compensatory growth rate influences metabolism and damage accumulation, and how thermal stress incurred by early environmental conditions affects growth and fitness in the next generation.

CHAPTER 4

CHANGES IN GROWTH RATE INDUCED BY EARLY DIET INCUR COSTS OVER MULTIPLE TIME SCALES

4.1 ABSTRACT

Early dietary conditions influence the tempo and pattern of growth and development. Whilst an episode of poor dietary conditions generally causes slower growth, normal adult size can still be reached if growth accelerates once conditions improve. However, it is known that such compensatory growth may have deleterious effects later in life. Using juvenile three-spined sticklebacks *Gasterosteus aculeatus*, I show that a brief period of food restriction imposed in early life slowed skeletal growth, with accelerated growth occurring when normal diet was resumed. Compensatory growth reduced pre-breeding swimming endurance and increased the decline in swimming endurance that occurs over the first breeding season. Reproductive investment (males: sexual ornaments and ability to build nests; females: first clutch size and mean egg size) was also negatively affected by compensatory growth. The magnitude of these effects was influenced by the perceived time until breeding. These results show that the costs of accelerated growth can last well beyond the period over which growth rates differ.

4.2 INTRODUCTION

It is obvious that diet (or nutritional condition) in animals continually affects life-history traits associated with growth, behaviour and reproduction. An imbalanced diet or poor nutrition can suppress growth and can have negative consequences later in life, although the fitness consequences are not always clear (Masoro 2005; Piper et al. 2005). For instance, although a diet that is supplied at less than the *ad libitum* amount can extend lifespan, the slower growth rate and development caused by this decreased diet can lead to delayed maturity at a smaller size and to an increased risk of predation (Roff 2002). Conversely, there may be costs associated with rapid growth (Metcalfe et al. 2002;

Metcalfe and Monaghan 2003). Natural selection will tend to favour growth rates that take account of these trade-offs and maximise long-term fitness, but these growth rates may be flexible to take account of current resource availability.

Compensatory growth is a well-known strategic adjustment that occurs when growth rate is accelerated upon re-feeding after a period of food restriction or starvation; if complete it results in normal adult size still being attained despite the earlier set-back (Arendt 1997). In populations that experience high rates of juvenile predation, this form of accelerated growth can increase survival (Arendt 1997; Sogard 1997). Moreover, larger individuals may have a greater competitive ability (Johnsson 1993) and an earlier age of maturation (Rowe and Thorpe 1990). Despite these benefits of compensatory growth, previous work has demonstrated that growth acceleration may also have negative effects in later life. The hyperphagic response needed for increased growth after food restriction manipulation could increase the risk of predation while foraging (Ali and Wootton 2000; De Block and Stoks 2008), while the physiological process of growth acceleration may cause increased cellular damage and metabolic costs which could, for instance, reduce future lifespan or reproductive capacity (Metcalfe and Alonso-Alvarez 2010). It has recently been documented that compensatory growth in fish induced by earlier food restriction causes a reduced ability to swim against fast flowing water (Álvarez and Metcalfe 2005) and reduced lifespan (Inness and Metcalfe 2008), plus effects on reproductive output (Auer et al. 2010). However, while several studies have documented the existence of a compensatory growth response during early life in fish (reviewed by Ali et al. 2003), there has been surprisingly little effort to study the interactions between environmental conditions and both the extent of growth compensation and its long-term effects.

Seasonal variations in factors such as temperature or photoperiod may affect several physiological and ecological processes in organisms (e.g. development, growth and reproduction) and so the perception of time of year is a further factor that could affect an animal's optimal growth and resource allocation strategy so as to maximise its fitness. Metcalfe et al. (2002) hypothesized that the amount of time available to catch up after a period of poor growth would influence compensatory growth rates: less time available until a key event such as breeding might result in increased pressure for growth acceleration (so-called 'time stress'). In these situations, however, why should animals opt to accelerate their growth as opposed to growing normally and breeding at a smaller size and/or continuing to grow through the breeding season? It has been shown that an increased body

size has reproductive benefits for both sexes, in terms of mate choice (Howard et al. 1998), which is important at the beginning of the breeding season. Moreover, rapid somatic growth prior to the breeding season would allow more time for gonad growth and hence fecundity or sperm production. Recently Metcalfe and Alonso-Alvarez (2010) argued that the extent of growth acceleration should be flexible under time stress since a reduced time available prior to a life history event such as reproduction would affect the ability of the animal to repair any molecular or tissue damage that had occurred as a result of the accelerated growth. Furthermore, while De Block et al. (2008) showed that compensatory growth was apparently unaffected by perceived time stress, Álvarez and Metcalfe (2005) found that compensatory growth caused a greater decrease in swimming performance in three-spined sticklebacks *Gasterosteus aculeatus* when this occurred close to the breeding season, and my work using temperature manipulations to alter growth showed that this cost was increased when the fish perceived a greater time stress due to photoperiod manipulation (Chapters 2 and 3).

The aim of the present study was to investigate how compensatory growth induced by poor early diet affects swimming endurance (=short-term consequence) and reproductive investment (=long-term consequence), and how the extent of the negative effects of compensatory growth are related to the level of time stress. Using three-spined sticklebacks, I altered growth trajectories by periods of dietary restriction, and the perceived time available until the breeding season by both running the same experiment in different seasons and by photoperiod manipulation. This experimental design has allowed me to investigate the effect of compensatory growth on locomotor and reproductive investment in both sexes and over multiple breeding seasons. While the negative effects of compensatory growth in sticklebacks have already been documented for some life-history traits, my approach has been to investigate in more detail and over longer time periods so as to produce a more complete analysis of the effects of compensatory growth on life-histories.

4.3 METHODS

The fish from the previously described experiments were examined during their development and in the breeding season. The methods for swimming and reproductive performance were described in Chapter 2 and 3. There treatment groups are described in Table 4.1; the same photoperiod treatment was applied as described previously.

Diet manipulation

Four replicate tanks of five fish were assigned randomly to each manipulation, defined in relation to dietary regime: restricted (R group, fed 2% of body mass) and control (C group, fed *ad libitum*). The diet of 2% of body mass was chosen since this has earlier been found to produce reduced growth at 10°C (Allen and Wootton 1984). At the end of the four week manipulation period (Period 1), all fish were returned to an *ad libitum* diet for the rest of the experiment. The temperature was held at 10°C during Periods 1 (manipulation) and 2 (compensation), but was raised to 14°C during each breeding season (defined as Period 3 for the first breeding season, starting on 16 May 2008 for the Winter and 3 July 2008 for the Spring experiment, and Period 5 for the second breeding season, which started on 6 May 2009 for the normal photoperiod treatment (see below) and 1 June 2009 for the delayed photoperiod treatment). Period 4 was the non-breeding phase between the first and second breeding seasons, when the temperature was again reduced to 10°C (Table 4.1) The start of the breeding season was fixed by the time when males had started to develop their breeding colouration (=reddish throats) and females to become gravid (see Chapter 3 for further details).

I re-measured the fish for length and mass every 2 weeks during the dietary manipulation period and every 3 weeks thereafter; all fish were starved for 24 h prior to measuring to prevent variation in the weight of stomach contents. Tanks were inspected daily in order to monitor mortality rates throughout the experiment.

TABLE 4.1 Description of experimental manipulations. Note that during Period 1 Restricted (R) fish were fed a restricted diet (2% of body mass) and Control (C) fish were fed *ad libitum*. After Period 1, all fish were fed *ad libitum*. Temperature was held at 10°C during Periods 1, 2 and 4, but was increased to 14 °C during the breeding periods in 2008 and 2009 (Period 3 and 5).

| Group | Food manipulation | | Photoperiod manipulation |
	Period 1	Period 2 to 5	
R Ambient	Restricted (2% of body mass)		Ambient
C Ambient	*Ad libitum* food ration	*Ad libitum* food	
R Delayed	Restricted (2% of body mass)		Delayed (35 days)
C Delayed	*Ad libitum* food ration		

Statistical analysis

To test for differences in body length and mass, multivariate analysis of variance (MANOVA) was used at the beginning of each experiment, before fish had been allocated to their treatment tanks, and at the end of the compensatory period. I used a general linear mixed model (GLMM) in order to analyze the effects of the dietary and photoperiod manipulations on compensatory growth rate (see detailed description in Chapter 5) in both experiments, with season (Winter or Spring), diet (restricted or control), photoperiod (ambient or delayed), sex (male or female) as fixed effects, fish length (manipulated length at the end of Period 1 for analysis of compensatory growth or breeding ornamentation, but length at time of first swimming test for analysis of swimming endurance, and length at time of spawning for analysis of egg investment in females) as a covariate and tank as a random effect, plus all interactions. The effects of diet and photoperiod manipulations on swimming and breeding performance were analyzed using a GLMM with age (first or second breeding season), season, diet, photoperiod, and sex as fixed effects, tank as a random factor and fish length (both at the end of the dietary manipulation period and at the time of spawning) and compensatory growth rate as covariates, plus all interactions. Fish identity was also included as a random factor in analyses with repeated measures from the same fish. I quantified the reproductive investment of both sexes in each breeding season as the duration (in weeks) that fish sustained the intensity of (a) their blue eyes above a threshold, taken to be an eye colour of 3 in males and 2 in females, and (b) their red throats (males only) above a threshold that was the mean red throat colouration of males in the first breeding season.

In all analyses non-significant variables were sequentially dropped so that the final models only included significant terms (or terms that were components of significant interactions). All means are presented with standard errors and all analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, Illinois).

4.4 RESULTS

Compensatory growth rate

At the beginning of both experiments there was no difference in the mean standard length or mass of sticklebacks allocated to the two dietary manipulation groups (MANOVA, Winter: Wilks' λ=0.987, $F_{2,77}$=0.52, P=0.132, Spring: Wilks' λ=0.988, $F_{2,77}$=0.46,

$P=0.636$) or the photoperiod treatments (Winter: Wilks' $\lambda=0.999$, $F_{2,77}=0.03$, $P=0.975$, Spring: Wilks' $\lambda=0.999$, $F_{2,77}=0.01$, $P=0.995$). In the Winter experiment, the mean standard length of R fish at the end of the manipulation period was significantly smaller than that of C fish (General Linear Mixed Model, $F_{1,12.69}=6.08$, $P=0.029$), while fish length did not differ between photoperiod treatments ($F_{1,11.68}=0.06$, $P=0.806$) or sexes ($F_{1,49.36}=0.41$, $P=0.523$). There was a positive effect of initial length at the start of the Winter experiment on the length at the end of the manipulation period (= manipulated length) ($F_{1,46.59}=123.89$, $P<0.001$). As with the analysis of length, mean wet mass of R fish at the end of the manipulation period of the Winter experiment (= manipulated mass) was significantly lighter than that of C fish ($F_{1,9.17}=109.01$, $P<0.001$), while there was no effect of photoperiod treatment on the mass ($F_{1,8.84}=0.64$, $P=0.445$). While manipulated mass did not different between sexes ($F_{1,56.37}=0.76$, $P=0.386$), there was a significant interaction between dietary regime and sex ($F_{1,57.85}=7.25$, $P=0.009$): males were lighter in the restricted diet regime group, but were heavier in the control groups. As with fish length, manipulated mass was positively related to body mass at the beginning of the Winter experiment ($F_{1,51.18}=521.64$, $P<0.001$).

The analysis of the Spring experiment showed that manipulated length was significantly different between not only the dietary treatment groups ($F_{1,12.95}=108.73$, $P<0.001$) but also the photoperiod treatments ($F_{1,12.94}=6.76$, $P=0.022$), with fish being larger under the ambient photoperiod. However, there was no difference between the sexes ($F_{1,67.09}=0.42$, $P=0.521$). Manipulated length was positively related to initial length ($F_{1,64.36}=5238.98$, $P<0.001$). While the manipulated mass was unaffected by photoperiod treatment ($F_{1,10.86}=1.86$, $P=0.200$) and did not differ between males and females ($F_{1,66.87}=0.66$, $P=0.420$), as expected there was a significant effect of the dietary manipulation ($F_{1,12.66}=263.72$, $P<0.001$). Manipulated mass in this experiment was also positively related to initial mass ($F_{1,62.37}=1919.56$, $P<0.001$).

When again given food *ad lib.*, R fish grew rapidly so that after 15 weeks in the Winter experiment and 12 weeks in the Spring experiment the differences in length and mass between R and C fish were no longer significant (Winter: Wilks' $\lambda=0.937$, $F_{2,64}=2.16$, $P=0.123$, Spring: Wilks' $\lambda=0.978$, $F_{2,69}=0.79$, $P=0.458$), nor were there differences between photoperiod groups (Winter: Wilks' $\lambda=0.996$, $F_{2,64}=0.12$, $P=0.885$, Spring: Wilks' $\lambda=0.968$, $F_{2,69}=1.14$, $P=0.325$).

Compensatory growth rates (= growth rate during the compensatory period) were significantly higher in the Winter experiment than in the Spring experiment (Table 4.2 and Fig. 4.1). While there was no effect of photoperiod on compensatory growth rate (GLMM, $F_{1,121.34}$=0.11, P=0.743), it was affected by dietary treatment and manipulated fish length (= length at the end of Period 1), the growth of R fish and of smaller fish being greatest (Table 4.2, Fig. 4.1). There was also a significant interaction between season and dietary regime (Table 4.2), with the growth rate of R fish in the Winter experiment being the fastest (Fig. 4.1).

TABLE 4.2 Growth rate during the compensation period in relation to dietary and photoperiod treatments in the Winter and Spring experiments. The full General Linear Mixed Model (GLMM) included season (Winter or Spring), dietary (restricted or control) and photoperiod (ambient or delayed) treatments as fixed effects, manipulated fish length (at the end of Period 1) as a covariate and tank as a random effect, plus interactions among variables. Non-significant variables were dropped from the final model.

Final model	F	d.f.	P
Season	19.42	1, 113.26	<0.001
Diet	363.77	1, 87.95	<0.001
Manipulated fish length	52.05	1, 123.60	<0.001
Season × diet	42.31	1, 88.09	<0.001
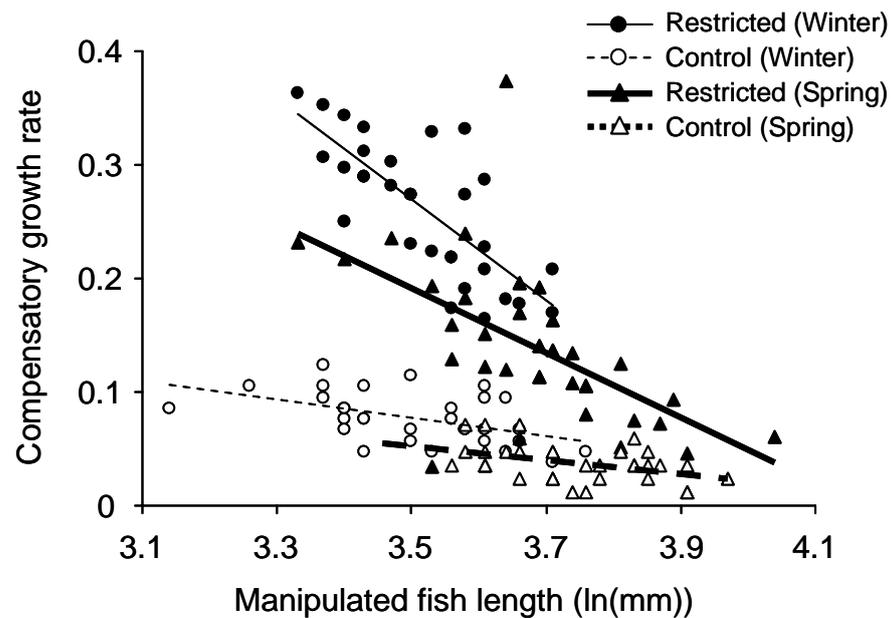
FIG. 4.1 Compensatory growth rate (i.e. growth rate in length during the compensatory period – see text) of three-spined sticklebacks in relation to their length at the end of the period of dietary manipulation (manipulated fish length, ln transformed). Data are plotted separately for the restricted diet group (black symbols and dashed line) and control group (white and solid line) in both experiments (Winter – thin line, and Spring – thick line).

Swimming performance

Swimming endurance prior to the first breeding season was not significantly different between fish from the Winter and Spring experiments (GLMM, $F_{1,25.23}=0.93$, $P=0.343$) nor between photoperiod treatment group ($F_{1,23.52}=3.68$, $P=0.067$). However, the endurance of R fish at this time was significantly lower than that of C fish ($F_{1,25.22}=18.07$, $P<0.001$). There was also an effect of fish length at time of first swimming test ($F_{1,118.89}=123.36$, $P<0.001$): the larger the length at the end of the dietary manipulation period, the greater the swimming endurance (Fig. 4.2a).
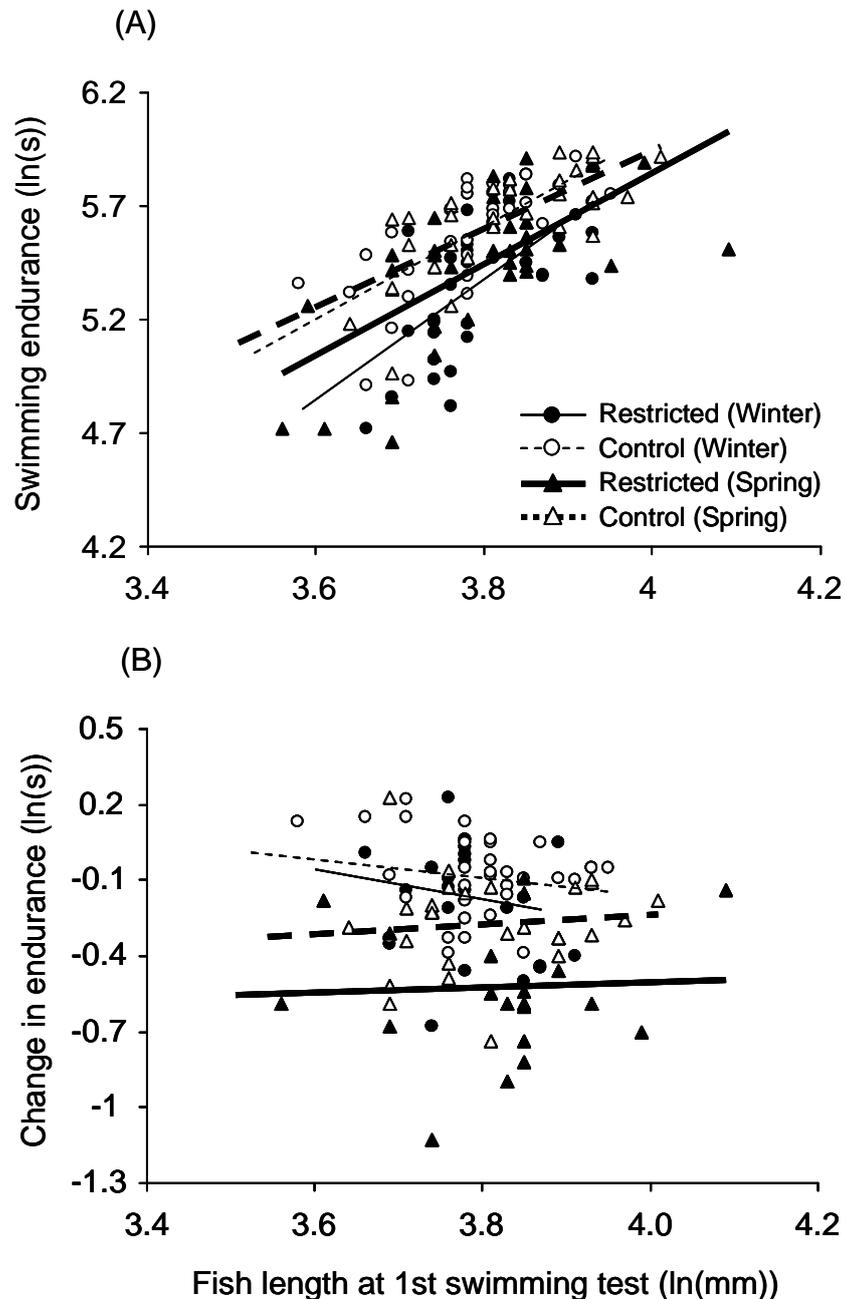
Fig. 4.2 Effects of dietary treatment on swimming performance in three-spined sticklebacks: (A) swimming endurance (ln(s)) at the end of the compensatory period in relation to fish length at time of first swimming test (ln(mm)) and (B) change in swimming endurance (ln(s)) over the breeding season in relation to fish length at time of first swimming test. Data are plotted according to diet treatment and experiment as in Fig. 4.1

The change in swimming endurance over the breeding season was greater in the Spring than the Winter experiment ($F_{1,22.40}$=21.95, P<0.001). There were significant effects of dietary treatment on the change in swimming endurance ($F_{1,22.48}$=9.41, P=0.006), with R fish showing a greater decline in endurance over the breeding season than C fish (Fig. 4.2b) whereas the change was not affected by photoperiod ($F_{1,21.81}$=0.79, P=0.384) or sex

($F_{1,92.42}=0.01$, $P=0.918$). There was no effect of fish length at time of first swimming test on the change ($F_{1,86.97}=0.21$, $P=0.645$).

TABLE 4.3 Mixed model analyses of blue eye colouration of male and female sticklebacks in relation to age (first or second breeding season), season (Winter or Spring experiment), dietary and photoperiod treatment, manipulated fish length (at the end of the dietary manipulation, ln transformed) and compensatory growth rate after the 4 weeks of dietary manipulation. The full GLMM included age, season, diet and photoperiod as fixed effects, manipulated fish length and compensatory growth rate as covariates and tank as a random effect, plus interactions. Non-significant variables were dropped from the final model.

	Final model	F	d.f.	P
Male	Age	248.23	1, 30.83	<0.001
	Season	20.82	1, 42.48	<0.001
	Diet	66.87	1, 53.37	<0.001
	Photoperiod	3.43	1, 45.00	0.071
	Compensatory growth rate	4.40	1, 57.72	0.040
	Season × photoperiod	19.10	1, 44.16	<0.001
Female	Age	164.41	1, 46.44	<0.001
	Season	3.69	1, 52.04	0.060
	Diet	7.52	1, 91.67	0.007
	Compensatory growth rate	6.04	1, 85.56	0.016
	Age × season	23.20	1, 45.79	<0.001
	Diet × compensatory growth rate	4.34	1, 94.94	0.040

Blue eye colouration of males and females

The period over which both sexes maintained their blue eye colour was longer in the first breeding season (= 2008) than in the second (= 2009), but in males there was also a significant effect of season of experiment, with the duration of blue eye colouration being longer in males from the Winter than the Spring experiment (there being no such effect in females; Table 4.3, Figs. 4.3 and 4.4). There was also a significant effect of dietary manipulation, with R fish of both sexes maintaining their blue eye colouration for a shorter

duration (Table 4.3, Figs. 4.3 and 4.4), whereas the photoperiod manipulation had no effect in either males (Table 4.3) or females ($F_{1,44.85}$=0.90, P=0.348). There was however an interaction between season of experiment and photoperiod in males (Table 4.3), with delayed photoperiod males in the Winter experiment maintaining their colouration for the shortest period of time. In females, there was a significant interaction between age and season (Table 4.3), with the duration of blue eye colour being shortest in Spring females in the second breeding season. The duration of blue eye colour was negatively affected by compensatory growth rate in both sexes (Table 4.3): the faster the compensatory growth rate (independent of diet treatment), the shorter the duration of blue eye colouration. In females there was also a slight effect of an interaction between dietary and compensatory growth (Table 4.3), with R females that had grown rapidly maintaining their blue eyes for shorter.
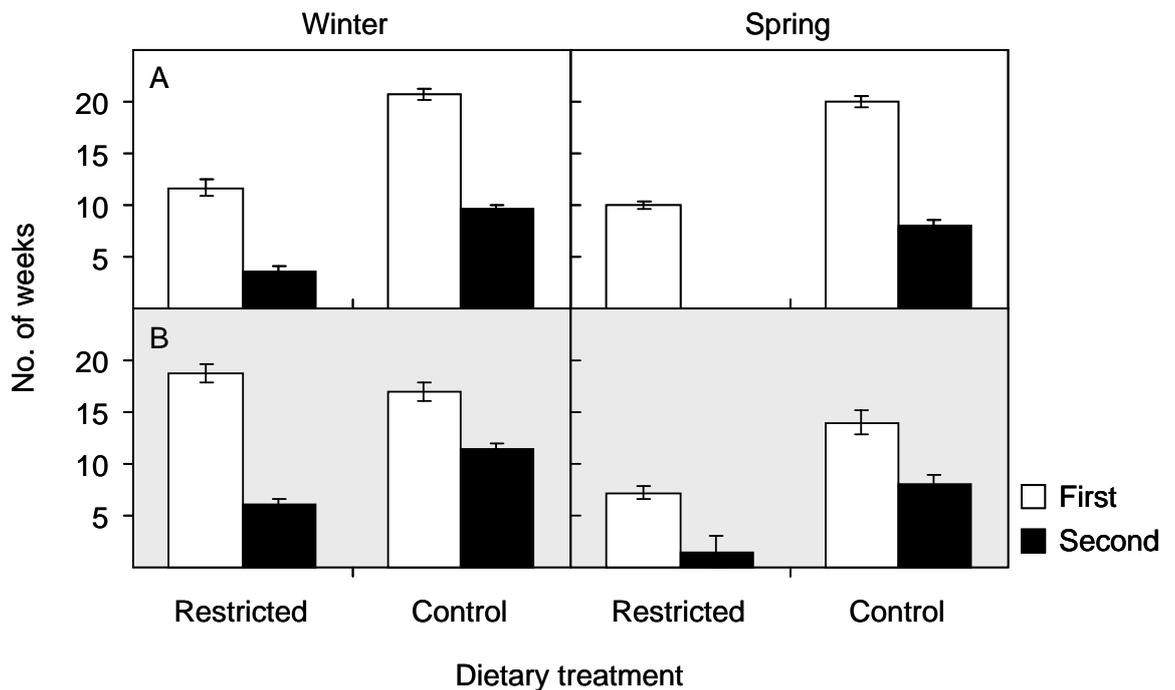
FIG. 4.3 No. of weeks that male three-spined sticklebacks maintained a strong blue eye colour (score 3 or 4) in their first (white bar) and second (grey bar) breeding seasons, in relation to dietary manipulation (restricted or control) and photoperiod regime (A – ambient and B – delayed) in both the Winter (left panels) and Spring (right panels) experiments. Data plotted as means + SE.
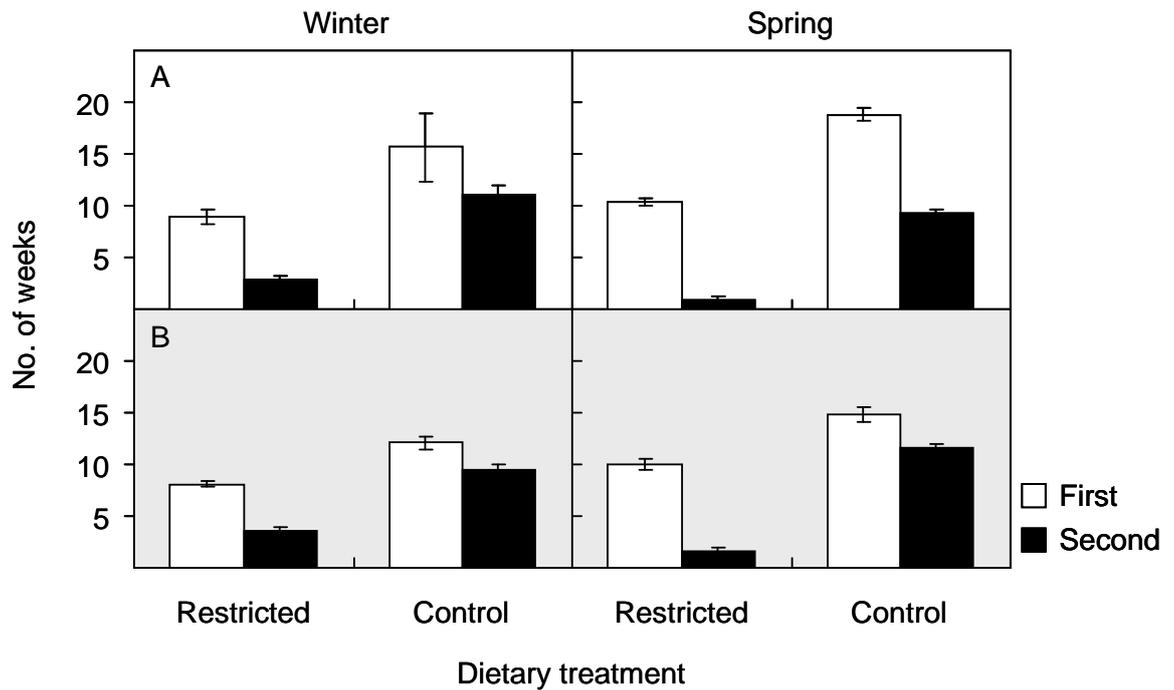
FIG. 4.4 No. of weeks that female three-spined sticklebacks maintained a blue eye colour (score of at least 2) in their first (white bar) and second (grey bar) breeding seasons, in relation to dietary manipulation (restricted or control) and photoperiod regime (A – ambient and B – delayed) in both the Winter (left panels) and Spring (right panels) experiments. Data plotted as means ± SE.

Reproductive investment in males

The duration for which males maintained a red throat colouration was longer in the first breeding season than in the second, and longer in the Winter experiment than in the Spring (Table 4.4 and Fig. 4.5). Dietary treatment affected the duration of red throat colouration (i.e. R males were red for a shorter period of time). While there was no overall effect of photoperiod, there was an interaction between age and photoperiod (R males maintaining the longest duration in the first breeding season, Table 4.4, Fig. 4.5). There was no effect of compensatory growth rate ($F_{1,51.23}=0.055$, $P=0.816$), but a male's length at the end of the period of dietary manipulation (= manipulated fish length) was positively related to the length of time he remained red and there was also an interaction between age and manipulated fish length (Table 4.4), the effect of fish size being more pronounced in the first breeding season.

TABLE. **4.4** Mixed model analyses of red throat colouration of male sticklebacks in relation to age, season, dietary and photoperiod treatment, manipulated fish length (at the end of the dietary manipulation, ln transformed) and compensatory growth rate after the 4 weeks of dietary manipulation, plus tank as a random effect. Non-significant variables were dropped from the final model.

Final model	F	d.f.	P
Age	10.18	1, 38.57	0.003
Season	4.81	1, 44.43	0.034
Diet	49.39	1, 41.37	<0.001
Photoperiod	3.28	1, 45.09	0.077
Manipulated fish length	6.70	1, 45.99	0.013
Age × photoperiod	8.96	1, 35.62	0.005
Age × manipulated fish length	9.61	1, 39.06	0.004
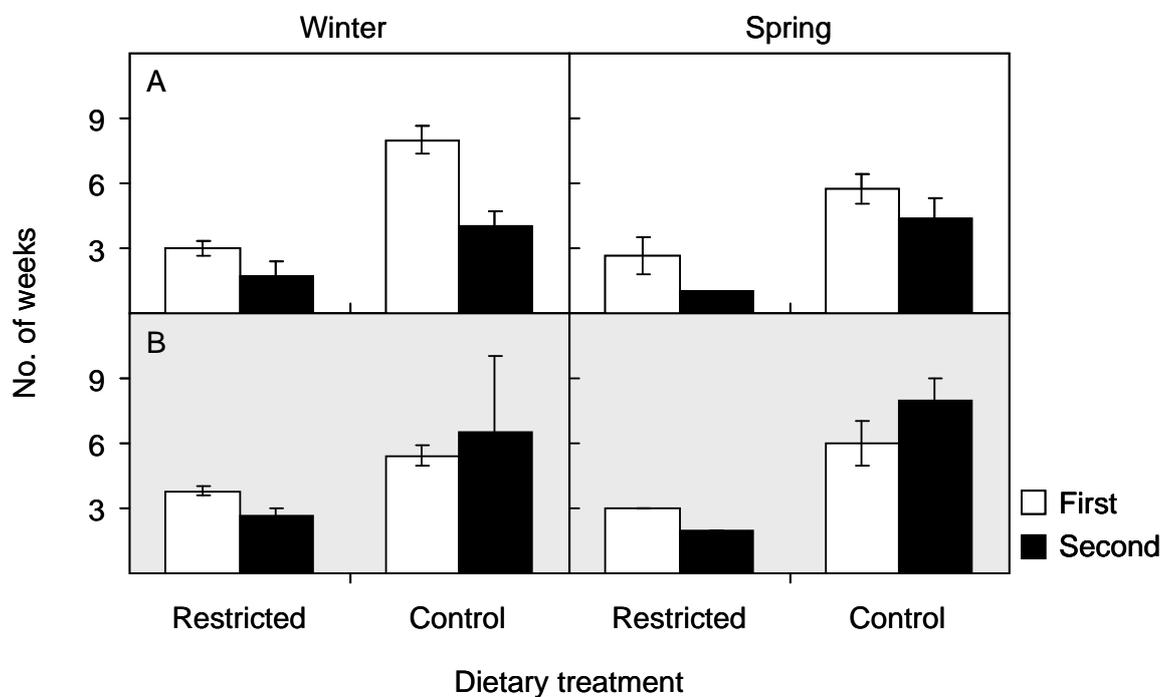
FIG. **4.5** No. of weeks that male sticklebacks exceeded the mean redness score in relation to dietary manipulation (restricted or control) and photoperiod regime ((A) ambient or (B) delayed).in the Winter and Spring experiments. Data plotted as means ± SE.

There was a significant difference in the rate of nest building between the first and the second breeding season, and between the Winter and Spring experiment (Table 4.5). Males completed nests within 3.3±0.2 days of receiving nest material in their first breeding season (Winter: 3.4±0.3 days and Spring: 3.1±0.2 days) but took longer (4.0±0.3 days) in their second breeding season (Winter: 4.0±0.3 days and Spring: 3.9±0.6 days), and males from the Winter experiment took longer than those from the Spring experiment. While there were no overall effects of diet ($F_{1,53.08}$=0.71, P=0.405) or photoperiod ($F_{1,57.71}$=0.02, P=0.891), there was an interaction between season and diet (Table 4.5): R males took longer than C males to complete nests in the Spring experiment whereas there was less effect of diet treatment (after controlling for growth rate – see below) in the Winter experiment (Fig. 4.6). There was a negative effect of manipulated fish length on duration, plus a significant interaction between age and manipulated fish length (Table 4.5): the larger the male at the end of dietary manipulation, the shorter the time of nest building. Compensatory growth rate negatively affected the rate of nest building and there was a significant interaction between season and compensatory growth rate (Table 4.5): the faster the compensatory growth rate, the longer the time needed to build a nest, particularly in the Spring experiment.

TABLE 4.5 Mixed model analyses of time required by male sticklebacks to build a nest in relation to age, season, dietary and photoperiod treatments, manipulated fish length (at the end of the dietary manipulation, ln transformed) and compensatory growth rate after the 4 weeks of dietary manipulation, plus tank as a random effect. Non-significant variables were dropped from the final model.

Final model	F	d.f.	P
Age	10.72	1, 35.66	0.002
Season	12.63	1, 54.29	0.001
Diet	0.71	1, 53.08	0.405
Manipulated fish length	13.44	1, 65.04	<0.001
Compensatory growth rate	5.16	1, 58.78	0.027
Season × diet	6.63	1, 54.05	0.013
Season × compensatory growth rate	10.53	1, 59.72	0.002
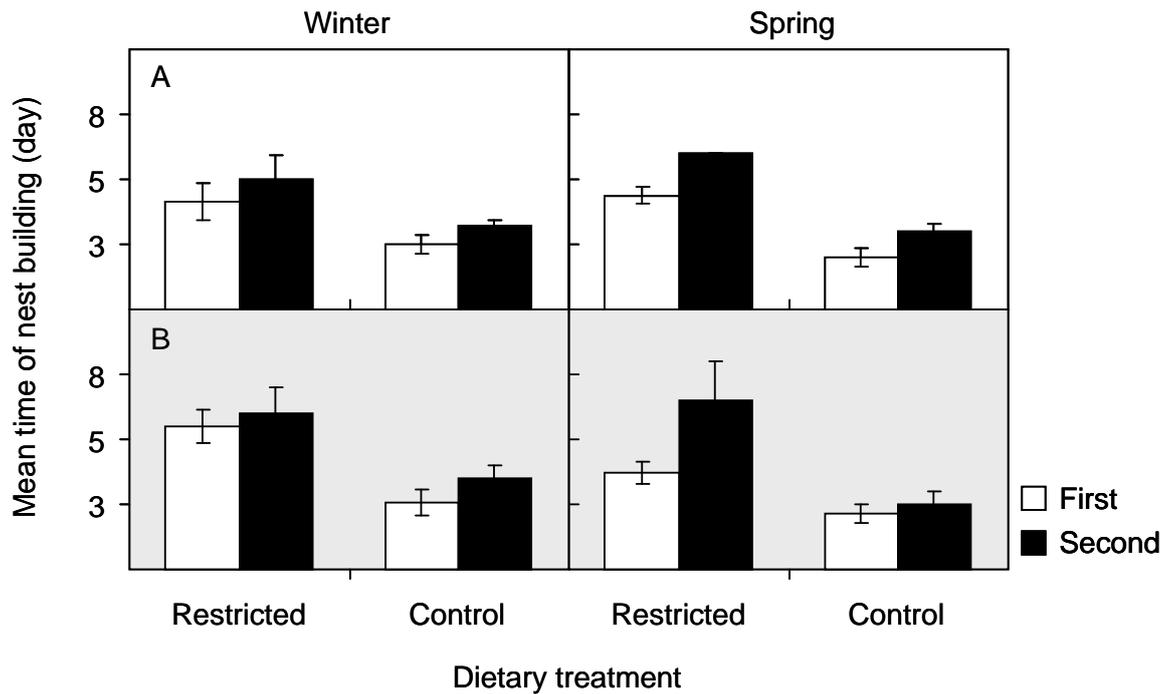
FIG. 4.6 Time taken by male three-spined sticklebacks to build a nest (days, mean ± SE) in relation to dietary manipulation (restricted or control) and photoperiod manipulation (A: ambient or B: delayed) in the Winter and Spring experiments. Data plotted as means ± SE.

Reproductive investment in females

A total of 24 and 25 females (out of 29 and 35 that were alive at the time) spawned during the first breeding season in the Winter and Spring experiments respectively, but only 9 females in the Winter experiment and 7 in the Spring experiment spawned in the second season (out of 23 and 31 that were still alive at that time). Given the low numbers of females spawning in the second season, the analysis of reproductive investment is based primarily on the first breeding season, and analysis of individual egg mass and number of eggs per clutch is only based on the first clutch since the number of clutches varied between females (mean (±standard deviation) number of clutches per female in the first season = 1.26±0.65). The mean mass per egg from the 1st clutch of each female was significantly heavier in the Winter experiment (3.3±0.1 mg) than in the Spring (2.4±0.2 mg; Table 4.6). While there was no effect of compensatory growth ($F_{1,31.72}=2.54$, $P=0.121$), the mass of an egg was related to a female's length at the time of spawning (Table 4.6), with larger fish producing heavier eggs. Dietary treatment also affected egg mass (with R females of a given size producing lighter eggs, Table 4.6) whereas there was no effect of

photoperiod treatment ($F_{1,21.76}$=0.53, P=0.475). Egg mass was affected by interactions between season and diet and between diet and length at time of spawning (Table 4.6): the effect of diet was strongest in the Spring experiment, and females from the R group showed less of an effect of fish size on egg size (Fig. 4.7a and c).

The number of eggs in the first clutch was not significantly different between the Winter (63.6±2.8) and Spring experiments (52.2±5.5) whereas there was an effect of dietary treatment (Table 4.6), with R fish producing fewer eggs than C fish (Fig. 4.7b and d). Females from the delayed photoperiod group spawned more eggs than those under an ambient photoperiod (Table 4.6, Fig. 7b and d). As with egg size, there was no effect on clutch size of compensatory growth ($F_{1,32.25}$=0.55, P=0.465) but a positive effect of the female's length at time of spawning (Table 4.6 and Fig. 4.7b and d). The interaction between season and photoperiod significantly affected the number of eggs, with delayed photoperiod fish in the Winter experiment spawning more eggs (Table 4.6).

TABLE 4.6 No. of eggs in 1st clutch and mean mass of an egg from that clutch in relation to season, dietary and photoperiod treatment, length at the time of spawning (ln transformed) and compensatory growth rate after the 4 weeks of dietary manipulation in the Winter and Spring experiments. The GLMM included season, diet and photoperiod as fixed effects, fish length at spawning and compensatory growth rate after 4 weeks manipulation as covariates and tank as random effects, plus all interactions. Non-significant variables were dropped from the final model.

	Final model	F	d.f.	P
Mass of each egg	Season	37.55	1, 30.00	<0.001
	Diet	4.98	1, 38.37	0.032
	Length at time of spawning	28.74	1, 38.40	<0.001
	Season × diet	7.76	1, 21.00	0.011
	Diet × length at time of spawning	6.13	1, 38.40	0.018
No. of eggs in 1st clutch	Season	1.77	1, 21.92	0.197
	Diet	5.89	1, 21.59	0.024
	Photoperiod	6.89	1, 21.58	0.016
	Length at time of spawning	10.72	1, 38.05	0.002
	Season × photoperiod	4.64	1, 21.09	0.043
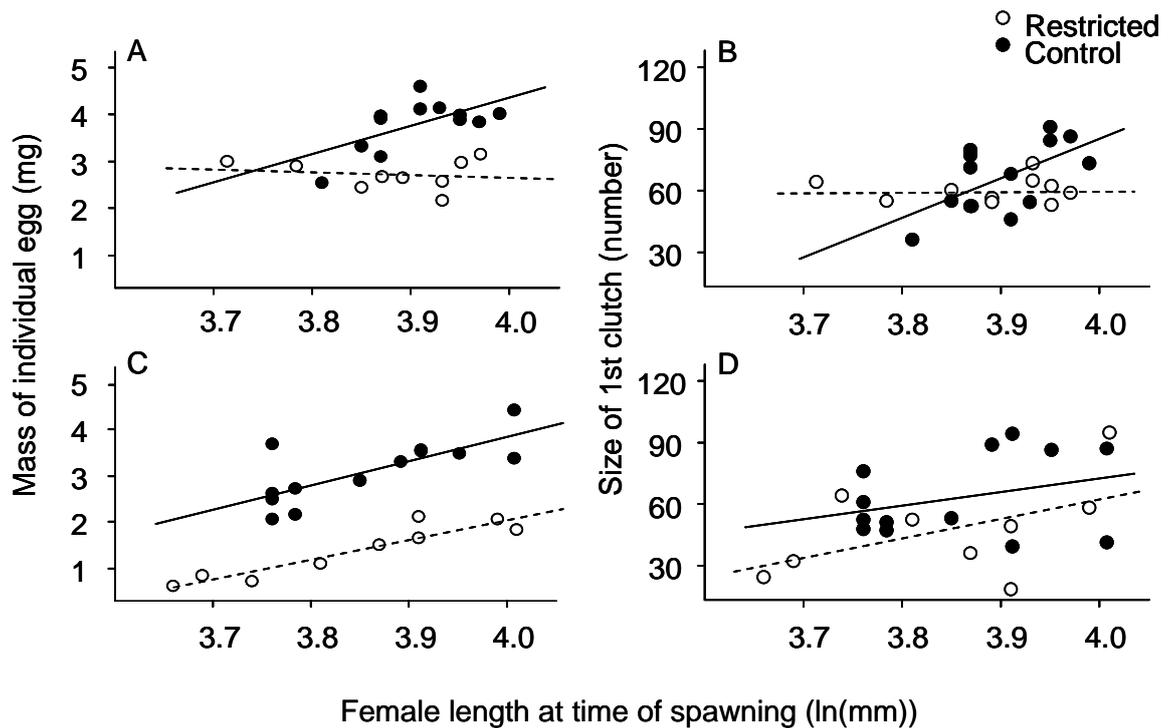
FIG. 4.7 Mean mass of individual eggs (mg, A and C) from the first clutch and size of the first clutch (number of eggs, B and D) produced by one year old female three-spined sticklebacks during the first breeding period in relation to their length at the time of spawning (mm, ln transformed). Values are plotted separately for the two dietary manipulation treatment groups (Restricted – open symbols and dashed line; Control – black symbols and line) in the Winter (A and B) and Spring (C and D) experiments.

The relative investment in the first breeding season (defined as the total number of eggs a female produced in Period 3 / total number of eggs she produced in Periods 3 plus 5 combined) was analysed to understand how growth trajectories influenced the investment by the female over time. There were significant differences between the Winter and Spring experiments in the proportion that the first season's eggs made up of the total egg production in the two years (Table 4.7), with females from the Spring experiment showing a greater relative investment in the first season (Fig. 4.8). While there was no effect of diet (Table 4.7) or photoperiod ($F_{1,39}$=0.01, P=0.919), there was a significant interaction between season and diet (Table 4.7): R females in the Spring experiment invested less in egg production in the second breeding season than did the corresponding females in the Winter experiment (Fig. 4.7). While there was no effect of length at time of first spawning ($F_{1,39}$=3.68, P=0.062), compensatory growth rate positively affected the proportion of eggs produced in the first year (Table 4.7).

TABLE 4.7 Proportion that the eggs produced in the first breeding season made up of the total number of eggs produced by a female over both the first and second breeding seasons, in relation to season, diet, photoperiod, length at time of spawning (ln transformed) and compensatory growth after the 4 weeks of dietary manipulation in the Winter and Spring experiments, plus tank as a random effect. Non-significant variables were dropped from the final model.

Final model	F	d.f.	P
Season	9.35	1, 40	0.004
Diet	2.73	1, 40	0.106
Compensatory growth rate	6.50	1, 40	0.015
Season × diet	4.20	1, 40	0.047
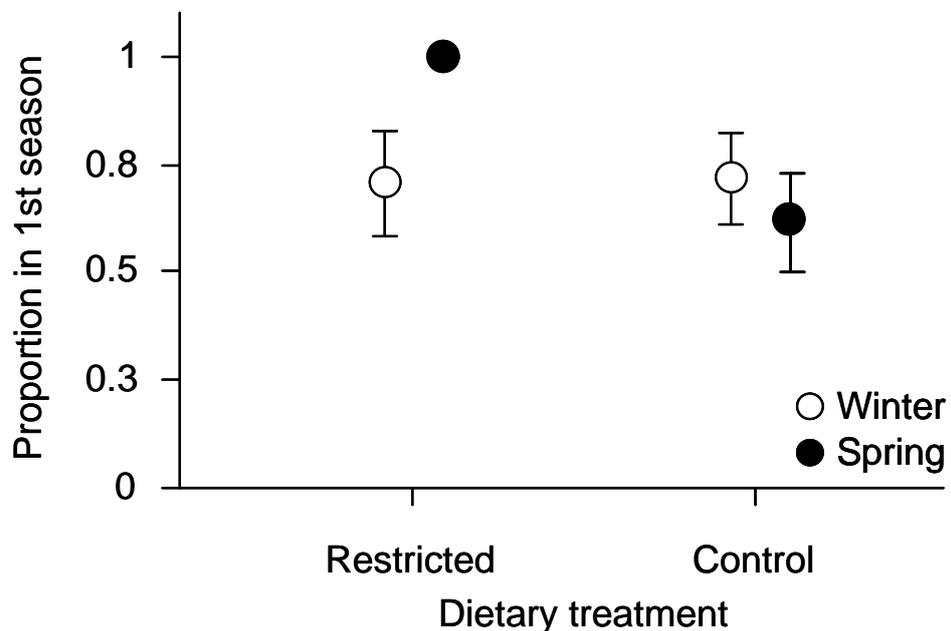
FIG. 4.8 Proportion that the eggs produced in the first breeding season made up of the total number of eggs produced by a female over both the first and second breeding seasons, in relation to dietary treatment (restricted or control) and experiment (Winter or Spring); data plotted as means ± SE.

4.5 DISCUSSION

In this experiment I have investigated how compensatory growth induced by dietary changes in early life has long-term consequences for locomotor performance and reproduction. A compensatory growth trajectory was successfully induced by restricting the availability of food during a short-term period in juvenile life. My findings showed that swimming performance and reproductive investment were negatively affected by the growth acceleration that was induced by this food manipulation. Interestingly, the negative long-term effects of a compensatory growth trajectory were strongly increased if the fish had a reduced amount of time available (either to catch up in size or to recover from that growth acceleration) until the breeding season. Unexpectedly, the growth rate during the phase of compensatory growth was faster in the Winter experiment (despite the fish being under less time stress than in the Spring experiment), but this may have been because fish in the Winter experiment were smaller in body length at the beginning of the experiment than in the Spring experiment. However, in general the subsequent performance of the R fish (in terms of both swimming and reproduction) was less affected in the Winter than the Spring experiment, despite the faster compensatory growth of this group of fish in the Winter experiment. This may have been because of the greater time available for fish to recover from any damage caused by fast growth in the winter experiment – a similar result for swimming performance was found by Álvarez and Metcalfe (2005).

It is clear that growth and development in animals may incur significant costs. The fish were able to accelerate their growth after the period of food restriction presumably through hyperphagia (Ali and Wootton 2000), but this may have incurred costs such as increased physiological damage due to oxidative stress associated with increased aerobic metabolism. In damselflies *Lestes viridis*, for instance, body levels of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were highest during a phase of accelerated growth, which was interpreted as a response to increased production of reactive oxygen species (ROS) (De Block et al. 2008). It is also well known that accelerated growth negatively affects muscle cellularity and development (Galloway et al. 1999; Johnston et al. 2002). Álvarez and Metcalfe (2005) showed that swimming endurance was lower in fish that had previously been subjected to food restriction and had then gone through a phase of compensatory growth, probably due to changes in cellular structure caused by the accelerated growth. The accumulation of damage can also negatively affect reproductive investment. Pike et al. (2007) showed that a reduced intake of dietary antioxidants in male sticklebacks led to increased oxidative damage and a reduced investment in breeding

ornaments. There is also increasing evidence of a negative relationship between oxidative stress and reproductive capacity in wild organisms (Bize et al. 2006; Perez et al. 2008), suggesting that oxidative stress may in some way constrain reproduction (Metcalfe and Alonso-Alvarez 2010). Therefore the reduction in endurance over the breeding season in the present study, especially in fish that had undergone accelerated growth, may be a consequence of increased levels of accumulated damage, with the addition of breeding costs on top of damage due to growth. However, it is still not clear how compensatory growth influences metabolic rates and hence ROS production.

Under conditions of finite resources, natural selection favours allocation strategies that will maximise long-term fitness over the organism's lifespan. It is well documented that reproductive effort and investment are affected by a trade-off between growth and reproduction (Stearns 1989; Green and Rothstein 1991). For instance, Poizat et al. (1999) showed that female sticklebacks lose somatic condition during the breeding season, but increase in gonad weight relative to body weight. Presumably a phase of restricted food availability in early life may affect this resource allocation between soma and gonads, since the subsequent growth acceleration can only be achieved by increasing use of resources. While this may partly be achieved through hyperphagia (Ali and Wootton 2000), as was clearly attempted in sticklebacks in a related experiment (i.e. faster rate of food consumption during the phase of compensatory growth, Chapter 5), this may not be sufficient to achieve the desired growth rate. A change in the allocation of resources in favour of skeletal growth could then negatively affect the development of reproductive tissues, in a similar manner to the way in which it is thought to interfere with the development of non-reproductive structures (Ricklefs et al. 1994; Arendt et al. 2001; Arendt 2003), but this is still unclear. While the number of eggs produced by a female fish is proportional to body size (Morita and Takashima 1998; Jonsson and Jonsson 1999), the present study shows that compensatory growth can reduce egg production and egg size, even after correction for the female's skeletal size at the time of spawning. A similar effect of compensatory growth on clutch size was found in guppies *Poecilia reticulata* by Auer et al. (2010). The different number of eggs produced by females of similar body size but differing growth trajectory may be caused by differences in their gonad size.

In addition to its effects on egg production, an accelerated (compensatory) growth trajectory caused a delay in the onset and reduced duration of sexual ornamentation (which suggests a shorter potential breeding season). This may again be due to oxidative stress, since reductions in dietary antioxidant intake (and hence presumed increases in oxidative

stress) have been shown to have effects on the intensity of nuptial colouration in sticklebacks (Pike et al. 2007). These results thus suggest that compensatory growth might cause a change in resource allocation such that there would be reduced resources devoted to both gonadal growth and repair of any oxidative damage to reproductive structure (including ornaments), but further experiments that include measurements of oxidative damage and repair rates are needed to confirm this hypothesis.

Growth and reproduction in ectotherms are sensitive to both temperature and photoperiod; while both of these are cues indicating the time of year, photoperiod is thought to be the one used most often as a time reference since it is not susceptible to temporal fluctuations. The results of the photoperiod manipulations showed that the time-stress (i.e. perceived amount of time available until the breeding season) affected the scale of the negative impact of compensatory growth on locomotor and reproductive performance. These results support the 'time-stress' hypothesis that both growth rates and resource allocation decisions can be affected by the perception of time of year, and in particular, the time available until key life history events (Metcalfe et al. 2002). The observed pattern for the negative effects of a given growth rate to be more pronounced when the perceived time stress was shorter (i.e. both in the Spring experiment, and under the ambient rather than delayed photoperiod) may be due to changes in the trade-off between growth and reproductive investment, since when time was short there was less time to repair any damage incurred by growth acceleration. In other words, an increased time-stress might induce more resources to be allocated to growth (with less to reproduction), so altering the effects of compensatory growth on subsequence swimming endurance and reproductive investment. The perception of reproductive timing in animals can alter the scheduled strategy in maturation (Dawson et al. 2000). For instance, Gotthard (Gotthard 2008) highlighted that butterfly larvae modify their growth strategies in response to information about a reliable cue of time of year (i.e. photoperiod). Therefore I suggest that the degree of time-stress interacts with prior growth trajectory to determine the animal's optimal current rate of growth, taking into account the trade-off between growth and reproduction and the effect of accelerated growth on performance in later life. However, this hypothesis requires more investigation since previous work has found mixed results (= no strong effect of time stress, De Block et al. 2008).

In summary, reproductive success in fish is often strongly correlated with body size and hence food rations (Rowland 1989; Maekawa et al. 1996; Lindström 1999). While compensatory growth (leading to an increased size after a period of poor food rations) was

expected to occur as a positive strategy to increase reproductive success, this study showed significant negative effects of such accelerated growth on both swimming endurance and the degree of reproductive investment, with effects becoming stronger rather than weaker over time (e.g. with faster growth leading to faster declines in swimming performance over the breeding season and a reduced investment in reproduction in the second year). Moreover the perception of amount of time available prior to breeding altered the documented costs of compensatory growth. However it is still unclear exactly how compensatory growth affects the relevant physiological mechanisms and rate of damage accumulation and how this translates into impaired performance, and also whether the negative impacts of compensatory growth are transferred to the next generation.

CHAPTER 5

A COMPARISON OF DYNAMIC STATE DEPENDENT MODELS OF THE TRADE-OFF BETWEEN GROWTH, DAMAGE AND REPRODUCTION

5.1 ABSTRACT

Environmental conditions early in life can alter subsequent growth and developmental trajectories. While animals can compensate to some extent for perturbations to growth trajectories, such changes in growth rate are known to have consequences for future performance. Strategically, organisms should therefore adjust growth trajectories to maximise their expected fitness under the given environmental conditions, taking into account any trade-offs between growth rate and fitness parameters such as future reproductive investment and rates of senescence (e.g. as measured through damage to biomolecules). I developed four models of increasing complexity with different growth-damage scenarios, ranging from assuming that the animal maximises growth regardless of any costs, through assuming a relationship between growth rate and mortality risk, to assuming growth leads to damage accumulation and that there allows the animal to apportion resources between somatic growth, gonadal growth and investment in repair of damage. The growth models were designed to fit the indeterminate growth patterns of ectotherms, and incorporated the concept that growth is sensitive to ambient temperature even when food is not limiting. I contrasted the predictions of the four models in terms of growth trajectories, feeding activity, reproductive investment and accumulation of damage. I also compared model predictions with experimental results from three-spined sticklebacks whose growth trajectories had been altered by temperature manipulations. All models predicted the observed pattern of compensatory growth (in both directions - accelerating and decelerating) in response to earlier temperature perturbations, but the more complex models provided the best fit to experimental data. Growth trajectories strongly influenced future reproductive investment irrespective of body size at the time of breeding, presumably due to the effects of damage accumulation in the run up to the breeding season; again the predictions of the most complex model were closest to the

experimental data on egg production. In conclusion, my findings suggested that it is possible to predict growth trajectories in variable environments using models that take account of the long-term fitness consequences of different growth rates. While simpler models can predict basic patterns of growth in stable conditions, they cannot capture the costly long-term effects of deviations from steady growth trajectories. In contrast, models in which the growth rate is optimised to take account of such effects are capable of predicting the complex patterns of feeding and growth seen in experimental animals.

5.2 INTRODUCTION

The growth rate of organisms influences their future survival and reproduction and is affected by energy supply and environmental conditions, in particular those pertaining when growth rates are normally fastest (usually in early life). Growth rate has known effects on life history traits: for instance, larger size in early life typically leads to higher survival and fecundity in later life (Quentin and Richard 2001). However, there may be costs induced by the rapid growth needed to reach large size, such as an increased risk of physiological damage to molecules, cells and tissues (Metcalfe and Monaghan 2001; Metcalfe and Monaghan 2003; Mangel and Munch 2005) and increased metabolic rate in adulthood (Criscuolo et al. 2008). Alternatively, or perhaps in addition, a faster overall rate of early growth might cause a mismatch in relative growth and development of component tissues/organs, producing a suboptimal adult phenotype (Martell et al. 2006) that would then fail sooner.

Compensatory growth is a well-known strategic adjustment that occurs when growth rate is accelerated upon normal condition after a period of poor episode (e.g. starvation or low temperature); if complete it results in normal adult size still being attained despite the earlier set-back (Arendt 1997). While compensatory growth leads to advantages in survival, feeding and mating (Rowe and Thorpe 1990; Johnsson 1993; Sogard 1997), recent work has shown that early accelerated growth after a period of food deprivation has negative effects on whole organism performance in later life. These include reductions in locomotor performance (Álvarez and Metcalfe Alvarez and Metcalfe 2005; Chapter 2), reproductive output (Auer et al. 2010; Chapter 3) and lifespan ((Inness and Metcalfe 2008; Chapter 6). In order to maximise Darwinian fitness, the growth strategy adopted by the individual faced with a finite energy resource will therefore depend

on allocation trade-offs which in turn often depend on the state of the individual (Mcnamara and Houston 1996).

In a theoretical examination of the consequences of such long term costs of growth rate, Mangel and Munch (2005) showed that if rapid growth increased the rate of accumulation of damage in body tissues, this could lead to different optimal long- or short-term growth strategies. However, their model did not consider other environmental impacts on growth rate. One such influence is temperature, which in ectotherms has diverse effects on organismal performance (Weatherley and Gill 1987; Wootton 1998; Quentin and Richard 2001): for instance, moderate increases in ambient temperature are associated with faster growth, but they also cause a higher metabolic rate and hence food requirement, leading to more active foraging behaviour and hence a greater risk of being detected by predators. Higher metabolism may also lead to a greater rate of damage accumulation due to an increased production of damaging reactive oxygen species (Metcalfe and Alonso-Alvarez 2010). Therefore environmental conditions that affect growth may have both benefits and costs, making it difficult *a priori* to predict the optimal rate of growth for a given set of environmental conditions.

In this chapter I develop a range of life-history models to understand the trade-off faced by ectotherms between early growth and damage in relation to both temperature and food supply, taking into account the level of activity required to obtain a given amount of food and the resulting pattern of energy allocation. I develop four models of increasing complexity with different growth-damage scenarios, ranging from assuming that the animal maximises growth regardless of any costs (the maximize growth model, MGM), through assuming a relationship between growth rate and mortality risk (optimize growth model, OGM), to assuming growth leads to damage accumulation (response to damage model, RDM) and to one that allows the animal to apportion resources between somatic growth, gonadal growth and investment in repair of damage (gonadal accumulation and repair model, GARM). I then compare the growth trajectories predicted by each model both with each other and with experimental data from three-spined sticklebacks, which were induced during their juvenile growth phase to follow three different growth trajectories (accelerating, decelerating, and steady) by temperature manipulations. The results also suggest how early growth rate is likely to cause long-term effects through the accumulation of physiological damage, and how this trade-off between growth tempo and damage level can influence optimal life-history strategies.

5.3 METHODS AND EXPERIMENTS

Dynamic state models

To find the activity level (and hence damage) that maximises expected fitness, I consider four possible dynamic models. Initially I consider a life history governed by one state variable, mass (W), but the later models also include two further state variables: the accumulation of oxidative or cellular damage (D) and of reproductive tissue (O). To model growth rates I combine a model of fish growth (Mangel and Munch 2005), a food consumption model for three-spined sticklebacks (Wootton et al. 1980), and a basal catabolic model for fish (Brett and Groves 1979). As with dynamic energy budget models, but without resorting to hidden state variables, I incorporate an interaction between activity levels and consumption using insights from optimal foraging theory (Clark and Mangel 2000; Satterthwaite et al. 2010).

I thus model the rate of change in mass $W(s)$ at time s and at activity level (measured as the fraction of the day active) i as

$$W(s+1) = W(s) + G(i) - C(i) \tag{1}$$

The amount of food G a fish consumes during a day when its activity level is i is

$$G(i) = i[c_0 + (c_1 W(s))^{0.75} + c_2 T(s)] \tag{2}$$

Consumption thus depends on fish size and temperature on that day $T(s)$, but the extent to which it reaches the maximal intake possible at that time depends on the fish's activity i. The basic catabolic costs of the fish,

$$C(i) = \alpha(i) e^{0.021 T(s)} W(s) \tag{3}$$

also depends on its mass, temperature and the specific metabolic cost $\alpha(i)$. Here, $e^{0.021T(s)}$ characterises the temperature dependence of growth costs (Brett and Groves 1979)). The specific metabolic cost for a given level of activity,

$$\alpha_s(i) = \alpha i + (1-i)\alpha m_r \tag{4}$$

depends on the weight-specific catabolic rate α and the multiplier for time spent resting m_r. Note that increases in i cause increases in both consumption and total catabolic costs but at

different rates (Mangel and Munch 2005), so that there is an intermediate, optimal level of activity. The basal catabolic term depends on the measure of weight-specific catabolic costs α and the effect of temperature $T(s)$.

Maximize Growth Model (MGM)

To begin, I assume that activity can be predicted by maximising growth rate, and find the activity level i^* that achieves this. Therefore the MGM model can predict the maximum possible net growth rate, but takes no account of other factors such as mortality risk through predation:

$$W(s+1) = W(s) + \max_i[G(i) - C(i)] \tag{5}$$

Over time, all organisms accumulate damage to molecules, cells and tissues, which will reduce their capacity in a range of ways. The rate of damage accumulation is not fixed but will vary with metabolic processes. In the MGM model, this is captured in as simple a way as possible, with damage accumulating passively (i.e. without the animal being able to repair it). I model the accumulated damage $D_p(S)$ in the individual at time S as

$$D_P(S) = k\sum_{s=1}^{S-1}(i^*(s) - i_d) \tag{6}$$

where the optimal activity level to maximize the net growth rate is indicated by $i^*(s)$, the coefficient for damage level by k and the activity level at zero net production of damage (i.e. repair=production) by i_d (Table 5.1).

Optimize Growth Model (OGM)

In contrast with the MGM model, in the OGM model, I assume that the aim is to optimise (rather than necessarily maximise) growth rate, taking account of its effect on survival. There is an intrinsic trade-off faced by organisms while foraging: a curtailment of foraging activity (i.e. increased time spent at rest) may reduce predation risk as well as food intake (Houston et al. 1993). That is, because of the impact of foraging on predation (and hence mortality) risk, growth should not necessarily always be maximised. I assume the probability of survival at each time s depends on activity level,

$$\beta(i) = e^{-\mu i - \mu_r(1-i)} \tag{7}$$

where the mortality rate for activity is μ, and the mortality rate for resting is μ_r (Table 5.1).

TABLE 5.1 Summary of variable and parameter definitions, and the range of values used in simulations.

		Range or values			
Variable	Description	MGM	OGM	RDM	GARM
W	Body mass (size)	1-2500	1-2500	1-2500	1-2500
s	Time	1-40	1-40	1-40	1-40
i	Activity	1-30	1-30	1-30	1-30
c_0	Constant by temperature	-12	-12	-12	-12
c_1	Weight coefficient for food consumption	0.388	0.388	0.388	0.388
c_2	Temperature coefficient for food consumption	19.312	19.312	19.312	19.312
c_3	Constant for catabolic cost	0.021	0.021	0.021	0.021
a	Weight-specific catabolic rate	0.125	0.125	0.125	0.125
m_r	Reduction in metabolic cost for resting	0.002	0.002	0.002	0.002
k	Parameter for damage accumulation	0.51	0.51	0.51	0.51
i_d	Activity level at zero damage	0.005	0.005	0.005	0.005
φ	Multiple for fitness value	1	1	1	1
μ	Mortality rate when active	-	0.015	0.015	0.015
μ_r	Mortality rate when resting	-	0.014	0.014	0.014
μ_d	Mortality rate due to damage	-	-	0.005	0.005
μ_b	Mortality rate during breeding season	-	-	0.002	0.002
ρ	Efficiency of repair	-	-	-	0.001

I next determine the optimal time- and state-dependent pattern of activity for the parameters (which then determines the pattern of growth and survival). I assume that expected reproductive success at the end of the fixed growth interval S, when mass is $W(S)$ is $(W(S) - w_c)^\varphi$, where w_c is a critical mass required for reproduction and and φ is a parameter (Table 5.1).

For previous times, I define fitness as

$$F(w,s) = \max E[(W(S) - w_c)^\varphi \mid W(s) = w] \tag{8}$$

where the maximum is taken over the level of activity and the expectation refers to the probability of surviving from the current time until the end of the growth interval. Then

$F(w,S) = (w - w_c)^\varphi$ and for previous times

$$F(w,s) = \max_i [\beta(i) F(w + G(i) - C(i), s+1)] \tag{9}$$

I solve Eqn. (9) using backward iteration (Mangel and Clark 1988; Clark and Mangel 2000). At each time and state, I generate the optimal level of activity $i^*(w,s)$ that maximises the fitness function. Given an initial mass, the trajectory of growth can then be calculated by forward Monte Carlo simulation, feeding the calculated values for optimal activity (given the animal's current size) at each time step into Eqn. (1).

As in the MGM model, the accumulated damage in the OGM model is also predicted passively (i.e. it is not taken into account when determining the optimal behaviour). Given the optimal activity level and associated mass determined from Eqn. (9), I model the accumulated damage as

$$D_P(S) = k \sum_{s=1}^{S-1} (i^*(W^*(s), s) - i_d) \tag{10}$$

where the optimal activity level is indicated by i^*, the optimal mass at time s is indicated by $W^*(s)$, the coefficient for damage level by k and the activity level at zero net production of damage by i_d. Thus the level of activity determines the level of damage, but in this model the accumulation of damage does not influence the optimal activity level, nor does it affect mortality rate and reproduction.

Response to Damage Model (RDM)

This model allows a more dynamic approach to damage: the level of physiological damage influences the optimal level of activity, and the animal can repair damage in order to reduce its impact on fitness (hence the animal 'responds' to the damage, in contrast to the two earlier models where damage can neither influence the animal's behaviour nor

decrease over time). Moreover, since damaged cells and tissues have diverse impacts, I let the level of damage influence mortality risk as well as reproductive output. I model damage levels D_R in relation to time s and activity level i as

$$D_R(s+1) = D_R(s) + k(i - i_d) \tag{11}$$

where the level of activity with zero net production of damage is i_d. Note that levels of activity below i_d result in a reduction in damage (i.e. repair).

I assume the probability of survival depends on the levels of both activity and current damage,

$$\beta(i) = e^{-\mu i - \mu_r(1-i) - \mu_d d} \tag{12}$$

where the mortality rate when resting is μ_r, the mortality rate due to damage is μ_d and the coefficient for mortality due to damage is d (Table 5.1).

I now define $F(w, d, s)$ in analogy to Eqn. (8) representing maximum expected terminal reproduction given the current mass and level of damage. The final condition becomes

$$F(w, d, S) = (w - w_c)^{\varphi} e^{-\mu_b d} \tag{13}$$

where μ_b is the parameter for mortality rate during the breeding season (note that this is multiplied by the level of damage d, so that damage reduces breeding lifespan and hence fitness), and for previous times

$$F(w, d, s) = \max_i [\beta(i) F(w + G(i) - C(i), d + k(i - i_d), s + 1)] \tag{14}$$

Gonadal Accumulation and Repair Model (GARM)

This model has a different structure to the first three, in that it allows the animal at each time step to decide on allocation of resources to the three options of growth, repair of damage or investment in reproduction. To do this, I rewrite the ingested amount of food G from Eqn. (2) and the basic catabolic costs of the fish C from Eqn. (3):

$$G(i) = ig(w, s) \tag{2a}$$

$$C(i) = \alpha(i + (1-i)m_r) \, h(w, s) = \alpha[i(1 - m_r) + m_r] \, h(w, s) \tag{3a}$$

where $g(w,s) = c_0 + (c_1 W(s))^{0.75} + c_2 T(s)$ and $h(w,s) = e^{0.021T(s)} W(s)$. So, the net gain of resources $R(i)$ at activity level i is described by

$$R(i) = G(i) - C(i) \qquad (14)$$

$$= ig(w,s) - \alpha i(1 - m_r)h(w,s) - \alpha m_r h(w,s)$$

$$= i[g(w,s) - \alpha(1 - m_r)h(w,s)] - \alpha m_r h(w,s)$$

$R(i)$ is then allocated to an increase in body mass, in reproductive tissue (e.g. oocyte), and/or repair of damaged tissue (Fig. 5.1).

If f_W is the fraction of resources allocated to mass gain, body mass $W(s)$ gained at time $s + 1$ from the net gain of resource $R(i)$ at activity level i is described by

$$W(s+1) = W(s) + f_W R(i) \qquad (15)$$

The reproductive tissue (here envisaged as oocytes) $O(s)$ at time $s + 1$ is described by

$$O(s+1) = O(s) + f_O R(i) \qquad (16)$$

where f_O is the fraction allocated to reproductive tissue.

The change in the level of damaged tissue $D(s)$ at time $s + 1$ is described by

$$D(s+1) = D(s) - (1 - f_W - f_O)R(i)\rho + k(i - i_d) \qquad (17)$$

where $(1-f_w-f_o)$ is the allocation to repair and ρ is the efficiency of repair (so is the parameter that links investment in repair to actual reduction in damage).

The fitness function is now $F(w,o,d,s)$ and so is dependent upon current mass, accumulated oocytes, and accumulated damage. The end condition is

$$F(w,o,d,S) = oe^{-\mu_b d} \qquad (18)$$

and for previous times

$$F(w,o,d,s) = \max_i \max_{f_w,f_o} [\beta(i)F(w + f_w R(i), \ o + f_o R(i), \ d - (1 - f_w - f_o)R(i)_\rho + ki, s+1)]$$

$$(19)$$

so that *F(w, o, d, s)* is the maximum, taken over activity levels, of fitness at the end of interval [*s, s+1*]. The fitness associated with a particular growth strategy is determined by the probability of surviving through the focal interval multiplied by the residual reproductive value associated with the final mass.
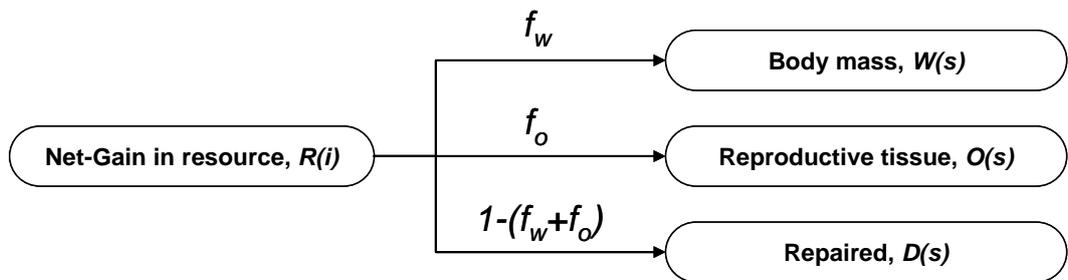
FIG. 5.1 Illustration of the resource allocation process in the Gonadal Accumulation and Repair Model (GARM).

Experimental data

In order to parameterise the growth models, I used data on the growth of the fish from the previously described experiments (Chapter 2), but only that collected in the Winter experiments due to reduce the seasonal effects on the growth. Food was provided *ad libitum* throughout in the form of a single meal per day of previously-frozen *Chironomid* larvae; by recording whether or not food was present at hourly intervals after feeding I produced an index of feeding activity in each tank using a 5 point scale from 5 (all food in the tank consumed in less than 1h) to 1 (still some food left after 4 h) for every week. (see detailed experiments description in Chapter 2).

Subsequent growth rate

In both models and experiments, subsequent growth rate (SGR, % per day) after the temperature manipulation was calculated as

$$SGR = 100 \frac{\ln[W(s_c)W(s_i)^{-1}]}{s_c - s_i} \tag{19}$$

where $W(s_i)$ is the initial wet-mass at the end of manipulation period and $W(s_c)$ is the wet-mass when fish in the different manipulation groups had finished the phase of compensatory growth and had appeared to converge on the same mean mass prior to breeding (based on inspection of growth trajectories).

Breeding investment by females

Only the GARM model includes explicit calculation of gonadal investment, so I used an indirect approach to predict final gonad size in all models (including the GARM model to allow comparison of the two approaches). The maximum mass of eggs that can be produced by female fish is known to be tightly related to body mass (Wootton 1998; Quentin and Richard 2001). I therefore assume this maximum reproductive tissue,

$$O_{max}(w) = 0.25w \tag{20}$$

where 0.25 is the mean observed ratio of total clutch mass (over the entire breeding season) to body mass (measured at the start of the breeding season) from the experimental data. However, if the individual has incurred significant damage during its earlier life, the total resources available to produce eggs may be reduced. I therefore assume that a fish's total reproductive mass will be reduced in proportion to the damage it has accumulated

$$O(W(S)) = 0.25W(S) - k_c D(S) \tag{21}$$

where the parameter for clutch damage k_c is set to 10.5.

Simulation and analysis

In order to compare the performance of the various growth models in relation to the observed data, I randomly generated a population of 20 juvenile fish with the same mean and standard deviation for initial body mass as in the observed data. The models were each run using this size frequency distribution of fish. The relative fit of each of the four models was compared by calculating a distance function d, based on the sum of squares of relative errors when comparing values for observed body mass at age with those predicted by each model

$$d = \sum_{s} \frac{[W_{obs}(s) - W_{predict}(s)]^2}{W_{obs}(s)}$$

(20)

I ran simulations of the MGM, OGM, and RDM in R (R development Core Team 2007) and the GARM in Microsoft Visual Basic 2008 Express (Microsoft 2008).

5.4 RESULTS

Optimum Growth and Activity Patterns

In order to find appropriate growth rate parameter values for the stickleback system, I ran the Maximize growth model (MGM), Optimize growth model (OGM), Response to damage model (RDM), and Gonadal accumulation and Repair model (GARM) with a wide range of parameter values until model outputs best matched the observed growth trajectories of fish kept at 10°C throughout. Using the parameter values in Table 5.1, the predicted growth trajectories from the models are similar to those observed in the experiment (Fig. 5.2). All models were able to broadly replicate the observed growth trajectories for fish in the Intermediate Temperature treatment (i.e. constant 10°C temperature), although they all predicted a greater reduction in the within-population variation in mass over time than was observed in the experimental data (Fig. 5.2). The GARM model provided the best fit to the experimental results obtained at 10°C, as indicated by the sum of squares of relative errors (Fig. 5.3). I did not use formal model comparison criteria because I am most interested in focusing on the kinds of information that the various models provide and the associated qualitative patterns rather than trying to find the "best" model (*cf* Clark and Mangel 2000, Ch 4).

Each model lead to the prediction of an acceleration in growth during the period after the temperature reduction (i.e. when the fish were transferred from 6°C to 10°C), relative to those kept at 10°C throughout, whereas those in the High temperature group (which had experienced a period at 14°C) were predicted to show decelerated growth when transferred to 10°C (Fig. 5.4). However, the predicted strength of this compensation differed between the models, with the least compensation (whether in terms of accelerated or decelerated growth) predicted by the MGM model and the strongest by the GARM model (which matched the experimental data closest). However, none of the models predicted as extreme an acceleration of growth as was observed in the experimental fish in the Low Temperature group (Fig. 5.4a).
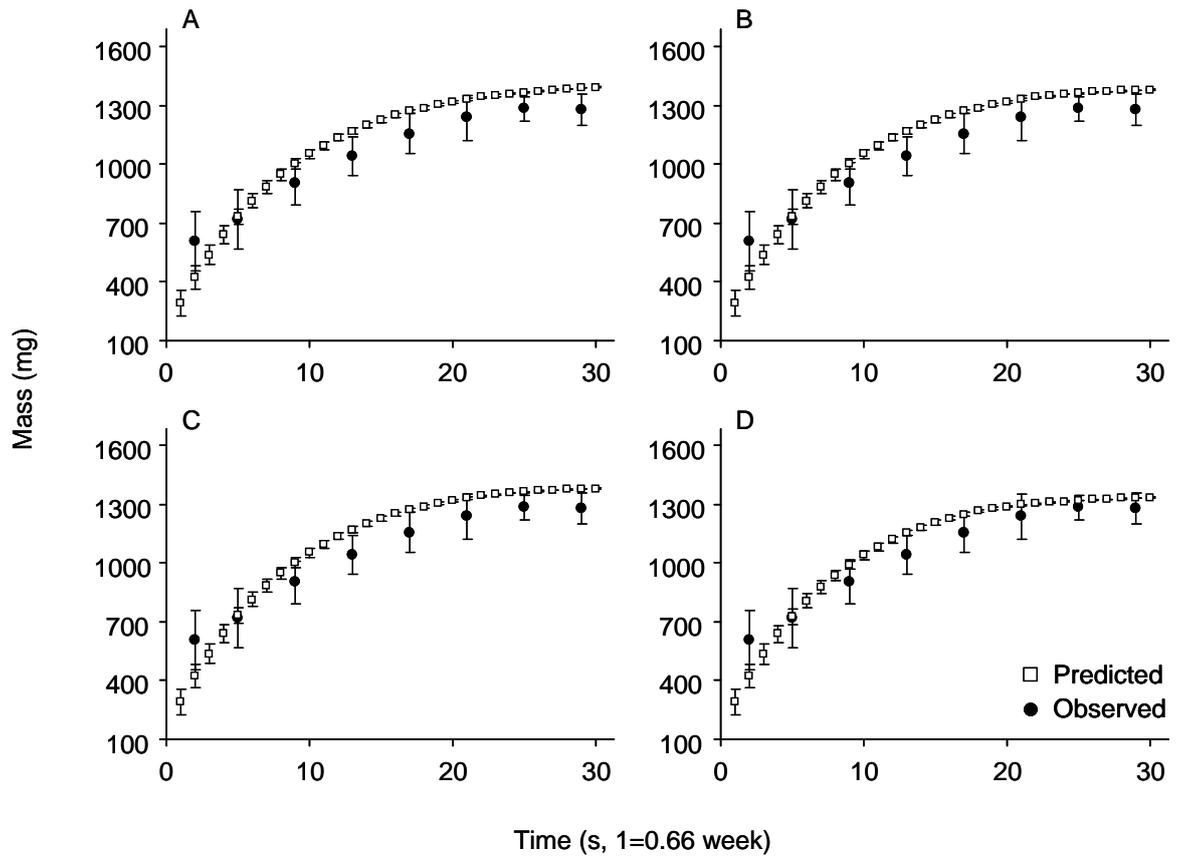
Fig. 5.2 Predicted and observed growth trajectories at time s = 1 to 30 for fish under conditions of *ad lib.* food and constant 10°C. The four plots show the predicted optimised growth trajectories (open squares, mean mass ± SD) for a simulated population of 20 fish with the same initial mean size and SD as the experimental population (see Methods) according to the four growth models: A = Maximize Growth Model, B = Optimize Growth Model, C = Response to Damage Model, and D = Gonadal Accumulation and Repair Model. Note that the error bars are indistinct at later time periods due to a predicted reduction in the variation in size among individuals over time. The closed circles and error bars show the observed mean size ± SD of three-spined sticklebacks in the Intermediate (i.e. constant 10°C temperature) group in the lab experiment.
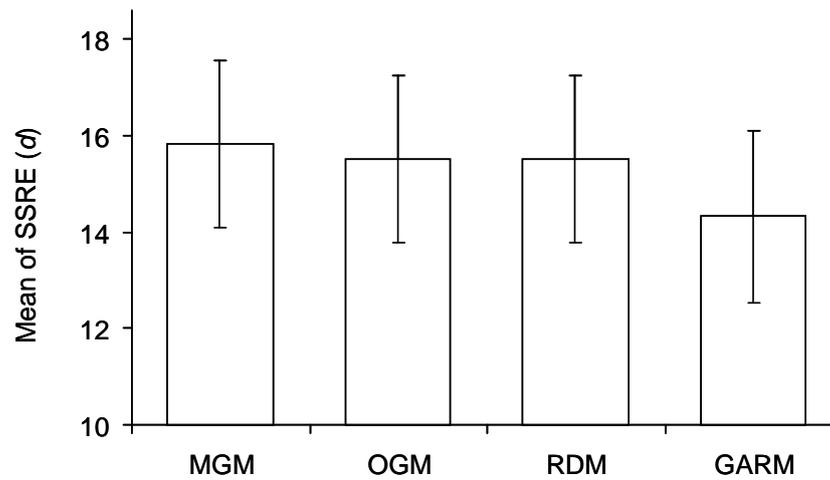
Fɪɢ. 5.3 Mean ± SE sum of squares of relative errors (SSRE, *d*) of the four growth models when compared to the observed growth data.

The MGM model predicted that activity levels of the three temperature treatment groups should remain at maximal levels (since in this model there is no cost to activity). However, the three other models (OGM, RDM and GARM) lead to predictions that activity levels would change over time and would differ between the treatment groups. The three models gave broadly similar patterns of maximal activity in all fish during the temperature manipulation period, but then a decline once all fish were at 10°C, with the activity of the high temperature treatment group declining first, followed by the intermediate and finally the low temperature treatment groups (Fig. 5.5). The GARM model predicted a smaller difference in activity between treatment groups than the OGM and RDM models, which predicted that the activity of the High temperature treatment fish would have dropped to very low levels by the onset of the breeding season (Fig. 5.5). The pattern predicted by the GARM model was the closest match to the observed data on feeding activity (i.e. time taken to consume each meal) (Fig. 5.5).

Accumulated damage

While the MGM model predicted the same value of accumulated damage for the three temperature treatment groups (since activity levels did not vary between them, and there was no repair of damage in this model), other models (OGM, RDM and GARM) predicted that the accumulated damage (measured at the final time point *S*, at reproduction) would

differ among the experimental temperature groups (Low, Intermediate or High temperature treatments; Fig. 5.6): in each of these three models the damage levels were predicted to be highest in animals initially subjected to the low temperature and least in those initially exposed to warmer temperatures; the predicted relative levels of accumulated damage were remarkably similar across these three models despite their different assumptions.
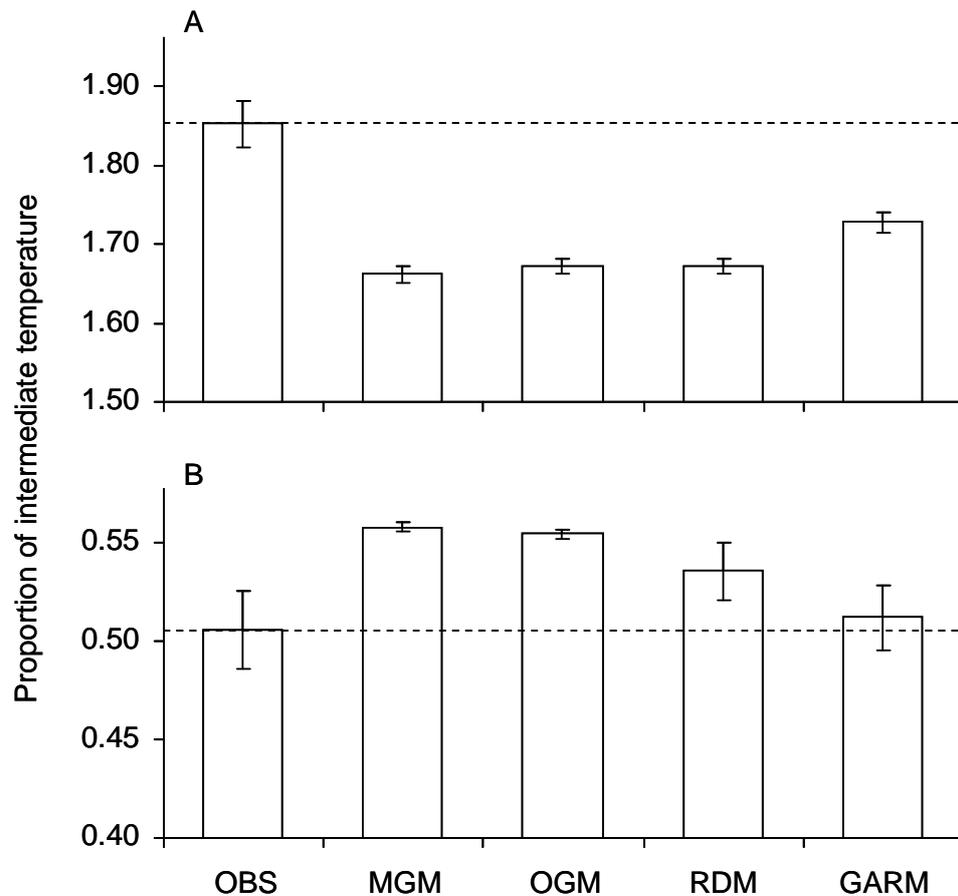
Fɪɢ. 5.4 Observed (OBS) and predicted growth rates of fish over the period from $s = 7 - 25$. This time corresponds to the period when experimental fish had just been returned to a temperature of 10°C after a 4 week period (from $s = 1 - 6$) when they were held at (A) 6°C or (B) 14°C. Values are expressed as a proportion of the growth rate of the Intermediate group of experimental and model fish held at a constant 10°C. Predicted growth rates are shown for the four separate models: maximize growth model (MGM), optimize growth model (OGM), response to damage model (RDM) and gonadal accumulation and repair model (GARM). Dashed lines indicate mean of observed values for each temperature to allow easy comparison. Data are shown as means values ± SD for the experimental and simulated populations (see text for explanation).
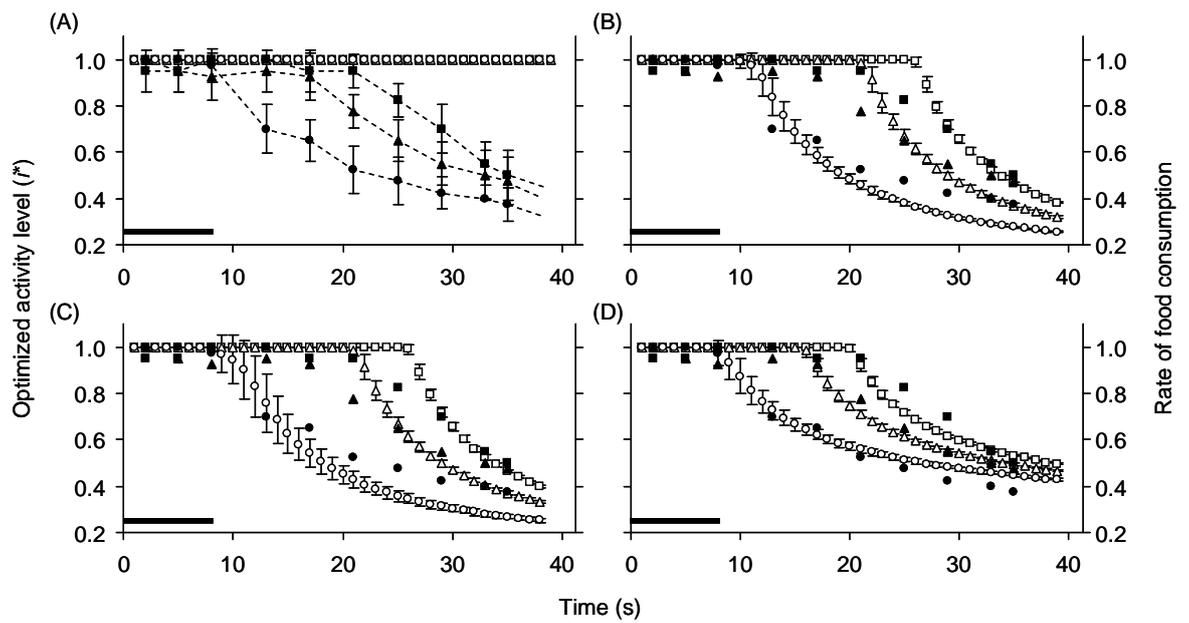
FIG. 5.5 The optimum activity levels (*i**) of fish in the three temperature treatment groups (high (14°C) – open circle, intermediate (10°C) – open triangle, low (6°C) – open square) as predicted by the four different models (A – maximize growth model, B – optimize growth model, C – response to damage model, and D – gonadal accumulation and repair model). Data show the mean ± SD predicted activity for the simulated populations of 20 fish per treatment. Also shown with closed symbols are the observed times taken by experimental fish to consume food after it had been presented. Data are shown separately for the three temperature treatment groups (high – circle, intermediate – triangle, low – square; values are plotted as means ± SD in A, but only the mean values are plotted in the other panels for clarity). The thick bar indicates the period during which temperatures differed between the groups, after which time all fish were at 10°C.
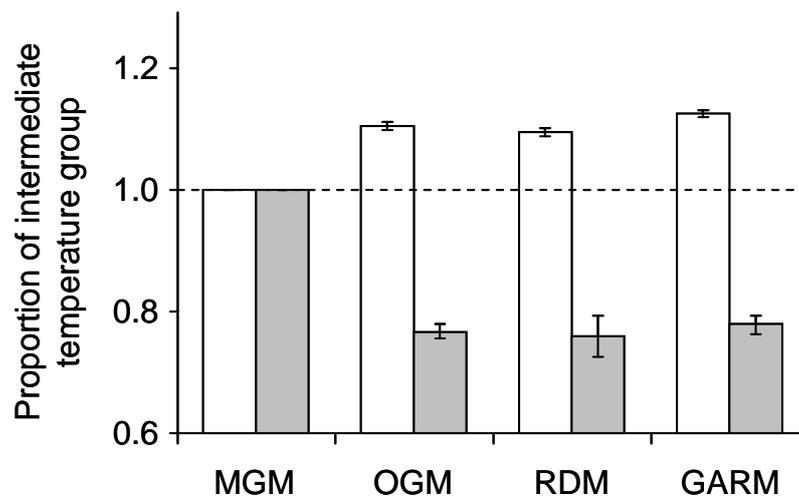
FIG. 5.6 Predicted accumulated damage at time *S* (i.e. onset of the breeding season) in Low (white) and High (grey) temperature treatment groups, expressed as a mean proportion (± SD) of the predicted mean damage in fish from the Intermediate group (indicated by the dashed line). Predicted values are shown for the four different models: MGM – maximize growth model, OGM – optimize growth model, RDM – response to damage model, and GARM – gonadal accumulation and repair model.

Investment in reproductive tissue

I used the indirect method (see Eqn. (21)) with all four models to predict the total reproductive investment of females, and compared this with the observed total production of eggs by the experimental fish (Fig. 5.7). The MGM model predicted a similar reproductive mass in all three treatment groups (Fig. 5.7), due to all fish having a similar final size and levels of accumulated damage. However, the observed data did not match this pattern: although fish in all three groups were seen to reach a similar mean size at the time of spawning (see Supplement Fig. S1), their egg production was different, with High Temperature females producing more, and Low Temperature females fewer, eggs than the Intermediate Temperature females (Fig. 5.7). This pattern was predicted by the OGM, RDM and GARM models, with the closest fit to the observed data being obtained by the GARM model. Interestingly, this model predicted similar results regardless of which of the two different methods (GARM$_a$ and GARM$_b$ in Fig. 5.7) were used to calculate reproductive mass.

The GARM model incorporates the concept of resources being allocated over time to gonadal growth. The prediction from this model is that initially there should

be no gonadal growth, but this accelerates later as the breeding season approaches (Fig. 5.8). Interestingly, there are effects of the mortality rate when active (μ) on the predicted growth of reproductive tissue, with reduced/suppressed investment in the gonads when the mortality risk during foraging is higher (Fig. 5.8). Furthermore, the temporal pattern of reproductive investment is predicted to differ between the temperature treatments, with an earlier onset of gonadal growth in the higher temperature treatments, but equal rates of growth thereafter (leading to larger final gonad size in the higher temperature treatments).
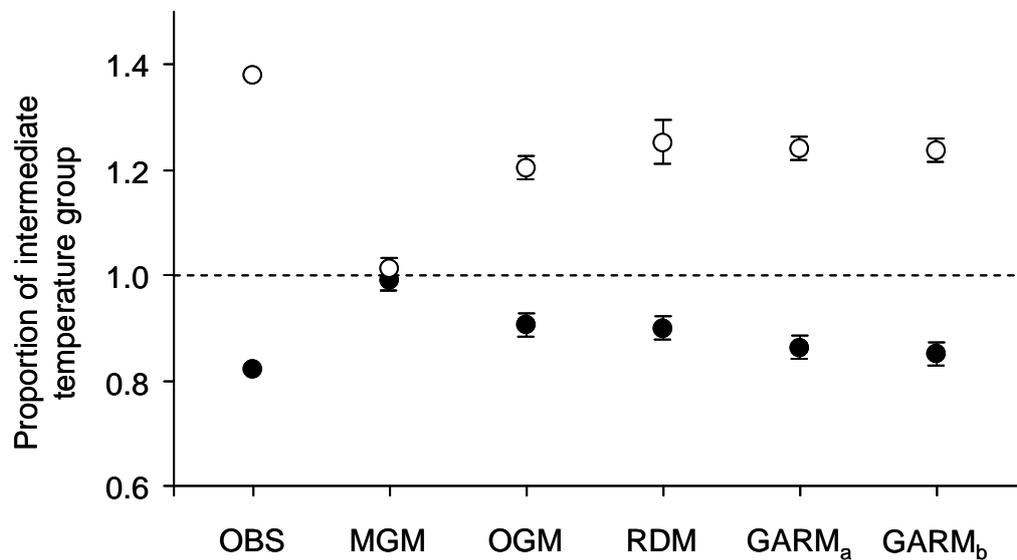
FIG. 5.7 Investment in reproduction, quantified as total mass of eggs produced during breeding season for the observed (OBS) experimental data and total reproductive mass for the four models. Values shown for Low (closed circle) and High (open circle) temperature treatment groups, expressed as a proportion of the mean value for fish in the Intermediate temperature group; data are plotted as a mean ± SD for observed or simulated population. MGM – maximize growth model, OGM – optimize growth model, RDM – response to damage model, and GARM – gonadal accumulation and repair model. The output for the GARM model is shown separately for the calculation based only on final somatic mass and accumulated damage (as for the other three models; GARM$_a$) and for the calculation based on modelled ovary growth (GARM$_b$; see text for explanation).
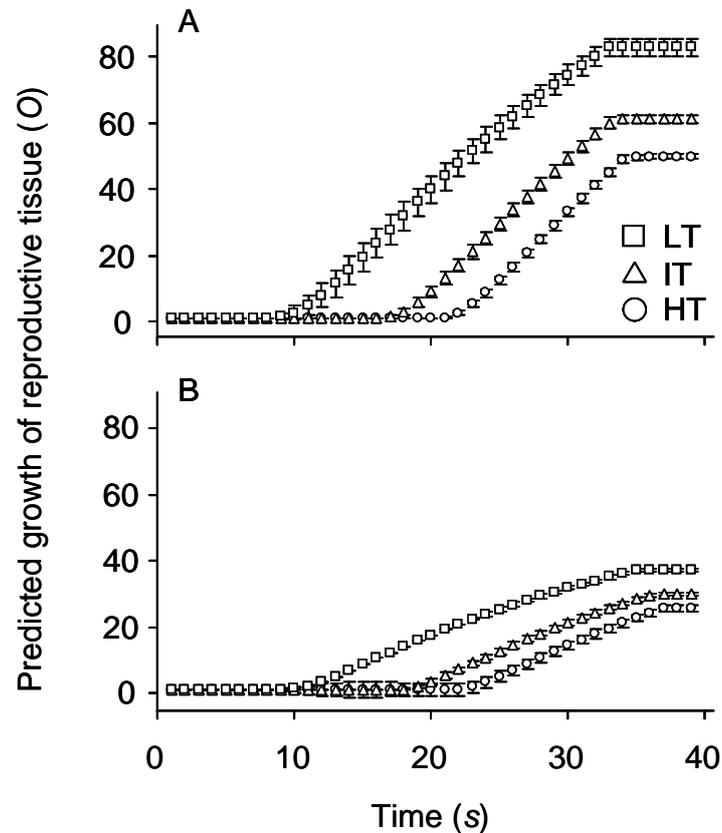
FIG. 5.8 Effects of mortality rate when active (μ) on the growth of reproductive tissue ($O(s)$) in the GARM model. The panels illustrate different values for the mortality parameter ($\mu = 0$ (A) and 0.015 (B)); in each case the predictions are plotted separately for the three temperature treatment groups – Low (square), Intermediate (triangle) and High (circle).

5.5 DISCUSSION

I have explored how early growth rate and the trade-off between growth tempo and damage level in relation to both temperature and food supply is likely to cause long-term effects and optimal life-history strategies through the accumulation of physiological damage. By the combination of dynamic state dependent models and experiments on the three-spined stickleback, I conclude that growth rate in early life affects later fitness. I have assumed that the cause of this reduction in fitness is an increased activity level (necessary to achieve a higher food intake), which causes both a higher mortality risk (e.g. through predation) and an accumulation of damage to molecules, cells and tissues. All but the simplest model were able to approximately replicate the different growth trajectories seen

in the experimental fish as a result of variations in activity levels: accelerated growth (induced by a period of lower temperatures) was associated with longer periods over which the activity level was maximised, whereas decelerated growth (seen in the high temperature treatment) was linked to reduced activity levels. These variations in growth trajectory were predicted to lead to differences in damage levels, with the accelerated growth trajectory having the highest expected damage and the decelerated trajectory the least. Therefore variations in growth rate induced by environmental temperatures in early life were predicted to cause differences in the accumulation of physiological damage as a consequence of changes in the optimal level of activity. In the more complex models this variation in damage levels influenced reproductive investment (i.e. the timing and extent of egg production), with greater accumulated damage level causing a lower expected reproductive rate and delayed breeding since animals would require more time and investment to repair tissues prior to breeding.

These theoretically predicted effects have a well-established empirical basis. It is well known that compensatory growth, particularly growth acceleration, has both costs and benefits in sticklebacks: it may increase the chance of reproduction (Wootton 1976; 1998), but also reduces locomotor performance (Álvarez and Metcalfe 2005; Chapter 2) and lifespan (Inness and Metcalfe 2008). Recent review (Monaghan et al. 2009) has highlighted the fact that levels of reactive oxygen species (ROS) may be elevated during juvenile development due to the high metabolic activities required for growth. It is possible that animals mount defences against such free radical attack - De Block and Stoks (2008) showed that levels of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were elevated during a phase of accelerated growth in damselflies *Lestes viridis* – but this increased investment will itself be a cost even if it prevents the faster accumulation of damage. My models and the experimental data also showed 'negative compensatory growth' after an earlier period of higher temperatures, with growth rates being suppressed in comparison with animals that had been at a constant temperature. The theoretical models predicted that this growth deceleration would be associated with a lower accumulated damage level; although damage levels were not measured as part of this study there is nonetheless indirect support for this from the finding that the growth deceleration after temperature manipulation had a positive effect on locomotor performance (Chapter 2) and reproductive investment (Chapter 3). Conversely, the models predicted an elevated level of accumulated damage in the fish that had undergone accelerated growth, as a consequence

of rapid growth rate incurring a higher level of oxidative stress as a result of an elevated metabolic rate (Metcalfe and Alonso-Alvarez 2010).

All but the MGM model predicted differences in reproductive investment between fish that had contrasting growth trajectories, even if their size by the time of the breeding season did not differ. Thus fish undergoing accelerated growth were predicted to have the lowest investment while the decelerated growth fish were expected to have the highest. The biggest effects of growth trajectory on reproduction were predicted by the GARM model, which also predicted that the onset of oocyte investment would be faster in the decelerated growth than in the accelerated growth. The predictions of the GARM model best matched the observed differences in egg production between growth treatment groups. These empirical results described in more detail in Chapter 3 have parallels in the recent study of Auer et al. (2010) who showed that accelerated compensatory growth (induced by prior food deprivation) reduced the rate of offspring production in female guppies *Poecilia reticulata*. While I did not explicitly include the effect of growth trajectory on male reproduction, I have experimental data showing that the duration of nuptial colouration in male sticklebacks varies with growth pattern, being shortest in accelerated growth groups (Chapter 3). This may again be associated with damage levels, since Pike et al. (2007) found that high levels of oxidative damage (in this case caused by reduced availability of dietary antioxidants) led to male sticklebacks being less able to invest in their nuptial signal. However, an unusual aspect of the present study was the positive effect of decelerated growth, with both models and data showing that fish undergoing rapid growth early in life but suppressed growth in the lead up to the breeding season would perform better than those growing steadily throughout their juvenile life. My models suggest that the positive as well as the negative long-term effects of early growth rate arise through changes to the rate of accumulation of physiological damage, but this remains to be tested by empirical measurements.

Environmental conditions affect both early development and their ecological consequences in later life. There are clear costs of impaired development in early life, but natural selection has led to a life-history strategy that reduces these negative effects so as to maximize expected reproductive success (e.g. through compensatory growth). This is illustrated by the predictions of my models, particularly those related to the optimal level of activity, which takes account of the trade-off between early growth rate and accumulated damage level. While the optimal activity level was predicted to be the maximum possible when the fish were still relatively small, all but the simplest model

predicted that the optimal activity level would then drop, especially in the decelerated growth group during the compensatory period. In contrast, the fish undergoing accelerated growth were predicted to continue for longer with a maximised level of activity (despite incurring a high level of accumulated damage) in order to increase their body size and hence their probability of overwinter survival and successful reproduction (Kraak et al. 1999; Garvey et al. 2004). Conversely, a decreased activity level may be selected for in fish that have previously experienced good growing conditions since it reduces the level of damage accumulation by the onset of the breeding season. If such females were instead to maintain a maximised activity level, they would be larger still by the time of the breeding season, but the high level of damage that they would accumulate would (according to the GARM model) reduce and delay their capacity to produce eggs, thus having a net negative effect on reproductive success. Therefore the decelerated growth fish are predicted to reduce activity (and hence damage, and the need to divert resources into repair), and to save the surplus resources for egg production rather than growth. Benefits are thus maximised over the long rather than the short term. In conclusion, these results suggest that the trade-off between early growth rate and accumulated damage level would result in the optimal activity level being determined by a maximisation of future reproductive investment.

It is well documented that early growth rates have long term consequences, but the pattern of growth observed (especially the degree of compensation for an earlier period of altered growth) still varies between different species and contexts. Some of this variation may be due to the current environmental conditions: for example, my models predicted that the differences in compensatory growth rate and accumulated damage level between temperature manipulation groups would disappear when the mortality rate (= predation pressure) was higher (Supplement Tables S1 and S2). Predation pressure affects feeding behaviour (Beukema 1968) since more active fish are more likely to be caught by a predator. An indirect cost of activity is its effect on damage. If this cost is increased (by altering the value of the parameter linking activity to damage accumulation, *k*), the accumulated damage level is predicted to sharply increase and the extent of growth compensation after an earlier growth retardation is predicted to be reduced (Supplement Tables S3 and S4). While there is no direct evidence of effects of predation risk on compensatory growth rate (Dmitriew and Rowe 2005; Stoks et al. 2005), my findings suggest that predation pressure may nonetheless influence (both directly and indirectly) the trade-off between compensatory growth rate and its long-term consequences.

The four models predicted similar patterns of growth, especially for animals in constant conditions, but they differed in the accuracy with which they matched the empirical data as a result of each model being based on different assumptions (see Methods). The predictions of the GARM model, which is the most complex, were the best match for the experimental data, while the simplest model (= MGM) was least able to match the observed patterns since it predicted the same levels of optimal activity, accumulated damage and reproductive investment irrespective of temperature treatment. Mangel and Munch (2005) found that growth models that excluded a consideration of the costs of growth (i.e. the damage level) could not adequately predict compensatory growth patterns, and the poor predictive power of the MGM model is likewise a consequence of it assuming that growth can be maximized without any effect on mortality rate. While the OGM and RDM models predicted similar growth trajectories for fish under constant conditions and similar activity levels, the pattern of compensatory growth and reproductive output was better predicted by the RDM model since a consideration of damage was included in the optimal decision process, whereas growth in the OGM model was determined independently of the damage level. The GARM model incorporated the idea of resource allocation between growth, damage and repair, and produced the most accurate predictions. This supports the approach of Mangel and Munch (2005) and also suggests that the greater the extent to which life-history theory is incorporated into growth models, the better the predictions.

In conclusion, I have shown by the use of four dynamic state-dependent models that a consideration of the costs of rapid growth (in terms of its effect on immediate mortality risk, long-term damage accumulation and future reproductive investment) allows prediction of complex growth trajectories that match empirical data. Moreover, the predictions are better when the models include more aspects of this trade-off between the benefits and costs of rapid growth. These results also emphasise that growth trajectories take account of life-history consequences as well as current ecological conditions.

5.6 SUPPLEMENT

Effects of mortality rate when active on growth and damage

In the OGM, RDM and GARM models, the mortality rate when active (μ) affected the predicted growth rates at the end of the temperature manipulation (Table S1). The predictions were qualitatively identical in the three models: when the mortality rate due to activity was either very low or relatively high, the optimal growth rate was low, but growth was predicted to increase to a peak at low levels of activity-related mortality. However, there is a greater difference in predicted subsequent growth rate between temperature treatment groups at the lowest mortality rates ($\mu < 0.015$) than at higher rates of mortality ($\mu > 0.15$), where temperature treatment has little effect on subsequent growth rate (Table S1). The accumulated damage levels predicted by the three models were also found to depend on the mortality rate when active (Table S2). Predicted damage levels declined with increasing mortality rate when active (μ) in all temperature treatment groups, with the Low temperature group having greater accumulated damage over all but the most extreme mortality rates (Table S2).

Sensitivity of parameter for damage accumulation on growth and damage

While the MGM and OGM model predictions of growth rate are unaffected by changes to the value of the parameter for damage accumulation (k), the RDM and GARM model growth predictions were sensitive to variation in this parameter when $k>3$ (Table S3): in both cases growth rates declined at high values for k and differences between temperature treatment groups became smaller (Table S3).

Variation in the value for k affected the predicted level of accumulated damage in all four models (Table S4). There was no difference between temperature treatment groups in the predicted damage accumulation when activity caused little damage ($k \rightarrow 0$), but the accumulated damage levels increased (most strongly in the MGM and least in the GARM models) when k increased above 0.1. Temperature treatment had no effect on damage levels in the MGM model but had significant effects in all three other models.
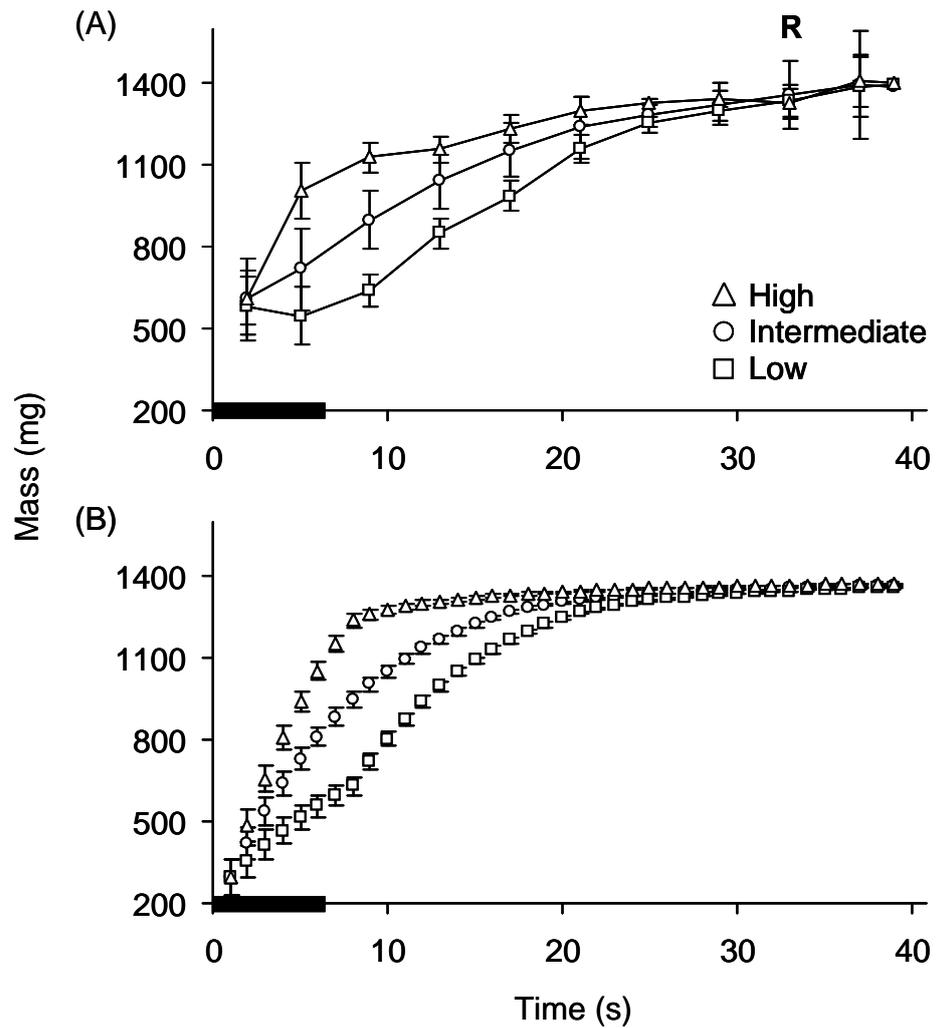
Fig. S1 Growth trajectories (wet mass in mg) of three-spined sticklebacks (A) observed in the experiment and (B) predicted by the GARM model. The thick horizontal line along the x axis indicates the period of temperature treatment manipulation (4 weeks). Values are plotted separately for the high (14°C; white circle), intermediate (10°C; white triangle) and low treatments (6°C; white square). After this treatment period, the temperature in all three groups was kept at 10°C until the start of the breeding season ('R'), at which point the temperature was raised to 14°C and male sticklebacks were isolated from female sticklebacks (see Chapter 3 for more details).

TABLE S1 A comparison among the OGM, RDM and GARM models of the sensitivity of the predicted growth rate after the period of temperature manipulation to parameter values for mortality rate when activity (μ). Predicted growth rates are shown for the Low, Intermediate and High temperature treatments (LT, IT and HT respectively).

μ	OGM			RDM			GARM		
	LT	IT	HT	LT	IT	HT	LT	IT	HT
0	4.6	2.7	1.6	4.6	2.7	1.5	4.6	2.7	1.4
0.005	4.6	2.7	1.6	4.6	2.7	1.5	4.6	2.7	1.4
0.015	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.03	6.3	6.1	6.0	6.5	6.3	6.1	6.1	5.5	5.0
0.05	4.9	4.7	4.5	5.1	4.7	4.7	4.8	4.5	4.2
0.15	1.7	1.7	1.7	1.8	1.7	1.7	2.1	2.0	1.9
0.25	1.2	1.2	1.2	1.3	1.3	1.3	1.3	1.3	1.3
0.35	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
0.45	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
0.55	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
0.65	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
0.75	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
0.85	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
0.95	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2

TABLE S2 A comparison among the OGM, RDM and GARM models of the sensitivity of the predicted accumulated damage after the period of temperature manipulation to parameter values for mortality rate when activity (μ). Predicted growth rates are shown for the Low, Intermediate and High temperature treatments (LT, IT and HT respectively).

	OGM			RDM			GARM		
μ	LT	IT	HT	LT	IT	HT	LT	IT	HT
0	19.3	19.3	19.3	20.8	20.8	20.8	20.9	20.9	20.9
0.005	19.3	19.3	19.3	20.8	20.8	20.8	20.9	20.9	20.9
0.015	16.7	15.1	11.6	18.2	16.6	12.6	18.6	16.5	12.8
0.03	9.3	9.2	9.2	10.9	10.9	10.8	9.3	9.0	8.8
0.05	5.8	5.8	5.8	7.5	7.4	7.4	7.7	7.6	7.4
0.15	1.8	1.7	1.7	3.4	3.3	3.3	3.8	3.7	3.6
0.25	0.8	0.8	0.8	2.4	2.4	2.3	2.8	2.8	2.7
0.35	0.6	0.6	0.6	2.1	2.1	2.1	2.2	2.2	2.2
0.45	0.6	0.5	0.5	2.1	2.0	2.0	2.2	2.2	2.2
0.55	0.5	0.5	0.5	2.1	2.0	2.0	2.2	2.2	2.2
0.65	0.5	0.5	0.5	2.0	2.0	2.0	2.2	2.2	2.2
0.75	0.5	0.5	0.5	2.0	2.0	2.0	2.2	2.2	2.2
0.85	0.5	0.5	0.5	2.0	2.0	2.0	2.1	2.1	2.1
0.95	0.5	0.5	0.5	2.0	2.0	2.0	2.1	2.1	2.1

TABLE S3 A comparison among the MGM, OGM, RDM and GARM models of the sensitivity of the predicted growth rate after the period of temperature manipulation to parameter values for damage accumulation (k). Predicted growth rates are shown for the Low, Intermediate and High temperature treatments (LT, IT and HT respectively).

	MGM			OGM			RDM			GARM		
k	LT	IT	HT	LT	IT	HT	LT	IT	HT	LT	IT	HT
0	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.1	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.2	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.3	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.4	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.5	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.51	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.6	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.7	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.8	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
0.9	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.3
1.0	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	4.5	2.6	1.5
3.0	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.7	1.5	5.1	4.5	4.0
5.0	4.6	2.7	1.5	4.6	2.7	1.5	4.6	2.6	1.4	3.9	3.6	3.3
10.0	4.6	2.7	1.5	4.6	2.7	1.5	3.1	2.0	1.3	1.6	1.6	1.5
15.0	4.6	2.7	1.5	4.6	2.7	1.5	1.8	1.3	1.0	1.2	1.2	1.2
20.0	4.6	2.7	1.5	4.6	2.7	1.5	1.0	0.8	0.6	1.2	1.2	1.2
25.0	4.6	2.7	1.5	4.6	2.7	1.5	0.5	0.5	0.4	1.2	1.2	1.2

TABLE S4 A comparison among the MGM, OGM, RDM and GARM models of the sensitivity of the predicted damage accumulation after the period of temperature manipulation to parameter values for damage accumulation (k). Predicted growth rates are shown for the Low, Intermediate and High temperature treatments (LT, IT and HT respectively).

	MGM			OGM			RDM			GARM		
k	LT	IT	HT	LT	IT	HT	LT	IT	HT	LT	IT	HT
0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	1.5	1.5	1.5
0.1	3.8	3.8	3.8	3.3	3.0	2.3	4.8	4.5	3.7	4.6	4.4	4.0
0.2	7.6	7.6	7.6	6.6	5.9	4.6	8.1	7.4	5.9	7.8	7.4	6.5
0.3	11.3	11.3	11.3	9.8	8.9	9.8	11.3	10.4	8.0	10.9	10.3	9.0
0.4	15.1	15.1	15.1	13.1	11.9	9.1	14.6	13.4	10.2	14.1	13.3	11.6
0.5	18.9	18.9	18.9	16.4	14.8	11.4	17.9	16.3	12.4	17.3	16.2	14.1
0.51	19.3	19.3	19.3	16.7	15.1	11.6	16.6	18.2	12.6	18.6	16.5	12.8
0.6	22.7	22.7	22.7	19.7	17.8	13.7	21.2	19.3	14.6	20.4	19.1	16.6
0.7	26.5	26.5	26.5	22.9	20.8	15.9	24.4	22.3	16.8	23.6	22.1	19.1
0.8	30.2	30.2	30.2	26.2	23.7	18.2	27.7	25.2	19.0	26.7	25.0	21.6
0.9	34.0	34.0	34.0	29.5	26.7	20.5	31.0	28.2	21.1	29.9	28.0	24.2
1.0	37.8	37.8	37.8	32.8	29.7	22.8	34.3	31.2	23.3	33.0	30.9	27.2
3.0	113.4	113.4	113.4	98.3	89.0	68.3	94.7	87.6	66.9	45.0	43.3	41.7
5.0	189.1	189.1	189.1	163.8	148.4	113.8	123.5	120.9	101.0	52.7	51.1	49.7
10.0	378.1	378.1	378.1	227.7	296.7	227.7	132.6	132.6	131.5	56.3	55.3	54.2
15.0	567.2	567.2	567.2	491.4	445.1	341.5	133.0	133.0	133.0	59.4	58.3	57.2
20.0	756.2	756.2	756.2	655.3	593.4	455.3	133.0	133.0	133.0	65.6	64.2	62.8
25.0	945.3	945.3	945.3	819.1	741.8	569.1	133.0	133.0	133.0	66.8	65.9	65.1

CHAPTER 6

THE EFFECT OF EARLY GROWTH RATE AND REPRODUCTIVE INVESTMENT ON LIFESPAN IN A SHORT-LIVED FISH

6.1 ABSTRACT

It has previously been shown that accelerated growth after an earlier period of food restriction can reduced adult lifespan, but it has been unclear whether this was partly due to the initial period of malnutrition. Here, I investigate how variation in growth trajectories independent of food availability may affect lifespan in three-spined sticklebacks, by altering growth through manipulation of environmental temperatures. Fish were exposed to one of three temperatures (high, intermediate and low) for 4 weeks in the non-breeding season, and were then all held at a common (intermediate) temperature. The two more extreme temperatures both induced compensatory growth trajectories (i.e. the low temperature regime induced growth acceleration when returned to the common temperature, while the high induced growth deceleration). I related subsequent lifespan to the temperature treatment and the fishes' growth pattern and reproductive investment (male – red throat colouration and female – egg production). Accelerated compensatory growth reduced lifespan whereas 'negative' compensatory growth (= growth deceleration) extended it. Moreover the effect of compensation on lifespan was strongest when the perceived time until the first breeding season was shortest. Within temperature treatment groups, female lifespan was positively related to investment in egg production. Lifespan in males was correlated positively with their growth rate between the first and second breeding seasons and with the extent to which they maintained the duration of red throat colouration in the second breeding season (an indicator of their ability to combat reproductive senescence). These results suggest that alterations to early growth rate induced by environmental conditions may disturb the balance of investment between growth, reproduction and survival and thereby modify lifespan.

6.2 INTRODUCTION

Environmental conditions can have profound effects on life-history traits such as growth, reproduction and lifespan. Sometimes these effects are counter-intuitive: while a reliable food supply is essential for the maintenance of life, it is less clear why a restriction in food intake below 'normal' levels generally extends lifespan (Kaeberlein et al. 2006; Bishop and Guarente 2007; Selesniemi et al. 2008). The effect of temperature is also not easy to predict: for instance, in ectotherms a reduced ambient temperature causes a slowing of metabolic rates, which can reduce growth rate and feed efficiency (Imsland et al. 2006) but may also lead to a slower rate of production of reactive oxygen species (ROS), which may reduce oxidative stress (Metcalfe and Alonso-Alvarez 2010).

However, if the environmental conditions result in a growth trajectory that deviates from the typical pattern, the animal may subsequently respond to a change in environmental conditions (e.g. warmer temperatures) by undergoing a phase of compensatory growth. In general a large body size has advantages in life, such as a reduced predation pressure, greater competitive ability, greater opportunity in choice of mates and an increased reproductive output. While catching up in size can therefore provide benefits, there is growing evidence that a rapid growth rate in early life (as often occurs with compensatory growth) can result in costs in later life, such as reduced locomotor performance (Álvarez and Metcalfe 2005, Chapter 2) and reproductive investment (Auer et al. 2010). Moreover Inness and Metcalfe (2008) showed that short phases of compensatory growth caused a reduction in lifespan. Interestingly it has also recently been shown that 'negative' compensatory growth (= decelerated growth) has positive effects on swimming endurance (Chapter 2); moreover, in contrast to most other studies of the effects of accelerated growth (which rely on earlier food deprivation) this effect was produced after perturbation of growth through temperature manipulations, so avoiding confounding effects of early food deprivation on later viability. However, it is still unclear how various growth trajectories (e.g. accelerated vs. decelerated growth rate) influence longevity.

It is increasingly thought that ageing (or senescence) is the result of cellular damage accumulation over time (Balaban et al. 2005). Many physiological processes (e.g. cell division, protein synthesis and storage for growth and reproduction, etc) also generate cellular damage as a result of ROS production and subsequent oxidative stress. Recent experimental evidence supports the view that life history processes (e.g. growth, breeding

and lifespan) are negatively affected by the level of oxidative stress (Alonso-Alvarez et al. 2010). Oxidative balance is thought to be affected by growth rate (e.g increased SOD and CAT induced by compensatory growth, De Block and Stoks 2008), possibly because of the elevation of ROS production due to the high metabolic activities required for growth (Monaghan et al. 2009; Metcalfe and Alonso-Alvarez 2010). While recent studies have concluded that oxidative stress is one of the major causes of reduced lifespan in animals (Cai et al. 2007; Csiszar et al. 2007; Droge and Schipper 2007; Metcalfe and Alonso-Alvarez 2010), it is still unclear what mechanisms link growth, life-history and longevity.

According to life-history theories, finite resources must be allocated to maintenance and repair processes to retain cellular integrity to the extent needed to ensure offspring birth and survival (Kirkwood 1977; Kirkwood and Holliday 1979). However, these resources must also be traded-off with the needs for growth and reproductive investment, so as to maximise expected lifetime fitness. For instance, the investment in skeletal growth and somatic condition in female sticklebacks *Gasterosteus aculeatus* decreases during the breeding season whereas the investment in gonads increases, in order to maximise reproductive output (Poizat et al. 1999). Loss of condition during a breeding season may have severe effects on future fitness, but may be part of a programmed life history for short-lived species (e.g. sticklebacks) which normally live for only one or two breeding seasons and have poor chances of surviving for a further breeding season (Poizat et al. 1999). Therefore the life-history strategy of short-lived species may be strongly affected by the trade-off between investment in reproduction versus body maintenance (and hence lifespan). Moreover the strategy may be different between males and females due to the different reproductive costs that they incur. For instance, while females incur costs of producing offspring, males may have to fight to gain access to females or to defend their breeding site, mate or offspring. There may also be pronounced sex differences in parental care – for instance, in many species this is provided entirely by the female and the male provides nothing but gametes, whereas in some species (e.g. the three-spined stickleback) it is the other way around. Moreover, in species with indeterminate growth (such as fish) a female's fecundity is strongly related to her body size whereas the link between body size and reproductive success is less clear in males. All of these factors potentially lead to different optimal trade-offs between growth, maintenance and reproductive investment in the two sexes.

These trade-offs are likely to be influenced by the timescale over which any re-allocation of resources takes place. The perception of time of year may thus affect the

fitness consequences of variation in growth rate. Metcalfe et al. (2002) hypothesized that the degree and rate of compensatory growth after a period of disturbed growth would be influenced by the amount of time available to restore body size prior to a key life history event such as migration, metamorphosis or reproduction (the so-called 'time stress'). So I can presume that the accumulated level of oxidative stress or other damage induced by compensatory growth may also be altered by the degree of time-stress. My own results support the concept that there may be an effect of time-stress, in that three-spined sticklebacks that had a shorter time available prior to the breeding season to compensate for an earlier growth perturbation showed an increased rate of locomotor senescence as a result of accelerated growth (Chapter 2 and 4).

The aims of the present study were to investigate by means of experimental manipulations the effect of differing growth trajectories and levels of 'available time' on lifespan. By using a short-lived ectotherm (the three-spined stickleback), I was able to alter growth trajectories and perceptions of time till the breeding season by means of temperature and photoperiod manipulations respectively; the effect of time available from the growth perturbation until breeding was also investigated directly by replicating the experiment in different seasons. In addition, by manipulating growth by means of ambient temperature rather than food, and by including decelerating as well as linear and accelerating growth trajectories under differing degrees of time stress, I am able for the first time to evaluate effects of growth trajectory independent of effects of nutrition. I also examined the relationships between reproductive investment and lifespan for both sexes within treatment groups. The results clearly demonstrate the strong effects of growth trajectory on lifespan.

6.3 METHODS

The fish from the previously described experiments were examined during both their development and breeding periods, but there was third non-breeding season after 2nd breeding season (Period 6, Table 6.1). During every period, I monitored fish's survival on everyday and recorded a number and reason when I found dead fish.

TABLE 6.1 Description of temperature and photoperiod treatments. Note that following the four week manipulation period (Period 1), all fish were kept at 10°C (Period 2) until the start of the first breeding season (Period 3). Fish were kept at 14°C during both the first and second breeding seasons (Periods 3 and 5 respectively), and at 10°C during the intervening non-breeding season (Period 4 and 6 respectively). Normal food rations (fed *ad libitum*) were provided throughout.

Group	Photoperiod manipulation	Temperature manipulation Period 1	Period 2	Period 3	Period 4	Period 5	Period 6
HA	Ambient	High (14°C)	10°C	14°C	10°C	14°C	10°C
IA		Intermediate (10°C)					
LA		Low (6°C)					
HD	Delayed	High (14°C)					
ID	(35 days)	Intermediate (10°C)					
LD		Low (6°C)					

Statistical analysis

I collected data on survival until 28 June 2010, when only 18 fish remained alive. I analysed the longevity data with the 18 fish still alive being included as censored cases using a Cox's regression analysis with season of experiment (Winter or Spring), temperature (high, intermediate or low), photoperiod (ambient and delayed) and sex (male or female) as main effects, and compensatory growth rate and manipulated length (= length at the end of temperature manipulation period) as covariates, plus all interactions. Non-significant terms were sequentially dropped (based on likelihood ratios) by a backwards stepwise method. I assumed the same nominal birth date (1 June 2007) for all fish for the purpose of statistical analysis. A second Cox's regression explored the effects of reproduction by females on longevity. In this analysis (limited to those females that survived to at least the start of the second breeding season), the breeding strategy of each female was categorised as having spawned eggs in: both the 1st and 2nd breeding season (BB), only the first season (1B), only the second season (2B), or not spawned in either season (NB).

An equivalent Cox's regression analyzed the relationships between a male's pattern of red throat investment, his non-breeding growth rate (i.e. growth between the 1st and 2nd breeding seasons) and his lifespan. The pattern of red throat investment was quantified as the difference between the two breeding seasons in the number of weeks that a male maintained a red throat above a threshold value (taken to be the mean value for the population in the first breeding season) - so that a positive value indicates that the male was redder for longer in the second than the first breeding season, while a negative value indicates he was redder in the first season. A General Linear Mixed Model was then used to explore the relationship between this non-breeding growth rate and difference in duration of sexual ornamentation (see later for details). In all cases non-significant variables were sequentially dropped (least significant first) so that the final models presented here only include significant terms. All means are presented with standard errors and all of the analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, Illinois).

6.4 RESULTS

Lifespan

The temperature manipulations of growth rate had negligible short-term effects on lifespan, with virtually all experimental fish still being alive at the start of the first breeding season. However, mortality increased substantially during each breeding season (Fig. 6.1). Lifespan was significantly affected by the time of year at which the temperature manipulation of growth trajectories took place (Table 6.2). Thus fish in the Spring experiment died significantly sooner than those in the Winter experiment (Fig. 6.1); the median lifespan of fish (i.e. when 50% had died) in the Spring experiment was 739 days, at the beginning of the second breeding season, whereas that of fish in the Winter experiment was 873 days, falling in the early fall following that second breeding season. However, in both experiments there were similar and highly significant effects of the experimental manipulations. Thus fish in high temperature treatment groups lived longer than those in either intermediate temperature (Table 6.2, Fig. 6.1a and b; Wald statistic=23.30, d.f.=1, $P<0.001$) or low temperature groups (Wald=4.30, d.f.=1, $P<0.001$). These effects were more pronounced in the Spring experiment, where 50% of the Low temperature fish had died before the second breeding season (and most of the remainder during that second season) whereas half of the High temperature fish were still alive by the end of that second breeding season (Fig. 1a and b; the median lifespan was 556 days in Low, 761 days in

Intermediate and 903 days in High temperature fish). Independent of these effects, fish under the ambient photoperiod regime died sooner than those under the delayed regime (Table 6.2), with the difference in mortality rate being apparent from the middle of the first breeding season onwards in both Experiments (Fig. 6.1c and d).

While there was no effect on lifespan of fish size at the end of the temperature treatment (i.e. manipulated length; Wald=0.24, d.f.=1, P=0.626), there was a negative effect of compensatory growth rate (i.e. growth rate during the subsequent period) even after controlling for treatment effects (Table 6.2): faster growth in body length was associated with a reduction in lifespan. Lifespan was also significantly different between males and females (Table 6.2). Over all treatments, 66% of male sticklebacks had died by the end of the second breeding season in comparison with only 47% of females (Fig. 6.1e and f). This sex difference in lifespan was much greater in the Spring experiment than in the Winter experiment, leading to a significant interaction between season of experiment and sex (Table 6.2).

TABLE 6.2 Results of Cox's regression analysis on lifespan of sticklebacks, showing the significant effects of season (Winter or Spring), temperature (high, intermediate or low) and photoperiod (ambient or delayed) treatment, and sex (male or female). Overall significance of model: $\chi_7^2 = 76.501$, P<0.001. Non-significant candidate variables were dropped from the model.

	Wald statistic	d.f.	P	Exp(B)
Season	4.49	1	0.034	0.582
Temperature	23.74	2	<0.001	
Photoperiod	12.36	1	<0.001	1.756
Sex	16.32	1	<0.001	2.322
Compensatory growth rate	4.15	1	0.042	3278.975
Season × sex	4.46	1	0.035	0.535
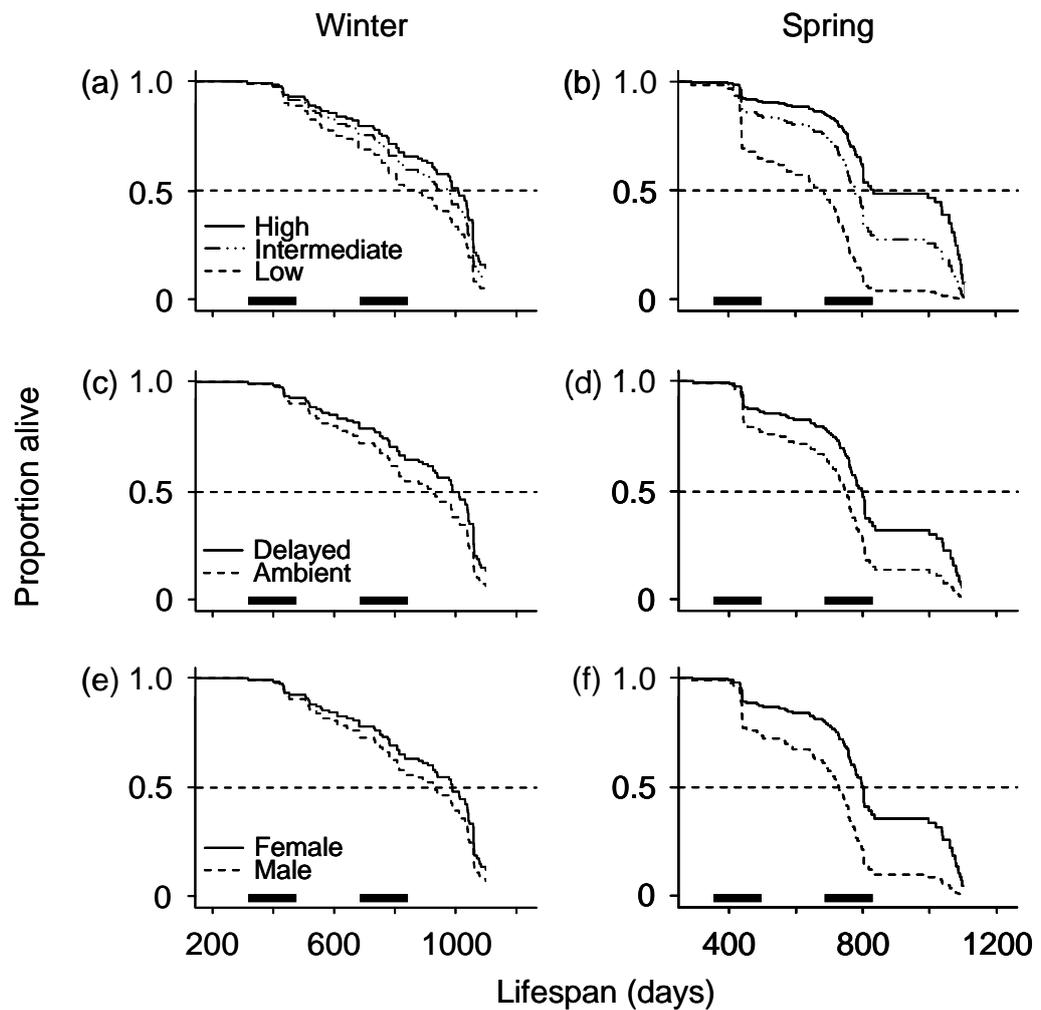
FIG. 6.1 Survival curves of three-spined sticklebacks in relation to temperature manipulation (a and b; High, solid line; Intermediate, double dashed line; Low, dashed line), photoperiod treatment (c and d; ambient, solid line; delayed, dashed line) or sex (e and f; female, solid line; male, dashed line) in the Winter (left panels) and Spring (right panels) experiments. The point at which each curve crosses the horizontal dashed line indicates the median lifespan. The two thick horizontal bars indicate the time of the 1st and 2nd breeding seasons. See text for statistical analysis.

Egg investment and lifespan in females

A total of 106 female fish were still alive at the start of the first breeding season (2008), of which 73 produced eggs. This had reduced to 80 females alive at the beginning of the second breeding season, of which 37 produced eggs (26 for the first time, while 12 spawned in both seasons). The relationships between breeding strategy and lifespan in

females that lived until at least the start of the 2nd breeding season were analyzed using a Cox's regression analysis, with breeding strategy (spawned in both breeding seasons (BB), only first season (1B), only second season (2B), or non-spawned (NB)) as a factor. Survival in females was significantly related to breeding strategy (Wald=11.34, d.f.=1, P=0.010). Females that lived as long as their 2nd breeding season but did not produce eggs in either the 1st or 2nd season died sooner than those that produced eggs, with the greatest lifespan being shown by females that spawned in both seasons (Fig. 6.2a). The biggest difference in mortality rates occurred during the second breeding season, with 63% of NB females that were alive at the start of the season having died by the end of it, in comparison with 50% of 2B females, 35% of 1B females, and 25% of BB females.

While the relatively small sample sizes precluded a detailed analysis of the links between temperature or photoperiod treatment and breeding strategy, it was clear that the three temperature treatments were not equally represented in the four breeding strategies, with females from the Low temperature treatment breeding less (e.g. being over-represented in the non-spawning category) and those from the High temperature treatment being disproportionately likely to spawn in both breeding seasons (Fig. 6.2b).
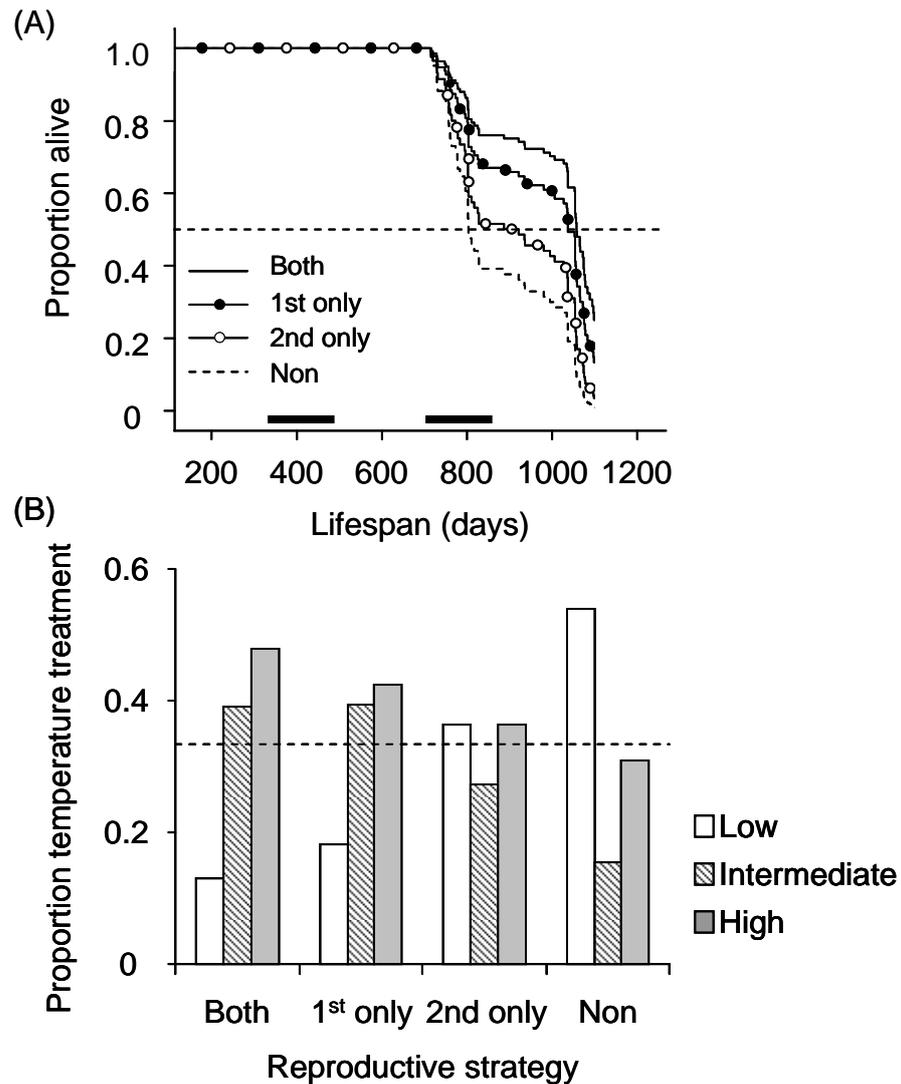
Fig. 6.2 (A) Survival curves of female sticklebacks that had survived to the start of the 2nd breeding season in relation to their reproductive strategy (spawned in both first and second breeding season, solid line; spawned in only first season, double dashed line; spawned in only second season, dashed line; failed to spawn in either season, thick line). The point at which each curve crosses the dashed horizontal line indicates the median lifespan, while the two thick horizontal bars indicate the two breeding seasons. See text for statistical analysis. (B) The proportion of females in a given breeding strategy that were from each of the three temperature treatments; the expected proportion is indicated by the horizontal line.

Reproductive investment and lifespan in males

A total of 90 males developed nuptial colouration (i.e. blue eye and/or red throat) during the first breeding season (2008), of which 3 males died before they had time to build a nest. 48 of these males entered the second breeding season (2009). The analysis of lifespan in

relation to non-breeding growth rate (= growth rate between the first and second breeding season) and the change in duration of red throat colouration is restricted to these males that lived to at least the start of the 2nd breeding season. The lifespan of these males was related to their non-breeding growth rate (Cox's regression, Wald=9.36, d.f.=1, P=0.002, Exp(B)<0.001): males that grew faster between the two breeding seasons also lived longer (Fig. 6.3A). While High temperature males tended to both grow more during the non-breeding season (Fig. 6.3a) and also (using the full data set) lived longer than Intermediate or Low temperature fish (Fig. 6.1a and b), there was no significant effect of temperature treatment groups on lifespan in this subset of males once their non-breeding growth rate had been taken into account (Wald=3.01, d.f.=2, P=0.222). Males tended to maintain their red coloration for a shorter period in the second breeding season compared to the first (so that the difference in red throat duration tended to be negative; Fig. 6.3b). There was a significant relationship between this difference in red throat duration and lifespan (Wald=10.64, d.f.=1, P=0.001, Exp(B)=0.854): the bigger the decline from 1st to 2nd breeding season in the duration of the red throat, the shorter the male's lifespan (Fig. 6.3b). This effect did not differ between temperature treatment groups (Wald=3.34, d.f.=2, P=0.188).

Given that both non-breeding growth rate and breeding coloration influenced lifespan, I examined the relationship between these two explanatory variables. I used a general linear mixed model with non-breeding growth rate as dependent variable, temperature (high, intermediate or low) and photoperiod treatments (ambient or delayed) as fixed effects, non-breeding tank identity as a random factor to control for the fact that several males were held in the same tank when not breeding, and the difference in red throat duration from 1st to 2nd breeding season and length at the end of first breeding season as covariates, plus all interactions. The non-breeding growth rate of males was positively related to the difference in red throat investment (GLMM, $F_{1,\ 30.55}$=7.76, P=0.009): males that grew least well over the non-breeding season also showed the biggest reduction in red ornamentation in their second breeding season (Fig. 6.4). While there was no effect of a male's length at the end of the first breeding season on his subsequent growth rate up to the next breeding season ($F_{1,\ 30.19}$=2.24, P=0.145), there was a significant interaction between his size at the end of the 1st season and the difference in red throat duration ($F_{1,\ 30.70}$=7.64, P=0.010): males grew fastest when they were already large at the end of the first season and where they were able to maintain their redness in the second

breeding season, indicating that the individual variation in non-breeding growth rate was primarily a consequence of variation in fish quality.
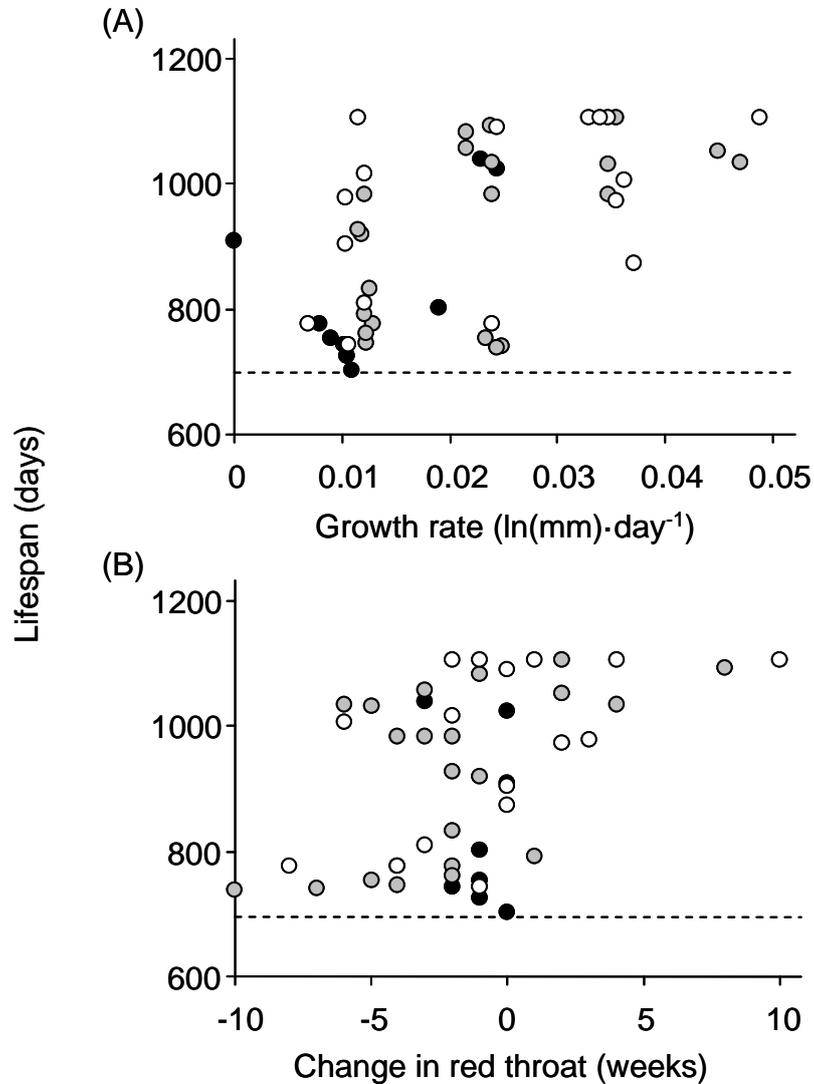
Fig. 6.3 Lifespan in male three-spined sticklebacks in relation to (A) growth rate during the non-breeding period (i.e. growth rate between end of first and beginning of second breeding season) and (B) the change in duration of red throat colouration above a threshold (see text) between the first and second breeding season (where positive values indicate the duration of the red throat was longer in the second season than the first). Data are shown separately for High (white symbols), Intermediate (grey) and Low (black) temperature manipulation groups. The dashed horizontal line indicates the age at the start of the 2nd breeding season. See text for statistical analysis.
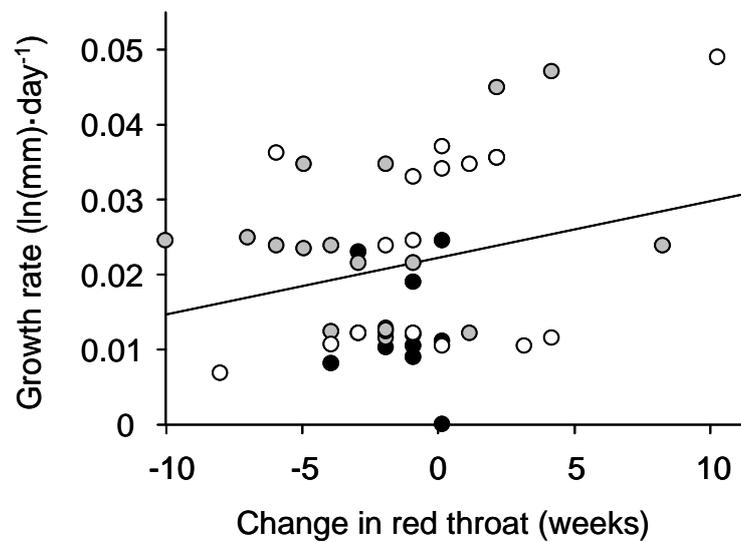
Fig. 6.4 Non-breeding growth rate (= growth between the 1st and 2nd breeding seasons) in male three-spined sticklebacks in related to difference in duration of red throat ornamentation between the 1st and 2nd breeding seasons in three temperature treatments (high – white circle, intermediate – grey, low – black); there was no effect of temperature treatment and so the regression line is based on the combined data for all treatment groups (see text for analysis).

6.5 DISCUSSION

I successfully demonstrated that environmental change in early life can influence lifespan, and that there can be relationships between reproductive investment and survival (although these may be in the opposite direction to those predicted by trade-offs). Temperature manipulation gave rise to a reduction in lifespan in the low temperature fish, and an increase in the high temperature fish, relative to the intermediate temperature group. The lifespan of the ambient photoperiod fish was on average shorter than that of the fish in the delayed photoperiod group. Female sticklebacks lived longer than males, and overall fish in the Spring experiment died earlier than those in the Winter experiment.

My recent results showed that the temperature manipulations clearly induced compensatory growth trajectory in both directions (= accelerated growth in response to an earlier period of low temperatures, and decelerated growth after a period of high temperatures). These compensatory growth trajectories have earlier been found to influence swimming endurance (Chapter 2) and reproductive investment (Chapter 3) in

opposite directions, with accelerated growth having negative effects in comparison to steadily growing control fish (= intermediate group), while a decelerating growth trajectory had improved performance relative to the controls. The same pattern has now been shown in terms of longevity. Although it has previously been documented that the longevity of ectotherms can be extended at cooler temperatures (Cailliet et al. 2001; Valenzano et al. 2006; Hsu and Chiu 2009) or through dietary restriction (Terzibasi et al. 2009), this study is the first to report that a period of warmer temperatures extends lifespan, moreover the differences in survival occurred without any food restriction. I found that, while females lived linger than males, lifespan was influenced by compensatory growth in the same way in both sexes despite their having different reproductive costs. Moreover the effects on lifespan were modified by the different amount of time available until breeding.

The mechanism underlying these trends is not known, but may relate to oxidative stress. An increase in ambient temperature after a cooler period can induce hyperphagia (e.g. juvenile brown flounde, Huang et al. 2008), which results in a more rapid growth rate but also an increased metabolic demand. Jennings et al. (Jennings et al. 2000) provided evidence that growth acceleration (= compensatory growth) increased oxidative stress levels and rates of cellular damage and senescence in mammals, which may be linked to organismal senescence due to the effects of growth rate on telomere lengths (Tarry-Adkins et al. 2008; 2009). Moreover, there may be effects on external causes of mortality as well as rates of senescence, since elevated levels of oxidative stress can impair the immune response and so make it more likely that survival will be reduced through disease. Conversely, the growth deceleration caused by earlier high temperatures in the high temperature treatment fish may have led to their accumulating lower levels of damage than the steadily-growing controls since a suppressed growth rate will have resulted in lower metabolic demands (hence lower production of ROS) and potentially greater allocation of resources to repair rather than new growth. However, further experiments are needed to test whether decelerating growth trajectories do result in reduced levels of cellular damage and improved immune responses.

It is generally assumed that animals face life history trade-offs, for instance between growth and reproduction, or reproduction and survival. These costs are usually different between males and females since the reproductive roles depend on sex (for instance, in sticklebacks nest building, egg fanning and defence are carried out by the male while females are only responsible for egg production), and hence the sexes will have different programs of resource allocation. In the present experiment the longevity of

females was greater than that of males, presumably because males paid greater reproductive costs. Sex differences in survival linked to differences in reproductive costs have also been found in other species (Hoffman et al. 2008). However, the present study does not provide evidence of trade-offs between reproduction and survival. Taking fish that had survived until at least the start of the second breeding season (to control for differences in opportunity to breed), the lifespan of females that never produced eggs was actually less than those that spawned, and the females that lived longest were those that had spawned in both breeding seasons. These data showing a positive relationship between reproduction and longevity in female might appear to conflict with predictions from evolutionary theories of ageing (Williams 1957; Kirkwood and Rose 1991) and, more generally life-history evolution (Westendorp and Kirkwood 1998). However, this is almost certainly because I did not manipulate reproductive effort in this experiment and so females were able to allocate resources to reproduction according to their current state or condition: females in better condition (e.g. because they were of better genetic 'quality') would have been able to both produce clutches and have enough resources left over to repair damage and maintain their somatic tissues, whereas the poorest quality animals would not have been able to invest adequately in either reproduction or self-maintenance. This would have led to a positive correlation between reproduction and survival (Reznick et al. 2000). It is only by carrying out experiments in which reproductive effort is manipulated that the true nature of the relationship is found – as was shown by Olsson et al. (Olsson et al. 2001), who found that the positive relationship between reproductive investment and survival in unmanipulated female lizards was reversed when females were allocated to treatment groups in which their reproductive effort was manipulated. Unlike long-lived species, short-lived species such as sticklebacks tend to invest significant resources in the first breeding season in which they are large enough to breed due to there being no guarantee that they will survive to the next breeding season. However, many of the females in the low temperature group (who had undergone growth acceleration) produced no eggs in the first breeding season. This may have been because the fish had built up a high level of damage in their phase of rapid growth just prior to the onset of the first breeding season, and so were not in good enough condition to breed. The reproductive output of those that did breed was also reduced (see Chapter 3), which further strengthens this hypothesis. Fish that did not breed in their first season often did so in their second, possibly because they had had time to recover condition, repair damage and build up gonadal tissue, but there were some fish that failed to breed in either season, presumably because of their poor condition (as was evident from their high mortality rate through the

second breeding season, despite not breeding). However, further manipulation experiments are needed to examine the true form of the relationships between a female's growth rate, egg production and life expectancy.

In male sticklebacks, skeletal growth either reduced or stopped at the onset of each breeding season but resumed at the end of the breeding season. There were positive relationships between a male's rate of growth between the first and second breeding season, the extent to which he maintained or enhanced his red throat colouration in that second breeding season (taken as an indicator of reproductive senescence) and his lifespan. Thus males that grew fast between seasons also managed to sustain their red throat colour in the second season, and also survived longer after the start of that second season. Since post-breeding growth, survival and future reproductive effort are all likely to depend on the animal's ability to acquire resources, this again suggests that there was significant variation in individual quality within each treatment group such that high quality fish (whether due to their genetic background or their early development) were able to simultaneously invest more in growth, sexual ornamentation and somatic maintenance than poorer quality males in the same treatment group. It is only by manipulating either resource intake and/or reproductive rate that it is possible to investigate these trade-offs – as in the recent study by Pike et al. (2010), who showed that the locomotor senescence of male sticklebacks was faster if their reproductive investment was increased (by making them re-build nests and court females), especially if they were also on a poorer quality diet. While my data cannot fully unravel these effects and so future work is needed, I suggest that the longevity of both sexes was affected by the tempo and degree of early growth trajectories in life but would also be related to levels of reproductive investment if this had been manipulated.

Most circadian and circannual systems in animals are involved in the temporal organization of a range of physiological and behavioural processes. Seasonal changes, particularly photoperiod and temperature, can prompt alterations to many physiological parameters, which may in turn influence growth rate and reproduction effort. Metcalfe et al. (2002) hypothesized that both the rate of compensatory growth and its impact would be influenced by the amount of time available until key life history events such as a breeding or migratory season (called the 'time-stress'). My findings support the 'time-stress' hypothesis. The negative effects of compensatory growth on lifespan were also dependent on photoperiod and time of season: effects were less if the time apparently available until the breeding season was increased (= delayed photoperiod, reduced time-stress), but increased if the time was apparently short (= the Spring experiment, increased time-stress).

While there is no evidence of a relationship between perception of time of year and lifespan, the modified rate of compensatory growth presumably affected both the level of accumulated damage and the time available in which to repair it. This had subsequent effects on locomotor performance (Chapter 2) and reproductive investment (Chapter 3) and the presumed trade-off between reproduction and survival, and resulted in alterations to lifespan. However, the mechanisms underlying the links between these traits cannot be verified without further study.

CHAPTER 7

GENERAL DISCUSSION

This thesis successfully demonstrated how early temperature and nutrition conditions can change growth trajectories and how such modified juvenile growth patterns can influence subsequent performance and lifespan. In addition, I developed dynamic state dependent models of trade-offs between growth, damage and reproduction in order to investigate how decision in life-history can strategically be changed to optimise fitness in relation to growth opportunities in early life. A manipulated episode of poor conditions during the juvenile period clearly induced compensatory growth, and interestingly trajectories of compensatory growth varied depending on the conditions during the short exposure period in early life: accelerated (brought about by low temperature or restricted diet), steady, and decelerated growth (brought about by high temperature). Overall, growth acceleration had negative effects on locomotory and breeding performance and on lifespan, whereas there were positive impacts of 'negative' compensatory growth (i.e. growth deceleration) on these outcomes. Moreover experimental evidence in this thesis strongly supported the time-stress hypothesis (Metcalfe et al. 2002) that the perception of time of year has consequences for juvenile growth patterns.

Large body size in animals has many advantages (e.g. mating, reproduction and survival) and so the fast growth to attain this may be favoured during development. Recent analyses in several taxa showed that growth is accelerated to reach a normal adult size if conditions improve after slow growth during an episode of poor conditions (e.g. Dobson and Holmes 1984; Miglavs and Jobling 1989; Quinton and Blake 1990). While there was an advantage in attaining large body size, such results showed negative effects on subsequent events: for instance, locomotory performance (Dawson et al. 2000; Álvarez and Metcalfe 2005; de la Hera et al. 2009), reproduction (Auer et al. 2010) and lifespan (Rollo 2002; Metcalfe and Monaghan 2003; Ricklefs 2006; Inness and Metcalfe 2008). Obviously animals need more resources when growth is accelerated and so usually show hyperphagia (Bull and Metcalfe 1997; Jobling and Johansen 1999; Ali and Wootton 2000; Gurney et al. 2003; Huang et al. 2008). However, rapid growth rate can increase cellular damage and metabolic rate (Jennings et al. 2000; Morgan et al. 2000; Pike et al. 2007; Criscuolo et al.

2008). In damselflies *Lestes viridis*, for instance, body levels of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were highest during a phase of accelerated growth (De Block et al. 2008). It is known that the causes of growth manipulation (dietary and temperature in this thesis) can directly influence the damage level. While Metcalfe and Alonso-Alvarez (2010) highlighted that changes in environmental temperatures (i.e. temperature manipulation) may cause oxidative stress, since animals may respond to temperature challenges by up-regulating antioxidant enzymes in tissues (Selman et al. 2000) and mobilising dietary antioxidants (Eraud et al. 2007), the damage will be repaired when conditions improve. However, the rapid growth itself can cause increased metabolic rate in adulthood (Criscuolo et al. 2008), and the resulting oxidative stress could then increase the rate of cellular damage (Jennings et al. 2000; Monaghan and Haussmann 2006), or muscle wastage (Kamel 2003). Hence life-history events can be continuously influenced by earlier growth patterns, even though environmental conditions may have improved.

During development, damage by accelerated growth may modify muscle structure and so reduce locomotor performance (Chapters 2 and 4). Differences in the timing of muscle fibre recruitment have been shown to lead to different compositions of white and red muscle fibres (Johnston 2006), which may be the cause of impaired locomotor performance. Effects of embryonic conditions on muscle development and subsequent motor performance have been reported in several taxa. For instance, effects of early growth rate on tail muscle fibre numbers and swimming performance have been found in tadpoles of both toads (Arendt and Hoang 2005) and frogs (Watkins and Vraspir 2006), and growth rate in birds can affect the size and total number of myofibres independently of muscle type (Remignon et al. 1995). Consequently, it has been suggested that such a trade-off between early growth rate and locomotor performance is common to all vertebrates (Arendt 2003).

Recent results also show that accumulated damage over the breeding season can negatively affect reproductive and locomotor performance (e.g. cost of sexual ornamentation (Pike et al. 2010 and Chapter 3 and 4), egg production (Chapter 3) and swimming endurance (Pike et al. 2010 and Chapter 2 and 4)). While the physiological processes of growth acceleration are expected to reduce reproductive capacity because of increased cellular damage and metabolic costs (Metcalfe and Alonso-Alvarez 2010), surprisingly little evidence of this has been reported. Studies in wild mammals have shown how oxidative damage caused by pollutants impairs fecundity or fertility (e.g. bonnet

monkeys *Macaca radiate* (Subramanian et al. 2006) and red deer *Cervus elaphus* (Reglero et al. 2009)). Recent results have shown negative effects of compensatory growth on reproductive effort in Trinidadian guppies *Poecilia reticulate* (Auer et al. 2010) and in three-spined sticklebacks (Chapters 3 and 4). Using dietary experiments, Pike et al. (2007) showed that a reduced dietary antioxidant intake in male sticklebacks led to reduced levels of defence against oxidative stress, with breeding investment then reducing due to the high level of oxidative stress. During the reproductive period, the accumulated damage could increase due to a rise in the risk of ovarian oxidative damage (Behrman et al. 2001): in zebra finches, for instance, the resistance to ROS (reactive oxygen species)-induced haemolysis is negatively related with clutch size during the breeding period (Bertrand et al. 2006). Moreover, the timing of death, the final event in life, can be related to this increased level of accumulated damage. Immune responses and protein levels in the body can be reduced by a high level of oxidative stress, and hence the increased probability of infectious disease may reduce survival rate. Most notably, accelerated telomere shortening by oxidative stress (von Zglinicki 2002) can negatively affect lifespan (Epel et al. 2004).

Obviously animals pay costs of growth and reproduction (Roff 1992). Moreover, the biotic and abiotic resources in nature are always finite, and the acquisition of resources may be costly in terms of predation risk. Natural selection will favour the best strategy in a given circumstance. Recent results (Metcalfe and Monaghan 2001; Metcalfe and Monaghan 2003; Mangel and Munch 2005; Auer et al. 2010) suggest that compensatory growth may be a well-adapted strategy induced by trade-offs among growth, reproduction and survival. While slow growth rate by survival-biased allocation may be selected as the optimal strategy during poor conditions (e.g. reduced food supply or unfavourable temperatures), accelerated growth by growth-biased allocation may be preferred when conditions recover so that the individual can reach a normal adult size with high reproductive potential. It is known that somatic growth in many species is reduced at the beginning of the breeding season while gonad weight increases relative to body weight (e.g. in fish: three-spined sticklebacks (Poizat et al. 1999) and round sardinella *Sardinella aurita* (Tsikliras et al. 2007); insects: burying beetle *Nicrophorus orbicollis* (Creighton et al. 2009)). When resource allocation is biased towards skeletal growth during compensatory growth, there can be negative impacts on development of non-reproductive structures (Ricklefs et al. 1994; Arendt et al. 2001; Arendt 2003) and hence the development of reproductive structures may also be affected. While experimental evidence in this thesis

(Chapters 3 and 4) support this implication, more evidence of negative effects on reproductive development and physiology is needed.

The change in allocation of resources towards reproductive functions at the beginning of the breeding season can disturb the balance between reproduction and survival, and thereby the outcomes are changed: therefore there may be negative effects of compensatory growth (rapid growth) on reproductive investment and gamete production (Auer et al. 2010 and Chapter 3 and 4). Post-breeding survival and future reproductive events depend on the level of surplus resource and the amount of accumulated damage at the end of the first breeding season. For instance, the lifespan of the female cricket *Gryllus lineaticeps* is longer when the number of matings is increased since males transfer a protein-rich spermatophore as a nuptial gift to the female during copulation (Wagner and Harper 2003). Moreover the degree of fat reserves in female Great tits *Parus major* sharply decreases over the breeding season, and birds may abandon one breeding season when their body condition is poor and instead recover their condition so as increase their chances of being successful in the next breeding season (Gosler and Harper 2000). While it is possible to abandon or reduce reproductive effort in long-lived species, short-lived species have to invest usable resources in reproduction since their chances of surviving to breed again are small. Reductions in available resources and increased damage levels could cause the decline in lifespan observed in this thesis, since sticklebacks are a short-lived species with only one or two breeding seasons. In relation to damage, particularly, Pike et al. (2010) showed that sticklebacks with low antioxidant levels had a rapid decline in swimming endurance during the breeding season whereas the endurance in fish with a high antioxidant diet remained stable. Elevated damage levels, particularly in accelerated growth groups, may have resulted in a decrease in reproductive investment and post-breeding growth rate and so longevity was reduced, whereas low levels of stress, which have been shown to be associated with growth deceleration, may have had a positive effect on immune function and so lifespan was extended.

In animal systems, the circadian rhythms of most physiological parameters (e.g. heart rate and body temperature) are related to the external environment. Because the time of year is recognized by length of day, environmental changes, particularly related to seasonal shifts, can alter biological rhythms and so influence growth rate by affecting the time available per day for feeding and reproductive activities. Photoperiod may also alter patterns of resource allocation: for instance, the development of reproductive tissue in birds (Jones 1986) and mammals (Steinlechner and Niklowitz 1992). It is possible that

changed rhythms may induce compensatory growth as a consequence of a perception of time of year since the time of the season may be a crucial factor in determining growth opportunity (Metcalfe and Monaghan 2001). Metcalfe et al. (2002) hypothesized that animals should be sensitive to the amount of time available when altering their growth trajectory to compensate for a period of perturbed growth, showing a stronger compensation (and hence potentially greater long term costs of compensation) when the time until an approaching life history event such as reproduction is shorter (so called the time-stress). Results in this thesis provide strong support for the hypothesis: negative effects of compensatory growth on whole life-history events (e.g. growth and reproduction) were reduced when the time available until the fish's breeding season was extended; unexpectedly lifespan was also increased. While there is no experimental evidence, a longer time in which to repair any damage in the run up to the breeding season may have led to a different balance of investment between somatic repair and gonad growth, hence a slower accumulation of cellular damage (Jennings et al. 2000). Because of the altered amount of accumulated damage, there may have been modified outcomes of subsequent reproductive investment and also revised solutions of the evolutionary trade-off between reproduction and survival (Metcalfe and Alonso-Alvarez 2010). However, it is still not clear that early growth rate and later consequence are affected by this time stress. In the Lestes damselfly, for instance, there was no evidence for effects of time stress on compensatory growth, in contrast to effects of food and thermal stress (De Block et al. 2008), and some mammals are not reproductively photo responsive (e.g. the red flying fox Pteropus scapulatus, O'Brien et al. 1993) so are unlikely to respond to photoperiodic evidence of time stress. Further study to examine the effects of time stress on early growth is needed, since stress-response strategies may vary between habitat features, feeding conditions and metabolism in species or populations.

This thesis has shown juvenile growth trajectory in the three-spined stickleback significantly influences subsequent performance and life history events (i.e. locomotion, reproduction and lifespan) and that time-stress can change the degree of impact of growth trajectory on the events. While it is well known that compensatory growth negatively affects subsequent events, much of the evidence has come from fragmentary studies of parts of the life history. This thesis has firstly shown that there are continuous impacts on major key events in life (i.e. pre-breeding, breeding and post-breeding season). Moreover, I have shown that a high temperature manipulation can induce 'negative' compensatory

growth which has 'positive' effects on the key events (resulting in better performance than that arising from steady growth under constant conditions).

These findings extend our knowledge of how early growth costs are related to subsequent performance. However, there are still unanswered questions. Firstly, while this thesis shows that 'negative' compensatory growth could be induced by an earlier period of higher temperature in which growth was increased, it is well known that temperatures greater than the optimum temperature for a given species can generally result in reduced growth rates due to elevated metabolic costs (Wootton 1998). So, presumably there may be a different pattern of compensatory growth if ectotherms are exposed to these high temperatures that are associated with poor growth. Secondly, in relation to reproductive performance, the experiments in this thesis did not include assessment of the incubating and hatching performance of the male sticklebacks, nor their ability to defend a nest site against rivals and so, unlike females, males in these experiments had reduced reproductive costs compared to the natural situation. The analyses of reproductive investment in this thesis are therefore not the complete picture, and there may be different effects between males and females in their locomotor performance over the breeding season since the reproductive behaviours in male sticklebacks include significant levels of activity (e.g. male spends more time fanning eggs mature, Wootton 1976). There are other still unresolved questions. For instance, further studies are needed to determine how compensatory growth rate influences metabolism and damage accumulation, and how thermal stress incurred by early environmental conditions affects growth and fitness in the next generation. This thesis showed that parents with high levels of accumulated damage have lower reproductive investment but also poorer locomotor function. Obviously there are negative effects for the offspring of a reduction in egg size, but if males are less able to fan the eggs then the resulting lower oxygen supply for the eggs during the incubation period may damage embryo development. So, further studies to show how the costs of early growth affect maternal condition and life history events in the next generation are desirable.

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