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Experimental demonstration of the growth rate–lifespan trade-off

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The hypothesized negative relationship between growth rate and lifespan has proved very difficult to test robustly because of potentially confounding variables, particularly nutrient availability and final size. Here we provide, to our knowledge, the first rigorous experimental test of this hypothesis, and find dramatic changes in lifespan in the predicted direction in response to both upward and downward manipulations of growth rates. We used brief (less than 4% of median lifespan) exposure to relatively cold or warm temperatures early in life to deflect juvenile three-spined sticklebacks *Gasterosteus aculeatus* from their normal growth trajectories; this induced catch-up or slowed-down growth when ambient temperatures were restored, and all groups attained the same average adult size. Catch-up growth led to a reduction in median lifespan of 14.5 per cent, while slowed-down growth extended lifespan by 30.6 per cent. These lifespan effects were independent of eventual size attained or reproductive investment in adult life. Photoperiod manipulations showed that the effects of compensatory growth on lifespan were also influenced by time available for growth prior to breeding, being more extreme when less time was available. These results demonstrate the growth–lifespan trade-off. While growing more slowly can increase longevity, the optimal resolution of the growth–lifespan trade-off is influenced by time constraints in a seasonal environment.

1. Introduction

Central to our understanding of the evolution of life histories is the fundamental concept that there are trade-offs in resource allocation among the key life-history traits of growth, reproduction and lifespan [1]. The focus of attention has been on the trade-offs involving reproduction, but there has long been a suggestion that growth rate may be negatively linked to lifespan, owing to a presumed link between rates of cell division, oxidative stress and rates of cellular senescence [2,3]. However, the extent to which growth rate *per se* is involved in such trade-offs is poorly understood. Correlative studies have provided only circumstantial evidence for an association between growth and longevity, owing to the confounding link between growth rate and adult body size [2,4,5]. Experimental studies in which growth rates are manipulated offer some support, but, since this manipulation has always been achieved by periods of dietary restriction, these studies suffer from the problem that negative effects on lifespan may be a consequence of early undernutrition rather than the tempo of growth [6]. Furthermore, such studies [7] have focused only on the effects of accelerated growth; the prediction that slowed growth leads to increased longevity has not, to our knowledge, hitherto been tested.

The resolution of such life-history trade-offs is likely to be influenced by the timescale over which any re-allocation of resources takes place [8]. In seasonal environments, the period of time available for growth may thus affect its fitness consequences [9]. For instance, it has been hypothesized [10] that the degree and rate of compensatory growth after a period of growth restriction 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 hypothesis'). If this is so, the consequences of any changes in growth rate should also be influenced by the degree of time stress under

which they occur. Recent experimental results provide some support for this hypothesis: animals given a shorter time period over which to attain a target size prior to reproduction showed reduced starvation resistance, poorer locomotor and breeding performance [9,11,12]. However, it is not known whether time-stress influences any effects of growth rate on potential lifespan.

Temperature manipulations can be used to manipulate growth trajectories in ectotherms such as fishes, with a brief cold spell inducing catch-up growth while a mild spell will induce 'slow-down' growth once animals are returned to their previous, intermediate, temperatures [12]. The degree of perceived time stress can be altered by using shifted photoperiod regimes [12]. By using both temperature and photoperiod manipulations, we examined here: (i) how perturbations of growth trajectories affect lifespan, and (ii) whether such effects are influenced by time stress. Our hypothesis was that catch-up growth (in which the rate of growth is faster than would normally be the case for the animal's age and prevailing conditions) should decrease lifespan, while a comparable slowing of growth should increase it. We predicted that both of these effects would be influenced by time stress, with the effects on growth rate and lifespan being more pronounced the shorter the time available for size recovery prior to a key life-history event (in this case the breeding season). Thus, the rate of catch-up growth and/or the subsequent cost to lifespan should be greater when there is a shorter period of time available to compensate prior to the breeding season. Since our predictions relate to the direct effects of growth rate on lifespan, and since the manipulations had no effect on final adult size [12], we expected that the growth–lifespan interrelationships would be independent of any indirect consequences for lifespan that come about through effects of growth rate on reproductive investment or adult body size.

2. Material and methods

(a) Fish and rearing conditions

On 1 November 2007, we collected juvenile (based on body length) three-spined sticklebacks from the River Endrick, Scotland, UK (56°04′ N, 4°23′ W) using a dip net and minnow traps. In the source population, the sticklebacks begin breeding in May. In order to analyse the effect of the amount of time available before the start of the breeding season on the extent and consequences of compensatory growth we repeated the experiment with new sticklebacks collected from the same population on 29 January 2008. There were therefore two experiments differing in the time at which the growth perturbation was conducted (= winter and spring experiment), potentially allowing the fish a long and short time, respectively, to recover from the growth perturbation prior to the breeding season. This enables us to test the time-stress hypothesis. However, because of the fact that the fish would also differ in maturational stage or size at the commencement of the two experiments, we also manipulated time stress experimentally by exposing the fish in both the winter and spring experiments to different photoperiods (see below), thereby within each experiment altering the perceived time to the onset of breeding in fish at the same stage. These photoperiod manipulations had the further advantage that, since they potentially alter growth trajectories without requiring any further changes to temperature regimes, they provide a control for the direct effects of exposure to warm or cold spells and additional test of the effect of growth compensation on lifespan. Our predictions were that when fish exposed to the same temperature regime perceive there to be less time available for growth compensation, the effect on growth rate will be greater, with correspondingly greater effects on lifespan.

Immediately following capture, all fish were initially held for three weeks in acclimatization aquaria (80 l and density two fish $\cdot l^{-1}$) at 9.7 $\pm$ 0.1°C under an ambient photoperiod. We changed 25 per cent of the total water every week, adding a small amount of seawater to prevent the risk of whitespot infection *Ichthyophthirius multifiliis*. Throughout the experiment, we fed the fish ad libitum with frozen chironomid larvae once per day: all tanks were checked daily and uneaten food removed in order to prevent any deterioration in water quality (all treatment groups had similar daily intakes after the end of the temperature manipulation and compensation; see [11]).

(b) Temperature and time-stress manipulation

On 21 November 2007 and 21 February 2008 (for the winter and spring experiments, respectively) fish were sorted into groups of five fish of different size, to aid within-group identification; regular measurements throughout the experiment confirmed that size ranks never changed within a tank. Each group of five fish was placed in a separate tank (335 × 170 × 185 mm) with aeration, a filter and artificial plants. For four weeks at the start of each experiment (period 1) we randomly assigned each tank to one of three temperature manipulations (14°C, simulating a mild spell; 6°C, simulating a cold spell and 10°C, representing constant conditions close to the mean annual water temperature in the source river at the fish capture site, [13]). The 4°C shift in the temperature experienced by the fish experiencing either a mild or cold spell was unlikely to have caused any physiological stress to the fish, since it is less than the range in temperatures experienced by fish every week at the collection site (Drumtian Ford) in the source river at the corresponding time of year (mean range from weekly minimum to maximum in November–December = 5.22 $\pm$ 0.50°C; February–March = 4.65 $\pm$ 0.61°C; [13]). However, these contrasting temperatures were sufficient to cause perturbations in growth, with fish experiencing the short mild spell growing faster than expected, and those experiencing the cold spell falling behind. (Note that the increased or decreased growth during this short stage is a direct consequence of the effects of the different temperatures on the food processing capacity of the fish, rather than the shift in resource allocation that would be required to fuel differences in growth at the same temperature, as in period 2.) Following this four-week temperature manipulation, all fish were kept at the same standard temperature regime for the rest of the experiment. They were thus held at 10°C during the non-breeding seasons (periods 2,4 and 6) and 14°C during breeding seasons (periods 3 and 5), these temperatures approximating local mean ambient conditions (this experimental protocol is illustrated in the electronic supplementary material, text S1 and figure S1).

During period 2, all fish experienced the same local ambient temperature, but their previous growth histories put them behind or ahead of schedule. We therefore predicted that fish in the three temperature-treatment groups would show different growth trajectories during period 2, despite all fish being under the same environmental conditions: those that had experienced the same temperature throughout (=control group) would grow at a constant rate, those that had fallen behind owing to the cold spell would attempt to compensate by growing faster than normal (=catch-up group), and those that were ahead of their growth schedule owing to experiencing a mild spell would subsequently grow more slowly than normal (=slow-down group). Unlike the differences in growth directly induced by environmental temperature during period 1, such differences in growth at the same temperature will require differences in resource allocation strategies.

We replicated the three temperature-treatment groups under two different photoperiod regimes in both the winter and spring experiments: a natural (ambient) photoperiod regime and a delayed photoperiod regime. Under the latter regime, fish were transferred to a day length which corresponded to a point 35 days earlier in the year. We used fluorescent lights controlled by electronic timers to produce simulated daylight for all fish, with blackout plastic sheeting around the tanks being used to achieve independent lighting regimes. After the initial adjustment of photoperiods we maintained the same seasonal rate of progression of the photoperiod for both the ambient and delayed groups, so that for the rest of the experiment the delayed group were continually at a stage 35 days earlier in the season (giving the delayed group in both the winter and spring experiments longer to recover prior to the breeding season from the growth perturbation caused by the temperature manipulation).

To summarize, in both the winter and spring experiments there were six manipulation groups (three growth × two photoperiod treatments), each with four replicate tanks. We randomly allocated the four replicate tanks of five fish to each of these manipulation groups in both experiments; the analyses in this study are therefore based on a total initial sample size of 240 fish. Details of the effects of the treatments on the swimming and breeding performance of the same fish are given in [11,12], respectively; here we examine the effects on lifespan.

(c) Survival and growth rates

Tanks were monitored daily and a record was made when each fish died (the last one dying on 20 June 2011). All fish were weighed and measured (standard length) every two weeks during the temperature-treatment period and every three weeks thereafter, being starved for 24 h prior to measuring. Compensatory growth rate was calculated as $100(\ln L_f - \ln L_i)/t$ for length, where L_i and L_f refer, respectively, to the manipulated fish length (length at the end of period 1) and the length at the time point in period 2 when the growth trajectories had converged such that there was no longer a significant difference in mean length between temperature-treatment groups; t is the time interval in days between these two measurements (105 and 84 days in the winter and spring experiments, respectively). The growth rate of adult fish between their first and second breeding season (referred to as the non-breeding growth rate) was calculated using the same equation as for compensatory growth rate, with L_i being the fish's length at the end of the first breeding season and L_f its length at the beginning of the second breeding season. The dates taken to be the end of one breeding season and start of the next were based on the changes in sexual ornamentation of the fish (see the electronic supplementary material and [11] for details).

(d) Reproduction

Analyses of the effect of the experimental manipulations on the reproductive investment of the fish (egg size, clutch size and the total number of eggs laid by females, intensity and duration of sexual ornamentation and nest-building rate of males) are presented elsewhere [11], and in this paper we consider only whether either the timing or investment in reproduction was related to lifespan.

On 16 May 2008 and 3 July 2008 (winter and spring experiments, respectively), we began to assign males that had started to develop the typical sexual ornamentation (blue eye coloration and reddish throats [14]) to individual tanks of the same size, with a Petri dish containing fine sand (i.e. a nesting dish) and nesting material (50 × 5 cm lengths of thread). For four weeks each male was shown a gravid female enclosed in a Plexiglas container for 5 min twice daily [15], in order to prompt full expression of nuptial coloration. Males were returned to their original group tank (of initially five fish) at the end of the first breeding season, but were again placed in individual breeding tanks for the second breeding season. We kept females in their original group tanks throughout the experiment, and stripped them of clutches of eggs whenever they became fully gravid.

The red throat of males was photographed weekly for 16 weeks in each breeding season in order to quantify temporal changes in their investment in sexual ornamentation; full details of the protocol are given in the electronic supplementary material, text S2 and in [11]. We re-measured the same measures of breeding investment during the second breeding season for any fish that survived that long; the very few fish (8%) that survived to the third breeding season showed little tendency to breed at that age [11] and so reproductive investment (i.e. egg production, sexual ornamentation) was only measured over the first two seasons.

(e) Statistical analysis

A linear mixed effect model (LME) was used to analyse effects of temperature and photoperiod manipulations on compensatory growth rate with experiment (winter or spring), temperature (cold, mild or control), photoperiod (ambient or delayed) and sex (male or female) as fixed effects, manipulated fish length (at the end of the temperature manipulation in period 1, ln-transformed) as a covariate, and tank as a random factor, plus all interactions. We analysed the longevity data using Cox proportional hazards models with season of experiment (winter or spring), growth treatment (catch-up, slow-down or control), photoperiod and sex as main effects, treatment growth rate (=growth rate during the four weeks of temperature manipulation in period 1), compensatory growth rate during period 2, and non-breeding growth rate (=growth rate between the first and second breeding season) as covariates, and tank as a random effect, plus all interactions. Since fish were captured as juveniles from the wild, and we could not therefore tell their precise birth date, we assigned all the same nominal birth date (1 June 2007) for the purpose of statistical analysis. Using the log-likelihood χ^2 test, we evaluated the overall importance of variables in the Cox models, while coefficients for each variable in the models were evaluated with the Wald test. In order to evaluate each model's goodness-of-fit, we considered the proportion of variance explained (r^2). Subsequent Cox models explored whether there was any evidence that fish which delayed breeding until the second season lived longer (i.e. through allocation of resources to repair rather than reproduction). These analyses were necessarily restricted to fish that survived to at least the start of the second breeding season; the results (presented in the electronic supplementary material, text S3 and figure S3) showed no evidence of a trade-off between reproduction and survival.

In all analyses non-significant variables were sequentially dropped (least significant first, on the basis of likelihood ratios), 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 the software R v. 2.15.0 [16] and the package *Survival* [17]. All experiments were performed under license from the UK Home Office (PIL 60/11377).

3. Results

(a) Growth trajectories

At the end of the short (4 week) temperature manipulation (period 1), the mean size of fish among temperature-treatment groups had, as expected, diverged. Those fish exposed to the cold spell were on average smaller than the control fish, while those exposed to the mild spell were larger (figure 1).

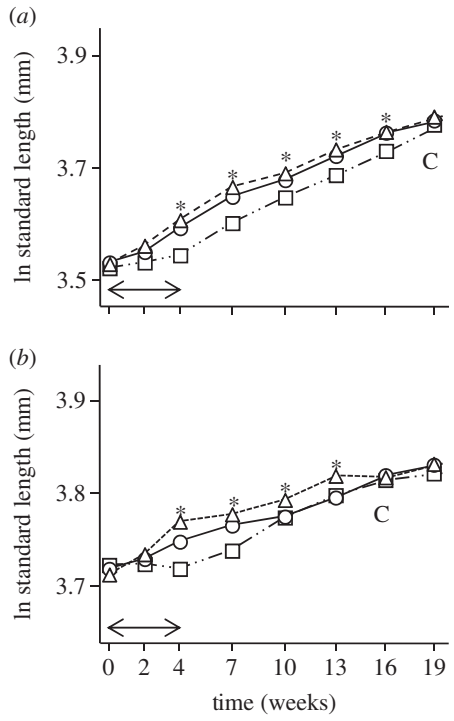

Figure 1. Growth trajectories (logarithm of standard length in mm) of three-spined sticklebacks (*Gasterosteus aculeatus*) over the early compensatory period in the (*a*) winter and (*b*) spring experiment. Note that the two experiments started on different days, so that day 1 is 21 November 2007 in (*a*) and 21 February 2008 in (*b*). The horizontal arrows indicate the period of temperature manipulation (slow-down (14°C)—triangle and dashed line; control (10°C)—circle and solid line; catch-up (6°C)—square and double dashed line). Asterisks indicate significant differences among all treatment groups ($p < 0.05$) and 'C' indicates the point when compensation was complete and there was no longer a significant difference in size between treatment groups. Adapted from Lee *et al*. [12].

All fish were then returned to the same ambient temperature conditions (period 2). However, the previous brief temperature perturbation affected subsequent growth trajectories: fish responded to the end of a cold spell by undergoing catch-up growth, while those that had experienced a mild spell subsequently slowed their growth, relative to the control fish, whose growth trajectory remained linear throughout periods 1 and 2 (effect of temperature treatment on compensatory growth rate: $F_{2,30.47} = 17.07$, $p < 0.001$). In general, growth rates during period 2 were significantly faster across all temperature treatments in the winter experiment ($n = 95$ fish) than in the spring experiment ($n = 98$; LME, $F_{1,42.66} = 118.07$, $p < 0.001$; figure 1). As a result of the treatment differences in compensation, the size of the fish within each experiment converged; the significant length differences among temperature-treatment groups found at the end of period 1 had disappeared after 15 weeks at the ambient temperature in the winter experiment and after 12 weeks in the spring experiment (figure 1). While there was no overall effect of photoperiod treatment on compensatory growth rate ($F_{1,29.97} = 1.25$, $p = 0.272$), there was a significant interaction between experiment and photoperiod ($F_{1,30.13} = 4.24$, $p = 0.048$; see the electronic supplementary material, figure S2*c*, *d* and *e*), with fish under the ambient photoperiod growing faster than those under the delayed photoperiod in the winter experiment, but not in the spring experiment. In addition, there was a significant interaction between temperature and photoperiod ($F_{2,30.55} = 8.10$, $p = 0.002$), with the differences in growth rate between temperature manipulation groups being much less under the delayed than the ambient photoperiod. Sex ($F_{1,173.55} = 9.85$, $p = 0.002$) and manipulated fish length ($F_{1,158.32} = 85.23$, $p < 0.001$) influenced compensatory growth: males grew slower than females, and growth rate was faster in fish that were smaller at the end of the temperature manipulation period (see [11,12] for more detailed statistical analysis).

From this point onwards (through the subsequent breeding and non-breeding seasons, periods 3, 4 and 5), there were no significant differences in fish length among the treatment groups within an experiment (see the electronic supplementary material, figure S2*a* and *b*). Growth trajectories for body mass showed the same patterns as those for body length (see the electronic supplementary material and [11,12] for statistical analysis of growth trajectories and size differences over time).

(b) Lifespan

No treatment differences were detected in mortality rates during or immediately after the period of temperature manipulations: only 1.25 per cent of fish (three out of 240) died during period 1 for non-accidental reasons; thus, the exposure to different temperatures did not have a direct effect on mortality patterns. Most experimental fish (88.4%) were still alive at the start of the first breeding season (a typical survival rate for juvenile fish under laboratory conditions), with no evident differences in pre-breeding survival among the treatment groups (figure 2). However, the breeding seasons were periods of increased mortality (figure 2).

There were highly significant treatment effects on lifespan in both experiments. Lifespan was unrelated to growth rate during the temperature manipulation period (i.e. growth rate during the four weeks temperature manipulation period, period 1; Cox proportional hazards model, coefficient (β) = −3.056, Wald = 2.12, $p = 0.15$). However, fish in the treatment groups that underwent catch-up growth in period 2 (i.e. catching up in period 2 after the short cold spell in period 1) had shorter lives than the control fish that had grown steadily (table 1; figure 2*a*,*b*), while those in the slow-down growth groups in period 2 lived for longer than the control fish (table 1). These effects were most pronounced in the spring experiment, where the median lifespan was 651 days in the catch-up, 761 days in the control and 994 days in the slow-down treatment groups, so that the catch-up fish experienced a 14.5 per cent reduction and the slow-down fish a 30.6 per cent increase in median lifespan compared to the control fish (figure 2*a*,*b*).

Lifespan was significantly affected by the season in which the temperature manipulation of growth trajectories took place (i.e. winter versus spring experiment; table 1): on average, fish in the spring experiment, which were under greater time stress, died at a younger age than those in the winter experiment. This is despite the spring experiment not having started until the fish were almost adult and so only involving fish that had already passed the stage associated with relatively high juvenile mortality (figure 2); the median lifespan of fish in the spring experiment was 739 days, whereas that of fish in the winter experiment was 873 days. The alternative method of inducing time stress (and so affecting growth trajectories) by altering the

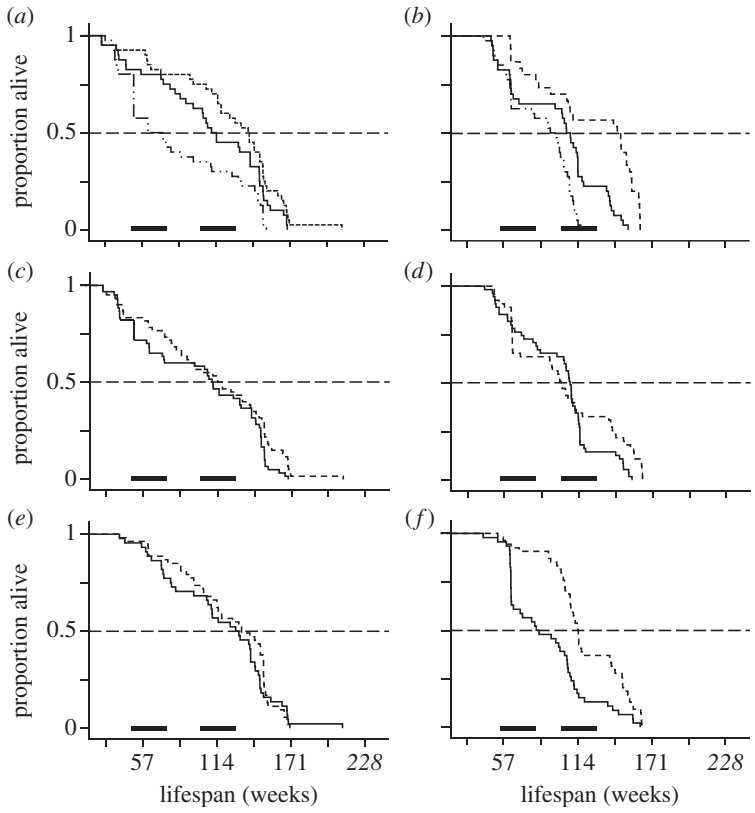

Figure 2. Survival curves of three-spined sticklebacks in relation to (*a*,*b*) the growth treatment (slow-down, dashed line; control, solid line; catch-up, double dashed line), (*c*,*d*) photoperiod treatment (ambient, solid line; delayed, dashed line) or (*e*,*f*) sex (female, dashed line; male, solid line) in the (*a*,*c*,*e*) winter and (*b*,*d*,*f*) spring 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 first and second breeding seasons. See table 1 for statistical analysis.

Table 1. Results of a Cox proportional hazard model of lifespan of sticklebacks, showing the significant effects of season of experiment (winter or spring), growth treatment (catch-up, slow-down or control) and photoperiod (ambient or delayed) treatment, sex (male or female), and compensatory growth rate. (Tank was included as a random factor. Overall significance of model: $r^2 = 0.341$, likelihood test = 80.2, d.f. = 7, $p < 0.001$. Growth rate during the temperature manipulation period was non-significant and was dropped from the model, as were non-significant interaction terms. s.e. is standard error; note that positive coefficients (β) for the hazard function are associated with shorter lifespans.)

	coefficient (β)	s.e.(β)	exp(β)	Wald	*p*
season (spring)	1.600	0.301	4.953	28.19	<0.001
growth treatment (catch-up)	0.601	0.200	1.825	9.08	0.003
growth treatment (slow-down)	−0.574	0.193	0.563	8.84	0.003
photoperiod (delayed)	−0.501	0.160	0.606	9.77	0.002
sex (female)	−0.149	0.222	0.862	0.45	0.500
compensatory growth rate	9.205	4.043	9946.739	5.18	0.023
season × sex	−0.708	0.303	0.493	5.45	0.020

perceived time of year through manipulations of photoperiod had similar effects on lifespan. Thus, fish under the greater time stress of the ambient photoperiod regime died sooner than those under the delayed photoperiod regime (table 1), with the difference in mortality rate being apparent from the middle of the first breeding season onwards in both experiments (figure 2*cd*); the median lifespan of fish in the ambient treatment in the winter and spring experiments was 774 and 741 days, respectively, whereas that of fish in the delayed treatment was 815 and 686 days, respectively.

In addition to the marked difference in average lifespan found among the treatment groups, we found that variation in lifespan within treatment groups was related to inter-individual variation in growth rate. Thus, over the period of compensation (period 2), when all fish were at the same temperature, those individual fish that had the faster growth rates within a treatment group were more likely to have shorter lifespans (table 1). There was no effect of growth rate before the period of growth compensation (i.e. during period 1), nor any effect of subsequent (adult) growth rate on survival (i.e. between the first and second breeding season: coefficient (β) = −7.167, Wald = 0.88, $p = 0.350$), possibly because adult growth rates were minimal compared to those in pre-breeding juvenile life. While there

Table 2. Maximum lifespan (defined as the age by which 90% of the population in a treatment group had died) in relation to photoperiod and growth treatments. (Also shown is the % difference compared to the value for control fish of the same photoperiod and experiment.)

	treatment			
experiment	photoperiod	growth	maximum lifespan (days)	% difference compared to intermediate value
winter	ambient	catch-up	886	−14.5[a]
		control	1036	
		slow-down	1132	+9.3[b]
	delayed	catch-up	1053	−10.8[a]
		control	1180	
		slow-down	1187	+0.6[b]
spring	ambient	catch-up	793	−1.1[a]
		control	802	
		slow-down	1064	+32.7[b]
	delayed	catch-up	777	−25.1[a]
		control	1037	
		slow-down	1137	+9.6[b]

[a]Decrease in maximum lifespan relative to the corresponding control fish.
[b]Increase in maximum lifespan relative to the corresponding control fish.

was no sex difference in lifespan in the winter experiment (median lifespan = 912 days in males, 935 days in females, figure 2*e*), males died sooner than females in the spring experiment (median lifespan = 581 days in males, 797 days in females, figure 2*f*), as shown by the significant interaction between season of experiment and sex (table 1).

The treatment effects on maximum lifespan (defined as the age at which 90% of the population had died) were similar to those on median lifespan: in both winter and spring experiments, and in both photoperiod treatments, the maximum lifespan of the catch-up fish was shorter than that of the control fish (with an average reduction in maximum lifespan over all treatment groups of 12.9 ± 5.0 (s.e.)%). By contrast, the slow-down fish had an average increase in maximum lifespan of 13.0 ± 6.9% (table 2). Fish under a greater time stress had a reduced maximum lifespan (with an average reduction of 13.6 ± 3.6% when comparing equivalent treatment groups in the spring versus winter experiments, and an average reduction of 10.0 ± 3.6% when comparing equivalent treatment groups in the ambient versus delayed photoperiods; table 2).

We tested whether the treatment effects on lifespan were confounded by any changes in reproductive schedules by examining links between lifespan and indicators of breeding investment in both sexes. A total of 106 female fish were alive at the start of the first breeding season, 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 (25 for the first time, while 12 spawned in both seasons). Survival in females was significantly related to breeding pattern: taking females that lived until at least the start of the second breeding season, those that produced eggs in both seasons lived longer than those only laying eggs in one season, and those that failed to lay eggs in either season had the shortest lives (see the electronic supplementary material).

In total, 90 males developed nuptial coloration (i.e. blue eye and/or red throat) during the first breeding season, of which 48 lived to at least the start of the second breeding season. The lifespan of these 48 males was related to both their growth rate between the first and second breeding seasons and their ability to retain sexual coloration in the second breeding season (see the electronic supplementary material); both relationships were positive, indicating that (as in females) they reflected differences in male quality.

4. Discussion

Since growth rate in ectotherms is temperature-dependent, subjecting juvenile fish to short cold or warm spells for four weeks (period 1) led to predictable changes in growth, with those kept at warmer temperatures becoming larger, and those kept at cooler temperatures smaller, than fish held at the intermediate temperature. Although exposure to different temperatures itself might have an impact on physiological processes that affect longevity, the temperature manipulations used in this experiment were unlikely to produce any marked physiological response; they involved a temperature shift that was well within the range naturally experienced by the fish every week at that time of year. The differences in growth rate during the manipulation period would be expected to arise from direct effects of temperature on resource acquisition (it being possible to digest and process food faster at warmer temperatures), hence we would not expect any effects on lifespan (since differences in growth were not achieved through diversion of resources away from e.g. somatic maintenance). As expected, therefore, lifespan was unrelated to growth rate during period 1.

This is not the case for period 2, when growth rates differed despite all fish living at the same intermediate temperature: fish that at the start of period 2 were larger than expected for their stage (because of the earlier favourable temperature for growth) slowed their rate of growth below that expected for their size, and so would have been able to divert more resources from growth to maintenance or reproduction. By contrast, those that were correspondingly smaller (because of the unfavourably cold conditions) at the

start of period 2 subsequently grew faster than expected for their stage, but could only have done so by reducing the proportion of resources allocated to maintenance or reproduction [8,18].

The contrasting rates of growth of the three groups under the same environmental conditions led to an eventual convergence in the size at the end of period 2, after which point all fish grew at the same average rate. However, the differences in growth trajectory during this period had significant and strong effects on patterns of longevity. Fish undergoing catch-up growth during period 2 had a shortened adult lifespan, while those showing a slow-down in growth over the same period had an extended lifespan. Although it has previously been documented that the longevity of ectotherms can be extended if they are continually kept at cooler temperatures [19–22], presumably because of temperature effects on cellular processes as mentioned above, this study is, to our knowledge, the first to report that brief exposures (equivalent to less than 4% of median lifespan) to episodes of atypical temperatures have important long-term effects on longevity.

The time over which the compensation took place (which affected both the rate of compensatory growth and the subsequent time for recovery) also had an impact: fish that underwent the growth perturbations in the spring had a shorter lifespan than those experiencing the perturbations in the winter, and those on the ambient photoperiod died sooner than those whose time stress was reduced (i.e. the delayed photoperiod group). In both cases, the fish that had longer to undertake any compensation before the breeding season had on average the longer subsequent lifespan, which supports the 'time-stress' hypothesis [10]. The effects of time stress were more apparent in males, which had a significantly shorter lifespan than females in the spring experiment. The fact that all three of the methods used to manipulate the compensatory growth response (i.e. temperature and photoperiod manipulations and repetition of the experiment in different seasons) produced similar effects on lifespan (with faster growing treatment groups having a shorter life) strengthen the conclusion that it is the catch-up growth rate that is the important factor, rather than temperature *per se*.

We have shown elsewhere that the manipulations of growth rate had effects on reproduction, with reproductive investment (measured in terms of egg production by females, nest building and sexual ornamentation in males) being negatively correlated with growth rate during the compensation period [11]. Sex differences in survival linked to differences in reproductive costs have been found in a diverse range of other species [23–25], but in this study there was no evidence in either sex of any trade-off between reproduction and survival; this is almost certainly because we did not manipulate reproductive effort, and so fish were able to allocate resources to reproduction according to their current state or condition, leading to positive relationships between reproduction and survival indicative of quality differences among individuals [26]. Relevant to this is the fact that while manipulated growth rates (i.e. during period 2) were negatively correlated with lifespan, growth rate during period 4 (i.e. after all fish had completed the compensation phase so that variation in growth more probably reflected individual differences in resource acquisition rather than allocation) was positively correlated with subsequent lifespan (see the electronic supplementary material, text S3 and figure S4).

We have also shown that these compensatory growth trajectories influenced swimming endurance [12] in a similar direction, with catch-up growth resulting in impaired locomotor performance in comparison to steadily growing control fish, while growth compensation in the opposite direction (i.e. a slowing of growth) led to improved performance relative to the controls. In combination, these effects on lifespan, locomotor and reproductive performance would have had a significant impact on fitness. Female three-spined sticklebacks can potentially produce a succession of clutches in each breeding season, which are then guarded by the males. The treatment effects on lifespan were already apparent at the start of the first breeding season (figure 2), and affected both the size and number of clutches each female was able to produce [11] and the period of time over which males could remain in breeding condition (and hence the number of clutches he could potentially rear). Moreover, since female sticklebacks have been shown to prefer mates that have a longer life expectancy [15], the effects on male reproductive success are likely to have been even more pronounced than is evident from this study.

The mechanism underlying these trends in lifespan is not known, but may relate to oxidative stress. Age-related deterioration in performance is generally held to be the result of cellular damage accumulation over time [27,28]. Rapid growth may lead to greater levels of cellular damage because the higher metabolic activity will lead to an increased production of reactive oxygen species and hence oxidative stress [29,30] and/or because rapid growth may only be achieved by diversion of resources away from maintenance and repair of damaged biomolecules [31,32]. Slowed growth is expected to have the opposite effect, allowing an increased allocation to maintenance and repair [18]. Jennings *et al.* [33] provided evidence from mammals that catch-up growth (in this case following earlier undernutrition) increased oxidative stress levels and rates of cellular damage and senescence, which may be linked to organismal senescence owing to the effects of growth rate on telomere lengths [34,35]. Similar links between growth rate, oxidative stress and changes in telomere length have recently been reported in penguin chicks [36].

The rate of compensatory growth presumably affected both the level of accumulated damage and the time available in which to repair it. The degree of time stress would also affect the time available for repair, and hence the probable rates of senescence. 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 probable that survival will be reduced through disease [37]. While the mechanisms underlying the links between growth rate and senescence require further study, it is nonetheless clear that there is indeed a trade-off between growth rate and other functions related to body state and later life senescence, which explains why growth rate is not normally maximized [6]. The lifespan advantages associated with slowed growth explains why animals might slow growth when they are ahead of target, as observed in this study, so sacrificing their potential size advantages in favour of delayed senescence. How animals are able to assess whether they are ahead or behind their target size for a given time of year is not known, but might relate to the attainment of

developmental milestones related to size. The optimal growth trajectory for a given set of environmental conditions will thus depend on the balance of costs and benefits to be accrued by increasing or decreasing growth at different times, and also the costs and benefits of being large or small.

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