

Experimentally manipulated growth rate in threespine sticklebacks: assessing trade offs with developmental stability

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Synopsis

The evolution of 'adaptive' growth rate and its influence on how other life history traits evolve is a neglected topic in biology. Growth rate influences life history because size strongly influences age-specific survival and fecundity, and because growth rate defines the relationship between age and size. Improved predictions about the evolution of life history traits may be possible with a greater understanding of the factors that influence the evolution of growth rate. We experimentally tested the hypothesis that a trade off may exist between growth rate and developmental stability in freshwater threespine sticklebacks, *Gasterosteus aculeatus*. We compared the degree of developmental instability (measured as fluctuating asymmetry = FA) in four lateral plate and two fin traits of fish reared under a high vs. low growth regime in response to food ration and temperature. We found evidence that symmetry was reduced (FA increased) in fast growing compared to slow growing fish, suggesting that a trade off between developmental stability and growth is possible. FA plausibly reflected developmental instability because of significant associations between rank FA levels across traits in individuals. These results are preliminary because of the possible confounding effects of temperature and food ration on asymmetry, and because we do not know if this trade off has fitness or other life history consequences. Our results also do not support the hypothesis of honest signaling sometimes invoked in studies of sexual selection because greater symmetry was found under poorer rather than better resource levels.

Introduction

The evolution of intrinsic growth rate and its influence on how other life history traits may evolve is a neglected topic in biology (reviewed in Case 1978, Arendt 1997). A better understanding of how evolutionary changes in growth rate can influence the evolution of other life history traits is important because while the optimal life history is expected to maximize a population's age-specific survival and fecundity (Roff 1992, Stearns 1992, Charnov 1993), it is

growth rate that defines the relationship between age and size in an organism (Gothard et al. 1994, Arendt 1997). Growth rate is often thought to be maximized in most nonreproductive organisms because energetic models often assume that the individual divides finite resources among a fixed maintenance cost and growth in the absence of reproduction (Arendt & Wilson 1997). However, empirical studies suggest that growth rate is frequently not maximized under natural conditions (Case 1978, Arendt 1997, Niewiarowski 2001). If growth is positively related to fitness as is widely

believed, then understanding the constraints on growth rate should provide a greater ability to predict the evolution of other life history characters.

The availability of resources is one factor that limits growth in most organisms. Yet it is unlikely that resource limitation constrains growth all the time and so alternative explanations must be considered. One alternative is that internal factors constrain growth through their influence on other fitness-related traits. Internal constraints can arise because growth is a polygenic trait that interacts with many other systems within an organism. Best known are trade offs between growth and age or size at maturity (reviewed in Roff 1992, Stearns 1992, Charnov 1993). Less widely appreciated are an array of other potential constraints on growth (which may influence life history) imposed by behavior, physiology, or development (Arendt 1997). For example, the nutritional and energetic demands of rapid growth may cause individuals to forage more actively causing increased risk of mortality due to predation (Werner & Arnholt 1993, Lachmansingh & Rollo 1994). When energy and nutrients are limited, trade offs may arise from the allocation of resources to somatic vs. reproductive tissues during development (Reznick 1983). The allocation of resources between immature tissue that grows fast and slower growing mature tissue can also constrain growth (Ricklefs et al. 1994). Lastly, development may impose a constraint if rapid growth results in developmental instability and reduced fitness (Sibly & Calow 1986). We report here on a preliminary experimental test of the relationship between growth rate and developmental stability using the freshwater threespine stickleback, *Gasterosteus aculeatus*.

In order to generate predictions about possible trade offs between growth and development, we resort to the following simplified model. We assume that the genotype and the environment (through resources and ambient conditions) each influence development (which is not directly measured). Development in turn influences two measurable traits, growth rate (measured as change in body size per unit time) and developmental stability (which we assume is reflected as bilateral symmetry). Our null hypothesis is that no trade off occurs between developmental stability and growth. In other words, developmental stability can be maintained in the face of rapid growth, and we predict that growth rate and developmental stability should be either unrelated or positively related. A positive relationship may exist if very slow growth instead results in developmental instability due to energetic stress (the 'honest signal' hypothesis

sometimes invoked in sexual selection theory, Nilsson 1994). The one-sided alternative hypothesis is that a trade off between development and rapid growth occurs because very rapid growth results in increased developmental instability and error, which may have fitness consequences. Thus, a negative relationship should exist between growth rate and developmental stability. We experimentally tested the null hypothesis of no trade off between growth and development by comparing developmental stability between sticklebacks reared in high and low growth treatments imposed by manipulating the environment.

We assumed that bilateral symmetry is a measure of developmental stability and that fluctuating asymmetry (FA) can be used as a measure of developmental instability. Departures from symmetry result from differences in the expression of a character on the right and left side of a bilaterally symmetric organism and are assumed to provide a measure of 'developmental noise' (Palmer & Strobeck 1986, Parsons 1990, 1992, but see Houle 1998). Such asymmetry is said to be fluctuating in a population when the asymmetry values are normally distributed around a mean of zero (e.g., not consistently biased to either the left or right side and referred to as directional asymmetry). A mean of zero, or perfect symmetry, is often considered the morphological ideal with departures from symmetry believed to be indicative of developmental stress. This is because perfect symmetry is 'the ability to execute developmental programs correctly and uniformly' (Watson & Thornhill 1994). Stress on the developmental system can result from internal genetic or external environmental factors (Palmer & Strobeck 1986). The assumption that perfect symmetry is ideal should be treated with some caution, however, because it is difficult to test and small asymmetries may have no measurable fitness consequence (Clarke 1998, Houle 1998).

For development to constrain growth rate we have to assume that developmental instability can have fitness consequences. This is not entirely unreasonable because FA may play a role in mate choice in many species, with symmetry on average thought to be preferred over asymmetry in potential mates (Moller 1991, 1992, 1997, Watson & Thornhill 1994, but see Clarke 1998, Houle 1998). A variety of traits exhibit fluctuating asymmetry in the freshwater threespine stickleback (Hatfield 1997, Bergstrom & Reimchen 2000), and in a close relative the brook stickleback, *Culea inconstans* (Moodie & Moodie 1996, Hechter et al. 2000). In sticklebacks morphological symmetry may plausibly influence fitness for a number of reasons.

Pectoral fins are functionally involved in swimming performance (Hart & Gill 1994) and in the fanning of eggs by nesting males. Hechter et al. (2000) found that female brook sticklebacks with symmetric fin ray counts averaged about 15% more eggs per clutch (and ovaries 6.5% heavier) than females that were asymmetric. The functioning of lateral plates as armor in defense against predation may be compromised with increasing bilateral asymmetry (Moodie & Reimchen 1976, Reimchen 1983, 1994, Bergstrom & Reimchen 2000). Thus, we can plausibly link asymmetry with fitness consequences for the traits that we have measured, although we do not quantify the fitness consequences of the asymmetry measured here.

Materials and methods

Rearing of sticklebacks and growth treatments

Sticklebacks used in this experiment were laboratory-reared progeny of mature fish collected in the same year from a population of low-plated morphs from Cranby Lake, British Columbia. Progeny were created *in vitro* following the procedure in Hatfield (1997) although our crosses involved parents from a single species. Fish were 2.5 months old at the start of the growth experiment. Prior to this they were reared in tanks maintained at 17°C and fed *ad libitum* with live brine shrimp nauplii and frozen bloodworm. Fifty fish were randomly assigned to either a high or low growth treatment aquarium (volume 102 l) each replicated three times (mean standard length high = 17.21 mm vs. low = 17.30 mm). Aquaria were housed in an environment chamber at 16.5°C with a 16:8 h light:dark cycle.

High and low growth treatments were achieved by varying two factors, the amount of food provided and water temperature (e.g., Wurtsbaugh & Cech 1983, Wootton 1994). Both treatments were maintained at temperatures within the natural range for threespine sticklebacks, and the low food ration allowed development (Wootton 1994, McPhail personal communication). A ration level of frozen bloodworm was chosen for each treatment based on a minimum percentage of average wet body weight (low = 3% and high = 9% per day), with low growth fish receiving approximately 29% of the ration fed to high growth fish. These are not unusual ration levels given that the natural rate of consumption is 2–10% of body weight depending on temperature (Wootton 1994). The temperature of the

high growth treatment tanks was maintained approximately 5°C above the low growth tanks with submersible heaters (mean°C ± SE for low = 16.3 ± 0.07, and high = 21.2 ± 0.04). Sticklebacks in coastal BC lakes normally experience a wide range of temperatures over the course of the year that range from 4°C in the winter to as high as 25°C in the summer (e.g., Bentzen et al. 1984). Tank temperature and mortality were monitored daily throughout the 62 day experiment. Environmental treatment did not result in significant differences in mortality between treatments (mean final surviving fish per tank, high = 26, low = 24; $t = 0.866$, $p = 0.50$).

Approximately 13 fish were removed from each aquarium after 39 days (hereafter time 1) with approximately 14 more removed after an additional 23 days of growth (hereafter time 2). The experiment was terminated at time 2 when the fish from the low growth treatment were approximately equal in size to the high growth treatment at time 1 (Figure 1). This design allowed three pair-wise comparisons of symmetry among the four treatment groups: between groups of fish that were the same age but different size (low vs. high at time 1, and again at time 2), and between groups that were similar in size but of different ages (high at time 1 vs. low at time 2). This last comparison was

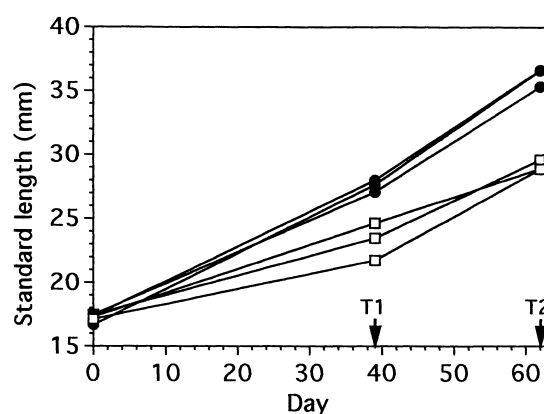


Figure 1. Difference in mean growth rate between the high (circles) and low (squares) growth aquaria over the 63-day experiment. A replicate aquarium was initially composed of 40 sticklebacks, with 13 removed at time 1 (T1) and approximately 14 removed at time 2 (T2). Mean body size at each time is the standard length (mm) averaged among individuals in each aquarium. Three pair-wise comparisons of FA were made: between high and low growth treatments at time 1, and again at time 2, and lastly between high at time 1 vs. low at time 2 when mean body sizes were similar.

intended to minimize any confounding effects of size on asymmetry as an alternative to statistically removing such size effects.

Measurement of morphology

All sticklebacks were anaesthetized in a lethal dose of MS222, fixed in 10% formalin for at least four weeks, rinsed in freshwater, stained with alazarin red in a 1% KOH solution to accentuate the lateral plates and fin rays and preserved in 40% isopropyl alcohol (Lavin & McPhail 1985). Both sides of each fish and a disc scale were photographed using a 35 mm camera fitted with a macro lens and the subsequent slides were scanned at 2000 dpi using Adobe Photoshop 3.0.4. The scale and the length of the lateral plates on both sides were digitized and measured using NIH Image. Plate identification and numbering followed Reimchen (1983). Plate length was measured from the midpoint of the top portion of the plate to the midpoint of the bottom (Figure 2). Only lateral plates 4 through 7 were measured on all fish (the presence of smaller plates 3 and 8 varied by body size and treatment resulting in low and heterogeneous sample sizes). Plate 6 in the low growth treatment at time 1 is not included in our analyses because we could not accurately determine

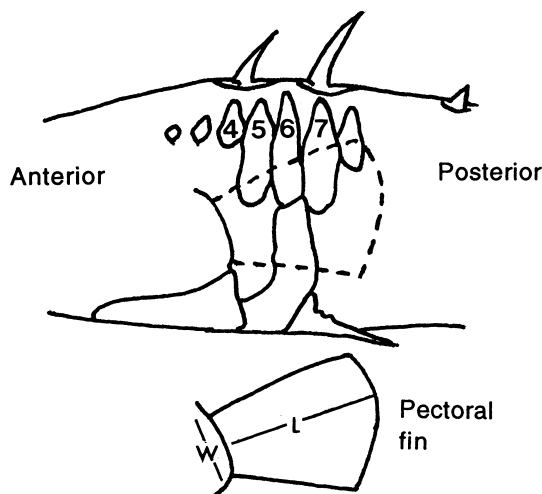


Figure 2. Lateral view of a typical low-plated form of the freshwater threespine stickleback, *Gasterosteus aculeatus*, showing the location and numbering of the lateral plates and the pectoral fin. Plate length was measured from the top to the bottom of each plate. The width at the base of the pectoral fin (w) at insertion and the length (l) of the longest fin ray were also measured.

its length as its ventral end appeared to be fused to the ascending process of the pelvic girdle. At larger sizes lateral plate 6 and the pelvic girdle were clearly separated. This fusion was not found in the other three treatment by time groups. The morphology of pectoral fins was measured at $25\times$ magnification using a dissecting microscope fitted with an ocular micrometer. Maximum length was measured as the longest pectoral fin ray and width was measured at the insertion of the fin to the body. Standard length was measured when the fish were removed from the aquaria.

We assessed repeatability by measuring the smallest and largest individuals (respectively from low at time 1 and high at time 2) 10 times and calculating the coefficient of variation for each trait. These individuals represented the extremes of body size in our samples and allowed us to test for an effect of size on the repeatability of our measurements. The coefficient of variation ranged among traits from 1.1% to 6.4% and was generally greater in the smaller fish. The width at insertion of the pectoral fin (6.4%) and the length of lateral plate 4 (5.4%) exhibited the greatest measurement error in the slower growing and smaller fish. Consequently, we expected that differences in FA among groups would be most difficult to detect for these two traits.

Calculation of asymmetry

Asymmetry was calculated as the value of the trait on the right side minus the left side. In a perfectly symmetrical fish this value would equal zero, with positive or negative departures representing increasing asymmetry. Traits were not corrected for size differences among individuals because absolute asymmetry (absolute value of right minus left) was not positively related to body size or mean trait size in either treatment using analysis of covariance (Table 1). A weak negative relationship between absolute asymmetry and size occurred only for fin width but this explained less than 4% of the total variation in asymmetry. As a further test, we experimentally minimized size effects on FA by comparing fish of similar size but at different ages (high at time 1 vs. low at time 2). We can test for an effect of size on differences in FA between groups by comparing the results of this size-constant analysis with those involving the high and low treatments at each time period (where fish were the same age but different size).

Table 1. The relationship between asymmetry (absolute value of right minus left) and two measures of size, average trait size (TS = right + left divided by two) and body size (SL = standard length), tested using ANCOVA with size as covariate for both growth treatments. Although size was related to asymmetry for fin width, the relationship was negative and the model explained less than 4% of the total variation in asymmetry. Nonsignificant (ns) p-values ranged from 0.18 to 0.93. Replicate aquaria were combined to maximize sample size in this analysis (i.e., individuals were assumed to be independent). Data on lateral plate 6 from the low growth treatment at time 1 was excluded because this plate appeared to be fused to the pelvic girdle.

Trait	n	R ²	Size measure	Effect (probability value)		
				Size	Treatment	Interaction
Fin width	151	0.038	SL	0.03	0.03	0.02
		0.036	TS	0.03	0.03	0.02
Fin length	151	0.013	SL	ns	ns	ns
		0.027	TS	ns	ns	ns
Plate 4	144	0.063	SL	ns	ns	ns
		0.053	TS	ns	ns	ns
Plate 5	151	0.028	SL	ns	ns	ns
		0.097	TS	ns	0.005	0.03
Plate 6	115	0.019	SL	ns	ns	ns
		0.044	TS	ns	ns	ns
Plate 7	151	0.030	SL	ns	ns	ns
		0.051	TS	ns	ns	ns

Analysis of asymmetry

The asymmetry for each trait on every individual was determined and the mean asymmetry (MA) calculated for each group of fish (e.g., treatment at time). We then tested whether the patterns of asymmetry in each group fluctuated around zero or were biased to the left or right side using two-tailed t-tests of the null hypothesis that MA equaled zero.

To test whether FA differed between pairs of treatment groups is equivalent to asking whether the variance in asymmetry values is greater in one treatment group than the other. We used four different equality of variance tests for each pair-wise contrast. Levene's test is an analysis of variance of the mean absolute deviations between treatments (Palmer & Strobeck 1986). A greater mean absolute value for a treatment indicates a greater level of FA for that group. Levene's test is generally robust despite any non-normality in the data. Second, we used Bartlett's test of homogeneity of group variances to test whether the variance in deviations (MA) differed between treatments. Third, we used Hartley's Fmax test that calculates an F-statistic based on the ratio of the largest to the smallest variance in

MA between two groups. Both Bartlett's and the Fmax test are sensitive to departures from normality that may result in spurious differences among group variances (Zar 1999). Therefore, we examined the distribution of MA for every trait in each group using normal quartile plots of the data. MA was generally normally distributed (except for plate 6 in the high growth group at time 1, which was not a concern as data on this plate was not available for comparison in the low growth group at time 1). Outliers in the high growth group also influenced the distribution of MA at time 2 for fin length and the plates 5 through 7 although the remaining data were normally distributed in each case. Lastly, because we were uncertain about the effect that these outliers may have had on our parametric analyses, we calculated bootstrap difference in MA using 1000 resamples of the data for each set of contrasts. The effective p-value for this test is the proportion of bootstrap differences in FA that fall below the actual observed difference for a given contrast. Two tailed significance levels were applied throughout. We did not adjust the alpha level to reflect multiple traits measured on each individual in these analyses following Palmer & Strobeck (1986).

We also tested if groups that exhibited elevated levels of asymmetry did so for just a few or most of the traits studied. We tested for a positive correlation in asymmetry among the six traits (4 lateral plates and 2 pectoral fin measures) using Kendall's coefficient w of concordance (Zar 1999) calculated by ranking the FA for each character among the four treatment by time groups (low and high growth at time 1 and 2). Kendall's w ranges from zero, where there is no association in rank FA levels among traits within a group (and little overall pattern of rank levels of asymmetry among groups) to one, indicating complete agreement among the rankings of all traits. The significance of the concordance was tested using Friedman's chi-square, and also by bootstrapping 1000 resamples of the ranked FA for each of the three pair-wise contrasts. The effective p-value in this case was the bootstrapped difference in ranked FA that fell below the actual difference in mean ranked FA between groups.

Results

Growth rate

Fish in the high growth treatment grew on average 63% faster than those in the low growth treatment

over the 62 days (Figure 1; mean \pm 1 SD of change in SL averaged over replicate means from a starting size of 17.2 mm, high: 0.31 mm per day, \pm 0.017; low: 0.19 mm per day, \pm 0.005). The mean standard length of the high growth fish at time 1 was 1.5 mm less than the mean size in the low growth treatment at time 2 when fish from replicate aquaria were combined (Figure 1; high time 1 mean = 27.54 mm vs. low time 2 mean = 29.08 mm; $t = 2.25$, $p = 0.04$). While this size difference was marginally significant, it represented only a 5% difference in body length between the high and low growth treatments between times. Mean growth rate was not significantly different among replicate aquaria within a treatment (ANOVA of mean final standard length, replicate $F_{1,4} = 1.15$, $p = 0.36$; treatment $F_{1,4} = 63.2$, $p = 0.004$).

Asymmetry

To increase the sensitivity of our FA analyses we treated fish within aquaria as independent units by combining replicate tanks within growth treatments. This was reasonable for three reasons. First, the non-independence of fish from the same aquarium would generally result in an increase in variance in growth rate and presumably asymmetry (with one fish taking food away from another), so that non-independence

is unlikely to result in growth rate (and FA) converging towards a single value within tanks. Second, there were no significant growth differences among replicate tanks within each treatment (see results above). Third, there were no significant differences in the FA of lateral plates or fin traits among replicate tanks within treatments (ranges of p-values for separate t-tests of each trait at time 1: low $0.96 > p > 0.11$, high $0.90 > p > 0.08$; and time 2: low $0.90 > p > 0.17$, high $0.62 > p > 0.16$).

In general the asymmetry of the traits was not a result of one side being consistently larger than the other side. One sample t-tests indicated that the MA of pectoral fin width and length did not differ from zero in each group (Table 2, Figure 3). Significant departures from zero were found in four out of 15 tests involving the MA of lateral plates, but there was no particular bias for the left or right side among these cases (two negative and two positive values).

Analyses of FA among treatment groups indicated a consistent pattern of greater FA in the lateral plates of the high compared to low growth treatments (Table 3). The Fmax test was the most sensitive to differences in FA between groups while Levene's test and our bootstrap analysis were the least sensitive. Nevertheless, all statistically significant results agreed on the same overall pattern of asymmetry being greatest in the high growth treatments. This pattern was strongest for the

Table 2. Mean asymmetry (MA = right minus left) and fluctuating asymmetry (FA = absolute value of right minus left) for the six characters and four treatment by time groups. The significance of the departure of MA from zero was determined using independent t-tests (superscripts indicate significance). All values of FA are significantly greater than zero based on t-tests ($p < 0.005$). Tests of differences in FA among treatment groups are presented in Table 3.

	Time 1				Time 2			
	n	Low	n	High	n	Low	n	High
<i>Mean asymmetry</i>								
Fin width	36	0.014	40	-0.011	36	0.007	39	-0.008
Fin length	36	0.009	40	-0.026	36	-0.012	39	0.040
Plate 4	36	0.045	40	0.059	36	0.089 ^b	39	-0.065
Plate 5	36	0.069	40	-0.052	36	0.128 ^c	39	-0.004
Plate 6	—	—	40	-0.140 ^b	36	-0.060	40	-0.241 ^a
Plate 7	36	0.017	41	-0.042	36	-0.034	39	-0.168
<i>Fluctuating asymmetry</i>								
Fin width	36	0.045	40	0.039	36	0.048	39	0.062
Fin length	36	0.087	40	0.091	36	0.087	39	0.131
Plate 4	36	0.243	40	0.263	36	0.185	39	0.339
Plate 5	36	0.267	40	0.329	36	0.244	39	0.335
Plate 6	—	—	40	0.217	36	0.171	40	0.305
Plate 7	36	0.304	41	0.335	36	0.239	39	0.424

^a $p < 0.05$; ^b $p < 0.02$; ^c $p < 0.01$.

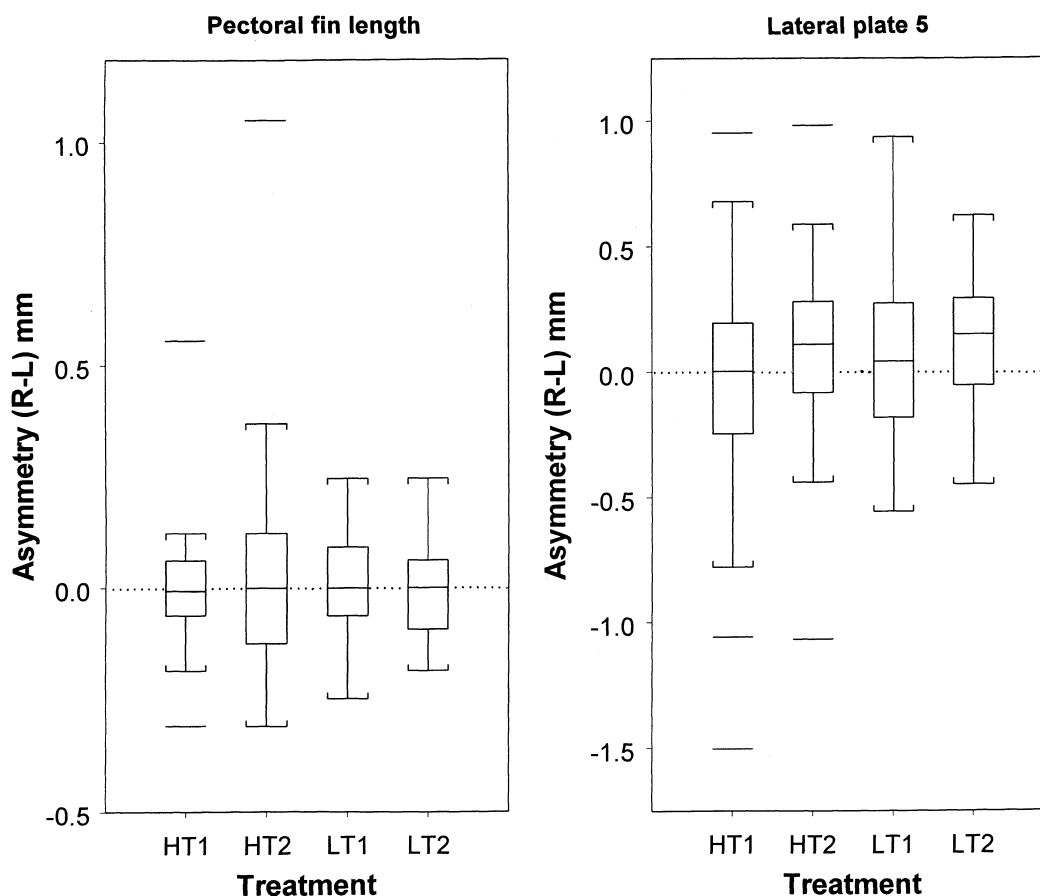


Figure 3. Asymmetry in pectoral fin length and lateral plate 5 for the four treatments of fish (high growth = H and low growth = L at time 1 = T1 and time 2 = T2, fish from replicate aquaria combined). Positive values indicate traits that are longer on the right side than on the left side. The spread of the data indicates the degree of FA for that treatment. Each boxplot shows the median (horizontal line within the rectangle), upper and lower quartiles (top and bottom of the rectangle), outer fences (the range of data), and any outliers. Note that the medians do not depart significantly from zero indicating that asymmetry is fluctuating randomly in each group.

plate data (especially plate 4) and weakest for the fin traits. The FA of fin length was greater in the high compared to the low growth treatments only at time 2 (Table 3) and no other comparisons of fin FA were significant.

The overall test of asymmetry involving all six traits together followed the same pattern, with body-wide FA greater in high compared to low growth treatments for high at time 1 vs. low at time 2, and between groups at time 2 (Table 4). The ranked values of FA across all four treatment by time groups were also significantly concordant although the ordering of the groups on pectoral fin width departed from the rest. The lowest and highest levels of FA were found respectively in the low and high growth fish at time 2.

Discussion

Using an experimental manipulation, we found that FA was higher in the fast growing compared to slow growing sticklebacks, and we conclude that a trade off may exist between growth and developmental stability in these fish. We reject the possibility that differences in asymmetry were due to larger body or trait sizes in the high growth treatment (Palmer & Strobeck 1986). FA was not positively related to body size or trait size (Table 1), and asymmetry was greatest in the high growth treatment when we experimentally controlled for body size (contrasting the high growth treatment at time 1 to low growth at time 2; Table 3c). Our interpretation is further supported by the fact that the

Table 3. Tests for differences in asymmetry between pairs of treatment groups: (a) time 1 between low and high growth, (b) time 2 between low and high, and (c) time 1 high growth vs. time 2 low growth. Four statistical tests were performed in each comparison. Levene's test (using absolute value of right minus left), Bartlett's and Fmax tests for homogeneity of group variances (both using right minus left), and a bootstrap of the observed asymmetry data. The rank order of asymmetry between groups is shown in the column labeled 'asymmetry' with H and L denoting high and low growth respectively.

Trait	Levene's		Bartlett's		Fmax		Bootstrap Prob.	Asymmetry
	F	Prob.	χ^2	Prob.	F	Prob.		
<i>(a) Time 1: low vs. high growth</i>								
Fin width	0.463	ns	1.4	ns	1.48	ns	ns	
Fin length	0.032	ns	0.777	ns	1.34	ns	ns	
Plate 4	0.07	ns	0.34	ns	1.59	ns	ns	
Plate 5	0.89	ns	3.38	ns	2.23	0.05	ns	H > L
Plate 6	—	—	—	—	—	—	—	
Plate 7	0.11	ns	0.03	ns	1.03	ns	ns	
<i>(b) Time 2: low vs. high growth</i>								
Fin width	1.46	ns	1.68	ns	1.53	ns	ns	
Fin length	1.98	ns	14.18	0.001	3.7	0.002	ns	H > L
Plate 4	9.51	0.006	11.6	0.002	2.69	0.01	0.008	H > L
Plate 5	0.03	ns	0.08	ns	1.18	ns	ns	
Plate 6	0.54	ns	0.25	ns	1.05	ns	ns	
Plate 7	1.44	ns	2.97	ns	1.96	0.05	ns	H > L
<i>(c) Time 1: high vs. time 2: low growth</i>								
Fin width	1.11	ns	2.61	ns	1.72	ns	ns	
Fin length	0.033	ns	0.999	ns	1.39	ns	ns	
Plate 4	4.06	0.05	7.16	0.015	2.49	0.02	0.004	H > L
Plate 5	1.89	ns	9.96	0.004	2.96	0.01	ns	H > L
Plate 6	0.65	ns	8.06	0.01	2.6	0.01	ns	H > L
Plate 7	2.89	ns	3.71	ns	1.92	ns	ns	

Table 4. Tests of differences in body-wide FA between treatment groups using Kendall's coefficient of concordance among the 6 traits (4 plates and 2 fin measures). The data are values of FA ranked among groups for each trait, with the rank value of 1 assigned to the treatment with the lowest asymmetry. Bootstrap p-values of concordance for each pair-wise contrast are also given. Note that high growth groups always had higher ranked asymmetry compared to low growth groups. Data on plate 6 are excluded at time 1 because this plate appeared to be fused to the pelvic girdle in the low growth treatment and so is excluded from analyses marked with a (*).

Treatment and time	Plate				Fin		Kendall's concordance test				Bootstrap Prob.
	4	5	6	7	W	L	Contrast	w	χ^2	Prob.	
Low (T1)	2	2	—	2	2	1.5	H1 and L1*	0.36	1.8	ns	ns
High (T1)	3	3	2	3	1	3	H1 and L2	0.44	2.67	ns	0.049
Low (T2)	1	1	1	1	3	1.5	H2 and L2	1.0	6.0	0.01	0.001
High (T2)	4	4	3	4	4	4	All 4 Groups*	0.72	10.86	0.001	—

lateral plates were actually larger in the low growth treatment despite the similarity in body size for this comparison (Table 5). Our findings must be treated as preliminary, however, for the four reasons that we discuss next.

First, our analyses demonstrated that the significance of FA differences between groups was test-dependent,

such that the more conservative Levene's and Bootstrap methods applied to single traits rarely indicated significant differences in pair-wise comparisons in contrast to the less conservative Bartlett's and Fmax tests. Differences between growth treatments were also more obvious when we analyzed body-wide measures of asymmetry combining all traits together (Table 4). This

Table 5. Tests of the difference in the size of traits between the high growth treatment at time 1 (T1) and the low growth treatment at time 2 (T2) when sticklebacks had similar mean standard lengths. Note that the low growth fish had larger plates and smaller fins compared to the high growth fish of the same standard length. This indicates that differential growth had an influence on the allometry of these traits. Estimates of least squares mean lengths (mm) of the traits are given for both treatments.

Trait	n	ANOVA		Mean trait size	
		F	Prob.	Low (T2)	High (T1)
Fin width	76	17.6	0.0001	1.48	< 1.71
Fin length	76	19.4	0.0001	3.86	< 4.58
Plate 4	75	0.001	ns	0.93	= 0.93
Plate 5	76	3.99	0.049	3.09	> 2.85
Plate 6	76	7.33	0.008	3.10	> 2.82
Plate 7	76	0.25	ns	2.86	= 2.79

is interesting because asymmetry is not often correlated between traits within individuals (Palmer & Strobeck 1986, Dufour & Weatherhead 1996, Bergstrom & Reimchen 2000). Such a correlation in FA levels among traits is consistent with our assumption that FA was an indication of overall developmental stability in this experiment.

Second, we have assumed throughout that developmental stability in these traits is positively related to fitness in sticklebacks. For example, if there is no reduction in fitness due to an increase in asymmetry then the positive relationship need not cause rapid growth to be constrained by development. That asymmetry has fitness consequences is controversial (e.g., Clarke 1998, Houle 1998). It is not clearly established that symmetry in the traits used here is positively related to fitness. For example, the relationship between lateral plate asymmetry (LPA) and fitness is poorly understood in sticklebacks. Moodie & Reimchen (1976) detected a negative correlation between LPA and the presence of predatory fishes suggesting that selection favors symmetry. On the other hand, other studies have shown a positive association between LPA and the probability of having young in a male's nest (Moodie & Moodie 1996), and a negative association between LPA and parasite load in juveniles (Reimchen 1997). Bergstrom & Reimchen (2000) suggest that the strength and direction of selection for symmetry may vary strongly among populations because the frequency of individuals with LPA varied from as little as 1% to as much as 76% with a mean of 42% among 60 natural populations. Less confusion may surround pelvic fin symmetry, which has been shown to be positively associated with female

fecundity (egg number and ovary size) in brook sticklebacks (Hechter et al. 2000). While this is perhaps the first demonstration of such a relationship in fishes, the cause of the association is unknown. Thus, asymmetry can be negatively, positively or unrelated to fitness in sticklebacks and further empirical work is required in sticklebacks and other fishes. In addition, we need to determine if the absolute levels of asymmetry generated by increased growth here and in future manipulative studies is different from levels of asymmetry found in natural populations. Although this may be difficult to interpret if levels of FA vary as dramatically among populations as recently found by Bergstrom & Reimchen (2000).

Third, our experimental methodology confounded two factors that may have independently influenced asymmetry, ration size and temperature. Our initial model viewed temperature and food ration as environmental factors that affect development, which in turn influences growth rate and developmental stability. It is possible that temperature may influence asymmetry independent of growth rate, particularly in poikilotherms. Any effects of temperature on asymmetry in fish are unlikely to be universal because they may be a function of the preferred body temperature of the particular species in question. As far as we know there is no information available to address this question in sticklebacks. Further experiments are therefore required to disentangle the effects of food ration and temperature on asymmetry in sticklebacks.

Fourth, trait asymmetry may be transitory if bilateral symmetry is restored by maturity (Teather 1996). While this would not mitigate the potential costs of asymmetric armor as a defensive structure during development, it could at maturity. It could also potentially reduce or eliminate any costs to mating success that are due to asymmetry. This is not as unlikely as it seems because sticklebacks exhibit a typical teleostean growth pattern with high growth early on in life followed by growth rate declining as size approaches an asymptote (Wootton 1994). If the asymmetry in lateral plates is initially due to a slight difference in the onset of plate growth between sides, and if lateral plates also grow in pattern similar to overall body size, then the lagging plate could catch up to the leading plate after the larger plate stops growing. We did not rear our fish to maturity and so cannot test how asymmetry varied over ontogeny, although FA did not change significantly over the duration of our experiment for most traits (the exception being

increased FA in fin width in the high growth treatment; Table 2).

Trade off between growth and developmental stability

Two methods are used to test for a relationship between rapid growth and development, those involving manipulating growth and measuring symmetry within a single generation as in our study, and those where growth is artificially selected and symmetry is compared among growth lines. As far as we know, fish have not been used in either method to assess such a trade off despite much work on the evolution of life histories in fishes. Leamy & Atchley (1985) selected for rapid growth in rats and found that the high growth line exhibited greater asymmetry than control lines. This was not simply a consequence of increased homozygosity (another possible source of developmental instability) because increased asymmetry was not found in similar lines selected for low growth. Developmental errors also appear more common in domestic animals selected for rapid growth (e.g., Riddell 1981, Yamasaki & Itakura 1988).

Experiments that have environmentally manipulated juvenile growth rate and subsequently assessed developmental stability show mixed results. Serafin (1982) demonstrated that one quarter of Greater Sandhill cranes fed diets promoting very rapid growth formed skeletal deformities in their limbs. Developmental disorders were also more common in the fastest growing chicks regardless of the food treatment. Hedhammar (1973) performed a similar experiment with Great Dane littermates divided into treatments either fed ad libitum or at two-thirds ad libitum. Developmental errors were more common in the faster growing treatment than in the treatment with restricted diet. However, inbreeding cannot be ruled out as a factor influencing the severity of the trade off in either of these cases. Contrary to these findings, Teather (1996) found that FA was uncorrelated with a variety of growth parameters distinguishing nestling tree swallows, although FA was related to peak growing periods within nestlings. On the other hand, Nilsson (1994) demonstrated that adult European nuthatches forced to slowly regrow feathers during periods of low food abundance (in winter) showed greater asymmetries in the size of the feathers compared to faster regrowth during periods of high food abundance (in late summer). A potentially exciting new method of assessing internal trade offs associated with rapid growth will be to study organisms

whose growth rate has been genetically enhanced by transgenic growth genes (e.g., Lachmansingh & Rollo 1994), as in numerous species of aquacultured fish.

Asymmetry as honest signals of mate quality

Recent research on asymmetry has focused on whether females can discriminate among potential mates on the basis of body symmetry (e.g., Moller 1991, 1992, 1997, Watson & Thornhill 1994). Under this hypothesis a male's ability to gain energy is assumed to be honestly signaled by his degree of symmetry, presumably because of constraints built into his energy budget. Nilsson (1994) found evidence to support this hypothesis using European nuthatches. He found that asymmetry was higher in the feathers regrown when food availability was low compared to feathers regrown when food was abundant. Our findings force us to reject the honest signal hypothesis at least for sticklebacks because symmetry was greatest under reduced resource levels.

There are at least three possible explanations for the different results. First, the growth of an organism and the regrowth of a feature of a mature organism are substantially different developmental processes, at least with respect to developmental stability. Maturity may change the relationship between growth rate and developmental stability because the allocation of limited resources to somatic vs. reproductive tissues can change upon reaching maturity. As far as we know, growth vs. regrowth has not been evaluated in this context. Second, the effect of resource level on asymmetry may be nonlinear. Unusually low or high food levels may influence asymmetry distinct from more moderate 'normal' resource levels. In our experiment the ration levels of 3% and 9% of wet body weight per day were not unusual for sticklebacks. For example, Allen & Wootton (1982) have estimated the maintenance ration for a 250 mg stickleback living at 15°C under laboratory conditions to be about 2% of its wet body weight per day (mean weight of fish in our experiment = 199 mg). Since food abundance did not appear to be unusually high or low in either experiment, a nonlinear response of asymmetry to ration level is an unlikely explanation for the different results.

Third, food abundance may not have been the only factor responsible for the different levels of asymmetry in either experiment. Confounding seasonal differences may have influenced Nilsson's (1994) results, just as temperature may have had direct effects on

asymmetry in our experiment. Nilssen attempted to control for the effects of season with two winter treatments, augmented food versus no extra food provided. The effect of food abundance on asymmetry was not supported by comparing patterns of asymmetry between these two treatments ($t = 0.656$, $DF = 15$, $p > 0.25$, determined using data from Nilssen 1994). This indicates that seasonal factors had a more important effect on asymmetry than did food abundance. While the difference in temperature between our high and low growth treatments was within the natural summer temperature range experienced by sticklebacks (e.g. Bentzen et al. 1984), ration level and temperature may still have different effects on the developmental process that underlies growth and symmetry. Nevertheless, these and other results (e.g., Teather 1996) do not provide strong support for the honest signal hypothesis, at least with respect to developmental stability during juvenile growth.

Our results support Case's (1978) and Arendt's (1994) contention that predictions about the evolution of life history traits will likely benefit from an explicit attention to the evolution of 'adaptive' growth rate (e.g., Abrams et al. 1996, Niewiarowski 2001). This is because size strongly influences age-specific survival and fecundity, and growth rate defines the relationship between age and size. Our focus on one specific trade off between growth and development involving developmental stability does not preclude other possible constraints on the evolution of growth rate. Development may constrain growth because rapid growth can cause shifts in the allometry of tissues and structures that affect fitness (Riddell 1981, Kemp & Bertness 1984, Ricklefs & Marks 1985, Boulding & Hay 1993). In our experiment, the size of pectoral fins and lateral plates relative to body size varied among growth treatments (Table 5), although we do not know if this can influence fitness. Rapid growth can also affect other aspects of tissue quality, such as bone or shell density (Kemp & Bertness 1984, Letierrier & Nys 1992, Boulding & Hay 1993). In other words, rapid growth may compromise development through increased rates of developmental error and changes in trait expression. These results provide one explanation for the growing body of evidence indicating that growth rate is rarely maximized in organisms that exhibit flexible growth. The consequences of this to the evolution of life history traits are just beginning to be explored, and fish lend themselves well to experimental tests in this developing field.

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