



## SYMPOSIUM

# Merging the “Morphology–Performance–Fitness” Paradigm and Life-History Theory in the Eagle Lake Garter Snake Research Project

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**Synopsis** The morphology–performance–fitness paradigm for testing selection on morphological traits has seen decades of successful application. At the same time, life-history approaches using matrix methods and perturbation studies have also allowed the direct estimate of selection acting on vital rates and the traits that comprise them. Both methodologies have been successfully applied to the garter snakes of the long-term Eagle Lake research project to reveal selection on morphology, such as color pattern, number of vertebrae, and gape size; and life-history traits such as birth size, growth rates, and juvenile survival. Here we conduct a reciprocal transplant study in a common laboratory environment to study selection on morphology and life-history. To place our results in the ecomorphology paradigm, we measure performance outcomes (feeding rates, growth, insulin-like growth factor 1 titers) of morphological variation (body size, condition) and their fitness consequences for juvenile survival—a trait that has large fitness sensitivities in these garter snake populations, and therefore is thought to be subject to strong selection. To better merge these two complementary theories, we end by discussing our findings in a nexus of morphology–performance–fitness–life history to highlight what these approaches, when combined, can reveal about selection in the wild.

## Introduction

The “morphology–performance–fitness” paradigm was put forth by Stevan J. Arnold in these pages (Arnold 1983) from a SICB (formerly American Society of Zoologists) 1981 symposium on snake feeding mechanisms. In that paper, Arnold presented a statistical method to test for the adaptive significance of morphological variation by tracing the influence of morphology on organismal performance and, in turn, on individual fitness. When framed in microevolutionary selection theory, the strength of natural selection on these phenotypes could be quantitatively assessed (Lande and Arnold 1983; Arnold and Wade 1984a, 1984b) and the adaptive

significance of this variation tested. Ensuing studies of morphology–performance–fitness (i.e., the fitness consequences of morphological variation as mediated through behavior and physiology) helped to change the field of micro-evolution from claims of adaptive story-telling (sensu Gould and Lewontin 1979) to a comprehensive and robust assessment of adaptation in diverse taxa (e.g., Losos 1990; Schluter 1995; Dudley 1996).

At the same time, the theory of life-history evolution (Stearns 1992) and the advent of matrix methods to test for the adaptive significance of variation in life-history traits (Caswell 2001)—complex traits that would not yield easily to the ecomorphology

paradigm—rapidly enhanced our ability to ask how and when variation in life-history traits (themselves mediated by morphology and performance) could reveal adaptation. Specifically, matrix models of population demography allowed for the quantification of the strength of natural selection on individual vital rates and life-history traits, as well as comprehensive tests for adaptive significance of such variation. Parallel questions could now be addressed both in the realms of morphology/behavior and life-history/demography. Because variation in vital rates (such as birth rates, juvenile survival, etc.) is underlain by variation in morphology and performance, these two theoretical constructs have a straightforward complementarity in their use to assess selection gradients (Lande 1982a).

The long-term Eagle Lake garter snake study system (Lassen County, California, USA) has figured prominently in empirical tests of ecomorphology theory and, more recently, in empirical tests of life-history theory. On the ecomorphology side, Arnold's pioneering work on the adaptive significance of morphological variation—feeding mechanisms, scale counts, and vertebral variation—produced now-classic examples of the utility of this framework to draw strong inferences on the adaptive significance of morphological variation (e.g., Ayres and Arnold 1983; Arnold and Wade 1984a; Arnold 1992; Kelley et al. 1997; Manier et al. 2007.) Furthermore, this paradigm was extended to link behavioral variation to subsequent variation in performance and fitness (Arnold 1988). At the same time, discovered through our decades of mark/recapture efforts, these same populations of Eagle Lake garter snakes harbor two distinct life-history phenotypes whose study has provided examples of the evolution of life histories within closely located populations (Bronikowski and Arnold 1999; Bronikowski 2000; Miller et al. 2011, 2014).

Conventionally, this ecomorphological paradigm has been concerned with measuring traditional morphology (e.g., coloration, shape, number of vertebrae) and mapping these traits in a statistical model with traditional performance traits (e.g., prey capture, slither speed) and fitness. We contend that the possibilities for considering performance are much broader and include traits that represent rates or endpoints of rates. For example, in our previous work on the Eagle Lake garter snake system, we have measured non-traditional estimates of performance such as innate and acquired immune function (measures of natural antibody production, bactericidal capability, and mitogen-stimulated lymphocyte proliferation; Palacios et al. 2011, 2013); hormonal titers of glucocorticoids (Palacios et al. 2012) and insulin-like growth factors 1

and 2 (IGF-1 and IGF-2; Sparkman et al. 2009; Reding et al. 2016); and metabolic function (Gangloff et al. 2015). While we recognize that immunological and hormonal responses are not in themselves a measure of performance, they are physiological mechanisms that contribute to variation in performance traits including growth, development, maturation, reproduction, and lifespan. Of these physiological traits, we focus on the endocrine system in this study, as it mediates relationships among morphology, fitness, and life-history traits similar to its role in mediating trade-offs among life-history traits (Ketterson and Nolan 1999; Zera and Bottsford 2001; Ricklefs and Wikelski 2002; Lailvaux and Husak 2014, Dantzer et al. 2016). We focus specifically on IGF-1, which is the primary hormone of the insulin/insulin-like signaling (IIS) system, and is a peptide hormone that is a paralog of insulin that has been highly conserved across amniotes (Duan 1998; Denley et al. 2005; Annunziata et al. 2011; Sparkman et al. 2012; McGaugh et al. 2015; Zhu et al. 2017) and facilitates variation in growth and maturation (e.g., Dantzer and Swanson 2012; Reding et al. 2016; Schwartz and Bronikowski 2016; Lewin et al. 2017).

Lailvaux and Husak (2014) argued convincingly for direct and explicit linkages between ecomorphology and life-history. Following their lead, we undertake here an explicit test of the relationships among morphology, performance, fitness, life-history, and demography in the Eagle Lake populations of garter snakes. Because of the clear function that IGF-1 has for organismal growth and its association with life-history phenotypes in the Eagle Lake garter snake system, we focus our performance measures on IGF-1, feeding rate, and growth. Specifically, we address the following three questions: (1) How does morphological variation at birth translate into variation in performance—measured as feeding rate, growth, and circulating IGF-1 concentration? (2) How does variation in these morphological and performance traits impact fitness—measured as survival from age 1 yr until sexual maturation (i.e., juvenile survival)? (3) How do phenotypes defined along specific morphology–performance–fitness trajectories map to variation in population demographic estimates of fitness, measured as population growth rates?

## Methods

### Study system

Populations of western terrestrial garter snakes (*Thamnophis elegans*) in the vicinity of Eagle Lake (Lassen County, CA, USA), are characterized by

**Table 1** Sample sizes and sampling dates for the full cohort of *T. elegans* neonates born and raised in captivity

Study	N neonates	Ecotype (litters, neonates)	Female- warm	Female- cool	Male- warm	Male- cool
<b>Cohort morphology, growth, feeding, and survival</b>						
Birth <sup>a</sup>	257 (65 released) <sup>b</sup>					
Assigned a rearing treatment	192	M-slow (19, 72)	18	20	18	16
		L-fast (21, 120)	31	29	30	30
Sampling dates						
November 29, 2010	189					
November 1, 2011 <sup>c</sup>	153					
November 15, 2012	66					
August 19, 2013	56					
December 10, 2014	28					
<b>IGF1-growth study<sup>d</sup></b>	130	M-slow (18, 47)	10	10	15	12
		L-fast (21, 83)	22	20	21	20

<sup>a</sup>Birth occurred August 12 to September 19, 2010.

<sup>b</sup>Sixty-five neonates from very large litters were released shortly after birth into their natal populations.

<sup>c</sup>Survival analysis included individuals surviving to 1 year (i.e., survival through the neonate stage) and used these 153 1-year olds.

<sup>d</sup>IGF1-growth study initiated September 14, 2011 on a subset of the cohort.

either slow or fast-paced life-history ecotypes. Lower elevation lakeshore snakes (“L-fast” ecotype) exhibit fast growth, large adult body size, early reproductive maturation with large reproductive effort, and low annual survival. Higher elevation meadow snakes (“M-slow” ecotype) exhibit slow growth, smaller adult body size, later maturation, low annual reproduction, high annual survival, and measurable demographic senescence with advancing adult age (Bronikowski and Arnold 1999; Sparkman et al. 2007; Miller et al. 2011; Schwartz et al. 2015). Selection gradients vary between these fast and slow ecotypes for survival and growth in the pre-adult stages (Miller et al. 2011). Many ecological factors differ between the two ecotypes: predation rates are higher in the lakeshore habitat (Sparkman et al. 2013) as is prey abundance and average temperature (Bronikowski and Arnold 1999). In wild-caught animals, variation in total plasma IGF-1 is driven by interactions among body length, ecotype, and life-history stage (juvenile or adult). At the same time, IGF-1 is sensitive to ecological variation: levels vary across year and covary with precipitation, likely due to the relationship between precipitation and prey availability (Sparkman et al. 2009).

### Study animals and treatment groups

In June 2010, we collected 40 pregnant females from six populations of *T. elegans* around Eagle Lake: three replicate populations of each of the L-fast and M-slow ecotypes. We transported these pregnant females to Iowa State University and housed them individually through gestation in 10-gallon glass aquaria.

Offspring ( $n = 257$ ) from these 40 pregnant females were born between August 12 and September 19, 2010 (Table 1). Within 24 h of birth, we determined offspring sex, measured their weight (g) and snout-vent length (SVL in mm), and moved each snake to an individual plastic cage. We divided litters randomly, with sexes split nearly evenly, into two temperature-rearing groups designed to mimic the differing thermal regimes of the warmer L-fast and cooler M-slow habitats (as in Bronikowski 2000; Gangloff et al. 2015). Snakes in the warm treatment received 16 h of supplemental heating per day to provide a gradient (22–32 °C) allowing thermoregulation, while the cool treatment received supplemental heating for only 8 h per day. Snakes were offered food (frozen/thawed pinky mice) once per week during the active season and hibernated at 4 °C for 4–5 months each winter (full captive care details in Gangloff et al. 2015).

### Repeated measures of IGF-1 and growth

A subset of the full colony, selected from each sex × ecotype × rearing treatment group, were sampled for short-term repeated measures analysis of total plasma IGF-1 concentration and growth to assess the relationship between IGF-1 titer and short-term growth rate. These 130 snakes from this 2010 cohort (Table 1) were weighed and measured on May 6, 2011; September 14, 2011; November 1, 2011; and January 12, 2012—i.e., at approximately 10, 14, 16, and 18 months of age. On the latter three dates, blood samples were collected from the caudal vein in heparin-rinsed syringes. Plasma was separated from red blood cells by centrifugation,

snap-frozen, and stored at  $-80^{\circ}\text{C}$ . Treatment of all experimental animals was in accordance with Iowa State University Institutional Animal Care and Use Committee protocol #3-2-5125-J.

Plasma IGF-1 concentrations were determined by radioimmunoassay (RIA) using GroPep Protocol #3002 (GroPep Ltd., Adelaide, Australia) via competitive binding with  $^{125}\text{I}$  labeled IGF-1 (PerkinElmer #NEZ033). This assay uses an antibody to human IGF-1 and was validated for the western terrestrial garter snake by Sparkman et al. (2009). IGF binding proteins were removed from IGF-1 by acid-ethanol extraction. All samples were measured in duplicate within a single assay. Intra-assay variation was 7.5%.

### Statistical analysis

All data were analyzed using PROC MIXED and PROC PHREG in SAS 9.4 (SAS Institute, Cary, NC, USA) with  $\alpha = 0.05$ . All data were first inspected for normality and homogeneity of variances and transformed to meet these assumptions as necessary (noted below). In all linear mixed model analyses of the captive-born cohort, we included population nested within ecotype as a fixed effect to account for among-population habitat heterogeneity within ecotypes (Palacios et al. 2013) and litter nested within population and ecotype as a random effect to account for among-litter variation within populations (Robert and Bronikowski 2010). Denominator degrees of freedom for  $F$ -tests were estimated using the Kenward–Roger Degrees of Freedom Approximation (Kenward and Roger 1997). For all models, we utilized a backward-selection approach toward interaction terms, retaining statistically significant interactions ( $P < 0.10$ ) and those that tested hypotheses of biological interest. Figures were made with the “ggplot” package (Wickham 2009) for R (R Core Team 2014).

### Morphology measures: body size

We analyzed body size at measurement points throughout lifetime as the SVL (in mm) of individual snakes, beginning at birth and until their last measurement before death or end-of-study. We used repeated-measures linear mixed models to test for the effects of ecotype, rearing treatment, age (in days), and interactions thereof, on body size. We also included sex as a fixed effect in this model but removed the non-significant interactions between sex and other fixed effects.

### Performance measures: growth, feeding, and plasma IGF-1 concentrations

To test for the main effects of ecotype and rearing treatment on growth rates during the short-term

IGF-1 sampling intervals, and then to test for the effects of ecotype, rearing treatment, and growth on IGF-1 levels, we used repeated measures linear mixed models. We modeled short-term growth (calculated as the specific growth rate, a measure of growth scaled to body size:  $\text{SGR} = 100 \times [\ln(\text{SVL}_2) - \ln(\text{SVL}_1)]/\text{days}$ ; Reid et al. 2011; Killen 2014) during the three intervals of IGF-1 sampling with a repeated-measures mixed model. This model included the main effects of interval (10–14, 14–16, and 16–18 months of age), ecotype, and rearing treatment. We also included sex as a fixed effect. Furthermore, we included the time-varying covariate of food consumed in the preceding interval.

IGF-1 values (ng/mL) were  $\log_{10}$  transformed to meet normality assumptions. Because IGF-1 is a main mediator of growth hormone effects, we considered three different proxies for growth in our analyses: body size (SVL), growth in the interval preceding the IGF-1 measure (change in SVL divided by the duration of the interval, mm/day; “prior interval growth”), and growth in the interval following the IGF-1 measure (“subsequent interval growth”). This latter variable of subsequent interval growth had far fewer observations available for the last IGF-1 measure because most animals were placed into hibernation after the last blood draw. Our models using each of these explanatory growth variables were in general agreement with each other (data not shown), but because we were interested in the effect of growth *per se* (and not body size) on IGF-1 we included growth in the preceding interval as the covariate in our final model. Our initial model included food consumption as a covariate over the same period as growth, but as it was not significant and collinear with growth, we removed it from the model.

We analyzed additional three additional measures of performance, included in Supplemental Materials: circulating IGF-1 in field-caught snakes and both lifetime growth and lifetime feeding rates in the captive-born cohort.

### Fitness measures: survival

We analyzed survivorship of captive-born snakes from ages 1 to 4 years (September 2011 to December 2014) with a semi-parametric Cox proportional hazards model. We included only individuals surviving to age 1 to correspond with the timing of the repeated measures of growth and IGF-1 study and because we were interested in the effects of morphology and performance on survival after the period of high neonatal mortality. We included the fixed factors of rearing treatment,

**Table 2** Repeated-measures linear mixed model analysis of body size (SVL in mm) from birth to December 2014 in captive-born *T. elegans*. Significant effects are indicated in bold.

Source of variation	Estimate	df <sub>n</sub> , df <sub>d</sub>	F	Pr > F	Direction of significant factors
Ecotype	10.22	1, 31.5	13.65	<b>0.0008</b>	L-fast > M-slow
Rearing treatment	-4.19	1, 137	1.45	0.23	-
Age	0.085	1, 140	1046.0	<b>&lt;0.0001</b>	Older > Younger
Sex	-4.36	1, 140	9.67	<b>0.0023</b>	Males > Females
Ecotype × Rearing treatment	5.22	1, 137	3.93	<b>0.049</b>	See Fig. 1
Ecotype × Age	0.035	1, 140	1046	<b>0.0015</b>	See Fig. 1
Rearing treatment × Age	0.038	1, 140	13.12	<b>0.0004</b>	See Fig. 1
Ecotype × Treatment × Age	-0.024	1, 140	2.77	0.098	-
Population(Ecotype)	-	5, 31.3	1.53	0.21	-

ecotype, and sex, as well as all possible two- and three-way interaction terms. To test for the effect of morphology on survival, we included body size at one year (SVL measured in September 2011) and body condition at one year (residual of the log-mass on log-SVL regression). To test for the effect of performance on survival, we included lifetime feeding rate (calculated as the amount of food consumed before death divided by the number of days alive) and lifetime growth rate (specific growth rate, SGR). No direct test of IGF-1 titer on survival was performed because the IGF-1 titers are plastic and can vary greatly over the lifetime; instead our analysis incorporates growth, for which we separately tested for an effect of IGF-1 concentration (above). We included litter as a random effect (PROC PHREG does not accept nested random effects).

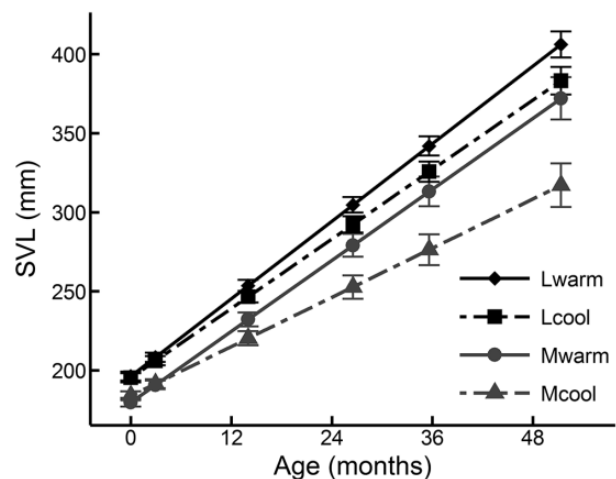
## Results

### Morphology measures: body size

For the repeated measures of body size from birth to 4 years of age, all two-way interactions of ecotype, rearing treatment, and age were significant in determining body size across lifetime, such that Mcool snakes increased size more slowly compared to the other ecotype-treatment group combinations (Table 2, Fig. 1). The main effect of ecotype was significant as well, with L-fast snakes maintaining larger body size across lifetime than M-slow snakes. Additionally, sex was a significant factor, with males being slightly larger than females after correcting for other effects.

### Performance measures: growth and plasma IGF-1 concentrations

Growth rate was determined by the interaction of time interval, ecotype, and rearing treatment such that growth rate was highest in the first interval compared to other intervals, while Mcool snakes



**Fig. 1** Size as a function of age by ecotype and rearing treatment for *T. elegans* juveniles born and raised in captivity through age 4 years. Depicted are least-square means ( $\pm$  SE) from the model in Table 2.

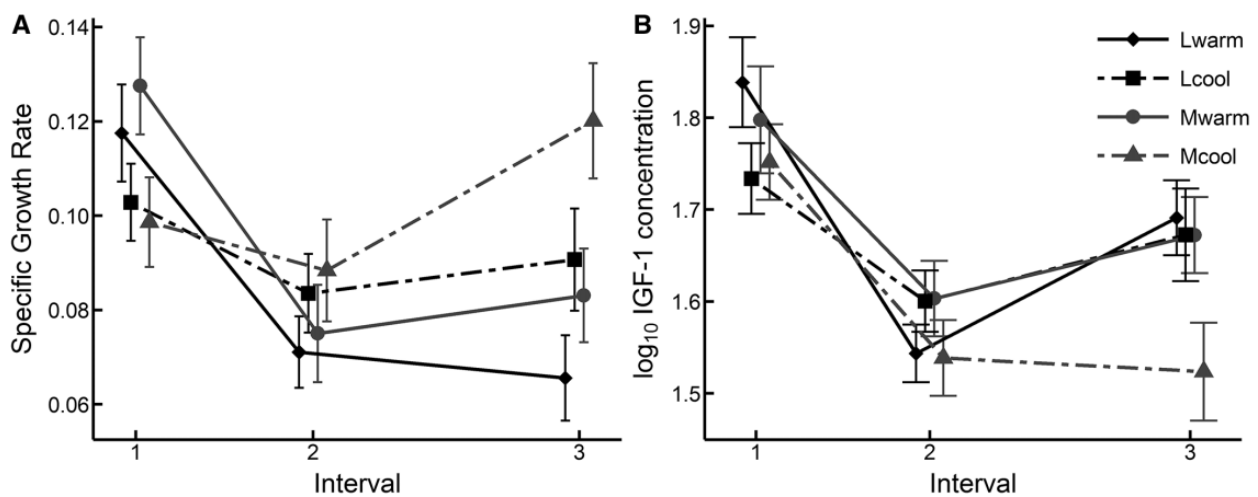
had higher growth rates in the third interval compared to other treatment groups (Table 3, Fig. 2A). Snakes that ate more also grew more (Table 3).

Higher growth rates in the interval preceding IGF-1 measurement corresponded with lower levels of circulating IGF-1 (Table 4, Fig. 2B). This negative correlation between previous growth and current IGF-1 was consistent across all three intervals. Accordingly, males grew slower than females across these three sampling intervals (Table 3, Fig. 2A) and males had overall higher levels of plasma IGF-1 (Table 4). In addition, snakes in the warm treatment had higher IGF-1 levels than those in the cool treatment (Table 4, Fig. 2B). Interestingly, the pattern of Mcool snakes diverged from other treatment groups in the third interval, exhibiting both higher growth rates and lower IGF-1 levels (Fig. 2).

Both ecotype and rearing treatment affected lifetime growth and feeding rates, such that L-fast

**Table 3** Repeated measures linear mixed model analysis of growth (SGR) over three time intervals measured between 10 and 18 months of age in captive-born *T. elegans*. Significant effects are indicated in bold.

Source of variation	Estimate	df <sub>n</sub> , df <sub>d</sub>	F	Pr > F	Direction of significant factors
Food consumed	0.0043	1, 169	127.36	<b>&lt;0.0001</b>	More consumed > Less consumed
Sex	0.0088	1, 109	4.52	<b>0.036</b>	Females > Males
Interval	–	2, 145	5.46	<b>0.0052</b>	Interval 1 > 3 > 2
Ecotype	–0.057	1, 39.3	2.03	0.16	–
Rearing treatment	–0.037	1, 96.8	2.78	0.099	–
Ecotype × Interval	–	2, 101	2.07	0.13	–
Rearing treatment × Interval	–	2, 107	10.05	<b>0.0001</b>	–
Ecotype × Rearing treatment	0.012	1, 81.5	0.00	0.96	–
Ecotype × Rearing treatment × Interval	–	2, 99.2	0.66	0.52	–
Population(Ecotype)	–	6, 30.8	2.09	0.083	–



**Fig. 2** Repeated measures of growth (SGR; Panel **A**) and plasma IGF-1 concentration ( $\log_{10}$ -transformed ng/mL; Panel **B**) by ecotype and rearing treatment for a subset of captive-born *T. elegans* juveniles measured across three time intervals (see “Methods” for details). In Panel **B**, the marginally significant three-way interaction of ecotype × treatment × interval is driven by the deviation of the Mcool values from the others during Interval 3. Depicted values are least-square means ( $\pm$  SE) from the models in Tables 3 and 4, respectively.

snakes ate and grew more than M-slow snakes and snakes in the warm rearing treatment ate and grew more than snakes in the cool rearing treatment (Supplementary Tables S2 and S3, Supplementary Fig. S2).

### Fitness measures: survival

Survivorship from age 1 to 4 years was dependent on the three-way interaction of ecotype, rearing treatment, and sex (Table 5, Fig. 3). Generally, L-fast snakes had higher survivorship than M-slow snakes, as did snakes in the warm rearing treatment relative to snakes in the cool treatment. The effect of sex was dependent on ecotype, with females experiencing higher survivorship in the L-fast ecotype and males in the M-slow ecotype. Additionally, snakes larger at

1 year of age and snakes with slower growth rates lived longer than small snakes and fast-growers. Overall, mortality rates were commiserate with previous work done in this system and with neonate mortality rates in wild populations (reviewed in Parker and Plummer 1987; Bronikowski 2000).

### Discussion

Life-history theory postulates trade-offs among suites of life-history traits—such as along a “pace-of-life” continuum—where species are arrayed along an axis from “slow” to “fast” living (Promislow and Harvey 1990). On the slow end of the continuum are species with slow growth and delayed sexual maturation, but with extended lifespan and iteroparity. On the fast end are species that exhibit fast growth, rapid

**Table 4** Repeated measures linear mixed model analysis of plasma  $\log_{10}$ IGF-1 concentration over three time intervals measured between 10 and 18 months of age in captive-born *T. elegans*. Significant effects are indicated in bold.

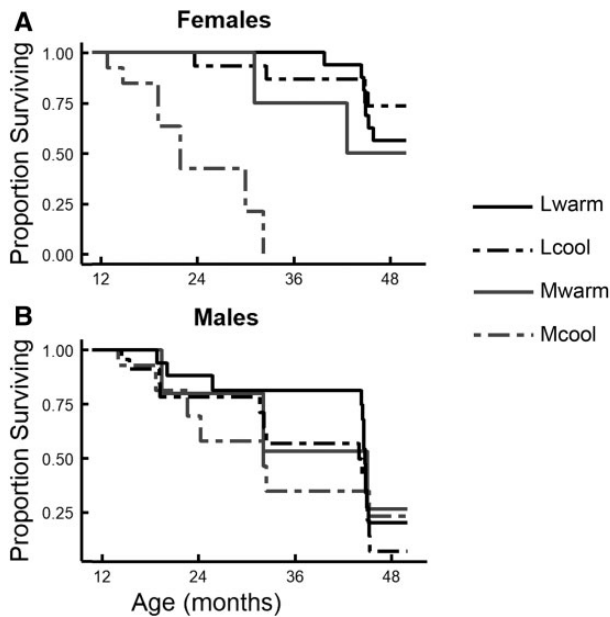
Source of variation	Estimate	df <sub>n</sub> , df <sub>d</sub>	F	Pr > F	Direction of significant factors
Prior interval growth	-0.0013	1, 236	4.41	<b>0.037</b>	Slower > Faster
Sex	-0.048	1, 95.3	5.54	<b>0.0021</b>	Males > Females
Interval	-	2, 209	9.73	<b>&lt;0.0001</b>	-
Ecotype	0.16	1, 46.9	1.34	0.26	-
Rearing treatment	0.15	1, 103	6.09	<b>0.015</b>	Warm > Cool
Ecotype × Interval	-	2, 178	1.34	0.26	-
Rearing treatment × Interval	-	2, 191	1.78	0.17	-
Ecotype × Rearing treatment	-0.13	1, 93.4	2.28	0.13	-
Ecotype × Rearing treatment × Interval	-	2, 183	2.45	0.089	-
Population(Ecotype)	-	6, 32.4	0.54	0.78	-

**Table 5** Semi-parametric Cox proportional hazards model of survivorship from age 1 to age 4 years in captive-born *T. elegans*. Significant effects are indicated in bold.

Source	$\chi^2$	Adj. df	P	Direction of significant factors
SVL at 1 year	5.23	0.88	<b>0.018</b>	Larger snakes > Smaller snakes
Body condition at 1 year	0.34	0.85	0.49	-
Lifetime feeding rate	0.29	0.89	0.54	-
Lifetime growth rate	20.23	0.88	<b>&lt;0.0001</b>	Slow growers > Fast growers
Sex	2.63	0.84	0.083	-
Ecotype	0.63	0.41	0.18	-
Rearing treatment	1.28	0.90	0.23	-
Sex × Ecotype	11.67	0.88	<b>0.0005</b>	Female L-fast > Male L-fast Male M-slow > Female M-slow
Sex × Rearing treatment	1.52	0.93	0.20	-
Sex × Ecotype × Rearing treatment	3.81	0.93	<b>0.046</b>	See Fig. 3
Population(Ecotype)	3.19	2.38	0.26	-

reproduction, semelparity or high reproductive effort in relatively fewer bouts, and a short lifespan (e.g., Lemaitre et al. 2015). This pattern is frequently documented across species; however, some studies have recognized a slow–fast continuum within a single species among populations that have experienced different selective pressures over generations (e.g., *Anolis heterodermis*, Moreno-Arias and Urbina-Cardona 2013); *T. elegans*, (this system); *Sceloporus grammicus*, Pérez-Mendoza and Zúñiga-Vega 2014). It is in such cases, where intra-specific variation in life-history strategies exists, that it is most fruitful to understand these polymorphisms in the ecomorphology framework. Specifically, when morphological and performance variation give rise to fitness differences among individuals, these fitness differences provide natural selection the basis to shape variation in population vital rates, including survivorship and reproduction.

In this garter snake system, we found that variation in body size—a morphological measure—can be accounted for by fixed differences between the ecotypes and interactions with thermal rearing treatment and age. Generally, older juvenile snakes growing in warmer conditions are larger than younger ones growing at cooler temperatures, particularly in the L-fast ecotype (Table 2, Fig. 1). In turn, variation in body size affects both short-term growth (a performance measure) as well as survival through the juvenile stage (a fitness measure): larger snakes grow more and experience higher survival than smaller snakes. Moreover, growth itself is predictive of survival, with slow growth (after age 1 year) predicting higher juvenile survival (Table 4, Fig. 3). In addition to growth, we considered two other performance measures: feeding rate and circulating IGF-1 concentration. Variation among individuals in feeding rate was not related to morphological variation directly.



**Fig. 3** Survival curves for captive-born *T. elegans* juveniles by ecotype and rearing treatment within each sex. Plots show the significant influence of ecotype, treatment, and sex on survivorship based on a semi-parametric Cox proportional hazards model (Table 5).

Generally, feeding rate was highly impacted by thermal rearing treatment, with snakes in the warmer treatment eating more, resulting in these animals growing more, and reaching larger body sizes. Such temperature-based plasticity of feeding behavior was further impacted by ecotype, with L-fast snakes eating disproportionately more than M-slow animals when reared in warm conditions (Supplementary Table S3, Supplementary Fig. S2B). In the short-term repeated measures growth and IGF-1 experiment, feeding rate and growth were essentially interchangeable, while food consumption and rearing treatment were confounded—animals in the warm treatment ate more and therefore grew more (Table 3, Fig. 2A).

Our final performance measure, IGF-1 plasma concentration, was considered over two time scales. First, we analyzed 5 years of field-collected blood plasma, finding a significant interaction among body size, ecotype, and life stage (juvenile versus adult). These results show that for adult snakes, the manner in which body size associates with IGF-1 titers is ecotype-dependent: L-fast adults have increasing IGF-1 with increasing body size, whereas M-slow adults show no dependence of circulating IGF-1 on body size (Supplementary Table S1, Supplementary Fig. S1). Our previous work has shown that M-slow adults essentially stop growing once they reach adulthood, whereas L-fast adults continue growing over their adult lifespans with ever

increasing size-associated fecundity (Bronikowski and Arnold 1999; Sparkman et al. 2007). Without additional data on individual growth rates, these field data cannot distinguish between competing functions of IGF-1 among growth, reproduction, and survival in juveniles and adults. Thus, we undertook here a short-term common-garden growth and IGF-1 study in which we documented, among other effects, a short-term negative association between IGF-1 levels and growth rates (Table 4, Fig. 2; see also the companion study Reding et al. 2016).

### IGF-1 as a mediator of ecomorphology to life-history transition

We originally envisioned IGF-1 as a measure of performance that may impact survival and that is itself potentially influenced by body size or body condition. Our results suggest it may be more appropriate to think of IGF-1 as a mediator of the morphology–performance–fitness–life history nexus. We found that variation in IGF-1 is not explained by food consumption *per se* and negatively relates to growth preceding IGF-1 measurement (Table 4). Others have found that IGF-1 responds to nutrition and/or food availability (Pierce et al. 2005; Beckman 2011; Hetz et al. 2015). For example, in the lizard *Sceloporus undulatus* both hepatic IGF-1 mRNA and circulating IGF-1 levels were reduced in response to food restriction but returned to normal levels at the cessation of food restriction (Duncan et al. 2015). We have hypothesized elsewhere that in this system, lower levels of circulating IGF-1 in M-slow snakes in the wild may be related to their unpredictable food availability (Sparkman et al. 2009; Robert and Bronikowski 2010).

In the short-term, prior food consumption was not correlated with IGF-1 levels beyond its positive association with warm rearing treatment. Yet, prior growth was negatively correlated with titers of IGF-1. These results suggest that the effects of food on growth are not directly mediated through IGF-1, similar to findings in the brown house snake (Sparkman et al. 2010) and Japanese quail (Ronning et al. 2009), but contrary to findings in salmon and lizards (Pierce et al. 2001; Duncan et al. 2015). It is possible that IGF-1 levels may only respond to extreme changes in food availability; since our experimental snakes were offered food at regular intervals and fed to satiation, there might have been insufficient variation in available food to affect IGF-1 levels. Another possible explanation could be that food affects transcription or translation of the IGF-1 gene, but we do not see those effects manifested into circulating IGF-1 protein concentration.



The negative correlation between prior growth and titers of circulating IGF-1 is opposite to the pattern found in brown house snakes (Sparkman et al. 2010), great tits (Lodjak et al. 2014), spiny lizards (Duncan et al. 2015), pied flycatchers (Lodjak et al. 2017), and spotted hyenas (Lewin et al. 2017, but note this study examined mass not growth), but similar to that found in M-slow *T. elegans* in the field (Sparkman et al. 2009, but note this study was looking at size not growth). More frequent measurements of size and IGF-1 could be more informative. For example, in salmon the strongest relationship between IGF-1 levels and growth was associated with the growth that occurred during 1 month preceding the hormone measurement (Beckman et al. 2004).

One key component of the IIS system the present study did not examine was localized expression of IGF-1. As we only looked at circulating levels of IGF-1, we do not know how temperature and food may have affected localized production of the hormone. A companion study by Reding et al. (2016) found that mRNA of IGF-1 in liver and muscle was higher in animals raised at cooler temperatures compared to those same tissues in animals in the warm rearing treatment. These results support our findings of higher levels of circulating IGF-1 in cool-treatment animals at our final time point (interval 3), although Reding et al. (2016) found that circulating IGF-1 levels were higher in warm animals (note that the current study had a larger sample size and more time intervals for growth and hormone measurement). The importance of paracrine and autocrine production versus endocrine release of IGF-1 is equivocal (e.g., Chauvigne et al. 2003; Eppler et al. 2007). Variation in the site of IGF-1 production, the density of the IIS receptors in various tissues, and potential interactions with growth hormone, warrant further study.

### Growth variation as a mediator of survival

As has been seen in snakes and other ectothermic vertebrates (Sinervo and Adolph 1989; Folkvord and Ottera 1993; Gangloff et al. 2015), initial size affects how much an individual grows. However, when considering growth over 4 years, the initial importance of birth size (or an interval's start size) disappears as animals reach sexual maturation. In the present study, this was true in both ecotypes and in both temperature treatments. Food consumption also positively predicted growth rate, with more food consumed in the warm treatment and as animals increased in size (as seen in other studies, such as Sparkman et al. 2010; Lodjak et al. 2014).

Previous work in this system, including a different subset of individuals from this same cohort, demonstrates that growth efficiency is higher in larger animals and animals in the warm rearing treatment (Gangloff et al. 2015). Whole organism resting metabolic rate—an additional performance measure—may shed light on long-term versus short-term trends in our study. In both ecotypes, snakes with higher mass-independent resting metabolic rates had lower growth efficiency (a measure of conversion of ingested food into body substance; Gangloff et al. 2015). Juvenile snakes that grew fastest after 1 year of age had lower probability of survival, despite that animals that started off larger had a higher probability of surviving their first 4 years. Although not a topic of this study, if faster growers were engaged in compensatory growth (e.g., due to cooler rearing temperatures), then these results would suggest a downstream cost in terms of survival (see also Bjørndal et al. 2003; Metcalfe and Monaghan 2003; Roark et al. 2009; Marcil-Ferland et al. 2013; Orizaola et al. 2014; reviewed in Lindstrom 1999).

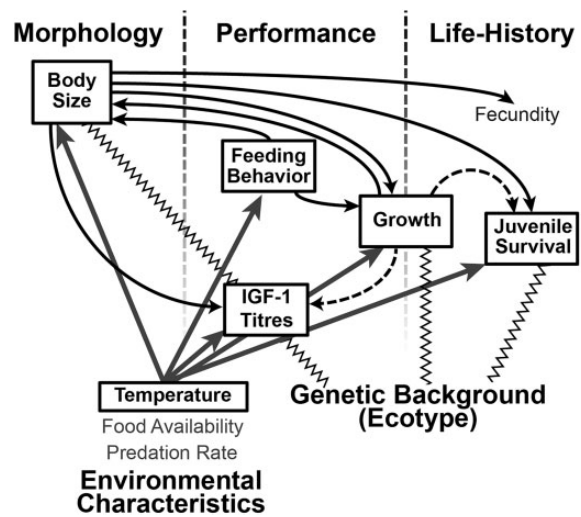
### Selection gradients: the direct link between ecomorphology and life history

One of the elements of the morphology–performance–fitness paradigm is the calculation of selection gradients and thus the ability to infer whether and how selection is operating on specific morphological/performance traits (Arnold 1983). Accomplished through regression techniques with a proxy for fitness and multiple phenotypes (Lande and Arnold 1983; Arnold and Wade 1984a, 1984b), the partial regression coefficients can be interpreted as the selection gradients. On the life history evolution side, population demography sensitivity analyses applied to a Leslie or Lefkovich matrix of vital rates can address how variation in vital rates affects population growth rates and how sensitive the fitness of a phenotype is to perturbations in vital rates (reviewed in Caswell 2001). At the same time, Lande (1982a, 1982b) made explicit that these estimated sensitivities are equivalent to selection gradients. Thus, selection gradients should be a direct link between ecomorphology and life-history theory.

Selection gradients vary between the L-fast and M-slow ecotypes for survival and growth in the pre-adult stages (Miller et al. 2011). Specifically, neonatal and juvenile survival are two vital rates that differ in their estimates of sensitivities (selection gradients) and elasticities (contribution to population growth rates) between M-slow and L-fast populations. In M-slow populations, survival increases in the juvenile stage to levels equivalent to that of adults (ca.

0.80 probability of annual survival), whereas in L-fast populations, juvenile survival is much lower than for adults ( $\sim 0.50$  probability of annual survival). Accordingly, the selection gradients for juvenile survival average 0.45 in M-slow populations and 0.35 in L-fast populations. Therefore, we sought to better understand the sources of variation in juvenile survival. Figure 4 presents the proposed contributions to juvenile survival not only through morphology and performance, but also through environmental variation and ecotype, which we use as a proxy for genetic background. Strong positive effects of body size and negative effects of growth on juvenile survival may buffer snakes from the negative effects of low-resource and high-temperature years. The relationships among feeding behavior, hormone levels, and growth are more complex, with larger snakes eating more and growing more, yet having lower IGF-1 plasma concentrations and negative association of growth with survival. We have argued elsewhere that growing maximally rather than optimally is likely very costly in this system where food availability is unpredictable (Bronikowski 2000). In M-slow habitats, any given year is only 50% likely to have adequate snowfall to provide for anuran (the primary prey of M-slow garter snakes) breeding in the spring. In L-fast habitats, although fish and leeches are the primary prey, fluctuations in water level and shoreline vegetation provide an annually changing gauntlet that snakes must traverse to get to their food source.

The results of the present study, combined with past work in the Eagle Lake garter snake system, present complex relationships among traits traditionally categorized as morphology, performance, and life history (Fig. 4). This web of mutually dependent traits belies simple categorization in one-way causal paths among different aspects of the phenotype. Rather, we see that traits feedback on each other through multiple pathways in which genetic background and environmental conditions interact to create distinct ecotypic syndromes at the population level. Importantly, our experimental results demonstrate that the hormone IGF-1 plays a central mediating role between body size and growth during the juvenile stage. Field observations complement this finding, showing that in adults of the L-fast ecotype plasma IGF-1 concentrations are correlated with size (See Fig. S1). Nonetheless, this relationship is not entirely straightforward and may shift during ontogeny, especially at life-history transitions when energetic allocation to growth begins to trade-off with allocation to reproduction. What is startling about the interdependent relationships among traits within individuals is that, despite this complexity, we



**Fig. 4** Schematic of the complex interplay of phenotypic traits involved in the morphology–performance–fitness–life history nexus in the Eagle Lake garter snake system. Black arrows represent relationships between aspects of the phenotype, with solid lines indicating positive and dashed lines indicating negative relationships. Solid gray arrows represent the direct impact of environmental characteristics on traits. Zig-zag lines show traits in which genetic canalization has resulted in differences between distinct life-history ecotypes. Traits and factors shown in bold black type are included in the present study; traits and factors in gray type have been established in previous studies (Bronikowski and Arnold 1999; Sparkman et al. 2007; Miller et al. 2011; Sparkman et al. 2013).

observe distinct life-history ecotypes in these natural populations. Traits of individuals within each ecotype—including metabolic and energetic functions, immune capacity, hormone titers, and life-history traits—can be categorized on the fast-slow pace-of-life continuum. Thus, ecotype differentiation is an emergent property of this biological system, relying not on a single causal pathway but multiple interwoven systems that regulate and counterregulate each other, resulting in suites of correlated phenotypic traits adapted to local environments.

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## Supplementary Data

Supplementary Data available at *ICB* online.

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