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Inconsistent evolution and growth–survival tradeoffs in *Gambusia affinis*

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Growth–survival tradeoffs may be a generalizable mechanism influencing trajectories of prey evolution. Here, we investigate evolutionary contributions to growth and survival in western mosquitofish (*Gambusia affinis*) from 10 populations from high- and low-predation ancestral environments. We assess (i) the degree to which evolutionary components of growth and survival are consistent or inconsistent across populations within ancestral predation environments, and (ii) whether growth and survival trade off at the population level. We measure growth and survival on groups of common-reared mosquitofish in pond mesocosms. We find that evolution of growth is consistent, with fish from low-predation ancestral environments showing higher growth, while the evolution of survival is inconsistent, with significant population-level divergence unrelated to ancestral predation environment. Such inconsistency prevents a growth–survival tradeoff across populations. Thus, the generalizability of contemporary evolution probably depends on local context of evolutionary tradeoffs, and a continued focus on singular selective agents (e.g. predators) without such local context will impede insights into generalizable evolutionary patterns.

1. Introduction

Contemporary prey evolution in the face of predators can determine the persistence and ecological dynamics of both prey and predators [1–8]. One key constraint commonly found in prey evolution is a growth–survival tradeoff, in which feeding ability and survival in the face of predators are negatively related [7–12]. Growth–survival tradeoffs have been observed in numerous taxa and can be generated by diverse underlying traits (table 1). Survival in the face of predators includes not only avoiding depredation, but also avoiding other hazards associated with predators or predation stress, such as starvation or parasitism [21,22]. Growth–survival tradeoffs facilitate prey evolution by tying prey traits to predator densities. When predators are abundant, prey may evolve defenses that enhance survival (at a cost to growth), driving down predator abundances [2]. When predator abundances are low and competition between prey increases, prey may evolve increased growth at a cost to survival [23], allowing predator abundances to climb. Thus, growth–survival tradeoffs have the potential to prevent a universal adaptive 'solution' to the joint problems of predator presence and resource limitation, leading dynamic environments to generate dynamic evolution along the growth–survival tradeoff axis [12]. In this article, we investigate two questions in contemporary evolution using western mosquitofish (*Gambusia affinis*): (i) the degree to which contemporary prey evolution of growth and survival are consistent with respect to ancestral predation environment, and (ii) whether an evolutionary tradeoff between growth and survival exists across multiple prey populations.

By definition, an interpopulation growth–survival tradeoff exists when populations that acquire resources and grow faster in the absence of predators suffer higher mortality when predators are present. Several genetic mechanisms can be responsible for an interpopulation growth–defence tradeoff. Direct

Table 1. Mechanistic examples of growth–survival tradeoffs across diverse study systems.

tradeoff type	taxa	mechanism	reference
physio-chemical	*Chlorella vulgaris*	algae that produce compounds reducing edibility grow more slowly	[12]
	Drosophila melanogaster	immunological defenses against parasitoids are associated with slower feeding	[13]
morphological	*Gambusia affinis*	mosquitofish have body forms hydrodynamically optimized for either efficient feeding or fast escape	[14]
	Rana sylvatica	head and body morphologies that enhance predator escape reduce feeding and digestive efficiency	[15,16]
life-historical	*Poecilia reticulata*	earlier maturation to avoid predation results in decreased reproductive output	[17]
	Rana lessonae	reduced activity and growth in the face of predators increases the risk of desiccation in temporary pool environments	[18]
behavioural	*Melanoplus femurrubrum*	hiding increases survival but decreases feeding rate	[19]
	Poecile atricapillus	travelling with food items to cover reduces predation risk but also reduces feeding efficiency	[20]

pleiotropy—when the same genes or gene networks underlie traits with inversely related growth and survival values—can generate persistent tradeoffs [24]. Separate genetic traits that pull from a limited pool of resources (e.g. time, materials or energy) can also lead to growth–survival tradeoffs (i.e. the 'Y-model' of tradeoffs) [25,26]. For example, limited time budgets dictate that increases in foraging time reduce time engaged otherwise, potentially decreasing time spent safe in hiding [19]. Finally, even otherwise independent traits can lead to growth–survival tradeoffs if selection causes them to be correlated. In this case, traits that confer higher growth would be positively correlated with traits that confer lower survival due to historical multivariate selection [27]. Such tradeoffs are less likely to persist across space and time because the selection that led to trait correlations may be inconsistent, and correlations can rapidly break down unless their underlying genes are closely chromosomally linked [28].

One key unknown in prey evolution is the degree to which growth–survival tradeoffs are generalizable across populations. Note that by generalizable here we mean consistent in nature across populations, not just common. Resolving this unknown will reveal whether prey evolution follows relatively universal growth–survival axes, at least within species, allowing more precise and straightforward prediction of prey evolution. To date, a combination of largely theoretical work [7,9,29] and limited population-level replication in foundational empirical examples [8,30,31] make species-wide generalization of growth–survival tradeoffs uncertain. While numerous examples of growth and survival evolution exist within populations [6,8,17,32–34], the extent to which this scales up to produce apparent growth and survival tradeoffs across numerous wild populations within a species remains largely unknown. Do traits that enhance growth have a similar survival cost across populations, in multiple contexts? Put another way, do populations showing higher individual growth rates consistently have decreased survival in the face of predators? If growth–survival tradeoffs are generalizable, then we would expect various populations of the same species to fall roughly along one growth–survival axis. However, if growth–survival tradeoffs are more population- and context-specific (i.e. inconsistent), then we would expect such an interpopulation growth–survival axis to be undetectable—even if growth–survival tradeoffs existed within some populations.

As highlighted earlier (table 1), the traits influencing growth and survival are incredibly diverse and include behaviours, morphology, life history and physiology. For example, just within the category of behaviour, defended traits include hiding, habitat shifts, freezing, fleeing, camouflage and predator inspections [35–37]. The environmental dependence of many traits suggests that tradeoffs present in some contexts might disappear in others [38]. For example, a nutritional tradeoff may only exist when nutrient resources are limiting [39]. Furthermore, in some contexts, there may be specific traits that successfully break the tradeoff. Continuing the example above, in environments rich with nutrients and energy, prey may be able to grow so fast that they quickly become inedible by gape-limited predators, thus generating a *positive* relationship between growth and survival—the opposite of a growth–survival tradeoff [40]. Even if growth or survival is consistently selected for, locally conflicting or confounding selection can prevent a consistent evolutionary response to selection. Finally, even if adaptive evolution of growth or survival occurs, such adaptation may be context-specific, and maladaptive in a different environment. For example, hiding survival behaviours require cover and may be habitat-specific [41,42]. Thus, inconsistency and context-specificity of contemporary evolution may prevent or obscure generalized growth–survival tradeoffs.

Here, we investigate the evolution of growth and survival in 10 populations of western mosquitofish (*Gambusia affinis*). We measured growth and survival of mosquitofish in mesocosms with largemouth bass predators (*Macropterus salmoides*) absent or present, respectively. Poeciliid fishes—like mosquitofish—have been model taxa for evolutionary ecology, showing strong phenotypic responses to predator introductions and removals [32–34,43,44], as well as strong ecological impacts of phenotypic change [2,6,8,45–48]. We test two sets of competing

Table 2. Mosquitofish source ponds in California. G = number of generations in our captive breeding facility.

population source	code	coordinates	ancestral predator environment	G	n (growth)	n (survival)
De Laveaga Pond	DeL	N 36.998071°, W 121.999344°	no bass or bluegill	2	5	5
Shorebirds Marsh	Sho	N 36.873470°, W 121.821673°	no bass or bluegill	1	2	3
Dow Wetland	PA	N 38.018818°, W 121.836500°	no bass, bluegill uncertain	1	5	5
Artesian Well	AW	N 37.350584°, W 118.326576°	no bass or bluegill	2	2	2
Antonelli Pond	Ant	N 36.956292°, W 122.060251°	bass	2	2	3
Neary Lagoon	NL	N 36.962687°, W 122.029602°	bass and bluegill	2	4	2
DeAnza Pond	DeA	N 36.951278°, W 122.061323°	bluegill	2	2	5
Spring Hills Pond	SpH	N 36.980472°, W 121.756520°	bluegill	2	9	10
Sac Yolo MVC	SY	N 38.424359°, W 121.383089°	MVC	1	5	4
Contra Costa MVC	CC	N 38.009202°, W 122.037591°	MVC	1	11	10

hypotheses regarding mosquitofish evolution, concerning (i) consistent and inconsistent evolution of growth and survival, and (ii) population-level growth–survival tradeoffs.

(a) Consistent and inconsistent evolution of growth and survival

We investigate patterns of growth and survival evolution across mosquitofish ancestral predation environments. By 'ancestral predation environment' here we mean multigenerational exposure or lack of exposure to various piscine predators in mosquitofish source ponds. We examine three competing hypotheses:

- There is no evolutionary divergence in mosquitofish growth and/or survival.
- Evolutionary divergence in growth and/or survival is inconsistent, i.e. varies widely across mosquitofish populations within ancestral predation environments.
- Evolutionary divergence in growth and/or survival is consistent, i.e. is driven primarily by ancestral predator environment and is relatively similar across mosquitofish populations within ancestral predation environments.

We note that here we are examining consistency of growth and survival—whether populations from similar ancestral predation environments exhibit similar growth and survival outcomes in a set of common environments. We are not testing for parallelism of specific traits underlying growth and survival. As highlighted earlier, these traits are numerous, and a complete accounting thereof is not the goal of this study. Thus, evolution that increases survival in the face of predators could be caused by the evolution of similar traits (parallel evolution) or of different traits that produce similar universal levels of survival (non-parallel, consistent evolution). Likewise, inconsistent evolution of survival could be due to evolution of different, context-dependent traits or different degrees of evolution of the same trait.

(b) Population-level growth–survival tradeoffs

We also test for interpopulation growth–survival tradeoffs in mosquitofish—whether populations with higher survival in the face of predators show lower growth when predators are absent. As noted above, such tradeoffs are only expected if contemporary evolution of both growth and survival respond consistently to predation risk in generalizable ways across populations. We examine two competing hypotheses:

- Population-level growth–survival tradeoffs are absent in mosquitofish.
- Populations with mosquitofish that grow faster have lower survival, and vice versa.

2. Methods

(a) Fish sources

We collected western mosquitofish from 10 ponds (i.e. populations) in central California in spring of 2017 (electronic supplementary material, figure S1; table 2). Western mosquitofish were introduced to California in the 1920s for mosquito control [49]. Although detailed stocking records and population genetic surveys are lacking, these original fish were widely stocked and translocated throughout the region over a period of decades. Thus, the available evidence points to relatively recent, common ancestry among our study populations. We collected individuals from at least two ponds from each of four predation regimes: captive propagated (mosquito vector control (MVC) hatcheries), wild predator-free, wild with bluegill (*Lepomis macrochirus*) predators and wild with largemouth bass (*Micropterus salmoides*) predators (table 2). For six of the 10 ponds, we collected fish from the wild in late winter of 2017, then held them in breeding stock mesocosms at the University of California Santa Cruz to facilitate ongoing mosquitofish experimentation needs. We collected the offspring of these wild fish for experimentation here, as the parental stocks were to be employed for other experiments. We also collected fish from two additional wild ponds and MVC 'pond' sources in central California in May 2017. The MVC sources are from two mosquitofish captive propagation facilities in central California, which breed mosquitofish completely in captivity for several generations with limited wild genetic input, resulting in some evidence of domestication [47]. We transported the fish to the University of Maine mosquitofish breeding facility after roughly one week of holding in Santa Cruz, CA. We held mosquitofish in captivity for roughly a year, then bred all populations for an additional generation in separate 300 l cattle tanks for each population. We used floating mesh refugia to passively separate fry from adults, after which we moved fry to separate 36 l tanks for growth and holding. We fed all fish an *ad libitum* mixture of tropical flake food and dried bloodworms. We used the offspring of the imported fish for experimentation. We accounted statistically for differences in numbers of generations in our captivity and breeding facilities (two generations total for mosquitofish collected in late winter, one generation for mosquitofish collected in May; see Analyses section below). However, it should be noted that all tested fish derived from parents that had reared most of their lives in our Maine rearing facility. The extended period of holding and breeding in a common laboratory environment was designed to minimize maternal and plastic influences on mosquitofish phenotypes, thus isolating the genetic components of growth and survival.

(b) Growth and survival trials

We placed mixtures of mosquitofish from multiple populations in mesocosms with or without bass to study survival and growth, respectively. We established 10 (five bass-present and five bass-absent) 1100 l mesocosms in the University of Maine Roger Clapp Greenhouses in October 2018. On 11 October we added to each mesocosm 3.6 l of benthic sediment from an unnamed pond in Orono, ME (N 44.900467°, W 68.724374°) and a mixture of zooplankton and whole water from Perch Pond (Mud Pond; N 44.946917°, W 68.777578°) and Pushaw Lake (N 44.946527°, W 68.801038°), both in Old Town, ME. We allowed mesocosms to equilibrate for 40 days prior to fish addition.

We tagged 100 mosquitofish on 15 November—5 days before introduction to mesocosms—using 1–2 mm of elastomer (VIE Northwest Marine Technology). We subcutaneously placed a single tag of either red, orange or yellow elastomer in one of four possible locations on each fish, creating unique identifiers within each mesocosm. Four out of the 100 mosquitofish died between tagging and experimentation. We measured length and towel-blotted wet mass of each fish before experimentation.

We introduced nine or 10 tagged adult mosquitofish into each mesocosm on 20 November 2017. Other work has shown that mosquitofish consumptive effects on zooplankton saturate above roughly five individuals per 1100 l mesocosm [8]; thus, we expected our stocking density of 9–10 individuals per mesocosm to generate intense intraspecific mosquitofish competition. Other work also shows that bass predation causes mosquitofish abundances to quickly drop below this saturation threshold, thus limiting the extent of competition in the bass-present mesocosms [2,8]. Therefore, competition and defense were considered the dominant forces driving mosquitofish success (i.e. growth and survival) in the bass-absent and bass-present mesocosms, respectively. Most mosquitofish populations were represented in every mesocosm, with some variability due to high or variable numbers of offspring production during breeding (electronic supplementary material, table S1).

We placed a single largemouth bass (length range: 11–15 cm) collected from either Pushaw Lake (see above) or Hermon Pond in Hermon, ME (N 44.779098°, W 68.950479°) in each of the five survival mesocosms roughly 1 h after mosquitofish introduction. Largemouth bass are a natural predator of mosquitofish, and while wild mosquitofish are not present in Maine [50], we habituated these bass to consuming mosquitofish for several weeks in the laboratory prior to experimentation. We also confirmed that each bass could readily consume mosquitofish from the entire size range present in our laboratory. We included a 15 cm diameter cylindrical mesh (1 cm square opening to allow mosquitofish entry but exclude bass) refuge filled with artificial macrophytes in the centre of each mesocosm.

We censused each mesocosm every 3 or 4 days for a period of 29 days. We removed bass from the survival mesocosms prior to censusing to ensure that census activities did not lead to mosquitofish depredation. We recorded which fish were present based on elastomer tags, using netting when necessary. We concluded the experiment when only one mosquitofish was remaining in total across all survival mesocosms. There was no mortality in the growth mesocosms. We measured the final mass (towel-blotted wet mass) of each mosquitofish in the growth mesocosms after the 29-day experimental period.

(c) Analyses

We tested our first set of hypotheses—consistent versus inconsistent evolution of growth and survival—by examining variation attributable to mosquitofish ancestral predation environment versus population within ancestral predation environment. We grouped mosquitofish with any piscine predators (bass or bluegill) in their source ponds into a ‘high-predation’ ancestral environment, and mosquitofish without any piscine predators in their source ponds (including captive propagated MVC fish) into a ‘low-predation’ ancestral environment for analysis. Variation attributable to ancestral predation environment and population within ancestral predation environment represent consistent and inconsistent evolution, respectively. We tested our second set of hypotheses—the existence of population-level growth–survival tradeoffs—by correlating population-specific estimates of growth and survival. We completed all analyses in R software version 4.1.0 [51].

(i) Growth

We assayed mosquitofish relative growth, which we calculated as

$$L ¼ \ln(M_F) - \ln(M_I) \qquad ð2{:}1Þ$$

where L = relative growth, M_F = final mass and M_I = initial mass. We used a set of nested linear mixed models (LMMs; *lme4* package version 1.1–27.1 [52]) to examine growth:

$$L ¼ b_X þ b_M \ln(M_I) þ b_F\ S þ s^2_R \qquad ð2{:}2Þ$$

where L = relative growth, $\beta_M \ln(M_I)$ = fixed effect of initial mass, $\beta_S S$ = fixed effect of sex (S is a dummy variable: male = 1; female = 0) and σ^2_R = random effect of mesocosm. β_X is an intercept (fixed effect) fitted at one of four nested levels, from lowest to highest: single intercept (null model), ancestral predation environment (high versus low predation), generations in our captive breeding facility and population (see electronic supplementary material, figure S2 for explanation). We included the random effect of mesocosm (σ^2_R) to account for variation due to mesocosm zooplankton dynamics, mosquitofish group composition, etc. We used type II likelihood ratio tests to examine the significance of all model fixed effects.

(ii) Survival

We used days survived around bass, number of conspecifics present at death and death order as assays for individual survival in the bass-present mesocosms. While these metrics of survival are expected to be correlated (electronic supplementary material, table S2), we included all three as each provides different information on mosquitofish survival. Days survived provides the absolute amount of time a mosquitofish survived in the face of bass and assumes a fish’s survival is independent of the identity and number of other fish present. Conspecifics present at death provides a relative metric of survival—if a fish was eaten with many conspecifics present, it was relatively undefended. Death order provides a relative metric of time survived, compared to that of other fish.

$$\frac{T_{\text{present}}\ þ\ T_{\text{absent}}}{2} D\ ¼ \qquad ð2{:}3Þ$$

$$C ¼ \frac{N_{T_{\text{present}}}\ þ\ N_{T_{\text{absent}}}}{2} \qquad ð2{:}4Þ$$

$$U ¼ \#_T\{(N_{T_{iþ1}} - N_{T_i}) > (T_i\ S\ T_{\text{present}})\} \qquad ð2{:}5Þ$$

where D = days survived, T_{present} = last day a fish was observed present; T_{absent} = first day a fish was observed absent; C =

number of mosquitofish present at death of the focal fish; $N_{T\text{present}}$ = number of fish alive in the focal mesocosm at the last census in which the focal mosquitofish was present; $N_{T\text{absent}}$ = number of fish alive in the focal mesocosms at the first census in which the focal mosquitofish was absent; U = death order; $\#_T$ = number of sampling periods; i indicates sampling period.

These equations assumed that deaths happened at the mid-point between the latest fish-present observation and the first fish-absent observation. A few fish in the bass-present mesocosms (five total across all five bass-present mesocosms) died from causes besides immediate bass consumption (i.e. were found dead in their mesocosms). We included these fish in our analyses under the assumption that they died due to stress, starvation or injuries from a bass attack [21]. We also compared results from the same analyses with these five fish excluded; the results were qualitatively the same. No fish died in the bass-free growth mesocosms.

As with growth (equation (2.2)), we used a LMM to examine survival:

$$\ln(D,C,U) = \beta_X + \beta_M \ln(M_I) + \beta_{♂} S + \sigma^2_R \qquad (2.6)$$

where D = days survived; C = conspecifics present at death; U = death order; $\beta_M \ln(M_I)$ = fixed effect of initial mass; $\beta_{♂} S$ = fixed effect of sex (S is a dummy variable: male = 1; female = 0); σ^2_R = random effect of mesocosm. Again, β_X is an intercept (fixed effect) fit at one of four nested levels, from lowest to highest: universal (null), ancestral predation environment (high- versus low-predation), generations in our captive breeding facility and population (see figure S2 for explanation). We included the random effect of mesocosm (σ^2_R) to account for variation due to mesocosm zooplankton dynamics, mosquitofish group composition, bass foraging variation, etc. We used type II likelihood ratio tests to examine the significance of all model fixed effects.

(iii) Consistency of growth and survival

We used the relative contribution of population versus ancestral predation environment to growth and survival to examine the degree of evolutionary consistency therein. To do so, we examined the role of four nested evolutionary units—ancestral predation environment, generations in our captive breeding facility, population and individual (i.e. residual)—in driving variation in growth and survival. We calculated the proportion of variation explained (PVE) by each unit for each growth and survival metric LMM. As we were only concerned with these four evolutionary units for this analysis, we did not include the effects of covariates sex, mass and experimental mesocosm in these PVE estimates. We calculated PVE using residual sum of squares (RSS) from full and reduced versions of our above LMMs (see electronic supplementary material, figure S2 for explanation):

$$\text{PVE}_B = \frac{\text{RSS}_{\{\ldots\}} - \text{RSS}_{\{B,\ldots\}}}{\text{RSS}_{\{\ldots\}}} \qquad (2.7)$$

$$\text{PVE}_G = \frac{\text{RSS}_{\{B,\ldots\}} - \text{RSS}_{\{B,G,\ldots\}}}{\text{RSS}_{\{\ldots\}}} \qquad (2.8)$$

$$\text{PVE}_P = \frac{\text{RSS}_{\{B,G,\ldots\}} - \text{RSS}_{\{B,G,P\ldots\}}}{\text{RSS}_{\{\ldots\}}} \qquad (2.9)$$

$$\text{PVE}_\varepsilon = \frac{\text{RSS}_{\{B,G,P,\ldots\}}}{\text{RSS}_{\{\ldots\}}} \qquad (2.10)$$

where PVE_B, PVE_G, PVE_P and PVE_ε = proportion of variation explained by ancestral predation environment, generations in our captive breeding facility, population and individual (residual), respectively; and $RSS_{\{\}}$ = residual sum of squares for models including the indicated combination of ancestral predation environment (B), generations in our captive breeding facility (G), population (P) and covariates (…).

If evolutionary divergence is largely consistent within ancestral predation environments, we would expect PVE_B to be large relative to PVE_P. However, if evolutionary divergence is largely inconsistent, we would expect PVE_B to be small and PVE_P to be relatively large. Finally, the sum of PVE_B and PVE_P provides an idea of the relative importance of evolution (i.e. divergence) in explaining individual growth and survival. If PVE_B and PVE_P are both close to zero, then evolution is likely to be playing a relatively small role in driving mosquitofish growth and survival.

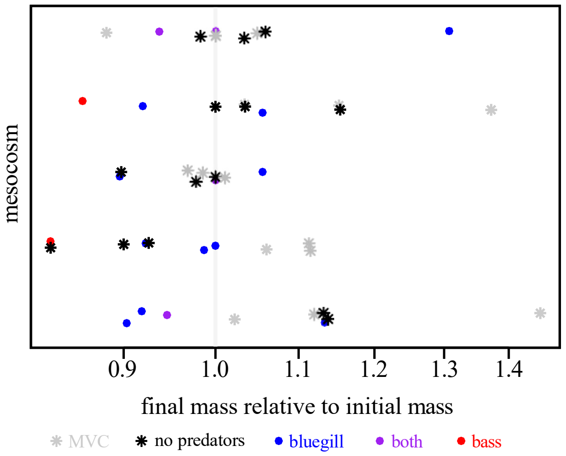

Figure 1. Mass change (in ratio of final mass to initial mass) of mosquitofish in mesocosms without bass over a 29-day period. Each point represents a fish; each row represents a mesocosm. The vertical line represents no mass change; points to the left of the line indicate fish that lost mass, while points to the right of the line represent fish that gained mass. Colours and symbols indicate source pond ancestral predation environment. (Online version in colour.)

(iv) Growth–survival tradeoffs

We tested for interpopulation growth–survival tradeoffs by correlating the population-specific β_X estimates from equation (2.2)—which represent the population-specific relative growth—with population-specific β_X estimates of survival (D, C and U) from equation (2.6). We used partial correlations, accounting for differences in the number of generations in our captive breeding facility, using the *ppcor* packaged version 1.1 in R [53]. We tested the significance of these correlations using Pearson tests.

3. Results

For model parameter estimates, see electronic supplementary material, table S3.

(a) Growth

There was broad variation in growth within our experimental growth mesocosms, with some fish gaining and losing mass in each mesocosm (figure 1). Some fish lost up to 20% of their mass over the study period, while others gained over 30%. Mosquitofish from low-predation ancestral environments (bass- and bluegill-free wild and hatchery populations) had significantly higher relative growth (final mass relative to initial mass) than mosquitofish from high-predation ancestral environments (figure 2 and table 3). Population identity within ancestral environments did not have a significant effect on relative growth, nor did sex or generations in captivity (table 3). Initial mass negatively affected relative growth (table 3), indicating that smaller individuals grew relatively faster than larger individuals. We conducted this experiment during winter, when mosquitofish are usually non-reproductive [54]

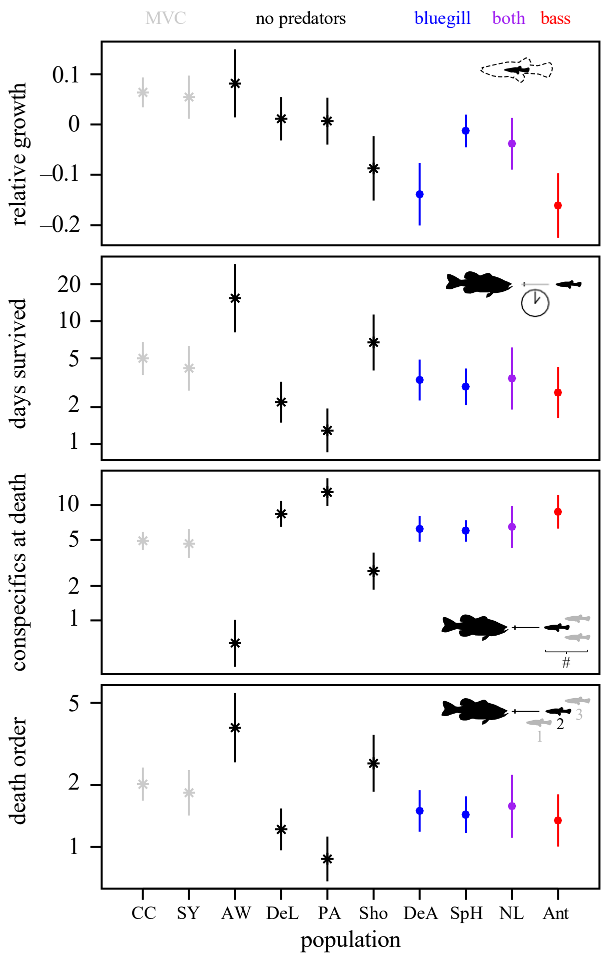

Figure 2. Population-level estimates of growth and survival metrics. Dots and lines show LMM estimates ± standard errors (see text), with variation from sex, mass and experimental mesocosm removed. Colours and symbols indicate source pond ancestral predation environment. (Online version in colour.)

and observed no fry in any of the experimental mesocosms, so it is unlikely that changes in mass were due to reproduction [55].

(b) Survival

Bass caused steady declines in mosquitofish abundance in the bass-present survival mesocosms, with some variation in the rate of those declines (figure 3). Declines in mosquitofish abundances were generally faster at the beginning of experimentation, resulting in quick knockdowns of mosquitofish abundance. All survival mesocosms dropped from nine or 10 to four or fewer fish by the end of the second week of experimentation.

Unlike with growth, ancestral predation environment did not significantly affect any of our three survival metrics (figure 2 and table 3). However, population did have an effect on all survival metrics (figure 2 and table 3). Both the most and least defended populations (in terms of all survival metrics) were from low-predation ancestral environments, while populations from ponds with various piscine predators had very similar levels of survival (figure 2). Sex and mass did not significantly affect any of our survival metrics (table 3), with the exception of conspecifics remaining at death, for which males died with significantly more conspecifics present than females. Generations in our captive breeding facility also did not have a significant effect on survival.

(c) Consistency of growth and survival

Evolutionary divergence played a strong role in shaping survival and growth, with ancestral predation environment and population together explaining 31–43% of variation in survival and 41% of variation in growth, excluding effects of sex, mass and experimental mesocosm (figure 4). Ancestral predation environment (i.e. consistent evolution) had nearly no effect on survival, explaining only 3–4% of variation therein, while population (i.e. inconsistent evolution) explained 28–39%. However, ancestral predation environment and population explained similar levels of variation in growth—19% and 22%, respectively.

(d) Growth–survival tradeoffs

At the population level, growth was positively correlated with survival, but these correlations were weak and non-significant - (figure 5 and table 4). These correlations were mainly driven by one bivariate outlier, without which the trends become even weaker (population AW, without which the above r-values became closer to zero and all p-values increased further).

4. Discussion

Our results reveal predictable, somewhat consistent evolutionary patterns of growth, but inconsistent evolutionary patterns of survival, across mosquitofish populations (figures 2 and 4). Low-predation mosquitofish populations grew faster than high-predation populations in the absence of predatory bass. However, high-predation mosquitofish populations did not necessarily survive better in the presence of bass. Thus, across populations, faster growth did not come at an obvious cost to survival, and we did not find evidence for an interpopulation growth–survival tradeoff (figure 5).

(a) Ancestral predation environment- and population-level adaptation

Relative growth was largely driven by size and ancestral predation environment (i.e. predator presence in the mosquitofish source ponds), with smaller individuals and those from low-predation ancestral environments (including captive propagated populations) having higher relative growth over the study period (figure 2). Selection for growth in these ancestral predation environments is intuitive, as the paucity of predators and higher density of competitors [56,57] should select for highly competitive individuals [6,23,58]. The generally higher relative growth of all low-predation populations suggests that evolution of growth in mosquitofish is somewhat consistent and generalizable—the evolution of increased growth in multiple high-competition contexts (natural ponds and MVC captive propagation facilities)—although a comparable chunk of variation in mosquitofish growth was explained by population (i.e. was inconsistent). This finding also suggests that competitive traits in mosquitofish are generally adaptive when predators are absent, regardless of other environmental contexts.

While variation in mosquitofish growth was significantly linked to ancestral predator environment, this does not imply that predators directly—or even indirectly—caused the observed evolution in mosquitofish traits. First, predator presence can be itself influenced by other environmental factors that can affect mosquitofish evolution. One such factor is proximity to urban environments affects both mosquitofish trait variation and the likelihood of predator introductions

Table 3. Type II likelihood ratio tests for effects of ancestral predation environment, population, generations in captivity, sex and mass on mosquitofish growth and survival. Note that generations in captivity indicates the generations in our captive breeding facility, specifically. Italics represent $p < 0.05$.

		population (d.f. = 7)		gen. in captivity (d.f. = 1)		ancestral pred. env. (d.f. = 1)		sex (d.f. = 1)		mass (d.f. = 1)		
response variable	experiment	x^2	p	x^2	p	x^2	p	x^2	p	x^2	p	N
days survived	survival	14.9	*0.037*	0.34	0.56	1.16	0.28	1.35	0.25	0.12	0.73	49
conspecifics remaining at death	survival	25.46	*< 0.01*	0.05	0.82	1.97	0.16	3.98	*0.046*	0.83	0.36	49
death order	survival	14.83	*0.038*	0.49	0.48	1.59	0.21	1.68	0.20	0.23	0.63	49
relative growth	growth	11.66	0.11	0.02	0.88	7.12	*< 0.01*	2.94	0.086	11.16	*< 0.01*	47

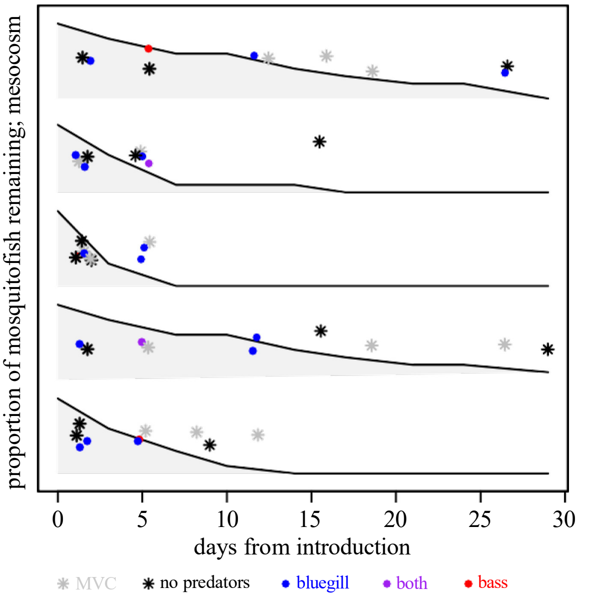

Figure 3. Proportion of mosquitofish remaining (out of ten) in five experimental mesocosms with bass predators over 29 days. Each line represents a mesocosm. Points represent inferred depredation times between fish checks; points are jittered vertically for easier viewing. Colours and symbols indicate source pond ancestral predation environment. (Online version in colour.)

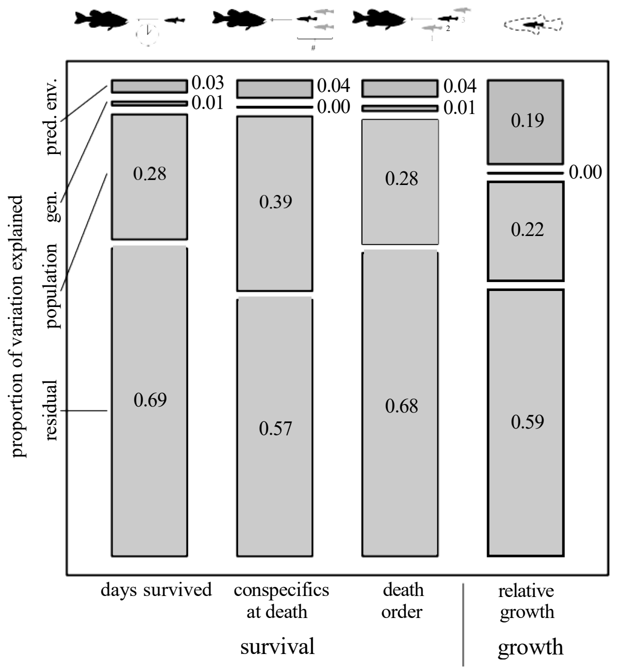

Figure 4. Proportion of mosquitofish growth and survival attributable to four sources of variation (nested bottom within top in the figure): ancestral predation environment, generations in our captive breeding facility, population and individual (i.e. residual). Totals ≠ 1 are due to rounding.

[59]. Second, predator presence can generate both ecological and evolutionary trophic cascades, which could change the composition of mosquitofish competitors, prey and abiotic environments in ways that generate selection in mosquitofish [7]. Third, predator presence could intuitively affect mosquitofish density, mediating intraspecific dynamics (e.g. competition) and leading to density-dependent evolution in mosquitofish [30]. Thus, while predators (or the lack thereof) could be directly driving selection in mosquitofish, a suite of indirect factors correlated with or driven by predator abundance could also be driving the mosquitofish trait variation linked with ancestral predator environment in this study. Nonetheless, we can conclude that the observed evolution in mosquitofish growth is a meaningful response to the predator *environment*, even without certainty that predators themselves are the direct cause of selection here.

Survival in mosquitofish was linked only to inconsistent population effects, with no clear signal of ancestral predation environment (figures 2 and 4). Inconsistent, population-specific evolution of survival suggests that predator exposure alone is not necessarily the sole determinant of genetic antipredator evolution in this system. The efficacy of common modes of mosquitofish survival—predator avoidance and hiding [60,61]—are strongly dependent on the local environment, including water clarity and vegetation [62]. Indeed, mosquitofish ponds are ecologically diverse, covering a large range of sizes, biomes and human influences on water quality [59]. Thus, environment-specific antipredator traits, rather than universal adaptation to specific predators, may be the main determinant of antipredator success in mosquitofish (see below).

Life-history antipredator strategies may also have contributed to our lack of observed contribution of ancestral predation environment to survival. In the case of non-gape-limited predation, earlier maturation and increased investment in early reproduction ensures that prey are more likely to reproduce before being eaten, thus increasing their fitness. Such life-

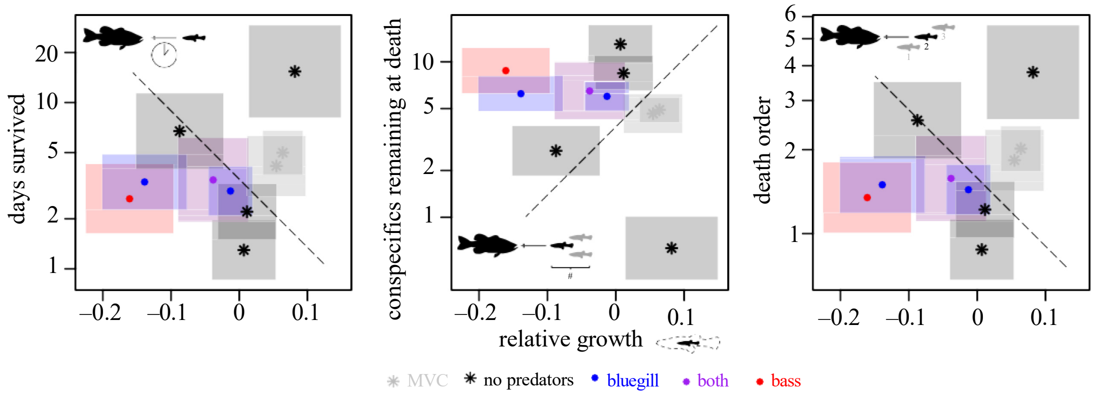

Figure 5. Population-level survival versus growth. Points and boxes show population-level general LMM estimate ± standard error. Colours and symbols indicate different ancestral predation environments. Dashed lines indicate the hypothesized—but not observed—growth–survival tradeoff. (Online version in colour.)

Table 4. Partial correlation Pearson tests for growth–survival tradeoffs, accounting for number of generations in our captive breeding facility.

metric of survival	estimate	s.e.	*t*	*p*
days survived	0.33	0.36	0.93	0.39
conspecifics remaining at death	−0.43	0.34	−1.25	0.25
death order	0.31	0.36	0.87	0.41

history evolution in response to increased mortality has been documented in mosquitofish [63,64] and guppies [34]. Early maturation and reproduction often come at a cost to growth investment [25]. Our experimental design would not have been able to directly test for the role of life-history adaptations as a form of defended phenotype. Interestingly, the observed lower growth rates in bass- and bluegill-adapted mosquitofish (figure 2) could partly integrate this life-history effect if defended life histories shift more resources to maturation and mating activities, rather than growth.

(b) Inconsistency and growth–survival tradeoffs

Interpopulation growth–survival tradeoffs require consistent and opposing patterns of both growth and survival, if not their underlying traits. Our work here shows that evolution of survival appears to be inconsistent—populations exposed to bass and bluegill predators did not have overall higher survival (by any metric) in mesocosms with bass (figure 2), and mosquitofish ancestral predation environment explained a mere 3–4% of variation in survival (figure 4). Nonetheless, relatively large population-level variation in mosquitofish survival (figure 4) suggests that there is at least some evolution of traits affecting survival outcomes in these populations. On the other hand, growth appeared to exhibit consistent evolution, with statistically and biologically significant variation among ancestral predation environments (figure 4 and table 3). Interestingly, decreased growth in mosquitofish from bass and bluegill ancestral predation environments implies that high-predation mosquitofish populations are paying a competitive cost.

Why, then, do high-predation mosquitofish populations not exhibit the highest levels of survival? We speculate that inconsistent evolution due to environmental context is the likely culprit. Though we have too few populations from each ancestral predation environment to provide a rigorous analysis of environmental factors here, we can explore some anecdotes. The population with the highest survival, AW, exists in a tiny, clear, spring-fed desert pond, and is probably the wild pond most similar in character to our mesocosms (electronic supplementary material, figure S1). The population with the next highest survival, Sho, is also a fairly small, very shallow pond (but is much less clear). The two MVC populations also had relatively high survival and are again relatively similar to mesocosms in character; the SY population is bred in small shallow outdoor ponds, while the CC population is bred in greenhouse tanks. These two MVC populations thus mimic the mesocosm environment in many environmental variables, but may experience some maladaptation via domestication [65,66], thus leading to their lower survival than the AW and Sho populations. On the other hand, our high-predation ancestral environment populations (DeA, SpH, NL and Ant) are all extremely macrophyte-rich environments. Our experimental mesocosms may have measured survival in a relatively open environment, as our refuge was relatively small, and we regularly observed mosquitofish outside of the refuge. As indicated earlier, antipredator adaptations are numerous, diverse and context-dependent (table 1), and there is unlikely to be a single panacea antipredator trait. For example, hiding is a useful behaviour only when cover is abundant [42]. Burst-swimming may only be adaptive when there is somewhere protected whither to swim. Shoaling requires sufficient numbers [67]. Fast growth life histories depend on adequate food resources [68]. While some survivals may be adaptive in open environments like our mesocosms [14], others may take advantage of shelter and camouflage [62] and thus not be adaptive in open environments. Indeed, other environmental variables like pond size, surrounding biome, and even urbanization drive mosquitofish phenotypic variation at least as much as predator regime [59]. Therefore, inconsistency of survival evolution, driven by environmental context or other selective agents acting on the same traits could be driving our observed patterns of survival.

5. Conclusion

Our work here suggests that growth–survival tradeoffs—while well documented within many populations—are unlikely to be generalizable across populations due to inconsistent evolution of survival. Instead, we argue that researchers should focus on elucidating context-specific tradeoffs and the traits that facilitate them to understand where and by what mechanisms tradeoffs are likely to arise. We also note that population-level replication is necessary to uncover meaningful patterns of contemporary evolution and avoid spurious conclusions.

More broadly, this study adds to the growing literature suggesting that inconsistency and non-parallelism in contemporary evolution may be more the rule, rather than the exception [38,48,69,70]. Indeed, while several studies on multiple mosquitofish and other poecilid populations show some parallelism in phenotypic responses to predator presence (most commonly morphology), predator presence explains relatively little variation in these phenotypes relative to variation between populations with the same predator regimes, leaving significant room for non-parallelism and inconsistency [32,34,43,46,48,59]. Incorporating environmental context and experimental evolution into studies of eco-evolutionary dynamics will help make the field robust to non-parallelism and will provide more specific clues to the drivers of eco-evolutionary dynamics.

Ethics. All animal care was approved and standards maintained under University of Maine Institutional Animal Care and Use Committee protocol A2016-02-03.

Data accessibility. All data and code are provided in the electronic supplementary material [71].

Authors' contributions. Z.T.W.: conceptualization, data curation, formal analysis, methodology and writing—original draft; E.P.P.: conceptualization, funding acquisition and writing—review and editing; M.T.K.: conceptualization, funding acquisition, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. The authors declare no conflicts of interest.

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