Variation in size and reproduction in Trinidadian guppies: ecological and evolutionary causes and effects

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Declaration of authorship

The work presented in this thesis is my own. In each of the chapters presented here, I have developed the aims, concepts, and methodology, performed the analyses, interpreted results, and written the manuscripts. I have been fortunate to collaborate with many excellent scientists during my DPhil. For each chapter, a full list of coauthors and statements of their contributions are given below.

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Contributions — TP co-conceived the study, developed and performed the analyses, wrote all drafts of the manuscript and incorporated co-author suggestions. TC co-conceived and supervised the study, and interpreted results with TP. RDB, DNR, and JT established, raised funds for, and ran the mark-recapture experiment. PB performed the genotyping and pedigree construction. CAH, JT-D, EWR and CKG performed the common garden experiments. All authors contributed to reviewing and editing the final version of the manuscript.

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Contributions — TP conceived the ideas, developed and performed the analyses, and wrote the manuscript; DNR designed and performed the common garden experiments and collected the data; TC supervised the study and interpreted results with TP. All authors contributed critically to the drafts.

Chapter 5

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Contributions — TP conceived the ideas, developed and performed the simulations and analyses, and wrote the manuscript; TC supervised the study and interpreted results with TP.

Chapter 6

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Abstract

Body size and reproductive rates are key characteristics of any organism. Size and reproduction are strongly influenced by environmental conditions, but individuals also influences their environment. The resulting feedback loop can generate ecological and evolutionary dynamics. In this thesis, I explore the causes and consequences of intraspecific variation in size and reproduction. Trinidadian guppies (*Poecilia reticulata*) are an ideal system for this purpose. Natural populations of guppies have evolved two distinct ecotypes that differ in terms of the sizes at which they are born and reach maturity, and in their reproductive rates. These ecotypes are adapted to different environments that vary in the intensity of predation risk, and in the availability of food. In this thesis, I use a combination of genetic, phenotypic, and environmental data from natural populations, experimental introductions, and controlled laboratory experiments. To better understand the causes and consequences of variation in body size and reproduction, I apply and test methods from three distinct approaches: quantitative genetics, dynamic energy budget theory, and life history theory. I quantify evolutionary change in a wild populations, and show that phenotypic change is masked by environmental degradation. I demonstrate why predictions of evolution can fail when environments change over time. I show how size and reproduction are linked through energetic assimilation, and demonstrate that smaller guppies are resource-limited in the wild. Using dynamic energy budget theory, I identify consistent differences in energetic allocation strategies between guppy ecotypes. Finally, I construct evolutionarily-explicit population simulations, where ecological and evolutionary dynamics are emergent properties of individual rates of growth, survival, and reproduction. I show that size-dependent competitive ability determines the outcome of life-history evolution in these simulations. My thesis presents solutions to problems relevant to many studies of the eco-evolutionary dynamics of wild populations.

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General Introduction

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1.1 Introduction

A female brown kiwi (*Apteryx australis*) lays a single egg, weighing around a fifth of her body weight (Calder, 1979). Because the chick hatches at such a large size, adults and chicks differ in mass only by a factor of six or seven. In contrast, the ocean sunfish (*Mola mola*) can produce 300 million eggs in one spawning event: juveniles that survive to adulthood grow at an astonishing rate, increasing in mass by a factor of 60 million (Pope et al., 2010).

Kiwis and sunfish illustrate the extraordinary range of variation in body size that can occur within species, and also the diversity in reproductive rates that occur across species. Body size and reproductive rates are key characteristics of any animal. Both are products of life histories – that is, the strategies generated by natural selection to optimise the conversion of energy into reproductive success (Stearns, 2000). Both are also sensitive to environmental conditions, such as resource availability. Furthermore, animals have a direct impact on their environment, for example by consuming food and depleting resource levels. The impact an individual has on its environment, and also on other members of the population, often depend on its size, and in the longer term, on its reproductive rate. As such, variation in body size and reproductive rates are both generated by and contribute to ecological and evolutionary dynamics.

In this thesis, I aim to disentangle the eco-evolutionary causes and consequences of intraspecific variation in size and reproduction. My thesis focuses on a classic

model system in evolutionary ecology – the Trinidadian guppy (*Poecilia reticulata*). In the following sections, I describe the core concepts that motivate the questions I pose, I introduce the study system with which I address my questions, and finally I provide an outline of my thesis, in which I briefly summarise the main findings of each chapter.

1.2 Core concepts and motivation

Predictions of evolution frequently fail for wild populations

Evolutionary change is often rapid. It is now accepted that evolutionary change can be concurrent to ecological change, rather than happening at the imperceptibly slow rate envisioned by Darwin (Carroll et al., 2007; Pelletier et al., 2009; Bassar et al., 2021; Grosklos and Cortez, 2021). Rapid evolutionary change has been documented in many natural systems (reviewed in Hendry and Kinnison 1999; Reznick et al. 2001a; Gienapp et al. 2008). This raises the possibility of studying the processes driving contemporary evolutionary change in the wild: understanding how natural populations evolve has been a long-standing goal of evolutionary biology (Lynch and Walsh, 1998). Describing how and why complex traits such as body size and reproductive rates evolve has traditionally been the domain of quantitative genetics (Lynch and Walsh, 1998). Quantitative genetics arose from the Modern Synthesis in the early 20th century, and was quickly and successfully employed and developed by animal breeders to predict responses to artificial selection (e.g. Lush 1940). Quantitative genetics is concerned with the variances of continuous traits, and aims to decompose phenotypic variation into contributions from genetic (i.e. heritable) and environmental (i.e. non-heritable) causes (Lynch and Walsh, 1998). A central assumption is that quantitative traits are controlled by a large, technically infinite, number of unlinked loci of small effect (Fisher, 1930). This is known as the infinitesimal model. One consequence of this assumption is that genetic variance underlying traits can be described without directly observing the genotype, provided that the relationship among individuals within a population is known. By considering unobserved genotypes, quantitative genetics has been described as

being "freed... from the tyranny of detail" (Queller, 2017), which has allowed for the development of elegant and general theorems of evolution. With the advent of molecular tools, it has become easier to determine the relatedness of individuals in wild populations, and as such, to apply the quantitative genetic framework to studies of evolution in natural systems. Research has typically focused on long-studied populations of birds (e.g. collared flycatchers (Sheldon et al., 2003) and great tits (Garant et al., 2004)) and large mammals (e.g. Soay sheep (Milner et al., 2000) and red deer (Kruuk et al., 2002)), although more recent studies of small mammals (e.g. voles (Bonnet et al., 2017) and squirrels (Lane et al., 2018)), lizards (Noble et al., 2014), and fish (Thériault et al., 2007) have broadened the scope of taxa covered.

A striking finding of this application to wild populations is that the refined models of quantitative genetics typically predict evolutionary change when none is observed in the study system (Merilä et al., 2001b; Gienapp et al., 2008). A review of 30 such studies found that the *direction* of evolutionary response predicted from quantitative genetic parameters matched the observed change in only 40% of cases (Gienapp et al., 2008): in this meta-analysis, quantitative genetic predictions of the direction of evolution in the wild were worse than predictions based on a coin toss. The absence of evolution despite evidence of selection and additive genetic variance has been termed the paradox of stasis, and remains an unresolved problem in our understanding of microevolutionary change in wild populations (Merilä et al., 2001b; Pujol et al., 2018). Several explanations for stasis have been proposed, which can broadly be classified as statistical failings or biological mechanisms (Pujol et al., 2018). Statistical failings include oversimplification of the genetic contributions to the phenotype (i.e. ignoring dominance and epistasic effects, which are difficult to quantify in unbalanced datasets typical of wild populations (Wilson et al., 2010)), assumptions of Gaussian distributions of traits (de Villemereuil, 2018), low precision of parameters as a consequence of the statistical power constraints when analysing limited datasets (Pujol et al., 2018), and not accounting for environmental covariance between traits and fitness (Rausher, 1992; Morrissey et al., 2012). Biological explanations include phenotypic plasticity (Alatalo et al.,

1990; Conover and Schultz, 1995; Merilä et al., 2001a), genetic correlations among traits that constrain evolutionary responses(Lande and Arnold, 1983; Morrissey et al., 2012), and fluctuating selection (Price et al., 1984; Hairston and Dillon, 1990; Grant and Grant, 2002; Siepielski et al., 2009).

Whilst these statistical and biological mechanisms provide explanations for specific instances of stasis, it is useful to consider why these explanations are required from a more general perspective. Most of these issues stem from the application of a body of theory – developed in the context of animal breeding in controlled environments – to wild populations. A key difference between captive-bred animals and wild populations is that of the degree of environmental variability. Wild populations are subject to greater variation in terms of environmental conditions – such as resource availability, predation risk, and exposure – relative to populations reared in captivity. Animals reared in captivity are typically provided with adequate resources and protection from harm: they experience a relatively stable, favourable environment, both within and across generations. By contrast, wild animals are subject to short- and long-term variation in environmental conditions, meaning that individuals may experience very different environmental effects, influencing both the expression of their traits and fitness. Although the quantitative genetic framework allows for the partitioning of environmental contributions to the phenotype, the implicit assumption is that environmental effects are constant within and across generations; that is, each generation experiences the same level of environmental variation (Lande and Arnold, 1983). In my first chapter, I investigate the consequences of this assumption for quantitative genetic predictions of the evolution of body size in a wild population.

Is the relationship between size and reproduction causal or correlative?

Body size and reproduction are inherently linked. The relationship between these traits poses challenges for both theoretical models of growth and quantitative genetic models of evolutionary change. For example, most theoretical models of growth

assume that reproduction scales proportionately with body size (e.g. Roff, 1983; West et al., 2001; Lester et al., 2004; Kooijman, 2010). However, in wild populations, larger individuals produce disproportionately more offspring than smaller conspecifics (Barneche et al., 2018; Marshall and White, 2019). This observation termed reproductive hyperllometry and seen in 95% of 342 species studied has prompted calls for new theoretical models of growth, in which allocation of energy to reproduction increases with body size (Barneche et al., 2018; Marshall and White, 2019). Similarly, body size is routinely found to be under positive selection – nearly 80% of studies identified selection for increased size (Kingsolver and Pfennig, 2004). The main question then is if larger individuals have disproportionately higher rates of reproduction and fitness, why isn't everything evolving larger body sizes?

Body size and reproductive rates are inherently plastic, food-dependant traits: in general, somatic growth and reproduction will both be reduced or stop entirely under conditions of starvation, and both will increase with increasing food availability. As such, individuals may be larger and have higher reproductive rates because they have been better able to acquire food, rather than through body size having a direct causal effect on fitness. The argument here is that individuals with optimal resource accrual traits will grow faster and have higher reproductive rates than those individuals with sub-optimal traits values (Coulson, 2020). If the value of the resource accrual trait is too low or too high, body size is reduced. For example, consider an individual searching for food: if its foraging rate is too low, individuals will not find sufficient food to grow to a large size; if foraging rate is too high, individuals will inefficiently expend energy searching for food, and so will have less energy to use for growth and reproduction. Individuals with the optimal foraging rate will have both the largest body size and the highest fitness. In this case, larger body size is always associated with higher reproduction and fitness, but the cause of both actually depends on foraging rate, which is under stabilising selection (Coulson, 2020). The absence of a causal effect of size on fitness could explain the evolutionary stasis often observed despite strong apparent selection for increased body size (e.g. Kingsolver and Pfennig, 2004). However, large individuals

often do have a size-dependent advantage in competitive interactions (Mittelbach, 1981; Post et al., 1999; Ward and Krause, 2001; Potter et al., 2019; Griffiths et al., 2020), meaning that body size may in fact have a causal impact on fitness, via increasing rates of energetic assimilation. When larger individuals are stronger competitors, this feedback would increase body size (and reproductive rates), and so further enhance the individual's competitive ability.

Food-dependent plasticity of traits and fitness presents an inherent problem to quantitative genetic methods applied to wild populations. When the same genotype – expressed under different environmental conditions – results in different phenotypes, the continuous relationship between the phenotype and environmental conditions is called a reaction norm (Stearns, 1989, provides an historical overview of the concept of reaction norms). If all individuals experience the same average environmental conditions, then quantitative genetic studies are justified in their focus on phenotypic traits. However, if individuals experience different conditions – as is almost certain to be the case in wild populations, where individuals differ in competitive ability – evolutionary studies should focus on reaction norms instead. Dobzhansky made this point early on when he wrote: "...what is inherited in a living being is not this or that morphological character, but a definite norm of reaction to environmental stimuli" (Dobzhansky, 1937). Nevertheless, identifying how individuals differ with respect to the environmental stimuli they experience – a crucial step for quantifying reaction norms – is challenging in wild populations. In my second chapter, I investigate how environmental effects of food availability influence the relationship between body size and reproduction, and assess support for two theoretical models that explain reproductive hyperallometry: one that predicts that larger individuals allocate a greater fraction of energy towards reproduction, and one that predicts that reproductive hyperallometry is due to differences among individuals in assimilation rates, i.e. that individuals that differ in size and reproduction experience different environmental stimuli, and so are at different points on the reaction norm.

Traits linked to energy budgets underpin size and reproduction

Body size and reproductive rates can be considered emergent properties of an animals ability to acquire and utilise resources (Coulson, 2020). As such, for evolutionary analyses it may be useful to focus instead on those traits that underpin this ability. Dynamic Energy Budget (DEB) theory is rooted in the concept of the acquisition and utilisation of resources. DEB theory provides a single framework to describe an animals development, growth, maintenance, reproduction, and ageing, by calculating the rates of assimilation and utilisation of energy for biological processes under varying environmental conditions (Nisbet et al., 2000; Kooijman, 2001, 2010). The flux of energy allocated to these processes describes an individuals life history in the context of its environment (Nisbet et al., 2000). The modelling framework is developed from first principles of physical and chemical laws, and can be parameterised using experimental data on feeding, growth, reproduction, and lifespan, measured at varying levels of food density (Kooijman et al., 2008; Kooijman, 2010). Individuals are defined by the parameter values of the model. DEB parameters are theoretically assumed to be constant over life, their values are independent of environmental conditions, yet they interact with environmental conditions to generate the full phenotype of the organism (Kooijman, 2010; Lika et al., 2011): in effect, the parameters of the DEB model describe the reaction norms of body size and reproductive rates, with respect to variation in food availability and temperature. As such, DEB parameters potentially provide an alternative set of traits for use in evolutionary models.

Although DEB models theoretically describe individuals, in practice, parameters are estimated at the level of the species. DEB parameters have been estimated for over 2500 species (AmP, 2020), and patterns of interspecific variation in parameter values have been used to describe broad patterns in life histories across the tree of life (Marques et al., 2018; Lika et al., 2019; Augustine et al., 2019). However, intraspecific variation in DEB parameters – a fundamental requirement if DEB theory can be of use in evolutionary models – has been little explored. In my third

chapter, I test whether dynamic energy budget theory can characterise intraspecific life history variation, and asses whether the DEB model could contribute to a framework for accurately predicting evolutionary change in wild populations.

Individuals impact their environment and each other

Individuals are not just passive recipients of environmental effects: they actively modify the environment that they and others experience through ecological interactions. One example of this is through density dependence (discussed in depth in Bassar et al., 2010; Travis et al., 2013). Density dependence occurs when population sizes decline when large, and increase when small. Higher population sizes may result in, for example, increased risks of predation or disease, or reduced levels of food availability. By definition, density dependence influences ecological dynamics, but it can also influence evolutionary dynamics. When the rank order of genotypes, with respect to fitness, changes with increases in population density, density-dependent selection occurs (Travis et al., 2013). Many ecological forces can generate density dependence, including predation, disease, and parasitism. In my fourth chapter, however, I focus on the effects of density dependent food limitation on the evolution of life histories.

Because all biological processes require energy, realised life histories are density dependent through resource limitation. Density dependence may act to suppress somatic growth and reproductive rates, delay maturation, or decrease survival probabilities (Mylius and Diekmann, 1995). How these effects influence life history evolution will depend on the stage or age class upon which the effects of density are most strongly felt (Charlesworth, 1994). A complicating factor is that population sizes fluctuate over time, meaning that the strength of density dependence also fluctuates (Lande et al., 2009; Engen et al., 2013; Sæther and Engen, 2015). In some cases, these dynamics may lead to balancing selection on life histories (Sæther et al., 2016).

Ultimately, density dependence through resource limitation occurs through the impact of individuals on local food availability. However, individuals are not equal

in terms of their impact on local food availability: some are better competitors than others, and some may produce more offspring, further increasing density-dependent effects on resource availability. When competitive ability depends on some aspect of the phenotype, competition is said to be asymmetric (Persson, 1985). The outcome of competition frequently depends on body size (Mittelbach, 1981; Persson, 1985; Ward et al., 2006). Larger individuals are often stronger competitors because they have higher feeding rates, have broader niches, and are better able to defend and monopolise resource patches (Mittelbach, 1981; Webster and Hixon, 2000; Scharf et al., 2000). In some cases, smaller individuals may have a competitive advantage: they have lower per capita energetic costs, and are better able to tolerate periods of reduced food availability (Persson, 1985), and they may be more efficient feeders on resources that are less accessible to larger individuals (Marshall and Keough, 1994; Byström et al., 2004). However, the higher food requirements of larger individuals mean that they will have a greater per-capita impact on food availability than smaller individuals.

Whether competitive asymmetry favours large or small individuals can have profound effects on the dynamics and structure of populations, and can modify vital rates such as development time, reproduction, and size-dependent survival probabilities (De Roos et al., 2003; Bassar et al., 2016). In my final chapter, I investigate how competitive asymmetry modifies the effects of density dependence, and how this influences life history evolution in fluctuating environments.

1.3 General aims of the thesis

In this thesis, I explore the causes and consequences of intraspecific variation in size and reproduction, in terms of their effect on life histories and their evolution. Here, I outline four general questions which I address in the chapters of my thesis:

1. How do changing environmental conditions influence quantitative genetic predictions of the evolution of body size?

2. How does the relationship between size and reproduction change as a consequence of food availability?

3. Can dynamic energy budget theory be used to quantify intraspecific variation in life histories?

4. How does the relationship between size and competitive ability modify life history evolution in density-regulated populations?

1.4 Trinidadian guppies as a model system

I address the general questions in my thesis with a classic model system in evolutionary ecology: natural populations of the Trinidadian guppy (*Poecilia reticulata*). Guppies are small, live-bearing, freshwater fish, that are native to montane streams and lowland rivers of Trinidad and Venezuela (Magurran, 2005).

Natural guppy populations differ in the intensity of predation risk that they experience. In downstream stretches of streams and rivers, guppies are the smallest species in a rich community of fishes (Gilliam et al., 1993). In these so-called "high-predation" habitats, guppies suffer high levels of mortality due to predation (Reznick et al., 1996a), in particular by the wolf fish (*Hoplias malabaricus*) and the pike cichlid (*Crenicichla alta*). Further upstream, barrier waterfalls restrict the movement of larger fish species. This leads to successively simpler fish communities along the transition from lowland to montane streams, and a gradient of decreasing predation risk for guppies (Deacon et al., 2018). In the simplest communities ("low-predation"), guppies coexist with one other fish species, Haart's killifish (*Rivulus hartii*). Killifish compete with guppies for food, and both species are occasional predators of the juveniles of the other (Fraser and Lamphere, 2013). The mortality risk of guppies due to predation is substantially lower in these habitats (Reznick et al., 1996a). This pattern of successions of fish communities (and gradients in predation-risk for guppies) is replicated across several river systems in Trinidad (Gilliam et al., 1993).

Trinidadian guppies are of particular interest to evolutionary ecologists, because they display locally adapted ecotypes that ultimately depend on the degree of

	Measurement	Difference	References
Life history	Size of offspring	Larger	1,2,3
	Number of offspring	Fewer	1,2,3
	Reproductive frequency	Slower	1,2
	Reproductive allotment	Lower	1
	Size/age at maturity	Larger/older	1,2,3
Physiology	Male colouration	Brighter	4,5
	Standard metabolic rate	Lower	6
	Rate of senescence	Higher	7,8
	Swimming speed and acceleration	Slower	9
Behaviour	Shoaling	More dispersive	10,11
	Response to predator cues	Reduced	10,11
	Male courtship display	Higher rate of display	12
Population	Absolute density	Higher	1
	Biomass density	Higher	13
	Mean body size	Larger	13
	Size structure	Fewer small individuals, more even distribution	13
	Monthly mortality rate	Much lower	14
	Regulation	Density dependent	15,16

Table 1.1: Examples of differences in the low-predation guppy ecotype, relative to the high-predation ecotype. References: 1. Reznick and Endler 1982; 2. Reznick 1982; 3. Reznick and Bryga 1987; 4. Endler 1980; 5. Gordon et al. 2015; 6. Auer et al. 2018; 7. Reznick et al. 2004; 8. Reznick et al. 2005; 9. Ghalambor et al. 2004; 10. Magurran et al. 1992; 11. Huizinga et al. 2009; 12. Seghers and Magurran 1990; 13. Reznick et al. 2001a; 14. Reznick et al. 1996b; 15. Reznick et al. 2012; 16. Bassar et al. 2013.

predation risk that they experience (Reznick and Endler, 1982; Reznick, 1982; Reznick and Bryga, 1987). Note that although different guppy populations exist along a continuum of predation risk (Deacon et al., 2018), in my thesis I focus on differences between populations at the extreme ends of this continuum, and so consider a dichotomy between high- and low-predation ecotypes. High-predation and low-predation guppy ecotypes display distinct life histories, physiological and behavioural differences, and differ in population demographics (Table 1.1). Increased predation rates in downstream localities leads to reduced densities of guppies compared to upstream locations: the absolute density of guppies in low-predation

habitats is approximately twice that of high-predation habitats (Reznick and Endler, 1982), whilst differences in population size structure mean that the biomass density of guppies in low-predation habitats is four-fold higher than in high-predation habitats (Reznick et al., 2001a). The lack of predators in low-predation locations shifts the nature of population regulation between these populations from top-down, via predation (as in high-predation) to bottom-up regulation through resource competition (as in low-predation) (Torres-Dowdall et al., 2012; Bassar et al., 2013). Guppies of both ecotypes are omnivorous, consuming invertebrates, detritus, and algae, however low-predation habitats have lower resource availability (Reznick et al., 2001a) and lower resource quality, i.e. fewer invertebrate prey available as food for guppies (Zandonà et al., 2011).

These changes in the predation risk and population densities ultimately drive differences in the life histories of these two ecotypes. Low-predation guppies are larger at maturity, commit fewer resources to reproduction, have lower reproductive frequency, and produce a smaller number of larger offspring per litter than high-predation guppies (Reznick and Endler, 1982). These differences have a genetic basis (Reznick, 1982; Reznick and Bryga, 1996) and are consistent across independent river watersheds (Reznick et al., 1996b). Genetic and genomic analyses support the parallel, independent evolution of the low-predation ecotype following invasion of guppies from ancestral high-predation habitats across multiple river watersheds in Trinidad (Alexander et al., 2006; Whiting et al., 2021): the life-histories of guppies in low-predation habitats have consistently, repeatedly, and independently diverged from those of the ancestral high-predation guppies. Furthermore, when high-predation guppies are introduced to low predation risk habitats, they rapidly evolve traits characteristic of the low-predation ecotype (Reznick and Bryga, 1987; Reznick et al., 1990; Travis et al., 2014; Reznick et al., 2019).

Changes in predation risk alone cannot explain the difference in life-histories between guppy ecotypes. Life-history evolution driven by predation requires a change in the pattern of mortality risk across age/size classes (Charlesworth, 1994). However, mark-recapture studies in natural high- and low-predation streams has

shown that whilst mortality risks are substantially higher for high-predation guppies, the difference is consistent across size/age classes, i.e. there is no difference in the pattern of mortality risk across size/age classes. However, it is worth noting that mortality rates of neonates and the smallest size classes of guppies could not be determined from these studies. Nevertheless, direct effects of predation cannot account for the differences in life-histories seen between high- and low-predation guppies (Reznick and Bryga, 1996). Instead, in low-predation guppies the increased population densities and strong density regulation of natural populations support the argument that limited resource availability drives the evolution of the low-predation life-history (Reznick and Bryga, 1996; Reznick et al., 2001b, 2012; Bassar et al., 2013; Reznick et al., 2019). Furthermore, low-predation guppies are less sensitive to increases in population density than high-predation guppies (Bassar et al., 2016), and life history differences such as larger offspring size confer a competitive advantage at high population densities (Bashey, 2008). Thus, the weight of evidence suggests that low-predation guppies are adapted to resource limitation. However, the exact mechanism through which the low-predation ecotype evolves remains unclear.

To summarise, guppies have repeatedly evolved distinct ecotypes, differing in a suite of life history traits, with natural populations characterised by differences in structure, density, and food availability (Table 1.1). In my thesis, I use mark-recapture data from a long-term introduction experiment (described in Travis et al., 2014; Reznick et al., 2019), as well as data on both ecotypes from free-living guppies, and those reared in common garden experiments. In short, Trinidadian guppies provide an excellent system for disentangling the relationships between size, reproduction, life histories, and their evolution.

1.5 Outline of thesis chapters

Chapter 2: Quantifying rapid evolution of body size in a wild population

Using quantitative genetic methods and data from a long-term experimental introduction, I show that male guppies evolved larger sizes at maturity following increases

in population density. Crucially, I verified predictions against observations from common garden experiments, allowing me to assess limitations to the application of quantitative genetic approaches in wild populations. I show that the evolutionary increase in male size was masked by a decrease in the plastic component of this trait. This is an example of cryptic evolution. This happened because as population densities increased, food levels decreased, meaning that male guppies could not grow to their full potential. More generally, I demonstrated that environmental drivers of selection (in this case population density) can inhibit our ability to detect the evolutionary change that they cause. This is likely to be problematic in any quantitative genetic studies of populations subject to long-term changes in environmental conditions. I show that models that do not account for changing environmental conditions can result in predictions of evolutionary change that are in the opposite direction to those observed. I describe the cause of this problem, and identify a simple solution: including environmental factors as fixed effects in the statistical models used to quantify additive genetic variance corrects for dissimilarity among relatives due to differences in the environment they experience. This reduces bias when estimating additive genetic variances and covariances, improving predictions of evolution in wild populations.

Chapter 3: Ecological causes of variation in body size and reproduction

In this chapter, I analysed the relationship between body size and reproductive output, using data from (i) wild guppies, sampled from 19 populations during both the wet and rainy season; and (ii) guppies from four populations reared in the lab under controlled levels of food availability. I found that reproduction was hyperallometric in the wild, and that this effect was strongest during the rainy season, when the effects of competition are more intense. I show that reproduction scales hyperallometrically when calculated across food treatments in the lab, but not when calculated within food treatments. I found no evidence that large guppies allocate a greater fraction of energy towards reproduction. My results suggest that

differences in size and reproductive output reflect differences among individuals in their feeding rates: individuals that attain larger sizes and produce more offspring do so because they have consumed more food. I conclude that the widespread phenomenon of reproductive hyperallometry in fishes may simply reflect that, in general, small individuals are more resource limited than large individuals in natural populations. This is consistent with research finding that larger individuals are generally stronger competitors in most fish species studied (Ward et al., 2006).

Chapter 4: Intraspecific variation in energy budgets: a comparative study of guppy populations

Dynamic energy budget (DEB) theory provides a framework for calculating how individuals allocate energy to different biological processes, such as growth, development, and reproduction. Differences between species in the parameter values of the DEB model are often used to quantify evolved life history differences. In this chapter, I investigated whether DEB theory could characterise intraspecific life history differences between guppy ecotypes. I calculated the DEB parameters for 16 populations of guppy, identifying consistent differences in patterns of energetic allocation between ecotypes. However, I found that using different combinations of data resulted in very different parameter sets for the same populations. Despite these differences, the alternative parameter sets reproduced the same life-history patterns – an example of getting the right answer for the wrong reasons. This artefact of parameter estimation appeared to be systematic: the type and amount of data used to fit the models affected different populations in a similar way. My results suggest that broad-scale comparisons of populations or species by differences in their DEB parameters may be invalid when models are fit with different types of data.

Chapter 5: Simulating life-history evolution: under what conditions can the low-predation ecotype evolve?

In my final data chapter, I combined elements from the three preceding chapters to investigate how size-dependent resource availability influences density-dependent life history evolution. I constructed individual-based models of guppy populations,

in which individuals were defined by DEB parameters. I made my simulations evolutionarily explicit by including heritable variation in a key DEB parameter, κ, which describes the fraction of energy allocated to somatic growth and maintenance over reproduction. In these simulations, ecological and evolutionary dynamics were emergent properties of the growth, reproduction, and survival of individuals competing for food. As such, my simulations recreate the establishment of populations of guppies in predator-free environments, much like the colonization of low-predation habitats by high-predation guppies that has occurred repeatedly in the wild and in introduction experiments. I show that when small individuals are resource limited, populations evolve slower life histories – similar to the evolution of low-predation ecotype guppies from high-predation ecotype ancestors – but the opposite is true when large individuals are resource limited. I show that changes in the way that competitive ability relates to body size alters the way that density dependence operates on the population. Specifically, fitness trade-offs emerge between juvenile survival and adult reproduction, but the balance favours enhanced juvenile survival when large individuals are stronger competitors. My results suggest that genetic variation in juvenile survival probability is likely to play a key role in the evolution of the low-predation ecotype in the wild.

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2

Environmental change, if unaccounted, prevents detection of cryptic evolution in a wild population

2. Environmental change, if unaccounted, prevents detection of cryptic evolution in a wild population *26*

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Abstract

Detecting contemporary evolution requires demonstrating that genetic change has occurred. Mixed-effects models allow estimation of quantitative genetic parameters and are widely used to study evolution in wild populations. However, predictions of evolution based on these parameters frequently fail to match observations. Here, we applied three commonly used quantitative genetic approaches to predict the evolution of size at maturity in a wild population of Trinidadian guppies. Crucially, we tested our predictions against evolutionary change observed in common garden experiments performed on samples from the same population. We show that standard quantitative genetic models underestimated or failed to detect the cryptic evolution of this trait as demonstrated by the common garden experiments. The models failed because: 1) size at maturity and fitness both decreased with increases in population density, 2) offspring experienced higher population densities than their parents, and 3) selection on size was strongest at high densities. When we accounted for environmental change, predictions better matched observations in the common garden experiments, although substantial uncertainty remained. Our results demonstrate that predictions of evolution are unreliable if environmental change is not appropriately captured in models.

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2.1 Introduction

Predictions of evolution based on quantitative genetic theory frequently fail to match observations of change (or lack thereof) in wild populations (Merilä et al., 2001; Gienapp et al., 2008; Pujol et al., 2018; Walsh and Lynch, 2018), Detecting contemporary evolution in natural populations is challenging enough (Merilä et al., 2001; Kruuk, 2004; Kruuk et al., 2008; Pemberton, 2010); understanding why predictions about evolution often fail creates a much larger, more important challenge (Pujol et al., 2018). There are three widely used quantitative genetic approaches for modelling contemporary evolution: 1) the Breeders equation, 2) the secondary theorem of selection, and 3) the change in estimated breeding values. The univariate Breeders equation (Lush, 1937) predicts the change in the population mean trait value due to selection and is formalized as:

$$\Delta z = h^2 S \tag{2.1}$$

where Δz is the response to selection (i.e. change in mean breeding value per generation), h^2 is the (narrow-sense) heritability of the trait, and S is the selection differential. The heritability is the proportion of population-level variation in a trait that can be explained by genetic differences among individuals, given by the ratio of the additive genetic variance $V_A(z)$ to the total phenotypic variance $V_P(z)$. The selection differential describes the relationship between the trait and fitness, which is given by their phenotypic covariance $cov_P(z, w)$. The Breeders equation in

its multivariate form allows predictions of responses to selection for any number of genetically correlated traits (Lande and Arnold, 1983). By accounting for the genetic covariance among multiple traits and their relationship to relative fitness, the multivariate Breeders equation should accurately predict evolutionary change – provided that (i) all correlated traits that influence fitness are included in the model, (ii) selection on the phenotype is equal to selection on the genotype, and (iii) that genetic and environmental determinants of variation are independent (i.e. no genotype by environment interactions) (Lande and Arnold, 1983). An alternative approach to describing evolutionary change was developed by Robertson (1966) and independently formalized by Price ((1970) shortly afterwards. The secondary theorem of natural selection (also known as the Robertson-Price identity) states that the expected change in mean trait value per generation, Δz, is equal to the additive genetic covariance cov_A of the trait z and relative fitness w:

$$\Delta z = cov_A(z, w) \tag{2.2}$$

The secondary theorem of selection considers only the genetic component of the relationship between a trait and relative fitness. As such, it considers the *net* association between the additive genetic components underlying the trait and relative fitness. Unlike the Breeder's equation, because the secondary theorem of selection makes no inference with regards to selection on the phenotype, it does not require all traits that influence fitness to be included in the model, nor does it assume that selection on the phenotype is equal to selection on the genotype (Morrissey et al., 2010, 2012). If the latter two conditions are met, then predictions from both models are equivalent (Morrissey et al., 2010). However, if these conditions are not met, predictions based on the breeder's equation and the secondary theorem of selection will differ, with the latter considered the more robust model of evolutionary change (Morrissey et al., 2010, 2012; Bonnet et al., 2017).

A third method of quantifying contemporary evolution is to describe the change in estimated breeding values over time. An individuals breeding value is the sum of the additive genetic effects on its phenotype (Lynch and Walsh, 1998). Whilst a

change in true breeding values over time is synonymous with evolutionary change, care is required when interpreting changes in estimated breeding values (Postma, 2006; Hadfield, 2008; Hadfield et al., 2010). Many applications of this approach in studies of wild populations have generated biased and anticonservative estimates of evolutionary change (reviewed and discussed in Hadfield et al., 2010). However, with appropriate modelling frameworks, contemporary evolution can be quantified by the temporal change in the population mean predicted breeding value.

All of these approaches rely on accurate decomposition of phenotypic variances and covariances into additive genetic and environmental components. The statistical foundation for this decomposition is the so-called animal model. Animal models are mixed-effects models for the phenotype that are structured with a relatedness matrix (Henderson, 1950; Lynch and Walsh, 1998; Kruuk, 2004). The basic animal model assumes an additive genotype-phenotype map, where an individuals phenotype P (with respect to the population mean value) is given by the sum of the breeding value G and the environmental component E, i.e. $P = G + E$. Animal models estimate G by assessing phenotypic similarity among all pairs of relatives, whilst E is described by the residual (Lynch and Walsh, 1998). As such, E is broadly defined, and captures not only the effect of environmental factors on the expression of traits (i.e. phenotypic plasticity), but also those of non-additive genetic effects (i.e. dominance and epistasis) (Lynch and Walsh, 1998). Animal models maximize the information on relatedness obtained from natural pedigrees and are robust to (randomly) missing and unbalanced data, and so are considered well-suited to inferring quantitative genetic parameters in natural systems (Lynch and Walsh, 1998; Kruuk, 2004; Wilson et al., 2010). Animal models are frequently used to quantify evolution in the wild, by estimating breeding values and parameters for the Breeders equation and the secondary theorem of selection (Merilä et al., 2001; Grant and Grant, 2002; Kruuk et al., 2008; Charmantier et al., 2014). However, the use of animal models in wild populations has revealed that despite the presence of heritable genetic variance and selection, there is frequently no observable change

in the mean phenotype, a phenomenon termed the paradox of stasis (Merilä et al., 2001; Gienapp et al., 2008; Pujol et al., 2018; Walsh and Lynch, 2018).

The crux of the paradox of stasis is that quantitative genetic approaches can accurately describe the response to selection in captive-bred organisms, but not in wild populations. A logical first step in resolving this paradox is to identify fundamental differences between artificial and natural selection. The main difference, undoubtedly, is that breeders target selection on specific traits, whereas natural selection acts on the full phenotype of the organism. The multivariate breeders equation addresses this issue to a degree by accounting for indirect selection through genetically correlated traits (Lynch and Walsh, 1998). The estimation of breeding values is also sensitive to the omission of correlated traits that are under selection; bias in estimation of breeding values is reduced when correlated traits are included in the animal model (Pollak et al., 1984). The secondary theorem of selection bypasses this problem by only considering selection at the genetic level, and making no inference about selection at the phenotypic level (Robertson, 1966; Price, 1970; Morrissey et al., 2012).

Another critical difference between artificial and natural selection is that of environmental variability (Walsh and Lynch, 2018, Chapter 20). Animal and plant breeders purposefully reduce environmental variation among individuals by making rearing conditions as similar as possible and providing nutrition, shelter, and protection from disease. In contrast, environmental factors in the wild may change substantially over time and space, generating considerable variation in phenotype and fitness within the population. In addition, dynamic environments may result in fluctuations in the target, magnitude, and direction of selection (e.g. Price et al., 1984; Hairston and Dillon, 1990; Grant and Grant, 2002); ignoring such effects could lead to erroneous predictions of evolution in the wild.

Not only does environmental variability have a direct influence on evolution in wild populations, it can also affect the results of statistical approaches we use to quantify evolutionary change. Here we consider how subgroup effects – environmental sources of phenotypic variation that differ in magnitude between

subgroups of a population – can bias estimates of quantitative genetic parameters from animal models. Subgroup effects vary spatiotemporally, such that there are broad-scale differences in the environmental conditions experienced among groups of individuals within the population as a whole. If subgroup effects are not explicitly accounted for, two distinct problems may arise: First, when relatives experience different subgroup effects, estimation of additive genetic (co)variances will be biased. This effect can occur within a generation between siblings or cousins or between generations between parents and offspring. Second, subgroup effects can lead to the regression model not capturing the causal relationship of traits on fitness, i.e. how variation in traits determines variation in fitness. Both of these problems may lead to misestimation of the evolutionary trajectory.

Regarding the first problem, if relatives consistently experience different subgroup effects the genotype-phenotype map is no longer strictly additive, because G and E are not independent. Estimation of V_A assumes that phenotypic similarity among relatives is due to shared genetic elements of additive effect on the phenotype (Lynch and Walsh, 1998). Non-genetic sources of similarity among relatives are routinely taken into consideration when estimating V_A using animal models (e.g. maternal effects (Wilson et al., 2010), birth year (Kruuk and Hadfield, 2007), common rearing environment (Kruuk et al., 2001), spatial autocorrelation (Stopher et al., 2012)). Doing so prevents upward bias when estimating V_A.

Non-genetic causes of dissimilarity (rather than similarity) among relatives are less often accounted for. If parents and offspring experience different environments their phenotypes and fitness will be less similar than if they had experienced the same conditions. For example, in a population growing towards carrying capacity, parents will experience higher per-capita resource availability then their offspring, i.e. there is a difference in subgroup effects between parents and offspring with respect to resource availability. This environmentally-induced dissimilarity among relatives could result in underestimation of V_A and misestimation of genetic covariances and breeding values.

When subgroup effects vary over time, a special case of the first problem occurs when estimating the change in breeding values. Estimation of breeding values in a simple animal model assumes that the environment is constant in time and space across the whole population (Mrode and Thompson, 2005; Postma, 2006). Under this assumption, estimated breeding values will partially reflect the environmental component of the phenotype (Postma, 2006). As such, when subgroup effects are not modelled, a change in estimated breeding values may reflect phenotypic rather than true genetic change (Postma, 2006; Hadfield et al., 2010).

Even when relatives experience the same subgroup effects, the second problem – of regression models capturing non-causal associations between traits and fitness – can arise when selection is estimated across the entire population, as is typical in studies of wild populations using animal models. Environmental factors that influence both traits and fitness can bias estimates of selection gradients (Rausher, 1992; Stinchcombe et al., 2002). For example, consider two adjacent plots of land, both sown with the same seeds. One plot receives fertiliser, while the other remains unfertilised. Plants on the fertilised plot will grow taller and produce more seed than plants on the unfertilised plot. Even if we found that there was no relationship between plant height and number of seeds within each plot, when this relationship is estimated across both plots, we will detect a positive covariance between plant height and fitness (i.e. number of seeds). This would suggest selection for increased plant height, even though no such selection pressure exists. In this case, the regression model captures a non-causal association between plant height and fitness; the phenotypic selection gradient is entirely dependent on environmental factors, rather than any real effect of the trait on fitness.

These statistical problems are likely inherent to studies of wild populations. A potential solution is to include subgroup-specific measurements of environmental factors as covariates in the animal model, to correct phenotypes and fitness prior to estimating breeding values, additive genetic variances, and selection. Including subgroup effects as covariates changes the mean part of the animal model. Instead of having a single population mean value around which G and E are estimated, the

model fits subgroup-specific mean values, accounting for subgroup-specific differences in environmental factors. This reduces the problems of non-independence of G and E, and of non-causal associations between traits and fitness as described in the previous paragraphs. However, for wild populations it can be difficult to identify and quantify the relevant environmental factors (Walsh and Lynch 2018). Nevertheless, not accounting for subgroup-effects may give rise to incorrect estimates of quantitative genetic parameters in wild populations, precluding accurate estimation of evolutionary change.

Regardless of which model is used to predict phenotypic change, quantitative genetic predictions and phenotypic observations from field data are rarely corroborated with independent data characterising evolutionary change of the same population (although see Van Asch et al., 2013 and Geerts et al., 2015) (although see Van Asch et al., 2013; Geerts et al., 2015). Common garden experiments, in which population samples from before and after selection are reared under identical environmental conditions, can determine if a change in the mean phenotype is due to evolution (Kawecki and Ebert, 2004; Pemberton, 2010). Common garden experiments remove the influence of subgroup effects on the phenotype, and thereby overcome the challenges posed by spatiotemporal environmental variation to quantitative genetic predictions of evolution. A difference in the phenotypic mean between ancestral and derived populations reared under common garden conditions is strong evidence for evolution, and thus, is considered the gold standard for demonstrating evolutionary change in natural populations (Pemberton, 2010; Walsh and Lynch, 2018). Although, by themselves, common garden experiments do not demonstrate the causes of evolutionary change (they do not distinguish changes caused by selection from those caused by drift or gene flow), they are, in principle, the standard against which model-based predictions of evolutionary change and field observations of changes in mean phenotype can be calibrated. Here we explore the problem of accurately predicting evolution under changing environmental conditions. We used animal models based on three years of mark-recapture data for a pedigreed, wild experimental population of Trinidadian guppies (*Poecilia reticulata*) to generate

predictions of evolutionary change based on the Breeders equation, the secondary theorem of selection, and the change in estimated breeding values. We then compared these predictions to observations of phenotypic change made in common garden experiments, as well as to the phenotypic change observed in the field data over the course of the study. The match was, in general, poor. However, when we accounted for environmental change when estimating quantitative genetic parameters, predictions based on the secondary theorem of selection and the change in estimated breeding values better matched observation of change derived from the common garden experiment. We discuss our results in the context of the challenges imposed by systematic environmental change when quantifying evolution.

2.2 Methods

Overview of Experimental Design

Our two objectives in this study were to assess 1) how well quantitative genetic predictions match observations of evolutionary change in a wild population, and 2) whether explicitly modelling subgroup effects improves the accuracy of predictions. Our study population is an experimentally-introduced, wild population of Trinidadian guppies. To generate predictions, we fit animal models with mark-recapture data from our study population, then parameterised quantitative genetic models of evolutionary change. We fit animal models both with and without subgroup effects as covariates. To observe evolutionary change, we performed annual common garden experiments comparing traits between the introduced and ancestral populations. If quantitative genetic parameters can be accurately estimated, predictions of evolution should match the changes observed in common garden experiments where environmental effects are controlled.

We used three quantitative genetic approaches to predict evolutionary change over a three-year period: the univariate Breeders equation, the secondary theorem of selection, and the change in estimated breeding values. We considered a single trait – male size at maturity – and used relative lifetime reproductive success as our measure of fitness. Using bivariate animal models, we estimated individual

breeding values and additive genetic and phenotypic variances and covariances among size at maturity and fitness. We fit animal models both with and without subgroup-specific environmental factors as covariates, to determine how changes in environmental conditions over the course of the study influenced the expression of traits and the estimation of quantitative genetic parameters and breeding values. To describe the temporal dynamics of evolutionary change, we used piecewise linear regression of the change in estimated breeding values with time, allowing different slopes in each year. Finally, we calculated the expected change in trait values according to our three quantitative genetic approaches, and compared these predictions to the observed change in trait values seen in (i) the study population field data (i.e. without correction of subgroup effects), and (ii) guppies reared under common garden conditions (i.e. corrected for subgroup effects). All analyses were conducted in R (R Core Team, 2018). Data and code underlying the analyses and figures in this manuscript are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.jm63xsj7k.

Study population

Trinidadian guppies are small live-bearing fish native to freshwater streams in Trinidad. Guppies display two distinct ecotypes that are defined by the intensity of local predation pressure. These ecotypes differ in a number of morphological, life-history, behavioural, and physiological traits. For example, the low-predation ecotype matures at larger sizes, has fewer, larger offspring per litter, and has lower reproductive rates than the high-predation ecotype (Reznick and Endler, 1982; Reznick, 1982b; Reznick and Bryga, 1987). These differences have a genetic basis, and have independently evolved in several different streams throughout Trinidad (Reznick, 1982a; Alexander et al., 2006). The evolution of the low-predation ecotype is driven by increased population density, rather than directly as a result of reduced predation pressure (Bassar et al., 2013; Reznick et al., 2019). Our study population is located in the Lower Lalaja, a tributary to the Guanapo river in the Northern Range mountains of Trinidad. This population is part of a long-standing introduction

experiment, described in detail elsewhere (Travis et al., 2014; Reznick et al., 2019). Briefly, high-predation ecotype guppies were introduced to a closed section of a previously guppy-free, low-predation-risk stream. This introduction replicates the upstream invasion of guppies, which has occurred naturally multiple times. The introduced population has since been censused monthly. Each founder and subsequent recruits received a unique identifying mark and two scales were removed from the caudal peduncle to sample for DNA. The mean monthly probability of capturing an individual in a census (given that it is alive) is 0.9 ((Reznick et al., 2019). At each census, standard length was recorded as the distance from the tip of the snout to the hypural plate, recorded to the nearest 0.1mm; mass was recorded to the nearest milligram; and the stage of sexual maturity in males was classified as either juvenile or mature, based on the development of the intromittent organ. Unfortunately, we do not have a similar metric for characterising maturity status in wild-caught females, so our study is limited to considering males only.

Mark-recapture and pedigree data

Our study focussed on the first three years of the experimental introduction, starting in March 2008. Because we were interested in male size at maturity, our dataset was restricted to those males caught and measured whilst in the juvenile stage ($\sim$14 mm standard length), and then caught and measured a month later and determined to have reached maturity. We recorded size at maturity as the standard length of males when first recorded as having reached maturity after having been observed to be immature in the preceding month. Using the individual DNA samples, we constructed a microsatellite-based pedigree for the introduced population. We used this pedigree to structure the animal models, and to calculate individual lifetime reproductive success. Full details on genotyping, pedigree construction, and estimation of lifetime reproductive success are provided in the supplementary material. Our final phenotypic dataset included 851 individuals, for which accurate estimates of lifetime reproductive success and size at maturity were available. We trimmed our pedigree to exclude lineages for which we had no phenotypic data.

Our final pedigree contained relatedness data for 1506 individuals (male and female) over three years, with a maximum depth of 7 generations. We estimated monthly population density as the number of individuals captured in a given months census, adjusted for capture probability, divided by the total area of the stream.

Common garden experiments

Juvenile guppies (∼12mm standard length) from the introduced and ancestral populations were collected at yearly intervals following the introduction, from 2009 to 2011. To account for maternal and environmental effects, guppies were reared for two generations under controlled environmental conditions. Fish were reared with a crossing design that sustained the genetic diversity of the founders, and avoided inadvertent selection by equally representing each line in each generation (Torres-Dowdall et al., 2012; Handelsman et al., 2013; Ruell et al., 2013; Reznick et al., 2019). In the common garden experiments, guppies were reared at either high or low food levels. Because there was no effect of any interaction between food level and population (ancestral or introduced) on size at maturity (Reznick et al., 2019), we pooled data across food treatments to increase sample sizes. Male size at maturity was recorded for second-generation lab-born guppies.

Observations of phenotypic change

We had two sources for observations of phenotypic change: the common garden data allowed us to quantify change between ancestral and introduced populations when subgroup effects were controlled, and the mark-recapture data (which were used to fit the animal models) provided measures of the phenotype in the wild, without any correction for subgroup effects. To calculate observations of change in the common garden data, we tested for phenotypic divergence between ancestral and derived populations with Bayesian generalized linear mixed effects models using *MCMCglmm* (Hadfield, 2010). Full details of the model fitting process are given in the supplementary material. We analyzed each year separately, including population (ancestral or derived) as a fixed effect. We accounted for relatedness among individuals within populations by fitting full-sibling family identity as a random

effect. Sample sizes from the ancestral (N_A) and introduced (N_I) populations in each year were: 2009, N_A=68, N_I=45; 2010, N_A=52, N_I=29; 2011, N_A=41, N_I=40. The difference in the mean trait in the introduced population, relative to the ancestral, is given by the coefficient for population.

For the mark-recapture data, we used *MCMCglmm* (Hadfield, 2010) to fit a piecewise linear regression of size at maturity against time (months since introduction), allowing different slopes in each year of the study. The intercept of the model provides the mean initial size at maturity, and estimates of mean size at maturity can be obtained for any month during the study.

Estimating quantitative genetic parameters

Using the field data (N=851) and pedigree, we fit bivariate animal models in the form:

$$[z, W] = \mathbf{bX} + \mathbf{D_1 a} + \mathbf{D_2 m} + \mathbf{Ie} \tag{2.3}$$

in which size at maturity (z) and lifetime reproductive success (fitness) (W) are estimated as functions of a matrix of fixed effects ($\mathbf{b}$) and random effects of breeding value ($\mathbf{a}$) and maternal identity ($\mathbf{m}$) which account for variation due to additive genetic and maternal effects, respectively. Breeding values were estimated based on a covariance matrix structured by pair-wise relatedness of all individuals in the pedigree (Lynch and Walsh, 1998). Maternal identity was included to account for phenotypic similarity among siblings arising from their shared maternal environment. $\mathbf{X}$, $\mathbf{D_1}$, and $\mathbf{D_2}$ are design matrices linking trait and fitness observations to the fixed and random effects. The residual environmental variation in traits and fitness is captured in $\mathbf{e}$, which is linked to individual observations by the incidence matrix ($\mathbf{I}$). Animal models were fit using *MCMCglmm* (Hadfield, 2010), generating 8000 samples of the posterior distribution. Full details and code for the model fitting process are given in the supplementary material.

By fitting bivariate models, we estimated the variance-covariance matrices among size at maturity and relative fitness due to additive genetic ($\mathbf{G}$), maternal

environment (**M**), and residual environmental effects (**E**). For example, the **G**-matrix is given by:

$$\mathbf{G} = \begin{bmatrix} V_A(z) & cov_A(z,W) \\ cov_A(W,z) & V_A(W) \end{bmatrix} \tag{2.4}$$

where V_A and cov_A are the additive genetic variances and covariances, respectively, of z and W. The same structure is used for **M** and **E**, and the total phenotypic variance-covariance matrix (**P**) is given by $\mathbf{P} = \mathbf{G} + \mathbf{M} + \mathbf{E}$. This bivariate decomposition allowed us to estimate breeding values and parameters for the Breeders equation and the secondary theorem of selection in the same model. To estimate the per-generation response predicted by the Breeders equation (Eqn. 1), we multiplied $V_A(z)$ by the selection gradient, which we calculated as the phenotypic covariance between the trait and fitness, divided by the total phenotypic variance, i.e.

$$\beta z = \frac{cov_A(z,W) + cov_M(z,W) + cov_E(z,W)}{V_A(z) + V_M(z) + V_E(z)} \tag{2.5}$$

Note that because we used a log-link function to transform absolute lifetime reproductive success (W), estimates on the latent scale are converted from absolute to relative lifetime reproductive success (w): this is because the log-link function essentially divides values of absolute fitness by the mean of absolute fitness (Smouse et al., 1999; Firth et al., 2015; Bonnet and Postma, 2018; Bonnet et al., 2019), meaning that our estimates of βz are equivalent to selection gradients as described by Lande and Arnold (1983). For the same reason, the additive genetic covariance between trait and fitness converts from absolute ($cov_A(z,W)$ to relative fitness ($cov_A(z,w)$) on the latent scale. For the secondary theorem of selection (Eqn. 2), the per-generation response is given by $cov_A(z,w)$.

Temporal dynamics in breeding values and drift

To assess the temporal dynamics of the evolution of size at maturity, we modelled the change in estimated breeding values over time. Using the estimated breeding values from our animal models, we fit piecewise linear regressions of estimated breeding values by monthly cohort. We chose this approach to allow the direction

and rate of genetic change (i.e. the slopes of the model) to differ in each year of the study, because our *a priori* expectation is of a delay in the onset of evolution of size at maturity (Reznick et al., 2019). We fit these models across all Bayesian posterior samples to account for uncertainty in estimated breeding values and the correlational structure of error terms (Hadfield et al., 2010), generating a posterior distribution of the monthly change in mean breeding value for each year (i.e. we used a piecewise linear regression modification of the approach used in e.g. Pigeon et al., 2016; Bonnet et al., 2019). To illustrate non-linear temporal dynamics of estimated breeding values, we also fit a general additive model with a smoothing function to each iteration of the Monte Carlo Markov chain used to sample from the posterior, as above, using the R-package *mgcv* (Wood, 2017).

A change in breeding values may arise from genetic drift. We simulated genetic drift by generating 8000 sets of random breeding values, based on the posterior distribution of $V_A(z)$ and the pedigree (Hadfield et al., 2010). We then fit the piecewise linear regression model described above using these random breeding values, to simulate the change in estimated breeding values that could be explained by genetic drift. We calculated the posterior probability that changes in mean estimated breeding values ($\Delta\overline{eBV}$) were greater than expected due to genetic drift ($\Delta\overline{eBV}_d$) as the proportion of posterior samples of $\Delta\overline{eBV}$ that were greater than the equivalent posterior samples of $\Delta\overline{eBV}_d$ (e.g. as in Hadfield et al., 2010).

Including subgroup effects as covariates

Because we wanted to test how subgroup effects can influence estimation of quantitative genetic parameters, we ran two types of model. In the first, the only fixed effects were the population mean values for each response variable. Our predictions for the non-corrected Breeders equation, secondary theorem of selection, and change in estimated breeding values were derived from this model. In the second, we also included subgroup-specific measures of environmental variables as fixed effects. This allowed us to test how the expression of traits and fitness are influenced by known environmental variables, and to determine how correcting phenotypes

for subgroup effects influenced estimation of breeding values and quantitative genetic parameters. We defined a subgroup of guppies as those that attained maturity in the same month, allowing us to account for broad-scale temporal changes in environmental factors influencing the population. Our subgroup-specific environmental factors were population density, season (rainy or dry), and their interaction, which we included as fixed effects in the animal model. We expect that population density and season affect resource availability, which in turn is likely to influence the means and variances of traits and fitness. Trinidad has a pronounced rainy season from June to December, during which flooding scours the streams of invertebrates and algae, reducing the availability of food for guppies. Population density is also likely to affect resource density, but we expect the relationship between population density and resource availability to change as the carrying capacity changes between seasons. A potential issue with correcting our measure of fitness for population density is that population density is a function of mean fitness: over a single time-step, population growth rate and mean absolute fitness are equivalent (Engen et al., 2009). However, our measure of population density applies to the month in which male guppies attained maturity, which is, by definition, before those individuals have contributed to population growth with their own offspring. This decouples individual lifetime reproductive success from the population density measure applied to that individual. We tested whether subgroup effects differed between generations by calculating the mean difference in population density experienced at maturity between sires and their offspring. We did this by fitting a linear model of population density with relation (offspring or sire) as a fixed effect, and included sire-offspring pair as a grouping factor (i.e. random effect). The intercept of the model is the mean population density experienced by offspring, and the slope gives the mean difference in population density experienced by sires. A negative value of the slope indicates that sires experienced lower population densities than their offspring. We fit this model using *MCMCglmm* (Hadfield, 2010), using default settings and priors.

Covariance between a trait and fitness may arise due to a.) a causal effect of the trait on fitness; b.) selection on a correlated (unmeasured) trait; and/or c.) environmental covariance between the trait and fitness (Walsh and Lynch, 2018). When the covariance is generated by b.) and/or c.) the estimate of the causal effect of the trait on fitness (i.e. the phenotypic selection gradient) will be biased. Bias can be detected by decomposition of the phenotypic selection gradient β_P into additive genetic ($\beta_A = cov_A(z,w)/V_A(z)$) and environmental ($\beta_E = cov_E(z,w)/V_E(z)$) selection gradients: selection is biased when $\beta_A \neq \beta_E$ (Rausher, 1992; Stinchcombe et al., 2002; Hadfield, 2008). If the focal trait is the sole determinant of variation in fitness (i.e. the true target of selection), then $\beta_P = \beta_A$, and predictions from the Breeders equation and the secondary theorem of selection will be equivalent (Morrissey et al., 2010, 2012; Walsh and Lynch, 2018). We assessed the bias in our estimates of selection by calculating the posterior probabilities that $\beta_E > \beta_A$ and $\beta_P > \beta_A$. By fitting an animal model with subgroup effects as covariates, we estimated β_P conditional on the subgroup effects, thus removing the effect of c.) due to changes in population density and season. Remaining bias would indicate that unmeasured correlated traits (or other environmental factors) also influence variation in fitness.

Comparing predictions and observations

We assume that the difference in trait values between ancestral and introduced guppies in the common garden experiments reflects the true genetic change. To compare predictions of change with observations of change in the field and the common garden experiments, we multiplied the per-generation predictions from the Breeders equation and the secondary theorem of selection by the mean number of generations elapsed after 3 years (mean generation time was 7.90 months (N=1457, SD = 3.15); see the supplementary material for details on this calculation). Because the common garden data and the field data used to fit the animal models are independent, we could directly calculate the posterior distribution of the differences between predictions and observations. For example, the difference

between prediction A and observation B was obtained by subtracting the posterior distribution of B from that of A. The resultant distribution of the difference, $|A-B|$, captures the magnitude and uncertainty of the difference between estimates. Note that due to slight skew in posterior distributions, the mode of $|A - B|$ may not be equal to the mode of A minus the mode of B. We define a difference (e.g. $|A - B|$) as statistically clear when the 95% credible intervals of the distribution do not include zero.

2.3 Results

Observations of phenotypic and environmental change

Parameter estimates provided in the following sections are the modes of the posterior distributions of estimates, with 95% credible intervals given in parentheses.

Male size at maturity as measured in the field was highly variable (Fig. 1A). At the onset of the experiment, mean size at maturity ($\bar{z}$) was 17.66 mm (17.17; 18.07); $\bar{z}$ declined after two years (after 1 year, $\bar{z}$ = 17.62 mm (17.47; 17.78); after 2 years, $\bar{z}$ = 16.29 mm (16.18; 16.43)), before increasing to slightly less than the initial size in the third year (after 3 years, $\bar{z}$ = 17.41 mm (17.21; 17.61)). Mean total population density increased each year following the introduction (Fig. 1B, year 1: mean=0.56 N m^{-2}, SD=0.25 N m^{-2}, N=11; year 2: mean=1.69 N m^{-2}, SD=0.38 N m^{-2}, N=12; year 3: mean=2.55 N m-2, SD=0.36 N m^{-2}, N=12). In the common garden experiments, mean size at maturity relative to the ancestral population increased slightly in the first year of the study, then decreased slightly in the second, followed by a clear increase in the third year (i.e. 95% credible intervals of the change do not overlap zero, Fig. 2). By contrast, mean size at maturity as measured in the field was only clearly lower in the second year of the experiment (Fig. 2). Observations of change in size at maturity clearly differed between the field and the common garden in years 2 and 3 ($|\Delta z_{CG2} - \Delta z_{F2}|$ =1.00 mm (0.36; 1.70); $|\Delta z_{CG3} - \Delta z_{F3}|$ =0.70 mm (0.07; 1.31)), but not in year 1 ($|\Delta z_{CG1} - \Delta z_{F1}|$ =0.34 mm (0.33; 1.03)) (Fig. 2).

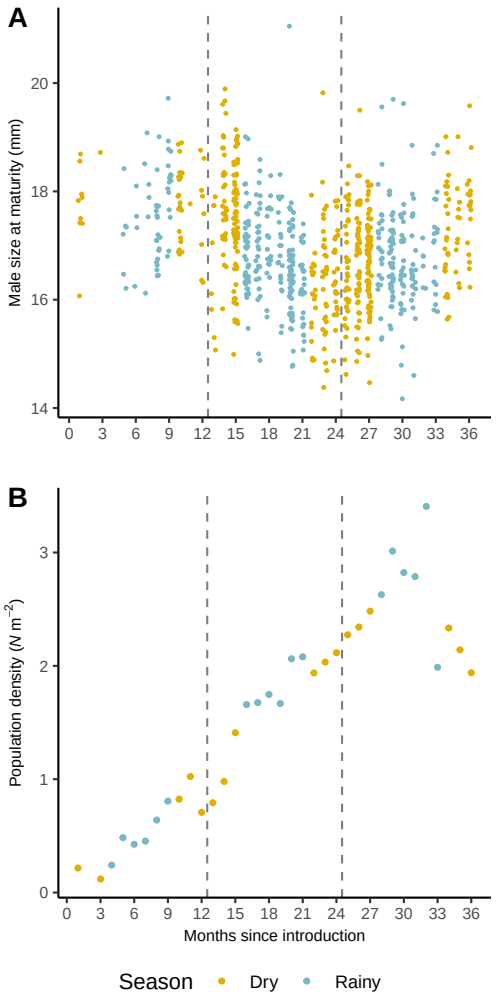

Figure 1: Raw data showing changes in (A) male size at maturity and (B) population density over time. Data are shown on a monthly basis for the first 3 years of the introduction experiment. Data points in (A) are jittered on the x-axis to facilitate visualisation of similar values. Vertical dashed lines highlight changes in year of study. Observations in the dry season are given in yellow, observations in the rainy season are in blue.

Environmental influence on size at maturity and fitness

The environmental subgroup effects that we measured population density and season strongly influenced the expression of both size at maturity and fitness. The size at which male guppies attained maturity decreased as population density increased ($\beta_{pop} = -0.66$ mm per N m^{-2}, $(-0.80; -0.51)$). Guppies tended to mature at smaller sizes during the rainy season ($\beta_{rainy} = -0.72$ mm, $(-1.09; -0.37)$). There was an interaction between population density and season, such that the negative effect of

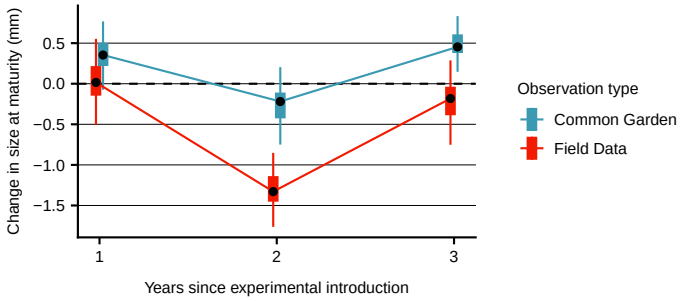

Figure 2: Annual observations of mean change size at maturity in the common garden experiments (blue) and in the mark-recapture field data (red). Changes in the common garden are based on comparisons between the grand-offspring of individuals sampled from the ancestral and experimental populations each year, reared under common conditions. Observations of change in the field data are of size at maturity, as measured in the experimental population, and fit with a piecewise linear model, allowing for different slopes in each year.

increasing population density on size at maturity was around 1.7 times greater during the dry season than in the rainy season ($\beta_{rainy:pop}$ =0.33 mm per N m^{-2}, (0.15; 0.51)).

The relative lifetime reproductive success of male guppies was influenced by the environmental conditions they experienced in the month prior to maturity. Guppies had lower fitness w if they attained maturity at times of increased population density ($\beta_{pop} = -0.59$ per N m^{-2}, (-0.85; -0.33)), and tended to have lower fitness if they attained maturity during the rainy season ($\beta_{rainy} = -0.67$, (-1.30; -0.03)). Again, there was an interaction between these factors, meaning that the decrease in w with increasing population density was strongest during the dry season ($\beta_{rainy:pop}$ =0.33 per N m^{-2}, (0.01; 0.65)).

Subgroup effects differed across generations: on average, the population densities experienced by offspring were ∼40% greater than those experienced by their sires (mean difference in population density at maturity of sire relative to that of offspring $= -0.59$ N m^{-2} (-0.63; -0.55); Fig. S3).

The impact of subgroup effects on estimation of quantitative genetic parameters

We ran two animal models which differed in whether subgroup effects were included as covariates. In both models, we detected significant additive genetic variance (V_A)

Table 1: Components of variances and covariance from animal models fit with and without subgroup effects SG as covariates. Posterior modes and 95% credible intervals (in parentheses) are provided for estimates of variances V and covariances cov relating to size at maturity z and relative fitness w. We used lifetime reproductive success as our measure of fitness. Subscripts A, M, and E denote, respectively, the additive genetic, maternal environment, and residual environmental components of the total phenotypic variance P. Note that calculations of V_P and $cov(z, w)$ were performed on the posterior distributions of their components; the sum of the modes of components does not necessarily equal the mode of V_P. Estimates for which 95% credible intervals do not overlap zero are given in bold.

Trait	Parameter	Estimates	
		Without SG	Including SG
Size at maturity (z)	V_A	**0.36** (0.21; 0.56)	**0.32** (0.17; 0.47)
	V_M	**0.10** (0.05; 0.20)	**0.09** (0.04; 0.16)
	V_E	**0.51** (0.39; 0.66)	**0.46** (0.35; 0.60)
	V_P	**1.01** (0.91; 1.13)	**0.89** (0.81; 0.99)
Relative fitness (w)	V_A	**0.47** (0.18; 0.84)	**0.73** (0.36; 1.31)
	V_M	**0.12** (0.04; 0.29)	**0.10** (0.04; 0.27)
	V_E	**1.24** (0.90; 1.63)	**0.97** (0.60; 1.38)
	V_P	**1.89** (1.61; 2.28)	**1.92** (1.62; 2.29)
$cov(z, w)$	$cov_A(z, w)$	0.09 (0.25; 0.08)	0.11 (0.07; 0.28)
	$cov_E(z, w)$	**0.20** (0.05; 0.35)	0.02 (0.18; 0.13)
	$cov_P(z, w)$	**0.17** (0.04; 0.28)	0.11 (0.01; 0.22)

for size at maturity z and relative fitness w (Table 1). Maternal effects accounted for small but significant proportions of the total phenotypic variance (V_P) in both z and w (Table 1). Residual environmental variance explained the majority of V_P in both size at maturity and relative fitness (Table 1). If relatives experience different subgroup effects, estimation of additive genetic (co)variances and breeding values may be biased when subgroup effects are not modelled (i.e. the first problem outlined in the Introduction). We found little difference between estimates of $V_A(z)$ between models fit with and without subgroup effects, but $V_A(w)$ was around 50% greater when subgroup effects were included in the animal model (Table 1). Accounting for subgroup effects had a pronounced effect on the estimation of the additive genetic covariance between size at maturity and relative fitness $cov_A(z, w)$:

when subgroup effects were included, the sign of $cov_A(z, w)$ changed from negative to positive (Table 1). Accordingly, when subgroup effects were not modelled, the secondary theorem of selection predicted a per-generation decrease in mean size at maturity of 0.09 mm (−0.07; 0.25), but predicted an increase of 0.11 mm (−0.07; 0.27) per generation when subgroup effects were accounted for. Note that while the credible intervals for both of these estimates span zero, the former indicates an 85.2% posterior probability that z will decrease, while the latter indicates an 86.6% posterior probability that z will increase.

When unmodeled subgroup effects vary over time, changes in estimated breeding values may reflect phenotypic, rather than genetic change. For both models, the change in estimated breeding values over the duration of the study was non-linear, but could be approximated as linear on an annual basis (Fig. 3). Without modelling subgroup effects, the change in estimated breeding values resembled the phenotypic change seen in the field data: there was no clear change in mean breeding value in the first year ($\Delta\overline{BV}_1$ = 0.01 mm month^{-1} (0.00; 0.02)), a decrease in the second year ($\Delta\overline{BV}_2$ = −0.03 mm month^{-1} (−0.05; −0.01)), and an increase in the third year ($\Delta\overline{BV}_3$ = 0.05 mm month^{-1} (0.02; 0.07)) (Fig. 3A, c.f. Fig. 2). By contrast, when we modelled subgroup effects, the pattern in the breeding values resembled that in the common garden experiments: there was no clear change in the first two years ($\Delta\overline{BV}_1$ = 0.00 mm month^{-1} (−0.01; 0.01); $\Delta\overline{BV}_2$ = −0.01 mm month^{-1} (−0.03; 0.01)), but there was a clear increase in the third year ($\Delta\overline{BV}_3$ = 0.03 mm month^{-1} (0.01; 0.05)) (Fig. 3B, c.f. Fig. 2). After three years, the posterior probability that the increase in estimated breeding values was greater than expected due to genetic drift was 98.0%.

If subgroup effects are present, but not modelled, the phenotypic selection gradient may capture a non-causal association between the trait and fitness (i.e. the second problem outlined in the Introduction). Without modelling subgroup effects, we found that male guppies that matured at a larger size had higher relative fitness, indicating positive phenotypic selection for this trait (β_P=0.16 mm^{-1}, (0.05; 0.28), Fig. S2); for comparison with other studies, the standardized selection gradient

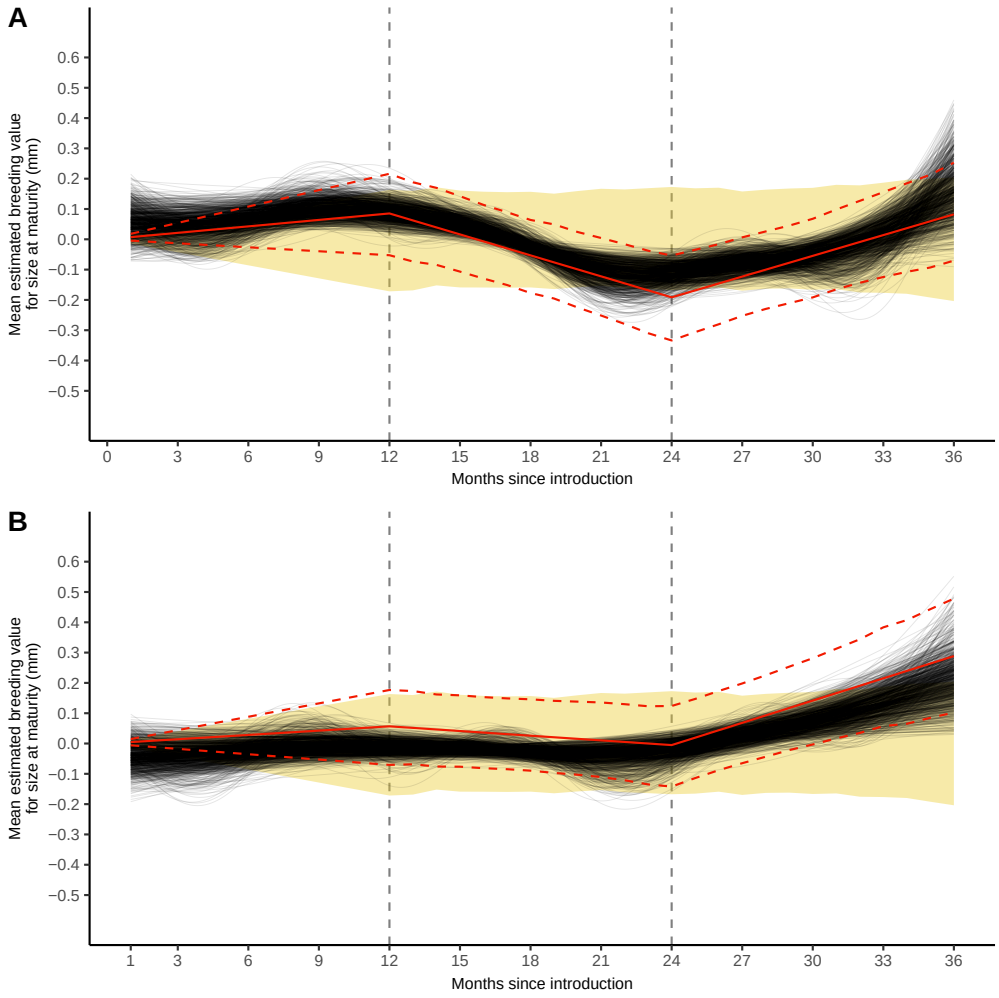

Figure 3: Change in mean estimated breeding value for size at maturity over the 36-month study period, (A) without correcting for environmental change, and (B) when changes in population density and season are accounted for when estimating breeding values. Solid red lines are the mean trend in estimated breeding values, dashed red lines indicate the 95% credible intervals of the trend, based on piecewise linear regression of the change in breeding value over time. Each grey line is the mean estimated breeding value fit with a general additive model to a single iteration of the Monte-Carlo Markov chain; 1000 lines are shown to illustrate the uncertainty around the non-linear trend in estimated breeding values with time. The shaded yellow region is the 95% credible interval of change in breeding values expected due to drift. Vertical dashed lines highlight changes in year of study.

(Kingsolver et al., 2001) was 0.16 (0.05; 0.29); the mean-standardised selection gradient (Hereford et al., 2004) was 2.69 (0.77; 4.93). However, decomposition of β_P revealed that the genetic (β_A) and environmental (β_E) selection gradients were not equal, meaning that this estimate of selection was biased (β_A=−0.22 mm^{-1} (−0.70, 0.22); β_E=0.37 mm^{-1} (0.07, 0.70); P($\beta_A < \beta_E$)=95.7%). After correcting for subgroup effects, the strength of phenotypic selection was slightly reduced (β_P)=0.12 mm^{-1}, (−0.01; 0.25)); although the confidence interval includes zero, there was a 97.0% posterior probability that selection was positive. This corrected estimate accounted for any potential non-causal covariance generated by subgroup effects, but was still biased. Phenotypic and genetic selection gradients were not equal (β_A=0.30 mm^{-1} (−0.24, 0.89); P($\beta_A > \beta_P$)=77.7%), implying that unmeasured genetically correlated traits (or other unmodeled environmental factors) caused variation in fitness.

Because there was little difference between models in our estimates of phenotypic selection and additive genetic variance, predictions of evolution according to the Breeders equation were similar whether subgroup effects were accounted for or not. Without accounting for subgroup effects, the Breeders equation predicted an increase of 0.06 mm per generation (0.01; 0.11); when subgroup effects were modelled, an increase of 0.04 mm per generation was expected (0.00; 0.09).

Comparing predictions with observations from the common garden

When we did not include subgroup effects as covariates in the animal model, all three predictions of change underestimated the change seen in the common garden experiments after three years (Fig. 4, in red). The predicted change according to the secondary theorem of selection was in the opposite direction to the change seen in the common garden experiments, and so underestimated the observed change by 0.93 mm (0.10; 1.73); the estimated breeding value approach underestimated the change by 0.40 mm (0.04; 0.78); and the Breeders equation underestimated the change by 0.22 mm (−0.20; 0.62), although this last difference was not statistically clear.

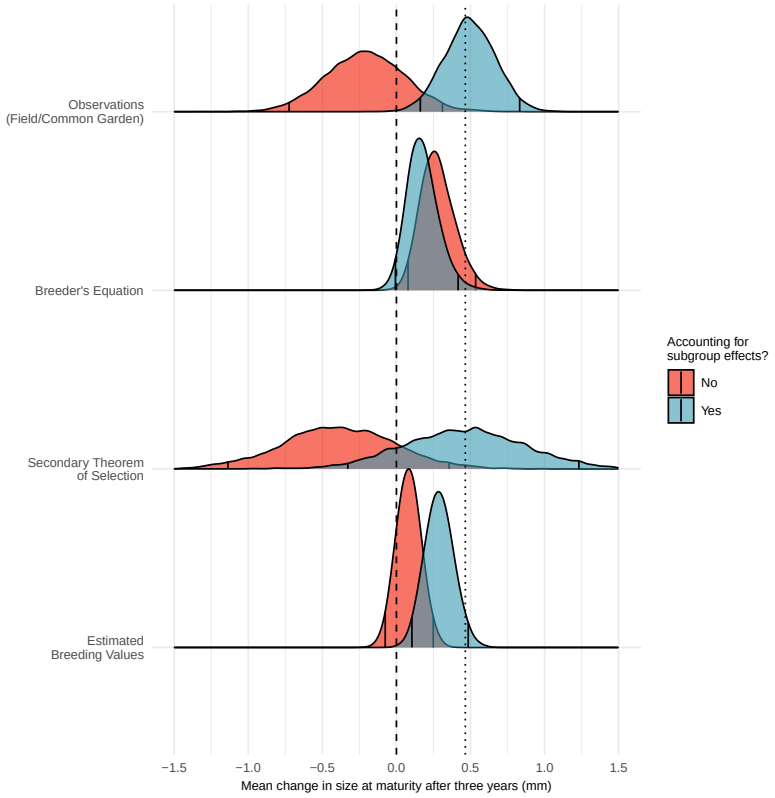

Figure 4: Observed and predicted change in mean size at maturity in male guppies, three years after their experimental introduction to a low-predation-risk environment. Observations and predictions that do not account for subgroup effects are given in red; those that do are in blue. Observations are of phenotypic change in the mark-recapture field data (in red) and in common garden experiments (in blue). The observed change in the field data is the mean change observed after 36 months, based on a piecewise linear regression of phenotypic change over time. The observed change in the common garden data is the mean difference between the grand-offspring of individuals sampled from the ancestral and experimental population three years after the introduction, and reared under common conditions. Predictions of change based on the Breeders equation and the secondary theorem of selection are based on 36 months of data, and assume a mean generation time of 7.90 months. Predictions based on the change in estimated breeding values are derived from piecewise linear regression of estimated breeding values over time. For predictions, subgroup effects were accounted for by including population density, season, and their interaction as fixed effects in the animal model used to estimate quantitative genetic parameters and breeding values. Full posterior distributions of observations and predictions of phenotypic change are shown, vertical black lines indicate the 95% credible intervals of the distributions. The vertical dotted line highlights the mean change in breeding value as determined by the common garden experiments.

When we accounted for subgroup effects, there were no statistically clear differences between predictions and observations in the common garden ($|\Delta\bar{z}_{pred} - \Delta\bar{z}_{obs}|$) (Fig. 4, in blue). For the secondary theorem of selection, $|\Delta\bar{z}_{STS} - \Delta\bar{z}_{obs}|$ was reduced to -0.02 mm (-0.93; 0.83); for the estimated breeding value approach, $|\Delta\bar{z}_{eBV} - \Delta\bar{z}_{obs}|$ was reduced to -0.19 mm (-0.59; 0.20). Correcting for subgroup-effects decreased the accuracy of the Breeders equation prediction: $|\Delta\bar{z}_{BE} - \Delta\bar{z}_{obs}|$ increased to -0.34 mm (-0.73; 0.07).

2.4 Discussion

Accounting for environmental effects alters predictions about evolution

Without accounting for subgroup effects, our results appeared to be a classic case of evolutionary stasis: we identified additive genetic variance and positive selection for size at maturity, but actually observed a slight decline in this trait in the field phenotypic data (Fig. 4, in red). Using the secondary theorem of selection, we then detected slightly negative selection occurring at the genetic level (albeit with considerable uncertainty), which agreed with the limited change seen in the field data (Fig. 4, in red). This type of discrepancy between predictions of change using the Breeders equation and the secondary theorem of selection has previously been attributed to unmeasured factors upwardly biasing estimates of selection at the phenotypic level; that is, if traits that influence fitness are not measured, the Breeders equation may predict evolution when none has occurred (Morrissey et al., 2012). Initial analysis of the estimated breeding values also indicated the absence of a clear evolutionary change after three years (Fig. 4, in red). However, results from the common garden experiments show that mean size at maturity in the experimental population increased relative to the ancestral population: this increase was roughly double that predicted by the Breeders equation (Fig. 4). Given that the natural low-predation guppy ecotype matures at larger sizes than the high-predation ecotype (Reznick and Endler, 1982), this change is consistent with the previously observed evolutionary transitions between ecotypes. Because environmental effects were controlled in the common garden, this increase must be

due to an increase in the mean breeding value of the population, i.e. evolution. This result begs the question: why did these quantitative genetic models underestimate or fail to predict the evolutionary change demonstrated by the common garden experiments? Our results suggest that not accounting for environmental change over the study period (i.e. subgroup effects) led us to predict an incorrect evolutionary trajectory. When we modelled subgroup effects (i.e. differences among subgroups in the population density and season experienced prior to maturity), predictions of change in size at maturity based on the secondary theory of selection and the change in breeding values better matched observations in the common garden experiments, although considerable uncertainty remained (Fig. 4, in blue). How then did changes in population density and season bias our estimation of quantitative genetic parameters? In the introduction we outlined two distinct problems when such subgroup effects are not modelled: (i) environmentally-induced dissimilarity among relatives, and (ii) non-causal associations between traits and fitness. In the following paragraphs, we discuss our results in the context of these problems.

Regarding the first problem, when we did not model subgroup effects, we implicitly assumed that relatives experienced the same environmental conditions. This was not the case: because our study population was growing, offspring experienced considerably higher population densities than their sires. At high population densities, guppies matured at smaller sizes and had lower fitness. By estimating the additive genetic covariance matrix across the full period of the study without modelling subgroup effects we failed to account for environmentally-induced dissimilarity in phenotypes and fitness among relatives. Although this had little effect on estimates of $V_A(z)$, there was a change in the sign of $cov_A(z, w)$ and a change in the temporal pattern of estimated breeding values when subgroup effects were modelled. In our first animal model, the secondary theorem of selection response was biased towards the phenotypic trend: after three years, the prediction of a slight decrease in mean size at maturity matched what we observed in the field measurements of this trait (Fig. 4). The change in estimated breeding values also resembled the phenotypic change over time observed in the field data (Fig 3A, c.f. Fig

1A). However, when we corrected traits and fitness for subgroup effects, predictions of evolution from both of these approaches more closely matched the changes seen in the common garden experiments, i.e. the genetic change (Fig 4, Fig 3B).

Regarding the second problem, accounting for subgroup effects had little effect on estimates of the phenotypic selection gradient, but the remaining mismatch between the genetic and environmental components of the gradient indicated that correlated traits that influence fitness were missing from the analysis. However, by correcting for subgroup effects, we showed that size at maturity had a (partial) causal effect on fitness. The omission of other traits under positive selection (and that are positively genetically correlated with size at maturity) from the analyses may explain the underestimation of evolutionary change predicted by the Breeders equation from both animal models (Fig. 4). This is supported by the underestimation we observed in the change in breeding values when subgroup effects were modelled (Fig. 4); estimation of breeding values is also sensitive to the omission of genetically correlated traits.

Both statistical problems that we have discussed are potential examples of the Yule-Simpson effect a change in the magnitude or sign of a covariance conditional on a third variable (Yule, 1903; Simpson, 1951). This is a well-known problem in regression analyses, whereby covariances estimated across aggregated data capture non-causal associations when the data is structured by a third confounding variable (Blyth, 1972; Pearl, 2014). With regards to estimating phenotypic selection gradients (problem (ii)), this issue is partially solved by the development of the multivariate Breeders equation (Lande and Arnold, 1983). This solution is only partial because fitness, which is convenient to think of as a trait in quantitative genetic analyses, is really an emergent property of the interaction between the phenotype and the environment (Coulson et al., 2006). As such, fitness is conditional not only on the traits that comprise an individuals phenotype, but also on the environmental conditions it experiences.

The strength of the animal model that it makes use of relatedness data across the entire population also makes it prone to the Yule-Simpson effect when

relatives experience different environmental conditions. In our study Yule-Simpson effects driven by two distinct unmodeled factors differences among relatives in environmental conditions experienced, and unmeasured traits influencing fitness reduced our ability to accurately quantify evolution. The secondary theorem of selection was sensitive to the former, the Breeders equation was sensitive to the latter, and the change in estimated breeding values was sensitive to both. Our results demonstrate that such effects, if unaccounted, can lead to incorrect inferences about both the magnitude and direction of evolutionary change.

Ecological feedback drives cryptic evolution

Our results support the hypothesis that the life-histories of guppies evolve in response to increased population density, rather than as a direct response to reduced predation risk (Reznick et al., 2019). If selection for increased size at maturity was due to release from predation pressure, selection should be strongest at the onset of the experiment (Reznick et al., 2019). Instead, several lines of evidence point towards size at maturity evolving in response to increases in population density: (i) the mean estimated breeding value for size at maturity only began to increase in the third year of the study, when population density was highest (Fig 3B); (ii) the monthly phenotypic trend shows a strong decline in size at maturity with increasing population density in the second year, but an increase in size at maturity in the third year despite higher average population densities than in the preceding years (Fig. 1); and (iii) the absence of a clear increase in size at maturity in the common garden experiments until the third year of the study (Fig. 2). Comparing change in size at maturity between the field and common garden data revealed cryptic evolution of this trait: an increase in the genetic component of the phenotype concealed by a decrease in the environmental component (Cooke et al., 1990). Under the additive genotype-phenotype map ($P = G + E$), increasing the breeding value G will increase the trait value P, provided that the environmental component E is constant (as in the common garden experiments). In the field data, a negative value of E masked the increase in G such that size at maturity

after three years did not differ from that at the onset of the experiment. The effect of the increase in G can be seen in Fig. 1A: in the third year, the phenotypic value began to increase, despite continued increases in population density (and by extension an increasingly negative value of E).

The population appeared to reach carrying capacity in the third year and then began to decline. This provided an opportunity for a comparison: population densities are approximately equal at months 23-24 and months 35-36 (Fig. 1B), yet there is an increase in mean size at maturity of around 1 mm between these time points. This increase is roughly equivalent to the increase in G in the common garden data over the same period (0.78 mm). The increase in population density led to a decrease in mean size at maturity due to changes in E, but also drove positive selection resulting in an increase in the breeding value G of this trait.

In biological terms, as the population approached carrying capacity, per-capita resource availability presumably declined, which had two conflicting effects on the populations mean phenotype. At the genetic level, decreased resource availability drove selection for increased size at maturity. This was likely because larger size in general is associated with higher competitive ability under resource limitation in guppies (Potter et al., 2019). This genetic change was only apparent in the common garden experiments, where resource levels were constant. At the same time, decreased per-capita resource availability likely had a direct effect that decreased size at maturity. This is consistent with experimental results on resource-dependent plasticity in guppies (Reznick, 1990). As such, at the phenotypic level, wild guppies could not attain the larger size that their breeding values would predict, because there were insufficient resources to do so. Thus, our study provides a novel example of ecological feedback driving cryptic evolution.

Generality and limitations of the study

Our results, like most quantitative genetic studies of natural systems, are based on a single population. This makes it difficult to assess the generality of our findings. However, our study population is one of four experimental introductions: we do

know that common garden studies indicated a similar evolutionary change driven by higher densities in the other three experimental populations (Reznick et al., 2019). Barriers to dispersal which bound the introduced populations limit the potential for evolution due to gene flow, and our analysis of estimated breeding values indicate that the observed change was greater than that expected due to genetic drift. As such, our results strongly suggest that the evolution of size at maturity is an adaptive response to resource limitation. It is possible that some component of the change seen in the common garden experiments is due to a genotype-by-environment interaction: differences in environmental conditions between the lab and field may exaggerate or minimize genetic differences between ancestral and introduced guppies. However, given the absence of any interaction between food level and population in the common garden experiments, in any of the years of study, the simplest explanation is of an increase in mean breeding value for size at maturity, occurring between years 2 and 3 of the experiment (as in Fig. 3B).

Systematic changes in resource availability generating subgroup effects are likely widespread, and may emerge from concurrent ecological and evolutionary dynamics. However, our experimental introduction replicated a colonisation event, and our focal population of guppies was not at equilibrium. In these first three years, we captured the beginning of the transition from one evolutionary optimum to another i.e. from the high-predation ecotype to the low-predation ecotype. Population density increased almost 30-fold in less than three years (Fig. 1B). Such large changes in population size are atypical of wild pedigreed populations. As such, the temporal trends we saw may have been more pronounced than in other systems. However, where systematic environmental change occurs, for example in studies of evolution in response to climate change, we argue that accounting for subgroup effects will improve predictions of evolutionary trajectories.

One problem with our study system is that it is not feasible to perform mark-recapture on juvenile guppies smaller than 14mm in length. This means that we have no record of individuals that died before we had an opportunity to sample

them. The survival rate of juvenile guppies to a size of 14mm in natural low-predation type streams is estimated at ∼20% (Reznick et al., 1996), meaning that we have a large invisible fraction (Grafen, 1988). This is further compounded by missing data from individuals that were initially sampled, but which died before attaining maturity. This is problematic, because individuals that did not survive to maturity are not included in the analysis (Hadfield, 2008). If, for example, viability selection favours larger juvenile size, and juvenile size is (positively) genetically correlated with size at maturity, then we will have underestimated the strength of (indirect) selection for size at maturity. This problem is offset to some extent because we also likely underestimated the change in size at maturity in the common garden experiments, relative to that which would occur in the field. In the common garden experiment, there was no invisible fraction lost between sampling (at ∼12 mm) and maturity. This means that smaller individuals that might have been lost to viability selection in the wild survived to maturity (at presumably smaller sizes) in the common garden experiment.

Estimation of genetic (co)variances is notoriously data-hungry, and subsequently there was considerable uncertainty around some of our predictions. In particular, predictions based on the secondary theorem of selection had very wide credible intervals spanning zero. Can we then justify saying that predictions improved when environmental effects were accounted for? We would argue that the accuracy of predictions improved, because the difference between predictions and observations decreased when environmental effects were accounted for. Our predictions, as is typical of such studies, lacked precision. However, by accounting for subgroup effects, predictions based on the secondary theorem of selection and the change in estimated breeding values more closely matched observations in the common garden, and less closely matched the phenotypic trend in the field data.

Implications for predicting evolution in changing environments

Unaccounted environmental effects are known to bias quantitative genetic predictions of evolution (Lande and Arnold, 1983; Rausher, 1992; Lynch and Walsh, 1998;

Postma, 2006). We were able to test for bias caused by the changing environment by comparing sets of different predictions for a wild population to observations in a common garden experiment, where environmental effects were controlled. How should researchers tackle the problem of environmental change in the absence of common garden data? Our results show that when relatives experience distinct environmental conditions, these environmental variables should be included as fixed effects in the animal model. This reduces Yule-Simpson effects by accounting for environmentally-caused differences in the expression of traits and fitness prior to estimating breeding values and the additive genetic variance-covariance matrix.

In our study system, we expected selection for the low-predation guppy ecotype to be driven by increased population densities and decreased resource availability (Bassar et al., 2013; Reznick et al., 2019). Given the many factors that may influence phenotypes and fitness in wild populations, selecting an appropriate environmental variable to include in quantitative genetic analyses may be challenging. One proposed solution to this problem is to simply account for all annual environmental differences by including year as a fixed effect in the animal model (Postma, 2006; Walsh and Lynch, 2018). However, this confounds environmental change with any true genetic change that occurs within a year, reducing the power to detect evolution (Postma, 2006; Walsh and Lynch, 2018). This is particularly problematic for organisms with short generation times. We suggest that researchers carefully consider which environmental variables might cause dissimilarity in phenotype and fitness among relatives, and to include those factors as covariates when estimating quantitative genetic parameters.

A general implication of our findings is that environmental drivers of selection can inhibit our ability to detect the evolutionary change that they cause. Not accounting for environmental drivers of selection in quantitative genetic models may result in false negative results, i.e. the failure to detect actual evolutionary change, as in this study. This is most likely when environmentally-induced phenotypic plasticity and selection act in opposing directions. Because evolutionary change is fastest when it is cryptic (Coulson et al., 2019), failure to detect instances of cryptic evolution

will result in underestimation of mean rates of evolution in wild populations. Not accounting for changing environmental influences on traits and fitness reduces the ability of quantitative genetic models to accurately detect evolutionary change.

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2.5 Supplementary information

Pedigree construction and lifetime reproductive success

Guppies in the experimental population were genotyped across 43 highly variable microsatellite loci (mean number of alleles per locus in founders=12.25), and parentage was assigned using *COLONY v2* (Jones and Wang, 2010). Individuals were excluded from the pedigree if they were successfully genotyped at fewer than 20 loci (8.4% of all genotypes), and parentage was unassigned if certainty was less than 95% (<1% of all genotypes). Lifetime reproductive success was determined as the number of offspring of each individual in the pedigree. Our measurement of lifetime reproductive success excludes juvenile offspring that died before reaching a standard length of approximately 14mm, because this is the size at which new recruits are first included in a census. Although we have full parentage information for the first 60 months of the introduction, the lifetime reproductive success of individuals born towards the end of that period cannot be determined, as any offspring of theirs born after the 60th month of the experiment are not included in the pedigree. We determined that all newly recruited guppies in months 58-60 were sired by males born after the first 36 months of the experiment (N=247). Therefore, we assume that measurements of lifetime reproductive success are reliable for males born in the first 36 months of the introduction, and so we restricted our analyses to this period.

Estimating generation time

To compare predictions of change with observations of change in the field and the common garden experiments, we multiplied the per-generation predictions from the Breeders equation and the secondary theorem of selection by the mean number of generations elapsed after 3 years. Generation time is the average age of parents at birth of offspring. Guppies are typically 6 weeks old at first capture. Because this applies to all guppies, we assume the interval between first capture of parent and first capture of an offspring is a reasonable measure of the age of parents at the birth of that offspring. With this assumption, we began the estimation of generation time by subtracting the date of first capture of each individual from

that of the first capture of both parents. For each parent, we calculated the mean age at offspring birth as the average of this interval for all of its offspring. We then took the average of these values for all parents. Mean generation time was 7.90 months (N=1457, SD=3.15), which is longer than previous estimates in a different population (6.9 months) (Reznick et al., 1997). This may be due to different methodology in calculating generation time, or may reflect biological or recruitment differences between populations. For models based on the full three-year dataset, we multiplied the posterior distribution of the response by the point estimate of 1.52 generations per year.

The relationship between size at maturity and fitness

In our manuscript, we are concerned with the relationship between size at maturity and fitness. Our measure of fitness is lifetime reproductive success. For our formal

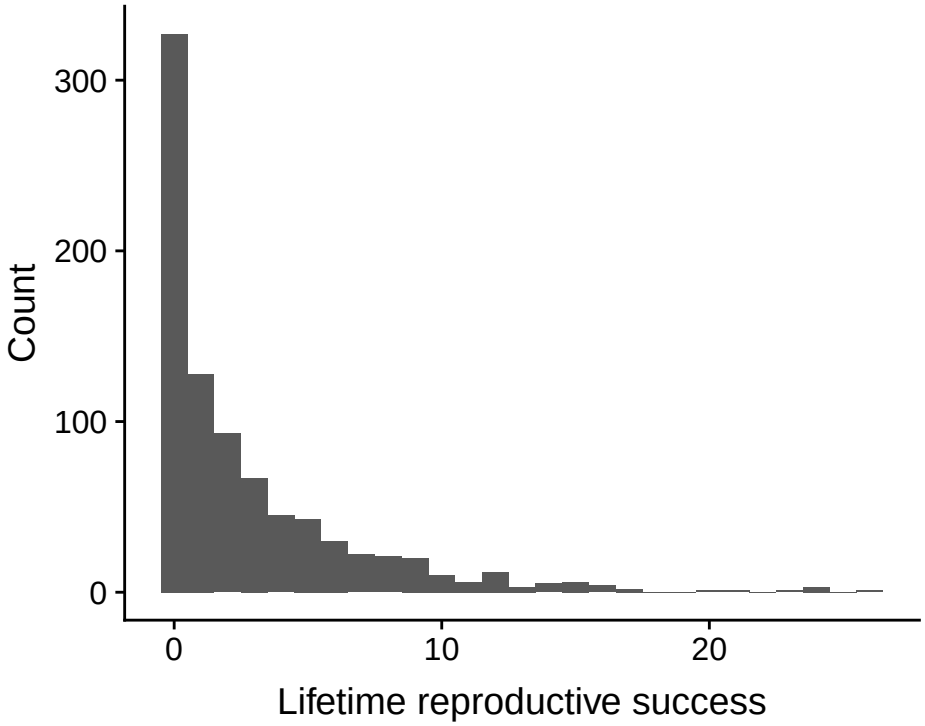

Figure S1: Histogram of lifetime reproductive success (LRS). In our study population, most males (62%) had at least one offspring in their lifetime. The Poisson distribution of LRS was over-dispersed (i.e. variance > mean), however *MCMCglmm* accounts for overdisperssion by default for Poisson distributed data

analyses, we calculated the phenotypic selection gradient as $\beta_P = cov_P(z, w)/V_P(z)$. By fitting a bivariate model of size at maturity z and lifetime reproductive success w, we directly estimated the variance-covariance matrix of z and w, allowing estimation of β_P. Because we used a log-link function to transform absolute lifetime reproductive success (count data), our estimates on the latent scale are equivalent to considering relative lifetime reproductive success. An alternative method to quantify β_P is to fit a linear univariate model of fitness, where $w = \beta_0 + \beta_P \cdot z$. In this case, the intercept β_0 is also estimated. The advantage of the latter approach is that, because the intercept is estimated, it is relatively straightforward to plot the regression line of β_P to the data on z. As such, we also fit a univariate linear model of fitness to illustrate the relationship between z and w in our dataset, using a log-link function that accounts for overdispersed Poisson data in *MCMCglmm* (Hadfield 2010), with default settings. Using this approach, our estimate of the phenotypic selection gradient was 0.18 mm-1 (0.07, 0.31). This is slightly higher than the estimate from the bivariate animal model (0.16 mm-1), likely because the non-independence of trait and fitness values between related individuals is accounted for in the bivariate animal model, but not in the univariate model of fitness.

Fitting animal models

In our analyses, we fit two bivariate animal models using the field phenotypic data on size at maturity (z) and lifetime reproductive success (fitness w): the first included only an intercept for z and w, whilst the second also included subgroup effects (population density and season, recorded in the month in which maturity was attained) and their interaction as fixed effects. Data and code under-lying the analyses and figures in this manuscript are deposited in the Dryad Digital Repository:https://doi.org/10.5061/dryad.jm63xsj7k

To speed up processing time and to better assess convergence, we used the mclapply() function of the parallel package (R Core Team, 2018) to run 10 MCMCs in parallel on separate processing cores, and then combined these chains. Each chain began with different starting values, generating unique samples of the posterior

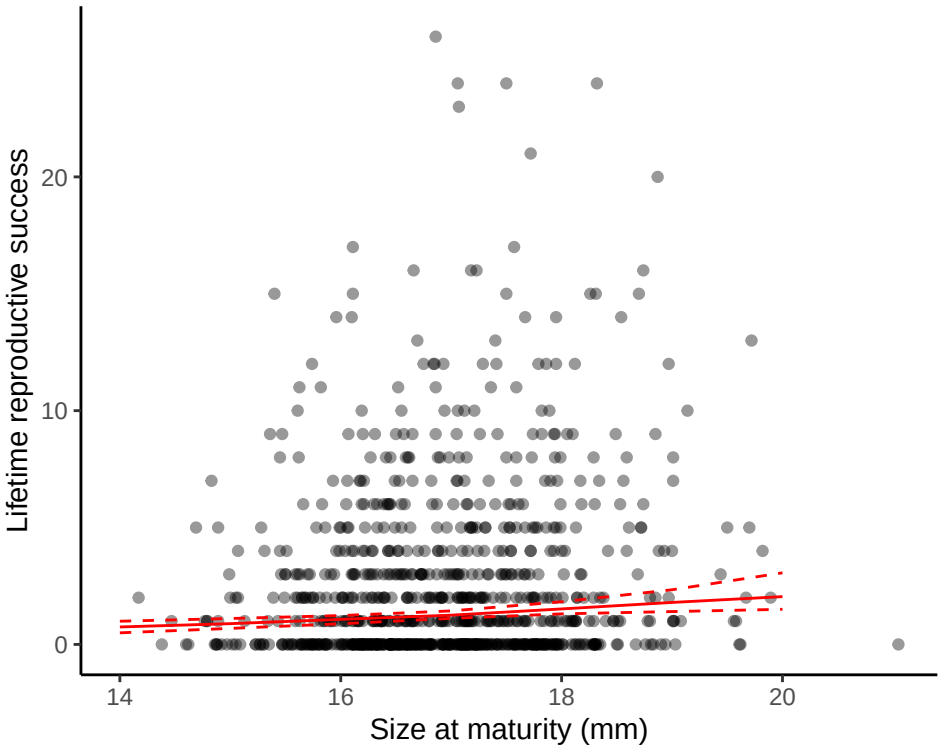

Figure S2: The relationship between size at maturity and absolute lifetime reproductive success. Note that the regression was fit with a Poisson distribution, meaning that the line is curved on the observed scale (absolute lifetime reproductive success, shown in plot), but is linear on the latent scale, where the regression coefficient estimates the phenotypic selection gradient β_P in terms of relative fitness.

probability distribution. After an initial burn in of 40,000 iterations, each chain ran for 400,000 iterations, with samples stored at a thinning interval of 500. Combining chains gave 8000 samples of the posterior distribution. We checked that parallel-run MCMCs had converged from plots of the traces of variances. Autocorrelation on variance components was less than 0.1 in the first lag period, and negligible thereafter. We used the default *MCMCglmm* Gaussian priors for fixed effects, and inverse-Wishart priors with nu=1.002 for multivariate random effects. Because Bayesian analyses can be sensitive to the choice of priors used, we also ran models with parameter-expanded priors, which are expected to improve the mixing properties of the chain and reduce autocorrelation in some data-sets (Gelman, 2006). The choice of priors did not influence the parameter estimates, however we found that parameter-expanded priors resulted in bimodal posteriors with a shoulder at zero

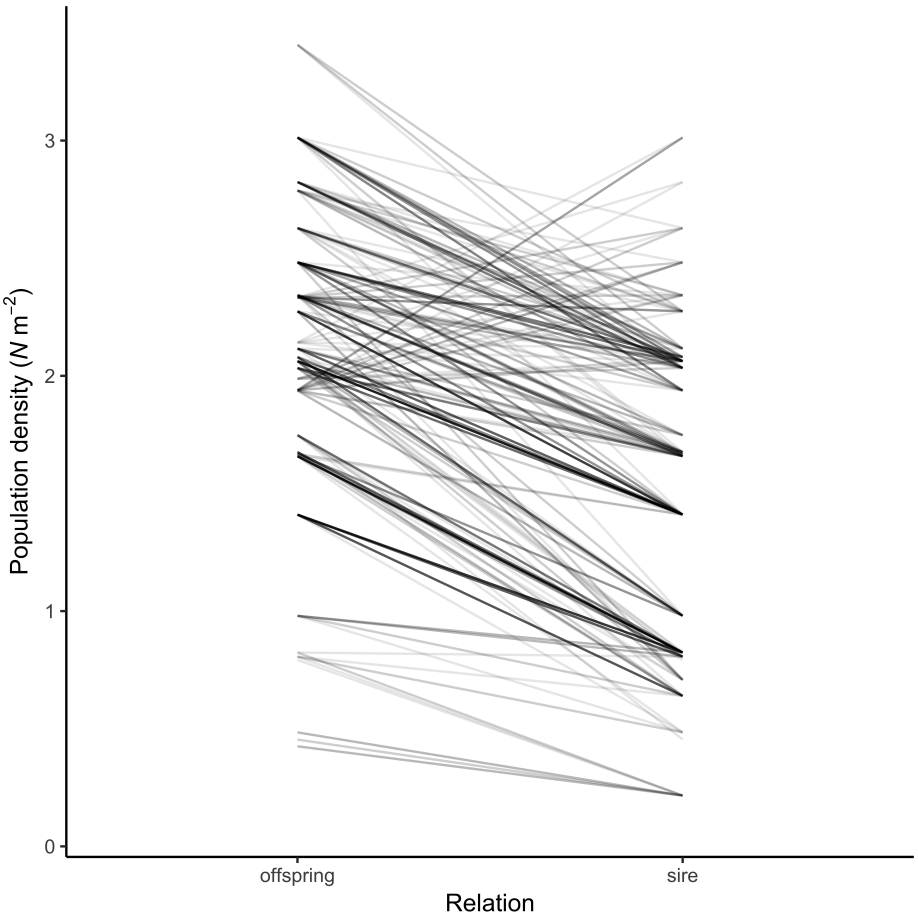

Figure S3: Data showing differences between sires and offspring in population density experienced prior to maturity. Lines connect individual sire-offspring pairs.

for variance in size at maturity due to maternal effects. We report on models fit using the inverse-Wishart priors for random effects here.

Assessing phenotypic change in the common garden and field data

Models assessing phenotypic divergence between ancestral and derived populations (using the common garden data) and the phenotypic trend over time (using the field data) were fit using *MCMCglmm*. For the common garden data, we analyzed each year separately, including population (ancestral or derived) as a fixed effect. We accounted for relatedness among individuals within populations by fitting full-sibling family identity as a random effect. For the phenotypic trend in the field data, we fit a piecewise linear regression of size at maturity against time (months since introduction), allowing different slopes in each year of the study. For both types

of model, we used a burn-in period of 40,000 iterations, and then ran chains for 800,000 iterations, with samples stored at a thinning interval of 100, yielding 8000 samples of the posterior. Autocorrelation on fixed effect and variance components was less than 0.1 in the first lag period, and negligible thereafter. We used the default *MCMCglmm* Gaussian priors for fixed effects, and parameter expanded priors for random effects. We confirmed that MCMCs had converged from plots of the traces of fixed effects and variances.

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3

An ecological explanation for hyperallometric scaling of reproduction

Abstract

In wild populations, large individuals have disproportionately higher reproductive output than smaller individuals. We suggest an ecological explanation for this observation: asymmetry within populations in rates of resource assimilation, where greater assimilation causes both increased reproduction and body size. We assessed how the relationship between size and reproduction differs between wild and lab-reared Trinidadian guppies. We show that (i) reproduction increased disproportionately with body size in the wild but not in the lab, where effects of resource competition were eliminated; (ii) in the wild, the scaling exponent was greatest during the wet season, when resource competition is strongest; and (iii) detection of hyperallometric scaling of reproduction is inevitable if individual differences in assimilation are ignored. We propose that variation among individuals in assimilation – caused by size-dependent resource competition, niche expansion, and chance – can explain patterns of hyperallometric scaling of reproduction in natural populations.

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3.1 Introduction

Across animal taxa, larger individuals reproduce more than smaller individuals of the same species (Honěk, 1993; Visman et al., 1996; Kingsolver and Huey, 2008; Lim et al., 2014; Ronget et al., 2018). While many theoretical models assume that reproduction is proportionate to body size (e.g. Roff, 1983; West et al., 2001; Lester et al., 2004; Kooijman, 2010), recent work in marine fishes has shown that larger individuals have disproportionately higher reproductive output: of 342 species, 95% display hyperallometric scaling of reproduction (Barneche et al., 2018). Reproductive hyperallometry – where large individuals produce more offspring per unit body weight than small individuals – appears to be the norm for free-living marine fishes, and has been reported in many other taxa (e.g. freshwater fish (King, 1998), crustaceans and marine invertebrates (Thompson, 1979), true bugs (Valle et al., 1987), turtles (Mueller et al., 1998), and frogs (Gibbons and McCarthy, 1986); reviewed in Marshall & White (2019)). These observations, seemingly at odds with theoretical predictions, beg the question: what process – absent from theoretical models that assume proportionate scaling of reproduction – generates these patterns?

One way to address this question is to consider a simple bioenergetic model of three processes central to any organism: assimilation of energy from the environment, and use of energy for either growth and somatic maintenance, or for reproduction (Fig. 1). Here we describe two versions of this model which both result in hyperallometry of reproduction.

In one version, proposed by Marshall and White (2019), allocation of energy to growth and reproduction is assumed to be size-dependent: individuals allocate an increasing fraction of energy towards reproduction as they grow (Fig. 1A). This increasing investment in reproduction can explain why larger individuals produce disproportionately more or larger offspring. Because a change in energetic allocation would presumably be under endocrinal control, we term this the physiological model.

An alternative version assumes that the fraction of energy allocated to reproduction is fixed within an individual, but rates of assimilation vary among individuals (Fig. 1B). Individuals with higher rates of assimilation attain both larger sizes and higher reproductive output than those with lower assimilation rates. This is essentially the model proposed by van Noordwijk and de Jong (1986) to explain why the detection of life-history trade-offs often fails at the level of individuals within populations: positive correlations between life-history traits will occur when variance in resource assimilation affects both traits. Following this model, we argue that variation among individuals in assimilation rates will result in reproductive hyperallometry at the level of the population. We term this the ecological model, because we propose that variance in assimilation rates – and therefore the degree of reproductive hyperallometry – is likely to be driven by the strength of ecological interactions.

Ecological interactions that will influence assimilation rates include resource competition, niche expansion, and the predation risk while foraging for food. A central assumption of the physiological model – that individuals (of different sizes) experience the same environmental conditions (Marshall and White, 2019) – is unlikely to hold true in natural systems, with respect to a key environmental variable: food availability. In wild populations, individuals are rarely equal in their ability to accrue resources, and such differences can drive divergence in realised life histories, generate population dynamics, and determine the strength of ecological interactions (Bassar et al., 2016; Coulson, 2020; Griffiths et al., 2020; Laskowski et al., 2021).

Evidence for intraspecific competition for resources is widespread among fishes, and larger body size is typically associated with stronger competitive ability (Ward

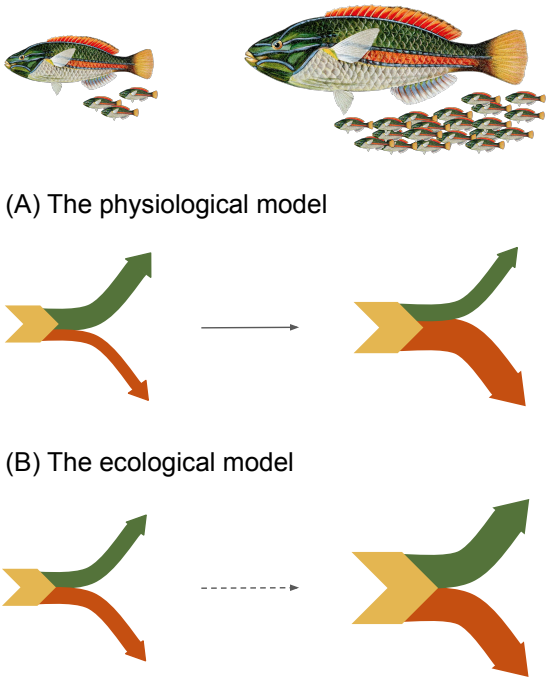

Figure 1: Two versions of a simple bioenergetic model of an organism which both predict hyperallometric scaling of reproduction. The organism is characterised by three energetic fluxes: assimilation of energy into the organism from the environment (yellow arrow), use of energy for growth and somatic maintenance (green arrow), and use of energy for reproduction (red arrow). The magnitude of each flux is depicted by the width of the arrows. In the physiological model (A) allocation to reproduction increases as the organism grows (growth of individual depicted by the solid black arrow). In the ecological model (B) the fraction of energy allocated to reproduction does not change with size (although this fraction will vary among individuals), but individuals with high rates of assimilation have more energy to allocate to both growth and reproduction than individuals with low rates of assimilation. Assimilation rates may increase as individuals grow (dotted arrow), but this is not a prerequisite for hyperallometric scaling of reproduction: variation in assimilation rates among individuals is sufficient for hyperallometric scaling of reproduction at the level of the population. The fish illustration (*Stetholojulis albovittata*) is in the public domain, accessed through the Biodiversity Heritage Library.

et al., 2006, and references therein). Individuals may become large because their local habitat is rich in food, or because they have certain behavioural or metabolic traits that increase their rates of energy assimilation in a given environment (McCarthy et al., 1992, 1994; Ward et al., 2004; Toscano et al., 2016; Auer et al., 2020). Furthermore, once an individual becomes large, it is likely to experience further size-dependent competitive advantages over smaller individuals, under scenarios of both exploitative and interference competition (Mittelbach, 1981; Post et al., 1999;

Ward and Krause, 2001; Potter et al., 2019; Griffiths et al., 2020). In addition, most fish are gape-limited feeders, which has two main consequences for size-dependent resource availability: larger fish can consume a broader range of food items (i.e. size-dependent niche expansion, Scharf et al., 2000), and they are at lower risk of predation, meaning that they can forage in areas too risky for smaller individuals (Werner and Hall, 1988; Byström et al., 2004; Magnhagen and Borcherding, 2008). As such, big fish inhabit a very different environment – in terms of food availability, past and present – to that experienced by small fish within the same population.

In wild populations, disentangling the causes and effects of resource competition on size and reproduction is extremely challenging. Observational data on size and fecundity alone cannot, for example, directly distinguish (i) whether larger individuals allocate a greater fraction of available energy to reproduction, or (ii) whether they are larger and have greater reproductive output because they have consumed more food than smaller individuals. By contrast, in the lab, the confounding effects of resource competition and niche expansion can be eliminated, allowing direct assessment of these questions.

Here, we assess factors that determine how reproductive output scales with body size in the Trinidadian guppy (*Poecilia reticulata*). Guppies are small, live-bearing, freshwater fish common throughout streams and rivers in Trinidad and Venezuela (Magurran, 2005). Resource competition is asymmetric in guppies, with larger fish being stronger competitors (Bassar et al., 2016; Potter et al., 2019; Griffiths et al., 2020). We compared reproductive scaling exponents from observations of wild populations with those of experimental populations in the absence of the effects of resource competition and niche expansion. Specifically, we assessed how reproduction scaled with female body size (i) for different measures of reproductive output both in the wild and lab, (ii) for wild fish sampled either in the dry or (resource-poor) wet season, and (iii) for lab-reared fish fed either a high-quantity or low-quantity diet. During the wet season in Trinidad, increased water flow and flooding scours the streams of the invertebrates, algae, and detritus that guppies feed on, leading to more intense competition for resources (Kohler et al., 2012; Auer

et al., 2012; Zandonà et al., 2015). In the laboratory, fish were housed individually and fed with quantified rations of food equally accessible to all size classes of fish, and so any effects of food level are independent of competitive ability. If larger guppies have disproportionately higher reproduction in both seasons (in the wild) and both food treatments (in the lab), this would support the physiological model, i.e. increasing allocation to reproduction with increasing body size. By contrast, if scaling is hyperallometric in the wild, but not in the lab (in the absence of effects of competition), this would support an ecological explanation for hyperallometric scaling of reproductive output in natural populations.

3.2 Methods

Data

The wild data that we used are from an observational study on variation in life histories among natural populations of guppies (Reznick, 1989), supplemented with additional, previously unpublished data. For full details on methodology, we refer the reader to the original study. Briefly, wild guppies were collected from 19 populations in the Northern Range mountains of Trinidad between the years 1981 and 1987 (the previously unpublished data were collected in 1986 and 1987). Sampling took place in both the dry season (in years 1981, 1984-1987) and wet season (1981-1983, 1985), with most populations sampled in more than 1 year (median = 3 years, range = 1-7 years, Table S1). Female guppies were dissected, and developing embryos counted. The weight of adults was recorded to the nearest mg, and the dry weight and lean dry weight of embryos were recorded to the nearest 0.1 mg. In total, the wild dataset included observations of 2547 individuals, of which 1483 contained developing embryos (Table S1).

The lab data are from an experimental study of rates of senescence in guppies (Reznick et al., 2004, 2005). In summary, guppies were collected from four natural populations and reared for two generations under lab conditions to control for differences in maternal environments. Experimental fish (females) were housed in individual tanks from 30 days of age (mean = 29.6 days, sd = 3.9 days, range

= 20-40 days) and fed twice daily with volumetrically quantified rations of brine shrimp nauplii or liver paste. Guppies were assigned to either a high or low food treatment. In both treatments, ration size gradually increased over the first 180 days, and was held constant thereafter. Juvenile females were paired with a mature male to ensure that they were mated as soon as they attained maturity. At each parturition event, females were weighed to the nearest mg, the number, dry weight and lean dry weight of neonates was recorded (to 0.1 mg), and females were re-mated. We calculated the mean somatic growth rate during pregnancy as the change in mass between parturition events, divided by the length of the interval (days) between parturition events. As an alternative measure of somatic growth, we also calculated the relative growth rate as $\ln(\text{mass}_{t2}/\text{mass}_{t1})/\text{interval}$. Data were collected over the entire reproductive lifespan of each individual (mean age at final parturition = 722 days, sd = 237 days). The common garden dataset included 4629 observations of 211 individual (Table S2).

We calculated three main measures of reproductive output: fecundity (number of offspring per litter), offspring mass (total dry weight of offspring in a litter, mg), and offspring energy (total energy content of litter, J). We calculated offspring energy by multiplying the total litter protein mass (lean dry weight) and fat mass (dry weight minus lean dry weight) by appropriate energy densities (protein = 17 kJ g^{-1}, fat = 37 kJ g^{-1}). Additionally, for the lab data only we were able to convert each of the three measures of reproductive output into reproductive powers (i.e. output per unit time) by dividing the measure of reproductive output by the interval between births (days).

Models

We used mixed-effects models to address five main questions: (1) How do different measures of reproduction scale with body size in the wild and in the lab, and do environmental differences change this relationship? (2) Do large guppies allocate less energy to growth than small guppies? (3) Do estimates of reproductive scaling change if differences among individuals in assimilation rates are ignored? (4)

What factors influence the interval between births in lab-reared guppies? (5) Does probability of pregnancy in wild guppies depend on size and season?

For questions 1-3, we modelled reproduction (R) as an exponential function of size (z), i.e. $R = az^{\beta_1}$. Reproduction is hyperallometric when the scaling exponent $\beta_1 > 1$, isometric (proportionate to body size) when $\beta_1 = 1$, and hypoallometric (smaller individuals have higher reproduction per unit body weight) when $\beta_1 < 1$. We estimated the scaling exponent by fitting linear mixed-effects models where the response was the natural log-transformed measure of reproduction, and the main fixed effect was the natural log-transformed measure of mass. The parameters a and β_1 are given by the intercept and slope, respectively, of these linear models.

To address question 1, we modelled all three measures of reproductive output for lab and wild data, and all three measures of reproductive power for the lab data. To test the relationship between environmental factors and the scaling exponent β_1, we included either season (wild data) or food treatment (lab data) as fixed effects, interacting with ln(mass). We used appropriate random effects structures to account for non-independence of data (Table S3).

For questions 2 and 3, we chose total offspring energy content per litter as our measure of reproduction, because this measure was available in both lab and wild data and is equivalent to the measure of reproduction used by Barneche and colleagues (2018). To assess whether allocation of energy to growth decreases with increasing size in the lab (question 2), we included an interaction between somatic growth rate and ln(mass) in our model of reproduction (Table S3). Under the physiological model, increased reproduction should be associated with decreased growth, and this effect should be strongest at larger body sizes. This would correspond to a negative main effect of growth and a negative interaction term in this linear model. To address question 3, we modelled reproduction of a cohort of guppies in the lab (aged 200-300 days old) as an exponential function of size, both with and without accounting for differences in food treatment (Table S3).

Questions 4 and 5 concern factors influencing components of reproduction other than output: the duration of the interval between successive births in the lab,

and the probability of being pregnant when sampled in the wild, respectively. For question 4, we included ln(mass) and the full interaction between age and food level as fixed effects in our model of the reproductive interval. For question 5, we estimated the probability of a wild-caught individual containing zero developing embryos, as a function of size and season. For this model we fit a logistic regression of the binary response (presence/absence of developing embryos), and included season and the natural log of mass as fixed effects (Table S3).

All analyses were performed using R version 3.5.1 (R Core Team, 2019). We fit our models in a Bayesian framework, using the R package *brms* version 2.14.4 (Bürkner, 2018), which makes use of the probabilistic programming language Stan (Carpenter et al., 2017). In the Gaussian models (Table S3), for the intercept and fixed effect coefficients we specified normal priors with a mean of 0 and standard deviation of 1 (sd = 5 for the intercept). For the standard deviation of random effects and residual variance, we specified cauchy priors with means of 0 and standard deviations of 1. For the correlation of random effects, we specified an LKJ prior with a value of 1, which assumes all correlational structures are equally likely (Bürkner, 2018). For the logistic regression model we used the "get_prior" function in *brms* to obtain appropriate default priors (Bürkner, 2018).

For each model, we ran four Monte Carlo Markov chains, with 2000 samples retained from each chain after a burn-in period of 1000 samples, yielding a combined total of 8000 samples of the posterior distribution. We confirmed that chains had converged by visually checking the trace-plots, and by confirming that scale reduction factors $\hat{R} < 1.01$. Effective samples sizes for all parameters were greater than 10% of the total number of samples.

Figures 2-4 were produced in R using the packages *ggplot2* (Wickham, 2011), *ggridges* (Wilke, 2018) and *patchwork* (Pederson, 2020).

3.3 Results

Parameter values reported in this section are the means of the posterior distribution, with the 95% credible intervals given in brackets. We define reproductive scaling

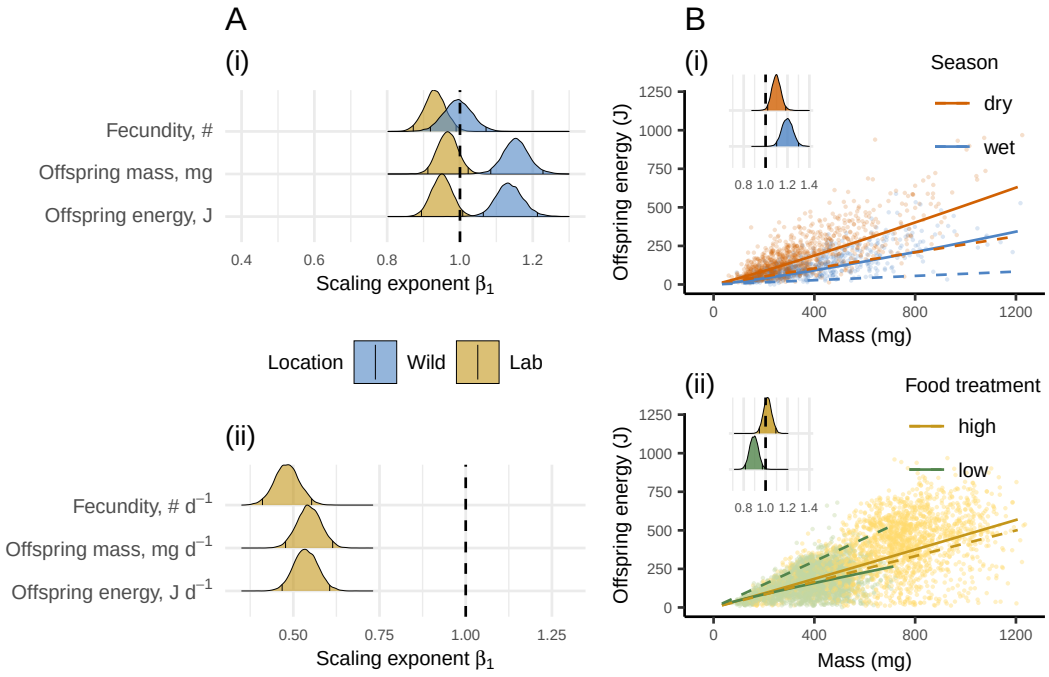

Figure 2: The scaling of reproduction with body size differs between wild and lab-reared populations of Trinidadian guppies, varies by reproductive measure, and is dependent on environmental conditions. (A) shows the posterior distributions of scaling exponents using three measures of reproduction: fecundity, offspring mass, and offspring energy content, expressed (i) per litter, and (ii) per unit time. Vertical black lines denote the 95% credible intervals. Posteriors are given for wild (blue) and lab (yellow) populations. Posteriors are the mean exponent across seasons (wild data) and food treatments (lab data), weighted by the number of observations in each season/food treatment. Measures of reproduction per unit time were not available for wild populations. (B) shows reproductive energy content per litter by body size in (i) wild guppies in the dry season (red) and wet season (blue) (1485 datapoints, one per individual), and (ii) guppies reared in the lab under high (yellow) or low (green) food treatments (4629 datapoints, from 211 individuals). Insets show the posterior distribution of the scaling exponents β_1. Solid lines show predicted reproduction given the estimated scaling exponents. Dashed lines show predicted reproduction assuming isometric scaling of reproduction, i.e. $\beta_1 = 1$.

as hyperallometric when the lower bound of the credible interval exceeds 1, as isometric when the interval contains 1, and as hypoallometric when the upper bound is less than 1.

Environmental effects on the scaling of reproduction with body size

In the wild, mean reproductive output across both seasons was hyperallometric when considering offspring mass ($\beta_{1_M} = 1.15$ [1.08; 1.23]) and offspring energy ($\beta_{1_E} = 1.14$

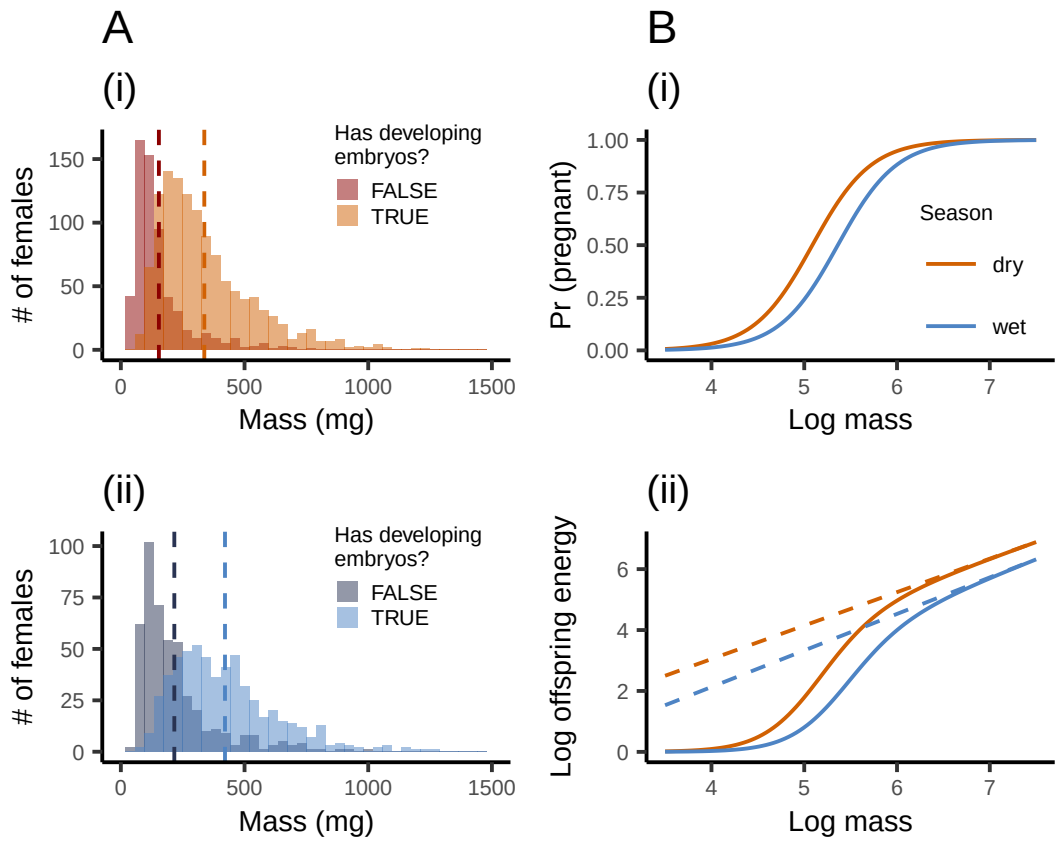

Figure 3: In wild populations, the probability of an individual being pregnant depended on size and season. (A) Histograms showing the size distribution of sampled fish that contained no developing embryos (dark) and those that contained at least one developing embryo (light) in (i) the dry season and (ii) the wet season. Dashed lines indicate the means of the distributions. (B.i) Probability of a wild fish containing developing embryos as a function of size (natural log of mass) in the dry season (red) and the wet season (blue). (B.ii) Log reproduction as a function of log size, when incorporating the probability of pregnancy by size (solid lines) in the dry season (red) and the wet season (blue). Dashed lines show the relationship between ln(size) and ln(reproduction) given that an individual is pregnant. The solid lines were calculated by multiplying the pregnancy probability function (B.i) by the season-specific log-linear function (dashed lines).

[1.06; 1.21]), but isometric in terms of fecundity (β_{1_F} = 0.99 [0.92; 1.07]) (Fig. 2Ai).

In the lab, mean reproduction across food treatments scaled hypoallometrically for fecundity (β_{1_F} = 0.93 [0.87; 0.99]), isometrically for offspring mass and energy (β_{1_M} = 0.97 [0.91; 1.02]; β_{1_E} = 0.95 [0.89; 1.01]; Fig. 2A.i), and hypoallometrically for all measures of reproductive power ($\beta_{1_{FP}}$ = 0.48 [0.41; 0.55]; $\beta_{1_{MP}}$ = 0.54 [0.48; 0.61]; $\beta_{1_{EP}}$ = 0.54 [0.47; 0.60]; Fig. 2A.ii). This reduction in how size scales with

reproductive power relative to reproductive output corresponded to a linear increase in the interval between births with age: in both food treatments, the interval was ∼40% longer in the oldest fish relative to the youngest (intercept = 25.4 d [23.8; 27.0], β_{age} =0.010 [0.008; 0.013]). There was no clear effect of the natural log of mass, food treatment, or the interaction between age and food on the duration of the reproductive interval ($\beta_{ln(mass)}$ = -0.13 [-1.14; 0.91], β_{food_low} = 0.75 [-0.39; 1.88] , $\beta_{ln(mass):food_low}$ = -0.001 [-0.004; 0.001]).

For the wild populations, reproduction increased disproportionately to body size in both seasons, but the magnitude of hyperallometry was greater in the wet season (offspring energy J, $\beta_{1_{dry}}$ = 1.10 [1.02; 1.18]; $\beta_{1_{wet}}$ = 1.20 [1.10; 1.30]; difference = 0.10 [0.00; 0.20], Fig. 2B.i). In the lab, reproduction scaled isometrically under the high food treatment, but hypoallometrically under the low food treatment (offspring energy J, $\beta_{1_{high}}$ = 1.02 [0.94; 1.09]; $\beta_{1_{low}}$ = 0.89 [0.82; 0.97], Fig. 2B.ii).

Correcting for likelihood of pregnancy in the wild

In the wild, pregnant females were larger on average than females without developing embryos: 337 mg [328;347] vs. 154 mg [141;167] in the dry season, and 420 mg [406; 436] vs. 216 mg [199;233] in the wet season (Fig. 3A). The probability that a wild fish contained developing embryos increased with size, and this effect was stronger in the wet season (logistic regression, intercept = -16.07 [-18.17; -14.12], β_{ln_size} = 3.16 [2.86; 3.50], β_{wet}= -0.87 [-1.20; -0.53], Fig. 3B.i). This meant that for a 200 mg guppy, there was a 45% chance of being pregnant in the wet season, but a 66% chance in the dry season. Accounting for size- and season-dependent pregnancy changed the predicted relationship between size and reproduction in the wild: large fish had an even greater relative advantage over small fish in terms of reproduction (Fig. 3B.ii). Accounting for the probability of being pregnant, the mean reproductive output (total energy content per litter) of a 200 mg fish was 19.2 J in the dry season, but only 5.3 J in the wet season; for a 1000 mg fish, mean reproductive output in the dry season was 503.9 J, and 263.9 J in the wet season. Per unit body weight,

the reproductive output of a 1000 mg guppy was 5.2 times higher than that of a 200 mg guppy in the dry season, and 10.0 times higher during the wet season.

Potential mechanisms underlying reproductive hyperallometry

In the lab, we found no evidence that larger fish allocate relatively more energy to reproduction over growth: when we included somatic growth rate interacting with size in our model, the main effect of growth was negative (β_G = -0.63 [-0.84; -0.44]), but the interaction with body size was positive ($\beta_{G:z}$ = 0.10 [0.07; 0.13]), meaning that growth rate and reproduction co-varied negatively in small individuals, but positively in large individuals. The same pattern occurred when we included relative growth rate in the model (instead of somatic growth rate): a negative main effect (β_{RGR} = -345.38 [-407.60; -285.55]), but a positive interaction term ($\beta_{RGR:z}$ = 57.88 [47.22; 68.32]).

Guppies reared under the high food treatment grew to larger sizes and had higher reproductive output than those in the same age cohort in the low food treatment (Fig. 4). Although reproduction scaled isometrically at high food (β_{1high} = 0.71 [0.36; 1.05]) and hypoallometrically at low food (β_{1low} = 0.62 [0.25; 0.98]), reproduction was hyperallometric when calculated across both food treatments ($\beta_{1high/low}$ = 1.30 [1.17; 1.43], Fig. 4).

3.4 Discussion

What mechanism can explain the patterns of reproductive hyperallometry that are so widespread among wild populations? We propose that such patterns emerge from differences among individuals in their history of energy assimilation (i.e. the ecological model, Fig. 1B), rather than as a consequence of an ontogenetic shift in allocation of resources within individuals (i.e. the physiological model, Fig. 1A (Marshall and White, 2019)). Our argument is supported by three main findings. First, in wild populations the strength of reproductive hyperallometry was environmentally dependent: large guppies had an even greater reproductive advantage over small guppies during the wet season, when resource competition

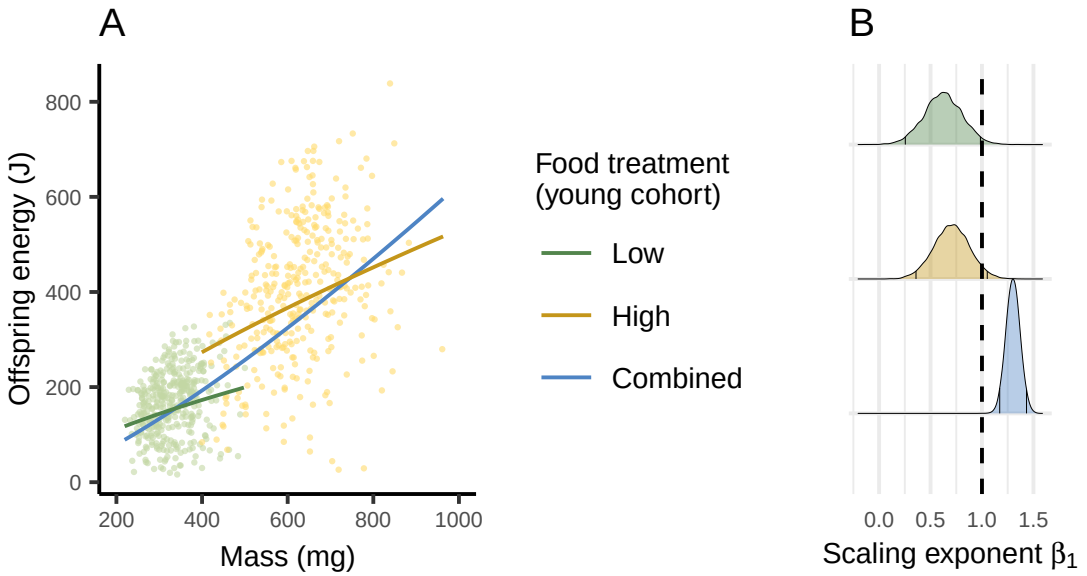

Figure 4: Hyperallometric scaling of reproduction was detected when differences in food availability within a cohort were ignored. (A) The relationship between reproduction (offspring energy content per litter, J) and body size (mass, mg) for a cohort of lab-reared guppies between the ages of 200 and 300 days old (total N = 729), reared under experimental treatments of either low (green) or high (yellow) levels of food availability. Lines show predicted reproduction when differences in food treatment are explicitly modelled (green and yellow) and when they are not (blue). (B) The reproductive scaling exponent β_1 was greater than 1 when data from both food treatments were combined (i.e. reproduction appeared hyperallometric), but mean values of the exponents were less than 1 when differences in food availability were included in the model.

was strongest (Fig. 2B.i, Fig 3). Second, in the lab, in the absence of the effects of resource competition and niche shifts, we found no evidence that large individuals allocated a greater fraction of available energy to reproduction: reproduction was either proportionate or less-than proportionate to body size (Fig. 2A, Fig 2B.ii). Third, we demonstrate that detection of reproductive hyperallometry is inevitable if differences in energetic assimilation are not taken into account (Fig. 4). We expand on these points in the following sections.

Environmental conditions affect how reproduction scales with body size

Our results add to the list of examples of reproductive hyperallometry in wild populations (Barneche et al., 2018; Marshall and White, 2019). Differences in reproductive output per unit body mass in wild guppies were driven by larger

individuals producing disproportionately larger offspring, rather than through disproportionately higher fecundity (Fig. 2A.i). We show that the strength of hyperallometry was dependent on environmental conditions: big guppies had an even greater reproductive advantage over small fish during the wet season (Fig. 2B.i). Increased levels of competition during the wet season lead to declines in recruitment and populations size (Reznick et al., 2019). When individuals are not equal competitors – which is the case in natural populations of guppies (Griffiths et al., 2020) – an increase in the intensity of resource competition will by definition result in increased variance in assimilation rates. Under the ecological model (Fig. 1B), increased variance in assimilation rates explains the increased value of the scaling coefficient β_1 in wild guppies during the wet season.

Not only did reproductive output decrease in pregnant guppies during the wet season, but the size-dependent probability of pregnancy also changed: when we accounted for the probability of wild fish being pregnant, the effects on reproduction of size and season increased drastically (Fig. 3B.ii). Although this measure of hyperallometry is not directly comparable with standard values of β_1 (our reproduction function was non-linear on the log scale), across a typical range of adult sizes, the pregnancy-corrected estimates corresponded to values of β_1 of $\sim$5 and $\sim$10: the reproductive output of large (1000 mg) guppies per unit body weight was five times that of a small (200 mg) guppy in the dry season, but ten times greater in the wet season. This degree of reproductive hyperallometry far exceeds those reported in other species (Barneche et al., 2018; Marshall and White, 2019), and highlights the strong effect of seasonality on the relationship between size and reproduction in wild guppies.

No evidence for increased allocation to reproduction with increased body size

The physiological model could explain the increased value of β_1 in the wet season if large individuals allocate even more energy to reproduction under conditions of resource limitation. Given that per-capita food availability is reduced in the wet season (Kohler et al., 2012; Auer et al., 2012), large fish may respond by allocating

an even greater fraction of assimilated energy towards reproductive efforts. Our results in the lab demonstrate that this is not the case: under conditions of low food reproductive output scaled hypoallometrically, i.e. smaller fish had higher reproductive output per unit body weight (Fig. 2B.ii). At high food, reproduction was proportionate to body size in lab-reared guppies (Fig. 2B.ii). However, when we considered measures of reproductive power (i.e. reproductive output per unit time), reproductive scaling was strongly hypoallometric in the lab (Fig. 2A.ii). This suggests that guppies became increasingly food-limited as they grew, and compensated for the increasing costs of somatic maintenance that come with larger body size by diverting energy away from reproduction. As such, this implies that energetic allocation is not fixed (as assumed in the ecological model), but here the direction of change was opposite to that predicted by the physiological model.

We detected a trade-off between somatic growth and reproduction in small fish, but these traits were positively correlated in large fish. Under the physiological model, we would expect the trade-off to be stronger in larger individuals, as they are assumed to allocate more energy to reproduction over growth. Instead, small fish that grew slowly had higher reproduction, but big fish that grew faster also had higher reproduction. This positive correlation in large fish was likely due to individual differences in assimilation rates (even within food treatments) due to differences that we could not control in the lab, e.g. individual variation in digestive efficiency. Because variation in digestive efficiency would have a cumulative effect on body size, size differences become more pronounced with time, such that the underlying trade-off between growth and reproduction becomes increasingly obscured by variation in assimilation rates (e.g. as in van Noordwijk and de Jong, 1986).

The expression of life-history traits in guppies is often highly dependent on food availability (Felmy et al., 2021). As such, it was unsurprising that guppies in the high food treatment grew to larger sizes and had higher reproductive outputs than those of the same in age in the low food treatment (Fig. 4A). Within food treatments, mean values of the reproductive scaling exponent β_1 were less than 1 (Fig. 4). However, when estimated across both treatments, β_1 was greater than

1 (Fig. 4). This illustrates how reproductive hyperallometry can be detected at the level of the population if differences among individuals in assimilation rates are not taken into account.

Causality and competition

The change from $\beta_1<1$ to $\beta_1>1$ shown in Figure 4, and more generally the explanation for positive correlations among life-history traits (van Noordwijk and de Jong, 1986), exemplify the Yule-Simpson effect: a change in a covariance conditional on a third factor (Yule, 1903; Simpson, 1951). Yule-Simpson effects are common in regression analyses, occurring when covariances are estimated over aggregated data structured by a third variable (Blyth, 1972; Pearl, 2014). In such cases, the covariance captures a non-causal association between the two variables of interest, which is in fact conditional on a third variable. Here, the variables of interest were size and reproduction, and the third variable was resource assimilation. The physiological model (Fig. 1A) assumes a causal effect of size on reproduction: bigger individuals allocate more energy to reproduction. By contrast, the ecological model (Fig. 1B) assumes a causal effect of assimilation on both growth and reproduction: individuals that assimilate more energy grow larger *and* produce more offspring.

In wild populations, the causal relationship between size and assimilation rates is more nuanced than that: large size may cause increased rates of assimilation (e.g. through increased competitive ability and niche shifts, as described in the Introduction), resulting in a positive feedback loop between assimilation rates and body size. In this case, size has an indirect causal relationship on reproduction, through size-dependent increases in assimilation. Furthermore, competition for finite resources is a zero-sum game: the assimilation rate of a focal individual (and subsequently its growth and reproduction) depends on the assimilation rates of its competitors. Here, size is key: in lab-based competition trials, large guppies suppressed the somatic growth rates of smaller individuals to a greater extent than vice-versa (Potter et al., 2019); in the wild, the growth and survival of individuals depended on their size relative to those of their competitors (Griffiths et al., 2020).

While size-dependent competitive ability will act to enhance the effect of reproductive hyperallometry, it is not a necessary condition. Differences in resource assimilation across a population could result from stochastic processes and environmental heterogeneity, without the need for resource competition among individuals. Nevertheless, given that size-structured competitive interactions are common in fish (Ward et al., 2006), the strength of resource competition is likely to be a key determinant of the magnitude of reproductive hyperallometry in wild populations.

Limitations, generality, and alternative explanations

Our study considered a single species. As such, whether reproductive scaling exponents are consistently higher in the wild than in the lab (i.e. under conditions that limit competition) remains to be tested. However, a recent aquaria based study of the European sea bass (*Dicentrarchus labrax*) reported hypoallometric scaling of reproduction ($\beta_1 = 0.84$) under constant food availability (Sadoul et al., 2020), whereas wild populations of this species show hyperallometric scaling of reproduction ($\beta_1 = 1.34$, Barneche et al., 2018). The same study demonstrated that, under the framework of dynamic energy budget theory (Kooijman, 2010), differences within a population in food availability or ambient temperature can generate patterns of reproductive hyperallometry (Sadoul et al., 2020). These empirical observations and theoretical predictions are consistent with our findings here.

The vast majority (98%) of data used by Barneche and colleagues (2018) were from wild populations. Without any additional information on relevant ecological and environmental conditions, we argue that one cannot infer the cause of reproductive hyperallometry from such data. Interestingly, the most extreme values of hyperallometric scaling reported by Marshall and White (2019) do not come from observations of wild populations: they come from a lab-based study on the effects of experimental "crowding" on the growth and reproduction of leafhoppers (Valle et al., 1987). This scenario – where scaling exponents were measured across multiple groups differing in the intensity of resource competition – is precisely

where the ecological model (and the Yule-Simpson effect) would predict the greatest degree of reproductive hyperallometry.

In our study there were, of course, many differences between the lab-reared and wild populations besides the effects of competition. One potential contribution to the observed difference in scaling exponents was selection bias in the wild-caught samples: while we obtained data from a random sample of individuals over their full lifespan in the lab, large individuals in the wild are rare, and likely represent a non-random subset (in terms of attributes) of the population as a whole. Indeed, in the wild dataset we observed very few large guppies (>800 mg) with low reproduction (<150 J per litter), compared to in the lab (48% of large lab-reared individuals (N = 60) had at least 1 litter with low reproductive output, compared to 15% of large wild guppies (N = 66), Fig. 2B). Nevertheless, this difference between lab and wild populations emphasises the role of ecological interactions in determining reproductive hyperallometry in natural populations: it is likely that those lab-reared large guppies with low reproduction (and presumably low assimilation/digestive efficiencies) would not have attained a large size under competitive conditions in the wild.

Conclusions and broader implications

Our results demonstrate that variation in assimilation rates among individuals cause patterns of reproductive hyperallometry at the level of the population. However, we also found evidence that the fraction of energy allocated to reproduction was not fixed: large individuals in the lab allocated less energy to reproduction, presumably to cover increasing somatic maintenance costs while available food levels remained constant. We cannot rule out that the opposite may be true – large individuals with excess energy may allocate more towards reproduction. Nevertheless, differences in assimilation rates ultimately drive differences in both size and reproductive output among members of a population.

What can we infer from observations of how reproduction scales with size in natural populations? The simplest interpretation is that it tells us how resource assimilation rates vary by size. For example, rare cases of hypoallometric scaling

in the wild, as in Arctic cod (*Boreogadus saida*, reported in Barneche et al., 2018), indicate that large fish are more food-deprived than smaller individuals. Given that reproductive hyperallometry is the rule in wild populations of fishes, rather than the exception (Barneche et al., 2018; Marshall and White, 2019), this implies that in most natural populations, small individuals are resource-limited relative to larger conspecifics. Although we cannot infer causality of size on assimilation rates from such observations – differences in assimilation rates may be due to chance – size-dependent asymmetric competition favouring large individuals will increase the strength of reproductive hyperallometry.

In natural populations, it is inevitable that individuals will differ in their rates of resource acquisition and assimilation. Whether through trait-mediated differences or chance, two individuals in a wild population will never have equivalent amounts of assimilated energy with which to fuel their metabolism and contribute to somatic growth, and with which to invest in reproduction. The effects of differences in food availability among individuals are non-trivial: resource-dependent expression of life-history traits often exceeds the effect of evolved differences between locally-adapted populations (Felmy et al., 2021), and trait-dependent differences in assimilation rates can flip predictions about life-history evolution (Laskowski et al., 2021) and generate eco-evolutionary dynamics (Coulson, 2020). Understanding how and why individuals differ in rates of resource assimilation is central to understanding variation in demographic rates that underpin ecological and evolutionary dynamics in wild populations.

Acknowledgements

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Environmental Research Doctoral Training Program, and from a Lamb and Flag scholarship from St Johns College, Oxford.

3.5 Supplementary information

Table S1: Sample sizes for the wild dataset, by population sampling code (19 locations) and year (7 sampling years). Numbers given in square brackets are samples collected during the wet season. Total sample size is 2547 sets of observations, one set per individual. Data collected from 1981 – 1985 are from Reznick (1989). Data collected in 1986 and 1987 have not previously been published.

Population code	Years sampled						
	1981	1982	1983	1984	1985	1986	1987
4	29	[36]		33	32 [34]	38	
5	36 [36]		[38]	29			
8	15 [27]		[25]	43		34	
10	12 [42]		[30]	39	37 [36]		45
15	11 [26]		[35]	25			22
18	17 [28]	[37]	[35]	27	22 [34]	39	34
19	19	[37]	[33]	32	46 [36]	34	34
20		[38]	[26]	31	44 [30]	56	15
22	40 [38]		[42]	29			36
23	9 [35]		[34]	30			
24	38 [32]		[28]	26			
25	14 [36]						
26			[34]	33	48 [36]	31	65
28					29		
29					28		
30					38		
31					26		
32					28		
39						25	30

Table S2: Sample sizes for the lab dataset, by population (4 locations) and experimental food treatment (high or low food). The founders of the lab populations were sampled from either high predation-risk (HP) or low predation-risk (LP) populations, from two distinct river drainage systems (the Oropuche and the Yarra). In total, the lab dataset included 4629 sets of observations of 211 individuals. Data are from Reznick et al (2004; 2005).

Population	Food reatment	Number of individuals	Total observations
Oropuche HP	High	22	620
	Low	26	718
Oropuche LP	High	27	450
	Low	30	599
Yarra HP	High	24	615
	Low	29	726
Yarra LP	High	26	428
	Low	27	473

Table S3: Overview of mixed-effects models used to address five main questions: (1) How do different measures of reproduction scale with body size, and do environmental differences (seasonality in wild populations, food level in the lab) change this relationship? (2) Do large guppies allocate less energy to growth than small guppies? (3) Do estimates of reproductive scaling change if differences among individuals in assimilation rates are ignored? (4) What factors influence the interval between births in lab-reared guppies? (5) Does probability of pregnancy in wild guppies depend on size and season? Models were fit with either the wild or lab datasets. Questions 1-3 modelled the natural log (ln) of reproduction. We had six different measures of reproduction: fecundity (number of offspring per litter), total offspring mass per litter (mg dry weight), total offspring energy content per litter (J), and the power equivalents of these measures (i.e. per unit time). Fixed effects included the intercept (int), the natural log of mass (ln(mass)), season (two levels: dry and wet, field data only), food (two levels: high and low, lab data only), population of origin (pop, four levels, lab data only), mean daily growth rate (gr, continuous, lab data only), and age (continuous, lab data only). Full interaction terms are denoted by an asterisk *. For all models fit with wild data, we allowed the intercept and slope to vary by sampling population (int + ln(mass) | pop), and we also included a random intercept for sampling year (int|year). For lab data, we allowed the intercept and slope to vary by individual ID (int + ln(mass) | id) to account for repeated measures on individuals, and we fit a random intercept to account for shared maternal identity among siblings in the study (int|mat). To answer question 3, we used a subset of the lab data, solely consisting of data on the size and reproduction of fish aged 200-300 days. As there were fewer observations per individual, we did not fit a random slope in these models. Models addressing questions 1-4 all used a Gaussian error structure. To address question 5 we fit a logistic regression with a Bernoulli error distribution. Unlike our other analyses of the wild dataset, the logistic regression included both pregnant and non-pregnant individuals.

Dataset	Question	Response	Fixed effects	Random effects	Error distribution
Wild	1	ln (fecundity)	int + ln (mass) * season	(int + ln (mass) | pop) + (int | year)	Gaussian
"	"	ln (offspring mass)	"	"	"
"	"	ln (offspring energy)	"	"	"
Lab	"	ln (fecundity)	int + ln (mass) * food + pop	(int + ln (mass) | id) + (int | mat)	"
"	"	ln (offspring mass)	"	"	"
"	"	ln (offspring energy)	"	"	"
"	"	ln (fecundity power)	"	"	"
"	"	ln (offspring mass power)	"	"	"
"	"	ln (offspring energy power)	"	"	"
"	2	ln (offspring energy)	int + ln (mass) * gr + pop + food	"	"
Lab	3	ln (offspring energy)	int + ln (mass) + pop	(int | id) + (int | mat)	"
"	"	ln (offspring energy)	int + ln (mass) * food + pop	"	"
Lab	4	interval length	int + age * food + ln (mass)	"	"
Wild	5	pregnant (true/false)	int + ln (mass) + season	(int + ln (mass) | pop) + (int | year)	Bernoulli

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4

Substantial intraspecific variation in energy budgets: biology or artefact?

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Abstract

Dynamic energy budget (DEB) theory provides a model of the intrinsic energetic trade-offs that shape life-histories, in terms of fluxes of energy through biological processes. In these models, life-histories are a function of environmental conditions and of fundamental traits of the organism relating to the acquisition, allocation, and use of energy. These traits are described by the parameters of the DEB model, which are typically estimated from multiple data sources. DEB parameters have been estimated for over 2500 species, and recent work has aimed to compare species on the basis of differences in parameter values. However, little is known about intraspecific variation in DEB parameters, and the reliability of comparisons between parameter sets is rarely tested. We estimated DEB parameters for 16 populations of Trinidadian guppy. Our objectives were to determine whether DEB theory could characterise known life-history differences between populations, and to assess whether differences in parameter values between populations were reliable indicators of biological differences. We found substantial intraspecific variation in parameter values. The models accurately reproduced biological processes, and also predicted differences in resource allocation and metabolic rate consistent with known life-history differences among these populations. However, parameter estimates varied substantially as an artefact of the amount and types of data used to fit the models. This bias appeared to be systematic, influencing estimates for multiple populations in a comparable fashion. We show that similar patterns of growth and reproduction can be reproduced with very different parameter sets. Our results imply that comparisons of DEB parameters between models fit with different types of data may reflect artefactual rather than biological differences.

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4.1 Introduction

The variety of life-history strategies observed in the natural world is truly astounding. From bristlecone pine that live for millenia, to mayflies that exist as adults for less than a day, evolution by natural selection has generated a vast array of solutions to lifes central problem: how best to convert energy into reproductive success. Classical theory proposes that life-history evolution is driven by the interaction between (i) extrinsic ecological effects on reproduction and survival; and (ii) intrinsic organismal constraints, arising from trade-offs in the allocation of energy to different biological processes (Stearns, 1989, 2000). Detection of these intrinsic energetic trade-offs has been successful across taxa (Stearns, 1983; Bauwens and Díaz-Uriarte, 1997; Salguero-Gómez et al., 2016; Healy et al., 2019), but less so within species (van Noordwijk and de Jong, 1986). This is problematic, because intraspecific variation in (ii) is a prerequisite for life-history evolution to occur. Furthermore, classical life-history theory lacks specification of the intra-organismal features that constrain the evolution of phenotypic traits, limiting the potential for quantitative predictions of life-history evolution (Stearns, 2013). For a mechanistic understanding of life-history evolution, a model that captures the intrinsic trade-offs involved in developing and expressing traits that underpin the acquisition, allocation, and use of resources is required.

Dynamic Energy Budget (DEB) theory offers such a model (Kooijman, 2010). The standard DEB model describes individual life-histories, derived from first

principles in accordance with the constraints of thermodynamic laws (Nisbet et al., 2000; Sousa et al., 2008; Jusup et al., 2017). The model provides a single framework to describe an animals development, growth, maintenance, reproduction, and ageing, under any range of environmental conditions (Kooijman, 2010). It is important to note that the standard DEB model does not capture extrinsic factors (such as competition and predation) that shape realised life-histories and drive evolution, but instead describes the intrinsic energetic trade-offs within an individual. Models are parameterised by quantifying the rates of assimilation, allocation, and efficiency of energy flow between biological processes under varying levels of food availability and/or temperature (van der Meer, 2006; Kooijman et al., 2008; Kooijman, 2010). The primary parameters of the standard DEB model represent fundamental (albeit abstract) traits of individuals, that, along with resource availability and temperature, determine the life-history of the organism (Kooijman, 2010). DEB parameters are assumed to be individual-specific, fixed throughout life, and independent of environmental conditions (except for those which are biological rates, which vary in a predictable way with temperature)(Kooijman, 2010). DEB models can be parameterised with a wide range of commonly collected empirical data, and to date parameters have been estimated for over 2500 species, covering all chordate classes (Marques et al., 2018; Augustine et al., 2019; AmP, 2020).

Differences in DEB parameter values between species are assumed to reflect evolved differences (Kooijman, 2010; Lika et al., 2011a; Marques et al., 2018; Lika et al., 2020). The reasoning underpinning this assumption is that the structure of the DEB model means that parameters should co-vary in a simple way with maximum body size: differences in parameter values between species (accounting for differences in maximum body size) therefore indicate departures from this null model and imply evolutionary adaptations of metabolic processes (Kooijman, 2010; Lika et al., 2011a; Marques et al., 2018). Recent work has begun to explore patterns of parameter variation across species, identifying several spectra of life-history strategies which are defined by the different ways in which species allocate energy to development, growth, and reproduction (Marques et al., 2018; Lika et al., 2019; Augustine et al., 2019).

Although theory defines DEB parameters as being individual-specific, individual-level differences are considered to be sufficiently small that mean values are taken to represent species-specific parameters (Marques et al., 2018). This assumption is central to analyses of interspecific patterns of variation in DEB parameters, yet little is known about how much these parameters actually vary within species. Few studies have employed DEB theory to quantify life-history differences between natural populations of the same species. Marn and colleagues (2019) found that DEB parameters differed between Mediterranean and North Atlantic loggerhead turtles (*Caretta caretta*), and that life-history differences between these populations could not be explained as a direct result of environmental differences between their respective habitats. In contrast, Guillaumot and colleagues (2020) were unable to determine differences in DEB parameters between intertidal and subtidal ecotypes of the Antarctic limpet (*Nacella concinna*), despite clear morphological divergence. There is little consensus on the extent to which DEB parameters vary within species, or on the causes of failure to detect differences in DEB parameters between ecologically and morphologically distinct populations.

Here, we assess the extent of intraspecific variation in energy budgets of the Trinidadian guppy (*Poecilia reticulata*). Guppies display well characterised variation in life-history strategies, evolving distinct ecotypes in the presence or absence of predators (Reznick and Endler, 1982; Reznick, 1982; Reznick and Bryga, 1987). Ancestral populations of guppies live in habitats characterised by the presence of voracious predators, in particular the pike cichlid (*Crenicichla alta*) (Magurran, 2005). In several rivers, guppies have invaded further upstream, beyond barrier waterfalls which restrict the movement of large predatory fish. In these upstream habitats, guppies coexist with only one other fish species, Harts killifish (*Anablepsoides hartii*), which is a competitor, and also an occasional predator of guppies (Magurran, 2005). On release from intense predation pressure, these newly founded upstream guppy populations grew rapidly, until diminishing resource availability regulates population growth, driving selection for a slower life-history (Bassar et al., 2013; Reznick et al., 2019; Potter et al., 2021a) . The

low-predation ecotype matures at larger sizes and older ages, and produces fewer, larger offspring per litter than their high-predation" ecotype ancestors (Reznick and Endler, 1982; Reznick, 1982; Reznick and Bryga, 1987). The low-predation ecotype has repeatedly and independently evolved several times in Trinidad, both in natural and experimentally introduced populations (Reznick, 1982; Shaw et al., 1992; Alexander et al., 2006; Reznick et al., 2019; Whiting et al., 2020). As such, Trinidadian guppies provide an excellent system with which to test the ability of DEB theory to distinguish intraspecific variation in the intrinsic trade-offs that underpin life-history evolution.

In this study, we have two main objectives. The first is to determine whether DEB models can characterise well known patterns of life-history variation among populations of Trinidadian guppies. The second is to ascertain whether parameterised DEB models reliably describe energy budgets, which would justify comparisons of species and populations by their DEB parameter values. Support for the second objective would also indicate that parameterised DEB models could play a role in broader life-history theory, by providing an appropriate model of the intrinsic energetic constraints that, together with extrinsic factors such as competition and predation risk, determine life-history evolution.

4.2 Methods

Overview of methods

We estimated the parameters of the standard dynamic energy budget model for twelve distinct guppy populations (three of which were sampled in multiple years), covering five independent evolutionary origins of the low-predation ecotype. According to DEB theory, differences in parameter values between populations indicate evolved differences in fundamental processes underlying the acquisition, allocation and use of energy. However, differences in parameter values may also arise from differences in data availability, i.e. the types of data (e.g. growth, feeding rate, O_2 consumption, etc) and amount of data (e.g. the number of individuals measured, whether data collection covers the full lifespan, etc) used to fit the models (Lika

et al., 2011b). To assess this effect, we fit DEB models for four focal populations with three different levels of data availability. To identify DEB parameters associated with adaptation to resource limitation, we quantified differences in parameters between guppy ecotypes, assessing the generality of these differences across all populations fit with the same degree of data availability. Finally, we compared the variance in parameter values within Trinidadian guppies with that reported across species within the order Cyprinodontiformes.

The standard DEB model

The standard dynamic energy budget model (hereafter 'DEB model') describes how an organism acquires, allocates, and uses energy over the course of its life, from its beginning as a fertilised egg until its death through ageing (Kooijman, 2001; Sousa et al., 2008; Kooijman, 2010). For a detailed description and derivations of the full model we refer the reader to Chapter 2 of Kooijman (2010). The DEB model defines three distinct life-history stages: embryonic (no eating; no reproduction), juvenile (eating; no reproduction), and adult (eating; reproducing). An individual is characterised by four state variables: reserve, structure, maturity level, and reproductive buffer (Fig. 1). Reserve can be considered as stored energy, obtained through feeding, which fuels metabolic processes. Structure describes the soma of the organism, and can be considered as the physical structure of the body produced and maintained through the metabolization of reserve. The transitions between life-history stages (i.e. embryonic to juvenile; juvenile to adult) are determined by maturity level. This somewhat abstract concept captures the cumulative energy spent on developing physiological complexity, theoretically including development of the immune system (Kooijman, 2010). The reproductive buffer describes the amount of mobilised reserve that is allocated specifically to reproduction. These states vary over the life of the organism as a function of the parameters of the model (Table 1) and their interactions with environmental conditions (namely, temperature and food availability). The model captures the biological processes of feeding, assimilation of food, defecation, mobilisation of assimilated energy, allocation of mobilised energy

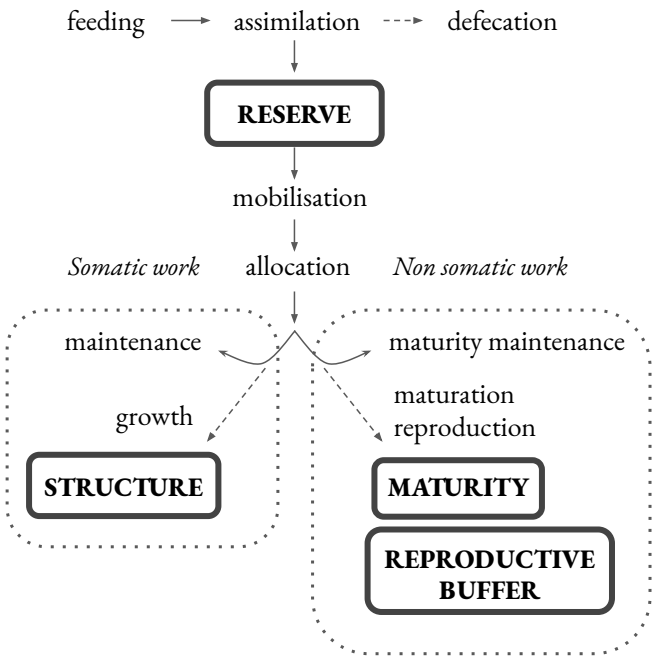

Figure 1: Schematic of the standard dynamic energy budget model. State variables are given in boxes, processes are shown in plain text. Processes (including ageing, not depicted) are determined by 14 parameters, described in Table 1. Arrows represent fluxes of energy through the system via biological processes and state variables. A fraction κ of mobilised reserve is allocated to somatic work, where somatic maintenance costs must first be met before the remainder is allocated to growth. Reserve allocated to reproductive work $(1 - \kappa)$ is first used to meet maturity maintenance costs, and then used to attain sexual maturity (in juveniles) or produce offspring (in adults).

to somatic or reproductive work, maintenance costs of somatic and reproductive work, somatic growth, attainment of maturity, reproduction, and ageing (Figure 1, Table 1, Table 2). Data on the growth and reproduction of individuals at more than one level of food availability are considered sufficient for estimation of most of the primary parameters of the DEB model (Lika et al., 2011a).

Estimating DEB parameters and predicting metabolic rate

The majority of the data that we used to fit population-specific DEB models came from three sets of common garden experiments, in which guppies sampled from sixteen populations (twelve geographically and four temporally distinct populations) were reared at either high or low levels of food availability (Reznick and Bryga, 1996; Reznick et al., 2004, 2005, and one unpublished dataset from D. Reznick and

Table 1: Parameters of the standard DEB model and the primary biological processes that they control. Two further parameters describe the shape and size of the organism: the shape coefficient δ_m links structural length L to physical measurements of length, and the zoom factor z, which is the maximum structural length of the organism at *ad lib* food (expressed in cm), allows comparisons between species of different sizes. Parameters without units are ratios, and therefore dimensionless. Symbols follow the standard DEB notation (Kooijman, 2010).

Symbol	Description	Units	Process
$\{\dot{F}_m\}$	Maximum specific searching rate	L d^{-1} cm^{-2}	Feeding
$\{\dot{p}_{Am}\}$	Maximum specific assimilation rate	J d^{-1} cm^{-2}	Assimilation
κ_X	Efficiency of converting food to reserve	-	Defecation
$\dot{v}$	Energy conductance	cm d^{-1}	Mobilisation of reserve
κ	Fraction of reserve allocated to somatic-work		Allocation of mobilised reserve
κ_R	Reproductive efficiency (reserve to egg)	-	Reproduction
$[\dot{p}_M]$	Volume-specific somatic maintenance cost	J d^{-1} cm^{-3}	Somatic maintenance
$\{\dot{p}_T\}$	Surface-area specific somatic maintenance cost	J d^{-1} cm^{-2}	Somatic maintenance
$\dot{k}_{\mathcal{J}}$	Maturity maintenance rate coefficient	d^{-1}	Maturity maintenance
$[E_G]$	Specific cost of structure	J cm^{-3}	Somatic growth
E_H^b	Maturity at birth	J	Life-history: onset of feeding
E_H^p	Maturity at puberty	J	Life-history: onset of allocation to reproduction
$\dot{h}_a$	Weibull ageing acceleration	d^{-2}	Ageing
S_G	Gompertz stress coefficient	-	Ageing

colleagues). Details of how these data were collected, including information on the sampled populations (location, sample size, sampling year etc), rearing protocols, and trait measurements, are provided in the supplementary material.

Data used to fit DEB models are of two types: zero-variate, and uni-variate (Lika et al., 2011b). Zero-variate data are single values, whereas uni-variate data captures the relationship between pairs of values. For our study, zero-variate data included the mean interval between births (d) and the mean dry weight of offspring (g), with both measures recorded at high and low food levels, and ultimate standard length (cm) at *ad lib* food. Univariate data included age (days since birth) versus standard

Table 2: Linking primary parameters to biological processes in the standard DEB model. Formulae are summarised from Table 1 in Lika & Kooijman (2011). The state variables (in red) are structural volume V (cm^3), energy in the reserve E (J), energy allocated to maturity E_H (J), and energy available for reproduction E_R (J). To simplify the presentation of equations, we also refer to two "compound" state variables: structural length L ($L = V^{1/3}$, cm), and reserve energy density $[E]$ ($[E] = E/V$, J cm^{-3}). Primary parameters (in blue) are described in Table 1. Environmental food density X is given in green. Compound parameters and state functions (in black) are defined within this table, and are functions of state variables, primary parameters, and food density. Symbols follow the standard DEB notation (Kooijman, 2010). Values that are expressed per unit of structural volume are in square brackets [ ], those expressed per unit of structural surface area are in braces {}.

Description	Formula	Process
Half-saturation coefficient	$K = \frac{\{\dot{p}_{Am}\}}{\kappa_X \{\dot{F}_m\}}$	Feeding
Scaled functional response	if $E_H < E_H^b$,	Feeding
	$f = 0$,	
	else $f = \frac{X}{K + X}$	
Assimilation flux	$\dot{p}_A = f\{\dot{p}_{Am}\}L^2$	Assimilation
Mobilisation flux	$\dot{p}_C = L^3[E](\dot{v}/L - \dot{r})$	Mobilisation of energy
Specific growth rate	$\dot{r} = \frac{\kappa[E]\dot{v}/L - [\dot{p}_S]}{[E]\kappa + [E_G]}$	Somatic growth
Somatic maintenance flux	$\dot{p}_S = [\dot{p}_M]L^3 + \{\dot{p}_T\}L^2$	Somatic maintenance
Maturity maintenance flux	$\dot{p}_J = k_J E_H$	Maturity maintenance
Reproductive/maturity flux	$\dot{p}_R = (1 - \kappa)\dot{p}_C - \dot{p}_J$	Allocation to non-somatic work
Reserve dynamics	$\frac{d}{dt}E = \dot{p}_A - \dot{p}_C$	**Change in reserve energy**
Structural dynamics	$\frac{d}{dt}V = \dot{r}V$	**Change in structural volume**
Maturity dynamics	if $E_H < E_H^p$,	**Maturation**
	$\frac{d}{dt}E_H = \dot{p}_R$,	
	else $\frac{d}{dt}E_H = 0$	
Reproductive buffer dynamics	if $E_H < E_H^p$,	**Reproduction**
	$\frac{d}{dt}E_R = 0$,	
	else $\frac{d}{dt}E_R = \dot{p}_R$	

Table 3: Types of data used to estimate DEB parameters in this study at each level of data availability tested. For twelve population samples, we used data at availability level 1 only. For four population samples, data was available at all three levels. The asterisk * indicates that level 1 univariate data were available for measurements at the first three parturition events only, i.e. 3 observation per individual. Univariate data at levels 2 and 3 were recorded at each parturition event over the full lifespan: mean number of observations per individual=22 (s.d.=8.5).

Data type	Units	Food treatment	Data level
Interval between births	d	high, low	1,2,3
Maximum standard length	cm	ad lib	1,2,3
Age vs weight	d, g	high, low	1*,2,3
Age vs length	d, cm	high, low	1*,2,3
Length vs weight	cm, g	high, low	1*,2,3
Age vs cumulative reproduction	d, -	high, low	1*,2,3
Dry weight at birth	mg	high, low	2,3
Age vs surviving fraction of population	d, -	high, low	2,3
Age vs food availability	d, J d^{-1}	high, low	3
O_2 consumption rate	mg hr^{-1}	ad lib	3

length (cm), weight (g), cumulative reproductive output (number of offspring), and length versus weight. Each of these measures were recorded at high and low food levels. For twelve of our study populations, these data were recorded at the first three parturition events only. For four populations, data were recorded at each parturition event over the entire lifespan of individuals. In addition, for these four populations we recorded mean dry weight (mg) of neonates at high and low food, the surviving fraction of the population as a function of time since birth, daily food ration (joules per day) used in the common garden experiments (Reznick et al., 2004), and we also incorporated data on basal metabolic rate (reported as O_2 consumption (mg hr^{-1}) at 25 °C, standardised for a body weight of 74 mg) from an independent study using the same populations (Auer et al., 2018).

For four populations (representing two independent evolutionry origins of the low-predation guppy ecotype), more types of data were available to estimate DEB parameters than in the other twelve. To assess how the use of different types of data may affect the estimation of parameters (Lika et al., 2011a), we first fit DEB models for the first four populations at three different levels of data availability (Table 3).

To compare populations across all datasets, we then fit sixteen population-specific DEB models using the same level of data availability (Table 3).

Both zero-variate and uni-variate observations can be described as functions of primary DEB parameters, state variables, temperature, and food availability. Because DEB parameters appear in multiple auxiliary models of observable relationships, model fitting is not a straight-forward process. All relationships must be modelled simultaneously because no single parameter is specific to a single type of observation (Marques et al., 2019). DEB parameters are derived from theory, meaning that there are substantial constraints on their values. For example, κ, the fraction of energy allocated to somatic work, must be bounded between 0 and 1, and the energy conductance rate $\dot{v}$ must be positive, etc. This means that parameter filters and psuedo-data – parameter values that correspond to those of a generalised animal – can be used to constrain the parameter space during estimation, in a manner that is analogous to the use of priors in Bayesian estimation (Lika et al., 2011a, 2014; Marques et al., 2019). The use of pseudo-data increases the identifiability of parameters, whilst their influence on final parameter estimates is restricted by low weighting of pseudo-data during estimation. Parameter estimation is based on the minimisation of a symmetric bounded loss function (i.e. a function that equally penalizes over- or underestimation when fitting the models), using a Nelder-Mead simplex minimization procedure (Marques et al., 2019).

We estimated parameters from data using the freely available software package *DEBtool* (available from github.com/add-my-pet/DEBtool_M, (Marques et al., 2018)) in MATLAB version R2020a. We used standard functions in the *DEBtool* package to predict the data for analyses with data availability levels 1 and 2. For analyses including data on food availability we built a custom function, consisting of a set of differential equations which described the rates of change of the reserve, structure, maturity level, reproductive buffer, and survival probability, accounting for the changes in food availability with age. We linked the changes in state variables to observations of traits via standard auxiliary theory (Kooijman, 2010). Biological rates are temperature dependent: for all analyses, we assumed an Arhennius

temperature of 8000 K. Some DEB parameters could not be estimated from the available data: we fixed the maximum specific searching rate $\{\dot{F}_m\}$ at 6.5 L d^{-1} cm^{-2}, and the reproductive efficiency κ_R at 0.95 in all analyses – these are typical parameter values, and are recommended for use when no data is available for estimation (Lika et al., 2011a). Without information on food availability (i.e. data levels 1 and 2), assimilation efficiency κ_X cannot be estimated: we fixed this value at 0.25 based on our estimates of κ_X at data availability level 3. When we could not directly estimate κ_X, we quantified feeding rates by estimating the scaled functional response f (Table 2) at high and low food levels. To achieve a better fit of the models to the data, we introduced two additional parameters: the first captured a delay in the onset of development, T_0 (measured in days) and the second was a low-food level specific assimilation efficiency $\kappa_{X_{low}}$. The parameter T_0 was included as an additive term in the auxiliary model for the interval between births only. For modelling growth, reproduction, and survival at low food levels, we used $\kappa_{X_{low}}$ in place of κ_X in the standard DEB model.

Although metabolic rate data (O_2 consumption) were only used to fit models with data availability level 3 (Table 3), we predicted metabolic rates from the parameter sets for each population, using the "scaled_power" function in *DEBtool*. For these predictions, we estimated the total power (i.e. energetic flux $J\ d^{-1}$) expended by a guppy weighing 74 mg, at a temperature of 25 °C. We excluded assimilation power from our analyses, because our reference data were collected from guppies that had fasted for 24 hours before measurement (Auer et al., 2018). We then converted this energetic flux into a mass flux (milligrams of O_2 per hour) following standard assumptions about the chemical composition and potential (J mol^{-1}) of structure and reserve, and assuming a molar weight for O_2 of 32 g mol^{-1} (Kooijman 2010, Chapter 3).

Each population-specific set of formatted data and the MATLAB scripts required to estimate the parameters presented in this manuscript are available to download

from the AmP online repository (AmP, 2020, see Table S1). Raw data are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.qv9s4mwf1 (Potter et al., 2021b).

Assessing model fit and differences among parameter sets

We assessed goodness of fit of the models to the data by calculating the mean error of the predictions relative to the data, for each set of observations used to fit the model (Marques et al., 2018). The overall fit of the model is described by the mean relative error, which is the grand mean of the relative error terms from each set of predictions and observations.

To determine whether there were significant differences in parameter sets between populations, we followed the approach described by Marn and collegaues (2019): we compared the fit of the model to the data when using the population-specific ("best") parameters with that when using a null-model parameter set. We compared the "best" parameters with two null models: "null 1", which was the parameter set of the alternative ecotype from the same stream, and "null 2", which was the parameter set of the same ecotype from the alternative stream. This allowed us to test whether the parameter sets were distinct between (i) ancestral and derived populations in the same stream, and (ii) between common ecotypes that have evolved in different streams. We assessed differences in parameter sets between the four populations (two ecotype pairs) for models fit with the highest level of data availability. We tested the difference in fit of DEB parameter sets using Wilcoxon's paired rank test (Marn et al., 2019).

Finally, we assessed how intraspecific variation in DEB parameters compares with interspecific variation. Parameter values are expected to be more similar among closely related species. We compared parameter values obtained in this study for Trinidadian guppies with parameter values of other species within the order Cyprinodontiformes. We retrieved all available Cyprinodontiformes parameter sets from the AmP database (AmP, 2020) (59 species), and visualised the variation among pairs of parameters. We performed F-tests to compare intra-

and interspecific variation in DEB parameters. Calculations were performed in the in the R environment (R Core Team, 2019), and all figures were produced using the "ggplot2" package (Wickham, 2011).

4.3 Results

Impact of data availability on parameter estimates

Our estimates of dynamic energy budget model parameters depended on the type and amount of data used to fit the models (i.e. data availability, Table 4). Estimates of the fraction of energy allocated to somatic work κ, the maximum assimilation rate $\{\dot{p}_{Am}\}$, and of the volume-specific somatic maintenance rate $[\dot{p}_M]$ were all substantially lower when estimated using level 3 data. Estimates of the maturity threshold at the onset of the adult stage, E_H^p, were highest when estimated using level 3 data. These patterns were consistent across all four populations tested (Table 4).

Despite substantial differences in parameter values by data level, the different parameter sets were able to reproduce how standard length, mass, and cumulative reproductive output changed as a function of age and food level (Fig. 2), as well as predicting age and dry weight at birth (at high and low food) and maximum length (Table S2). The overall fits of the models to the data, as quantified by the mean relative error, were poorest for models fit with data level 1, and were reasonable (MRE<0.1) and comparable between models fit at data levels 2 and 3 (Table 4).

Ecotype-specific parameter differences

Our initial assessment of ecotype-specific differences in DEB parameters considered two ecotype pairs (four populations) for which level 3 data were available. In general, parameter sets were significantly different between populations and ecotypes: the population-specific parameter sets gave a significantly better fit to the data compared to parameter sets from the other population (different ecotype) within the same river systems, or the from the same ecotype from a different river system (Table S3). There was one exception: for data from the low-predation Oropuche population,

Table 4: Estimates of dynamic energy budget model parameters vary depending on the type and amount of data used to fit the models. We estimated parameters using three levels of data availability (see Table 3). Parameters are shown for four populations of Trinidadian guppy: the high-predation (HP) and low-predation (LP) ecotypes from the Oropuche and Yarra rivers. Parameters which were fixed and therefore not estimated during model fitting are denoted with (n. e.). Parameters that were fixed across all data levels were: the maximum specific searching rate $\{\dot{F}_m\} = 6.5$ L d^{-1} cm^{-2}; the reproductive efficiency $\kappa_R = 0.95$; the surface area specific somatic maintenance $\{\dot{p}_T\} = 0$ J d^{-1} cm^{-2}; and the Arhennius temperature = 8000 K. Models were fit assuming an environmental temperature of 26 °C; following convention, we report temperature-adjusted parameters for a reference temperature of 20 °C. The mean relative error (MRE) quantifies the fit of the models to the data.

	Population:	Oropuche HP			Oropuche LP			Yarra HP			Yarra LP		
	Data level:	1	2	3	1	2	3	1	2	3	1	2	3
Parameter	Unit												
Assimilation efficiency, κ_X	-	0.25^{ne}	0.25^{ne}	0.27	0.25^{ne}	0.25^{ne}	0.23	0.25^{ne}	0.25^{ne}	0.26	0.25^{ne}	0.25^{ne}	0.21
Assimilation efficiency at low food, $\kappa_{X\,low}$	-	-	-	0.34	-	-	0.30	-	-	0.34	-	-	0.26
Max specificassimilation rate, $\{\dot{p}_{Am}\}$	J d^{-1} cm^{-2}	116.7	117.8	63.1	108.2	132.1	52.5	175.0	138.1	74.6	156.4	110.9	56.1
Energy conductance, $\dot{v}$	cm d^{-1}	0.028	0.028	0.029	0.029	0.033	0.025	0.029	0.028	0.030	0.027	0.030	0.030
Allocation fraction to soma, κ	-	0.55	0.73	0.43	0.70	0.78	0.58	0.61	0.70	0.40	0.69	0.78	0.54
Vol-spec somatic maint, $[\dot{p}_M]$	J d^{-1} cm^{-3}	68.2	93.5	27.31	95.1	119.4	35.32	120.1	114.5	33.78	121	105.7	34.31
Maturity maint rate coefficient, $\dot{k}_J$	d^{-1}	0.0019	0.0020	0.0020	0.0020	0.002098	0.0017	0.0020	0.0020	0.0020	0.0020	0.0021	0.0021
Spec cost for structure, E_G	J cm^{-3}	5220	5220	5223	5228	5219	5185	5225	5221	5213	5222	5220	5232
Maturity at birth, E_H^b	J	36.9	6.4	26.6	12.8	6.7	17.9	25.2	5.5	21.6	8.8	5.3	18.1
Maturity at puberty, E_H^p	J	298.3	63.0	501.8	146.5	222.6	401.5	382.5	264.9	515.3	238.9	227.7	524.5
Weibull aging acceleration, $\dot{h}_a$	d^{-2}	-	2.65×10^{-8}	2.03×10^{-8}	-	3.98×10^{-8}	4.10×10^{-8}	-	6.15×10^{-8}	4.41×10^{-8}	-	6.71×10^{-8}	5.62×10^{-8}
Gompertz stress coefficient, S_G	-	-	0.070	0.062	-	0.074	0.075	-	0.029	0.037	-	0.048	0.049
Zoom factor, z	-	0.94	0.92	0.99	0.80	0.87	0.86	0.89	0.84	0.88	0.89	0.82	0.88
Shape coefficient, δ_M	-	0.24	0.24	0.26	0.24	0.25	0.26	0.23	0.23	0.25	0.23	0.24	0.26
Scaled functional response high food, f_{high}	-	0.86^{ne}	0.92	-	0.86^{ne}	0.93	-	0.86^{ne}	0.90	-	0.86^{ne}	0.96	-
scaled functional response low food, f_{low}	-	0.79	0.72	-	0.79	0.74	-	0.81	0.72	-	0.76	0.75	-
Developmental delay, t_0	d	5.2	8.171	6.7	9.0	10.7	7.7	6.0	8.149	7.0	9.8	10.32	8.9
Model fit, MRE	-	0.111	0.076	0.076	0.113	0.087	0.099	0.122	0.089	0.086	0.120	0.089	0.085

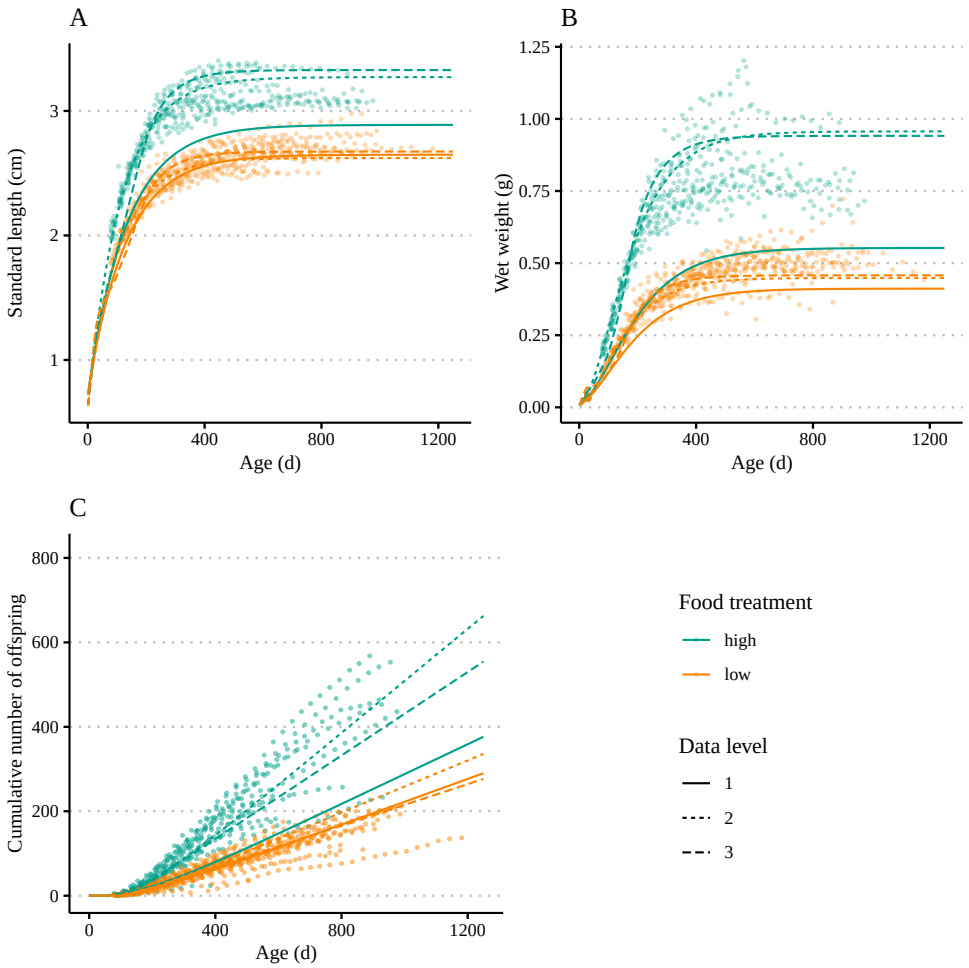

Figure 2: Observations and predictions of (A) length, (B) weight, and (C) cumulative number of offspring, as a function of age. Individual trajectories of growth and reproduction (points) and predictions (lines) are of low-predation ecotype guppies from the Oropuche river, under high (blue) and low (orange) food treatments. Lines show predictions based on different parameter sets, estimated with different levels of data availability (defined in Table 3).

there was no significant difference in fit when using the population-specific parameter set, or the parameter set from the low-predation Yarra population (Table S3).

Guppy ecotypes adapted to high and low predation-risk environments differ in how they obtain, allocate, and use resources: we found that DEB parameters (estimated with level 3 data availability) differed between ecotype pairs, and that these differences were often consistent between independent evolutionary origins of ecotype pairs in the Oropuche river and Yarra rivers. (Table 4, Fig. 3). Six parameters varied consistently between ecotypes: for both rivers, compared to the

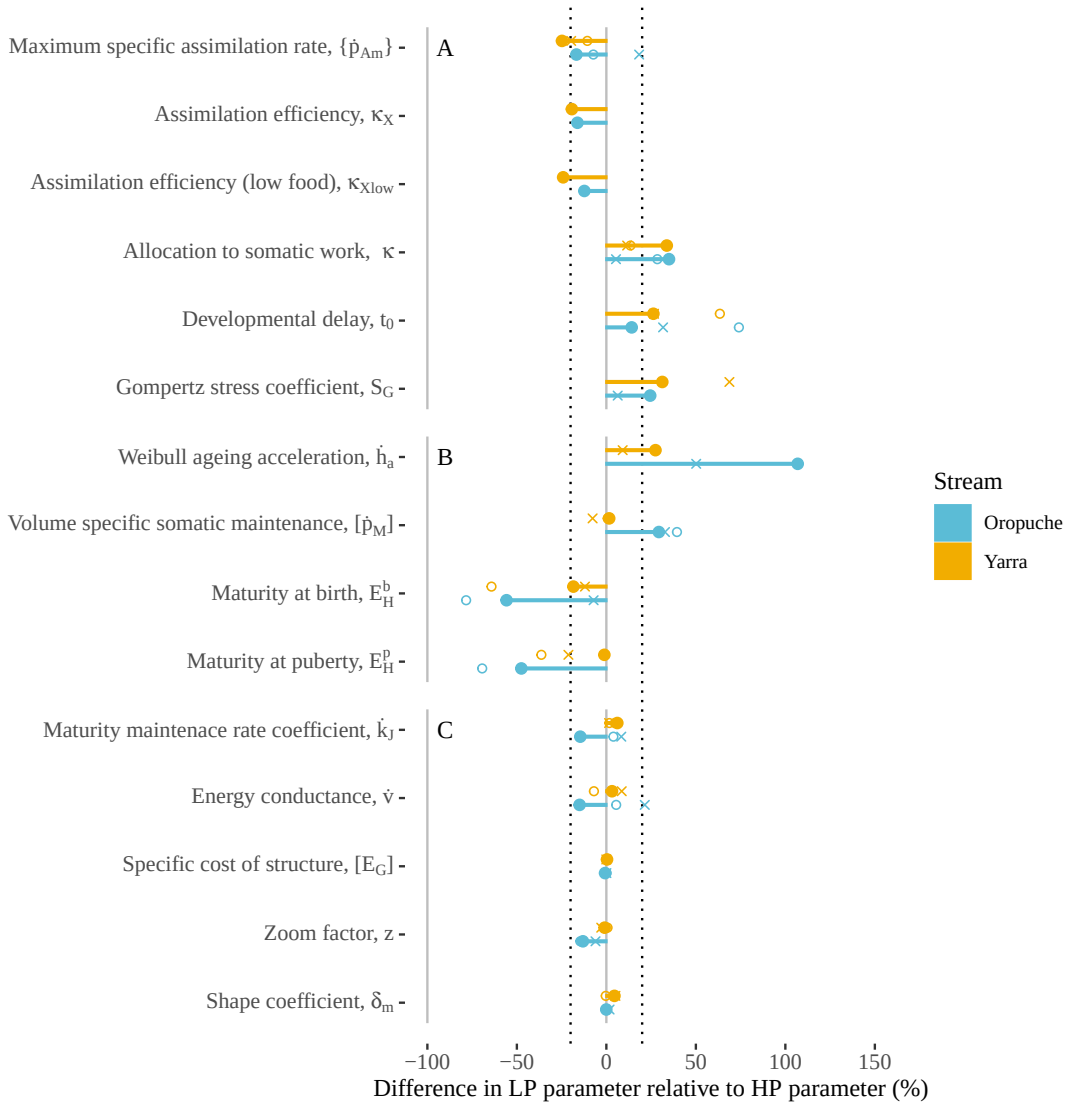

Figure 3: Percentage differences in DEB parameter values for the low-predation (LP) ecotypes relative to the high-predation (HP) ecotype. Differences between ecotypes from the Oropuche are given in blue; those from the Yarra are given in yellow. Values are given for values fit at data availability levels 1 (circles), 2 (crosses), and 3 (filled circles with bars). Parameters are categorised into three groups with respect to relationships observed at data availability level 3: (A) Parameters differ between ecotypes, and the direction of the effect is consistent between streams; (B) Parameters differ between ecotypes, and the direction and/or magnitude of the effect differs between streams; and (C) Parameters do not differ between ecotypes or streams. Dotted lines indicate a difference of plus or minus 20%, representing the threshold used to categorise parameters as falling in A, B, or C. N.B The ecotype difference for E_H^p for the Oropuche at data level 2 was extreme (+548%) and is not shown in this figure.

high-predation ecotype, low-predation guppies had lower maximum assimilation rates ($\{\dot{p}_{Am}\}$) and assimilation efficiencies (κ_X and $\kappa_{X\,low}$), allocated a greater

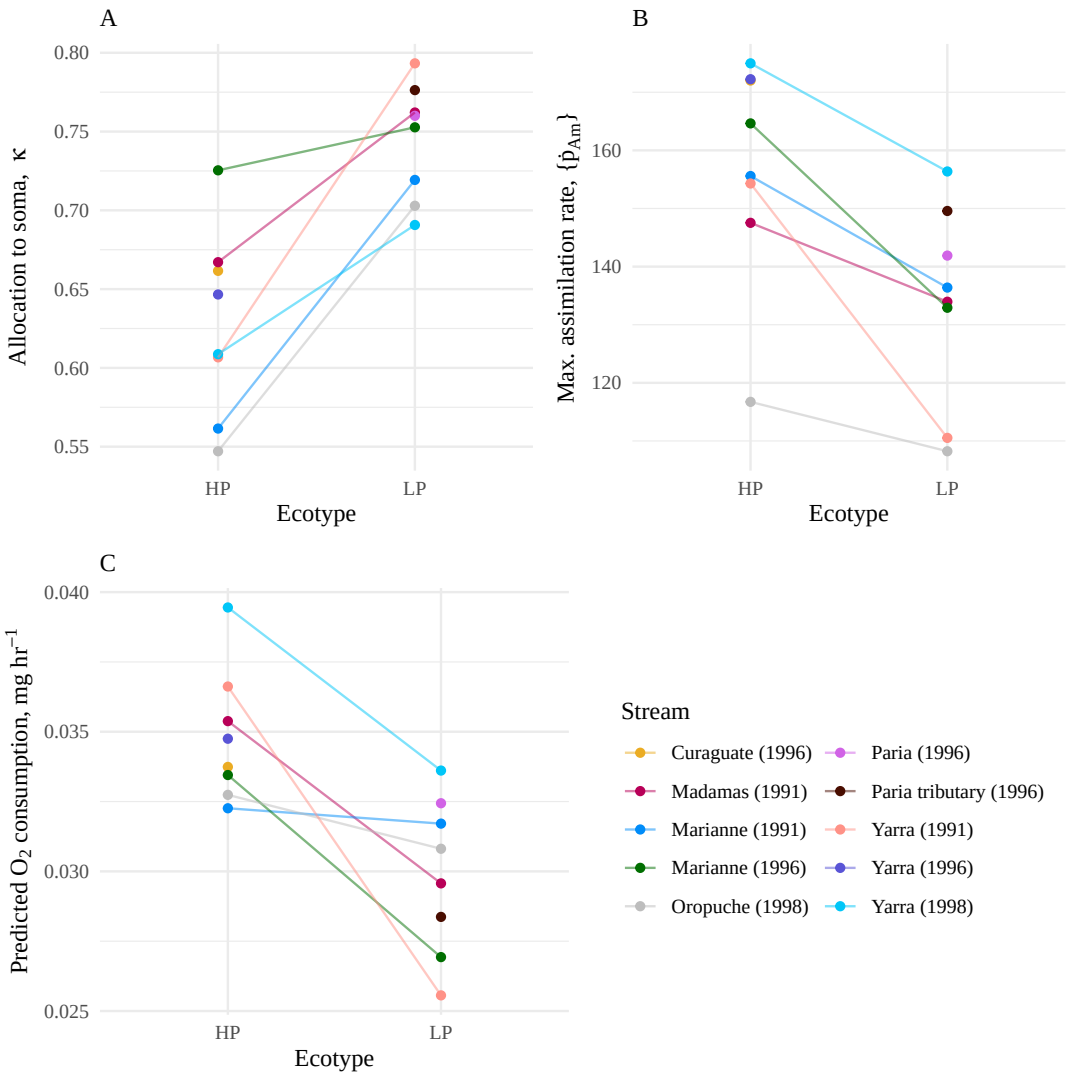

Figure 4: Differences between high-predation (HP) and low-predation (LP) ecotype populations in (A) the fraction of energy allocated to somatic work κ, (B) the maximum specific assimilation rate $\{\dot{p}_{Am}\}$, and (C) predicted basal metabolic rate (oxygen consumption) for an individual weighing 74 mg, based on the full population-specific parameter set. Parameters were estimated using level 1 data availability for 16 populations. Lines connect ecotype pairs sampled from the same stream in the same year, i.e. they connect the ancestral (HP) ecotype to the derived (LP) ecotype.

fraction of available energy to somatic work (κ), had a longer delay period at the onset of development (t_0), and had higher values for the Gompertz stress coefficient (S_G) (Fig. 3A).

The magnitude of ecotype differences were inconsistent between streams for somatic maintenance costs ($[\dot{p}_M]$), the Weibull ageing acceleration parameter ($\dot{h}_a$),

and for maturity thresholds (E_H^b and E_H^p) (Fig. 3B). For five parameters, there were negligible differences between ecotypes, and this was consistent between streams. These parameters were the maturity maintenance coefficient ($\dot{k}_{\mathcal{J}}$), energy conductance ($\dot{v}$), the energetic cost of structure ($[E_G]$) the shape coefficient (δ_m), and the zoom factor z (Fig. 3C).

In general, the ecotype differences observed when models were fit with level 3 data availability were qualitatively similar when estimated with data levels 1 and 2 (Fig. 3A). The exception was for $\{\dot{p}_{Am}\}$, which followed the same trend as at data levels 2 and 3 in the Yarra river, but not in the Oropuche river (Table 4, Fig. 3A). We found that parameters relating to the maturity thresholds (E_H^b and E_H^p) varied considerably by data availability and population (Table 4).

We then fit DEB models at level 1 data availability for these four populations, plus an additional twelve populations. The overall fits of the models (MRE) ranged from 0.076 to 0.137, with a grand mean MRE of 0.107. Full parameter sets and model fit scores are provided in Table S4. We again found that the low-predation ecotype allocated a greater fraction of energy to somatic work, and had lower maximum assimilation rates than the high-predation ecotype (Fig. 4). Using the parameter sets for each population, we predicted basal metabolic rate in terms of O_2 consumption rates. Note that metabolic rate data was not used to fit models at data level 1. The low-predation ecotype was predicted to have lower rates of oxygen consumption than the high-predation ecotype (Fig. 4C) – a pattern consistent with previously published work (Auer et al., 2018). However, the estimates of oxygen consumption for both ecotypes were typically about 50% greater than the values of those reported in (Auer et al., 2018), i.e. the values used to fit models at level 3 in this study.

Intra- and interspecific variation

We compared the intraspecific variation in six DEB parameters reported in this study with the interspecific variation reported within the order Cyprinodontiformes (AmP, 2020) (Fig. 5). The variance in κ for Trinidadian guppies (across all data levels in this study) was comparable to that reported in 59 species within

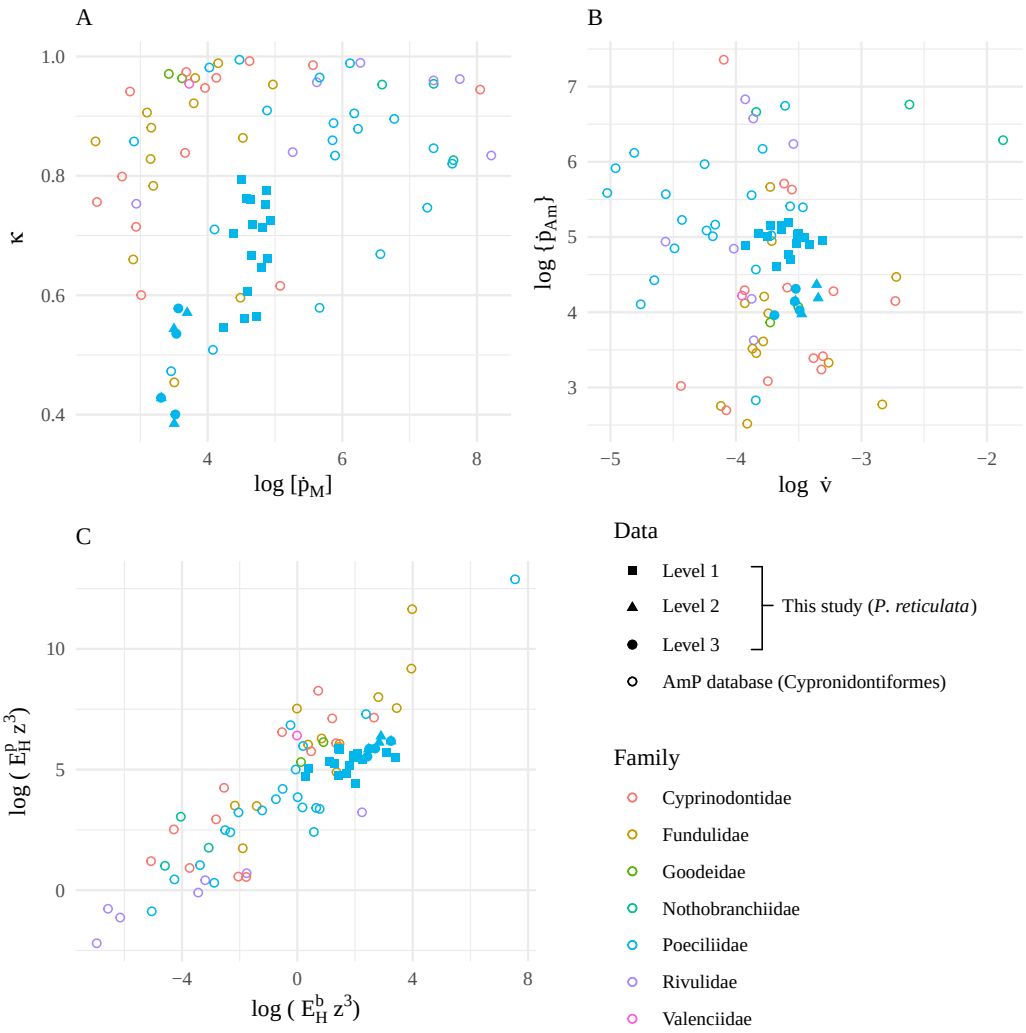

Figure 5: Comparison of intra- and interspecific variation in primary DEB parameters: Pairwise plots are of (A) the fraction of reserve allocated to somatic work κ and the volume-specific somatic maintenance cost $[\dot{p}_M]$, (B) the maximum specific assimilation rate $\{\dot{p}_{Am}\}$ and the energy conductance $\dot{v}$, and (C) the maturity level at birth E_H^b and at puberty E_H^p. Note that to account for parameter variation due to differences in maximum body size between species, values for E_H^b and E_H^p are multiplied by z^3 (Kooijman, 2010). Axes are on the log-scale, except for the y-axis in (A). Filled points are parameter values obtained for Trinidadian guppies in this study, using data availability at level 1 (squares), level 2 (triangles) and level 3 (circles). For details on data levels, see Table 3. Empty circles are parameter estimates for 59 species from 7 families within the order Cyprinodontiformes, obtained from the AmP online database (AmP, 2020).

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the order Cyprinodontiformes ($F_{23,58} = 0.77$, p=0.49). This variance in guppy κ values was largely an artefact due to differences in the data levels used to fit these models. When we considered only parameters from models fit with data level

1, the intraspecific variance in κ across 16 populations of Trinidadian guppy was significantly less than that seen within 20 species in the family Poeciliidae ($F_{15,19}$ = 0.24, p=0.008). Similarly, for the five other DEB parameters ($[\dot{p}_M]$, $\{\dot{p}_{Am}\}$, $\dot{v}$, E_H^b, and E_H^p), intraspecific variation was significantly less than variation within the family Poeciliidae (F-tests, $p<0.05$).

4.4 Discussion

We identified considerable variation in dynamic energy budget (DEB) parameters among populations of Trinidadian guppies. Although variation in DEB parameters are theoretically predicted to reflect evolved, biological differences (Kooijman, 2010; Lika et al., 2011a; Marques et al., 2018; Lika et al., 2020), our results suggest that systematic bias during parameter estimation – an artefact of the type and amount of data used to fit the models – may generate substantial variation. Nevertheless, population-specific parameter sets were able to characterise known life-history variation in our study species.

Data type influences estimation of DEB parameters

Fitting models with different types of data resulted in very different parameter estimates for the same populations. This variation was clearly an artefact of the estimation procedure, whereby the amount and type of data used to fit models had substantial effects on estimates of DEB parameters. For example, including data on metabolic rate and daily food availability halved estimates of the maximum rate at which energy is assimilated ($\{\dot{p}_{Am}\}$). Estimates of κ varied such that guppies in one population were predicted to allocate as much as 70% of available energy to somatic work, or as little as 40%, depending on the type of data used to fit the models (Table 4). Do these fish invest more energy in reproduction, or in somatic growth and maintenance? Had we used a single level of data availability when fitting our models, we may have felt confident in our answer. That confidence would have been misplaced: when fitting models with different types of data, our results gave conflicting answers to this basic question of energy allocation.

When we included data only from juveniles and young adults (level 1 data, Table 3), the models attained a reasonable fit but did not accurately predict growth and reproduction of older adults (Fig. 2). This suggests that greater data coverage of growth and reproduction trajectories is required to accurately predict energy budgets over the full lifespan. However when models were fit with full lifespan data (levels 2 and 3), despite substantial differences among parameter sets, the net combination of parameter values reproduced remarkably similar patterns of growth and reproduction, reasonably fitting the observed data (Fig. 2). This is an example of getting the right answer for the wrong reasons. But which reason is least wrong? The nature of a balanced energy budget means that, for example, a decrease in somatic maintenance costs must be balanced by, say, a decrease in assimilation efficiency, if patterns of growth are to remain fixed. The interactive and parameter-rich structure of the DEB model allows many possible routes through which this balance can manifest, but those routes become increasingly constrained as more different types of data are used to fit the models.

When we included data on O_2 consumption and daily food availability (i.e. level 3 data availability, Table 3), estimates of several parameters changed substantially, and these changes were consistent across all four populations tested: maximum assimilation rate, the fraction of energy allocated to somatic work, and somatic maintenance costs all decreased, while the maturity threshold at which the adult stage is met increased (Table 4). To balance the food availability with O_2 consumption rates, this required a set of parameters where: less energy was assimilated from the environment; less was allocated to somatic growth; somatic overheads were reduced; and more energy was invested in attaining, and also in maintaining maturity. This happened because initial fits (without using O_2 consumption data) generated parameter sets that overestimated basal metabolic rate by about a half (Table S2). When the fitting process was forced to account for the measured rates of O_2 consumption, the total dissipation power had to decrease: data on growth and reproduction constrained this to decreases in energy assimilation rates, with more investment in non-somatic work balanced by decreases in somatic maintenance

costs, and increases in the costs of maturation. As such, in the absence of O_2 consumption data, we overestimated the energetic costs of somatic maintenance by a factor of around 2, resulting in overestimation of basal metabolic rate. Note that the data on O_2 consumption were measures of basal metabolic rate from a separate study to the main data-sets used, whereas our predictions include the somatic costs associated with every day activity. As such, it may be expected that predicted metabolic rates would be higher than measured basal metabolic rate. However, DEB theory assumes that energetic costs associated with routine movement are generally negligible with respect to the total energy budget (Kooijman, 2010), meaning that DEB predictions of metabolic rate should closely match measurements of basal metabolic rate. Our results could be interpreted as a violation of this assumption, which acts to modify the estimates of a suite of parameters when basal metabolic rate data are used to fit DEB models.

Does increasing data availability improve the likelihood of identifying the "true" DEB parameters? Although in our study using level 3 data generally gave a better fit than lower levels (Table 4), and including more data during parameter estimation should reduce overfitting, we cannot rule out the possibility that parameter values at lower levels are more realistic. A confounding factor in our analyses is that the population-specific O_2 consumption data came from an independent study (Auer et al., 2018). Although combining data from different studies is an appealing aspect of the DEB philosophy, and has facilitated the estimation of parameter sets for several thousand species (AmP, 2020), this approach will introduce additional sources of variation – both biological and environmental – that could reduce the reliability of parameter estimates. We suspect that somatic and maturity maintenance costs are generally poorly determined by data on reproduction and growth alone, but data on O_2 consumption (from the same study that reproduction and growth data are obtained) would be needed to test this. As such, in the present analyses we cannot determine which parameter values are closest to the "true" values describing population-specific energy budgets.

Biological variation and DEB parameters

For most parameters, differences between our four focal populations were qualitatively similar when fit with different levels of data availability (Fig. 3). This suggests that the level of data availability has a systematic effect on parameter estimation, implying that comparison of populations fit with the same level of data availability may be valid for assessing relative (rather than absolute) differences in energy budgets. To test this, we assessed whether the DEB approach could identify and predict known life-history variation among sixteen populations of Trinidadian guppies, comparing predicted responses and parameters within levels of data availability.

Our results demonstrate that life history variation among populations of guppies can be characterised by differences in dynamic energy budget parameters. Low-predation ecotype guppies allocated a greater fraction of energy to somatic work (higher values of κ), and had lower maximum assimilation rates (lower values of $\{\dot{p}_{Am}\}$) than their high-predation ecotype ancestors. These differences were consistent across sixteen spatiotemporally distinct populations, representing five independent evolutionary origins of the low-predation ecotype (Fig. 4). For the four populations that we were able to fit at higher levels of data availability, we also found that the low-predation ecotype were less efficient at assimilating energy (lower values of κ_X) than their high-predation counterparts (Fig. 3). Population-specific parameter sets gave significantly better fits to the data than parameter sets from the other ecotype, indicating that these parameter differences reflect biological differences between ecotypes.

Apart from predicting the patterns of growth and reproduction used to fit the models, how do these differences in DEB parameters compare with known life-history variation in Trinidadian guppies? Low-predation guppies invest less in reproduction (Reznick and Endler, 1982), which corresponds with the higher values of κ we identified here. Low-predation ecotypes also have slower metabolic rates (Auer et al., 2018)] – DEB estimates of metabolic rate from all populations predicted this pattern (Fig. 4C), despite metabolic rate data not being used to fit these models.

A surprising result is lower rates and efficiencies of assimilation in low-predation guppies. Given that the low-predation ecotype evolves in response to reduced resource availability (Bassar et al., 2013; Reznick et al., 2019; Potter et al., 2021a), it would appear maladaptive to evolve a reduced capacity for energy assimilation. Furthermore, low-predation guppies have increased gut length relative to the high-predation ecotype, which should facilitate higher rates of energy assimilation (Zandonà et al., 2015). However, our parameter estimates (κ_X and $\{\dot{p}_{Am}\}$) were specific to the high-protein diet used in the experiments. One possible explanation is that the experimental diet more closely resembled the natural diet of high-predation guppies, which are richer in high quality invertebrate prey (Zandonà et al., 2011). Digestion of protein-rich food is energetically costly (Cho et al., 1982), and high efficiencies may not be maintained through selection in natural low-predation populations, where diets are dominated by low-quality algae and detritus (Zandonà et al., 2011). Had a lower quality diet been used in these experiments, we may have seen higher assimilation rates and efficiencies in the low-predation ecotype.

To improve model fits, we introduced two additional parameters to the standard DEB model. First, we included a second assimilation efficiency parameter, specific to the low food treatment ($\kappa_{X\,low}$). Assimilation efficiencies were consistently higher in the low food treatment than they were in the high food treatment. This could indicate that in the high food treatment not all of the daily food ration was consumed, meaning that we overestimated the amount of food eaten by individuals in this group. However, a daily record of whether each fish had consumed their full ration was kept for these experiments: failure to eat the full ration was rare, and there was no systematic tendency for fish in the high food treatment to not consume full rations. A more plausible explanation is that our DEB model failed to capture how total energetic flux changed as a function of food availability in guppies. One empirical measure of energetic flux - standard metabolic rate - has been shown to increase at higher food levels (Auer et al., 2015). Although this should be captured in the DEB model – reserve density should be greater, and therefore every process downstream of the assimilation flux (Table 2) should have higher rates at high food

levels – in our study, this difference was partially attributed to different assimilation efficiencies (κ_X) at different food levels. The second introduced parameter was the developmental delay, t_0. This parameter represented a delay in the onset of development of the fertilised embryo, and was an additional term in the sub-model for the interval between births. However, there is no empirical support for such a delay in guppy development (Martyn et al., 2006). The requirement for these additional parameters in our models suggest that some assumptions of the standard DEB model – that parameters are independent of food levels, and that they are constant throughout life – may be unrealistic for guppies.

Differences in parameter values between ecotypes were not always consistent between different streams. For four parameters, the magnitude of ecotype differences varied considerably between streams (Fig. 3B). Furthermore, there was substantial variation in parameter values and predicted metabolic rates within ecotypes for models fit with level 1 data (Fig. 4, Table S4). Because each stream represents an independent evolutionary origin of the low-predation ecotype, these differences may reflect alternative mechanisms through which adaptation to competitive, resource-limited environments has occurred. The distinction between guppy ecotypes is well characterised (Reznick and Endler, 1982; Reznick, 1982; Reznick and Bryga, 1987). However, there is substantial life-history variation among low-predation populations from different streams, including differences in rates of senescence (Reznick et al., 2004, 2005), juvenile growth rates (Arendt and Reznick, 2005), basal metabolic rate (Auer et al., 2018), how competitive ability scales with body size (Potter et al., 2019), and in genes associated with living in a low-predation habitat (Whiting et al., 2020). Natural populations of guppies display considerable variation in organismal stoichiometry, including within ecotypes (El-Sabaawi et al., 2012b,a): our results here support the notion that there are multiple mechanistic routes through which the low-predation ecotype can evolve.

Implications of variation in DEB parameters

One of the strengths of the DEB approach – that models can be fit with a wide range of data from different studies – may come at a cost in terms of the reliability of comparisons of parameters across species. Although the sensitivity of parameter estimation to the types of data used to fit DEB models has been noted in a simulation study (Lika et al., 2011b), we feel that the potential scale of this problem may not be generally appreciated. Our results show that variation in DEB parameters in a single species can match the variation seen across an entire order, if differences in the types of data used to fit the models are not taken into account (Fig. 5A). This type of variation in estimated parameter values, which does not reflect true biological variation, is potentially problematic for studies that aim to quantify life-history strategies across taxa on the basis of DEB parameters (Marques et al., 2018; Lika et al., 2019; Augustine et al., 2019). However, if this type of data-type-dependent variation is truly systematic, as it appears to be in this study, then it may be possible to correct for differences in data type between parameter sets and improve the ability to detect such broad-scale patterns of life-history strategies.

DEB theory predicts that closely related species should share more similar parameter values, and our results support this: guppy parameters (when fit with the same data types) tended to cluster together when compared to interspecific variation in parameters (Fig. 5), and intraspecific variation in guppies was significantly lower than the variation seen within the family Poeciliidae. However, in our system, ecotype pairs (i.e. high-predation and low-predation ecotypes) within a stream are more closely genetically related than two populations of the same ecotype, yet DEB parameters were more similar within ecotypes than between. Our results suggest that convergent evolution can result in similarity of DEB parameters that do not reflect genetic relatedness.

Conclusions

Our objectives in this study centred around two questions: (1.) Can DEB theory characterise well known life-history variation among populations of the Trinidadian

guppy?; and (2.) Do parameterised DEB models reliably describe energy budgets, such that populations and species can be compared by the difference in their parameter values? The answer to the first question is "yes", in that DEB models reproduced population-specific patterns of growth, development, and reproduction, and qualitatively predicted known differences between guppy ecotypes in resource allocation and metabolic rate. However, the answer to the second question, at least in this study, is less clear. We showed that variation among sets of DEB parameters can result not only from biological differences (i.e. between individuals, populations, or species), but also from artefacts of the parameter estimation process. Different parameter sets can generate the same biological patterns. In some instances this may not matter, for example, if the goal is to predict a biological process based on limited data. In our study, DEB models predicted O_2 consumption rates reasonably well, considering that the models were fit using data only on age, weight, length, and number of offspring. Furthermore, qualitative differences between parameter sets were retained at different levels of data availability, suggesting that comparisons of parameter values for models fit with the same type of data can yield information on relative differences in energy budgets between populations or species. However, the goal of mechanistic modelling is to determine the causal relationships among interacting components of a system. In the context of DEB theory, this means accurately and reliably quantifying the primary parameters of the dynamic energy budget model – a feat that we did not achieve here, given the data-type dependency of estimates. Estimation of DEB parameters is not a straightforward process, but many important developments to estimation procedures have been achieved, and are on-going (Lika et al., 2011b,a, 2014; Marques et al., 2018, 2019; Lika et al., 2020). We hope that future research will establish the extent to which differences in data availability cause systematic bias in parameter estimation, and identify solutions for comparative analyses of DEB parameters. If these issues can be overcome such that DEB models accurately quantify intrinsic energetic trade-offs, dynamic energy budget theory could play a key role in the development of predictive models of life-history evolution.

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4.5 Supplementary information

Data used to fit the models

The majority of the data used to fit population-specific DEB models came from three sets of common garden experiments (Table S1). All of these experiments followed the same general protocol, described in detail in (Reznick and Bryga, 1987). Briefly, gravid female guppies were sampled from natural high- and low-predation ecotype populations and reared for two generations under standard lab conditions. Guppies were collected in 1992, 1996, and 1998, with each year representing a different dataset: we refer to each dataset by its sampling year. Male and female guppies were crossed following a breeding plan designed to avoid inadvertent selection, and to maintain the original level of genetic variation in the wild-caught females. The data used in this study are from second-generation lab-born females. Experimental treatments started 25-30 days after the birth of the focal females. After this time, females were housed individually in 8 L aquaria and subject to either high or low levels of food. For both treatments, guppies were fed twice daily with brine shrimp and liver paste. For each fish, a daily record was kept recording whether the full ration had been consumed. There was no systematic tendency for fish in the high food treatment to not consume full rations. The exact food levels differed between the three sets of experiments (details below). Focal females were paired with mature males one week into the experiment, ensuring that they mated as soon as they attained maturity, and were re-mated following each subsequent birth. Age and standard length were recorded at the onset of the experiment, and age, standard

Table S1: Population location, sampling year, and sample sizes used to estimate sixteen population-specific sets of DEB parameters. High-predation (HP) and low-predation (LP) ecotype guppies were sampled from six different river systems in three different years. Sample sizes are shown for common garden experiments in which individually housed female guppies were subject to either high or low levels of food availability. Data from fish sampled in 1991 are from (Reznick and Bryga, 1996); those sampled in 1996 are from a previously unpublished dataset (D. Reznick); data from fish sampled in 1998 are from (Reznick et al., 2004, 2005). Raw data are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.qv9s4mwf1 (Potter et al., 2021b). Formatted data and the Matlab scripts used to fit DEB models are archived on the freely-accessible online AmP database (AmP, 2020). Links to population-specific archives are provided in the table.

River system	Sampling site name	Ecotype	Year sampled	Sample size per treatment		Data and code archive
				High food	Low food	
Oropuche	Oropuche river	HP	1998	22	25	tinyurl.com/y5d7a2jo
	Campo river	LP	1998	27	30	tinyurl.com/y36qq26b
Yarra	Yarra river	HP	1991	16	16	tinyurl.com/y3rc9foa
		HP	1996	20	20	tinyurl.com/y68n2l35
		HP	1998	24	29	tinyurl.com/yyudxc8g
	Limon tributary	LP	1991	15	15	tinyurl.com/y5hq5dmc
		LP	1998	26	26	tinyurl.com/y6qlykas
Madamas	Madamas river	HP	1991	18	18	tinyurl.com/y4wolu6f
	Miguel tributary	LP	1991	17	17	tinyurl.com/yy92s7to
Marianne	Marriane river	HP	1991	15	15	tinyurl.com/y5erknxb
		HP	1996	14	14	tinyurl.com/yxsynbfm
	Marianne tributary	LP	1991	16	16	tinyurl.com/y4h5xdxt
	Marianito tributary	LP	1996	16	16	tinyurl.com/y2x97a35
Curaguate	Ricon river	HP	1996	16	16	tinyurl.com/y6q6vd5d
Paria	Paria river	LP	1996	15	15	tinyurl.com/y6cmedu3
	Paria tributary	LP	1996	14	14	tinyurl.com/yxb3zqdt

length, and wet weight were recorded at each reproductive event. Offspring number and dry weight were recorded for each litter produced. For the 1991 and 1996 datasets, data were recorded for the first three reproductive events only. For the 1998 dataset, data were recorded at each reproductive event for the entire lifespan of experimental fish (mean age at death across all treatments and populations = 842 days). Sample sizes for each experimental population are given in Table S1.

High and low food treatment levels differed between datasets. For the 1998 dataset, ration size for both food treatments increased periodically over the first 5 months of the assays, and low and high food levels were designed to allow 65% and 90% of maximum growth attainable at *ad lib* food, respectively (Figure S1, (Reznick, 1980)), replicating the food availability in natural low- and high-predation

habitats, respectively (Reznick, 1980, 1982). We estimated the energy content of the food rations based on calorific measurements of representative samples of both food types (Reznick, 1980). Data on the exact food levels used were not available for the 1991 and 1996 datasets, but we were able to estimate relative food treatment levels. Because the high-predation Yarra population is included in each of the three datasets (Table S1), we compared the average length at the first three partuitions across all datasets and food treatment levels. Mean standard length was greatest in the high food treatment of the 1996 dataset (mean SL=2.46 cm), indicating that this food treatment level was the highest. We then divided mean length in the other treatments by 2.46 cm to estimate relative food levels in terms of differences in patterns of growth between treatments. For the 1996 dataset, the estimated relative food abundances were 1 at high food and 0.94 at low food; for the 1991 dataset, estimated relative food abundance was 0.85 at high food, and 0.81 at low food.

The parameter estimation procedure (details in main text) requires information on maximum length at *ad lib* food levels. For the 1998 dataset, we estimated maximum length at *ad lib* food by multiplying the mean maximum length of guppies in the high-food treatment by 1/0.9, because the high food level in this dataset was designed to allow 90% of maximum growth. Because the 1991 and 1996 datasets do not contain data on maximum size, we had to first estimate maximum growth and then adjust estimates to account for the different food levels used in these experiments. To do this, we used the 1998 dataset to fit a linear model predicting maximum length as a function of age and length at the third partuition. This model fit the data with an adjusted R^2 value of 0.86. We then used the parameters of this model to predict the maximum size of each individual in the 1991 and 1996 datasets, based on their sizes and ages at the third partuition. We adjusted the predicted values by multiplying each value by $1/f$, where f is the relative food level in each experimental treatment (see previous paragraph). Finally, we took the mean value of our individual-level estimates, calculated for each population, as the population-specific value of maximum length at ad-lib food.

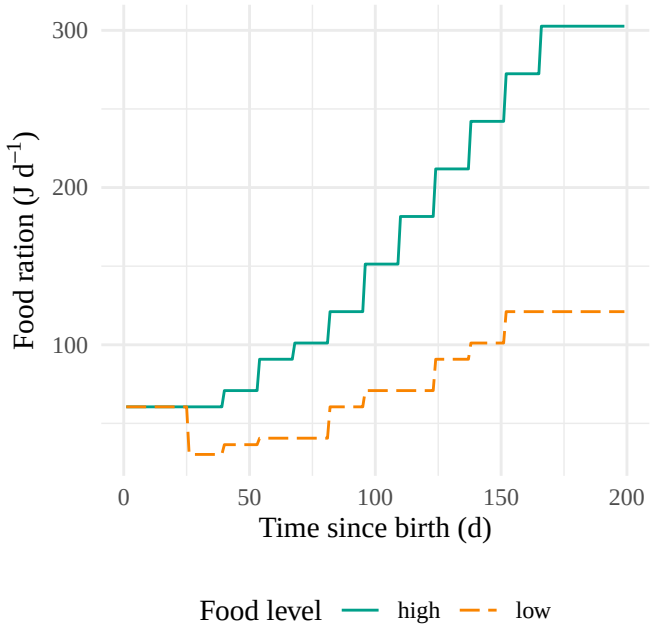

Figure S1: Quantified rations used in the 1998 experiments. 25-30 days after birth, guppies were assigned to either high (green) or low (orange) food rations. Rations increased over the first 5 months then remained constant.

Table S2: Zero-variate observations and corresponding DEB model predictions for high-predation (HP) and low-predation (LP) ecotype guppies from the Oropuche and Yarra rivers in Trinidad. Predictions (pred) and relative error (RE) are given based on models that were fit with data availability levels 1, 2, or 3. Note that O_2 consumption data were not used to fit models at data levels 1 and 2, and so the respective RE terms in this table did not contribute to the overall fit.

			Data level:	1		2		3	
Population	Data type	Unit	Observation	Pred	RE	Pred	RE	Pred	RE
Oropuche HP	Mean age at birth, high food	d	26.01	26.05	0.002	25.96	0.002	25.87	0.006
	Mean age at birth, low food	d	26.34	26.30	0.001	26.40	0.002	26.69	0.013
	Ultimate standard length	cm	3.80	3.91	0.030	3.82	0.005	3.81	0.002
	Mean dry weight at birth at high food	mg	1.09	-	-	1.11	0.020	1.07	0.016
	Mean dry weight at birth at low food	mg	1.01	-	-	0.99	0.017	0.97	0.035
	O2 consumption	mg hr^{-1}	0.025	0.033	0.315	0.018	0.280	0.025	0.015
Oropuche LP	Mean age at birth, high food	d	29.03	29.51	0.016	29.37	0.012	29.90	0.030
	Mean age at birth, low food	d	30.18	29.69	0.016	29.72	0.015	30.86	0.022
	Ultimate standard length	cm	3.51	3.36	0.044	3.53	0.004	3.31	0.057
	Mean dry weight at birth at high food	mg	1.38	-	-	1.49	0.079	1.36	0.012
	Mean dry weight at birth at low food	mg	1.45	-	-	1.35	0.072	1.22	0.157
	O2 consumption	mg hr^{-1}	0.018	0.031	0.750	0.034	0.934	0.020	0.117
Yarra HP	Mean age at birth, high food	d	23.59	24.61	0.043	24.48	0.038	24.69	0.046
	Mean age at birth, low food	d	25.76	24.73	0.040	24.81	0.037	25.20	0.022
	Ultimate standard length	cm	3.59	3.88	0.080	3.63	0.012	3.56	0.009
	Mean dry weight at birth at high food	mg	0.81	-	-	0.85	0.052	0.81	0.003
	Mean dry weight at birth at low food	mg	0.81	-	-	0.77	0.054	0.75	0.078
	O2 consumption	mg hr^{-1}	0.029	0.039	0.360	0.033	0.146	0.030	0.029
Yarra LP	Mean age at birth, high food	d	27.33	29.00	0.061	28.91	0.058	28.86	0.056
	Mean age at birth, low food	d	31.08	29.22	0.060	29.33	0.056	29.78	0.042
	Ultimate standard length	cm	3.33	3.92	0.175	3.36	0.008	3.37	0.011
	Mean dry weight at birth at high food	mg	1.02	-	-	1.12	0.098	1.10	0.074
	Mean dry weight at birth at low food	mg	1.10	-	-	1.01	0.087	0.98	0.105
	O2 consumption	mg hr^{-1}	0.023	0.034	0.436	0.030	0.274	0.022	0.040

Table S3: Comparison of the mean relative error (MRE) when models are fit using the population-specific parameter set and null parameter sets. We determined the significance of the differences in fit using Wilcoxon's signed rank tests of the relative error terms of predictions to data when fit with different parameter sets.

Population	Parameter set	MRE	Wilcoxon signed rank test	
			t	p-value
High Predation Oropuche (HPO)	Best (HPO)	0.076	-	-
	Null 1 (LPO)	0.203	3.92	0.0014
	Null 2 (HPY)	0.191	6.71	7.0×10^{-6}
High Predation Yarra (HPY)	Best (HPY)	0.086	-	-
	Null 1 (LPY)	0.199	4.06	0.0010
	Null 2 (HPO)	0.206	6.71	7.0×10^{-6}
Low Predation Oropuche (LPO)	Best (LPO)	0.099	-	-
	Null 1 (HPO)	0.245	6.34	1.3×10^{-5}
	Nul 2 (LPY)	0.142	1.09	0.2900
Low Predation Yarra (LPY)	Best (LPY)	0.085	-	-
	Null 1 (HPY)	0.257	5.19	0.0001
	Null 2 (LPO)	0.147	4.06	0.0010

Table S4: Dynamic energy budget parameters for sixteen populations of Trinidadian guppy, estimated using level 1 data availability (see Table 3 in the main text for details). Parameters that were fixed, and therefore not estimated, were: the assimilation efficiency κ_X=0.25; the maximum specific searching rate $\{\dot{F}_m\} = 6.5$ L d^{-1} cm^{-2}; the reproductive efficiency $\kappa_R = 0.95$; the surface area specific somatic maintenance $\{\dot{p}_T\} = 0$ J d^{-1} cm^{-2}; and the Arhennius temperature = 8000 K. Parameters are given for a reference temperature of 20 °C. The mean relative error (MRE) quantifies the fit of the models to the data.

River system	Sampling site name	Ecotype	Year sampled	Parameter: MRE	$\{\dot{p}_{Am}\}$	$\dot{v}$	κ	$[\dot{p}_M]$	$\dot{k}_{\mathcal{J}}$	E_G	E_H^b	E_H^p	z	δ_M	f_{high}	f_{low}	t_0
Oropuche	Oropuche river	HP	1998	0.109	116.7	0.028	0.55	68.2	0.0019	5220	36.9	298.3	0.94	0.24	0.86	0.79	5.2
	Campo river	LP	1998	0.119	108.2	0.029	0.70	95.1	0.0020	5228	12.8	146.5	0.80	0.24	0.86	0.79	9.0
Yarra	Yarra river	HP	1991	0.099	154.3	0.030	0.61	98.2	0.0021	5223	8.2	306.7	0.95	0.22	0.85	0.69	7.0
		HP	1996	0.089	172.2	0.026	0.65	120.7	0.0021	5220	5.4	446.2	0.92	0.21	1.00	0.83	8.0
		HP	1998	0.119	175.0	0.029	0.61	120.1	0.0020	5225	25.2	382.5	0.89	0.23	0.86	0.81	6.0
	Limon tributary	LP	1991	0.118	110.5	0.028	0.79	90.4	0.0020	5220	4.6	126.2	0.97	0.24	0.85	0.71	8.6
		LP	1998	0.137	156.4	0.027	0.69	121.0	0.0020	5222	8.8	238.9	0.89	0.23	0.86	0.76	9.8
Madamas	Madamas river	HP	1991	0.100	147.5	0.032	0.67	104.4	0.0020	5233	11.6	269.5	0.94	0.23	0.85	0.70	7.0
	Miguel tributary	LP	1991	0.134	134.0	0.033	0.76	96.5	0.0021	5191	6.0	203.6	1.06	0.24	0.85	0.67	7.4
Marianne	Marianne river	HP	1991	0.109	155.6	0.022	0.56	94.7	0.0022	5232	10.4	374.2	0.92	0.22	0.85	0.74	5.6
		HP	1996	0.106	164.6	0.026	0.73	138.5	0.0020	5241	5.6	301.9	0.86	0.22	1.00	0.86	7.8
	Marianne tributary	LP	1991	0.123	136.4	0.030	0.72	105.9	0.0020	5216	7.0	157.0	0.93	0.24	0.85	0.67	7.0
	Marianito tributary	LP	1996	0.081	132.9	0.020	0.75	128.5	0.0020	5230	2.8	234.3	0.78	0.21	1.00	0.94	8.7
Curaguate	Ricon river	HP	1996	0.076	172.0	0.024	0.66	132.3	0.0021	5229	4.7	331.1	0.86	0.21	1.00	0.86	7.1
Paria	Paria river	LP	1996	0.101	141.9	0.036	0.76	102.9	0.0021	5219	3.8	294.0	1.05	0.25	1.00	0.83	8.9
	Paria tributary	LP	1996	0.090	149.6	0.024	0.78	130.8	0.0020	5225	2.1	218.5	0.89	0.22	1.00	0.87	8.5

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5

Size-dependent resource limitation determines the direction and pace of life history evolution: a simulation study

Abstract

Density dependence through food limitation is widespread in natural populations. When the competitive ability of individuals depends on an aspect of an individual's phenotype, such as body size, competition is said to be asymmetric, and the effects of density dependence will also be asymmetric. Here, we investigate how density dependence and competitive asymmetry influence life history evolution. We constructed individual-based models, in which individuals were defined by the phenotypic traits used in dynamic energy budget theory (DEB-IBMs). We included heritable variation in κ, which is the fraction of energy that an individual allocates to growth and somatic maintenance (rather than to reproduction). In our simulations, ecological and evolutionary dynamics were emergent properties of the demographic rates of individuals in the context of their environment. The degree of competitive asymmetry had strong effects on the size, dynamics, and structure of populations, altering the direction and pace of life history evolution. Whether selection favoured increased allocation to somatic growth or to reproduction depended, respectively, on whether large or small individuals were superior competitors. Differences in the degree of competitive asymmetry altered how heritable variation in κ generated variation in demographic rates. Ultimately, the direction of life history evolution depended on the balance of the trade-off between juvenile survival and adult reproductive rates: changing the degree of competitive asymmetry changed this balance, by modifying the way that density dependence operated on the population.

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5.1 Introduction

The unpleasant fact that we usually know little about the way density dependence operates in real populations should not seduce us to pursue an ostrich-policy.

Mylius & Diekmann, 1995

When populations are large, they tend to decline in size; when they are small, they tend to increase. This process – known as density dependence – captures the self-regulation of populations, and has long been of interest to both ecologists and evolutionary biologists (e.g. Nicholson and Bailey, 1935; Smith, 1935; Dobzhansky, 1950; Haldane, 1953; Ricker, 1954; Lewontin, 1955; Pimentel, 1961, — discussed in Bassar et al 2010 and Travis et al 2013). In the context of the effects of density dependence on fitness, MacArthur and Wilson (1967) introduced the concepts of r- and K-selection: at low population densities, selection should maximise population growth rate r, whereas at high population densities, selection should acts to increase the carrying capacity K of the population. Pianka (1970) extended this concept to define life history strategies as representing suites of r- or K-selected traits: r-strategists were defined as having rapid development and high investment in reproduction (leading to increases in population growth rates), while K-strategists were characterised by increased competitive ability, delayed development, larger body sizes, and lower rates of reproduction (facilitating increases in the population

carrying capacity). Although initially highly influential, the framework fell from grace in the 1980s and 1990s (Reznick et al., 2002). The chief criticism was that the theory over-simplified life history evolution (Stearns, 1976, 1977). Under Pianka's (1970) classification, K-selected life history traits (i.e. those favoured in density-dependent populations) were simply assumed to be the opposite of r-selected traits (i.e. those favoured in density-independent populations), rather than being derived logically from MacArthur and Wilson's (1967) concepts of r- and K-selection (Reznick et al., 2002). This theoretical weakness was underscored by inconclusive empirical results: although some observational studies supported the notion of r-K life histories (Gadgil and Solbrig, 1972; McNaughton, 1975), others did not (Tinkle and Hadley, 1975; Wilbur, 1976). Experimental evolution studies demonstrated density dependent selection for increased competitive ability (validating MacArthur and Wilson's concept of K-selection), but without the predicted r-K life history differences (Mueller, 1988; Bierbaum et al., 1989; Mueller et al., 1991).

The concept of r-K life histories was superseded by demographic life history theory, which incorporated age- and stage-dependent mortality as drivers of life history evolution (Stearns, 1977; Charlesworth, 1994). The concept of fitness trade-offs – where an increase in once component of fitness results in a decrease in another – is central to demographic life history theory (Stearns, 1989). Given that individuals have a finite amount of energy, investment in reproduction should come at a cost to survival. Density dependence acting on different life-history stages can alter the balance of fitness trade-offs, changing the optimum life-history strategy. In general, selection will maximise the number of individuals in the life-history stage that is subject to density dependence (Charlesworth, 1994). For example, in a population structured by adult and juvenile stages, when density dependence acts to reduce survival in juveniles, selection will favour earlier maturity at smaller sizes and increased investment in reproduction – leading to an increase in the number of juveniles in the population (Charlesworth, 1994; Reznick et al., 2002). As such, selection for a so-called r-strategist (sensu Pianka 1970) can in fact result from density dependence. The failure to reliably identify K-strategists was

ultimately due to the simplification of the effects of density dependence: to predict life history evolution in response to density dependence, the crucial factor is to identify which stage (or which demographic rate in that stage) density dependence acts upon (Charlesworth, 1994; Mylius and Diekmann, 1995). As the epigraph above warns, identifying how density dependence acts is challenging yet essential for understanding life history evolution (Mylius and Diekmann, 1995).

It is important to distinguish between density dependent fitness and density dependent selection (Reznick et al., 2002; Travis et al., 2013). The former occurs when fitness changes with population density; the latter requires that the rank order of fitness of different genotypes changes with increasing density (Travis et al., 2013). When density dependent selection occurs, the strength and possibly the direction of selection fluctuates with the dynamics of the population, which may result in balancing selection that acts to constrain life-history evolution (Lande et al., 2009; Engen et al., 2013; Sæther and Engen, 2015). Balancing density dependent selection has been demonstrated to be operating on clutch size in great tits (*Parus major*), where females that produced larger clutches had a fitness advantage at low population densities, but suffered reduced fitness at high population densities: overall, selection favoured an intermediate clutch size (Sæther et al., 2016). Life history evolution in a fluctuating environment ultimately depends on how the demographic rates of different genotypes respond to changes in population density. Fitness – i.e. the net product of individual demographic rates – is an emergent property of the interaction between the expressed phenotype and the environment (Coulson et al., 2006). To understand how fitness trade-offs arise in a fluctuating environment, it is therefore crucial to identify how changing environmental factors interact with the genotypic variation underlying phenotypes, to alter demographic rates.

In this study we investigate how size-dependent food limitation affects fitness trade-offs in fluctuating populations. Food limitation is often central to density dependent regulation of populations, and when the outcome of competition for food depends on trait variation, competition is said to be asymmetric (Persson, 1985). Competitive asymmetry often depends on body size in many natural systems.

For example, in fishes, competitive ability often increases with body size (Ward et al., 2006), meaning that larger individuals have access to more food than smaller individuals. Larger fish often have higher feeding rates, giving them an advantage under scramble competition (e.g. Mittelbach, 1981), are better able to defend territories and monopolise resources through interference competition (e.g. Webster and Hixon, 2000), and in gape-limited feeders, are able to occupy a broader resource niche (Scharf et al., 2000). However, smaller fish have lower energetic requirements, and will have a competitive advantage if food availability scale with size to a lesser degree than energetic maintenance costs (Persson, 1985). As such, the impact of density dependence is likely to vary among individuals dependent on the degree and direction of size-dependent resource limitation. Theoretic work has demonstrated that population dynamics and demographic rates are drastically altered depending on whether competitive asymmetry favours large or small individuals (De Roos et al., 2003; Bassar et al., 2016), but how these effects manifest in a fluctuating environment – and their consequences for life history evolution – have been little tested.

Here, we use population simulations to assess how size-dependent resource limitation influences fitness and selection to shape life history evolution in a fluctuating environment. Life histories depend on the interaction between: (i) the intrinsic energetic trade offs within an organism that govern how energy is used and allocated to different biological processes, reflecting (heritable) covariation among traits; and (ii) the extrinsic, ecological factors that impact survival and reproduction, such as resource competition and predation risk (Stearns, 2000). To simulate life history evolution, the underlying model must capture the interaction between these intrinsic and extrinsic factors in an evolutionarily explicit framework.

In our simulations, the intrinsic energetic trade-offs within an individual follow from dynamic energy budget (DEB) theory (Kooijman, 2010). DEB theory provides a generic model for an individual's life history in terms of fluxes of energy to different biological processes such as growth, somatic maintenance, the attainment of sexual maturity, and reproduction (Kooijman, 2001; Sousa et al., 2008; Kooijman, 2010). The balance of fluxes between different biological processes depends on the primary

parameter values of the model, which represent fundamental traits of the individual relating to the acquisition, allocation, and use of energy. In theory, an individual's set of DEB parameter values is analogous to it's genotype, in that parameter sets: (i) interact with environmental conditions (food density, temperature) to create the individual's phenotype; (ii) are assumed to be fixed over an individual's life; and (iii) are assumed to be more similar among related individuals (Kooijman, 2010; Lika et al., 2011; Marques et al., 2018). DEB parameters can be estimated from data on the growth, development, and reproduction of organisms reared under varying environmental conditions (van der Meer, 2006; Kooijman et al., 2008; Kooijman, 2010). A paramaterized DEB model characterises the intrinsic energetic trade-offs that constrain the expression of an individual's life history. However, it does not capture the extrinsic ecological factors – such as resource competition – that drive life history evolution.

Population models in which individuals are characterised by DEB parameters (DEB-IBMs, Martin et al., 2012) can bridge the gap between the intrinsic constraints that DEB theory describes, and the extrinsic forces that drive life history evolution. In DEB-IBMs, individuals compete for food indirectly by feeding on a shared resource generating population dynamics that are predicted by the traits (i.e. DEB parameters) of individuals (Martin et al., 2013; Beaudouin et al., 2015). This framework generates density dependence and fluctuations in population size through the demographic rates of individuals and their effects on resource availability. However, DEB theory inherently assumes that individuals are competitively superior when they are small, because energetic assimilation rates scale with surface area (L^2) while maintenance costs scale with volume (L^3) (Kooijman, 2010; van der Meer, 2016). This means that the ratio of assimilated energy over maintenance costs decreases with increasing size (L), such that smaller individuals acquire more energy relative to their energetic demands. As a result, in the absence of extrinsic regulation of juveniles, populations become dominated by juveniles who deplete resource availability, suppressing the reproduction and survival of adults (van der Meer, 2016). In our simulations, we introduce extrinsic variation in size-dependent

resource limitation, which acts to shift competitive asymmetry such that smaller, rather than larger individuals are limited by resource availability. We also expand the standard DEB-IBM by introducing heritable variation in DEB parameters, thus allowing ecological and evolutionary dynamics to occur simultaneously. Together, these additions allow us to assess how fitness trade-offs manifest under size-mediated density dependence in a fluctuating environment.

We show that when resource availability was independent of size, selection favoured earlier maturation and increased reproductive effort. As the strength of size-dependent resource limitation increased, the fitness trade-off between juvenile survival and adult reproduction changed such that selection favoured increased somatic growth rates and decreased investment in reproduction. In all cases, fitness was density dependent, but in some scenarios selection was density dependent too. Our results demonstrate that size-dependent resource limitation can drastically alter how density dependence operates, generating diverging trajectories of life history evolution.

5.2 Methods

Overview of methods

We constructed an evolutionarily explicit DEB-IBM for a freshwater fish species, the Trinidadian guppy (*Poecilia reticulata*, described below) . In our model, individuals differ only in the value of one fundamental trait, κ, which is the fraction of energy that is allocated to somatic work (i.e. growth and maintenance) rather than to non-somatic work (i.e. maturation and reproduction). Individuals inhabit a landscape grid, and consume food within the patch that they are occupying. Feeding depletes the available food in the patch, and food regrows following a logistic growth function. When individuals experience low food levels, they move at random to one of the adjacent patches. Once individuals are mature, and if they have sufficient energy, they produce offspring. Reproduction is asexual and offspring inherit the parameter values of their mother. Individuals can die through starvation or senescence.

In our simulations, ecological dynamics (changes in total population size) and evolutionary dynamics (changes in the distribution of the heritable trait κ) are emergent properties of the vital rates (i.e. growth, maturation, reproduction, and survival) of individuals. Variation among individuals in vital rates depends on variation in κ, and also on variation in resource availability. The dynamics of resource availability are driven by total rates of consumption (which depends on the number and size of individuals occupying a patch) and by the rate at which food grows back.

We tested how size-dependent resource limitation influenced ecological and evolutionary dynamics by running our model over four different scenarios: a null scenario of no size-dependence of resource availability, and three scenarios ranging from weakly to strongly size-dependent resource limitation. These scenarios represent a shift in competitive asymmetry from favouring small to large individuals. We analysed our simulated data to determine how components of fitness (survival and reproduction) varied across our experimental scenarios, as functions of the heritable trait κ and population density. Finally, we characterised the balance of emergent trade-offs between fitness components, and described how these determined life history evolution in our simulations.

Study species: Trinidadian guppies

We used Trinidadian guppies (*Poecilia reticulata*) as a model system in our simulations. Guppies are small, live-bearing, freshwater fish endemic to Trinidad and Venezuela, and are a classic system for studying life history evolution (Magurran, 2005). Natural guppy populations have evolved distinct life histories which ultimately depend on the strength of predation pressure that they experience (Reznick and Endler, 1982; Reznick, 1982; Reznick and Bryga, 1987). In lowland streams and rivers, guppies live in communities characterised by high levels of resource availability and the presence of large predatory fish (Reznick et al., 2001). These "high-predation" guppies have fast life histories, with high reproductive rates and rapid maturation (Reznick and Endler, 1982; Reznick, 1982; Reznick and Bryga, 1987). Further upstream, above waterfalls which act as barriers to the movement of

large predators, guppies live in low predation-risk environments, establishing large, density-regulated populations that experience low levels of resource availability (Reznick et al., 2001; Bassar et al., 2013). "Low-predation" guppies have slower life histories than their high-predation ancestors, producing fewer but larger offspring per litter, and maturing at larger sizes and greater ages (Reznick and Endler, 1982; Reznick, 1982; Reznick and Bryga, 1987). We have previously estimated DEB parameters for multiple populations of high- and low-predation guppies (Potter et al., 2021b), showing that the fraction of energy allocated to somatic work (i.e. growth and somatic maintenance) – the primary DEB parameter κ – is consistently higher in low-predation guppies relative to their high-predation ancestors. The evolution of the low-predation life history (associated with high values of κ) has occurred repeatedly and independently in several streams across Trinidad (Reznick, 1982; Shaw et al., 1992; Alexander et al., 2006; Reznick et al., 2019; Whiting et al., 2020). Experimental introductions, field manipulations, common garden experiments, and quantitative genetic analyses have demonstrated that the low-predation life history evolves in response to high population densities (and lower resource availability)(Bassar et al., 2013; Reznick et al., 2019; Potter et al., 2021a), rather than as a direct result of reduced predation pressure (Reznick et al., 1996, 2019).

Empirical work and predictions based on DEB parameters show that, even at low food levels, high-predation guppies always produce more offspring than low-predation guppies (Reznick et al., 2004, 2005; Potter et al., 2021b). However, these predictions and observations do not consider how density dependence (through resource competition) alters fitness trade-offs in high- and low-predation guppies. If reproduction trades off against competitive ability, low-predation guppies may have a fitness advantage at high population densities when resource competition is intense. Larger size confers a competitive advantage in guppies (Bashey, 2008; Potter et al., 2019; Griffiths et al., 2020). Given that low-predation guppies are larger at birth and maturity than high-predation guppies, it seems likely that increased investment in offspring size – which presumably acts to increase juvenile survival – represents an adaptation to living in competitive environments under density

dependence. However, it is unclear how the strength of size-dependent competition alters the trade-off between juvenile survival and adult reproduction. In this study, our simulations recreate the establishment of populations of guppies in predator-free environments, while allowing us to test how the strength of size-dependent resource limitation – an environmental factor that determines the outcome of size-dependent competition – modifies the direction and pace of life history evolution.

The individual based model

Our model builds on the standard dynamic energy budget individual based model (the DEB-IBM, Martin et al., 2012). In the DEB-IBM, each individual is defined by four main state variables: structure, reserve, maturity level, and the reproductive buffer. Structure describes the soma of the individual, and so determines body size. Reserve is the amount of energy assimilated from the environment which is available to fuel metabolic processes. The maturity level quantifies the amount of energy invested in attaining maturity, and determines the transitions between embryo, juvenile, and adult life history stages. The reproductive buffer quantifies the amount of energy available for producing offspring. A fifth state variable (which is not central to the standard DEB model) is the probability of mortality due to senescence. Rates of change to these state variables are determined by the system of differential equations which define the standard DEB model (Kooijman et al., 2008; Kooijman, 2010). Under the standard DEB model, an individual assimilates energy from their environment and into their reserve by feeding. Energy in the reserve is then mobilised to fuel metabolic processes. A fraction (κ) of the mobilised energy is allocated to somatic work, with the remainder (1 - κ) allocated to non somatic work. Somatic work includes somatic growth and maintenance costs, whereas non somatic work includes the energy invested in maintaining maturity, increasing maturity level (i.e. development in embryos and juveniles), and reproduction (i.e. offspring production in adults). For both somatic and non somatic work, maintenance costs must be paid before energy can be used for growth, development, or reproduction (Fig. 1).

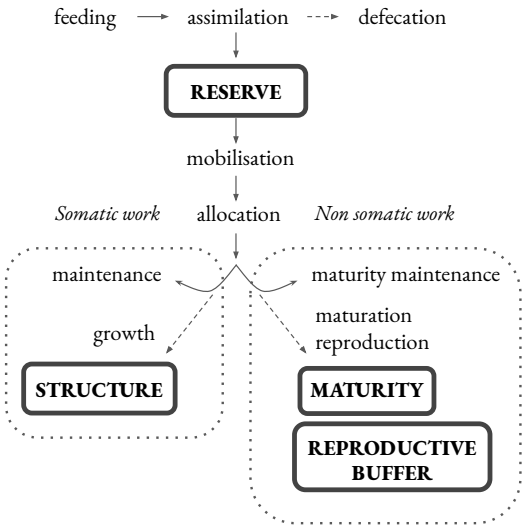

Figure 1: Schematic of the standard dynamic energy budget model, reproduced from Potter et al (2021b). In the DEB-IBM, each individual is defined by four state variables (in boxes). Arrows depict energetic fluxes through different biological processes (plain text) over the full life history of the organism. Individuals begin life as embryos (no feeding, no reproduction), developing into juveniles (feeding, no reproduction) and then mature adults (feeding and reproducing). The rates of change of the state variables are determined by the individual's primary parameter values (see Table 1) and the density of food in its environment.

Table 1: Primary DEB parameters, the main biological processes that they control, and parameter values used in this study. Symbols follow the standard DEB notation (Kooijman, 2010). The parameter values used in this study are for high-predation ecotype guppies (the "Oropuche HP" population from Potter et al., 2021b). * Note that individual values of the allocation fraction to soma, κ were drawn from a normal distribution with a mean of 0.43 and a standard deviation of 0.05. All other primary parameter values were identical among individuals.

Primary DEB parameter	Primary biological process	Value	Units
Max searching rate, $\{\dot{F}_m\}$	Feeding	6.5	$m^2\ cm^{-2}\ d^{-1}$
Assimilation efficiency, κ_X	Assimilation / defecation	0.27	-
Energy conductance, $\dot{v}$	Mobilisation of energy	0.029	$cm\ d^{-1}$
Allocation fraction to soma, κ	Allocation of energy	* 0.43	-
Vol-spec somatic maint, $[\dot{p}_M]$	Somatic maintenance	27.3	$J\ d^{-1}\ cm^{-3}$
Specific cost for structure, E_G	Growth	5223	$J\ cm^{-3}$
Maturity maint rate coefficient, $\dot{k}_J$	Maturity maintenance	0.002	d^{-1}
Maturity at birth, E_H^b	Maturity threshold: embryo to juvenile	26.6	J
Maturity at puberty, E_H^p	Maturity threshold: juvenile to adult	501.8	J
Reproductive efficiency, κ_R	Reproduction	0.95	-
Weibull ageing acceleration, $\dot{h}_a$	Ageing	2.03×10^{-8}	d^{-2}
Gompertz stress coefficient, S_G	Ageing	0.062	-

In the DEB-IBM, individuals can be specified by twelve primary DEB parameters: ten that determine the ten biological processes depicted in Figure 1, and two that determine ageing through senescence (Table 1). Rates of change to the state variables of an individual depend on its primary DEB parameters, and on the density of food in the environment (Martin et al., 2012). During the simulation, the state of each organism – its structure, reserve, maturity level, reproductive buffer, and probability of mortality due to senescence – is updated each time step. Changes in the state of the organism can result in discrete outcomes relating to development, reproduction, and survival. At specific thresholds, changes in the maturity level will change the status of an individual from embryo (which do not feed), to juvenile (which feed but do not reproduce), and finally to mature adult (which feed and reproduce). If the reproductive buffer of mature adults contain sufficient energy, they produce one embryo. If there is insufficient energy in the reserve to pay somatic maintenance costs, the individual dies of starvation. Finally, if the probability of death due to ageing exceeds the value of a randomly drawn number, the individual senesces and dies.

We made four main additions to the standard DEB-IBM: 1.) Our model is spatially explicit, consisting of a grid of 16 x 16 habitat patches – the dynamics of food density operate independently in each patch. 2.) Individuals can move to adjacent patches following a food-dependent sub-model: when an individual's assimilation rate is less than 50% of its maximum, it will move at random to one of the eight adjacent patches in the next time step. 3.) We include a model of inheritance: reproduction is asexual, so offspring inherit their parent's primary DEB parameter values. 4.) In our model, all individuals share the same primary DEB parameters, except for κ. The "founders" of the population are assigned values of κ drawn from a normal distribution. This is the sole source of heritable variation among individuals in the model, generating variation in offspring and adult size, size and age at maturity, and rates of reproduction (Fig. 2).

By making the model spatially explicit and allowing individuals to move, additions 1 and 2 means that individuals on different patches will experience different

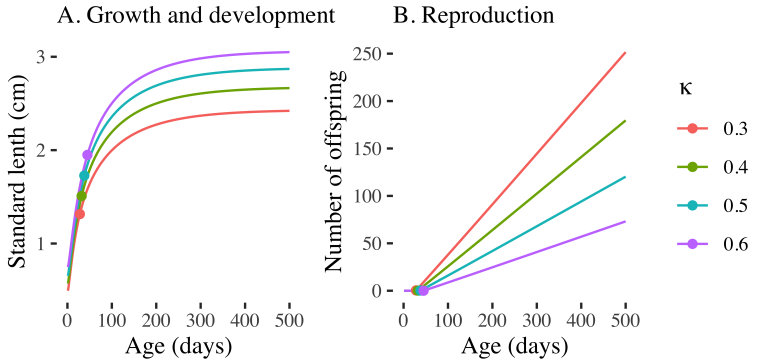

Figure 2: Variation in intrinsic demographic rates as a function of κ. Predictions of (A) growth and (B) reproduction, from dynamic energy budget models fit with the parameter values in Table 1 (except for κ, values for which are given in the plot legend). (A) shows change in standard length (cm) with age (days) for individuals with different values of κ (given in different colours). The y-intercepts indicate standard length at birth, and the points indicate the size and age at sexual maturity. (B) shows the cumulative number of offspring produced with age for individuals with different values of κ. These simulations assume no effects of competition, constant food density at 150 J m^{-2}, and a constant environmental temperature of 26 °C.

levels of food availability, introducing a degree of environmental heterogeneity into the model. By including asexual inheritance of a single quantitative trait (additions 3 and 4), the model becomes evolutionarily explicit in simple, tractable terms.

Running simulations: parameters, experimental scenarios, data output

We parameterised our model to simulate the introduction of high-predation ecotype guppies to a predator-free environment, replicating *in silico* the foundation of real-world experimental populations (Travis et al., 2014; Reznick et al., 2019). We used DEB parameters that accurately reproduce the resource-dependent growth, development, reproduction, and senescence of high predation guppies (Potter et al., 2021b, Table 1). Simulations were initiated with 100 "founders", with individual-specific values of κ drawn from a normal distribution with a mean of 0.43 and a standard deviation of 0.05. We chose to use 100 founders, as this is comparable to the number of founders used in the real-world experimental introduction that we are emulating (Travis et al., 2014).

We defined the general environment as a grid of 16 x 16 habitat patches, with each habitat patch representing an area of 2 m^2. The regrowth of food in each

patch followed a logistic growth function, with growth rate $r_X = 15$ J d^{-1}, and carrying capacity $K_X = 150$ J m^{-2}.

To determine how size-dependent food limitation influences the ecological and evolutionary dynamics that emerge from our model, we defined four experimental environments in which to run the simulation. These environments differed in the extent to which food availability depends on body size: at the start of the simulation, we assigned a value to each patch which describes the minimum body size an individual must have to be able to feed on that patch. These values were drawn at random from a gamma distribution with rate parameter β=0.5, and shape parameters α ranging from 0 to 2 (Fig. 3). The four different levels of α define the four scenarios tested in this study, and we refer to these values to distinguish trials throughout the manuscript. When $\alpha = 0$, there is no size-dependence of resource availability: individuals of any size can feed in any patch in the environment – this is the null scenario in our study. As the value of α increases, the proportion of patches in which small individuals can feed decreases: size-dependent resource limitation ranges from weak to strong as α increases (Fig. 3).

Models ran for a simulated period of 100 months (1 month = 30 days), with 12 time steps per day. We ran each experimental scenario (i.e. different values of α) five times. We recorded data from the simulations after every thirty days. At the population level, we recorded the total number of embryos, juveniles, and adults alive at the time of data recording. At the individual level, for each juvenile and adult alive at the time of sampling, we recorded the values of each state variable, their value of kappa, the total number of embryos produced in each thirty day period, and the identification code of their parent. All simulations were performed in the agent-based modelling framework NetLogo, version 6.0.4 (Wilensky, 1999).

Analyses of simulated data

In our simulations, κ was the sole source of heritable variation upon which selection could act. To determine how fitness trade-offs arose in fluctuating environments, we first described the link between κ and the phenotype at quasi-equilibrium in each of

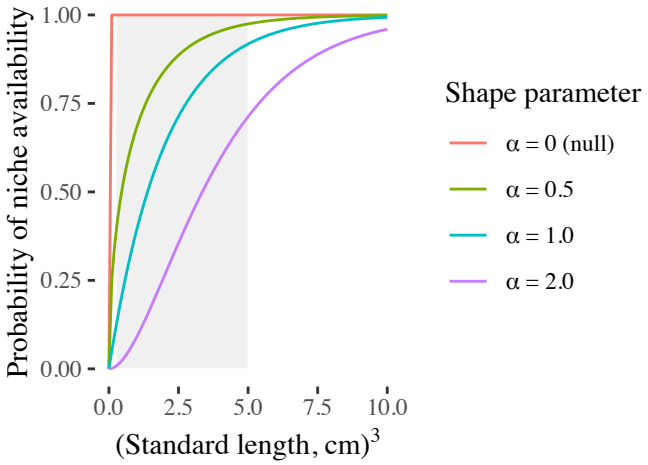

Figure 3: Probability of resource availability (y-axis) by body size (x-axis) under the different scenarios in our simulations (shape parameters α). Lines are the cumulative distribution of gamma functions with rate parameter $\beta = 0.5$, and four different values for the shape parameter α, which define the four levels of size-dependent resource limitation tested in this experiment. In our simulations, the ability to feed in a given patch depends on standard length cubed, which is proportional to body mass. The grey region highlights the juvenile size range in our simulations, from newborn individuals up to maturity.

the scenarios of size-dependent resource limitation. Specifically, we determined how variation in κ influenced variation in monthly somatic growth rates and variation in fecundity (the number of embryos produced by an individual over a month) at the mean population density in each scenario. We then linked variation in κ to variation in components of fitness across the range of population densities, describing how survival and reproduction (i.e. the number of embryos that successfully recruit into the population) vary as a function of population density, body size, and κ.

We fit models to describe the phenotype and demographic rates of individuals over a single timestep, i.e. over one month. For each scenario of size-dependent food limitation, we estimated somatic growth rates (the absolute change in standard length), the probability of survival, fecundity, and reproduction. Each of these regression models included size (standard length, cm), κ, population density (N m^{-2}), and their interactions as fixed effects, and separate models were fit for each scenario of α. We fit models with Gaussian (growth), Poisson (fecundity, reproduction), or binomial (survival) error structures. All analyses were conducted in R version 4.0.5 (R Core Team, 2019).

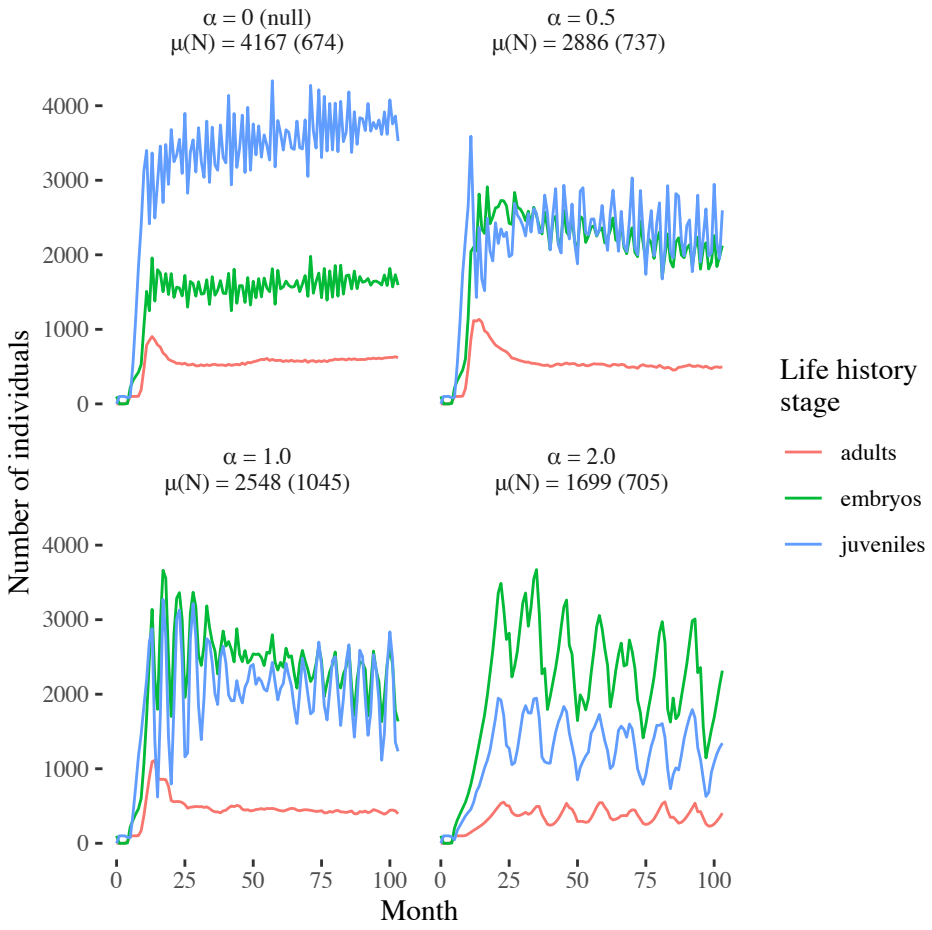

Figure 4: Population dynamics over a 100-month period under different scenarios of size-dependent resource limitation (α). Lines describe the total number of embryos (green), juveniles (blue), and adults (red) sampled at monthly intervals. Monthly values are the means of five simulations per scenario. The mean population size after the initial growth period (>24 months) is given for each scenario ($\mu(N)$, standard deviation in parentheses, population size is the combined number of adults and juveniles).

5.3 Results

Population dynamics

The strength of size-dependent resource limitation strongly influenced the size, dynamics, and stage structure of the populations (Fig. 4). The mean population size $\mu(N)$ was highest in the null scenario, and decreased as the strength of size-dependent resource limitation (α) increased. This change in population size across scenarios was driven by a decrease in the number of juveniles as α increased. However, the decrease in juveniles was not due to a decrease in adult fecundity: embryo

production increased across scenarios with increasing α. In all scenarios, adults made up the smallest demographic, and the mean number of adults was similar across scenarios. The mean body size of individuals in the population increased as α increased (mean body size $\bar{z}$ for each scenario, standard deviation given in parentheses: $\bar{z}_{\alpha=0}$=11.0 mm (0.5 mm), $\bar{z}_{\alpha=0.5}$=12.2 mm (0.8 mm), $\bar{z}_{\alpha=1}$=12.7 mm (0.8 mm), $\bar{z}_{\alpha=2}$=13.7 mm (1.0 mm)). Fluctuations in the size of each life history stage increased in periodicity across scenarios with increasing α. Trends in the stage structure of the population (e.g. increasing number of juveniles at α=0, decreasing number of embryos at α=2, Fig. 4) reflected ongoing evolutionary change over the course of the study. These trends meant that population size increased over time in the null model of no size-dependent resource limitation (α=0), whereas population size declined over time in the other scenarios (α= 0.5, 1, 2).

Evolutionary change

Over the duration of the simulations, the direction and rate of evolutionary change (i.e. the change in the population mean values of κ) differed between scenarios of size-dependent resource limitation, but was broadly consistent among repeated runs within scenarios (Fig. 5). In all scenarios, the direction and magnitude of evolutionary change varied over time. The mean value of κ declined sharply in the first few months in all scenarios, reflecting the earlier maturity and higher rates of reproduction of individuals with low values of κ during the initial stage of population growth. Size-dependent resource limitation determined the overall trend in evolutionary dynamics: when size-dependent resource limitation was absent (α= 0), the mean value of κ declined over time; at weak to intermediate size-dependent resource limitation (α= 0.5, 1), the mean value of κ initially decreased, then began increasing after about 36 months; when size-dependent resource limitation was strongly limiting for small individuals (α=2), the mean value of κ increased over time (Fig. 5).

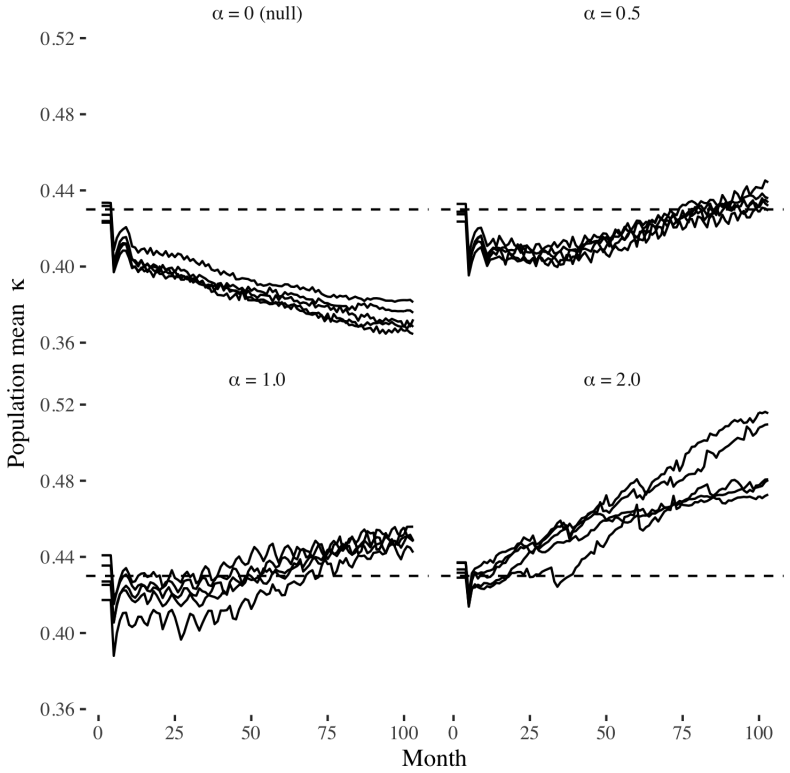

Figure 5: Evolutionary dynamics of κ over the course of the simulations, for different scenarios of size-dependent resource limitation (α). We ran each scenario five times: lines trace the population mean value of κ every month for each of the simulations. The dashed horizontal line at $\kappa = 0.43$ highlights the initial mean value of κ.

Linking κ to traits, demographic rates, and density dependence

Across all scenarios, higher values of κ led to increased somatic growth rates and decreased fecundity, reflecting the energetic trade-off between growth and reproduction inherent to DEB theory (Fig. 6). Growth and fecundity both increased as α increased. This reflected the decrease in mean population density (and increase in mean food density) across scenarios as α increased.

Juvenile survival probability decreased with increasing population density in all scenarios (Fig. 7A). Variation in κ had little effect on juvenile survival in the null scenario, but as α increased, survival was highest for juveniles with high values of κ, and this advantage increased with increasing population density. Across the typical range of population densities in each scenario (grey shaded regions in Fig. 7A), adult survival was relatively insensitive to changes in population

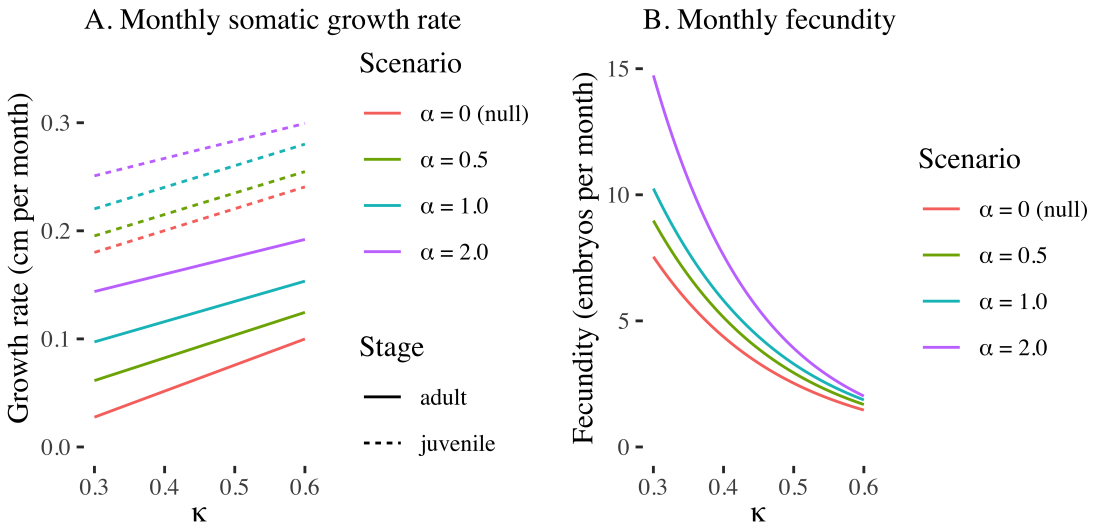

Figure 6: Linking κ to traits at the mean population density in each scenario of size-dependent resource limitation (α). Somatic growth rate (A) is the monthly change in standard length (mm) for juveniles (dashed lines, initial length = 8 mm) and adults (solid lines, initial length = 24 mm), as a function of κ and population density. Fecundity (B) is the number of embryos produced by an adult (standard length = 24 mm) as a function of κ and population density. The mean density of food (μ_X) increased across scenarios with increasing α. When: α=0, μ_X=45.1 J m^{-2}; α=0.5, μ_X=49.3 J m^{-2}; α=1, μ_X=59.8 J m^{-2}; α=2, μ_X=92.9 J m^{-2} .

density, although in general adults with high values of κ had slightly lower survival probability at higher densities.

The reproductive rate of adults (i.e. the number of offspring produced in a month that were alive at the time of sampling) declined with increasing population density (Fig. 7B). As with fecundity, low values of κ led to higher rates of reproduction. In contrast to fecundity (Fig. 6), reproductive rates declined with increasing α, reflecting a decreases in the successful recruitment of neonates to the population as the strength of size-dependent resource limitation increased. The effects of variation in κ on reproductive rates was strongest in the null scenario, and declined as α increased across scenarios. The effects of population density on the relationship between variation in κ and variation in reproductive rates differed between scenarios. In the extreme scenarios (α=0, 2), the relative advantage of low values of κ increased with increasing population density, i.e. the effects of variation in κ on reproductive rates increased at high densities. The opposite was true at weak and intermediate

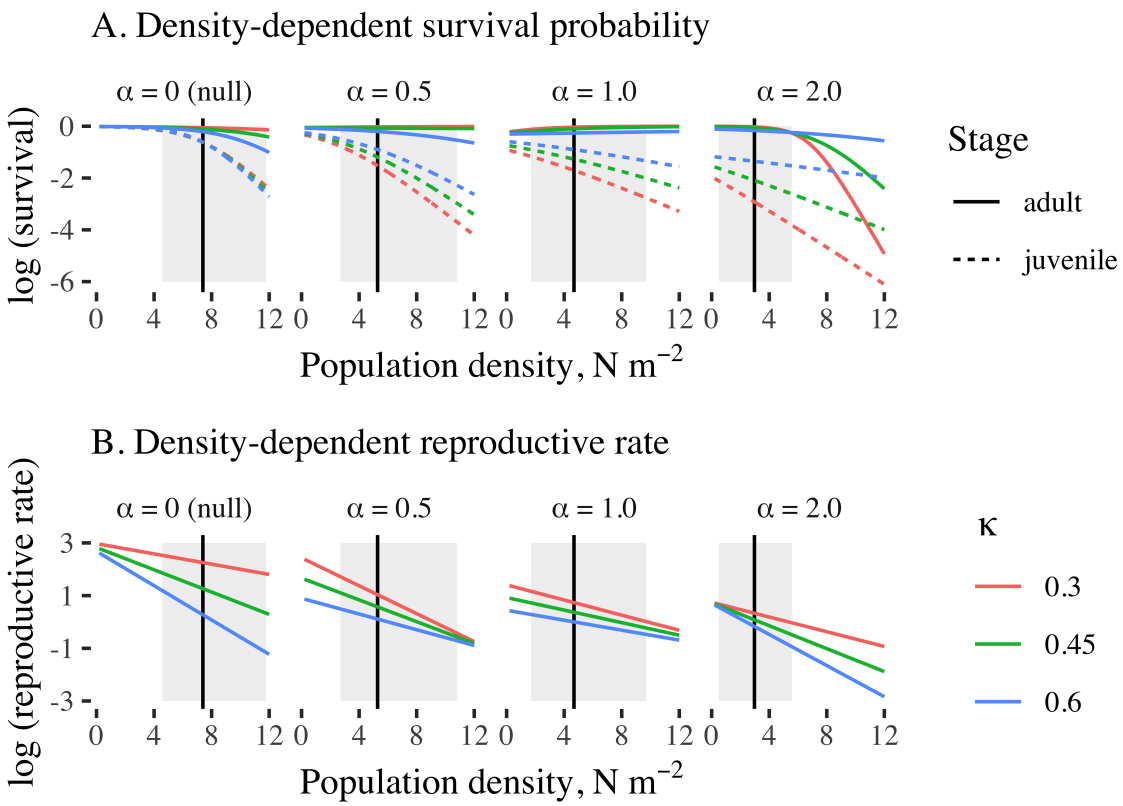

Figure 7: Density dependence of components of fitness: (A) survival and (B) reproduction, for low (red), medium (green), and high (blue) values of κ. Panels depict different scenarios of size-dependent resource limitation (α). (A) Monthly survival probabilities are functions of length, κ, and population density, and are shown for juveniles (dashed lines, standard length = 8 mm), and adults (solid lines, standard length = 24 mm). (B) Reproductive rates are the total number of offspring produced in a month that survived to sampling period. This is distinct from the measure of fecundity given in Figure 6 (i.e. the monthly production of embryos) - the difference between these measures reflects neonate mortality prior to sampling, i.e. a failure to recruit to the juvenile stage. Components of fitness are log-transformed, and are presented on the same scale in (A) and (B), allowing direct comparison of the relative effects of κ and population density on components of fitness. Vertical black lines indicate the mean population density in each scenario, grey boxes highlight the range of population densities in each scenario at quasi-equilibrium (i.e. after the first 24 months, following initial population growth).

strengths of size-dependent resource limitation (α=0.5, 1), where the effects of variation in κ on reproductive rates declined with increasing population density.

5.4 Discussion

Life history evolution depends on the interaction between intrinsic (heritable) energetic constraints, and extrinsic factors that influence reproduction and survival. We demonstrated how variation in two parameters – one intrinsic, one extrinsic – can generate complex patterns of density dependent fitness and selection on life histories. Variation in the extrinsic parameter – the strength of size-dependent resource limitation – altered trade-offs between components of fitness (Fig. 7), modifying the dynamics, size, and stage structure of populations (Fig. 4), and the direction and pace of life history evolution (Fig. 5).

When resource limitation was independent of size (α=0), selection favoured faster life histories with greater investment in reproduction (i.e. lower values of κ, Fig. 5), leading to large population sizes (Fig. 4). By contrast, as the strength of size-dependent resource limitation increased, selection increasingly favoured slower life histories, with decreased allocation of energy to reproduction, and increased allocation to growth and somatic maintenance (i.e. higher values of κ, Fig. 5, Fig. 6), resulting in smaller population sizes (Fig. 4). Increasing α changed the way that density dependence operated on the population, modifying fitness trade-offs between juvenile survival and adult reproduction. In general, as α increased, density dependence acted to increase the potential for selection on juvenile survival, while decreasing the potential for selection on adult reproductive rates (Fig. 7).

To understand why the balance of fitness trade-offs changed as α increased, we need to consider (i) how increasing α modified the nature of size-dependent resource competition that regulated demographic rates, and (ii) how heritable variation in κ linked to variation in components of fitness under different scenarios of α, generating differences in the opportunity for selection on these fitness components.

How did changing α modify ecological interactions in our simulations? As α increased there was a shift in the relationship between size and competitive ability: at low values of α, smaller individuals were competitively superior, whereas at high values of α, larger individuals were competitively superior. In the absence of extrinsic regulation, dynamic energy budgets (DEB) models – that we used to

characterise the intrinsic energetic constraints on life histories – inherently assume that competitive asymmetry favours smaller individuals (van der Meer, 2016). Our results demonstrated this effect: when resource availability was independent of size (i.e. there was no extrinsic size-dependent regulation of competitive ability), the total population was dominated by the juvenile stage, and the competitive superiority of smaller individuals suppressed the fecundity of adults (Fig. 4, Fig. 6). When we increased the strength of size-dependent resource limitation, competitive asymmetry shifted to favour larger individuals. In these scenarios, juveniles were increasingly constrained by resource limitation: there was less space in which they were able to feed, and as such competition among juveniles for food increased and juvenile survival decreased. By contrast, for adults, increasing α had the effect of increasing the space in which they could feed without competing with juveniles. This meant that adults experienced increasing levels of resource availability as α increased. As such, the fecundity of adults increased, but the size of the juvenile stage declined, due to decreased recruitment and a reduction in juvenile survival due to starvation of small individuals (Fig. 4, Fig. 7).

How did these changes to juvenile survival and reproductive rates link to variation in κ across different scenarios of α? When small individuals were strong competitors (α=0), variation in κ had little effect on variation in juvenile survival (Fig. 7A): an increase in κ under this scenario would have little impact on juvenile fitness. However, variation in κ had a large effect on adult fitness: adults with low κ had substantially higher rates of reproduction, and their probability of survival was less sensitive to increases in population density (Fig. 7). As such, there was little opportunity for selection on juvenile survival, but ample opportunity for selection on reproductive rates: selection favoured a decrease in κ, maximising fitness through increasing rates of adult reproduction.

When competitive asymmetry shifted to favour larger individuals, variation in κ had an increasingly strong effect on juvenile survival: high values of κ increased juvenile fitness. This effect increased at high population densities (Fig. 7A). This happened because resource availability increased with body size in these scenarios,

and individuals with high values of κ are born at larger sizes and grow more rapidly, meaning that they can escape resource limitation more easily than individuals with low values of κ. When large individual were strong competitors (α=2), the effects of variation in κ on juvenile survival exceeded those on reproductive rates (Fig. 7). Although there was a trade-off between juvenile survival and reproduction with respect to κ – high values of κ increased juvenile survival but decreased reproduction – because the influence of variation in κ was always greater on juvenile survival, selection favoured an increase in κ.

At low and moderate values of α, the effects of variation in κ at low population densities were greater on reproductive rates than on juvenile survival, but the opposite was true at high population densities (Fig. 7). This meant that there was density-dependent selection for κ in these scenarios: the balance of the fitness trade-off between juvenile survival and adult reproduction – and so the direction of selection on κ – depended on population density. Low values of κ led to higher fitness (through increased reproduction) at low densities, but high values of κ led to higher fitness (through juvenile survival) at high population densities.

In our simulations, fluctuations in population size were due to density dependent fitness acting through resource limitation. At high population densities, food levels were reduced, leading to starvation, reduced reproductive rates, and population decline. After the decline, food levels recovered, competition was less intense, and survival and reproductive rates increased. When competitive asymmetry favoured small individuals, there was greater heritable variation in the effects of density on fitness among adults; when competitive asymmetry favoured larger body sizes, juveniles experienced greater heritable variation in the effects of density on fitness. Although density dependent regulation is stabilising by definition, in our simulations evolutionary change in κ fed back into density dependent fitness (e.g. through food dependent changes in survival and reproductive rates), causing long-term trends in population size. The direction of these trends depended on the balance of competitive asymmetry. When small individuals were strong competitors (α=0), population size increased over time and there was runaway selection for decreasing

κ: as the mean value of κ decreased, adults evolved intrinsically higher reproductive rates, producing more juveniles which in turn out-competed the adults for food, diminishing their fecundity and so maintaining selection for low κ. By contrast, when large individuals were strong competitors (α=2), population size declined over the long term. This happened because of selection for higher κ operating through juvenile survival, which meant a decline in intrinsic reproductive rates of adults as the mean value of κ increased, acting to lower population density as adults with low values of κ – and higher intrinsic rates of reproduction – became less common.

In both of these scenarios, while fitness was density dependent, selection was not (or only weakly so). This led to runaway selection that in our simulations was only constrained by the variation in κ in the founding population. However, at weak and intermediate strengths of size-dependent resource limitation, selection for κ was density dependent: the rank order of genotypes (i.e. values of κ) with respect to fitness changed with changing density. Hypothetically, this could have led to stabilising selection and long-term population stability: any long-term increase in population density should select for slower life-histories, which would act to reduce population density; any long-term decrease in population densities should select for faster life-histories, leading to an increase in population density, and so on. In practice, this is not what we observed in our simulations. In both scenarios in which density-dependent selection occurred, the mean population size decreased over the course of the simulations, but this did not alter the evolutionary trajectory of κ. However, density-dependent selection in these scenarios did slow the rate of evolutionary change (Fig. 5), and so decreased the rate of decline in genetic variation in κ relative to the scenarios without density-dependent selection.

Links to broader theory

In classical models of density-dependent life history evolution, populations are structured by discrete age classes (Charlesworth, 1994). Although our study differs in this respect (we consider populations structured by size and the heritable energetic allocation trait κ), our results agree with classical theory. For example, theory

predicts that selection should favour earlier maturity when density regulates adult reproductive rates, but should favour decreased reproductive investment when density increases juvenile mortality (Charlesworth, 1994, , Chapter 5). This matches our main results: when density impacted adult reproductive rates (i.e. α=0), selection favoured earlier maturity through decreasing the mean value of κ; by contrast, when density impacted juvenile survival (e.g. α=2), we saw the evolution of higher values of κ, which corresponds to a reduction in reproductive investment. Note that classical theory is agnostic with respect to *why* density operates on any particular demographic rate. Here, our results are consistent with classical theory, but we additionally show that modifying size-based competitive ability (i.e. changing α) alters how density influences demographic rates and so life history evolution.

Modern density-dependent selection theory predicts that there will be a trade-off between density-independent population growth and density-dependent factors that limit reproduction and survival. (Lande et al., 2009; Sæther and Engen, 2015; Lande et al., 2017; Wright et al., 2019, 2020; Engen et al., 2020). This means that genotypes with high fitness at low densities suffer greater reductions in fitness as population densities increase. In our models, contributions to density independent population growth traded off against body size: individuals with low values of κ had higher intrinsic rates of fecundity, but had reduced somatic growth rates and smaller body sizes than individual with high values of κ (Fig. 2, Fig. 6). Under scenarios where resource availability increased with increasing body size, individual with lower values of κ suffered reduced fitness as population densities increased. As such, our simulations are in agreement with predictions that mean body size should increase under density dependence when genotypes that generate larger body sizes (but with lower intrinsic fecundity) have higher fitness at high population densities (Engen et al., 2020).

Limitations

Our simulations resulted in runaway selection. In natural populations, guppies do not evolve ever slower life histories: populations reach an evolutionary equilibrium.

One such mechanism that could maintain this equilibrium is stabilising selection. Even though some of our simulations generated density-dependent life history trade-offs, such that individuals favoured at low densities were disadvantaged at high densities (and *vice versa*), these did not generate stabilising selection (as observed in e.g. Sæther et al. (2016)). One explanation is that our simulations lacked sufficient environmental stochasticity: theoretical work has demonstrated that both density-dependence and environmental stochasticity are required to generate stabilising selection in a fluctuating environment (Lande et al., 2009; Sæther and Engen, 2015). Environmental stochasticity favours fast reproducing life histories (e.g. low κ in our simulations): including environmental stochasiticity in our simulations could potentially shift the balance of fitness so as to favour intermediate values of κ in our simulations, and prevent the runaway selection for higher values of κ observed in three of four of our experiments.

Another possible explanation is that we considered heritable variation in only a single trait, κ. We chose to focus on variation in κ, because low- and high-predation guppy populations consistently differ in this parameter (Potter et al., 2021b). Had we included heritable variation in other parameters of the standard DEB model, we may have imposed additional constraints and fitness trade-offs in our populations, at a cost of increased complexity.

In this study, we replicated *in silico* the establishment of populations of guppies in predator-free environments. We used DEB parameters from high-predation guppies with fast life histories, and we identified conditions under which a slower life history, more typical of low-predation guppies, could evolve. However, our models assumed asexual reproduction, a single type of food, and single-species communities. In reality, guppies in low-predation risk habitats experience strong sexual selection, consume a wide range of food items, and compete with and suffer from intra-guild predation by Haart's killifish (*Rivulus hartii*). Each of these factors will contribute to variation in fitness in natural populations. Nevertheless, our results shed light on the impact of size-dependent resource availability on the evolution of the low-predation life history.

Implications for life history evolution in guppies

Natural low-predation guppies populations are regulated by density (Bassar et al., 2013), and have evolved a slower pace of life history: they mature later and produce fewer, larger offspring per litter than their high-predation ancestors (Reznick and Endler, 1982; Reznick, 1982). There is strong evidence that the evolution of the low-predation life history occurs through density-dependent selection: high-predation guppies have higher fitness at low population densities, but low-predation guppies have an advantage at high population densities (Bassar et al., 2013), and the low-predation life-history evolves after population densities reach carrying capacity (Reznick et al., 2019; Potter et al., 2021a). Our results suggest that size-dependent resource limitation – which acts to focus density-dependent mortality on small size classes – is likely to play a key role in generating density-dependent selection for slower life-histories in guppies.

Several lines of evidence point to the pervasiveness of size-dependent resource limitation and the importance of size with respect to competitive ability in guppy populations. Analysis of data on size and fecundity in wild caught adults suggest that smaller guppies are more food limited, and the advantage of being large increases in the rainy season, when resource competition is more intense (Potter and Felmy, 2021). Large size confers a competitive advantage in neonate, juvenile, and adult guppies, both in the lab and in the wild (Bashey, 2008; Potter et al., 2019; Griffiths et al., 2020). Guppies display adaptive plasticity in offspring size: mothers that are subject to resource limitation produce larger and leaner (but fewer) offspring, increasing the fitness of offspring born into resource limited environments (Bashey, 2006). Our results suggest that competitive asymmetry favouring larger body sizes may be a prerequisite condition for the evolution of slower life histories in guppies. Our simulations show how increased body size can enhance fitness – even at the cost of reduced fecundities – through increasing survival of small guppies in high density environments.

The same pattern of life-history evolution could also occur if size-dependent mortality was instead driven by cannibalism. In lab experiments, guppies often

display cannibalistic tendencies, where large adults prey on small juveniles, and juveniles actively seek refuge to evade predation (Nilsson et al., 2011; Nilsson and Persson, 2013). Although dissection analyses conclude that cannibalism in wild guppies is rare (Rodd and Reznick, 1997), the threat of cannibalism may force juvenile guppies to inhabit limited refuges away from adults. Indeed, in wild population of guppies, neonates and small juveniles are often found in shallow side-pools away from adults (personal observation). This could potentially cause the type of size-dependent resource limitation described in this paper, leading to increased effects of density dependence acting on the small size-classes of guppy that are at risk of cannibalism.

Conclusions

Our study examined the connections between density dependence, competitive asymmetries, and life history evolution. We constructed individual-based population simulations, including heritable variation in energetic allocation strategies. Whether selection favoured increases in allocation to somatic growth or to reproduction depended on whether small or large individuals were superior competitors. We show that the degree of size-dependent resource limitation – and so the direction and magnitude of competitive asymmetry – can alter the pace and direction of life-history evolution, by modifying the way that density operates on the population. More generally, our results suggest that selection maximises the component of fitness in which there is the greatest genetic variance in the mean environment.

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6
General discussion

Contents

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6.1 Environmental feedback drives ecological and evolutionary dynamics, presenting challenges for classical analyses of wild populations

A central theme of my thesis is of the reciprocity between individuals and their biotic environment. To understand ecological and evolutionary dynamics, I show that it is crucial to account for differences among individuals in (i) the environmental effects that they experience (Chapters 2 and 3), and (ii) the change in environmental conditions that they cause through ecological interactions (Chapter 5). Variation in phenotypic traits within a population are both influenced by, and have an effect on (i) and (ii). In natural systems, the environment that individuals experience differ not only over time and space, but also vary as a function of the individual's phenotype. Furthermore, phenotypic variation means that individuals differ with respect to the impact that they have on the environment, and so on other members of the population through ecological interactions. Phenotypic and environmental variation interact to generate differences in demographic rates, such as size-dependent growth, reproduction, or survival probabilities. When genetic variation underpins variation in demographic rates, evolutionary change occurs concurrently with ecological change, which may simultaneously alter the biotic environment in an inherent feedback loop.

It is no surprise that wild animals are subject to variation in biotic environmental conditions. What perhaps is surprising is the extent to which variation in environmental conditions are overlooked in studies of wild populations. Standard

quantitative genetic approaches assume that environmental conditions are constant across time and space, and across the entire population (Lande and Arnold, 1983; Mrode and Thompson, 2005; Postma, 2006). In Chapter 2, I demonstrated the consequences of this assumption. Differences among related individuals in terms of the environment that they experienced led to misestimation of additive genetic variances, covariances, and breeding values, leading to incorrect predictions of evolutionary change (Chapter 2). I showed how this problem could be reduced by including environmental covariates in the regression models used to estimate quantitative genetic parameters.

In Chapter 3 I identified a similar problem, whereby the statistical association between two variables (body size and reproduction) was influenced by a third factor, in this case food availability. Although my results were limited to observations in a single species, I argue that widespread patterns of reproductive hyperallometry (Barneche et al., 2018) – i.e. a disproportionate increase in reproductive output with increased body size – likely reflect the structuring of wild populations by food availability. More generally, I demonstrated that a statistical association between body size and reproduction at the level of the population is not necessarily explained by an ontogenetic relationship between size and reproduction at the level of the individual (i.e. as proposed by Marshall and White, 2019). The statistical problems identified in Chapters 2 and 3 emerged from regression analyses performed across aggregated data (i.e. the entire population) structured by a confounding environmental variable. Both of these are examples of Yule-Simpson effects, i.e. covariances that are conditional on a third unmeasured factor (Yule, 1903; Simpson, 1951). I argue that such confounding environmental effects are likely common in analyses of wild populations. Accounting for these effects drastically altered the interpretation of both evolutionary (e.g. Chapter 2) and physiological/ecological (e.g. Chapter 3) analyses of wild populations of guppies.

Body size and reproduction are inherently plastic traits. Both depend on the amount of food that an individual has consumed, but wild animals will never experience the *exact* same conditions of food availability. Ideally, individual measures

of feeding rates, or some other measure of energetic assimilation, would be included in regression analyses to prevent Yule-Simpson effects. Individual based measures of assimilation rates could also be used to calculate reaction norms, which would help to truly characterise "what is inherited in a living being" rather than "this or that morphological character" (Dobzhansky, 1937). However, quantification of individual assimilation rates presents a major challenge for long-term evolutionary studies of wild populations.

6.2 A role for dynamic energy budgets in life history theory?

Dynamic energy budget (DEB) theory has developed a sizeable body of literature, but there is relatively little cross-communication (or cross-citation) between DEB theory, quantitative genetics, and life history theory. I find this quite surprising, given their shared common goal of understanding evolved differences among populations and species. In my thesis, I have sought to combine insights from these branches of theory, and have attempted to identify how these different approaches may compliment each other.

DEB theory is not eco-evolutionarily explicit in itself: it does not consider extrinsic sources of mortality, nor does it consider inheritance. Nevertheless, it could prove to be a useful piece of the puzzle when it comes to understanding eco-evolutionary dynamics and life history evolution. I believe it offers two main contributions. First, the parameters of the DEB model define reaction norms (with respect to food density and temperature) for growth, somatic maintenance, maturation, reproduction, offspring size, metabolic rate, and senescence. As such, the primary parameters of the DEB model, as abstract as they may be, seem close to what Dobzhansky (1937) had in mind when it comes to what is truly passed on from parents to offspring through inheritance. Second, life history theory has been searching for a generic model of the energetic trade-offs that define the intrinsic factors governing life history evolution (Stearns, 2013): DEB theory offers precisely that, by defining individuals in terms of how they allocate limited energy

to fundamental biological processes (Kooijman, 2010). The identification of such a model of the "intra-organismal features that constrain the evolution of phenotypic traits" would allow quantitative, rather than qualitative predictions of life history evolution (Stearns, 2013). Given that DEB parameters have been estimated for over 2500 species (AmP, 2020), combining DEB theory with estimates of extrinsic sources of mortality from the field could potentially generate quantitative predictions of evolution for thousands of different species.

There are three main attributes of DEB parameters which make the DEB model appropriate as an intermediary structure between genetic and phenotypic variation, and which could allow characterisation of genetic variation in the reaction norms of biological processes. These are that DEB parameter values are theoretically assumed to: (i) be independent of environmental conditions; (ii) be constant throughout life; and (iii) reflect evolved differences between species, thus being more similar among more closely related individuals (Kooijman, 2010). In practice, I found that these three assumptions were not met when I quantified DEB parameters for guppy populations, at least for some of the parameter values (Chapter 4). However, it isn't clear whether this occurred because of a failure of theory, or because of a failure of the parameter estimation process, the latter of which I found to be a major cause for concern. Crucially, differences in the type and amount of data used to fit the models seriously biased estimates of DEB parameter values (Chapter 4). This meant that – even though different parameter sets fit observations equally well – it was not possible to determine which parameter values were accurate, which is problematic if DEB theory is to play a role in truly quantitative predictions of life history evolution. Nevertheless, there are continued efforts to improve methods for the estimation of DEB parameters and their uncertainty, which could contribute to resolving this issue in future (e.g. Boersch-Supan and Johnson, 2019; Lika et al., 2020).

In Chapter 5, I expanded individual based models structured by DEB theory (DEB-IBMs, Martin et al., 2012) by including heritable variation in DEB parameters. To my knowledge, this is the first example of incorporating DEB theory into models that generate life history evolution. The results of these simulations – in terms of

which size class increased in population size most in response to density-dependent selection – conflicted with predictions from classical demographic life history theory (Charlesworth, 1994). These contradictions occurred because in the simulations evolution was constrained by the amount of genetic variation in demographic rates. However, the emergent trade-offs observed between adult reproductive rates and juvenile survival probability were consistent with the assumptions of modern density-dependent selection theory (Lande et al., 2009; Sæther and Engen, 2015; Lande et al., 2017; Wright et al., 2019, 2020; Engen et al., 2020). This suggests that evolutionarily explicit DEB-IBMs, similar to those developed in Chapter 4, could be used to generate generic predictions about how changes in environmental conditions will alter evolutionary trajectories of life histories. Although the simulations in Chapter 5 were paramterised for guppies (based on my results in Chapter 4), they could easily be run for any species for which DEB parameters are available, and could be extended to incorporate interspecific competition and the effects of predation. However, with increasing complexity of models and simulations comes increasing difficulty in interpretation, as well as cascading effects of the assumptions of the model. As with life history evolution, the value of these approaches will be depend on the balance between their potential benefits and costs.

6.3 Relevance for our understanding of guppies, and beyond

The results of the detailed quantitative genetic analyses presented in Chapter 2 are consistent with observations of delayed evolution of a slower life history, following increases in population density, as seen in common garden experiments (Reznick et al., 2019). This provides additional support for the hypothesis that the low-predation guppy ecotype is selected in response to density dependence, rather than as a direct response to lower risks of predation (Bassar et al., 2013; Reznick et al., 2019). The conclusion that larger guppies are less affected by resource limitation – as determined by the degree of reproductive hyperallometry in 19 wild populations (Chapter 3) – supports experimental and observational studies that competitive

ability increases with body size in guppies (Bashey, 2006; Potter et al., 2019; Griffiths et al., 2020; Anaya-Rojas et al., 2021). These findings build on observations of the resource-dependent expression of life-history traits in guppies (Felmy et al., 2021). Estimates of DEB parameters for 16 populations of guppies (Chapter 4) are in agreement with different methodologies that show that the high-predation ecotype allocates more energy to reproduction, and have higher metabolic rates than the low-predation ecotype (Reznick and Endler, 1982; Auer et al., 2018). Finally, the simulations in Chapter 5 illustrate the importance of density dependence operating on juvenile survival for the evolution of the low-predation ecotype. This theoretical finding demonstrates how reduced reproductive rates can lead to higher fitness when fecundity trades off against offspring size and survival. This result consolidates the findings from Chapters 2, 3, and 4, to illustrate how selection can favour a slower life history when guppies are regulated by population density.

Trinidadian guppies are an extremely well-studied system in evolutionary ecology. This fact, and the wealth of data that this focus has generated, have allow me to test different theories and methodologies for understanding life-history evolution and eco-evolutionary dynamics. In particular, through combining insight from analyses of field observations, genetic data, and controlled laboratory experiments, I have been able to benchmark the accuracy of methods that have recently been applied to wild populations. In doing so, I have identified weaknesses, sources of bias, and solutions to these problems, that are likely to be relevant to many studies of natural populations.

6.4 Conclusions

Variation in traits, such as size and reproduction, are reciprocally linked with variation in the biotic environment, and the interaction of both can generate eco-evolutionary dynamics. Disentangling cause and effect in a system of feedback loops is challenging. Nevertheless, to improve our understanding of the processes that generate the patterns we observe in nature, unravelling causality is a must. Complementary field observations and controlled lab experiments can enhance our

insight into the underlying causes of these patterns. This thesis has been an exercise in reductionism, which sits at odds with the elegant and general theorems of evolution developed by quantitative genetics. The challenge in balancing reductionism with practicality and clarity is in identifying those critical assumptions that hinder our understanding of natural systems. Simplicity is beautiful, but life is complex.

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