**Life history evolution under high and low food regimes**

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**Abstract**

The role of resource availability in shaping life histories has an august history but our capacity to make generalisations is limited by the dearth of experimental evolution studies. Marine copepods are a key link in the global carbon budget, and face shifting food regimes, but we cannot predict the consequences of these changes. We used an experimental evolution approach to explore how a 10-fold difference in food availability for ∼30 generations affects life history evolution in the copepod, *Tisbe sp*.. We observed evolution in body size, size-fecundity relationships and maternal investment strategies in response to different food regimes. Our results suggest that changes to food regimes reshape life histories and that cryptic evolution in traits such as body size is likely. Evolution in response to changes in ocean productivity change consumer life histories, altering trophic links in marine foodchains in ways that have not been anticipated.

**Introduction**

Resource availability shapes ecological dynamics and evolutionary trajectories (Chesson 2000; Macarthur & Levins 1967; Tilman 1982). Classic studies on adaptive radiation have shown that changes in resources can induce rapid trait evolution (Abzhanov et al. 2006; Grant & Grant 2006), but traits can evolve in unpredictable ways (Pianka 1970). Key life history traits such as body size, fecundity, and offspring size, can all evolve in response to changes in resource availability, but the directions of evolution are not easily anticipated (Reznick et al. 2002). There is a notable lack of consensus across theoretical and macroecological studies of life history evolution in response to changes in resource availability, and while there have been valuable foundational case studies (e.g. Grant & Grant 2006; Reznick & Travis 2019), there are too few to generalise.

Classic theory invoked density-dependence as the driver of evolution in response to changes in resource availability (MacArthur & Wilson 1967). Pianka (1970) and then Greenslade (1983) predicted how distinct life history strategies should evolve under density-dependent and -independent conditions. Known as r/K selection theory, it was ultimately unable to explain the complexities of life history evolution observed in nature, ignoring other possible agents of selection and found limited empirical support (Stearns 1992).

Following r/K selection theory, demographic theory examines how selection acts on the entire life cycle, how vital rates (such as growth, survival, and reproduction) are affected, and which individual life history traits evolve in response (Charlesworth 1994). While early models focused on density-independent dynamics and extrinsic age-specific mortality (Charlesworth 1994; Stearns 1992), natural populations are generally resource-limited at some point (Hassell 1986; Hixon et al. 2002; Turchin 1995)**.** Predictions from demographic theory are mixed e.g., resource limitation can select for either late age at maturity and lower reproductive effort (Gadgil & Bossert 1970) or the opposite (Kozłowski & Uchmanski 1987; Kozłowski & Wiegert 1987), depending on the assumptions used. Resource abundance can alter life histories whether directly or indirectly via mortality (e.g. predation) or density (e.g. Abrams 1983; Gadgil & Bossert 1970; Reznick et al., 2002; Walsh & Reznick 2009) but again there are conflicting predictions across studies. For example, food scarcity can drive life history evolution due to a trade-off between egg size and fecundity in fish, such that mothers produce fewer, larger larvae under food-limited conditions (Winemiller & Rose 1993). Consequently, there is no theoretical consensus as to exactly how resource availability affects life history evolution (Walsh & Reznick 2008).

Macroecological studies are similarly ambivalent about the role of resource availability in shaping life histories. For example, adults are often larger but offspring are smaller in higher productivity environments (Huston & Wolverton 2011; Marshall & Burgess 2015; Segers & Taborsky 2011). But exceptions to these patterns are common however because of trade-offs between population size and body size (Damuth 1991), or the influence of factors that covary with resource availability. For example, marine copepods exhibit decreasing body-size with increasing productivity, possibly due to the covariance with predation and food-chain length (Brun et al. 2016). Ultimately the conclusions that can be drawn from comparative work are limited owing to such confounding effects, particularly predation (Reznick et al. 2002).

Thanks to well-studied systems such as foundational work on systems such as Trinidadian guppies, there are excellent examples of how life histories can respond to resource regimes (Reznick & Travis 2019). Controlling for predation pressure, guppies in high primary-productivity exhibit higher growth rates, larger adult size, as well as smaller broods of larger young relative to low primary productivity sites (Grether et al. 2001). Despite such exemplars, there are too few case studies to make generalisations about the role of resource availability in life history evolution – we seek to address this issue. Experimental evolution approaches can provide insights into life history evolution by minimising potentially confounding environmental correlates (Fox & Wolf 2006). We evaluated the effects of food abundance on life history evolution in a marine copepod. An understanding of how copepods are likely to evolve in response to changes in resource regimes is particularly important given the predicted changes in primary productivity under climate change and the role of copepods in the global carbon pump (Turner 2015). Future oceans are predicted to be warmer, more acidic but also much less productive due to thermal shoaling. Copepods have a history of being studied from an experimental evolution perspective (Kelly et al. 2012), particularly with regards to the direct drivers of global change such as temperature and thermal tolerance but they have been less studied from perspective of indirect drivers of global change effects, namely how changes in resources affect their evolution. We subjected copepod populations to either high-food or low-food environments for 2 years and then used multigenerational common garden experiments to examine how life histories have evolved independently of any cross-generational parental effects (Burgess & Marshall 2014).

**Methods**

*STUDY ORGANISM*

*Tisbe sp.* is a littoral marine copepod from the Tisbidae family (Arthropoda: Harpacticoida) that has not been resolved to species level in the Southern Ocean (Gangur & Marshall 2020). Our laboratory cultures were originally sampled in 2017 from wild populations from Port Phillip Bay, Australia. These ancestral cultures were maintained in 500 mL mason jars with biweekly cleaning and gentle oxygenation, and water replacement every two months with freshly pasteurised sea water (FSW). All copepods were reared on a marine microalga (*Dunaliella tertiolecta*) which in turn was cultured with F/2 medium (Guillard & Ryther 1962), and ancestral stocks were fed at a rate of 2.475 x 109 algae cells per litre of copepod culture per week.

Algae concentrate was prepared three times a week from freshly cultured *D. tertiolecta*, and density of each algae culture was determined spectroscopically using a SPECTROstar Nano. Algae was concentrated into a pellet with centrifugation, the supernatant consisting of F/2 media was removed to minimise bacterial growth in copepod cultures, and the pellets were reconstituted in FSW at a fixed concentration of approximately 1.1 x 1010 *D. tertiolecta* cells/L. Food dosing was performed manually using a serological pipette controller for ancestral stocks. For experimental cultures housed in flow-through culture vessels (see Experimental Evolution), feeding was automated using Kamoer X4 peristaltic dosing pumps. Pumps provisioned algae concentrate semi-continuously (12 times a day), while manual feeding was performed in a single pulse once a day, on weekdays only (no dosing on weekends).

*EXPERIMENTAL EVOLUTION*

*Experimental design*

Ancestral stocks were divided between two treatments – high-food and low-food environments – which differed in their rate of food supply (of *D. tertiolecta cells*) by an order of magnitude. In total, 20 copepod cultures were subjected to experimental evolution, consisting of 10 high-food and 10 low-food replicates reared in separate glass pressure-equalising dropping funnels. One low-food replicate went extinct one year into the experiment due to bacterial contamination.

High and low food rates were determined through pilot experiments in 2018*.* Accordingly, three food supply rates were used throughout experimental evolution and common gardening: high (4.5 x 109 algae cells per litre of copepod culture per week) intermediate/ancestral (2.475 x 109 algae cells per litre of copepod culture per week), and low (4.5 x 108 algae cells per litre of copepod culture per week). Cultures were organised into 5 feeding blocks of four (in a randomised sequence of 2 high-food and 2 low-food replicates) due to spatial constraints. Each block was provisioned food from a separate algae reservoir and separate peristaltic dosing pumps for redundancy (e.g., in case of pump failure or food contamination). Algae and FSW reservoirs were covered to minimise evaporation and cleaned weekly to minimise build-up of waste and pathogens, and algae was kept well-mixed using magnetic stirrers. Both high-food and low-food treatments received a total inflow of 80mL per litre of culture per day (on weekdays only, no dosing on weekends). High-food treatments were dosed with 80mL of algae concentrate per day, while low-food treatments received 8 mL of algae concentrate and 72mL of FSW (i.e. 10% of the high food regime). Pilot work indicated that very few adult copepods were lost at this rate of inflow-outflow.

*Initiating evolutionary lineages*

Experimental evolution commenced on the 13th of October 2018 at Monash University Clayton Campus, Melbourne, Australia. Starting one month prior to experimental evolution, ancestral stocks were reared in 1L Schott bottles at an intermediate food supply rate (2.475 x 109 cells per litre per week). When experimental evolution commenced, these founding cultures were mixed and then divided into 12 equal fractions using a Folsom plankton splitter. 10 fractions were split again into 20 replicates and randomly allocated to high-food or low-food treatments. The fractions were transferred to 1L glass flow-through culture vessels and topped up to 1L with FSW. Differential food supply was ramped up gradually, with high and low food treatments receiving the same intermediate rate of supply for the first week, partial treatments in week two (3.5 x 109 and 1.5 x 109 *D. tertiolecta* cells per week, respectively), and final treatments of high or low food supply by week three after initiating the experiment. These treatment differences were then maintained for the next 16 months.

*COMMON GARDENS*

*Experimental design*

To disentangle genetic responses from plastic responses in experimental evolution, individuals need to be sampled from divergent evolutionary lineages and reared in a common environment (Huey & Rosenzweig 2009). Because environmental effects can persist between generations, such common environment (or ‘common garden’) experiments must also be performed over multiple generations to minimise any lingering parental and grandparental effects on offspring phenotypes (Burgess & Marshall 2014). To evaluate the evolutionary response to high- and low-food environments, we performed a common garden experiment wherein copepods were sampled from their treatment cultures (G0) and their descendants were reared (separately) under the same environmental conditions over two generations (G1 and G2). With a generation time of ∼17 days, our *Tisbe sp.* cultures had undergone approximately 30 generations of evolution prior to common gardening, which commenced on 18 February 2020. Evolutionary responses in five traits were measured across the three generations. Maternal size, mean egg size, and fecundity were measured in G0, G1, and G2, while survival and age at maturity were measured in G1 and G2 only. Maternal size was measured as length between end of urosome to tip of prosome. We used mean egg size from 10 randomly measured eggs within each clutch, and fecundity was estimated by dividing egg sac diameter by mean egg size for each clutch of eggs. Maternal body size, egg size, and fecundity were recorded with photographs using a Motic Moticam 1080 camera mounted on an Olympus SZ61 dissecting microscope and digitally measured using FIJI version 1.53c (Schindelin et al. 2012).

G1 offspring were collected from G0 copepods, which were sampled from 19 cultures and photographed. These G1 offspring across 19 replicates were reared in intermediate food environments in subreplicates of 25 ± 5 individuals (to normalise copepod densities) until they too produced a first clutch of eggs and were photographed. The first clutch of G2 offspring was collected from each G1 mother and in turn reared in intermediate food environments in subreplicates of 25 ± 5 individuals. Within each replicate, subreplicates were re-normalised to 25 ± 5 individuals at sexual maturity to ensure mates were available.

Throughout the experiment, *D. tertiolecta* was provisioned at an intermediate level of food supply. For G0, G1, and G2 juveniles and adults, food was provisioned each weekday (5 times a week) in a 176 uL pulse from a 1.1 x 107 cells/mL stock representing an intermediate food supply of approximately 2.475 x 109 cells per litre per week. Due to their lower feeding rate, larvae were provisioned a single 176uL (at 1.1 x 107 cells/mL) pulse of food to achieve the same maximum ambient food density experienced by adults under an intermediate feed regime (approx. 5 x 106 *D tertiolecta* cells per mL each day).

Paired high- and low-food cultures were randomly sampled between the 18th and 20th of February 2020. 10 gravid G0 mothers were collected from each culture, photographed, and transferred to sterile plastic culture trays containing 4 mL FSW. 16uL of 10000 units mL-1 (approximately 6 mg mL-1) penicillin G and 10 mg ml-1 streptomycin solution (Sigma-Aldrich) was added to each tray to inhibit the growth of pathogens (Gangur & Marshall 2020). Food was provisioned, and G0 mothers were monitored daily and returned to their cultures after releasing their G1 eggs, until all eggs had hatched and all G0 mothers had been removed (generally 3-5 days after initial collection). All common garden replicates commenced G1 with >100 larvae.

*Data collection*

Freshly hatched G1 larvae within each replicate were counted and randomly allocated to subreplicates. Individuals were transferred into new culture trays with 4 mL FSW, antibiotics, and food. Replicates were censused (all surviving individuals were counted) when metamorphosis was first observed in any subreplicate (generally 3-7 days after hatching), and juveniles were transferred again into new culture trays with 4 mL FSW, antibiotics, and food. Water was then changed and censusing was conducted weekly, until sexual maturity was first observed within a replicate. When sexual maturity first occurred within a replicate, all larval subreplicates were censused, pooled, mixed, and reallocated into new adult subreplicates of 25 ± 5 individuals. Water changes and censusing occurred fortnightly for adult subreplicates. All G1 mothers were removed from their subreplicates upon producing their first clutch of eggs. Mothers and their egg sacs were photographed, then pooled at the replicate level into fresh culture trays containing 4 mL FSW, antibiotics, and food. At least 5 G1 mothers and 50 offspring were obtained for 17 of 19 replicates (8 high-food and 9 low-food cultures), and these G2 offspring were collected for the final stage of the experiment. Replicates containing copepods from the two remaining high-food cultures were accidentally dropped before reaching G2.

G2 larvae were collected from culture trays containing gravid G1 mothers in a similar fashion to G1 larvae collection from G0 mothers. For each replicate, G2 larval collection took place over a week after the first clutch hatched. Larvae collected over the course of this week were continually transferred to subreplicates of 25 ± 5 individuals in new sterile culture trays with 4 mL FSW, antibiotics, and food. At the end of the collection week these larvae (some of which had metamorphosed) were re-pooled, mixed, and randomly allocated to new subreplicates of 25 ± 5 individuals in fresh sterile culture trays of 4 mL FSW, antibiotics, and food. G2 larvae that hatched outside this initial collection week were retained but reared in separate subreplicates. G2 larvae were then reared to sexual maturity following the same protocol used for G1. When metamorphosis was first observed within a replicate, the replicate was censused and individuals were transferred to fresh trays with 4 mL FSW, antibiotics, and food. For juveniles, water was then changed and censusing was conducted weekly until sexual maturity was first observed within a replicate. Then subreplicates were censused, pooled, mixed, and reallocated into new adult subreplicates of 25 ± 5 with fortnightly water changes and censusing. All reproductive G2 mothers were collected and photographed with their first clutch, until all G2 individuals had either produced a first clutch or died.

*STATISTICAL ANALYSES*

All phenotypes were analysed using linear mixed effects models. Full models for size, survival, and age at maturity included treatment and generation as fixed effects, as well as their interaction. Fecundity and egg size were modelled separately for each generation due to complex interactions and included mother size as a fixed covariate, as well as its interaction with treatment. G0 feeding block was included as a fixed effect in all models due to insufficient replication to treat as a random effect. All models also included culture nested within treatment as a random intercept term. Where interaction terms were nonsignificant they were removed and the analysis was repeated. Models were evaluated using type III tests due to imbalance of high-food and low-food replicates. *p* values for relevant fixed effects were obtained with *F* tests using Sattertwaithe’s approximation.

Analyses were performed with R version 4.1.2 (R Core Team 2021) and RStudio version 2021.09.1 (RStudio Team 2021), using dplyr (Wickham et al. 2021) to prepare the data. Linear mixed effect models were fitted with lme4 (Bates et al. 2015)**.** The lmerTest package (Kuznetsova et al. 2017) was used to perform Sattertwaithe’s approximations andtype III tests on fixed effects, and likelihood ratio tests on random effects. Bootstrapped 95% confidence intervals were obtained between cultures using merTools (Knowles & Frederick 2020), and plots were built using ggplot2 (Wickham 2016). Diagnostic residuals vs fits and QQ plots were visually assessed as per Keough & Quinn (2002), and VIF calculated to check for collinearity. Predictors were plotted against each other to visually assess acceptable domain/range overlap.

**Results**

*SURVIVAL AND BODY SIZE*

Copepod body sizes evolved in response to the food regime, but the direction of difference changed from one generation to another. Prior to common gardening, G0 females were slightly larger in the high-food environments but in the common-gardened generations (G1 and G2) females were consistently larger in the low-food regimes relative to the high food regimes (Table 1, Figure 1a). There was also an increase in body size over course of the common gardening relative to G0 (Figure 1a). Survival in the common garden was unaffected by food regime (Table S1 and Figure S1, see Supporting Information).

*AGE AT MATURITY*

Age at maturity differed between food regimes in G1 copepods but converged in G2 (Table 2, Figure 1b). In G1, copepods that had evolved in high-food regimes matured later despite being smaller than copepods that had evolved in low-food regimes, but by G2, these differences had disappeared.

*REPRODUCTIVE OUTPUT*

The relationships between maternal size and reproduction evolved in response to food regime but these effects only manifested in the second generation of common gardening (Egg size: Table 3, Fecundity: Table 4). Larger mothers from the low-food lineages produced larger (Figure 1c) but slightly fewer offspring (Figure 1d), whereas from the high-food lineages larger mothers produced smaller (Figure 1c) but more offspring (Figure 1d). Combining these two components of reproduction, mothers from the low-food lineages showed a steeper positive relationship between body size and reproductive output than mothers from the high food lineages (Figure 2).

**Discussion**

Copepods under different resource regimes evolved different life histories: body size, fecundity, and per-offspring investment all evolved, while age at maturity also changed but appeared to be driven by a strong parental environment effects that dissipated across generations. Copepods evolved to be slightly larger in low food regimes, and larger mothers invested more in their individual offspring in this environment. Meanwhile, in high food regimes, copepods evolved to smaller and larger mothers invested less in offspring but were much more fecund. Interestingly, we found evidence for differences in evolved responses to different resource environments relative to the expressed phenotypes in those environments, indicating countergradient evolution. Overall, our results match the findings of some previous studies on life history evolution in response to resource regime but not others.

Copepods were smaller in the low food regime but their offspring grew to be larger when transferred to common environment – indicating countergradient evolution in body size. Assuming cubic scaling with length, copepods from low-food environments were only 5.9% smaller by volume. Once released from the low-food conditions, they were 3.5%-5.6% larger than copepods from high-food lineages, suggesting that the impact of food scarcity was moderated by genetic compensation (Grether 2005). Such countergradient variation is observed in field studies as well and seems particularly common in fish (Arendt & Wilson 1999; Conover et al. 2009). Phytoplankton productivity is predicted to decline on average with future climate change, due to thermal shoaling and changes in nutrient availability at the sea surface. Our results imply that such changes will invoke evolutionary change in copepod body sizes but that these changes might be masked by countergradient evolution. Studies seeking to understand how copepod body sizes have changed and continue to change should consider common garden experiments to disentangle phenotypic and genetic responses, which may counteract each other, resulting in what is sometimes called ‘cryptic evolution’ (Grether 2005).

The relationship between body size and reproductive investment evolved, albeit in subtle ways. In low food environments, egg size increased with maternal size at the expense of fecundity while, while clutch size increased with maternal size at the expense of egg size in high-food lineages. These results are in keeping with general offspring size theory whereby in poor environments, mothers make larger offspring in order to buffer them from harsh conditions (Parker & Begon 1986). Such phenotypic effects have been observed in other taxa (e.g. Allen et al. 2008; Fox & Czesak 2000; see Marshall et al. 2018 for a review) but to our knowledge, ours is one of the few unequivocal demonstrations that differences in reproductive investment strategies can rapidly evolve.

Regardless of evolutionary lineage, larger mothers reproduced more than smaller mothers, implying larger mothers have more resources to invest in offspring. Larger individuals may be better competitors, able to acquire more resources for reproduction (e.g. Bassar et al. 2016). Larger mothers may also alter their allocation of resources among fitness components, or may simply be physically able to brood more or larger eggs (Bernardo 1996). We find that the way in which larger mothers use this resource advantage depends on the resource regime they evolved – larger mothers from low resource regimes use size advantages to make better provisioned offspring, while larger mothers from high resource regimes make more offspring. Ultimately, this yields an evolved difference in reproductive scaling – reproductive scaling was steeper in low resource environments than high resource environments. Interestingly, low resource lineages also evolved larger body sizes (at least genetically) – it may be that they evolved to be larger so as to gain the fitness advantages of increased body size that come from steeper reproductive scaling but this remains speculative. Nevertheless, to our knowledge our study is the first to demonstrate experimental evolution in reproductive scaling, which has clear consequences for population dynamics (Marshall et al. 2022).

Our findings show similarities and differences with regards to previous work. Some of the best explorations of the role of different food regimes on the evolution of life histories are in Trinidadian guppies (Felmy et al. 2022; Reznick & Travis 2019) and Bahamas mosquitofish (Hulthén et al. 2021). Both the fishes and *Tisbe sp.* exhibit similar genetic responses to food regimes, including cryptic evolution in body size and different egg size-fecundity trade-offs, although time to maturity also evolved in guppies. In contrast, mosquitofish evolved larger offspring and quicker maturation in high-food environments, in direct contrast with copepods. These variable results suggest to us at least that generalisations are not yet possible.

Interestingly, we found differences in the timing of maturity in G1 copepods but not G2, suggesting that age at maturity is a transgenerational effect rather than an evolved response. It seems to us at least that the low food parental environment programs offspring to mature sooner than offspring whose parents experience high food levels. While transgenerational plasticity in key life history traits is relatively common, we are unaware of studies that have shown such effects on the timing of maturity specifically. That this effect disappears after a single generation suggests that this parentally program trait has evolved to track environmental variation closely, with minimal persistent lags as predicted by some theory (Burgess & Marshall 2014).

Ocean primary productivity is expected to decline due to climate change (Fu et al. 2016). Warming increases upper ocean stratification , impacting nutrient fluxes and light availability for photosynthesis (Hannon et al. 2001). The effects of stratification are exacerbated as higher temperatures select for smaller phytoplankton cell size, which yield a lower total biovolume relative to larger cells at a given nutrient flux (Malerba et al. 2018). Higher temperatures may also reduce the efficiency of energy transfer from phytoplankton to copepod grazers (Barneche et al. 2021). Copepods play a vital role in ocean foodwebs, comprising the major (>75%) component of zooplankton biomass and facilitating the flow of nutrients and energy through marine food chains (Conover & Huntley 1991; Pakhomov et al. 2002). Experimental evolution in *Tigriopus* has provided some insight as to how planktonic communities may respond to the direct temperature effects of climate change, suggesting a limited capacity to evolve greater thermal tolerance (Kelly et al. 2012). Our results show that climate change-induced food scarcity will have additional evolutionary impacts – altering body size and fecundity. Given the pivotal role that copepods play in marine foodwebs, the knock effects of food-mediated evolution are unlikely to be trivial – copepods will evolve in response to changing food regimes, and this evolution is likely to have consequences for the foodchains that rely on them.

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**Table 1:** Linear mixed effects model for the effect of evolutionary treatment on adult female body size across three generations (G0, G1, G2), with ancestral G0 cultures reared within feeding blocks of four. *p* values are provided for tests of interest, significant effects are specified in bold, and random effect is specified in italics. Degrees of freedom (df) reported as numerator df, denominator df.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Effect** | **Df** | **Mean squares** | **F** | ***p*** |
| Treatment | 1, 11.67 | 5.69 x 10-1 | 0.16 | 0.70 |
| Generation | 2, 691.43 | 3.20 x 10-4 | 287.52 | **<10-15** |
| Block | 4, 11.76 | 1.95 x 10-3 | 0.98 | 0.45 |
| Treat x Gen | 2, 686.54 | 1.01 x 10-2 | 5.10 | **0.006** |
| *Culture(Treat)* |  | 1.93 x 10-3 |  |  |

**Table 2:** Linear mixed effects model for the effect of evolutionary treatment on age at maturity across two generations (G1, G2), with ancestral G0 cultures reared within feeding blocks of four. *p* values are provided for tests of interest, significant effects are specified in bold, and random effect is specified in italics. Degrees of freedom (df) reported as numerator df, denominator df.

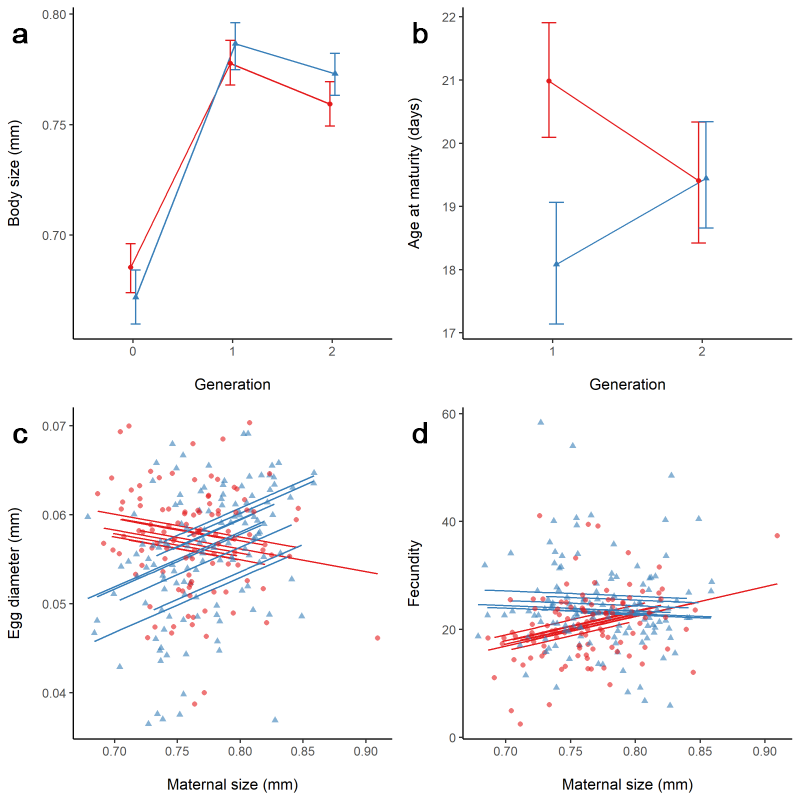
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Effect** | **Df** | **Mean squares** | **F** | ***p*** |
| Treatment | 1, 10.26 | 95.65 | 6.31 | **0.03** |
| Generation | 1, 517.99 | 2.39 | 0.16 | 0.69 |
| Block | 4, 10.44 | 15.20 | 1.00 | 0.45 |
| Treat x Gen | 1, 517.91 | 517.91 | 18.33 | **<10-4** |
| *Culture(Treat)* |  | 14.74 |  |  |

**Table 3:** Linear mixed effects models for the effects of evolutionary treatment and maternal size on mean egg size across three generations (G0, G1, G2), with ancestral G0 cultures reared within feeding blocks of four. *p* values are provided for tests of interest, significant effects are specified in bold, and random effect is specified in italics. Degrees of freedom (df) reported as numerator df, denominator df.

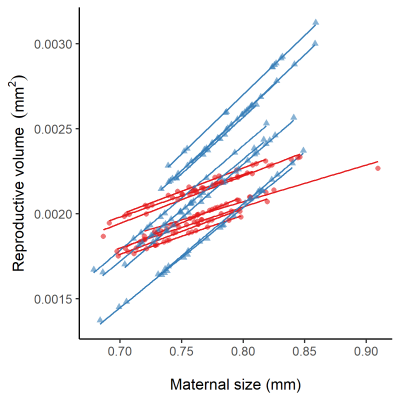
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Effect** | **Df** | **Mean squares** | | **F** | ***p*** |
| **G0** |  |  | |  |  |
| Treatment | 1, 12.98 | 1.36 x 10-4 | | 3.00 | 0.11 |
| Maternal Size | 1, 77.59 | 7.36 x 10-5 | | 1.62 | 0.21 |
| Treat x Size | 1, 95.48 | 3.12 x 10-5 | | 0.69 | 0.41 |
| Block | 4, 12.45 | 3.87 x 10-5 | | 0.85 | 0.52 |
| *Culture(Treat)* |  | 4.14 x 10-5 | |  |  |
| **G1** |  |  | |  |  |
| Treatment | 1, 12.32 | 1.83 x 10-6 | | 0.07 | 0.80 |
| Maternal Size | 1, 184.00 | 2.24 x 10-5 | | 0.84 | 0.36 |
| Treat x Size | 1, 184.00 | 1.39 x 10-5 | | 0.52 | 0.47 |
| Block | 4, 13.11 | 8.57 x 10-6 | | 0.32 | 0.86 |
| *Culture(Treat)* |  | 2.48 x 10-5 | |  |  |
| **G2** |  |  | |  |  |
| Treatment | 1, 232.57 | 6.11 x 10-4 | | 17.69 | **<10-4** |
| Maternal Size | 1, 231.65 | 9.87 x 10-4 | | 2.85 | 0.09 |
| Treat x Size | 1, 233.32 | 5.99 x 10-3 | | 17.32 | **<10-4** |
| Block | 4, 11.35 | 9.57 x 10-4 | | 2.77 | 0.08 |
| *Culture(Treat)* |  | 3.25 x 10-5 | |  |  |
|  | | |
|  | | |

**Table 4:** Linear mixed effects models for the effects of evolutionary treatment and maternal size on fecundity across three generations (G0, G1, G2), with ancestral G0 cultures reared within feeding blocks of four. *p* values are provided for tests of interest, significant effects are specified in bold, and random effect is specified in italics. Degrees of freedom (df) reported as numerator df, denominator df.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Effect** | **Df** | **Mean squares** | **F** | ***p*** |
| **G0** |  |  |  |  |
| Treatment | 1, 11.15 | 0.83 | 0.05 | 0.83 |
| Maternal Size | 1, 102.98 | 206.72 | 11.38 | **0.001** |
| Treat x Size | 1, 114.55 | 3.11 | 0.17 | 0.68 |
| Block | 4, 10.98 | 10.98 | 0.48 | 0.75 |
| *Culture(Treat)* |  | 16.10 |  |  |
| **G1** |  |  |  |  |
| Treatment | 1, 9.99 | 8.75 | 0.27 | 0.62 |
| Maternal Size | 1, 183.48 | 863.73 | 26.38 | **<10-6** |
| Treat x Size | 1, 183.95 | 0.99 | 0.03 | 0.86 |
| Block | 4, 10.57 | 9.39 | 0.29 | 0.88 |
| *Culture(Treat)* |  | 30.30 |  |  |
| **G2** |  |  |  |  |
| Treatment | 1, 234 | 383.72 | 7.44 | **0.007** |
| Maternal Size | 1, 234 | 165.44 | 3.86 | 0.051 |
| Treat x Size | 1, 234 | 342.10 | 6.69 | **0.01** |
| Block | 4, 234 | 271.44 | 5.36 | **<10-3** |
| *Culture(Treat)* |  | 49.71 |  |  |



**Figure 1:** Key life history responses to high (red, circular points) and low (blue, triangular points) food regimes. Panels show a: mean adult female body length across all three generations, b: mean cohort age at first observation of sexual maturity (within each replicate) in G1 and G2, c: mean first-clutch egg diameter in G2 mothers, d: mean first-clutch fecundity in G2 mothers. In a and b: errors bars show between-culture bootstrapped 95% confidence intervals. In c and d: points show raw data at the sub-replicate (individual female) level, lines show regressions at the culture level.



**Figure 2:** Predicted relationships between reproductive volume (product of mean egg volume and fecundity) and maternal size in G2 mothers from high-food (solid red lines, circular points) and low-food (dotted blue lines, triangular points) regimes. Points show data at the individual level, lines show regressions at the culture level, data obtained from G2 egg-size and G2 fecundity model coefficients.