Copepod life history evolution under high and low food regimes

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Abstract

Copepods play a critical role in the carbon cycle of the planet – they mediate the sequestration of carbon into the deep ocean, and are the trophic link between phytoplankton and marine foodwebs. Global change stressors that decrease copepod productivity create the potential for catastrophic positive feedback loops. Accordingly, a growing list of studies examine the evolutionary capacity of copepods to adapt to the two primary stressors associated with global change: warmer temperatures and lower pH. But the evolutionary capacity of copepods to adapt to changing food regimes, the third major stressor associated with global change, remains unknown. We used experimental evolution to explore how a 10-fold difference in food availability affects life history evolution in the copepod, *Tisbe sp*. over two years, and spanning 30+ generations. Different food regimes evoked evolutionary responses across the entire copepod life history: we observed evolution in body size, size-fecundity relationships and offspring investment strategies. Our results suggest that changes to food regimes reshape life histories and that cryptic evolution in traits such as body size is likely. We demonstrate that evolution in response to changes in ocean productivity will alter consumer life histories, and may distort trophic links in marine foodchains. Evolution in response to changing phytoplankton productivity may alter the efficacy of the global carbon pump in ways that have not been anticipated until now.

Introduction

26 The oceans account for around 50% of carbon sequestration (Sabine *et al.* 2004). Phytoplankton are
27 the primary producers that account for most marine carbon fixation (del Giorgio & Duarte 2002), but
28 only a small proportion is sequestered into the deep sea (Dunne *et al.* 2007). Copepods play a key
29 role here, they feed on phytoplankton, and excrete them in faecal pellets that much sink faster than
30 individual phytoplankton cells. Of the total biomass fixed every year, up to 15-58% is consumed by
31 copepods during some times of the year, and 7.4-29 gigatonnes of carbon are annually consumed on
32 average (Steinberg & Landry 2017). Given the massive biomass turnover between phytoplankton
33 and copepods, anything that affects the dynamics of this trophic link has significant consequences
34 for the efficacy global carbon pump. If global change affects copepods, then the carbon pump will
35 also be affected. Worse still, a positive feedback loop could be generated: increasing atmospheric
36 CO_2 increases stressors (e.g. higher temperatures, lower seawater pH), decreasing copepod
37 productivity, decreasing carbon sequestration rates, leading to more rapid increases in atmospheric
38 CO_2. The capacity for copepods to adapt to global change represents a key uncertainty in making
39 predictions for the future of the global carbon pump.

40 For organisms with relatively short lifespans, predicting their capacity to adapt to global change
41 requires an understanding of their evolutionary capacity to adapt to future conditions (Munday *et al.*
42 2013; Kelly & Griffiths 2021). Accordingly, a growing number of studies estimate how copepods
43 adapt to higher temperature regimes and lower pH – the two primary stressors associated with
44 global change in the sea (Brennan *et al.* 2021, 2022; deMayo *et al.* 2021; Sasaki & Dam 2021). While
45 temperature and pH are undoubtedly important, they are not the only factors that will change in
46 future oceans – food availability is likely to be dramatically different for copepods under future
47 regimes (Fu *et al.* 2016), and indeed is already changing (Capuzzo *et al.* 2018).

48 Phytoplankton depend on sunlight for photosynthesis, so they are most productive in the surface
49 waters of the ocean – the productivity of these surface waters are likely to alter with global change.
50 The upper, sunlit layers of the ocean are depleted of nutrients by growing phytoplankton and must

51 replenished by cooler, nutrient-rich water from deeper, darker layers. Unfortunately, global warming
52 can create more stratification in the water column, generating more intense thermoclines, and
53 reducing the rate of mixing between water layers (Hannon *et al.* 2001). This thermal shoaling is likely
54 to reduce phytoplankton productivity in some places by up to 40% (Jang *et al.* 2011). On the other
55 hand, some regions are predicted to experience more intense storms, which will actually enhance
56 mixing of surface waters with deeper layers, possibly increasing the productivity in those places
57 (Nicholson *et al.* 2016). Thus, copepods must not only evolve to cope with warmer, more acidic
58 oceans, they are also likely to experience massive changes to their food regimes. The capacity for
59 evolution in response to such changes remains unclear.

60 Food regimes have classically been recognised as key drivers of life history evolution across a broad
61 range of species (Macarthur & Levins 1967; Tilman 1982; Chesson 2000; Grant & Grant 2006), but
62 the direction and nature of such effects remain debated. For example, guppies in food-scarce
63 environments evolve larger body sizes (Felmy *et al.* 2022) while flies get smaller (Kolss *et al.* 2009).
64 As such, it is difficult to predict how, or even if, copepods will evolve in response to changes in food
65 based on studies in other systems. Biogeographical studies are similarly ambiguous. For example,
66 some imply that copepods should become larger under low productivity regimes but whether body
67 size is decreasing because of food availability *per se* or other covarying factors such as predation risk
68 remains unclear (Brun et al. 2016). As far as we are aware, no study has directly addressed the
69 capacity of copepods to evolve in response to different food regimes. We sought to address this
70 important knowledge gap here. We subjected copepod populations to either high-food or low-food
71 environments for 2 years and then used multigenerational common garden experiments to examine
72 how life histories have evolved independently of any cross-generational parental effects (Burgess &
73 Marshall 2014).

74 **Materials and Methods**

75 *STUDY ORGANISM*

76 *Tisbe sp.* is a littoral marine copepod from the Tisbidae family (Arthropoda: Harpacticoida) that has
77 not been resolved to species level in the Southern Ocean. Mothers brood clutches of approximately
78 10-40 eggs on abdominal egg-sacs for 1-3 days. The naupliar larvae are facultative planktotrophs,
79 and pass through 6 stages before metamorphosing into a juvenile copepodid approximately 3-5 days
80 after hatching with *ad libitum* food. Juveniles pass through a further 6 copepodid stages before
81 reaching the final adult and sexually mature stage at approximately 18-20 days old. We collected
82 copepods from Port Phillip Bay, Australia in 2017 and maintained these 'ancestral' populations in
83 gently oxygenated 500 ml mason jars in freshly pasteurised seawater (FSW). Ancestral populations
84 were reared on the marine microalga *Dunaliella tertiolecta* and fed at a rate of 2.475 x 10^9 algae cells
85 per litre of copepod culture per week.

86 *Dunaliella* cultures were maintained using F2 media (Guillard & Ryther 1962) and algae concentrate
87 for feeding copepod populations was prepared three times a week. Density of algal cultures was
88 determined spectroscopically using a SPECTROstar Nano and algal concentrations were adjusted to
89 approximately 1.1 x 10^{10} *D. tertiolecta* cells/L in FSW after centrifuging and removing media.
90 Ancestral populations were fed manually 3 times a week, while for experimental cultures housed in
91 flow-through culture vessels feeding was automated (see Experimental Evolution).

92 *EXPERIMENTAL EVOLUTION*

93 *Experimental design*

94 Experimental evolution commenced on the 13th of October 2018 at Monash University Clayton
95 Campus, Melbourne, Australia. Copepods from ancestral stocks were randomly assigned to either
96 high-food or low-food environments, which differed in their rate of food supply (of *D. tertiolecta*
97 *cells*) by an order of magnitude. A Folsom plankton splitter was used to ensure equal numbers of
98 copepods were assigned to each culture and the 1L glass culture vessels were topped up with FSW.
99 In total, 20 copepod cultures were subjected to experimental evolution, consisting of 10 high-food

100 and 10 low-food replicates reared in separate glass pressure-equalising dropping funnels. One low-
101 food replicate went extinct one year into the experiment due to bacterial contamination.

102 High and low food rates were determined through pilot experiments in 2018 and were set as
103 follows: high food replicates received 4.5 x 10^9 algae cells per litre of copepod culture per week, and
104 low food replicates received 4.5 x 10^8 algae cells per litre of copepod culture per week. An
105 intermediate feeding rate (2.475 x 10^9 algae cells per litre of copepod culture) was used for the
106 ancestral populations and the common garden. Differential food supply in experimental treatments
107 was ramped up gradually, with high and low food treatments receiving the same intermediate rate
108 of supply for the first week, partial treatments in week two (3.5 x 10^9 and 1.5 x 10^9 *D. tertiolecta* cells
109 per week, respectively), and final treatments of high or low food supply by week three after
110 initiating the experiment. These treatment differences were then maintained for the next 16
111 months.

112 Experimental cultures were organised into 5 feeding blocks of four (in a randomised sequence of 2
113 high-food and 2 low-food replicates) due to spatial constraints. Each block received food from a
114 separate algae reservoir using a Kamoer X4 peristaltic dosing pump. Both high-food and low-food
115 treatments received a total inflow of 80mL per litre of culture per day (on weekdays only, no dosing
116 on weekends). High-food treatments were dosed with 80mL of algae concentrate per day, while low-
117 food treatments received 8 mL of algae concentrate and 72mL of FSW (i.e. 10% of the high food
118 regime). Pumps provisioned algae concentrate semi-continuously by dosing 12 times a day. Pilot
119 work indicated that very few adult copepods were lost at this rate of inflow-outflow. Algae and FSW
120 reservoirs were covered to minimise evaporation and cleaned weekly to minimise build-up of waste
121 and pathogens, and algae was kept well-mixed using magnetic stirrers.

122 *COMMON GARDENS*

123 *Experimental design*

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

142 Paired high- and low-food cultures were randomly sampled between the 18th and 20th of February
143 2020. 10 gravid G0 mothers were collected from each culture, photographed, and transferred to
144 sterile plastic culture trays containing 4 mL FSW. 16uL of 10000 units mL^{-1} (approximately 6 mg mL^{-1})
145 penicillin G and 10 mg ml^{-1} streptomycin solution (Sigma-Aldrich) was added to each tray to inhibit
146 the growth of pathogens (Gangur & Marshall 2020). G0 mothers were monitored daily and returned
147 to their cultures after releasing their G1 eggs, until all eggs had hatched and all G0 mothers had been
148 removed (generally 3-5 days after initial collection). All common garden replicates commenced G1
149 with >100 larvae.

150 Throughout the experiment, *D. tertiolecta* was provisioned at an intermediate level of food supply.
151 For G0, G1, and G2 juveniles and adults, food was provisioned each weekday (5 times a week) in a
152 176 uL pulse from a 1.1 x 10^7 cells/mL stock representing an intermediate food supply of
153 approximately 2.475 x 10^9 cells per litre per week. Due to their lower feeding rate, larvae were
154 provisioned a single 176uL (at 1.1 x 10^7 cells/mL) pulse of food to achieve the same maximum
155 ambient food density experienced by adults under an intermediate feed regime (approx. 5 x 10^6 *D*
156 *tertiolecta* cells per mL each day).

157 *Data collection*

158 Freshly hatched G1 larvae within each replicate were counted and randomly allocated to individual
159 culture trays of 25 ± 5 individuals with 4 mL FSW, antibiotics, and food. When metamorphosis was
160 first observed within a replicate, the replicate was censused and individuals were transferred to
161 fresh trays with 4 mL FSW, antibiotics, and food. For juveniles, water was then changed and
162 censusing was conducted weekly until sexual maturity was first observed within a replicate. Then
163 subreplicates were censused, pooled, mixed, and reallocated into new adult subreplicates of 25 ± 5
164 with fortnightly water changes and censusing. All reproductive mothers were collected and
165 photographed with their first clutch. Mothers and their egg sacs were photographed, then pooled at
166 the replicate level into fresh culture trays containing 4 mL FSW, antibiotics, and food. At least 5 G1
167 mothers and 50 offspring were obtained for 17 of 19 replicates (8 high-food and 9 low-food
168 cultures), and these G2 offspring were collected for the final stage of the experiment. Replicates
169 containing copepods from the two remaining high-food cultures were accidentally dropped before
170 reaching G2.

171 G2 larvae were collected from culture trays containing gravid G1 mothers in a similar fashion to G1
172 larvae collection from G0 mothers, but we also accounted for temporal staggering. For each
173 replicate, G2 larval collection took place over a week after the first clutch hatched. Larvae collected
174 during this week were continually transferred to culture trays at a density of 25 ± 5 individuals with 4

175 mL FSW, antibiotics, and food. At the end of the collection week these larvae (some of which had
176 metamorphosed) were re-pooled, mixed, and randomly allocated to new culture trays of 25 ± 5
177 individuals in 4 mL FSW, antibiotics, and food. G2 larvae that hatched outside this initial collection
178 week were retained but reared in separate trays. G2 larvae were then reared to sexual maturity and
179 reproductive G2 mothers were measured following the same protocol used for G1.

180 *STATISTICAL ANALYSES*

181 All phenotypes were analysed using linear mixed effects models. Full models for size, survival, and
182 age at maturity included treatment and generation as fixed effects, as well as their interaction.
183 Fecundity and egg size were modelled separately for each generation due to complex interactions
184 and included mother size as a fixed covariate, as well as its interaction with treatment. G0 feeding
185 block was included as a fixed effect in all models due to insufficient replication to treat as a random
186 effect. All models also included culture nested within treatment as a random intercept term. Where
187 interaction terms were nonsignificant they were removed and the analysis was repeated. Models
188 were evaluated using type III tests due to imbalance of high-food and low-food replicates. *p* values
189 for relevant fixed effects were obtained with *F* tests using Sattertwaithe's approximation
190 (Kuznetsova *et al.* 2017).

191 Analyses were performed with R version 4.1.2 (R Core Team 2021) and RStudio version 2021.09.1
192 (RStudio Team 2021), using dplyr (Wickham et al. 2021) to prepare the data. Linear mixed effect
193 models were fitted with lme4 (Bates et al. 2015). The lmerTest package (Kuznetsova et al. 2017) was
194 used to perform Sattertwaithe's approximations and type III tests on fixed effects, and likelihood
195 ratio tests on random effects. Bootstrapped 95% confidence intervals were obtained between
196 cultures using merTools (Knowles & Frederick 2020), and plots were built using ggplot2 (Wickham
197 2016). Diagnostic residuals vs fits and QQ plots were visually assessed as per Keough & Quinn (2002),
198 and VIF calculated to check for collinearity. Predictors were plotted against each other to visually
199 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 the 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 many 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

224 by a strong parental environment effects that dissipated across generations. Copepods evolved to be
225 slightly larger in low food regimes, and within those regimes, larger mothers invested more in their
226 individual offspring in this environment. In high food regimes, copepods evolved to be smaller and
227 within those regimes, larger mothers invested less in their individual offspring but were much more
228 fecund. Interestingly, we found evidence for differences in evolved responses to different resource
229 environments relative to the expressed phenotypes in those environments, indicating
230 countergradient evolution. Overall, our results suggest that the changes in food regimes predicted to
231 occur in future oceans will generate life history evolution in copepods but not in straightforward
232 ways. Our results also imply that biogeographical patterns in life history and covariance between
233 productivity and phenotypes may provide very little information or predictive power about the
234 underlying genetic clines in these traits due countergradient variation.

235 Copepods were smaller in the low food regime but their offspring grew to be larger when
236 transferred to common environment – indicating countergradient evolution in body size. Assuming
237 cubic scaling with length, copepods from low-food environments were only 5.9% smaller by volume.
238 Once released from the low-food conditions, they were 5.6% larger than copepods from high-food
239 lineages, suggesting that the impact of food scarcity was moderated by genetic compensation
240 (Grether 2005). Such countergradient variation is observed in field studies as well and seems
241 particularly common in fish (Arendt & Wilson 1999; Conover et al. 2009). We suggest that global
242 changes to phytoplankton productivity will evoke evolutionary change in copepod body sizes but
243 that these changes may be masked by countergradient evolution. Studies seeking to understand
244 how copepod body sizes have changed, and continue to change should consider common garden
245 experiments to disentangle phenotypic and genetic responses, which may counteract each other,
246 resulting in what is sometimes called 'cryptic evolution' (Grether 2005).

247 The relationship between body size and reproductive investment evolved, albeit in subtle ways. In
248 low food environments, egg size increased with maternal size at the expense of fecundity while,

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

255 The way reproduction scaled with maternal size showed clear evolutionary responses to food
256 regime. Regardless of evolutionary lineage, larger mothers reproduced more than smaller mothers,
257 implying larger mothers have more resources to invest in offspring. Larger individuals may be better
258 competitors, able to acquire more resources for reproduction (e.g. Bassar et al. 2016). Larger
259 mothers may also alter their allocation of resources among fitness components, or may simply be
260 physically able to brood more or larger eggs (Bernardo 1996). We find that the way in which larger
261 mothers deploy this resource advantage relative to smaller mother depends on the resource regime
262 in which they evolved – in low resource regimes, they make better provisioned offspring, in high
263 resource regimes, they make more offspring. These different allocation patterns yield an evolved
264 difference in reproductive scaling – reproductive scaling was steeper in low resource environments
265 than high resource environments. Interestingly, low resource lineages also evolved larger body sizes
266 (at least genetically) – it may be that they evolved to be larger so as to gain the fitness advantages of
267 increased body size that come from steeper reproductive scaling but this remains speculative.
268 Nevertheless, to our knowledge our study is the first to demonstrate experimental evolution in
269 reproductive scaling, which has clear consequences for population dynamics (Marshall et al. 2022).
270 Generally theory predicts that when reproductive scaling is steeper, population replenishment and
271 productivity depends more strongly on having larger females in population (Marshall et al. 2022).
272 Given smaller body sizes are expected under higher temperatures (Atkinson & Sibly 1997), our
273 results suggest that combining high temperature regimes (the redaction of larger females) and low
274 food conditions (steeper reproductive scaling) could synergise to generate catastrophic reductions in

275 population productivity. An important next step will be to examine how combined stressor regimes
276 (e.g. temperature and food) affect evolutionary trajectories.

277 We found differences in the timing of maturity in G1 copepods but not G2, suggesting that age at
278 maturity is a transgenerational plasticity effect rather than an evolved response. It seems to us at
279 least that the low food parental environment programs offspring to mature sooner than offspring
280 whose parents experience high food levels. While transgenerational plasticity in key life history traits
281 is relatively common (Yin *et al.* 2019), we are unaware of studies that have shown such effects on
282 the timing of maturity specifically. That this effect disappears after a single generation suggests that
283 this parentally program trait has evolved to track environmental variation closely, with minimal
284 persistent lags as predicted by some theory (Burgess & Marshall 2014).

285 In summary, we show that differing food regimes induce rapid (in less than 50 generations)
286 evolutionary responses, affecting every aspect of their life history from offspring size, through to
287 growth and reproduction. These evolutionary responses may maximise the fitness of individuals in
288 their particular food regimes but will undoubtedly wreak changes to the productivity of whole
289 populations. None of the responses we observed were entirely predictable based on existing theory
290 or studies in other systems. Our findings emphasise that evolution will alter and complicate
291 biological responses to global change – with concomitant changes to global food webs that cannot
292 be anticipated based on ecological experiments alone. An important next step is to understand the
293 eco-evolutionary consequences of copepod life history evolution for higher trophic links – how do
294 planktivores that depend on copepods evolve in response to copepod evolution themselves?

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407 **Table 1:** Linear mixed effects model for the effect of evolutionary treatment on adult female body
408 size across three generations (G0, G1, G2), with ancestral G0 cultures reared within feeding blocks of
409 four. *p* values are provided for tests of interest, significant effects are specified in bold, and random
410 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	5.69×10^{-1}	0.16	0.70
Generation	2, 691.43	3.20×10^{-4}	287.52	**$<10^{-15}$**
Block	4, 11.76	1.95×10^{-3}	0.98	0.45
Treat x Gen	2, 686.54	1.01×10^{-2}	5.10	**0.006**
Culture(Treat)		1.93×10^{-3}		

411

412 **Table 2:** Linear mixed effects model for the effect of evolutionary treatment on age at maturity
413 across two generations (G1, G2), with ancestral G0 cultures reared within feeding blocks of four. *p*
414 values are provided for tests of interest, significant effects are specified in bold, and random effect is
415 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		

416

417

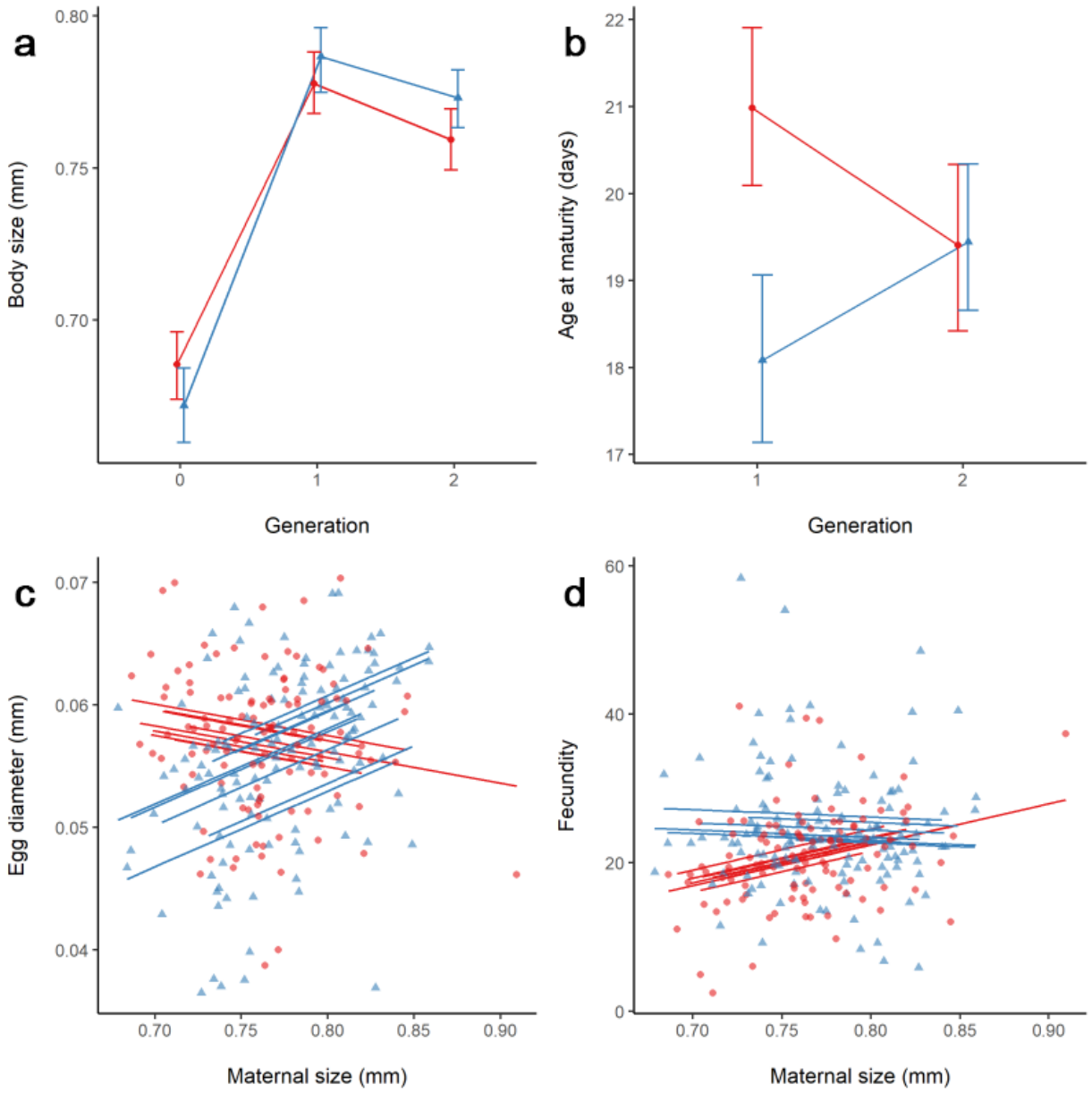
418 **Table 3:** Linear mixed effects models for the effects of evolutionary treatment and maternal size on
419 mean egg size across three generations (G0, G1, G2), with ancestral G0 cultures reared within
420 feeding blocks of four. *p* values are provided for tests of interest, significant effects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

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

423 **Table 4:** Linear mixed effects models for the effects of evolutionary treatment and maternal size on
424 fecundity across three generations (G0, G1, G2), with ancestral G0 cultures reared within feeding
425 blocks of four. *p* values are provided for tests of interest, significant effects are specified in bold, and
426 random effect is specified in italics. Degrees of freedom (df) reported as numerator df, denominator
427 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		

428



429

430 **Figure 1:** Key life history responses to high (red, circular points) and low (blue, triangular points) food

431 regimes. Panels show a: mean adult female body length across all three generations, b: mean cohort

432 age at first observation of sexual maturity (within each replicate) in G1 and G2, c: mean first-clutch

433 egg diameter in G2 mothers, d: mean first-clutch fecundity in G2 mothers. In a and b: errors bars

434 show between-culture bootstrapped 95% confidence intervals. In c and d: points show raw data at

435 the sub-replicate (individual female) level, lines show regressions at the culture level.

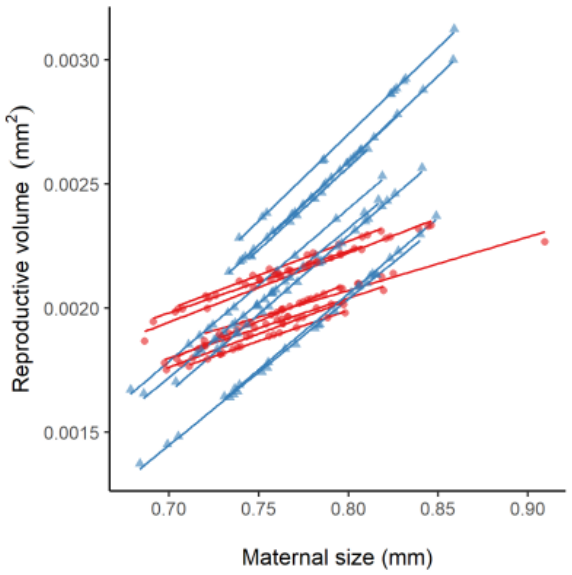


Figure 2: Predicted relationships between reproductive volume (product of mean egg volume and fecundity) and maternal size in G2 mothers from high-food (solid red lines, circular points) and low-food (dotted blue lines, triangular points) regimes. Points show data at the individual level, lines show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